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# Chromatography Applications

Searchable by technique, industry, and analyte

**Volume 1: HPLC, UHPLC, LC-MS, SFC, SPE**
Volume 2: GC, GC-MS, SPME, Air Monitoring
Volume 3: Chiral

# Explore the Solutions Within

Method development can be a time-consuming part of the chromatographic process. Finding the best column, sample prep technique, mobile phase or carrier gas, and detection method is a challenging process. Just having a starting point can go a long way in reducing method development time.

Sigma-Aldrich has developed thousands of applications showing commonly analyzed compounds across many fields of research and testing.

This tool is interactive, searchable, and organized by research market focus within analytical technique, analyte, compound class, and key word. Each application includes links to the website which features additional information and convenient ordering.

This tool demonstrates how Sigma-Aldrich's analytical brands—Supelco, Fluka, Cerilliant, and RTC—are uniquely able to provide customers with a wide spectrum of chromatographic components across the analytical workflow:

- Sample collection and sample prep devices
- UHPLC, HPLC, LC-MS columns
- GC columns
- Chiral columns
- Mobile phase solvents
- Buffers and additives
- Derivitization reagents
- Analytical standards

Sigma-Aldrich continually adds new applications to its portfolio. If you have a suggestion for an application not found in this notebook, contact **techservice@sial.com**.

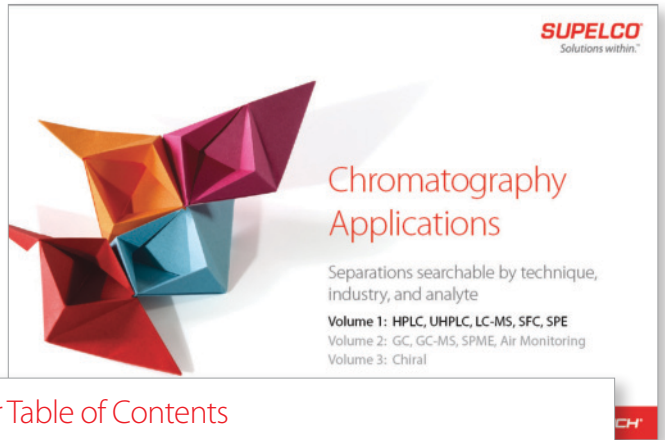
# Expanded Publication

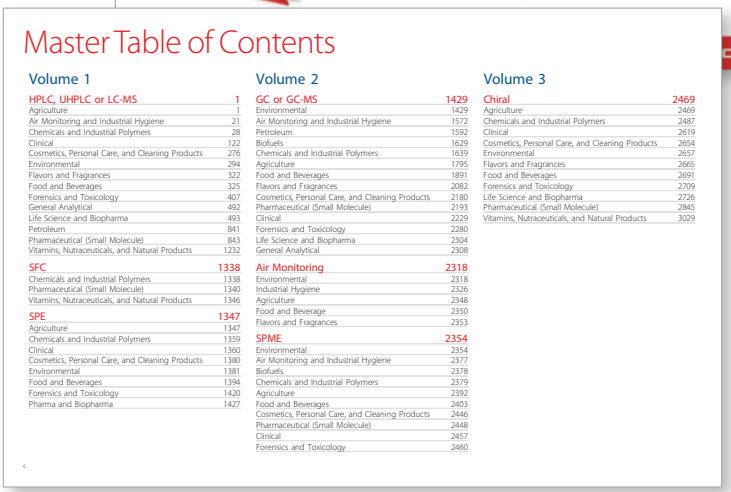
## Learn How to Navigate the New Format

This expanded edition of the Chromatography Applications Notebook consists of three separate, but linked, volumes. Note that the application pages are numbered consecutively, **Volume 1** through **Volume 3**, beginning with 1.

Inside each volume is a **Master Table of Contents** and a comprehensive **Analyte Index**. These pages can be used to navigate from one volume to another.

When searching in the index, the page number(s) will help indicate the correct volume to visit. Volume page number keys are found in the index footer.



## Master Table of Contents

# Master Table of Contents

## Volume 1

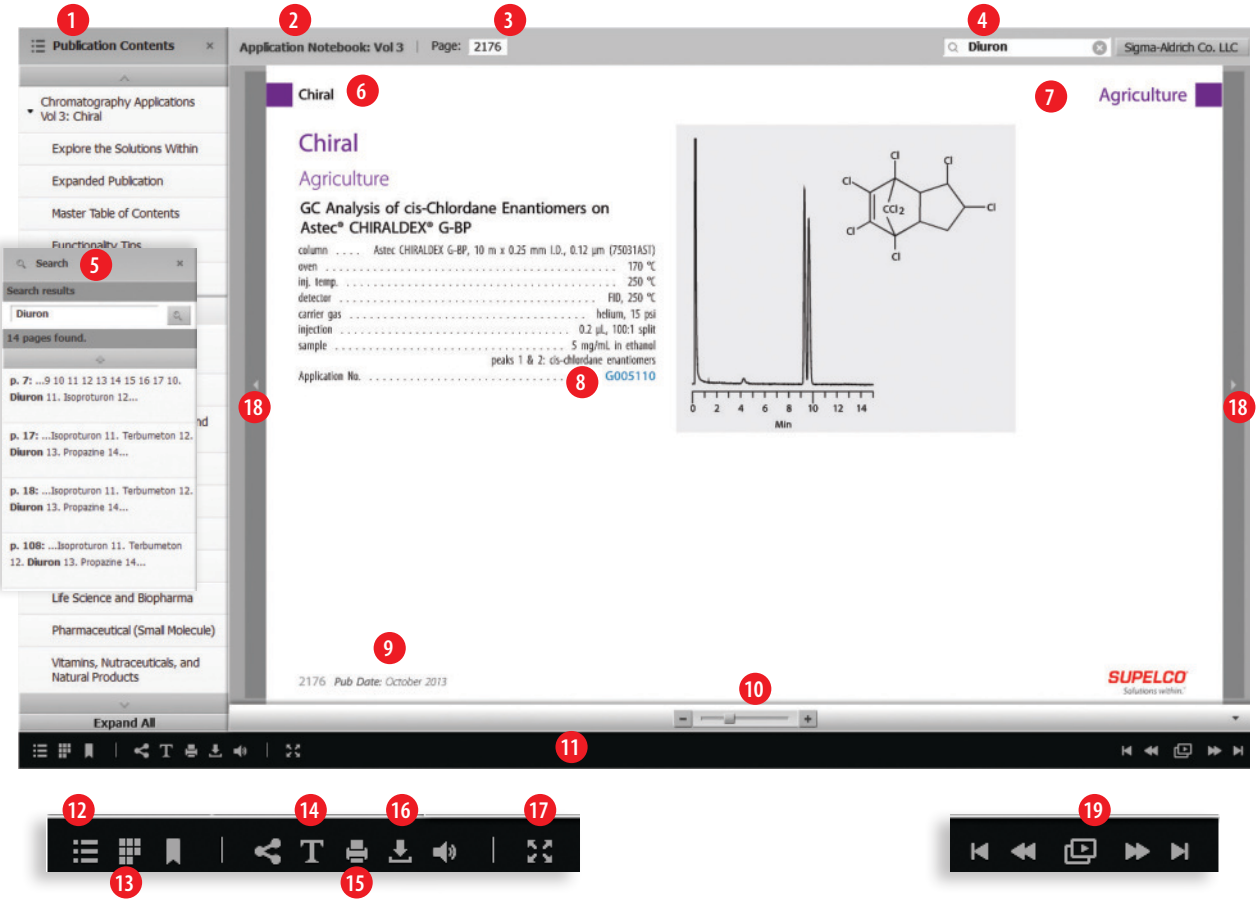


## Volume 2



## Volume 3

# Functionality Tips




## Legend

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# HPLC, UHPLC or LC-MS

## Agriculture

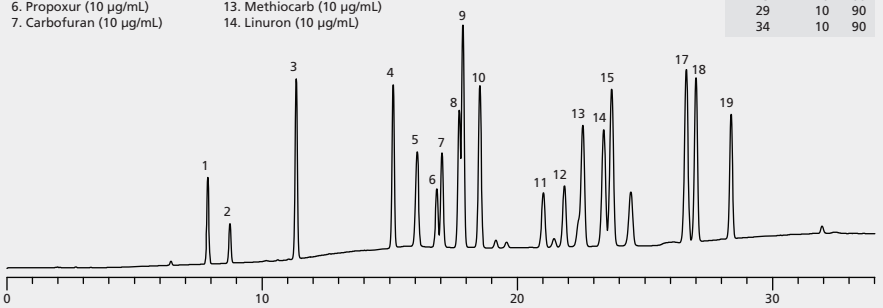
### EPA Method 632: HPLC Analysis of Pesticides on Ascentis® C18

column . . . . . Ascentis C18, 25 cm x 4.6 mm I.D., 5 µm particles (581325-U)
mobile phase . . . . . (A) water; (B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 16% acetonitrile in water
Application No. . . . . G003194

1. Oxamyl (10 µg/mL)
2. Methomyl (10 µg/mL)
3. Fenuron (10 µg/mL)
4. Monuron (10 µg/mL)
5. Aminocarb (10 µg/mL)
6. Propoxur (10 µg/mL)
7. Carbofuran (10 µg/mL)
8. Fluomethuron (10 µg/mL)
9. Carbaryl (10 µg/mL)
10. Diuron (10 µg/mL)
11. Propham (10 µg/mL)
12. Siduron (10 µg/mL)
13. Methiocarb (10 µg/mL)
14. Linuron (10 µg/mL)
15. Swep (10 µg/mL)
16. Mexacarbate (10 µg/mL)
17. Chlorpropham (10 µg/mL)
18. Barban (10 µg/mL)
19. Neburon (10 µg/mL)

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 13 | 50 | 50 |
| 23 | 40 | 60 |
| 29 | 10 | 90 |
| 34 | 10 | 90 |



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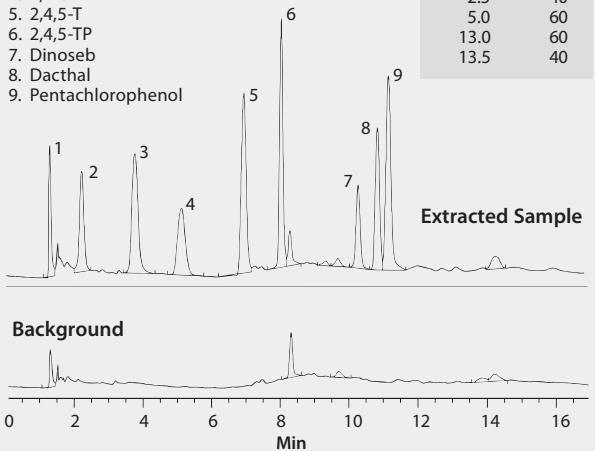
## HPLC Analysis of Acidic Herbicides in Water on a Polymeric C18 Column after SPE using Supelclean™ ENVI™-Carb

*using Zymark AutoTrace Extraction WorkStation 1.20*

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . Fresh 1 L water samples, dechlorinated with sodium thiosulfate when necessary, at ambient temperature and pH.
SPE tube/cartridge . . . . . Supelclean ENVI-Carb, 250 mg/6 mL (57092)
condition . . . . . 10 mL DI water at 20 mL/min
sample addition . . . . . 0.9 L water sample at 20 mL/min
drying . . . . . 10 min using clean nitrogen
washing . . . . . 10 mL DI water at 20 mL/min
elution . . . . . 10 mL 0.1% phosphoric acid in methylene chloride:acetonitrile (80:20) at 5 mL/min
column . . polymeric-coated silica-based PAH specialty column, 20 cm × 3 mm I.D., 5 µm (Supelco equivalent, SUPELCOSIL LC-PAH, available upon request)
mobile phase . . gradient, (A): 0.05% phosphoric acid in DI water; (B): acetonitrile
flow rate . . . . . 0.5 mL/min
column temp. . . . . 50 °C
detector . . . . . photodiode array- peak width: 0.053 min, sampling interval: 0.320 sec, monitor 210 nm & 225 nm
injection . . . . . 10 µL of extract (4-5 ppb each analyte in water)
Application No. . . . . 796-0150

1. Tetrachloroterephthalic acid
2. Picloram
3. Dicamba
4. 2,4-D
5. 2,4,5-T
6. 2,4,5-TP
7. Dinoseb
8. Dacthal
9. Pentachlorophenol

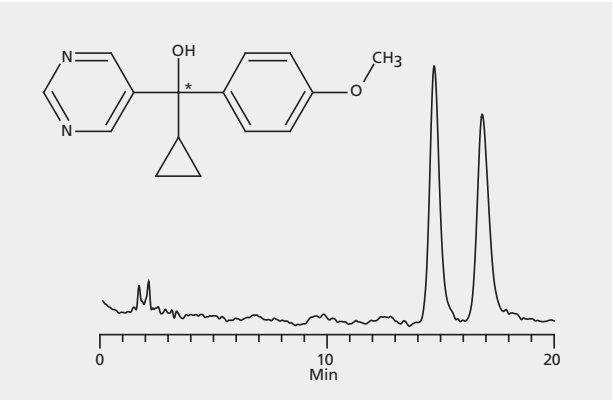
| Gradient Program | |
|---|---|
| Time (min) | %B |
| 2.5 | 40 |
| 5.0 | 60 |
| 13.0 | 60 |
| 13.5 | 40 |




Figures provided by A. Lichtman, Nassau County Dept. of Health, Hempstead, New York, USA. Request Application Note No. T196100.

## HPLC Analysis of Ancymidol Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

| | |
|---|---|
| column | CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST) |
| mobile phase | (A) 5 mM ammonium acetate, pH 6.0; (B) acetonitrile; (80:20, A:B) |
| flow rate | 1 mL/min |
| column temp. | 35 °C |
| detector | UV, 215 nm |
| injection | 5 µL |
| sample | 1 mg/mL in methanol |
| Application No. | G004684 |



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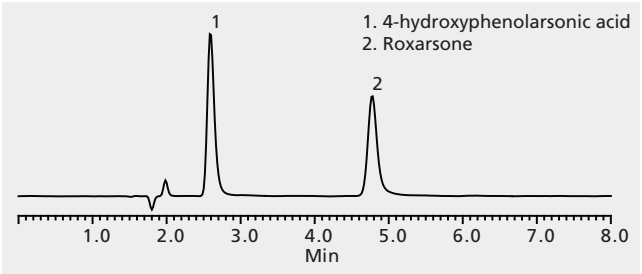
## HPLC Analysis of Animal Feed Additives on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formic acid); (B) methanol; (78:22, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 225 nm
injection . . . . . 5 µL
sample . . . . . as indicated in mobile phase A
Application No. . . . . G003712



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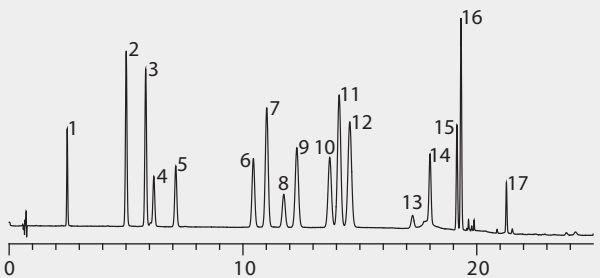
## HPLC Analysis of Atrazine Herbicides on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of 17 triazine pesticides.

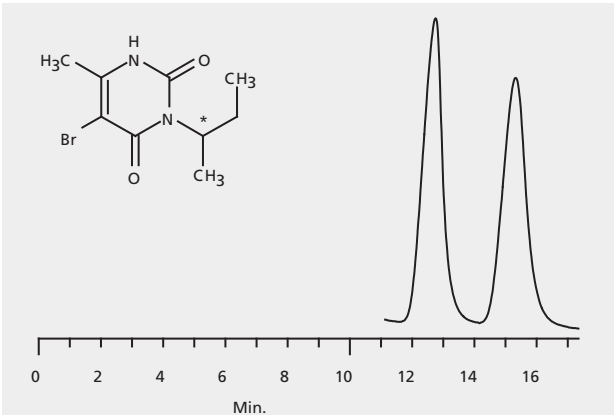
column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . (A) 20 mM ammonium acetate, pH 6.4 unadjusted; (B) acetonitrile
gradient . . 20 to 28% B in 11 min; 28 to 65% B in 5 min; held at 65% B for 4 min
flow rate . . . . . 0.6 mL/min
column temp. . . . . 46 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . 10-25 µg/mL in 10:90, water: acetonitrile
Application No. . . . . G005654

1. Desethylatrazine
2. Metoxuron
3. Hexazinone
4. Simazine
5. Cyanazine
6. Methabenzthiazuron
7. Chlorotoluron
8. Atrazine
9. Monolinuron
10. Diuron
11. Isoproturon
12. Metobromuron
13. Metazachlor
14. Sebuthylazine
15. Terbutylazine
16. Linuron
17. Metolachlor



## HPLC Analysis of Bromacil Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . (A) water; (B) ethanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004595



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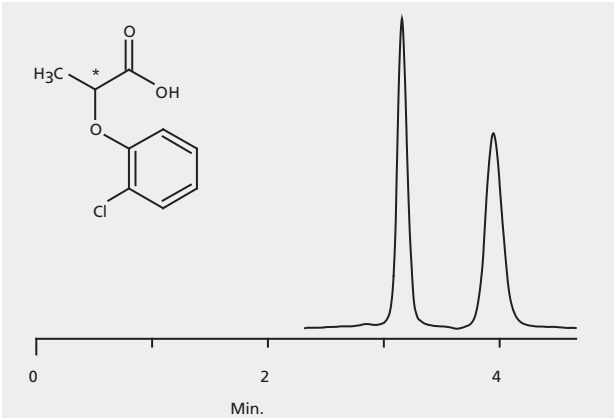
## HPLC Analysis of 2(2-Chlorophenoxy) Propionic Acid Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 0.1 wt% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 235 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004579



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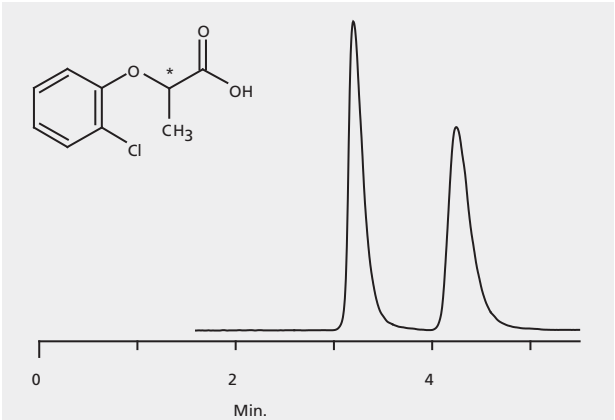
## HPLC Analysis of 2(2-Chlorophenoxy) Propionic Acid Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . 0.1 wt% ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004606



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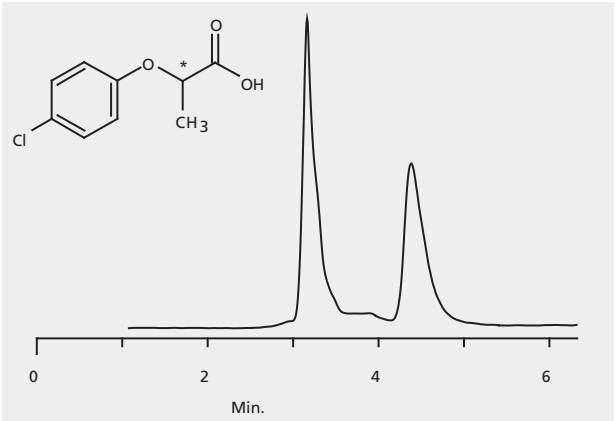
## HPLC Analysis of 2(4-Chlorophenoxy) Propionic Acid Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . 0.1 wt% mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004607



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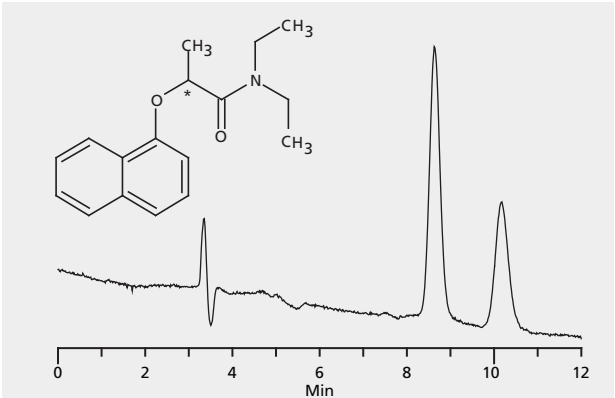
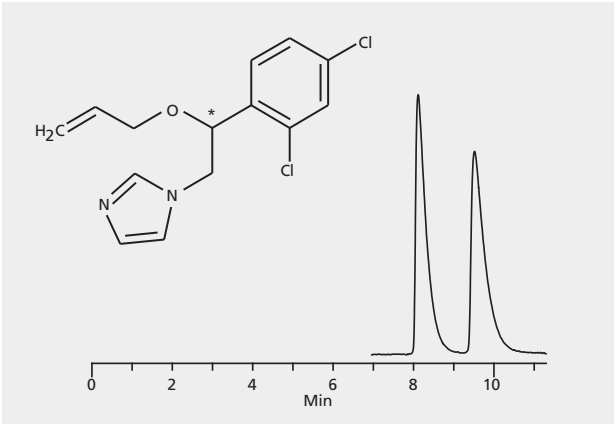
## HPLC Analysis of Devrinol (Napropamide) Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 0.1% triethylamine, pH 5.0; (B) THF; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004622



## HPLC Analysis of Enilconazol (Imazalil) Enantiomers on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . (A) 0.1% triethylamine acetate, pH 4.1; (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 0.7 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004690



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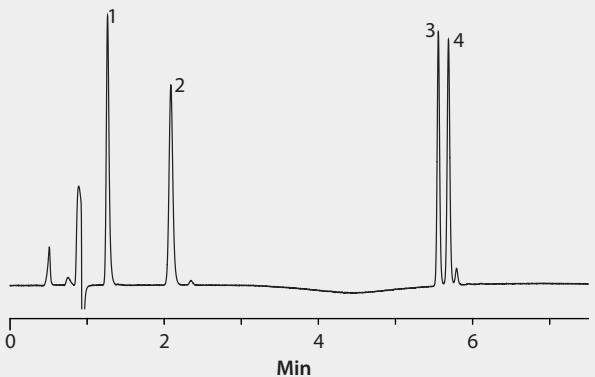
## HPLC Analysis of Fusarium Mycotoxins on Ascentis® Express Phenyl-Hexyl

The trichothecene vomitoxins of Fusarium are significant plant pathogens of grain and forage crops that can lead to anorexic effects with livestock. This application demonstrates resolution of deoxynivalenol and three of its analogs.

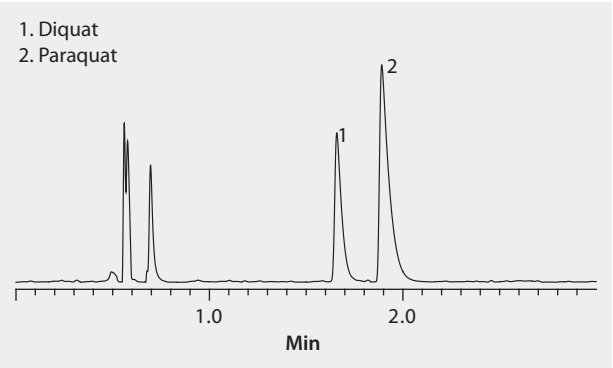
| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 10 cm x 2.1 mm I.D., 2.7 µm particles (53336-U) |
| mobile phase | (A) water, acetonitrile, 90:10; (B) acetonitrile |
| gradient | 0% B held for 0.5 min; to 15% B in 1 min; held at 15% B for 6 min |
| flow rate | 0.3 mL/min |
| pressure | 192 bar (2780 psi) |
| column temp. | 30 °C |
| detector | UV, 218 nm |
| injection | 10 µL |
| sample | 5 mg/L ea. in 95:5, water:acetonitrile |
| Application No. | G005539 |

1. Nivalenol
2. Deoxynivalenol
3. 15-Acetyldeoxynivalenol
4. 3-Acetyldeoxynivalenol



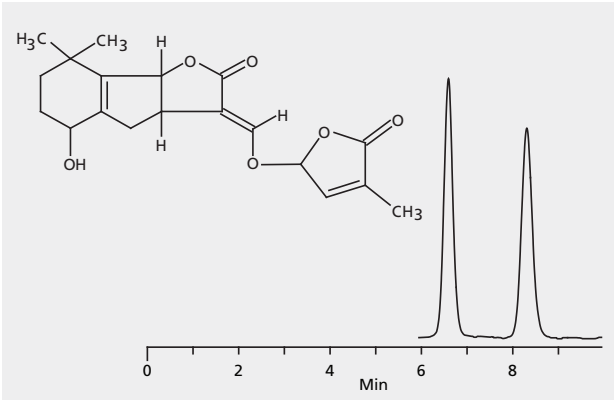
## HPLC Analysis of Paraquat and Diquat on Ascentis® Express HILIC (UV Detection)

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 200 mM ammonium TFA; (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 60 °C
detector . . . . . UV, 257 nm (paraquat) and 308 nm (diquat)
injection . . . . . 1 µL
sample . . . . . 50 mg/L in mobile phase
Application No. . . . . G005718



## HPLC Analysis of Strigol Enantiomers on Astec® CYCLOBOND® I 2000 RSP

column . . CYCLOBOND I 2000 RSP, 25 cm x 4.6 mm I.D., 5 µm particles (20324AST)
mobile phase . . . . . (A) 0.1% triethylamine, pH 4.1; (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004707

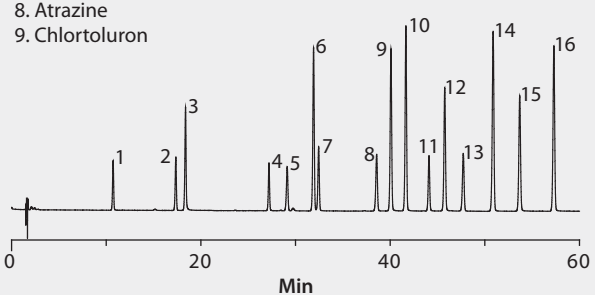


## HPLC Analysis of 16 Triazine and Phenyl Urea Compounds on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of 16 triazine and phenyl urea compounds. Lower column temperature can improve resolution of the critical pair.

column . . Ascentis Express RP-Amide, 15 cm x 3 mm I.D., 2.7 µm particles (53919-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . . . . 5 to 53% B in 70 min
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . 20 mg/L (except terbuthylazin desethyl and terbuthylazine 60 mg/L) in 60:40, water: acetonitrile
Application No. . . . . G005651

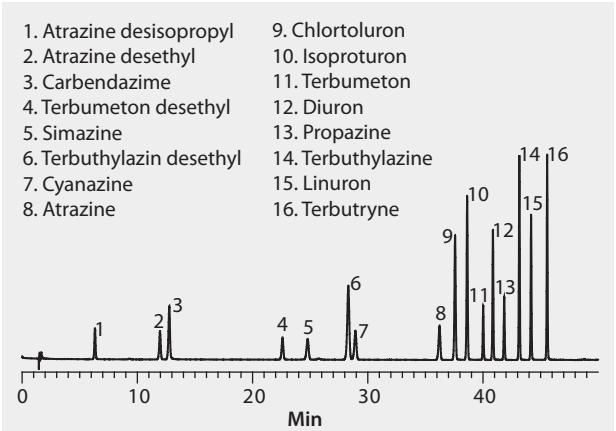
1. Atrazine desisopropyl
2. Atrazine desethyl
3. Carbendazime
4. Terbumeton desethyl
5. Simazine
6. Terbuthylazin desethyl
7. Cyanazine
8. Atrazine
9. Chlortoluron
10. Isoproturon
11. Terbumeton
12. Diuron
13. Propazine
14. Terbuthylazine
15. Linuron
16. Terbutryne



## HPLC Analysis of 16 Triazine and Phenyl Urea Compounds on Ascentis® Express RP-Amide, Modified Gradient

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of 16 triazine and phenyl urea compounds. Lower column
temperature can improve resolution of the critical pair.

column . . Ascentis Express RP-Amide, 15 cm x 3 mm I.D., 2.7 µm particles (53919-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . 10 to 25% B in 30 min; 25 to 60% B in 15 min; held at 60% B for 5 min
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . 20 mg/L each (except terbuthylazin desethyl and terbuthylazine 60 mg/L) in 60:40, water: acetonitrile
Application No. . . . . G005652



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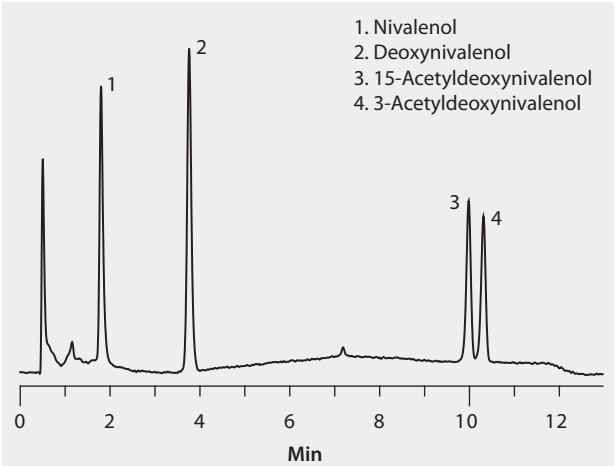
## HPLC Analysis of B-Trichothecenes on Ascentis® Express C18

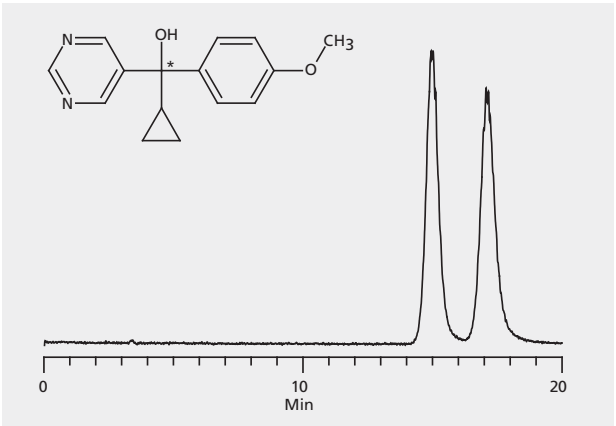
Mycotoxins are toxic secondary metabolites produced by fungi. Those chemicals exist in food as a result of fungal infection of crops. Mycotoxins have strong resistance to decomposition and digestion, so they remain in the food chain in meat and dairy products. They also resist to temperature, such as cooking and freezing. Their effects on human and animal health include death, cancer, weakened immune systems and as allergens or irritants.

column . . . Ascentis Express C18, 10 cm x 3 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) water:acetonitrile:methanol (92:4:4); (B) acetonitrile
gradient . . 0% B held for 5 minutes, to 8% B in 3 minutes, 8% B held for 2 minutes
flow rate . . . . . 0.8 mL/min
pressure . . . . . 4061 psi (280 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 40 µL
sample . . . . . 2 ppm each in water:methanol:acetonitrile (92:4:4)
Application No. . . . . G005561



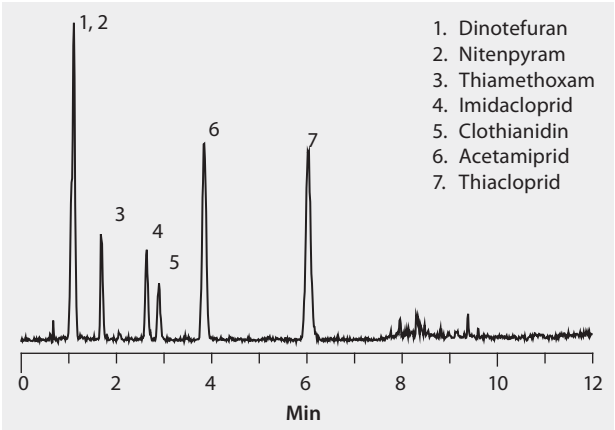
## LC-MS Analysis of Ancymidol Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)
mobile phase . . (A) 5 mM ammonium acetate, pH 6.0; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . MS (m/z = 257)
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004683



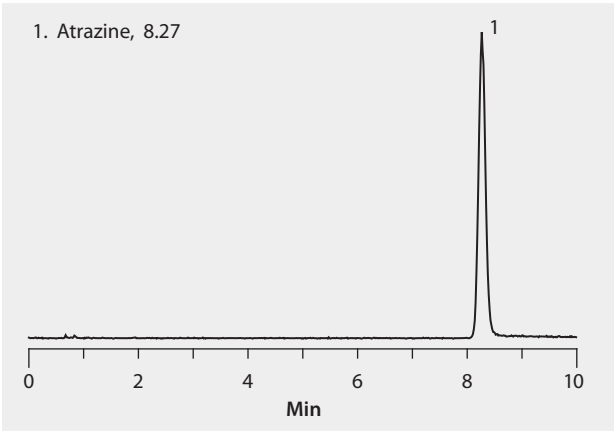
## LC-MS/MS Analysis of Neonicotinoid Pesticides in Dandelion Blossoms on Ascentis® Express C18 after Dispersive SPE (QuEChERS) using Supel™ QuE

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . 3 g pulverized dandelion blossoms, homogenized in 10 mL water
extraction tube . . . . . Supel QuE Acetate extration tube (55234-U)
extraction process . . add 25 mL of acetonitrile; add contents of Supel QuE acetate extraction tube; shake immediately for 1 minute; centrifuge at 3400 rpm for 5 minutes
clean-up tube . . . . . Supel QuE PSA/C18 tube (55288-U)
clean-up process . . . . . Transfer 1 mL of the acetonitrile layer into a Supel QuE PSA/C18 cleanup tube; shake for 1 minute; centrifuge at 3500 rpm for 3 minutes; (draw off 700 uL of supernatant, evaporate to dryness at 50 °C under nitrogen; reconstitute in 200 µL of 50:50 0.1% formic acid:0.1% formic acid in methanol)
column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in methanol
gradient . . 30% B from 0 to 5 min; to 100% B in 0.2 min; held at 100% B for 5.3 min; to 30% B in 0.5 min; held at 30% B for 5 min
flow rate . . . . . 500 µL/min
pressure . . . . . 3800 psi (262 bar)
column temp. . . . . 25 °C
detector . . . . . MS, ESI(+), MRM, m/z 203.2/129.2, 271.2/225.0, 292.1/211.0, 256.0/175.2, 250.0/132.0, 223.2/126.0, 253.0/125.8
injection . . . . . 3 µL
Application No. . . . . G006046



## UHPLC Analysis of Atrazine on Titan™ C18

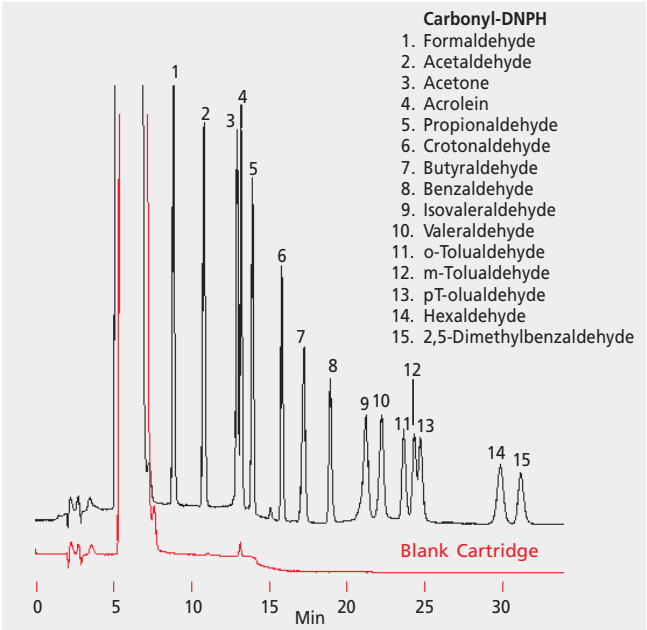
column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 216.10
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006089



## Air Monitoring and Industrial Hygiene

### EPA Method TO-11/IP-6A and ASTM® D5197: HPLC Analysis of Aldehydes and Ketones on SUPELCOSIL™ LC-18 after Collection/Desorption using LpDNPH

sample/matrix . . . . . 5 µg each carbonyl-DNPH on LpDNPH cartridge. Cartridge eluted with 5.0 mL acetonitrile
adsorbent tube . . . . . LpDNPH Cartridge (21024-U)
column . . . . . SUPELCOSIL LC-18, 25 cm × 4.6 mm I.D., 5 µm particles (58298)
mobile phase . . . . . (A): acetonitrile:tetrahydrofuran:water, 30:10:60; (B): acetonitrile:water, 60:40 gradient 0% B for 1 min, linear gradient to 100% B over 10 min
flow rate . . . . . 1.5 mL/min
detector . . . . . VIS, 360 nm
injection . . . . . 25 µL of extract
Application No. . . . . 795-0298

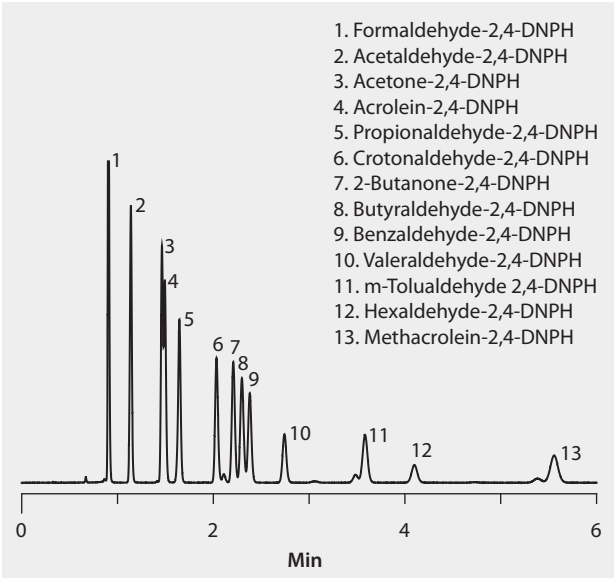





SUPELCO® Solutions within.™

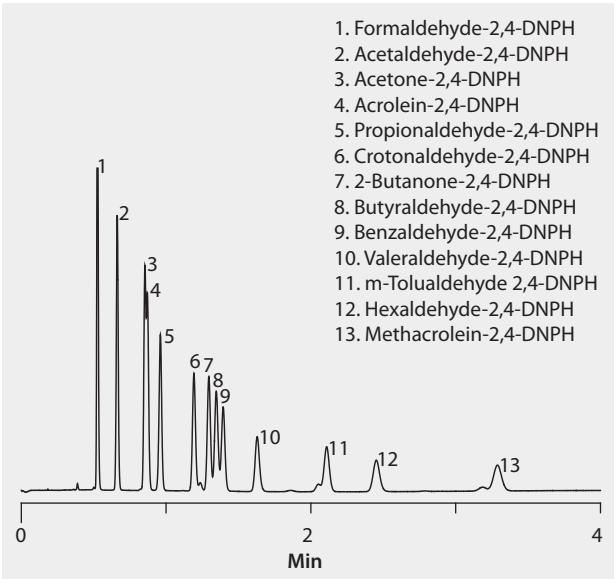
## HPLC Analysis of 13 Aldehyde/Ketone DNPH Derivatives on Ascentis® Express C18

column . . Ascentis Express C18, 10 cm x 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (40:60 v/v, A:B)
flow rate . . . . . 2 mL/min
pressure . . . . . 4685 psi (323 bar)
detector . . . . . UV, 360 nm
injection . . . . . 10 µL
sample . . . . . carb Method 1004 DNPH Mix 2 (47651-U) dilute 3:2 with water
Application No. . . . . G005635



## HPLC Analysis of 13 Aldehyde/Ketone DNPH Derivatives on Ascentis® Express C18 (High Flow Rate)

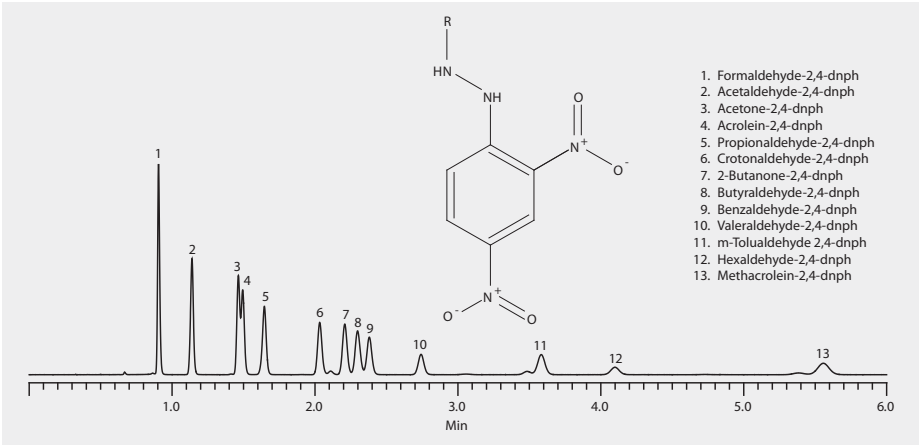
column . . Ascentis Express C18, 10 cm x 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (40:60, v/v, A:B)
flow rate . . . . . 3.5 mL/min
pressure . . . . . 8296 psi (572 bar)
detector . . . . . UV, 360 nm
injection . . . . . 10 µL
sample . . . . . Carb Method 1004 DNPH Mix 2 (47651-U) dilute 3:2 with water
Application No. . . . . G005636



## HPLC Analysis of Aldehydes and Ketones as DNPH Derivatives on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the separation of aldehyde/ketone-DNPH derivatives.

column . . Ascentis Express C18, 10 cm x 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (40:60, A:B)
flow rate . . . . . 2 mL/min
pressure . . . . . 4685 psi (323 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 360 nm
injection . . . . . 5 µL
sample . . . . . Sample in water
Application No. . . . . G005382

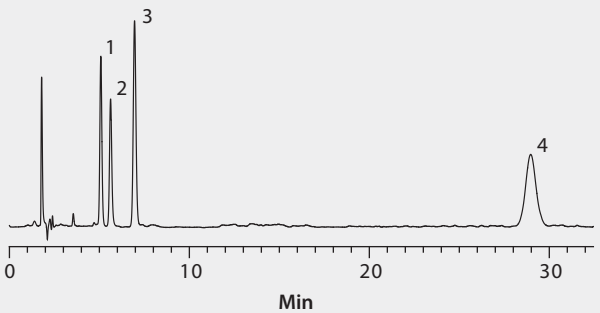


## HPLC Analysis of Isocyanate Derivatives on Ascentis® ES-Cyano

This application demonstrates the suitability of Ascentis Cyano for the efficient separation of isocyanate derivatives.

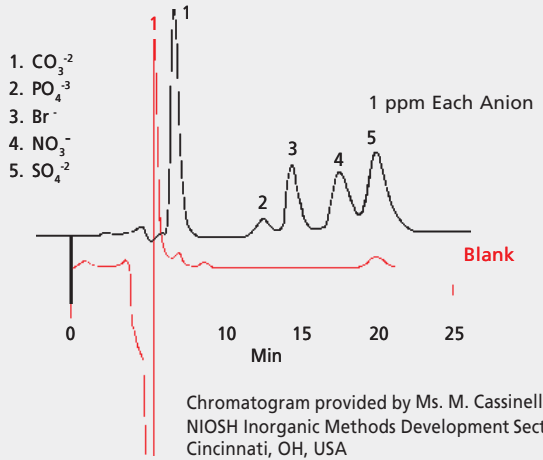
column . . . . . Ascentis Cyano, 15 cm x 4.6 mm I.D., 5 µm particles (577306-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water; (B) 0.1% ammonium acetate in acetonitrile; (70:30, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 20 µg/mL in 80:12:8, water: acetonitrile: dimethylsulfoxide
Application No. . . . . G005707

1. *N,N'*-(2-Methyl-1,3-phenylene)bis(4-(2-pyridinyl)-1-piperazinecarboxamide)
2. *N,N'*-1,6-Hexanediylbis(4-(2-pyridinyl)-1-piperazinecarboxamide)
3. *N,N'*-(4-Methyl-1,3-phenylene)bis(4-(2-pyridinyl)-1-piperazinecarboxamide)
4. *N,N'*-(Methylenediphenylene)bis(4-(2-pyridinyl)-1-piperazinecarboxamide)



## NIOSH Method 7903: IC Analysis of Inorganic Acids after Collection/Desorption using ORBO™-53

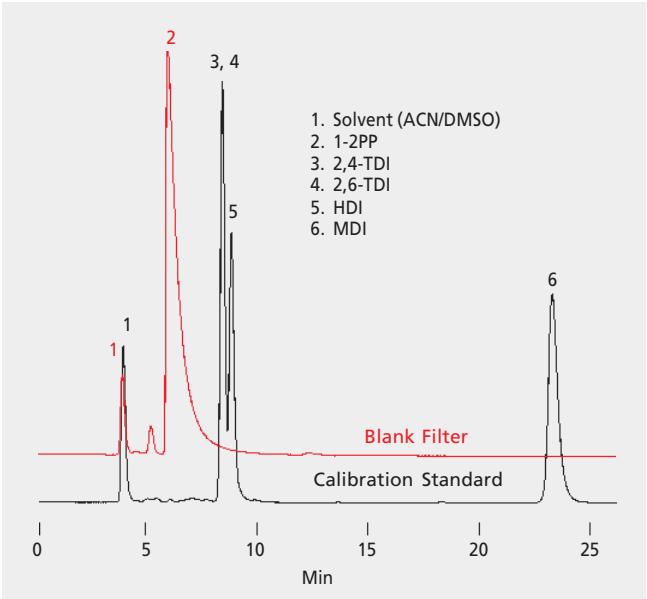
| | |
|---|---|
| sample/matrix | 1 ppm of each component |
| adsorbent tube | ORBO-53 (20265) |
| column | Dionex S2 Fast Run Anion, 25 cm × 3 mm I.D. |
| mobile phase | 3 mM $NaHCO_3$/2.4 mM $Na_2CO_3$ |
| flow rate | 3 mL/min |
| detector | conductivity (10µSiemans/ cm) |
| Application No. | 713-0873 |




Chromatogram provided by Ms. M. Cassinelli, NIOSH Inorganic Methods Development Section, Cincinnati, OH, USA

## OSHA Method 42/47 and ASTM® D5836: HPLC Analysis of Isocyanates on SUPELCOSIL™ LC-8 after Collection/Desorption using ORBO™-80

sample/matrix . . calibration standard: isocyanate derivatives, 5 µg/mL in acetonitrile
blank: 1-(2-pyridyl)piperazine (1-2PP) coated glass fiber filter desorbed in acetonitrile/DMSO, 9:1
adsorbent tube . . . . . ORBO-80 (20811)
column . . . . . SUPELCOSIL LC-8, 25 cm × 4.6 mm I.D., 5 µm particles (58297)
mobile phase . . . . . 0.05 M ammonium acetate in water:acetonitrile, 70:30 (pH 6-6.2 with acetic acid)
flow rate . . . . . linear gradient, 0.7 mL/min to 2 mL/min in 15 min
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . 796-0376



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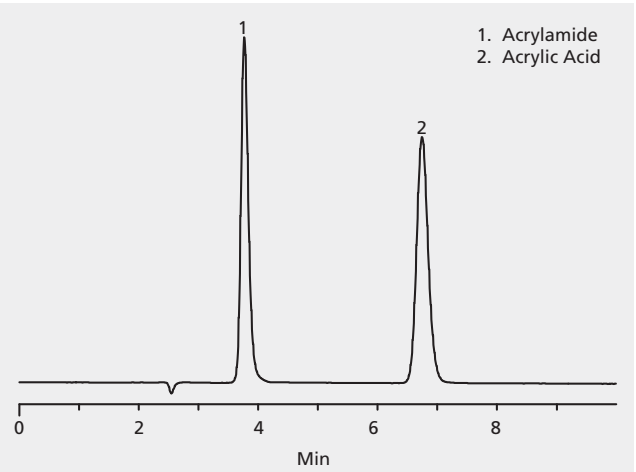
## Chemicals and Industrial Polymers

### HPLC Analysis of Acrylamide and Acrylic Acid on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . 0.05% formic acid
flow rate . . . . . 1.0 mL/min
column temp. . . . . ambient
detector . . . . . UV, 206 nm
injection . . . . . 1 µL
sample . . . . . 1.0 mg/mL each in mobile phase
Application No. . . . . G002569

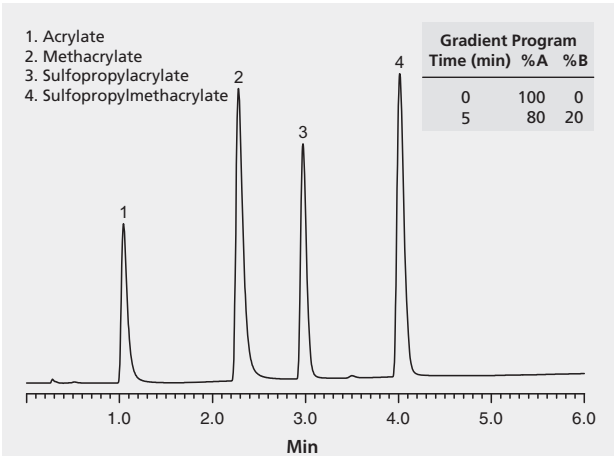





SUPELCO® Solutions within.™

## HPLC Analysis of Acrylic Acid Derivatives on Discovery® Zr-Carbon

column . . . Discovery Zr-Carbon, 7.5 cm × 2.1 mm I.D., 3 µm particles (65726-U)
mobile phase . . . . . (A) 30 mM phosphoric acid, pH 1.9; (B) acetonitrile
flow rate . . . . . 0.42 mL/min
column temp. . . . . 50 °C
detector . . . . . UV, 210 nm (200 nm will enhance sensitivity ~2x)
injection . . . . . 1 µL
sample . . . . . acrylate @ 0.05 g/L, methacrylate @ 0.05 g/L, sulfopropylacrylate @ 0.02 g/L, sulfopropylmethylacrylate @ 0.02 g/L in 30 mM $H_3PO_4$, pH 1.9
Application No. . . . . G002143

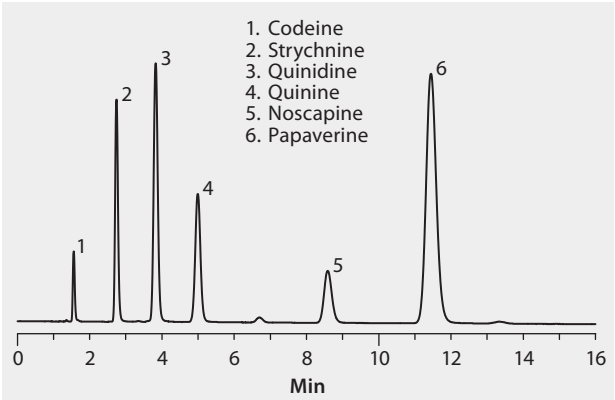





SUPELCO® Solutions within.™

## HPLC Analysis of Amides on Discovery® RP-Amide C16

column . . . Discovery RP-Amide C16, 15 cm × 4.6 mm, 5 µm particles (505013)
mobile phase . . . . . (A) methanol; (B) 25mM monopotassium phosphate, pH 3.0; (20:80, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000691

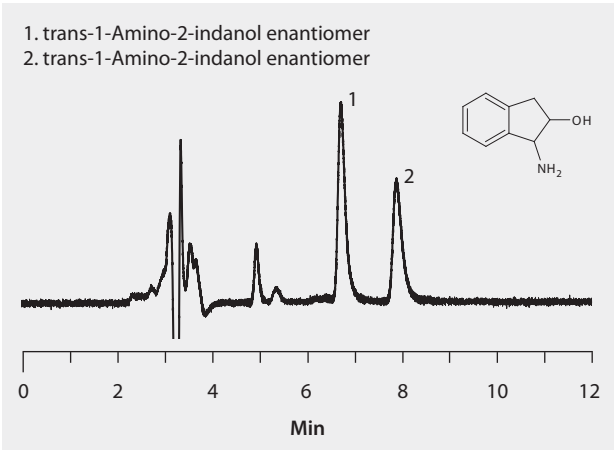





SUPELCO® Solutions within.™

## HPLC Analysis of trans-1-Amino-2-Indanol Enantiomers on LARIHC™ CF6-P

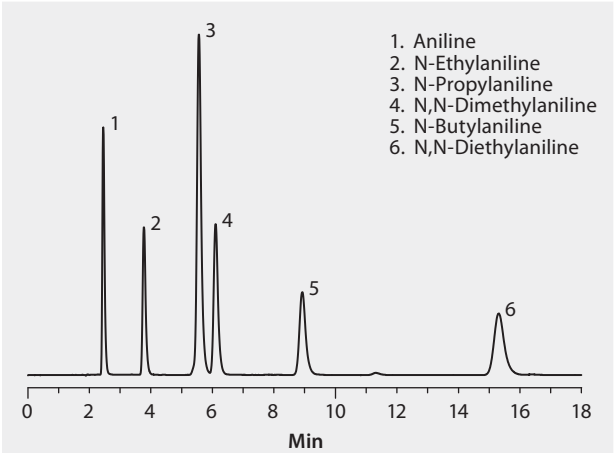
column . . LARIHC CF6-P, 25 cm x 4.6 mm I.D., 5 µm particles (AZYP Part No.L1001, available from Supelco/Sigma-Aldrich as a custom item.)
mobile phase . . . (A) methanol; (B) acetonitrile; (C) acetic acid; (D) triethylamine, (70:30:0.3.0.2, A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . Trans-1-Amino-2-indanol, 0.3 mg/mL in ethanol
Application No. . . . . G005256




**Ref:** 1. Ping Sun, Chunlei Wang, Zachary S. Breitbach, Ying Zhang, and Daniel W. Armstrong, Development of New HPLC Chiral Stationary Phases Based on Native and Derivatized Cyclofructans *Anal. Chem.* **81**, 10215 (2009)
2. Ping Sun and Daniel W. Armstrong, Effective enantiomeric separations of racemic primary amines by the isopropyl carbamate-cyclofructan6 chiral stationary phase *J. Chromatogr. A.* **1217**, 4904 (2010)

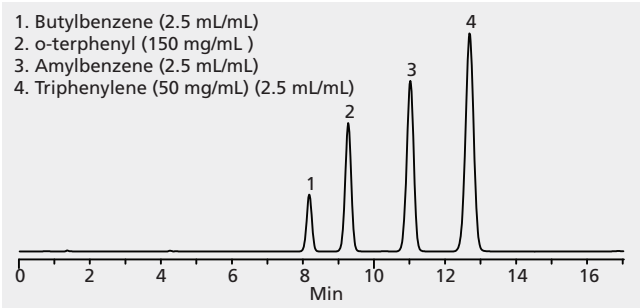
## HPLC Analysis of Aniline Homologs on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) methanol; (B) water (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
Application No. . . . . G000295



## HPLC Analysis of Aromatics on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (30:70, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . as indicated in acetonitrile
Application No. . . . . G002607

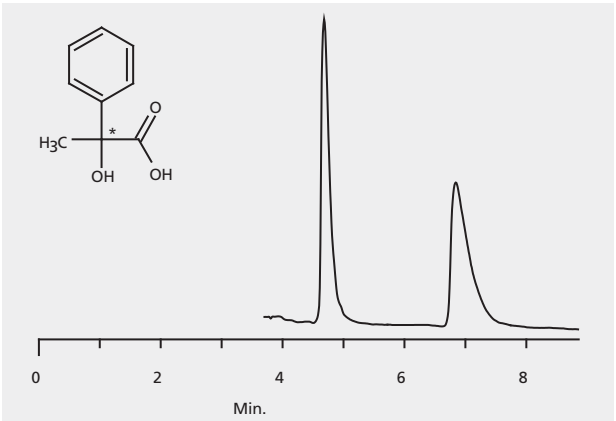





SUPELCO® Solutions within.™

## HPLC Analysis of Atrolactic Acid Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1 wt% ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004594

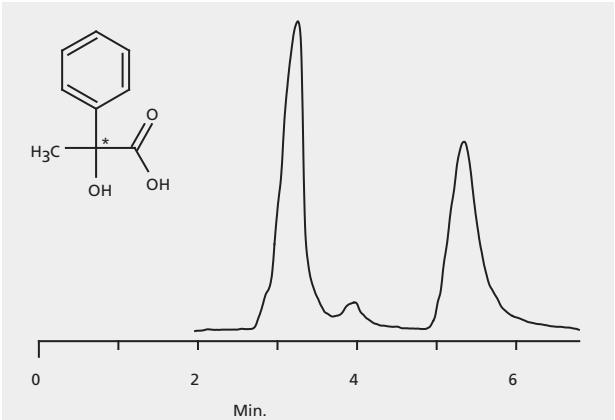





SUPELCO® Solutions within.™

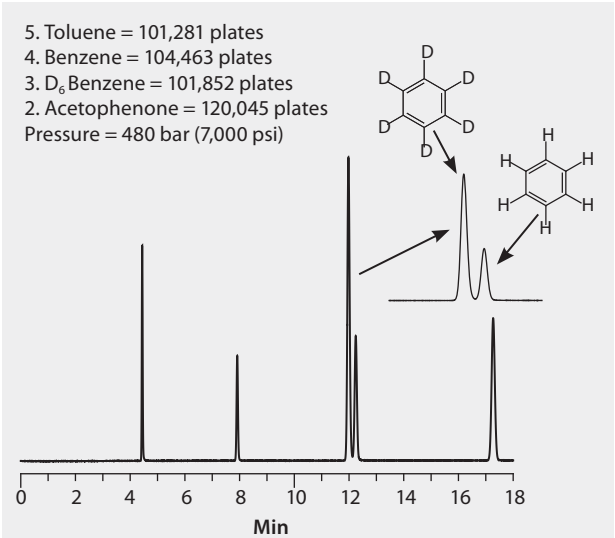
## HPLC Analysis of Atrolactic Acid Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . 0.1 wt% ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004604



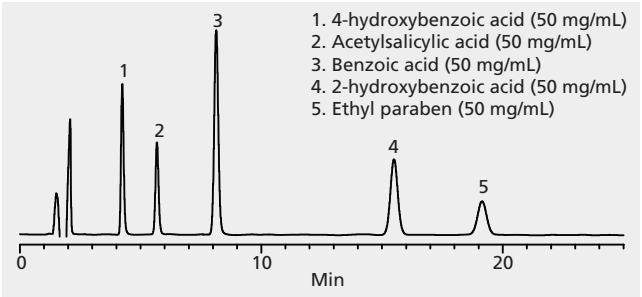
## HPLC Analysis of Benzene and Deuterated Benzene on Ascentis® Express C18

column . . . . . Ascentis Express C18, 15 cm × 4.6 mm I.D., 2.7 µm particles
mobile phase . . . . . (A) acetonitrile; (B) water (55:45, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 50 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G004046



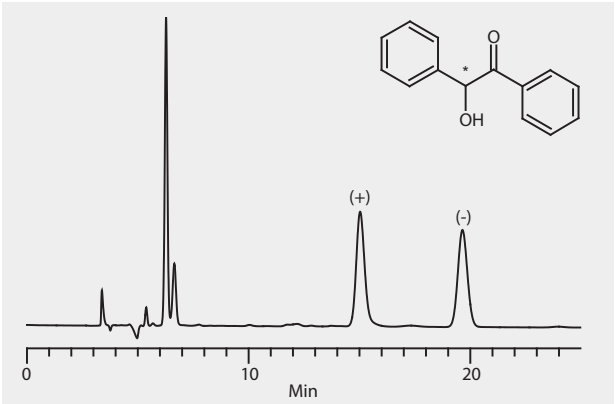
## HPLC Analysis of Benzoic Acid Deriviatives on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . (A) 0.1% TFA in water; (B) 0.1% TFA in acetonitrile; (70:30, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 0.1% TFA in water
Application No. . . . . G002627



## HPLC Analysis of Benzoin Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) TFA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004986

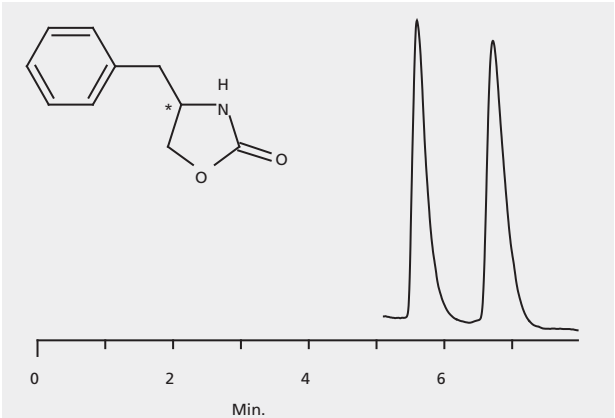





SUPELCO® Solutions within.™

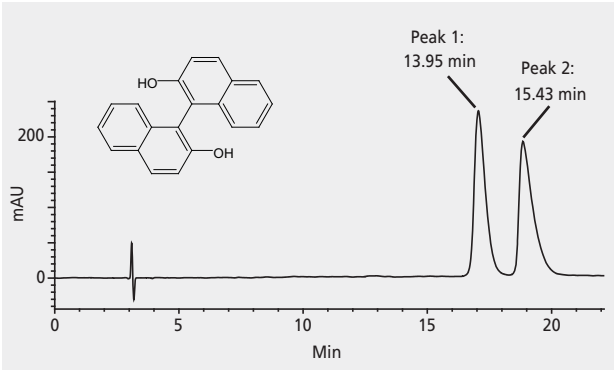
## HPLC Analysis of 4-Benzyl-2-oxazolidinone Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004605



## HPLC Analysis of Binaphthol Enantiomers on Astec® CYCLOBOND® I 2000 DNP

column . . CYCLOBOND I 2000 DNP, 25 cm × 4.6 mm I.D., 5 µm particles (25024AST)
mobile phase . . (A) 0.1% triethyl ammonium acetate, pH 4.1; (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
Application No. . . . . G004028

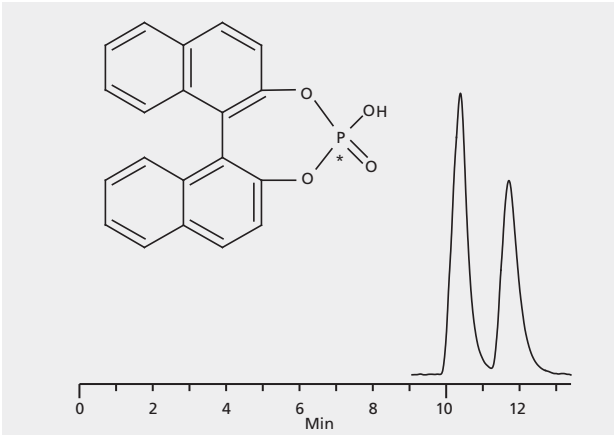





SUPELCO® Solutions within.™

## HPLC Analysis of 1,1′-Binaphthyl-2,2′-diyl Hydrogenphosphate Enantiomers on Astec® (R,R) P-CAP™

column . . . . Astec (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
mobile phase . . . . . 20 mM ammonium acetate in: (A) acetonitrile; (B) methanol; (70:30, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004718



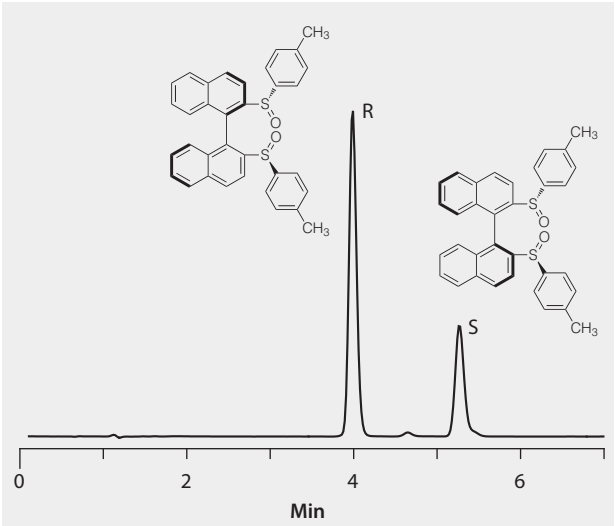



SUPELCO® Solutions within.™

## HPLC Analysis of 2,2′-Bis[(*S*)-(4-methylphenyl) sulfinyl]-1,1′-binapthalene Diastereomers on Ascentis® Express C18

This application demonstrates the suitability of the Ascentis Express C18 for the separation of the diastereomers of by HPLC in reversed phase mode.

column . . Ascentis Express C18, 10 cm x 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) water; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.8 mL/min
pressure . . . . . 2450 psi (169 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . (1R)-2,2′-Bis[(S )-(4-methylphenyl)sulfonyl]-1,1′-binapthalene, 25 µg/mL in methanol; (1S)-2,2′-Bis[(S)-(4-methylphenyl)sulfonyl]-1,1′-binapthalene, 50 µg/mL in methanol
Application No. . . . . G005612

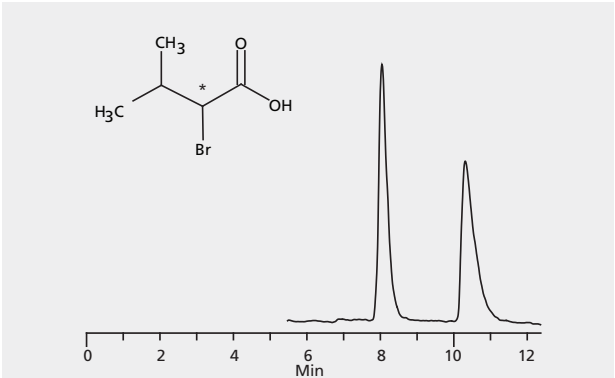





SUPELCO® Solutions within.™

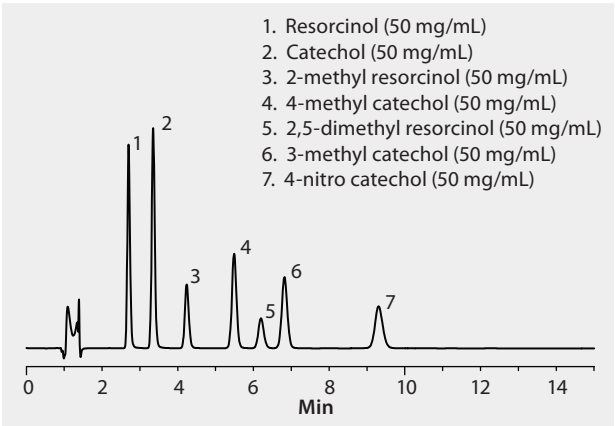
## HPLC Analysis of 2-Bromo-3-Methylbutyric Acid Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . (A) 20 mM ammonium acetate; (B) methanol; (15:85, A:B)
(alternate mobile phase of 0.1% ammonium acetate in methanol gives 3.5 and 4.3 min retention)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004667



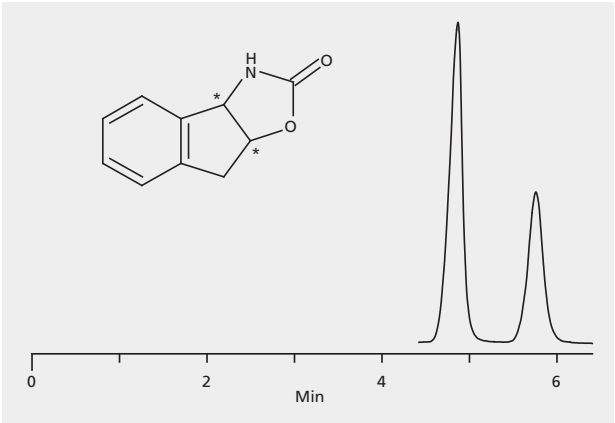
## HPLC Analysis of Catechols and Resorcinols on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0 unadjusted): (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 270 nm
injection . . . . . 25 µL
sample . . . . . as indicated in 20 mM phosphoric acid (pH 2.0)
Application No. . . . . G002597



## HPLC Analysis of Cis-(+/-)-3,3a,8,8a-Tetrahydro-2H-indeno[1,2-d]oxazol-2-one Enantiomers on Astec® (R,R) P-CAP™-DP

column . . Astec (R,R) P-CAP-DP, 25 cm x 4.6 mm I.D., 5 µm particles (35024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (98:2, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004729

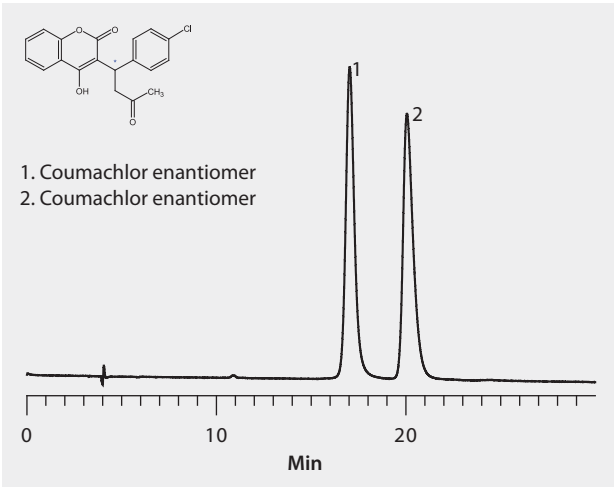





SUPELCO® Solutions within.™

## HPLC Analysis of Coumachlor Enantiomers on Astec® CYCLOBOND® I 2000 DM

column . . CYCLOBOND I 2000 DM, 25 cm x 4.6 mm I.D., 5 µm particles (20924AST)
mobile phase . . (A) acetonitrile; (B) 20 mM ammonium acetate, pH 2.9; (20:80, A:B)
flow rate . . . . . 0.8 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 3 µL
sample . . . . . coumachlor, 1 mg/mL in acetonitrile:water (50:50)
Application No. . . . . G005160

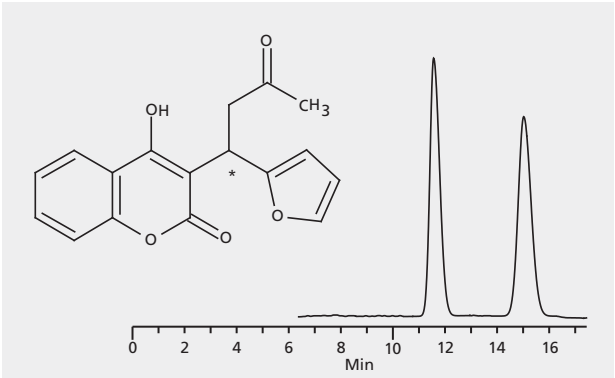





SUPELCO® Solutions within.™

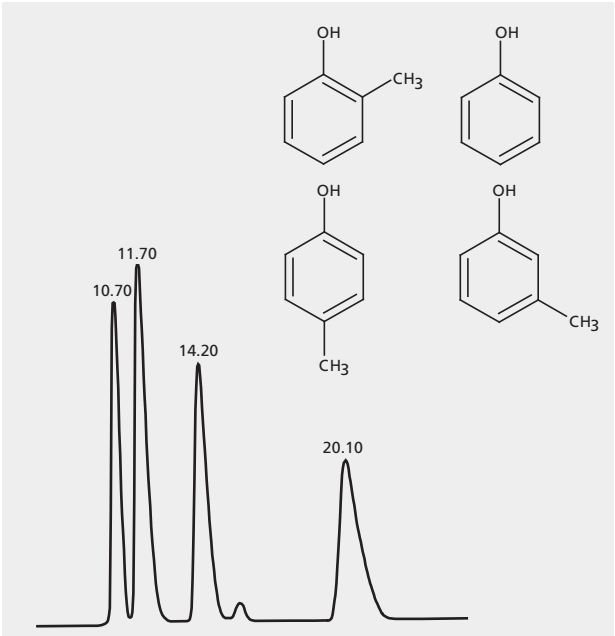
## HPLC Analysis of Coumafuryl Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM ammonium nitrate, pH 5.5; (B) THF; (90:10, A:B)
flow rate . . . . . 1.2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004639



## HPLC Analysis of Cresols and Phenol on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . . . . (A) water; (B) methanol; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 0.5 mg/mL in methanol
peak 1: o-cresol (10.70 min)
peak 2: phenol (11.70 min)
peak 3: m-cresol (14.20 min)
peak 4: p-cresol (20.10 min)
Application No. . . . . G004731

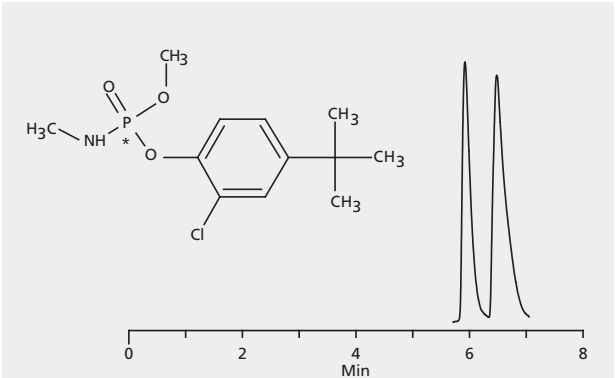





SUPELCO® Solutions within.™

## HPLC Analysis of Crufomate (Ruelene) Enantiomers on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . (A) acetonitrile; (B) acetic acid; (C) triethylamine; (100:0.2:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004703

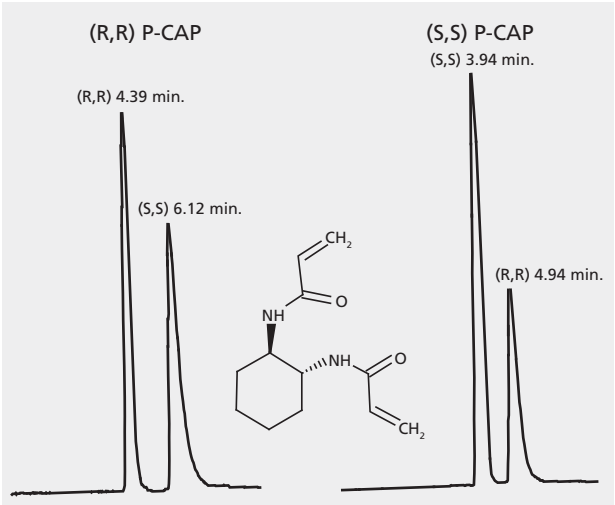





SUPELCO® Solutions within.™

## HPLC Analysis of DACH-ACR Enantiomers on Astec® P-CAP™

column . . . . . Astec (R,R) P-CAP or (S,S) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST, 33024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (97:3, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . (R,R)- and (S,S)-DACH-ACR (N-(2-acryloylamino-(1R,2R)-cyclohexyl)-acrylamide)
Application No. . . . . G004401

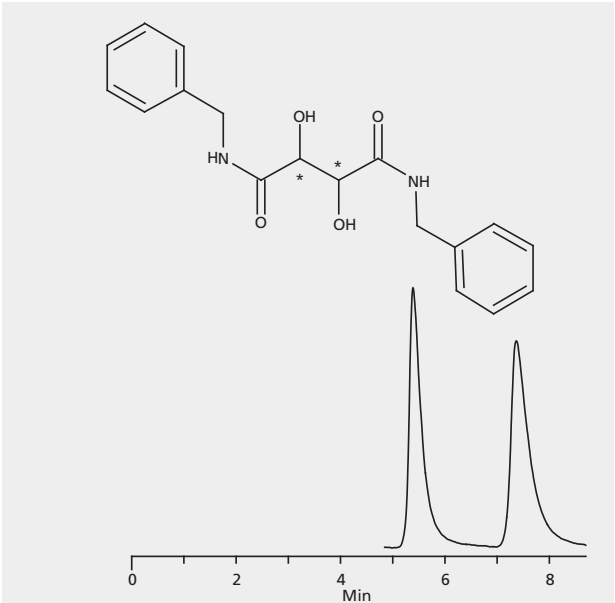





SUPELCO® Solutions within.™

## HPLC Analysis of (+/-)-N,N-Dibenzyltartaric Diamide Enantiomers on Astec® (R,R) P-CAP™-DP

column . . Astec (R,R) P-CAP-DP, 25 cm x 4.6 mm I.D., 5 µm particles (35024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004727

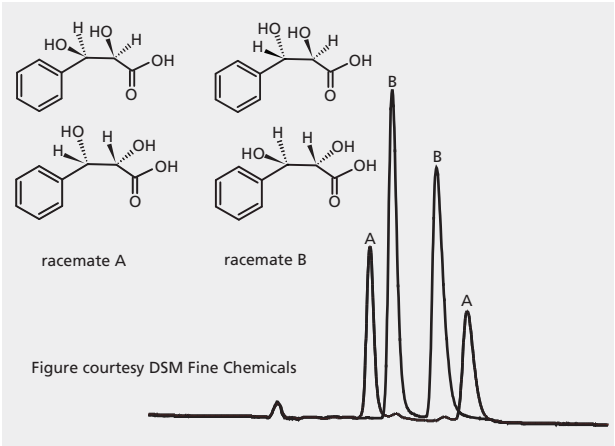





SUPELCO® Solutions within.™

## HPLC Analysis of 2,3-Dihydroxy-3-Phenylpropionic Acid Enantiomers on CHIROBIOTIC R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . (A) 0.1% ammonia, pH 4.1 with formic acid;
(B) methanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 258 nm
injection . . . . . 10 µL
sample . . . . . 2,3-dihydroxy-3-phenyl-propionic acid isomers:
(A) racemate A (4.85 and 6.95 min)
(B) racemate B (5.33 and 6.29 min)
Application No. . . . . G004409

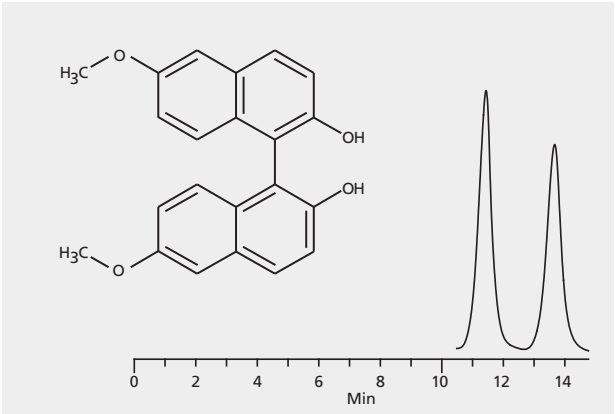





SUPELCO® Solutions within.™

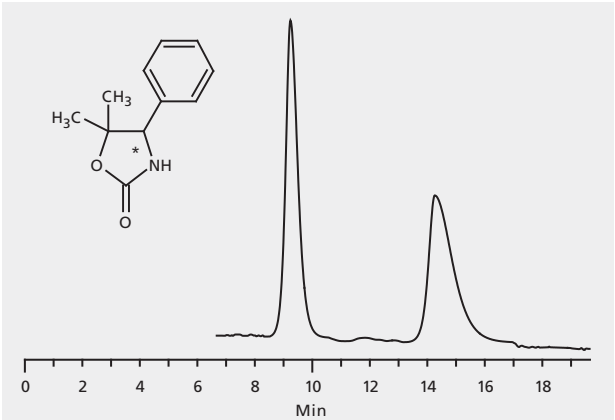
## HPLC Analysis of Di-6,6′-Methoxy-bi(2-Naphthol) Enantiomers on Astec® (R,R) P-CAP™

column . . . . Astec (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
mobile phase . . . . . (A) ethanol; (B): heptane; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004724



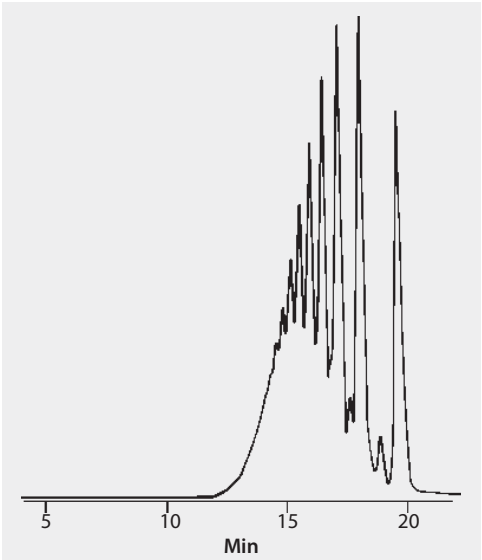
## HPLC Analysis of 5,5-Dimethyl-4-phenyl-2-oxazolidinone Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 0.5% triethylamine acetate, pH 4.1; (B) methanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004547



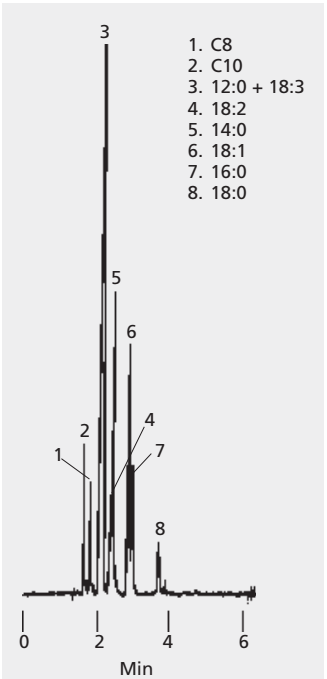
## HPLC Analysis of Epoxy resin, Epikote® 1001 on TSKgel® G3000HXL

column . . . . . TSKgel G3000HXL, 30 cm × 7.8 mm I.D., 6 µm particles (816136)
mobile phase . . . . . tetrahydrofuran
flow rate . . . . . 1 mL/min
detector . . . . . UV, 254 nm
Application No. . . . . 713-0783



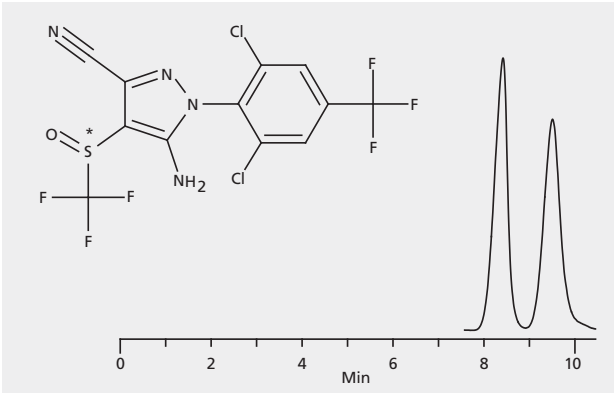
## HPLC Analysis of Fatty Acid Methyl Esters (FAMES) on SUPELCOSIL™ LC-18

column . . . . . SUPELCOSIL LC-18, 25 cm × 4.6 mm I.D., 5 µm particles (58298)
mobile phase . . . . . (A) acetonitrile; (B) acetone; (59.0:41.0, v/v, A:B)
flow rate . . . . . 1 mL/min
detector . . . . . RI
injection . . 10 µL of 9% C8 to C18 saturated and unsaturated FAMEs in mobile phase
Application No. . . . . 797-0501



## HPLC Analysis of Fipronil Enantiomers on Astec® (R,R) P-CAP™

column . . . . Astec (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
mobile phase . . . . . (A) heptane; (B) ethanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 235 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004720



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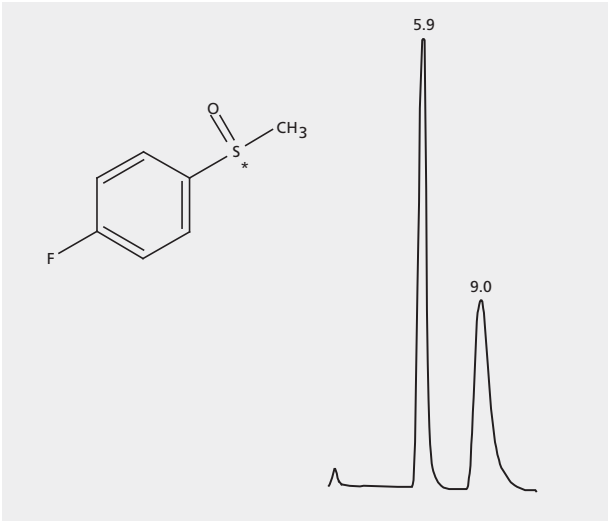
## HPLC Analysis of p-Fluorophenyl Methyl Sulfoxide Enantiomers on Astec® CHIROBIOTIC® TAG

Details: A. Berthod *et al*, J. Chromatogr A, 955 (2002) 53-69.

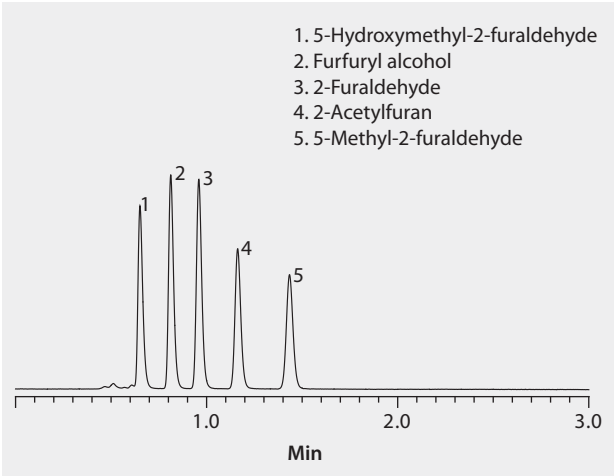
column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . (A) hexane; (B) ethanol; (50:50, A:B)
flow rate . . . . . 2 mL/min
detector . . . . . UV, polarimetry to confirm elution order
Application No. . . . . G004735



## HPLC Analysis of Furans on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of furans as listed in ASTM method 05837-99.

column . . Ascentis Express C18, 5 cm x 3.0 mm I.D., 2.7 µm particles (53811-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 20 µg/mL in 80:20, water: acetonitrile
Application No. . . . . G005700



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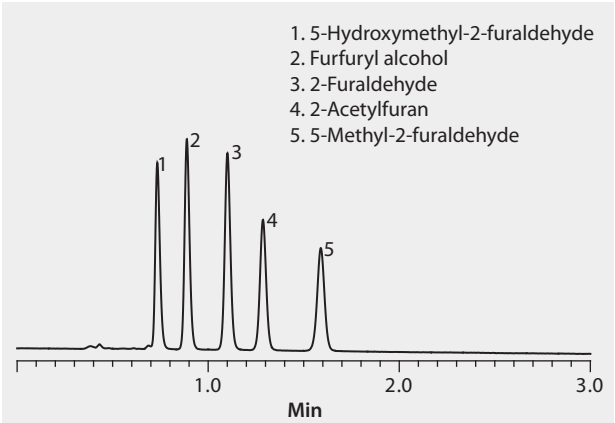
## HPLC Analysis of Furans on Ascentis® Express Phenyl-Hexyl

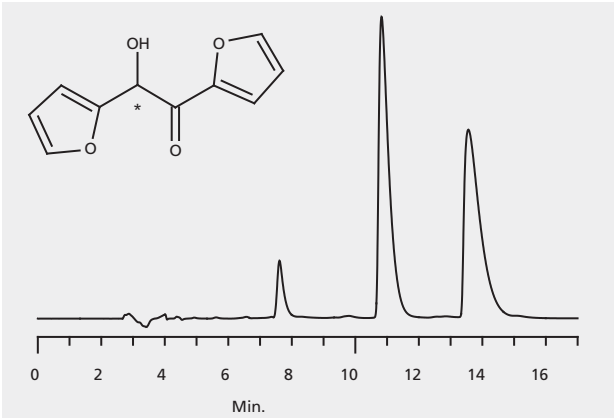
This application demonstrates the suitability of Ascentis Express Hexyl-Phenyl for the efficient separation of furans as listed in ASTM method D5837-99.

| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 5 cm x 3.0 mm I.D., 2.7 µm particles (53342-U) |
| mobile phase | (A) water; (B) acetonitrile; (80:20, A:B) |
| flow rate | 0.4 mL/min |
| column temp. | 35 °C |
| detector | UV, 230 nm |
| injection | 1 µL |
| sample | 20 µg/mL in 80:20, water: acetonitrile |
| Application No. | G005704 |



## HPLC Analysis of Furoin Enantiomers on Astec® P-CAP™-DP

column . . . . . (R,R) P-CAP-DP, 25 cm x 4.6 mm I.D., 5 µm particles (35024AST)
mobile phase . . . . . (A) hexane; (B) ethanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 235 nm
injection . . . . . 10 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004587



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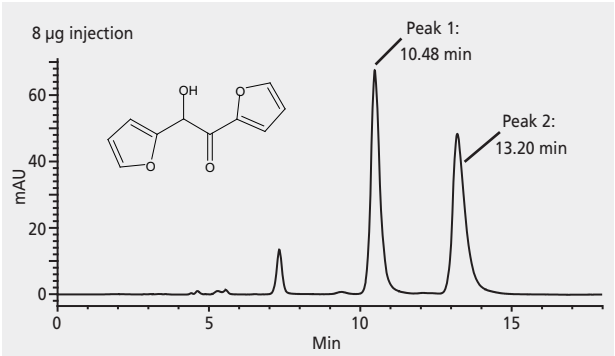
## HPLC Analysis of Furoin on Astec® (R,R) P-CAP™-DP

column . . . . . (R,R) P-CAP-DP, 25 cm × 4.6 mm I.D., 5 µm particles (25024AST)
mobile phase . . . . . (A) hexane; (B) ethanol; (80:20, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 235 nm
injection . . . . . 0.2 mL
sample . . . . . 4 µg/mL
Application No. . . . . G004031



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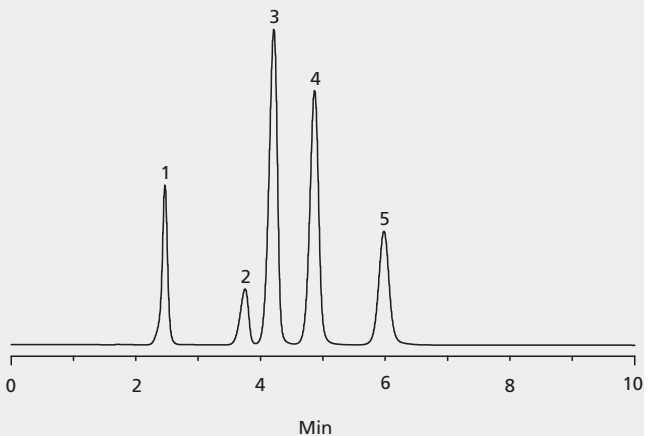
## HPLC Analysis of Heterocyclic Amines (HCAs) on Discovery® HS F5

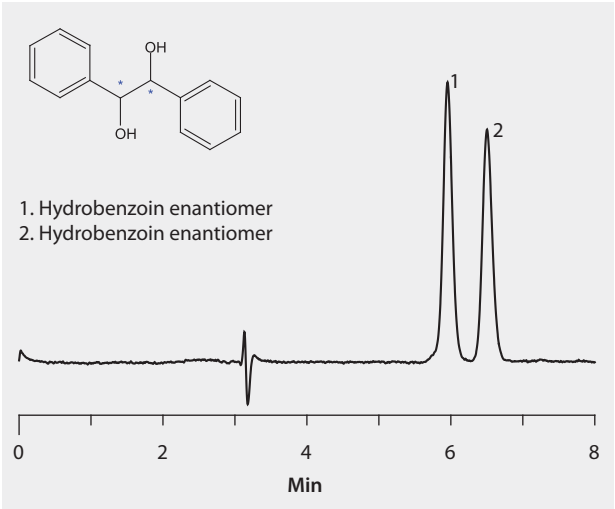
column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . (A) water (10 mM ammonium acetate, pH 4.0 with acetic acid); (B) methanol (v/v); (15:85, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µg/mL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002333

1. 2-Amino-9H-pyrido[2,3-b]indole (AC)
2. 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PHIP)
3. 2-Amino-3-methyl-3H-imidazo[4,5-f]quinoline (IQ)
4. 2-Amino-3,4-dimethyl-3H-imidazo [4,5-f]quinoline (MeIQ)
5. 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)



## HPLC Analysis of Hydrobenzoin Enantiomers on Astec® CYCLOBOND® I 2000 RSP

column . . CYCLOBOND I 2000 RSP, 25 cm x 4.6 mm I.D., 5 µm particles (20324AST)
mobile phase . . (A) acetonitrile; (B) 10 mM ammonium acetate, pH 4.0; (25:75, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . hydrobenzoin, 1 mg/mL in acetonitrile:water (50:50)
Application No. . . . . G005163



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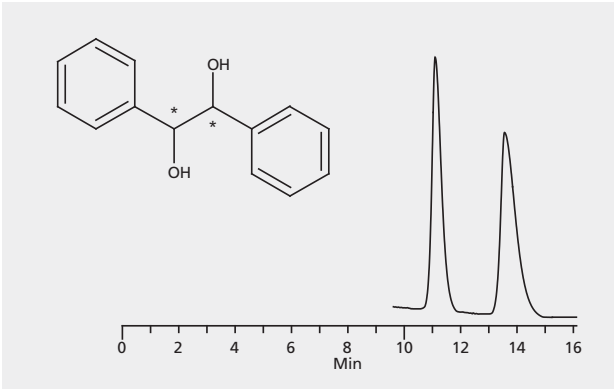
## HPLC Analysis of Hydrobenzoin Enantiomers on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004692

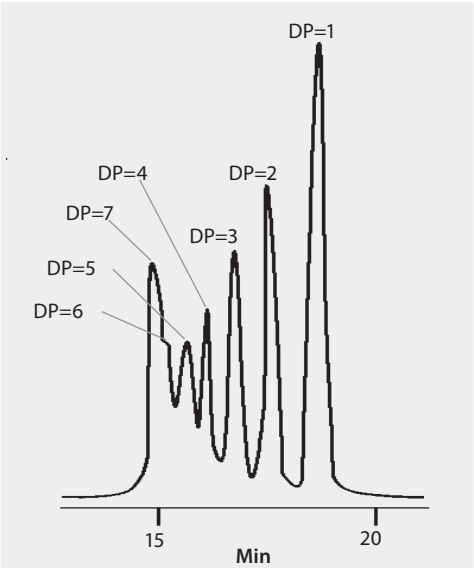





SUPELCO® Solutions within.™

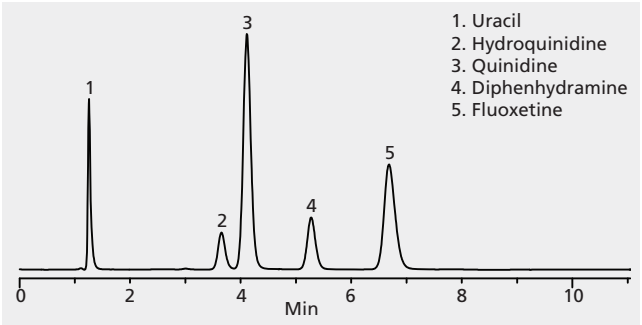
## HPLC Analysis of Hydrolyzed β-Cyclodextrin on TSKgel® G-Oligo-PW

column . . . . TSKgel G-Oligo-PW, 30 cm × 7.8 mm I.D., 6 µm particles (808031)
mobile phase . . . . . distilled water
flow rate . . . . . 1 mL/min
column temp. . . . . 60 °C
detector . . . . . RI
Application No. . . . . 713-0917



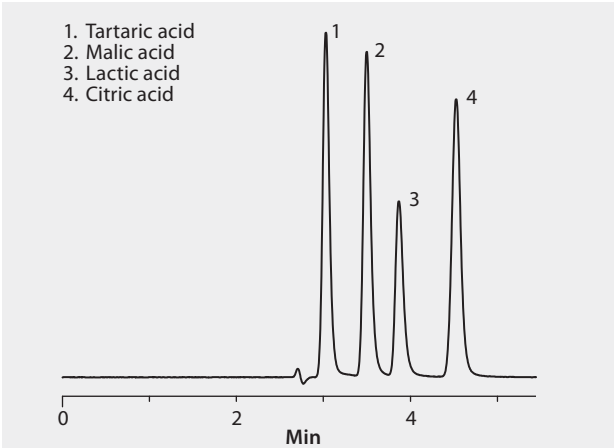
## HPLC Analysis of Hydrophobic Bases on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . (A) 25 mM ammonium phosphate, pH 7; (B) methanol; (35:65, A:B)
flow rate . . . . . 1.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
sample . . . . . N05158
Application No. . . . . G002612



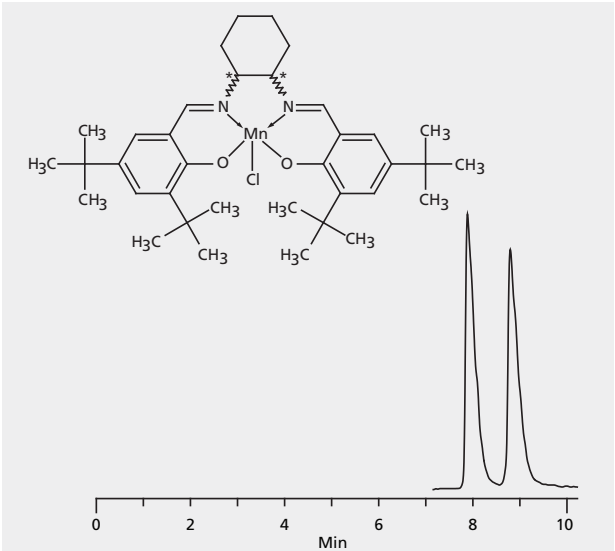
## HPLC Analysis of α-Hydroxy Aliphatic Acids on Discovery® RP-Amide C16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . 25 mM potassium phosphate, pH 3.0
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G000624



## HPLC Analysis of Jacobsen Catalyst Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP (Ammonium Hydroxide Mobile Phase)

| | |
|---|---|
| column | CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST) |
| mobile phase | (A) acetonitrile; (B) methanol; (C) acetic acid; (D) ammonium hydroxide; (85:15:0.4:0.1, A:B:C:D) |
| flow rate | 0.9 mL/min |
| column temp. | 25 °C |
| detector | UV, 254 nm |
| injection | 5 µL |
| sample | 2 mg/mL in methanol |
| Application No. | G004714 |



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## HPLC Analysis of Jacobsen Catalyst Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP (TEA mobile phase)

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)

mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) triethylamine; (95:5:0.2:0.1, A:B:C:D)

flow rate . . . . . 1.5 mL/min

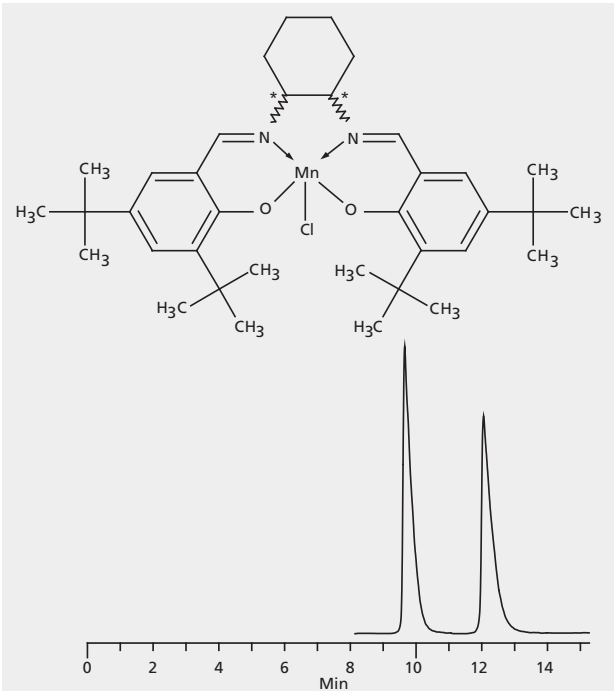
column temp. . . . . 25 °C

detector . . . . . UV, 254 nm

injection . . . . . 2 µL

sample . . . . . 5 mg/mL in methanol

Application No. . . . . G004715

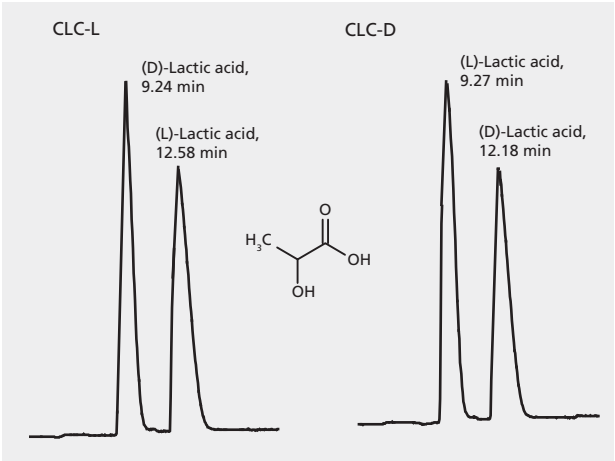





SUPELCO® Solutions within.™

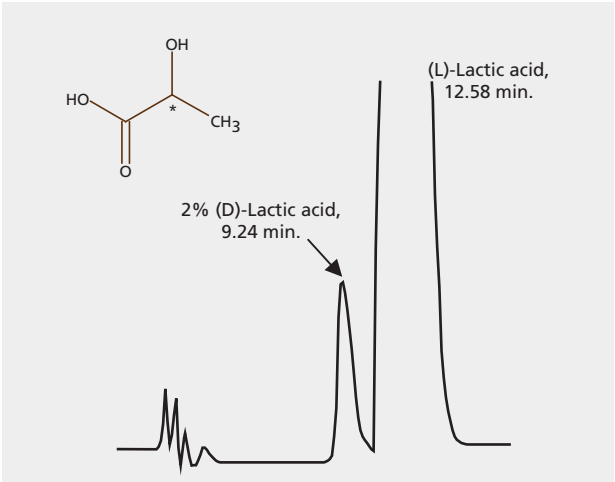
## HPLC Analysis of Lactic Acid Enantiomers on Astec® CLC-L and CLC-D

| | |
|---|---|
| column | Astec CLC-L and CLC-D, 15 cm x 4.6 mm I.D., 5 µm particles (53023AST, 53123AST) |
| mobile phase | 5 mM copper sulfate |
| flow rate | 1 mL/min |
| column temp. | 25 °C |
| detector | UV, 254 nm |
| injection | 10 µL |
| Application No. | G004399 |



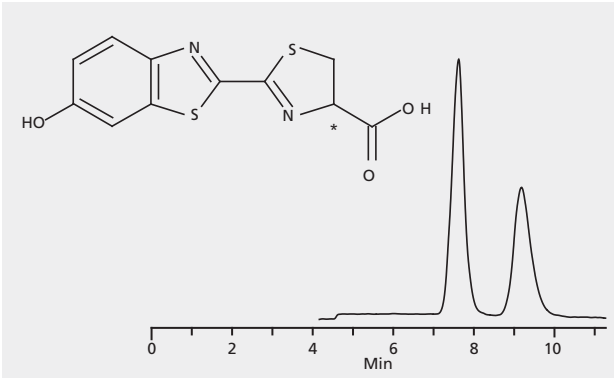
## HPLC Analysis of Lactic Acid Enantiomers on Astec® CLC-L, Trace Level Detection

column . . . . . Astec CLC-L, 15 cm x 4.6 mm I.D., 5 µm particles (53123AST)
mobile phase . . . . . 5 mM copper sulfate
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL in water
Application No. . . . . G004663



## HPLC Analysis of Luciferin Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM ammonium nitrate, pH 5.5; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 6 µL
sample . . . . . 0.5 mg/mL in methanol
Application No. . . . . G004641

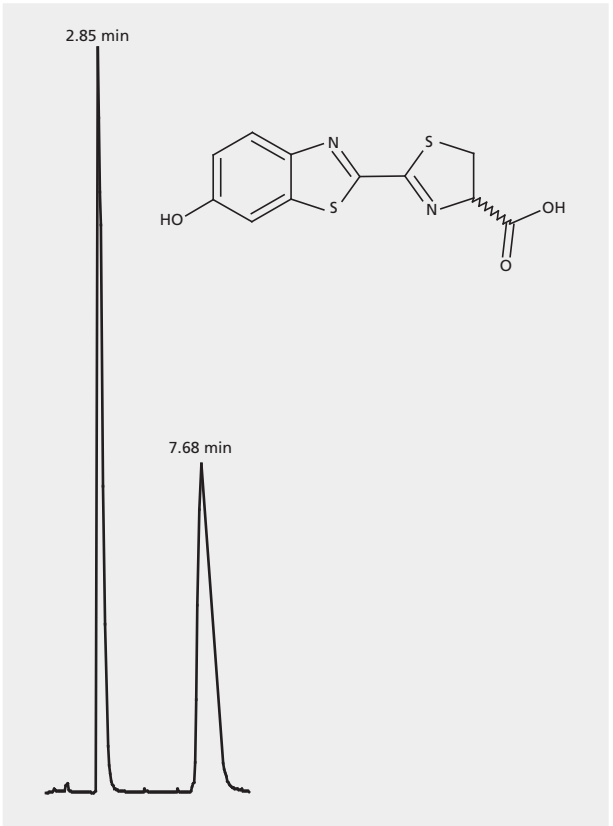





SUPELCO® Solutions within.™

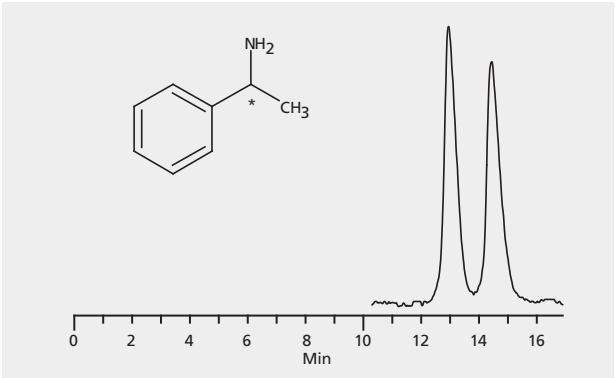
## HPLC Analysis of Luciferin Enantiomers on CHIRALPAK® AGP

column . . . . . CHIRALPAK AGP, 10 cm x 4 mm I.D., 5 µm particles (58150AST)
mobile phase . . . . . 10 mM sodium phosphate, pH 6.0
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 225 nm
sample . . . . . luciferin
Application No. . . . . G004398



## HPLC Analysis of α-Methylbenzylamine Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) hexane; (B) isopropanol; (90:10, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004642



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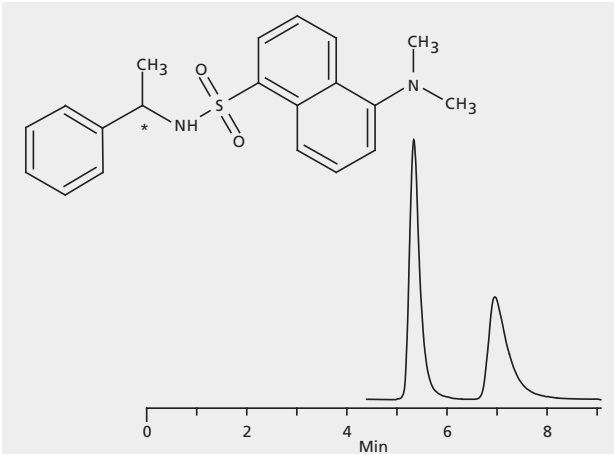
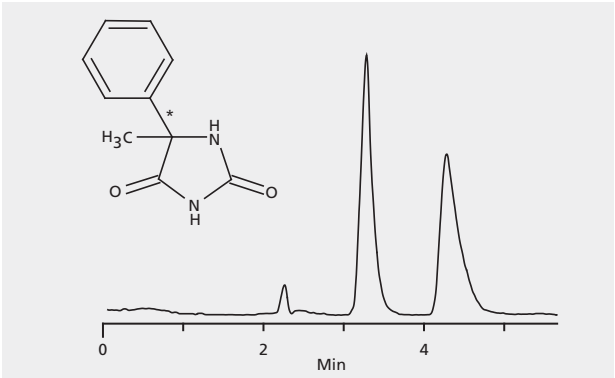
## HPLC Analysis of α-Methylbenzylamine Enantiomers on Astec® CYCLOBOND® I 2000 DMP

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . . . . ethanol
flow rate . . . . . 1.2 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 3 mg/mL in methanol
Application No. . . . . G004698



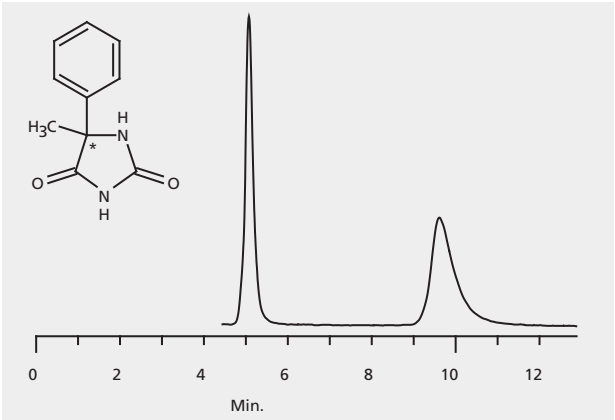
## HPLC Analysis of 5-Methyl-5-Phenylhydantoin Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . ethanol
flow rate . . . . . 1.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 4 mg/mL in methanol
Application No. . . . . G004643



## HPLC Analysis of 5-Methyl-5-Phenylhydantoin Enantiomers on Astec® CHIROBIOTIC® TAG (Fast Flow)

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . methanol
flow rate . . . . . 0.8 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004613



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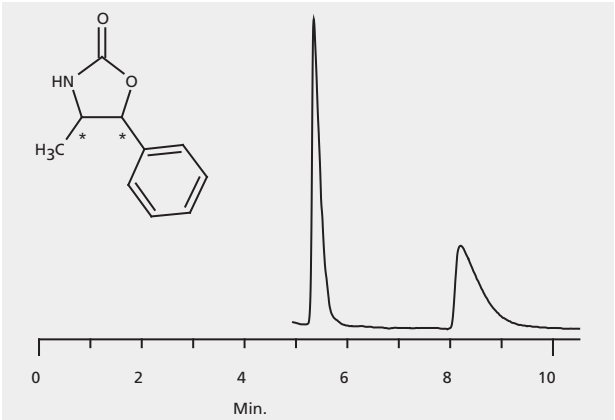
## HPLC Analysis of 4-Methyl-5-Phenyl-2-Oxazolidone Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . methanol
flow rate . . . . . 0.8 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004612



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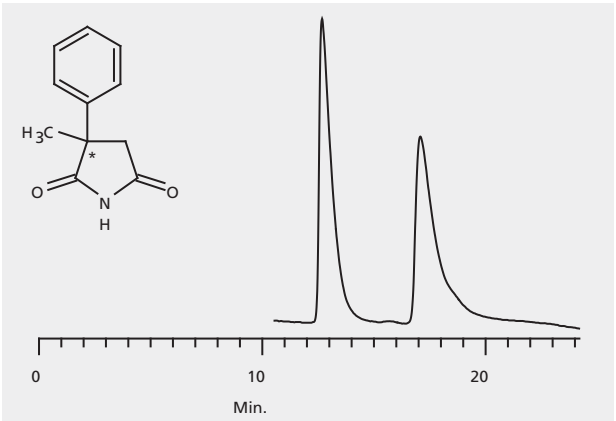
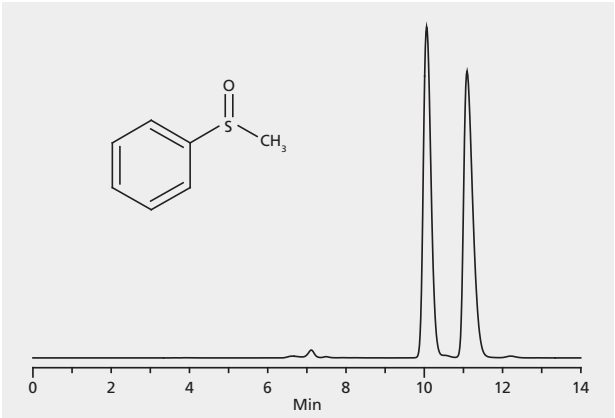
## HPLC Analysis of α-Methyl-α-Phenylsuccinimide Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . (A) hexane; (B) ethanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004611



## HPLC Analysis of Methyl Phenyl Sulfoxide Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . methanol
flow rate . . . . . 0.48 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in mobile phase
Application No. . . . . G004407



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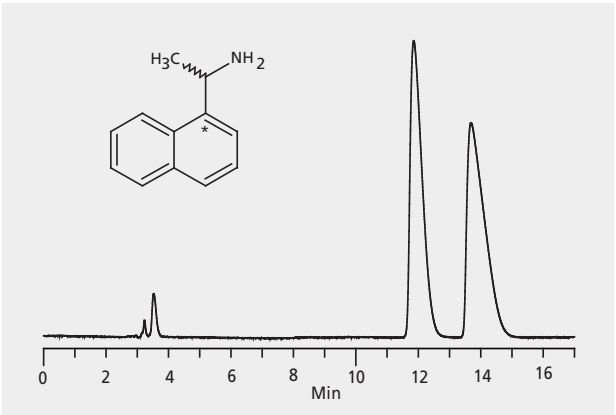
## HPLC Analysis of 1-(1-Naphthyl)ethylamine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . (A) methanol; (B) 20 mM ammonium acetate; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 1 mgl/mL in methanol
Application No. . . . . G004458

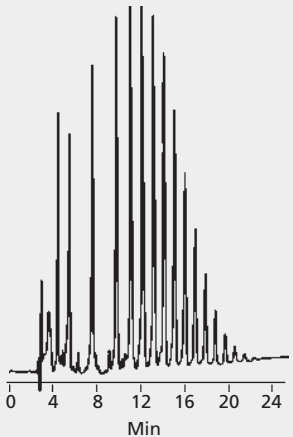





SUPELCO® Solutions within.™

## HPLC Analysis of Nonionic Surfactants, 9-10 Ethoxy Groups (Octylphenol) on SUPELCOSIL™ LC-Diol

column . . . . . SUPELCOSIL LC-Diol, 25 cm × 4.6 mm I.D., 5 µm particles (58201)
mobile phase . . . . . (A) hexane:methylene chloride (95:5);
(B) hexane:methylene chloride:methanol (50:40:10)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL methylene chloride containing 20 µg surfactant
Application No. . . . . 713-0782

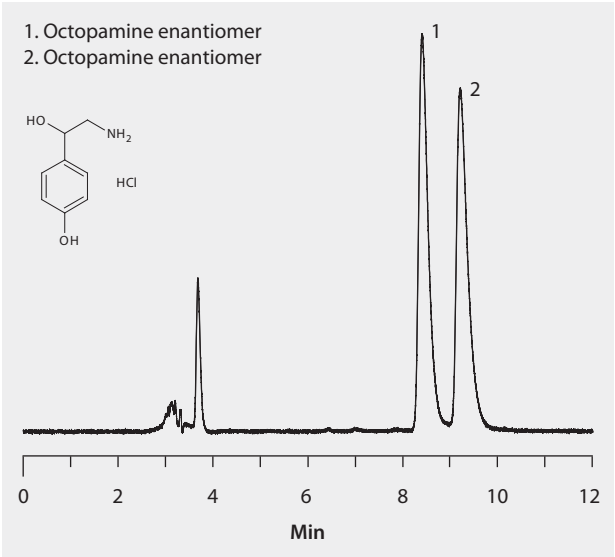



9-10 Ethoxy Groups
(Octylphenol)

| Gradient Program Time (min) | %B |
|---|---|
| 0 | 30 |
| 8 | 50 |
| 15 | 60 |
| 25 | 80 |
| 35 | 100 |

## HPLC Analysis of Octopamine Enantiomers on LARIHC™ CF6-P

| | |
|---|---|
| column | LARIHC CF6-P, 25 cm x 4.6 mm I.D., 5 µm particles (AZYP Part No.L1001, available from Supelco/Sigma-Aldrich as a custom item.) |
| mobile phase | (A) methanol: (B) acetonitrile: (C) acetic acid: (D) triethylamine, (70:30:0.3.0.2, A:B:C:D) |
| flow rate | 1 mL/min |
| column temp. | 20 °C |
| detector | UV, 254 nm |
| injection | 5 µL |
| sample | Octopamine, 0.3 mg/mL in ethanol |
| Application No. | G005258 |

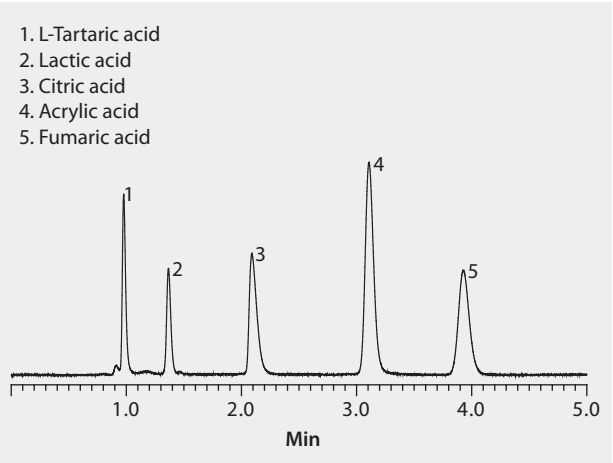



**Ref:** 1. Ping Sun, Chunlei Wang, Zachary S. Breitbach, Ying Zhang, and Daniel W. Armstrong, Development of New HPLC Chiral Stationary Phases Based on Native and Derivatized Cyclofructans *Anal. Chem.* **81**, 10215 (2009)
2. Ping Sun and Daniel W. Armstrong, Effective enantiomeric separations of racemic primary amines by the isopropyl carbamate-cyclofructan6 chiral stationary phase *J. Chromatogr. A.* **1217**, 4904 (2010)

## HPLC Analysis of Organic Acids on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the analysis of organic acids under 100% aqueous conditions.

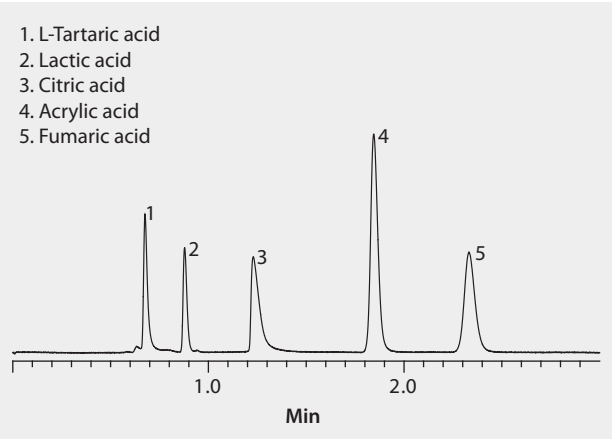
| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U) |
| mobile phase | (A) 0.1% (v/v) TFA in (B) water |
| flow rate | 0.3 mL/min |
| column temp. | 35 °C |
| detector | UV, 210 nm |
| injection | 1 µL |
| sample | 0.2 - 4 g/L in mobile phase |
| Application No. | G005714 |



## HPLC Analysis of Organic Acids on Ascentis® RP-Amide

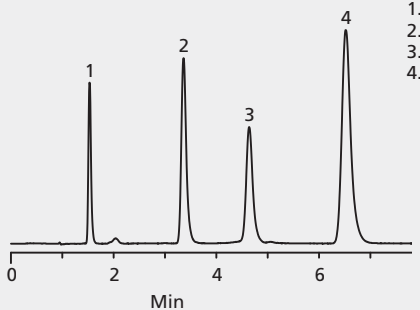
This application demonstrates the suitability of Ascentis RP-Amide for the analysis of organic acids under 100% aqueous conditions.

column . . . Ascentis RP-Amide, 10 cm x 2.1 mm I.D., 3 µm particles (565301-U)
mobile phase . . . . . (A) 0.1% (v/v) TFA in (B) water
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 1 µL
sample . . . . . 0.2 - 4 g/L in mobile phase
Application No. . . . . G005715



## HPLC Analysis of Organic Acids on Discovery® C18

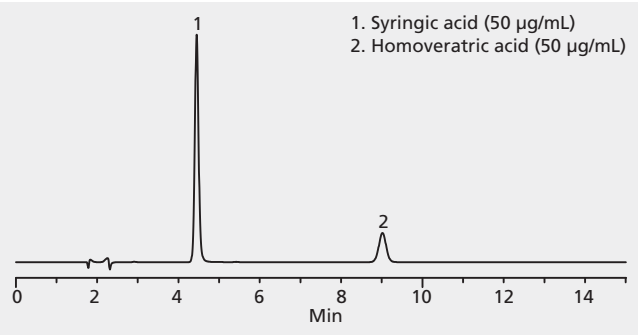
column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) methanol; (B) water, 0.1% TFA (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL each analyte
Application No. . . . . 797-0657




1. Homovanillic acid
2. Sorbic acid
3. Salicylic acid
4. p-Toluic acid

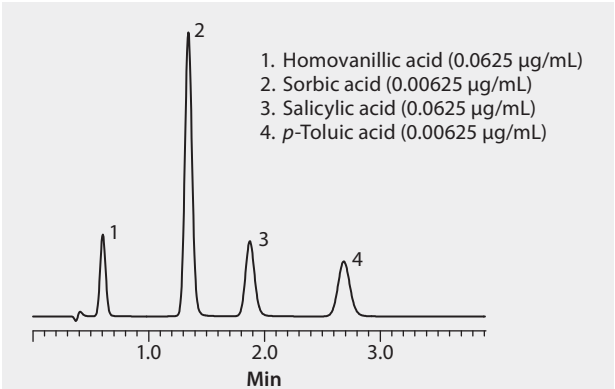
## HPLC Analysis of Organic Acids on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) 10 mM ammonium phosphate monobasic (pH 2.50 with phosphoric acid): (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003160



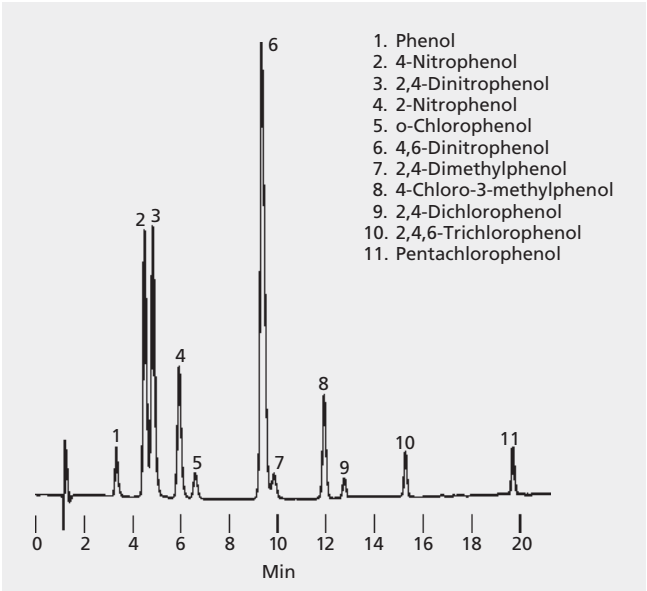
## HPLC Analysis of Organic Acids on Discovery® C18

| | |
|---|---|
| column | Discovery C18, 5 cm × 4.6 mm I.D., 5 µm particles (504947) |
| mobile phase | (A) 0.1% TFA in (B) water:methanol; (60:40, A:B) |
| flow rate | 2.0 mL/min |
| column temp. | 20 °C |
| detector | UV, 254 nm |
| injection | 10 µL |
| Application No. | G001431 |



## HPLC Analysis of Phenols on SUPELCOSIL™ LC-8

column . . . . . SUPELCOSIL LC-8, 15 cm × 4.6 mm I.D., 5 µm particles (58220-U)
mobile phase . . . . . (A) 1% acetic acid/methanol: (B) 1% acetic acid/water
gradient . . . . . 35:65 to 100:0 (20 min), return to 35:65 (5 min)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 280 nm
Application No. . . . . 713-1031



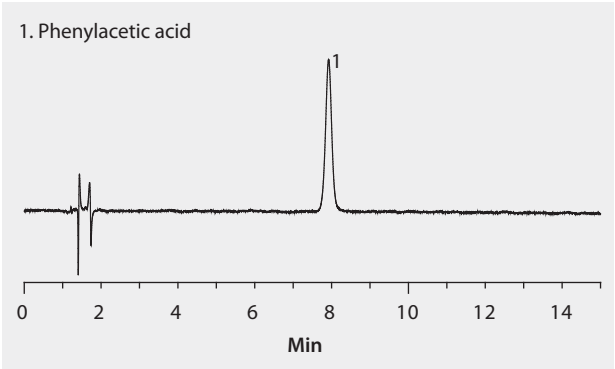



SUPELCO® Solutions within.™

## HPLC Analysis of Phenylacetic Acid on Ascentis® C18

This application demonstrates the suitability of Ascentis C18 for the analysis of phenylacetic acid.

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid; (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL in water: acetonitrile: methanol (75: 20: 5)
Application No. . . . . G005720



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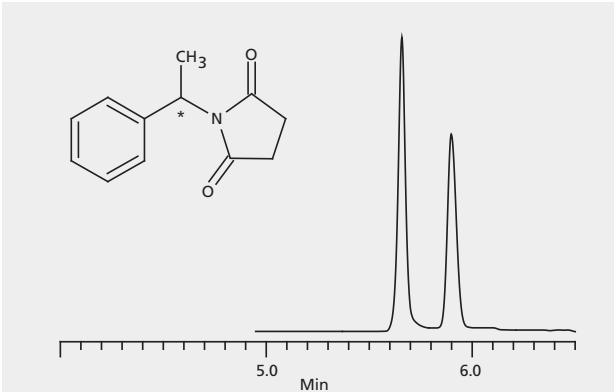
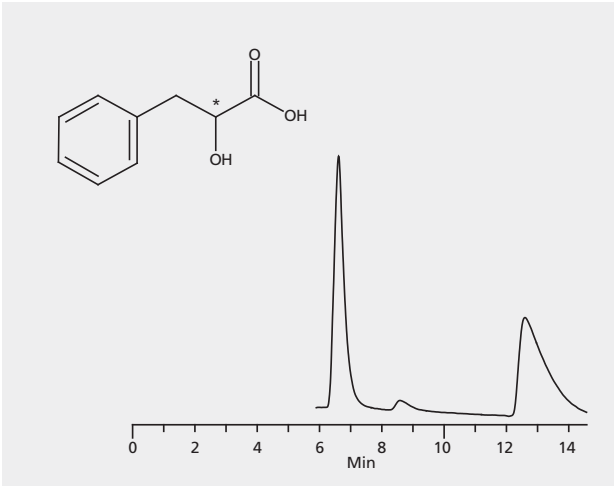
## HPLC Analysis of N-(1-Phenylethyl)maleimide Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . (A) 0.1% triethylamine acetate, pH 4.1; (B) methanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 3 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004668



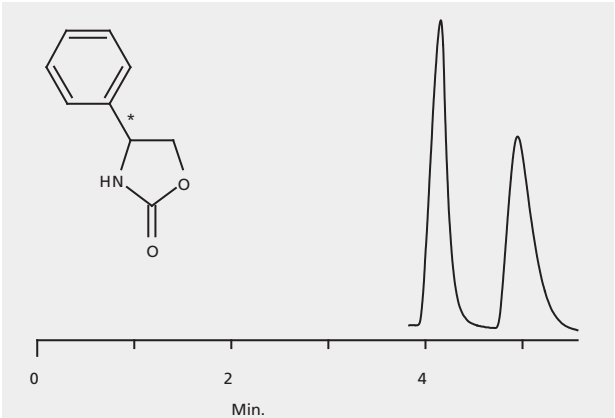
## HPLC Analysis of 3-Phenyllactic Acid Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1% ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 0.5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004680



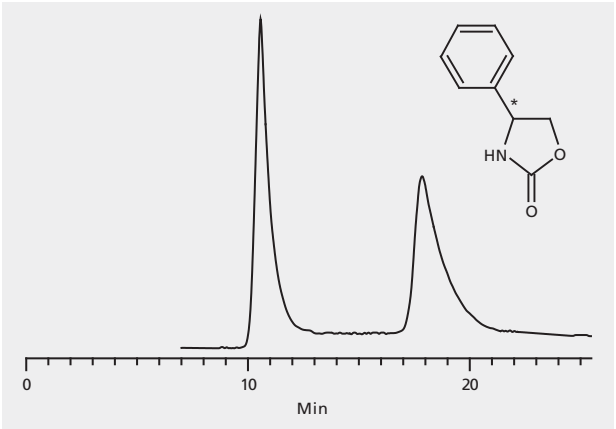
## HPLC Analysis of 4-Phenyl-2-Oxazolidinone Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004599



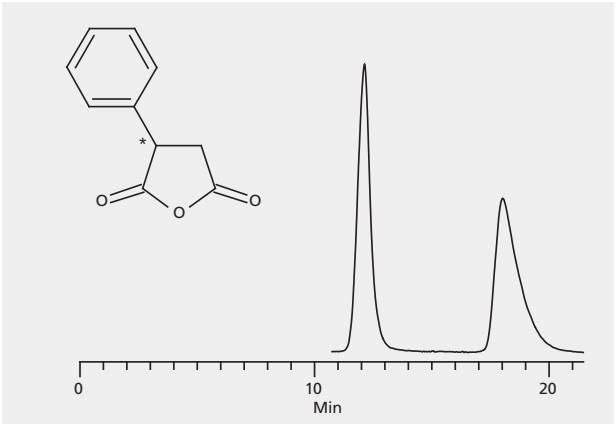
## HPLC Analysis of 4-Phenyl-2-oxazolidinone Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 0.5% triethylamine acetate, pH 4.1; (B) methanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004548



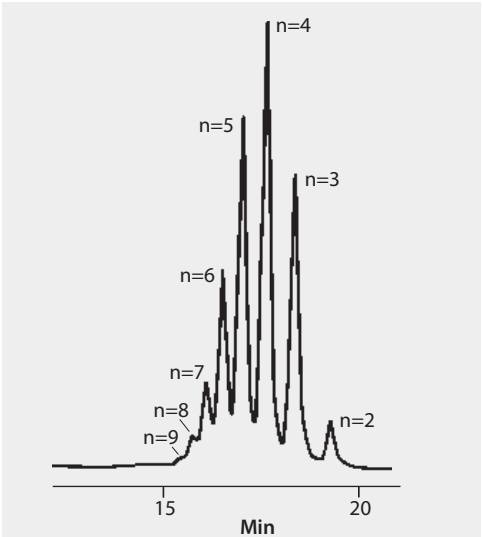
## HPLC Analysis of Phenylsuccinic Anhydride Enantiomers on Astec® (R,R) P-CAP™-DP

column . . Astec (R,R) P-CAP-DP, 25 cm x 4.6 mm I.D., 5 µm particles (35024AST)
mobile phase . . (A) acetonitrile; (B) methanol; (C) trifluoroacetic acid; (99:1/0.1 (A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004728



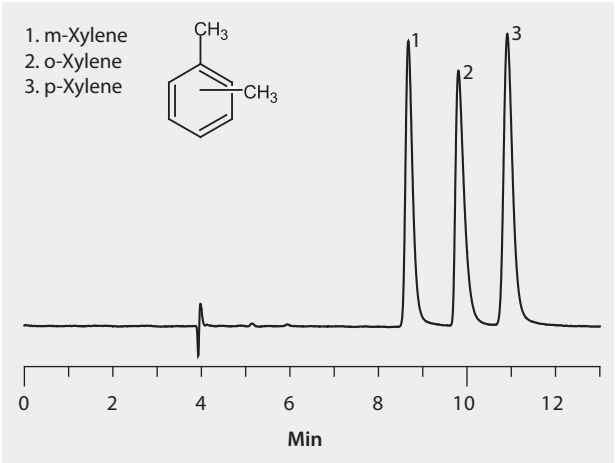
## HPLC Analysis of Polyethylene Glycol 200 on TSKgel® G-OLIGO-PW

column . . . TSKgel G-OLIGO-PW, 30 cm × 7.8 mm I.D., 6 µm particles (808031)
mobile phase . . . . . distilled water
flow rate . . . . . 1 mL/min
detector . . . . . RI
Application No. . . . . 713-0778



## HPLC Analysis of Positional Isomers (Xylenes) on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . . . . (A) acetonitrile; (B) water; (15:85, A:B)
flow rate . . . . . 0.8 mL/min
column temp. . . . . 55 °C
detector . . . . . UV, 230 nm
injection . . . . . 3 µL
sample . . . . . xylene isomers, each compound, 0.1 mg/mL in acetonitrile:water (50:50) ((elution order: m, o, p))
Application No. . . . . G005166



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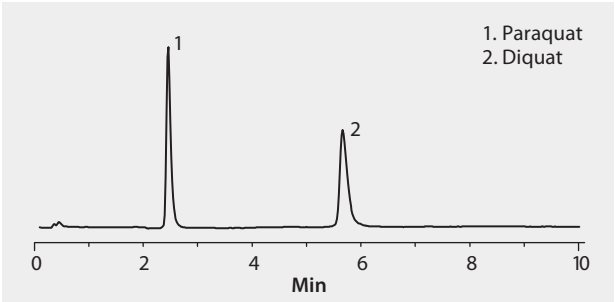
## HPLC Analysis of Quaternary amines on Discovery® Zr-PS

column . . . . . Discovery Zr-PS, 7.5 cm × 4.6 mm I.D., 3 µm particles (65741-U)
mobile phase . . (A) 25 mM phosphoric acid, 25 mM ammonium fluoride (pH with ammonium hydroxide): (B) acetonitrile, (50:50, A:B)
flow rate . . . . . 3 mL/min
column temp. . . . . 65 °C
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
sample . . . . . Paraquat, diquat (50 µg/mL)
Application No. . . . . G001853



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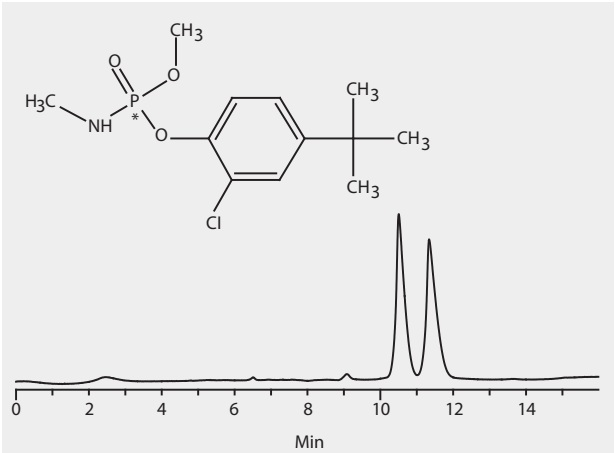
## HPLC Analysis of Ruelene (Cruformate) Enantiomers on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . . . . (A) acetonitrile; (B) acetic acid; (C) TEA; (100:0.3:0.2, A:B:C)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . Ruelene (cruformate), 1 mg/mL in acetonitrile
Application No. . . . . G005158

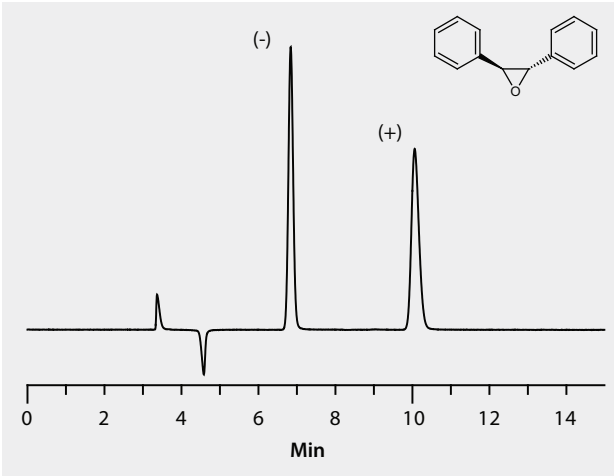





SUPELCO® Solutions within.™

## HPLC Analysis of trans-Stilbene Oxide Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (90:10, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004995

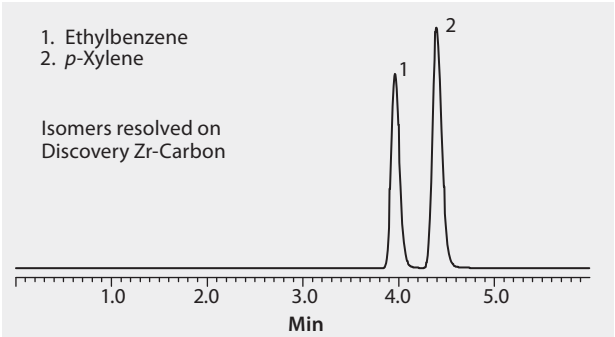





SUPELCO® Solutions within.™

## HPLC Analysis of Structural Isomers on Discovery® Zr-Carbon

column . . . Discovery Zr-Carbon, 15 cm × 4.6 mm I.D., 3 µm particles (65730-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 60 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G001874





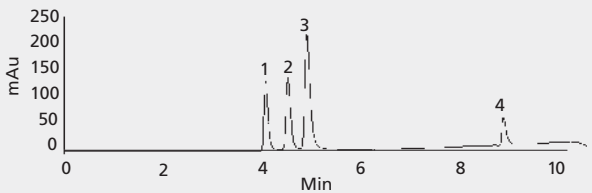

SUPELCO® Solutions within.™

## HPLC Analysis of Sulfonamide Positional Isomers on Discovery® Zr-Carbon C18

column . . Discovery Zr-Carbon C18, 15 cm × 4.6 mm I.D., 3 µm particles (65706-U)
mobile phase . . . . . (A) 10mM diethylamine, pH 10.8; (B) acetonitrile;
flow rate . . . . . 1.5 mL/min
column temp. . . . . 80 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
Application No. . . . . G001866

1. Isomer 1
2. Isomer 2
3. Parent drug
4. Isomer 3

| Gradient | | |
|---|---|---|
| Time (min) | %A | %B |
| 0.0 | 55 | 45 |
| 5.0 | 55 | 45 |
| 7.5 | 25 | 75 |
| 10.0 | 25 | 75 |

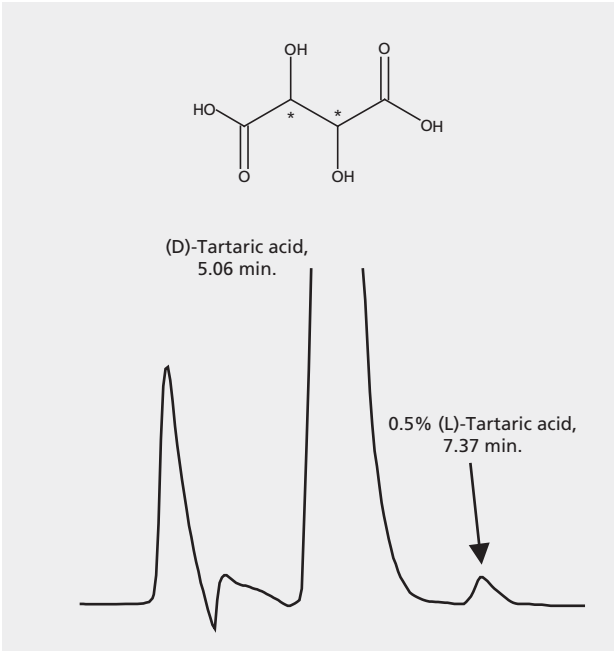





SUPELCO® Solutions within.™

## HPLC Analysis of Tartaric Acid Enantiomers on Astec® CLC-D, Trace Level Detection

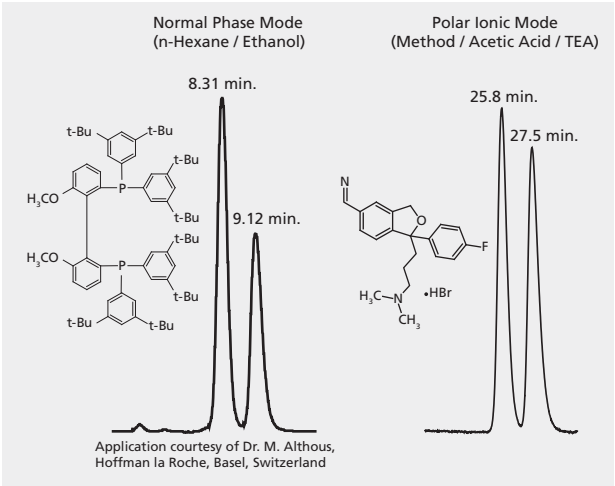
column . . . . . Astec CLC-D, 15 cm x 4.6 mm I.D., 5 µm particles (53023AST)
mobile phase . . . . . 3 mM copper sulfate, pH 3.2
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL in water
Application No. . . . . G004665



## HPLC Analysis of 3,5-tBu-MeOBIPHEP Enantiomers on Astec® CHIROBIOTIC® V in Normal Phase and Polar Ionic Modes

Demonstration of CHIROBIOTIC® column utility in both normal phase and polar ionic modes. The same CHIROBIOTIC® column can be used in all four modes, from aqueous to organic, without memory effects or loss of performance.
Column: CHIROBIOTIC® V, 25 cm x 4.6 mm, 5 µm particles (11024AST).
Left: **Normal phase mode**. 3,5-tBu-MeOBIPHEP enantiomers. n-Hexane:10% ethanol in n-hexane (75:25), 1.5 mL/min.
Right: **Polar ionic mode**. Citalopram enantiomers. Methanol:acetic acid:TEA (99.8:0.1:0.1), 0.5 mL/min.

column . . . . . CHIROBIOTIC® V, 25 cm x 4.6 mm, 5 µm particles (11024AST)
Application No. . . . . G004430




Application courtesy of Dr. M. Althous, Hoffman la Roche, Basel, Switzerland



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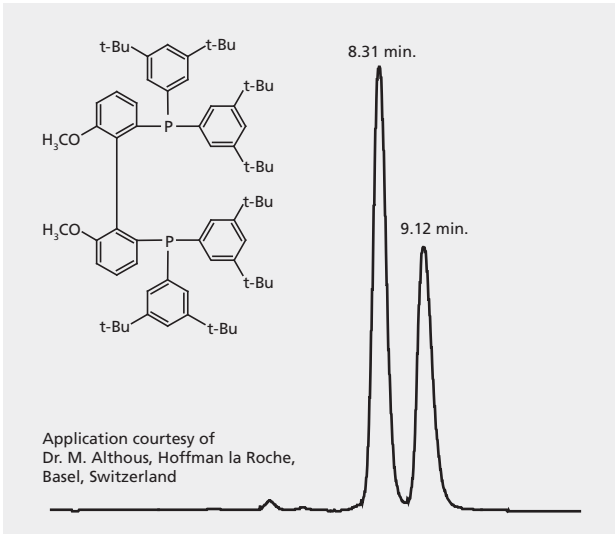
## HPLC Analysis of 3,5-tBu-MeOBIPHEP Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) n-hexane; (B) 10% ethanol in n-hexane; (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 18 °C
detector . . . . . UV, 230 nm
sample . . . . . 3,5-tBu-MeOBIPHEP ((6,6′-dimethoxybiphenyl-2,2′-diyl)bis[bis(3,5-di-*tert*-butylphenyl)phosphine]), 1 mg/mL in ethanol with 5 drops of $H_2O_2$ added for oxidation
Application No. . . . . G004402



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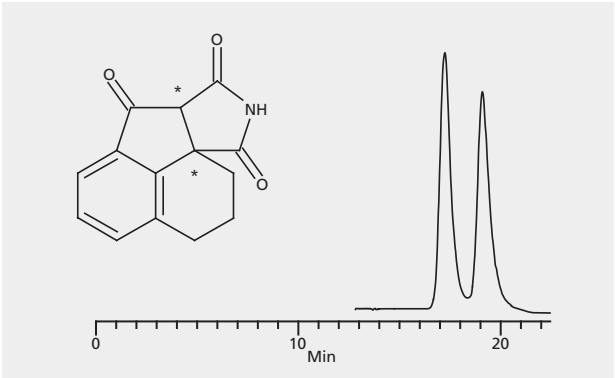
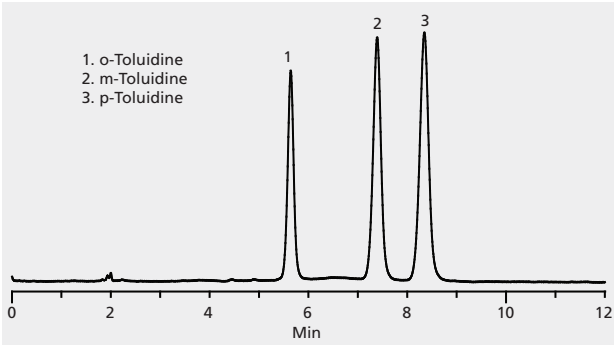
## HPLC Analysis of 3a,4,5,6-Tetrahydrosuccinimido [3,4-b]acenaphthen-10-one Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) hexane; (B) ethanol; (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 4 mg/mL in methanol
Application No. . . . . G004648



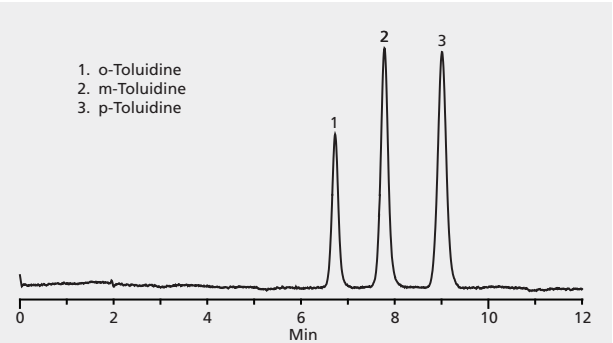
## HPLC Analysis of Toluidine Isomers on Ascentis® Si (Hexane: 2-Propanol)

column . . . . . Ascentis Si, 15 cm × 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) hexane; (B) 2-propanol; (96:4, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 245 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in mobile phase
Application No. . . . . G003799



## HPLC Analysis of Toluidine Isomers on Ascentis® Si (Silica), (80:20 Hexane:Ethyl Acetate)

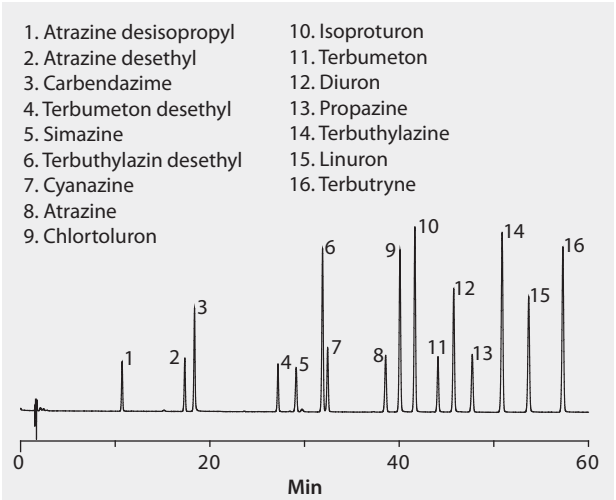
column . . . . . Ascentis Si, 15 cm × 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) hexane; (B) ethyl acetate; (80:20, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 245 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in mobile phase
Application No. . . . . G003697



## HPLC Analysis of 16 Triazine and Phenyl Urea Compounds on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of 16 triazine and phenyl urea compounds. Lower column temperature can improve resolution of the critical pair.

column . . Ascentis Express RP-Amide, 15 cm x 3 mm I.D., 2.7 µm particles (53919-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . . . . 5 to 53% B in 70 min
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . 20 mg/L (except terbuthylazin desethyl and terbuthylazine 60 mg/L) in 60:40, water: acetonitrile
Application No. . . . . G005651



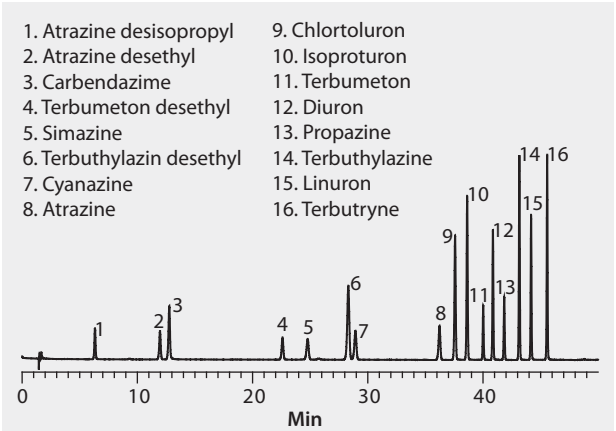



SUPELCO® Solutions within.™

## HPLC Analysis of 16 Triazine and Phenyl Urea Compounds on Ascentis® Express RP-Amide, Modified Gradient

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of 16 triazine and phenyl urea compounds. Lower column
temperature can improve resolution of the critical pair.

column . . Ascentis Express RP-Amide, 15 cm x 3 mm I.D., 2.7 µm particles (53919-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . 10 to 25% B in 30 min; 25 to 60% B in 15 min; held at 60% B for 5 min
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . 20 mg/L each (except terbuthylazin desethyl and terbuthylazine 60 mg/L) in 60:40, water: acetonitrile
Application No. . . . . G005652



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## HPLC Analysis of Triazole Antifungal Agent Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

column . . . . . Astec CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)

mobile phase . . . . . (A) 0.1% formic acid; (B) acetonitrile; (70:30, A:B)

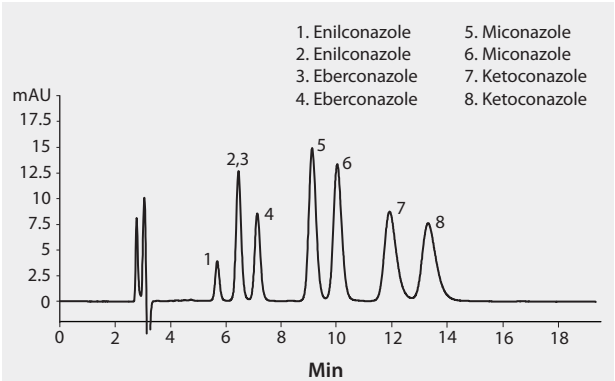
flow rate . . . . . 1 mL/min

detector . . . . . UV, 230 nm

injection . . . . . 1 µL

sample . . . . . 0.1 mg/mL in methanol
peaks 1&2: enilconazole
peaks 2&3: eberconazole
peaks 4&5: miconazole
peaks 6&7: ketoconazole

Application No. . . . . G004737



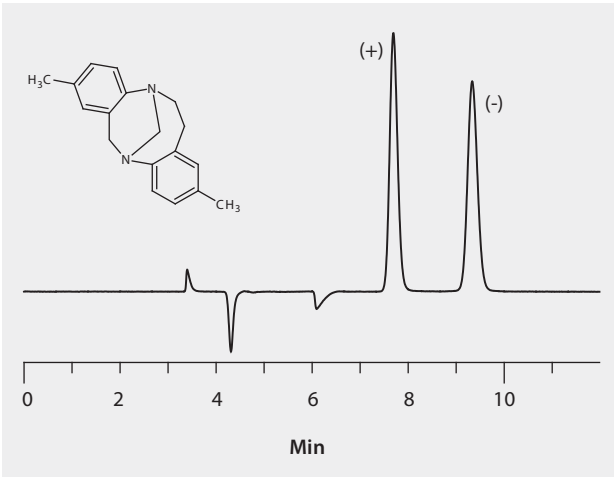



SUPELCO® Solutions within.™

## HPLC Analysis of Tröger's Base Enantiomers on Astec® Cellulose DMP

Tröger's base is an organic compound that exhibits chirality due to the presence of two bridgehead stereogenic nitrogen atoms in its structure.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) DEA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004996



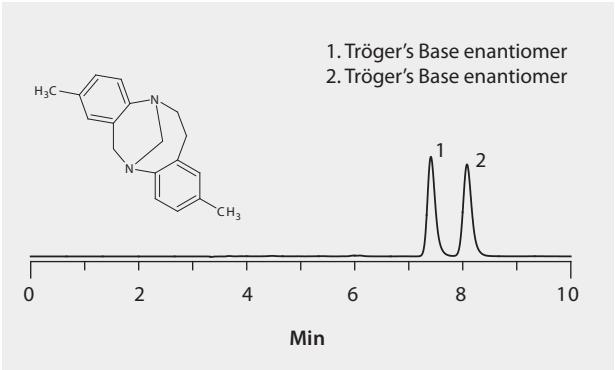



SUPELCO® Solutions within.™

## HPLC Analysis of Tröger's Base Enantiomers on Astec® Cellulose DMP (LC/MS Mobile Phase)

Tröger's base is an organic compound that exhibits chirality due to the presence of two bridgehead stereogenic nitrogen atoms in its structure.

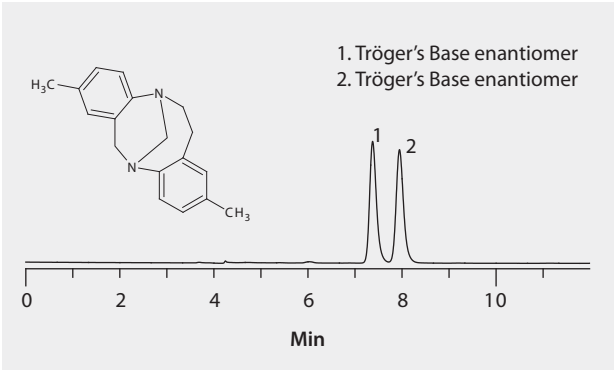
column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) ammonium formate; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
Application No. . . . . G005002



## HPLC Analysis of Tröger's Base Enantiomers on Astec® Cellulose DMP (Polar Organic Mode)

Tröger's base is an organic compound that exhibits chirality due to the presence of two bridgehead stereogenic nitrogen atoms in its structure.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) DEA; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005001

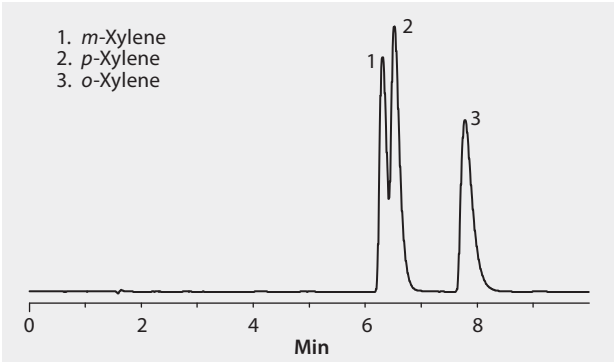





SUPELCO® Solutions within.™

## HPLC Analysis of Xylene Isomers on Discovery® Zr-Carbon

column . . . Discovery Zr-Carbon, 15 cm × 4.6 mm I.D., 3 µm particles (65730-U)
mobile phase . . . . . (A) water: (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . o-xylene, m-xylene, p-xylene (290 µg/mL)
Application No. . . . . G001880

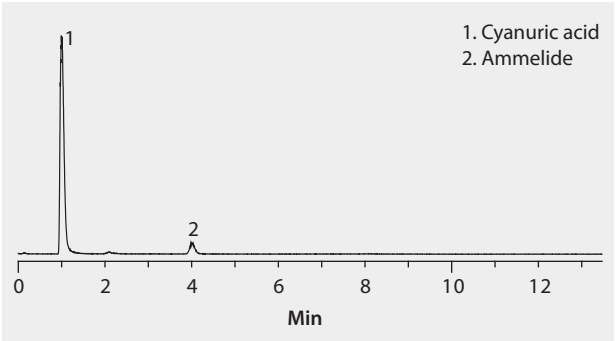





SUPELCO® Solutions within.™

## LC-MS Analysis of Cyanuric Acid on Ascentis® Express HILIC

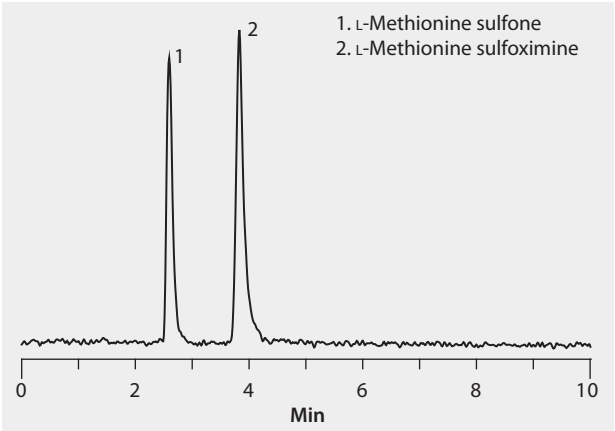
column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 5 mM ammonium formate in 95:5 (v/v) acetonitrile:water
flow rate . . . . . 0.2 mL/min
pressure . . . . . 328 psi (22.6 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(-), SIR mode
injection . . . . . 2 µL
sample . . . . . 1 µg/mL in mobile phase
Application No. . . . . G005690



## LC-MS Analysis of L-Methionine Sulfoximine and Sulfone on Ascentis® Express OH5

L-methionine sulfoximine and L-buthionine sulfoximine are used in are used to prevent additional enzyme activity in Chinese hampster ovary (CHO) cell lines with supplemental Glutamine Synthase (GS). It is of interest to be able to monitor both purity of such molecules in order to control addition to cell media as well as for the assay of parent molecules during use. The analytes are highly polar and thus should be amenable to HILIC-LC-MS analysis. This first installment investigates the ability of Ascentis Express HILIC and Ascentis Express OH5 stationary phases for the separation of the methionine analog and related compounds as well as methionine and buthionine separations.

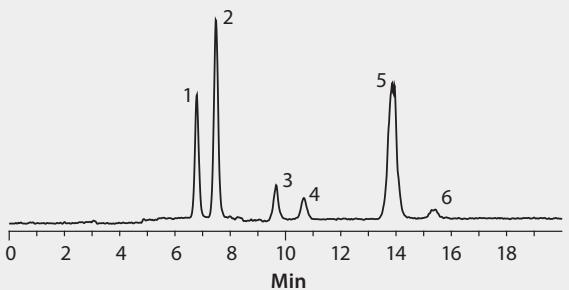
column . . Ascentis Express OH5, 10 cm x 3.0 mm I.D., 2.7 µm particles (53769-U)
mobile phase . . . . . (A) acetonitrile; (B) water with 0.1% formic acid, pH 3.5 with ammonium formate; (75:25, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1350 ppi (93 bar)
column temp. . . . . 25 °C
detector . . . . . MS, ESI (+), TIC, m/z 150-300
injection . . . . . 5 µL
Application No. . . . . G005791



## LC-MS Analysis of Quarternary Ammonium Compounds on Ascentis® Si (Silica)

column . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U) (65741-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted):
(B) 0.1% ammonium acetate in acetontrile; (10:90, A:B)
flow rate . . . . . 1 mL/min. split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 10:90:water:acetonitrile
Application No. . . . . G003724

1. Propantheline (1 µg/mL) (M =368.22)
2. Oxyphenonium (1 µg/mL) (M = 348.25)
3. Mepenzolate (1 µg/mL) (M= 340.19)
4. Scopolamine (10 µg/mL) (M+H)+ = 304.14
5. Ipratropium (1 µg/mL) (M = 332.22)
6. Methscopolamine (1 µg/mL) (M= 318.16)



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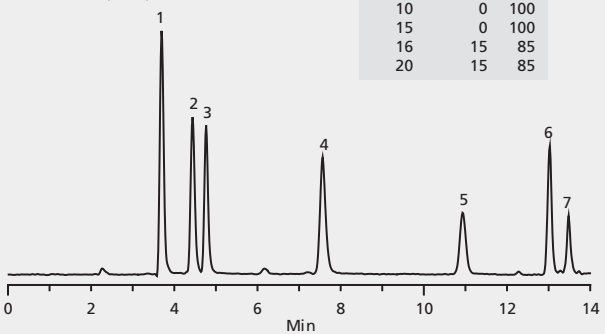
## LC-MS Analysis of Sudan Red Dyes on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm × 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 0.1% formic acid in water (pH unadjusted): (B) acetonitrile; (15:85, A:B)
flow rate . . . . . 1 mL/min, split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, APCI (+) in selected ion recording (SIR) mode
injection . . . . . 15 µL
sample . . 1 µg/mL in 30:70, 0.1% formic acid in water (pH unadjusted):acetonitrile
Application No. . . . . G003860

1. Sudan Orange G (M+H)+= 215.07
2. Sudan Red G (M+H)+= 279.10
3. Sudan I (M+H)+= 249.09
4. Sudan II (M+H)+= 277.12
5. Sudan III (M+H)+= 353.13
6. Sudan Red 7B (M+H)+= 380.17
7. Sudan IV (M+H)+= 381.16

Gradient Program

| Time (min) | %A | %B |
|---|---|---|
| 0 | 5 | 85 |
| 8 | 15 | 85 |
| 10 | 0 | 100 |
| 15 | 0 | 100 |
| 16 | 15 | 85 |
| 20 | 15 | 85 |



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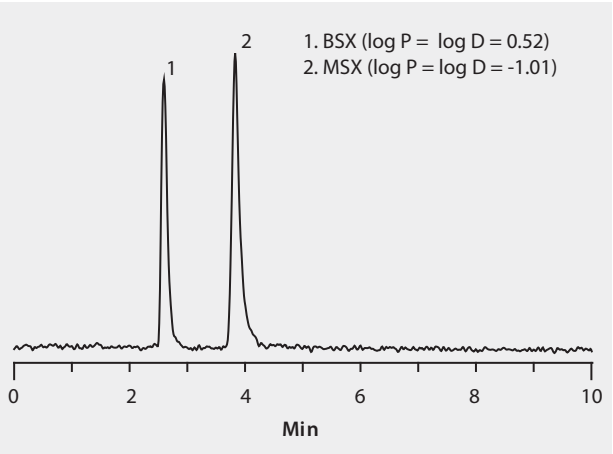
## LC-MS Extracted Ion Chromatogram of MSX, Sulfone, and Sulfoxide on Ascentis® Express OH5 (HILIC Mode)

This figure shows the extracted ion currents for methionine sulfoximine-related compounds on Ascentis Express OH5. The OH5 shows excellent peak shape compared to traditional HILIC on bare silica.

column . . . Ascentis Express OH5, 10 cm x 3 mm I.D., 2.7 µm particles (53769-U)
mobile phase . . . . . (A) 0.1% formic acid, pH 3.5 with ammonium formate; (B) acetonitrile; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1350 psi (93 bar)
column temp. . . . . ambient
detector . . . . . ESI (+), scan m/z 150-300
injection . . . . . 5 µL
sample . . . . . 10 µg/mL in 90% methanol
Application No. . . . . G005803

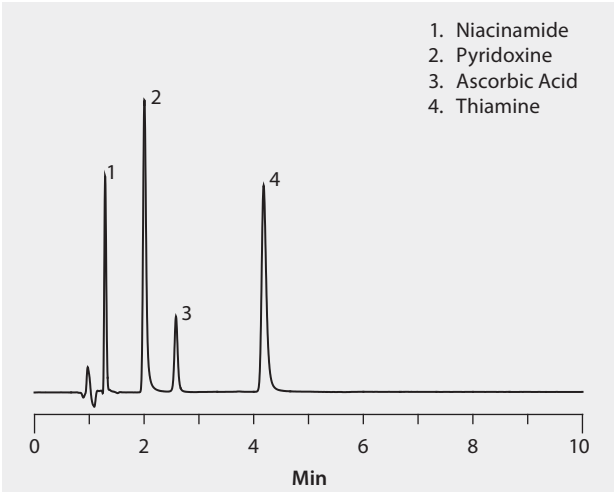


## Clinical

### HILIC Analysis of Polar Vitamins on Ascentis® Express 2.7 µm OH5

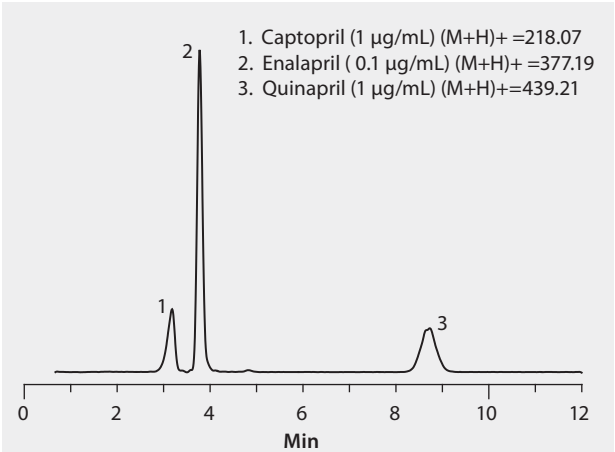
This application demonstrates good retention and selectivity for polar vitamins on Ascentis® Express OH5 in HILIC mode.

column . . Ascentis Express OH5 10 cm x 3.0 mm I.D. x 2.7 µm particles (53769-U)
mobile phase . . . . . 100 mM ammonium formate:Acetonitrile (15:85, v/v), pH adjusted to 5.0 with formic acid
flow rate . . . . . 0.5 mL/min
pressure . . . . . 850 psi (58.6 bar)
column . . . . . 35 °C
detector . . . . . UV, 276 nm
injection . . . . . 2.0 µL
sample . . . . . 100 ug/mL in 50:50 methanol:acetonitrile
Application No. . . . . G006253



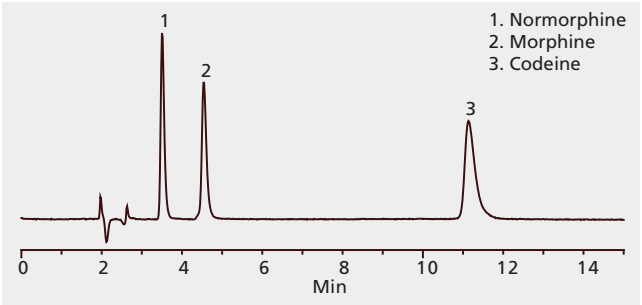
## HPLC Analysis of ACE Inhibitors on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 13 mM ammonium formate (pH 2.0 with concentrated formic acid): (B) methanol; (40:60, A:B)
flow rate . . . . . 1 mL/min, split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 40:60, 13 mM ammonium formate (pH 2.0 with concentrated formic acid):methanol
Application No. . . . . G003702



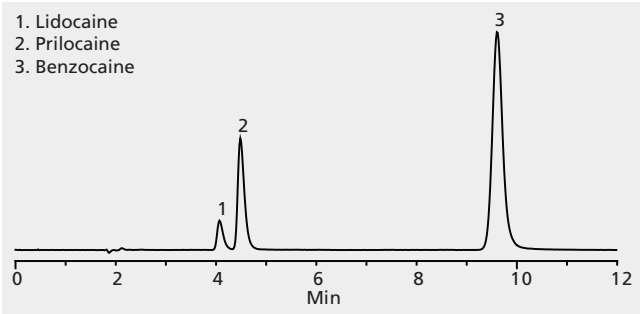
## HPLC Analysis of Analgesics, Narcotics on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formic acid); (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 90:10, water:methanol
Application No. . . . . G003709



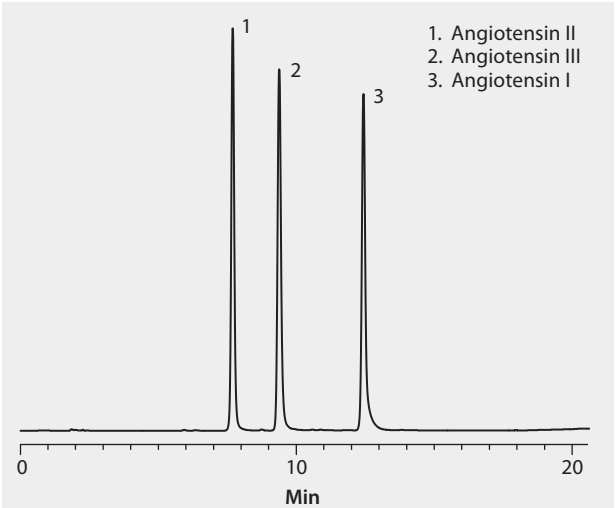
## HPLC Analysis of Anesthetics, Local on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formic acid); (B) methanol; (55:45, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 85:15, water:methanol
Application No. . . . . G003708



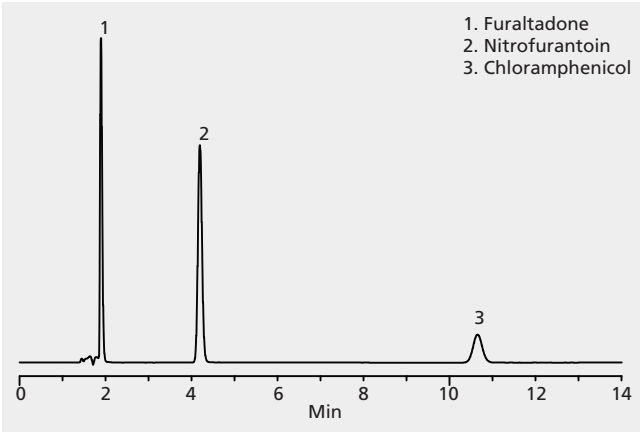
## HPLC Analysis of Angiotensins on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) 5 mM $(NH_4)H_2PO_4/NH_4OH$, pH 7.0;
(B) 5 mM $(NH_4)H_2PO_4/NH_4OH$, pH 7.0: $CH_3CN$ (50:50, A:B)
gradient . . . . . 30-60% B in 15 min
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
Application No. . . . . G001085



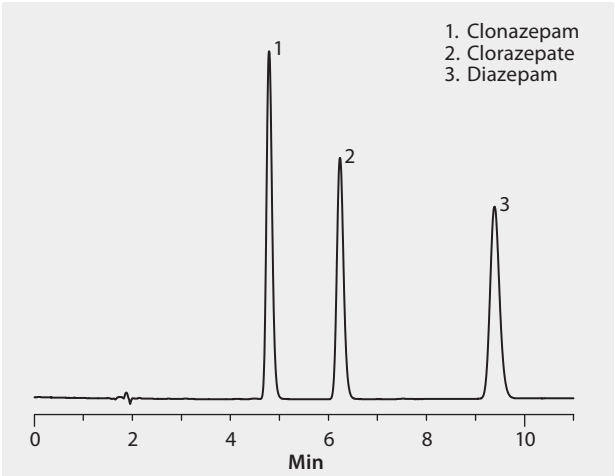
## HPLC Analysis of Antibacterials/Antifungals on Ascentis® RP-Amide (Formate Mobile Phase)

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formic acid); (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002602



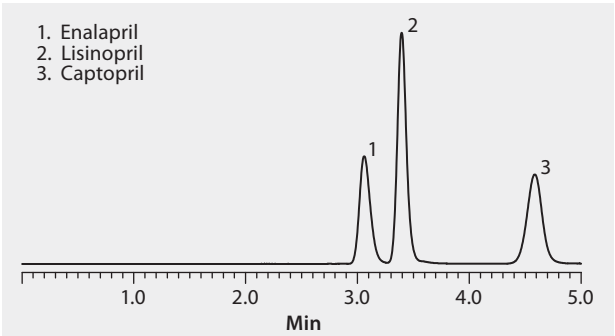
## HPLC Analysis of Anticonvulsant Drugs on Discovery® RP-AmideC16

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . (A) acetonitrile; (B) 25 mM potassium phosphate, pH 7.0 (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000188



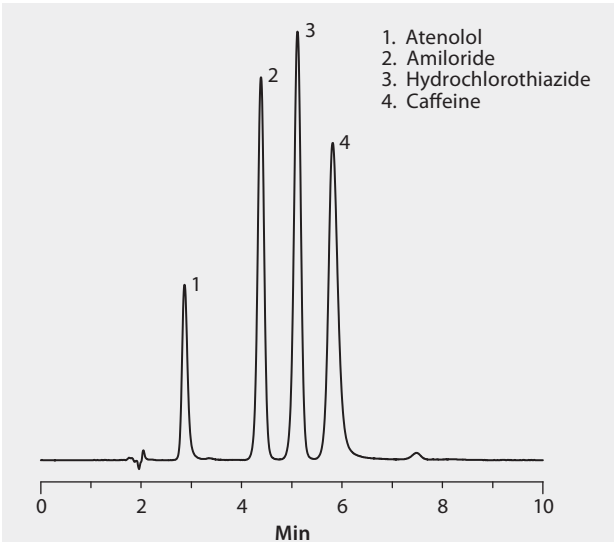
## HPLC Analysis of Antihypertensive ACE Inhibitors on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . (A) acetonitrile; (B) 25 mM potassium phosphate, pH 2.3 (33:67, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 3 µL
Application No. . . . . G000279



## HPLC Analysis of Antihypertensive and Diuretic Drugs on Discovery® RP-Amide C16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) methanol; (B) 25 mM potassium phosphate, dibasic, pH 7.0 (22:78, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000280

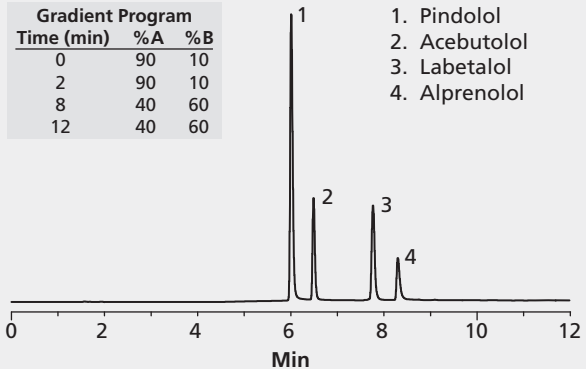


## HPLC Analysis of Antihypertensive Drugs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 7.0 with potassium hydroxide); (B):acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002383

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 2 | 90 | 10 |
| 8 | 40 | 60 |
| 12 | 40 | 60 |



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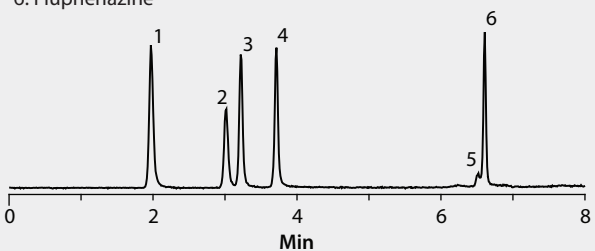
## HPLC Analysis of Antipsychotic Drugs on Ascentis® Express RP-Amide

column . . . . . Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U)
mobile phase . . . . . (A) 5 mM ammonium formate (in water); (B) 5 mM ammonium formate (in 95:5 methanol:water)
flow rate . . . . . 3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), TIC 100 - 1000 m/z
injection . . . . . 1 µL
sample . . . . . 200 µg/L in water
Application No. . . . . G005373

1. Desmethyl venlafaxine
2. Hydroxybupropion
3. Hydroxyrisperidon
4. Bupropion
5. Zuclopehthixol
6. Fluphenazine



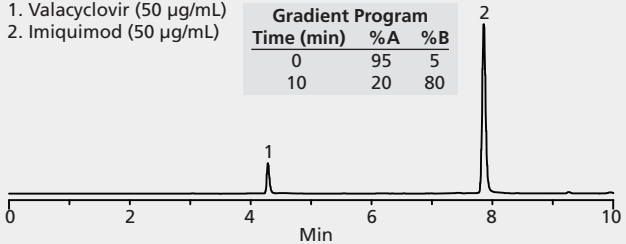
## HPLC Analysis of Antiviral Drugs on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 2.1 mm I.D., 3 µm particles (581402-U)
mobile phase . . (A) 10 mM ammonium phosphate (pH 2.5 with phosphoric acid); (B) acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003201

1. Valacyclovir (50 µg/mL)
2. Imiquimod (50 µg/mL)

**Gradient Program**

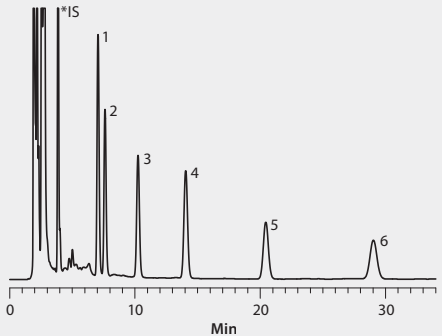
| Time (min) | %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 10 | 20 | 80 |



## HPLC Analysis of Barbituates in Serum on Discovery® C18 after SPE using Discovery® DSC-18Lt

A Zymark® RapidTrace® SPE Workstation was used for this analysis.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . 0.5 mL porcine serum spiked with 0.5 µg/mL or 1.0 µg/mL each analyte then diluted with 0.5 mL water
SPE tube/cartridge . . . . . Discovery DSC-18Lt, 500 mg/3 mL (52613-U)
condition . . . . . 2 mL methanol; 2 mL DI water
sample addition . . . . . 1 mL at 0.75 mL/min
washing . . . . . 2 mL 5% methanol, then vacuum or air dry for 5-10 min
elution . . . . . 1-2 mL methanol
eluate post-treatment . . . . . dry eluate with nitrogen purge (40 °C; 15-20 min), reconstitute in 20 µL mobile phase
column . . . . . Discovery C18, 15 cm × 4.6 mm, 5 µm preceded by a 2 cm C18 guard column and 0.5 µm frit filter (504955)
mobile phase . . . . . (A) methanol; (B) water (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 214 nm
injection . . . . . 30 µL, diluted porcine serum extract
Application No. . . . . G001056




Barbiturates from serum using 500 mg/3 mL Discovery DSC-18Lt SPE tubes and Zymark's RapidTrace SPE Workstation.
*IS = Barbital (internal standard).

Sample Info: 0.5 mL porcine serum spike with 0.5 µg/mL or 1.0 µg/mL each analyte then diluted with 0.5 mL water.

**Efficiency of Recovery**

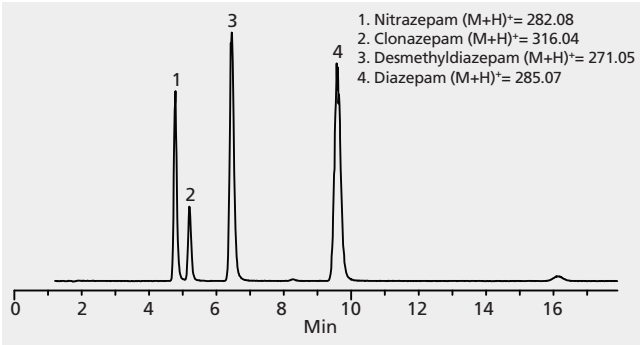
| Compound | Concentration (µg/mL) | %Recovery | %RSD (n=6) |
|---|---|---|---|
| 1. Phenobarbital | 0.5 | 96.2 | ±1.6 |
| | 1.0 | 94.9 | ±1.7 |
| 2. Aprobarbital | 0.5 | 98.5 | ±2.1 |
| | 1.0 | 100.8 | ±0.8 |
| 3. Butabarbital | 0.5 | 97.2 | ±1.9 |
| | 1.0 | 98.7 | ±1.8 |
| 4. Mephobarbital | 0.5 | 99.7 | ±2.4 |
| | 1.0 | 101.0 | ±2.0 |
| 5. Pentobarbital | 0.5 | 96.4 | ±1.7 |
| | 1.0 | 96.4 | ±1.9 |
| 6. Secobarbital | 0.5 | 98.2 | ±1.7 |
| | 1.0 | 97.7 | ±1.8 |

**SPE Method For RapidTrace SPE Workstation Application**

1. Condition & equilibrate each tube/well with 2 mL MeOH & 2 mL DI Water
2. Load sample
3. Wash each tube/well with 2 mL 5% MeOH
4. Vacuum or air dry for 5–10 min
   *This removes any excess water from the sorbent. The presence of water in the final eluent may prolong eluent evaporation.*
5. Elute with 1–2 mL MeOH
6. Dry eluate with nitrogen purge (40 °C; 15–20 min)
7. Reconstitute with 200 µL mobile phase
8. Quantify against internal or external standards via HPLC analyses

## HPLC Analysis of Benzodiazepines on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 2.1 mm I.D., 3 µm particles (581402-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 6.98 unadjusted); (B) acetonitrile; (55:45, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+), Selected Ion Recording Mode
injection . . . . . 5 µL
sample . . . . . 1 µg/mL each in 10 mM ammonium acetate (pH 6.98 unadjusted)
Application No. . . . . G003309



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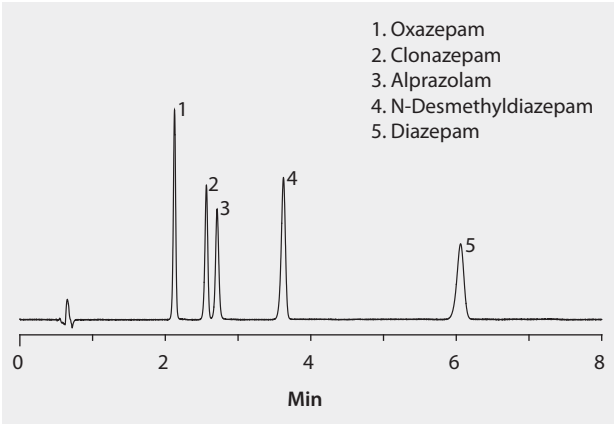
## HPLC Analysis of Benzodiazepines on Ascentis® Express C18, Acetonitrile Mobile Phase

column . . . Ascentis Express C18, 10 cm x 3 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (66:34, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 250 nm
injection . . . . . 2 µL
sample . . . . . 0.1 g/L each in 20% methanol
Application No. . . . . G005673

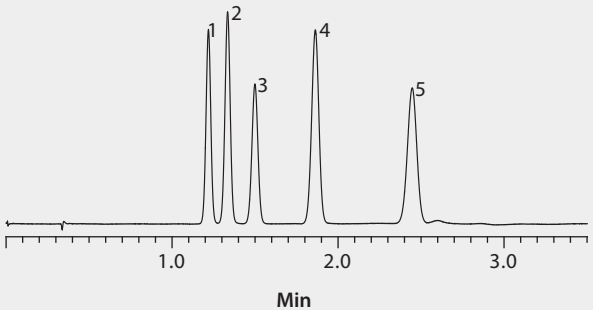


## HPLC Analysis of Benzodiazepines on Ascentis® Express Phenyl-Hexyl, Methanol Mobile Phase

The Phenyl-Hexyl phase of the Ascentis Express product line displays the best selectivity of this sample; selectivity is markedly affected by choice of organic modifier. The application also demonstrates feasibility of fast LC with Ascentis Express by virtue of low backpressure and high efficiency.

| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 5 cm x 4.6 mm I.D., 2.7 µm particles (53348-U) |
| mobile phase | (A) water; (B) methanol; (35:65, A:B) |
| flow rate | 1.4 mL/min |
| pressure | 3600 psi (248 bar) |
| column temp. | 35 °C |
| detector | UV, 250 nm |
| injection | 2 µL |
| sample | 0.1 g/L in 20% methanol |
| Application No. | G005674 |

1. Clonazepam
2. Oxazepam
3. Alprazolam
4. N-Desmethyldiazepam
5. Diazepam

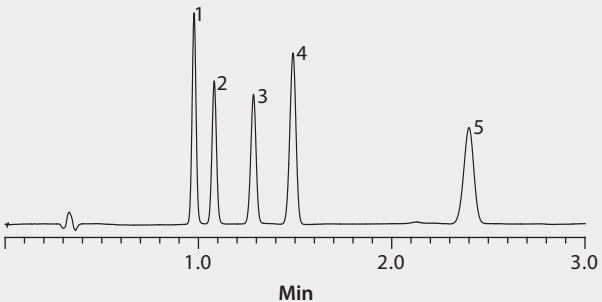


## HPLC Analysis of Benzodiazepines on Ascentis® Express Phenyl-Hexyl with Acetonitrile Mobile Phase

The Phenyl-Hexyl phase of the Ascentis Express product line displays the best selectivity of this sample; selectivity is markedly affected by choice of organic modifier. The application also demonstrates feasibility of fast LC with Ascentis Express by virtue of low backpressure and high efficiency.

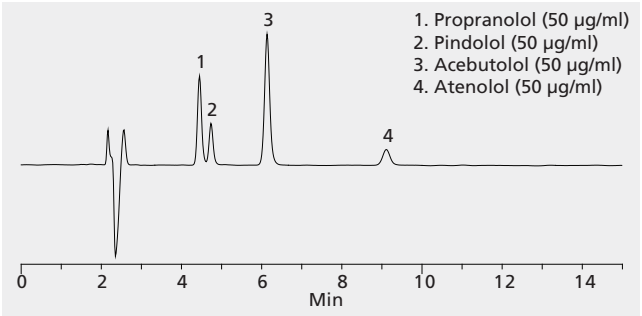
| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 5 cm x 4.6 mm I.D., 2.7 µm particles (53348-U) |
| mobile phase | (A) water; (B) acetonitrile; (62:38, A:B) |
| flow rate | 1.4 mL/min |
| pressure | 2600 psi (179 bar) |
| column temp. | 35 °C |
| detector | UV, 250 nm |
| injection | 2 µL |
| sample | 0.1 g/mL of each in 20% methanol |
| Application No. | G005675 |

1. Oxazepam
2. Alprazolam
3. Clonazepam
4. N-Desmethyldiazepam
5. Diazepam



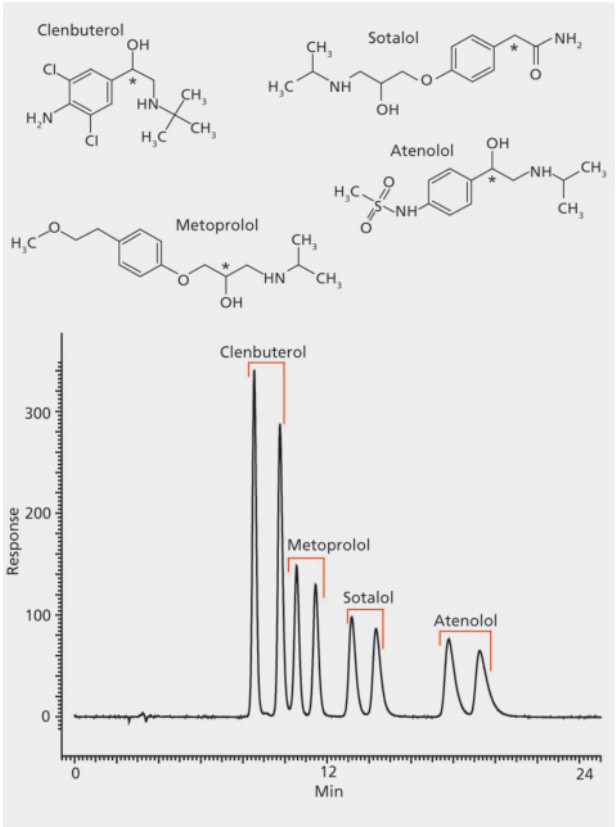
## HPLC Analysis of Beta Blockers on Ascentis® Si (Silica)

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted); (B) 0.1% ammonium acetate in acetonitrile; (15:85, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 225 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 10:90:water:acetonitrile
Application No. . . . . G003735



## HPLC Analysis of Beta-Receptor Agonist Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm, 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
Application No. . . . . G004337

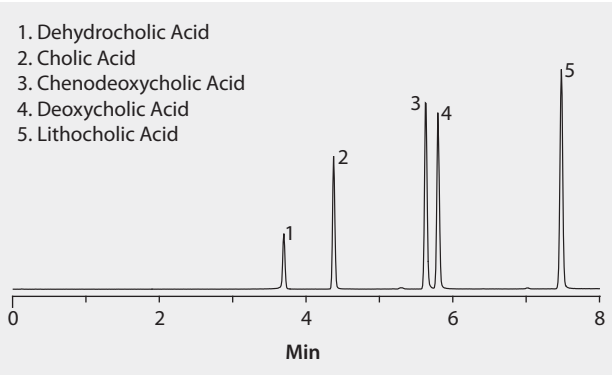





SUPELCO® Solutions within.™

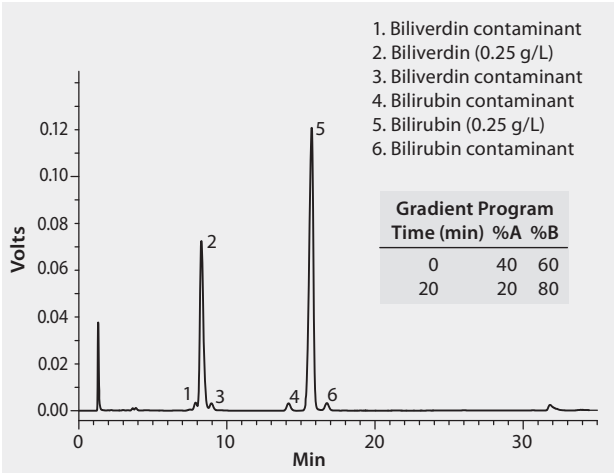
## HPLC Analysis of Bile Acids on Ascentis® Express C18

column . . Ascentis Express C18, 5 cm x 4.6 mm I.D., 2.7 µm particles (53826-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 4 (adjusted with acetic acid); (B) acetonitrile; (80:20, A:B)
gradient . . . . . 20 to 100% B in 10 min
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(-) in SIR mode
injection . . . . . 5 µL
sample . . . . . 10 µg/mL
Application No. . . . . G005679



## HPLC Analysis of Bilirubin and Biliverdin on Discovery® C18

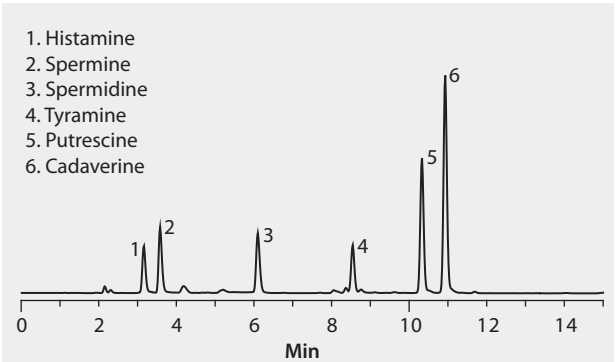
column . . . . . Discovery C18, 10 cm × 2.1 mm I.D., 5 µm particles (569220-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH unadjusted)
(B) 90:10, methanol:(100 mM ammonium acetate)
flow rate . . . . . 0.2 mL/min
column temp. . . . . ambient
detector . . . . . UV, 405 nm
injection . . . . . 1 µL
sample . . . . . as indicated (in 50% MeOH, 1% $NH_4OH$)
Application No. . . . . G002490



## HPLC Analysis of Biogenic Amines on Ascentis® RP-Amide

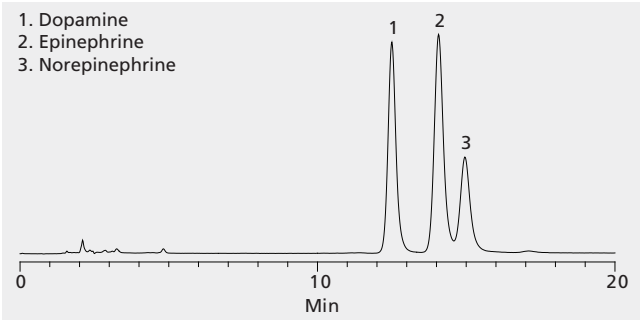
Following pre-column derivatization of primary amino groups using o-phthaldialdehyde/thiol reagent, the Ascentis RP-Amide was used for the analysis of biogenic amines commonly found in wines. These include histamine, spermine, spermidine, tyramine, putrescine and cadaverine. Structures along with an optimized chromatogram are presented.

column . . . Ascentis RP-Amide, 15 cm x 2.1 mm I.D., 5 µm particles (565305-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted); (B) acetonitrile
gradient . . . . . 25 to 75% B in 10 min; held at 75% B for 5 min
flow rate . . . . . 0.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 340 nm
injection . . . . . 10 µL
Application No. . . . . G005680



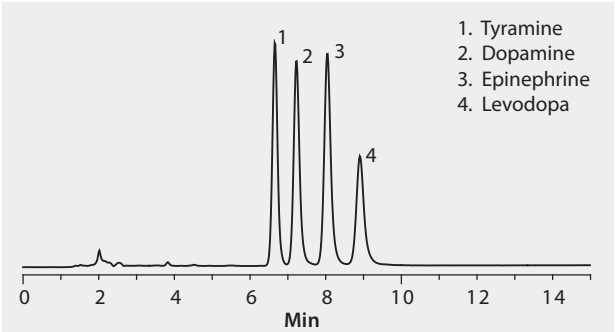
## HPLC Analysis of Biogenic Amines on Ascentis® Si, HILIC Mode, (90 % Acetonitrile)

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted); (B) 0.1% ammonium acetate in acetonitrile; (10:90, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . 50 *u*g/mL each in 0.1% ammonium acetate in 10:90:water:acetonitrile
Application No. . . . . G003717



## HPLC Analysis of Biogenic Amines on Ascentis® Si, HILIC Mode (85 % Acetonitrile)

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted);
(B) 0.1% ammonium acetate in acetonitrile; (15:85, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 0.1% ammonium acetate in 10:90:water:acetonitrile
Application No. . . . . G003716



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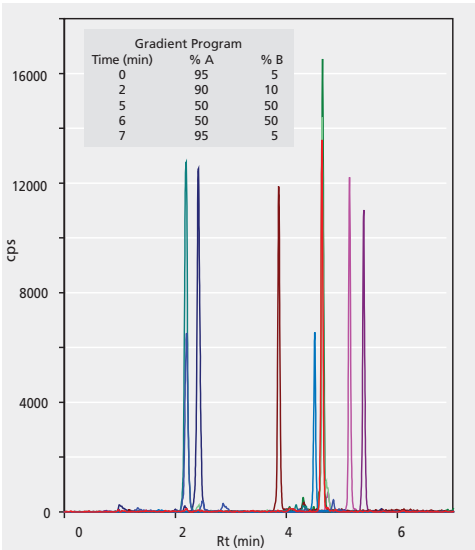
# HPLC Analysis of β-Blockers and β-Agonists in Urine and Wastewater on a C18 Column after SPE using SupelMIP® SPE-Beta-Receptor

**Sample Pre-treatment:**

Horse urine was centrifuged at 3000 g for 10 min, diluted 1:1 (v/v) with DI water, adjusted to pH 7. Wastewater was filtered with 1 µm filter paper and adjusted to pH 6-7. Samples were spiked with 10 beta-agonists and beta-blockers at the level of 1 ng/mL.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . horse urine or waste water spiked with 10 beta-agonists and beta-blockers at 1 ng/mL
SPE tube/cartridge . . SupelMIP SPE - Beta-Receptor, 25 mg/10 mL (LRC) (53223-U)
condition . . . . . 1 mL acetonitrile; 1 mL DI water
sample addition . . . . . 1 mL
washing . . . 3 x 1 mL DI water; 1 mL acetonitrile; 1 mL 60% acetonitrile/40% DI water; apply 2 min of full vacuum to dry the tube after each step
elution . . . . . 2 x 1 mL 1% formic acid in acetonitrile
eluate post-treatment . . . . . evaporate under nitrogen and reconstitute with 150 µL 5% acetonitrile in 10 mM ammonium acetate, pH 4.6 prior to LC-MS-MS analysis
column . . . . . C18, 5 cm x 3 mm I.D., 3 µm particles
mobile phase . . . . . (A) 10 mM ammonium acetate, pH 4.6 (adjusted with acetic acid); and (B) acetonitrile
flow rate . . . . . 0.5 mL/min
column temp. . . . . ambient
detector . . . . . MS/MS, ESI(+)
injection . . . . . 20 µL
Application No. . . . . G004059



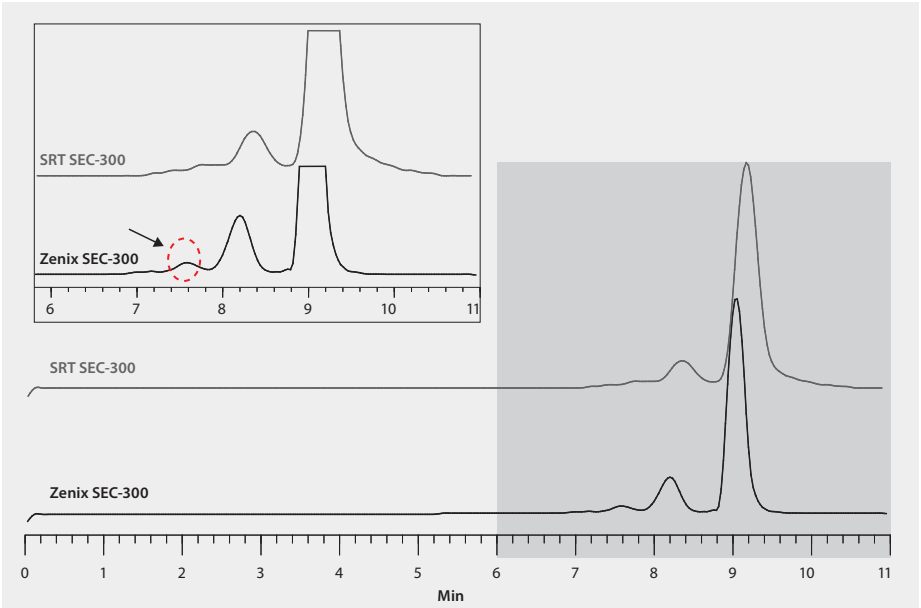

Note: Clenbuterol and Tulobuterol were spiked at the levels of 0.1 ng/mL.

| Analyte | Rt (min.) | Q1/Q3 |
|---|---|---|
| Atenolol | 3.0 | 267.2/145 |
| Carazolol | 6.2 | 299.1/194.2 |
| Metoprolol | 5.6 | 268.2/133 |
| Propranolol | 6.5 | 260.2/154.9 |
| Timolol | 5.5 | 317.2/188.1 |
| Clenbuterol | 5.6 | 277.1/202.9 |
| Ritodrine | 3.9 | 288.2/121 |
| Salbutamol | 2.6 | 240.2/147.9 |
| Terbutaline | 2.6 | 226.2/152 |
| Tulobuterol | 5.6 | 228.2/154.1 |

| | Lower Limit of Quantitation (ng/mL, ppb, or µg/kg) | |
|---|---|---|
| Analyte | 1 mL Horse Urine | 10 mL Wastewater |
| Atenolol | 0.1 | 0.01 |
| Carazolol | 0.1 | 0.01 |
| Metoprolol | 0.1 | 0.01 |
| Propranolol | 0.1 | 0.01 |
| Timolol | 0.1 | 0.01 |
| Clenbuterol | 0.02 | 0.002 |
| Ritodrine | 0.05 | 0.005 |
| Salbutamol | 0.1 | 0.01 |
| Terbutaline | 0.2 | 0.02 |
| Tulobuterol | 0.005 | 0.0005 |

## HPLC Analysis of Bovine Serum Albumin (BSA) on Zenix® SEC-300 versus SRT® SEC-300, Effect of Particle Size on Resolution

column . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm, 300 Å particles (Z777051)
column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL, BSA
Application No. . . . . G006119



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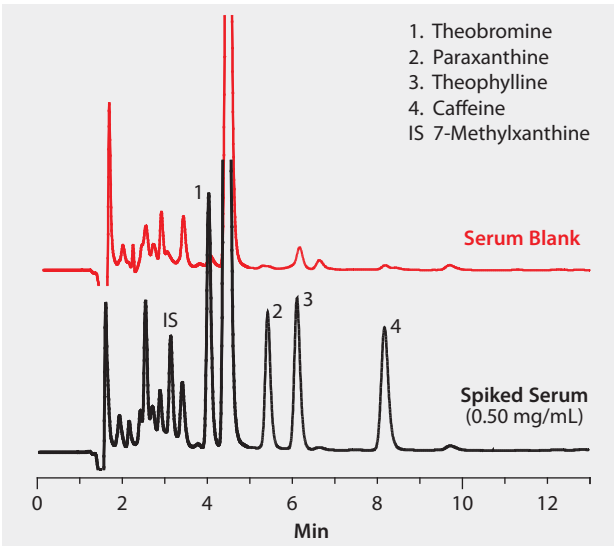
# HPLC Analysis of Bronchodilators Theophylline and Other Caffeine Metabolites from Serum on Discovery® RP-AmideC16 after SPE using Discovery® DSC-18

sample preparation . . . . . SPE (Solid Phase Extraction)
SPE tube/cartridge . . . . . Discovery® DSC-18, 500 mg/3 mL (52603-U)
condition . . . . . 2 mL methanol, then 2 mL water
sample preparation . . . . . 1 mL porcine serum spiked with 0.1 µg/mL, 0.50 µg/mL, or 1.0 µg/mL each analyte
sample preparation . . . . . 1 mL methanol; evaporate to dryness with nitrogen stream at room temperature; reconstitute in 200 µL mobile phase containing 0.2 µg/mL or 7-methylxanthine (IS)
sample preparation . . . . . 2 mL 5% methanol in water; dry tube 10 min with nitrogen stream
column . . Discovery RP-AmideC16, 15 cm × 4.6 mm, 5 µm particles, preceded by a 2 cm RP-AmideC16 guard column and 0.5 µm frit filter (505013)
mobile phase . . . . . (A) Methanol; (B) 1% acetic acid (17:38, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 272 nm
injection . . . . . 20 µL reconstituted porcine serum extract
Application No. . . . . G000594

Efficiency of Recovery

| Compound | Concentration (µg/mL) | % Recovery | % RSD (n=6) |
|---|---|---|---|
| 1. Theobromine | 0.1 | 97.4 | ±6.8 |
| | 0.5 | 96.4 | ±8.5 |
| | 1.0 | 96.1 | ±5.0 |
| 2. Paraxanthine | 0.1 | 96.2 | ±8.4 |
| | 0.5 | 95.2 | ±8.7 |
| | 1.0 | 95.0 | ±8.7 |
| 3. Theophylline | 0.1 | 97.8 | ±8.5 |
| | 0.5 | 97.8 | ±8.8 |
| | 1.0 | 98.5 | ±5.7 |
| 4. Caffeine | 0.1 | 98.8 | ±3.9 |
| | 0.5 | 95.6 | ±6.7 |
| | 1.0 | 97.6 | ±5.8 |






SUPELCO® Solutions within.™

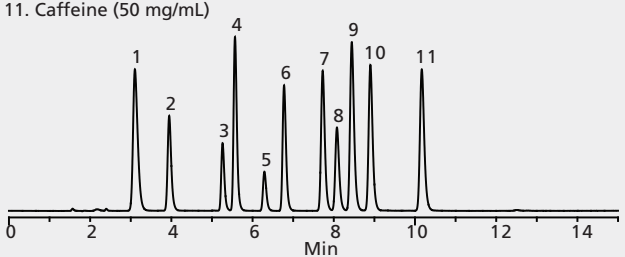
## HPLC Analysis of Caffeine and Metabolites on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . (A) 0.1% ammonium acetate in water (pH 4.5 with acetic acid );
(B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 275 nm
injection . . . . . 10 µL
sample . . as indicated in 0.1% ammonium acetate in water (pH 4.5 with acetic acid)
Application No. . . . . G003750

1. Uric acid (50 mg/mL)
2. Xanthine (30 mg/mL)
3. 7-methylxanthine (20 mg/mL)
4. 1-methyluric acid (40 mg/mL)
5. 1-methylxanthine (20 mg/mL)
6. Theobromine (30 mg/mL)
7. 1,7-dimethyluric acid (40mg/mL)
8. 1,7-dimethylxanthine (50 mg/mL)
9. Theophylline (50 mg/mL)
10. beta-(hydroxyethyl) theophylline (50 mg/mL)
11. Caffeine (50 mg/mL)

**Gradient Program**

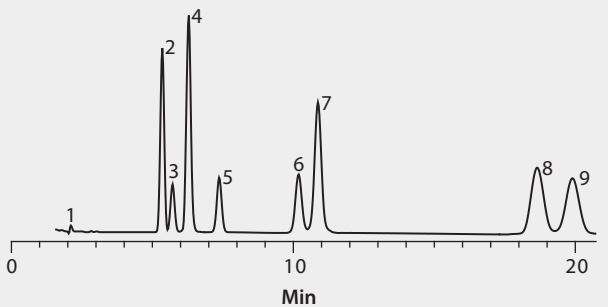
| Time (min) | %A | %B |
|---|---|---|
| 0 | 98 | 2 |
| 3 | 92 | 8 |
| 12 | 80 | 20 |
| 14 | 80 | 20 |
| 14.1 | 98 | 2 |
| 15 | 98 | 2 |



## HPLC Analysis of Caffeine Metabolites and Analogs on Discovery® C18

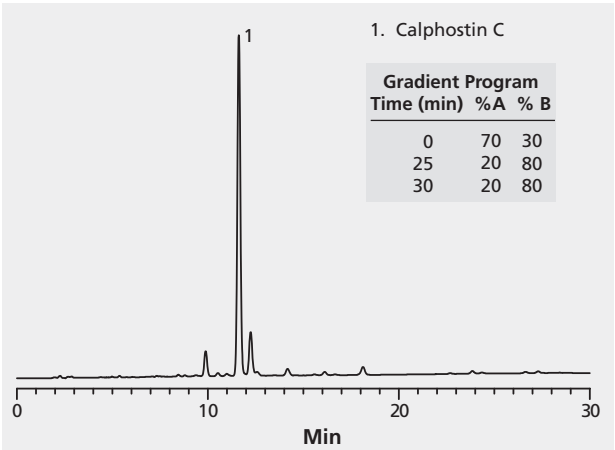
column . . . . . Discovery C18, 15 cm x 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) 1% acetic acid in water; (B) methanol; (92:8, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
Application No. . . . . G005684

1. Unknown
2. 7 -Methylxanthine
3. 1-Methyluric acid
4. 1-Methylxanthine
5. 3-Methylxanthine
6. 1,3-Dimethyluric acid
7. 1,3-Dimethylxanthine
8. 1,7-Dimethylxanthine
9. 3,7-Dimethylxanthine



## HPLC Analysis of Calphostin C on Discovery® HS C18

column . . . . Discovery HS C18, 25 cm × 2.1 mm I.D., 5 µm particles (568503-U)
mobile phase . . . (A) (0.1% phosphorus acid in water):(0.1% phosphorus acid in acetonitrile); (B) 0.1% phosphorus acid in acetonitrile; (50:50, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 24 °C
detector . . . . . UV, 254 nm
injection . . . . . 3 µL
sample . . . . . Calphostin C (0.25 g/L) in 40% MeOH, 0.025% TFA
Application No. . . . . G002574



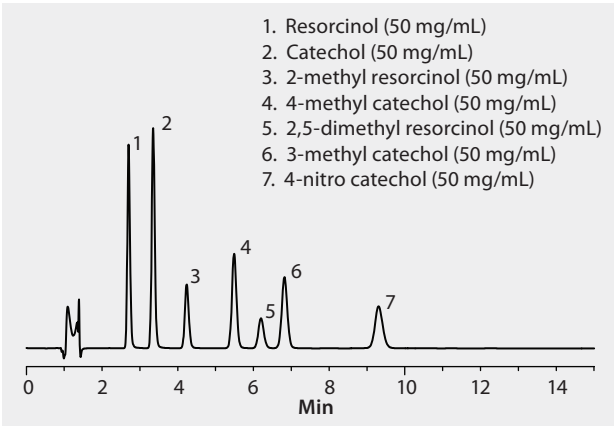

1. Calphostin C

**Gradient Program**

| Time (min) | %A | % B |
|---|---|---|
| 0 | 70 | 30 |
| 25 | 20 | 80 |
| 30 | 20 | 80 |

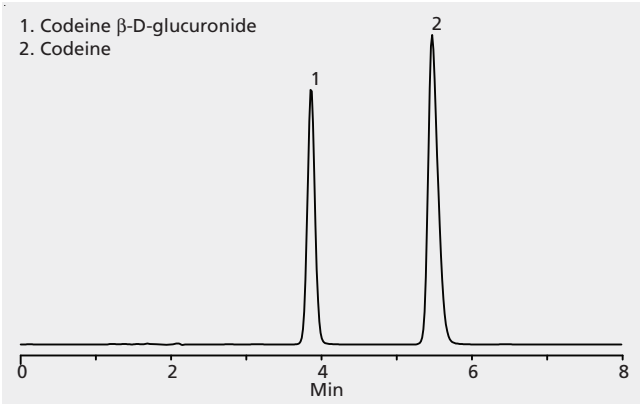
## HPLC Analysis of Catechols and Resorcinols on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0 unadjusted): (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 270 nm
injection . . . . . 25 µL
sample . . . . . as indicated in 20 mM phosphoric acid (pH 2.0)
Application No. . . . . G002597



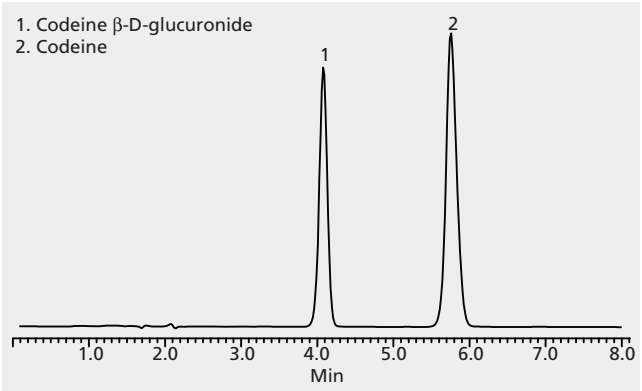
## HPLC Analysis of Codeine and Metabolite on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002417



## HPLC Analysis of Codeine and Metabolite on Ascentis® RP-Amide

column . . . . Ascentis RP-Amide 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002599



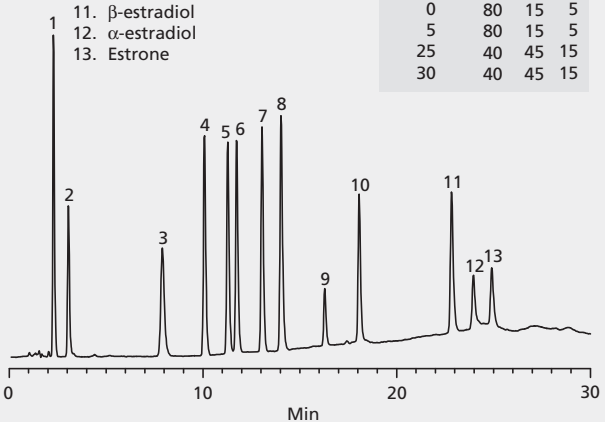
## HPLC Analysis of Conjugated Estrogens on Discovery® HS C18

column . . . . Discovery HS C18, 15 cm × 4.6 mm I.D., 5 µm particles (568522-U)
mobile phase . . . . . (A) 10 mM potassium phosphate (pH 7.01 unadjusted); (B) acetonitrile; (C) methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 10 µg/mL each in 60/40, water:acetonitrile
Application No. . . . . G002155

1. Estriol 3-(β-D-glucuronide)
2. β-estradiol 3-(β-D-glucuronide) 17-sulfate
3. Estriol 3-sulfate
4. β-estradiol 3,17-disulfate
5. β-estradiol 3-(β-D-glucuronide)
6. β-estradiol 17-(β-D-glucuronide)
7. Estrone 3-(β-D-glucuronide)
8. Estriol
9. β-estradiol 3-sulfate
10. Estrone 3-sulfate
11. β-estradiol
12. α-estradiol
13. Estrone

**Gradient Program**

| Time (min) | %A | %B | %C |
|---|---|---|---|
| 0 | 80 | 15 | 5 |
| 5 | 80 | 15 | 5 |
| 25 | 40 | 45 | 15 |
| 30 | 40 | 45 | 15 |

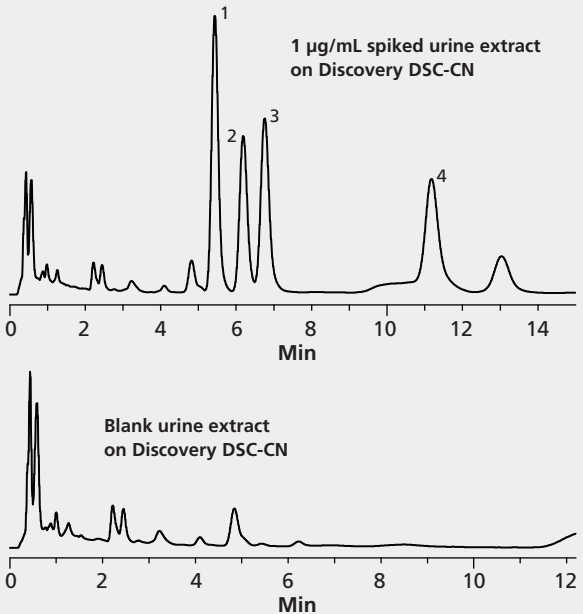


## HPLC Analysis of Cortocisteroids in Urine on Discovery® HS F5 after SPE using Discovery® DSC-CN

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | human urine spiked with corticosteroids at 0.5 and 1.0 µg/mL and diluted in DI water (1:1, v/v) |
| SPE well plate | Discovery DSC-CN 96-well SPE, 100 mg/well (575636-U) |
| condition | 1 mL methanol; 1 mL DI water. |
| sample addition | 1 mL spiked urine |
| washing | 1 mL 20% methanol |
| elution | 1 mL methanol |
| eluate post-treatment | Evaporate eluate with nitrogen at 30 °C. Reconstitute in 200 µL mobile phase. |
| column | Discovery HS F5, 5 cm x 4.6 mm I.D., 3 µm particles (567504-U) |
| mobile phase | (A) methanol; (B) DI water; (40:60, A:B) |
| flow rate | 1.5 mL/min |
| column temp. | 35 °C |
| detector | UV, 240 nm |
| injection | 5 µL |
| Application No. | G003763 |

**Efficiency of Recovery**

| Compound | %Recovery ± RSD (n=3) 0.5 µg/mL spike level | 1.0 µg/mL spike level |
|---|---|---|
| 1. Hydrocortisone | 123.3±1.4% | 95.9±1.7% |
| 2. Prednisilone | 107.2±1.1% | 91.9±1.1% |
| 3. Prednisone | 103.2±1.0% | 88.4±1.8% |



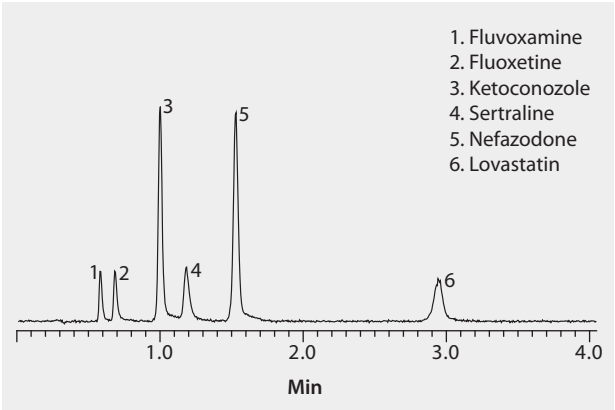



SUPELCO® Solutions within.™

## HPLC Analysis of CYP3A Inhibitors on Ascentis® Express RP-Amide

This application demonstrates the suitability of the Ascentis Express RP Amide for the analysis of CYP3A Inhibitors.

column . . . . . Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U)
mobile phase . . . (A) 20 mM ammonium formate in 50:50 (v/v) water:acetonitrile
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), m/z 100-600
injection . . . . . 1 µL
Application No. . . . . G005750

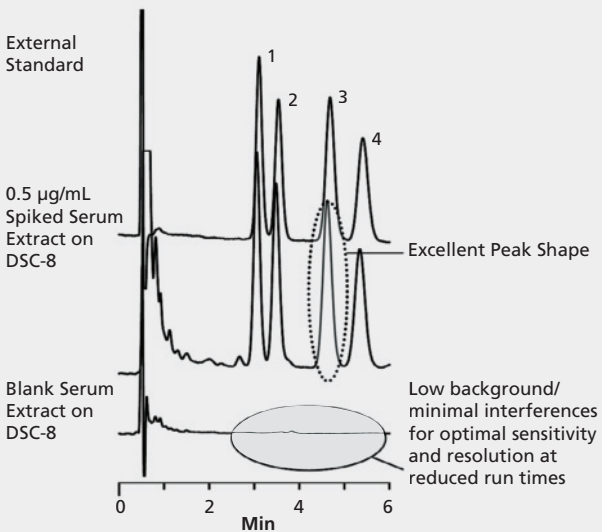


## HPLC Analysis of Diazepam and Metabolites in Serum on Discovery® C18 after SPE using Discovery® DSC-8

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . goat serum spiked with diazepam and metabolites at 0.5 µg/mL and diluted 1:1 in 25 mM ammonium formate, pH 7.1
SPE well plate . . . . Discovery DSC-8 96-well SPE Plate, 100 mg/well (575627-U)
condition . . . . . 1 mL methanol followed by 1 mL DI water.
sample addition . . . . . 1 mL spiked goat serum
washing . . . . . 1 mL 25% methanol in 25 mM ammonium formate, pH 2.75
elution . . . . . 1 mL 60% methanol in 25 mM ammonium formate, pH 2.75
elution . . . . . Evaporate eluate with nitrogen purge (30 °C; ~15 min.). Reconstitute in 200 µL mobile phase.
column . . . . . Discovery C18, 5 cm x 4.6 mm I.D., 5 µm particles (504947)
mobile phase . . (A) Methanol; (B)10 mM ammonium acetate, pH 4.5;(45:55, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 25 µL
Application No.Application No. . . . . G003766

**Efficiency of Recovery**

| Compound | %Recovery ± RSD (n=3) Systematically Developed Method on Discovery DSC-8 | Generic Method On Conventional C18 |
|---|---|---|
| 1. Oxazepam | 94.7 ± 1.2% | 82.8 ± 4.0% |
| 2. Temazepam | 99.9 ± 1.1% | 89.1 ± 4.0% |
| 3. Desmethyl diazepam | 94.2 ± 1.8% | 82.4 ± 5.0% |
| 4. Diazepam | 90.0 ± 3.4% | 68.5 ± 9.1% |

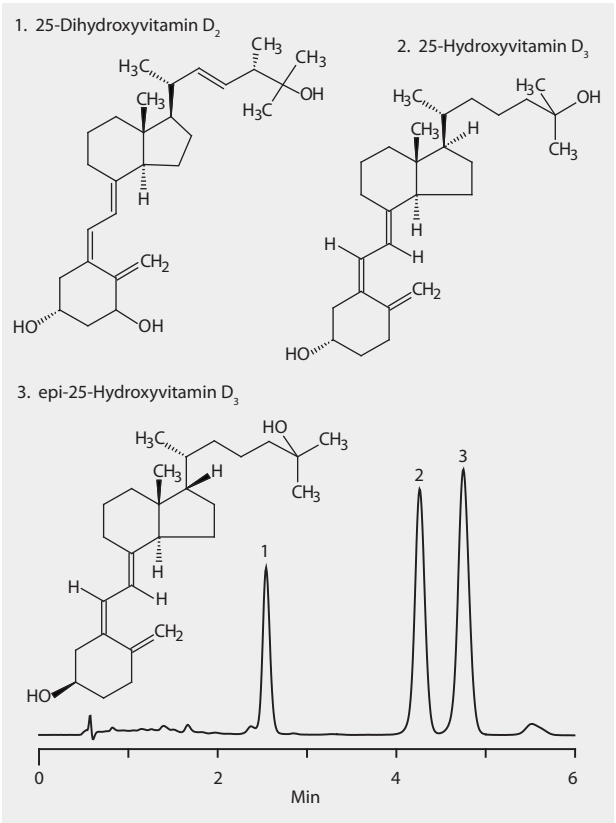





SUPELCO® Solutions within.™

## HPLC Analysis of 25-Dihydroxyvitamin D2, 25-Hydroxyvitamin D3 and 3-epi-25-Hydroxyvitamin D3 on Ascentis® Express F5, 20 °C

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) 5 mM ammonium formate; (B) methanol:water (75:25)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 3553 psi (245 bar)
column temp. . . . . 20 °C
detector . . . . . (UV, 265 nm, ESI(+), m/z 100 - 1000)
injection . . . . . 1 µL
sample . . . . . 500 mg/L in 25:75, water:methanol
Application No. . . . . G005375

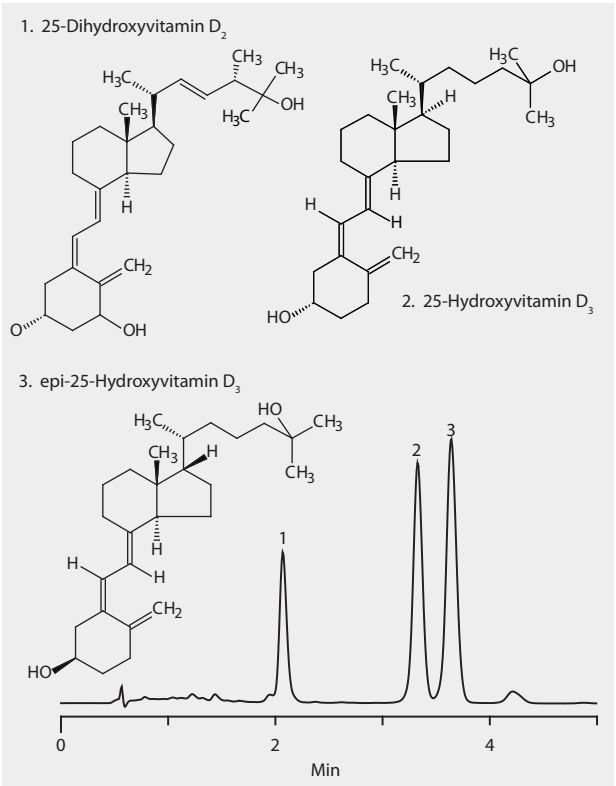





SUPELCO® Solutions within.™

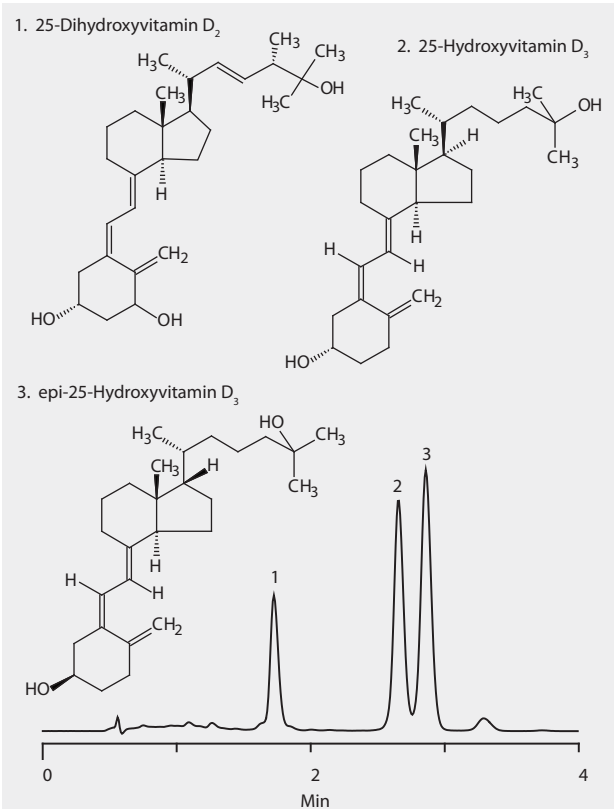
## HPLC Analysis of 25-Dihydroxyvitamin D2, 25-Hydroxyvitamin D3 and 3-epi-25-Hydroxyvitamin D3 on Ascentis® Express F5, 30 °C

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . (A) 5 mM ammonium formate in methanol; (B) water (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 4293 psi (296 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 265 nm
ESI(+), m/z 100 - 1000
injection . . . . . 1 µL
sample . . . . . 500 µg/mL in methanol
Application No. . . . . G005376



## HPLC Analysis of 25-Dihydroxyvitamin D2, 25-Hydroxyvitamin D3 and 3-epi-25-Hydroxyvitamin D3 on Ascentis® Express F5, 40 °C

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . (A) 5 mM ammonium formate in methanol; (B) water (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 5221 psi (360 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 265 nm
ESI(+), m/z 100 - 1000
injection . . . . . 1 µL
sample . . . . . 300 mg/L in 25:75, water:methanol
Application No. . . . . G005377

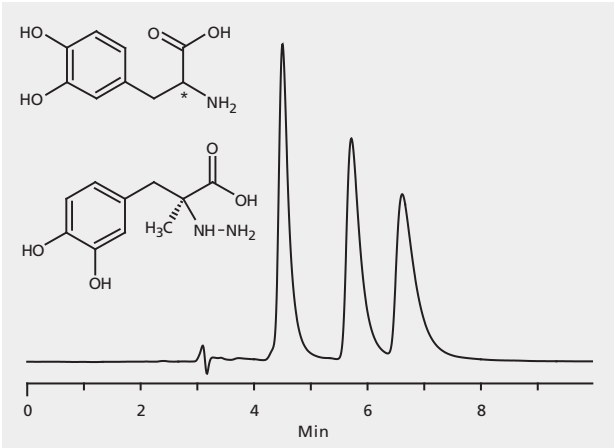





SUPELCO® Solutions within.™

## HPLC Analysis of Dopa and Carbidopa Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4; (B) methanol; (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004529

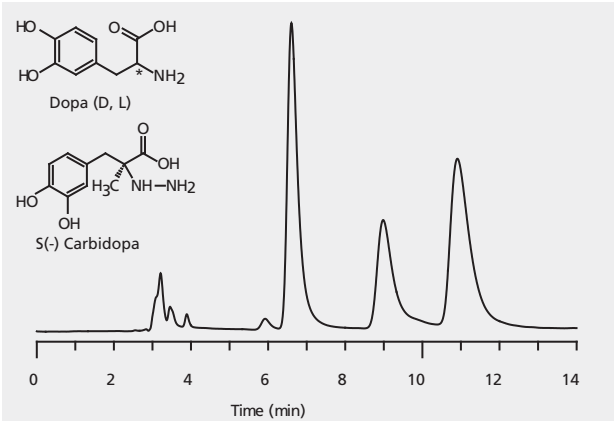





SUPELCO® Solutions within.™

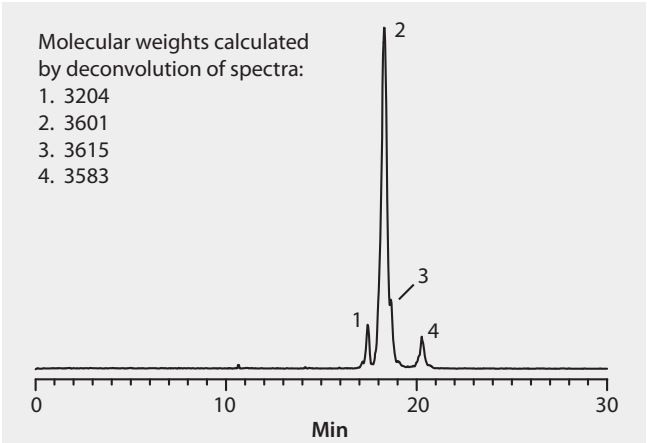
## HPLC Analysis of Dopa and Carbidopa Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4; (B) methanol; (10:90, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004481



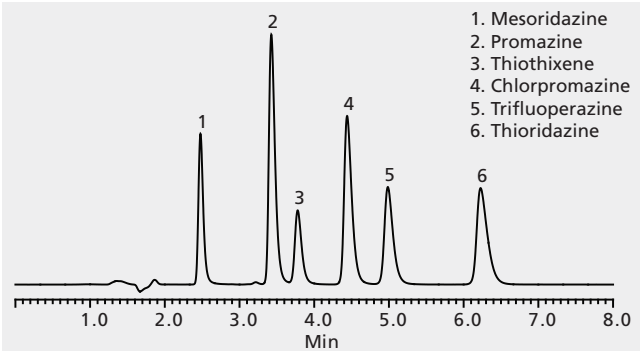
## HPLC Analysis of Dopamine and Related Compounds on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with concentrated formic acid), (B) acetonitrile
flow rate . . . . . 1 mL/min, split to the MS
column temp. . . . . 35 °C
detector . . . MS in SIR (Selected Ion Recording) mode using (+) or (-) ESI mode
injection . . . . . 25 µL
sample . . . . . 50 µg/mL in 10 mM ammonium formate (pH 3.0 with formic acid)
Application No. . . . . G002482



## HPLC Analysis of Dopamine Receptor Antagonists on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 50 mM ammonium formate (pH 3.0 with formic acid); (B) acetonitrile; (45:55, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 50:50, water:methanol
Application No. . . . . G003707



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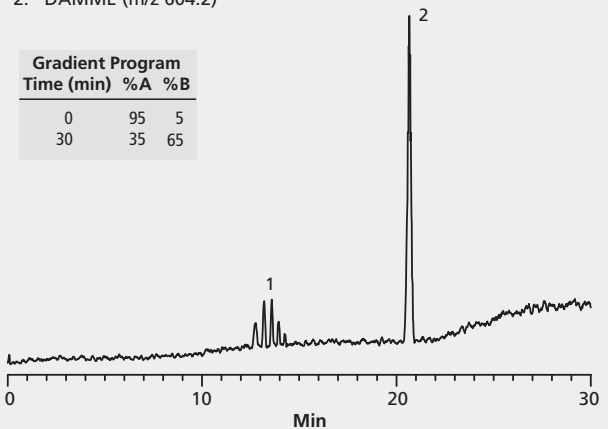
## HPLC Analysis of Enkephalin Derivative (DAMME) on Discovery® HS F5

column . . . . Discovery HS F5, 10 cm × 2.1 mm I.D., 5 µm particles (568501-U)
mobile phase . . . . (A) 25 mM $HCO_2H$ in water; (B) 25 mM $HCO_3H$ in acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 22 °C
detector . . . . . ESI (+), full scan
injection . . . . . 2 µL
sample . . . . . 10 mg/L in 0.1% $HCO_2H$
Application No. . . . . G002572

1. PEG contaminants (m/z 520.1, 564.1, 608.2, 652.2, 696.2)
2. DAMME (m/z 604.2)

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 30 | 35 | 65 |

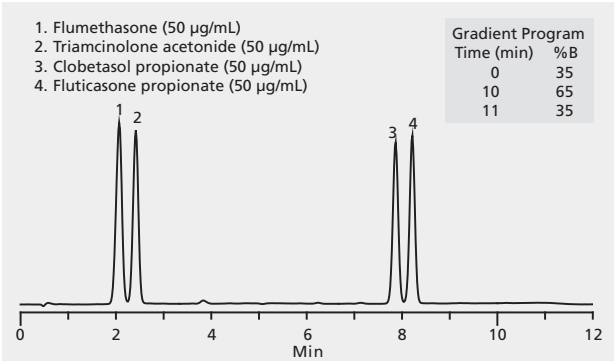





SUPELCO® Solutions within.™

## HPLC Analysis of Fluorinated Corticosteroids on Ascentis® C18

column . . . . . Ascentis C18, 5 cm × 4.6 mm I.D., 5 µm particles (581323-U)
mobile phase . . . . . (A) water; (B) acetonitrile;
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . as indicated in acetonitrile
Application No. . . . . G003939

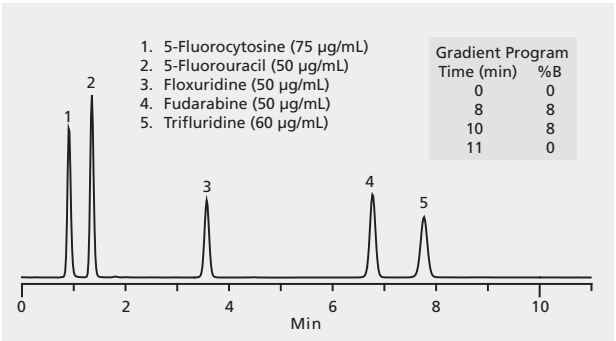





SUPELCO® Solutions within.™

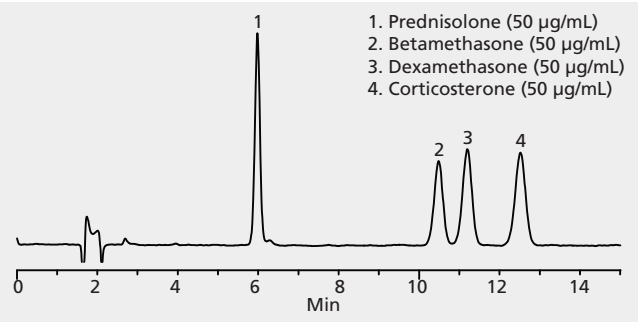
## HPLC Analysis of Fluorinated Pyrimidine Nucleosides on Ascentis® RP-Amide

column . . . . Ascentis RP-Amide, 5 cm × 4.6 mm I.D., 5 µm particles (565323-U)
mobile phase . . . . . (A) water with 0.1% ammonium formate (pH 3.04 with formic acid), (B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 260 nm
injection . . . . . 5 µL
sample . . . . . as indicated in mobile phase A
Application No. . . . . G003940



## HPLC Analysis of Glucocorticoids on Ascentis® C8

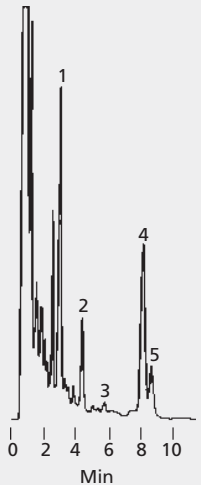
column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (68:32, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003148



## HPLC Analysis of Hippuric and Methylhippuric Acids in Urine on SUPELCOSIL™ LC-18

Urinary excretion of hippuric acid and m-or p-methylhippuric acid in the urine is a test of exposure to toluene and m-or p-xylene vapors.

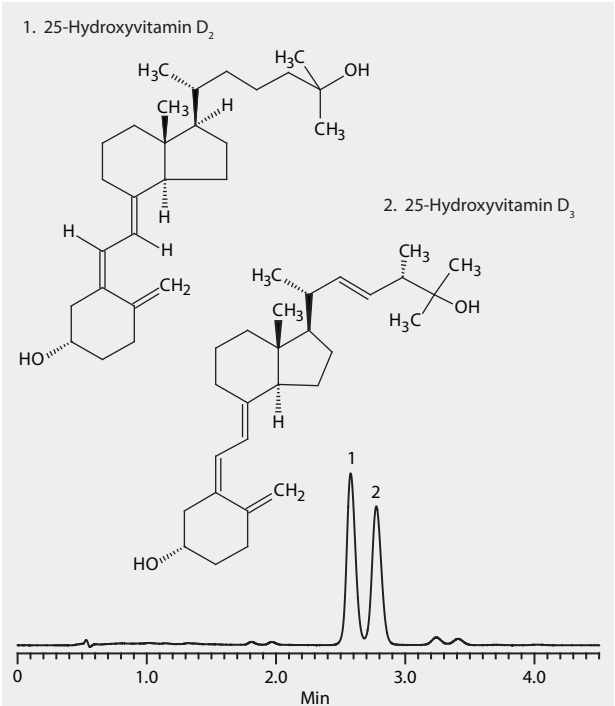
column . . . . . SUPELCOSIL LC-18, 7.5 cm × 4.6 mm I.D., 3 µm particles (58984)
mobile phase . . . . . (A) tetrahydrofuran:10 mM potassium phosphate, pH 3.0 (B) with phosphoric acid (3:97, A:B)
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 260 nm
injection . . 10 µL spiked urine (300 µg/mL HA, o-MHA; 150 µg/mL m-MHA, p-MHA)
Application No. . . . . 713-0961A




1. Hippuric acid
2. o-Methylhippuric acid
3. p-Methylhippuric acid impurity
4. p-Methylhippuric acid
5. m-Methylhippuric acid

## HPLC Analysis of 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 on Ascentis® Express F5

mobile phase . . . . . (A) 7 mM ammonium formate; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 250 nm
ESI(+), TIC m/z 100 - 1000
injection . . . . . 1 µL
sample . . . . . 300 mg/L in 25:75, water:methanol
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
Application No. . . . . G005374



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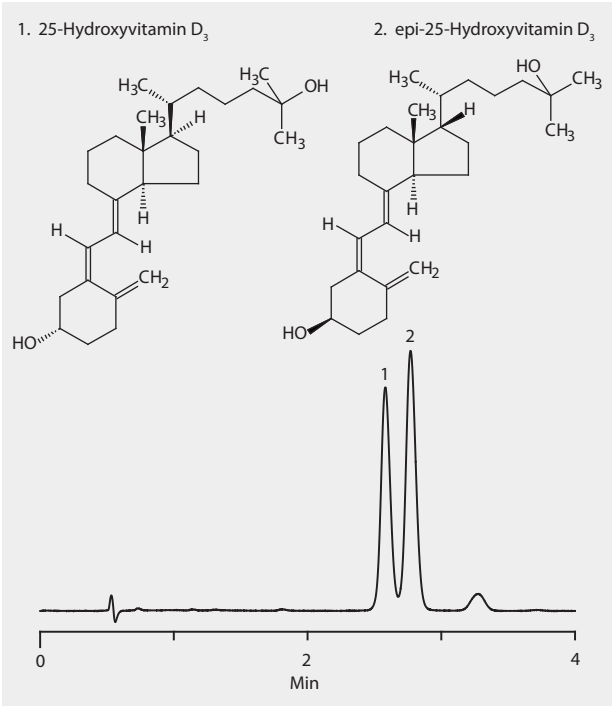
## HPLC Analysis of 25-Hydroxyvitamin D3 and epi-25-Hydroxyvitamin D3 on Ascentis® Express F5

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) 7 mM ammonium formate; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 265 nm
ESI(+), m/z 100 - 1000
injection . . . . . 1 µL
sample . . . . . 300 mg/L in 25:75, water:methanol
Application No. . . . . G005378



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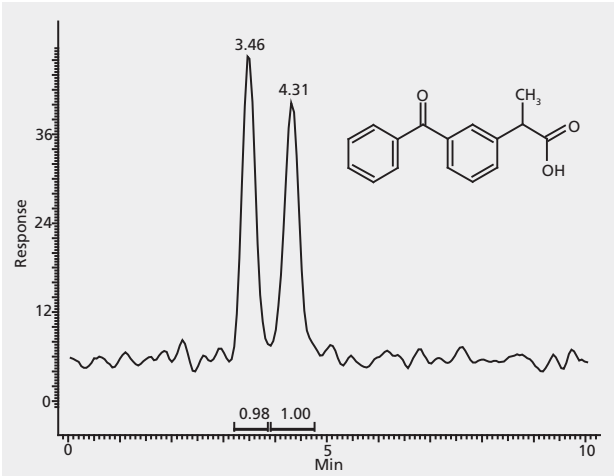
## HPLC Analysis of Ketoprofen Enantiomers on Astec® CHIROBIOTIC® R (MS Detection)

column . . . . . CHIROBIOTIC R, 15 cm x 2.1 mm, 5 µm particles (13019AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 5.6 (B) methanol; (70:30, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(-)
Application No. . . . . G004331



## HPLC Analysis of Levothyroxine and Liothyronine on Ascentis® Express ES-Cyano

This application demonstrates the suitabilty of Ascentis Express ES-Cyano for the analysis of Levothyroxine according to USP Monograph 3301 with Liothyronine as a reference standard.

column . . . . . Ascentis Express ES-Cyano, 10 cm x 2.1 mm I.D., 2.7 µm particles (53491-U)

mobile phase . . . . . (A) water with 0.05% phosphoric acid; (B) acetonitrile with 0.05% phophoric acid; (60:40, A:B)

flow rate . . . . . 1.5 mL/min

pressure . . . . . 3270 psi (225 bar)

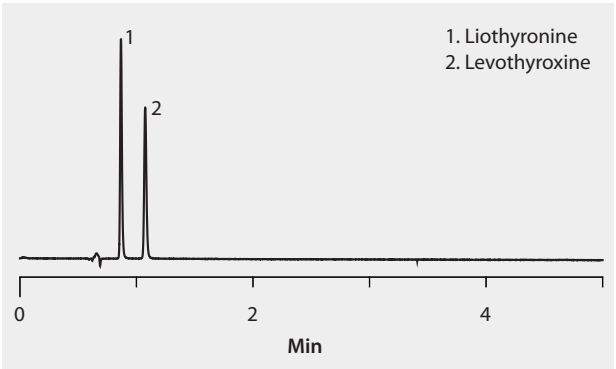
column temp. . . . . 30 °C

detector . . . . . UV, 225 nm

injection . . . . . 5 µL

sample . . . . . 20 µg/mL in mobile phase

Application No. . . . . G005601



## HPLC Analysis of Metabolites of 7,12-Dimethylbenz[a]anthracene on SUPELCOSIL™ LC-18

column . . . . . SUPELCOSIL LC-18, 25 cm × 4.6 mm I.D., 5 µm particles (58298)
mobile phase . . (A) methanol:(B) water, (50:50, A:B), 10 min to 100:0 at 2.5% /min
flow rate . . . . . 0.8 mL/min
detector . . . . . fluorescence
sample . . . . rat liver, 9000 × g supernatant fraction from Aroclor-treated animals
Application No. . . . . 713-1043

1. 2-OH-DMBA(t-diol)
2. 7-OHM-12-MBA-(t-10,11-diol)
3. 7-M-12-OHMBA-(t-8,9-diol)
4. 7-OHM-12-MBA-(t-8,9-diol)
5. 7-OHM-(t-3,4-diol)
6. Mixed diols, incl. DMBA-(t-8,9-diol & t-10, 11-diol)
7. Position of 7,12-bis-OHMBA
8. 7-OHM-12-MBA-2-ol
9. 7-OHM-12-MBA-(3-ol)
10. 7-M-12-OHMNA-4-ol
11. 7-OHM-12-MBA-4-ol
12. 7-M-12-OHMBA-(3-ol)
13. 7-M-12-OHMBA-2-ol
14. 7-OHM-12-MBA
15. 7-M-12-OHMBA
16. DMBA-2-ol
17. DMBA-3-ol
18. DMBA-4-ol
19. DMBA

(parentheses = tentative identifications)

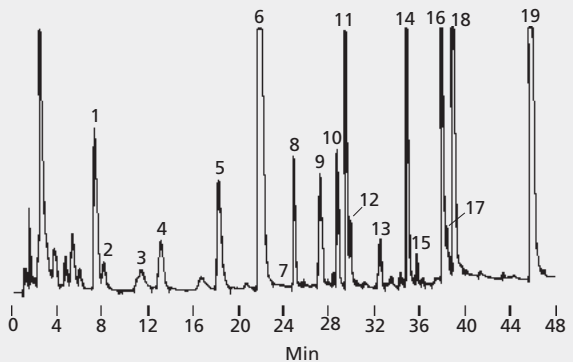
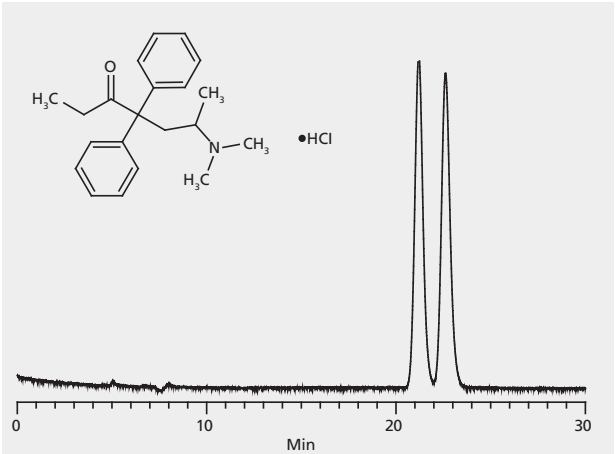



Figure provided by Drs. J. Milner and J. Grunau, University of Illinois, Urbana, Illinois, USA.

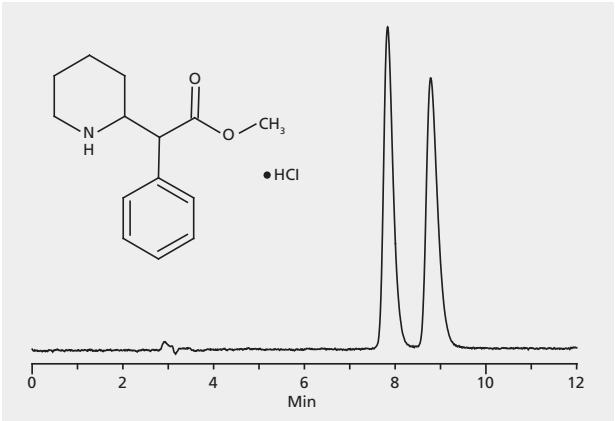
## HPLC Analysis of Methadone Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . (A) methanol; (B) 20 mM ammonium formate; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 205 nm
injection . . . . . 2 µL
sample . . . . . methadone, 1 mg/mL in mobile phase
Application No. . . . . G004403



## HPLC Analysis of Methylphenidate (Ritalin) Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . methylphenidate, 1 mg/mL in mobile phase
Application No. . . . . G004405

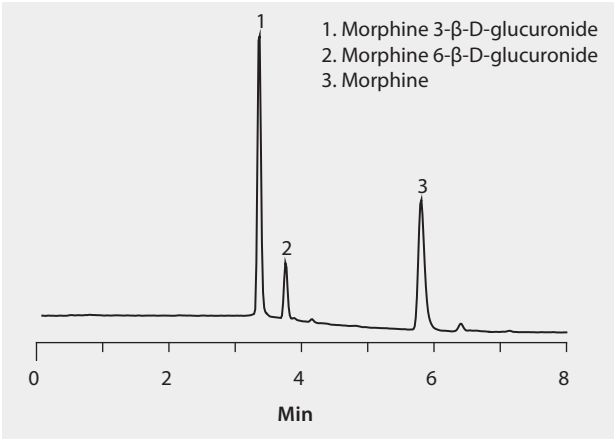





SUPELCO® Solutions within.™

## HPLC Analysis of Morphine and Glucuronide Metabolites on Discovery® HS F5

column . . . . . Discovery HS F5, 5 cm x 4.6 mm I.D., 5 µm particles (567513-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 6.8 (unadjusted); (B) acetonitrile
gradient . . . . . 0% B for 1 min, to 30% B in 4 min, held 3 min
flow rate . . . . . 1 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in final mobile phase
Application No. . . . . G006123

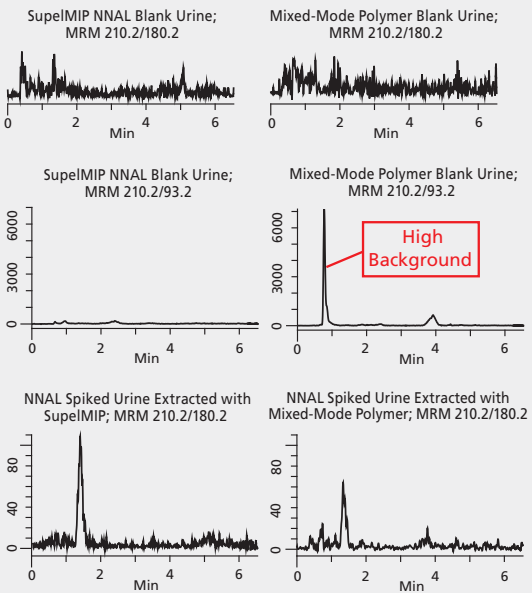


## HPLC Analysis of NNAL in Urine on Ascentis® Express C18 after SPE using SupelMIP® SPE-NNAL

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . human urine spiked with 60 or 100 pg/mL NNAL
SPE tube/cartridge . . . . . SupelMIP SPE - NNAL, 25 mg/10 mL (LRC) (53206-U)
condition . . . . . 1 mL dichloromethane; 1 mL methanol; 1 mL DI water
sample addition . . . . . 2 mL (60 pg/mL sample), 5 mL (100 pg/mL sample)
washing . . . . . 2 x 1 mL DI water, 10 min vacuum; mL toluene; 1 mL toluene: dichloromethane (9:1, v/v); 1 mL toluene:dichloromethane (4:1, v/v), 2 min. vacuum
elution . . . . . 2 x 1 mL 10% methanol in dichloromethane, apply gentle vacuum between each fraction
eluate post-treatment . . . . . evaporate under nitrogen and reconstitute in 0.15 - 0.25 mL mobile phase
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (581307-U)
mobile phase . . . . . (A) 10 mM ammonium acetate; (B) 10 mM ammonium acetate in acetonitrile
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS/MS, MRM Transitions, ESI(+) (210.2/180 and 210.2/93.2 m/z)
injection . . . . . 20 µL
Application No. . . . . G004434

Gradient Program

| Time (min.) | % A | % B |
|---|---|---|
| 0.0 | 90 | 10 |
| 1.5 | 70 | 30 |
| 2.5 | 70 | 30 |
| 2.6 | 90 | 10 |
| 6.0 | 90 | 10 |




| SPE Procedure | % Recovery 50 pg/mL Spike | 60 pg/mL Spike | 100 pg/mL Spike |
|---|---|---|---|
| SupelMIP SPE – NNAL | * | 67% | 87% |
| Mixed-Mode Polymer SPE | 47% | * | * |

* Not analyzed



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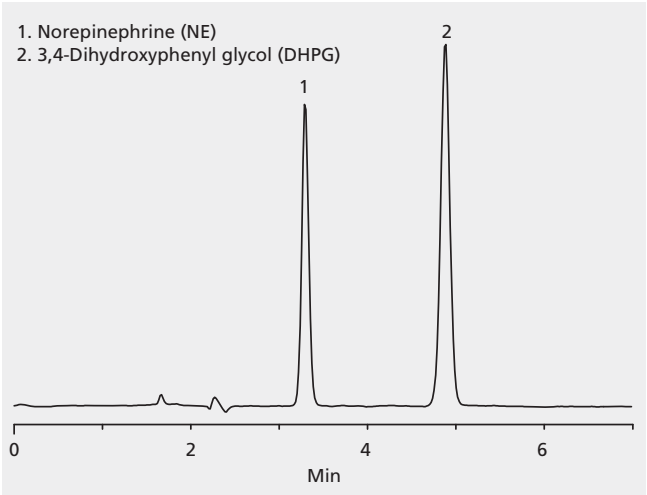
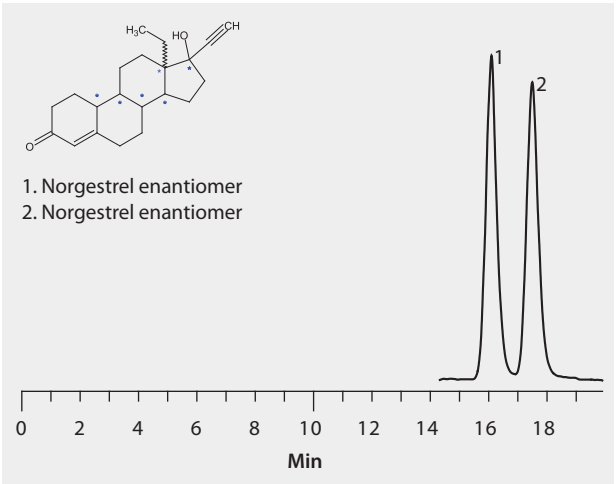
## HPLC Analysis of Norepinephrine and 3,4-Dihydroxyphenylglycol on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567561-U)
mobile phase . . . . . 50 mM ammonium formate, pH to 3.0 with formic acid
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 266 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each (NE and DHPG) in 5% methanol in mobile phase
Application No. . . . . G002096



## HPLC Analysis of Norgestrel Enantiomers on Astec® CYCLOBOND® II

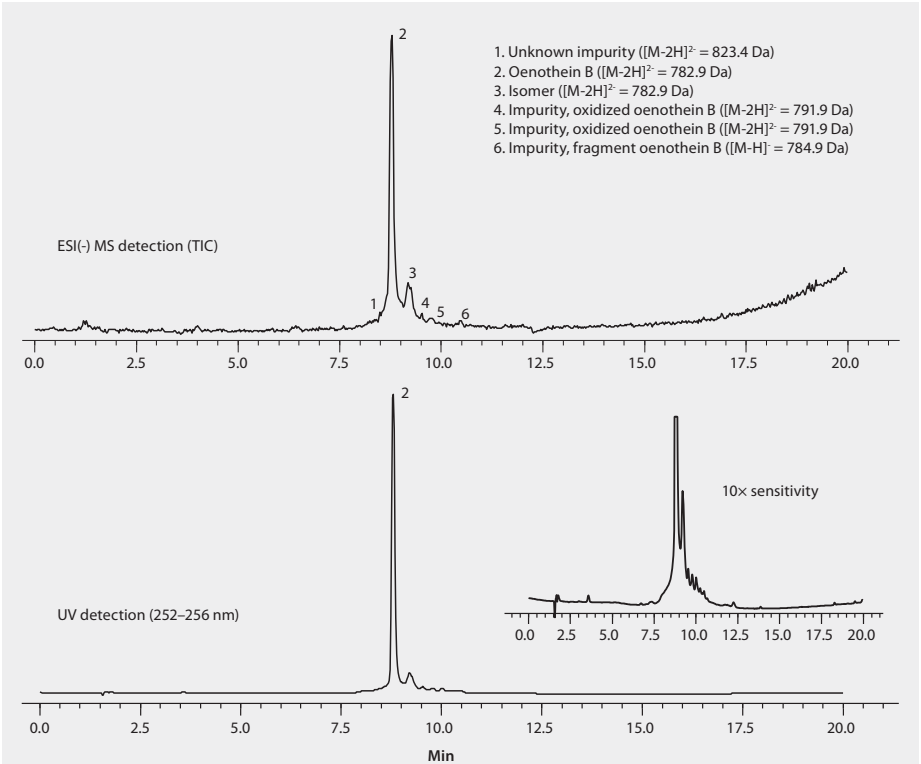
column . . . . . CYCLOBOND II, 25 cm x 4.6 mm I.D., 5 µm particles (41020AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.8 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . norgestrel, 1 mg/mL in methanol
Application No. . . . . G005165



## HPLC Analysis of Oenothein B on Ascentis® Express RP-Amide, UV and MS Detection

Optimization of a published method showing much better retention, peak shape and resolution on Supelco Ascentis Express RP-Amide 2.7 µm compared to a C18 phase. Isomers and small amounts of homologues are resolved and detectable with higher sensitivity.

column . . . . . Ascentis Express RP-Amide, 10 cm x 4.6 mm I.D., 2.7 µm particles (53929-U)
mobile phase . . . . . (A) 0.2% formic acid; (B) methanol
gradient . . . 5% B for 2 min; to 90% B over 18 min; held at 90% B for 10 min
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
detector . . . . . MS (ion trap), m/z 100-1500
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G006164



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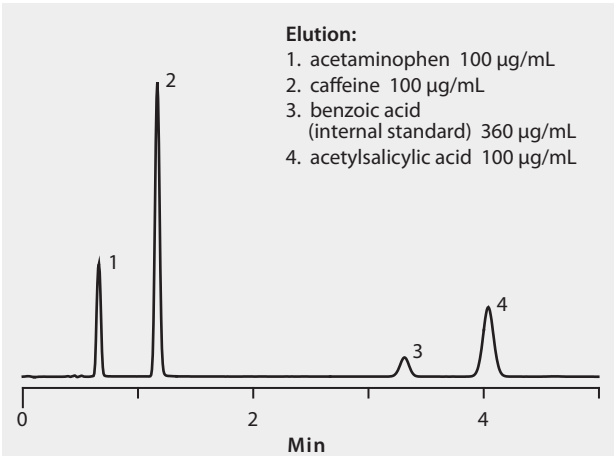
## HPLC Analysis of Pain Relievers on Ascentis® Express C18 5µm

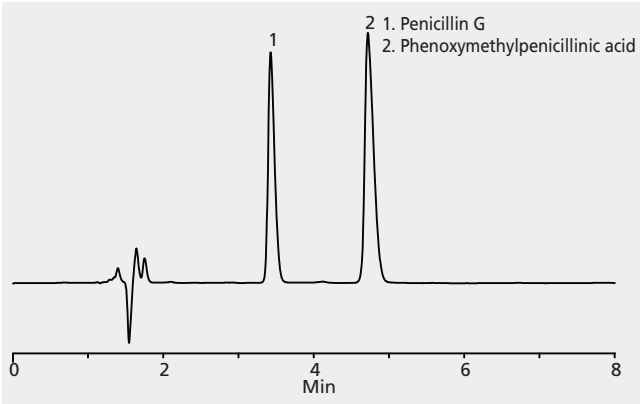
This application shows the improvements in efficiency that may be obtained for USP methods using modern Fused-Core technology.

column . . . Ascentis Express C18, 10 cm x 4.6 mm I.D., 5 µm particles (50536-U)
mobile phase . . . . . (A) water with 3% acetic acid;
(B) methanol with 3% acetic acid; (79:21, A:B)
flow rate . . . . . 2.0 mL/min
pressure . . . . . 3290 psi (227 bar)
column temp. . . . . 45 °C
detector . . . . . UV,275 nm
injection . . . . . 10 µL
sample . . . . . 100 - 360 µg/mLin mobile phase
Application No. . . . . G005894



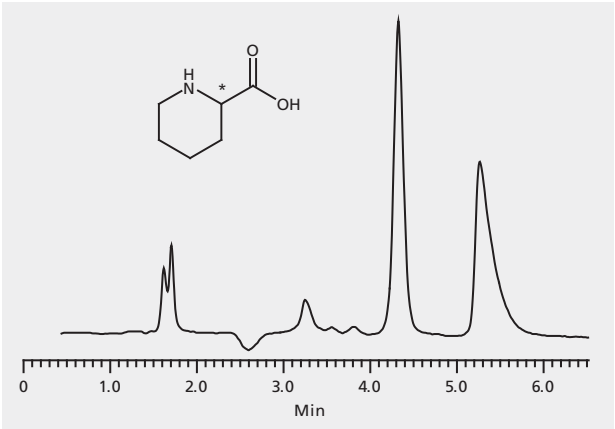
## HPLC Analysis of Penicillin G and Phenoxymethylpenicillinic Acid on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 4.5 acetic acid): (B) acetonitrile, (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 100 µg/mL each in mobile phase
Application No. . . . . G002427



## HPLC Analysis of Pipecolic Acid Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004554



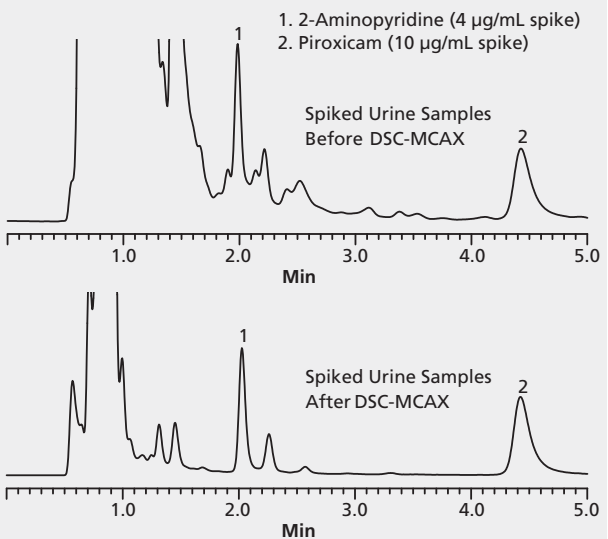
# HPLC Analysis of Piroxicam and 2-Aminopyridine in Urine on Discovery® HS F5 after SPE using Discovery® DSC-MCAX

**Sample Pre-Treatment:**
Piroxicam and 2-aminopyridine (piroxicam's polar metabolite) was spiked into human urine at the levels of 4 and 10 µg/mL, respectively. The urine sample was diluted 1:1 with 10 mM potassium phosphate, pH 3.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . human urine spiked with piroxicam (4 µg/mL) and 2-aminopyridine (10 µg/mL)
SPE tube/cartridge . . . . . Discovery DSC-MCAX, 100 mg/3 mL (52783-U)
condition . . . . . 1 mL methanol; 1 mL 10 mM potassium phosphate, pH 3
sample addition . . . . . 1 mL
washing . . . . . 1 mL 10 mM potassium phosphate, pH 3; 1 mL methanol
elution . . . . . 1 mL 5% ammonium hydroxide in methanol
eluate post-treatment . . evaporate to dryness with nitrogen at room temperature, reconstitute in 1 mL mobile phase
column . . . . . Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . (A) 10 mM potassium phosphate, pH 6: (B) acetonitrile (85:15, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G003762

**Efficiency of Recovery**

| | %Recovery ± RSD (n=4) | |
|---|---|---|
| | 2-Aminopyridine | Piroxicam |
| Discovery DSC-MCAX | 102 ± 3.5% | 101 ± 1.2% |
| Leading Competitor | A30 ± 52.5% | 98 ± 3.2% |
| Leading Competitor | B36 ± 24.2% | 83 ± 4.3% |



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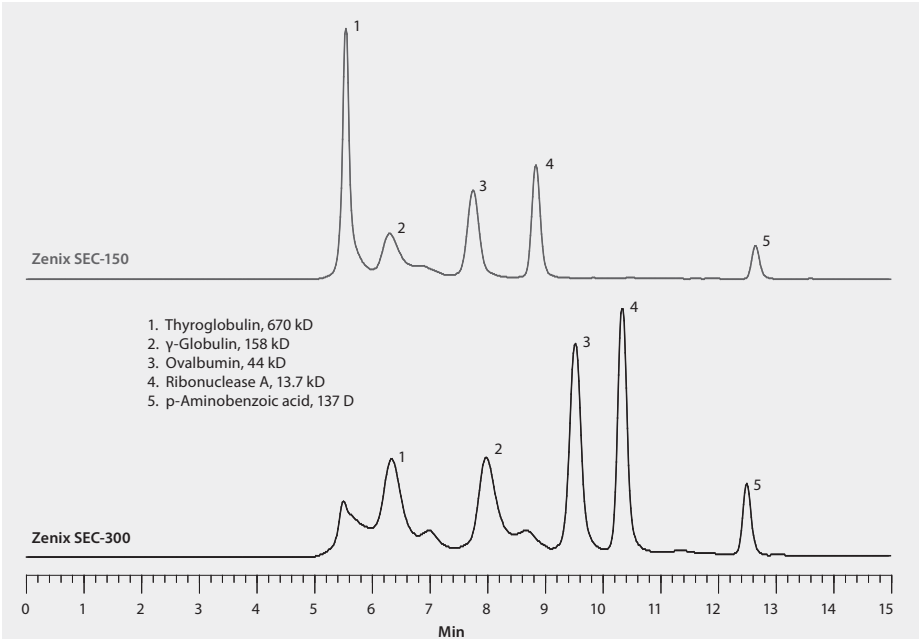
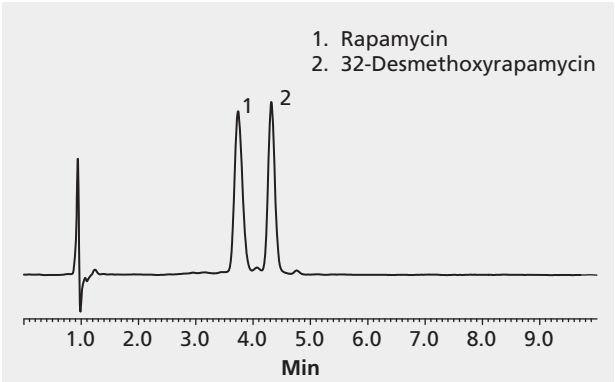
## HPLC Analysis of a Protein Mixture on Zenix® SEC-150 versus Zenix® SEC-300, Effect of Pore Size on Resolution

column . . Zenix SEC-150, 30 cm x 7.8 mm I.D., 3 µm, 150 Å particles (Z777018)
column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . protein mixture
Application No. . . . . G006118



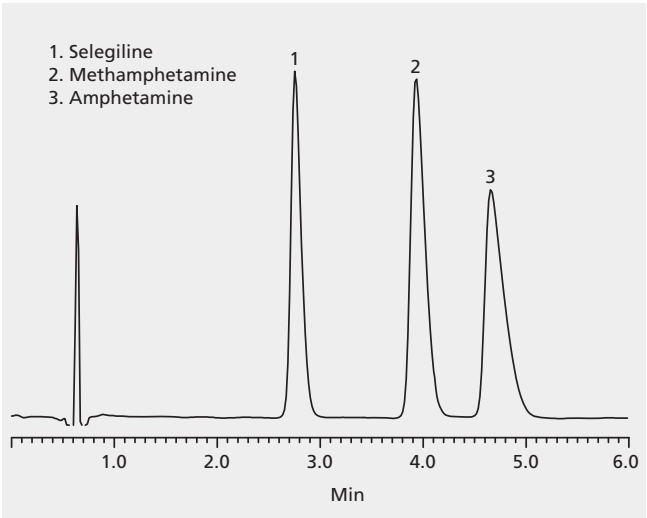
## HPLC Analysis of Rapamycin and 32-Desmethoxyrapamycin on Discovery® HS C18

column . . . . . Discovery HS C18, 15 cm × 4.6 mm ID, 3µm particles (568522-U)
mobile phase . . . . . (A) 10 mM ammonium acetate, pH unadjusted: (B) acetonitrile (25:75, v/v, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 60 °C
detector . . . . . UV, 268 nm
injection . . . . . 10 µg/mL
Application No. . . . . G002015



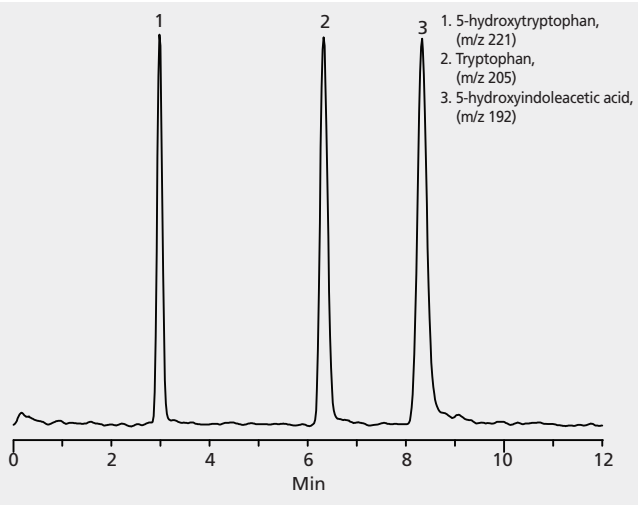
## HPLC Analysis of Selegiline and Amphetamine Metabolites on Discovery® HS F5

column . . . . . Discovery HS F5, 5 cm × 4.6 mm I.D., 5 µm particles (567513-U)
mobile phase . . . (A) water (10 mM ammonium acetate, pH 4.0 with acetic acid: (B) acetonitrile (v/v), (25:75, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002136



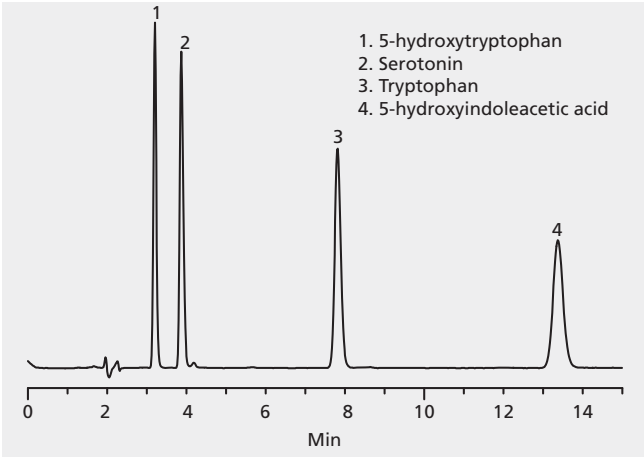
## HPLC Analysis of Serotonin Metabolites on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . (A) 10 mM formic acid/acetate (pH 3.0): (B) methanol; (80:20, A:B)
flow rate . . . . . 0.7 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI (+); overlay of extracted ion chromatograms of individual (M+H)+ species
injection . . . . . 10 µL
sample . . . . . 25 mg/L in 90:10, 10 mM formic acid/acetate (pH 3.0):methanol
Application No. . . . . G002449



## HPLC Analysis of Serotonin Synthetic Intermediates and Metabolites on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . (A) water (10 mM ammonium formate, pH 3.0 with formic acid): (B) acetonitrile; (95:5, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each
Application No. . . . . G002533



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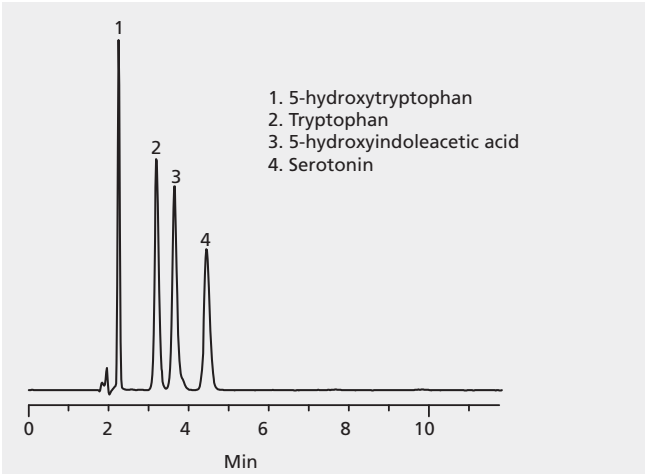
## HPLC Analysis of Serotonin Synthetic Intermediates and Metabolites on Discovery® HS F5

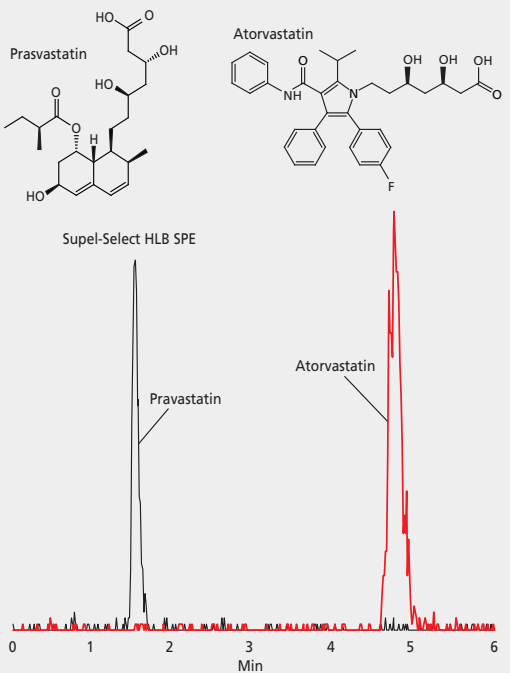
column . . . . Discovery HS F5, 15 cm × 4.6 mm A.D., 5 µm particles (567516-U)
mobile phase . . . (A) water (10 mM ammonium sulfate, pH 3.0 with formic acid): (B)acetonitrile; (80:20, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each
Application No. . . . . G002561



## HPLC Analysis of Statins in Rat Plasma on Ascentis® Express C18 after SPE using Supel™-Select HLB

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . rat plasma spiked with provastin and atorvastin at 5 and 100 ng/mL
SPE tube/cartridge . . . . . Supel-Select HLB SPE, 30 mg/1 mL (54181-U)
condition . . . . . 0.5 mL methanol:acetonitrile (1:1, v/v); 0.5 mL DI water
sample addition . . . . . 0.5 mL
washing . . . . . 0.5 mL 5% methanol
elution . . . . . 0.5 mL methanol:acetonitrile (1:1, v/v)
eluate post-treatment . . . . . evaporate to dryness with nitrogen at 37 °C, reconstitute in 0.5 mL DI water
column . . . . . Ascentis Express C18, 5 cm x 2.1 mm, 2.7 µm particles (53822-U)
mobile phase . . . . . 0.1% acetic acid diluted in 60% methanol
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . ABI 3200 QT; ESI(+), MRM (423.3/321.3, 423.3/101.0, 557.3/397.2 and 557.3/453.4)
injection . . . . . 5 µL
Application No. . . . . G004432

Total Ion Chromatogram (MRM, 4 pairs: 557.3/397.2)
Rat Plasma spiked with 5 ng/mL Statins Extracted by Supel-Select HLB SPE

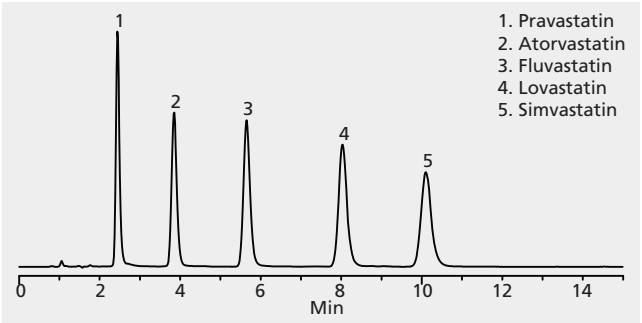



Absolute Recovery ± RSD (n=3)

| | 5 ng/mL spike | | 100 ng/mL spike | |
|---|---|---|---|---|
| | Pravastatin | Atorvastatin | Pravastatin | Atorvastatin |
| Supel-Select HLB | 84 ± 8% | 92 ± 5% | 103 ± 4.2% | 89 ± 3.9% |
| Competitor W | 83 ± 17% | 92 ± 2% | 104 ± 2.2% | 87 ± 1.1% |
| Competitor P | 77 ± 5% | 93 ± 2% | 102 ± 3.0% | 91 ± 1.3% |

## HPLC Analysis of Statins on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . (A) water with 0.1% formic acid (34673): (B) methanol; (25:75, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in mobile phase
Application No. . . . . G003270



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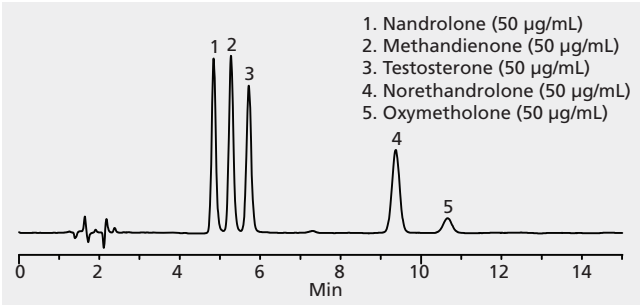
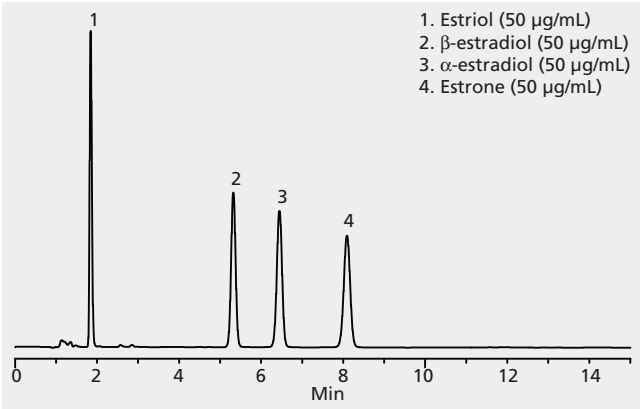
## HPLC Analysis of Steroids, Anabolic, on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003238



## HPLC Analysis of Steroids on Ascentis® C18 (Mobile phase: 55:45)

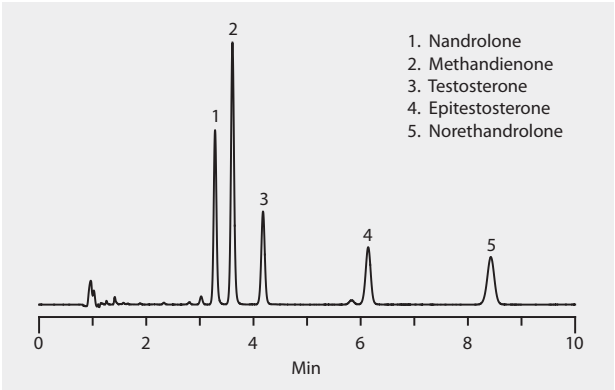
column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (55,45:A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 55:45, water:acetonitrile
Application No. . . . . G002330



## HPLC Analysis of Steroids on Ascentis® Express C18 (10 cm x 2.1 mm I.D., 2.7 µm)

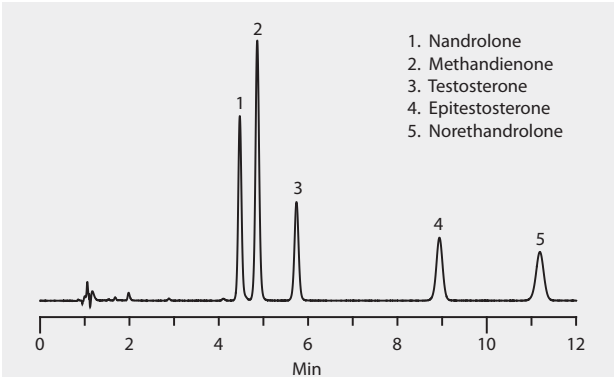
This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of 5 steroids.

column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 0.2 mL/min
pressure . . . . . 2944 psi (203 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 50 mg/L in 75:25, water:methanol
Application No. . . . . G005401



## HPLC Analysis of Steroids on Ascentis® Express C18 with Methanolic Mobile Phase

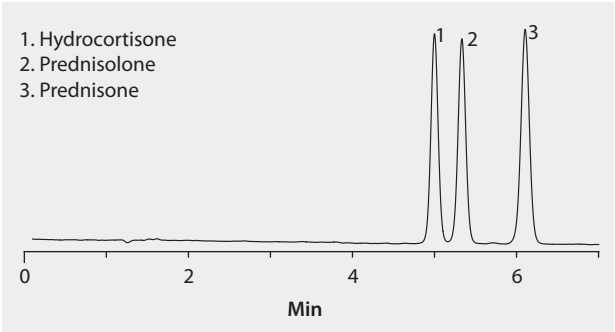
column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) water; (B) methanol; (60:40, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 50 mg/L in 75:25, water:methanol
Application No. . . . . G005404



## HPLC Analysis of Steroids on Ascentis® Express F5

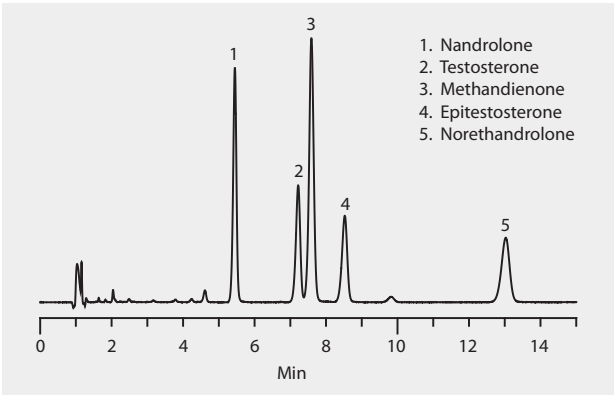
Sample concentrations at 10 ug/mL in 90:10 water:methanol. Acetonitrile does not provide adequate selectivity on this phase. Mass spec was used for peak identification.

column . . . Ascentis Express F5, 15 cm x 4.6 mm I.D., 2.7 µm particles (53591-U)
mobile phase . . . . . (A) water; (B) methanol; (50:50, A:B)
flow rate . . . . . 0.8 mL/min
pressure . . . . . 2393 psi (165 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . 10 µg/mL of each in 90: 10, water: methanol
Application No. . . . . G005743



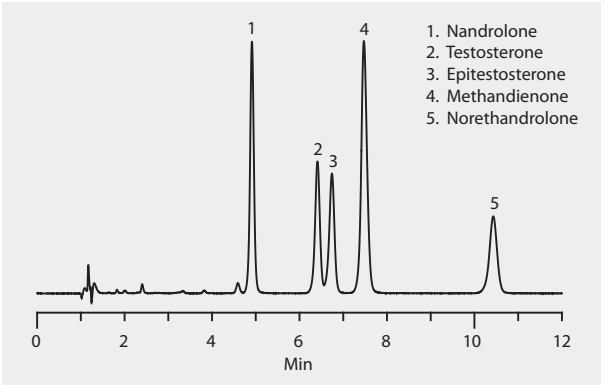
## HPLC Analysis of Steroids on Ascentis® Express F5, Acetonitrile Mobile Phase

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 50 mg/L in 75:25, water:methanol
Application No. . . . . G005409



## HPLC Analysis of Steroids on Ascentis® Express F5 with Methanolic Mobile Phase

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) water (B) methanol; (40:60, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 0.2 mL/min
sample . . . . . 50 mg/L in 75:25, water:methanol
Application No. . . . . G005410



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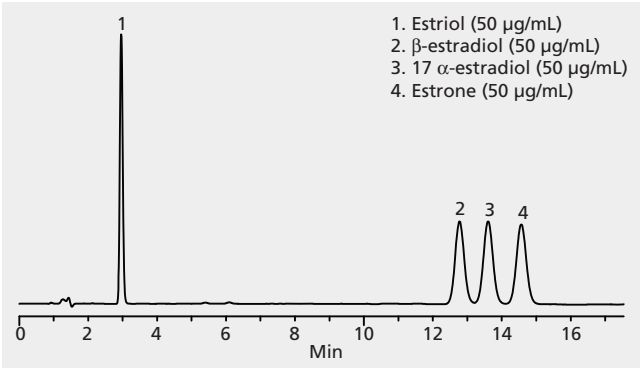
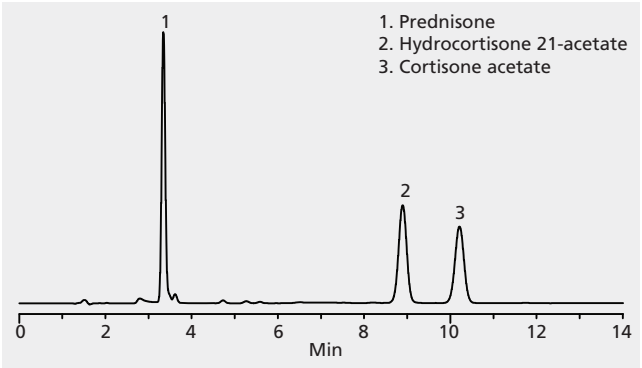
## HPLC Analysis of Steroids on Ascentis® RP-Amide (Mobile phase: 55:45)

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (55:45, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 55:45, water:acetonitrile
Application No. . . . . G002672



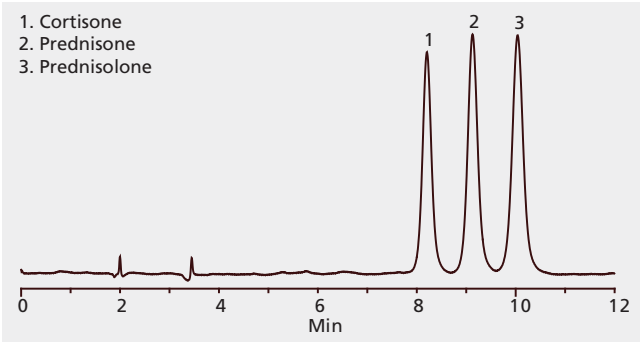
## HPLC Analysis of Steroids on Ascentis® RP-Amide (Mobile phase: 60:40)

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002674



## HPLC Analysis of Steroids on Ascentis® Si

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) hexane; (B) ethanol; (88:12, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 245 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 85:15, hexane:2-propanol
Application No. . . . . G003728



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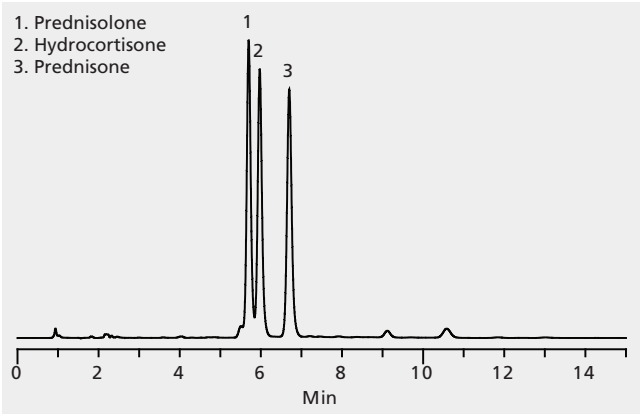
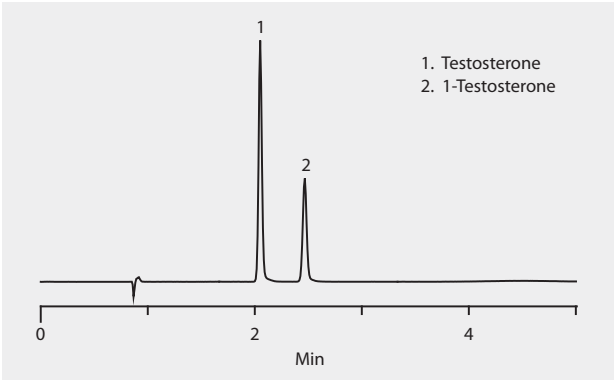
## HPLC Analysis of Steroids on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 3 µm particles (581610-U)
mobile phase . . . . . (A) water: (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 85:15, water:methanol
Application No. . . . . G003698



## HPLC Analysis of Testosterone and 1-Testosterone on Ascentis® Express C18

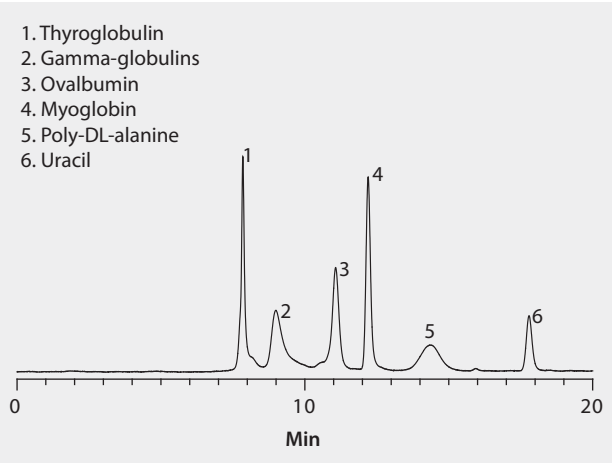
column . . Ascentis Express C18, 10 cm x 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) water; (B) methanol; (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL in 10:90 water:methanol
Application No. . . . . G005413



## HPLC Analysis of Thyroglobulin, gamma-Globulins, Ovalbumin, Myoglobin, and Poly-DL-Alanine on Zenix® SEC-150, 30 cm x 7.8 mm I.D., 3 µm particles

This application demonstrates the suitability of Discovery BIO GFC 150 for the separation of proteins. Separation is by inclusion into the pore volume with larger molecules being excluded and therefore eluting earlier.

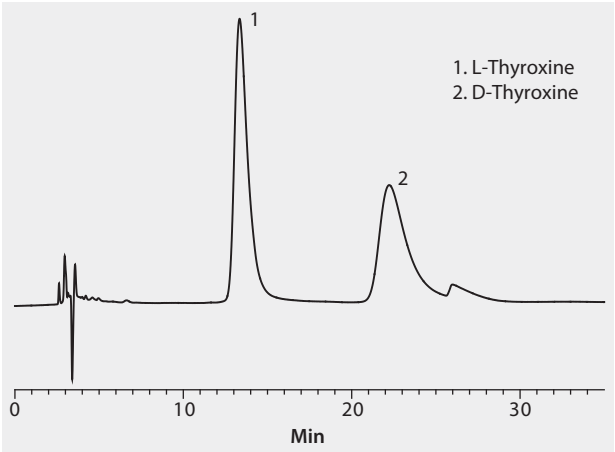
column . . . . . Zenix® SEC-150, 30 cm x 7.8 mm I.D., 3 µm particles (Z777018)
mobile phase . . . . . 150 mM potassium phosphate monobasic, pH 7.0 (adjusted with potassium hydroxide)
flow rate . . . . . 0.7 mL/min
pressure . . . . . 890 psi (61.4 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
sample . . . . . 1 g/L each (except uracil, 0.1 g/L ) in mobile phase A
Application No. . . . . G005727



## HPLC Analysis of Thyroxine Enantiomers on Astec® CHIROBIOTIC® TAG

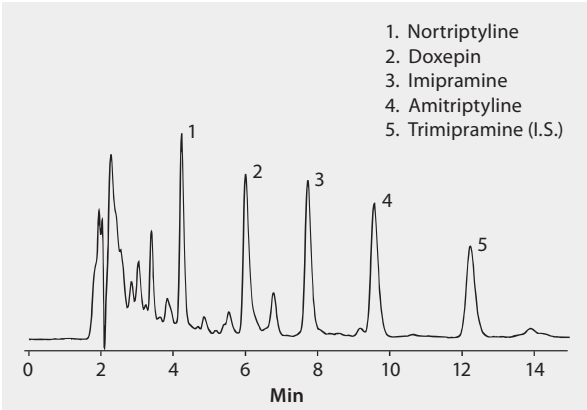
Baseline resolution of the chiral enantiomers of DL-Thyroxine is achieved by polar ionic mode on the Astec CHIROBIOTIC TAG with the use of an LC/MS-compatible mobile phase.

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . 1% ammonium formate in methanol
flow rate . . . . . 1 mL/min
pressure . . . . . 826 psi (57 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . each enantiomer 0.1 mg/L in methanol
Application No. . . . . G005515



## HPLC Analysis of Tricyclic Antidepressants in Serum on Discovery® C18 after SPE using Discovery® DSC-18

sample preparation . . . . . SPE (Solid Phase Extraction)
SPE tube/cartridge . . . . . Discovery DSC-18, 100 mg/1 mL (52602-U)
column . . . . . Discovery C18, 15 cm × 4.6 mm, 5 µm preceded by a 2 cm C18 guard column and 0.5 µm frit filter (504955)
mobile phase . . . (A) acetonitrile: (B) methanol: (C) 25 mM potassium phosphate, monobasic (pH 7 with triethlylamine) (45:25:30, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 50 µL, diluted porcine serum extract
Application No. . . . . G000595




### Efficiency of Recovery

| Compound | Concentration (µg/mL) | % Recovery | % RSD (n=6) |
|---|---|---|---|
| 1. Nortriptyline | 0.10<br>0.50 | 103.6<br>97.5 | ±4.5<br>±4.5 |
| 2. Doxepin | 0.10<br>0.50 | 102.2<br>100.8 | ±3.0<br>±1.8 |
| 3. Imipramine | 0.10<br>0.50 | 92.0<br>97.5 | ±1.5<br>±1.7 |
| 4. Amitriptyline | 0.10<br>0.50 | 93.6<br>95.7 | ±1.2<br>±1.4 |

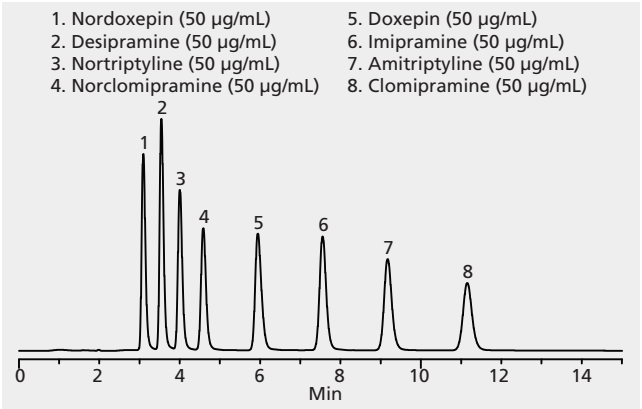
### SPE Procedure, Using Zymark RapidTrace SPE Workstation

| Step | Solvent/ Solution | Volume (mL) | Flow Rate (mL/min) | Comments |
|---|---|---|---|---|
| 1. Condition | MeOH | 2.0 | 5.0 | conditions sorbent |
| 2. Condition | $H_2O$ | 2.0 | 5.0 | conditions sorbent |
| 3. Load | spiked porcine serum | 2.0[A] | 0.75 | applies serum sample |
| 4. Rinse | 20% MeOH in $H_2O$ | 2.0 | 5.0 | washes sorbent |
| 5. Purge-Cannula | $H_2O$ | 4.0 | 30.0 | cleans sample cannula |
| 6. Rinse | vent | 0.1 | 2.0 | positions SPE tube over waste port |
| 7. Dry | $N_2$ | Time = 10 min | | dries sorbent |
| 8. Purge-Cannula | MeOH | 4.0 | 30.0 | cleans sample cannula |
| 9. Collect | MeOH | 1.0 | 1.0 | elutes analytes into collection vessel |
| 10. Collect | vent | 6.0 | 3.0 | pushes residual eluent into vessel[B] |
| 11. Purge-Cannula | $H_2O$ | 4.0 | 30.0 | cleans sample cannula |

[A]1 mL porcine serum spiked with 0.1 µg/mL each analyte basified with 3 µL 10 N KOH, then diluted with 1 mL water
[B]350 µL water added per mL methanolic eluent before analysis

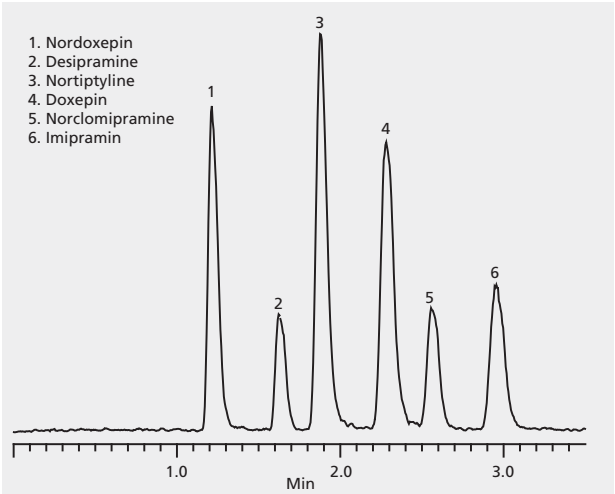
## HPLC Analysis of Tricyclic Antidepressants on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 2.1 mm I.D., 3 µm particles (581402-U)
mobile phase . . . . . (A) 25 mM ammonium phosphate dibasic (pH 7.0 with phosphoric acid): (B) methanol; (25:75, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 60:40 water:methanol
Application No. . . . . G003156



## HPLC Analysis of Tricyclic Antidepressants on Ascentis® Express C18

column . . Ascentis Express C18, 10 cm × 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) 100 mM ammonium acetate (pH 7.0; titrated with ammonium hydroxide), (B) water, (C) methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 55 °C
detector . . . . . Thermo LCQ Advantage; ESI(+), m/z 250-320
injection . . . . . 1 µL
instrument . . . . . Jasco X-LC
Application No. . . . . G004062



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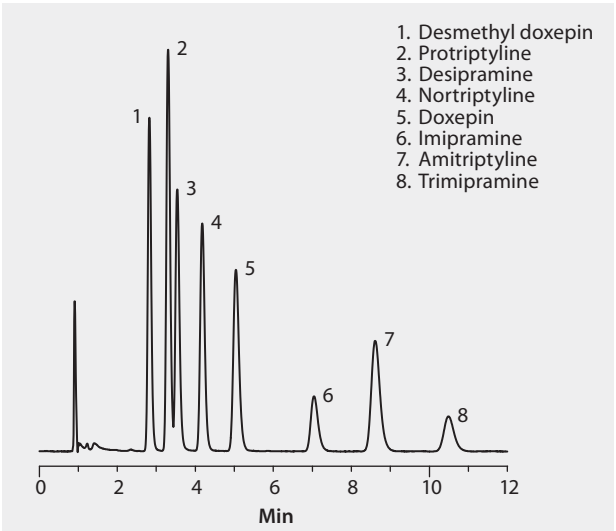
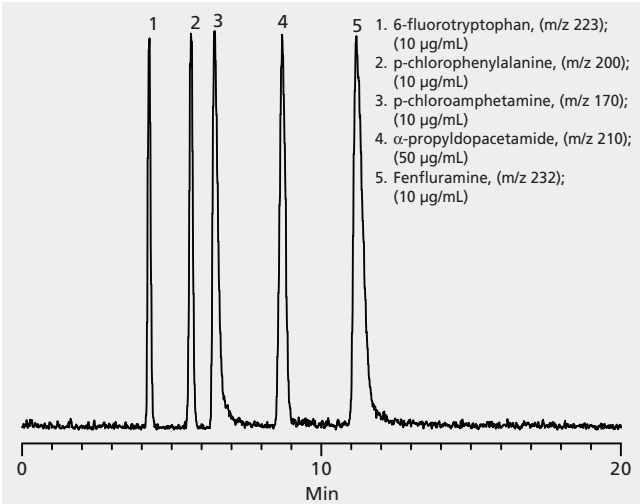
## HPLC Analysis of Tricyclic Antidepressants on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . (A) water/acetonitrile (40:60 v/v); (B) 10 mM potassium phosphate
flow rate . . . . . 2 mL/min
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
Application No. . . . . G000163



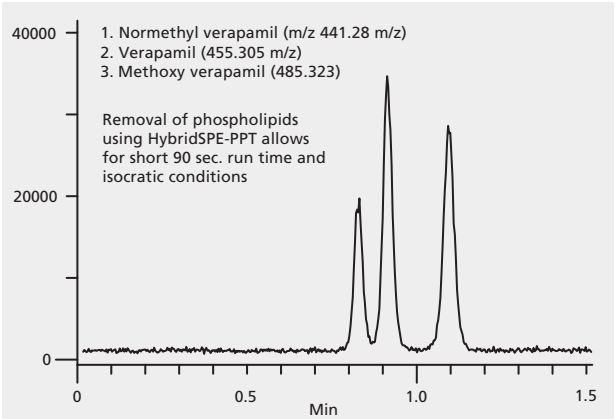
## HPLC Analysis of Tryptophan Hydroxylase Inhibitors on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . (A) 40 mM formic acid, (pH ~2.5): (B) methanol; (65:35, A:B)
flow rate . . . . . 0.7 mL/min
column temp. . . . . 35 °C
detector . . . . . full scan MS; overlay of extracted ion chromatograms of individual (M+H)+ species
injection . . . . . 10 µL
sample . . . . . as indicated in 90:10, 10 mM formic acid:methanol
Application No. . . . . G002453



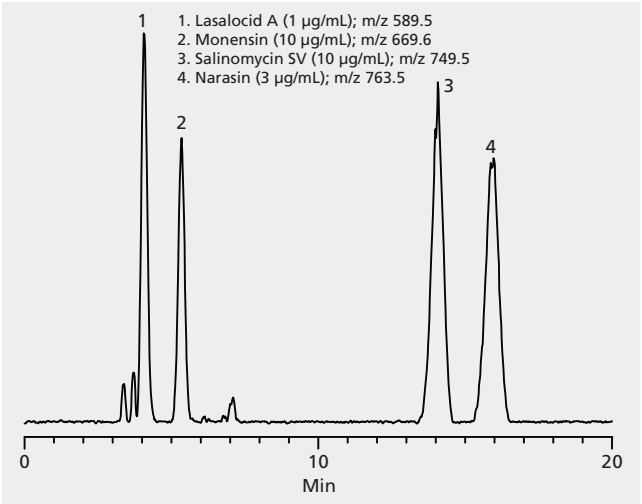
## HPLC Analysis of Verapamil and Metabolites in Rat Plasma on Ascentis® Express C18 after Phospholipid Removal using HybridSPE®-Phospholipid

SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample preparation . . . . . SPE (Solid Phase Extraction)
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
sample/matrix . . . Rat plasma spiked with verapamil and metabolites at 10 ng/mL
mobile phase . . . . . (A) water; (B) acetonitrile, pH 2.55 adjust with formic acid (30:70, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . TOF/MS
injection . . . . . 1 µL
sample addition . . . . . 100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile
elution . . . . . apply vacuum
Application No. . . . . G004366



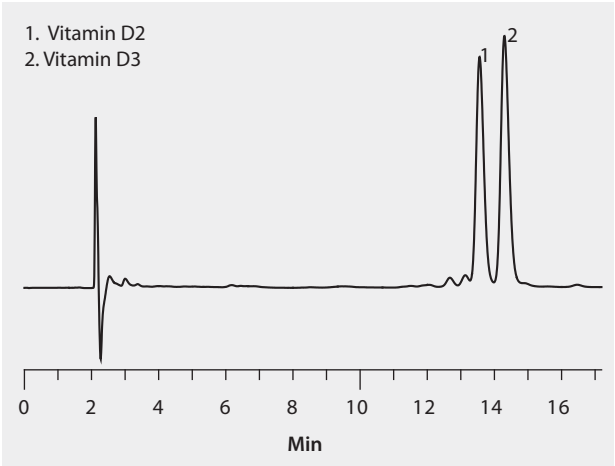
## HPLC Analysis of Veterinary Antibiotics on Ascentis® C18

column . . . . . Ascentis C18, 5 cm x 2.1 mm I.D., 3 µm particles (581300-U)
mobile phase . . (A) 50 mM ammonium acetate (pH 6.0): (B) methanol; (20:80, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 22 °C
detector . . . . . ESI(-), full scan
injection . . . . . 2 µL
sample . . . . . as indicated in 50:50, water:methanol
Application No. . . . . G002459



## HPLC Analysis of Vitamin D2 and D3 on Ascentis® Express C18

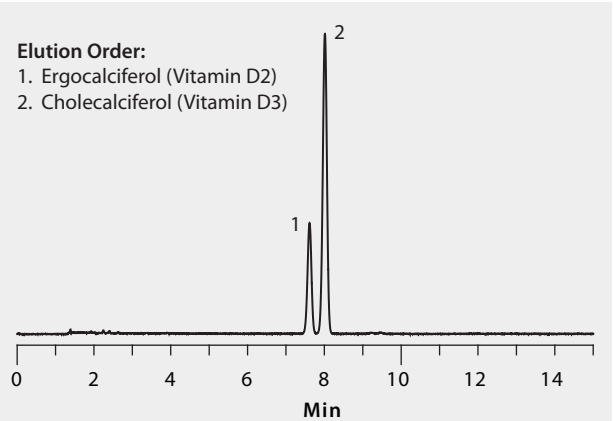
column . . Ascentis Express C18, 15 cm x 2.1 mm I.D., 2.7 µm particles (53825-U)
mobile phase . . . . . acetonitrile
flow rate . . . . . 0.15 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
sample . . . . . 10 mg/L each (vitamins D2 and D3) in methanol
Application No. . . . . G005615



## HPLC Analysis of Vitamin D2 and D3 on Ascentis® Express C18 5 µm

This application demonstrates the suitability of the Ascentis Express C18, 5 µm, for the routine analysis of the fat-soluble vitamins D2 and D3 . Structures along with the optimized chromatogram obtained on the Ascentis Express C18, 5 µm are presented.

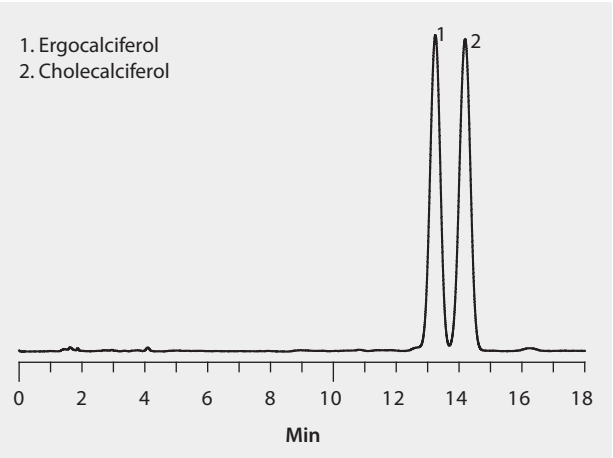
column . . Ascentis® Express C18, 25 cm x 4.6 mm I.D., 5 µm particles (50538-U)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (95:5, A:B)
flow rate . . . . . 1.5 mL/min
pressure . . . . . 1726 psi (119 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 265 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 90:10, acetonitrile:methanol
Application No. . . . . G005891



## HPLC Analysis of Vitamin D2 and Vitamin D3 on Ascentis® C18

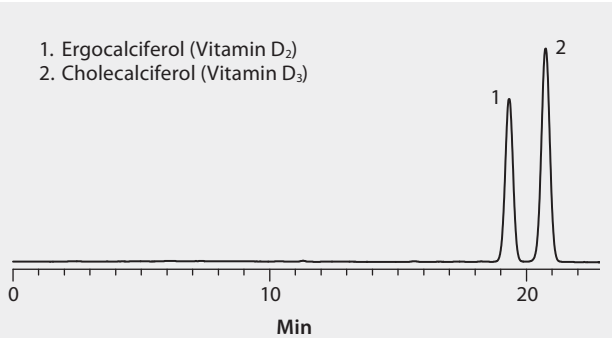
This application demonstrates the suitability of the Ascentis C18 for the analysis of the fat-soluble vitamins D2 and D3 . Structures along with the optimized chromatogram obtained on the Ascentis C18 are presented.

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 290 nm
sample . . . . . 50 µg/mL each in acetonitrile
Application No. . . . . G005614



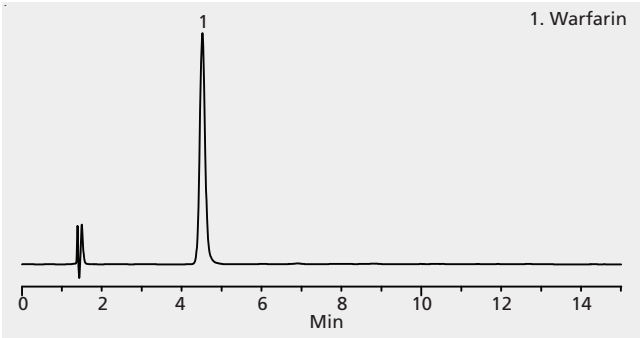
## HPLC Analysis of Vitamins, Fat Soluble (D2 and D3), on Discovery® HS C18

column . . . . Discovery HS C18, 15 cm × 4.6 mm I.D., 3 µm particles (569252-U)
mobile phase . . . . . 100% acetonitrile
flow rate . . . . . 0.8 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each analyte
Application No. . . . . G001419



## HPLC Analysis of Warfarin™ Anticoagulant on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0, unadjusted): (B) methanol; (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002340



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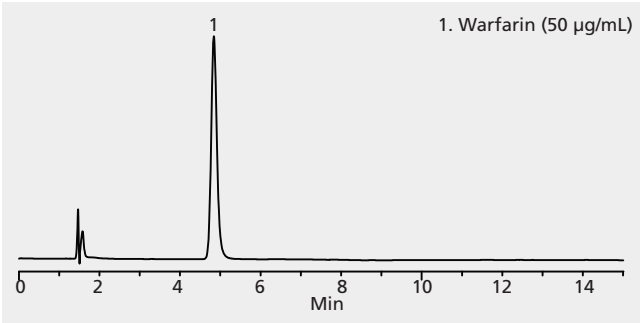
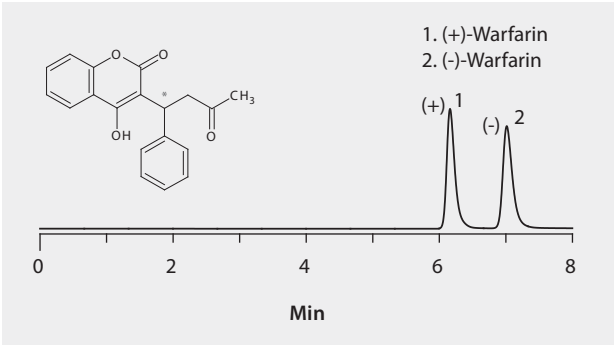
## HPLC Analysis of Warfarin™ Anticoagulant on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0 unadjusted): (B) methanol; (30:70, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 20 mM phosphoric acid (pH 2.0)
Application No. . . . . G002638



## HPLC Analysis of Warfarin™ Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) TEA; (100:0.2:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 278 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005007



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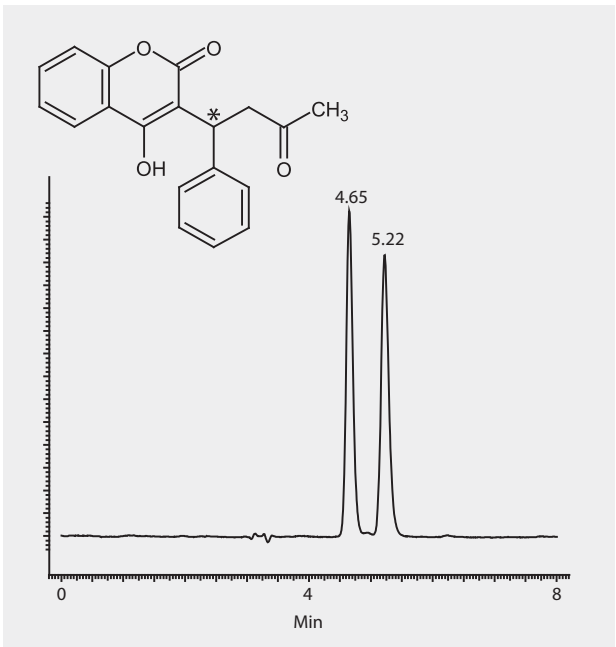
## HPLC Analysis of Warfarin™ Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) 5 mM ammonium acetate, pH 4.1; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004349



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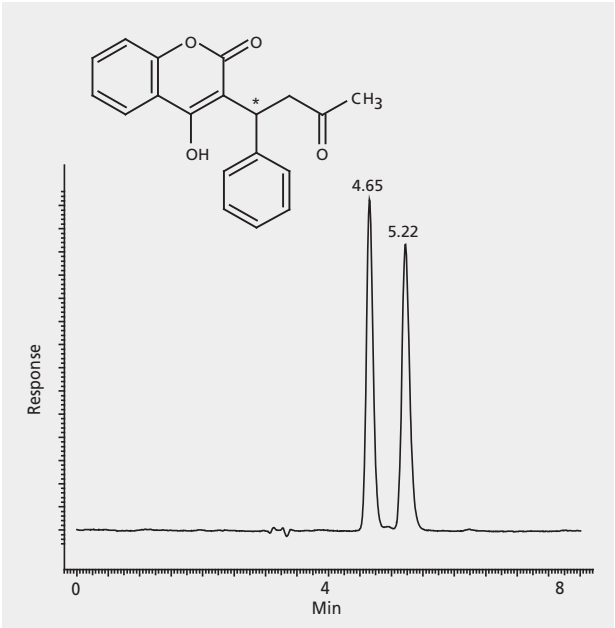
## HPLC Analysis of Warfarin™ Enantiomers on Astec® CHIROBIOTIC® V (Method 1)

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) acetonitrile; (B) 5 mM ammonium acetate, pH 4.1; (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in mobile phase
Application No. . . . . G004472



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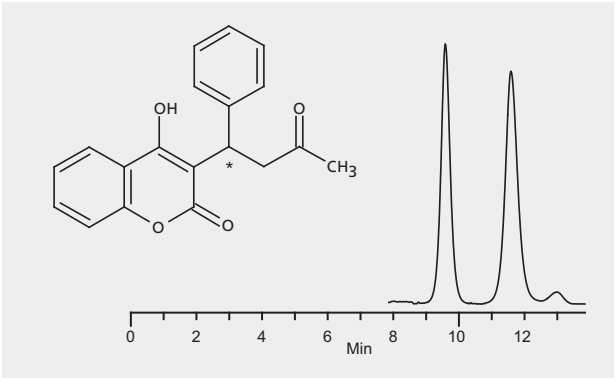
## HPLC Analysis of Warfarin™ Enantiomers on Astec® CHIROBIOTIC® V (Method 2)

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . (A) 0.1% triethylamine acetate, pH 5.0; (B) THF; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 4 mg/mL in methanol
Application No. . . . . G004649



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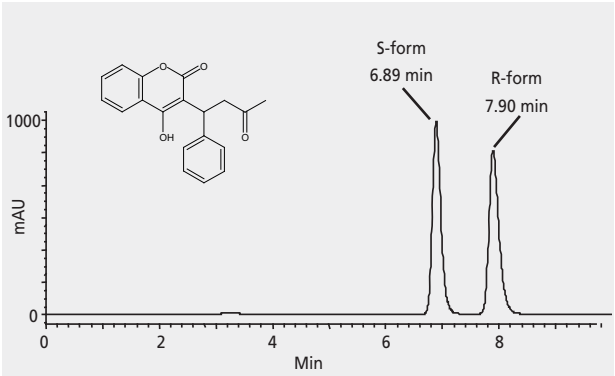
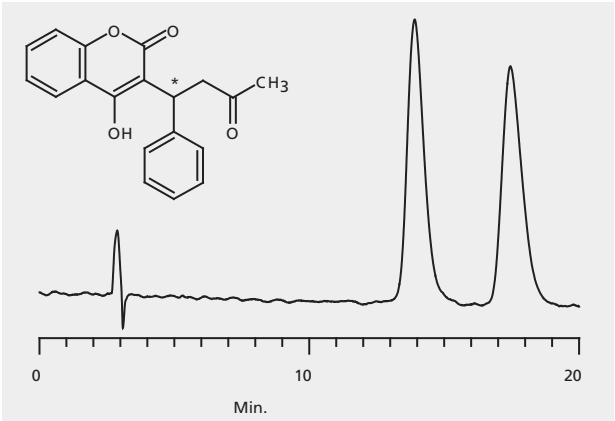
## HPLC Analysis of Warfarin™ Enantiomers on Astec® CYCLOBOND® I 2000 (UV at 254 nm)

column . . CYCLOBOND I 2000, 25 cm × 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . . . . (A) acetonitrile: (B) acetic acid: (C) triethylamine; (100:0.3:0.25, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
Application No. . . . . G004027



## HPLC Analysis of Warfarin™ Enantiomers on Astec® CHIROBIOTIC® V2

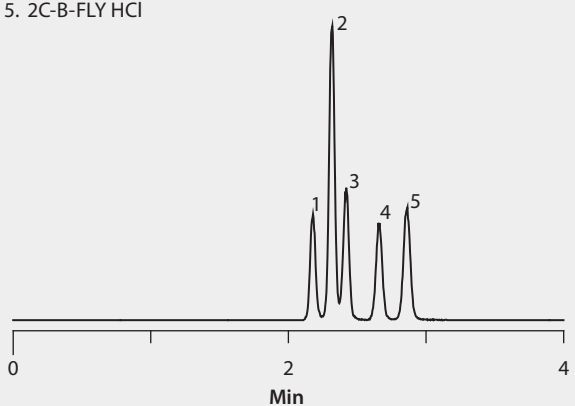
column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004475



## LC-MS Analysis of 2C Amines Drugs of Abuse on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . 5 mM ammonium formate in acetonitrile:water (90:10, v/v)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . TOF/MS
detector . . . . . 2.0 µL
sample . . . . . 300 ng/mL in acetonitrile
Application No. . . . . G006166

1. *R*-(-)-Bromo-DragonFLY HCl
2. (±)-2,5-Dimethoxy-4-Iodoamphetamine HCl (DOI HCl)
3. (±)-2,5-Dimethoxy-4-Bromoamphetamine HCl (DOB HCl)
4. 4-Bromo-2,5-dimethoxyphenethylamine HCl (2C-B)
5. 2C-B-FLY HCl

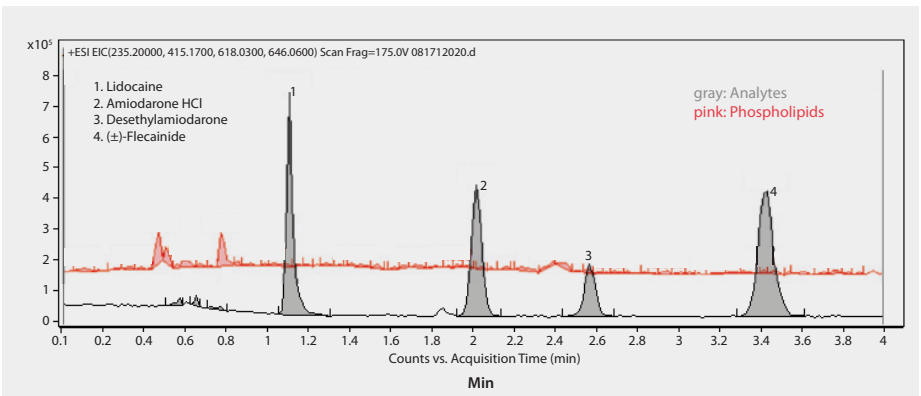


## LC-MS Analysis of Antiarrhythmic Drugs and Metabolites in Plasma on Ascentis® Express HILIC following Sample Prep using HybridSPE®-Phospholipid

For efficient therapeutic drug monitoring, it is important for clinicians to have access to fast and robust analytical methods for accurate assessment of drug efficacy. Industrial trends toward highly specific LC/MS applications over traditional ELISA type immunoassay have resulted in the need for high-speed chromatographic assays along with simplified sample preparation methods. Often the limitation of a bioanalytical technique is based upon the effectiveness of the sample preparation technique. Plasma and serum samples are often susceptible to assay irregularities due to matrix-induced interferences. In this study the impact of matrix interference is investigated with respect to precision and accuracy of antiarrhythmic cardiac drugs from plasma samples. A robust bioanalytical method was developed using a combination of fast HILIC chromatography on Ascentis Express along with a selective sample preparation using HybridSPE-Phospholipid. Solvents were LC-MS Ultra CHROMASOLV® and Certified Reference Material grade standards were from Cerilliant.

Endogenous phospholipids can dramatically impact the precision and accuracy of a bioanalytical method. During development, the sample matrix impact on analyte detection should be priority in method validation. Failure to do so can result in inaccuracies in reported levels, thus impacting the assessment of patient health. Eliminating matrix effects in LC/MS is imperative to producing reliable and accurate bioanalytical methods. The targeted phospholipid selectivity of the HybridSPE-Phospholipid technique enables simplified sample processing with no detected phospholipid matrix interference, while exhibiting excellent recovery from plasma and serum samples. This application demonstrates how combining selectivity in both sample preparation and chromatographic separation allows for a simplified and efficient bioanalytical method resulting in a high precision and accurate assay.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . rabbit plasma, unfiltered K2-EDTA, spiked with each compound at 100 ng/mL (3:1, plasma:1% formic acid in acetonitrile)
SPE tube/cartridge . . . . . HybridSPE-Phospholipid, 96-well plate (575656-U)
sample addition . . . . . To each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes. Collect filtrate and analyze directly.
column . . . . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile, 5:95 (A:B), adjusted to pH 7.0 with formic acid
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1305 psi (90 bar)
column temp. . . . . 35 °C
detector . . . . . ESI+, 100-1000 m/z
injection . . . . . 0.5 µL
Application No. . . . . G005854



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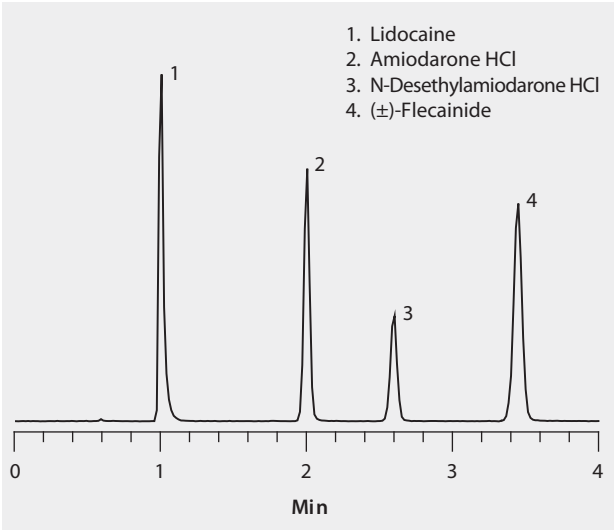
## LC-MS Analysis of Antiarrhythmic Drugs and Metabolites on Ascentis® Express HILIC

The basic nature of these antiarrhythmic agents makes them ideal candidates for HILIC (hydrophilic interaction) chromatographic separation. The benefit of HILIC over traditional reversed-phase chromatography is two-fold for both sample introduction and analyte detection. First, the high acetonitrile concentration of HILIC mobile phases allows for direct analysis of precipitated plasma samples without the need for additional sample solvent exchange. Second, the high acetonitrile content provides increased analyte response in positive ESI MS detection. Of the various HILIC-mode columns tested, method development for this assay determined the Ascentis Express HILIC, 2.7 µm particles, provided the best chromatographic resolution of the antiarrhythmic drugs while maintaining high peak efficiency for enhanced detection levels.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile, 5:95 (A:B), adjusted to pH 7.0 with formic acid
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1305 psi (90 bar)
column temp. . . . . 35 °C
detector . . . . . ESI+, 100-1000 m/z
injection . . . . . 0.5 µL
sample . . each compound, 300 ng/mL in 75:25 (1% formic acid acetonitrile:water)
Application No. . . . . G005855



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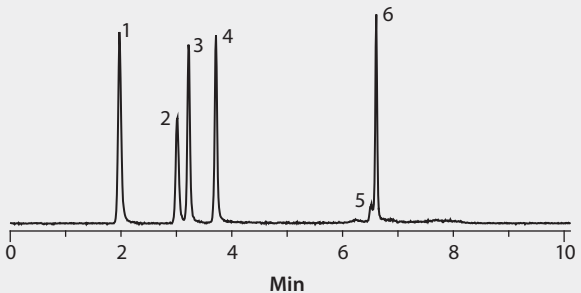
## LC-MS Analysis of Antipsychotic Drugs on Ascentis® Express RP Amide

| | |
|---|---|
| column | Ascentis Express RP Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U) |
| mobile phase | (A) 5 mM ammonium formate water; (B) 5mM ammonium formate in 95:5 (v/v) methanol:water |
| gradient | held at 35% B for 0.5 min; 35 to 85% B in 5.5 min; held at 85% B for 1 min |
| flow rate | 0.3 mL/min |
| pressure | 4641 psi (320 bar) |
| column temp. | 35 °C |
| detector | ESI(+), m/z 100-1000 |
| injection | 1 µL |
| sample | 200 ng/mL in waler |
| Application No. | G005668 |

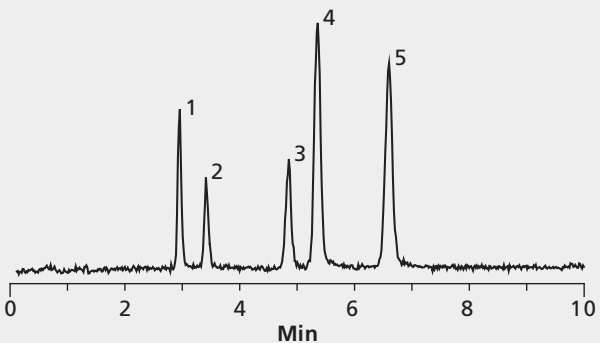
1. Desmethyl venlafaxine
2. Hydroxybupropion
3. Hydroxyrisperidon
4. Bupropion
5. Zuclopenthixol
6. Fluphenazine



## LC-MS Analysis of Benzodiazepines on Ascentis® Phenyl (Analyte Mix 1)

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted), (B): 0.1% ammonium acetate in acetonitrile, (50:50, A:B)
flow rate . . . . . 1 mL/min., split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 90:10:water:acetonitrile
Application No. . . . . G003705

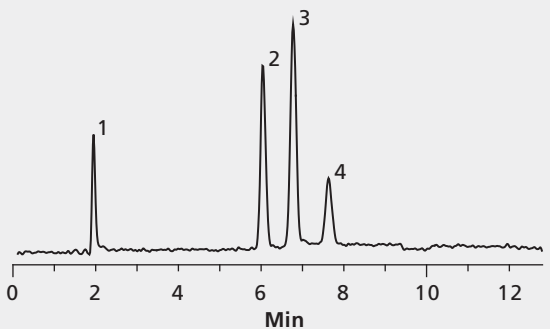
1. Demoxepam (1 µg/mL) (M+H)+ = 287.05
2. Oxazepam (1 µg/mL) (M+H)+ = 287.05
3. Temazepam (1 µg/mL) (M+H)+ = 301.06
4. Clobazam (1 µg/mL) (M+H)+ = 301.06
5. Diazepam (1 µg/mL) (M+H)+ = 285.07



## LC-MS Analysis of Benzodiazepines on Ascentis® Phenyl (Analyte Mix 2)

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted); (B) 0.1% ammonium acetate in acetonitrile; (60:40, A:B)
flow rate . . . . . 1 mL/min., split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 90:10:water:acetonitrile
Application No. . . . . G003706

1. Fenoldopam (10 µg/mL) (M+H)+ = 306.08
2. Lorazepam (10 µg/mL) (M+H)+ = 321.01
3. Nitrazepam (10 µg/mL) (M+H)+ = 282.08
4. Clonazepam (10 µg/mL) (M+H)+ = 316.04



## LC-MS Analysis of Bile Acids and Their Conjugates on Ascentis® Express C18

A fast robust LC-MS/MS method was developed on a C18 Ascentis Express Fused-CoreR Particle Column allowing the 15 bile acid species to be measured individually rather than the alternative measurement of a total concentration by colourimetric kinetic enzyme assays.

sample/matrix . . . . . Plasma proteins were removed with addition of 900 µL of acetonitrile, containing deuterated internal standards, to 250 µL of human EDTA plasma. The mixture was vortexed, (centrifuged and the supermatant evaporated before being reconstituted in a 50:50 solution of methanol and water. 10 µL (corresponding to 8.57 µL of plasma) was injected into the HPLC.)

column . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)

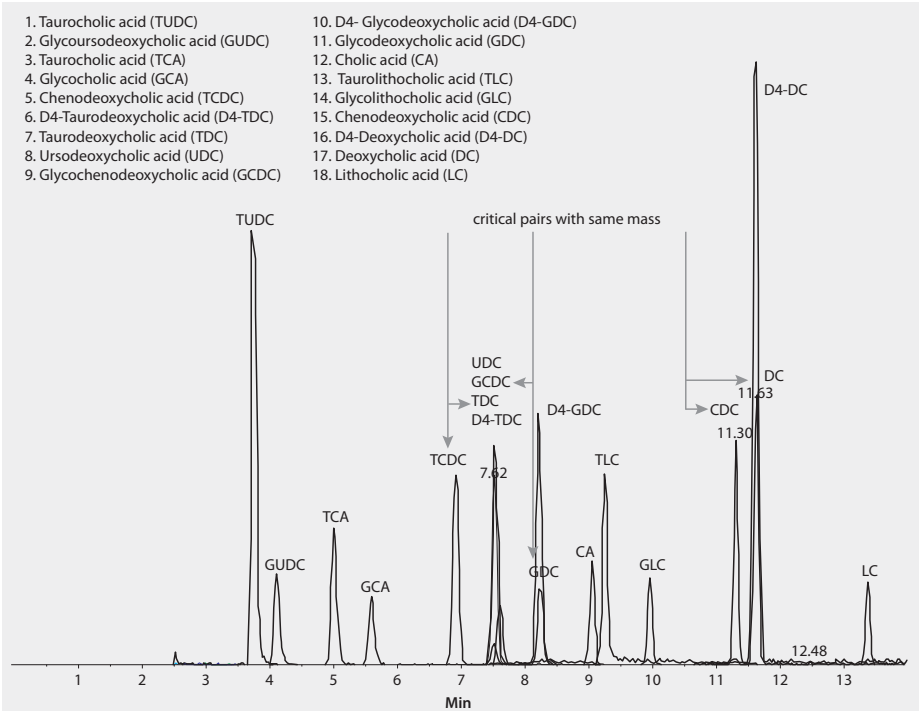
gradient . . . . . 70 to 95% B in 10 minutes held 4 minutes

flow rate . . . . . 0.6 mL/min

column temp. . . . . 40 °C

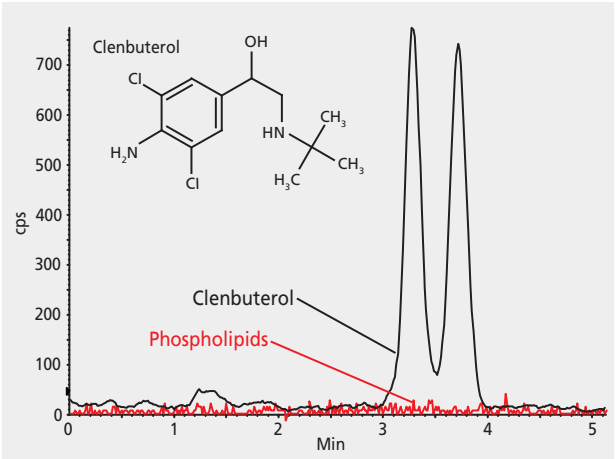
detector . . . . . ESI(-), MRM Mode

Application No. . . . . G005599



## LC-MS Analysis of Clenbuterol Enantiomers in Plasma on Astec® CHIROBIOTIC® T after SPE using HybridSPE®-Phospholipid

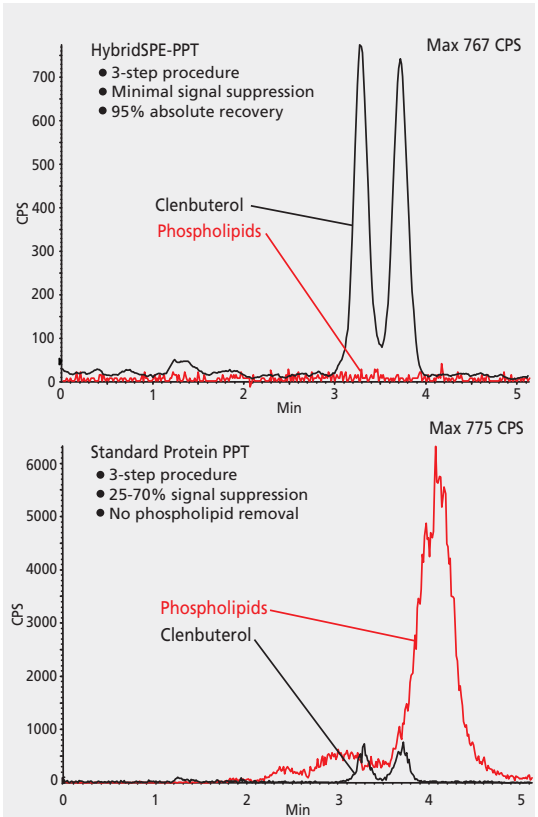
column . . . . . CHIROBIOTIC T, 10 cm x 2.1 mm I.D., 5 µm particles (12018AST)
mobile phase . . . . . 10 mM ammonium formate in methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . ESI(+)
sample . . clenbuterol, 10 ng/mL in rat plasma (phospholipids removed by extraction with HybridSPE-Phospholipid)
Application No. . . . . G004245



## LC-MS Analysis of Clenbuterol In Plasma on Astec® CHIROBIOTIC® T with Phospholipid Removal Using HybridSPE® Phospholipid

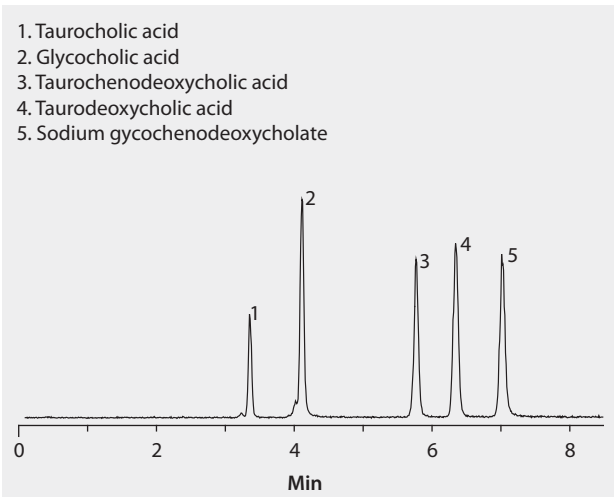
The HybridSPE method provides significant improvement in LC-MS baseline.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . rat plasma spiked with clenbuterol enantiomers at 10 ng/mL
SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample addition . . 100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile. Mix by vortexing the HybridSPE-PPT plate briefly.
elution . . . . . apply vacuum
column . . . . . CHIROBIOTIC T, 10 cm x 2.1 mm I.D., 5 µm particles (12018AST)
mobile phase . . . . . 10 mM ammonium formate in methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . ABI 3200 QT; ESI(+), MRM: 184/104 m/z (phospholipids); 277.2/203.1 m/z (clenbuterol)
injection . . . . . 10 µL
Application No. . . . . G004431



## LC-MS Analysis of Conjugated Bile Acids on Ascentis® Express C18

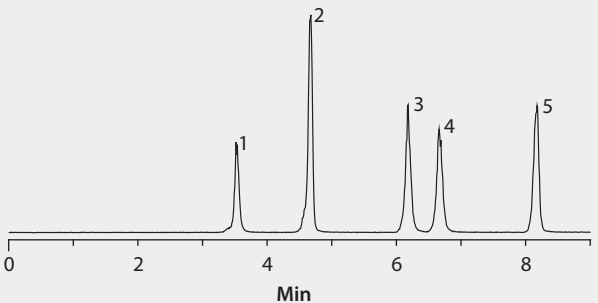
column . . Ascentis Express C18, 5 cm x 4.6 mm I.D., 2.7 µm particles (53826-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 4 with acetic acid; (B) acetonitrile
gradient . . . . . 25 to 40% B in 10 min
flow rate . . . . . 1.0 mL/min (postcolumn split to MS)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(-), SIR
injection . . . . . 5 µL
sample . . . . . 10 mg/L each in mobile phase A
Application No. . . . . G005688



## LC-MS Analysis of Conjugated Bile Acids on Ascentis® Express RP-Amide

column . . Ascentis Express RP-Amide, 5 cm x 4.6 mm I.D., 2.7 µm particles (53922-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 3 with acetic acid; (B) acetonitrile
gradient . . . . . 25 to 40% B in 10 min
flow rate . . . . . 1.0 mL/min (postcolumn split to the MS)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(-), SIR
injection . . . . . 5 µL
sample . . . . . 10 mg/L in mobile phase A
Application No. . . . . G005689

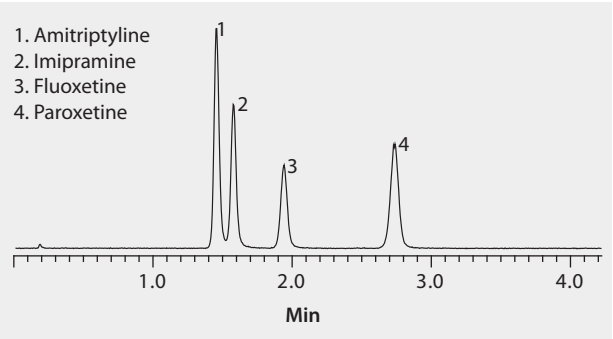
1. Taurocholic acid
2. Glycocholic acid
3. Taurochenodeoxycholic acid
4. Taurodeoxycholic acid
5. Sodium glycochenodeoxycholate



## LC-MS Analysis of CYP2D6 Inhibitors on Ascentis® Express HILIC

This application demonstrates the suitability of the Ascentis Express HILIC for the analysis of CYP2D6 Inhibitors.

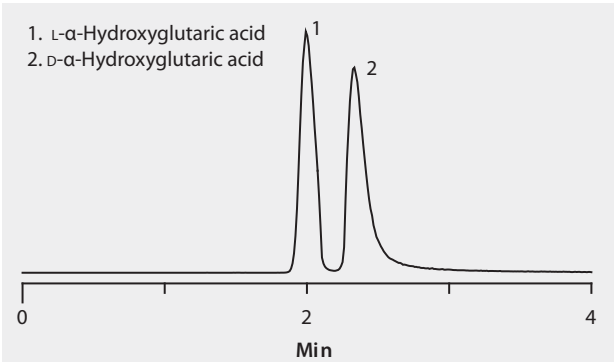
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . 5 mM ammonium formate in 2:98 (v/v) water:acetonitrile
flow rate . . . . . 1.2 mL/min
column temp. . . . . 50 °C
detector . . . . . ESI(+), m/z 100-400
injection . . . . . 0.5 µL
Application No. . . . . G005691



## LC-MS Analysis of DL-α-Hydroxyglutaric acid on Astec® CHIROBIOTIC® R

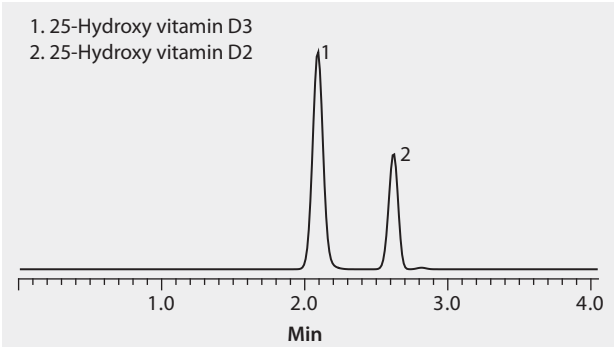
Chiral 2-hydroxyglutarates (2-OHG) are important molecular signatures of both healthy and diseased biological cells, their specific biochemical pathways and inborn errors of metabolism. The chiral differentiation and quantification of D-2-OHG and L-2-OHG is key for characterizing neuro-metabolic disorders like the 2-hydroxyglutaric acidurias that cause neuro-logical impairment early in life. In this application, an Astec® CHIROBIOTIC® R column run under polar ionic mobile phase conditions achieved a rapid separation of the D and L enantiomers of 2-OHG under conditions amenable for sensitive LC-MS/MS detection.

column . . Astec CHIROBIOTIC® R, 10 cm x 4.6 mm I.D., 5µm particles (13022AST)
mobile phase . . . . . 5:95, (water:methanol) with 0.3% acetic acid and 0.1% ammonium hydroxide
flow rate . . . . . 1 mL/min
pressure . . . . . 1700 psi (117 bar)
column temp. . . . . 25 °C
detector . . . . . MS, ESI(-) TOF, SIM, m/z 147.03, split 100:1
injection . . . . . 10 µL
sample . . . . . DL-a-Hydroxyglutaric acid, 1 mg/mL in 50:50, water:methanol
Application No. . . . . G005887



## LC-MS Analysis of 25-Hydroxyvitam D3 and 25-Hydroxyvitamin D2 on Ascentis® Express C18

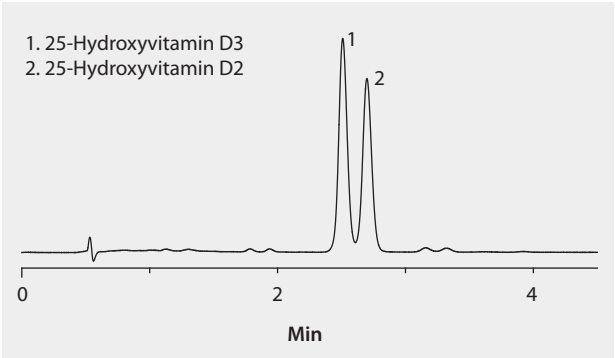
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 10mM ammonium formate in water; (B) 10mM ammonium formate in acetonitrile; (32:68, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1711 psi (118 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+)
injection . . . . . 2 µL
sample . . . . . 10 µg/mL in 25:75, water:methanol
Application No. . . . . G005658



## LC-MS Analysis of 25-Hydroxyvitamin D2 and 25-Hydroxyvitamin D3 on Ascentis® Express F5

The 25-OH forms of the secosteroids vitamin D2 and D3 are indicators of the vitamin D status of an individual. However, because they have different biological activity and clinical interest, they should be distinguishable. The F5 phase was able to resolve the two compounds under highly LC-MS/MS compatible conditions.

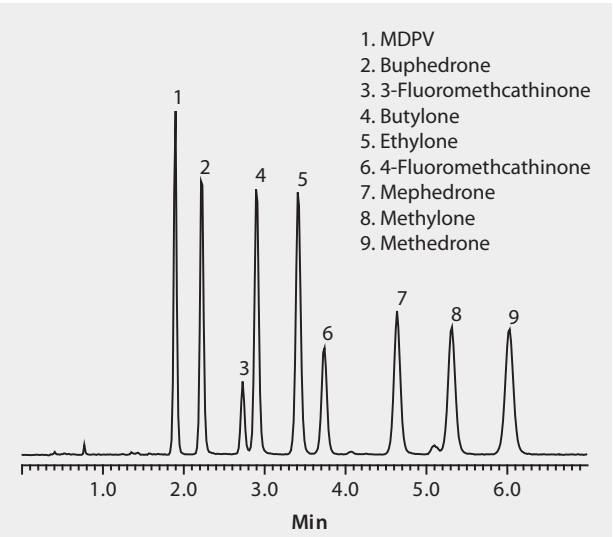
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . (A) 5 mM ammonium formate in (B) 75:25 (v/v) methanol:water
flow rate . . . . . 0.4 mL/min
detector . . . . . UV, 265 nm (MS, ESI(+), m/z 100-1000)
column temp. . . . . 40 °C
injection . . . . . 1 µL
sample . . . . . 20 µg/mL in methanol
Application No. . . . . G005858



## LC-MS Analysis of Illicit Bath Salts in Urine on Ascentis® Express HILIC after Solid Phase Extraction (SPE) on Supel™-Select SCX

The designer drugs known as "bath salts" are synthetic stimulants and illegal in most states as of 2011. This application demonstrates the analysis of bath salts extracted from human urine samples using polymeric solid phase extraction (SPE) sample preparation followed by hydrophilic interaction liquid chromatography (HILIC) analysis with TOF-MS detection. HILIC conditions on the Ascentis Express HILIC (Si) phase are used for fast, high-resolution separation of nine synthetic bath salts. Recoveries greater than 65% were observed for all analytes except MDPV (43.7%). The Figure illustrates the detection of bath salts in the spiked urine sample after SPE sample cleanup. Notice there are no interfering peaks in the chromatogram, demonstrating the effectiveness of the SPE sample cleanup.

sample/matrix . . Urine samples were spiked to a level of 100 ng/mL with each target analyte. (To ensure full ionization of the analytes, spiked samples were treated with formic acid to a final concentration of 0.1% formic acid.)
SPE tube/cartridge . . . . . Supel-Select SCX, 30 mg/1 mL (54240-U)
condition . . . . . 1 mL 1% formic acid acetonitrile then 1 mL water
sample addition . . . . . 1 mL spiked water blank or urine
washing . . . . . 1 mL water, 1 mL 1% formic acid acetonitrile, 1 mL water
elution . . . . . 2 mL 10% ammonium hydroxide in acetonitrile
eluate post-treatment . . thoroughly mix via vortex agitation, evaporate 1 mL aliquot to dryness, reconstitute in 100 µL water:methanol
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 5 mM ammonium formate acetonitrile; (B) 5 mM ammonium formate water; (98:2, A:B); premixed
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1842 psi (127 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+), TIC, m/z 100-1000
injection . . . . . 1 µL
sample . . . . . 200 µg/L ea. in acetonitrile
Application No. . . . . G005796

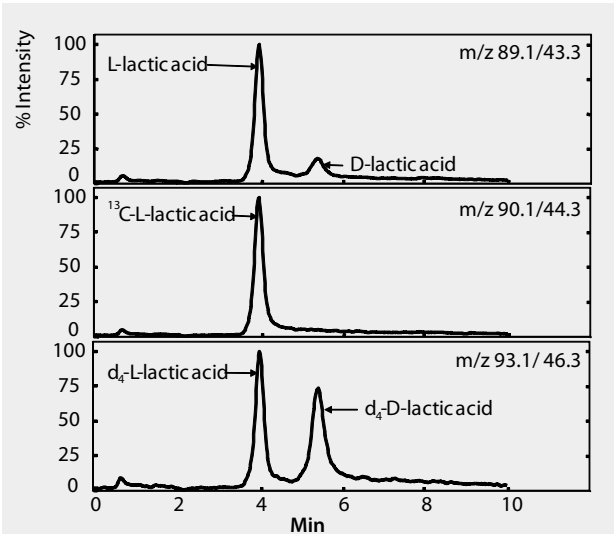


## LC-MS Analysis of Lactic Acid Enantiomers in Urine on Astec® CHIROBIOTIC® R

Chromatogram supplied courtesy of H. Henry and associates at the Clinical Chemistry Laboratory, Centre Hospitalier Universitaire Vaudois, University of Lausanne, CH-1011, Switzerland. View the related full-length contributed article in Reporter 30.1.

Reference: Biomed. Chromatogr. 2011 "Sensitive determination of D-lactic acid and L-lactic acid in urine by high-performance liquid chromatography–tandem mass spectrometry" H. Henry, N. Marmy Conus, P. Steenhout, A. Béguin and O. Boulat, Published online in Wiley Online Library, wileyonlinelibrary.com/journal/bmc.

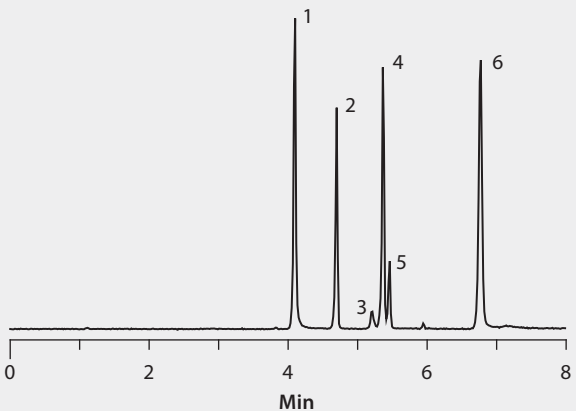
column . . Astec CHIROBIOTIC R, 15 cm x 2.1 mm I.D., 5 µm particles (13019AST)
mobile phase . . . . (A) 33.3 mM ammonium acetate; (B) acetonitrile; (15:85, A:B)
flow rate . . . . . 0.7 mL/min
column temp. . . . . 4 °C
detector . . . . . (-)ESI
injection . . . . . 20 µL
sample . . . . . Urine extracts in 5% (v/v) acetic acid, 10% (v/v) $H_2O$ and 85% (v/v) acetonitrile
Application No. . . . . G005482



## LC-MS Analysis of Nicotine and Metabolites on Kromasil® Eternity™ C18 (Analyte Set 1)

column . . Kromasil Eternity C18, 10 cm x 3.0 mm I.D., 2.5 µm particles (K0897111)
mobile phase . . . . . (A) 10 mM ammonium acetate in water:methanol (95:5);
(B) 10 mM ammonium acetate in water:methanol (10:90)
gradient . . . 0% B held for 1 min; to 100% B in 8.5 min;100% B held for 1 min
flow rate . . . . . 0.4 mL/min
pressure . . . . . 332 psi (23 bar)
column temp. . . . . 45 °C
detector . . . . . MS, ESI(+) TOF, EIC
injection . . . . . 5 µL
sample . . . . . 100 ng/mL in mobile phase A
Application No. . . . . G005924

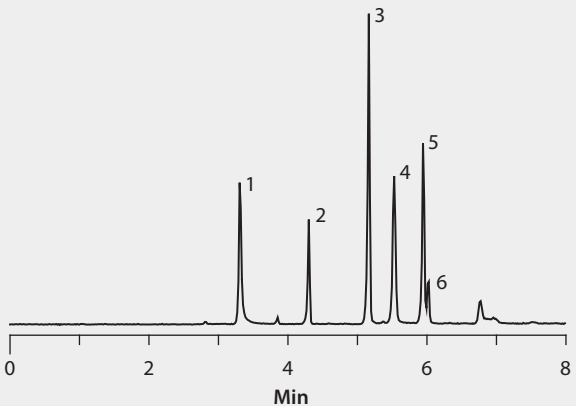
1. Anabasine
2. Norcotinine
3. Nicotine
4. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) isomer 1
5. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) isomer 2
6. (*S*)-N-Nitrosoanabasine (NAB)



## LC-MS Analysis of Nicotine and Metabolites on Kromasil® Eternity™ C18 (Analyte Set 2)

column . . Kromasil Eternity C18, 10 cm x 3.0 mm I.D., 2.5 µm particles (K08971111)
mobile phase . . . . . (A) 10 mM ammonium acetate in water:methanol (95:5); (B) 10 mM ammonium acetate in water:methanol (10:90)
gradient . . . 0% B held for 1 min; to 100% B in 8.5 min;100% B held for 1 min
flow rate . . . . . 0.4 mL/min
pressure . . . . . 332 psi (23 bar)
column temp. . . . . 45 °C
detector . . . . . MS, ESI(+) TOF, EIC
injection . . . . . 5 µL
sample . . . . . 100 ng/mL in mobile phase A
Application No. . . . . G005925

1. Nornicotine
2. *trans*-3'-Hydroxycotinine
3. Cotinine
4. (±)-*N*'-Nitrosonornicotine (NNN)
5. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) isomer 1
6. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) isomer 2

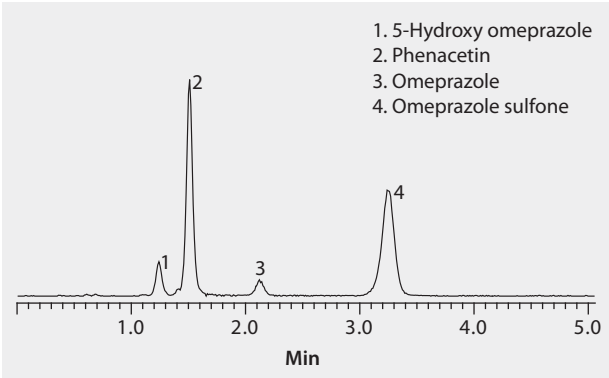


## LC-MS Analysis of Omeprazole and Metabolites in Plasma on Ascentis® Express RP Amide after Sample Prep using HybridSPE®-Phospholipid

This application demonstrates the suitability of HybridSPE and the Ascentis Express RP Amide for the analysis of omeprazole metabolites.

Sample was vorlexed and centrifuged for 2 minutes at 15000 rpm. Supemate was then passed through HybridSPE 96 well plate. Elutent was collected, evaporated and reconstituted in mobile phase.

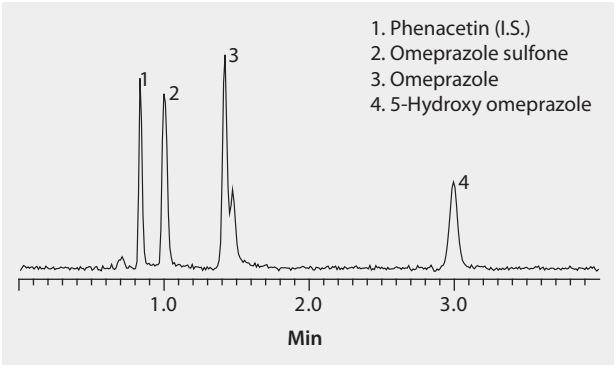
sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . rat plasma spiked with omeprazole, phenacetin and metabolites at 2 µg/mL
SPE well plate . . HybridSPE-Phospholipid, 96-well plate, 15 mg/0.8 mL (575656-U)
sample addition . . . . . 100 µL plasma to each well, followed by 300 mL of 1% formic acid in acetonitrile
elution . . . . . apply vacuum
eluate post-treatment . . . . . eluate was collected, evaporated, and reconstituted in mobile phase.
column . . Ascentis Express RP-Amide, 5 cm x 2.1 mm I.D., 2.7 µm particles (53911-U)
mobile phase . . . . . (A) water, 10 mM ammonium formate, pH 3.4; (B) methanol, 10 mM ammonium formate, pH 3.4; (65:35, A:B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), m/z = 50-400
injection . . . . . 1 µL
Application No. . . . . G005711



## LC-MS Analysis of Omeprazole and Metabolites on Ascentis® Express HILIC

This application demonstrates the suitability of the Ascentis Express HILIC for the analysis of omeprazole metabolites.

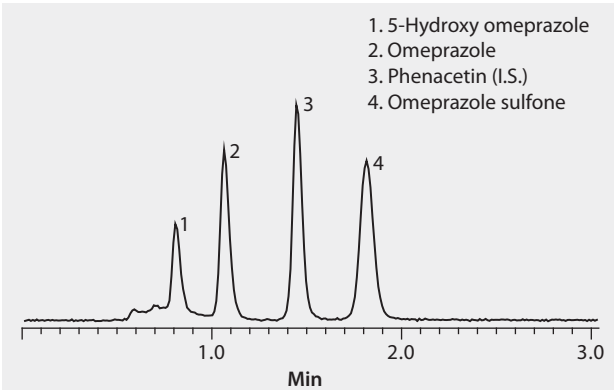
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 5 mM ammonium formate in water, pH 6.5;
(B) 5 mM ammonium formate in acetonitrile, pH 6.5; (2:98, A:B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), m/z 50-400
injection . . . . . 0.1 µL
sample . . . . . 1 mg/L each in mobile phase
Application No. . . . . G005710



## LC-MS Analysis of Omeprazole and Metabolites on Ascentis® Express RP Amide (pH 2.7)

This application demonstrates the suitability of the Ascentis Express RP Amide for the analysis of omeprazole metabolites.

column . . Ascentis Express RP-Amide, 5 cm x 2.1 mm I.D., 2.7 µm particles (53911-U)
mobile phase . . . . . (A) 10 mM ammonium formate in water, pH 2.7;
(B) 10 mM ammonium formate in methanol, pH 2.7; (65:35, A:B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), m/z 50-400
injection . . . . . 1 µL
sample . . . . . 1 mg/L in mobile phase
Application No. . . . . G005713



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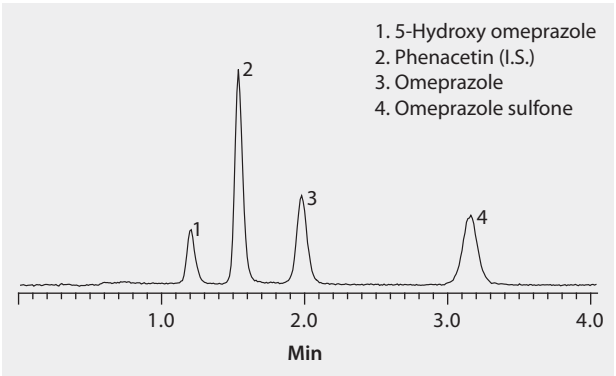
## LC-MS Analysis of Omeprazole and Metabolites on Ascentis® Express RP Amide (pH 3.4)

This application demonstrates the suitability of the Ascentis Express RP Amide for the analysis of omeprazole metabolites.

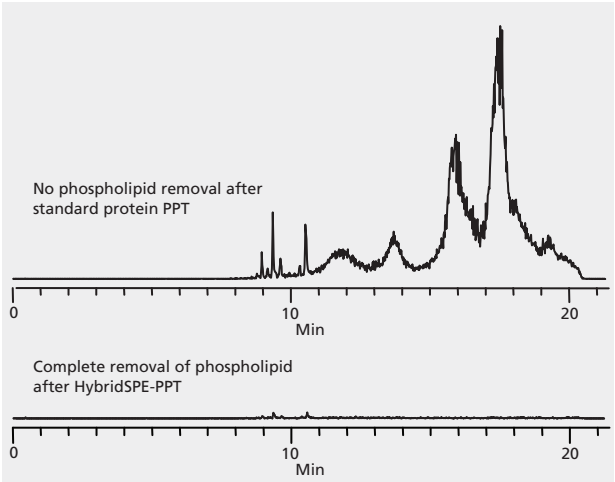
column . . Ascentis Express RP-Amide, 5 cm x 2.1 mm I.D., 2.7 µm particles (53911-U)
mobile phase . . . . . (A) 10 mM ammonium formate in water, pH 3.4;
(B) 10 mM ammonium formate in methanol, pH 3.4; (65:35, A:B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), m/z 50-400
injection . . . . . 1 µL
sample . . . . . 1 mg/L in mobile phase
Application No. . . . . G005712



## LC-MS Analysis of Plasma Samples on Ascentis® Express C18 Showing Baseline Improvements after Phospholipid Removal using HybridSPE®-Phospholipid

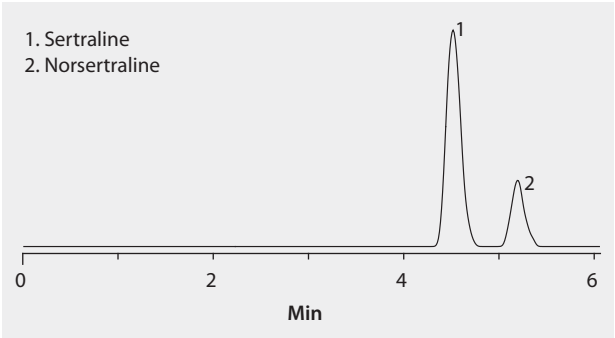
HybridSPE-Precipitation:
**Precipitate** proteins by first adding 100 µL rat plasma to the HybridSPE-PPT plate followed by 300 µL 1% formic acid in acetonitrile. Do not apply vacuum.
**Mix** by vortexing the HybridSPE-PPT plate briefly.
**Apply vacuum** using a 96-well vacuum manifold and collect the resulting eluate for analysis.
Standard Protein Precipitation:
**Combine** 100 µL rat plasma with 300 µL 1% formic acid in acetonitrile.
**Mix** by vortexing and centrifuge.
**Isolate** resulting supernatant for analysis.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . rat plasma
SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample addition . . . . . 100 µL rat plasma followed by 300 µL 1% formic acid in acetonitrile
elution . . . . . apply vacuum
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 10 mM ammonium acetate; (B) 10 mM ammonium acetate in acetonitrile
gradient . . . . . 0 min - 95% (A); 10 min - 50% (A); 18 min - 50% (A); 18.1 min - 95% (A); 22 min - 95% (A)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 35 °C
detector . . . . . ABI 3200QT; ESI (+), MRM (184/103 m/z)
injection . . . . . 5 µL
Application No. . . . . G004243



## LC-MS Analysis of Sertraline and Norsertraline on Ascentis® Express RP-Amide

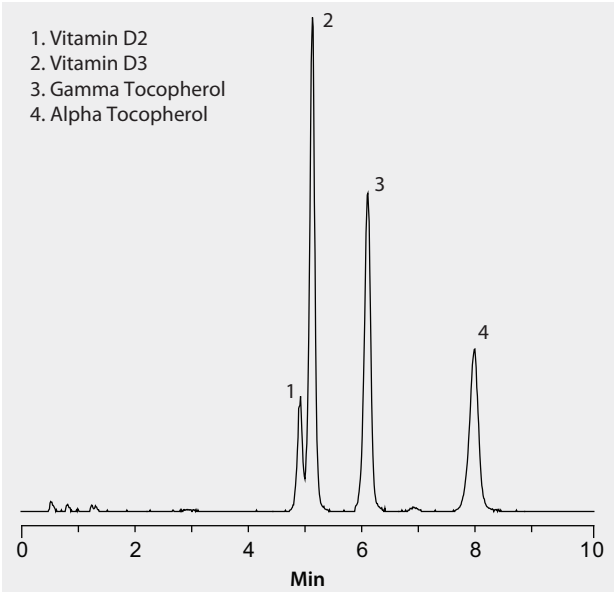
column . . . . . Ascentis Express RP Amide, 10 cm x 3.0 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . . . . (A) 10 mM ammonium formate, water, pH 3.6; (B) 10 mM ammonium formate, acetonitrile, pH 3.6; (65:35, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 55 °C
detector . . . . . ESI(+), 100-1000 m/z
injection . . . . . 2 µL
sample . . . . . 200 ng/mL in 25:75, water: acetonitrile
Application No. . . . . G005736



## LC-MS Analysis of Vitamin D2, D3, Gamma- and Alpha-Tocopherol on Ascentis® Express C18

Analysis was conducted using standard solution, detection on LC/MS system.

column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (5:95, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 40 °C
detector . . . . . MS, ESI+
injection . . . . . 2 µL
sample . . . . . 1 µg/mL in acetonitrile
Application No. . . . . G005806



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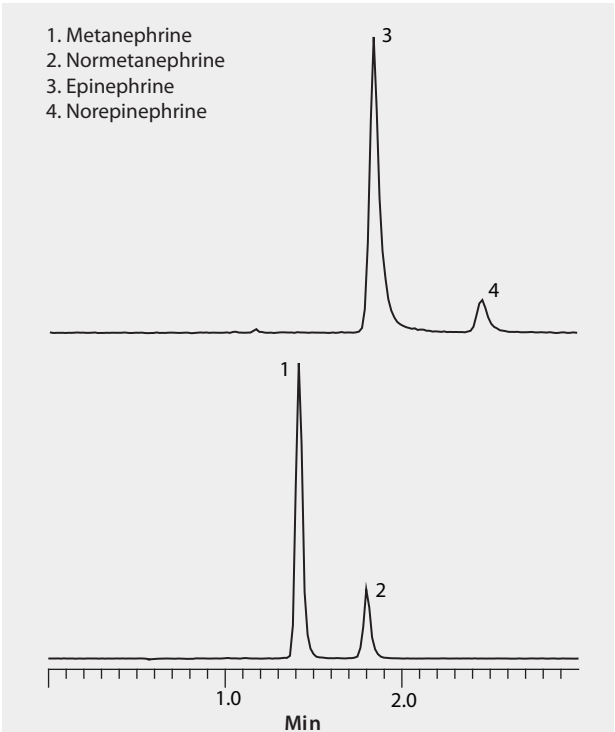
## LC-MS/MS Analysis of Epinephrine, Metanephrine and Metabolites on Ascentis® Express OH5

HILIC mode on Ascentis Express OH5 provided rapid separation of neurotransmitters in highly MS-compatible mobile phases.

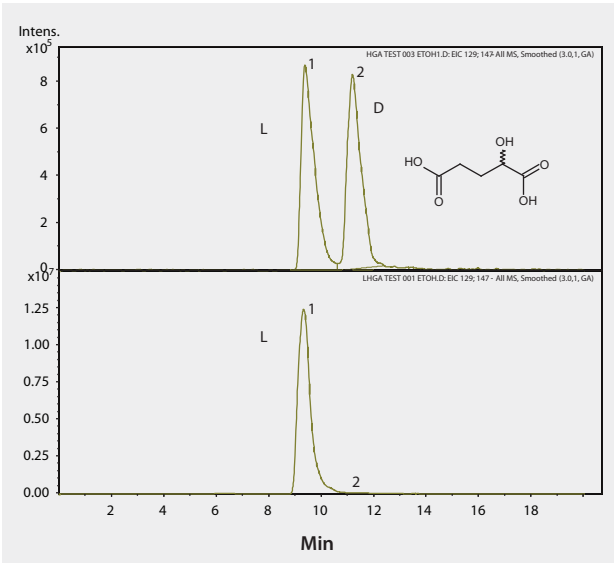
column . . Ascentis Express OH5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53757-U)
mobile phase . . . . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile; (15:85, A:B)
flow rate . . . . . 0.3 mL/min
pressure . . . . . 1189 psi (82 bar)
column temp. . . . . 35 °C
detector . . . . . MS ESI(+), 100-1000 m/z
injection . . . . . 1 µL
sample . . . . . 300 ng/mL each in acetonitrile
Application No. . . . . G005879



## LC-MS/MS Analysis of 2-Hydroxyglutaric Acid Enantiomers on Astec® CHIROBIOTIC® R

Chiral 2-hydroxyglutarates (2-OHG) are important molecular signatures of both healthy and diseased biological cells, their specific biochemical pathways and inborn errors of metabolism. The chiral differentiation and quantification of D-2-OHG (R(-)-2-OHG) and L-2-OHG (S(+)-2-OHG) is key for characterizing neurometabolic disorders like the 2-hydroxyglutaric acidurias that cause neurological impairment early in life. The ability to distinguish and quantify the enantiomers of 2-OHG is therefore important to be able to track its disposition throughout the metabolic pathway of normal vs. mutated cells. We show here a rapid and sensitive method to separate and measure D-2-OHG and L-2-OHG using LC-MS/MS detection. An Astec® CHIROBIOTIC R (ristocetin chiral selector) column was run under polar ionic mobile phase conditions. Using tandem MS eliminated two fundamental analytical challenges with these compounds: the lack of a UV-absorbing chromophore in the 2-OHG molecule, and the abundance of competing low molecular weight acids in urine. The upper panel in the chromatogram shows the separation of D-2-OHG and L-2-OHG obtained during method development. The lower panel shows the pure L-2-hydroxyglutaric acid. Besides providing the necessary enantioselectivity, Astec® CHIROBIOTIC columns have the advantage of operating in aqueous and polar organic mobile phases that are amenable to polar drugs and metabolites. Their ionic interactions promote analyte ionization which enhances sensitivity in ESI detection. In addition to the CHIROBIOTIC columns, Sigma-Aldrich also provides the high purity mobile phase agents, racemic and pure chiral 2-OHG enantiomers and other compounds in the isocitrate dehydrogenase (IDH) pathway.

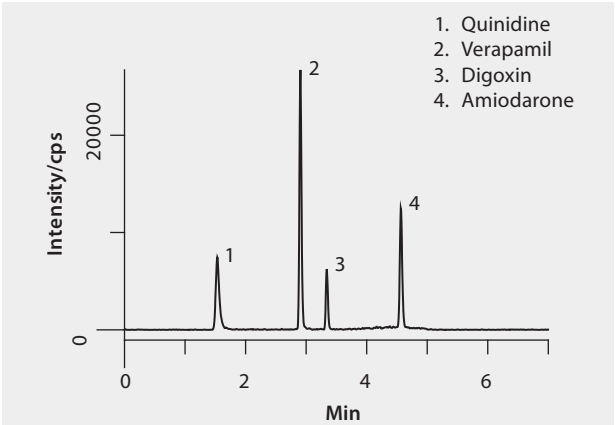
column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . (A) ethanol:methanol (3:1, v:v); (B) 0.1% v/v TEA in water, adjusted to pH 4.5 with acetic acid; 75:25 (A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 20 °C
detector . . . . . MS, ESI(-), 100-1000 m/z
injection . . . . . 5 µL
sample . . . . . each enantiomer, 100 µg/mL in water
Application No. . . . . G005848



## LC-MS/MS Analysis of Interacting Cardiac Drugs Digoxin, Quinidine, Amiodarone and Verapamil on Titan™ C18

**Sample pretreatment**: Sample spiked into rat plasma
**Detector:**
MRM transitions:
Peak MRM
1. Quinidine 325.2/81.1, 307.1
2. Verapamil 455.3/165.1
3. Digoxin 798.5/651.5
4. Amiodarone 646.1/58.1, 100.1

| | |
|---|---|
| sample/matrix | Sample spiked into rat plasma |
| SPE well plate | HybridSPE®-PLus 96-Well Plate (575659-U) |
| condition | 100 uL of spiked rat plasma was added to the HybridSPE plate well, followed by 300 uL of protein precipitation solvent, acetonitrile with 1% formic acid. (The plate, sealed, secured on a vibrater, agitated by vibration at 1000 rpm for 2 min. The plate was transfered to a vacuum manifold. Vacuum was applied at 10 in Hg for 4 min.) |
| column | Titan C18, 5 cm x 2.1 mm I.D., 1.9 µm particles (577122-U) |
| mobile phase | (A) Water; (B) Methanol, both with 0.1% formic acid/10 mM ammonium formate |
| gradient | 0-1 min, 45%B; 1.5-4 min 90%B, 4.5-8 min 45%B |
| flow rate | 0.2 mL/min |
| pressure | 2900 psi |
| column temp. | 35 °C |
| detector | MS, ESI(+), MRM mode |
| injection | 1.0 µL |
| Application No. | G006304 |



## LC-MS/MS Analysis of Steroid Hormones in Plasma on Ascentis® Express C18 after Sample Prep using HybridSPE®-Phospholipid

Immunoassay approaches for steroid determination are typically hindered due to the lack of specificity of antibodies for the measurement of the particular steroids. With the wide acceptance of LC-MS/MS in the clinical setting, there is a growing trend toward converting traditional IA techniques toward more specific and robust LC-MS/MS approaches. Though LC-MS/MS improves assay specificity and allows of multiplexed analyte assays to be conducted simultaneously, it is not without limitations specifically toward interferences form endogenous sample matrix. Often LC-MS/MS assays can be hindered due to ionization effects due to endogenous matrix from biological samples; this can result in random and arbitrary discrimination in analyte response. The use of HybridSPE-Phospholipid prior to LC-MS/MS analysis removes the endogenous phospholipids and precipitated proteins in one step, enabling high analyte recovery, negligable matrix background and long column lifetime.

SPE tube/cartridge . . . . . HybridSPE®-Phospholipid, 96-well plate, 50 mg/well (575656-U)

sample addition . . . . To each well add 100 µL of plasma followed by 300 µL of precipitation solvent (1% formic acid or 0.5% citric acid in acetonitrile)0. (Agitate via vortex for 4 minute, place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.)

column . . Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)

mobile phase . . (A) 5 mM ammonium formate pH 4.0 with formic acid; (B) methanol

gradient . . 60% B for 3 min, 60% B to 95% B in 5 min, held at 95% B for 2 min

flow rate . . . . . 0.3 mL/min

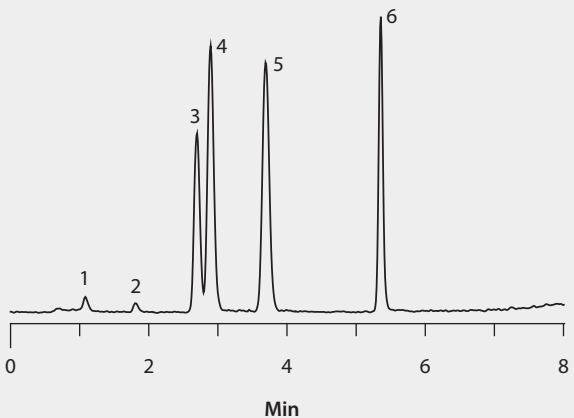
column temp. . . . . 50 °C

detector . . . . . ESI+, MRM

injection . . . . . 2 µL

Application No. . . . . G005895

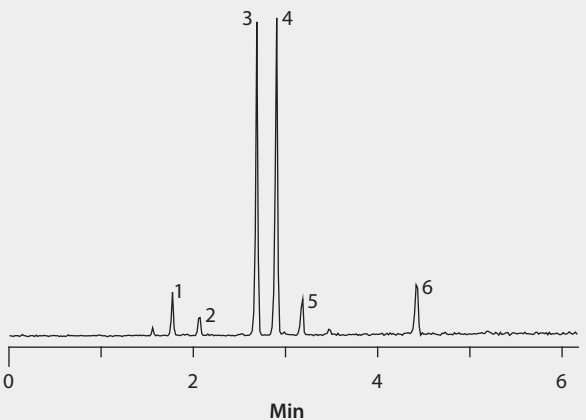
| | MRM Transition |
|---|---|
| 1. Aldosterone | 361.0/343.1 |
| 2. Corticosterone | 347.6/109.0 |
| 3. 11-Deoxycorticosterone | 331.1/109.0 |
| 4. Testosterone | 289.0/109.0 |
| 5. 17α-Methyltestosterone | 303.1/97.0 |
| 6. Progesterone | 315.0/109.1 |



## LC-MS/MS Analysis of Testosterone and Metabolites on Ascentis® Express C18

column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 5 mM ammonium formate water pH 4.0 formic acid; (B) 0.1% formic acid acetonitrile
gradient . . . . . 10 to 70% B in 5 min; held at 70% B for 3 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 3698 psi (255 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+), TIC, m/z 100-1000
injection . . . . . 5 µL
sample . . . . . 1000 ng/mL in 40:60 (water:methanol)
Application No. . . . . G005882

1. Testosterone glucuronide
2. 5α-Dihydrotestosterone glucuronide
3. Testosterone
4. 17α-Methyltestosterone
5. 5α-Dihydrotestosterone
6. 6β-Hydroxytestosterone



## LC-MS/MS Analysis of Warfarin™ in Plasma on Ascentis® Express C18 after SPE using HybridSPE®-Phospholipid

Warfarin is a widely prescribed oral anticoagulant for treatment and prevention of thrombosis and thromboembolism. The pharmacological effect of warfarin is derived from its ability to inhibit the enzyme vitamin K epoxide reductase, thereby reducing circulating levels of vitamin K which is required in the clotting process. In spite of its popularity, there are some downsides to warfarin, including a relatively narrow therapeutic index, interactions with other clinically important drugs, side effects, genetic variation in warfarin metabolism, frequent migration of blood levels outside the therapeutic range, bleeding events, and variability in time to reach therapeutic levels. These, and the fact that warfarin is the second leading cause of drug-related emergency room visits make it a commonly analyzed compound in the clinical lab. The clinical implications and importance of therapeutic drug monitoring of warfarin necessitate reliable and sensitive analytical methods, like UHPLC-MS/MS, to detect and quantify warfarin and its enantiomers in serum.

sample/matrix . . . . . rabbit plasma, unfiltered K2-EDTA, spiked with warfarin at 100 ng/mL (3:1, plasma:1% formic acid in acetonitrile)

SPE tube/cartridge . . . . . HybridSPE®-Phospholipid, 96-well plate (575656-U)

sample addition . . . . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes

elution . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes

column . . Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)

mobile phase . . . . . (A) 5mM ammonium formate, pH 4.2 with formic acid; (B) 5mM ammonium formate in 95:5 acetonitrile:water, 50:50 (A:B)

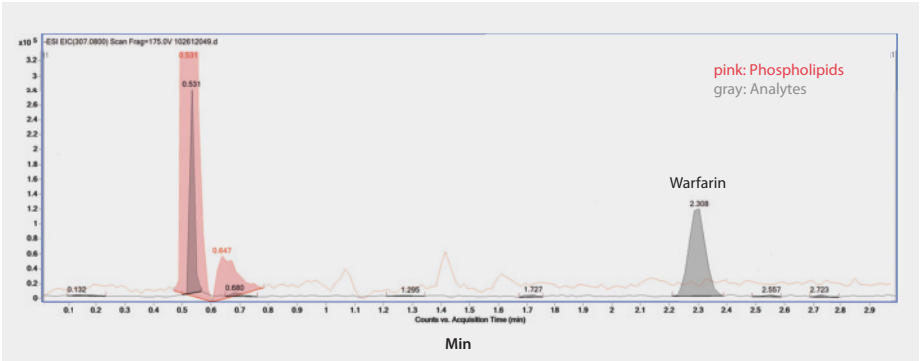
flow rate . . . . . 0.3 mL/min

column temp. . . . . 35 °C

detector . . . . . ESI+, 100-1000 m/z

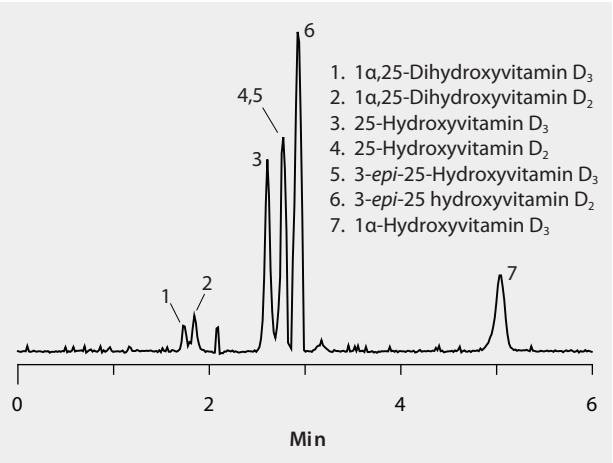
injection . . . . . 2 µL

Application No. . . . . G005850



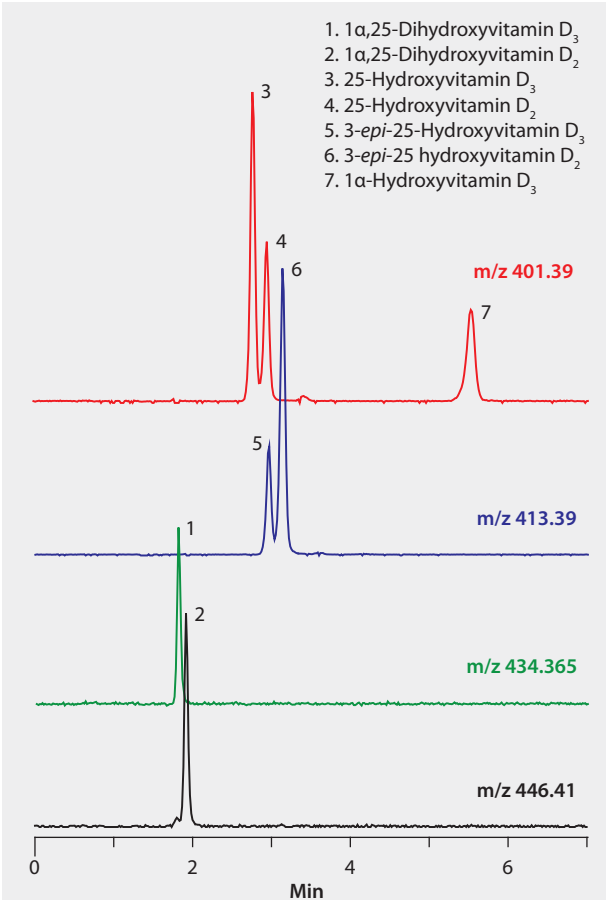
## LC-MS/MS Analysis of Vitamin D Metabolites on Ascentis® Express F5

column . . Ascentis Express F5, 15 cm x 2.1 mm I.D., 2.7 µm particles (53571-U)
mobile phase . . (A) 5 mM ammonium formate water; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 5178 psi (357 bar)
column temp. . . . . 40 °C
detector . . . . . ESI(+), 100-1000 m/z
injection . . . . . 2 µL, each compound 300 ng/mL in 40:60 (water:methanol)
Application No. . . . . G005878



## LC-MS/MS Analysis of Vitamin D Metabolites on Ascentis® Express F5 (Monitored Ions)

column . . Ascentis Express F5, 15 cm x 2.1 mm I.D., 2.7 µm particles (53571-U)
mobile phase . . (A) 5 mM ammonium formate water; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 5178 psi (357 bar)
column temp. . . . . 40 °C
detector . . . . . ESI(+), 100-1000 m/z
injection . . . . . 2 µL, each compound 300 ng/mL in 40:60 (water:methanol)
Application No. . . . . G005876



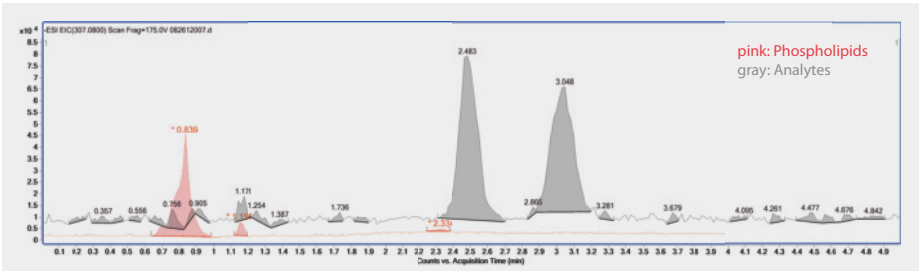



SUPELCO® Solutions within.™

## LC-MS/MS Analysis of Warfarin™ Enantiomers in Plasma on Astec® CHIROBIOTIC® V after SPE using HybridSPE®-Phospholipid

Warfarin is a widely prescribed oral anticoagulant for treatment and prevention of thrombosis and thromboembolism. The pharmacological effect of warfarin is derived from its ability to inhibit the enzyme vitamin K epoxide reductase, thereby reducing circulating levels of vitamin K which is required in the clotting process. In spite of its popularity, there are some downsides to warfarin, including a relatively narrow therapeutic index, interactions with other clinically important drugs, side effects, genetic variation in warfarin metabolism, frequent migration of blood levels outside the therapeutic range, bleeding events, and variability in time to reach therapeutic levels. These, and the fact that warfarin is the second leading cause of drug-related emergency room visits make it a commonly analyzed compound in the clinical lab. Warfarin is a chiral compound comprising an equal mixture of (R) and (S) enantiomers. (S)-Warfarin is considerably more potent and pharmacologically active than (R)-warfarin. Astec CHIROBIOTIC V columns resolve warfarin enantiomers under LC-MS conditions and hold up well to biological samples. The clinical implications and importance of therapeutic drug monitoring of warfarin necessitate reliable and sensitive analytical methods, like UHPLC-MS/MS, to detect and quantify warfarin and its enantiomers in serum.

sample/matrix . . . . . rabbit plasma, unfiltered K2-EDTA, spiked with warfarin at 100 ng/mL (3:1, plasma:1% formic acid in acetonitrile)
SPE tube/cartridge . . . . . HybridSPE-Phospholipid, 96-well plate (575656-U)
sample addition . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes
elution . . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes
column . . Astec CHIROBIOTIC® V, 10 cm x 4.6 mm I.D., 5 µm particles (11022AST)
mobile phase . . (A) 0.1% formic acid (pH unadjusted); (B) acetonitrile, 75:25 (A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI+, 100-1000 m/z
injection . . . . . 2 µL
Application No. . . . . G005849



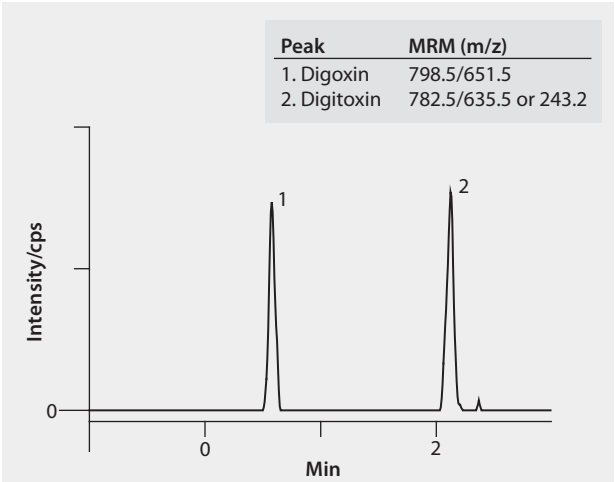



SUPELCO® Solutions within.™

## LC/MS/MS of Digoxin and Digitoxin in Serum or Plasma on Titan™ C18 after SPE with HybridSPE®-Phospholipid, Minimization of Matrix Effects

The cardiac glycosides digitoxin and digoxin are widely prescribed for treating congestive heart failure. Digitoxin differs from digoxin only in that digoxin possesses an additional OH group. In fact, digoxin is one of the OH-metabolites of digitoxin. Therefore, there is a need for simultaneous and sensitive determination of digitoxin and digoxin in biological fluids using LC-MS. This chromatogram shows the compounds as standards at very low level of 0.05 ng/mL.

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . 10 mM ammonium formate in methanol:water (80:20)
flow rate . . . . . 0.2 mL/min
pressure . . . . . 4550 psi (314 bar)
detector . . . . . ESI-MS/MS
detector . . . . . MRM Transitions: Peak MRM(m/z) (1.) Digoxin 798.5/651.5 (2.) Digitoxin 782.5/635.5 or 243.2
injection . . . . . 2 µL
sample . . . . . 0.05 ng/mL
Application No. . . . . G006306

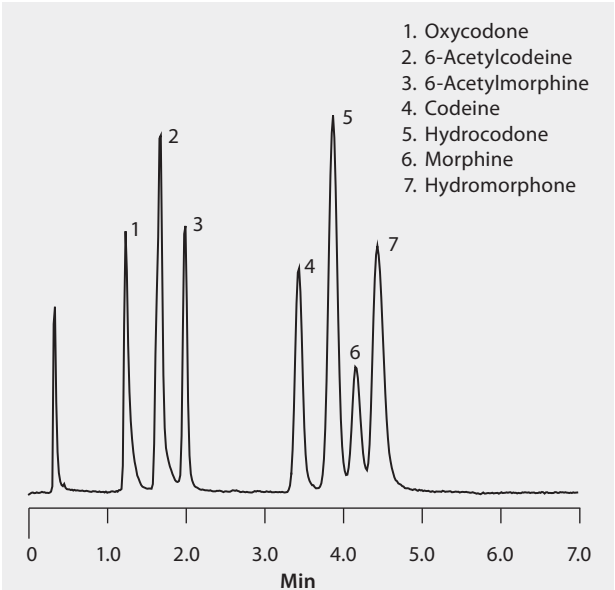





SUPELCO® Solutions within.™

## LC-MS (TOF) Analysis of Opioids and Opiate-Dependence Management Drugs on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 2 mM ammonium formate in 90:10 (v:v) acetonitrile:water
flow rate . . . . . 0.3 mL/min
column temp. . . . . 55 °C
detector . . . . . TOF/MS
injection . . . . . 2 µL
sample . . . . . 400 ng/mL in acetonitrile
Application No. . . . . G006153



## LC-MS (TOF) Analysis of Warfarin™ Enantiomers in Plasma on Astec® CHIROBIOTIC® V after SPE Using HybridSPE®-Phospholipid

This application demonstrates the benefit of using Sigma-Aldrich Analytical (Supelco, Fluka, and Cerilliant) consumables for rapid, accurate, sensitive and reliable MS detection in a clinical or bioanalytical setting.

Warfarin was originally developed as a rodenticide, but soon found medical application as an anticoagulant to prevent the formation of blood clots, especially after heart attacks. At the time of this writing, it is the most widely prescribed oral anticoagulant and is sold under Coumadin and various other brand names. Warfarin is a chiral molecule. The R- and S- forms are cleared by the body via different pathways and have different pharmacokinetic and pharmacodynamic effects. S-warfarin is more potent than the R-warfarin in producing the anticoagulant response.

The Astec® CHIROBIOTIC V column provided rapid resolution of the enantiomers under conditions that are compatible with mass spectrometric (MS) detection. Considering the rise of MS in the clinical laboratory, this is an important consideration. The CHIROBIOTIC family of chiral columns are based on the macrocyclic glycopeptide molecule and affect enantioresolution under mobile conditions that are compatible with reversed-phase and MS conditions. Their durability allows them to stand up to repeated injections of biological extracts.

By using HybridSPE-Phospholipid for the sample prep, ion-suppressing phospholipids were removed for improved sensitivity and longer column lifetime. LC-MS Ultra CHROMASOLV solvents were used to supply low background interference and particulate contaminants. Cerilliant reference material grade standards provided reliable quantitation.

system: Agilent 1290, 6210 TOF

sample/matrix . . . . . Rat plasma stabilized with K2EDTA was acquired from Lampire Biological Laboratories, (Pipersville PA). (Plasma was spiked directly from stock standard to a level of 100 ng/mL in 3:1 (plasma:1% formic acid acetonitrile).)

SPE tube/cartridge . . Pk1 50 mg/96-Well HybridSPE-Phospholipid Plate (575656-U)

condition . . Apply 100 µL of plasma to plate, followed by 300 µL of 1% formic acid acetonitrile. Agitate via vortex for 4 min.

elution . . . . . Place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

column . . . . . CHIROBIOTIC V,10 cm x 4.6 mm I.D., 5 µm particles (11022AST)

mobile phase . . . . . (A) 0.1% formic acid water; (B) acetonitrile, (75:25, A:B)

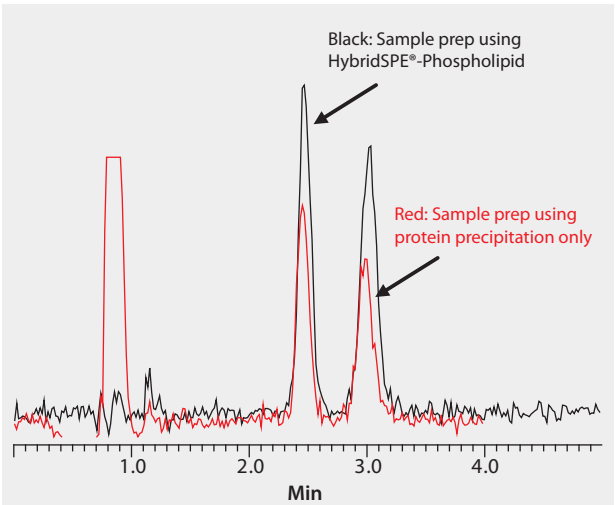
flow rate . . . . . 1 mL/min

column temp. . . . . 35 °C

detector . . . . . ESI+, 100-1000 m/z

injection . . . . . 2 µL

Application No. . . . . G005827



## LC-TOF Analysis of Antiarrhythmic Drugs and Metabolites in Plasma on Ascentis® Express HILIC after Sample Prep using HybridSPE®-Phospholipid

The basic characteristics of antiarrhythmic cardiac drugs and associated metabolites make them targets for HILIC chromatographic separation. Because HILIC mobile phases consist of a high composition of acetonitrile, this can also facilitate the direct analysis of precipitated plasma samples without the need for additional sample solvent exchange. In most cases, the high organic mobile phase also facilitates increased analyte response in ESI (+) MS detection. This application used HybridSPE-Phospholipid sample prep to remove phospholipids and precipitated proteins prior to LC-MS analysis on Ascentis Express HILIC. The resulting method was robust and sensitive.

sample/matrix . . . Rat plasma stabilized with K2EDTA was acquired from Lampire Biological Laboratories, (Pipersville PA). Plasma was spiked directly from stock standard to a level of 400 ng/mL

SPE tube/cartridge . . HybridSPE-Phospholipid 96-Well Plate, 50 mg/2 mL (575656-U)

sample addition . . Apply 100 µL of plasma to plate, followed by 300 µL of 1% formic acid acetonitrile. Agitate via vortex for 4 min

elution . . . . Place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)

mobile phase . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile; (5:95, A:B, pH 7.0 with formic acid) (LC-MS CHROMASOLV Ultra grade)

flow rate . . . . . 0.4 mL/min

pressure . . . . . 1305 psi (90 bar)

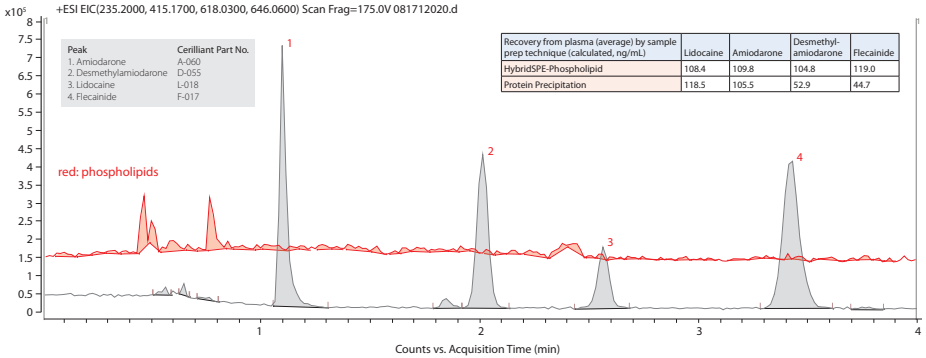
column temp. . . . . 35 °C

detector . . . . . ESI(+), full scan, m/z 100-1000

injection . . . . . 0.5 µL

sample . . . . . plasma extract, analyte concentration of final sample work up is equivalent to 100 ng/mL

Application No. . . . . G005818

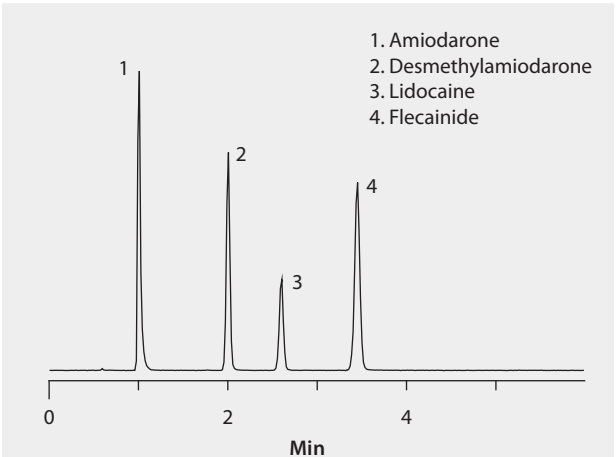



| Recovery from plasma (average) by sample prep technique (calculated, ng/mL) | Lidocaine | Amiodarone | Desmethyl-amiodarone | Flecainide |
|---|---|---|---|---|
| HybridSPE-Phospholipid | 108.4 | 109.8 | 104.8 | 119.0 |
| Protein Precipitation | 118.5 | 105.5 | 52.9 | 44.7 |

## LC-TOF Analysis of Antiarrhythmic Drugs and Metabolites on Ascentis® Express HILIC

The basic characteristics of these compounds make them targets for HILIC chromatographic separation. Because HILIC mobile phases consist of a high composition of acetonitrile, this can also facilitate the direct analysis of precipitated plasma samples without the need for additional sample solvent exchange. In most cases, the high organic mobile phase also facilitates increased analyte response in ESI+ MS detections.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile; (5:95, A:B, pH 7.0 with formic acid) (LC-MS CHROMASOLV Ultra grade)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), full scan, m/z 200-800
injection . . . . . 0.5 µL
sample . . each compound, 300 ng/mL in 1% formic acid acetonitrile:water, 75:25
Application No. . . . . G005817



## LC-TOF Analysis of Antiarrhythmic Drugs and Metabolites on Ascentis® Express HILIC: Improvement in Analyte Response after Sample Prep using HybridSPE®-Phospholipid

The basic characteristics of antiarrhythmic cardiac drugs and associated metabolites make them targets for HILIC chromatographic separation. Because HILIC mobile phases consist of a high composition of acetonitrile, this can also facilitate the direct analysis of precipitated plasma samples without the need for additional sample solvent exchange. In most cases, the high organic mobile phase also facilitates increased analyte response in ESI (+) MS detection. This application used HybridSPE-Phospholipid sample prep to remove phospholipids and precipitated proteins prior to LC-MS analysis on Ascentis Express HILIC. These chromatograms compare the response with HybridSPE-Phospholipid vs. standard protein precipitation sample prep methods.

sample . . . . . Rat plasma stabilized with K2EDTA was acquired from Lampire Biological Laboratories, (Pipersville PA). Plasma was spiked directly from stock standard to a level of 400 ng/mL

SPE tube/cartridge . . HybridSPE-Phospholipid 96-Well Plate, 50 mg/2 mL (575656-U)

sample addition . . Apply 100 µL of plasma to plate, followed by 300 µL of 1% formic acid acetonitrile. Agitate via vortex for 4 min.

elution . . . . Place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)

mobile phase . . . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile; (5:95, A:B, pH 7.0 with formic acid) (LC-MS CHROMASOLV Ultra grade)

flow rate . . . . . 0.4 mL/min

pressure . . . . . 1305 psi (90 bar)

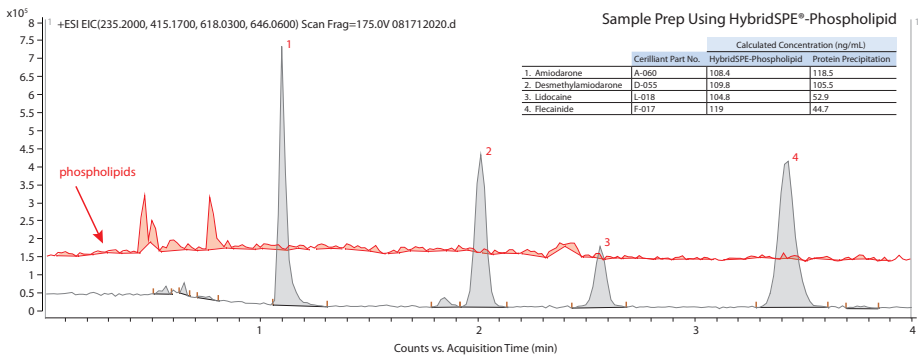
column temp. . . . . 35 °C

detector . . . . . ESI(+), full scan, m/z 100-1000

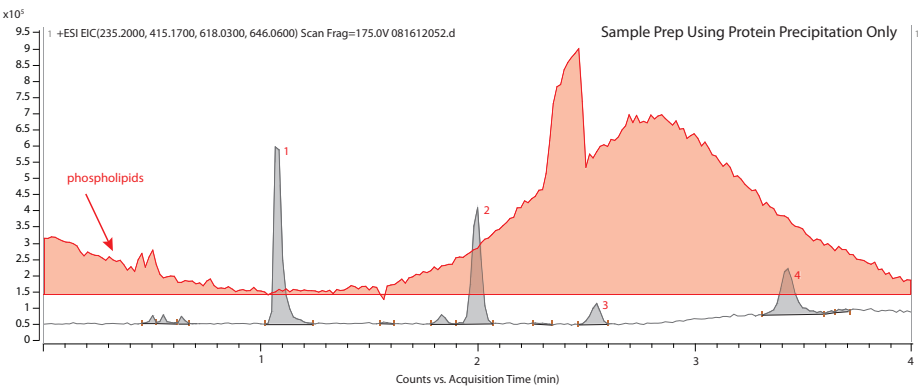
injection . . . . . 0.5 µL

sample . . . . . plasma extract, analyte concentration of final sample work up is equivalent to 100 ng/mL

Application No. . . . . G005819




| | Cerilliant Part No. | Calculated Concentration (ng/mL) HybridSPE-Phospholipid | Protein Precipitation |
|---|---|---|---|
| 1. Amiodarone | A-060 | 108.4 | 118.5 |
| 2. Desmethylamiodarone | D-055 | 109.8 | 105.5 |
| 3. Lidocaine | L-018 | 104.8 | 52.9 |
| 4. Flecainide | F-017 | 119 | 44.7 |



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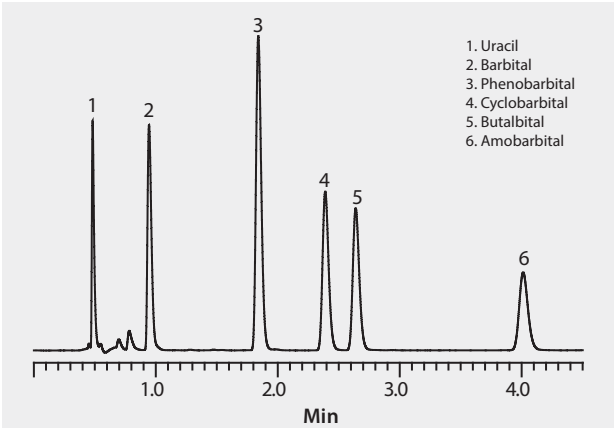
## UHPLC Analysis of Barbituates on Titan™ C18

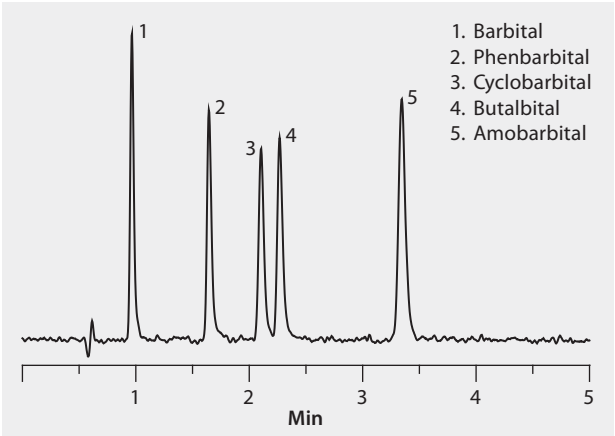
Analysis of a Barbituate Mix using a MS compatible mobile phase.

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% ammonium acetate (not adjusted); (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 7540 psi (520 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
Application No. . . . . G006073



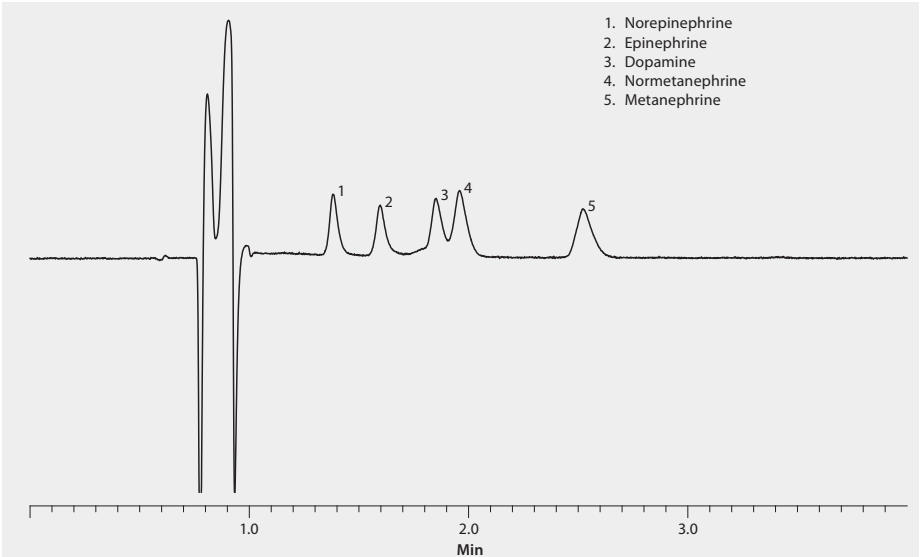
## UHPLC Analysis of Barbituates on Titan™ C18 with MS Detection

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% ammonium acetate (not adjusted); (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 6796 psi (468 bar)
column temp. . . . . 35 °C
detector . . . . . ESI (-) MS TIC MRM
injection . . . . . 1 µL
sample . . . . . 1000 ng/ml in mobile phase
Application No. . . . . G006137



## UHPLC Analysis of Catecholamines on Ascentis® Express F5

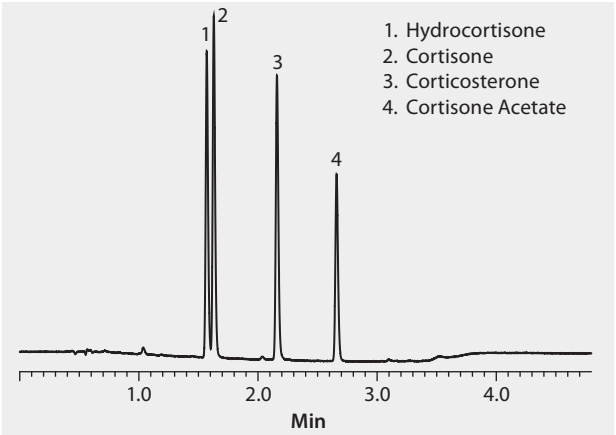
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . (A) water; (B) 100 mM ammonium acetate (pH 4.1 with acetic acid); (C) acetonitrile; (88:2:10, A:B:C)
flow rate . . . . . 0.3 mL/min
pressure . . . . . 1880 psi (130 bar)
column temp. . . . . 60 °C
detector . . . . . UV, 250 nm
injection . . . . . Catecholamines 25 µg/mL in 90:10, water:methanol
Application No. . . . . G006281



## UHPLC Analysis of Corticosteroids on Titan™ C18

Analysis of Corticosteroids on a Titan C18 UPLC column with MS detection in ESI (+) Mode

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (70:30, A:B)
gradient . . . . . 30% to 60% B in 3 minutes, hold for 0.2 min
flow rate . . . . . 0.4 mL/min
pressure . . . . . 7423 psi (512 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 1 µL
sample . . . . . 1000 ng/ml in mobile phase
detector . . . . . ESI (+) MS TIC SIR
Application No. . . . . G006147

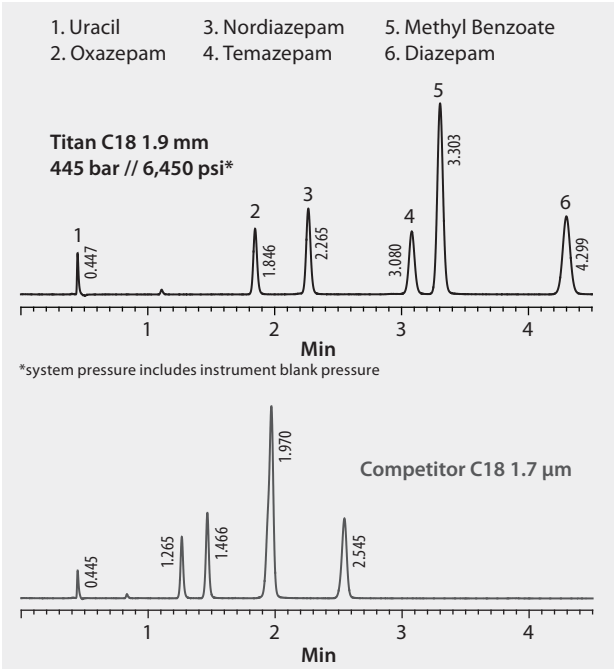


## UHPLC Analysis of Diazepam and Metabolites on Titan™ C18, Competitive Comparison

This application shows a comparison between Titan™ C18 and a competitive column for diazepam and its metabolites using a MS compatible mobile phase.

Used 75 µm I.D. x 250 mm L tubing in system.

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid; (B) acetonitrile; (60:40, A:B)
pressure . . . . . 6450 psi (445 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
Application No. . . . . G006001




*system pressure includes instrument blank pressure



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## UHPLC Analysis of Riboflavin and Impurities on Titan™ C18 with MS Detection

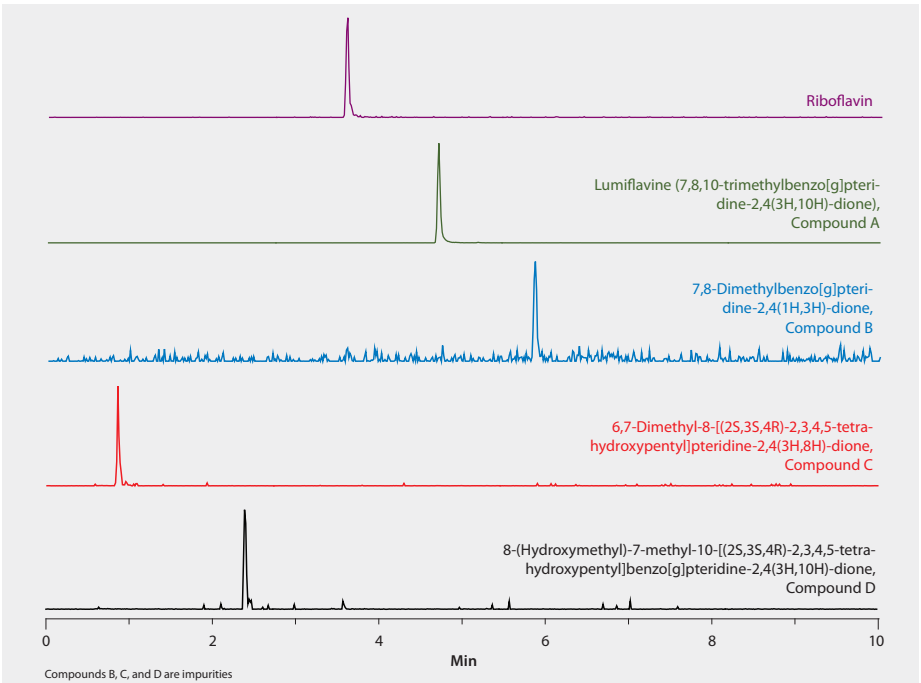
Riboflavin (vitamin B2) is a water-soluble vitamin. It is synthesized by all plants and many microorganisms, but it is not produced by higher animals. It is a precursor of coenzymes that are required for the enzymatic oxidation of carbohydrates, so it is essential to basic metabolism. Riboflavin is used as an additive to food and feed and is also used in fortification of baby food and cereal. The current USP method for impurity analysis is non-quantitative, and uses an ion pair reagent in the mobile phase. An alternative to USP and EP methods using LC-MS/MS to identify and quantify the riboflavin and impurities is presented. This new proposed method is also compatible with UV-Vis detection.

A riboflavin certified reference material (CRM) was used in this study. MRM transitions for the determination of ions for the parent and four impurites were made. Three transitions were used for each ion. Structures for each of these major impurities were confirmed by MS. In addition to improving the specificity of the method with MS/MS detection, two additional goals were to decrease analysis time and eliminate the need for ion pairing reagents that are not compatible with MS.

### Other MS Conditions

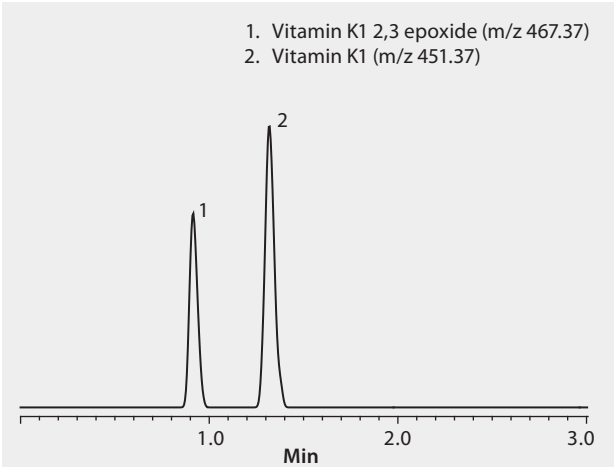
Capillary (kV) 2.29
Cone (V) 54.95
Extractor (V) 5.13
Source Temperature (°C) 151
Desolvation Temperature (°C) 349
Cone Gas Flow (L/Hr) 2
Desolvation Gas Flow (L/Hr) 646
Collision Gas Flow (mL/Min) ON

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile (95:5)
gradient . . . . . 5% to 25% in 6 min, return to 5% b in 0.1 min, and re-equilibrate for 4 min
flow rate . . . . . 500 µL/min
pressure . . . . . 5000 psi (345 bar)
column temp. . . . . 35 °C
detector . . . . . ESI (+), MRM and TIC
detector . . . . . Diode Array at 276 nm
injection . . . . . 2.0 µL
Application No. . . . . G006138



## UHPLC-MS Analysis of Vitamin K1 and Related Compound on Ascentis® Express 2.7 µm C18

column . . . Ascentis Express C18 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) methanol; (B) 2-propanol; (90:10, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1870 psi (129 bar)
column temp. . . . . 35 °C
detector . . . . . MS-APCI+, full scan 425-525 m/z
injection . . . . . 2.0 µL
sample . . . . . 50 ng/mL 90:10, methanol:2-propanol
Application No. . . . . G006309

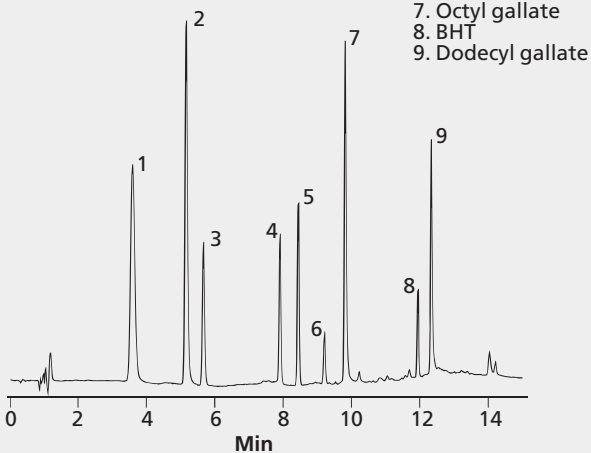


# Cosmetics, Personal Care, and Cleaning Products

## HPLC Analysis of Antioxidants on SUPELCOSIL™ LC-18

column . . . . SUPELCOSIL LC-18, 15 cm × 4.6 mm I.D., 5 µm particles (58230-U)
mobile phase . . (A) 5% acetic acid in deionized water; (B) acetonitrile:methanol (1:1)
70% A/30% B to 100% B, linear gradient over 10 min, hold 10min
flow rate . . . . . 2 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 10µL, 20 µg/mL each antioxidant
Application No. . . . . 795-0438

| Gradient Program | | |
|---|---|---|
| Time (min) | %A | %B |
| 0 | 70 | 30 |
| 10 | 0 | 100 |
| 20 | 70 | 30 |

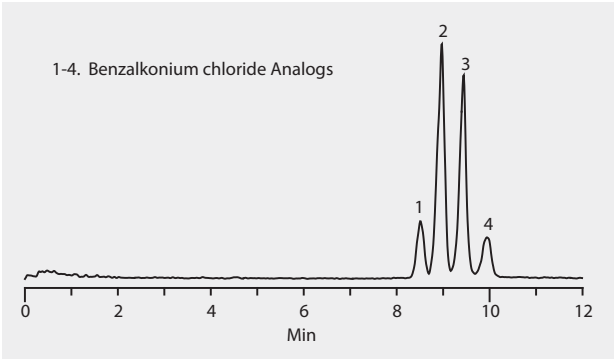





SUPELCO® Solutions within.™

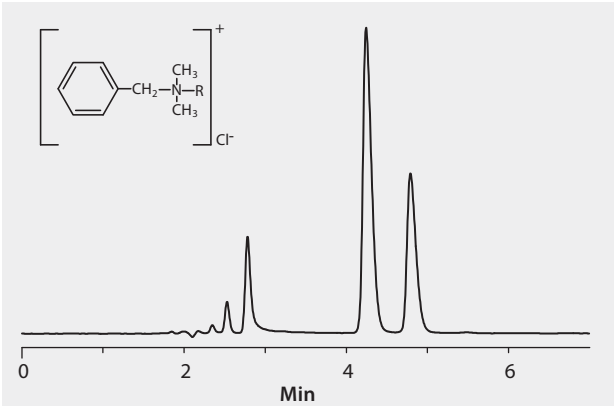
## HPLC Analysis of Benzalkonium Chloride in Commercial Disinfecting Wipes on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . . . . (A) 20 mM ammonium acetate; (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), TIC 150 - 500 m/z
injection . . . . . 2 µL
sample . . . . . Single wipe sheet, extracted with 10 mL methanol
Application No. . . . . G005414



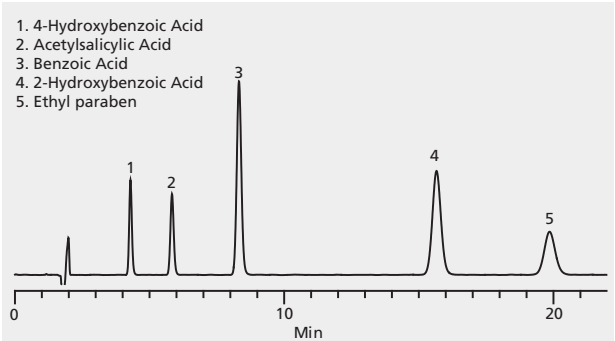
## HPLC Analysis of Benzalkonium Chlorides on Discovery® Cyano

column . . . . . Discovery Cyano, 15 cm × 4.6 mm I.D., 5 µm particles (59356-U)
mobile phase . . . . . (A) acetonitrile; (B) acetate buffer, pH 4.5, (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
sample . . . . . undefined C8 to C18 Benzalkonium Chlorides
Application No. . . . . G000726



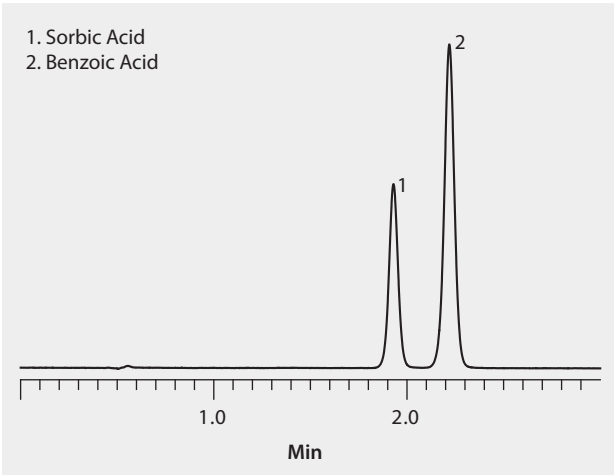
## HPLC Analysis of Benzoic Acid and Related Compounds on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm × 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) water with 0.1% TFA; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G004094



## HPLC Analysis of Benzoic Acid and Sorbic Acid on Ascentis® Express RP-Amide ("Green" Mobile Phase Conditions)

column . . Ascentis Express RP-Amide, 5 cm x 3 mm I.D., 2.7 µm particles (53916-U)
mobile phase . . . . . (A) 10 mM ammonium phosphate monobasic, pH 2.5 with phosphoric acid; (B) ethanol; (75:25, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 138 bar (2000 psi) column, 248 bar (3600 psi) total
column temp. . . . . 55 °C
detector . . . . . UV, 215 nm
injection . . . . . 1 µL
sample . . . . . 50 µg/mL in mobile phase
Application No. . . . . G005588



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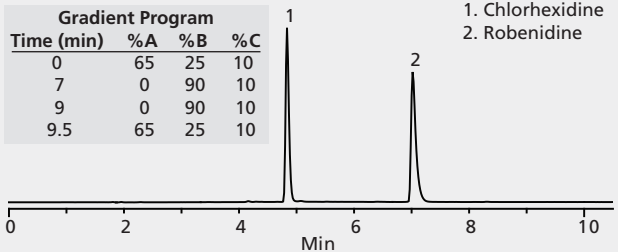
## HPLC Analysis of Chlorhexidine and Robenidine on Ascentis® Phenyl

column . . . . . Ascentis Phenyl 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (C) 500 mM ammonium formate, pH 3.0, with formic acid
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 273 nm
injection . . . . . 10 µL
sample . . 100 *u*g/mL each in 70:30, 25 mM ammonium formate pH 3.0:acetonitrile
Application No. . . . . G003711

**Gradient Program**

| Time (min) | %A | %B | %C |
|---|---|---|---|
| 0 | 65 | 25 | 10 |
| 7 | 0 | 90 | 10 |
| 9 | 0 | 90 | 10 |
| 9.5 | 65 | 25 | 10 |



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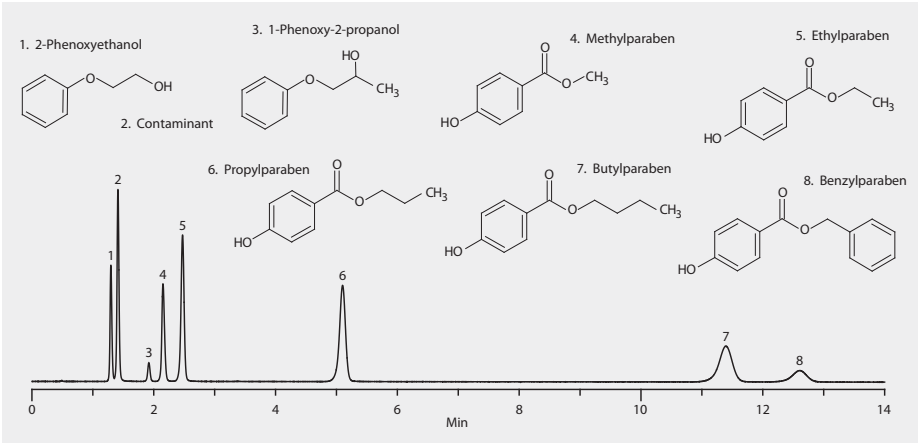
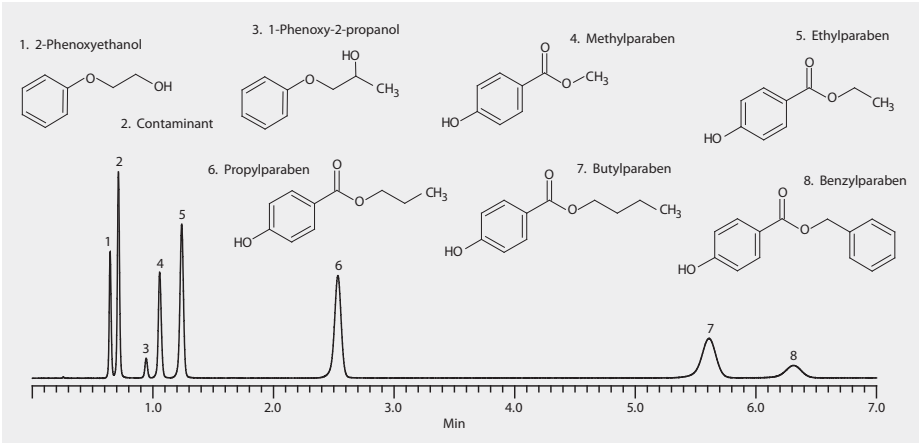
## HPLC Analysis of Cosmetic Preservatives on Ascentis® Express C18 (0.6 mL/min)

column . . Ascentis Express C18, 7.5 cm x 3 mm I.D., 2.7 µm particles (53812-U)
mobile phase . . . . . (A) water; (B) methanol; (55:45, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 250 nm
injection . . . . . 1 µL
sample . . . . . methyl-, benzylparaben, 50 mg/L; ethylparaben, 30 mg/L; propyl-, butylparaben, 100 mg/L; 2-phenoxyethanol, 1.5 g/L; 1-phenoxy-2-propanol, 3 g/L; all in 40% methanol
Application No. . . . . G005386



## HPLC Analysis of Cosmetic Preservatives on Ascentis® Express C18 (1.2 mL/min)

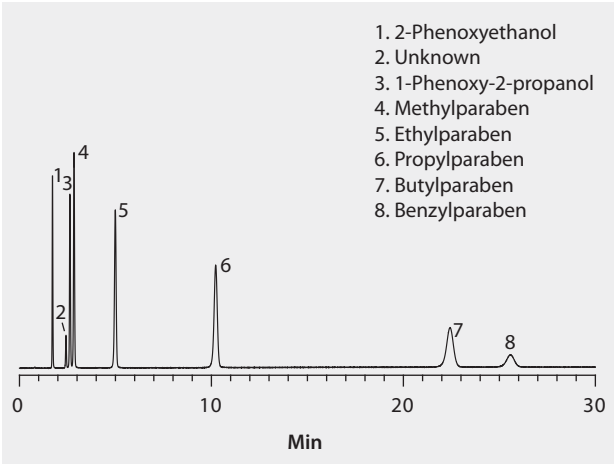
column . . Ascentis Express C18, 7.5 cm x 3 mm I.D., 2.7 µm particles (53812-U)
mobile phase . . . . . (A) water; (B) methanol; (55:45, A:B)
flow rate . . . . . 1.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 250 nm
injection . . . . . 1 µL
sample . . . . . methyl-, benzylparaben, 50 mg/L; ethylparaben, 30 mg/L; propyl-, butylparaben, 100 mg/L; 2-phenoxyethanol, 1.5 g/L; 1-phenoxy-2-propanol, 3 g/L; all in 40% methanol
Application No. . . . . G005385



## HPLC Analysis of Cosmetic Preservatives on Ascentis® Express RP-Amide

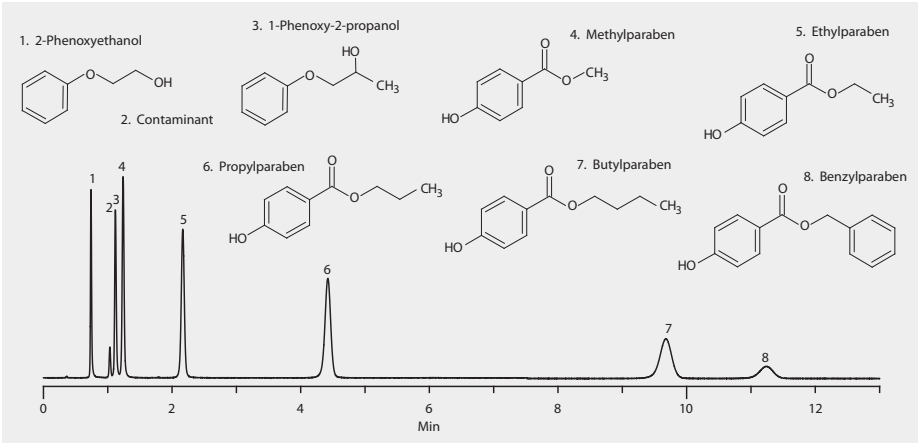
With the publication Borremans, M. et.al. 2004, Chromatographia 59: 47-53 as a reference, this method is 10% faster, but with superior resolution of critical pairs; in this case, the minimum USP resolution is 2.4.

column . . Ascentis Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . . . . (A) water; (B) methanol; (60:40, A:B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 2900 psi (200 bar)
column temp. . . . . 50 °C
detector . . . . . UV, 250 nm
injection . . . . . 1 µL
sample . . . . . methyl-, benzylparaben (50 mg/L), ethylparaben (30 mg/L), propyl-, butylparaben (100 mg/L), 2-phenoxyethanol (1.5 g/L), 1-phenoxy-2-propanol (3 g/L), all in 40% methanol
Application No. . . . . G005738



## HPLC Analysis of Cosmetic Preservatives on Ascentis® Express RP-Amide at Elevated Flow and Temperature (1.2 mL/min)

column . . Ascentis Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . . . . (A) water; (B) methanol; (60:40, A:B)
flow rate . . . . . 1.2 mL/min
column temp. . . . . 50 °C
detector . . . . . UV, 250 nm
injection . . . . . 1 µL
sample . . . . . methyl-, benzylparaben, 50 mg/L; ethylparaben, 30 mg/L; propyl-, butylparaben, 100 mg/L; 2-phenoxyethanol, 1.5 g/L; 1-phenoxy-2-propanol, 3 g/L; all in 40% methanol
Application No. . . . . G005384



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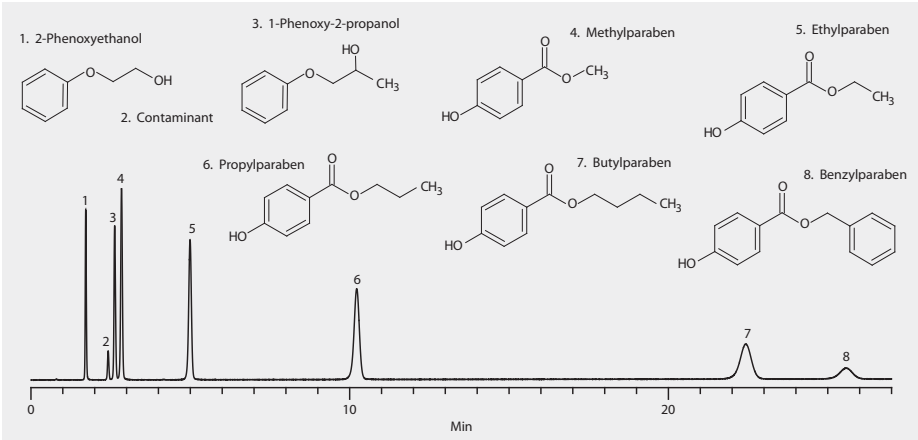
## HPLC Analysis of Cosmetic Preservatives on Ascentis® Express RP-Amide at Elevated Temperature (0.6 mL/min)

column . . Ascentis Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . . . . (A) water; (B) methanol; (60:40, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 50 °C
detector . . . . . UV, 250 nm
injection . . . . . 1 µL
sample . . . . . methyl-, benzylparaben, 50 mg/L; ethylparaben, 30 mg/L; propyl-, butylparaben, 100 mg/L; 2-phenoxyethanol, 1.5 g/L; 1-phenoxy-2-propanol, 3 g/L; all in 40% methanol
Application No. . . . . G005383



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## HPLC Analysis of Hydrocortisone in Topical Cream on Discovery® HS C18 after SPE using Discovery® DSC-Si

**Sample Pre-treatment:**

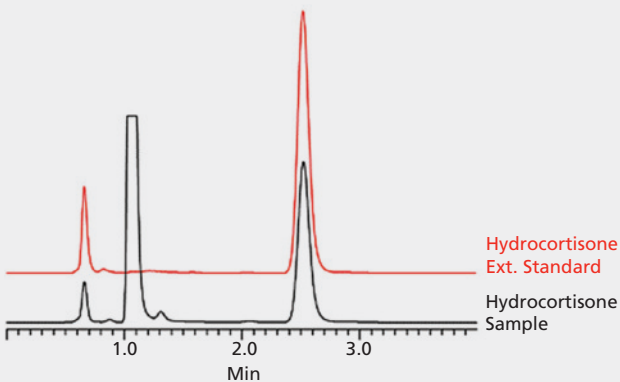
Dissove 1 g 1% hydrocortisone topical hand cream in 10 mL ethyl acetate. Dilute 2 mL of ethyl acetate-cream sample with 8 mL hexane:ethyl acetate (2:1, v/v).

sample preparation . . . . . SPE (Solid Phase Extraction)
sample addition . . . . . hydrocortisone topical hand cream in hexane:ethylacetate
SPE tube/cartridge . . . . . Discovery DSC-Si, 500 mg/3 mL (52695-U)
condition . . . . . 1 mL hexane:ethyl acetate (2:1, v/v)
sample addition . . . . . 1 mL
washing . . . . . 1 mL hexane:ethyl acetate (2:1, v/v)
elution . . . . . 1 mL methanol:DI water (1:1, v/v)
eluate post-treatment . . . dilute eluate to 2 mL with methanol:DI water (1:1, v/v)
column . . . . Discovery HS C18, 15 cm x 4.6 mm I.D., 5 µm particles (568520-U)
mobile phase . . . . . (A) methanol; (B) DI water (1:1, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G003765

**Efficiency of Recovery**

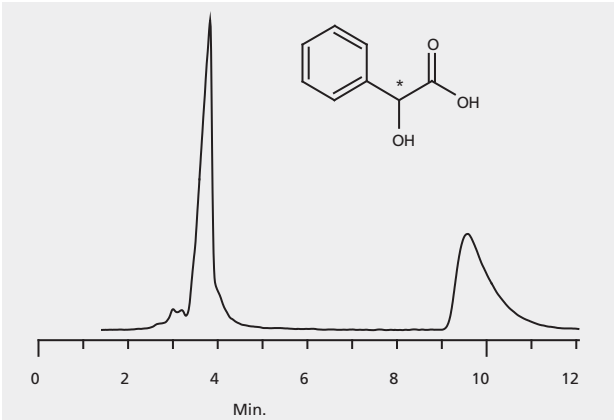
| Sample No. | Sample Weight (g) | Theoretical Hydrocortisone Weight (g)[1] | Actual Hydrocortisone weight recovered (g) | Absolute Recovery (%) | Actual % Hydrocortisone in Cream |
|---|---|---|---|---|---|
| 1 | 1.0258 | 0.0103 | 0.0092 | 89.32 | 0.9003 |
| 2 | 1.1078 | 0.0118 | 0.0111 | 94.07 | 0.9975 |
| 3 | 1.1151 | 0.0112 | 0.0119 | 106.25 | 1.0424 |
| 4 | 1.1369 | 0.0114 | 0.0119 | 104.39 | 1.0442 |
| 5 | 1.1390 | 0.0114 | 0.0113 | 99.12 | 1.0785 |
| 6 | 1.1634 | 0.0116 | 0.0123 | 106.03 | 1.0567 |
| | | | Average: | 99.86 | 1.02 |
| | | | RSD: | 6.99 | 0.06 |

[1]Based on manufacturer's label description of 1% hydrocortisone (w/w)



## HPLC Analysis of Mandelic Acid Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . 0.1 wt% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004610



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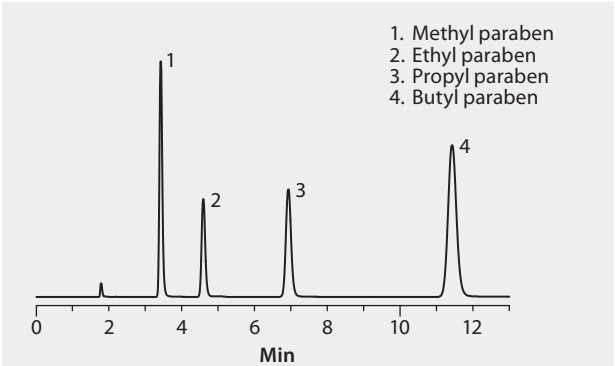
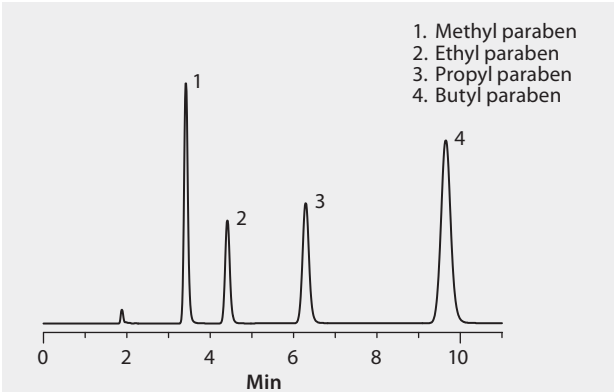
## HPLC Analysis of Paraben Preservatives on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) acetonitrile: (B) water, (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G001234



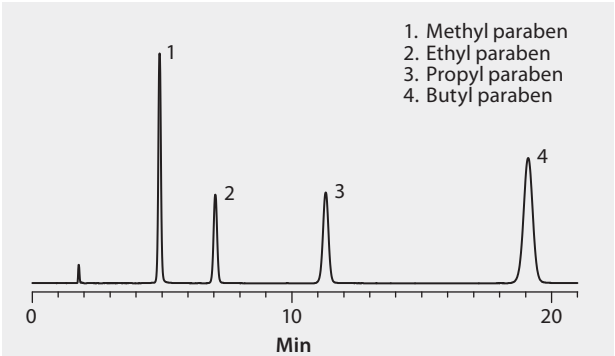
## HPLC Analysis of Paraben Preservatives on Discovery® C8

column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . . . . (A) acetonitrile: (B) water, (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G001236



## HPLC Analysis of Paraben Preservatives on Discovery® RP-AmideC16

column . . Discovery RP-Amide C16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) acetonitrile: (B) water, (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G001235



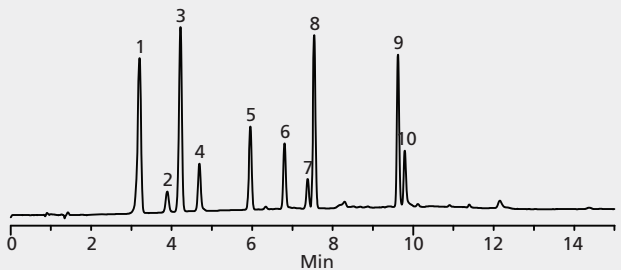
## HPLC Analysis of Phenolic Antioxidants on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) 5% acetic acid; (B) (50:50) methanol:acetonitrile
flow rate . . . . . 2.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 50:50 acetonitrile:2-propanol
Application No. . . . . G003054

1. Propyl gallate (10 µg/mL)
2. Ethoxyquin (10 µg/mL)
3. 2,4,5-trihydroxybutyrophenone (10 µg/mL)
4. tert-butylhydroquinone (10 µg/mL)
5. Nordihydroguaiaretic acid (10 µg/mL)
6. 3-tert-butyl-4-hydroxyanisole (10 µg/mL)
7. 2,6-di-tert-butyl-4-hydroxymethylphenol (10 µg/mL)
8. Octyl gallate (10 µg/mL)
9. Lauryl gallate (10 µg/mL)
10. 3,5-di-tert-butyl-4-hydroxytoluene (10 µg/mL)

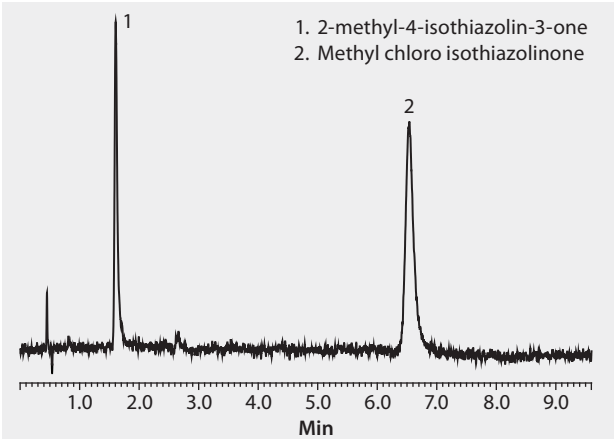
**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 70 | 30 |
| 10 | 0 | 100 |
| 15 | 0 | 100 |



## UHPLC Analysis of Kathon on Titan™ C18 Using MS Detection

column . . . . . Titan C18 50 mm x 2.1 mm, I.D, 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 0.1% formic acid in water (B):methanol
flow rate . . . . . 500 µL/min
detector . . . . . MS, ESI (+) MRM TIC
detector . . . . . Diode Array at 275 nm
injection . . . . . 1.0 µL
sample . . Diluted Kathon at 1 ug/ml in 95:5 0.1% formic acid in water::methanol
Application No. . . . . G006148



# Environmental

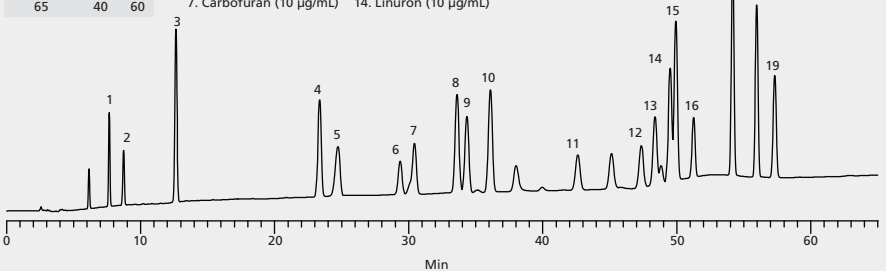
## EPA Method 632: HPLC Analysis of Pesticides on Ascentis® C8

column . . . . . Ascentis C8, 25 cm x 4.6 mm I.D., 5 µm particles (581425-U)
mobile phase . . . . . (A) DI water; (B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 16% acetonitrile in DI water
Application No. . . . . G003239

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 85 | 15 |
| 4 | 75 | 25 |
| 44 | 55 | 45 |
| 50 | 40 | 60 |
| 65 | 40 | 60 |

1. Oxamyl (10 µg/mL)
2. Methomyl (10 µg/mL)
3. Fenuron (10 µg/mL)
4. Monuron (10 µg/mL)
5. Aminocarb (10 µg/mL)
6. Propoxur (10 µg/mL)
7. Carbofuran (10 µg/mL)
8. Fluomethuron (10 µg/mL)
9. Carbaryl (10 µg/mL)
10. Diuron (10 µg/mL)
11. Propham (10 µg/mL)
12. Siduron (10 µg/mL)
13. Methiocarb (10 µg/mL)
14. Linuron (10 µg/mL)
15. Swep (10 µg/mL)
16. Mexacarbate (10 µg/mL)
17. Chlorpropham (10 µg/mL)
18. Barban (10 µg/mL)
19. Neburon (10 µg/mL)



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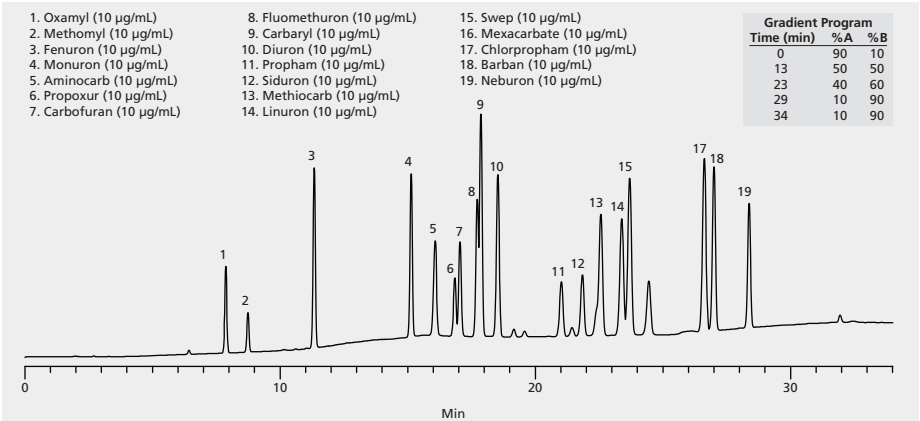
## EPA Method 632: HPLC Analysis of Pesticides on Ascentis® C18

column . . . . . Ascentis C18, 25 cm x 4.6 mm I.D., 5 µm particles (581325-U)
mobile phase . . . . . (A) water; (B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 16% acetonitrile in water
Application No. . . . . G003194



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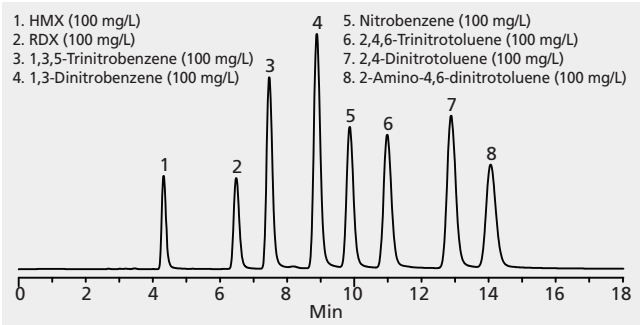
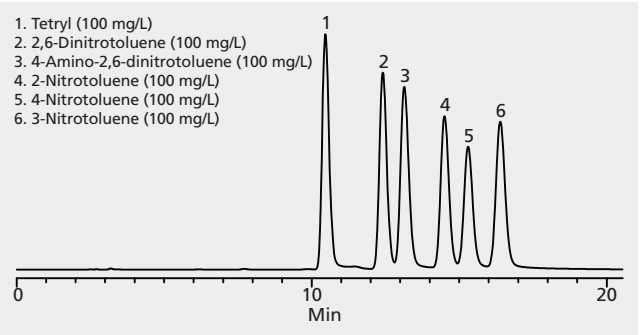
## EPA Method 8330: HPLC Analysis of Nitroaromatics and Nitramines, Mix A on Ascentis® C18

| | |
|---|---|
| column | Ascentis C18, 25 cm x 2.1 mm I.D., 5 µm particles (581305-U) |
| mobile phase | (A) water: (B) methanol; (50:50, A:B) |
| flow rate | 0.2 mL/min |
| column temp. | 60 °C |
| detector | UV, 254 nm |
| injection | 1 µL |
| sample | acetonitrile |
| Application No. | G003278 |



## EPA Method 8330: HPLC Analysis of Nitroaromatics and Nitroamines, Mix B on Ascentis® C18

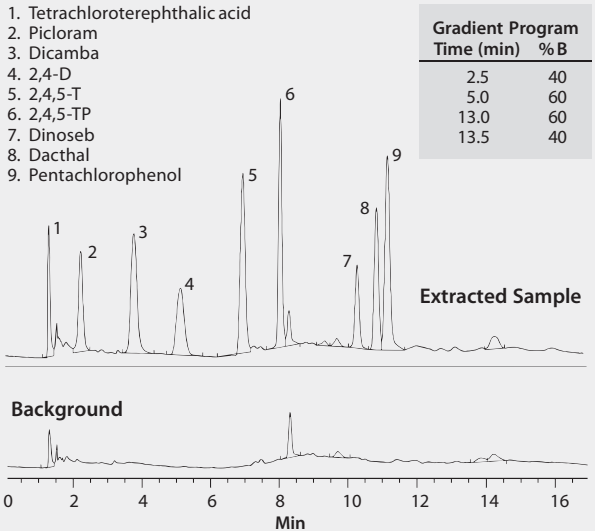
| | |
|---|---|
| column | Ascentis C18, 25 cm x 2.1 mm I.D., 5 µm particles (581305-U) |
| mobile phase | (A) water;(B) methanol;(50:50, A:B) |
| flow rate | 0.2 mL/min |
| column temp. | 60 °C |
| detector | UV, 254 nm |
| injection | 1 µL |
| sample | as indicated in acetonitrile |
| Application No. | G003285 |



## HPLC Analysis of Acidic Herbicides in Water on a Polymeric C18 Column after SPE using Supelclean™ ENVI™-Carb

*using Zymark AutoTrace Extraction WorkStation 1.20*

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . Fresh 1 L water samples, dechlorinated with sodium thiosulfate when necessary, at ambient temperature and pH.
SPE tube/cartridge . . . . . Supelclean ENVI-Carb, 250 mg/6 mL (57092)
condition . . . . . 10 mL DI water at 20 mL/min
sample addition . . . . . 0.9 L water sample at 20 mL/min
drying . . . . . 10 min using clean nitrogen
washing . . . . . 10 mL DI water at 20 mL/min
elution . . . . . 10 mL 0.1% phosphoric acid in methylene chloride:acetonitrile (80:20) at 5 mL/min
column . . polymeric-coated silica-based PAH specialty column, 20 cm × 3 mm I.D., 5 µm (Supelco equivalent, SUPELCOSIL LC-PAH, available upon request)
mobile phase . . gradient, (A): 0.05% phosphoric acid in DI water; (B): acetonitrile
flow rate . . . . . 0.5 mL/min
column temp. . . . . 50 °C
detector . . . . . photodiode array- peak width: 0.053 min, sampling interval: 0.320 sec, monitor 210 nm & 225 nm
injection . . . . . 10 µL of extract (4-5 ppb each analyte in water)
Application No. . . . . 796-0150

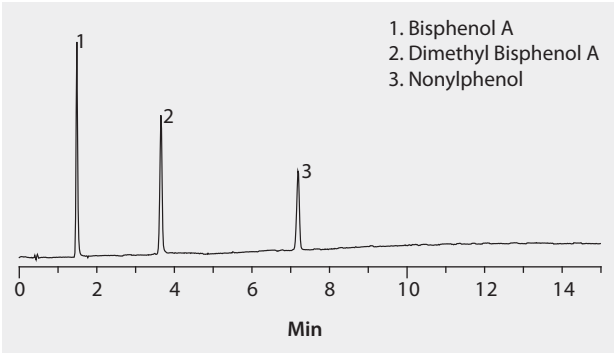



Figures provided by A. Lichtman, Nassau County Dept. of Health, Hempstead, New York, USA. Request Application Note No. T196100.

## HPLC Analysis of Bisphenol A, Dimethylbisphenol A and Nonylphenol on Ascentis® Express RP-Amide (UV Detection)

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of milk thistle related compounds

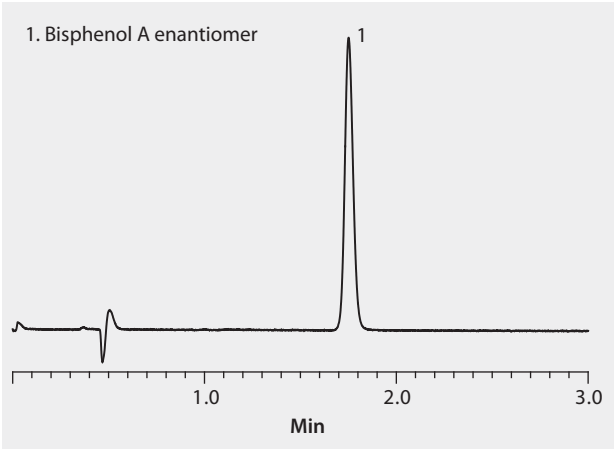
column . . Ascentis Express RP-Amide, 5 cm x 4.6 mm I.D., 2.7 µm particles (53922-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . . . . 50 to 100% B in 10 min
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 50 mg/L of each in 50:50, water: acetonitrile
Application No. . . . . G005681



## HPLC Analysis of Bisphenol A in Water on Ascentis® Express C18 after SPE using Supelclean™ ENVI-18

Bisphenol A is a contaminant that can leach into aqueous solutions from polycarbonate plastics or metal cans; it may impact human development and reproduction. This application demonstrates the extraction and analysis of bisphenol A from water.

SPE tube/cartridge . . Supelclean ENVI-18, 500 mg/6 mL glass SPE tube (54331-U)
condition . . . . . 1 mL 1% formic acid in acetonitrile, then 1 mL water
sample . . . . . 5 mL spiked water blank, concentration of 0.2 µg/mL
elution . . . . . 2 mL 1% formic acid in acetonitrile
eluate post-treatment . . . . . 1 mL aliquot of eluent, and evaporate/reconstitute to 0.5 mL in acetonitrile
column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 3270 psi (256 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
Application No. . . . . G005530

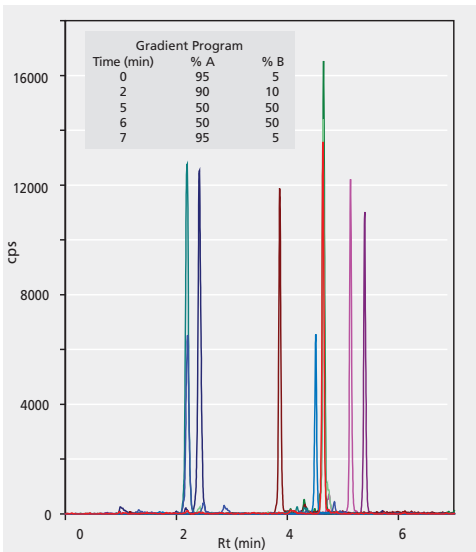


# HPLC Analysis of β-Blockers and β-Agonists in Urine and Wastewater on a C18 Column after SPE using SupelMIP® SPE-Beta-Receptor

**Sample Pre-treatment:**

Horse urine was centrifuged at 3000 g for 10 min, diluted 1:1 (v/v) with DI water, adjusted to pH 7. Wastewater was filtered with 1 µm filter paper and adjusted to pH 6-7. Samples were spiked with 10 beta-agonists and beta-blockers at the level of 1 ng/mL.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . horse urine or waste water spiked with 10 beta-agonists and beta-blockers at 1 ng/mL
SPE tube/cartridge . . SupelMIP SPE - Beta-Receptor, 25 mg/10 mL (LRC) (53223-U)
condition . . . . . 1 mL acetonitrile; 1 mL DI water
sample addition . . . . . 1 mL
washing . . . 3 x 1 mL DI water; 1 mL acetonitrile; 1 mL 60% acetonitrile/40% DI water; apply 2 min of full vacuum to dry the tube after each step
elution . . . . . 2 x 1 mL 1% formic acid in acetonitrile
column . . . . . C18, 5 cm x 3 mm I.D., 3 µm particles
eluate post-treatment . . . . . evaporate under nitrogen and reconstitute with 150 µL 5% acetonitrile in 10 mM ammonium acetate, pH 4.6 prior to LC-MS-MS analysis
mobile phase . . . . . (A) 10 mM ammonium acetate, pH 4.6 (adjusted with acetic acid); and (B) acetonitrile
flow rate . . . . . 0.5 mL/min
column temp. . . . . ambient
detector . . . . . MS/MS, ESI(+)
injection . . . . . 20 µL
Application No. . . . . G004059




Note: Clenbuterol and Tulobuterol were spiked at the levels of 0.1 ng/mL.

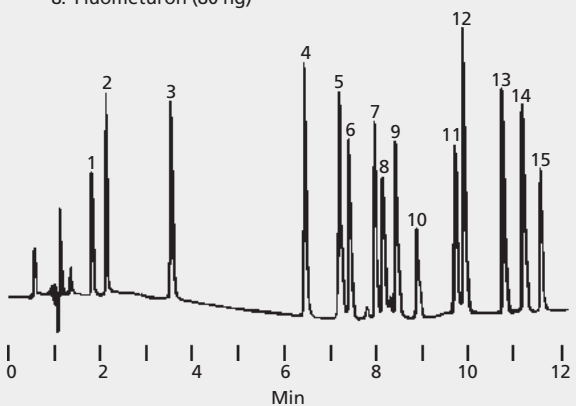
| Analyte | Rt (min.) | Q1/Q3 |
|---|---|---|
| Atenolol | 3.0 | 267.2/145 |
| Carazolol | 6.2 | 299.1/194.2 |
| Metoprolol | 5.6 | 268.2/133 |
| Propranolol | 6.5 | 260.2/154.9 |
| Timolol | 5.5 | 317.2/188.1 |
| Clenbuterol | 5.6 | 277.1/202.9 |
| Ritodrine | 3.9 | 288.2/121 |
| Salbutamol | 2.6 | 240.2/147.9 |
| Terbutaline | 2.6 | 226.2/152 |
| Tulobuterol | 5.6 | 228.2/154.1 |

| | Lower Limit of Quantitation (ng/mL, ppb, or µg/kg) | |
|---|---|---|
| Analyte | 1 mL Horse Urine | 10 mL Wastewater |
| Atenolol | 0.1 | 0.01 |
| Carazolol | 0.1 | 0.01 |
| Metoprolol | 0.1 | 0.01 |
| Propranolol | 0.1 | 0.01 |
| Timolol | 0.1 | 0.01 |
| Clenbuterol | 0.02 | 0.002 |
| Ritodrine | 0.05 | 0.005 |
| Salbutamol | 0.1 | 0.01 |
| Terbutaline | 0.2 | 0.02 |
| Tulobuterol | 0.005 | 0.0005 |

## HPLC Analysis of Carbamate and Urea Pesticides on SUPELCOSIL™ LC-8

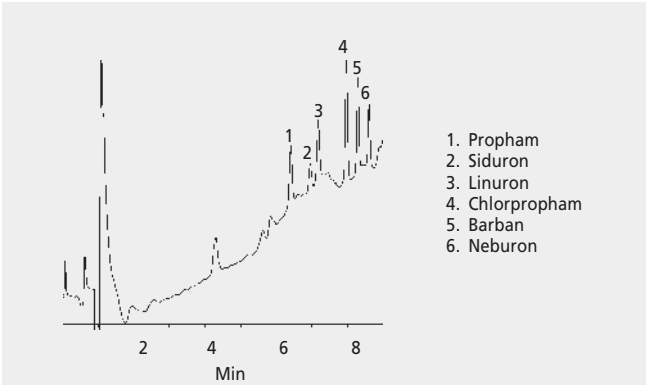
column . . . . . SUPELCOSIL LC-8, 15 cm × 4.6 mm I.D., 5 µm particles (58220-U)
mobile phase . . . . . (A) acetonitrile; (B) water
gradient . . . . . 18:82 to 65:35 in 9 min, hold 3 min
flow rate . . . . . 2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 10 µL
Application No. . . . . 794-0803

1. Methomyl (80 ng)
2. Oxamyl (80 ng)
3. Fenuron (80 ng)
4. Monuron (80 ng)
5. Carbofuran (1000 ng)
6. Propoxur (800 ng)
7. Carbaryl (200 ng)
8. Fluometuron (80 ng)
9. Diuron (80 ng)
10. Propham (80 ng)
11. Siduron (80 ng)
12. Linuron (80 ng)
13. Chlorpropham (80 ng)
14. Barban (80 ng)
15. Neburon (80 ng)



## HPLC Analysis of Carbamate and Urea Pesticides on SUPELCOSIL™ LC-8 after SPME using 60 µm PDMS/DVB Fiber

sample/matrix . . . . 3 mL water containing 8ng/mL of each analyte in 10% NaCl
SPME fiber . . . . . PDMS/DVB, 60 µm (57317)
extraction . . . . . immersion, 40 min, rapid stirring
desorption process . . . . . static, 5 min in acetonitrile:water (65:35); dynamic, valve open during run
column . . . . . SUPELCOSIL LC-8, 15 cm × 4.6 mm I.D., 5 µm particles (58220-U)
mobile phase . . . . . (A) acetonitrile; (B) water
gradient . . . . . 18:82 to 65:35 in 9 min, hold 3 min
flow rate . . . . . 2.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
Application No. . . . . 797-0049






SUPELCO® Solutions within.™

## HPLC Analysis of Metabolites of 7,12-Dimethylbenz[a]anthracene on SUPELCOSIL™ LC-18

column . . . . . SUPELCOSIL LC-18, 25 cm × 4.6 mm I.D., 5 µm particles (58298)
mobile phase . . (A) methanol:(B) water, (50:50, A:B), 10 min to 100:0 at 2.5% /min
flow rate . . . . . 0.8 mL/min
detector . . . . . fluorescence
sample . . . . rat liver, 9000 × g supernatant fraction from Aroclor-treated animals
Application No. . . . . 713-1043

1. 2-OH-DMBA(t-diol)
2. 7-OHM-12-MBA-(t-10,11-diol)
3. 7-M-12-OHMBA-(t-8,9-diol)
4. 7-OHM-12-MBA-(t-8,9-diol)
5. 7-OHM-(t-3,4-diol)
6. Mixed diols, incl. DMBA-(t-8,9-diol & t-10, 11-diol)
7. Position of 7,12-bis-OHMBA
8. 7-OHM-12-MBA-2-ol
9. 7-OHM-12-MBA-(3-ol)
10. 7-M-12-OHMNA-4-ol
11. 7-OHM-12-MBA-4-ol
12. 7-M-12-OHMBA-(3-ol)
13. 7-M-12-OHMBA-2-ol
14. 7-OHM-12-MBA
15. 7-M-12-OHMBA
16. DMBA-2-ol
17. DMBA-3-ol
18. DMBA-4-ol
19. DMBA

(parentheses = tentative identifications)

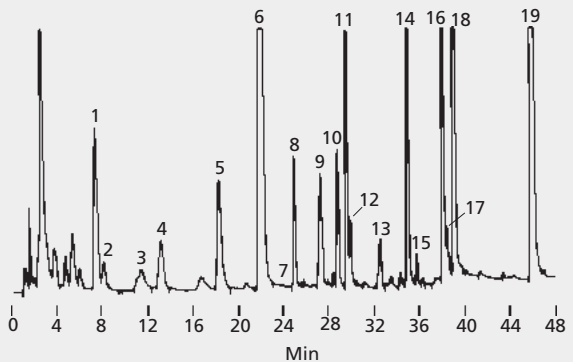
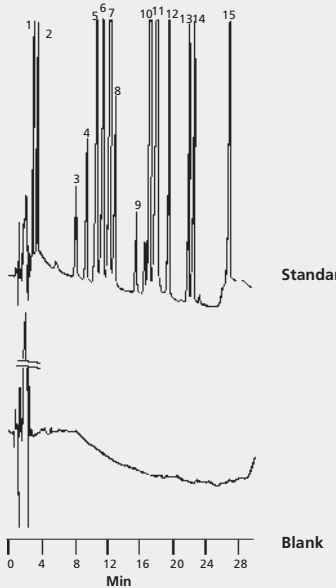



Figure provided by Drs. J. Milner and J. Grunau, University of Illinois, Urbana, Illinois, USA.

## HPLC Analysis of Nonvolatile Pesticides in Water on SUPELCOSIL™ LC-18-DB after SPE using Supelclean™ ENVI-Carb™

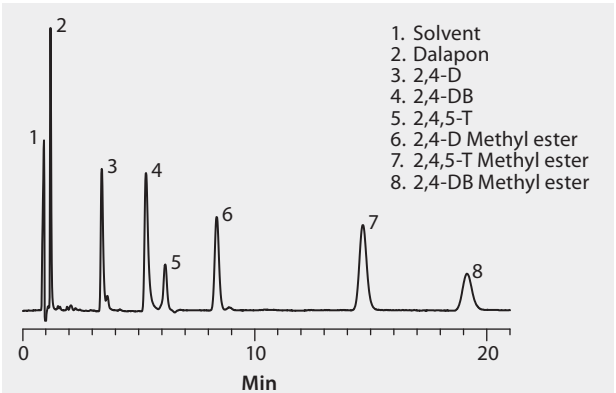
sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . water spiked with pesticides at 10–50 µg/L
SPE tube/cartridge . . . . . Supelclean ENVI-Carb, 250 mg/3 mL (57088)
condition . . . . . 5 mL methylene chloride:methanol (80:20); 1 mL methanol; 10 mL 2% acetic acid in water (keep bed moist until sample addition)
sample addition . . . . . 100 mL sample at 5 mL/min
drying . . . . . 1 minute with vacuum
elution . . . . . 0.8 mL methanol; 2 x 35 mL methylene chloride:methanol (80:20)
eluate post-treatment . . . . . dry eluate to 500 µL under gentle nitrogen stream, reconstitute to 1 mL with methanol
column . . SUPELCOSIL LC-18-DB, 25 cm × 4.6 mm I.D., 5 µm particles (58355-U)
mobile phase . . . . . (A) water:acetonitrile (90:10), (B) acetonitrile; gradient 80% A for 5 min then to 30% A over 30 min
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 220 nm
injection . . . . . 20 µL
Application No. . . . . 85-438




| Analyte | % Recovery (± RSD, n = 5) |
|---|---|
| 1. Oxamyl | 111 ± 9.6 |
| 2. Methomyl | 105 ± 5.0 |
| 3. N-1-Naphthylthiourea | — |
| 4. Aldicarb | 92 ± 0.8 |
| 5. Simazine | 91 ± 6.5 |
| 6. Monuron | 99 ± 3.2 |
| 7. Cyanazine | 90 ± 5.4 |
| 8. Metribuzin | 97 ± 3.9 |
| 9. Carbofuran | 106 ± 6.2 |
| 10. Atrazine | 89 ± 5.7 |
| 11. Carbaryl | 97 ± 3.5 |
| 12. Diuron | 88 ± 5.7 |
| 13. Propham | 95 ± 3.2 |
| 14. Propachlor | 96 ± 3.8 |
| 15. Linuron | 88 ± 5.4 |

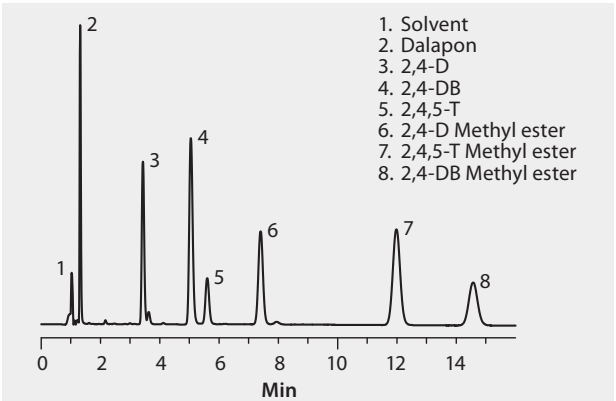
## HPLC Analysis of Organo-acid Pesticides on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) 25 mM potassium phosphate, (pH 2.3): (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
Application No. . . . . G001042



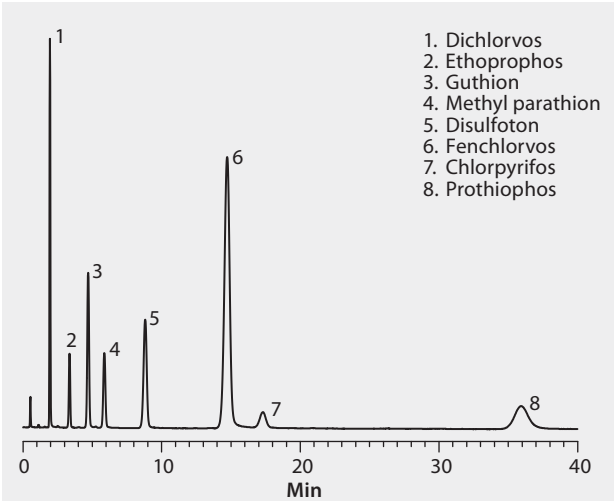
## HPLC Analysis of Organo-acid Pesticides on Discovery® C8

column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . . . . (A) 25 mM potassium phosphate, (pH 2.3): (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
Application No. . . . . G001041



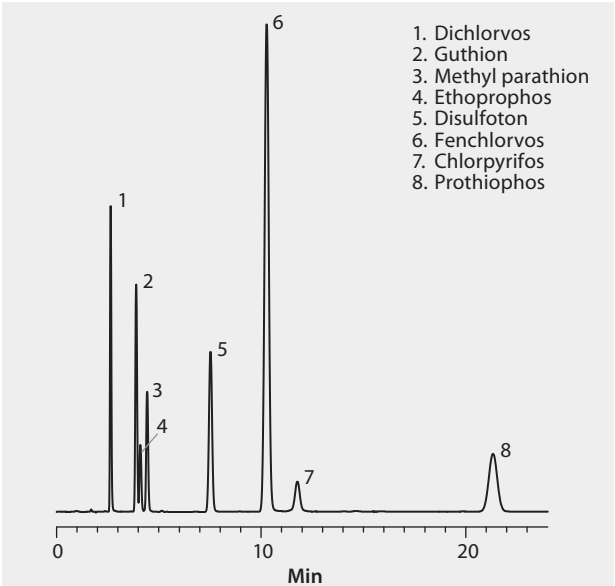
## HPLC Analysis of Organophosphorus Pesticides on Discovery® Cyano

column . . . . . Discovery Cyano, 15 cm × 4.6 mm I.D., 5 µm particles (59356-U)
mobile phase . . . . . (A) water: (B) acetonitrile, (75:25, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
Application No. . . . . G001030



## HPLC Analysis of Organophosphorus Pesticides on Discovery® RP-Amide C16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) acetonitrile: (B) water; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
Application No. . . . . G001031



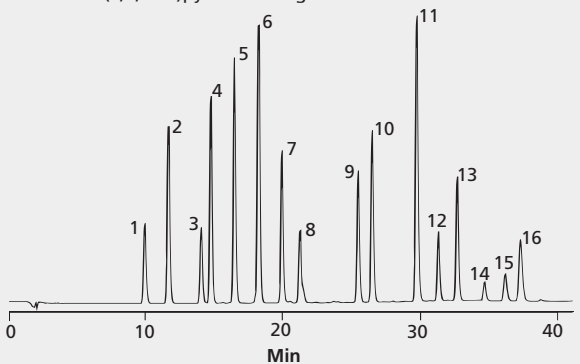
## HPLC Analysis of PAHs in Water on SUPELCOSIL™ LC-PAH after SPME using 100 µm PDMS Fiber

sample/matrix . . . . . 5 µL PAH mix (Cat. No. 4-8743) in 5 mL water
SPME fiber . . . . . polydimethylsiloxane, 100 µm (57301)
extraction . . . . . immersion, 30 min (rapid stirring)
desorption process . . . . . static, 200 µL acetonitrile:water, 40:60, 2 min
column . . . . SUPELCOSIL LC-PAH, 15 cm × 4.6 mm I.D., 5 µm particles (58318)
gradient . . 0 min - 50% A; 5 min - 50% A; 30 min - 100% A; 45 min - 100% A
flow rate . . . . . 0-2 min: 0.2 mL/min 2-45 min: 1.0 mL/min
mobile phase . . . . . (A) acetonitrile:(B) water
detector . . . . . UV, 254 nm
Application No. . . . . 796-0086

1. Naphthalene 1,000 ng/mL
2. Acenaphthylene 2,000 ng/mL
3. Acenaphthene 1,000 ng/mL
4. Fluorene 200 ng/mL
5. Phenanthrene 100 ng/mL
6. Anthracene 100 ng/mL
7. Fluoranthene 200 ng/mL
8. Pyrene 100 ng/mL
9. Benzo(a)anthracene 100 ng/mL
10. Chrysene 100 ng/mL
11. Benzo(b)fluoranthene 200 ng/mL
12. Benzo(k)fluoranthene 100 ng/mL
13. Benzo(a)pyrene 100 ng/mL
14. Dibenzo(a,h)anthracene 200 ng/mL
15. Benzo(ghi)perylene 200 ng/mL
16. Indeno(1,2,3-cd)pyrene 100 ng/mL

| Gradient Program | |
|---|---|
| Time (min) | % ACN |
| 0 | 50 |
| 5 | 50 |
| 30 | 100 |
| 45 | 100 |

Flow increased at 2.0 min



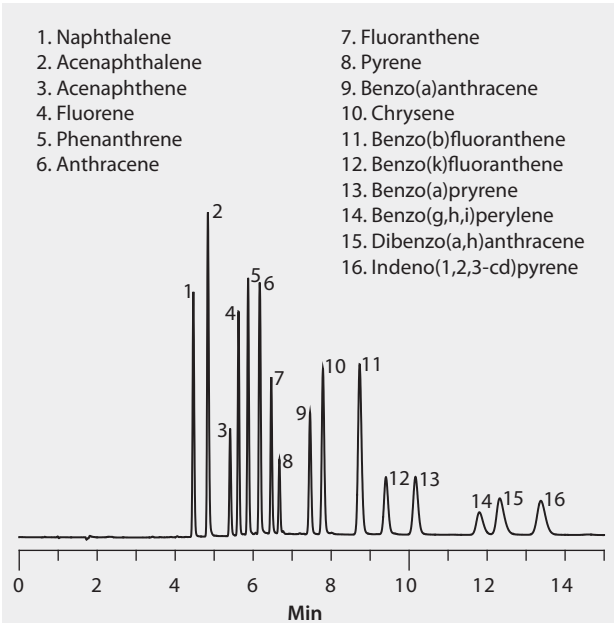



SUPELCO® Solutions within.™

## HPLC Analysis of PAHs on SUPELCOSIL™ LC-PAH

PAHs are traditionally evaluated using an acetonitrile/water gradient. Recently customers are looking for alternatives to this method using a methanol/water gradient due to the risk of an another acetonitrile shortage, solvent costs, and toxicity to the environment. This analysis demostrates the separation using a methanol gradient.

column . . . . . SUPELCOSIL LC-PAH, 15 cm x 4.6 mm I.D., 5 µm particles (58318)
mobile phase . . . . . (A) water, (B) methanol
gradient . . . . . 55 to 100% B in 5 min; held at 100%B for 10 min
flow rate . . . . . 1.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . EPA 610 mix
Application No. . . . . G005559






SUPELCO® Solutions within.™

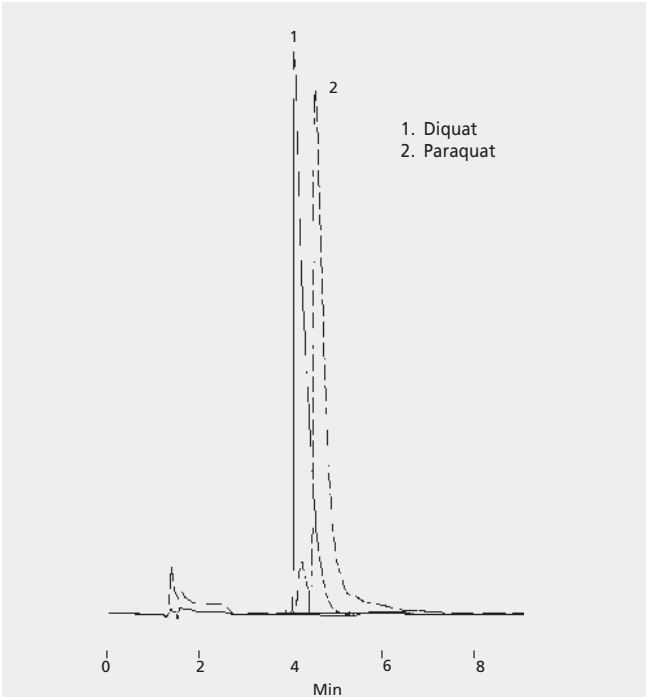
## HPLC Analysis of Paraquat and Diquat on SUPELCOSIL™ LC-18 after SPE using ENVI™-8 DSK

*Refer to US EPA Method 549.1 for full details*

**Sample Pre-treatment:**

250 mL drinking water. Adjust water sample to pH to 10.5 ± 0.2 with 10% sodium hydroxide or 10% hydrochloric acid

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . drinking water adjusted to pH to 10.
sample preparation . . . . . ENVI-8 DSK, 47 mm (57172)
SPE tube/cartridge . . . . . ENVI-8 DSK SPE Disk, 47 mm (57172)
condition . . . . 10 mL acetonitrile; 2 × 10 mL reagent water; 10 mL conditioning solvent A (5 g cetyl trimethyl ammonium bromide and 5 mL conc. ammonium hydroxide in 500 mL DI water, dilute to 1 L); ( 2 × 10 mL reagent water; 10 mL conditioning solvent B (10 g hexanesulfonic acid, sodium salt and 10 mL conc. ammonium hydroxide in 250 mL DI water, dilute to 500 mL))
sample addition . . . . . 250 mL water 100 mL/min
elution . . 0.5-1.0 mL acetonitrile (to cover/solvate disk); 2 × 4 mL eluting solution (13.5 mL orthophosphoric acid and 10.3 mL diethylamine in 500 mL DI water, dilute to 1 L)
column . . . . SUPELCOSIL LC-18, 15 cm × 4.6 mm I.D., 5 µm particles (58230-U)
mobile phase . . 3.5 mL triethylamine and 1.0 g 1-hexane-sulfonic acid, sodium salt to 800 mL DI $H_2O$ add orthophosphoric acid to pH 2.5, dilute to 1 L
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . photodiode array, diquat - 308 nm, paraquat - 257 nm
injection . . . . . 100 µL
Application No. . . . . 794-0715

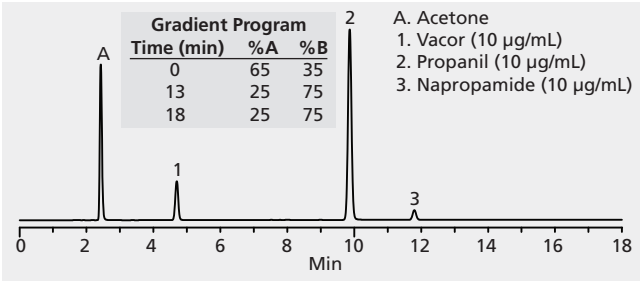





SUPELCO® Solutions within.™

## HPLC Analysis of Pesticides on Ascentis® C8 (15 cm x 4.6 mm x 5 µm)

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) DI water; (B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 100 µL
sample . . . . . as indicated in 2.2% 90:10 acetonitrile:acetone in DI water
Application No. . . . . G003121





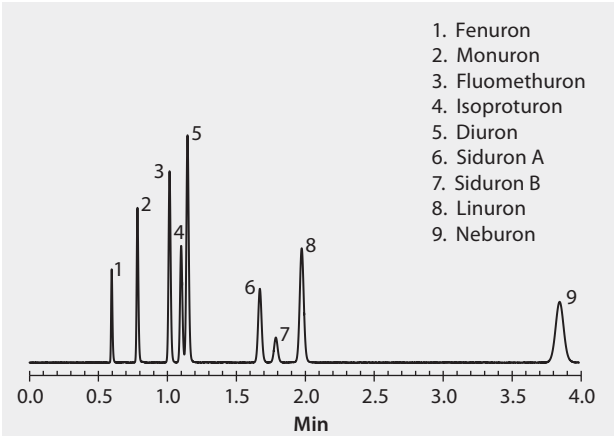

SUPELCO® Solutions within.™

## HPLC Analysis of Phenyl Urea Pesticides on Ascentis® Express C18 Phase

This separation illustrates the use of the highly efficient 2.7 um Ascentis Express Fused-Core C18 stationary phase in the analysis of some popular herbicides. The short run times allow analyses using isocratic conditions so that column equilibration time is not required between runs.

**Response Time**: 0.02 sec.
**Flow Cell**: 2.5 µL semi-micro

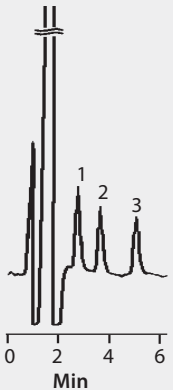
column . . Ascentis® Express C18, 10 cm x 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) 0.025 M potassium phosphate buffer, adj. to pH = 2.5, (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 2.0 mL/min
pressure . . . . . 300 bar
column temp. . . . . 30 °C
detector . . . . . UV 245 nm, VWD
injection . . . . . 0.5 µL
sample . . . . . acetonitrile
Application No. . . . . G006189



## HPLC Analysis of Triazine Herbicides in Grass on SUPELCOSIL™ LC-8-DB after SPE using Supelclean™ LC-SCX

**Sample Pre-treatment:**
5 g fresh grass clippings spiked with 2 ppm each herbicide. Add 4 g anhydrous sodium sulfate and 20 mL methylene chloride:acetone (80:20). Shake 20 min and allow mixture to stand 1 min.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . fresh grass clippings spiked with herbicides at 2 ppm
SPE tube/cartridge . . . . . Supelclean LC-SCX, 500 mg/3 mL (57018)
condition . . . . . 1 mL methylene chloride
sample addition . . . . . 2 mL grass extract. Wash with 2 x 2 mL acetonitrile.
drying . . . . . Dry packing for 5 min. under nitrogen purge
washing . . Dry packing for 5 min under nitrogen purge; wash with 2 x 2 mL DI water
elution . . . . . 1.5 mL methanol
eluate post-treatment . . . . . dilute to 2 mL with DI water
column . . . . . SUPELCOSIL LC-8-DB, 15 cm × 4.6 mm I.D., 5 µm with guard column (58347)
mobile phase . . . . . (A) acetonitrile; (B) water; (45:55, A:B)
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 254 nm
injection . . . . . 100 µL
Application No. . . . . 84-109

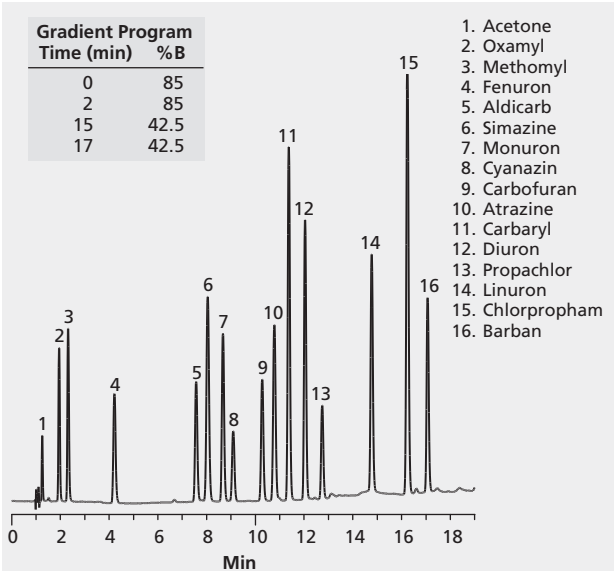



| Recovery (± SD, n = 3) | | |
|---|---|---|
| 102.0 | ± | 2.9 |
| 102.2 | ± | 1.6 |
| 100.5 | ± | 1.8 |

1. Simazine
2. Atrazine
3. Propazine

## HPLC Analysis of Urea, Carbamate, Triazine, Aniline Pesticides on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) acetonitrile; (B) water
flow rate . . . . . 2 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
Application No. . . . . G000948

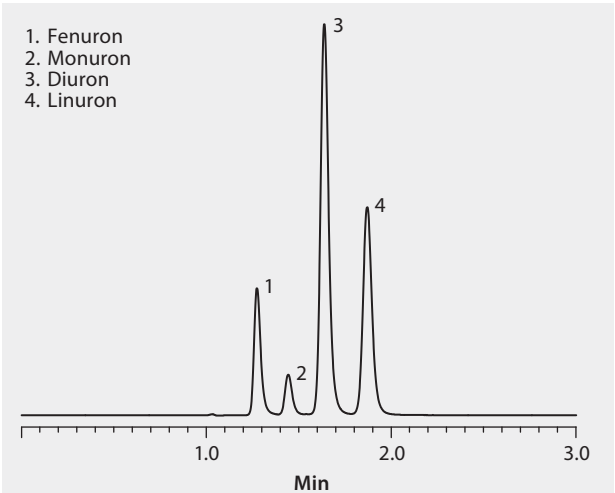





SUPELCO® Solutions within.™

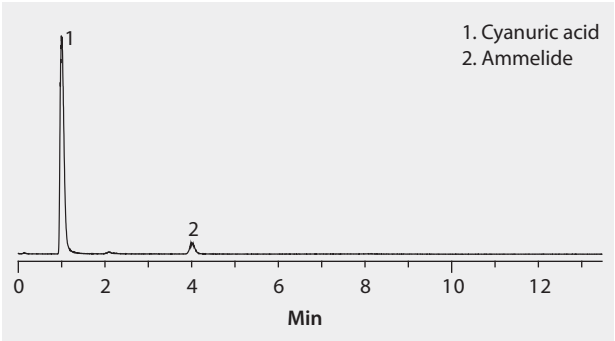
## HPLC Analysis of Urea Pesticides on Discovery® Cyano

column . . . . . Discovery Cyano, 15 cm × 4.6 mm I.D., 5 µm particles (59356-U)
mobile phase . . . . . (A) water: (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
Application No. . . . . G001067



## LC-MS Analysis of Cyanuric Acid on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 5 mM ammonium formate in 95:5 (v/v) acetonitrile:water
flow rate . . . . . 0.2 mL/min
pressure . . . . . 328 psi (22.6 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(-), SIR mode
injection . . . . . 2 µL
sample . . . . . 1 µg/mL in mobile phase
Application No. . . . . G005690

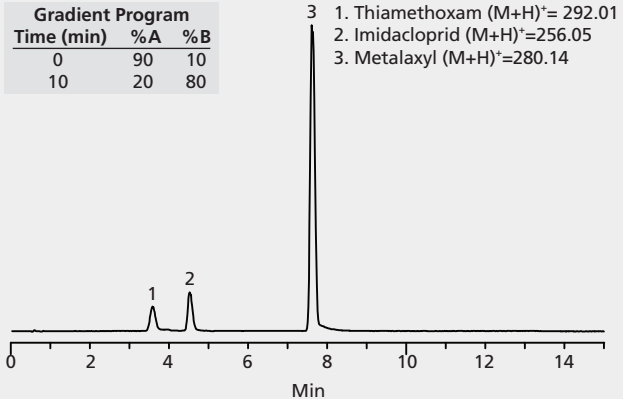


## LC-MS Analysis of Pesticides on Ascentis® C18 (5 cm x 2.1 mm x 3 µm)

column . . . . . Ascentis C18, 5 cm x 2.1 mm I.D., 3 µm particles (581300-U)
mobile phase . . . . . (A) 0.1% ammonium acetate, pH unadjusted; (B) acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, (+) ESI, Selected Ion Recording Mode
injection . . . . . 5 µL
sample . . . . . 1 µg/mL each in 90:10 water:acetonitrile
Application No. . . . . G003130

**Gradient Program**

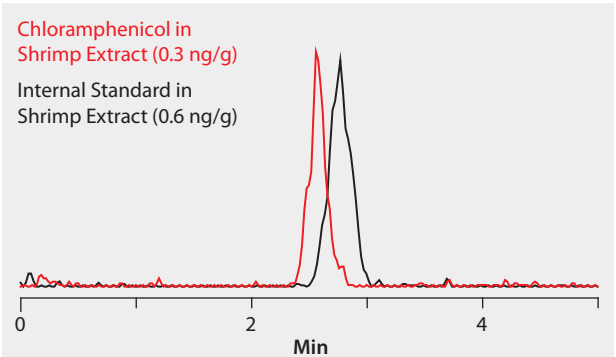
| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 10 | 20 | 80 |



## LC-MS Anaysis of Chloramphenicol in Shrimp on Ascentis® Express C18 Using Z-Sep+ Cleanup

This application demonstrates the superior cleanup associated with Z-Sep+ using QuEChERS approach.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . 1.0 g shrimp
extraction . . . . . 9 mL acetonitrile, then 90 µL of formic acid
extraction process . . post treatment: 8 mL supernatant evaporated to dryness and reconstituted with 1 mL water
clean-up tube . . . . . Supel™ Que Z-Sep+ tube, 12 mL, 500 mg (55296-U)
clean-up process . . . . . clean-up volume: 9 mL
column . . . . . Ascentis Express C18, 5 cm X 2.1mm, 2.7 um particles (53822-U)
mobile phase . . . . . (A) Water; (B) Acetonitrile
gradient . . . . . 15% B for 0.1 min, 15-80% in 1.9 min, held at 80% for 1.5 min, 80-15% in 0.5 min, at 15% for 3 min
flow rate . . . . . 0.5 mL/min
column temp. . . . . Ambient
detector . . . . . MS, ESI(+), MRM, m/z 320.9/151.9, 325.9/157.0
injection . . . . . 10 µL
Application No. . . . . G006249

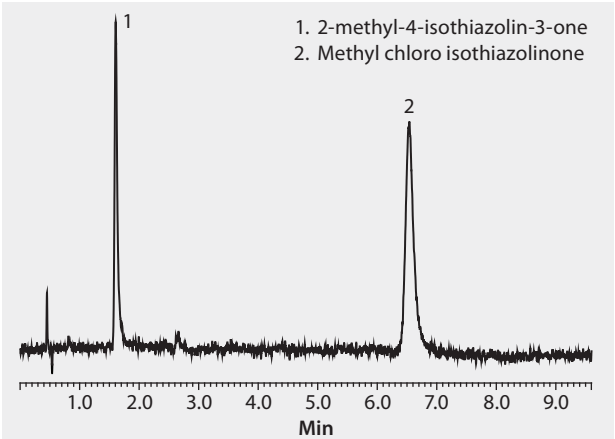





SUPELCO® Solutions within.™

## UHPLC Analysis of Kathon on Titan™ C18 Using MS Detection

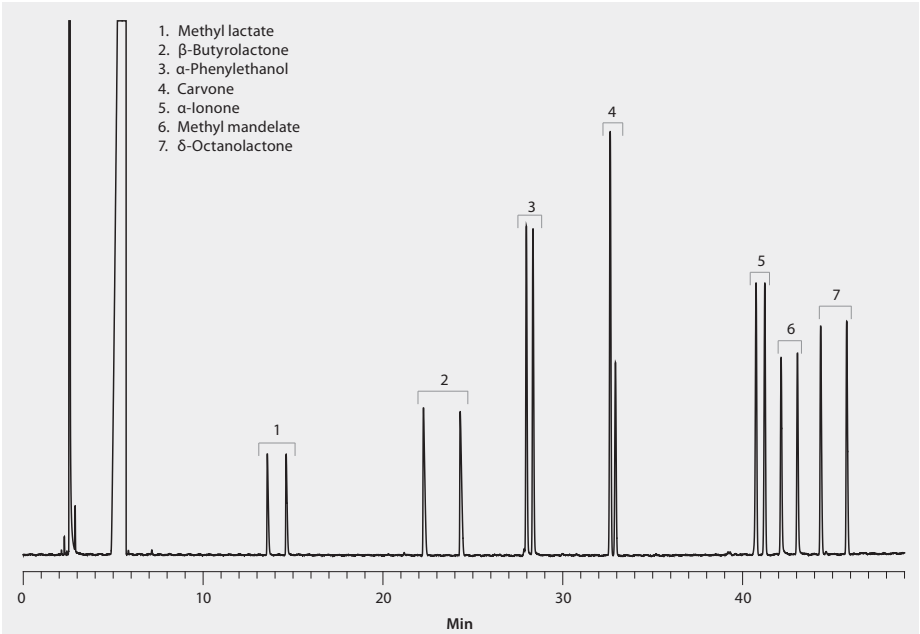
column . . . . . Titan C18 50 mm x 2.1 mm, I.D, 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 0.1% formic acid in water (B):methanol
flow rate . . . . . 500 µL/min
detector . . . . . MS, ESI (+) MRM TIC
detector . . . . . Diode Array at 275 nm
injection . . . . . 1.0 µL
sample . . Diluted Kathon at 1 ug/ml in 95:5 0.1% formic acid in water::methanol
Application No. . . . . G006148



## Flavors and Fragrances

### GC Analysis of Volatile Flavor Compound Enantiomers on Astec CHIRALDEX® G-TA

column . . . . Astec CHIRALDEX G-TA, 30 m x 0.25 mm I.D., 0.12 µm (73033AST)
oven . . . . . 60 °C, 2 °C/ min to 160 °C, hold 20 min
inj. temp. . . . . 250 °C
detector . . . . . FID, 250 °C
carrier gas . . . . . helium, 30 cm/sec, set at 75 °C
injection . . . . . 1 µL, split 100:1
Application No. . . . . G006117

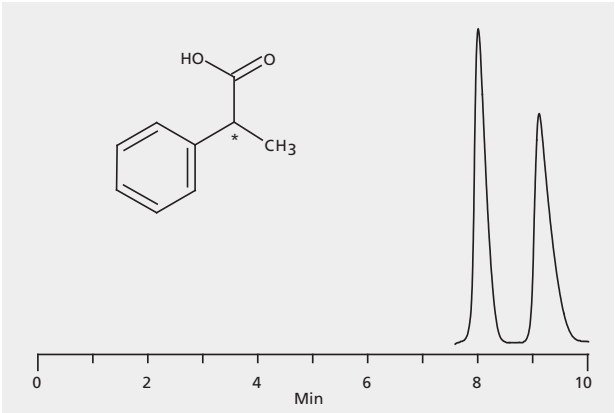





SUPELCO® Solutions within.™

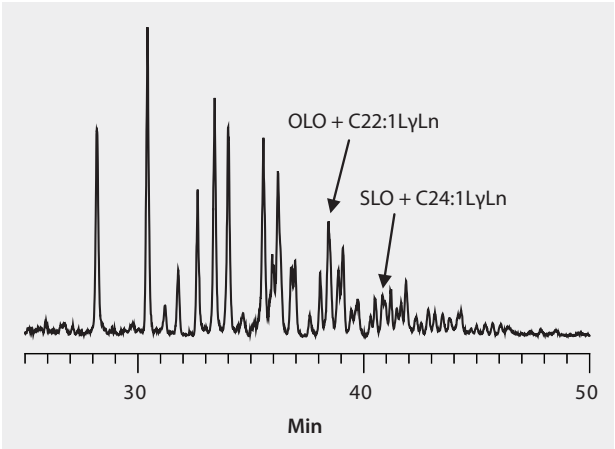
## HPLC Analysis of 2-Phenylpropionic Acid Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

| | |
|---|---|
| column | CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST) |
| mobile phase | (A) 10 mM ammonium acetate, pH 4.0; (B) acetonitrile; (75:25, A:B) |
| flow rate | 1 mL/min |
| column temp. | 25 °C |
| detector | UV, 235 nm |
| injection | 1 µL |
| sample | 1 mg/mL in methanol |
| Application No. | G004717 |



## HPLC Analysis of Triacylglycerols (TAG) of Borago Officinalis Oil on Ascentis® Express C18

column . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)
mobile phase . . . . . (A) acetonitrile; (B) isopropyl alcohol
gradient . . . . . 0% B held for 50 min; to 70% B in 5 min; to 0% B in 1 min
flow rate . . . . . 1 mL/min
detector . . . . . MS, APCI(+); mass spectral range, 250-1200 m/z; interval, 0.5 s; scan speed, 2143 amu/s; nebulizing gas (N2) flow, 4.0 L/min; interface temperature, 400 °C; (heat block, 230 °C; desolvation line (DL) temperature, 250 °C; DL voltage, 34 V; probe voltage, +4.5 kV; Qarray voltage, 1.0 V; RF voltage, 90 V; detection gain, 1.05 kV)
injection . . . . . 2 µL
sample . . . . . Borago Officinalis, 40 mg diluted in 10 mL of hexane
Application No. . . . . G005602



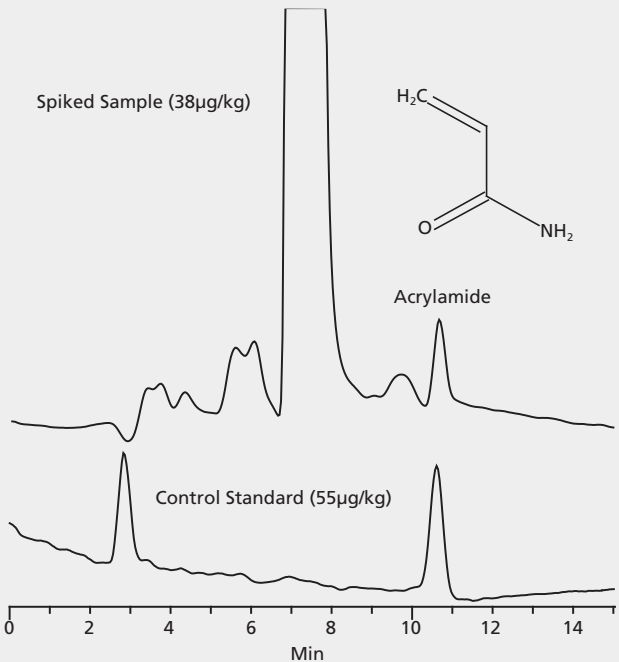
# Food and Beverages

## HPLC Analysis of Acrylamide in Fried Potato Chips on Discovery® HS F5 after SPE using Discovery® DSC-MCAX and DSC-18

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . Finely grind 2.0 g of potato chips, place in 20 mL vial and add 10 mL water. Mix using vortex until mixture forms a thick paste. (Place sample in centrifuge tubes and centrifuge at 16.1 rcf for 5 min. Extract aqueous portion of sample leaving oil layer and solids.)
SPE tube/cartridge . . . . . Discovery DSC-MCAX, 300 mg/3 mL (52784-U)
SPE tube/cartridge . . . . . Discovery DSC-18, 1g/6mL (52606-U)
condition . . connect a Discovery DSC-MCAX SPE tube to the top of a Discovery DSC-18 SPE tube using an SPE tube adapter, pass 1 mL methanol, then 1 mL DI water through both SPE tubes until the tubes are dry
sample addition . . . . . 1 mL of aqueous extract
washing . . . . . 1 mL DI water
elution . . . . . removing upper MCAX SPE tube, pass 2 mL methanol through lower DSC-18 SPE tube
eluate post-treatment . . . . . evaporate to dryness with nitrogen at 30 °C, reconstitute in 0.5 mL DI water
column . . . . . Discovery HS F5, 15 cm x 4.6 mm I.D., 3 µm particles (567507-U)
mobile phase . . . . . 100% ultra-pure water
column temp. . . . . 35 °C
detector . . . . . ESI+
injection . . . . . 5 µL
Application No. . . . . G003759

**Efficiency of Recovery**

| Concentration (µg/mL) | %Recovery ± RSD (n=3) |
|---|---|
| 10 | 78 ± 7% |
| 55 | 93 ± 5% |



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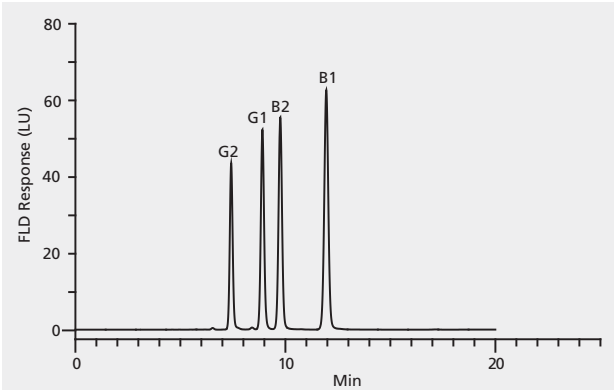
## HPLC Analysis of Aflatoxin Analogs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm × 4.6 mm I.D., 3 µm particles (581322-U)
mobile phase . . . . . (A) acetonitrile: (B) methanol: (C) water; (20:20:60, A:B:C)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C or 50 °C
detector . . . . . fluorescence, 360 nm excitation, 455 nm emission
injection . . . . . 20 µL
sample . . . . . Aflatoxin Standard Mix, diluted 1:1 with water
Application No. . . . . G004166



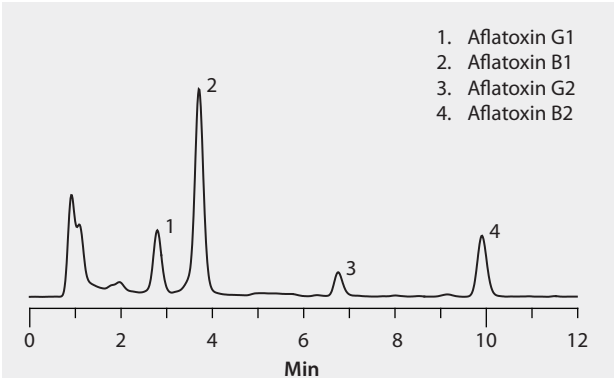



SUPELCO® Solutions within.™

## HPLC Analysis of Aflatoxins B1, B2, G1 and G2 in Corn on Ascentis® Express C18 after SPE using Supel™ Tox AflaZea and Fluorescence Detection

Aflatoxins are closely related compounds produced as secondary metablolites of fungi. They are found in grains and nuts like corn, wheat or peanuts. Shown here is a rapid, sensitive method to extract these compounds from corn using SPE cartridges specifically designed for aflatoxins, Supel Tox AflaZea (aflatoxin zearalenone), which removes interferences from grains, feeds, TMR samples, peanuts, peanut products and aqueous solutions. Analysis was via HPLC on Ascentis Express Fused-Core C18 colums.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . 25 g corn meal in 100 mL acetonitrile:water (84:16)
SPE tube/cartridge . . . . . Supel™ Tox AflaZea, 6 mL (55314-U)
sample addition . . . . . 2 mL
elution . . . . . collect sample in 5 mL silanized glass vial
eluate post-treatment . . . . transfer 200 µL sample, add 300 µL TFA derivatization solution (70:20:10 water:TFA:acetic acid), heat in microreactor 20 min at 65 °C, cool, add 580 µL deionized water, vortex
column . . Ascentis Express C18, 15 cm x 2.1 mm I.D., 2.7 µm particles (53825-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 0.350 mL/min
pressure . . . . . 325 psi (22.4 bar)
column temp. . . . . 35 °C
detector . . . . . FL, Ex 360 nm, Em 440 nm
injection . . . . . 20 µL
Application No. . . . . G005922



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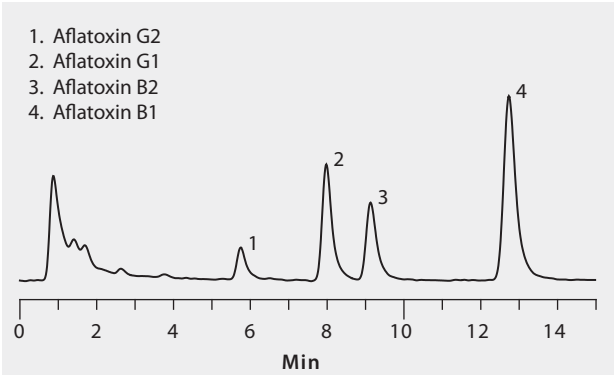
## HPLC Analysis of Aflatoxins in Wheat on Ascentis® Express C18 after SPE using Supel™ Tox AflaZea

**Sample/matrix:**
Weigh 25 grams of wheat meal into a 500 mL Erlenmeyer flask. Add 100 mL of 84:16 acetonitrile:water, seal, and place on an orbital shaker at med-high speed for 90 minutes. Filter the sample through a #2 qualitative filter. Spike sample at 2 mg/mL using analytical standards.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | 25 g wheat meal spiked with pesticide standards |
| SPE tube/cartridge | Supel™Tox AflaZea, 6 mL (55314-U) |
| sample addition | 2 mL |
| elution | collect eluate in 5 mL silanized culture tubes |
| eluate post-treatment | combine 200 µL of sample eluate and 880 µL of DI water in a 2 mL autosampler vial |
| column | Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) |
| mobile phase | (A) water; (B) methanol; (C) acetonitrile; (720:120:120, A:B:C) containing 0.780 g/L potassium bromide, 230 µL/L nitric acid |
| flow rate | 0.400 mL/min |
| pressure | 4568 psi (315 bar) |
| column temp. | 35 °C |
| detector | FLD 360 ex; 440 em |
| injection | 20 µL |
| derivatization | via KOBRA electrochemical cell, 50 µA |
| Application No. | G005907 |



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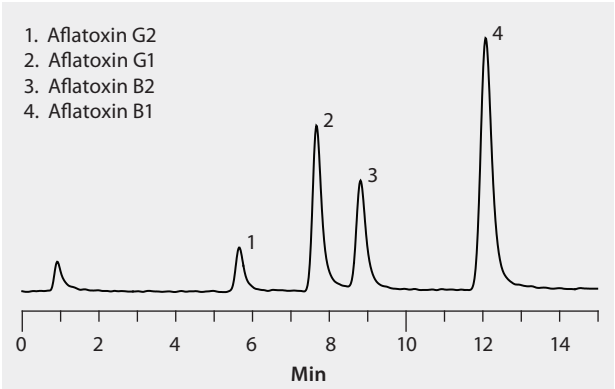
## HPLC Analysis of Aflatoxins on Ascentis® Express C18

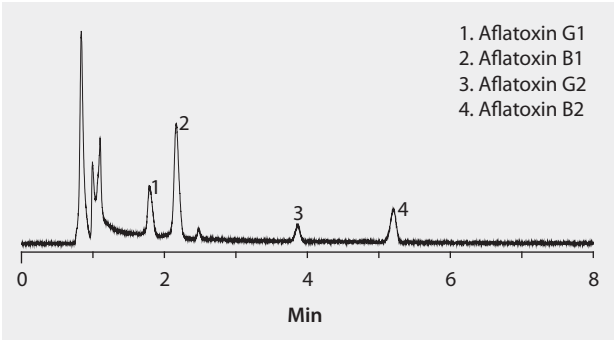
Aflatoxins are a complex of closely related toxins produced as secondary metablolites of fungi. They are found in grains and nuts such as corn, wheat, or peanuts. They are produced by aspergillus flavus and a. Parasiticus. Members of the group include aflatoxin b1, aflatoxin b2, aflatoxin g1, aflatoxin g2, aflatoxin m1, and aflatoxin m2.

column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . (A) water; (B) methanol; (C) acetonitrile; (720:120:120, A:B:C) containing 0.780 g/L potassium bromide, 230 µL/L nitric acid
flow rate . . . . . 0.400 mL/min
pressure . . . . . 4568 psi (315 bar)
column temp. . . . . 35 °C
detector . . . . . FLD excitation = 360 nm, emission = 440 nm
injection . . . . . 20 µL
sample . . . . . 200 µL spiked 84:16 acetonitrile:di water in 880 µL di water, Spike concentrations: B1 = 16 ppb; B2 = 4 ppb; G1 = 16 ppb; B2 = 4 ppb
Application No. . . . . G005908



## HPLC Analysis of Aflatoxins on Ascentis® Express C18 with Fluorescence Detection

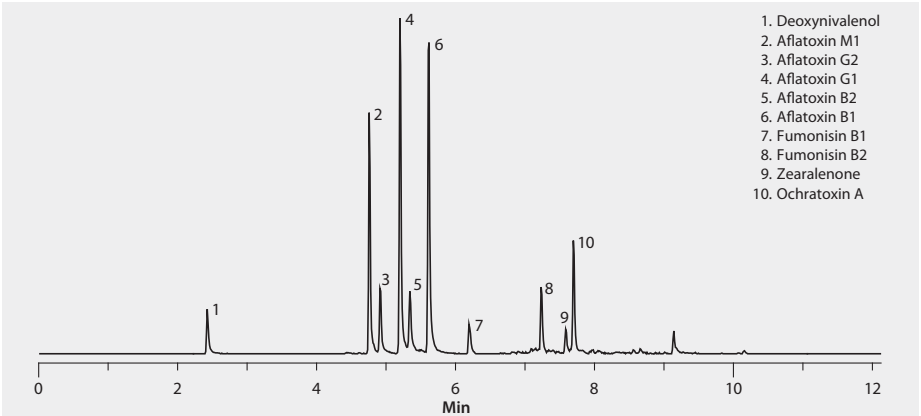
column . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 2.0 mL/min
pressure . . . . . 6381 psi (440 bar)
column temp. . . . . 35 °C
detector . . . . . Fluorescence, Ex 360 nm, Em 440 nm
injection . . . . . 50 µL
sample . . . . . 200 µL aflatoxin standard in acetonitrile; 0.5 µg/mL B2 and G2; 2 µg/mL B1 and G1; 800 µL delivatization solution: 70% water, 20% TFA and 10% acetic acid heat for 9 min at 65 °C
Application No. . . . . G005662



## HPLC Analysis of Aflatoxins on Ascentis® Express C18

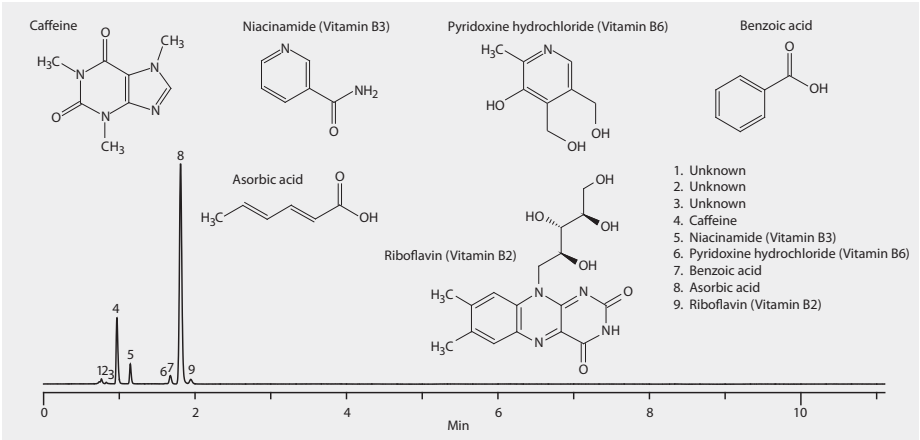
This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of various toxins.

column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) 5 mM ammonium formate, pH 2.5 (titrated with formic acid); (B) methanol
gradient . . . . . 5 to 100% B in 10 min
flow rate . . . . . 0.4 mL/min
pressure . . . . . 4714 psi (325 bar)
column temp. . . . . 35 °C
detector . . . . . APCI (+)
injection . . . . . 5 µL
sample . . . . . 0.15 - 30 mg/L in 90:10, water:acetonitrile
Application No. . . . . G005441



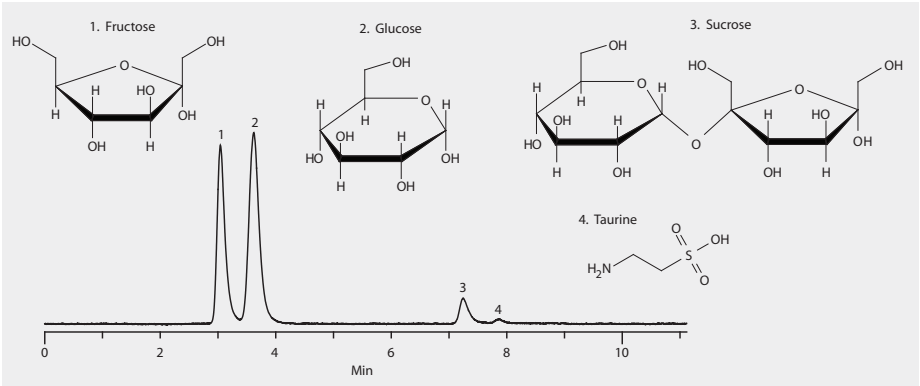
## HPLC Analysis of an Energy Drink on Ascentis® Express HILIC, 10 cm (UV Detection)

column . . . Ascentis Express HILIC, 5 cm x 3 mm I.D., 2.7 µm particles (53967-U)
mobile phase . . (A) 100 mM ammonium acetate, pH 5 (titrated with acetic acid); (B) water; (C) acetonitrile; (9:1:90, A:B:C:)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . Energy Drink Diluted 1:9 in acetonitrile
Application No. . . . . G005387



## HPLC Analysis of an Energy Drink on Ascentis® Express HILIC (ELSD Detection)

column . . . Ascentis Express HILIC, 5 cm x 3 mm I.D., 2.7 µm particles (53967-U)
mobile phase . . . . . (A) 100 mM ammonium acetate, pH 5 (titrated with acetic acid) (B) water (C) acetonitrile; (9:1:90, A:B:C:)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . ELSD, 55° C, 3.5 bar nitrogen
injection . . . . . 2 µL
sample . . . . . Energy Drink Diluted 1:9 in acetonitrile
Application No. . . . . G005388



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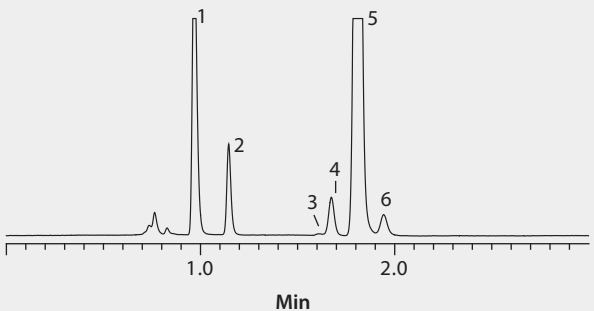
## HPLC Analysis of an Energy Drink on Ascentis® Express HILIC (UV Detection)

This application demonstrates the suitability of Ascentis Express HILIC for the efficient separation of common drink additives used in caffeinated energy drinks. The analysis was performed with UVand ELSD detectors in series, this chromatogram being from the UV.

column . . Ascentis Express HILIC, 10 cm x 3.0 mm I.D., 2.7 µm particles (53970-U)
mobile phase . . . . . (A) 100 mM ammonium acetate, pH 5.0 with acetic acid; (B) water; (C) acetonitrile; (09:01:90, A:B:C)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 812 psi (56 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . Dilute 1:9 in acetonitrile
Application No. . . . . G005753

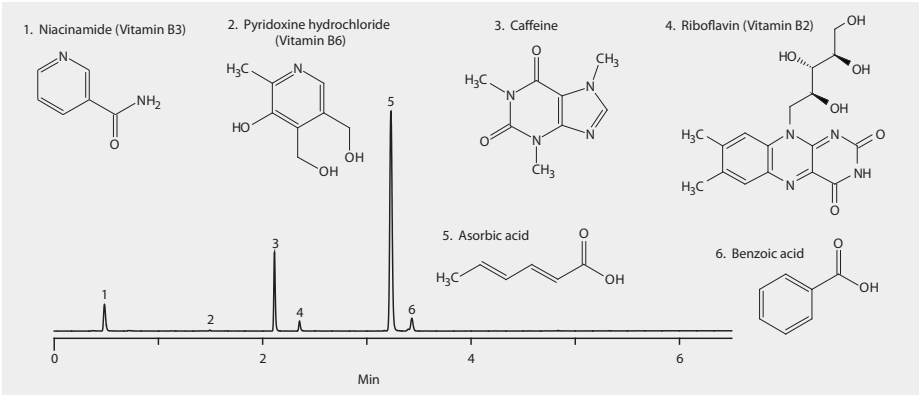
1. Caffeine
2. Niacinamide (Vitamin B3)
3. Pyridoxine hydrochloride (Vitamin B6)
4. Benzoic acid
5. Sorbic acid
6. Riboflavin (Vitamin B2)



## HPLC Analysis of an Energy Drink on Ascentis® Express RP-Amide (UV detection)

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of common drink additives used in caffeinated energy drinks.

column . . Ascentis Express RP-Amide, 5 cm x 3 mm I.D., 2.7 µm particles (53916-U)
mobile phase . . . . . (A) water with 0.1% TFA; (B) acetonitrile with 0.1% TFA
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1146 psi (79 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . Energy Drink Diluted 1:9 in acetonitrile
Application No. . . . . G005389



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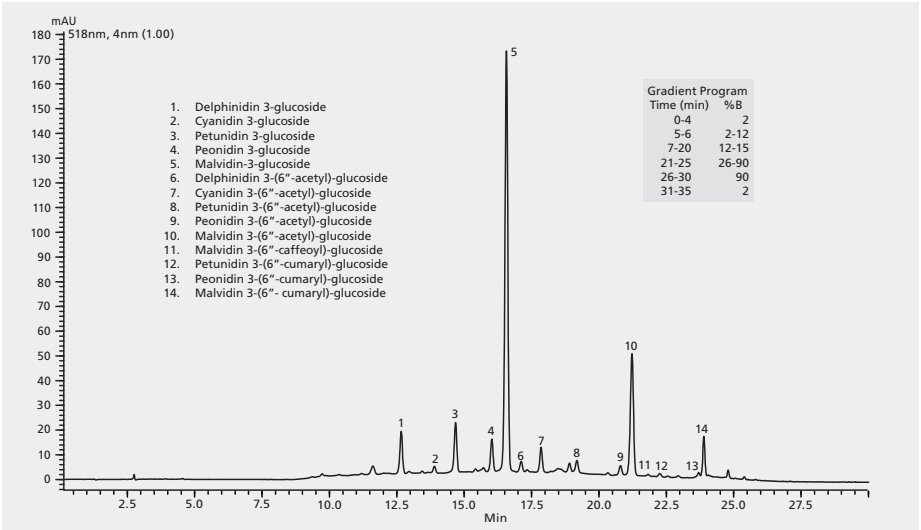
## HPLC Analysis of Anthocyanins in Red Wine on Ascentis® C18

column . . . . . Ascentis C18, 25 cm × 4.6 mm I.D., 5 µm particles (581325-U)
mobile phase . . (A) water:formic acid (9:1), (B) acetonitrile:water:formic acid (5:4:1)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . VIS, 518 nm
injection . . . . . 10 µL
sample . . . . . red wine diluted with water, centrifuged and passed through a 0.45 µm filter
Application No. . . . . G003922



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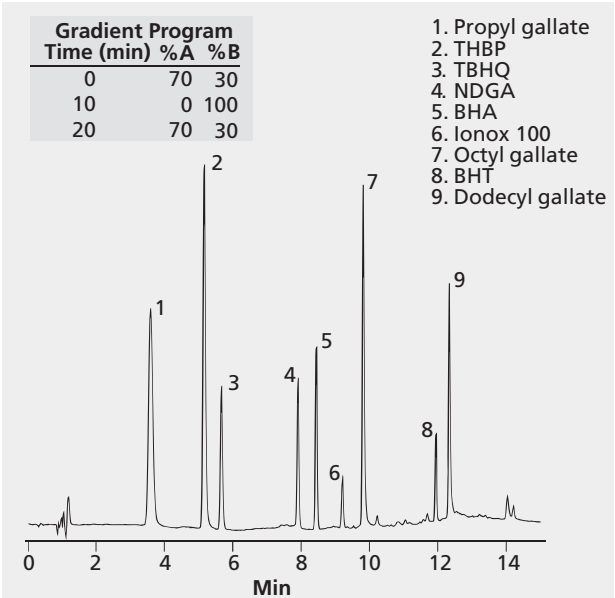
## HPLC Analysis of Antioxidants on SUPELCOSIL™ LC-18

column . . . . SUPELCOSIL LC-18, 15 cm × 4.6 mm I.D., 5 µm particles (58230-U)
mobile phase . . (A) 5% acetic acid in deionized water; (B) acetonitrile:methanol (1:1)
70% A/30% B to 100% B, linear gradient over 10 min, hold 10min
flow rate . . . . . 2 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 10µL, 20 µg/mL each antioxidant
Application No. . . . . 795-0438

| Gradient Program | | |
|---|---|---|
| Time (min) | %A | %B |
| 0 | 70 | 30 |
| 10 | 0 | 100 |
| 20 | 70 | 30 |



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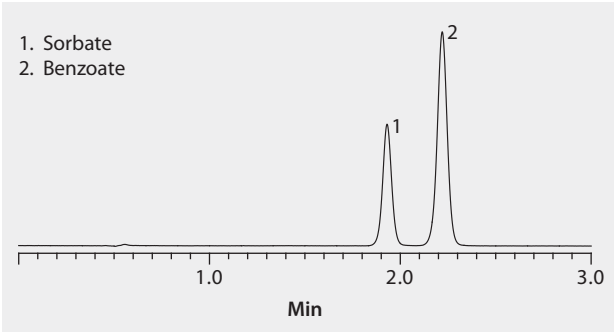
## HPLC Analysis of Benzoate and Sorbate on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of sorbate and benzoate.

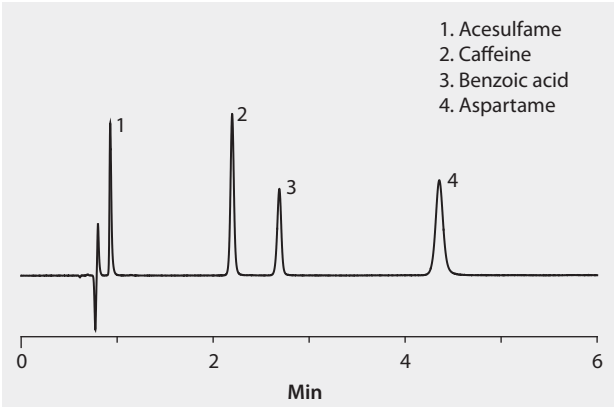
column . . Ascentis Express RP-Amide, 5 cm x 3 mm I.D., 2.7 µm particles (53916-U)
mobile phase . . . . . (A) 10 mM ammonium phosphate monobasic, pH 2.5 with phosphoric acid; (B) ethanol; (75:25, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 1 µL
sample . . . . . 50 mg/L each
Application No. . . . . G005672



## HPLC Analysis of Beverage Additives in Diet Cola on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of common drink additives used in diet soda.

column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) 20 mM ammonium acetate, pH 4.7 with acetic acid: (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
sample . . . . . 100 - 500 µg/mL in 95:5, buffer: acetonitrile
Application No. . . . . G005677



## HPLC Analysis of Beverage Additives in Diet Cola on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of common drink additives used in diet cola.

column . . . . . Ascentis Express RP-Amide, 10 cm x 3.0 mm I.D., 2.7 µm particles (53918-U)

mobile phase . . . . . (A) 20 mM ammonium acetate, pH 4.7 with acetic acid; (B) acetonitrile; (92:8, A:B)

flow rate . . . . . 0.6 mL/min

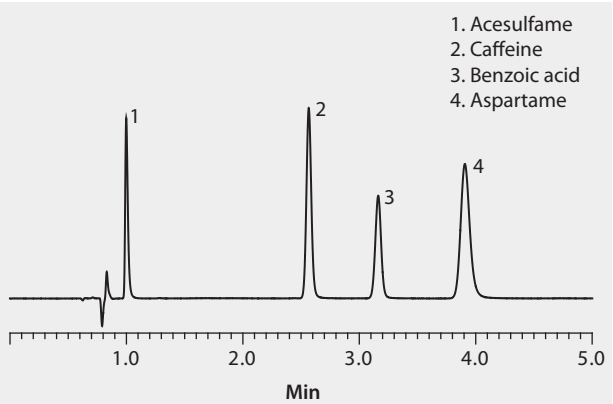
column temp. . . . . 40 °C

detector . . . . . UV, 214 nm

injection . . . . . 1 µL

sample . . . . . 500 - 1000 µg/mL in 95:5, buffer: acetonitrile

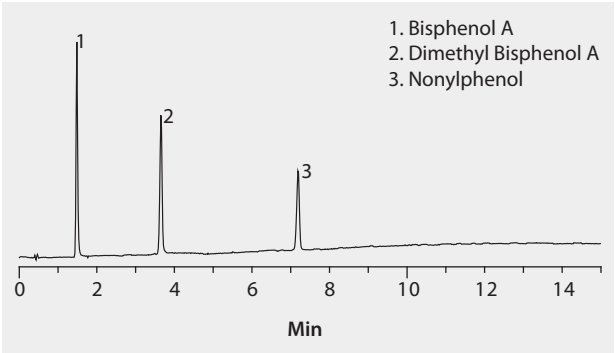
Application No. . . . . G005678



## HPLC Analysis of Bisphenol A, Dimethylbisphenol A and Nonylphenol on Ascentis® Express RP-Amide (UV Detection)

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of milk thistle related compounds

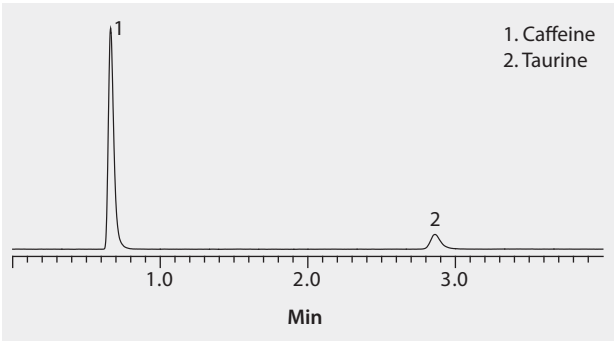
column . . Ascentis Express RP-Amide, 5 cm x 4.6 mm I.D., 2.7 µm particles (53922-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . . . . 50 to 100% B in 10 min
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 50 mg/L of each in 50:50, water: acetonitrile
Application No. . . . . G005681



## HPLC Analysis of Caffeine and Taurine on Ascentis® Express HILIC Column using Evaporative Light Scattering Detection (ELSD)

This application demonstrates the suitability of Ascentis Express HILIC for the efficient separation of caffeine and taurine. Elution is by aqueous normal phase (ANP) as an increase in organic will increase retention.

column . . Ascentis Express HILIC, 5 cm x 3.0 mm I.D., 2.7 µm particles (53967-U)
mobile phase . . . . . (A) 100 mM ammonium formate, pH 3.0 with formic acid; (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 25 °C
detector . . . . . ELSD, 55 °C, 3.5 bar nitrogen
injection . . . . . 2 µL
sample . . . . . 1000 µg/mL in 10:90, water: acetonitrile
Application No. . . . . G005682



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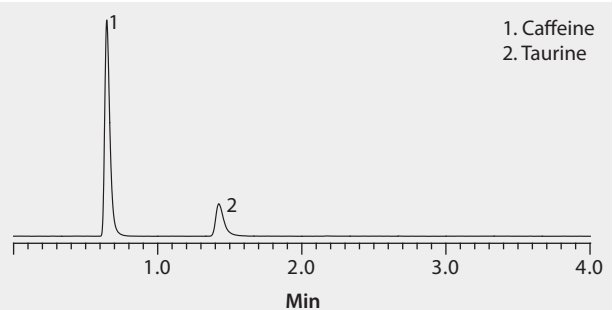
## HPLC Analysis of Caffeine and Taurine on Ascentis® Express HILIC Column using Evaporative Light Scattering Detection (ELSD, Fast Analysis)

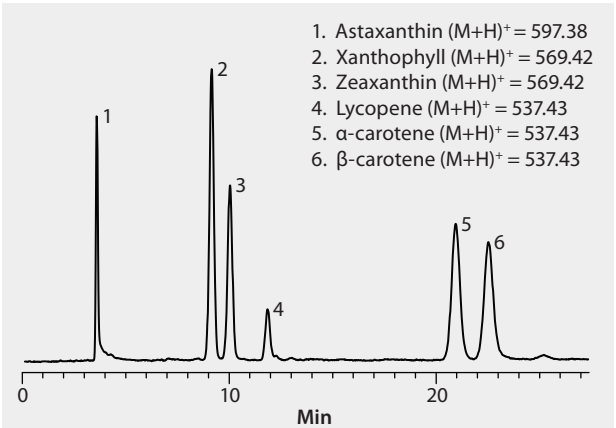
This application demonstrates the suitability of Ascentis Express HILIC for the efficient separation of caffeine and taurine. Elution is by aqueous normal phase (ANP) as an increase in organic will increase retention.

column . . Ascentis Express HILIC, 5 cm x 3.0 mm I.D., 2.7 µm particles (53967-U)
mobile phase . . . . . (A) 100 mM ammoniumformate, pH 3.0 with formic acid; (B) acetonitrile; (15:85, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 25 °C
detector . . . . . ELSD, 55 °C, 3.5 bar nitrogen
injection . . . . . 2 µL
sample . . . . . 1000 µg/mL in 10:90, water: acetonitrile
Application No. . . . . G005683



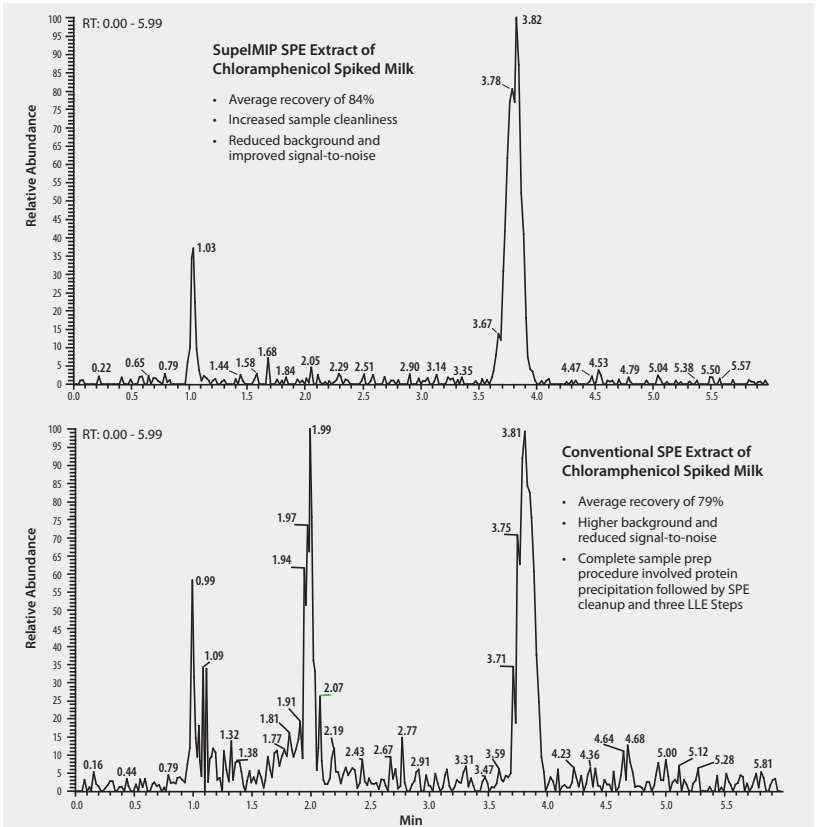
## HPLC Analysis of Carotene Compounds on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . 100% acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+) in selective ion monitoring (SIR) mode
injection . . . . . 5 µL
sample . . . . . 10 µg/mL each in 100% acetonitrile
Application No. . . . . G003138



## HPLC Analysis of Chloramphenicol in Milk on Ascentis® C18 after SPE using SupelMIP® SPE-Chloramphenicol

sample preparation . . . . . SPE (Solid Phase Extraction)
sample addition . . . . . whole pasteurized milk spiked with chloramphenicol at 15 ng/mL and 38 ng/mL
SPE tube/cartridge . . SupelMIP SPE - Chloramphenicol, 25 mg/10mL (LRC) (53210-U)
condition . . . . . 1 mL methanol;1 mL DI water
sample addition . . . . . 1 mL
washing . . . 2 x 1 mL MS-grade water, 1 mL 5% acetonitrile in 0.5% acetic acid, 2 x 1 mL MS-grade water, 1 mL 20% acetonitrile in 1% ammonium hydroxide. (dry SPE tubes for 15 min. under gentle vacuum; 3 x 1 mL dichloromethane; dry SPE tubes for 1 min. under gentle vacuum)
elution . . . . elute chloramphenicol with 2 x 1 mL methanol:acetic acid:MS-grade water (89:1:10, v/v/v)
eluate post-treatment . . . . evaporate combined eluate to dryness at 50 °C under nitrogen. reconstitute in 150 mL LC mobile phase prior to LC-MS analysis
column . . . . . Ascentis C18, 2.1 mm x 10 cm I.D., 3 µm particles (581301-U)
mobile phase . . . . . (A) 100 mM ammonium acetate: (B) MS-grade water: (C) acetonitrile (10:60:30, A:B:C)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI(-), 320-323 m/z range
injection . . . . . 5 µL
Application No. . . . . G004433



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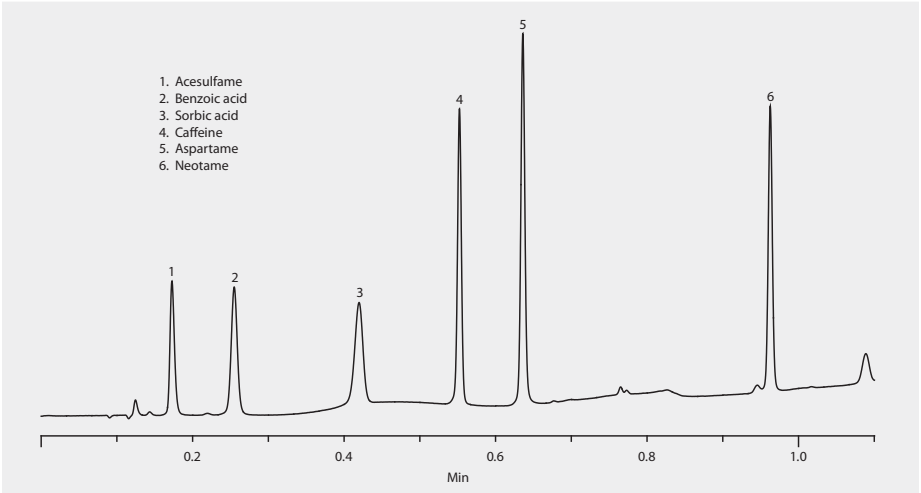
## HPLC Analysis of Diet Soda Additives on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of common drink additives used in diet cola.

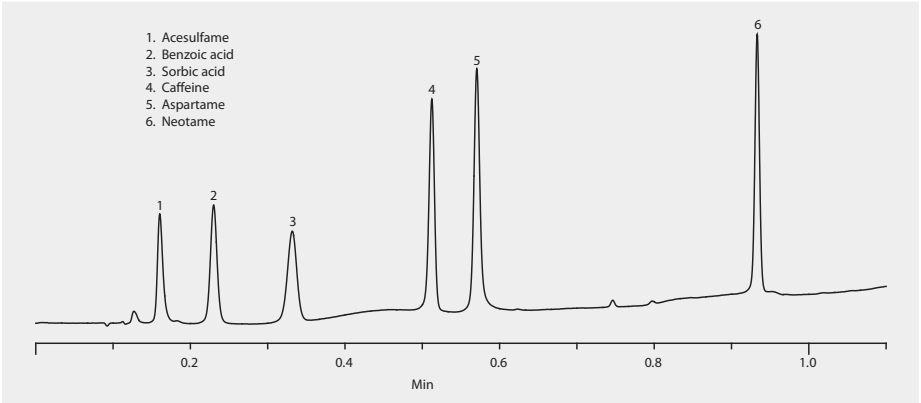
column . . Ascentis Express C18, 3 cm x 4.6 mm I.D., 2.7 µm particles (53818-U)
mobile phase . . . . . 20% A constant; 75 to 20% B, 5 to 60% C in 1 min; held at 20% B, 60% C for 0.1 min
pressure . . . . . 3191 psi (220 bar)
flow rate . . . . . 3 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
sample . . . . . (Diet Soda 100 - 500 µg/mL in buffer)
Application No. . . . . G005392



## HPLC Analysis of Diet Soda Additives on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of common drink additives used in diet cola.

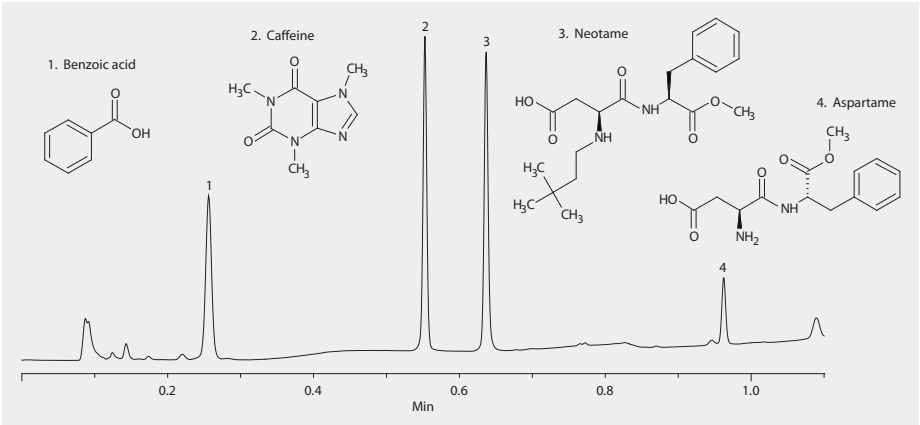
column . . Ascentis Express RP-Amide, 3 cm x 4.6 mm I.D., 2.7 µm particles (53921-U)
mobile phase . . . . . (A) 20 mM ammonium acetate, pH 5.6 with acetic acid; (B) water; (C) acetonitrile
gradient . . . . . 20% A constant; 75 to 20% B, 5 to 60% C in 1 min; held at 20% B, 60% C for 0.1 min
pressure . . . . . 3336 psi (230 bar)
flow rate . . . . . 3.0 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
sample . . . . . Diet Soda 100 - 500 µg/mL in buffer
Application No. . . . . G005393



## HPLC Analysis of Diet Soda Spiked with Neotame on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of common drink additives used in diet soda.

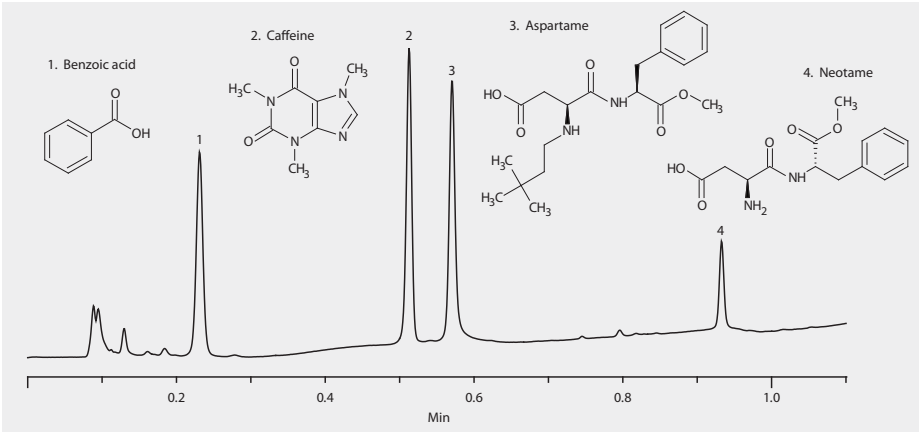
column . . Ascentis Express C18, 3 cm x 4.6 mm I.D., 2.7 µm particles (53818-U)
mobile phase . . . . . (A) 100 mM ammonium acetate, pH 5.6 (titrated with acetic acid); (B) water; (C) acetonitrile
gradient . . . . . 20% A constant; 75 to 20% B, 5 to 60% C in 1 min; held at 20% B, 60% C for 0.1 min
flow rate . . . . . 3 mL/min
pressure . . . . . 3191 psi (220 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
sample . . . . . neotame 350 µg/mL in diet cola
Application No. . . . . G005390



## HPLC Analysis of Diet Soda Spiked with Neotame on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of common drink additives used in diet cola.

column . . Ascentis Express RP-Amide, 3 cm x 4.6 mm I.D., 2.7 µm particles (53921-U)
mobile phase . . . . . (A) 100 mM ammonium acetate, pH 5.6 with acetic acid; (B) water; (C) acetonitrile
gradient . . . . . 20% A constant; 75 to 20% B, 5 to 60% C in 1 min; held at 20% B, 60% C for 0.1 min
flow rate . . . . . 3 mL/min
pressure . . . . . 3336 psi (230 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 mL/min
sample . . . . . diet soda spiked with neotame 350 µg/mL
Application No. . . . . G005391



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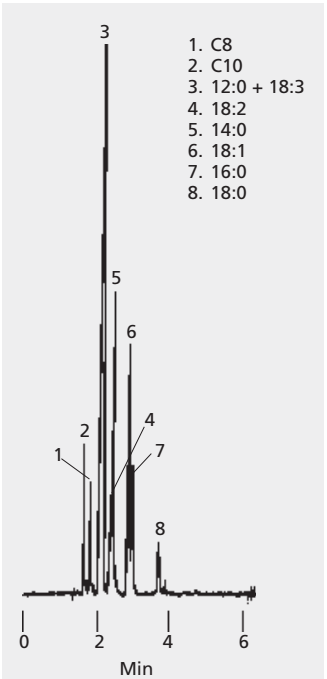
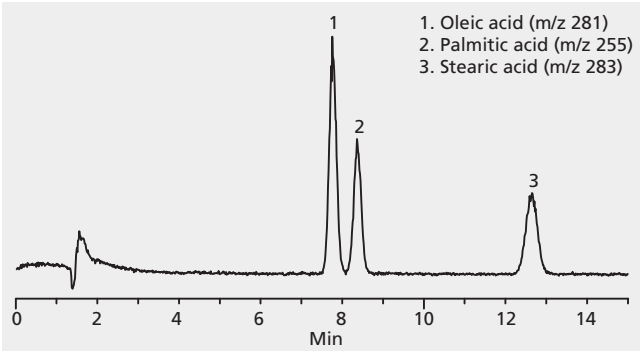
## HPLC Analysis of Fatty Acid Methyl Esters (FAMES) on SUPELCOSIL™ LC-18

column . . . . . SUPELCOSIL LC-18, 25 cm × 4.6 mm I.D., 5 µm particles (58298)
mobile phase . . . . . (A) acetonitrile; (B) acetone; (59.0:41.0, v/v, A:B)
flow rate . . . . . 1 mL/min
detector . . . . . RI
injection . . 10 µL of 9% C8 to C18 saturated and unsaturated FAMEs in mobile phase
Application No. . . . . 797-0501



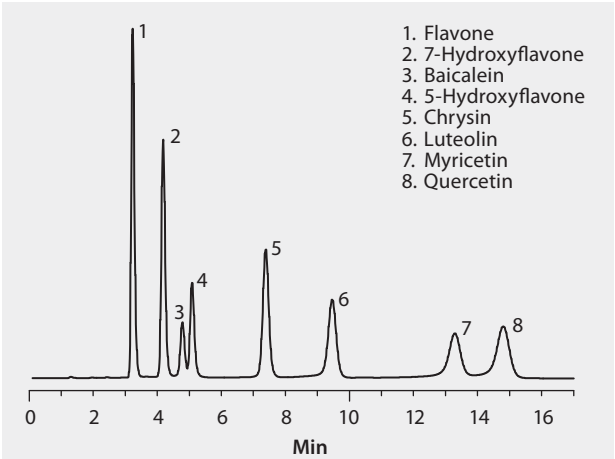
## HPLC Analysis of Fatty Acids on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 0.1% formic acid in water: (B) acetonitrile; (5:95, A;B)
flow rate . . . . . 1.0 mL/min., split to detector
column temp. . . . . 35 °C
detector . . . . . ESI(-)
injection . . . . . 5 µL
sample . . . . . 10 µg/mL in acetonitrile
Application No. . . . . G002905



## HPLC Analysis of Flavones on Discovery® HS PEG

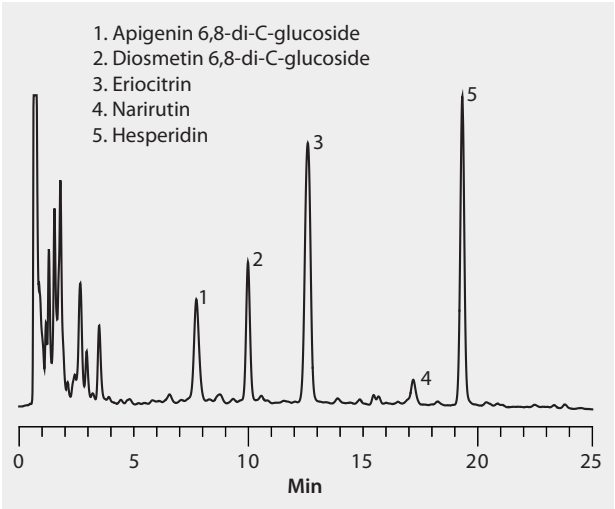
column . . . . Discovery HS PEG, 15 cm × 4.6 mm I.D., 5 µm particles (567416-U)
mobile phase . . . . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in methanol; (45:55, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL, each compound 50 µg/mL
Application No. . . . . G001665



## HPLC Analysis of Flavonoids in Lime Juice on Ascentis® Express C18

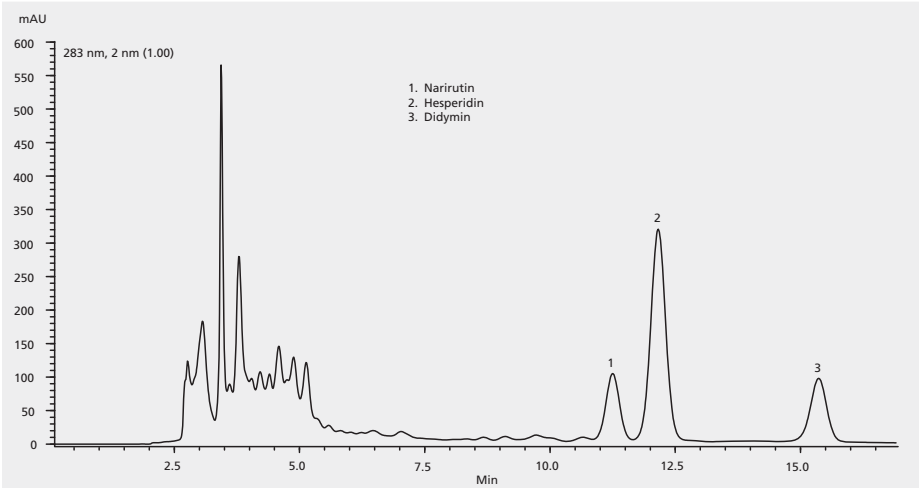
Flavonoids are polyphenolic compounds ubiquitous in nature and classified, according to their chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. They are widely present in fruits, vegetables, leaves, nuts, seeds, bark and beverages (tea, coffee, beer, wine and fruit drinks).

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . lime juice hand-squeezed in laboratory, centrifuged and filtered through a 0.45 µm nylon filter disc (Acrodisc)
column . . Ascentis Express C18, 5 cm x 4.6 mm I.D., 2.7 µm particles (53826-U)
mobile phase . . . . . (A) water/formic acid (99.9:0.1); (B) water/acetonitrile/isopropanol/formic acid (39.9:20:40:0.1)
gradient . . . 10% B for 3 min; 10 to 34% B in 30 min; 34 to 100% B in 1 min; held at 100% B for 6 min; 100 to10% B in 1 min
flow rate . . . . . 0.8 mL/min
column temp. . . . . ambient
detector . . . . . PDA: 190–370 nm; chromatogram extracted at 283 nm
detector . . . . . ESI-MS: mass spectral range, 250-700 m/z; interval, 0.5 s; scan speed, 938 amu/s; nebulizing gas (N2) flow, 1.5 L/min; (interface temperature, 350 °C; heat block, 300 °C; desolvation line (DL) temperature, 300 °C; DL voltage, 34 V; probe voltage, +4.5 kV; Qarray voltage, 1.0 V; RF voltage, 90 V; detection gain, 1.05 kV)
injection . . . . . 2 µL
Application No. . . . . G005593



## HPLC Analysis of Flavonoids in Orange Juice on Ascentis® C18

column . . . . . Ascentis C18, 25 cm × 4.6 mm I.D., 5 µm particles (581325-U)
mobile phase . . . . . (A) formic acid: (B) 2-propanol: (C) acetonitrile: (D) water (0.1:10:12:78, A:B:C:D:)
flow rate . . . . . 0.8 mL/min
column temp. . . . . 25 °C
detector . . . . . PDA, 283 nm
injection . . . . . 10 µL
sample . . . . . orange juice, passed through a 0.45 µm filter
Application No. . . . . G003936



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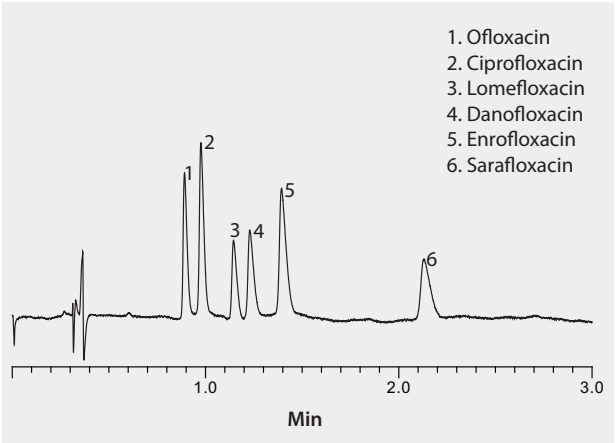
## HPLC Analysis of Fluoroquinolone Antibiotics on Ascentis® Express C18

This application demonstrates the suitability of the Ascentis Express C18 for the analysis of fluoroquinolones.

column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) 0.1% formic acid in water;
(B) 0.1% formic acid in acetonitrile; (85:15, A:B)
flow rate . . . . . 1.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 4 µL
sample . . . . . 200 ng/mL in 25:75, water: acetonitrile
Application No. . . . . G005699



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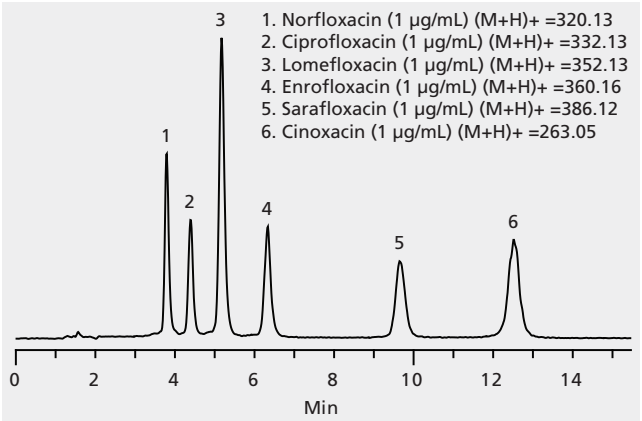
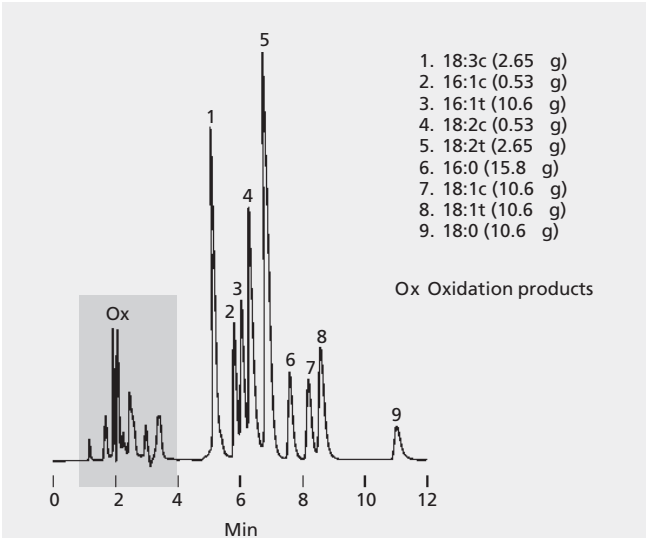
## HPLC Analysis of Fluoroquinolone Antibiotics on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 13 mM ammonium formate (pH 3.0 with concentrated formic acid): (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1 mL/min, split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 80:20, 13 mM ammonium formate (pH 3.0 with concentrated formic acid):acetonitrile
Application No. . . . . G003713



## HPLC Analysis of Free Fatty Acids on SUPELCOSIL™ LC-8

column . . . . . SUPELCOSIL LC-8, 15 cm × 4.6 mm I.D., 3 µm particles (58983)
mobile phase . . . acetonitrile:tetrahydrofuran: 0.1% phosphoric acid (50.4:21.6:28)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 10 µL
Application No. . . . . 713-0969





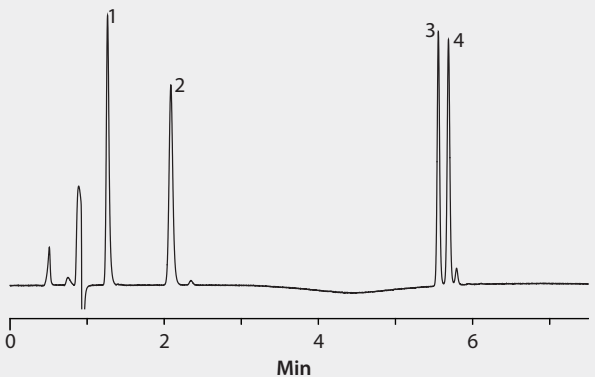

SUPELCO® Solutions within.™

## HPLC Analysis of Fusarium Mycotoxins on Ascentis® Express Phenyl-Hexyl

The trichothecene vomitoxins of Fusarium are significant plant pathogens of grain and forage crops that can lead to anorexic effects with livestock. This application demonstrates resolution of deoxynivalenol and three of its analogs.

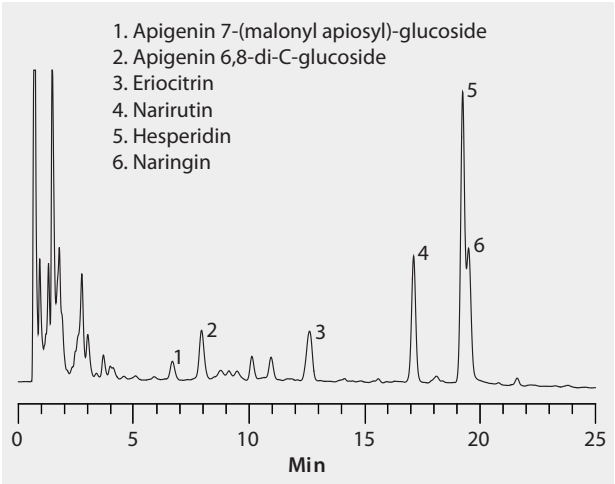
| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 10 cm x 2.1 mm I.D., 2.7 µm particles (53336-U) |
| mobile phase | (A) water, acetonitrile, 90:10; (B) acetonitrile |
| gradient | 0% B held for 0.5 min; to 15% B in 1 min; held at 15% B for 6 min |
| flow rate | 0.3 mL/min |
| pressure | 192 bar (2780 psi) |
| column temp. | 30 °C |
| detector | UV, 218 nm |
| injection | 10 µL |
| sample | 5 mg/L ea. in 95:5, water:acetonitrile |
| Application No. | G005539 |

1. Nivalenol
2. Deoxynivalenol
3. 15-Acetyldeoxynivalenol
4. 3-Acetyldeoxynivalenol



## HPLC Analysis of Grapefruit Juice on Ascentis® Express C18 Column

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . grapefruit juice hand-squeezed in laboratory, centrifuged and filtered through a 0.45 µm nylon filter disc (Acrodisc)
column . . Ascentis Express C18, 5 cm x 4.6 mm I.D., 2.7 µm particles (53826-U)
mobile phase . . . . . (A) water/formic acid (99.9:0.1); (B) water/acetonitrile/isopropanol/formic acid (39.9:20:40:0.1)
gradient . . . 10% B for 3 min; 10 to 34% B in 30 min; 34 to 100% B in 1 min; held at 100% B for 6 min; 100 to10% B in 1 min
flow rate . . . . . 0.8 mL/min
column temp. . . . . ambient
detector . . . . . PDA: 190–370 nm; chromatogram extracted at 283 nm
detector . . . . . ESI-MS: mass spectral range, 250-700 m/z; interval, 0.5 s; scan speed, 938 amu/s; nebulizing gas (N2) flow, 1.5 L/min; interface temperature, 350 °C; (heat block, 300 °C; desolvation line (DL) temperature, 300 °C; DL voltage, 34 V; probe voltage, +4.5 kV; Qarray voltage, 1.0 V; RF voltage, 90 V; detection gain, 1.05 kV.)
injection . . . . . 2 µL
Application No. . . . . G005592

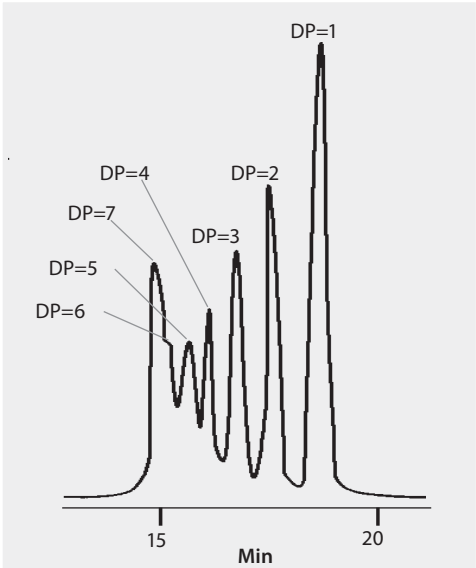





SUPELCO® Solutions within.™

## HPLC Analysis of Hydrolyzed β-Cyclodextrin on TSKgel® G-Oligo-PW

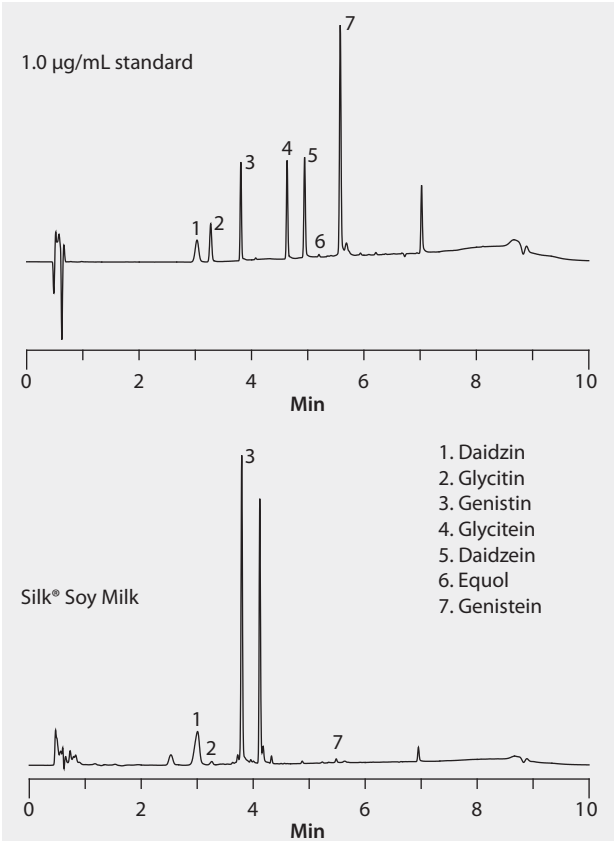
column . . . . TSKgel G-Oligo-PW, 30 cm × 7.8 mm I.D., 6 µm particles (808031)
mobile phase . . . . . distilled water
flow rate . . . . . 1 mL/min
column temp. . . . . 60 °C
detector . . . . . RI
Application No. . . . . 713-0917



## HPLC Analysis of Isoflavones in Soy Milk on Ascentis® Express RP Amide (Milk Brand 1)

This application demonstrates the separation of seven isoflavones on Ascentis Express RP Amide

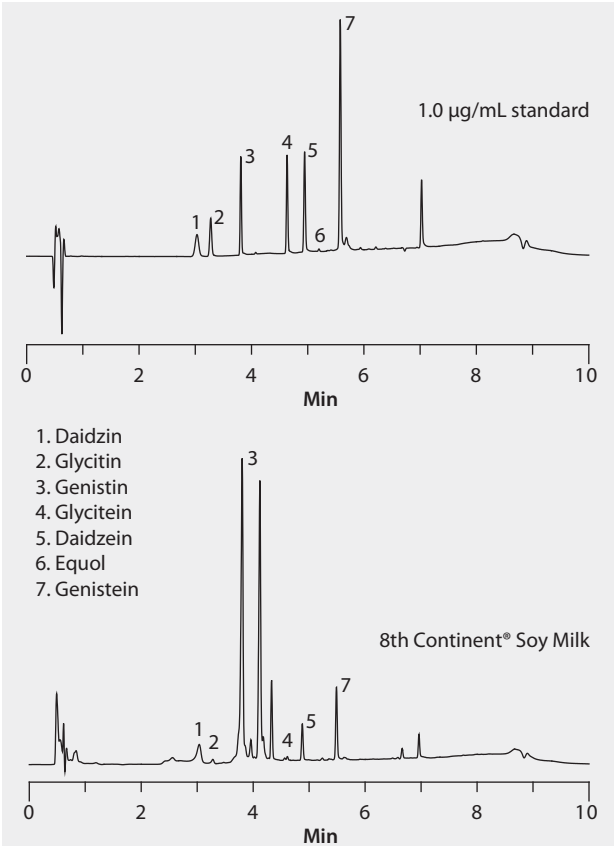
column . . Ascentis Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in methanol
gradient . . . . . held at 15% B for 1 min; 15 to 100% in 5 min; held at 100% B for 1 min; 100-15% B in 0.1 min; held at 15% B for 2.9 min
flow rate . . . . . 0.8 mL/min
pressure . . . . . 2900 psi (200 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 260 nm
injection . . . . . 10 µL
sample . . soy milk, methanol extract (1. Measure 2 mL of each brand of soy milk (6 samples) 2. Add 8 mL of methanol and shake for 1 hour 3. Centrifuge tubes at 10000 rpm for 30 minutes 4. Transfer aliquot to autosampler vial)
Application No. . . . . G005918



## HPLC Analysis of Isoflavones in Soy Milk on Ascentis® Express RP Amide (Milk Brand 2)

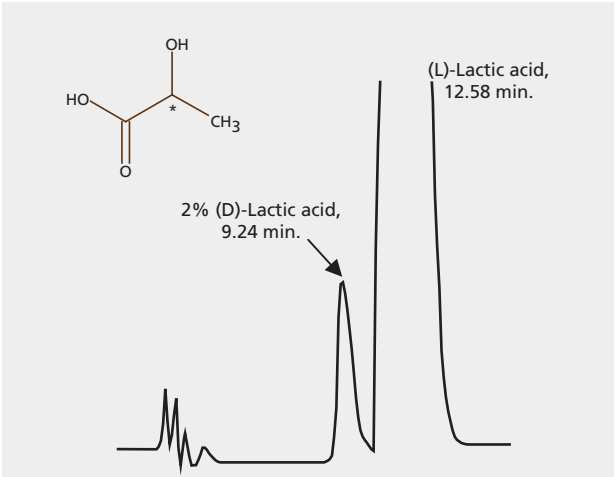
This application demonstrates the separation of seven isoflavones on Ascentis Express RP Amide

column . . Ascentis Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in methanol
gradient . . . . . held at 15% B for 1 min; 15 to 100% in 5 min; held at 100% B for 1 min; 100-15% B in 0.1 min; held at 15% B for 2.9 min
flow rate . . . . . 0.8 mL/min
pressure . . . . . 2900 psi (200 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 260 nm
injection . . . . . 10 µL
sample . . . . . soy milk, methanol extract (1. Measure 2 mL of each brand of soy milk (6 samples) 2. Add 8 mL of methanol and shake for 1 hour 3. Centrifuge tubes at 10000 rpm for 30 minutes 4. Transfer aliquot to autosampler vial)
Application No. . . . . G005917



## HPLC Analysis of Lactic Acid Enantiomers on Astec® CLC-L, Trace Level Detection

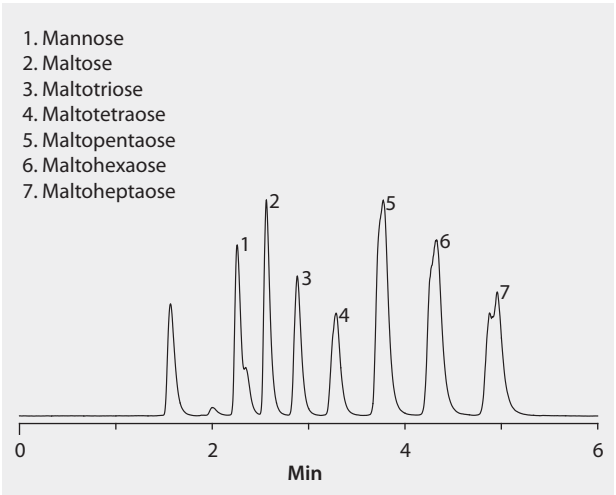
column . . . . . Astec CLC-L, 15 cm x 4.6 mm I.D., 5 µm particles (53123AST)
mobile phase . . . . . 5 mM copper sulfate
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL in water
Application No. . . . . G004663



## HPLC Analysis of Mannose and Maltose Sugars on Ascentis® Express HILIC

This application demonstrates the suitability of the Ascentis Express HILIC for the analysis of sugars. Structures along with the optimized chromatogram obtained on the Ascentis Express HILIC are presented. Separation is by normal-phase mechanism as a decrease in mobile phase water content results in increased retention.

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (25:75, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . ELSD, 55 °C, 3.5 bar nitrogen
injection . . . . . 10 µL
Application No. . . . . G005745

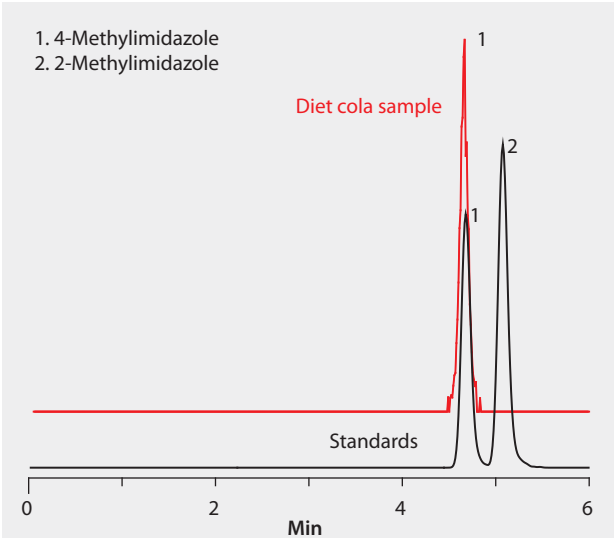


## HPLC Analysis of Methylimidazoles in Diet Cola on Ascentis® Express HILIC

This method features fast analysis of methylimidazole derivatives used in caramel-colored carbonated beverages.

The purpose of this study was to develop a fast analytical method for analyzing for 2-methylimidazole and 4-methylimidazole in caramel colored carbonated beverages. The FDA has recently increased the awareness of the potential hazards of methylimidazole. These compounds can be found in caramel additives used in a broad range of food stuff. In this study the focus is the levels of methylimidazole in various colas. Methylimidazoles are a difficult group of compounds to analyze due their polar nature and low molecular weight. Traditional reversed phase separation are unsuccessful in retaining these small polar compounds, with most methods utilizing ion-exchange resins for analysis. Other common methods are GC analysis with sample derivitization.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . 2 mM ammonium formate in 5:95, water:acetonitrile, to pH 4.4 with formic acid
flow rate . . . . . 0.6 mL/min
column temp. . . . . 50 °C
detector . . . . . ESI(+), 50-800 m/z
injection . . . . . 1 µL
sample . . . . . 200 mg/L in methanol
Application No. . . . . G005469



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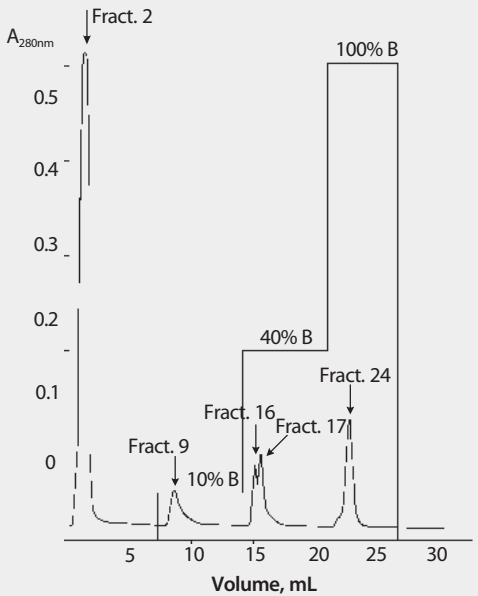
## HPLC Analysis of Milk Proteins on HiTrap® SP

column . . . . . HiTrap SP, 1 mL (54817)
mobile phase . . . . . (A) 50 mM sodium acetate, pH 6.0;
(B) 50 mM sodium acetate/1.0 M sodium chloride, pH 6.0
flow rate . . . . . 1 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 0.5 mL filtered casein-precipitated human milk
(buffer exchanged to mobile phase A)
Application No. . . . . 795-0493

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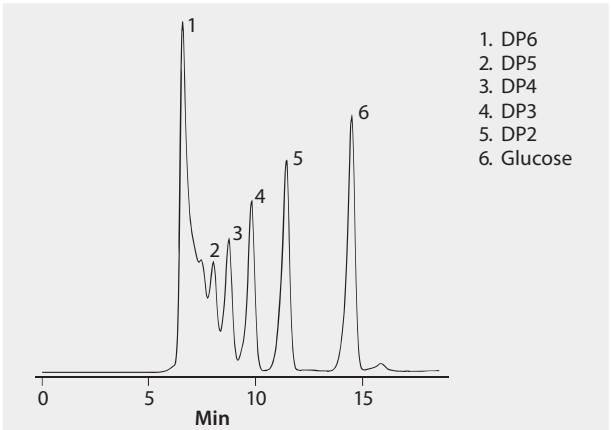
## HPLC Analysis of Oligosaccharides from Dark Corn Syrup on SUPELCOGEL™ Ag

column . . . . . SUPELCOGEL Ag, 30 cm × 7.8 mm I.D. (59315)
mobile phase . . . . . water
flow rate . . . . . 0.5 mL/min
column temp. . . . . 85 °C
detector . . . . . RI
injection . . . . . 10 µL of 1g syrup/10 mL DI water, filtered (0.20 µm filter)
Application No. . . . . 794-0029



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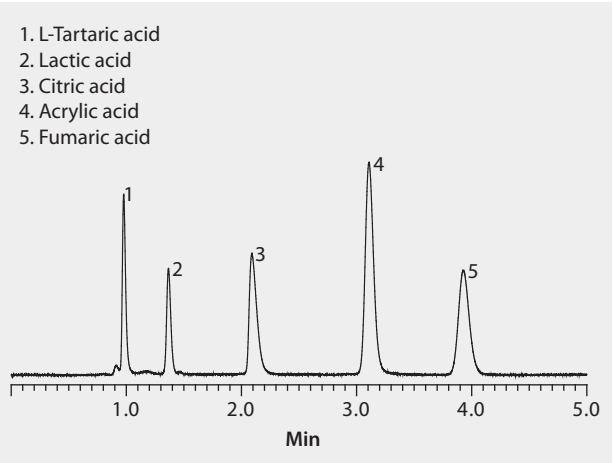
## HPLC Analysis of Organic Acids on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the analysis of organic acids under 100% aqueous conditions.

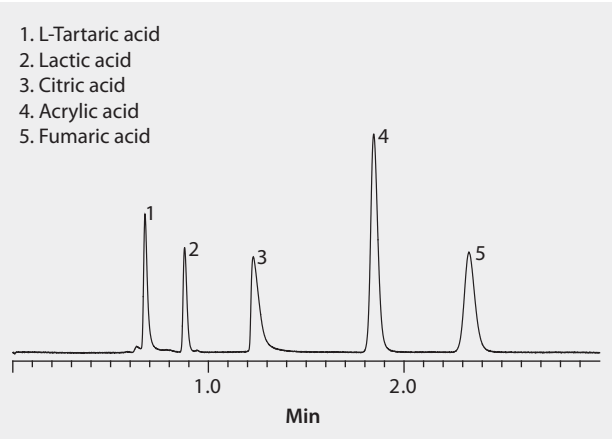
| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U) |
| mobile phase | (A) 0.1% (v/v) TFA in (B) water |
| flow rate | 0.3 mL/min |
| column temp. | 35 °C |
| detector | UV, 210 nm |
| injection | 1 µL |
| sample | 0.2 - 4 g/L in mobile phase |
| Application No. | G005714 |



## HPLC Analysis of Organic Acids on Ascentis® RP-Amide

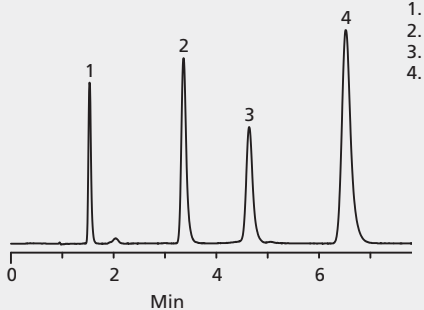
This application demonstrates the suitability of Ascentis RP-Amide for the analysis of organic acids under 100% aqueous conditions.

column . . . Ascentis RP-Amide, 10 cm x 2.1 mm I.D., 3 µm particles (565301-U)
mobile phase . . . . . (A) 0.1% (v/v) TFA in (B) water
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 1 µL
sample . . . . . 0.2 - 4 g/L in mobile phase
Application No. . . . . G005715



## HPLC Analysis of Organic Acids on Discovery® C18

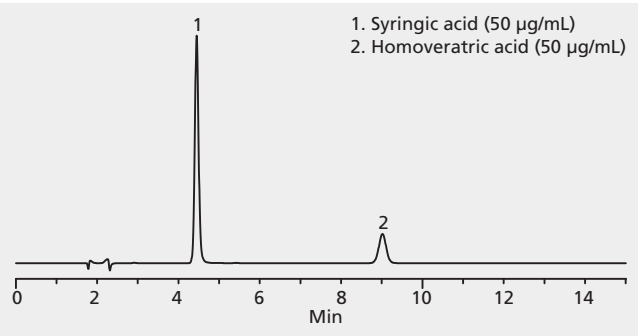
column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) methanol; (B) water, 0.1% TFA (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL each analyte
Application No. . . . . 797-0657




1. Homovanillic acid
2. Sorbic acid
3. Salicylic acid
4. p-Toluic acid

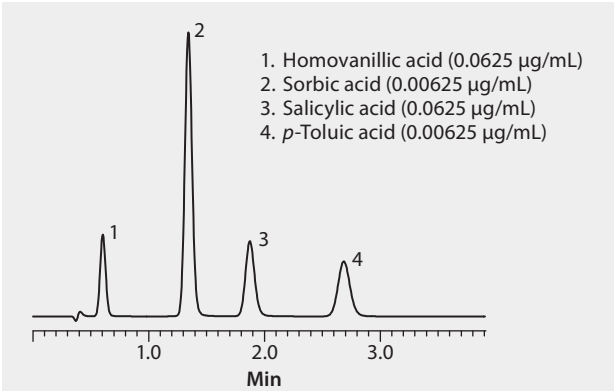
## HPLC Analysis of Organic Acids on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) 10 mM ammonium phosphate monobasic (pH 2.50 with phosphoric acid): (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003160



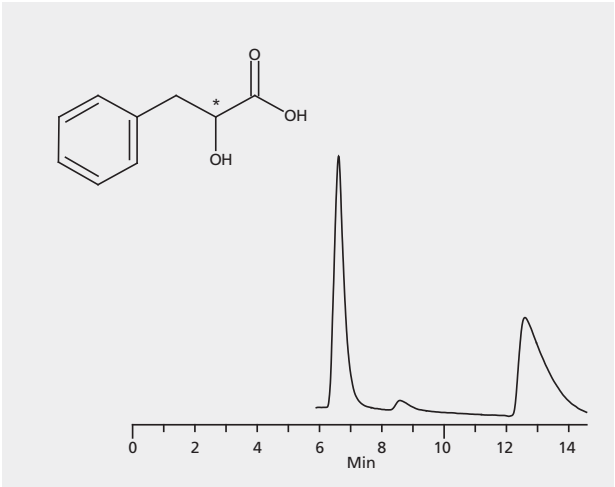
## HPLC Analysis of Organic Acids on Discovery® C18

| | |
|---|---|
| column | Discovery C18, 5 cm × 4.6 mm I.D., 5 µm particles (504947) |
| mobile phase | (A) 0.1% TFA in (B) water:methanol; (60:40, A:B) |
| flow rate | 2.0 mL/min |
| column temp. | 20 °C |
| detector | UV, 254 nm |
| injection | 10 µL |
| Application No. | G001431 |



## HPLC Analysis of 3-Phenyllactic Acid Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1% ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 0.5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004680



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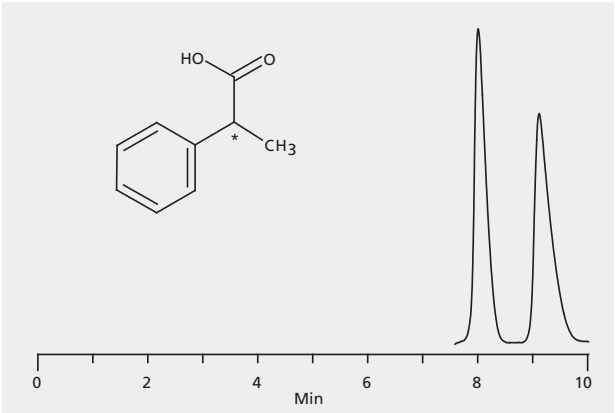
## HPLC Analysis of 2-Phenylpropionic Acid Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.0; (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 235 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004717



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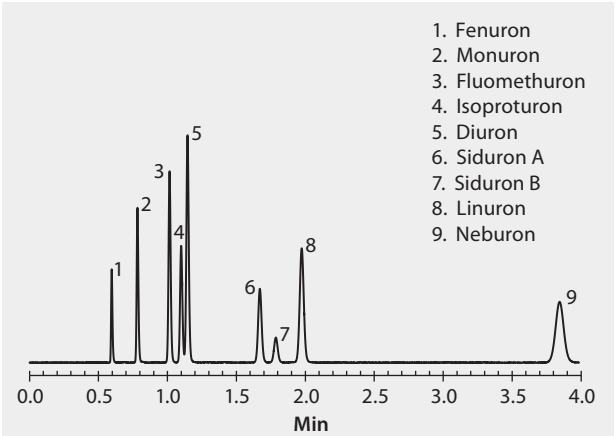
## HPLC Analysis of Phenyl Urea Pesticides on Ascentis® Express C18 Phase

This separation illustrates the use of the highly efficient 2.7 um Ascentis Express Fused-Core C18 stationary phase in the analysis of some popular herbicides. The short run times allow analyses using isocratic conditions so that column equilibration time is not required between runs.

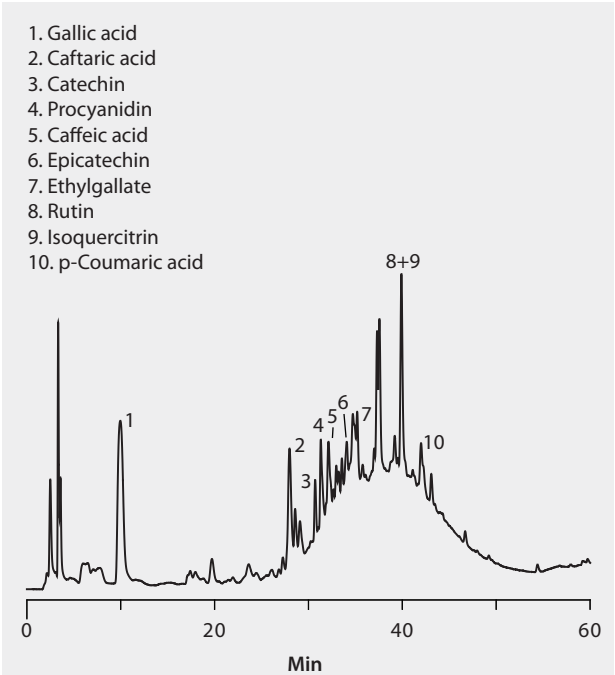
**Response Time**: 0.02 sec.
**Flow Cell**: 2.5 µL semi-micro

column . . Ascentis® Express C18, 10 cm x 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) 0.025 M potassium phosphate buffer, adj. to pH = 2.5, (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 2.0 mL/min
pressure . . . . . 300 bar
column temp. . . . . 30 °C
detector . . . . . UV 245 nm, VWD
injection . . . . . 0.5 µL
sample . . . . . acetonitrile
Application No. . . . . G006189



## HPLC Analysis of Polyphenols in Nero d'Avola Red Wine on Discovery® HS C18 (UV 280 nm)

sample/matrix . . . . . Nero d'Avola wine sample filtered prior injection through a 0.45 µm nylon membrane
column . . . . Discovery HS C18, 15 cm x 2.1 mm I.D., 3 µm particles (569255-U)
mobile phase . . . . . (A) water, pH 3 with formic acid; (B) acetonitrile, pH 3 with formic acid
flow rate . . . . . 0.2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 280 nm
injection . . . . . 5 µL
Application No. . . . . G005595

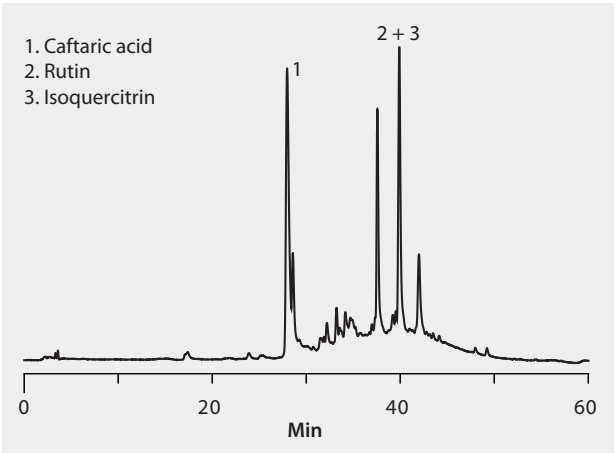





SUPELCO® Solutions within.™

## HPLC Analysis of Polyphenols in Nero d'Avola Red Wine on Discovery® HS C18 (UV 354 nm)

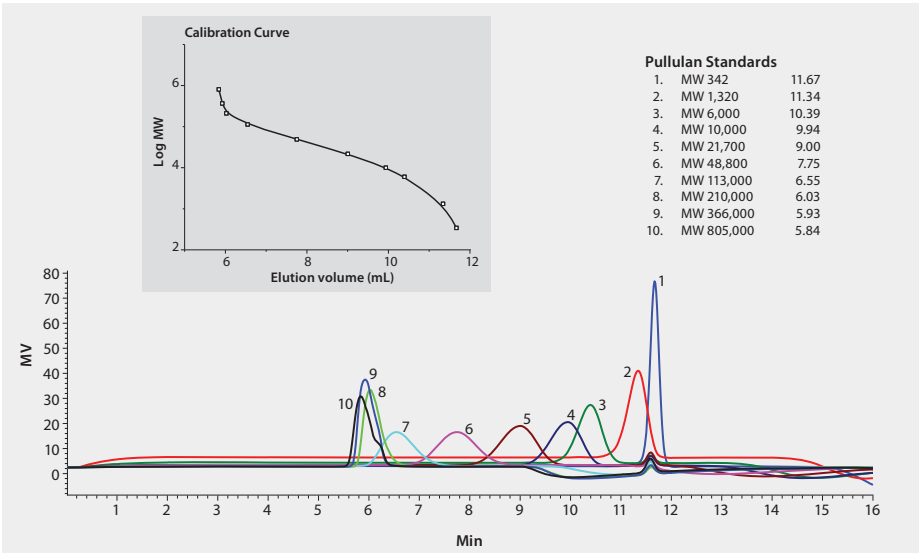
sample/matrix . . . . . Nero d'Avola wine sample filtered prior injection through a 0.45 µm nylon membrane
column . . . . Discovery HS C18, 15 cm x 2.1 mm I.D., 3 µm particles (569255-U)
mobile phase . . . . . (A) water, pH 3 with fomic acid; (B) Acetonitrile, pH 3 with fomic acid
flow rate . . . . . 0.2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 354 nm
injection . . . . . 5 µL
Application No. . . . . G005596



## HPLC Analysis of Pullulan on an SRT® SEC-300 Gel Filtration Column

Pullulan is a water-soluble polysaccharide polymer consisting of glucan units. Pullulan is used in the manufacture of edible films that are used in breath fresheners and mints and is also used as a food additive. Another use of pullulan is as calibration standards in gel filtration chromatography.
Gel filtration chromatography (GFC) is used to determine the molecular mass composition of pullulan and many other water-soluble polymers using silica- or polymer-based particles that have been modified with a chemically bonded hydrophilic coating.
In this particular example, a SRT SEC-300 column from Sepax Technologies was used to analyze several pullulan standards. In the accompanying table shows retention volumes for the pullulan standards and the molecular mass of each; this data was then used to construct the calibration curve which allows the calculation of the molecular mass and distribution of an unknown pullulan polymer or other polymers of similar geometrical shape.

column . . . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm, 300 Å (Z777051)
mobile phase . . . . . 150 mM phosphate Buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
pressure . . . . . 600 psi (41.4 bar)
column temp. . . . . 25 °C
detector . . . . . RI (30° C)
injection . . . . . 20 µL
sample . . . 10 mg/mL in mobile phase 0.342 kD, 1.32 kD, 6 kD, 10 kD, 21.7 kD, 48.8 kD 113 kD, 210 kD, 366 kD and 805 kD
Application No. . . . . G006202

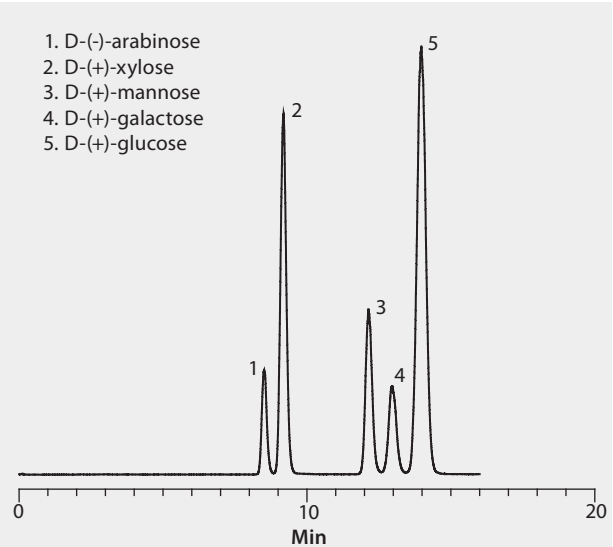





SUPELCO® Solutions within.™

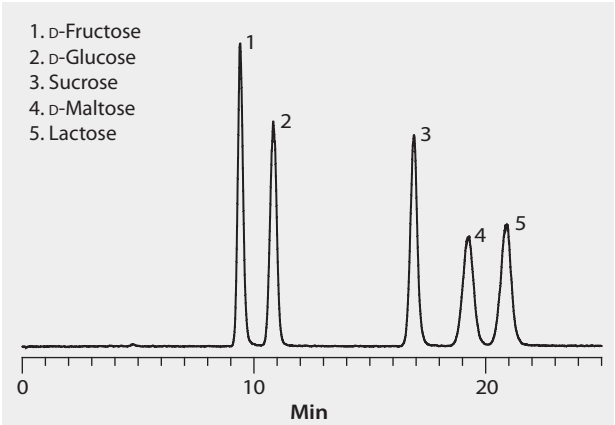
## HPLC Analysis of Simple Underivatized Sugars on apHera™ NH2

column . . . . . apHera NH2, 15 cm × 4.6 mm I.D., 5 µm particles (56401AST)
mobile phase . . . . . (A) water: (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . ELSD, 45 °C, 3.5 psi nitrogen
injection . . . . . 10 µL
sample . . . . . 500 µg/mL in 30:70, water: acetonitrile
Application No. . . . . G003996



## HPLC Analysis of Sugars and Sugar Enantiomers on Astec® CYCLOBOND® I 2000

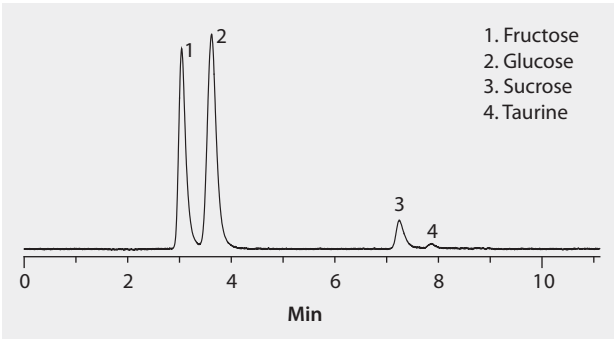
column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (15:85, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . ELSD at 35°C and 3.2 bar nitrogen
injection . . . . . 10 µL
sample . . . . . 0.5 mg/mL each in water:acetonitrile, 25:75
Application No. . . . . G004682



## HPLC Analysis of Sugars and Taurine in an Energy Drink on Ascentis® Express HILIC using UV and ELSD Detection in Series

This application demonstrates the suitability of Ascentis Express HILIC for the efficient separation of common drink additives used in caffeinated energy drinks. The analysis was performed with UV and ELSD detectors in series, this chromatogram being from the ELSD.

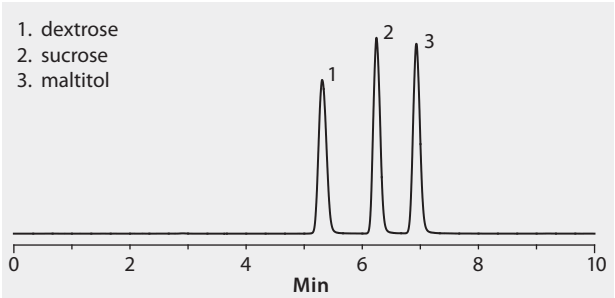
column . . Ascentis Express HILIC, 10 cm x 3.0 mm I.D., 2.7 µm particles (53970-U)
mobile phase . . . . . (A) 100 mM ammonium acetate, pH 5.0 with acetic acid; (B) water; (C) acetonitrile; (09:01:90, A:B:C)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 812 psi (56 bar)
column temp. . . . . 35 °C
detector . . . . . ELSD, 55 °C, 3.5 bar nitrogen
injection . . . . . 2 µL
sample . . . . . dilute1:9 in acetonitrile
Application No. . . . . G005751



## HPLC Analysis of Sugars by HILIC Chromatography using Ascentis® Express OH-5 Column with ELSD Detection

This application shows the analysis of sugars by HILIC Chromatography with ELSD detection. The purpose of the work shown here was to develop a HILIC method that could quickly separate mono- and di-saccharides with good sensitivity and reproducibility utilizing the Ascentis Express OH-5 column and an Evaporative Light Scattering Detecctor (ELSD) detector.

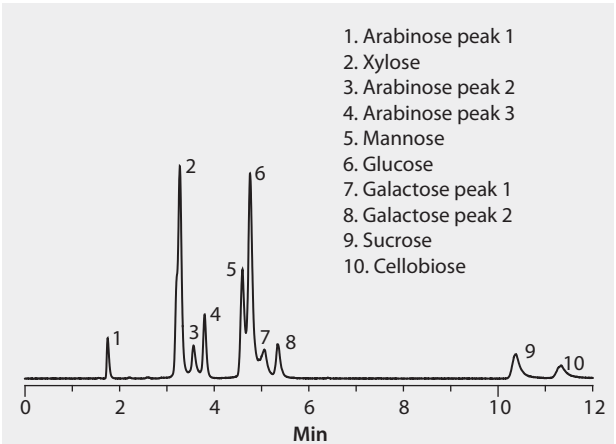
column . . Ascentis Express OH-5, 15 cm x 4.6 mm, I.D., 2.7 µm particles (53778-U)
mobile phase . . . . . (A) 0.005 M ammonium formate, pH 3.15 with concentrated TFA (B) acetonitrile (30:70, A:B)
flow rate . . . . . 0.5 mL/min
pressure . . . . . 841 psi (58 bar)
column temp. . . . . 35 °C
detector . . . . . ELSD (detector other: Nebulizer 60º C, Evaporator 80º C, Gas flow 1.60 SLM (Standard Liters/ Minute), LED 100%, Smoothing 5.0 S, PMT ( Photomultiplier) Gain 1.0)
injection . . . . . 10 µL
sample . . . . . 0.5 mg/mL each prepared in 50:50 Water:Acetonitrile
Application No. . . . . G005902



## HPLC Analysis of Sugars on Ascentis® Express HILIC (Mobile Phase 5:95)

This application demonstrates the suitability of the Ascentis Express HILIC for the analysis of sugars. Structures along with the optimized chromatogram obtained on the Ascentis Express HILIC are presented. Separation is by normal-phase mechanism as a decrease in mobile phase water content results in increased retention

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (5:95, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . ELSD, 55 °C, 3.5 bar nitrogen
injection . . . . . 10 µL
Application No. . . . . G005744



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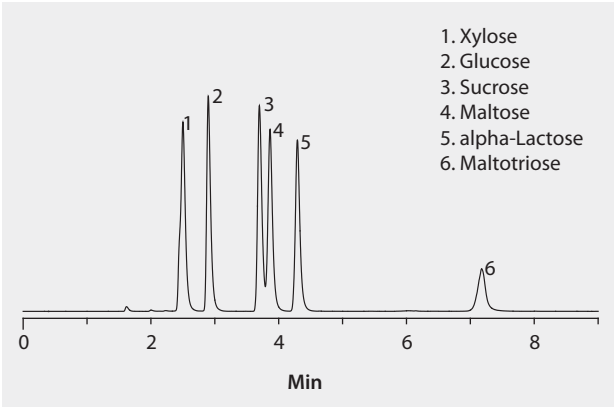
## HPLC Analysis of Sugars on Ascentis® Express HILIC (Mobile Phase 15:85)

This application demonstrates the suitability of the Ascentis Express HILIC for the analysis of sugars. Structures along with the optimized chromatogram obtained on the Ascentis Express HILIC are presented. Separation is by normal-phase mechanism as a decrease in mobile phase water content results in increased retention.

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (15:85, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . ELSD, 55 °C, 3.5 bar nitrogen
injection . . . . . 10 µL
Application No. . . . . G005746



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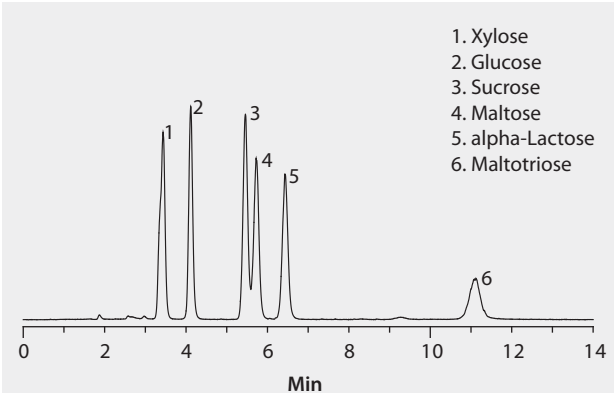
## HPLC Analysis of Sugars on Ascentis® Si

This application demonstrates the suitability of the Ascentis Si for the analysis of sugars. Structures along with the optimized chromatogram obtained on the Ascentis Si are presented. Separation is by normal-phase mechanism as a decrease in mobile phase water content results in increased retention.

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (15:85, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . ELSD, 55 °C, 3.5 bar nitrogen
injection . . . . . 10 µL
Application No. . . . . G005747



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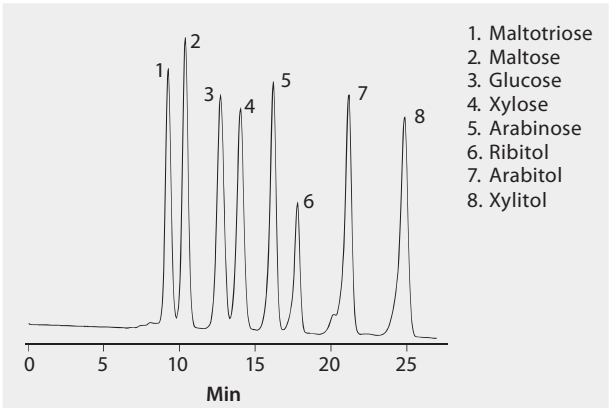
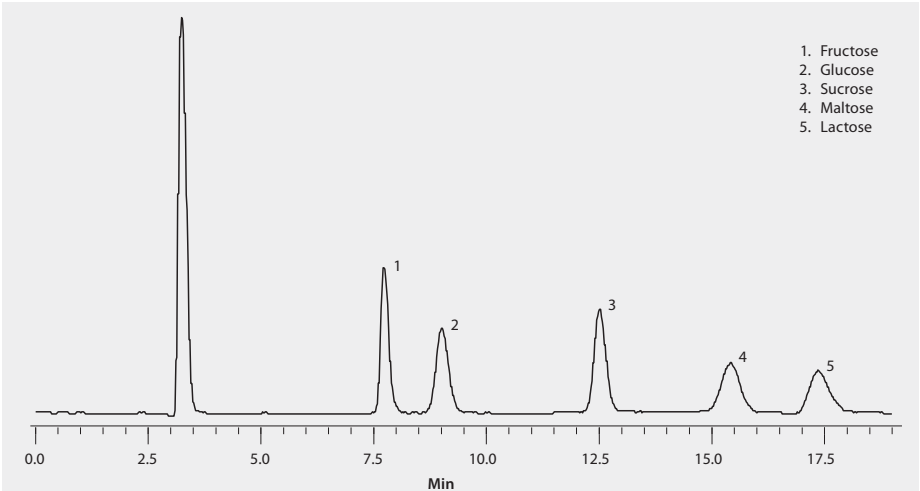
## HPLC Analysis of Sugars on SUPELCOGEL™ Ca

column . . . . . SUPELCOGEL Ca, 30 cm × 7.8 mm I.D., 9 µm particles (59305-U)
mobile phase . . . . . water
flow rate . . . . . 1.5 mL/min
column temp. . . . . 80 °C
detector . . . . . RI
injection . . . . . 10 µL, 2.5 mg/mL each analyte in water
Application No. . . . . 795-0016



## HPLC Analysis of Sugars on SUPELCOSIL™ LC-NH2

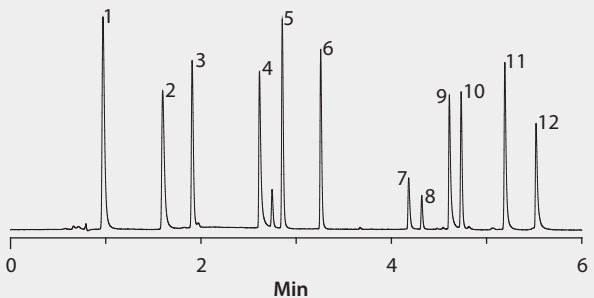
column . . . . . SUPELCOSIL LC-NH2, 25 cm x 4.6 mm I.D., 5 µm particles (58338)
mobile phase . . . . . (A) water; (B) acetonitrile; (25:75, A:B)
flow rate . . . . . 1 mL/min
temp. . . . . ambient
detector . . . . . refractive index (RI)
injection . . . . . 2 µL
sample . . . . . 10 mg/mL in water
Application No. . . . . G006195



## HPLC Analysis of Synthetic Food Dyes on Ascentis® Express C18

column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) 100 mM ammonium phosphate monobasic, pH 7.0 with ammonium hydroxide; (B) water; (C) acetonitrile
gradient . . . . . 10% A constant; 85 to 25% B, 5 to 65% C in 6 min; 25% B, 65% C held for 5 min
flow rate . . . . . 0.3 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 20 µg/mL each in water
Application No. . . . . G005650

1. Tartrazine
2. Amaranth
3. Indigo carmine
4. New coccine (Acid Red 18)
5. Sunset yellow FCF
6. Allura red
7. Fast green
8. Erioglaucine
9. Erythrosin B
10. Acid red
11. Phloxine B
12. Rose bengal



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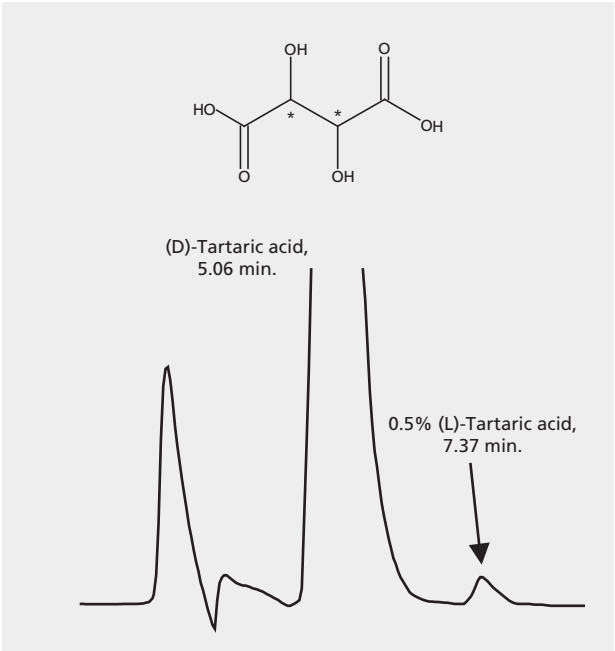
## HPLC Analysis of Tartaric Acid Enantiomers on Astec® CLC-D, Trace Level Detection

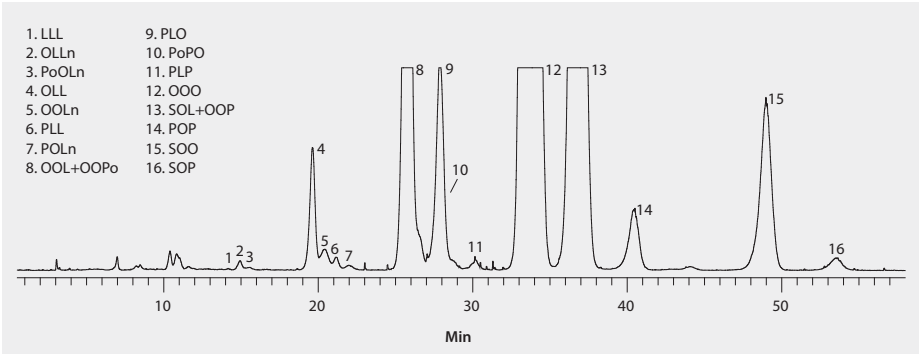
column . . . . . Astec CLC-D, 15 cm x 4.6 mm I.D., 5 µm particles (53023AST)
mobile phase . . . . . 3 mM copper sulfate, pH 3.2
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL in water
Application No. . . . . G004665



## HPLC Analysis of Triacylglycerols in Olive Oil on Discovery® HS C18 after Sample Prep using Discovery® DSC-Si (Acetone-Acetonitrile Mobile Phase, ELSD Detection)

Olive oil was pre-cleaned using Silica SPE, analyzed as 5% sample in acetone (w/v).

SPE tube/cartridge . . . . . Discovery DSC-Si, 1 gm/6 mL (52656-U)
condition . . . . . 6 mL hexane
sample addition . . . . . 0.12 g oil in 0.5 mL hexane
elution . . . . . 10 mL hexane-diethyl ether 87-13 (v/v)
eluate post-treatment . . . evaporation to dryness, reconstituted into 2 mL acetone
column . . . . Discovery HS C18, 25 cm x 4.6 mm I.D., 5 µm particles (568523-U)
mobile phase . . . . . (A) acetone; (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 1.5 mL/min
pressure . . . . . 914 psi (63 bar)
column temp. . . . . 30 °C
detector . . . . . ELSD
injection . . . . . 5 µL
Application No. . . . . G005863



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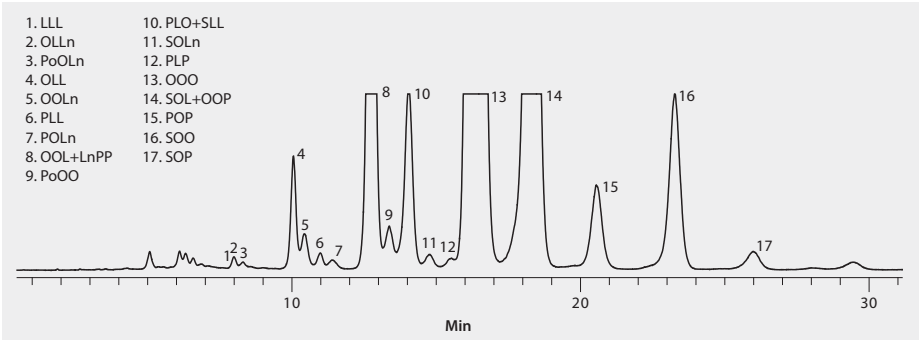
## HPLC Analysis of Triacylglycerols in Olive Oil on Discovery® HS C18 after Sample Prep using Discovery® DSC-Si (Propionitrile Mobile Phase, ELSD Detection)

Olive oil was pre-cleaned using Silica SPE, analyzed as 5% sample in acetone (w/v).

SPE tube/cartridge . . . . . Discovery DSC-Si, 1 gm/6 mL (52656-U)
condition . . . . . 6 mL hexane
sample addition . . . . . 0.12 g oil in 0.5 mL hexane
elution . . . . . 10 mL hexane-diethyl ether 87-13 (v/v)
eluate post-treatment . . . evaporation to dryness, reconstituted into 2 mL acetone
column . . . . Discovery HS C18, 25 cm x 4.6 mm I.D., 5 µm particles (568523-U)
mobile phase . . . . . propionitrile
flow rate . . . . . 1.5 mL/min
pressure . . . . . 1117 psi (77 bar)
column temp. . . . . 30 °C
detector . . . . . ELSD
injection . . . . . 5 µL
Application No. . . . . G005864



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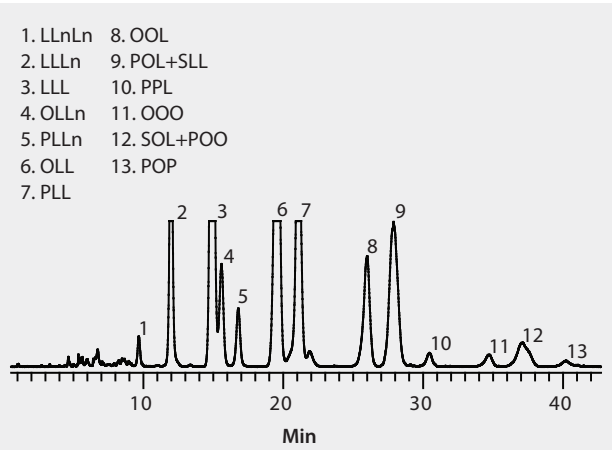
## HPLC Analysis of Triacylglycerols in Soybean Oil on Discovery® HS C18 (Acetone-Acetonitrile Mobile Phase, ELSD Detection)

Soybean oil was analyzed as 5% diluted sample in acetone (w/v)

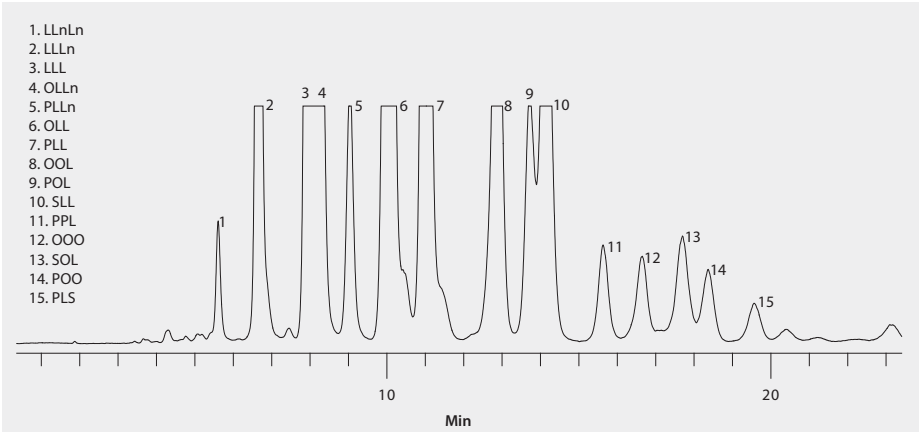
column . . . . Discovery HS C18, 25 cm x 4.6 mm I.D., 5 µm particles (568523-U)
mobile phase . . . . . (A) acetone; (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 1.5 mL/min
pressure . . . . . 914 psi (63 bar)
column temp. . . . . 30 °C
detector . . . . . ELSD
injection . . . . . 5 µL
sample . . . . . 5% soybean oil in acetone (w/v)
Application No. . . . . G005877



## HPLC Analysis of Triacylglycerols in Soybean Oil on Discovery® HS C18 after Sample Prep using Discovery® DSC-Si (Propionitrile Mobile Phase, ELSD Detection)

Soybean oil was cleaned using Silica SPE and analyzed as 5% sample in acetone (w/v).

condition . . . . . 6 mL hexane
SPE tube/cartridge . . . . . Discovery DSC-Si, 1 gm/6 mL (52656-U)
sample addition . . . . . 0.12 g oil in 0.5 mL hexane
elution . . . . . 10 mL hexane-diethyl ether 87-13 (v/v)
eluate post-treatment . . . . vaporation to dryness, reconstituted into 2 mL acetone
column . . . . Discovery HS C18, 25 cm x 4.6 mm I.D., 5 µm particles (568523-U)
mobile phase . . . . . propionitrile
flow rate . . . . . 1.5 mL/min
pressure . . . . . 1117 psi (77 bar)
column temp. . . . . 30 °C
detector . . . . . ELSD
injection . . . . . 5 µL
Application No. . . . . G005865



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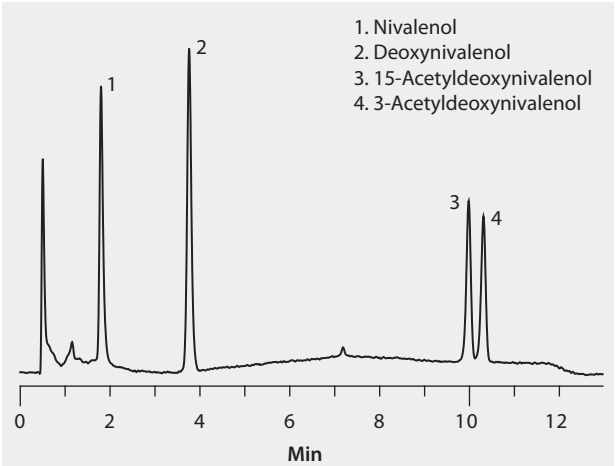
## HPLC Analysis of B-Trichothecenes on Ascentis® Express C18

Mycotoxins are toxic secondary metabolites produced by fungi. Those chemicals exist in food as a result of fungal infection of crops. Mycotoxins have strong resistance to decomposition and digestion, so they remain in the food chain in meat and dairy products. They also resist to temperature, such as cooking and freezing. Their effects on human and animal health include death, cancer, weakened immune systems and as allergens or irritants.

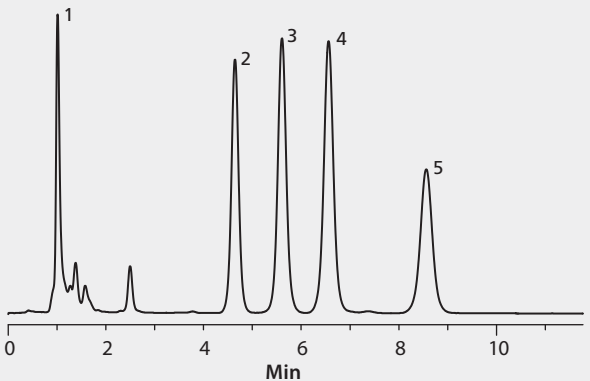
column . . . Ascentis Express C18, 10 cm x 3 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) water:acetonitrile:methanol (92:4:4); (B) acetonitrile
gradient . . 0% B held for 5 minutes, to 8% B in 3 minutes, 8% B held for 2 minutes
flow rate . . . . . 0.8 mL/min
pressure . . . . . 4061 psi (280 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 40 µL
sample . . . . . 2 ppm each in water:methanol:acetonitrile (92:4:4)
Application No. . . . . G005561



## HPLC Analysis of Vitamins, Fat Soluble (A and E) on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) methanol: (B) water (95:5, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
Application No. . . . . G000911A

1. Retinol acetate (Vitamin A acetate), 50 µg/mL
2. δ-Tocopherol, 165 µg/mL
3. γ-Tocopherol, 200 µg/mL
4. α-Tocopherol (Vitamin E), 292 µg/mL
5. α-Tocopherol acetate (Vitamin E acetate), 405 µg/mL

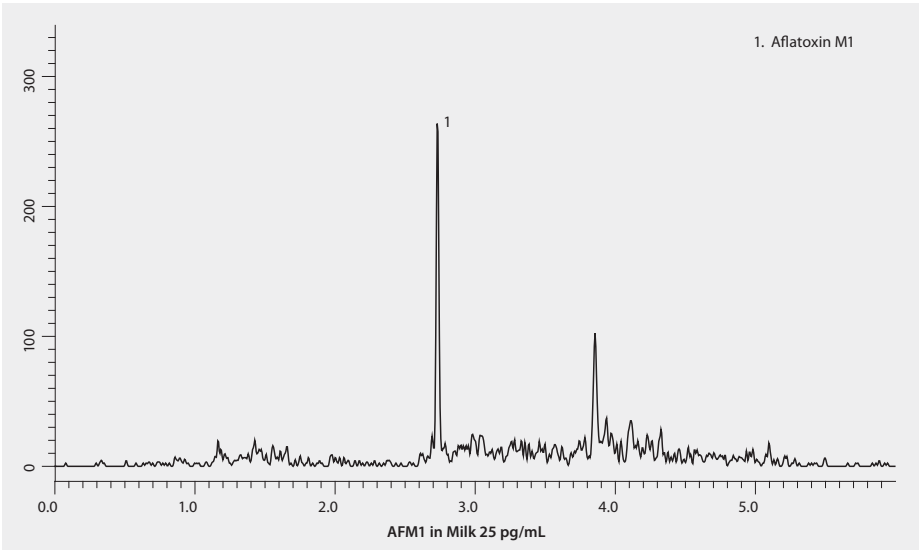


## LC-MS Analysis of Aflatoxin M1 in Milk on Ascentis® Express HILIC after SPE using Supel™ Tox AflaZea, Quantifying Picogram Concentrations

Aflatoxin M1 is the metabolite of Aflatoxin B1 and can be found in milk products when animals have been fed alatoxin-contminated feeds. Aflatoxins are positively associated with hepataocellular carcinomas. Aflattoxin M1 was detected in milk and quantified at concentrations fo 25 pg/mL. Sample preparation included a protein precipitation with solvent partitionng and sample clean-up using Supel Tox AflaZea SPE tubes. Analysis was perfomed via LC-MS using an Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm HPLC column for the separation. Detection and quanitification of aflatoxin M1 at 25 pg/mL satisfies the current regulatory requirements for aflatoxin M1 for EU treaty participants as stated in EC 1881/2006.

**Sample pretreatment:** centrifuge 45 g liquid milk for 60 min at 5000 rpm (RCF 4863 xg), cool at 0 °C for 30 minutes, remove fat layer, transfer middle layer to collection flask, add 20 mL acetonitrile to 50 mL centrifuge tube, add 20 mL of sample to acetonitrile, add contents of 1 Supel Q non-buffered tube, immedately disperse salt, quicky place on shaker for 5 minutes, centrifuge at 5000 rpm for 20 min, remove top layer (loading solution)

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . cow milk
SPE tube/cartridge . . . . . Supel™ Tox AflaZea, 6 mL (55314-U)
sample addition . . . . . 10 mL, 2 drop/second using vacuum
washing . . . . . 2 x 2 mL acetonitrile (collect with sample)
eluate post-treatment . . . . . evaporate to dryness at 70 °C using nitrogen
sample preparation . . . . . reconstitut in 500 µL 20:80 acetonitrile:water, filter into 750 µL poly vial using PVDF filter, 0.2 µm
column . . Ascentis Express C18, 50 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 0.1% (v/v) formic acid; (B) acetonitrile
gradient . . . . 90% A for 0.5 min; to 10% A in 0.5 min; held at 10% for 3 min; to 90% A in 0.10 min; held at 90% A for 3 min
flow rate . . . . . 0.4 mL/min
column temp. . . . . ambient
detector . . . . . MS/MS (MRM transitions)
injection . . . . . 10 µL
Application No. . . . . G006300



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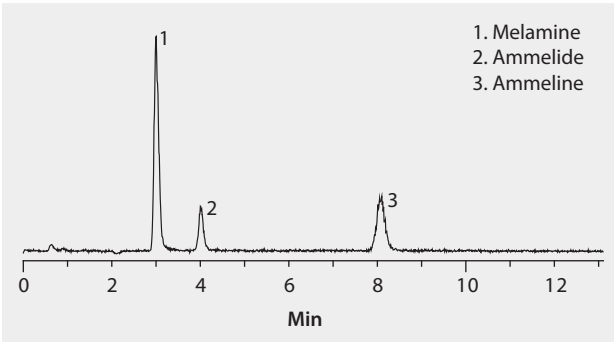
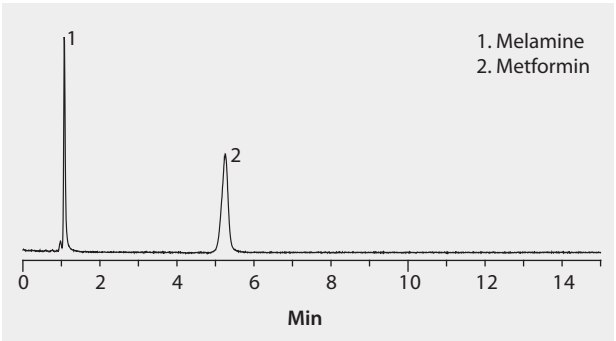
## LC-MS Analysis of Melamine and Hydrolysis Products on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 5 mM ammonium formate in 95:5 (v/v) acetonitrile:water
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+), full scan
injection . . . . . 2 µL
sample . . . . . 1 mg/L in mobile phase
Application No. . . . . G005708



## LC-MS Analysis of Melamine and Metformin on Ascentis® Express HILIC

column . . . Ascentis Express HILIC, 5 cm x 3 mm I.D., 2.7 µm particles (53967-U)
mobile phase . . (A) 10 mM ammonium formate in water; (B) acetonitrile; (5:95, A:B)
flow rate . . . . . 1.0 mL/min
pressure . . . . . 551 psi (38 bar)
column temp. . . . . 25 °C
detector . . . . . ESI(+), SIR, m/z 127, 130
injection . . . . . 2 µL
sample . . . . . 1 mg/L
Application No. . . . . G005754



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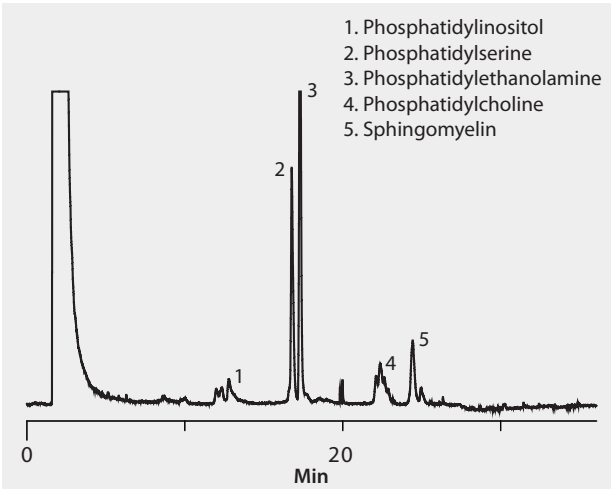
## LC-MS Analysis of Phospholipids in Milk on Ascentis® Express HILIC after SPE using Supelclean™ Si, Shotgun HPLC Analysis

Extraction of the lipid fraction was carried out from 10 mL of the cow's milk sample, according to the Folch method to ensure the exhaustive extraction of the whole lipid content. The total extract was evaporated under vacuum, and the final dry residue (400 mg) was re-dissolved in chloroform/methanol 2:1 (v/v). Lipid extract (100 mg) was afterward dissolved in 1 mL mixture of chloroform/methanol (2:1, v/v). After the cartridge has been conditioned with hexane, the non-polar lipids were eluted with 3 mL of hexane/diethyl-ether (8:2, v/v) and 3 mL of hexane/diethyl-ether (1:1, v/v). Recovery of PLs from the cartridge was obtained by two-step elution, using 4 mL of methanol as first extraction solvent, and subsequently 2 mL of methanol followed by 2 mL of chloroform/methanol/water (3:5:2, v/v/v). The recovered fraction was dried under a gentle stream of nitrogen, yielding 49.7 mg dry residue. The residue was finally re-dissolved in chloroform/methanol (2:1, v/v).

sample/matrix . . . Phospholipids from 10 mL cow's milk (Folch method extraction)
SPE tube/cartridge . . . . . Supelclean LC-Si, 1 g/6 mL (57051)
condition . . . . . hexane
sample addition . . . . . 1 mL extract in chloroform/methanol (2:1, v/v)
washing . . . . . 3 mL of hexane/diethylether (8:2, v/v) and 3 mL of hexane/diethylether (1:1, v/v) to remove nonpolar lipids
elution . . . . . 1 x 4 mL methanol, followed by 1 x 2 mL methanol, followed by 1 x 2 mL chloroform/methanol/water (3:5:2, v/v/v).
eluate post-treatment . . dry under a gentle stream of nitrogen, redissolved residue in chloroform/methanol (2:1, v/v)
column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . . . . (A) acetonitrile; (B) acetonitrile:water (2:1)
gradient . . . . . 0% B held for 5 min; to 30% B in 5 min; to 100% B in 20 min; held at 100% B for 10 min; to 0% B in 1 min
flow rate . . . . . 0.7 mL/min
column temp. . . . . ambient
detector . . . . . ELSD. nebulizing gas (nitrogen) flow: 2 mL/min (180 KPa); drift tube temperature: 50 °C
injection . . . . . 5 µL
Application No. . . . . G005600



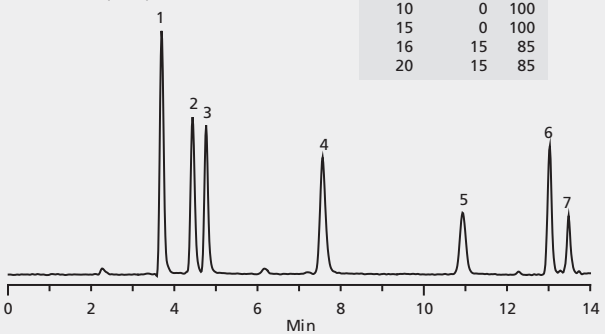
## LC-MS Analysis of Sudan Red Dyes on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm × 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 0.1% formic acid in water (pH unadjusted): (B) acetonitrile; (15:85, A:B)
flow rate . . . . . 1 mL/min, split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, APCI (+) in selected ion recording (SIR) mode
injection . . . . . 15 µL
sample . . 1 µg/mL in 30:70, 0.1% formic acid in water (pH unadjusted):acetonitrile
Application No. . . . . G003860

1. Sudan Orange G (M+H)+= 215.07
2. Sudan Red G (M+H)+= 279.10
3. Sudan I (M+H)+= 249.09
4. Sudan II (M+H)+= 277.12
5. Sudan III (M+H)+= 353.13
6. Sudan Red 7B (M+H)+= 380.17
7. Sudan IV (M+H)+= 381.16

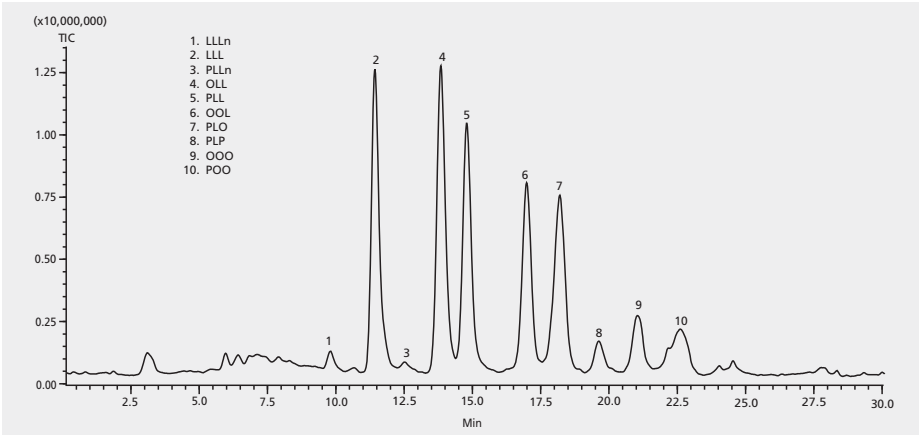
Gradient Program

| Time (min) | %A | %B |
|---|---|---|
| 0 | 5 | 85 |
| 8 | 15 | 85 |
| 10 | 0 | 100 |
| 15 | 0 | 100 |
| 16 | 15 | 85 |
| 20 | 15 | 85 |



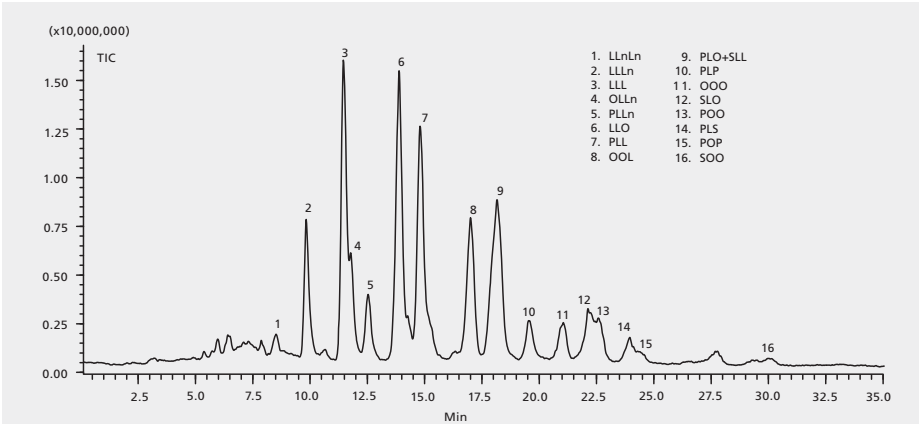
## LC-MS Analysis of Triglycerides in Corn Oil on Ascentis® C18

column . . . . . two Ascentis C18, 15 cm × 2.1 mm I.D., 3 µm particles connected in series (581302-U)
mobile phase . . . . . (A) 2-propanol: (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 25 °C
detector . . . . . APCI/MS positive ion mode
injection . . . . . 2 µL
sample . . . . . corn oil weighed and diluted in acetone (10 mg in 1 ml w/v)
Application No. . . . . G003921



## LC-MS Analysis of Triglycerides in Soybean Oil on Ascentis® C18

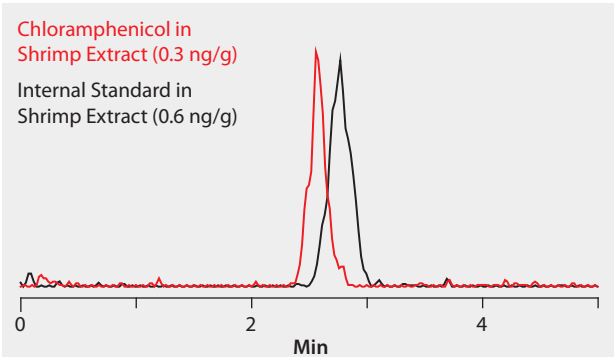
column . . . . . two Ascentis C18, 15 cm × 2.1 mm I.D., 3 µm particles connected in series (581302-U)
mobile phase . . . . . (A) 2-propanol: (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 25 °C
detector . . . . . MS APCI mode
injection . . . . . 2 µL
sample . . . . . soybean oil weighed and diluted in acetone (10 mg in 1 ml w/v)
Application No. . . . . G003923



## LC-MS Anaysis of Chloramphenicol in Shrimp on Ascentis® Express C18 Using Z-Sep+ Cleanup

This application demonstrates the superior cleanup associated with Z-Sep+ using QuEChERS approach.

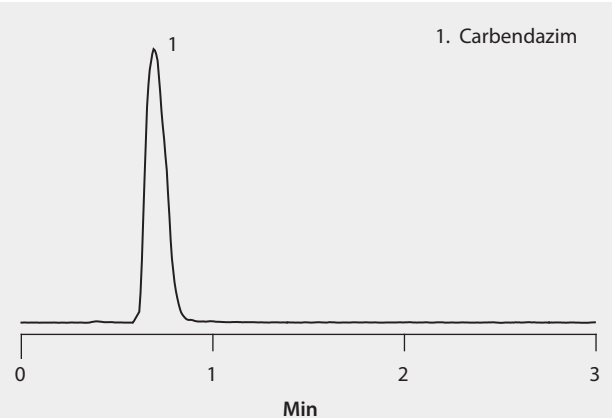
sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . 1.0 g shrimp
extraction . . . . . 9 mL acetonitrile, then 90 µL of formic acid
extraction process . . . . . post treatment: 8 mL supernatant evaporated to dryness and reconstituted with 1 mL water
clean-up tube . . . . . Supel™ Que Z-Sep+ tube, 12 mL, 500 mg (55296-U)
clean-up process . . . . . clean-up volume: 9 mL
column . . . . . Ascentis Express C18, 5 cm X 2.1mm, 2.7 um particles (53822-U)
mobile phase . . . . . (A) Water; (B) Acetonitrile
gradient . . . . . 15% B for 0.1 min, 15-80% in 1.9 min, held at 80% for 1.5 min, 80-15% in 0.5 min, at 15% for 3 min
flow rate . . . . . 0.5 mL/min
column temp. . . . . Ambient
detector . . . . . MS, ESI(+), MRM, m/z 320.9/151.9, 325.9/157.0
injection . . . . . 10 µL
Application No. . . . . G006249



## LC-MS/MS Analysis of Carbendazim in Orange Juice (Spiked) on Ascentis® Express C18 after Supel™ QuE PSA/C18 QuEChERS Cleanup

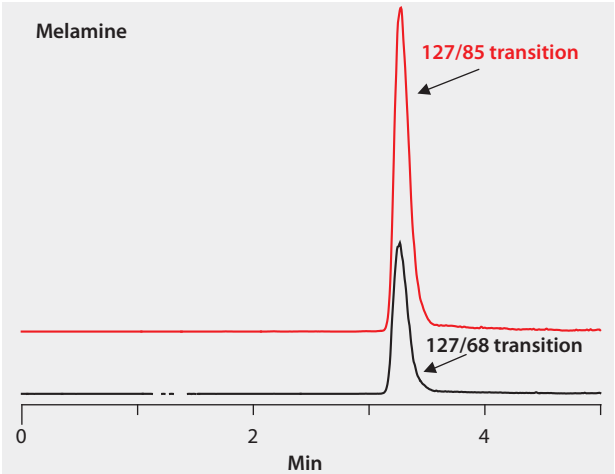
Simple and fast extraction and analytical method were developed for analysis of fungicide carbendazim in orange juice samples. QuEChERS extraction method was used with PSA/C18 cleanup. Ascentis Express C18 column chromatography was coupled to MS/MS detection with limits of detection at 0,1 ppb.

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . . . . orange juice spiked with carbendazim at 1 ppb
extraction tube . . . . . Supel™ QuE Citrate (EN) Tube (55227-U)
extraction process . . . . . add 10 mL of orange juice with pulp to a 50 mL empty extraction tube (55248-U); add carbendazim at 1 ppb; add 10 mL acetonitrile; (shake for 1 minute; add contents of citrate extraction tube (55227-U); shake for 1 min; centrifuge at 3200 rpm for 5 minutes)
clean-up tube . . . . . Supel-Q™ PSA/C18 (55288-U)
clean-up process . . transfer 0.7 mL of the acetonitrile layer to the PSA/C18 cleanup tube (55288-U); shake 1 minute; (centrifuge for 3 minutes at 3400 rpm; remove 0.25 mL of the supernatant; mix with 0.25 mL water for LC-MS analysis)
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . A) 10 mM ammonium acetate in water; (B) 10 mM ammonium acetate in methanol
gradient . . . . . 0-1 min: 30% B; 1.5-3.5 min: 100% B; 3.5-7 min: 30% B
flow rate . . . . . 0-1 min: 0.3 mL/min; 1.5-7 min: 0.5 mL/min
column temp. . . . . 30 °C
detector . . . . . MS, ESI(+), MRM, m/z 192/160, 192/132
injection . . . . . 5 µL
Application No. . . . . G005619



## LC-MS/MS Analysis of Melamine Extracted from Milk on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . (A) 10 mM ammonium formate in 10:90 (v/v) water:acetonitrile;
(B) 10 mM ammonium formate in 30:70 (v/v) water:acetonitrile
gradient . . held at 0% B for 5 min; 0 to 100% B in 0.1 min; held at 100% B for 4.9 min; 100 to 0% B in 0.1 min; held at 0% B for 4.9 min
flow rate . . . . . 0.2 mL/min
column temp. . . . . 30 °C
detector . . . . . ABI 3200 Q-Trap
injection . . . . . 2 µL
sample . . . . . SPE extract from spiked milk
Application No. . . . . G005607






SUPELCO® Solutions within.™

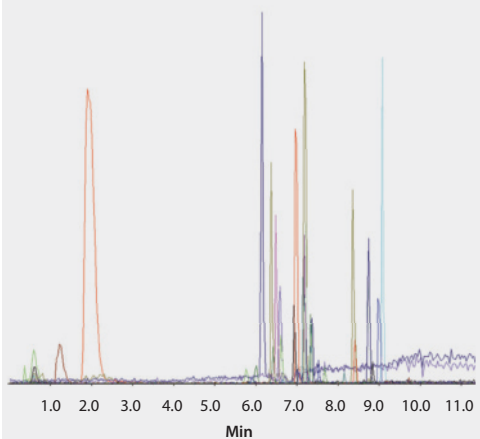
## LC-MS/MS of Pesticides in Oranges on Ascentis® Express C18 after Sample Prep using Supel™ Que Z-Sep/C18 QuEChERS sorbent

**Sample pretreatment:** 10 g of pureed oranges homogenized with rind; spike at 50 ppb by adding 16.75 µL of a pesticide mix containing each analyte at 30 µg/mL

This demonstrates the application of the Z-Sep/C18 sorbent to the cleanup of fruits in the LC-MS-MS analysis of polar pesticides including organophosphates, carbamates and benzoylureas

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . 10 g of pureed oranges (homogenized with rind); spike at 50 ppb (add 16.75 µL of a custom made pesticide mix, each analyte at 30 µg/mL)
extraction tube . . . . . Supel™ QuE Citrate (EN) Tube (55227-U)
extraction process . . add 10 mL acetonitrile; shake for 1 minute; add contents of a Supel QuE citrate extraction tube (55227-U); shake immediately for 1 minute; centrifuge at 3200 rpm for 5 minutes
clean-up tube . . . . . Supel-Q™ PSA/C18 (55284-U)
clean-up process . . transfer 0.7 mL of the acetonitrile layer into a Supel QuE Z-Sep/C18 cleanup tube (55284-U); shake for 1 minute; centrifuge at 5000 rpm for 5 minutes; (transfer 0.2 mL of the supernatant into an empty 1.5 mL centrifuge tube; add 0.2 mL of water; centrifuge at 5000 rpm for 2 minutes.)
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 10 mM ammonium acetate in water; (B) 10 mM ammonium acetate in acetonitrile
gradient . . Hold at 30% B for 1 min; 30% to 80% B in 2 min; hold at 80% B for 4 min; hold at 100% B for 3 min; hold at 30% B for 3 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 2730 psi (188 bar)
column temp. . . . . 30 °C
detector . . . . . MS/MS, ESI positive
Application No. . . . . G005645

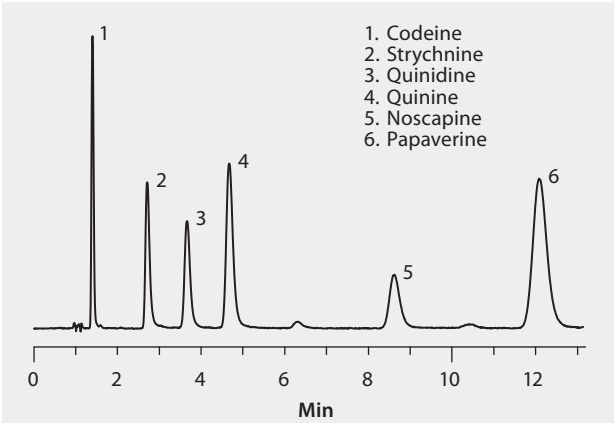
| | $t_R$(min) | | $t_R$(min) |
|---|---|---|---|
| 1. Methomyl | 0.60 | 20. Quinalphos | 7.23 |
| 2. Trichlorfon | 1.22 | 21. Edifenphos | 7.28 |
| 3. Carbendazim | 1.94 | 22. Etrimfos | 7.33 |
| 4. Aldicarb | 2.70 | 23. Fenthion | 7.34 |
| 5. Parathion-methyl | 5.87 | 24. Fenitrothion | 7.36 |
| 6. Methabenzthiazuron | 6.16 | 25. Diazinon | 7.37 |
| 7. Naled | 6.37 | 26. Tolclofos-methyl | 7.52 |
| 8. Methidathion | 6.38 | 27. Phorate | 7.53 |
| 9. Clethodim | 6.42 | 28. Chlorpyrifos-methyl | 7.67 |
| 10. Phosmet | 6.50 | 29. EPN | 7.68 |
| 11. Ametryn | 6.58 | 30. Terbufos | 8.14 |
| 12. Sethoxydim | 6.61 | 31. Ethion | 8.36 |
| 13. Anilazine | 6.78 | 32. Lufenuron | 8.40 |
| 14. Fenxehamid | 6.94 | 33. Spiromesifen | 8.74 |
| 15. Mecarbam | 7.00 | 34. Octhilinone | 8.74 |
| 16. Oryzalin | 7.06 | 35. Pyraclostrobin | 8.75 |
| 17. Diflubenzuron | 7.18 | 36. Carbophenothion | 8.83 |
| 18. Fenoxycarb | 7.19 | 37. Flufenoxuron | 9.02 |
| 19. Iprobenfos | 7.21 | 38. Fenpyroximate | 9.10 |



# Forensics and Toxicology

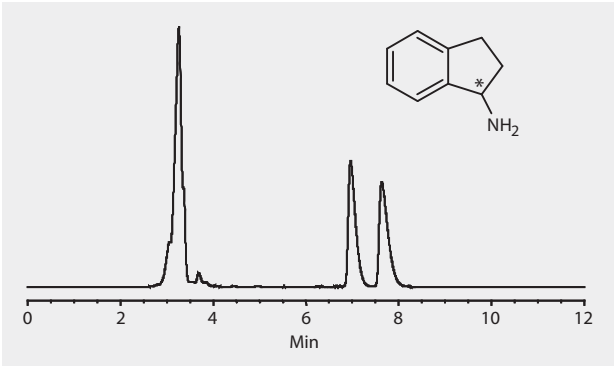
## HPLC Analysis of Alkaloids on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) methanol; (B) 25 mM potassium phosphate, dibasic, pH 3.0; (20:80, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000235



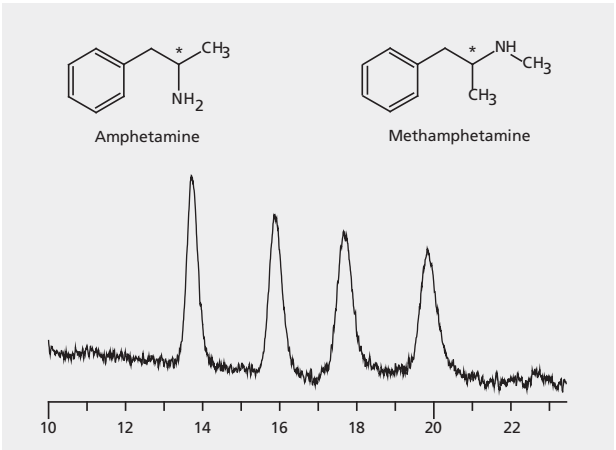
## HPLC Analysis of 1-Aminoindan Enantiomers on LARIHC™ CF6-P

column . . LARIHC CF6-P, 25 cm x 4.6 mm I.D., 5 µm particles (AZYP Part No.L1001, available from Supelco/Sigma-Aldrich as a custom item)
mobile phase . . . . . (A) methanol: (B) acetonitrile: (C) acetic acid: (D) triethylamine, (70:30:0.3.0.2, A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1-Aminoindan, 0.3 mg/mL in ethanol
Application No. . . . . G005255



## HPLC Analysis of Amphetamine and Methamphetamine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) ammonium hydroxide; (99.88:0.1:0.01, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004467



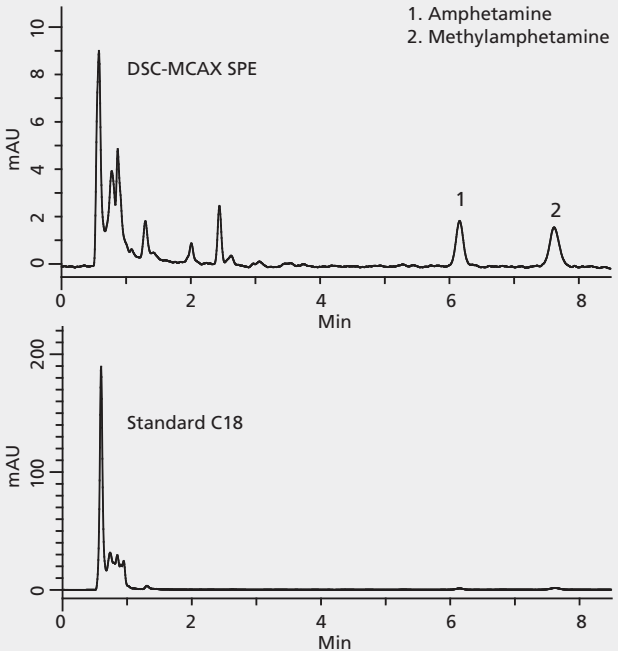



SUPELCO® Solutions within.™

## HPLC Analysis of Amphetamines in Urine on Discovery® HS F5 after SPE using Discovery® DSC-MCAX and Standard C18

**Sample Pre-Treatment:**
1 mL human urine was spiked with 2 mg/mL amphetamine and methylamphetamine. The spiked sample was diluted 1:1 with 1:1 with 50 mM ammonium acetate, pH 6.0.

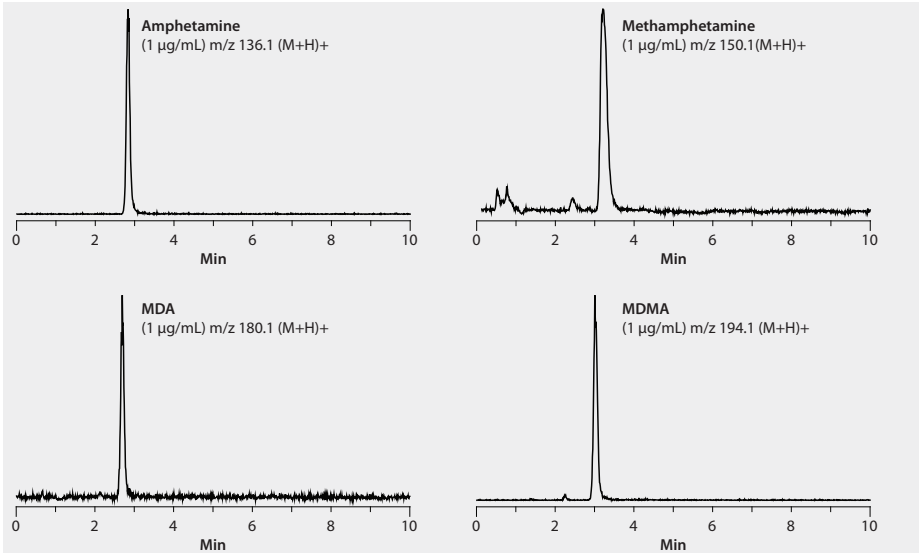
| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | human urine spiked amphetamine and methylamphetamine at with 2 mg/mL |
| SPE tube/cartridge | Discovery DSC-MCAX, 100 mg/3mL (52783-U) |
| condition | DSC-MCAX SPE tube: 1 mL methanol; 1 mL 50 mM ammonium acetate, pH 6.0; C-18 SPE tube: 1 mL methanol; 1 mL DI water |
| sample addition | 1 mL |
| washing | DSC-MCAX SPE tube: 1 mL 50 mM ammonium acetate, pH 6.0; 1 mL 1M acetic acid; 1mL methanol; C-18 SPE tube: 1 mL DI water; 1 mL 20% methanol |
| elution | DSC-MCAX SPE tube: 1 mL 5% ammonium hydroxide in methanol; C-18 SPE tube: 1 mL methanol |
| eluate post-treatment | evaporate to dryness with nitrogen at room temperature, reconstitute in mobile phase |
| column | Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (567516-U) |
| mobile phase | (A) 10 mM ammonium acetate, pH 4.5: (B) methanol; (35:65, A:B) |
| flow rate | 2 mL/min |
| column temp. | 40 °C |
| detector | UV, 210 nm |
| injection | 10 µL |
| Application No. | G003761 |




Discussion:
Note the Y-axis scale difference between DSC-MCAX and C18 SPE. DSC-MCAX SPE offered a maximum background height of ~9 mAU. In contrast, standard C18 background levels were 20 times greater than DSC-MCAX.
Also, on DSC-MCAX absolute recovery averaged at 100.3 and 101.7%, for amphetamine and methylamphetamine, respectively. On standard C18, absolute recovery averaged at 48 and 79% for the two compounds.

## HPLC Analysis of Amphetamines on Discovery® HS F5

column . . . . . Discovery HS F5, 5 cm x 2.1 mm I.D., 3 µm particles (567500-U)
mobile phase . . (A) 0.1% ammonium acetate in water; (B) 0.1% ammonium acetate in acetonitrile; (C) acetonitrile; (10:30:60, A:B:C)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in Single Ion Recording (SIR) Mode
injection . . . . . 5 µL
sample . . . . . 1 ug/mL in acetonitrile
Application No. . . . . G003731

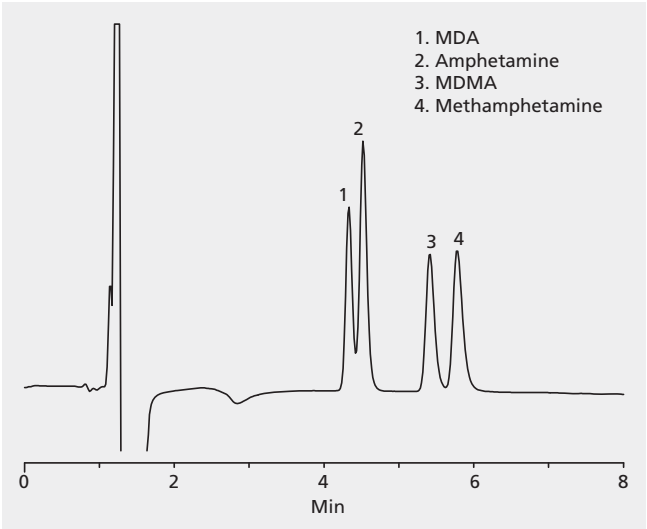





SUPELCO® Solutions within.™

## HPLC Analysis of Amphetine, Methamphetamine, MDA, and MDMA on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . (A) 10 mM ammonium acetate in (B) water: acetonitrile (10:90)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL each (amphetamine, methamphetamine, MDA and MDMA) in methanol
Application No. . . . . G002081

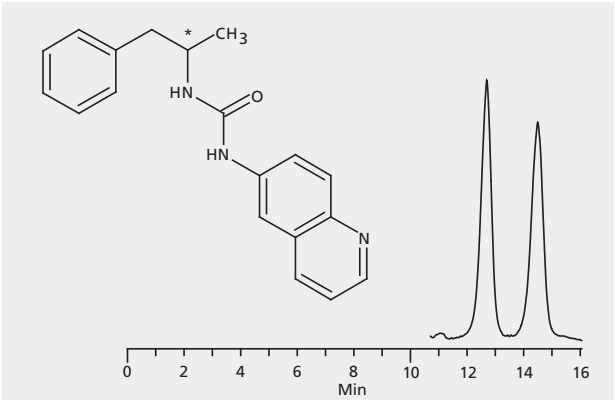





SUPELCO® Solutions within.™

## HPLC Analysis of AQC-Amphetamine Enantiomers on Astec® CYCLOBOND® I 2000 DMP

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004696

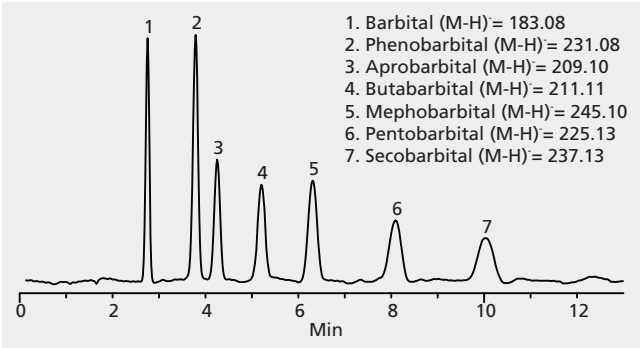





SUPELCO® Solutions within.™

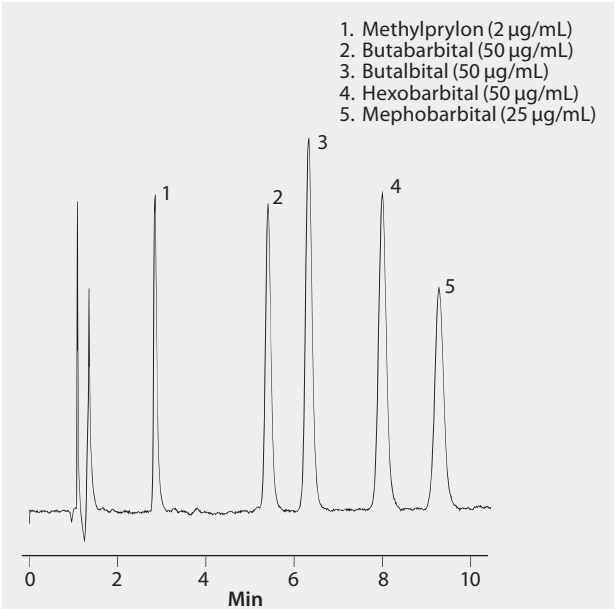
## HPLC Analysis of Barbiturates on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) methanol; (B) water (45:55, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . 10 µg/mL each in 50:50 water:methanol
Application No. . . . . G003307



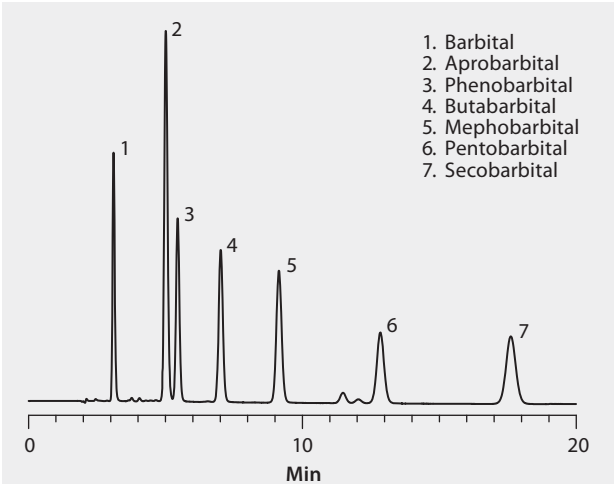
## HPLC Analysis of Barbiturates on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . (A) acetonitrile; (B) 25 mM potassium phosphate buffer, pH 6.9 (25:75, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 25 µL
Application No. . . . . 713-0141



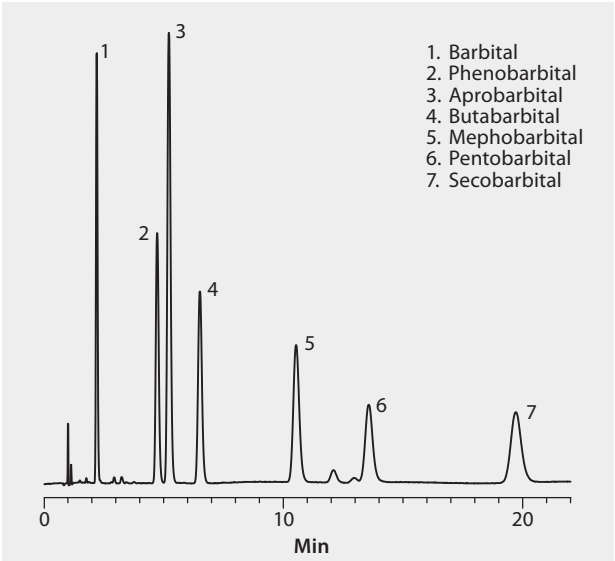
## HPLC Analysis of Barbiturates on Discovery® C18, Unbuffered Mobile Phase

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) methanol; (B) water (45:55, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
Application No. . . . . G000190



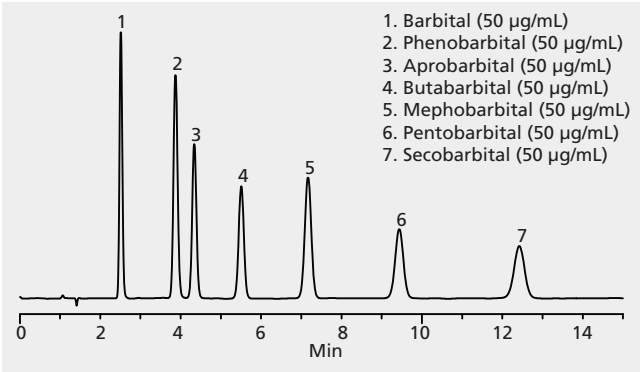
## HPLC Analysis of Barbiturates on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) methanol:acetonitrile, 60:40; (B) water; (25:75, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 214 nm
injection . . . . . 2 µL
Application No. . . . . G000193



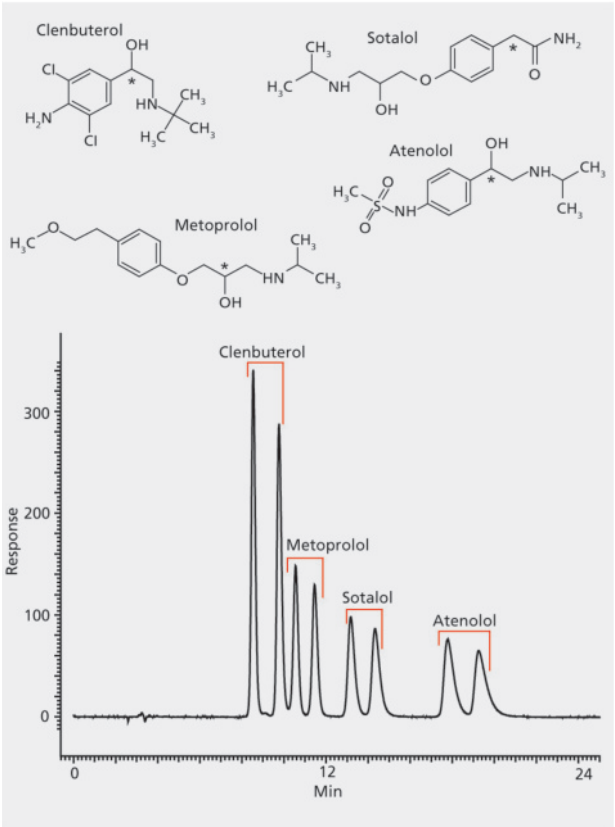
## HPLC Analysis of Barbiturates on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) methanol; (B) water (50:50, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 65:35 water:methanol
Application No. . . . . G002877



## HPLC Analysis of Beta-Receptor Agonist Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm, 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
Application No. . . . . G004337



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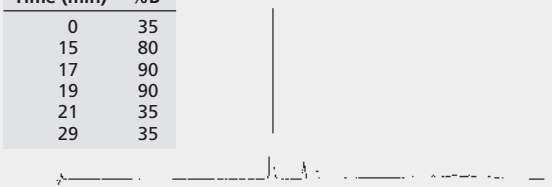
## HPLC Analysis of Bufalin Toxin on Discovery® BIO Wide Pore C18

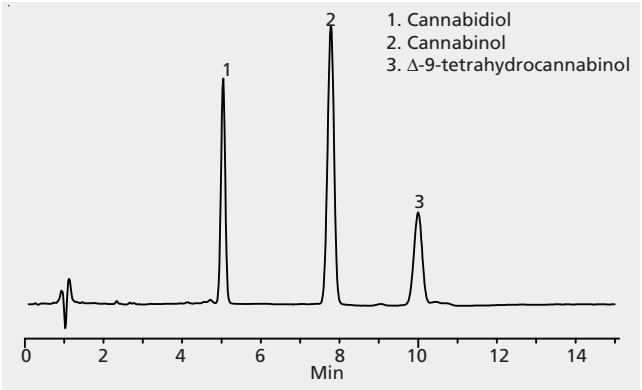
column . . . . . Discovery BIO Wide Pore C18, 25 cm × 4.6 mm I.D., 5 µm particles (568223-U)
mobile phase . . (A) 0.1% TFA in water; (B) 0.1% TFA in acetonitrile:water (3:1, A:B)
flow rate . . . . . 1.3 mL/min
column temp. . . . . ambient
detector . . . . . UV, 298 nm
injection . . . . . 10 µL bufalin, 0.5 mg/mL in acetonitrile
Application No. . . . . G001722

| Gradient Program | |
|---|---|
| Time (min) | %B |
| 0 | 35 |
| 15 | 80 |
| 17 | 90 |
| 19 | 90 |
| 21 | 35 |
| 29 | 35 |



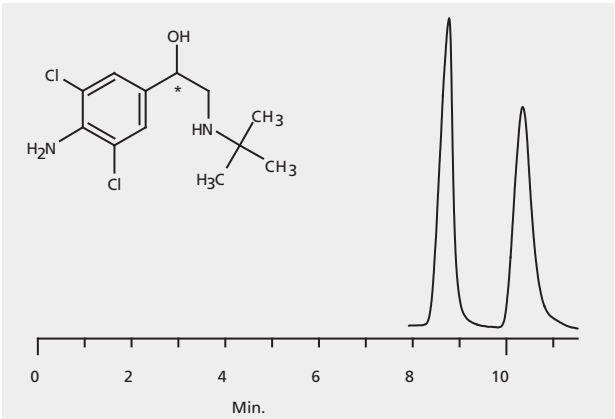
## HPLC Analysis of Cannabinoids on Ascentis® C18

column . . . . . Ascentis C18, 10 cm x 4.6 mm I.D., 3 µm particles (581321-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 4.5 with acetic acid); (B) acetonitrile; (25:75, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 30 µg/mL each in mobile phase
Application No. . . . . G002326



## HPLC Analysis of Clenbuterol Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004596



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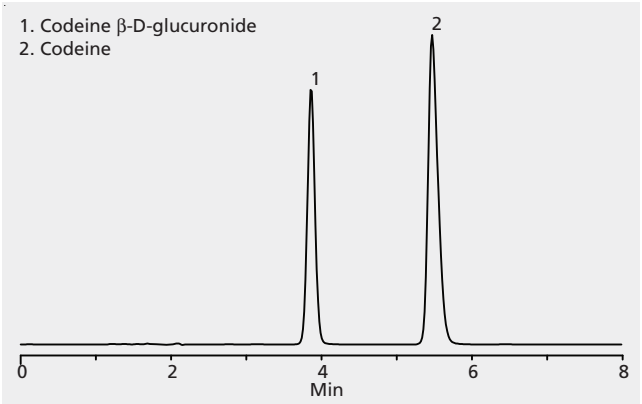
## HPLC Analysis of Codeine and Metabolite on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002417



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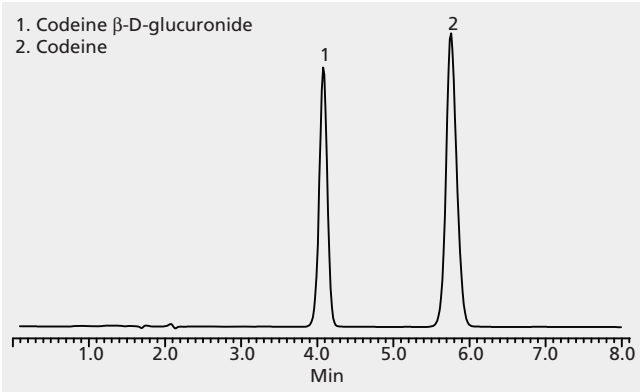
## HPLC Analysis of Codeine and Metabolite on Ascentis® RP-Amide

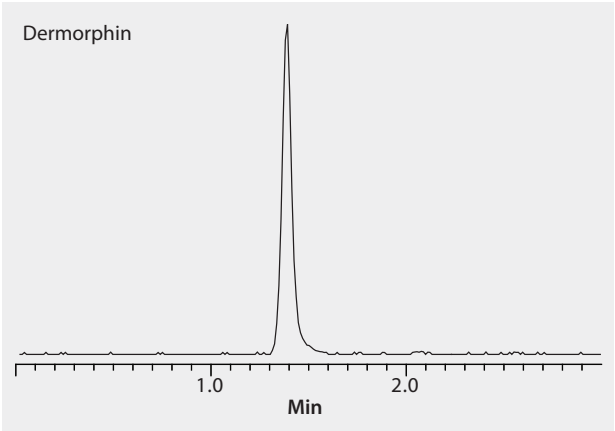
column . . . . Ascentis RP-Amide 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002599



## HPLC Analysis of Dermorphin in Horse Plasma on Ascentis® Express F5 after SPE with HybridSPE®

Dermorphin is a natural heptapeptide opioid. Because of poor recovery from horse plasma following protein precipitation, alternate methods of extraction are sought. While the HybridSPE protocol successfully removes proteins and phospholipids, dermorphin solubility in plasma remains a limitation. Dermorphin is effectively retained on the pentafluorophenyl phase under aqueous normal phase conditions.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . horse plasma spiked at 10 µg/mL
SPE tube/cartridge . . [TM"HybridSPE"]-Phospholipid Plate, 50mg/96-Well (575656-U)
sample addition . . . . . 3:1, (1% formic acid in acetonitrile); plasma
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) acetonitrile (B) 20 mM ammonium formate; (90:10, A:B); pH 4.5 with formic acid, after mixing A & B
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1305 psi (90 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+), TIC, m/z 100-1000
injection . . . . . 1 µL
Application No. . . . . G005540

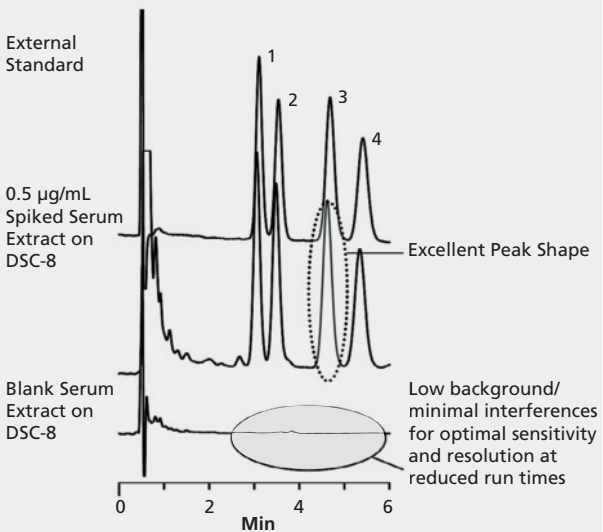


## HPLC Analysis of Diazepam and Metabolites in Serum on Discovery® C18 after SPE using Discovery® DSC-8

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . goat serum spiked with diazepam and metabolites at 0.5 µg/mL and diluted 1:1 in 25 mM ammonium formate, pH 7.1
SPE well plate . . . . Discovery DSC-8 96-well SPE Plate, 100 mg/well (575627-U)
condition . . . . . 1 mL methanol followed by 1 mL DI water.
sample addition . . . . . 1 mL spiked goat serum
washing . . . . . 1 mL 25% methanol in 25 mM ammonium formate, pH 2.75
elution . . . . . 1 mL 60% methanol in 25 mM ammonium formate, pH 2.75
elution . . . . . Evaporate eluate with nitrogen purge (30 °C; ~15 min.). Reconstitute in 200 µL mobile phase.
column . . . . . Discovery C18, 5 cm x 4.6 mm I.D., 5 µm particles (504947)
mobile phase . . (A) Methanol; (B)10 mM ammonium acetate, pH 4.5;(45:55, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 25 µL
Application No.Application No. . . . . G003766

**Efficiency of Recovery**

| Compound | %Recovery ± RSD (n=3) Systematically Developed Method on Discovery DSC-8 | %Recovery ± RSD (n=3) Generic Method On Conventional C18 |
|---|---|---|
| 1. Oxazepam | 94.7 ± 1.2% | 82.8 ± 4.0% |
| 2. Temazepam | 99.9 ± 1.1% | 89.1 ± 4.0% |
| 3. Desmethyl diazepam | 94.2 ± 1.8% | 82.4 ± 5.0% |
| 4. Diazepam | 90.0 ± 3.4% | 68.5 ± 9.1% |



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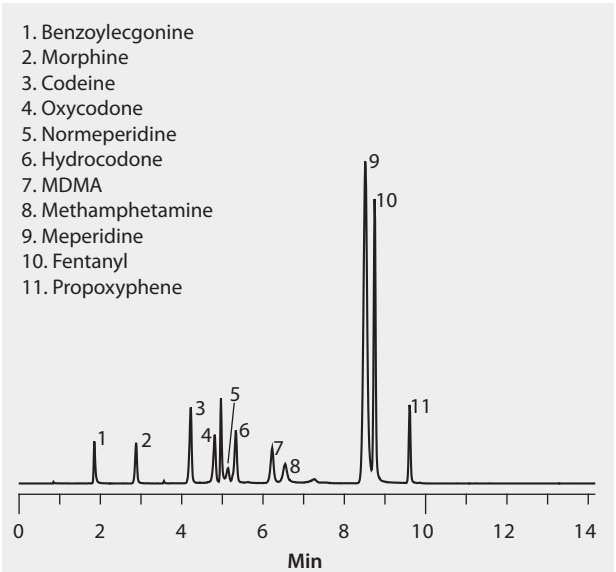
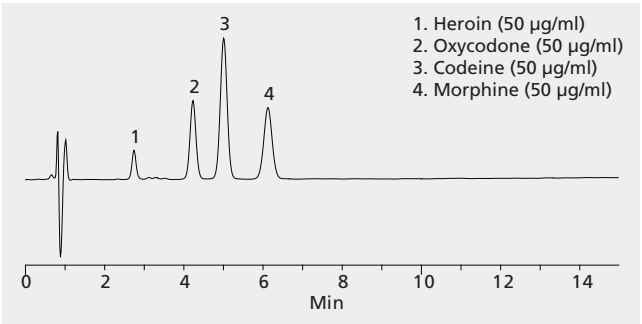
## HPLC Analysis of Drugs of Abuse on Ascentis® Express F5

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in 95:5 (v/v) acetonitrile:water
gradient . . . . . held at 20% B for 0.5 min; 20 to 100% B in 5.5 min; held at 100% B for 4 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 175 bar
column temp. . . . . 35 °C
detector . . . . . ESI(+), m/z 100-1000
injection . . . . . 1 µL
sample . . . . . 1 µg/mL in 50:50, water: methanol
Application No. . . . . G005634



## HPLC Analysis of Drugs of Abuse on Ascentis® Si (HILIC Mode)

column . . . . . Ascentis Si, 5 cm x 4.6 mm I.D., 5 µm particles (581511-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted): (B) 0.1% ammonium acetate in acetontrile; (10:90, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 10:90:water:acetonitrile
Application No. . . . . G003720



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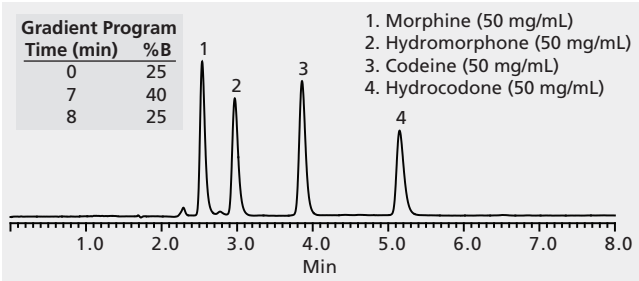
## HPLC Analysis of Drugs of Abuse on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . (A) water with 0.1% ammonium acetate (pH 6.6, unadjusted);
(B) acetonitrile with 0.1% ammonium acetate
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase A
Application No. . . . . G003701



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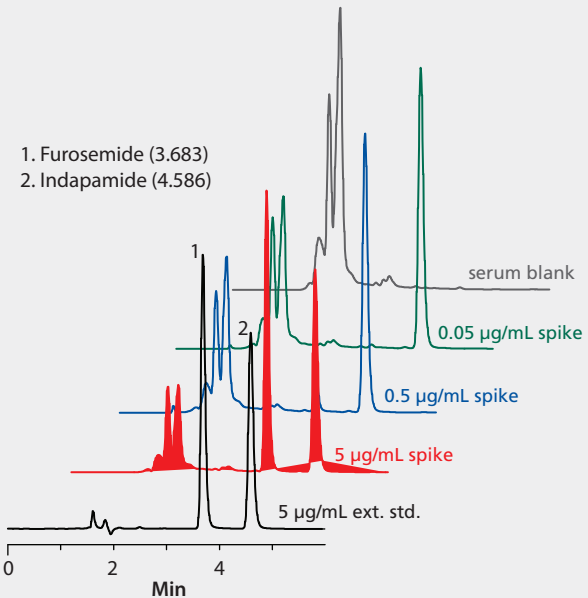
## HPLC Analysis of Furosemide (Lasix) in Horse Serum on Discovery® C18 after SPE using Discovery® DSC-18

**Sample Pre-treatment:**
Neat horse serum was acidified with 10 mL 6 M HCl/mL serum. Internal standard (I.S.) blank was prepared by spiking acidified horse serum with indapamide at 10 mg/mL. Sample A = 10 mg/mL furosemide in I.S. blank. Sample B = 5 mg/mL furosemide in I.S. blank. Sample C = 0.5 mg/mL furosemide in I.S. blank. Sample D = 0.1 mg/mL furosemide in I.S. blank. Sample E = 0.05 mg/mL furosemide in I.S. blank.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . acidified horse serum spiked with indapamide at 10 mg/mL
SPE tube/cartridge . . . . . Discovery DSC-18, 50 mg/1 mL (52601-U)
condition . . . . . 1 mL methanol; 1 mL 10 mM potassium phosphate, pH 3
sample addition . . . . . 1 mL
washing . . . . . 10 mM potassium phosphate, pH 3
elution . . . . . 1 mL 60% methanol in DI water.
column . . . . . Discovery C18, 15 cm x 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) 10 mM $KH_2PO_4$, pH 3 (adjusted with $H_3PO_4$): (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 234 nm
injection . . . . . 10 µL
Application No. . . . . G003764

**Efficiency of Recovery**

| Sample | Concentration (µg/mL) | Avg. Response Factor | %Recovery ± RSD (n=3) |
|---|---|---|---|
| A | 10.00 | 2.307 | 93.1 ± 3.1 |
| B | 5.00 | 1.168 | 100.8 ± 1.4 |
| C | 1.00 | 0.107 | 97.4 ± 2.8 |
| D | 0.50 | 0.065 | 120.7 ± 1.3 |
| E | 0.10 | 0.009 | 132.8 ± 8.3 |



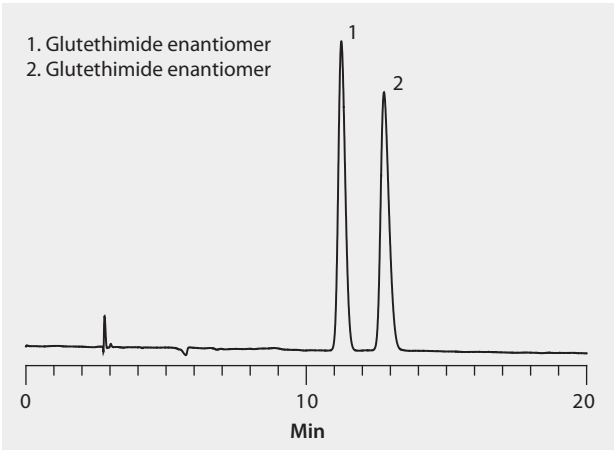



SUPELCO® Solutions within.™

## HPLC Analysis of Glutethimide Enantiomers on Astec® Cellulose DMP

Glutethimide enantiomers are resolved by normal phase chromatography. Glutethimide is used to treat insomnia.

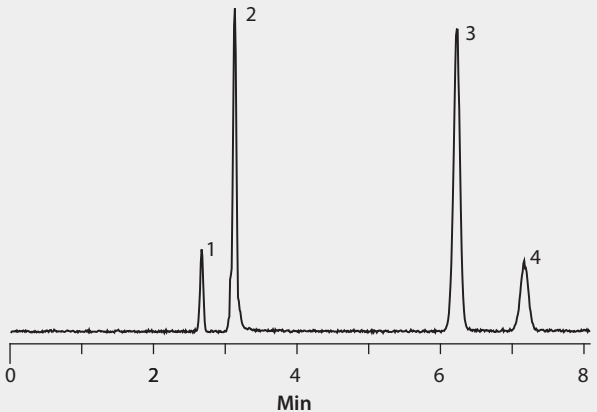
column . . Astec Cellulose DMP, 25 cm x 4.6 mm I.D., 5 µm particles (51099AST)
mobile phase . . . . . (A) heptane; (B) isopropanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 15 µL
sample . . . . . glutethimide, 1 g/L in heptane:isopropanol (50:50)
Application No. . . . . G005514



## HPLC Analysis of Heroin, Cocaine, Amphetamine and MDMA on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 10 mM ammonium acetate in water, pH 3.5; (B) 10 mM ammonium acetate in acetonitrile; (2.98, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 50 °C
detector . . . . . ESI(+), m/z 100-400
injection . . . . . 1 µL
Application No. . . . . G005513

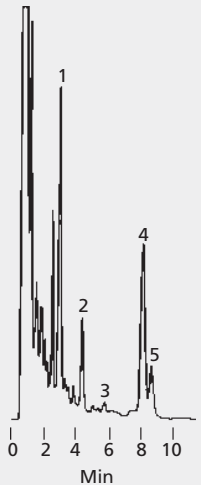
1. Heroin
2. Cocaine
3. Amphetamine
4. MDMA (3,4-Methylenedioxymethamphetamine hydrochloride)



## HPLC Analysis of Hippuric and Methylhippuric Acids in Urine on SUPELCOSIL™ LC-18

Urinary excretion of hippuric acid and m-or p-methylhippuric acid in the urine is a test of exposure to toluene and m-or p-xylene vapors.

column . . . . . SUPELCOSIL LC-18, 7.5 cm × 4.6 mm I.D., 3 µm particles (58984)
mobile phase . . . . . (A) tetrahydrofuran:10 mM potassium phosphate, pH 3.0 (B) with phosphoric acid (3:97, A:B)
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 260 nm
injection . . 10 µL spiked urine (300 µg/mL HA, o-MHA; 150 µg/mL m-MHA, p-MHA)
Application No. . . . . 713-0961A

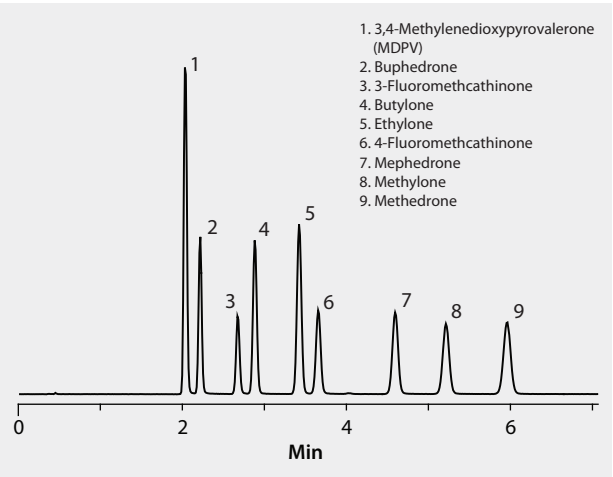



1. Hippuric acid
2. o-Methylhippuric acid
3. p-Methylhippuric acid impurity
4. p-Methylhippuric acid
5. m-Methylhippuric acid

## HPLC Analysis of Illicit Bath Salts on Ascentis® Express HILIC

With the rapid development of unregulated designer and synthetic compounds, the field of illicit drug testing has recently been met with a changing environment. Of most concern has been the development of a class of phenethylamine and cathinone compounds being marketed as "bath salts," "jewelry cleaner," or "plant food." Though sold as "not for human consumption," these compounds are reported to generate stimulating affects similar to that of methamphetamine, heroin, and 3,4- methylene-dioxypyrovalerone (MDMA). For a period of time, these compounds could be acquired legally through the internet and head shops due to no direct legal control. In the US, both state and local governments have instituted bans on the sale of these bath salt compounds. Forensic testing facilities often experience difficulty in testing these compounds due to the fact that they are not detected under normal ELISA testing methods; additional more specific LC-MS methods are necessary. The challenge for LC-MS detection of these particular bath salts resides in three sets of isobaric compounds that require chromatographic resolution for positive confirmation. For example, both butylone and ethylone have the same mono isotopic mass, making these compounds indistinguishable, even when using accurate mass time of flight TOF-MS. Efficient chromatographic separation is necessary for accurate quantitation of these compounds. The polar basic nature of the bath salts makes these compounds difficult to retain on traditional reversed-phase C18 and even polar embedded stationary phases, making them prime subjects for HILIC chromatographic separation. Shown here is the fast, high-resolution separation of nine synthetic bath salts on Ascentis Express HILIC.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) acetonitrile; (B) 0.25 M ammonium formate; (98:2, A:B); premixed
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1842 psi (127 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+), TIC, m/z 100-1000
injection . . . . . 1 µL
sample . . . . . 200 µg/L ea. in acetonitrile
Application No. . . . . G005537



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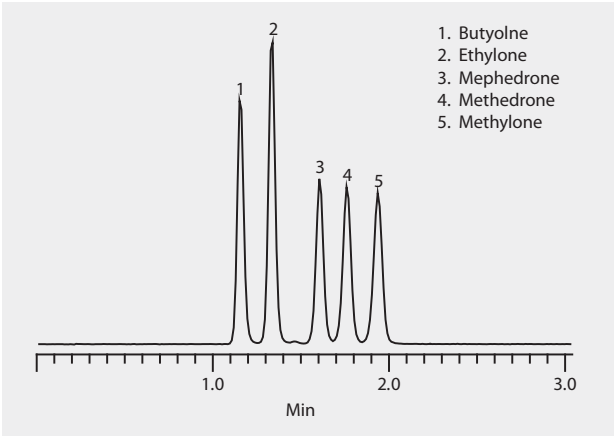
## HPLC Analysis of Illicit Bath Salts on Ascentis® Express HILIC, Fast Analysis

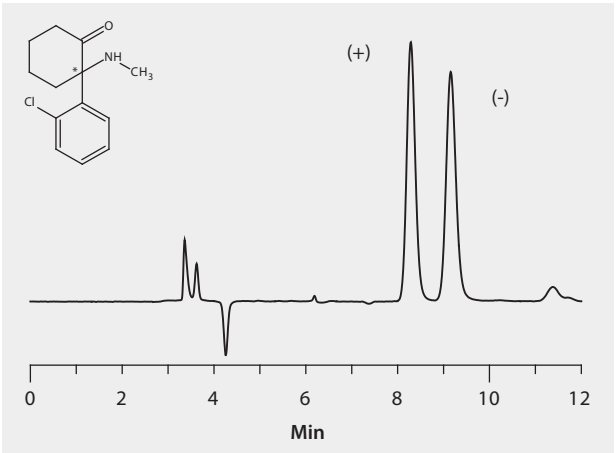
With the rapid development of unregulated designer and synthetic compounds, the field of illicit drug testing has recently been met with a changing environment. Of most concern has been the development of a class of phenethylamine and cathinone compounds being marketed as "bath salts," "jewelry cleaner," or "plant food." Though sold as "not for human consumption," these compounds are reported to generate stimulating affects similar to that of methamphetamine, heroin, and 3,4- methylene-dioxypyrovalerone (MDMA). For a period of time, these compounds could be acquired legally through the internet and head shops due to no direct legal control. In the US, both state and local governments have instituted bans on the sale of these bath salt compounds. Forensic testing facilities often experience difficulty in testing these compounds due to the fact that they are not detected under normal ELISA testing methods; additional more specific LC-MS methods are necessary. The challenge for LC-MS detection of these particular bath salts resides in three sets of isobaric compounds that require chromatographic resolution for positive confirmation. For example, both butylone and ethylone have the same mono isotopic mass, making these compounds indistinguishable, even when using accurate mass time of flight TOF-MS. Efficient chromatographic separation is necessary for accurate quantitation of these compounds. The polar basic nature of the bath salts makes these compounds difficult to retain on traditional reversed-phase C18 and even polar embedded stationary phases, making them prime subjects for HILIC chromatographic separation. Shown here is the fast, high-resolution separation of nine synthetic bath salts on Ascentis Express HILIC.

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . A: 5 mM ammonium formate (95:5 acetonitrile:water)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), TIC 100 - 1000 m/z
sample . . . . . 1 µL
Application No. . . . . G005444



## HPLC Analysis of Ketamine Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) DEA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004990



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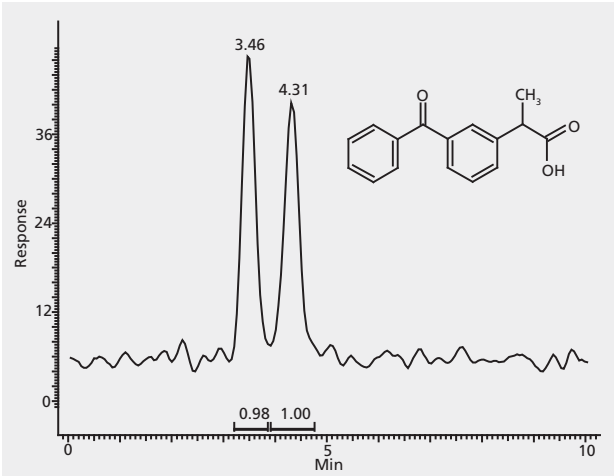
## HPLC Analysis of Ketoprofen Enantiomers on Astec® CHIROBIOTIC® R (MS Detection)

column . . . . . CHIROBIOTIC R, 15 cm x 2.1 mm, 5 µm particles (13019AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 5.6 (B) methanol; (70:30, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(-)
Application No. . . . . G004331



## HPLC Analysis of Metabolites of 7,12-Dimethylbenz[a]anthracene on SUPELCOSIL™ LC-18

column . . . . . SUPELCOSIL LC-18, 25 cm × 4.6 mm I.D., 5 µm particles (58298)
mobile phase . . (A) methanol:(B) water, (50:50, A:B), 10 min to 100:0 at 2.5% /min
flow rate . . . . . 0.8 mL/min
detector . . . . . fluorescence
sample . . . . rat liver, 9000 × g supernatant fraction from Aroclor-treated animals
Application No. . . . . 713-1043

1. 2-OH-DMBA(t-diol)
2. 7-OHM-12-MBA-(t-10,11-diol)
3. 7-M-12-OHMBA-(t-8,9-diol)
4. 7-OHM-12-MBA-(t-8,9-diol)
5. 7-OHM-(t-3,4-diol)
6. Mixed diols, incl. DMBA-(t-8,9-diol & t-10, 11-diol)
7. Position of 7,12-bis-OHMBA
8. 7-OHM-12-MBA-2-ol
9. 7-OHM-12-MBA-(3-ol)
10. 7-M-12-OHMNA-4-ol
11. 7-OHM-12-MBA-4-ol
12. 7-M-12-OHMBA-(3-ol)
13. 7-M-12-OHMBA-2-ol
14. 7-OHM-12-MBA
15. 7-M-12-OHMBA
16. DMBA-2-ol
17. DMBA-3-ol
18. DMBA-4-ol
19. DMBA

(parentheses = tentative identifications)

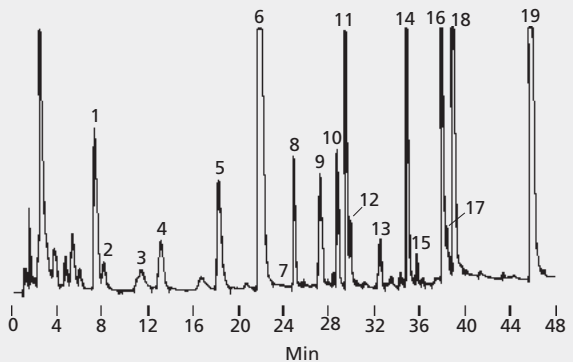
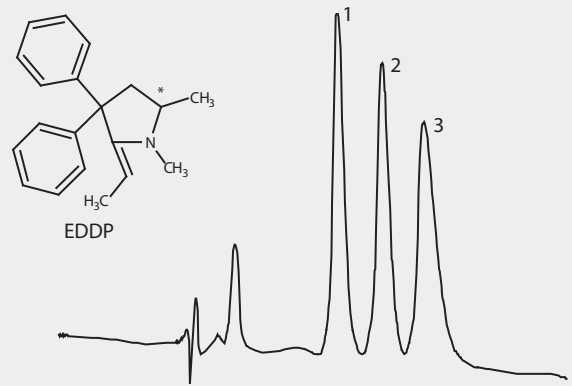



Figure provided by Drs. J. Milner and J. Grunau, University of Illinois, Urbana, Illinois, USA.

## HPLC Analysis of Methadone Enantiomers and Methadone Metabolite EDDP on Astec® CYCLOBOND® I 2000 HP-RSP

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)
mobile phase . . . . . (A) 0.1% acetic acid; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 0.8 mL/min
detector . . . . . UV, 292 nm
Application No. . . . . G004734

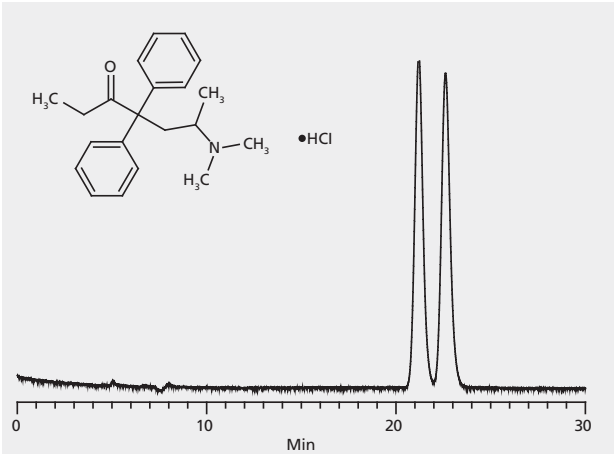
| Peak | Rt (min) | Peak ID |
|---|---|---|
| 1 | 8.5 | R-Methadone |
| 2 | 9.7 | S-Methadone |
| 3 | 11.0 | R,S-EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine) |




EDDP

## HPLC Analysis of Methadone Enantiomers on Astec® CHIROBIOTIC® V2

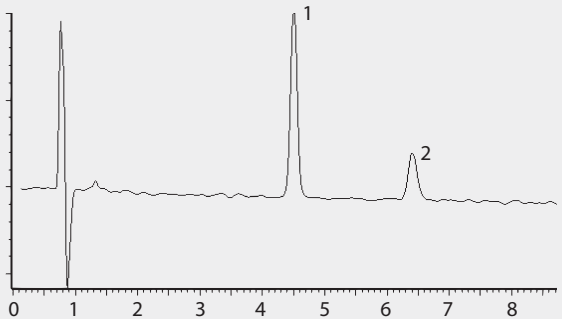
column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . (A) methanol; (B) 20 mM ammonium formate; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 205 nm
injection . . . . . 2 µL
sample . . . . . methadone, 1 mg/mL in mobile phase
Application No. . . . . G004403



## HPLC Analysis of Methcathinone and Ephedrine on Discovery® HS F5

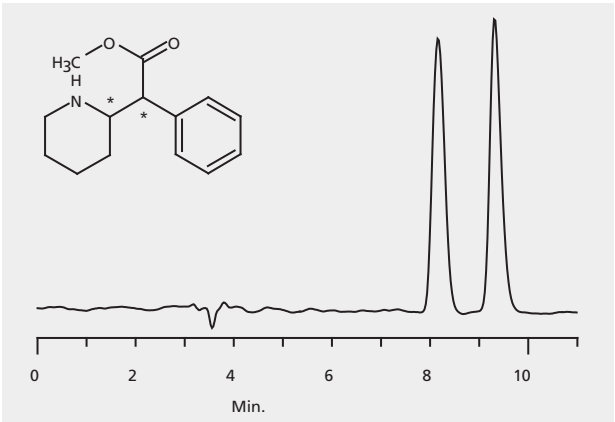
column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 6.98); (B) acetonitrile (30:70, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 35 °C
detector . . . . . photodiode array
injection . . . . . 5 µL
Application No. . . . . G001673

1. Methcathinone (100 µg/mL)
2. (+/-) Ephedrine (200 µg/mL)



## HPLC Analysis of Methylphenidate Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) methanol; (B) 1% triethylamine, pH 4/1; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004505



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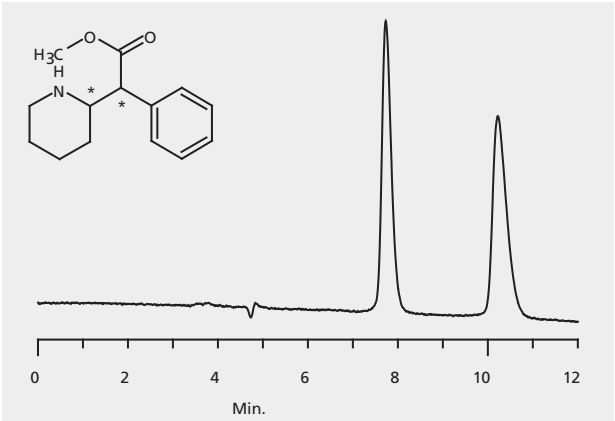
## HPLC Analysis of Methylphenidate (Ritalin) Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 15 mM ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 215 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004504





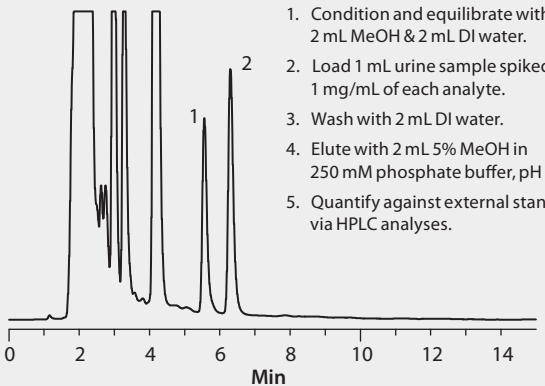

SUPELCO® Solutions within.™

## HPLC Analysis of 3-Methylpyrazole and 4-Methylpyrazole in Urine on Discovery® C18 after SPE using Discovery® DSC-SCX

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . urine spiked with 3- and 4-methylpyrazole at 1 mg/mL
SPE tube/cartridge . . . . . Discovery DSC-SCX, 500 mg/3 mL (52686-U)
condition . . . . . 2 mL methanol; 2 mL DI water
sample addition . . . . . 1 mL
washing . . . . . 2 mL DI water
elution . . . . 2 mL 5% methanol in 250 mM potassium phosphate buffer, pH 7.4
column . . . . . Discovery C18, 15 cm × 4.6 mm, 5 µm preceded by a 2 cm guard column and 0.5 µm frit filter (504955)
mobile phase . . . . . MeOH:5 mM phosphate buffer, pH 6 (20:80)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 25 µL, dilute urine extract
Application No. . . . . G001635

**Efficiency of Recovery**

| Compound (mg/mL) | Discovery DSC-SCX (n=3) % Recovery | %RSD | Leading Competitor SCX (n=2) %Recovery | %RSD |
|---|---|---|---|---|
| 1. 3-methylpyrazole (1.0) | 89.4 | ±10.2% | 67.1 | ±20% |
| 2. 4-methylpyrazole (1.0) | 79.4 | ±6.8% | 50.5 | ±30% |

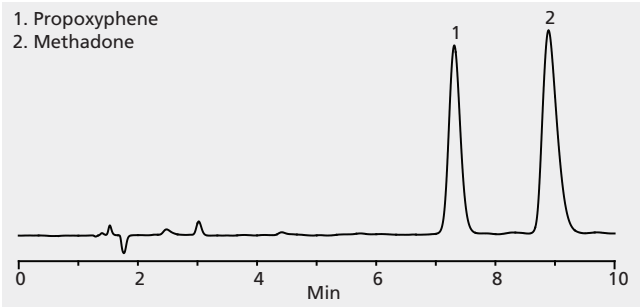



**SPE Procedure**

1. Condition and equilibrate with 2 mL MeOH & 2 mL DI water.
2. Load 1 mL urine sample spiked with 1 mg/mL of each analyte.
3. Wash with 2 mL DI water.
4. Elute with 2 mL 5% MeOH in 250 mM phosphate buffer, pH 7.4.
5. Quantify against external standards via HPLC analyses.

## HPLC Analysis of Propoxyphene and Methadone on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . (A) water with 0.1% ammonium acetate: (B) methanol; (20:80, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 20:80, water:methanol
Application No. . . . . G003699



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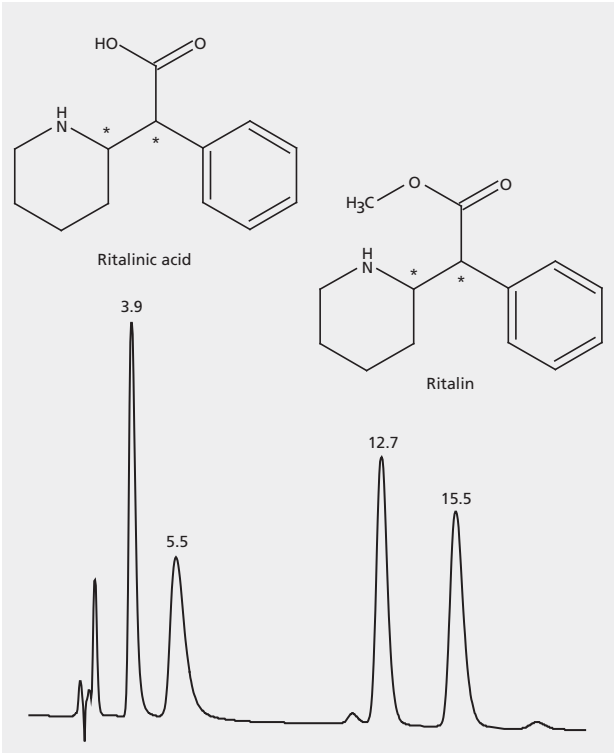
## HPLC Analysis of Ritalinic Acid and Ritalin (Methylphenidate) Enantiomers on Astec® CHIROBIOTIC® V2 with T2 Guard Column

| | |
|---|---|
| column | CHIROBIOTIC T2 guard column, 2 cm x 4 mm I.D., 5 µm particles (16100AST (requires holder 21150AST)) |
| column | CHIROBIOTIC V2, 15 cm x 4.6 mm I.D., 5 µm particles (15023AST) |
| mobile phase | (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (7:93, A:B) |
| flow rate | 1 mL/min |
| column temp. | 25 °C |
| detector | UV, 230 nm |
| injection | 2 µL |
| sample | 1 mg/mL in methanol |
| Application No. | G004733 |



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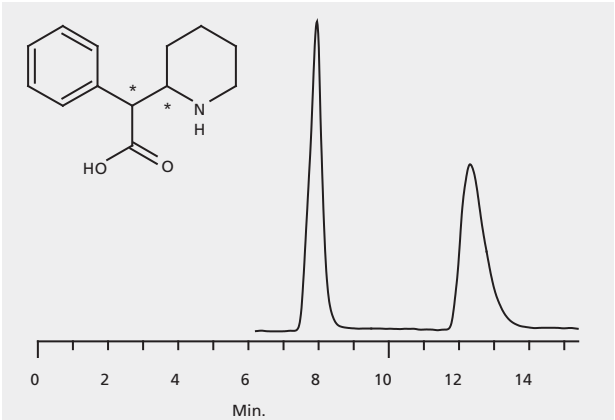
## HPLC Analysis of Ritalinic Acid Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 5.5; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004600



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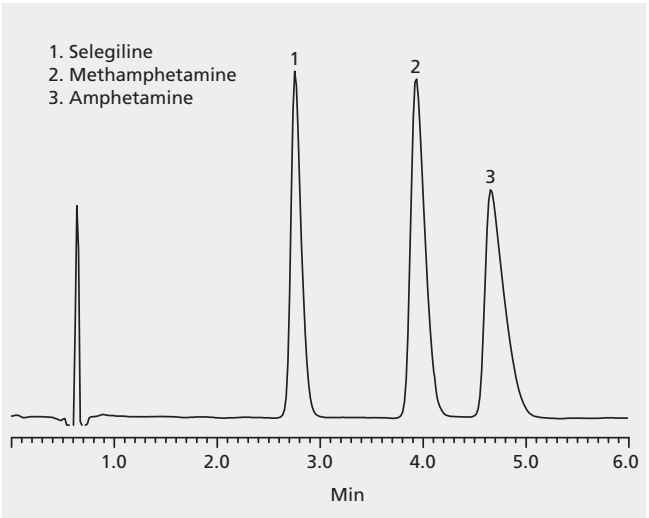
## HPLC Analysis of Selegiline and Amphetamine Metabolites on Discovery® HS F5

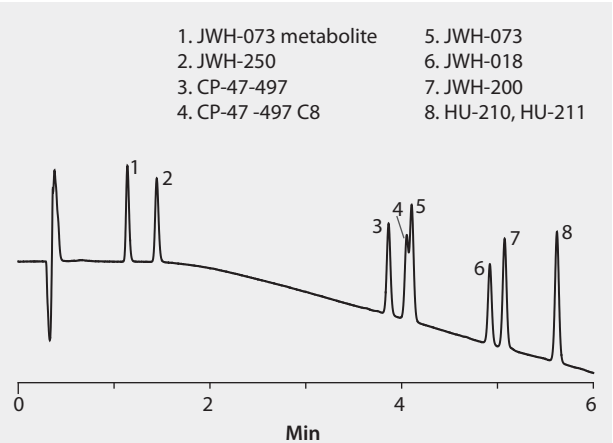
column . . . . . Discovery HS F5, 5 cm × 4.6 mm I.D., 5 µm particles (567513-U)
mobile phase . . . (A) water (10 mM ammonium acetate, pH 4.0 with acetic acid: (B) acetonitrile (v/v), (25:75, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002136



## HPLC Analysis of Spice Cannabinoids on Ascentis® Express C18

Synthetic cannabinoids (Spice) are a relatively new type of designer drug used as a pseudo-legal means to get a cannabis-type high. New synthetic cannabinoids are continually being introduced as suppliers tweak the molecular structures. The ability to rapidly and reliably identify the continually changing population of these compounds in the blood or urine suspected users is a significant analytical challenge facing forensic chemists.

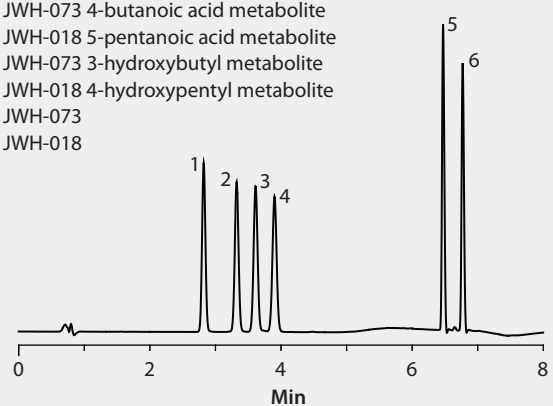
column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) 5 mM ammonium formate water; (B) 5 mM ammonium formate (95:5, acetonitrile:water)
gradient . . . . . 68 to 100% B in 8 min; held at 100% B for 2 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 4351 psi (300 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 265 nm
injection . . . . . 3 µL
Application No. . . . . G005740



## HPLC Analysis of Spice Cannabinoids JWH-018, JWH-073 and Metabolites on Ascentis® Express C18, 2.7 µm particles

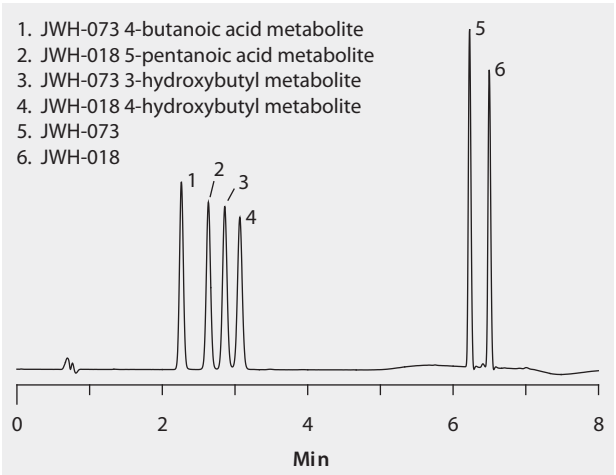
column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) 0.1% formic acid in water;
(B) 0.1% formic acid in water:acetonitrile (10:90)
gradient . . 20% B held for 3 min; 20 to 100% B in 0.1 min; 100% B held for 2 min
flow rate . . . . . 0.5 mL/min
pressure . . . . . 2150 psi (148 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 315 nm
injection . . . . . 2 µL
sample . . . . . 17 µg/mL each in acetonitrile
Application No. . . . . G005833

1. JWH-073 4-butanoic acid metabolite
2. JWH-018 5-pentanoic acid metabolite
3. JWH-073 3-hydroxybutyl metabolite
4. JWH-018 4-hydroxypentyl metabolite
5. JWH-073
6. JWH-018



## HPLC Analysis of Spice Cannabinoids JWH-018, JWH-073 and Metabolites on Ascentis® Express C18, 5 µm particles

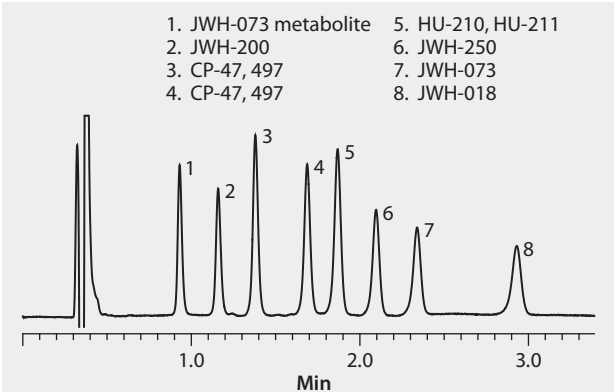
column . . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 5 µm particles (50526-U)
mobile phase . . . . . (A) 0.1% formic acid in water;
(B) 0.1% formic acid in water:acetonitrile (10:90)
gradient . . 20% B held for 3 min; 20 to 100% B in 0.1 min; 100% B held for 2 min
flow rate . . . . . 0.5 mL/min
pressure . . . . . 1037 psi (71.5 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 315 nm
injection . . . . . 2 µL
sample . . . . . 17 µg/mL each in acetonitrile
Application No. . . . . G005834



## HPLC Analysis of Spice Cannabinoids on Ascentis® Express F5

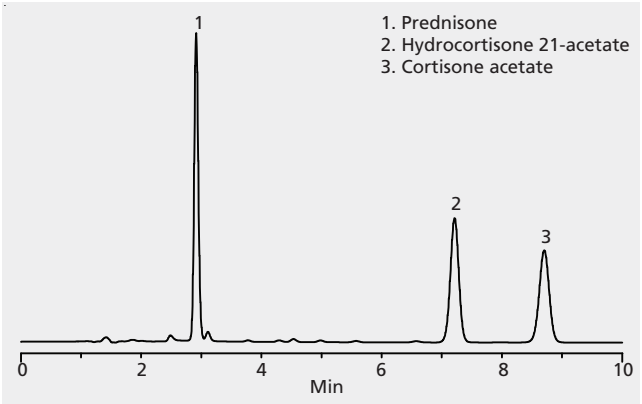
Synthetic cannabinoids (Spice) are a relatively new type of designer drug used as a pseudo-legal means to get a cannabis-type high. New synthetic cannabinoids are continually being introduced as suppliers tweak the molecular structures. The ability to rapidly and reliably identify the continually changing population of these compounds is a significant analytical challenge facing forensic chemists. A rapid separation of nine of these compounds on Ascentis Express F5 column is shown here.

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) 50 mM ammonium formate; (B) water; (C) acetonitrile; (10:35:55, A:B:C)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 4075 psi (281 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 200 nm
injection . . . . . 3 µL
sample . . . . . 100 µg/mL in 45:55 water:acetonitrile
Application No. . . . . G005446



## HPLC Analysis of Steroids on Ascentis® C18 (Mobile phase: 60:40)

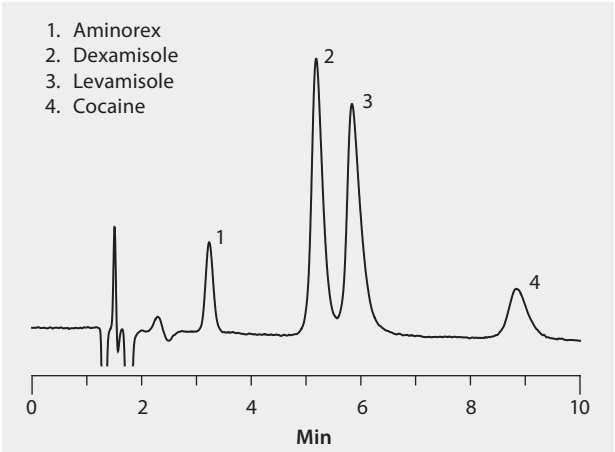
column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002432



## HPLC Analysis of Tetramisole Enantiomers and Related Compounds on Astec® Cyclobond® I 2000 DMP

Cocaine is often found laced with levamisole (one of the tetramisole enantiomers) and aminorex is a metabolite of levamisole known to cause disorders.

| | |
|---|---|
| column | Astec CYCLOBOND I 2000 DMP 10 cm X 2.1 mm |
| mobile phase | (A) 100 mM ammonium acetate, pH 5 with acetic acid (B) acetonitrile, 90:10, v/v |
| flow rate | 0.2 mL/min |
| pressure | 360 psi |
| column temp. | 35 °C |
| detector | UV, 220 nm |
| injection | 2.0 µL |
| sample | 100 ug/mL tetramisole and 50 ug/mL each cocaine and aminorex in water:methanol, 80:20, v/v |
| Application No. | G006305 |



## HPLC Analysis of Tetramisole Enantiomers on Astec® Cyclobond® I 2000 DMP

Cyclobond I 2000 DMP is effective at chiral recognition of tetramsiole enantiomers. This method appears to be a suitable starting point for further development towards the LC/MS analysis of tetramisole in various matrices. The method is also a suitable replacement for published methods utilizing Cyclobond I 2000 SN (no longer available) for the analysis of Levamisole.

column . . . . . ASTEC CYCLOBOND I 2000 DMP 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)

mobile phase . . . . . (A) 100 mM ammonium acetate, pH 5 with acetic acid (B) acetonitrile, 80:20, v/v

flow rate . . . . . 1.0 mL/min

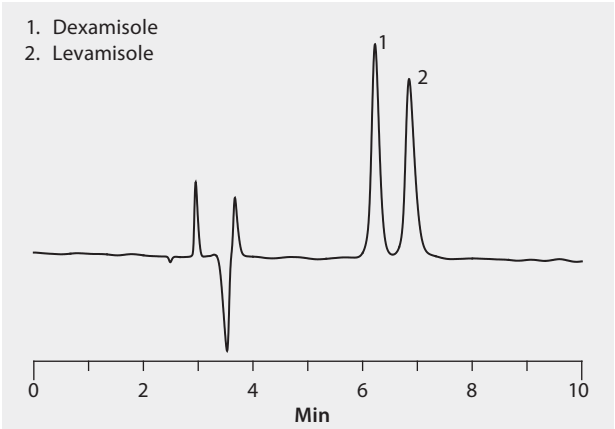
column temp. . . . . 35 °C

detector . . . . . UV, 230 nm

injection . . . . . 10 µL

sample . . . . . 200 ug/mL tetramisole in mobile phase A:methanol, 80:20, v/v

Application No. . . . . G006293

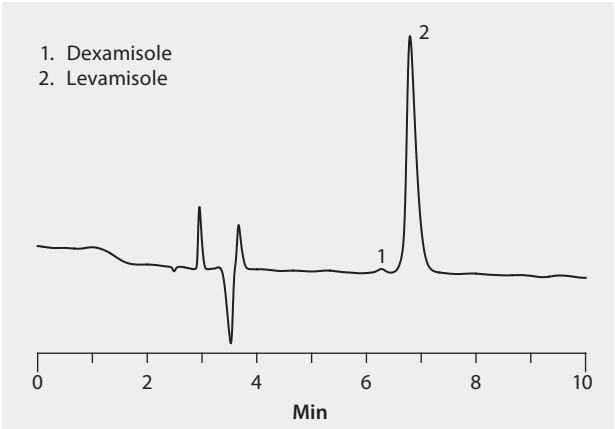



**Ref:** 1. D. W. Armstrong, C. D. Chang, S. H Lee, *J. Chromatogr. A* **539**, 83-90 (1991)
2. M. Dolezalova, M. Tkaczykova, *J. Pharm. Biomed. Anal.* **25**, 407-415 (2001)

## HPLC Chiral Analysis of Levamisole on Astec® Cyclobond® I 2000 DMP

Cyclobond I 2000 DMP is effective at chiral recognition of dexamisole from levamisole formulations. This method appears to be a suitable starting point for further development towards the analysis of levamisole chiral purity. The method is also a suitable replacement for published methods utilizing Cyclobond I 2000 SN which is no longer available.

column . . . . . ASTEC CYCLOBOND I 2000 DMP 25 cm x 4.6 mm I.D., 5µm particles (20724AST)
mobile phase . . . . . (A) 100 mM ammonium acetate, pH 5 with acetic acid (B) acetonitrile, 80:20, v/v
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1600 psi (110 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
sample . . . . . 200 ug/mL tetramisole in mobile phase A:methanol, 80:20, v/v
Application No. . . . . G006294

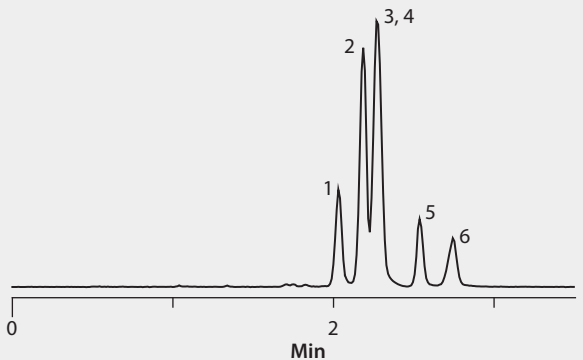



**Ref:** 1. D. W. Armstrong, C. D. Chang, S. H Lee, *J. Chromatogr. A* **539**, 83-90 (1991)
2. M. Dolezalova, M. Tkaczykova, *J. Pharm. Biomed. Anal.* **25**, 407-415 (2001)

## LC-MS Analysis of 2C Amines Drugs of Abuse in Urine on Ascentis® Express HILIC following Sample Prep using Supel™-Select SCX SPE

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . spiked urine, 1 mL
SPE tube/cartridge . . Supel-Select SCX SPE 96-well Plate, 30 mg/well (575664-U)
condition . . . . . 1 mL methanol, then 1 mL 0.1% formic acid in water
washing . . 2 x 1 mL 10% methanol in water, 2 x 1 mL 1% formic acid in methanol
elution . . . . . 2 mL 5% ammonium hydroxide in acetonitrile
eluate post-treatment . . evaporate to 500 µL with nitrogen at room temperature, reconstitute in 250 µL acetonitrile
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . 5 mM ammonium formate in acetonitrile:water (90:10, v/v)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1570 psi (108 bar)
column temp. . . . . 35 °C
detector . . . . . TOF/MS
injection . . . . . 2.0 µL
Application No. . . . . G006193

| Peak | Compound | Rentention | m/z |
|---|---|---|---|
| 1 | Bromo Dragonfly | 2.03 | 294 |
| 2 | DOI | 2.18 | 322 |
| 3 | 2C-T-7 | 2.27 | 256.1 |
| 4 | DOB | 2.29 | 274.1 |
| 5 | 2C-B | 2.54 | 260 |
| 6 | 2C-B-Fly | 2.74 | 284 |



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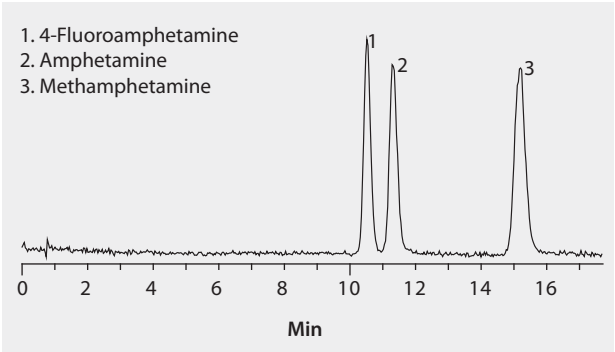
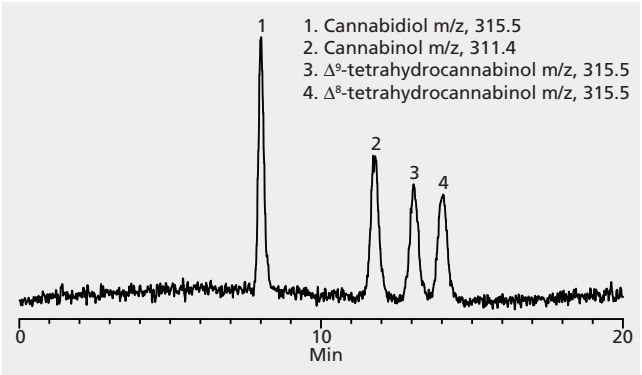
## LC-MS Analysis of Amphetamines on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 10 cm x 3 mm I.D., 2.7 µm particles (53970-U)
mobile phase . . (A) 1 mM ammonium acetate in (B) 10:90 (v/v) water:acetonitrile
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1189 psi (82 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+), full scan, m/z 110-200
injection . . . . . 2 µL
sample . . . . . 10 mg/L each in acetonitrile
Application No. . . . . G005667



## LC-MS Analysis of Cannabinoids on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 5.0 with acetic acid): (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 1 mL/min, split to the MS
column temp. . . . . 35 °C
detector . . . . . MS ESI (+), SIR Mode
injection . . . . . 5 µL
sample . . . . . 1 µg/mL in water:acetonitrile (1:1)
Application No. . . . . G002681



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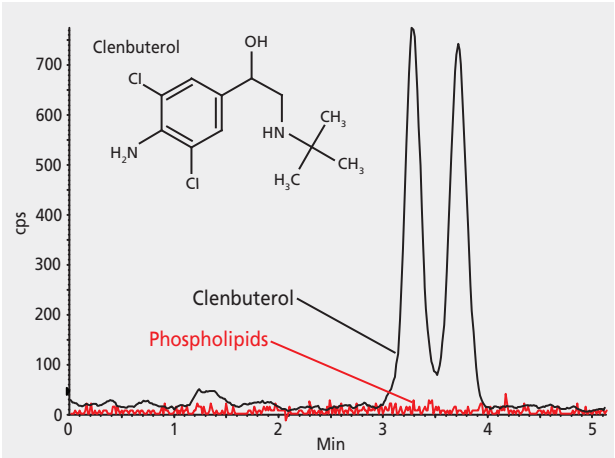
## LC-MS Analysis of Clenbuterol Enantiomers in Plasma on Astec® CHIROBIOTIC® T after SPE using HybridSPE®-Phospholipid

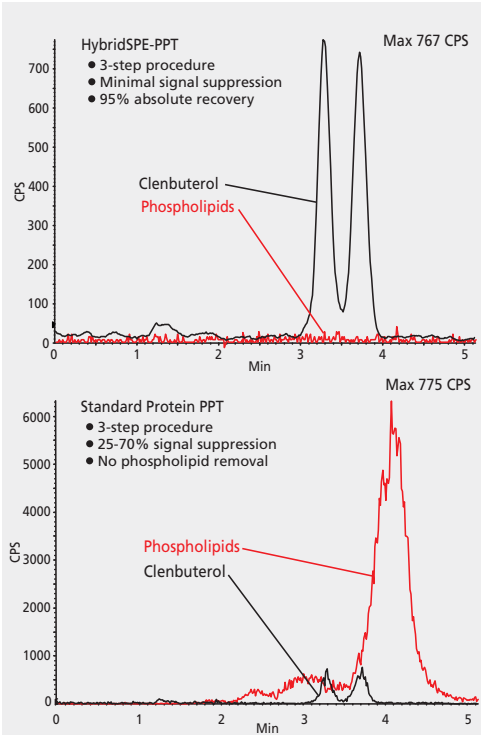
column . . . . . CHIROBIOTIC T, 10 cm x 2.1 mm I.D., 5 µm particles (12018AST)
mobile phase . . . . . 10 mM ammonium formate in methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . ESI(+)
sample . . . . . clenbuterol, 10 ng/mL in rat plasma (phospholipids removed by extraction with HybridSPE-Phospholipid)
Application No. . . . . G004245



## LC-MS Analysis of Clenbuterol In Plasma on Astec® CHIROBIOTIC® T with Phospholipid Removal Using HybridSPE® Phospholipid

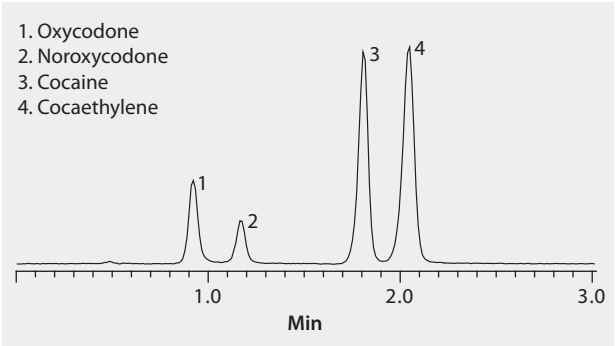
The HybridSPE method provides significant improvement in LC-MS baseline.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . rat plasma spiked with clenbuterol enantiomers at 10 ng/mL
SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample addition . . 100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile. Mix by vortexing the HybridSPE-PPT plate briefly.
elution . . . . . apply vacuum
column . . . . . CHIROBIOTIC T, 10 cm x 2.1 mm I.D., 5 µm particles (12018AST)
mobile phase . . . . . 10 mM ammonium formate in methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . ABI 3200 QT; ESI(+), MRM: 184/104 m/z (phospholipids); 277.2/203.1 m/z (clenbuterol)
injection . . . . . 10 µL
Application No. . . . . G004431



## LC-MS Analysis of Cocaine, Cocaethylene, Oxycodone, and Noroxycodone on Discovery® HS F5

column . . . . . Discovery HS F5, 5 cm x 2.1 mm I.D., 3 µm particles (567500-U)
mobile phase . . . . . (A) 10 mM ammonium formate in water, pH 6.8; (B) 10mM ammonium formate in acetonitrile, pH 6.8; (10:90, A:B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+)
injection . . . . . 2 µL
sample . . . . . 200 ng/mL in 25:75, water:acetonitrile
Application No. . . . . G005687



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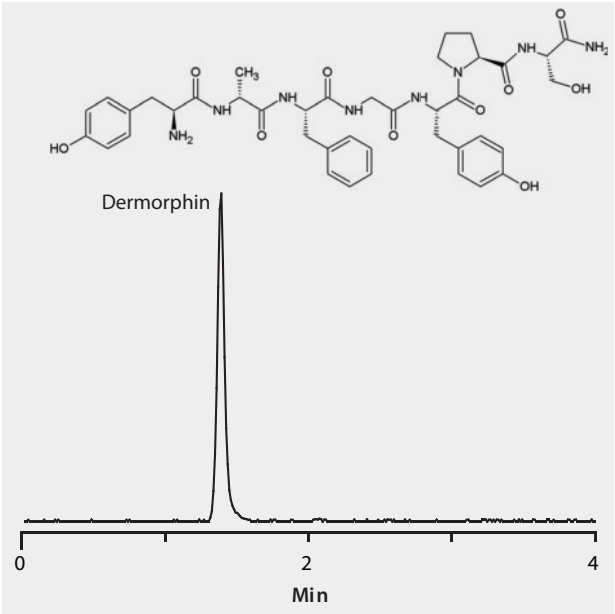
## LC-MS Analysis of Dermorphin in Horse Plasma on Ascentis® Express F5

Dermorphin is a heptapeptide derived from frogs. It is a painkiller, and used illicitly in racehorses so they continue to run through the pain.

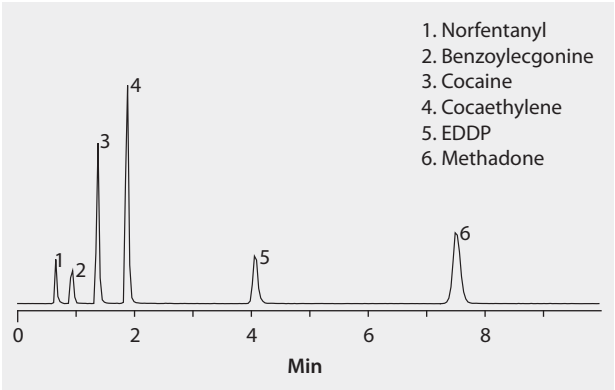
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
sample . . . . . 2 mM ammonium formate (90:10 acetonitrile:water) adjusted to pH 4.5 with formic acid
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . ESI(+), TIC 100 – 1000 m/z; APCI (+), XIC m/z = 150.1, 203.7, 378.2
injection . . . . . 1 µL
sample . . . . . 10 µg/mL in 75:25 acetonitrile:water
Application No. . . . . G005826



## LC-MS Analysis of Drugs of Abuse on Ascentis® Express RP-Amide

This applic.ation demonstrates the suitability of Ascentis Express RP-Amide for the efficient separation of common drink additives used in diet cola

| | |
|---|---|
| column | Ascentis Express RP Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U) |
| mobile phase | (A) 10 mM ammonium formate in water; (B) 10mM ammonium formate in acetonitrile; (75:25, A:B) |
| flow rate | 0.2 mL/min |
| column temp. | 35 °C |
| detector | ESI(+) |
| injection | 2 µL |
| sample | 500 ng/mL in water |
| Application No. | G005695 |



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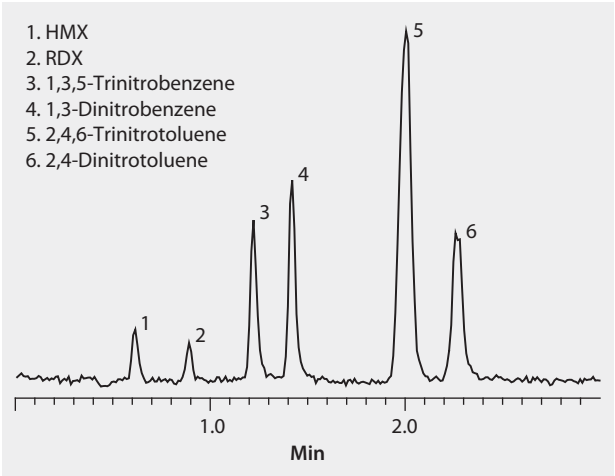
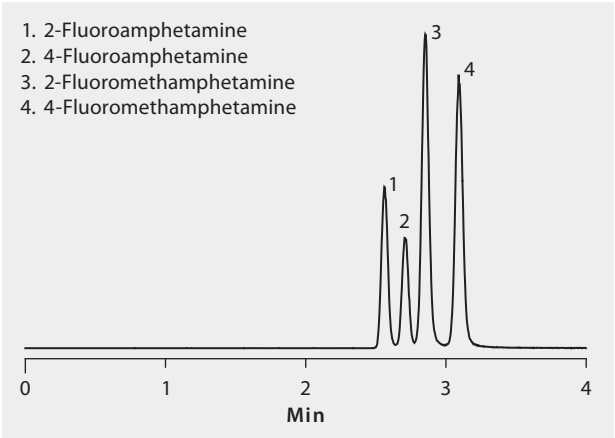
## LC-MS Analysis of Explosives on Ascentis® Express C18

column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) methanol; (B) water; (50:50, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 50 °C
detector . . . . . MS: APCI(-)
injection . . . . . 5 µL
sample . . . . . 100 ng/mL in 50:50, methanol:water
Application No. . . . . G005608



## LC-MS Analysis of Fluoroamphetamine Drugs of Abuse on Ascentis® Express HILIC

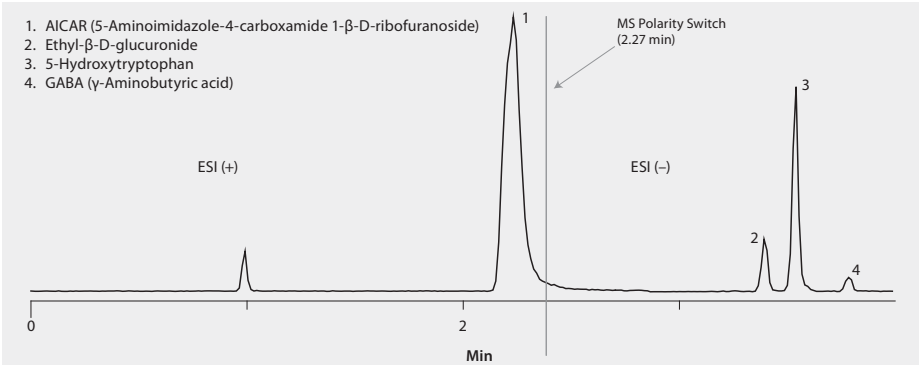
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
gradient . . . . . 5 mM ammonium formate in acetonitrile:water (90:10, v/v)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . TOF/MS
injection . . . . . 2.0 µL
sample . . . . . 300 ng/mL in acetonitrile
Application No. . . . . G006165



## LC-MS Analysis of GABA and AICAR on Ascentis® Express HILIC

This study focuses on the analysis of possible dopants used in equine racing, polar compounds requiring HILIC chromatographic separation.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . rat plasma or human serum spiked at 0.05 ng/mL with digitoxin and digoxin
SPE well plate . . . . . HybridSPE-PLus 96-well Plate, 50 mg/well (575659-U)
sample addition . . . . . 100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile
elution . . . . . apply vacuum
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 5 mM ammonium formate in acetonitrile:water (95:5); (B) 5 mM ammonium formate in acetonitrile:water (70:30)
gradient . . . . . 0% to 70%B in 3 min, held for 1 min
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI+/ESI-
injection . . . . . 2.0 µL
sample . . . . . 1 µg/mL in acetonitrile
Application No. . . . . G006301



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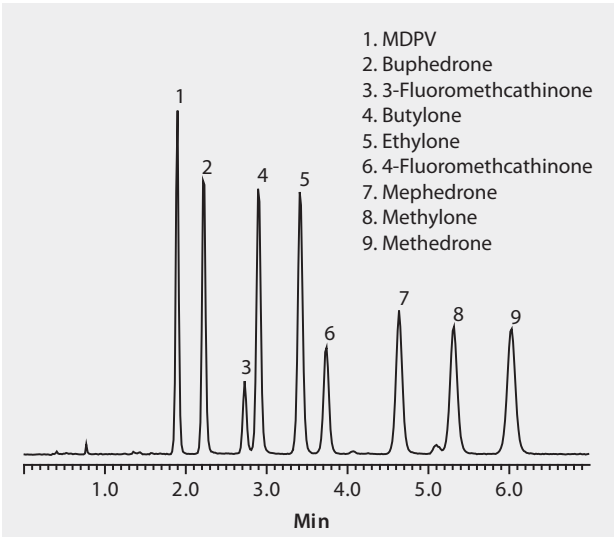
## LC-MS Analysis of Illicit Bath Salts in Urine on Ascentis® Express HILIC after Solid Phase Extraction (SPE) on Supel™-Select SCX

The designer drugs known as "bath salts" are synthetic stimulants and illegal in most states as of 2011. This application demonstrates the analysis of bath salts extracted from human urine samples using polymeric solid phase extraction (SPE) sample preparation followed by hydrophilic interaction liquid chromatography (HILIC) analysis with TOF-MS detection. HILIC conditions on the Ascentis Express HILIC (Si) phase are used for fast, high-resolution separation of nine synthetic bath salts. Recoveries greater than 65% were observed for all analytes except MDPV (43.7%). The Figure illustrates the detection of bath salts in the spiked urine sample after SPE sample cleanup. Notice there are no interfering peaks in the chromatogram, demonstrating the effectiveness of the SPE sample cleanup.

sample/matrix . . Urine samples were spiked to a level of 100 ng/mL with each target analyte. (To ensure full ionization of the analytes, spiked samples were treated with formic acid to a final concentration of 0.1% formic acid.)
SPE tube/cartridge . . . . . Supel-Select SCX, 30 mg/1 mL (54240-U)
condition . . . . . 1 mL 1% formic acid acetonitrile then 1 mL water
sample addition . . . . . 1 mL spiked water blank or urine
washing . . . . . 1 mL water, 1 mL 1% formic acid acetonitrile, 1 mL water
elution . . . . . 2 mL 10% ammonium hydroxide in acetonitrile
eluate post-treatment . . thoroughly mix via vortex agitation, evaporate 1 mL aliquot to dryness, reconstitute in 100 µL water:methanol
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 5 mM ammonium formate acetonitrile; (B) 5 mM ammonium formate water; (98:2, A:B); premixed
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1842 psi (127 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+), TIC, m/z 100-1000
injection . . . . . 1 µL
sample . . . . . 200 µg/L ea. in acetonitrile
Application No. . . . . G005796



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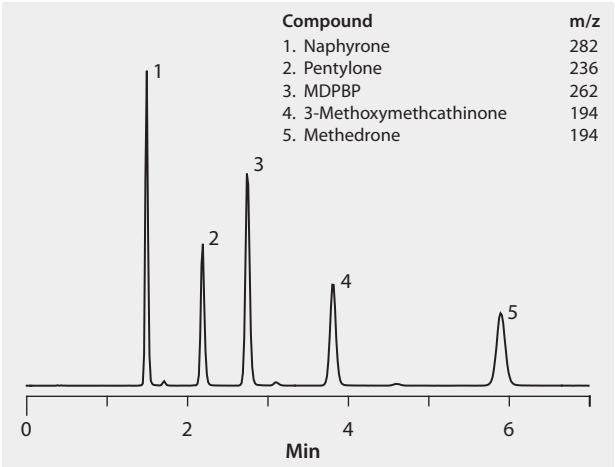
## LC-MS Analysis of Illicit Bath Salts on Ascentis® Express HILIC, Set 1

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . 5 mM ammonium formate in (A) acetonitrile; (B) water; (98:2, A,B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 267 psi (18.4 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+) TOF, Summed Ion Chromatogram
injection . . . . . 2 µL
sample . . . . . 500 ng/mL in acetonitrile
Application No. . . . . G005910



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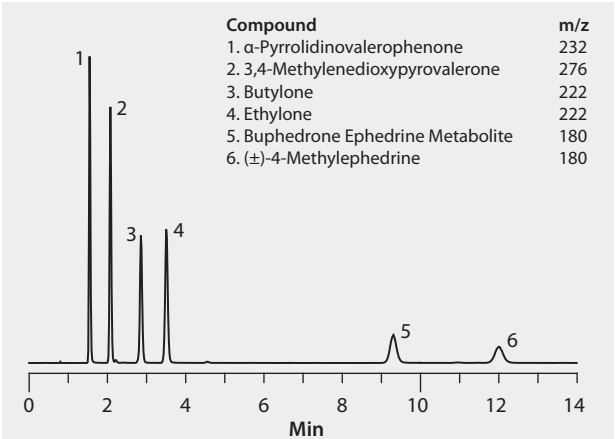
## LC-MS Analysis of Illicit Bath Salts on Ascentis® Express HILIC, Set 2

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . (A) 5 mM ammonium formate in acetonitrile: (B) water; (98:2, A;B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 267 psi (18.4 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+) TOF, Summed Ion Chromatogram
injection . . . . . 2 µL
sample . . . . . 500 ng/mL in acetonitrile
Application No. . . . . G005911



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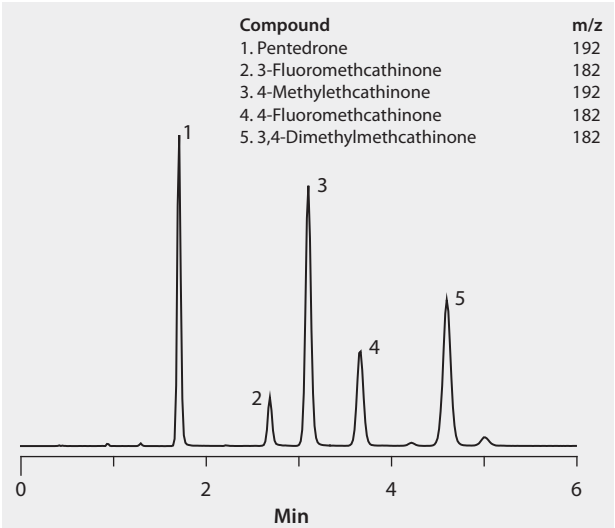
## LC-MS Analysis of Illicit Bath Salts on Ascentis® Express HILIC, Set 3

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . (A) 5 mM ammonium formate in acetonitrile; (B) water; (98:2, A:B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 267 psi (18.4 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+) TOF, Summed Ion Chromatogram
injection . . . . . 2 µL
sample . . . . . 500 ng/mL in acetonitrile
Application No. . . . . G005912



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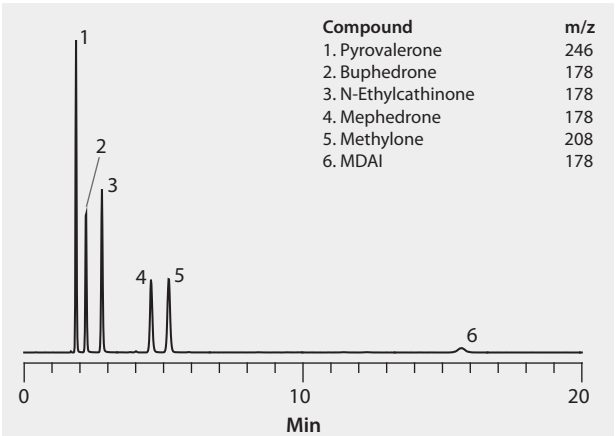
## LC-MS Analysis of Illicit Bath Salts on Ascentis® Express HILIC, Set 4

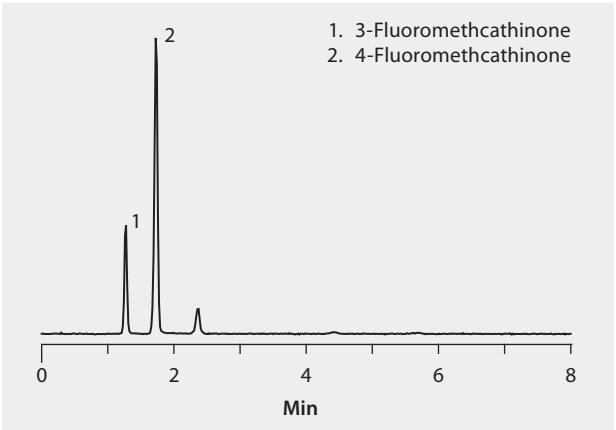
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . 5 mM ammonium formate in (A) acetonitrile; (B) water; (98:2, A:B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 267 psi (18.4 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+) TOF, Summed Ion Chromatogram
injection . . . . . 2 µL
sample . . . . . 500 ng/mL in acetonitrile
Application No. . . . . G005913




| Compound | m/z |
|---|---|
| 1. Pyrovalerone | 246 |
| 2. Buphedrone | 178 |
| 3. N-Ethylcathinone | 178 |
| 4. Mephedrone | 178 |
| 5. Methylone | 208 |
| 6. MDAI | 178 |

## LC-MS Analysis of Isobaric Bath Salts 3- and 4-Fluoromethcathinone on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 5 mM ammonium formate (98:2 acetonitrile:water)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1262 psi (87 bar)
detector . . . . . ESI(+), 182 m/z
injection . . . . . 1.0 µL
sample . . . . . 20 ng/mL
Application No. . . . . G006224



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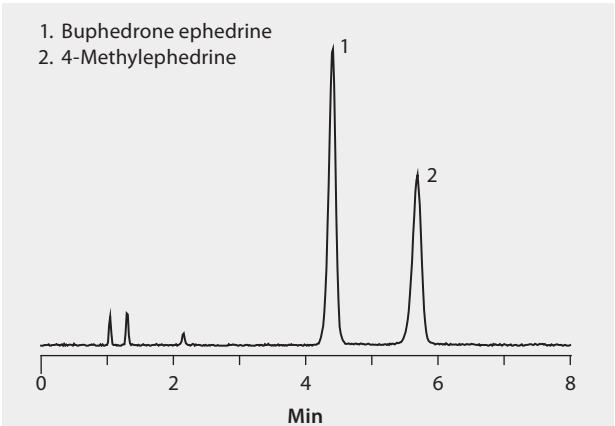
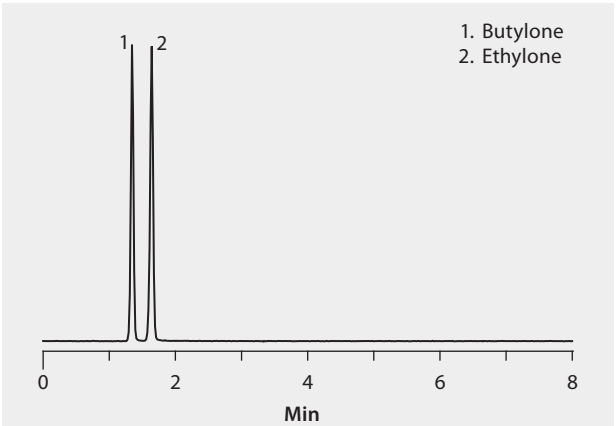
## LC-MS Analysis of Isobaric Bath Salts Buphedrone Ephedrine and 4-Methylephedrine on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 5 mM ammonium formate (98:2 acetonitrile:water)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1262 psi (87 bar)
detector . . . . . ESI(+), 180 m/z
injection . . . . . 1.0 µL
sample . . . . . 20 ng/mL
Application No. . . . . G006226



## LC-MS Analysis of Isobaric Bath Salts Ethylone and Butylone on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 5 mM ammonium formate (98:2 acetonitrile:water)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1262 psi (87 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+), 222 m/z
injection . . . . . 1.0 µL
sample . . . . . 20 ng/mL
Application No. . . . . G006228



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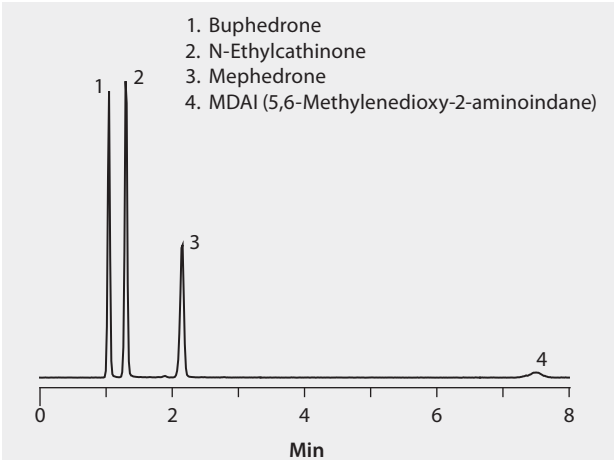
## LC-MS Analysis of Isobaric Bath Salts Mephedrone, Buphedrone, N-Ethylcathinone and MDAI on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 5 mM ammonium formate (98:2 acetonitrile:water)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1262 psi (87 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+), 178 m/z
injection . . . . . 1.0 µL
sample . . . . . 20 ng/mL
Application No. . . . . G006227



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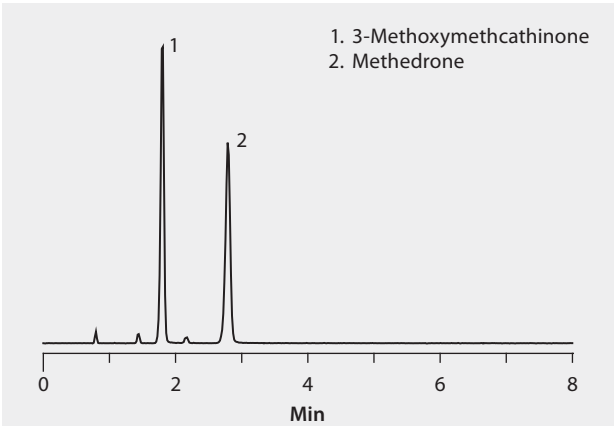
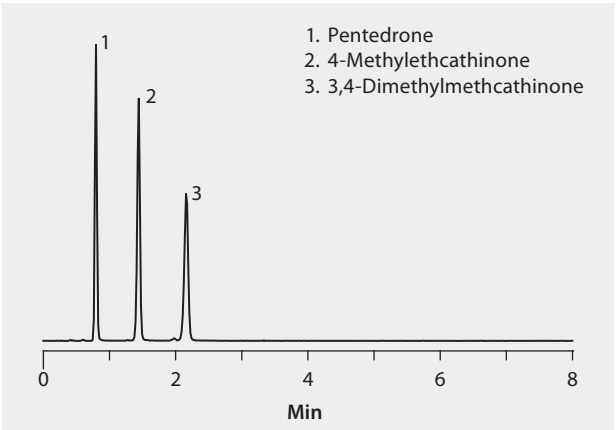
## LC-MS Analysis of Isobaric Bath Salts 3-Methoxymethcathinone and Methedrone on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . (5 mM ammonium formate (98:2 acetonitrile:water))
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1262 psi (87 bar)
detector . . . . . ESI(+), 194 m/z
injection . . . . . 1.0 µL
sample . . . . . 20 ng/mL
Application No. . . . . G006225



## LC-MS Analysis of Isobaric Bath Salts Pentedrone, 4-Methylethcathinone and 3,4-Dimethylmethcathinone on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 5 mM ammonium formate (98:2 acetonitrile:water)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1262 psi (87 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+), 192 m/z
injection . . . . . 1.0 µL
sample . . . . . 20 ng/mL
Application No. . . . . G006229



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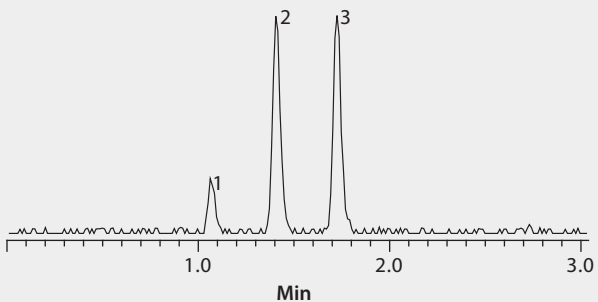
## LC-MS Analysis of Methadone and Metabolites EDDP and EMDP on Ascentis® Express RP Amide

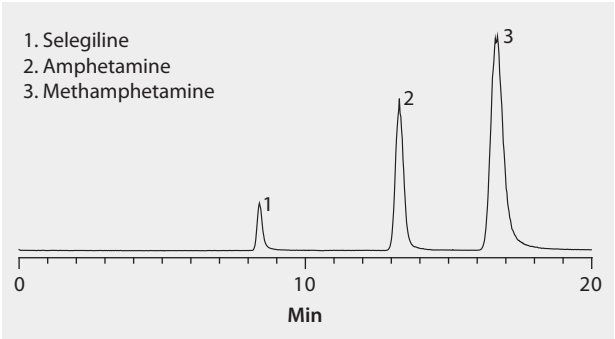
| | |
|---|---|
| column | Ascentis Express RP Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U) |
| mobile phase | (A) 10 mM ammonium formate in water, pH 3.6; (B) 10 mM ammonium formate in acetonitrile, pH 3.6; (65:35, A:B) |
| flow rate | 0.5 mL/min |
| column temp. | 35 °C |
| detector | ESI(+), m/z 100-1000 |
| injection | 0.5 µL |
| sample | 200 µg/L in 25:75, water: acetonitrile |
| Application No. | G005709 |

1. EDDP (2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)
2. Methadone
3. EMDP (2-Ethyl-5-methyl-3,3-diphenylpyrroline)



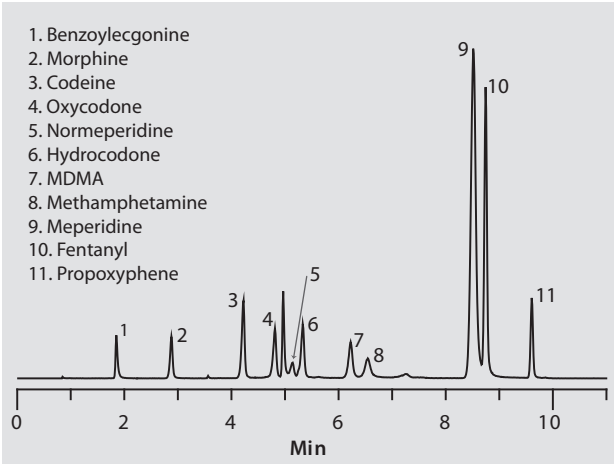
## LC-MS Analysis of Selegiline and Amphetamines on Ascentis® Express F5

column . . . Ascentis Express F5, 10 cm x 4.6 mm I.D., 2.7 µm particles (53590-U)
mobile phase . . . . . (A) 10 mM ammonium acetate, pH 4.0 with acetic acid; (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 1.0 mL/min
pressure . . . . . 2320 psi (160 bar)
column temp. . . . . 25 °C
detector . . . . . MS, ESI(+), SIR, m/z 136, 150, 188
injection . . . . . 2 µL
sample . . . . . 10 µg/mL in methanol
Application No. . . . . G005734



## LC-MS/MS Analysis of Drugs of Abuse: Generic Screening on Ascentis® Express F5

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) 5 mM ammonium formate water pH 4.0 formic acid;
(B) 5 mM ammonium formate (95:5 acetonitrile:water)
gradient . . . . . 20%B for 0.5 min; to % B in 5.5 min; held at 100%B for 3 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 2538 psi (175 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+), TIC, m/z 100-1000
injection . . . . . 1 µL
sample . . . . . each compound 1000 ng/mL in 40:60 (water:methanol)
Application No. . . . . G005881



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## LC-MS/MS Analysis of Spice Compounds from Plasma on Ascentis® Express F5 after SPE using HybridSPE®-Phospholipid

Synthetic cannabinoids (Spice) are a relatively new type of designer drug used as a pseudo-legal means to get a cannabis-type high. New synthetic cannabinoids are continually being introduced as suppliers tweak the molecular structures. The ability to rapidly and reliably identify the continually changing population of these compounds in the blood or urine suspected users is a significant analytical challenge facing forensic chemists. A four prong approach using column selectivity, high purity solvents, effective sample prep, and reference standards, was used to develop a method to rapidly isolate and identify Spice cannabinoids from plasma. The Ascentis Express F5 column gave the necessary resolution, and the LC-MS CHROMASOLV solvents and additives gave adduct-free response for maximum sensitivity. Sample prep employing the HybridSPE-Phospholipid was rapid and effective and the Cerilliant reference standards enabled confident identification.

sample/matrix . . . . . rabbit plasma, unfiltered K2-EDTA spiked with Spice cannabinoids (5 ng/mL each)

SPE tube/cartridge . . . . . HybridSPE-Phospholipid, 96-well plate, 50 mg bed wt., 2 mL well vol (575656-U)

sample addition . . . . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 2 minutes

elution . . . . . attach collection plate and apply vacuum at 10" Hg for 4 minutes

column . . . Ascentis Express F5, 5 cm x 2.1 mm I.D., 2.7 µm particles (53567-U)

mobile phase . . . . . (A) 10 mM ammonium formate in water, pH 6.8 (unadjusted); (B) acetonitrile; (50:50; A:B)

flow rate . . . . . 0.3 mL/min

pressure . . . . . 1296 psi (89 bar)

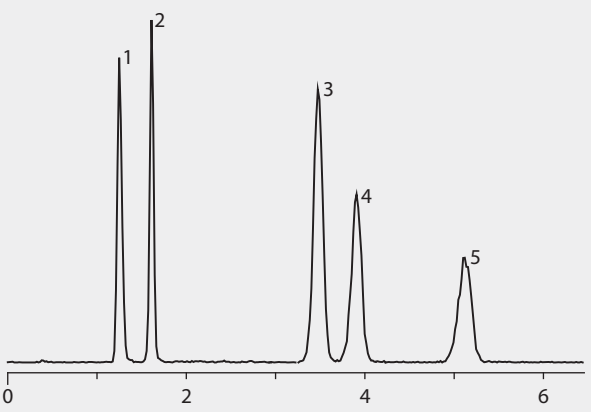
column temp. . . . . 35 °C

detector . . . . . MS, ESI(+), MRM, m/z 344/155 (JWH-073 metabolite), 385/155 (JWH-200), 336/121 (JWH-250), 328/155 (JWH-073), and 342/155 (JWH-018)

injection . . . . . 2 µL

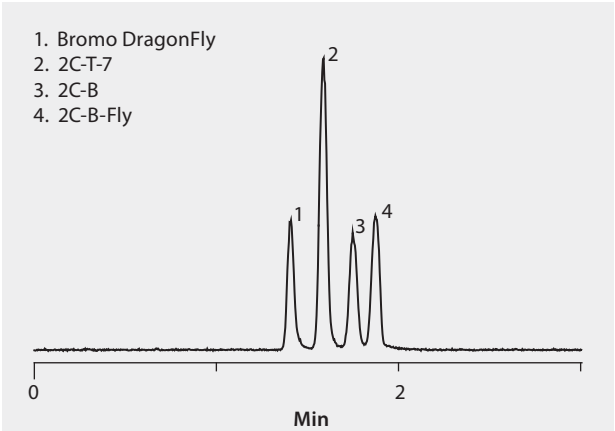
Application No. . . . . G005786

1. JWH-073 3-hydroxybutyl metabolite
2. JWH-200
   ((1-(2-Morpholin-4-ylethyl)indol-3-yl)-naphthalen-1-ylmethanone)
3. JWH-250
   (2-(2-Methoxyphenyl)-1-(1-penylindol-3-yl)ethanone)
4. JWH-073
   (Naphthalen-1-yl-(1-butylindol-3-yl)methanone)
5. JWH-018
   (Naphthalen-1-yl-(1-pentylindol-3-3yl)methanone)



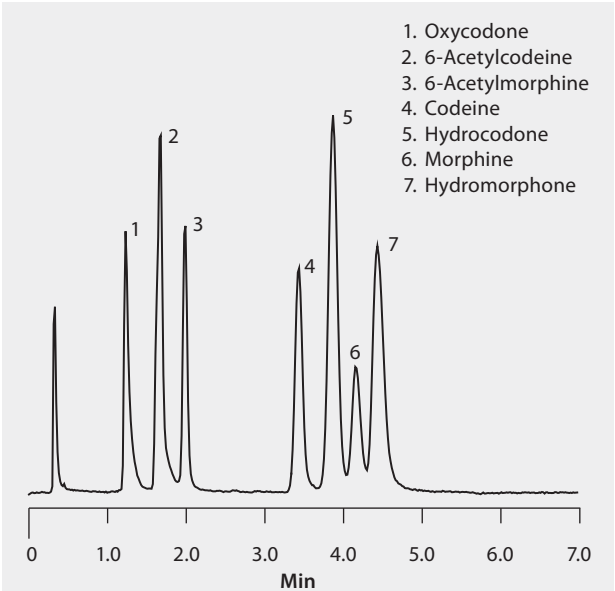
## LC-MS (TOF) Analysis of 2C Amine Drugs of Abuse on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 2 mM ammonium formate in 90:10 (v:v) acetonitrile:water
flow rate . . . . . 0.4 mL/min
pressure . . . . . 943 psi (65 bar)
column temp. . . . . 35 °C
detector . . . . . TOF/MS
injection . . . . . 2 µL
sample . . . . . 300 ng/mL in acetonitrile
Application No. . . . . G006152



## LC-MS (TOF) Analysis of Opioids and Opiate-Dependence Management Drugs on Ascentis® Express HILIC

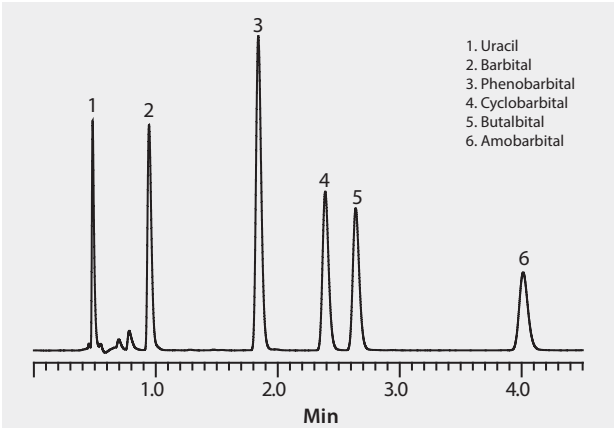
column . . Ascentis Express HILIC, 5 cm x 2.1 mm I.D., 2.7 µm particles (53934-U)
mobile phase . . . . . 2 mM ammonium formate in 90:10 (v:v) acetonitrile:water
flow rate . . . . . 0.3 mL/min
column temp. . . . . 55 °C
detector . . . . . TOF/MS
injection . . . . . 2 µL
sample . . . . . 400 ng/mL in acetonitrile
Application No. . . . . G006153



## UHPLC Analysis of Barbituates on Titan™ C18

Analysis of a Barbituate Mix using a MS compatible mobile phase.

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% ammonium acetate (not adjusted); (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 7540 psi (520 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
Application No. . . . . G006073



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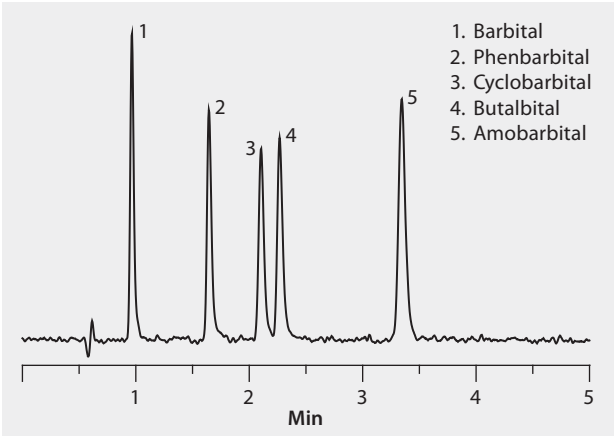
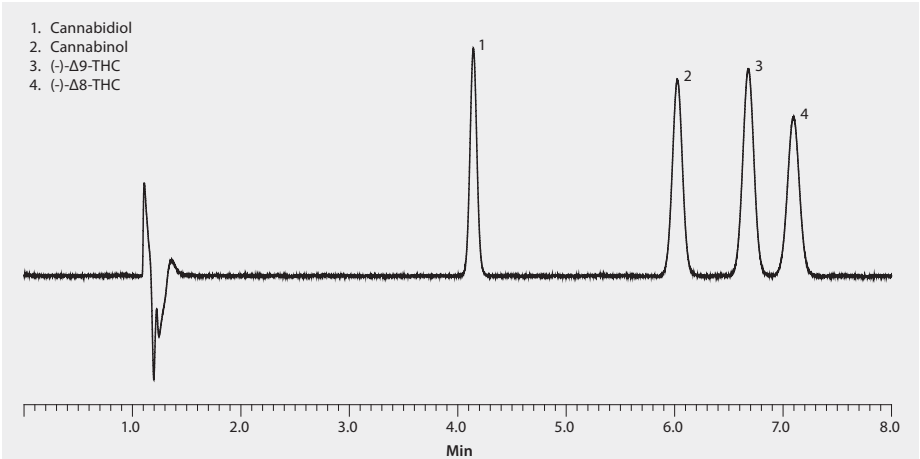
## UHPLC Analysis of Barbituates on Titan™ C18 with MS Detection

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% ammonium acetate (not adjusted); (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 6796 psi (468 bar)
column temp. . . . . 35 °C
detector . . . . . ESI (-) MS TIC MRM
injection . . . . . 1 µL
sample . . . . . 1000 ng/ml in mobile phase
Application No. . . . . G006137



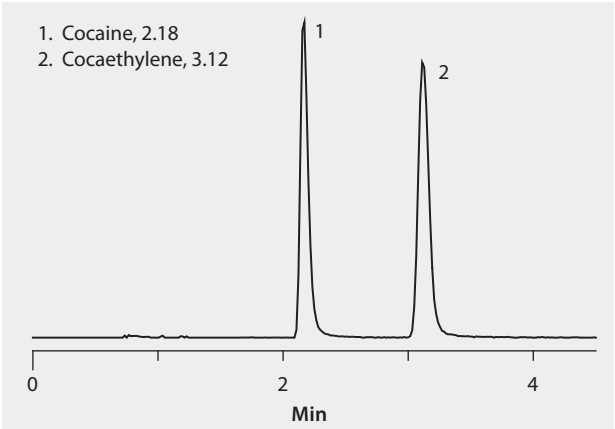
## UHPLC Analysis of Cannabinoids Using Ascentis® Express 5µm RP-Amide

column . . Ascentis Express RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (50774-U)
mobile phase . . . . . 5 mM ammonium acetate (pH 4.5 with acetic acid) in 20:80, acetonitrile:water
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 5.0 µL
sample . . . . . 4 cannabinoids 50 µg/mL 50:50, water:methanol
Application No. . . . . G006303



## UHPLC Analysis of Cocaine and Cocaethylene on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 304.15 and 318.17
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006091



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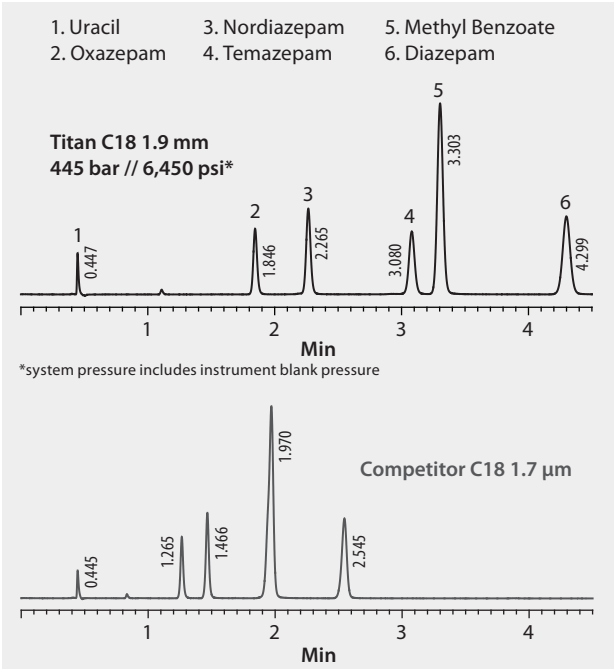
## UHPLC Analysis of Diazepam and Metabolites on Titan™ C18, Competitive Comparison

This application shows a comparison between Titan™ C18 and a competitive column for diazepam and its metabolites using a MS compatible mobile phase.

Used 75 µm I.D. x 250 mm L tubing in system.

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid; (B) acetonitrile; (60:40, A:B)
pressure . . . . . 6450 psi (445 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
Application No. . . . . G006001



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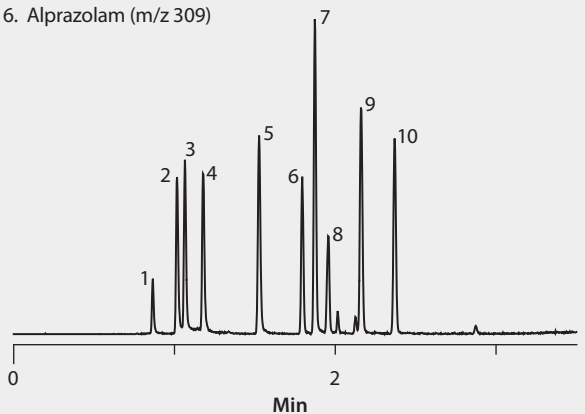
## UHPLC Analysis of Heroin and Related Compounds on Titan™ C18

column . . . . . Titan C18 5 cm x 2.1 mm I.D., 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 5 mM ammonium acetate in 95:5, water:acetonitrile;
(B) 5 mM ammonium acetate in 5:95, water:acetonitrile
gradient . . . . . 0 to 100% B in 3 min, held at 100% B for 1 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 6770 psi (467 bar)
column temp. . . . . 35 °C
detector . . . . . MS-TOF, XIC
injection . . . . . 0.5 µL
sample . . . . . 1 µg/mL in 95:5, water:methanol
Application No. . . . . G006116

1. Morphine (m/z 286)
2. Procaine (m/z 237)
3. Codeine (m/z 300)
4. 3-Monoacetylmorphine (m/z 328)
5. Heroin (m/z 370)
6. Alprazolam (m/z 309)
7. Papaverine (m/z 340)
8. Flunitrazepam (m/z 314)
9. Diazepam (m/z 285)
10. Noscapine (m/z 414)



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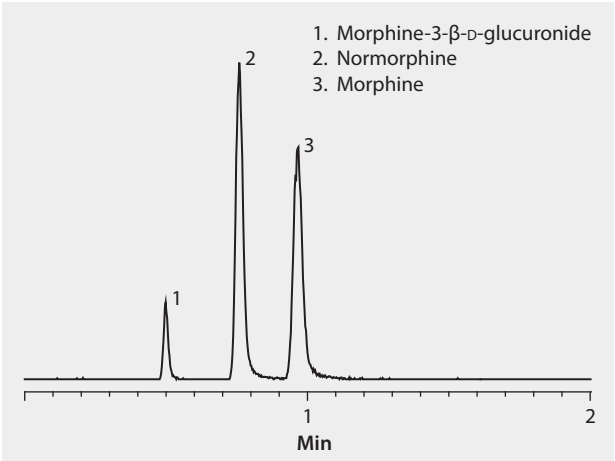
## UHPLC Analysis of Morphine and Metabolites on Titan™ C18 using MS Detection

column . . . . . Titan C18, 5 cm x 2.1 mm I.D., 1.9 µm particles (577122-U)
mobile phase . . . . . (A) water with 0.1% formic acid;
(B) acetonitrile with 0.1% formic acid ; (95:5, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 4960 psi (342 bar)
column temp. . . . . 25 °C
detector . . . . . MS-TOF, XIC
injection . . . . . 0.5 µL
sample . . . . . 1 µg/mL in 99:1, water:methanol
Application No. . . . . G006115



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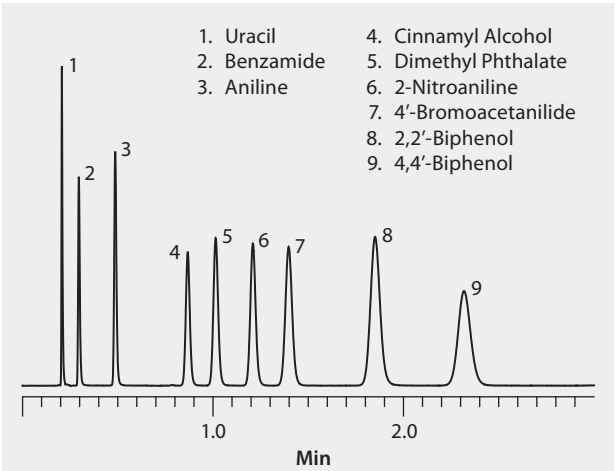
# General Analytical

## HPLC Analysis of Column Test Mixture on Ascentis® Express 5µm RP-Amide

This application shows a test mixture of compounds of widely varying polarity on the 5µm RPA Ascentis Express column.

column . . . . . Ascentis Express RPA 15 cm x 4.6 mm, 5 µm particles (50773-U)
mobile phase . . (A) 20 mM potassium phosphate, pH 7; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 4.0 mL/min
pressure . . . . . 4467 psi (308 bar)
column temp. . . . . 26 °C
detector . . . . . UV, 254 nm
injection . . . . . 5.0 µL
sample . . . . . 1. Uracil (0.016 mg/mL), 2. Benzamide (0.161 mg/mL), 3. Aniline (0.161 mg/mL), 4. Cinnamyl Alcohol (0.016 mg/mL), 5. Dimethyl Phthalate (0.323 mg/mL), (6. 2-Nitroaniline (0.081 mg/mL), 7. 4′-Bromoacetanilide (0.040 mg/mL), 8. 2,2′-Biphenol (0.161 mg/mL), 9. 4,4′-Biphenol (0.040 mg/mL), in 50/50 Water/Acetonitrile )
Application No. . . . . G006279



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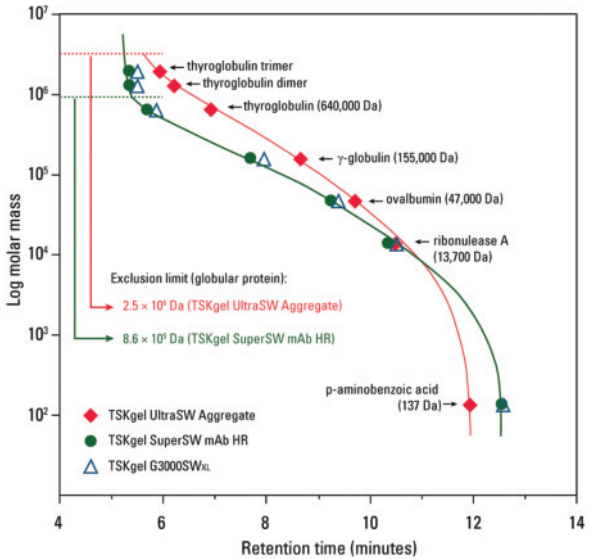
# Life Science and Biopharma

## Calibration Curves for TSKgel® SW mAb Columns

In gel fitration chromatography, the calibration curve of a column shows the relationship between retention time or retention volume of a set of water-soluble polymer or protein standards vs. the logarithm of their molecular masses. The linear portion of a calibration curve is used to estimate the molecular mass of an unknown protein. An expanded linear range is desirable as it allows for a wider variety of molecular masses to be analyzed with that column. This example shows the calibratuion curves for TSKgel® SuperSW mAb HTP, TSKgel SuperSW mAb HR, TSKgel UltraSW Aggregate and the industry standard TSKgel G3000SWxl column using several commonly available globular proteins and p-aminobenzoic acid as a small MW marker to indicate the void volume of the column. The exclusion volume of the column is indicated by a trimer of thyroglobulin. [Note that the calibration curve of the 15 cm x 4.6 mm ID TSKgel SuperSW mAb HTP column is not included in the Figure as the column volume is every small and the packing material in the column is the same as the TSKgel SuperSW mAb HR column].

column . . TSKgel SuperSW mAb HR, 30 cm x 7.8 mm I.D., 4 µm particles (822854)
column . . TSKgel UltraSW Aggregate, 30 cm x 7.8 mm I.D., 3 µm particles (822856)
column . . . . . TSKgel G3000SWxl, 30 cm x 7.8 mm I.D., 5 µm particles (808541)
mobile phase . . 0.2 M sodium phosphate buffer, pH 6.7 and 0.05% sodium azide
flow rate . . . . . 1.0 mL/min
column temp. . . . . Ambient
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
Application No. . . . . G006277



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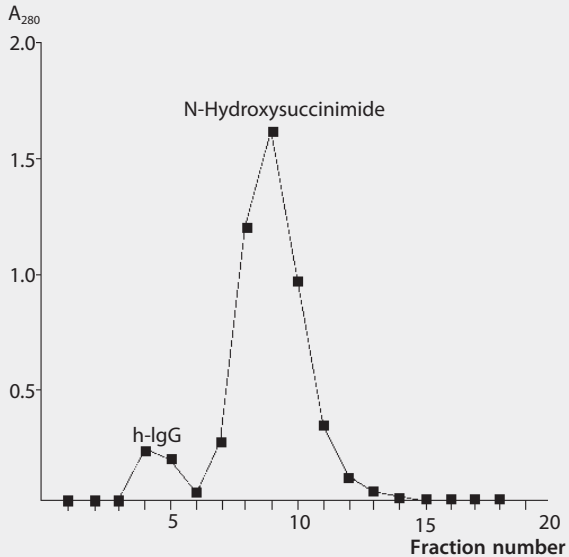
## Desalting of Immunoglobulins (IgG, Human) on HiTrap® Desalting Cartridge

column . . . . . HiTrap Desalting Cartridge, 5 mL (54822)
mobile phase . . . . . 0.2 M sodium carbonate buffer, pH 8.5
detector . . . . . UV, 280 nm
injection . . . 0.5 mL unbound human IgG in 0.2 M sodium carbonate buffer after coupling to a HiTrap NHS 1 mL column. Fraction volume 0.5 mL
Application No. . . . . 796-0403

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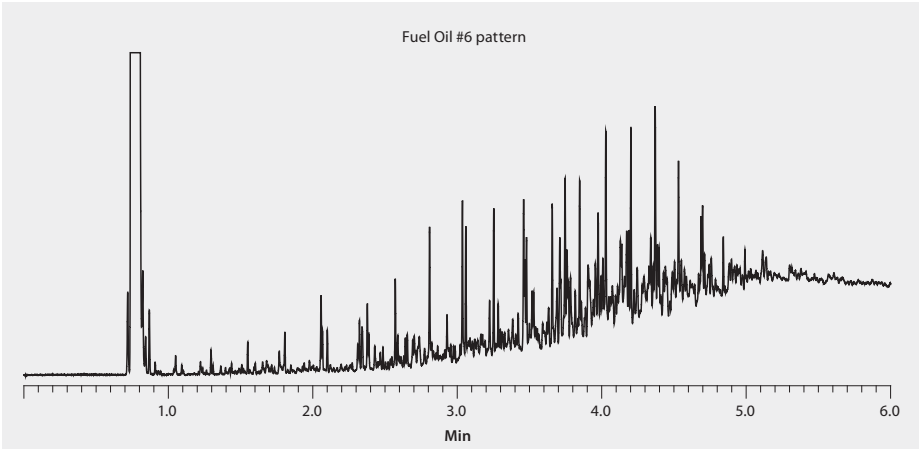
## GC Analysis of Fuel Oil #6 on Equity®-1 (15 m x 0.10 mm I.D., 0.10 µm), Fast GC Analysis

column . . . . . Equity-1, 15 m x 0.10 mm I.D., 0.10 µm (28039-U)
oven . . . . . 80 °C, 50 °C/min to 325 °C
inj. temp. . . . . 250 °C
detector . . . . . FID, 350 °C
carrier gas . . . . . hydrogen, 45 cm/sec constant
injection . . . . . 0.3 µL, 100:1 split, 0.1 min pre-injection dwell time
liner . . . . . 2 mm I.D., straight
sample . . . . . No.6 Fuel Oil standard, 20 mg/mL in hexane:chloroform (47536-U)
Application No. . . . . G003908



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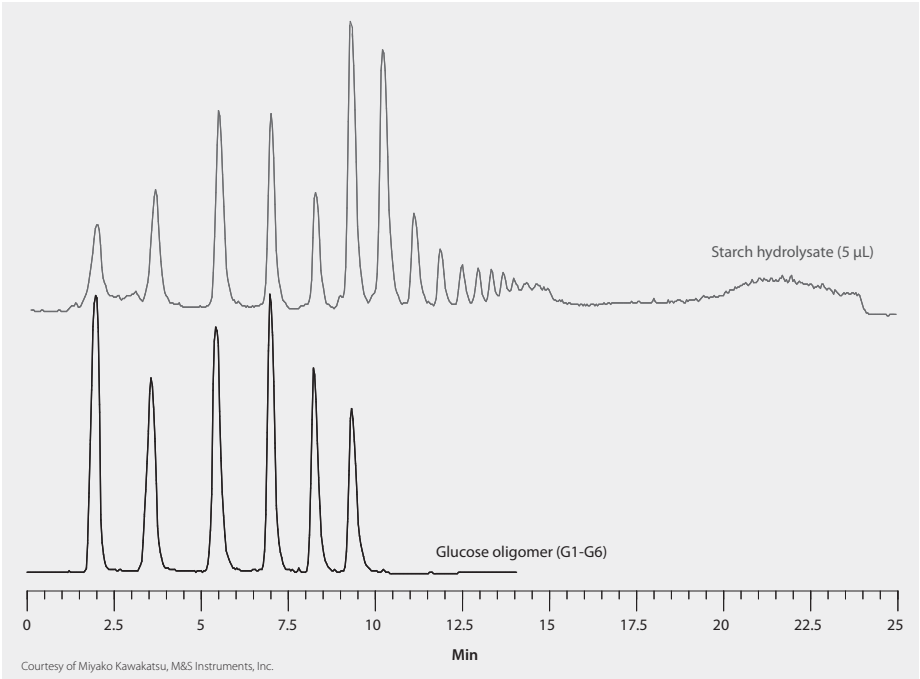
## HPLC Analysis Carbohydrates on Proteomix® SAX-NP5

column . . . . Proteomix SAX-NP5, 15 cm x 4.6 mm I.D., 5 µm particles (Z777228)
mobile phase . . . . . (A) 0.05% (25% ammonium hydroxide) in acetonitrile; (B) 0.05% (25% ammonium hydroxide) in water
gradient . . . . . 15 to 80% B in 20 min
flow rate . . . . . 0.7 mL/min
temp. . . . . 35 °C
detector . . . . . ELSD
sample . . . . . Corn starch hydrolysate; glucose oligomer (G1 to G6)
Application No. . . . . G005958

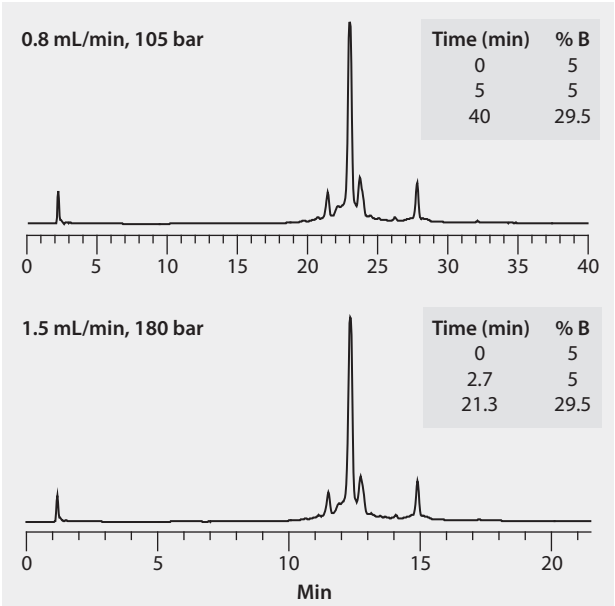



Courtesy of Miyako Kawakatsu, M&S Instruments, Inc.

## HPLC Analysis MAb Variants on Proteomix® SCX-NP5, Fast Analysis

Due to the low pressure of Proteomix SCX, faster analysis and high resolution can be achieved with a higher flow rate.

column . . . . Proteomix SCX-NP5, 25 cm x 4.6 mm I.D., 5 µm particles (Z777164)
mobile phase . . (A) 2.4 mM Tris, 1.5 mM imidazole, 11.6 mM piperazine, pH 6.0; (B) A + 0.5 M NaCl, pH 10.5
gradient . . . . . top (0.8 mL/min): 5% B held for 5 min; to 29.5% B in 35 min; bottom (1.5 mL/min): 5% B held for 2.7 min; to 29.5% B in 18.6 min
flow rate . . . . . 0.8 mL/min or 1.5 mL/min
detector . . . . . UV 280 nm
injection . . . . . 20 µL
sample . . . . . MAb 321, 5 mg/mL
Application No. . . . . G006032



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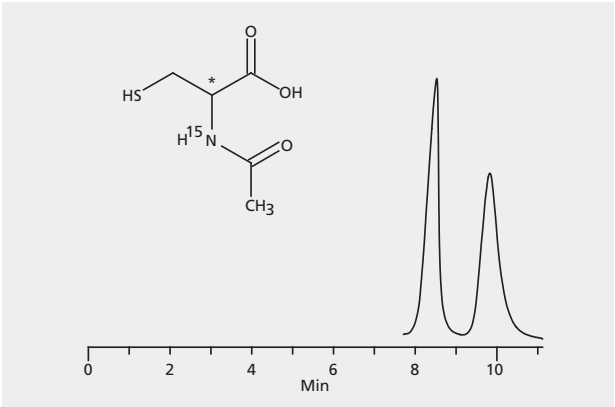
## HPLC Analysis of $^{15}$N-Acetylcysteine Enantiomers on Astec® (R,R) P-CAP™

column . . . . Astec (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
mobile phase . . . . . 20 mM ammonium acetate in: (A) acetonitrile; (B) methanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004719

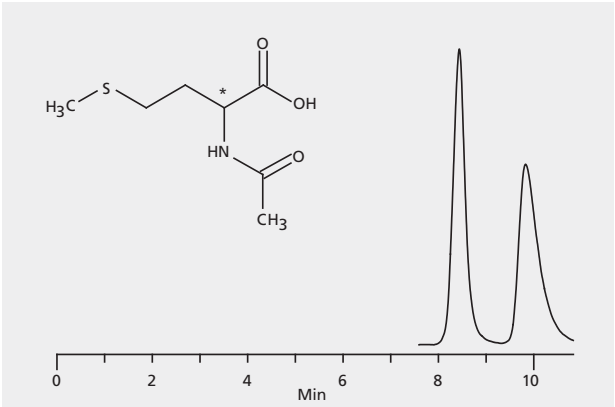





SUPELCO® Solutions within.™

## HPLC Analysis of N-Acetylmethionine Enantiomers on Astec® (R,R) P-CAP™

column . . . . Astec (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
mobile phase . . . . . 20 mM ammonium acetate in: (A) acetonitrile; (B) methanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004722



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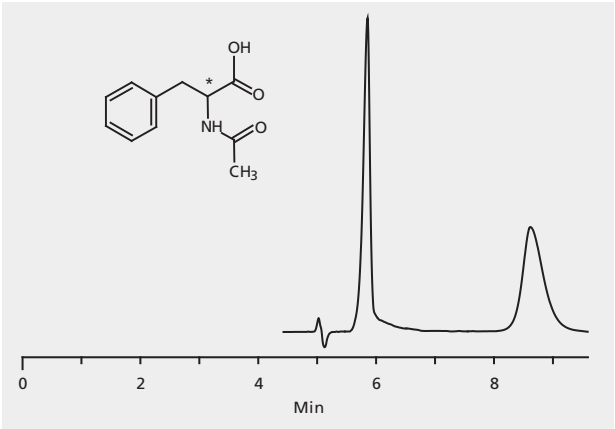
## HPLC Analysis of N-Acetylphenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 0.1% triethylamine acetate, pH 3.8; (B) ethanol; (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 225 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004556



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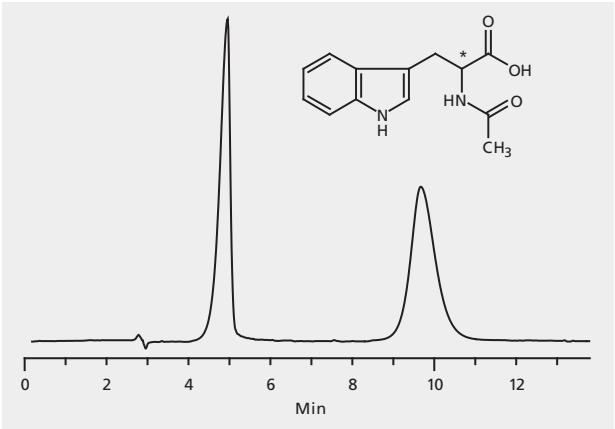
## HPLC Analysis of N-Acetyltryptophan Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) 20 mM ammonium acetate; (B) methanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004555



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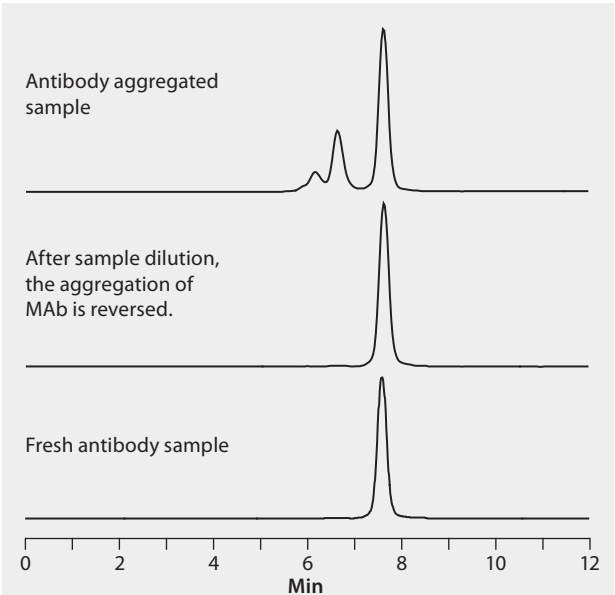
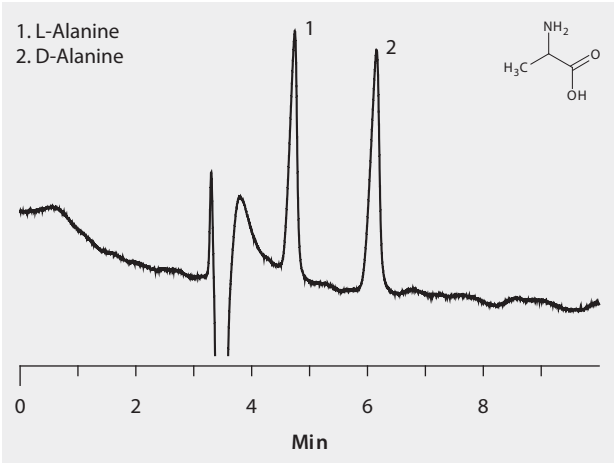
## HPLC Analysis of Aggregated Antibodies on Zenix® SEC-300, Fast Analysis

column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles, 300 Å (Z777033)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 2.9 µL
sample . . . . . antibody sample (17 mg/mL)
Application No. . . . . G006174



## HPLC Analysis of Alanine Enantiomers on Astec® CHIROBIOTIC® T (Formic Acid Mobile Phase Modifier)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 mg /mL in 30:70 water:methanol
Application No. . . . . G005330

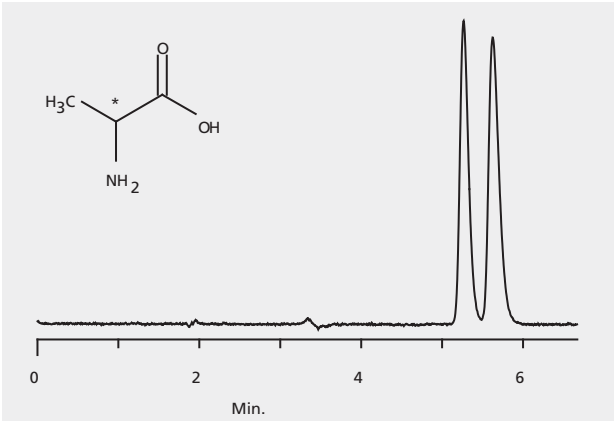





SUPELCO® Solutions within.™

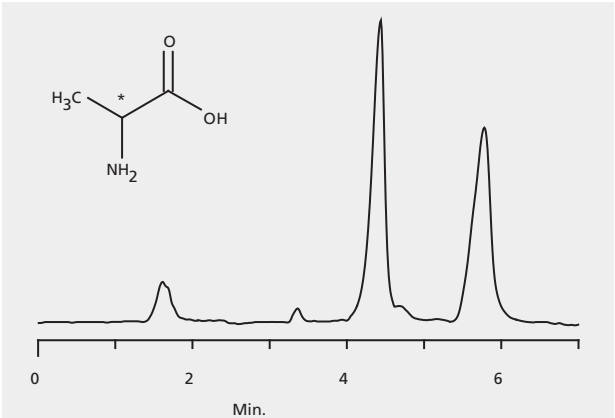
## HPLC Analysis of Alanine Enantiomers on Astec® CHIROBIOTIC® T (No Mobile Phase Modifier)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004485



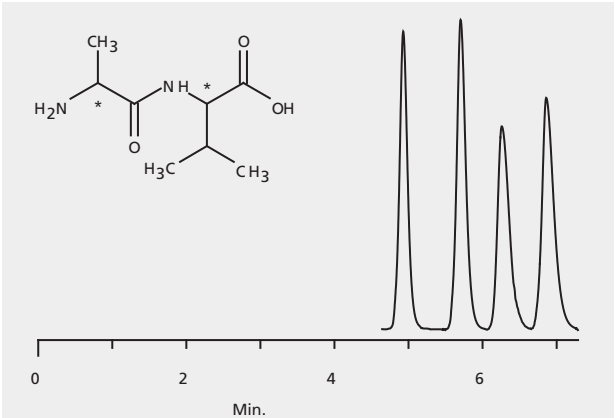
## HPLC Analysis of Alanine Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 0.8 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004603



## HPLC Analysis of Alanylvaline Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004582

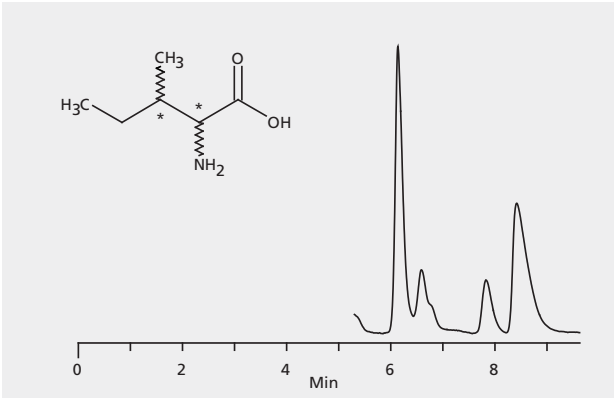





SUPELCO® Solutions within.™

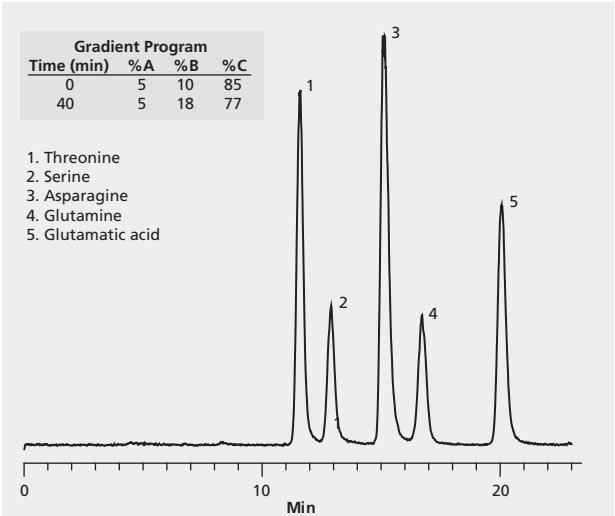
## HPLC Analysis of Alloisoleucine Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . (A) water; (B) methanol; (40:60, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004676



## HPLC Analysis of Amino Acids on Ascentis® Si (Silica), HILIC Mode

column . . . . . Ascentis Si, 15 cm × 2.1 mm I.D., 5 µm particles (581509-U)
mobile phase . . . . . (A) 100 mM ammonium formate (pH 3.0, with formic acid), (B) water, (C) acetonitrile
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI (+), full scan
injection . . . . . 2 µL
sample . . . in 10:90, (50 mM ammonium formate/formic acid, pH 3.0):acetonitrile
Application No. . . . . G004088



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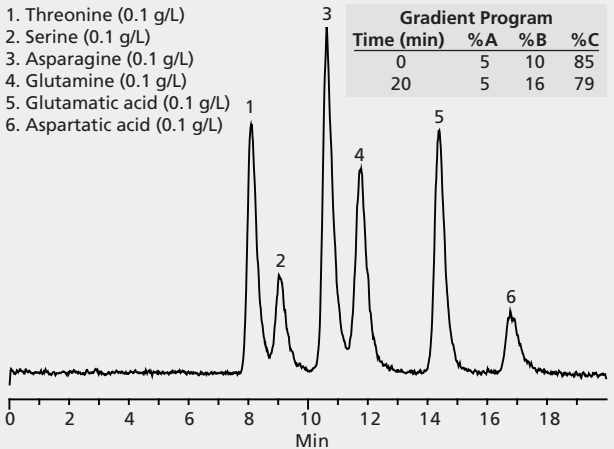
## HPLC Analysis of Amino Acids, Polar Neutral and Acidic on Ascentis® Si

column . . . . . Ascentis Si, 10 cm x 2.1 mm I.D., 5 µm particles (581508-U)
mobile phase . . . . . (A) 100 mM ammonium formate (pH 3.0, with formic acid); (B) water; (C) acetonitrile
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI (+), full scan
injection . . . . . 2 µL
sample . . . . . as indicated, in 10:90, (50 mM ammonium formate/formic acid, (pH 3.0):acetonitrile
Application No. . . . . G003722

1. Threonine (0.1 g/L)
2. Serine (0.1 g/L)
3. Asparagine (0.1 g/L)
4. Glutamine (0.1 g/L)
5. Glutamatic acid (0.1 g/L)
6. Aspartatic acid (0.1 g/L)

**Gradient Program**

| Time (min) | %A | %B | %C |
|---|---|---|---|
| 0 | 5 | 10 | 85 |
| 20 | 5 | 16 | 79 |

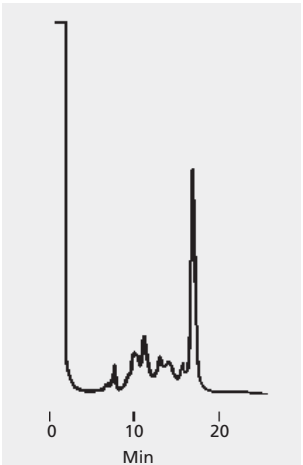





SUPELCO® Solutions within.™

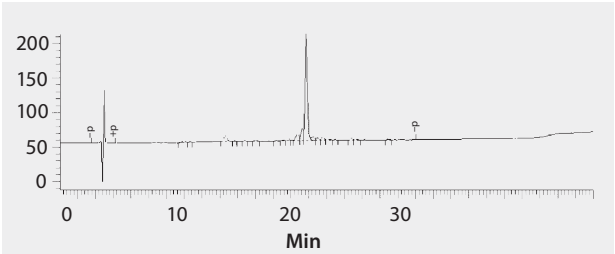
## HPLC Analysis of α-Amylase (Crude) on TSKgel® Ether-5PW

column . . . . TSKgel Ether-5PW, 7.5 cm × 7.5 mm I.D., 10 µm particles (808641)
mobile phase . . . . . (A) 0.1 M phosphate buffer + 1.1 M sodium sulfate, pH 7.0
(B) 0.1 M phosphate buffer A to B over 60 min
flow rate . . . . . 1.0 mL/min
detector . . . . . UV, 280 nm
Application No. . . . . 7130961B



## HPLC Analysis of Amyloid β-Protein Fragment 10-35 on Discovery® BIO Wide Pore C18

column . . . . . Discovery BIO Wide Pore C18, 25 cm × 4.6 mm I.D., 5 µm particles (568223-U)
mobile phase . . . . . (A) 0.1% TFA in water; (B) 0.1% TFA in water:0.1% TFA in acetonitrile; (25:75)
flow rate . . . . . 1.3 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 215 nm
injection . . . . . 10 µL, 1 mg/mL in water
Application No. . . . . G001589



## HPLC Analysis of Angiotensins on Discovery® BIO Wide Pore C8

column . . . . . Discovery BIO Wide Pore C8, 15 cm × 4.6 mm I.D., 5 µm particles (568322-U)

mobile phase . . . . . (A) 10 mM $NH_4H_2PO_4$/ $NH_4OH$, pH 7; (B) 50:50, (20 mM $NH_4H_2PO_4$/ $NH_4OH$, pH 7):$CH_3CN$

gradient . . . . . 30-60% B in 15 min

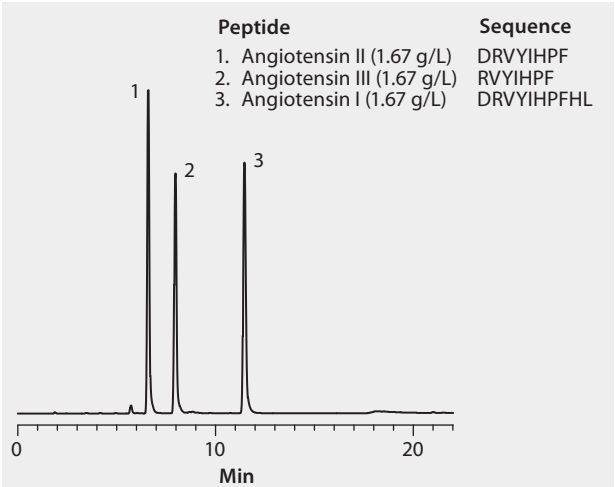
flow rate . . . . . 1 mL/min

column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

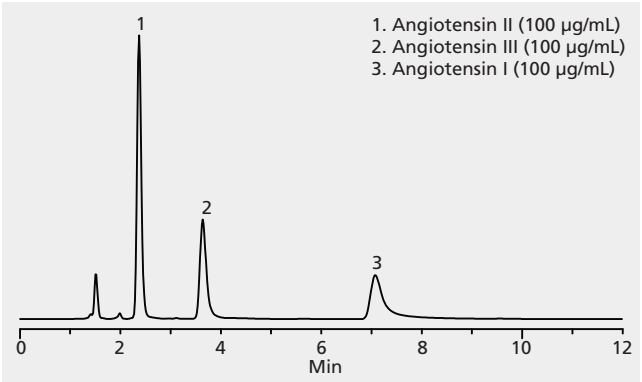
injection . . . . . 6 µL in water

Application No. . . . . G001503B



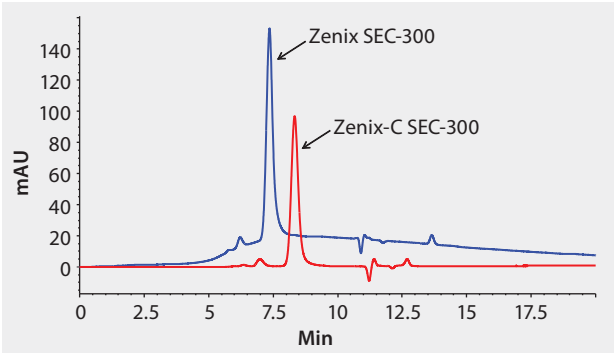
## HPLC Analysis of Angiotensins on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 5 mM ammonium phosphate (pH 7.0 with ammonium hydroxide); (B) acetonitrile; (77.5:22.5, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G002914



## HPLC Analysis of an mPEG-peptide on Zenix® SEC Phases

column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles, 300 Å (Z777033)
column . . Zenix C SEC-300, 30 cm x 7.8mm I.D., 3 µm particles, 300 Å (Z777092)
mobile phase . . . . . 150 mM Sodium Phosphate Buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 20 µL
sample . . . . . 20kD mPEG-MAL (methoxy-PEG-Maleimide) (1mg/mL)
Application No. . . . . G006178



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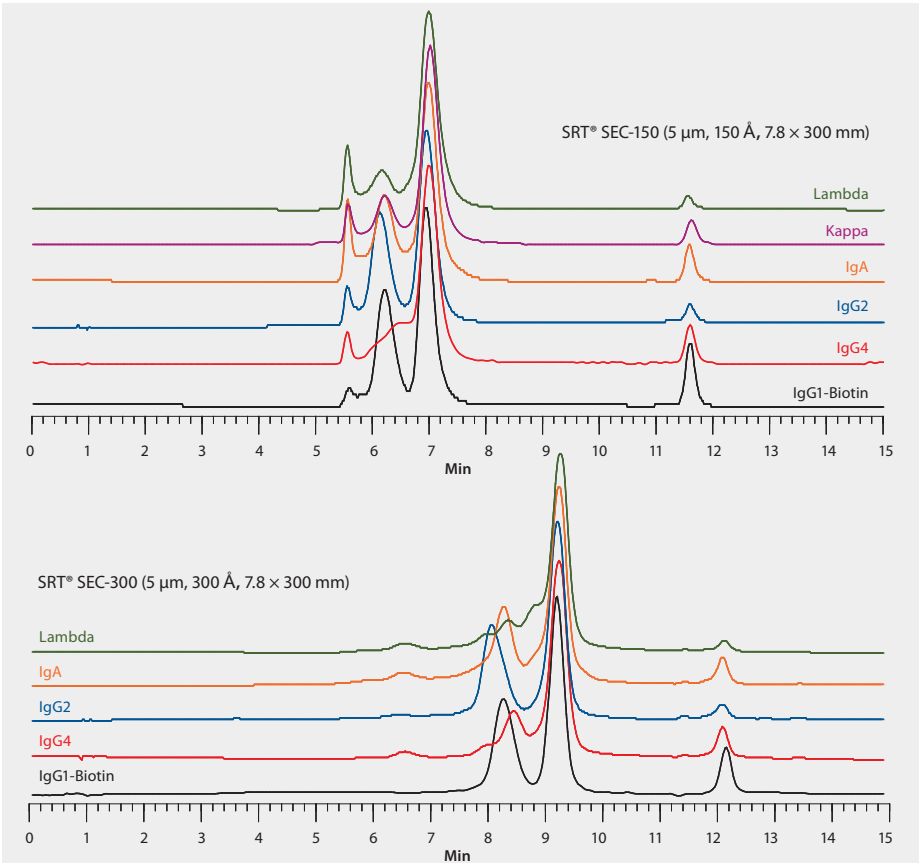
## HPLC Analysis of Antibody Isotypes on SRT® Gel Filtration Columns

A change in the relative concentration of IgG subclasses (IgG1, IgG2, IgG3 and IgG4) in tissue indicates a change in immunological response. These chromatograms show the elution profiles of several IgG subclasses, including lambda and Kappa light chains and biotinylated IgG1. Note that sme of the aggregate peaks in front of the main component are partially excluded on the smaller (SEC-150) pore size column, but not on the 300 Angstrom pore size column.

column . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm particles, 300 Å (Z777051)
column . . . SRT SEC-150, 30 cm x 7.8 mm I.D., 5 µm particles, 300 Å (Z777045)
mobile phase . . . . . 150 mM phosphate buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . Ambient
detector . . . . . UV, 214 nm
Application No. . . . . G006180



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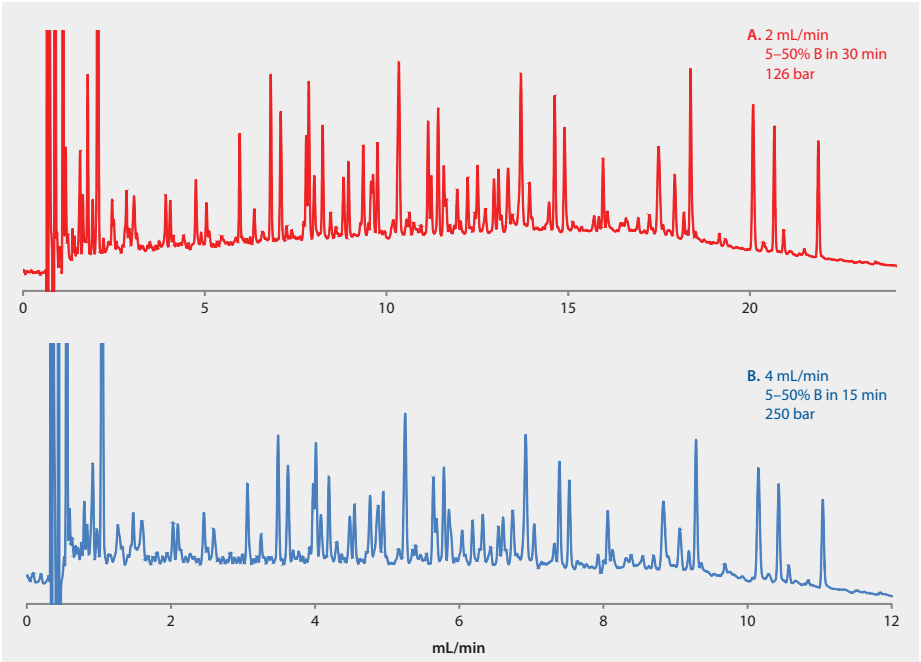
## HPLC Analysis of Apotransferrin Tryptic Digest on BIOshell™ A160 Peptide C18, Comparison of Different Flow Rates and Pressures

BIOshell columns packed with five micron Fused-Core particles feature modest back pressures, which allows the use of very high flow rates as is demonstrated in the two chromatograms, one of which was obtained at 2mL/min, the other at 4mL/min. Note that at 4mL/min the gradient time was reduced in half to keep the gradient volume constant. At a flow rate of 4mL/min the back pressure for this 15cm x 4.6mm column is 250 bar (approx. 3500 psi), which illustrates that these high efficiency columns can be operated on standard HPLC systems.

| | |
|---|---|
| column | BIOSHELL A160 Peptide C18, 15 cm x 4.6 mm I.D., 5 µm particles (67015-U) |
| mobile phase | (A): water/0.1% TFA (B): acetonitrile/0.1% TFA |
| gradient | 5-50%B in 30 minutes; 5-50%B in 15 minutes; |
| flow rate | 2 mL/min or 4/mL minute |
| pressure | 126 bar, 250 bar |
| column temp. | 60 °C |
| detector | UV, 215 nm |
| injection | 15 µL |
| sample | 1 mg/mL in water/0.1% TFA |
| Application No. | G006220 |



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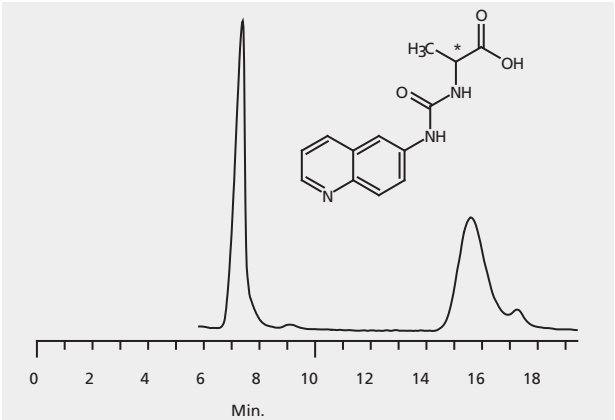
## HPLC Analysis of AQC-Alanine Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (50:50, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 0.5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004602

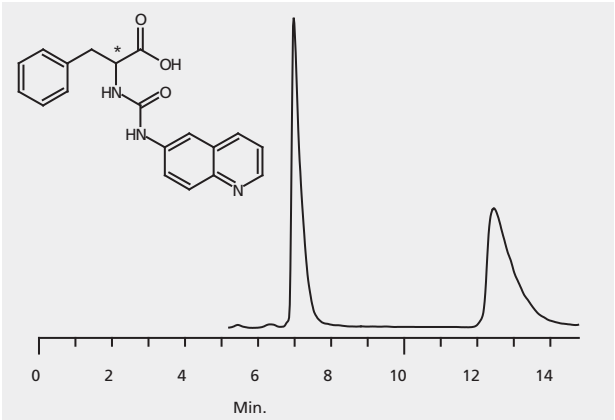





SUPELCO® Solutions within.™

## HPLC Analysis of AQC-Phenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) methanol; (50:50, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004583



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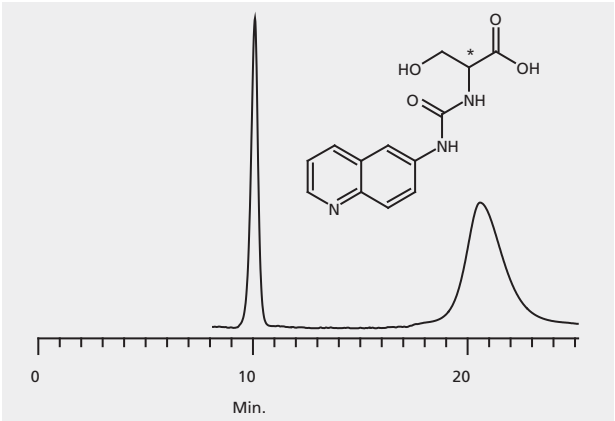
## HPLC Analysis of AQC-Serine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) methanol; (70:30, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004576



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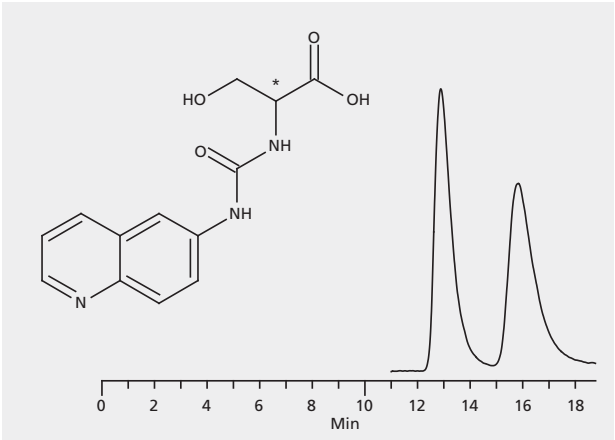
## HPLC Analysis of AQC-Serine Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (70:30, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 0.5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004739



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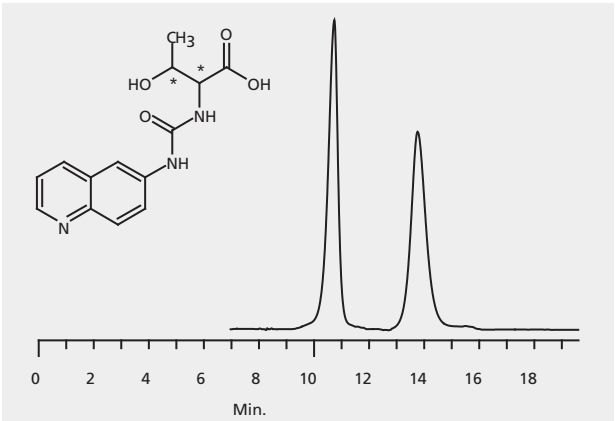
## HPLC Analysis of AQC-Threonine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (90:10, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004578



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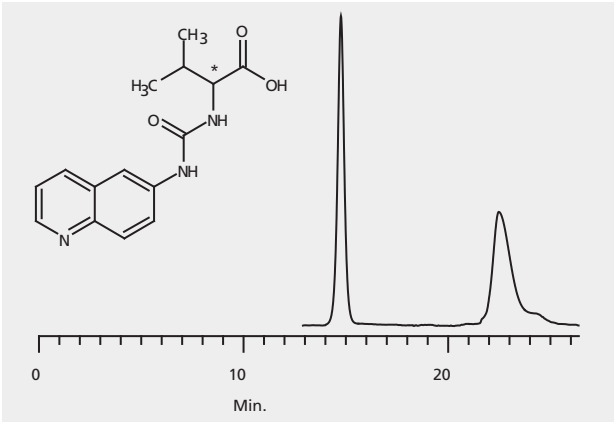
## HPLC Analysis of AQC-Valine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) methanol; (70:30, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004577



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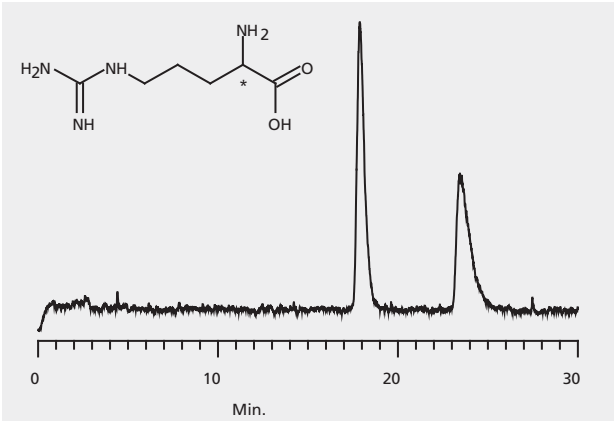
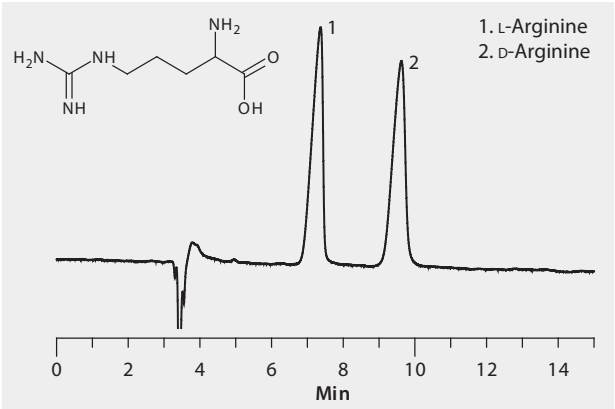
## HPLC Analysis of Arginine Enantiomers on Astec® CHIROBIOTIC® T (23 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004487



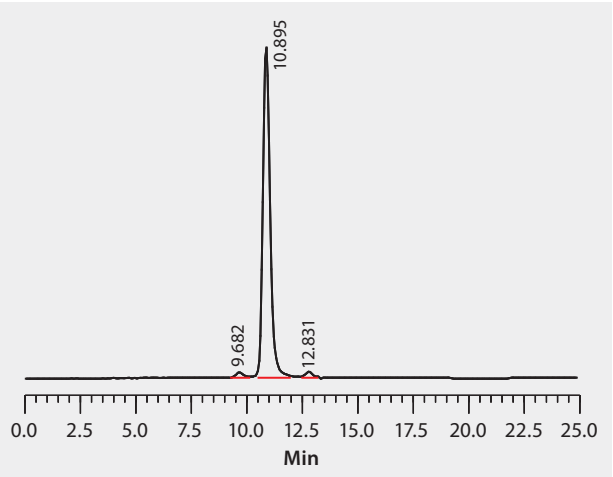
## HPLC Analysis of Arginine Enantiomers on Astec® CHIROBIOTIC® T (25 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water, (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005331



## HPLC Analysis of Asparaginase on Zenix® SEC-150

column . . . . . Zenix SEC-150, 30 cm x 7.8 mm I.D., 3 µm particles (Z777018)
mobile phase . . . . . 150 mM sodium phosphate, pH 7.0
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1015 psi (70 bar)
column temp. . . . . ambient
detector . . . . . UV, 280 nm
inj. temp. . . . . 5 µL
sample . . . . . asparaginase (2.0 mg/mL in water), RT 10.895 minute, MW 5000-6000, pI 4.85
Application No. . . . . G005973



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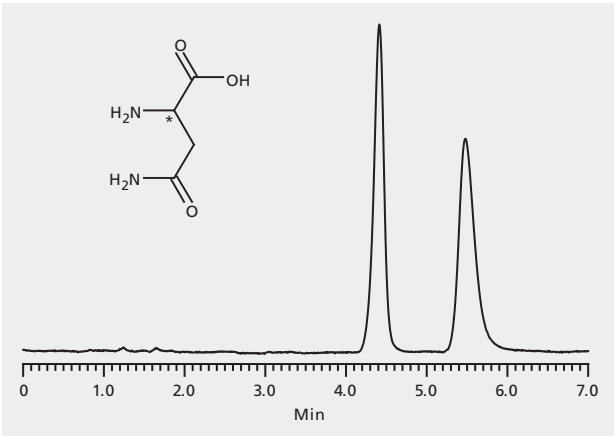
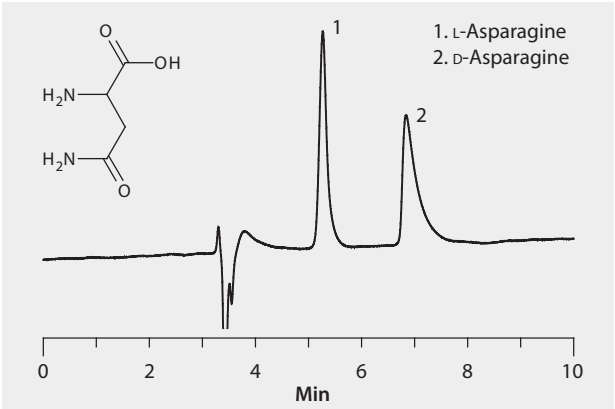
## HPLC Analysis of Asparagine Enantiomers on Astec® CHIROBIOTIC® T (23 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample preparation . . . . . 1 mg/mL in methanol
Application No. . . . . G004488



## HPLC Analysis of Asparagine Enantiomers on Astec® CHIROBIOTIC® T (25 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . (A) water); (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
detector . . . . . UV, 205 nm
column temp. . . . . 25 °C
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005332



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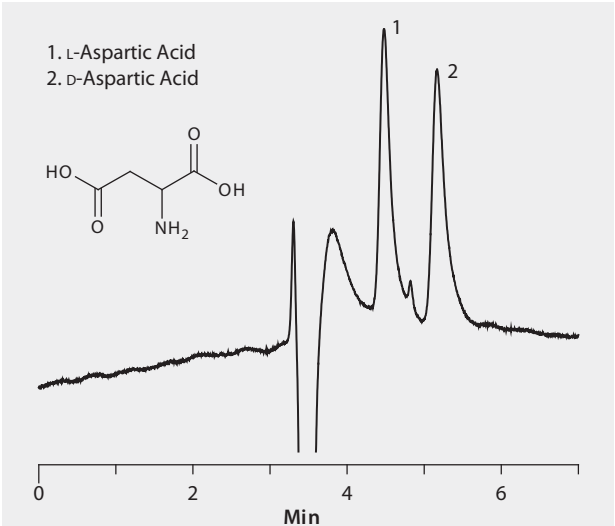
## HPLC Analysis of Aspartic Acid Enantiomers on Astec® CHIROBIOTIC® T (Methanol Mobile Phase)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005333

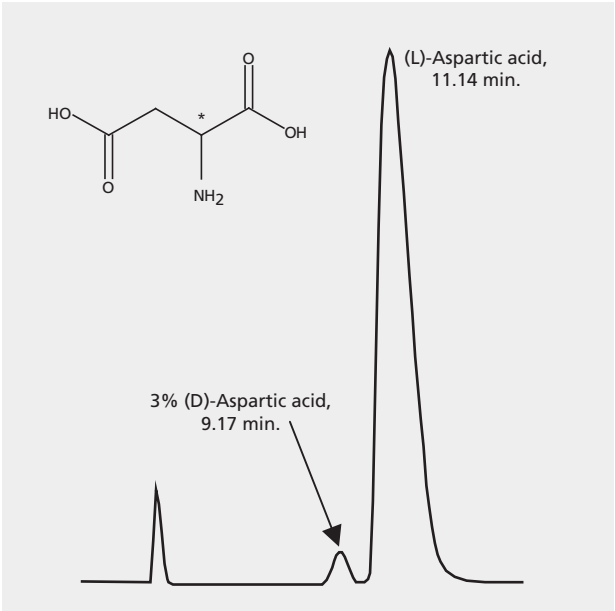





SUPELCO® Solutions within.™

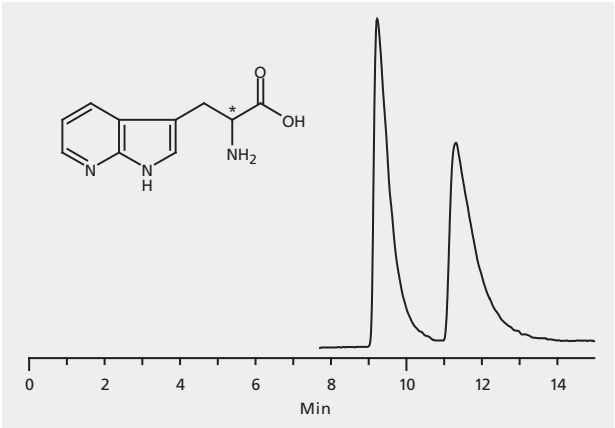
## HPLC Analysis of Aspartic Acid Enantiomers on Astec® CLC-L, Trace Level Detection

column . . . . . Astec CLC-L, 15 cm x 4.6 mm I.D., 5 µm particles (53123AST)
mobile phase . . . . . 5 mM copper sulfate
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL in water
Application No. . . . . G004664



## HPLC Analysis of 7-Azatryptophan Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (70:30, A:B)
flow rate . . . . . 0.8 mL/min
column temp. . . . . ambient
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol/water/ammonium hydroxide
Application No. . . . . G004538

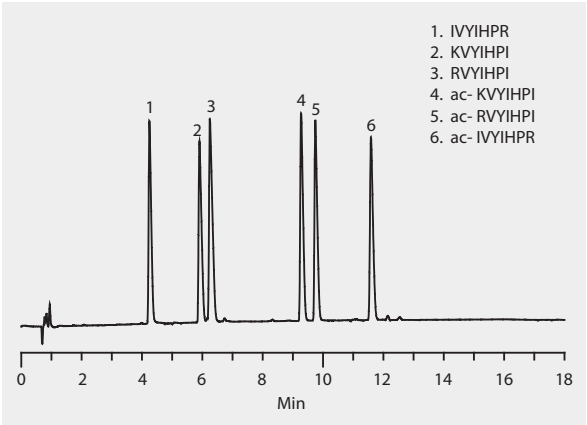





SUPELCO® Solutions within.™

## HPLC Analysis of Basic Peptides (Angiotensin Analogs) on Ascentis® Express Peptide ES-C18

column . . . . . Ascentis Express Peptide ES-C18, 10 cm x 3 mm I.D., 2.7 µm particles (53313-U)
mobile phase . . . . . (A) 0.1% (v/v) formic acid, pH 4.0 (titrated with ammonium hydroxide); (B) 50:50, (0.175% formic acid in water, pH 4.0) : acetonitrile
gradient . . . . . 20 to 50% B in 15 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 3002 psi (207 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 2.0 µL
sample . . . . . 0.5 g/L ea. peptide
Application No. . . . . G005379





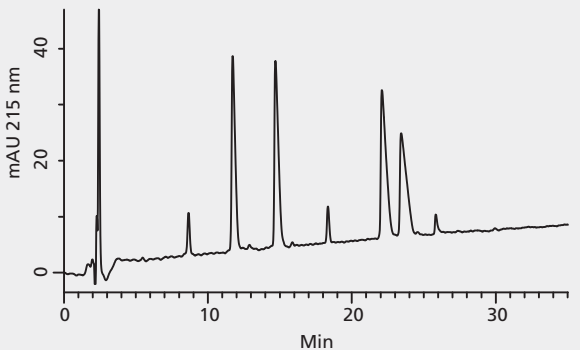

SUPELCO® Solutions within.™

## HPLC Analysis of Basic Peptides on Discovery® BIO Wide Pore C18, No TFA

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 2.1 mm I.D., 5 µm particles (568202-U)
mobile phase . . . . . (A) 25 mM formic acid in water; (B) 50:50 (25 mM formic acid in water):(20 mM formic acid in acetonitrile)
flow rate . . . . . 0.20 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 0.5 µL (~0.25 µg each peptide)
Application No. . . . . G001642A

Peptide 1: ac-GGGLGGAGGLK-amide
Peptide 2: ac-KYGLGGAGGLK-amide
Peptide 3: ac-GGALKALKGLK-amide
Peptide 4: ac-KYALKALKGLK-amide

| Gradient Program Time (min) | %B |
|---|---|
| 0 | 15 |
| 45 | 60 |

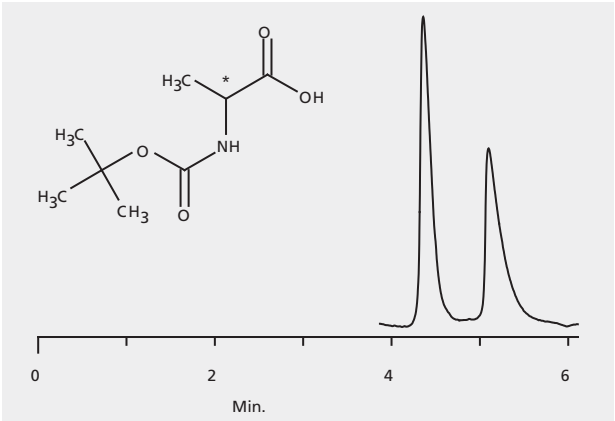





SUPELCO® Solutions within.™

## HPLC Analysis of t-BOC-Alanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 6; (B) methanol; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004573

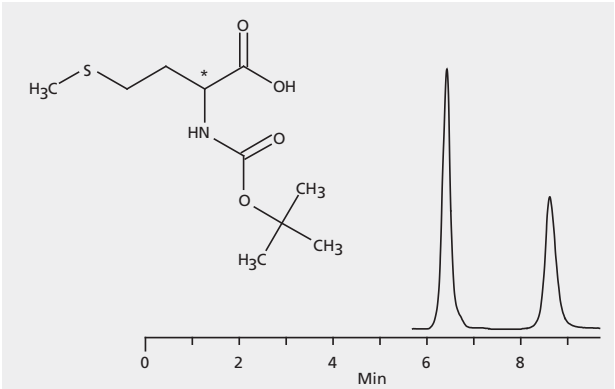





SUPELCO® Solutions within.™

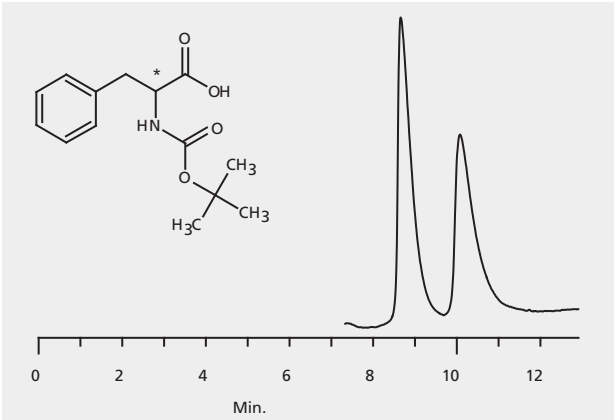
## HPLC Analysis of t-BOC-Methionine Enantiomers on Astec® (R,R) P-CAP™

column . . . . Astec (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
mobile phase . . . . . 20 mM ammonium acetate in: (A) acetonitrile; (B) methanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004723



## HPLC Analysis of t-BOC-Phenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 6; (B) methanol; (90:10, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004585

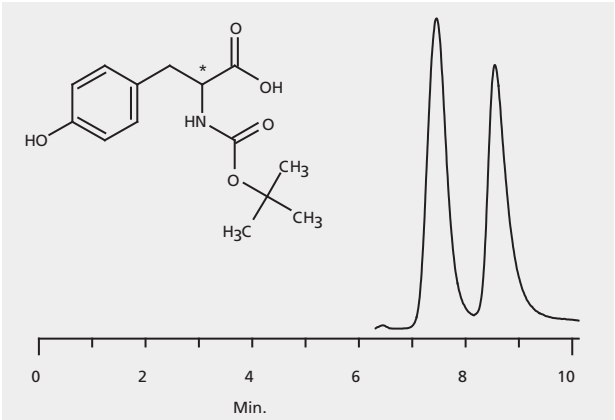





SUPELCO® Solutions within.™

## HPLC Analysis of t-BOC-Tyrosine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 6; (B) methanol; (90:10, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004572

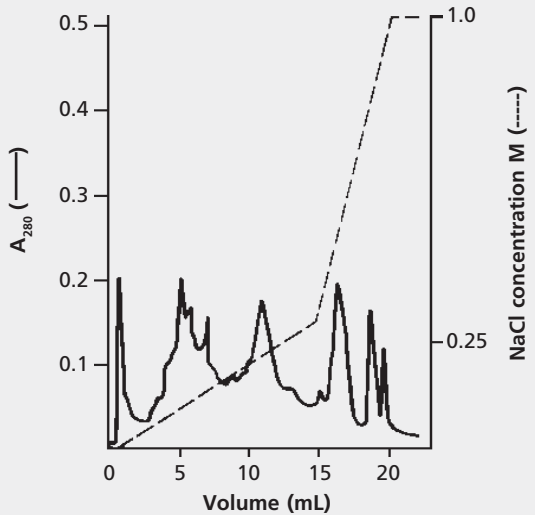





SUPELCO® Solutions within.™

## HPLC Analysis of Bovine Liver Extract on Mono Q® 5/50 GL

column . . . . . Mono Q 5/50 GL, 5 cm x 5 mm I.D., 10 µm particles (5-4807)
mobile phase . . . . . (A) 50 mM diethanolamine, pH 8.8;
(B) 50 mM diethanolamine + 1.0M NaCl, pH 8.8
flow rate . . . . . 1.0 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 100 µL 1.6 mg protein
Application No. . . . . 794-0376

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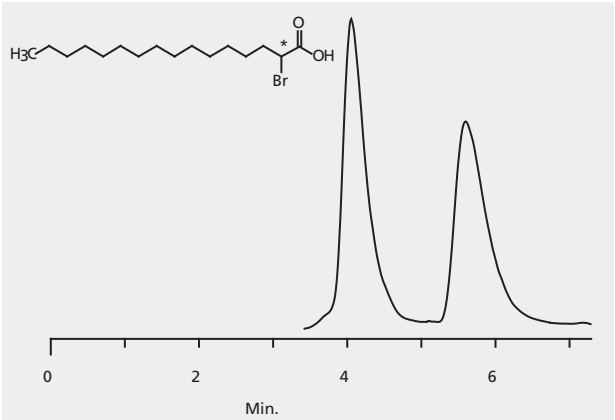
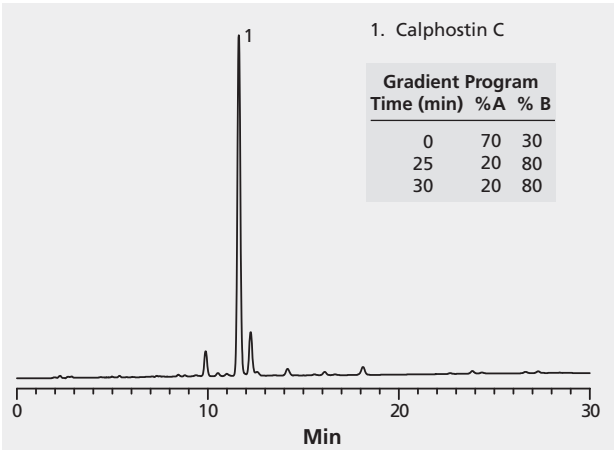
## HPLC Analysis of α-Bromopalmitic Acid Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 5.5; (B) methanol; (40:60, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004601



## HPLC Analysis of Calphostin C on Discovery® HS C18

column . . . . Discovery HS C18, 25 cm × 2.1 mm I.D., 5 µm particles (568503-U)
mobile phase . . . (A) (0.1% phosphorus acid in water):(0.1% phosphorus acid in acetonitrile); (B) 0.1% phosphorus acid in acetonitrile; (50:50, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 24 °C
detector . . . . . UV, 254 nm
injection . . . . . 3 µL
sample . . . . . Calphostin C (0.25 g/L) in 40% MeOH, 0.025% TFA
Application No. . . . . G002574

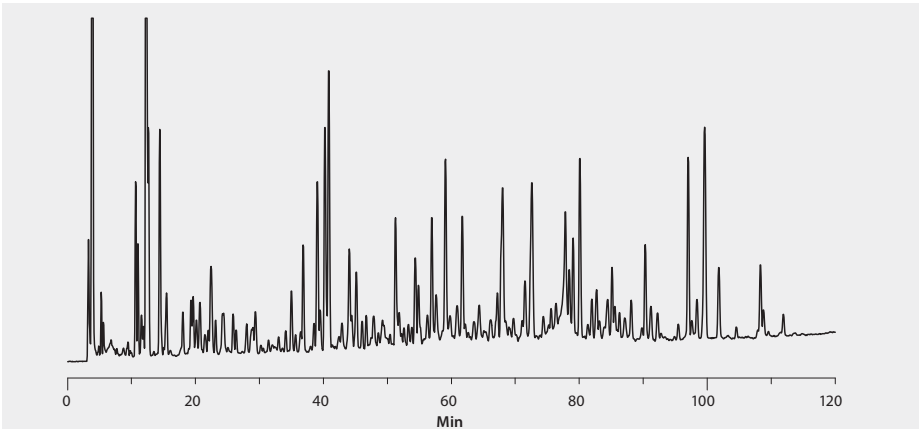





SUPELCO® Solutions within.™

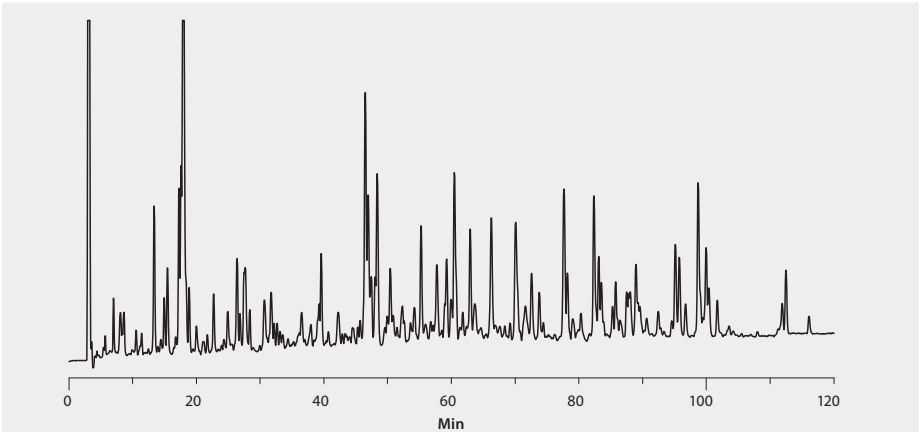
## HPLC Analysis of Carboxyamido Methylated BSA Tryptic Digest on Discovery® RP-Amide C16

column . . Discovery RP-AmideC16, 25 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) 0.1% TFA, 1% acetonitrile in water; (B) 0.1% TFA, 40% acetonitrile
gradient . . . . . 0-100% B in 130 min
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
Application No. . . . . G001087



## HPLC Analysis of Carboxyamido-Methylated BSA Tryptic Digest on Discovery® C18

column . . . . . Discovery C18, 25 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) 0.1% TFA, 1% acetonitrile in water; (B): 0.1% TFA, 40% acetonitrile
gradient . . . . . 0-100% B in 130 min
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
Application No. . . . . G001086



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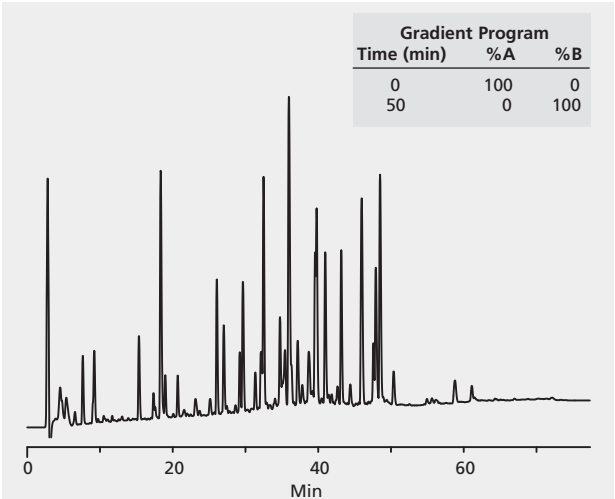
## HPLC Analysis of Carboxymethylated Apohemoglobin (Horse) Tryptic Digest on Discovery® BIO Wide Pore C18

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 2.1 mm I.D., 3 µm particles (568222-U)
mobile phase . . . . . (A) 0.1% TFA in water; (B) 0.1% TFA in water:0.1% TFA in acetonitrile, 50:50;
gradient . . . . . 0-100% B in 50 min
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35°C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL, each at 0.8 mg/mL in 0.1% TFA
Application No. . . . . G001719






SUPELCO® Solutions within.™

## HPLC Analysis of Carboxymethylated Apohemoglobin Tryptic Digest on Discovery® BIO Wide Pore C18

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 4.6 mm I.D., 5 µm particles (568222-U)

mobile phase . . (A) (0.1% TFA in water):(0.1% TFA in acetonitrile) 95:5; (B) (0.1% TFA in water):(0.1% TFA in acetonitrile):50:50;

gradient . . . . . 0-100% B in 65 min

flow rate . . . . . 1 mL/min

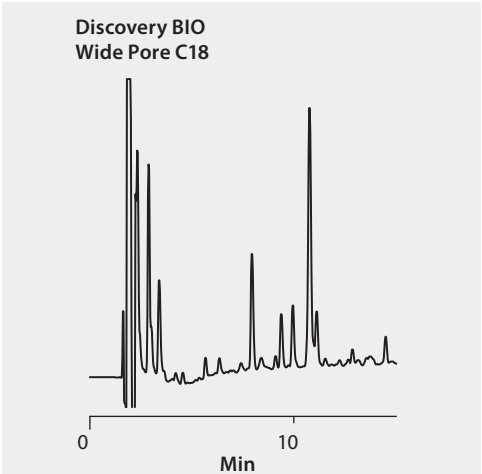
column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 50 µL

sample . . . . . carboxymethylated apohemoglobin tryptic digest in 50 mM ammonium bicarbonate

Application No. . . . . G001505A



## HPLC Analysis of Carboxymethylated Apohemoglobin Tryptic Digest on Discovery® BIO Wide Pore C5

column . . . . . Discovery BIO Wide Pore C5, 15 cm × 4.6 mm I.D., 5 µm particles (568422-U)

mobile phase . . . . . (A) 0.1% TFA in water:0.1% TFA in acetonitrile, 95:5; (B) 0.1% TFA in water:0.1% TFA in acetonitrile; 50:50

gradient . . . . . 0-100% B in 65 min

flow rate . . . . . 1 mL/min

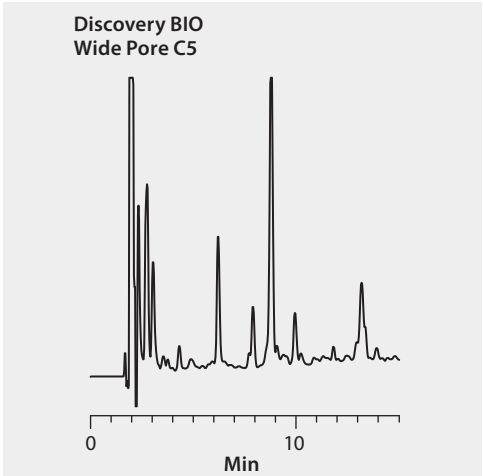
column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 50 µL

sample . . . . . carboxymethylated apohemoglobin tryptic digest in 50 mM ammonium bicarbonate

Application No. . . . . G001506A



## HPLC Analysis of Carboxymethylated Apohemoglobin Tryptic Digest on Discovery® BIO Wide Pore C8

column . . . . . Discovery BIO Wide Pore C8, 15 cm × 4.6 mm I.D., 5 µm particles (568322-U)

mobile phase . . (A) 0.1% TFA in water:0.1% TFA in acetonitrile, 95:5; (B) 0.1% TFA in water::0.1% TFA in acetonitrile, 50:50.

gradient . . . . . 0-100% B in 65 min

flow rate . . . . . 1 mL/min

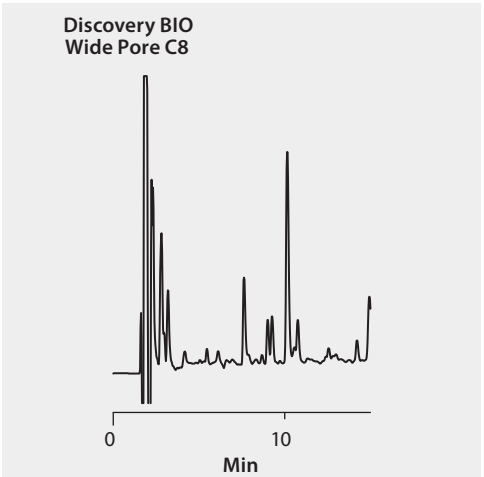
column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 50 µL

sample . . . . . carboxymethylated apohemoglobin tryptic digest in 50 mM ammonium bicarbonate

Application No. . . . . G001504A



## HPLC Analysis of Carboxymethylated Cytochrome c (Chicken) Tryptic Digest on Discovery® BIO Wide Pore C18

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 2.1 mm I.D., 3 µm particles (567202-U)

mobile phase . . . . . (A) water/0.1% TFA; (B) (water/0.1%TFA):(acetonitrile/0.1% TFA); 50:50

flow rate . . . . . 0.20 mL/min

column temp. . . . . 35 °C

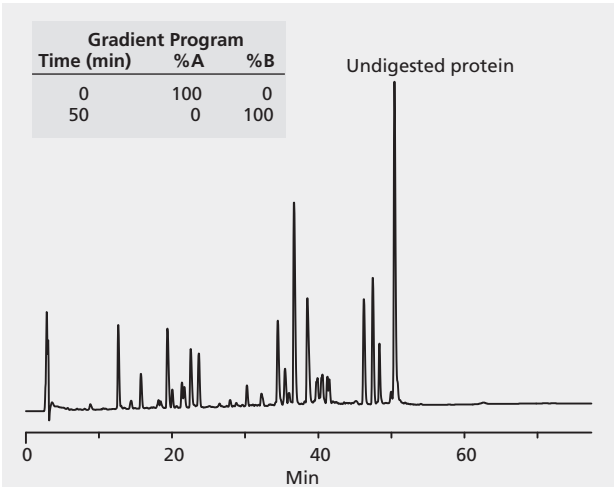
detector . . . . . UV, 215 nm

injection . . . . . 5 µL

Application No. . . . . G001721

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 100 | 0 |
| 50 | 0 | 100 |



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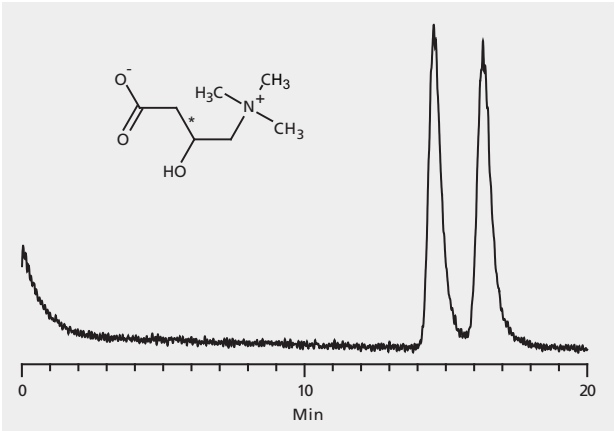
## HPLC Analysis of Carnitine Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . (A) 5 mM ammonium acetate, pH 6; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+)-MS (m/z = 162)
injection . . . . . 5 µL
sample . . . . . 1 µg/mL in mobile phase
Application No. . . . . G004524



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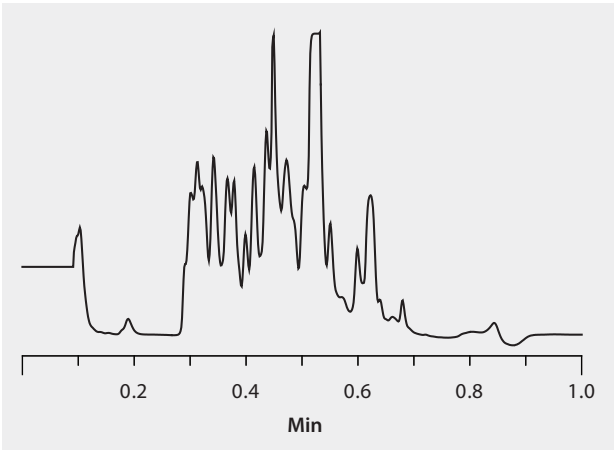
## HPLC Analysis of α-Casein Tryptic Digest on Ascentis® Express Peptide ES-C18

LC-MS-based shotgun proteomics relies both on the power of the separation techniques, and the sensitivity of detection methods. As a viable alternative to classical approaches in this field, we developed a fully automated, comprehensive 2D LC system, in which RP LC x RP LC was coupled to MS detection, for the first time, and applied for the analysis of tryptic digests obtained from a-casein and dephosphorylated a–casein. The use of a significantly different pH in the two dimensions allowed to attain high peak capacity, despite the employment of novel identical stationary phases. Furthermore, such a combination addresses compatibility issues, thus allowing straightforward interfacing in on-line 2D LC configuration, as well as direct linkage to mass spectrometer. A theoretical peak capacity of approximately 8500 was calculated for the set-up employing four serially coupled C18 columns in the first dimension (4 columns, each 15 cm x 2.1 mm I.D., 2.7 µm particles), operated under basic conditions, and 3 cm length of the same stationary phase (3 cm x 4.6 mm I.D., 2.7 µm particles), under acidic conditions for fast second dimension analysis.

**Sample Prep**: one-tenth gram of α-casein was dissolved in 10 mL of 0.01 M ammonium formate buffer, and the pH was adjusted to 8.0 with ammonium hydroxide; the solution was heated in a boiling water bath for 6 min. After the solution cooled, 2.0 mg of trypsin from bovine pancreas was added, and the mixture was allowed to react for 4 h at 37 °C; the reaction was quenched by adding 0.1% trifluoroacetic acid to pH 2. The digest were stored at 4°C and filtered prior to injection through a 0.45 µm nylon membrane.

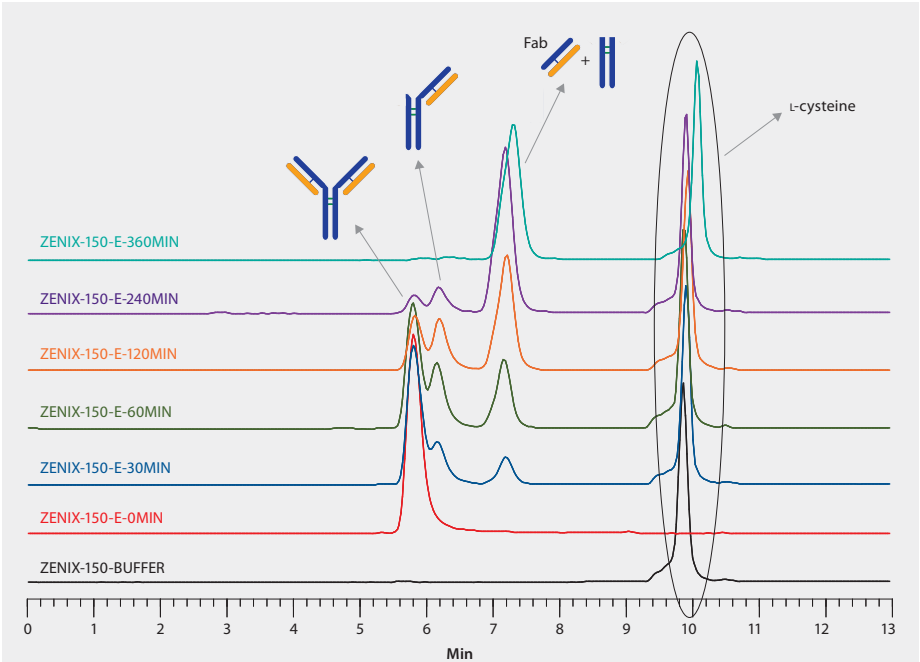
sample/matrix . . . . . tryptic digested α-casein
column . . . . . Ascentis Express Peptide ES-C18, 3 cm x 4.6 mm I.D., 2.7 µm particles (53316-U)
mobile phase . . . . . (A) 0.1% TFA in water; (B) 0.1% TFA in water:acetonitilre (10:90, A:B)
gradient . . 0-0.05 min, 0-20% B; 0.05-0.40 min, to 40% B; 0.40-0.50 min, to 50% B; 0.50-0.69 min, to 90% B; 0.69-0.70 min, to 0% B; held for 0.3 min
flow rate . . . . . 4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 2 µL
Application No. . . . . G005597



## HPLC Analysis of Cetuximab Antibody Fragments from Papain Digestion on Zenix® SEC-150

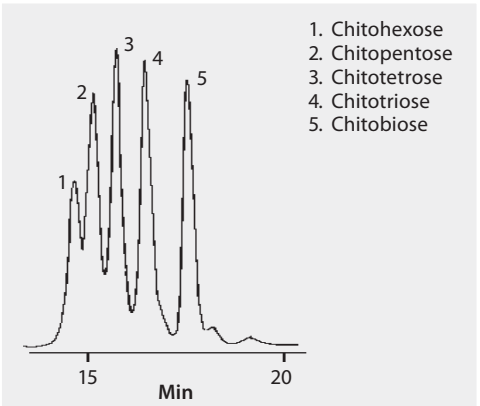
Papain digestion conditions: Buffer: 5.0 mM L-Cysteine, 2.0 mM EDTA and 300 mM NaCl; Enzyme: 80 µg/mL papain; Temperature: 37 °C; Time: 30 min, 60 min, 120 min, 240 min, and 360 min.

column . . Zenix SEC-150, 30 cm x 7.8 mm I.D., 3 µm, 150 Å particles (Z777018)
mobile phase . . . . . 20 mM sodium phosphate, pH 7.0, 150 mM NaCl
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . Cetuximab ERBITUX®, papain digest
Application No. . . . . G006172



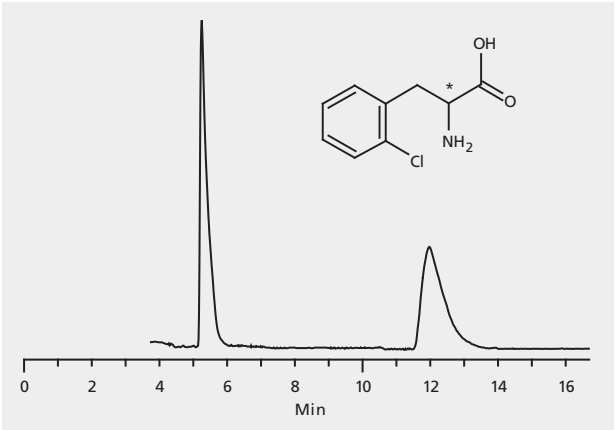
## HPLC Analysis of Chito-Oligosaccharides on TSKgel® G-Oligo-PW

column . . . . TSKgel G-Oligo-PW, 30 cm × 7.8 mm I.D., 6 µm particles (808031)
flow rate . . . . . 1 mL/min
column temp. . . . . 60 °C
detector . . . . . RI
sample preparation . . . . . distilled water
Application No. . . . . 794-0325



## HPLC Analysis of o-Chlorophenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 210 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004550

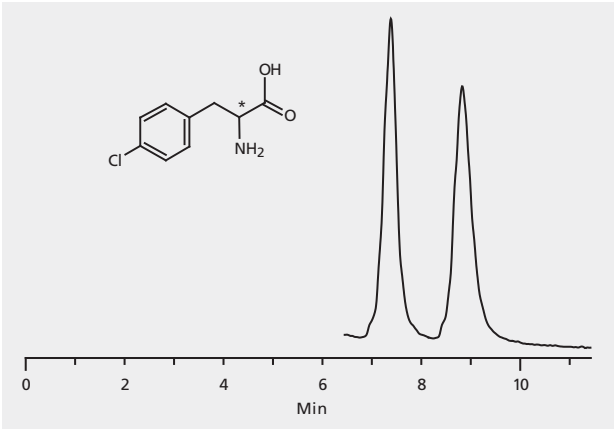





SUPELCO® Solutions within.™

## HPLC Analysis of p-Chlorophenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 3 mg/mL in methanol
Application No. . . . . G004546



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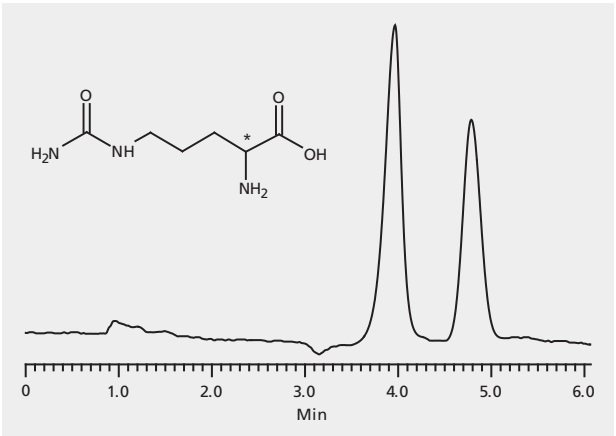
Solutions within.™

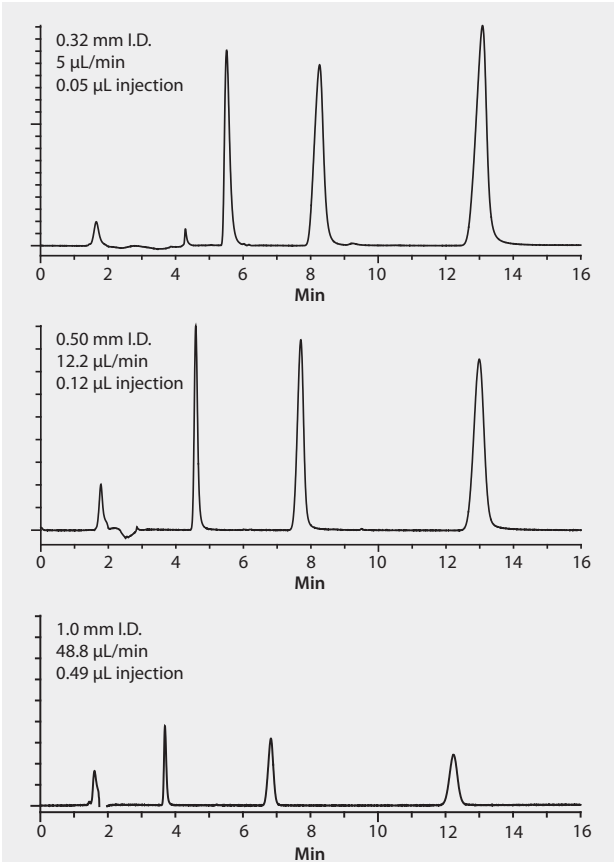
## HPLC Analysis of Citrulline Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004531



## HPLC Analysis of Column Test Mix on Discovery® BIO Wide Pore C18, Effect of HPLC Column I.D. on Sensitivity

column . . . . . Discovery BIO Wide Pore C18, 10 cm x various I.D., 3 µm particles
mobile phase . . . . . (A) water; (B) acetonitrile; (65:35, A:B)
flow rate . . . . . as indicated
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . as indicated
sample . . . . . acetophenone (0.1 µg), benzene (1 µg), toluene (1 µg)
Application No. . . . . G004416



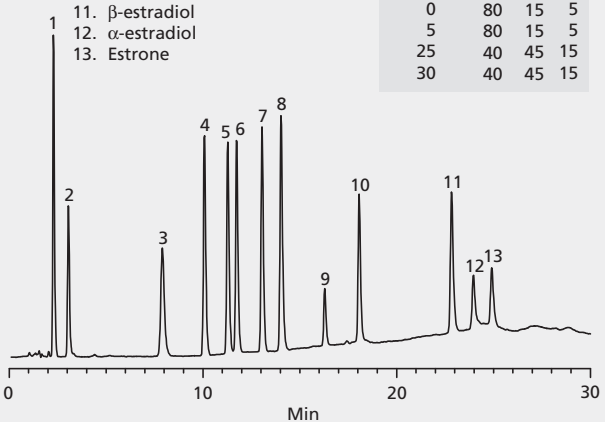
## HPLC Analysis of Conjugated Estrogens on Discovery® HS C18

column . . . . Discovery HS C18, 15 cm × 4.6 mm I.D., 5 µm particles (568522-U)
mobile phase . . . . . (A) 10 mM potassium phosphate (pH 7.01 unadjusted); (B) acetonitrile; (C) methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 10 µg/mL each in 60/40, water:acetonitrile
Application No. . . . . G002155

1. Estriol 3-(β-D-glucuronide)
2. β-estradiol 3-(β-D-glucuronide) 17-sulfate
3. Estriol 3-sulfate
4. β-estradiol 3,17-disulfate
5. β-estradiol 3-(β-D-glucuronide)
6. β-estradiol 17-(β-D-glucuronide)
7. Estrone 3-(β-D-glucuronide)
8. Estriol
9. β-estradiol 3-sulfate
10. Estrone 3-sulfate
11. β-estradiol
12. α-estradiol
13. Estrone

**Gradient Program**

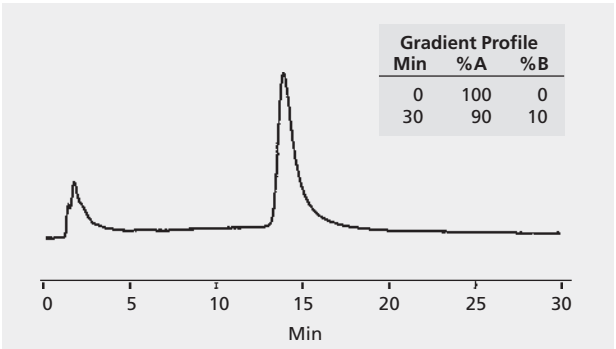
| Time (min) | %A | %B | %C |
|---|---|---|---|
| 0 | 80 | 15 | 5 |
| 5 | 80 | 15 | 5 |
| 25 | 40 | 45 | 15 |
| 30 | 40 | 45 | 15 |



## HPLC Analysis of Creatine Kinase on Discovery® BIO PolyMA-WAX

Discovery BIO PolyMA-SCX and PolyMA-WAX columns give sharp, efficient peaks for a wide variety of proteins.

column . . Discovery BIO PolyMA-SCX, 5 cm × 4.6 mm, 5 µm particles (59601-U)
mobile phase . . . . . (A) 20 mM sodium phosphate, pH 7.0;
(B) 20 mM sodium phosphate, 0.5M NaCl, pH 7.0
flow rate . . . . . 0.5 mL/min
injection . . . . . 10 µL
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
Application No. . . . . G001836

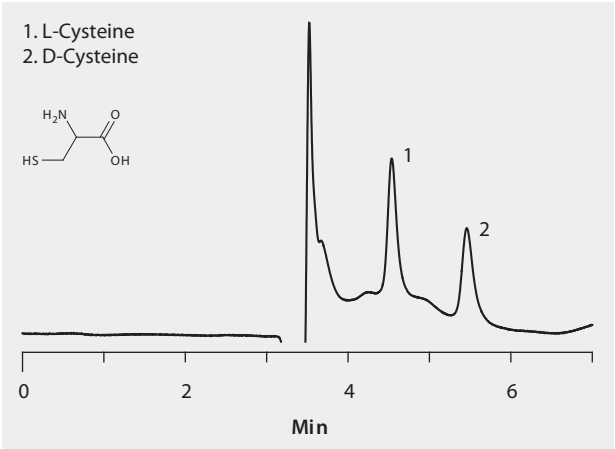





SUPELCO® Solutions within.™

## HPLC Analysis of Cysteine Enantiomers on Astec® CHIROBIOTIC® T (Formic Acid Mobile Phase Additive)

column . . Astec CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (40:60:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . (200 µg /mL in 50:50 water:methanol)
Application No. . . . . G005334

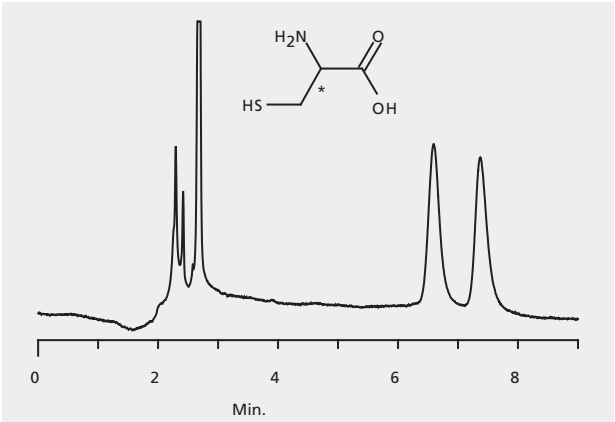





SUPELCO® Solutions within.™

## HPLC Analysis of Cysteine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (30:70, A:B)
extraction tube . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004495



## HPLC Analysis of Cytochrome c Species Variants on Discovery® BIO PolyMA-SCX

column . . Discovery BIO PolyMA-SCX, 5 cm × 4.6 mm I.D., 5 µm particles (56901-U)
mobile phase . . . . . (A) 20 mM Bis-Tris HCl, pH 7.0;
(B) A + 0.5 M NaCl 24-69% B over 20 min
flow rate . . . . . 0.5 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 10 µg each variant
Application No. . . . . 796-0477B

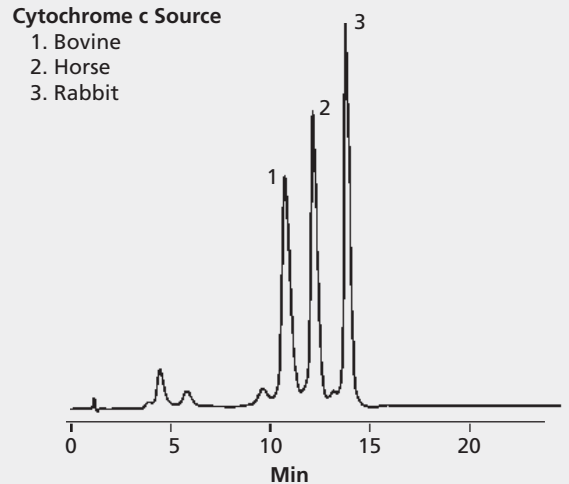



Figure used with permission of Mitsubishi Chemical Co.

## HPLC Analysis of Cytochrome c Species Variants on Discovery® BIO PolyMA-SCX (Ion Exchange)

This Figure shows the separation of three cytochrome c variants on Discovery BIO PolyMA-SCX. Note the difference in elution order compared to the reversed-phase separation on Discovery BIO Wide Pore C18 shown in product G001581.

column . . . Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U)

mobile phase . . . . . (A) 20 mM Bis-Tris HCl, pH 7.0;
(B) 20 mM Bis-Tris HCl, 0.5M NaCl, pH 7.0;
24 to 69% B in 20 min. (linear)

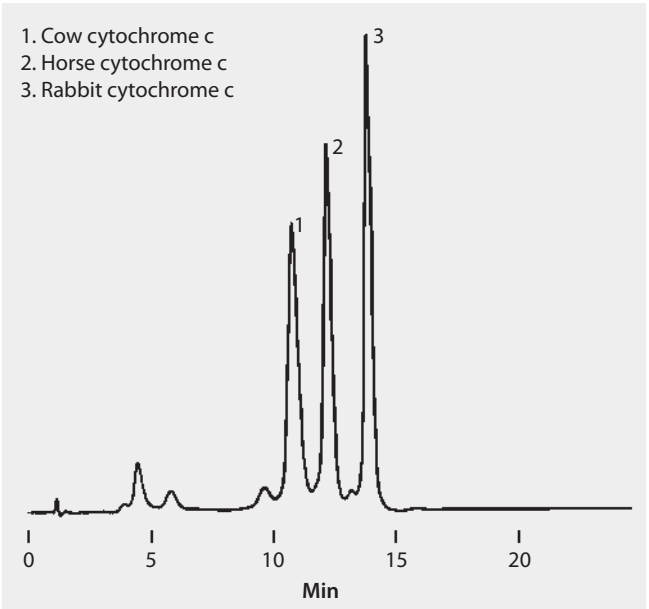
flow rate . . . . . 0.5 mL/min

column temp. . . . . 25 °C

detector . . . . . UV, 280 nm

injection . . . . . 10 µg each variant

Application No. . . . . 796-0477



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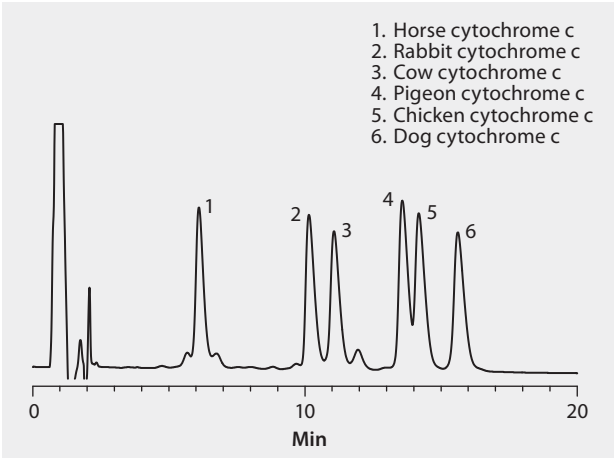
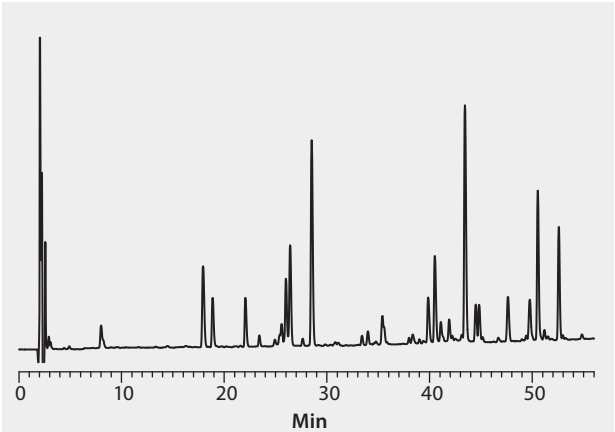
## HPLC Analysis of Cytochrome c Species Variants on Discovery® BIO Wide Pore C18

| | |
|---|---|
| column | Discovery BIO Wide Pore C18, 15 cm × 4.6 mm I.D., 5 µm particles (568222-U) |
| mobile phase | (A) (0.1% TFA in water):(0.1% TFA in acetonitrile); (70:30), (B) (0.1% TFA in water):(0.1% TFA in acetonitrile), 64:36 |
| gradient | 0-100% B in 30 min |
| flow rate | 1 mL/min |
| column temp. | ambient |
| detector | UV, 220 nm |
| injection | 12 µL each at 0.8 mg/mL in 0.1%TFA |
| Application No. | G001581 |



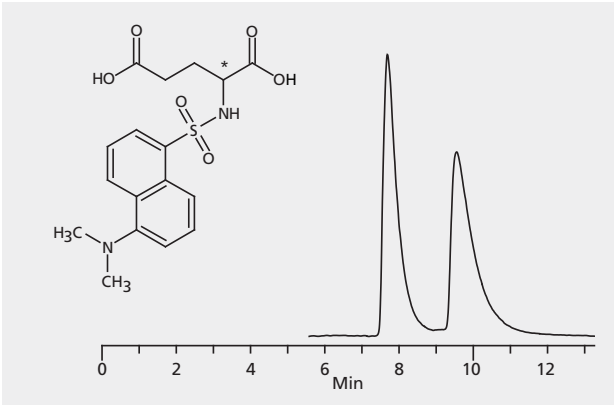
## HPLC Analysis of Cytochrome c Tryptic Digest on Discovery® C18

column . . . . . Discovery C18, 15cm × 4.6mm I.D., 5µm particles (504955)
mobile phase . . . . . (A) 0.1% TFA, 3% n-propanol in water;
(B) 0.1% TFA, 3% n-propanol in acetonitrile
gradient . . . . . 1-37% B in 72 min
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
Application No. . . . . G000307



## HPLC Analysis of Dansyl-Glutamic Acid Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004669



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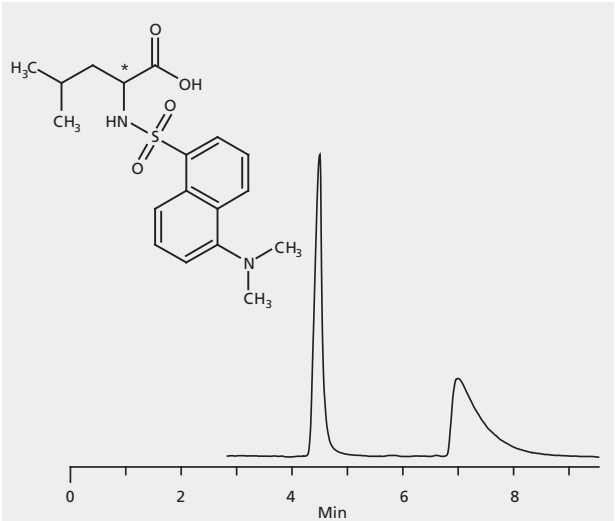
## HPLC Analysis of Dansyl-Leucine Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004670



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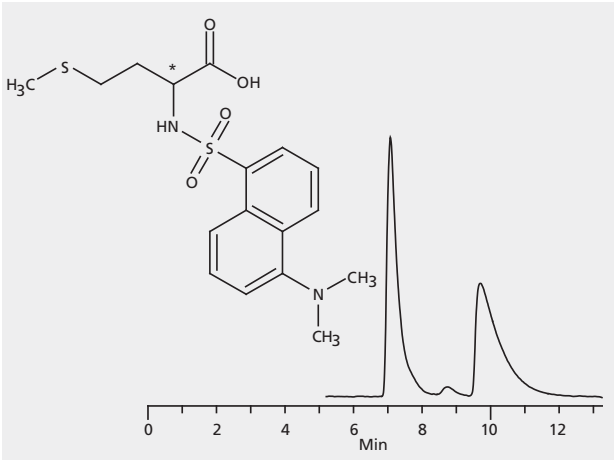
## HPLC Analysis of Dansyl-Methionine Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004671



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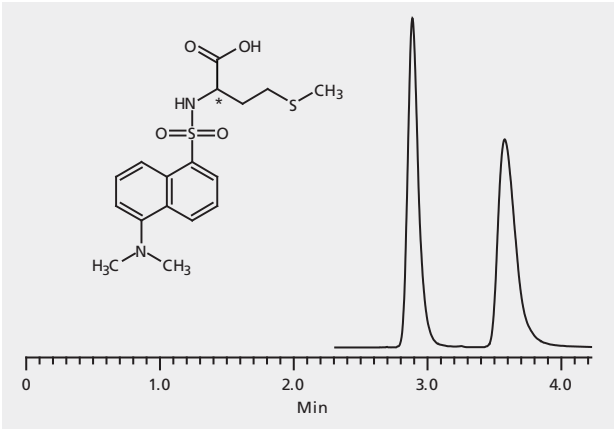
## HPLC Analysis of Dansyl-Methionine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 0.1 wt% ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 3 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004558

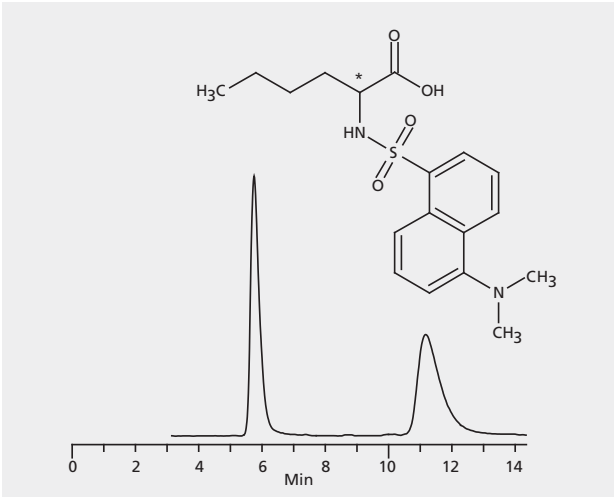





SUPELCO® Solutions within.™

## HPLC Analysis of Dansyl-Norleucine Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (70:30. A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004672



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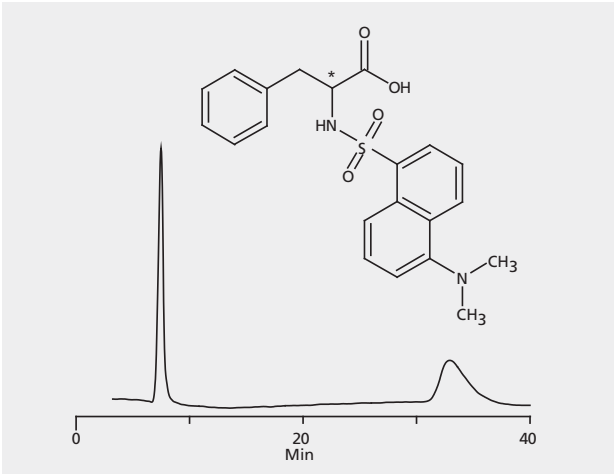
## HPLC Analysis of Dansyl-Phenylalanine Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . (A) 20 mM ammonium acetate; (B) methanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004673



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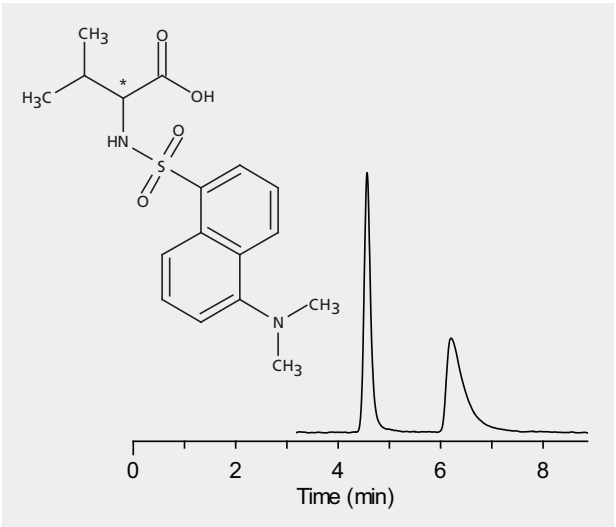
## HPLC Analysis of Dansyl-Valine Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004675

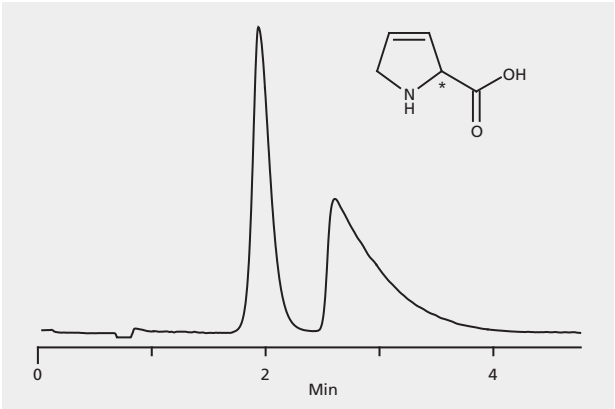





SUPELCO® Solutions within.™

## HPLC Analysis of Dehydroproline Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . water
flow rate . . . . . 2.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 200 nm
injection . . . . . 100 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004528



## HPLC Analysis of Deoxyribonucleosides and Ribonucleosides on SUPELCOSIL™ LC-18-S

column . . . . SUPELCOSIL LC-18-S, 15 cm × 4.6 mm I.D., 5 µm particles (58931)
mobile phase . . 0.05 M $K_2HPO_4/KH_2PO_4$, pH 4.0:methanol (A) 97.5:2.5; (B) 80:20
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
sample . . . . . nucleoside standards in water
Application No. . . . . 713-0955

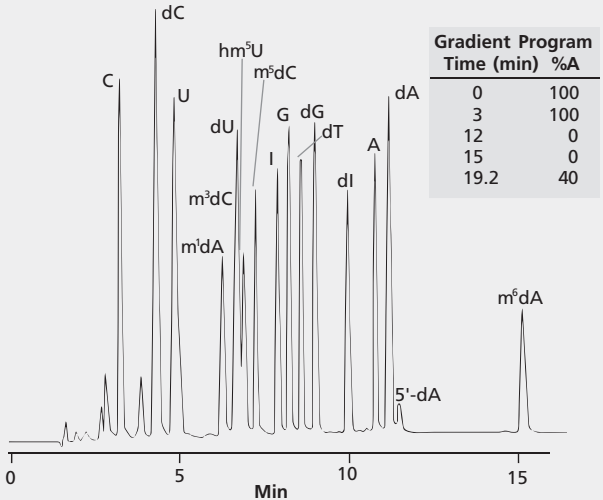
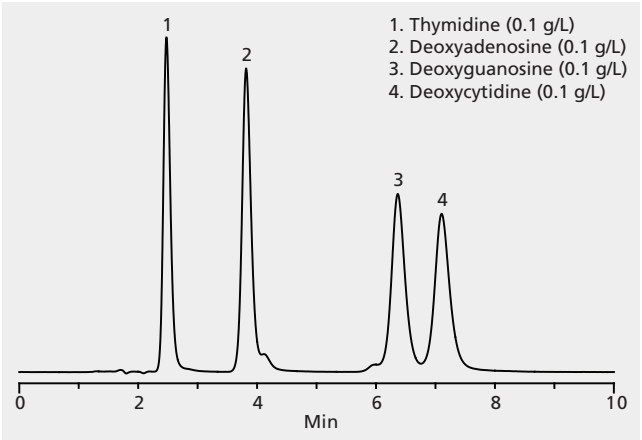



Figure provided by Dr. C. W. Gehrke and Mr. K. C. Kuo, University of Missouri-Columbia, Experimental Station Chemical Laboratories, Columbia, MO USA.

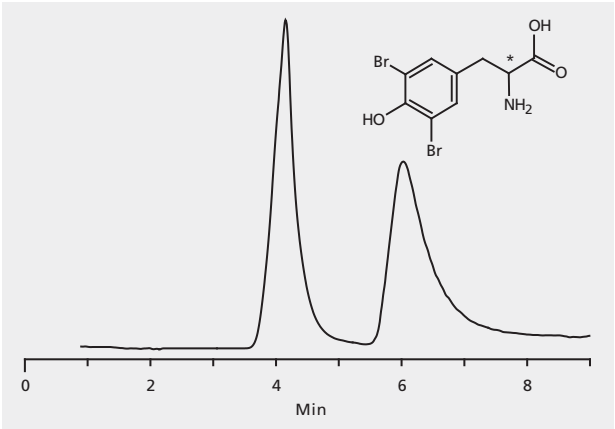
## HPLC Analysis of Deoxyribonucleosides on Ascentis® Si

column . . . . . Ascentis Si, 10 cm x 2.1 mm I.D., 5 µm particles (581508-U)
mobile phase . . . . . (A) 150 mM ammonium formate (pH 3.0, with formic acid); (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 270 nm
injection . . . . . 1 µL
sample . . . . . as indicated, in mobile phase
Application No. . . . . G003719



## HPLC Analysis of 3,5-Dibromo-p-Tyrosine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (50:50, A:B)
flow rate . . . . . 0.8 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004552



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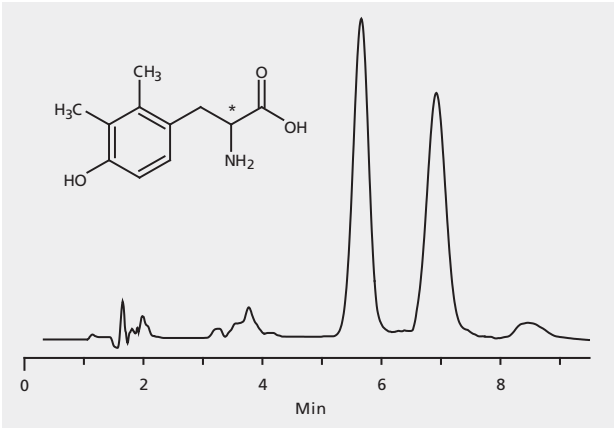
## HPLC Analysis of 2,3-Dimethyltyrosine Enantiomers on Astec® CHIROBIOTIC® T

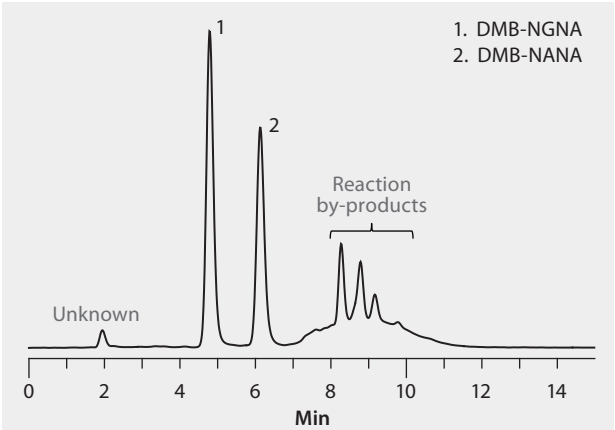
column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004532



## HPLC Analysis of DMB-Labeled Sialic Acids on Ascentis® Express RP-Amide: Comparison of Biosimilars to Reference Materials

Sialic acids affect the bioavailability, function, stability, and metabolism of glycoproteins. Two forms of sialic acid are commonly present in therapeutic glycoproteins: N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid (NGNA). One of the most common quantification methods involves releasing sialic acids from the glycoprotein, derivatizing NANA and NGNA with 1,2-diamino-4, 5-methylenedioxybenzene (DMB), and analyzing by C18-HPLC with fluorescence detection. This procedure is subject to interference from peaks originating from excess reagent and other derivatized impurities, limiting sensitivity and reproducibility. The objectives of this study were to develop a significantly improved HPLC-fluorescence method for DMB-NANA and DMB-NGNA, and to apply this method to compare two candidate biosimilar therapeutic proteins to their respective reference materials.

column . . . . . Ascentis® Express RP-Amide, 10 cm x 2.1 mm I,D., 2.7 µm particles (53913-U)
mobile phase . . . . . (A) Water, 0.1% formic acid, (B) ACN, 0.1% formic acid
gradient . . . . . 0-1 min 6% B; 1.01-4 min 20% B; 4.01-12 min 6% B, total run time 15 min
flow rate . . . . . 0.2 mL/min
pressure . . . . . 1300 psi (89.6 bar)
column temp. . . . . 30 °C
detector . . . . . fluorescence, λ excitation = 373 nm, λ emission = 448 nm
injection . . . . . 0.5 µL
sample . . . . . Mix of DMB-labeled NGNA and NANAsialic acid, 5 mg/ml each
Application No. . . . . G006199



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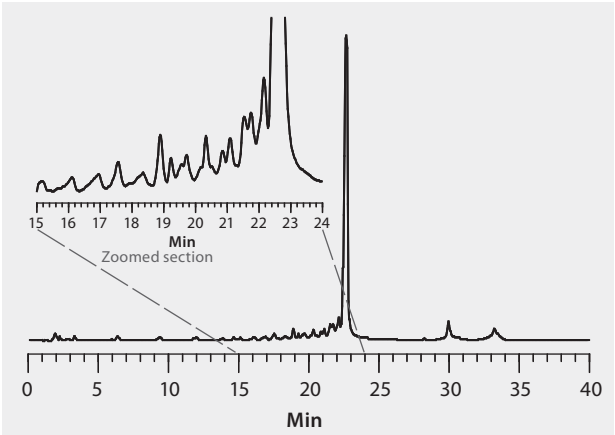
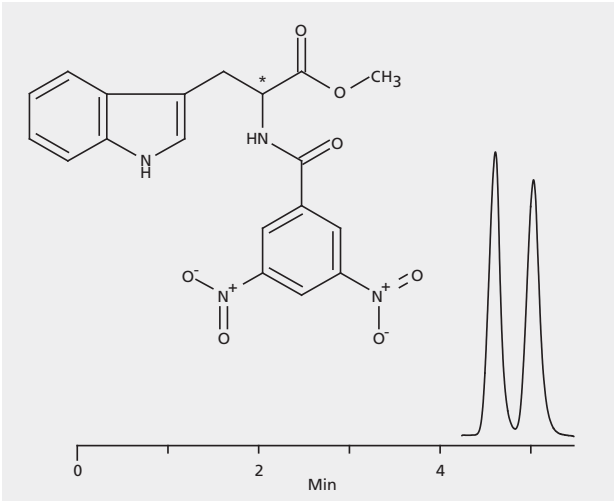
## HPLC Analysis of DNA on Proteomix® WAX-NP5

column . . . Proteomix WAX-NP5, 15 cm x 4.6 mm I.D., 5 µm particles (Z777262)
mobile phase . . . . . (A) 20 mmol Tris, pH 6.0; (B) A + 1.0 M NaCl
gradient . . . . . 20 to 100% B in 40 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1015 psi (70 bar)
temp. . . . . ambient
detector . . . . . UV, 260 nm
injection . . . . . 5 µL
sample . . . . . DNA
Application No. . . . . G005963



## HPLC Analysis of 3,5-DNB-Tryptophan Methyl Ester Enantiomers on Astec® CYCLOBOND® I 2000 SN

column . . CYCLOBOND I 2000 SN, 25 cm x 4.6 mm I.D., 5 µm particles (20524AST)
mobile phase . . . . . ethanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004709



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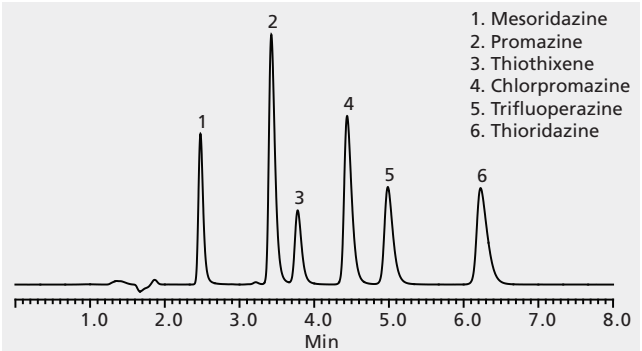
## HPLC Analysis of Dopamine Receptor Antagonists on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 50 mM ammonium formate (pH 3.0 with formic acid); (B) acetonitrile; (45:55, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 50:50, water:methanol
Application No. . . . . G003707



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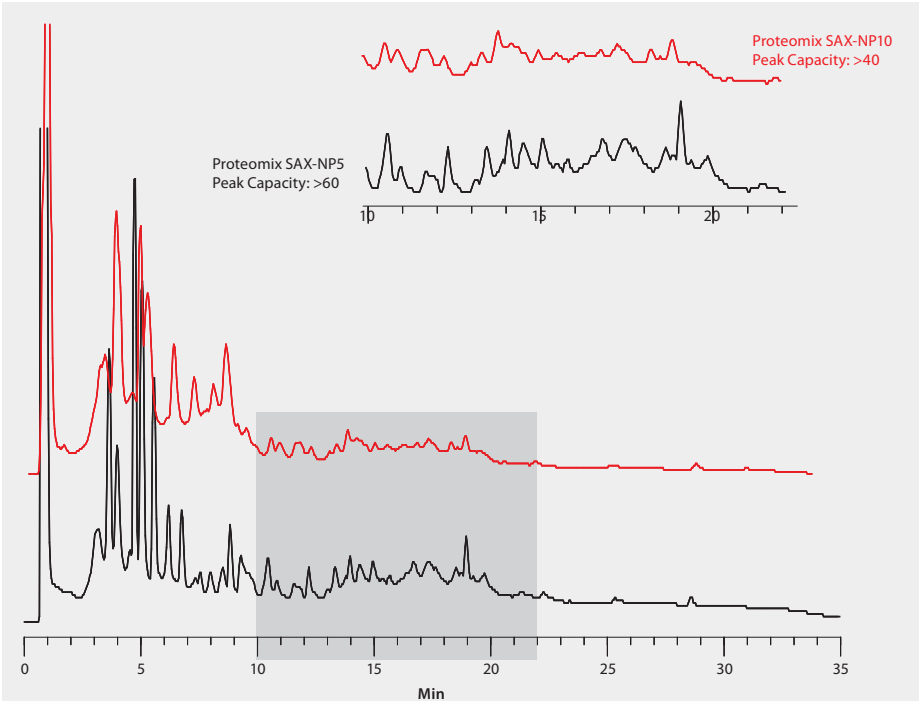
## HPLC Analysis of E. coli Lysate on Proteomix® SAX Columns, Particle Size Comparison

flow rate . . . . . 0.5 mL/min
injection . . . . . 10 µL
mobile phase . . . . . (A) 20 mM Tris, pH 9.0; (B) A + 0.5 M NaCl
column temp. . . . . 25 °C
column . . . . . Proteomix SAX-NP5, 5 cm x 4.6mm I.D., 5 µm particles (Z777227)
column . . . Proteomix SAX-NP10, 5 cm x 4.6 mm I.D., 10 µm particles (Z777203)
gradient . . . . . 0 - 100%B in 30 min
sample . . . . . 2.5 mg/mL
detector . . . . . UV, 280 nm
Application No. . . . . G006129



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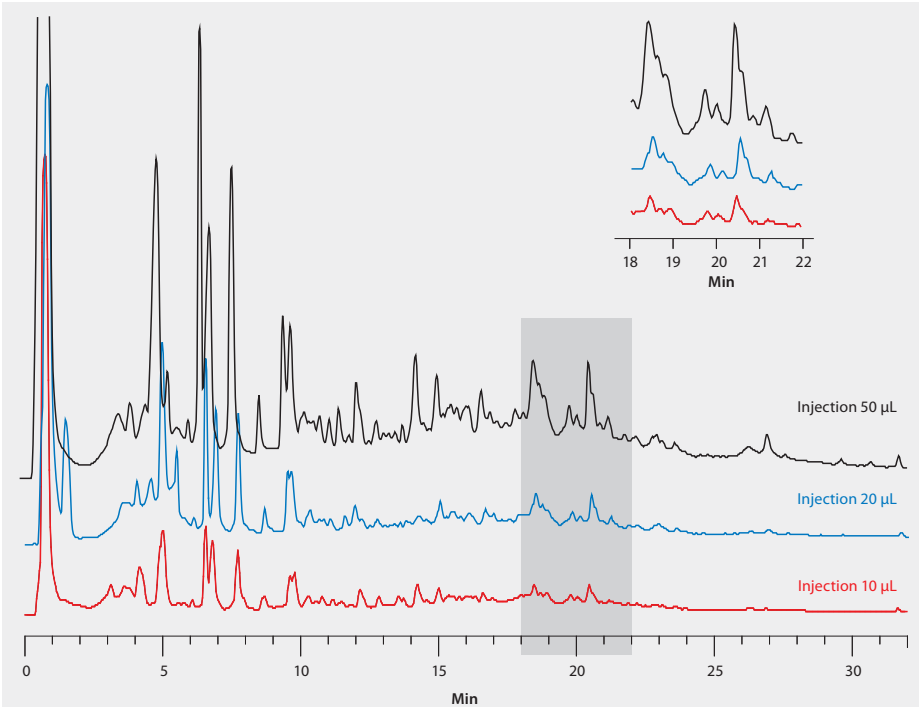
## HPLC Analysis of E. coli Lysate on Proteomix® SAX-NP3, Demonstration of High Loading Capacity

column . . . . . Proteomix SAX-NP3, 5 cm x 4.6mm I.D., 3 µm particles (Z777219)
mobile phase . . . . . (A) 20 mM Tris, pH 9.0; (B) A + 0.5 M NaCl
gradient . . . . . 0 - 100% B in 30 min
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . as indicated in figure
sample . . . . . 2.5 mg/mL
Application No. . . . . G006130



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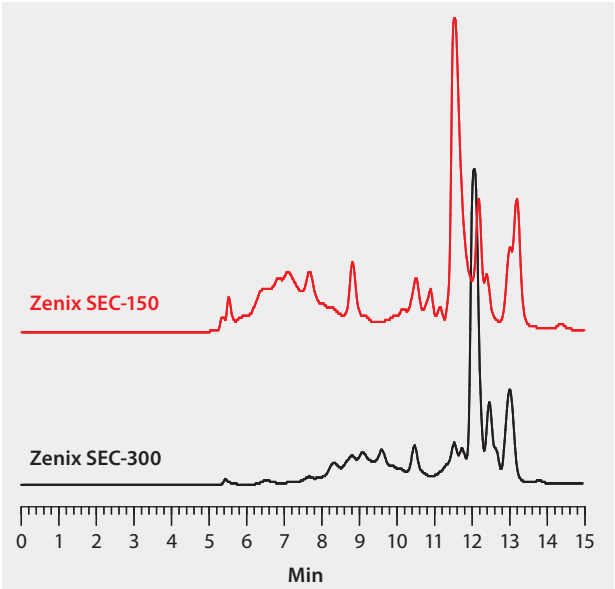
## HPLC Analysis of E. coli lysate with various pore size Zenix® columns

An cell lysate, produced by destroying the membranes of E. Coli cells, is analyzed on two Zenix gel filtration columns packed with 3 micron particles. The top chromatogram shows the peak profile on a 150 Å pore size Zenix SEC-150 gel filtration column, while the bottom chromatogram shows the same sample on a 300 Å pore size Zenix SEC-300 column. As expected, the peaks shift to longer retention times on the SEC-300 column. Although roughly the same number of components are visible in each chromatogram, the SEC-150 column does a better job of pulling the peaks apart, which helps when fractions need to be collected for further analysis.

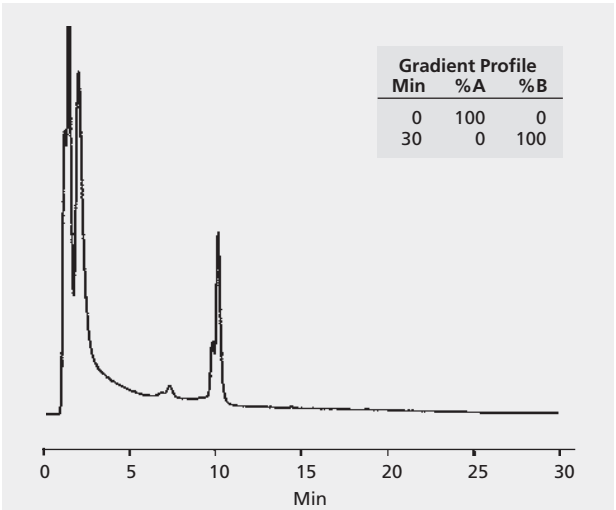
| | |
|---|---|
| column | Zenix SEC-150, 30 cm x 7.8 mm I.D., 3 µm particles (Z777018) |
| column | Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033) |
| mobile phase | 150 mM Phosphate Buffer, pH 7 |
| flow rate | 1.0 mL/min |
| column temp. | Ambient |
| detector | UV, 214nm |
| injection | 10 µL |
| sample | E. coli lysate, 2.5 mg/mL |
| Application No. | G006183 |



## HPLC Analysis of Elastase on Discovery® BIO PolyMA-SCX, Ion Exchange

Discovery BIO PolyMA-SCX and PolyMA-WAX columns give sharp, efficient peaks for a wide variety of proteins.

column . . . . Discovery BIO PolyMA-SCX, 5cm × 4.6mm, 5µm particles (59601-U)
mobile phase . . . . . (A) 20 mM sodium phosphate, pH 7.0;
(B) 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0
gradient . . . . . 0 to 100% B in 30 min. (linear)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
Application No. . . . . G001833



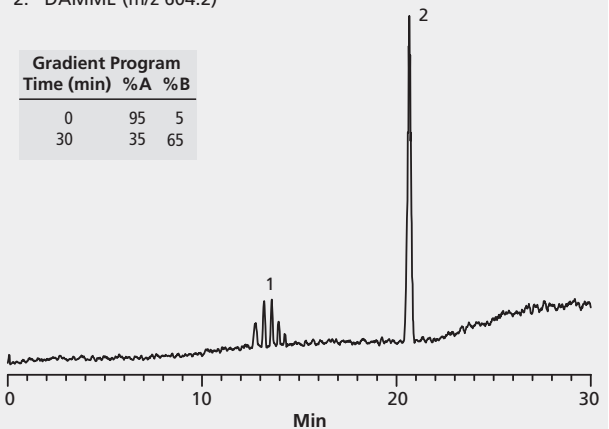
## HPLC Analysis of Enkephalin Derivative (DAMME) on Discovery® HS F5

column . . . . Discovery HS F5, 10 cm × 2.1 mm I.D., 5 µm particles (568501-U)
mobile phase . . . . (A) 25 mM $HCO_2H$ in water; (B) 25 mM $HCO_3H$ in acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 22 °C
detector . . . . . ESI (+), full scan
injection . . . . . 2 µL
sample . . . . . 10 mg/L in 0.1% $HCO_2H$
Application No. . . . . G002572

1. PEG contaminants (m/z 520.1, 564.1, 608.2, 652.2, 696.2)
2. DAMME (m/z 604.2)

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 30 | 35 | 65 |



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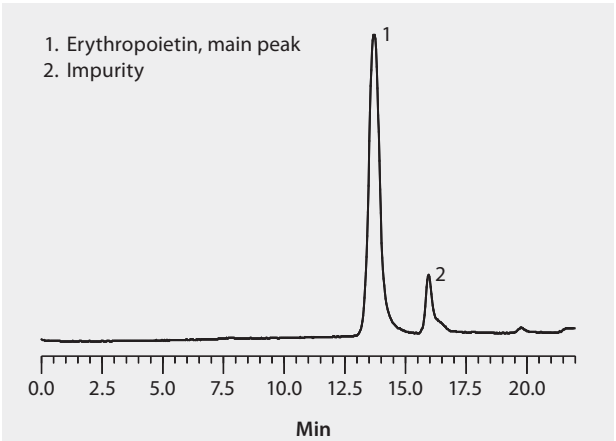
## HPLC Analysis of Erythropoietin on SRT® SEC-300 Gel Filtration Column

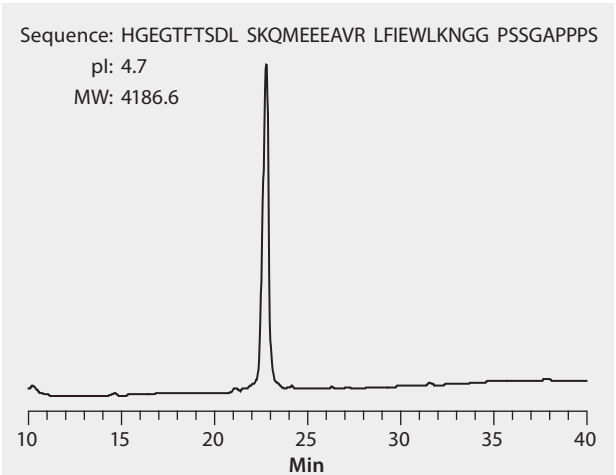
Erythropoietin (EPO) is a glycoprotein hormone that controls red blood cell production. EPO was one of the first genetically engineered therapeutic proteins. It is available under such brand names as Epogen and Aranesp for the treatment of anemia. Human EPO has a molecular mass of 34 kDa. The 5 micron SRT SEC-300 column is operated at a flow rate of 0.7 mL/min. Note that after the main peak, an impurity elutes at about 16 minutes.

column . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm partices, 300 Å, (Z777051)
mobile phase . . . . . $Na_2HPO_4$-$KH_2PO_4$-NaCl
flow rate . . . . . 0.7 mL/min
column temp. . . . . Ambient
detector . . . . . UV, 280 nm
injection . . . . . 20.0 µL
sample . . . . . EPO (Recombinant Human Erythropoietin)
Application No. . . . . G006182



## HPLC Analysis of Exenatide Acetate on Proteomix® SCX-NP5

column . . . . Proteomix SCX NP5, 25 cm x 4.6mm I.D., 5 µm particles (Z777161)
mobile phase . . . . . (A) 10 mM monosodium phosphate:acetonitrile (56:44, v/v), adjusted to pH to 2.0 with phosphoric acid;
((B) 0.5 M NaCl/10 mM monosodium phosphate:acetonitrile (56:44, v/v), adjusted to pH to 2.0 with phosphoric acid)
gradient . . . . . 0 to 65% B in 35 min; held for 5 min; to 0% B in 10 min
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1653 psi (114 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 235 nm
injection . . . . . 20 µL
sample . . . . . 0.325 mg/mL exenatide acetate in mobile phase A
Application No. . . . . G006163



## HPLC Analysis of Ferredoxin NADP Reductase on TSKgel® Phenyl-5PW

column . . . . . TSKgel Phenyl-5PW column, 7.5 cm × 7.5 mm I.D., 10 µm particles (807573)

mobile phase . . . 60 min decreasing linear gradient from 1.5M to 0M ammonium sulfate in 0.1M sodium phosphate buffer, pH7

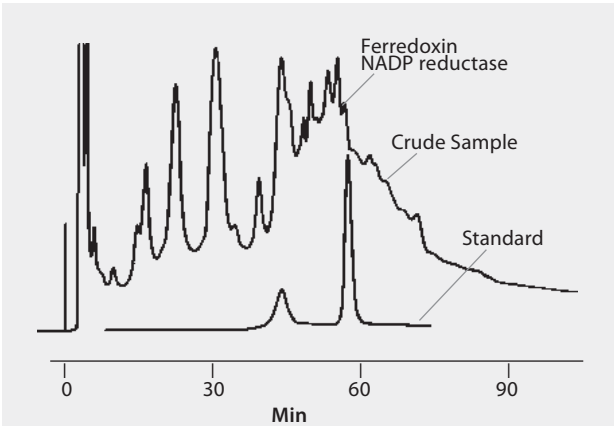
flow rate . . . . . 0.5 mL/min

column temp. . . . . 25 °C

detector . . . . . UV, 280 nm

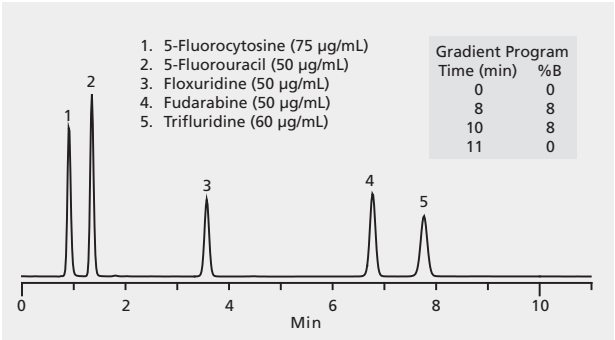
injection . . . . . 3mg (crude) or 0.2mg (standard)

Application No. . . . . 713-0922



## HPLC Analysis of Fluorinated Pyrimidine Nucleosides on Ascentis® RP-Amide

column . . . . Ascentis RP-Amide, 5 cm × 4.6 mm I.D., 5 µm particles (565323-U)
mobile phase . . . . . (A) water with 0.1% ammonium formate (pH 3.04 with formic acid), (B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 260 nm
injection . . . . . 5 µL
sample . . . . . as indicated in mobile phase A
Application No. . . . . G003940



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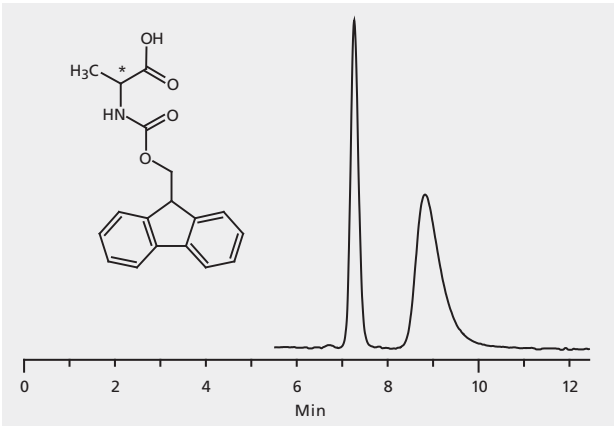
## HPLC Analysis of FMOC-Alanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 5.0; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004559



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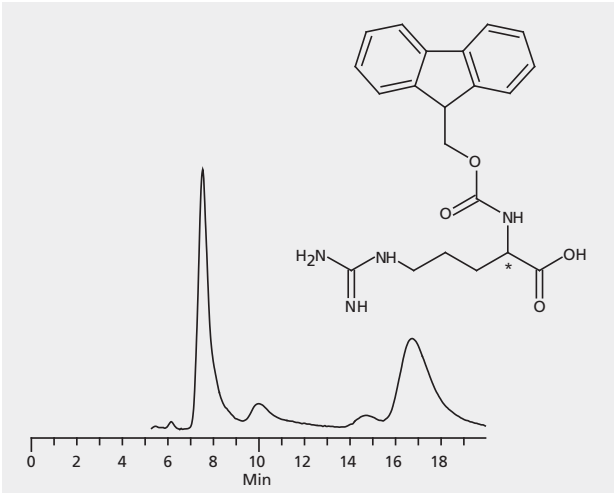
## HPLC Analysis of FMOC-Arginine Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 260 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004678



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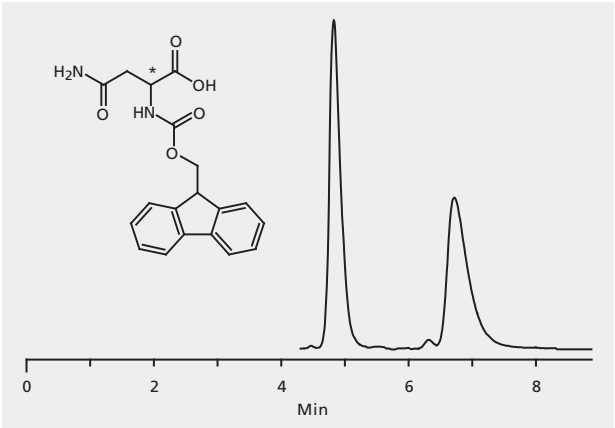
## HPLC Analysis of FMOC-Asparagine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004562



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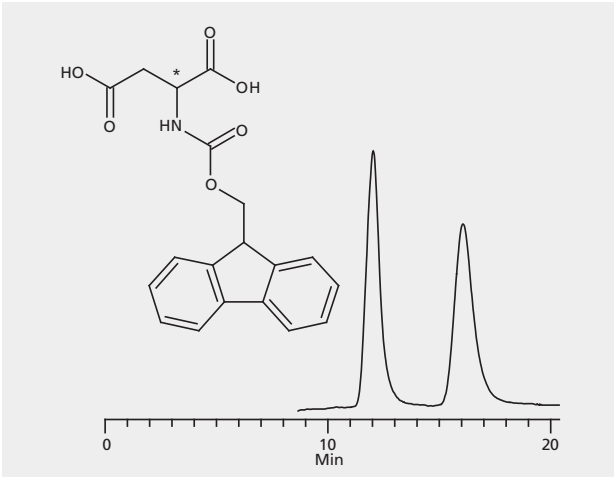
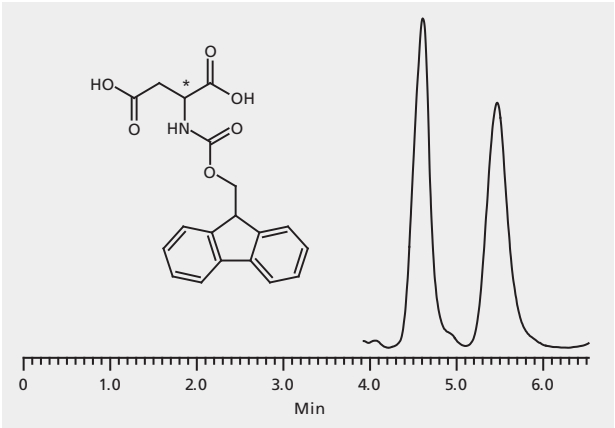
## HPLC Analysis of FMOC-Aspartic Acid Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 5.0; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004677



## HPLC Analysis of FMOC-Aspartic Acid Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004560



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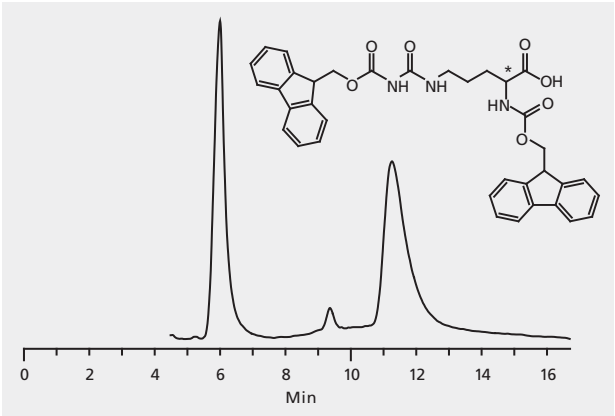
## HPLC Analysis of FMOC-Citrulline Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
sample . . . . . 1 mg/mL in methanol
injection . . . . . 1 µL
Application No. . . . . G004563



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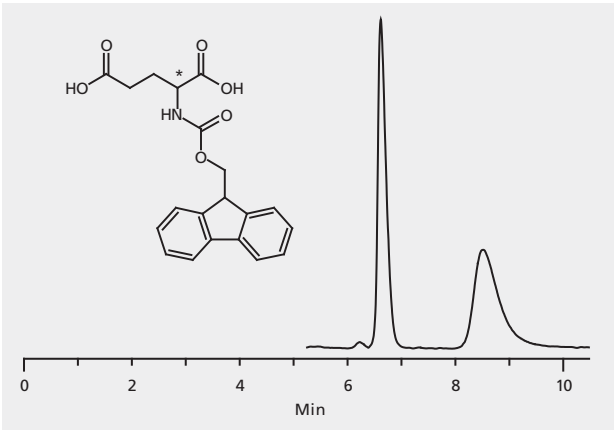
## HPLC Analysis of FMOC-Glutamic Acid Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004561



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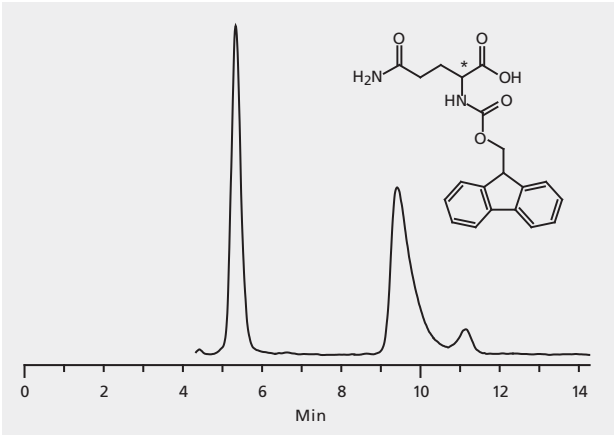
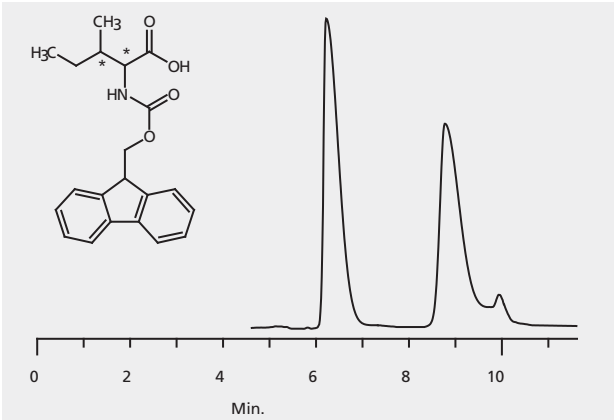
## HPLC Analysis of FMOC-Glutamine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004565



## HPLC Analysis of FMOC-Isoleucine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 3 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004575



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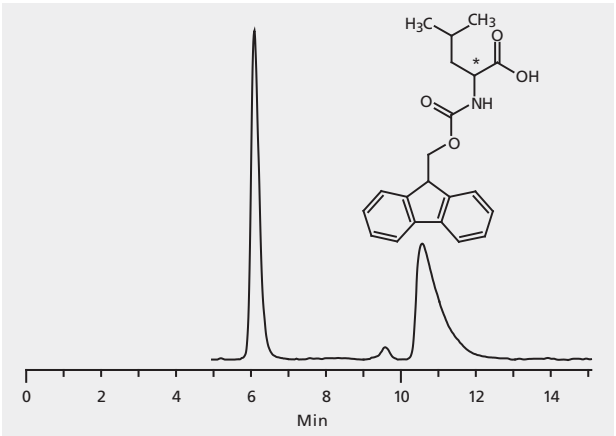
## HPLC Analysis of FMOC-Leucine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004564



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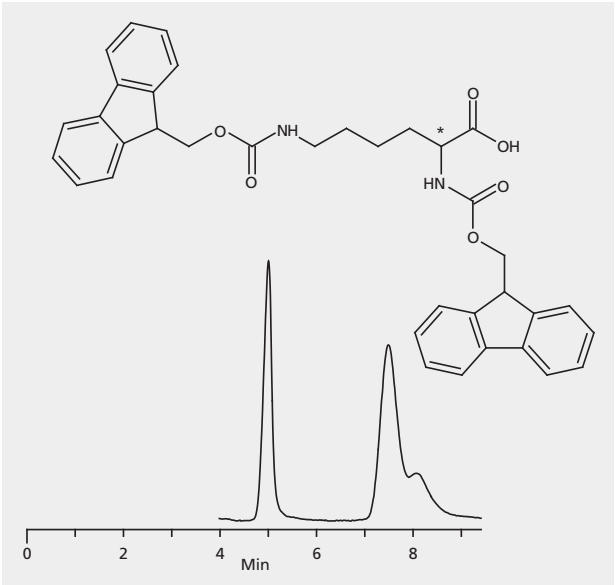
## HPLC Analysis of FMOC-Lysine Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004679



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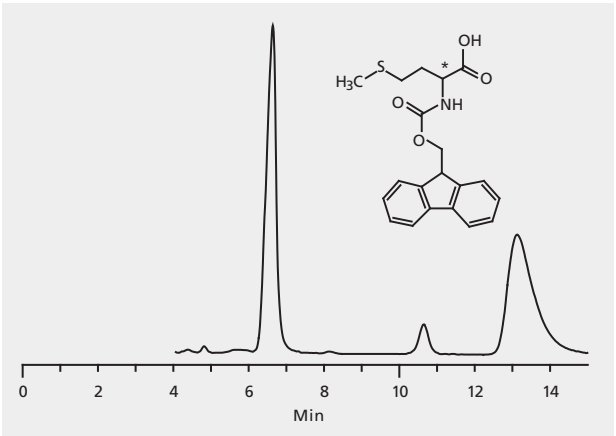
## HPLC Analysis of FMOC-Methionine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004566



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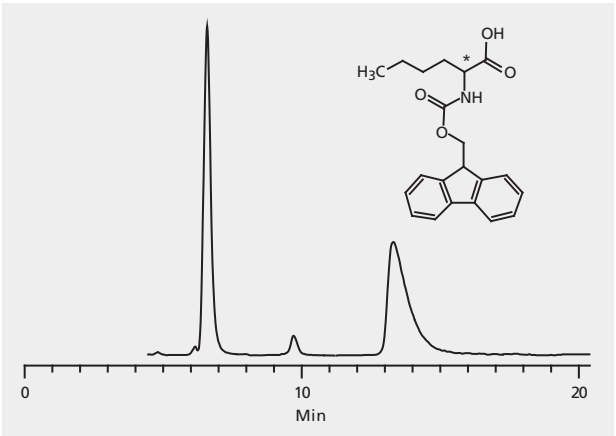
## HPLC Analysis of FMOC-Norleucine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004567

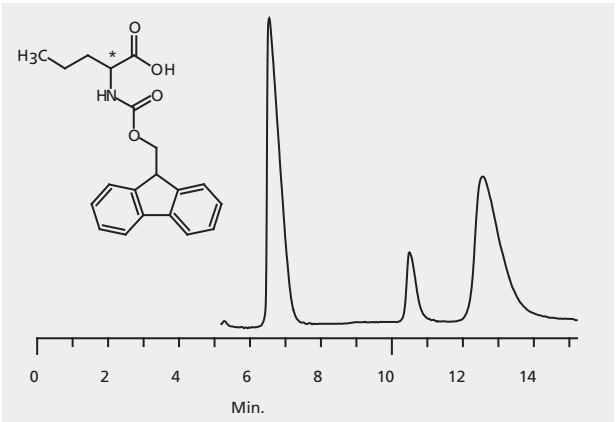





SUPELCO® Solutions within.™

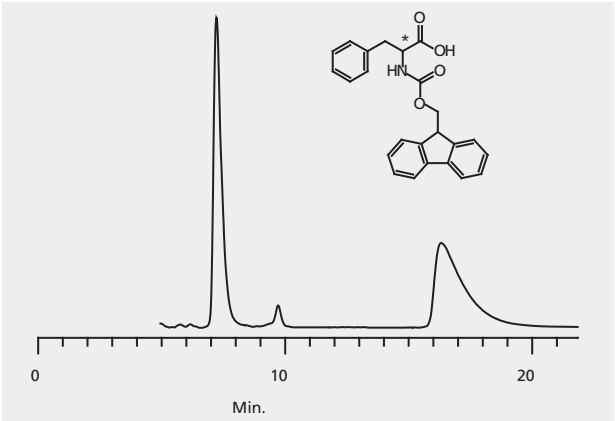
## HPLC Analysis of FMOC-Norvaline Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 0.5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004568



## HPLC Analysis of FMOC-Phenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004569

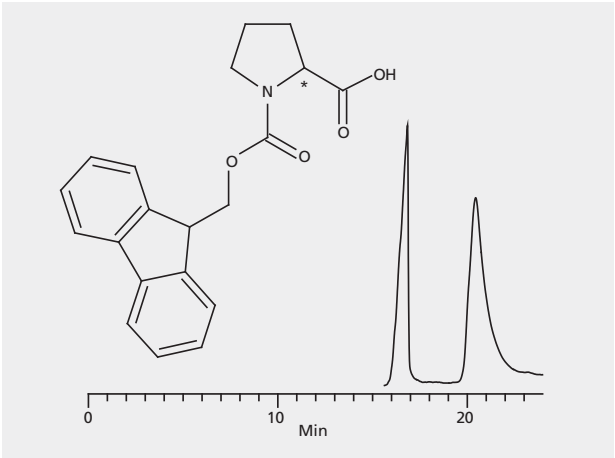





SUPELCO® Solutions within.™

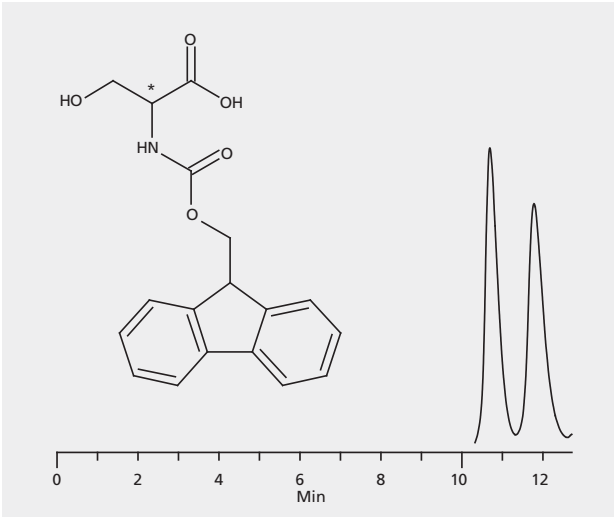
## HPLC Analysis of FMOC-Proline Enantiomers on Astec® CYCLOBOND® I 2000 SN

column . . CYCLOBOND I 2000 SN, 25 cm x 4.6 mm I.D., 5 µm particles (20524AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) ammonium hydroxide; (85:15:0.4:0.1, A:B:C:D)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004710



## HPLC Analysis of FMOC-Serine Enantiomers on Astec® (R,R) P-CAP™

column . . . . Astec (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
mobile phase . . . . . 20 mM ammonium acetate in: (A) acetonitrile; (B) methanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 0.5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004725

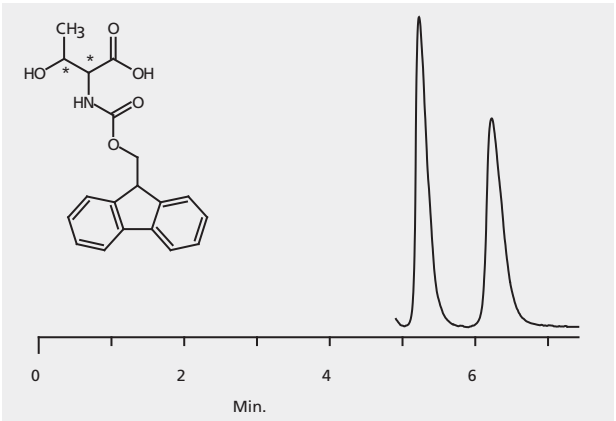





SUPELCO® Solutions within.™

## HPLC Analysis of FMOC-Threonine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 0.8 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004571

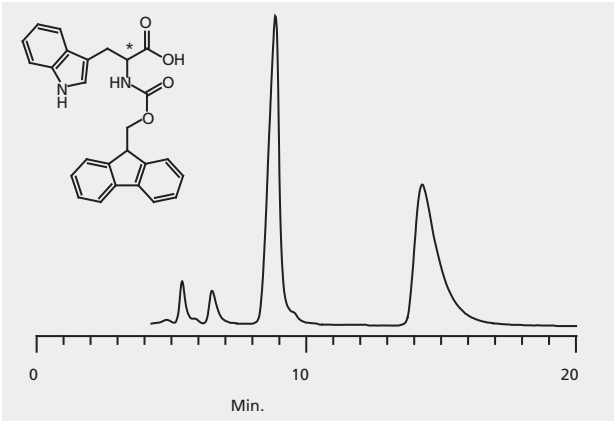





SUPELCO® Solutions within.™

## HPLC Analysis of FMOC-Tryptophan Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004570



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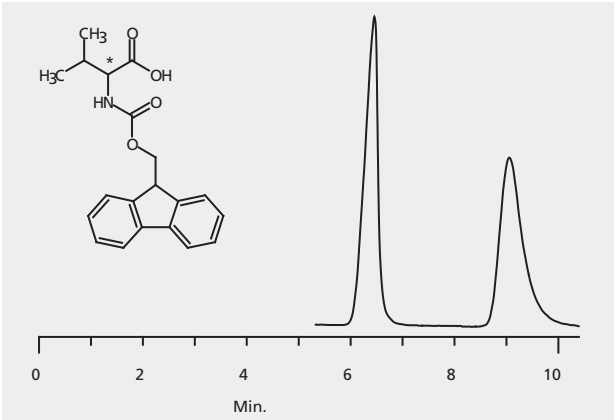
## HPLC Analysis of FMOC-Valine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 260 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004589

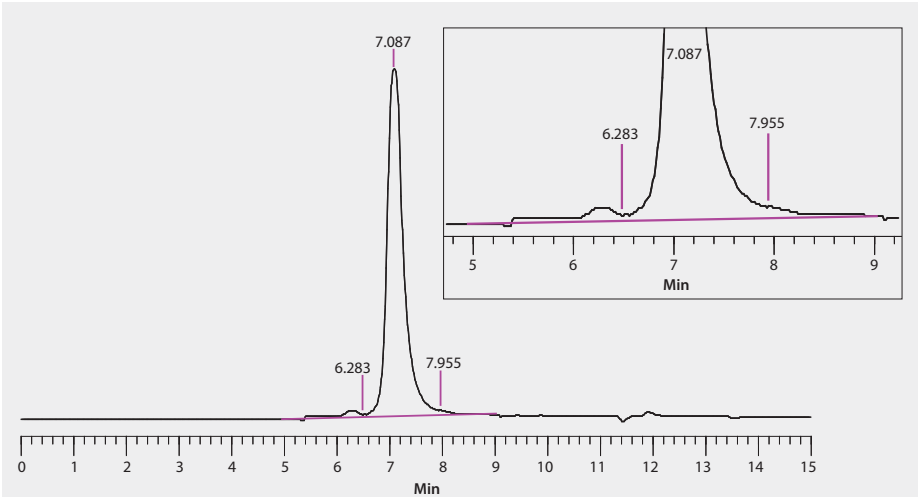





SUPELCO® Solutions within.™

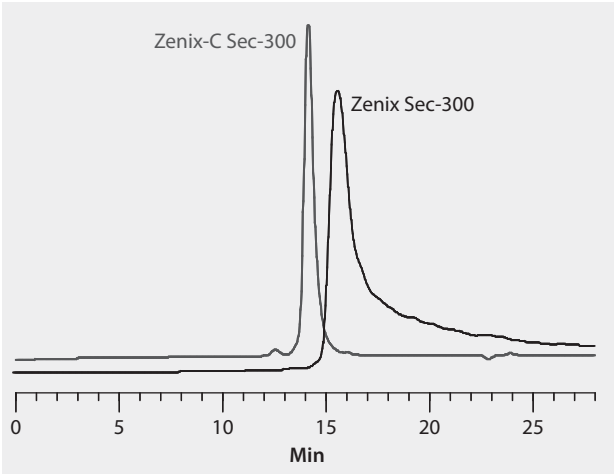
## HPLC Analysis of Fusion Protein on Zenix®-C SEC-300

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
mobile phase . . . . . 150 mM sodium phosphate buffer, pH 7.0 + 200 mM NaCl
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1102 psi (76 bar)
column . . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL fusion protein
Application No. . . . . G005953



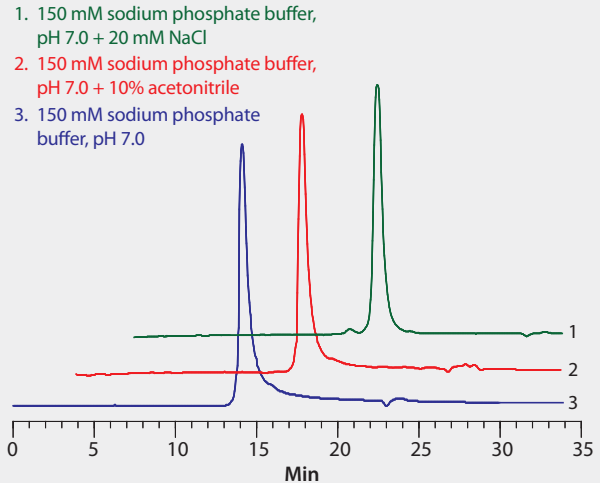
## HPLC Analysis of Fusion Protein on Zenix®-C SEC-300 vs. Zenix® SEC-300

column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
mobile phase . . . . . 150 mM sodium phosphate buffer, pH 7.0 + 200 mM NaCl
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL fusion protein, MW 170 kD, pI 6.8-7.0
Application No. . . . . G005951



## HPLC Analysis of Fusion Protein on Zenix®-C SEC-300, Effect of Mobile Phase Composition

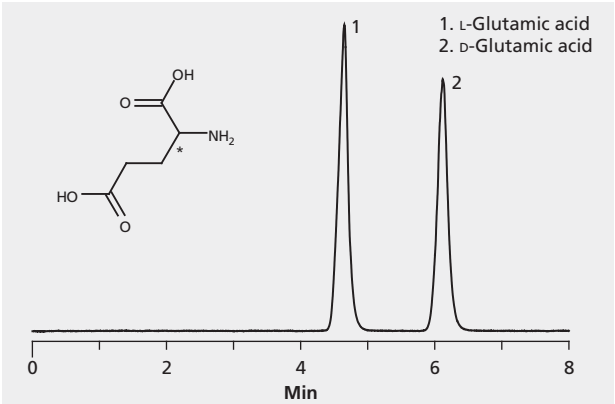
column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
gradient . . . . . (A) lower, 150 mM sodium phosphate buffer, pH 7.0
(B) middle, 150 mM sodium phosphate buffer, pH 7.0 + 10% acetonitrile
(C) top, 150 mM sodium phosphate buffer, pH 7.0 + 20 mM NaCl
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL fusion protein, MW 170 kD, pI 6.8-7.0
Application No. . . . . G005952




**Note:** 10% retention time offset for presentation purpose

## HPLC Analysis of Glutamic Acid Enantiomers on Astec® CHIROBIOTIC® T (23 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (20:80:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004496



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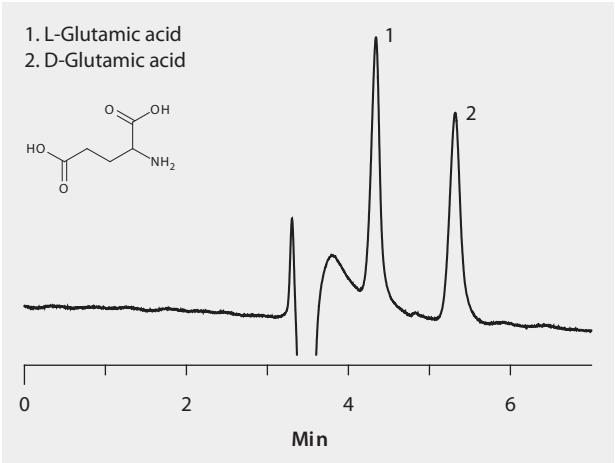
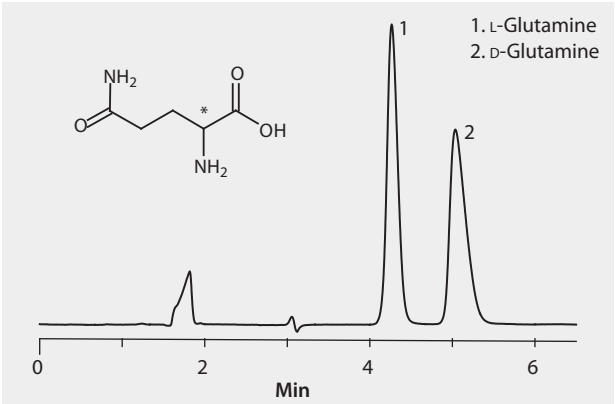
## HPLC Analysis of Glutamic Acid Enantiomers on Astec® CHIROBIOTIC® T (25 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005335



## HPLC Analysis of Glutamine Enantiomers on Astec® CHIROBIOTIC® T (23 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample gas . . . . . 1 mg/mL in methanol
Application No. . . . . G004497



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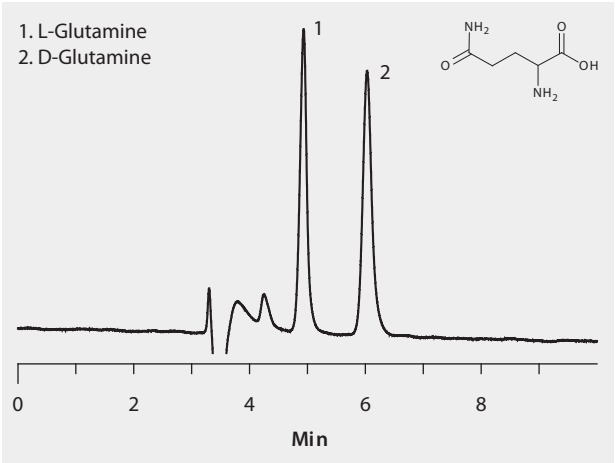
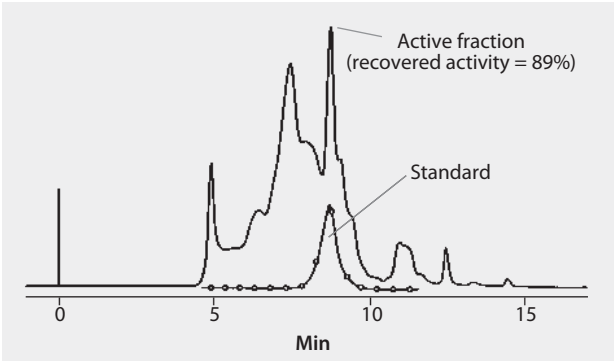
## HPLC Analysis of Glutamine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005336



## HPLC Analysis of Glutathione S-Transferase on TSKgel® G3000SW$_{XL}$

column . . . . TSKgel G3000SW$_{XL}$, 30 cm × 7.8 mm I.D., 5 µm particles (808541)
mobile phase . . 0.3M sodium chloride/0.05M sodium dihydrogen phosphate, pH 7.0
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220nm and enzyme assay
injection . . . . . 100µL crude glutathione S-transferase preparation from guinea pig liver (0.7mg)
Application No. . . . . 713-0939



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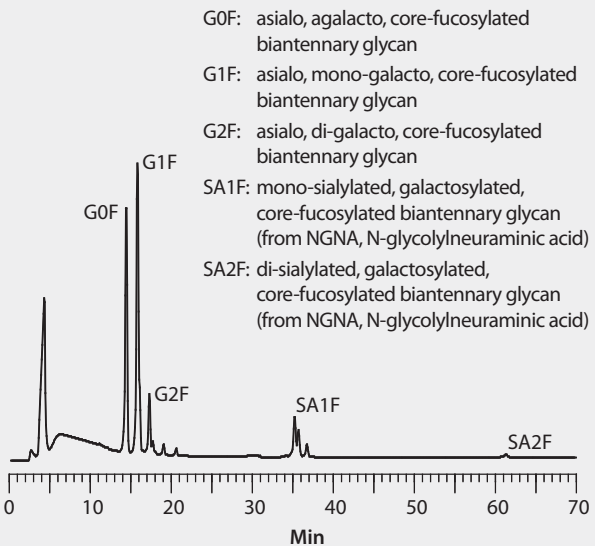
## HPLC Analysis of Glycan and Isomers on Proteomix® SAX-NP5, High Resolution Separation

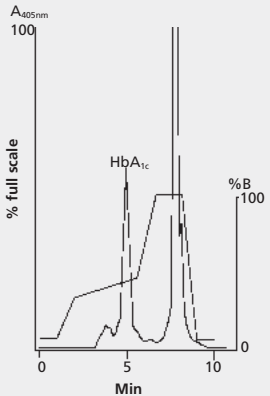
column . . . . Proteomix SAX-NP5, 15 cm x 4.6 mm I.D., 5 µm particles (Z777228)
mobile phase . . . . . (A) 2.5% (v/v) acetic acid, 0.5% TEA in water; 0.5% acetic acid in acetonitrile
gradient . . . . . 0-100% B in 60 min
flow rate . . . . . 0.3 mL/min
detector . . . . . Fluorescence, Ex/Em=360/425 nm
sample . . . . . 2-AA (anthranilic acid) labeled N-linked oligosaccharide profiling of an IgG1 sample
Application No. . . . . G005959




Courtesy of Miyako Kawakatsu, M&S Instruments Inc

## HPLC Analysis of Hemoglobin $HbA_{1C}$ on Mono S® HR5/5

column . . . . . Mono S HR5/5, 5 cm x 5 mm I.D., 10 µm (54808)
mobile phase . . (A) 0.01M malonate-NaOH, pH 5.7; (B) 0.3M LiCl in mobile phase A
flow rate . . . . . 2.0 mL/min
detector . . . . . VIS, 405 nm
injection . . . . . 50 µL hemolysate, diluted 1:5
Application No. . . . . 794-0412

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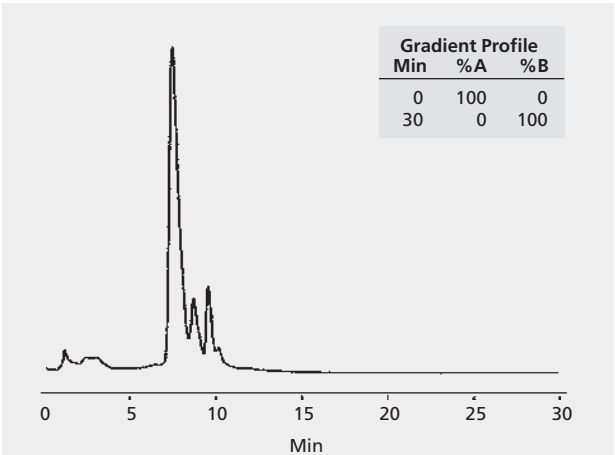
## HPLC Analysis of Hemoglobin S on Discovery® BIO PolyMA-SCX, Ion Exchange

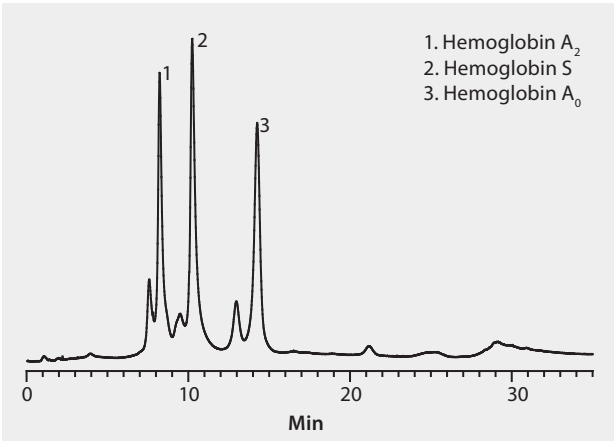
Discovery BIO PolyMA-SCX and PolyMA-WAX columns give sharp, efficient peaks for a wide variety of proteins.

column . . . . Discovery BIO PolyMA-SCX, 5cm × 4.6mm, 5µm particles (59601-U)
mobile phase . . . . . (A) 20 mM sodium phosphate, pH 7.0;
(B) 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0
gradient . . . . . 0 to 100% B in 30 min. (linear)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
Application No. . . . . G001834



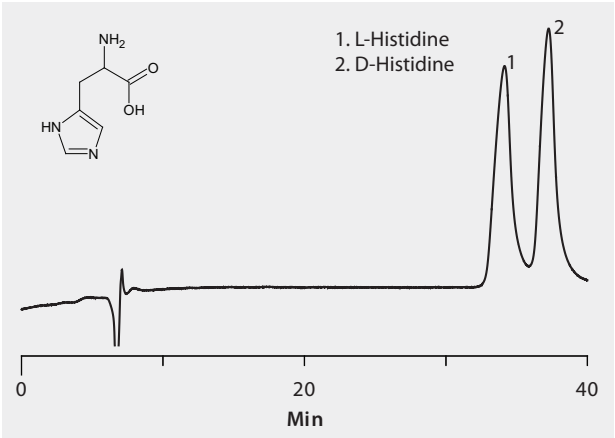
## HPLC Analysis of Hemoglobin Variants on Discovery® BIO PolyMA-WAX

column . . Discovery BIO PolyMA-WAX, 5 cm x 4.6 mm, 5 µm particles (59602-U)
mobile phase . . . . . (A) 10 mM Tris, pH 8.0 with acetic acid;
(B) 10 mM Tris, 0.25 M KCl, pH 8.0 with acetic acid
gradient . . . . . 0 to 32% B in 20 min. (linear)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 50 µg each Hb variants (A2, S and A0)
Application No. . . . . G001830



## HPLC Analysis of Histidine Enantiomers on Astec® CHIROBIOTIC® T (15 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.006, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 15 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 200 µg /mL in 50:50 water:methanol
Application No. . . . . G005337



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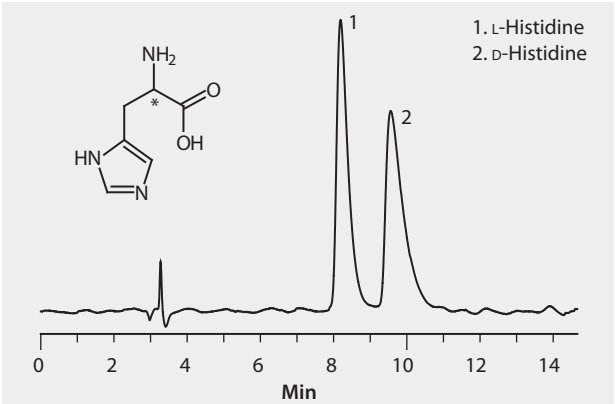
## HPLC Analysis of Histidine Enantiomers on Astec® CHIROBIOTIC® T (23 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 160 mM sodium phosphate, pH 4.5; (B) ethanol; (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004498



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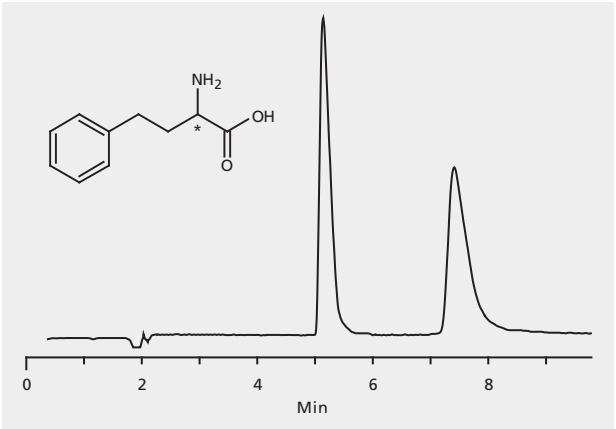
## HPLC Analysis of Homophenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 10 mg/mL in methanol
Application No. . . . . G004533



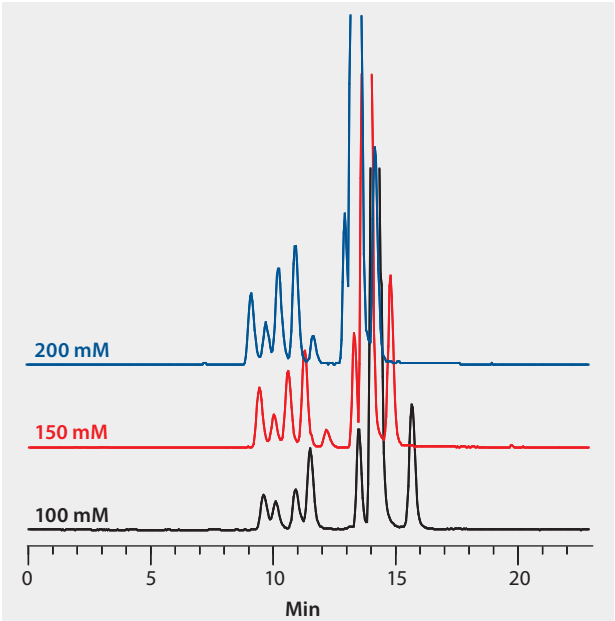
## HPLC Analysis of Horse Serum Proteins in Acetonitrile as a Function of Ammonium Formate Concentration

Although horse serum proteins can be analyzed under standard SEC conditions, the resolution is poor. However, very high efficiency separations can be achieved when using pure acetonitrile to which ammonium formate has been added. About 200 mM ammonium formate is required for all proteins to elute within the pore volume of SRT SEC-150 column. The position of the components can be modulated by decreasing the ammonium formate concentration.

Ref.: V. McKay; X. Huang, LC-GC North America;Sep2006 Supplement, Vol. 24, p25, 2D Chromatography of Proteins: Horse Serum Analysis

Courtesy of Miyako Kawakatsu, M&S Instruments Inc.

column . . . SRT SEC-150, 30 cm x 4.6 mm I.D., 5 µm particles, 150 Å (Z777043)
mobile phase . . . . . 100–200 mM CH3COONH4/CH3CN, pH 6.3
flow rate . . . . . 0.25 mL/min
detector . . . . . SofTA ELSD
sample . . . . . Molecular weight marker proteins, horse serum
Application No. . . . . G006212



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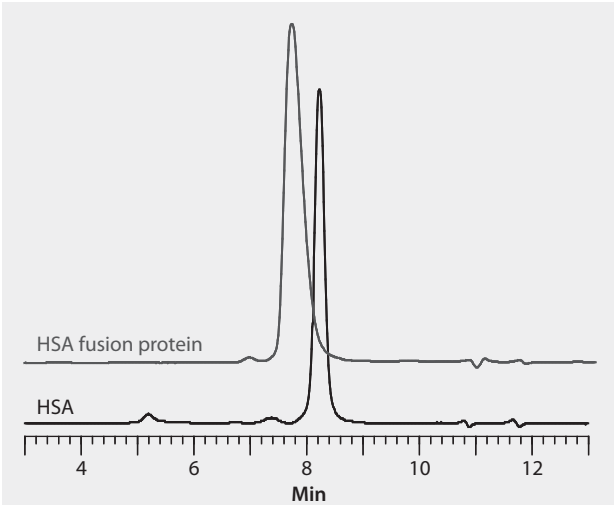
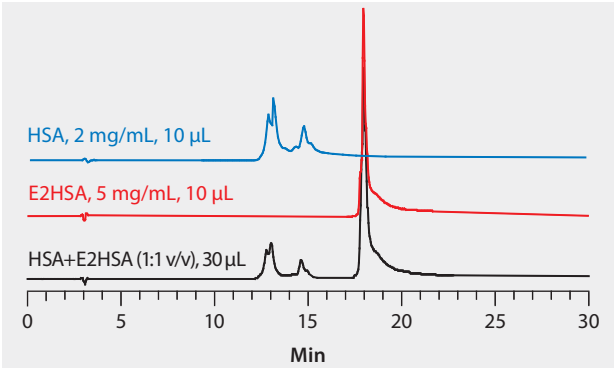
## HPLC Analysis of HSA and HSA Fusion Protein on Zenix®-C SEC-300

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
mobile phase . . . . . (A) 150 mM sodium phosphate buffer, pH 7.0; (B) IPA; (95:5, v/v, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . HSA fusion peptide 5 mg/mL (MW 75 kD, pI 5.0, HSA conjugated peptide in diabetes treatment)
Application No. . . . . G005956



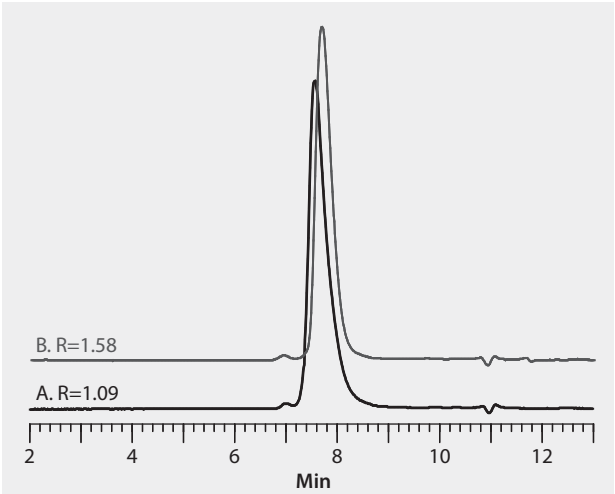
## HPLC Analysis of HSA Fusion Protein on Proteomix® SAX-NP5

column . . . . . Proteomix SAX-NP5 25 cm x 4.6mm I.D., 5 µm particles (Z777229)
detector . . . . . UV, 280 nm
flow rate . . . . . 0.5 mL/min
column temp. . . . . 30 °C
mobile phase . . Piperazine, Triethanolamine, bis-tris propane and N-methylpiperazine, 20 mM each,(A) pH 9.7; (B) pH 3.0 ( adjust by HCl )
sample . . . . . HSA fusion peptide 5 mg/mL, 10 µL (MW 75 kD, pI 5.0, HSA conjugated peptide in diabetes treatment)
pressure . . . . . 2016 psi (139 bar)
Application No. . . . . G006133



## HPLC Analysis of HSA Fusion Protein on Zenix®-C SEC-300, Effect of Mobile Phase Composition

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
mobile phase . . 150 mM sodium phosphate buffer, pH 7.0 or (A) 150 mM sodium phosphate buffer, pH 7.0; (B) IPA; (95:5, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . HSA fusion peptide 5 mg/mL (MW 75 kD, pI 5.0, HSA conjugated peptide in diabetes treatment)
Application No. . . . . G005955



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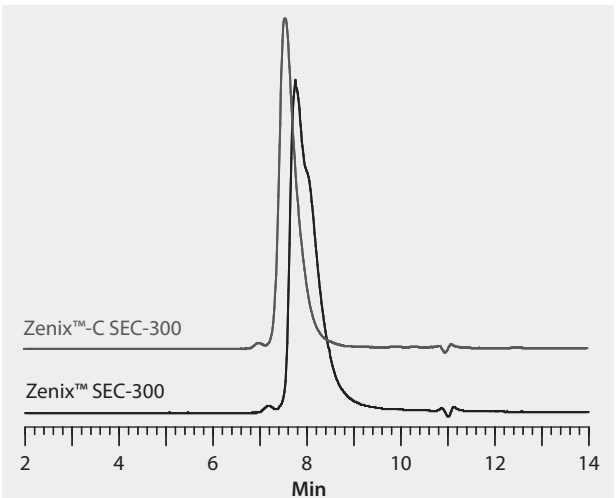
## HPLC Analysis of HSA Fusion Protein on Zenix®-C SEC-300 vs. Zenix® SEC-300

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate buffer, pH 7.0
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . HSA fusion peptide 5 mg/mL (MW 75 kD, pI 5.0, HSA conjugated peptide in diabetes treatment)
Application No. . . . . G005957



## HPLC Analysis of Human Growth Hormone (hGH) on Discovery® BIO PolyMA-WAX

This Figure shows the degradation products of human growth hormone (hGH) well-separated on a Discovery BIO PolyMA-WAX anion-exchange column. The difference between the resolved compounds is the conversion of protein amide(s) to carboxylate(s), demonstrating both the power of the ion-exchange technique, and the efficiency of the Discovery BIO PolyMA-WAX column.

column . . Discovery BIO PolyMA-WAX, 5 cm × 4.6 mm I.D., 5 µm particles (59602-U)
mobile phase . . (A) 20 mM Tris-HCl, pH 8.0; (B) 20 mM Tris-HCl, 0.5 M NaCl, pH 8.0
gradient . . . . . 5 to 70% B in 30 min. (linear)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 mg hGH
Application No. . . . . 796-0659

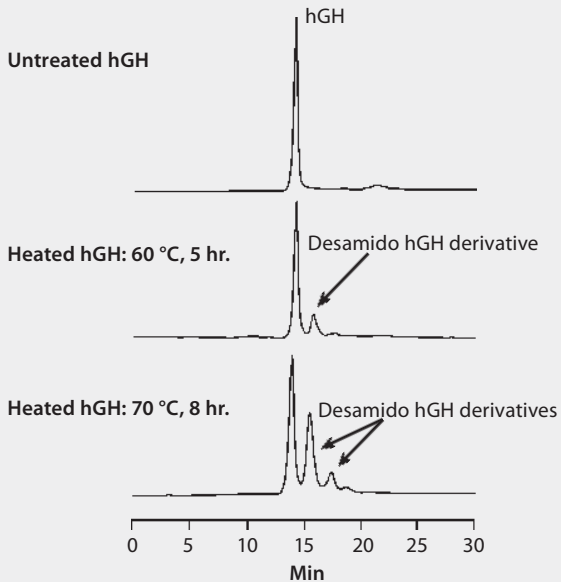
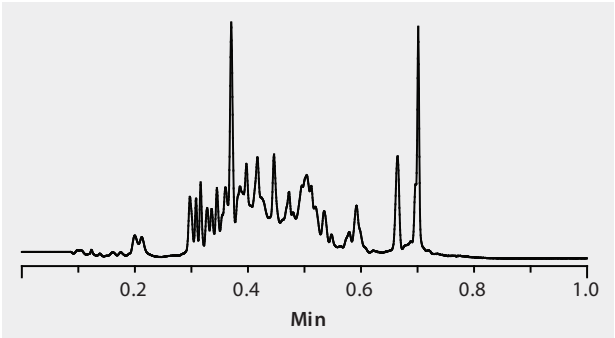



Figure used with permission of Mitsubishi Chemical Co.

## HPLC Analysis of Human Serum Albumin Tryptic Digest on Ascentis® Express C18

**Sample Prep**: 0.1 gram of albumin was dissolved in 10 mL of 0.01 M ammonium formate buffer, and the pH was adjusted to 8.0 with ammonium hydroxide; the solution was heated in a boiling water bath for 6 min. After the solution cooled, 2.0 mg of trypsin from bovine pancreas was added, and the mixture was allowed to react for 4 h at 37 °C; the reaction was quenched by adding 0.1% trifluoroacetic acid to pH 2. The digest was stored at 4 °C and filtered prior to injection through a 0.45 ìm nylon membrane

sample/matrix . . . . . human serum albumin, tryptic digest
column . . Ascentis Express C18, 3 cm x 4.6 mm I.D., 2.7 µm particles (53818-U)
mobile phase . . . . . (A) 10 mM ammonium acetate in water, pH 9; (B) 10 mM ammonium acetate in methanol/water, pH 9; (90:10, A:B)
gradient . . . . 0% B in 0.50 min; 55% B in 0.25 min; 90% B for 0.50-0.69 min; 0% B for 0.70-1.00 min
flow rate . . . . . 4.0 mL/min
column temp. . . . . 45 °C
detector . . . . . UV, 254 nm
injection . . . . . 20 µL
Application No. . . . . G005598



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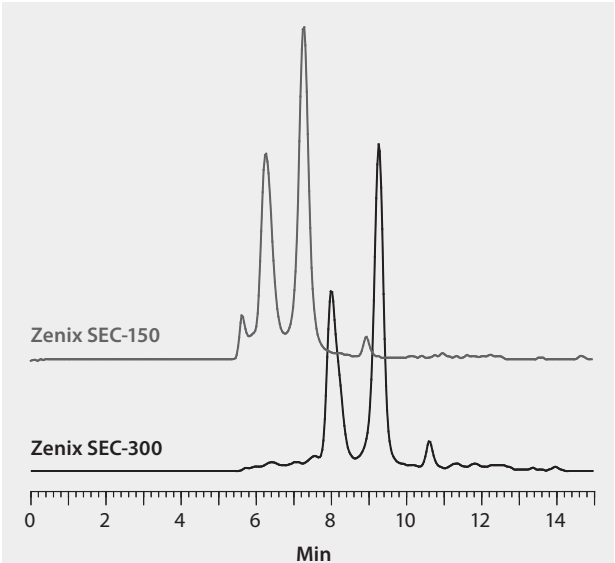
## HPLC Analysis of Immunoglobulin G (IgG) on Zenix® SEC-150 versus Zenix® SEC-300, Effect of Pore Size on Resolution

column . . Zenix SEC-150, 30 cm x 4.6 mm I.D., 3 µm, 150 Å particles (Z777016)
column . . Zenix SEC-300, 30 cm x 4.6 mm I.D., 3 µm, 300 Å particles (Z777028)
mobile phase . . . . . 150 mM sodium phosphate, pH 7.0
flow rate . . . . . 1 mL/min
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL, IgG
Application No. . . . . G006120

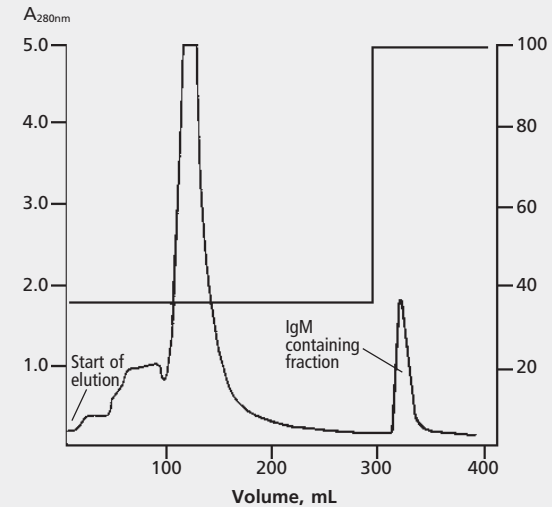





SUPELCO® Solutions within.™

## HPLC Analysis of Immunoglobulins (IgM) on Q Sepharose® Fast Flow

media . . . . . Q Sepharose Fast Flow (Q1126-100ML)
column . . . . . 30 cm × 50 mm I.D. (Q1126-100ML)
mobile phase . . . . . (A) 0.02 M $NaH_2PO_4$, pH 6.5; (B) A + 0.5 M NaCl, pH 6.5
flow rate . . . . . 25 cm/hr
detector . . . . . UV, 280 nm
injection . . . . . cell culture supernatant
Application No. . . . . 794-0370

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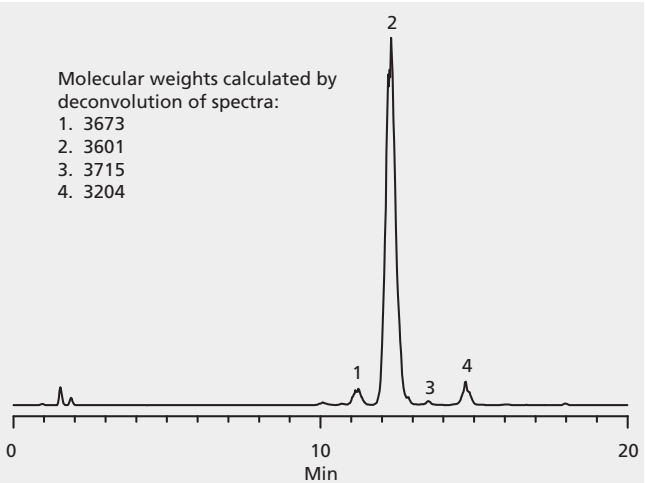
## HPLC Analysis of Inhibin α-Subunit Fragment 1-32 on Discovery® HS C18

column . . . . Discovery HS C18, 10 cm × 2.1 mm ID, 5 µm particles (568501-U)
mobile phase . . (A) 20 mM formic acid in water; (B) (20 mM formic acid in water): (20 mM formic acid in acetonitrile), 50:50
flow rate . . . . . 0.2 mL/min
injection . . . . . 2 µL
column temp. . . . . 22 °C
detector . . . . . ESI (+), full scan
sample . . . . . 20 mg/L in 0.1 % formic acid
Application No. . . . . G002581

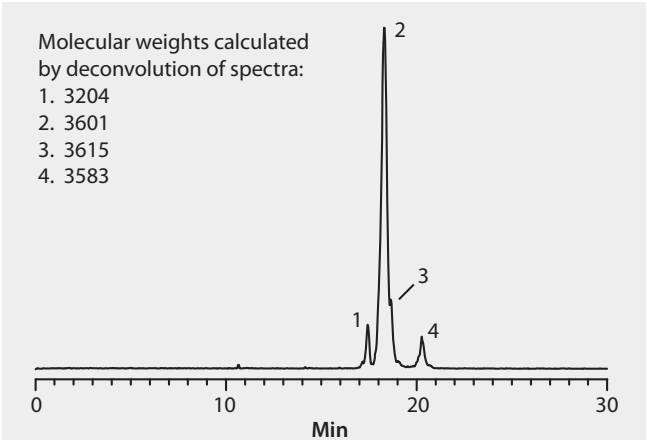





SUPELCO® Solutions within.™

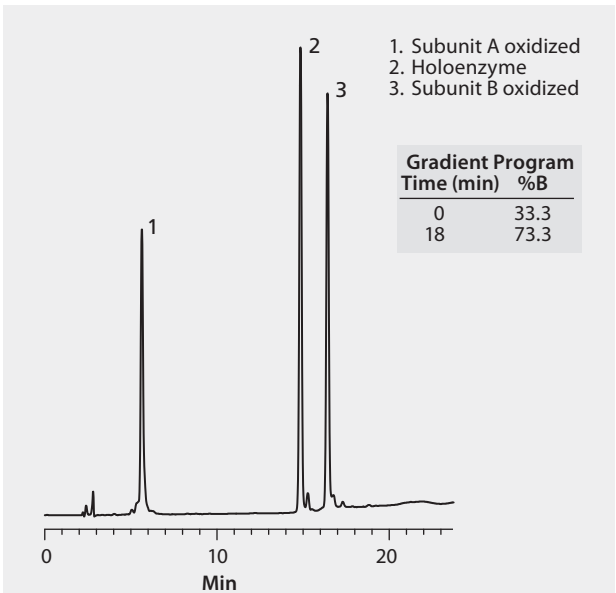
## HPLC Analysis of Inhibin α-Subunit Fragment 1-32 on Discovery® HS F5

column . . . . . Discovery HS F5, 10 cm × 2.1 mm ID, 5 µm particles (567510-U)
mobile phase . . (A) 80 mM formic acid, 40 mM ammonium hydrate (pH ~3.5) in water; (B) 80 mM formic acid, 40 mM ammonium hydrate in 80% acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 22 °C
detector . . . . . ESI (+), full scan
injection . . . . . 2 µL
sample . . . . . 50 mg/L in 0.1 % formic acid
Application No. . . . . G002580



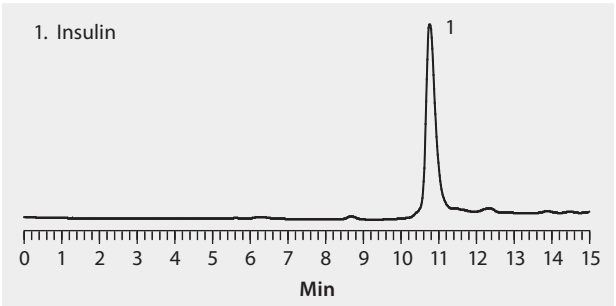
## HPLC Analysis of Insulin, Bovine on Discovery® C8

column . . . . . Discovery C8, 5 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . . . 50 mM sodium phosphate, monobasic, 50 mM sodium sulfate, pH 3.0 (w/phosphoric acid), (A) +5% acetonitrile, (B) +50% acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
Application No. . . . . G000765



## HPLC Analysis of Insulin on SRT®-C SEC-300

column . . . . . SRT-C SEC-300, 30 cm x 7.8 mm I.D., 5 µm particles (Z777110)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1.0 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . insulin
Application No. . . . . G006167



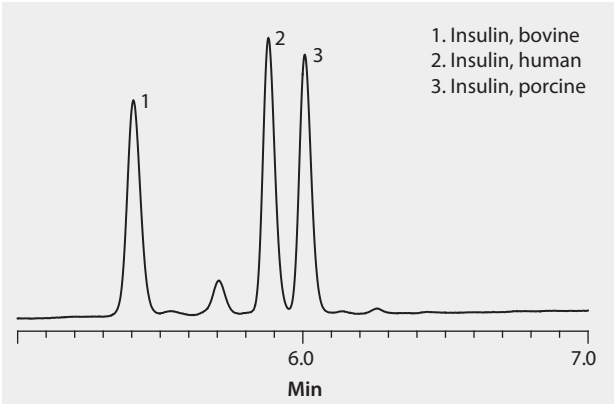



SUPELCO® Solutions within.™

## HPLC Analysis of Insulin (Species Variants) on Ascentis® Express Peptide ES C18

These three insulins have more than 76% identical positions in a primary structure alignment. The human and porcine proteins are most similar with 85% identity. Baseline resolution of these insulins in achieved on Ascentis Express Peptide ES-C18, with relative band spacing of these three peaks approximating their relative sequence similarities. Ascentis Express Peptide ES-C18 is ideally suited for RP chromatography of small proteins or large peptides.

column . . . . . Ascentis Express Peptide ES C18, 15 cm x 2.1 mm I.D., 2.7 µm particles (53307-U)
mobile phase . . . . (A) 70:30, water with 0.1% TFA:acetonitrile with 0.09% TFA; (B) 55:45, water with 0.1% TFA:acetonitrile with 0.09% TFA
gradient . . . . . 0 to 100% B in 7.5 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 4040 psi (278 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 215 nm
injection . . . . . 0.5 µL
sample . . . . . 200 µg/mL insulin (bovine, human, porcine) in 0.1% formic acid
Application No. . . . . G005794



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## HPLC Analysis of Insulin (Species Variants) on Discovery® BIO Wide Pore C5

column . . . . . Discovery BIO Wide Pore C5, 15 cm × 4.6 mm I.D., 5 µm particles (568422-U)

mobile phase . . . . . (A): 71:29, (0.1% TFA in water):(0.1% TFA in acetonitrile); (B): 68:32, (0.1% TFA in water):(0.1% TFA in acetonitrile)

gradient . . . . . 0-100% B in 30 min

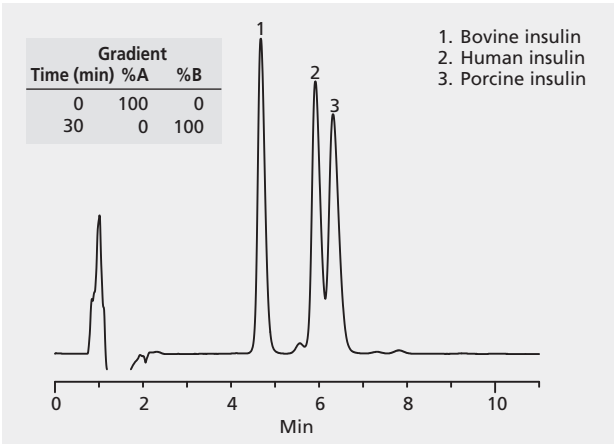
flow rate . . . . . 1 mL/min

column temp. . . . . ambient

detector . . . . . UV, 215 nm

injection . . . 5 µL 5 µL in 0.1% TFA containing 5ug each of three insulin variants

Application No. . . . . G001580

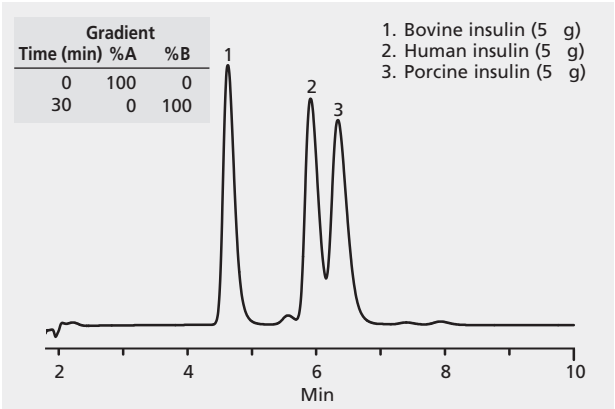





SUPELCO® Solutions within.™

## HPLC Analysis of Insulin (Species Variants) on Discovery® BIO Wide Pore C8

column . . . . . Discovery BIO Wide Pore C8, 15 cm × 4.6 mm I.D., 5 µm particles (568322-U)
mobile phase . . . . . (A) (0.1% TFA in water):(0.1% TFA in acetonitrile), 71:29 (B): (0.1% TFA in water):(0.1% TFA in acetonitrile), 68:32,
gradient . . . . . 0-100% B in 30 min
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 215 nm
injection . . . . . 5 µL in 0.1% TFA containing 5ug each of three insulin variants
Application No. . . . . G001507






SUPELCO® Solutions within.™

## HPLC Analysis of Insulin (Specirs Variants) on Ascentis® Express Phenyl-Hexyl

These three insulins have more than 76% identical positions in a primary structure alignment. The human and porcine proteins are most similar with 85% identity. The Phenyl-Hexyl phase provides an alternate retention and selectivity to a C18 phase, particularly the C18 phase on a larger pore silica, Ascentis Express ES-C18

column . . . . . Ascentis Express Phenyl-Hexyl, 15 cm x 2.1 mm, 2.7 µm particles (53338-U)

mobile phase . . . . (A) 70:30, water with 0.1% TFA:acetonitrile with 0.09% TFA; (B) 55:45, water with 0.1% TFA:acetonitrile with 0.09% TFA

gradient . . . . . 0 to 100% B in 7.5 min

flow rate . . . . . 0.3 mL/min

pressure . . . . . 3960 psi (273 bar)

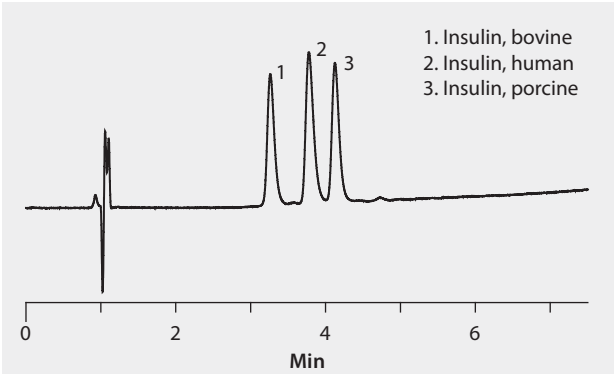
column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 0.5 µL

sample . . . . . 200 µg/mL in 0.1% formic acid

Application No. . . . . G005795

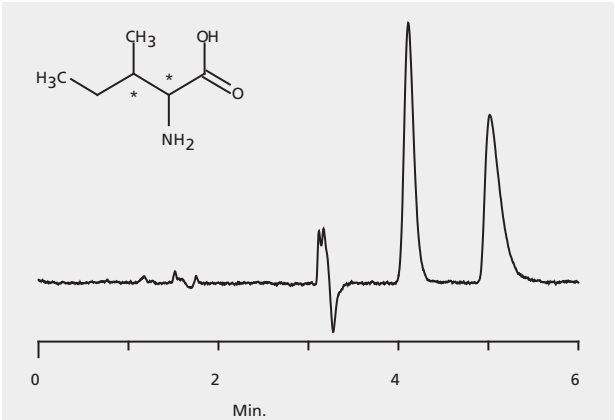





SUPELCO® Solutions within.™

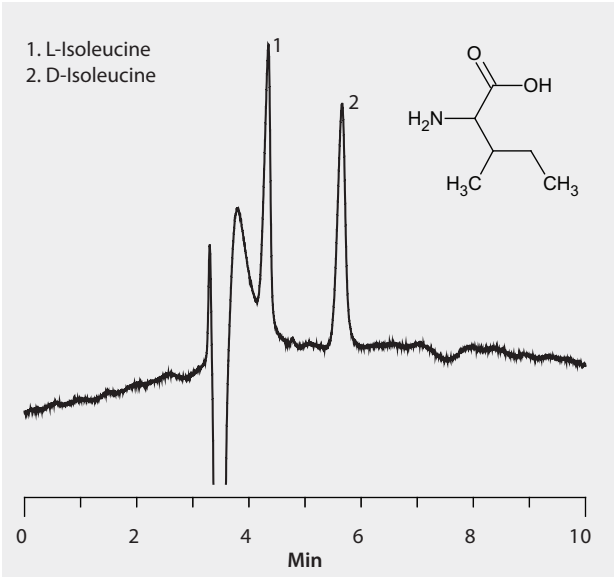
## HPLC Analysis of Isoleucine Enantiomers on Astec® CHIROBIOTIC® T (23 °C )

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004500



## HPLC Analysis of Isoleucine Enantiomers on Astec® CHIROBIOTIC® T (25 °C )

column . . Astec CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005338



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## HPLC Analysis of β-Lactoglobulin, Carboxymethylated, Tryptic Digest on Discovery® BIO Wide Pore C18

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 2.1 mm I.D., 3 µm particles (567202-U)

mobile phase . . . . . (A) water/0.1% TFA; (B) (water/0.1% TFA):(acetonitrile/0.1% TFA), 50:50

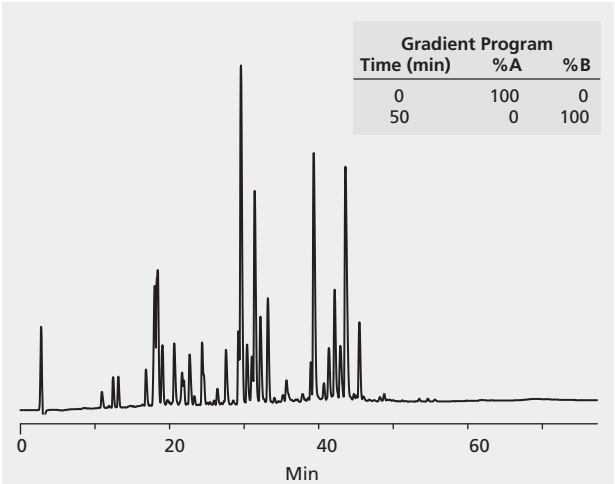
flow rate . . . . . 0.20 mL/min

column temp. . . . . 35 °C

detector . . . . . UV, 215 nm

injection . . . . . 5 µL

Application No. . . . . G001720

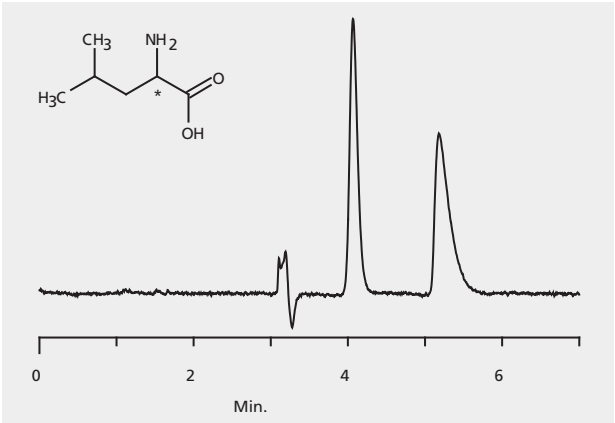





SUPELCO® Solutions within.™

## HPLC Analysis of Leucine Enantiomers on Astec® CHIROBIOTIC® T (23 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004501

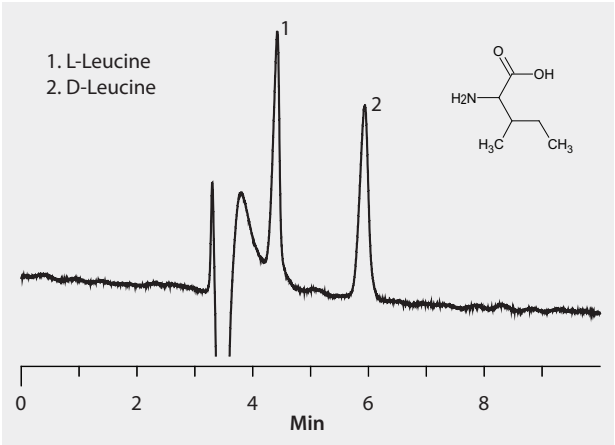





SUPELCO® Solutions within.™

## HPLC Analysis of Leucine Enantiomers on Astec® CHIROBIOTIC® T (25 °C)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005339



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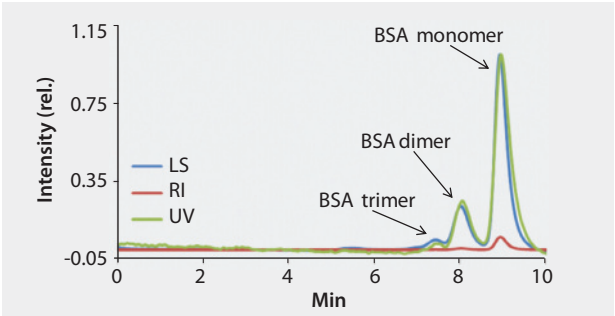
## HPLC Analysis of Light Scattering Detection of BSA on Zenix® SEC-300

The chromatogram shows an overlay of the ultraviolet (UV), differential refractive index (RI) and multi angle light scattering data (MALS) for a sample of bovine serum albumin; showing the prsenece of dimer and trimer as well as the monomer. The chromatogram illustrates the power of light scattering used with Sepax's Zenix® SEC in protein characterization. The light scattering data makes it possible to determine the molecular mass for the monomer, dimer and trimer.

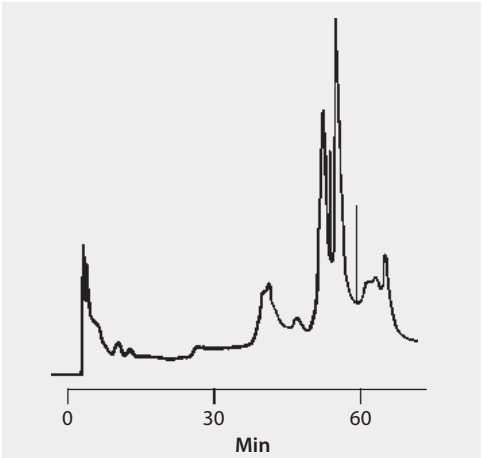
Acknowledgements and thanks to Matthew Thompson for this data.
Postdoctoral Researcher - Gianneschi Lab, Department of Chemistry & Biochemistry University of California, San Diego

column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles, 300 Å (Z777033)
mobile phase . . . . . 0.1M phosphate buffer
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . Hitachi-Elite LaChromL-2130 pump equipped with amulti-angle light scattering detector (DAWN-HELIOS: Wyatt Technology), a refractive index detector (Hitachi L-2490) and a UV-Vis detector (Hitachi 2420)
injection . . . . . 20 µL
sample . . . . . BSA (20mg/mL)
Application No. . . . . G006198



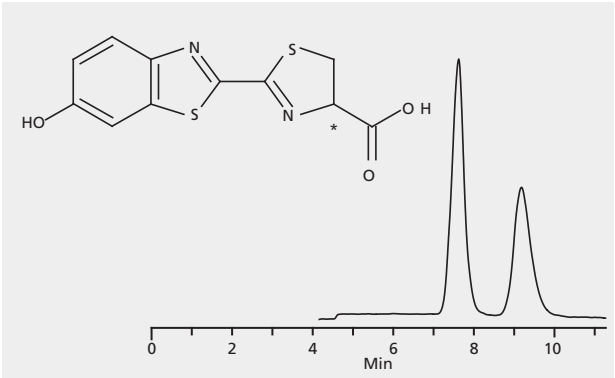
## HPLC Analysis of Lipoxidase on TSKgel® Phenyl-5PW

column . . . TSKgel Phenyl-5PW, 7.5 cm × 7.5 mm I.D., 10 µm particles (807573)
mobile phase . . . . . (A) 0.1M phosphate buffer, pH 7.0 + 1.5M $(NH_4)_2SO_4$;
(B) 0.1M phosphate buffer, pH 7.0;
gradient . . . . . linear, 0% B to 100% B over 60 min
flow rate . . . . . 0.5 mL/min
detector . . . . . UV, 280 nm
Application No. . . . . 794-0320



## HPLC Analysis of Luciferin Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM ammonium nitrate, pH 5.5; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 6 µL
sample . . . . . 0.5 mg/mL in methanol
Application No. . . . . G004641



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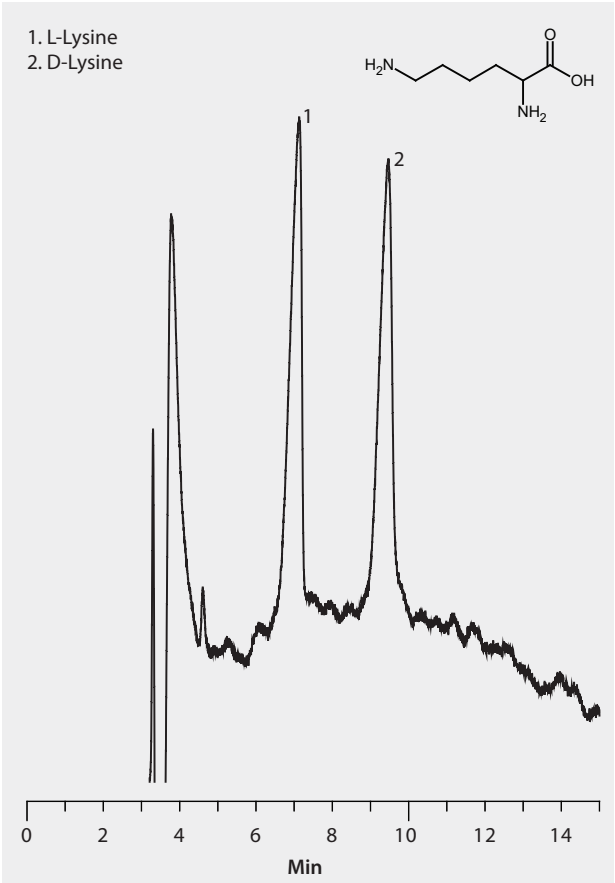
## HPLC Analysis of Lysine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005340

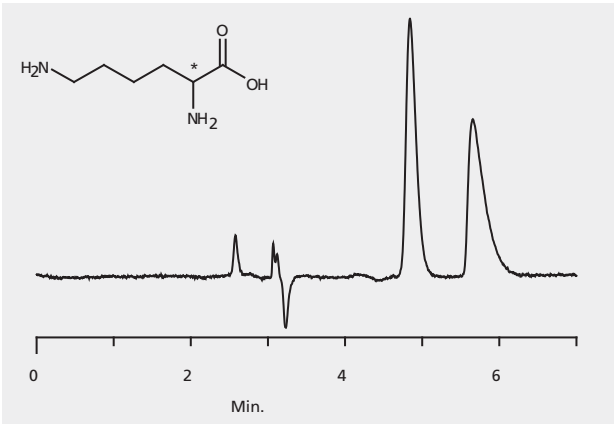





SUPELCO® Solutions within.™

## HPLC Analysis of Lysine Enantiomers on Astec® CHIROBIOTIC® T (Ethanol Mobile Phase)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 100 mM sodium phosphate, pH 4.5; (B) ethanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004502

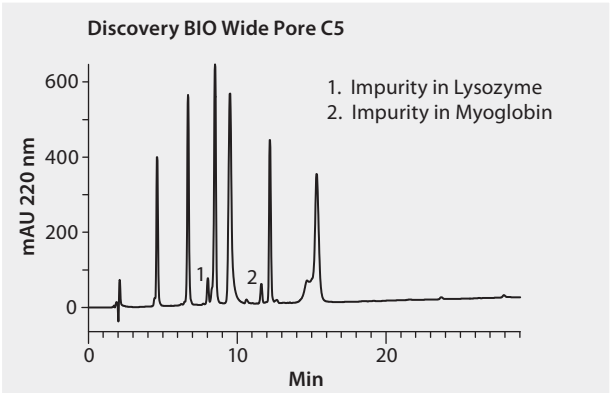





SUPELCO® Solutions within.™

## HPLC Analysis of Lysozyme, Myoglobin and Impurities on Discovery® BIO Wide Pore C5

column . . Discovery BIO Wide Pore C5, 15cm × 4.6mm I.D., 5µm particles (568422-U)
mobile phase . . . . . (A) (75:25) 0.1% TFA in water:0.1% TFA in acetonitrile;
(B) (25:75) 0.1% TFA in water:0.1% TFA in acetonitrile
gradient . . . . . 0-100% B in 25 min
flow rate . . . . . 1.0 mL/min
column temp. . . . . ambient
detector . . . . . UV, 220 nm
injection . . . . . 12 µL in 0.1% TFA
Application No. . . . . G001488A



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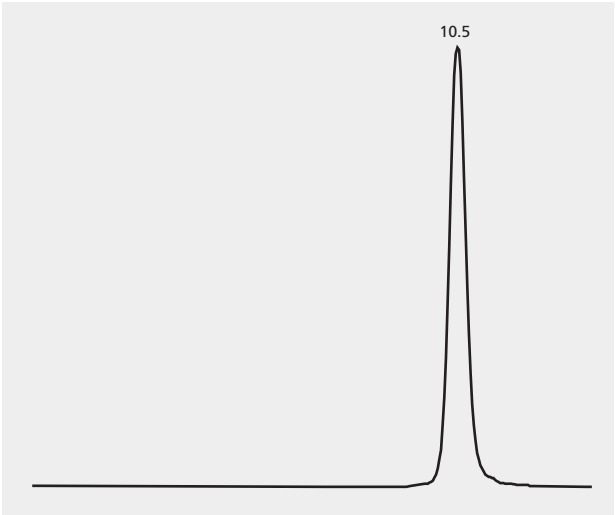
## HPLC Analysis of Lysozyme on SRT® SEC-300, 30 cm x 7.8 mm I.D., 5 µm particles

The positively-charged protein, lyzoyme (pI=11), exhibits excellent peak shape and recovery on Discovery BIO GFC columns due to its highly-inert surface. Low protein recovery is usually attributed to 1) hydrophobic interactions with the stationary phase and 2) electrostatic interactions with the silica suface. Discovery BIO GFC surface is hydrophilic and densely bonded to silica, therefore providing high mass recovery and good peak shape.

column . . . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm particles (Z777051)
mobile phase . . . . . 50 mM phosphate buffered saline, 200 mM $Na_2SO_4$, pH 7.0
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in 50 mM phosphate buffered saline
Application No. . . . . G004736

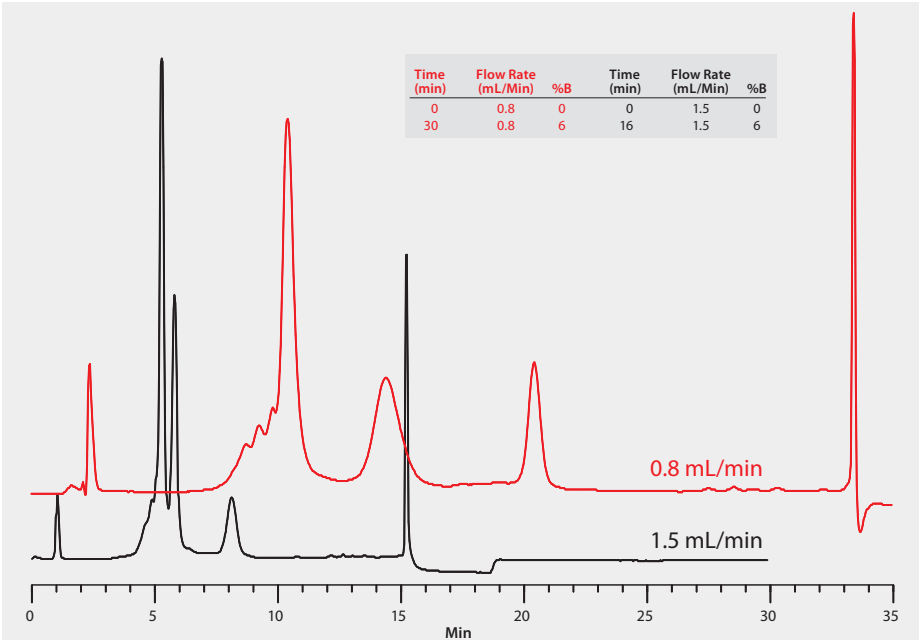


## HPLC Analysis of mAb 016 on Proteomix® SCX-NP5 Column at Different Flow Rates

This figure shows the how flow rate (F) affects analysis time and resolution during gradient analysis. When comparing the performance of different column formats or flow rates or gradient times, the rule of thumb is to keep the gradient volume, i.e. the product of gradient time and flow rate, constant. When gradient volume is kept constant, the compounds elute from the column in the same eluent composition.

Note that in this example the gradient time (tg) was reduced by the same factor as the increase in flow rate, thereby proportionaly increasing the gradient steepness, since gradient steepness is equal to the change in composition per unit time; in this case 6%B/16 vs. 6%B/30. It is now easy to see that the gradient volume was kept constant, since 30 min x 0.8 mL/min = 15 min x 1.6 mL/min = 24 mL. Clearly, the separation deteriorated substantially at the higher flow rate.

column . . Proteomix SCX-NP5, 25 cm x 4.6mm I.D., 5 µm particles, PEEK (Z777164)
mobile phase . . . . . (A) 20 mM phosphate buffer, pH 7.5 and (B) A + 1 M NaCl
flow rate . . . . . 0.8 mL/min
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 280 nm
sample . . . . . mAb 016 (11.8 mg/mL) 4 µL
Application No. . . . . G006173



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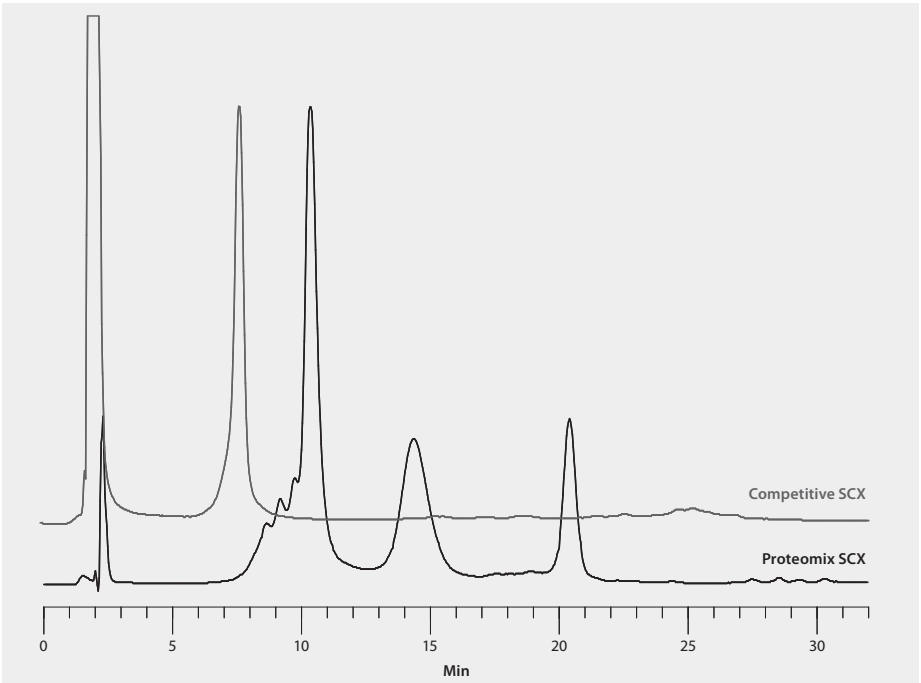
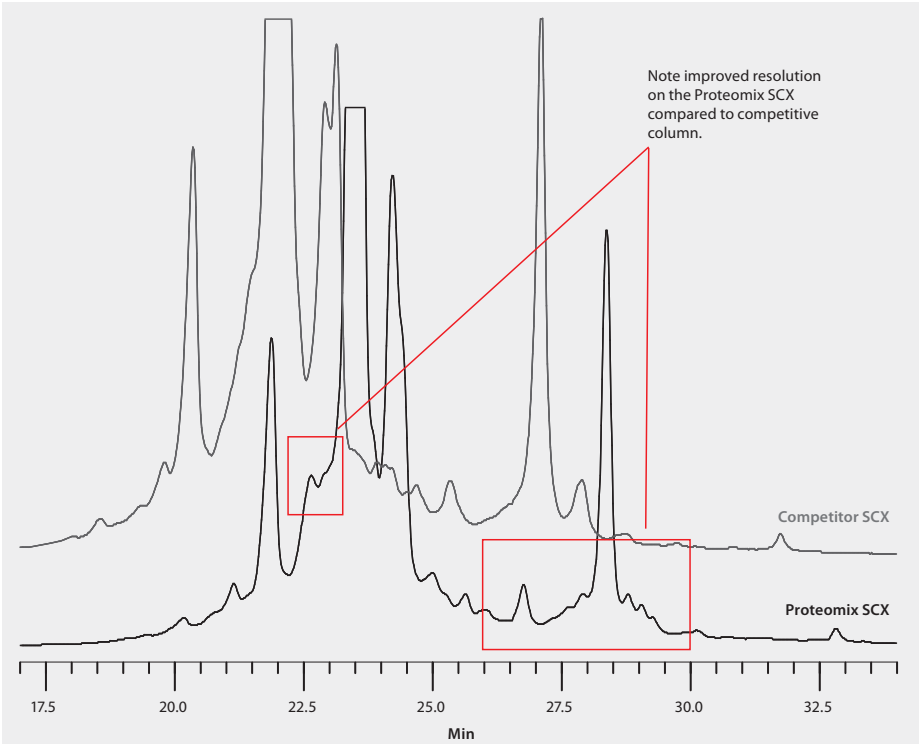
## HPLC Analysis of MAb 016 on Proteomix® SCX NP5 versus Competitive SCX using a Salt Gradient

column . . . . . Proteomix SCX-NP5, 25 cm x 4.6 mm I.D. (PEEK hardware), 5 µm particles (Z777164)
mobile phase . . . . . (A) 20 mM phosphate buffer, pH 7.5; (B) A + 1 M NaCl
gradient . . . . . 0 to 6% B in 30 min
flow rate . . . . . 0.8 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 4 µL
sample . . . . . MAb 016 (11.8 mg/mL)
Application No. . . . . G006107



## HPLC Analysis of MAb 321 on Proteomix® SCX NP5 versus Competitive SCX using pH and Salt Gradients

column . . . . . Proteomix SCX-NP5, 25 cm x 4.6 mm I.D. (PEEK hardware), 5 µm particles (Z777164)
mobile phase . . . . . (A) 2.4 mM Tris, 1.5 mM imidazole, 11.6 mM piperazine, pH 6.0; (B) A + 0.5 M NaCl, pH 10.5
flow rate . . . . . 0.8 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 20 µL
sample . . . . . MAb 321 (5 mg/mL)
Application No. . . . . G006098



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## HPLC Analysis of mAb Charge Variants on TSKgel® CM-STAT

A TSKgel CM-STAT weak cation exchange (WCX) column was applied to separate charge variants of several monoclonal antibodies. The typical analysis time on conventional 25 cm long WCX columns of about eighty minutes could be significantly reduced when separation was performed on a 10 cm TSKgel CM-STAT column, filled with 7 µm particles. The analysis profiles for five antibodies show that high resolution analysis can be obtained in about 20 minutes analysis time.

From "Ion Exchange Chromatography for the Characterization of Biotherapeutics" in The Supelco Reporter, Vol. 29.3, page 20.

column . . . . . TSKgel CM-STAT, 10 cm x 4.6 mm I.D., 7 µm particles (821966)

mobile phase . . (A) 20 mM MES buffer, pH 6.0; (B) 0.5 M NaCl in buffer A, pH 6.0

gradient . . . . . 0% B (0 min), 30% B (15 min), 100% B (15 min), 0% B (17 min), 10% B (17 min), 10% B (21 min)

flow rate . . . . . 1 mL/min

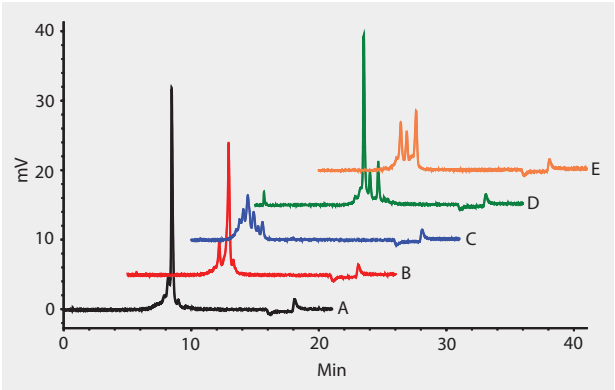
column temp. . . . . ambient

detector . . . . . UV, 280 nm

injection . . . . . 20 µL

sample . . . . . monoclonal antibodies (mAb A through E)

Application No. . . . . G005458



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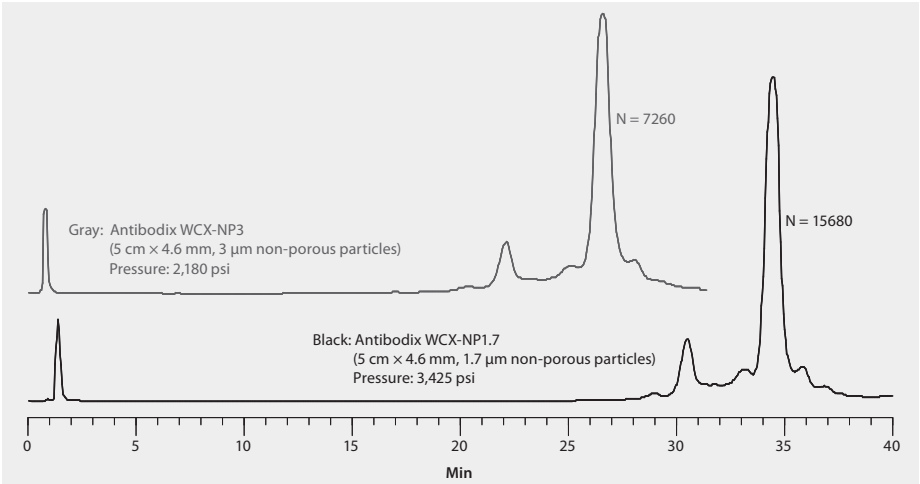
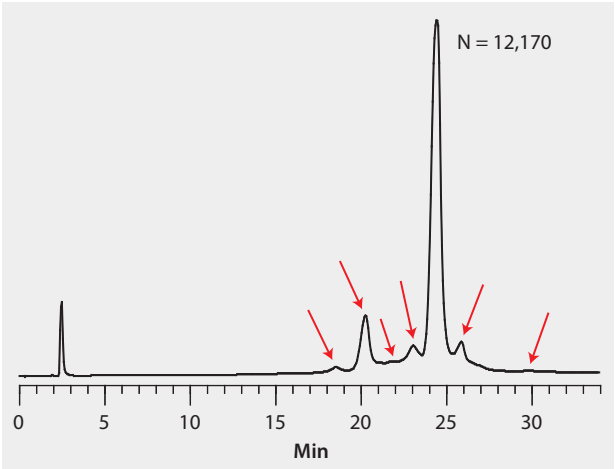
## HPLC Analysis of mAb on Antibodix® WCX, Effect of Particle Size (5 cm columns)

column . . Antibodix WCX-NP3 PEEK, 5 cm x 4.6 mm I.D., 3 µm particles (Z777288)
column . . . . . Z777280 (Antibodix WCX-NP1.7, 5 cm x 4.6 mm, 1.7 µm particles)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 7.5; (B) A + 100 mM NaCl
gradient . . . . . NP3 column: 15 to 55% B in 30 min, held for 3 min;
NP1.7 column: 15 to 100% B in 60 min, held for 1.7 min
flow rate . . . . . 0.5 mL/min for 3 min; 0.3 mL/min for 1.7 min
pressure . . . . . 2180 psi (3 min), 3425 psi (1.7 min)
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . Monoclonal antibody, 5 mg/mL
Application No. . . . . G005941



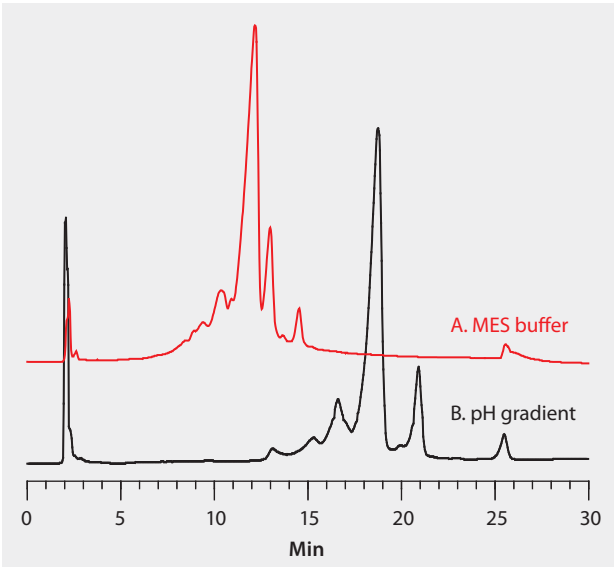
## HPLC Analysis of mAb on Antibodix® WCX-NP5, 25 cm Columns

column . . . . Antibodix WCX-NP5, 25 cm x 4.6 mm I.D., 5 µm particles (Z777294)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 7.5; (B) A + 100 mM NaCl
gradient . . . . . 15 to 55% B in 30 min
flow rate . . . . . 0.8 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . monoclonal antibody
Application No. . . . . G005942



## HPLC Analysis of MAb on Proteomix® SCX-NP5 Comparing Different Buffer Systems and pH Gradients

column . . . . Proteomix SCX-NP5, 25 cm x 4.6 mm I.D., 5 µm particles (Z777161)
mobile phase . . . . . Mobile phase A: (A) 20 mM MES, pH 6.0; (B) A + 1 M NaCl, pH 6.0Mobile phase B: (A) 2.4 mM Tris, 1.5 mM imidazole, 11.6 mM piperazine, pH 6.0; (B) A, pH 10.5
gradient . . . . . Gradient A: 7% B held for 2 min; to 14% B in 20 min, Gradient B: 0 to 30% B in 2 min; to 55% B in 30 min
flow rate . . . . . 0.8 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 5 µL
sample . . . . . 10 mg/mL
Application No. . . . . G006141



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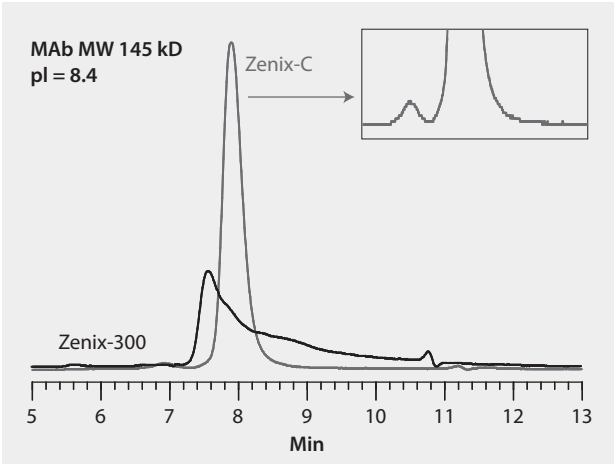
## HPLC Analysis of mAb F on Zenix®-300 and Zenix®-C 300

column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
mobile phase . . . . . 150 mM sodium phosphate, pH 7.0
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1073 psi (74 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1.23 mg/mL mAb F in 10 mM sodium succinate, pH 5.0
Application No. . . . . G005950



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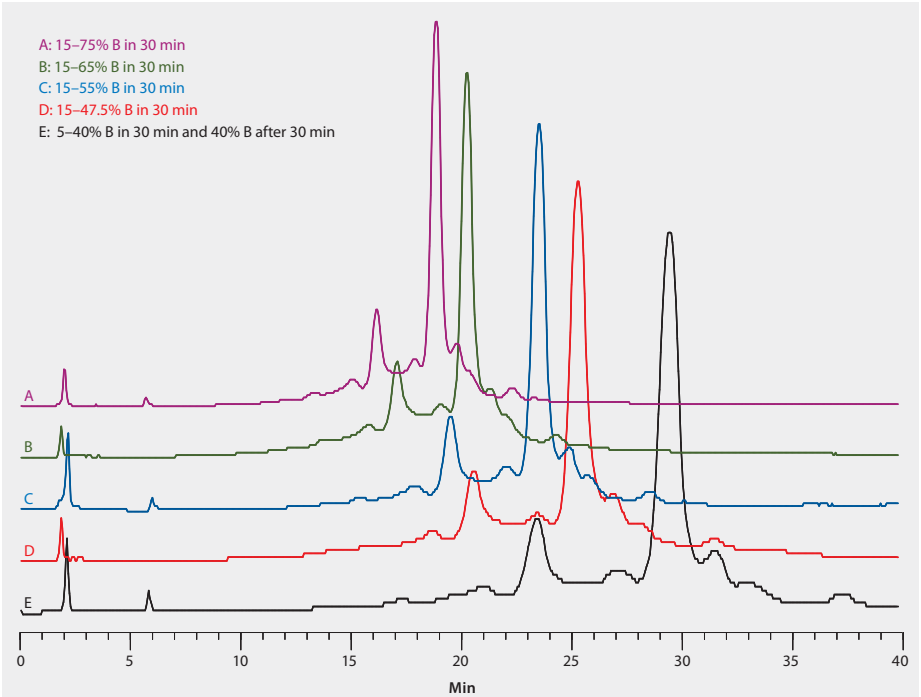
## HPLC Analysis of mAb-X22 on Antibodix® WCX-NP10, Effect of Gradient Profile

column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 7.5; (B), A + 100 mM NaCl
gradient . . . . . (A) 15-75% B in 30 min
(B) 15-65% B in 30 min
(C) 15-55% B in 30 min
(D) 15-47.5% B in 30 min
(E) 15-40% B in 30 min and 40% B after 30 min
flow rate . . . . . 0.8 mL/min
temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . MAb-X22, 1.5 mg/mL
Application No. . . . . G005943



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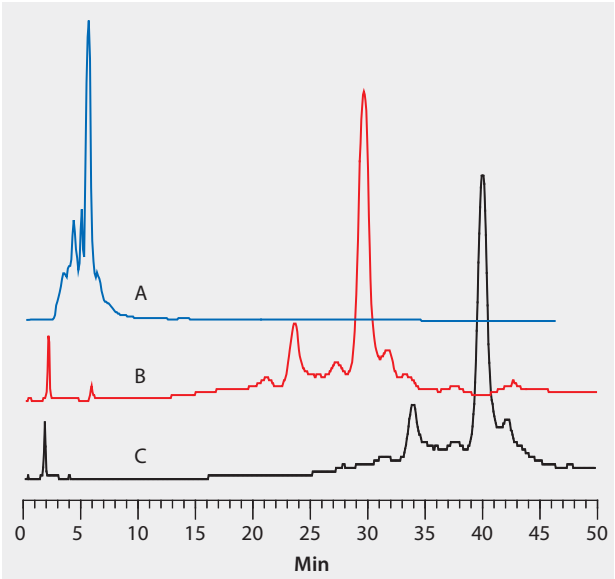
## HPLC Analysis of mAb-X22 on Antibodix® WCX-NP10, Impact of Initial Salt Concentration

column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 7.5; (B), A + 100 mM NaCl
gradient . . . . . 15 to 65% B in 60 min
flow rate . . . . . 0.8 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . MAb-X22, 1.5 mg/mL
Application No. . . . . G005944



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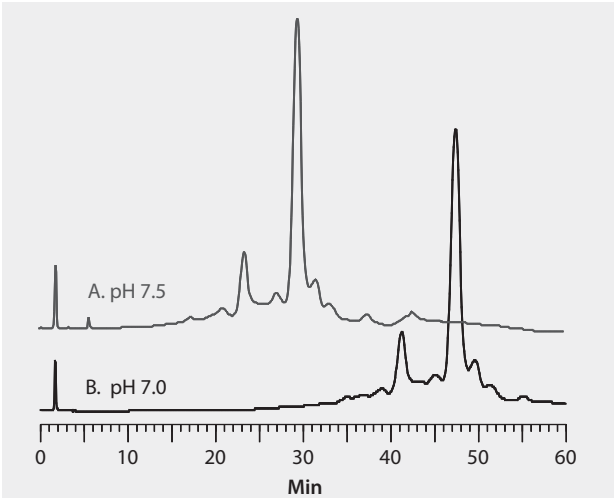
## HPLC Analysis of mAb-X22 on Antibodix® WCX-NP10, Impact of Mobile Phase pH

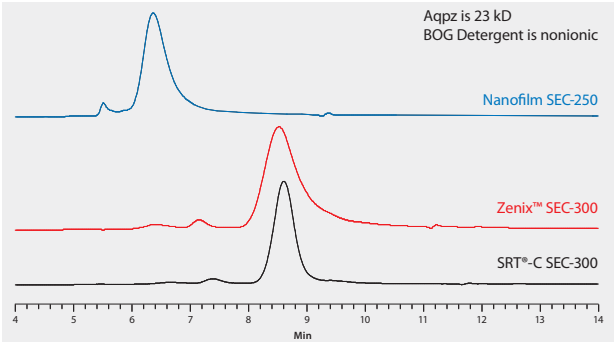
column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . . . . (A) 10 mM sodium phosphate buffer at 7.5 pH; (B), A + 100 mM NaCl at 7.0 pH
gradient . . . . . 15 to 65% B in 60 min
flow rate . . . . . 0.8 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . MAb-X22, 1.5 mg/mL
Application No. . . . . G005945



## HPLC Analysis of Membrane Protein Aquaporin-Z (AqpZ) on SRT® SEC-300, Octyl Glucoside Mobile Phase Additive

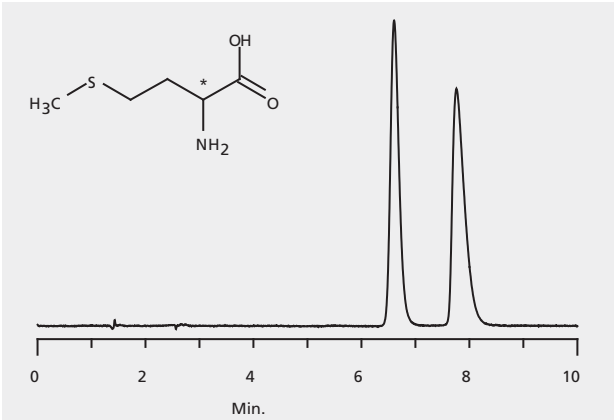
SRT SEC-300 gave the best separation of the three columns tested. It exhibited fewer secondary interactions and provided baseline separation of the high molecular weight proteins.

column . . . . . SRT-SEC 300, 30 cm x 7.8mm I. D., 5 µm particles (Z777110)
mobile phase . . . . . 20 mM tris-HCl pH 7.0, 190 mM NaCl, 10 mM KCl, 40 mM octyl glucoside
flow rate . . . . . 1.0 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 2 µL
sample . . . . . aqpZ, 6 mg/mL
Application No. . . . . G006169



## HPLC Analysis of Methionine Enantiomers on Astec® CHIROBIOTIC® T (No Additives)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004503



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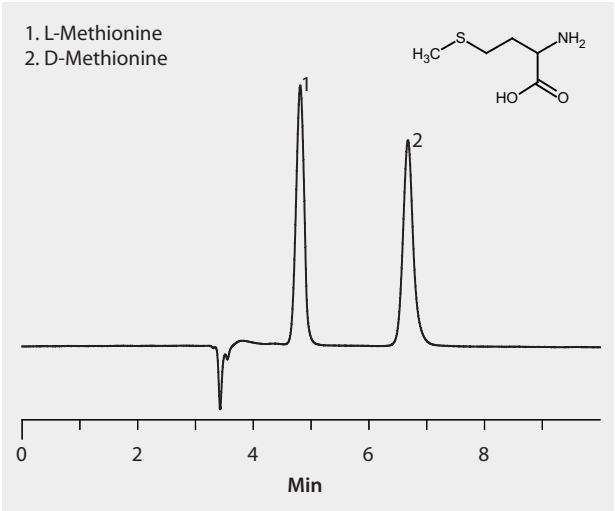
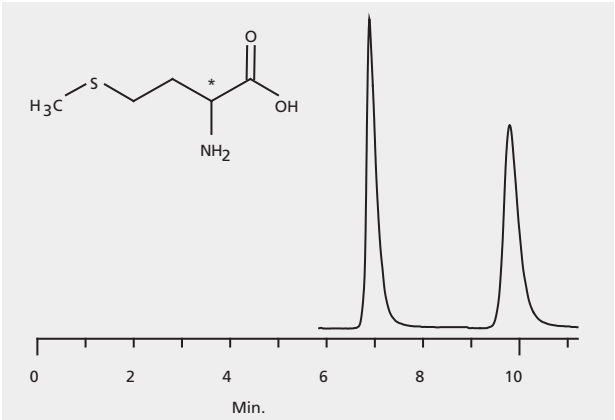
## HPLC Analysis of Methionine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005341



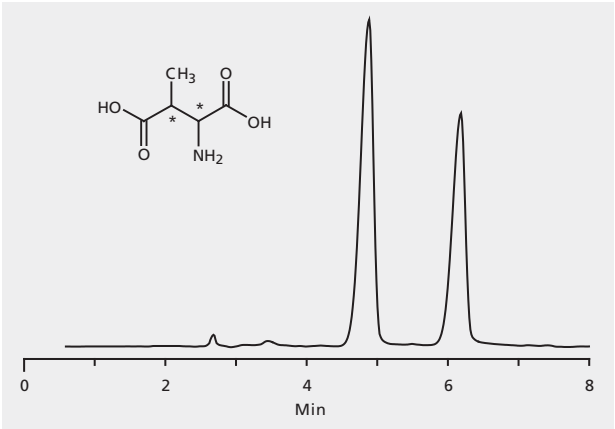
## HPLC Analysis of Methionine Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (25:75, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004598



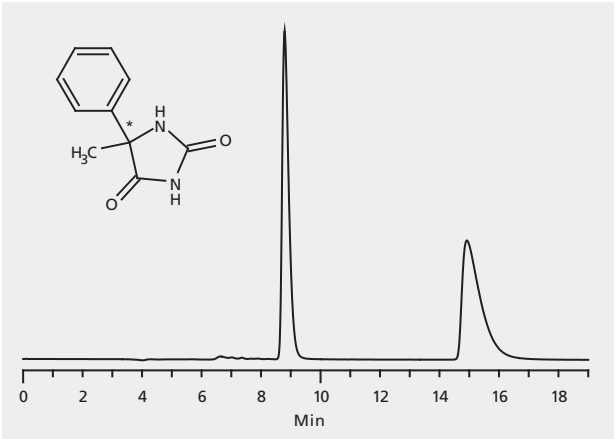
## HPLC Analysis of 3-Methylaspartic Acid Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) methanol; (B) water; (C) formic acid; (80:20:0.02, A:B:C)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . ELSD
injection . . . . . 4 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004553



## HPLC Analysis of 5-Methyl-5-Phenylhydantoin Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . methanol
flow rate . . . . . 0.48 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004525



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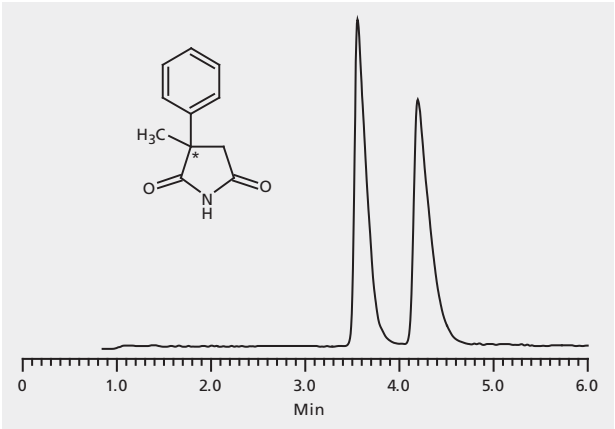
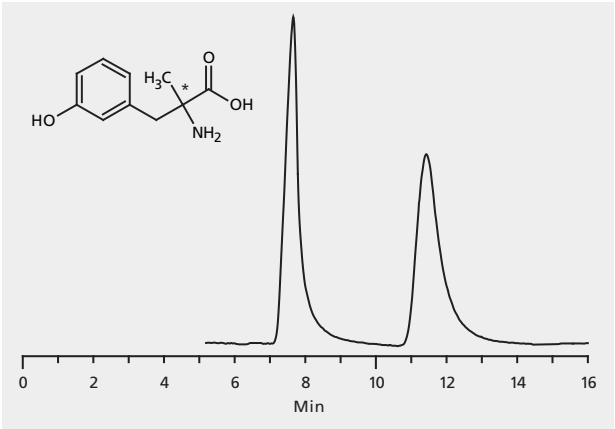
## HPLC Analysis of α-Methyl-α-Phenylsuccinimide Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) ethanol; (B) hexane; (20:80, A:B)
flow rate . . . . . 3 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004544



## HPLC Analysis of α-Methyl-m-Tyrosine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . methanol
extraction tube . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 1 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004557



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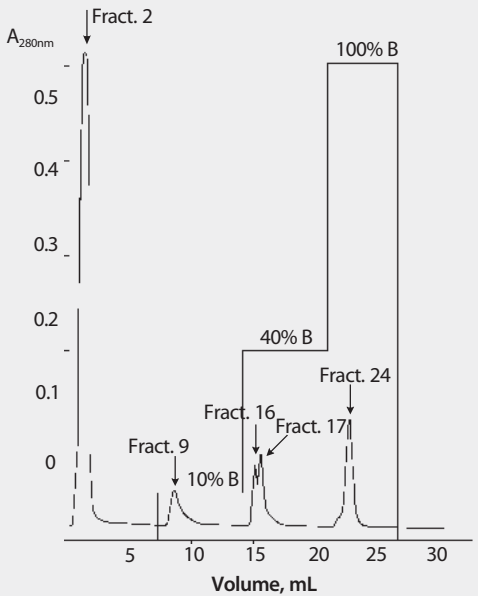
## HPLC Analysis of Milk Proteins on HiTrap® SP

column . . . . . HiTrap SP, 1 mL (54817)
mobile phase . . . . . (A) 50 mM sodium acetate, pH 6.0;
(B) 50 mM sodium acetate/1.0 M sodium chloride, pH 6.0
flow rate . . . . . 1 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 0.5 mL filtered casein-precipitated human milk
(buffer exchanged to mobile phase A)
Application No. . . . . 795-0493

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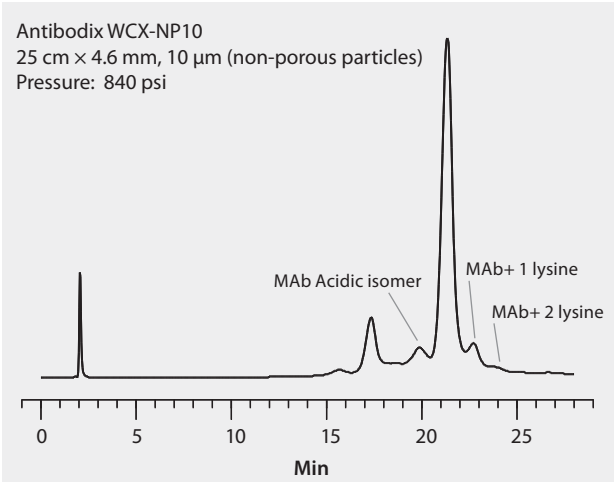
## HPLC Analysis of Monoclonal Antibodies (mAb) with Charge Variance on Antibodix® WCX-NP10

column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 7.5; (B) A + 100 mM NaCl
gradient . . . . . 15 to 55% B in 30 min
flow rate . . . . . 0.8 mL/min
pressure . . . . . 840 psi (58 bar)
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . monoclonal antibody
Application No. . . . . G005940






SUPELCO® Solutions within.™

## HPLC Analysis of Monoclonal Antibody Fragments on BIOshell™ A400 Protein C4

Digesting an antibody into a few large fragments facilitates a middle-down approach for subsequent structural characterization. The IdeS is a Streptococcal protease (commercialized as FabRICATOR) that cleaves IgG in the hinge region resulting in two fragments: F(ab')2 and scFc.

Monoclonal antibody SO57 (0.5 mg) was digested by IdeS protease according to Genovis (www.genovis.com) FragIT kit protocol. The digest was then diluted 1:5 with mobile phase A, before injection; final protein conc. <0.3 g/L.

Genovis FragIT kit is available from Sigma, outside the USA.

column . . . . . BIOshell A400 Protein C4, 10 cm x 2.1 mm I.D., 3.4 µm particles (66825-U)

mobile phase . . . . . (A) 80:20, [0.1% TFA in water]:[0.1% TFA in acetonitrile] ; (B) 50:50, [0.1% TFA in water]:[0.1% TFA in acetonitrile]

gradient . . . . . 30 to 70% B in 12 min

flow rate . . . . . 0.3 mL/min

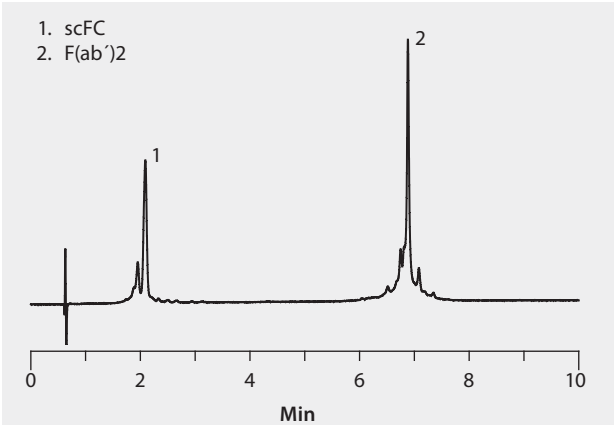
pressure . . . . . 1000 psi initial (69 bar initial)

column temp. . . . . 90 °C

detector . . . . . UV, 215 nm

injection . . . . . 1.0 µL

Application No. . . . . G006204

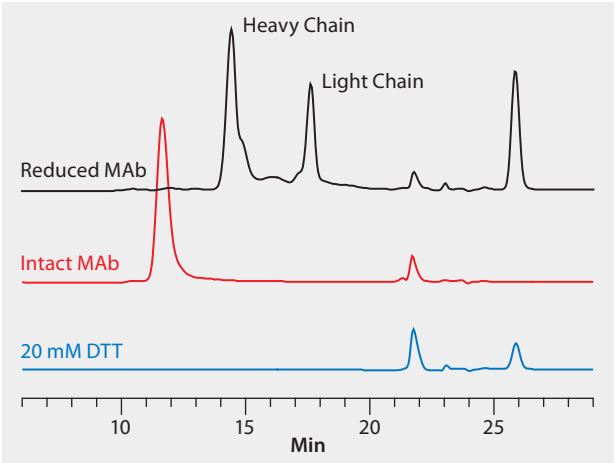





SUPELCO® Solutions within.™

## HPLC Analysis of Monoclonal Antibody Heavy and Light Chains on Zenix® SEC-300

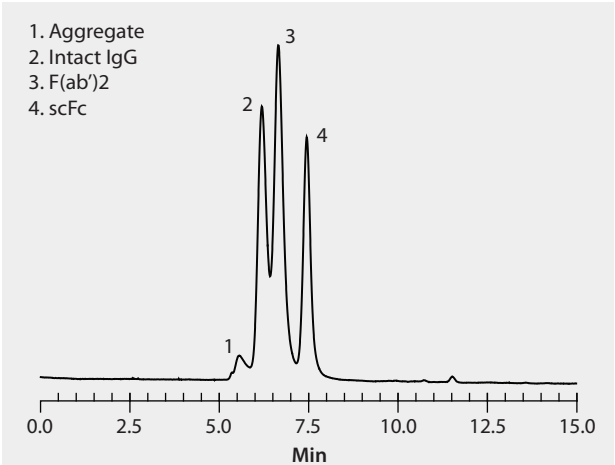
column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
mobile phase . . . . . 0.1% TFA, 0.1% formic acid, 20% acetonitrile
flow rate . . . . . 0.5 mL/min
detector . . . . . UV, 280nm
injection . . . . . 20 µL
sample . . . . . Monoclonal antibody (1 mg/mL) reduced with 20 mM DTT
Application No. . . . . G006171



## HPLC Analysis of a Monoclonal Antibody on Discovery® BIO GFC 150 after Partial Proteolysis with IdeS Protease

Proteolysis of antibodies can be useful for simplifying further analysis. This application demonstrates separation of the two fragments, F(ab')2 and scFc from the intact IgG, after partial digestion with IdeS protease.

column . . Discovery® BIO GFC 150, 30 cm X 4.6 mm I.D., 3 µm particles (567329-U)
mobile phase . . . . . 0.15 M potassium phosphate monobasic, pH 7.0 with potassium hydroxide (567329-U)
flow rate . . . . . 0.35 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . ambient
detector . . . . . UV, 215 nm
injection . . . . . 1 µL
sample . . . . . digested SO57 mAb, ~0.5 g/L
Application No. . . . . G005896



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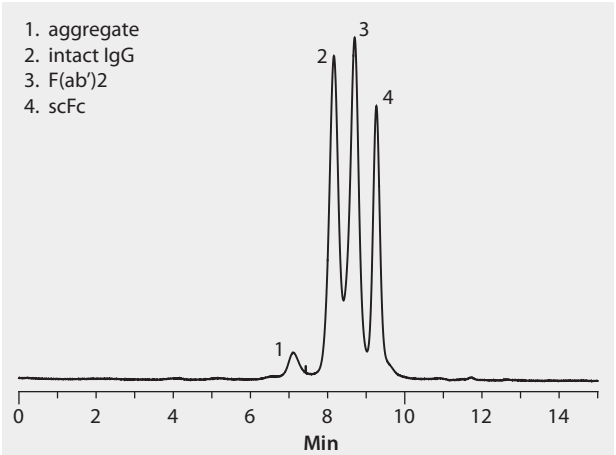
## HPLC Analysis of a Monoclonal Antibody on Discovery® BIO GFC 300 after Partial Proteolysis with IdeS Protease

Proteolysis of antibodies can be useful for simplifying further analysis. This application demonstrates separation of the two fragments, F(ab')2 and scFc from the intact IgG, after partial digestion with IdeS protease.

column . . Discovery® BIO GFC 300, 30 cm X 4.6 mm I.D., 3 µm particles (567335-U)
mobile phase . . . . . 0.15 M potassium phosphate monobasic, pH 7.0 with potassium hydroxide
flow rate . . . . . 0.35 mL/min
pressure . . . . . 1350 psi (93 bar)
column temp. . . . . ambient
detector . . . . . UV, 215 nm
injection . . . . . 1 µL
sample . . . . . digested SO57 mAb, ~0.5 g/L
Application No. . . . . G005899

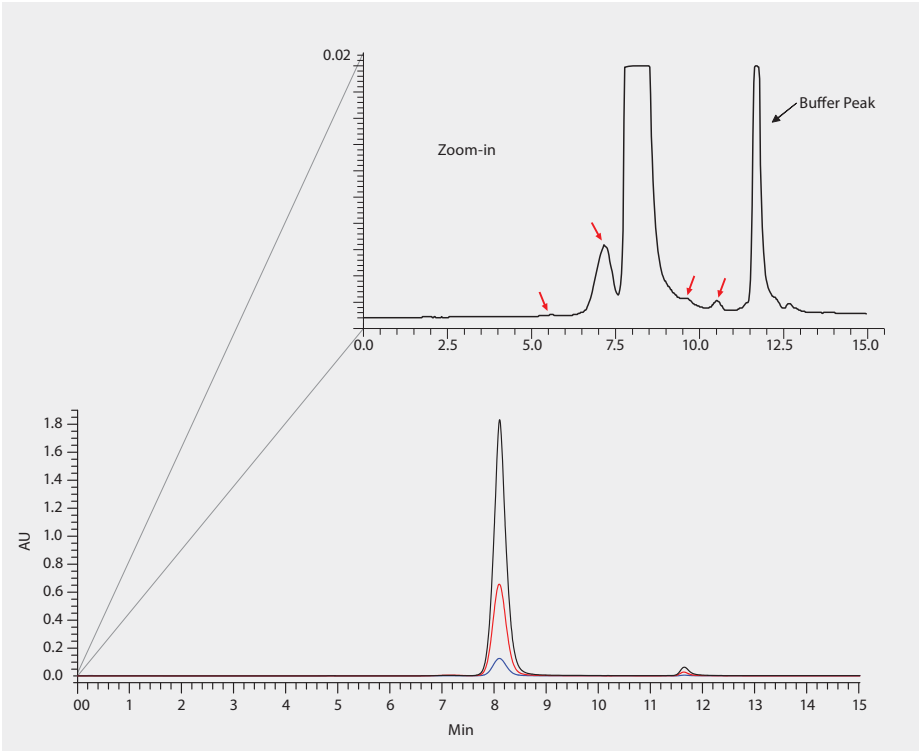





SUPELCO® Solutions within.™

## HPLC Analysis of a Monoclonal Antibody on SRT® SEC-300

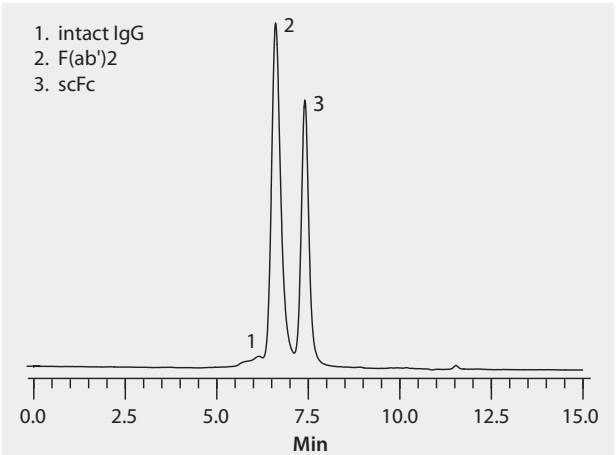
column . . . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm particles (Z777051)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1.0 mL/min
column temp. . . . . ambient
injection . . . . . 10 µL
sample . . . . . MAb 10, 2.5, 0.5 mg/mL
Application No. . . . . G006170



## HPLC Analysis of a Monoclonal Antibody on Zenix® SEC-150, 30 cm X 4.6 mm I.D., 3 µm particles after Proteolysis with IdeS Protease

Proteolysis of antibodies can be useful for simplifying further analysis. This application demonstrates separation of the two fragments, F(ab')2 and scFc, after digestion with IdeS protease

column . . . . . Zenix® SEC-150, 30 cm X 4.6 mm I.D., 3 µm particles (Z777018)
mobile phase . . . . . 0.15 M potassium phosphate monobasic, pH 7.0 with potassium hydroxide
flow rate . . . . . 0.35 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . ambient
detector . . . . . UV, 215 nm
injection . . . . . 1 µL
sample . . . . . digested SO57 mAb, ~0.5 g/L
Application No. . . . . G005889

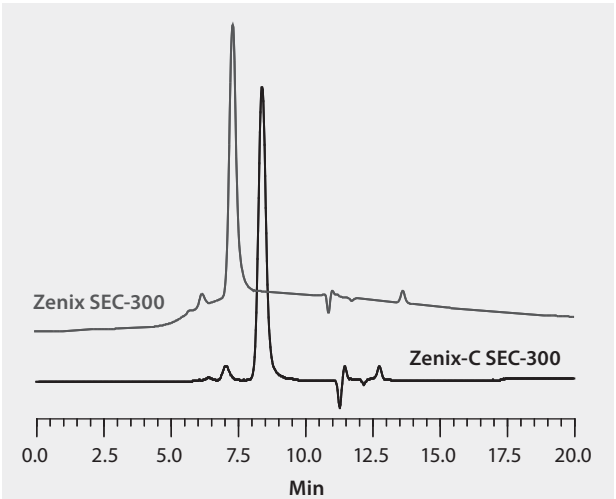





SUPELCO® Solutions within.™

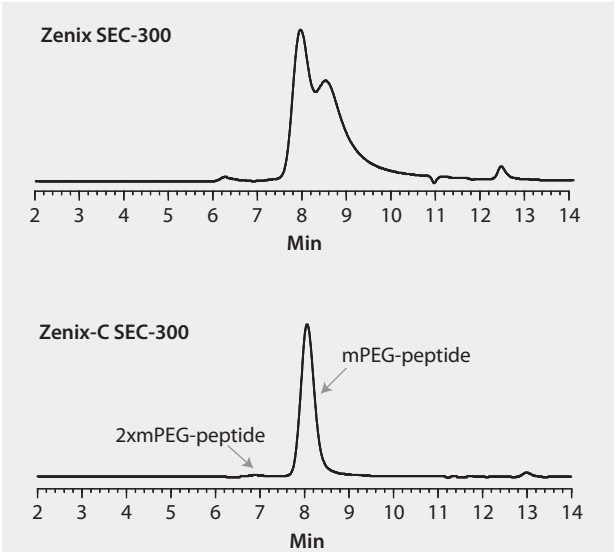
## HPLC Analysis of mPEG and mPEG-Peptide (Methoxy-PEG-Maleimide) on Zenix® and Zenix®-C SEC

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm, I.D., 3 µm particles (Z777092)
column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 20 µL
sample . . . . . 20kD peptide mPEG-MAL (methoxy-PEG-Maleimide), 1 mg/mL
Application No. . . . . G005948



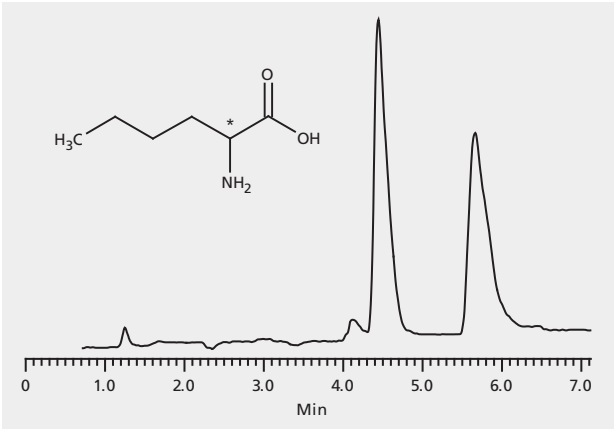
## HPLC Analysis of mPEG and mPEG-Peptide on Zenix® and Zenix®-C SEC

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm, I.D., 3 µm particles (Z777092)
column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 20 µL
sample . . 4kD peptide and 20kD mPEG-MAL(methoxy-PEG-Maleimide) (6 mg/mL)
Application No. . . . . G005947



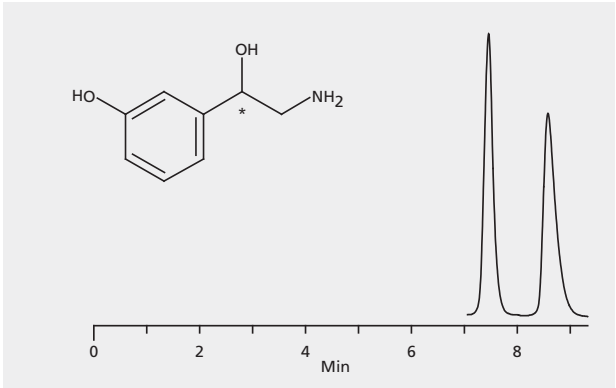
## HPLC Analysis of Norleucine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (80:20, A:B)
flow velocity . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004534



## HPLC Analysis of Norphenylephrine Enantiomers on Astec® CYCLOBOND® I 2000 AC (pH 4.1)

column . . CYCLOBOND I 2000 AC, 25 cm x 4.6 mm I.D., 5 µm particles (20124AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (93:7, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004693

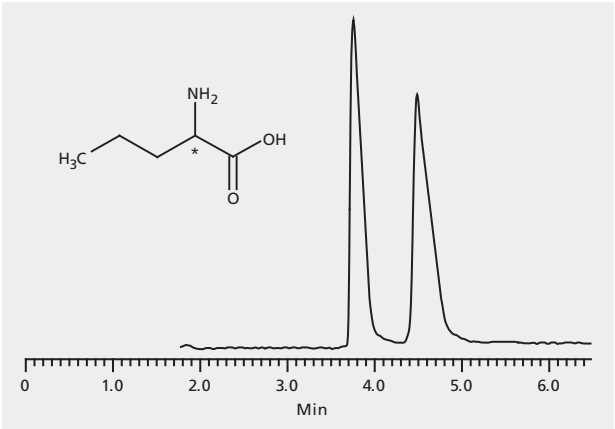





SUPELCO® Solutions within.™

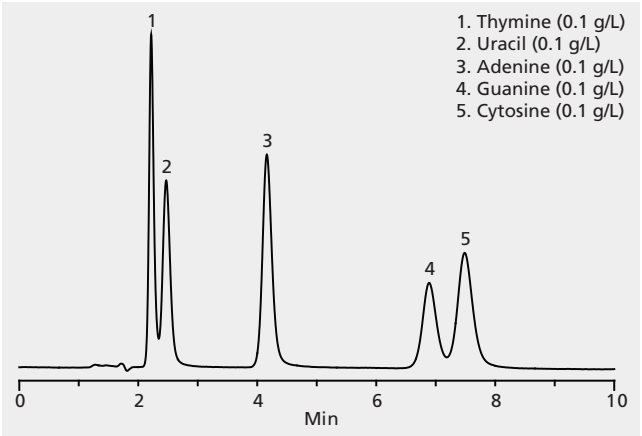
## HPLC Analysis of Norvaline Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004535



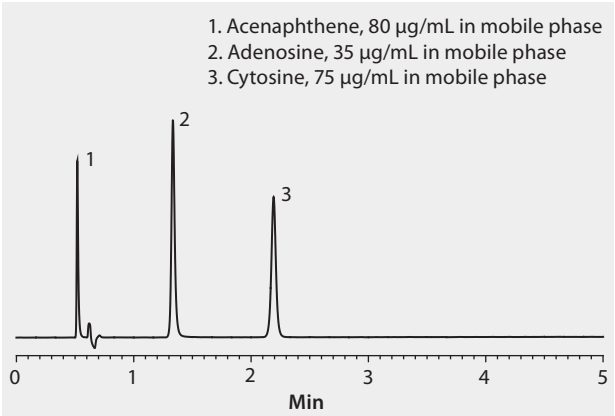
## HPLC Analysis of Nucleic Acid Bases on Ascentis® Si

column . . . . . Ascentis Si, 10 cm x 2.1 mm I.D., 5 µm particles (581508-U)
mobile phase . . . . . (A) 200 mM ammonium formate (pH 3.0, with formic acid): (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 270 nm
injection . . . . . 1 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003723



## HPLC Analysis of Nucleosides on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 10 cm × 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 100 mM ammonium formate, pH 3.0 with concentrated formic acid: (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
Application No. . . . . G004183






SUPELCO® Solutions within.™

## HPLC Analysis of Nucleosides on Ascentis® Express OH5 (HILIC Mode)

Nucleosides are involved in many aspects of metabolism. Analogs of nucleosides have medical application in antivirals or anticancer treatments. This application demonstrates the utility of hydrophilic interaction chromatography (HILIC) for the retention and resolution of nucleosides and analogs thereof. Elution is not solely explained by logD but by other ionic and solvophobic effects.

column . . Ascentis Express OH5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53757-U)

mobile phase . . . . . (A) 5 mM ammonium acetate, pH 5.0 with acetic acid in 95:5, acetonitrile:water; (B) 5 mM ammonium acetate, pH 5.0 with acetic acid in 80:20, acetonitrile:water

gradient . . 0% B held for 1 min; to 100% B in 10 min; held at 100% B for 1 min

flow rate . . . . . 0.3 mL/min

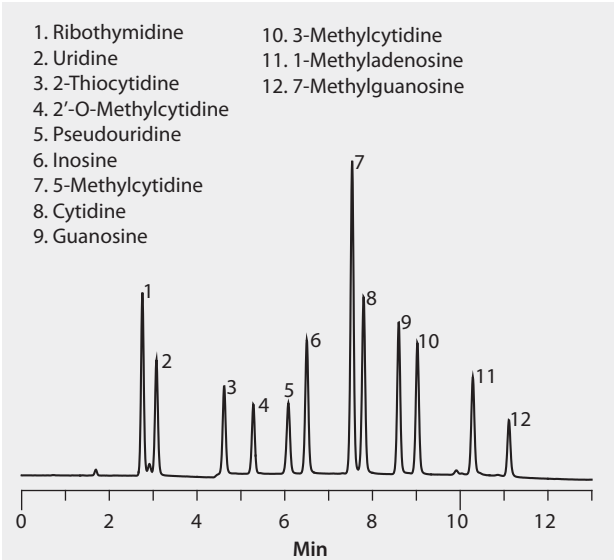
column temp. . . . . 25 °C

detector . . . . . UV, 250 nm

injection . . . . . 2 µL

sample . . 10 - 100 µg/mL in 95:5, acetonitrile:water (pH of buffer stock (in water) was adjusted before further dilution with water and/or acentonitrile)

Application No. . . . . G005644

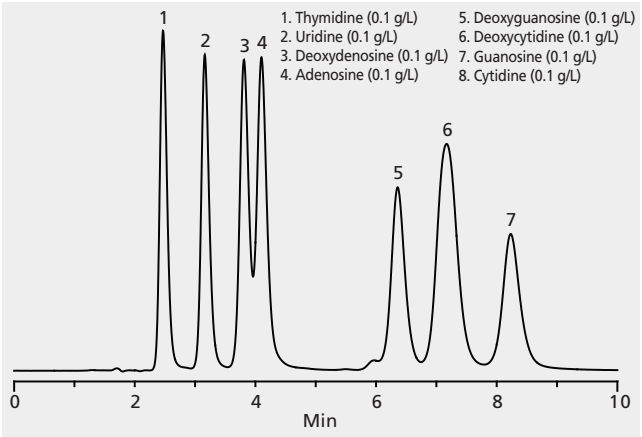





SUPELCO® Solutions within.™

## HPLC Analysis of Nucleosides on Ascentis® Si (HILIC Mode)

column . . . . . Ascentis Si, 10 cm x 2.1 mm I.D., 5 µm particles (581508-U)
mobile phase . . . . . (A) 150 mM ammonium formate (pH 3.0, with formic acid): (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 270 nm
injection . . . . . 1 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003721

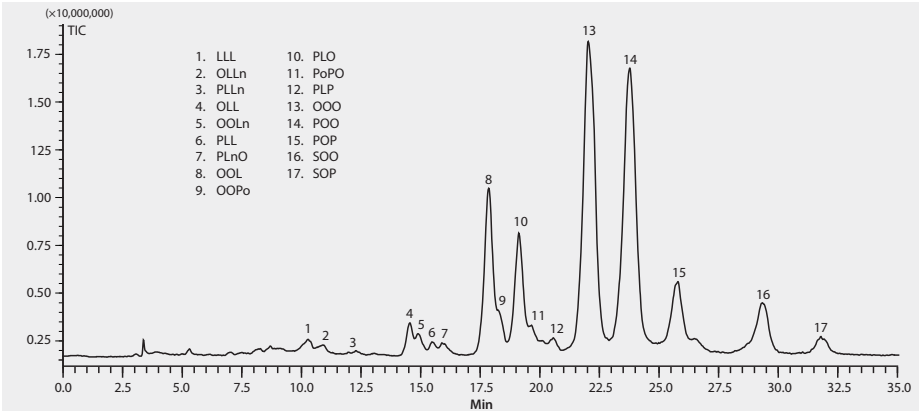





SUPELCO® Solutions within.™

## HPLC Analysis of Nucleosides on Ascentis® Si (Silica)

column . . . . . Ascentis Si, 10 cm × 2.1 mm I.D., 5 µm particles (581508-U)
mobile phase . . . . . (A) 150 mM ammonium formate (pH 3.0, with formic acid): (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 270 nm
injection . . . . . 1 µL
sample . . . . . 100 µg/mL in mobile phase
Application No. . . . . G003680

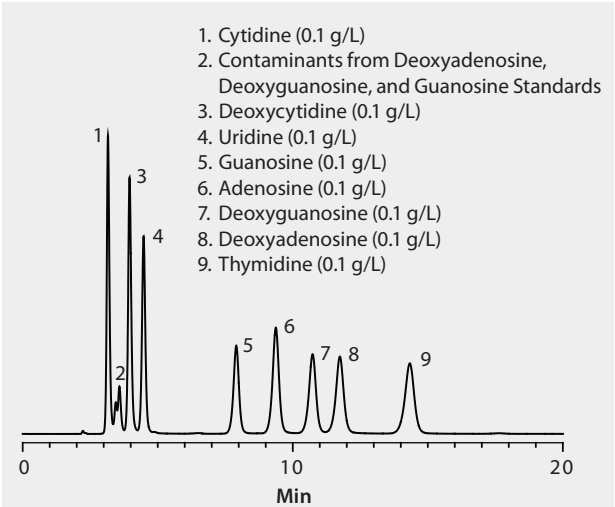





SUPELCO® Solutions within.™

## HPLC Analysis of Nucleosides on Ascentis® Phenyl

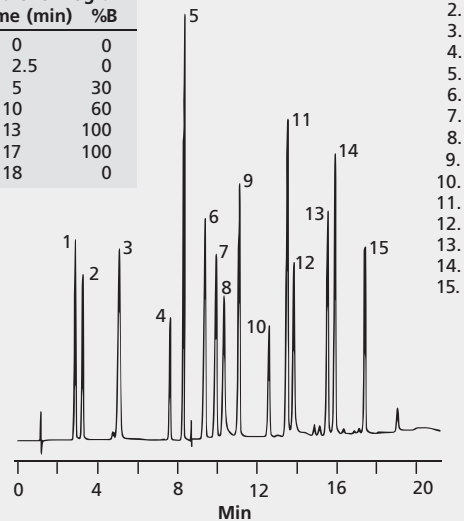
column . . . . . Ascentis Phenyl, 15 cm x 2.1 mm I.D., 5 µm particles (581613-U)
mobile phase . . . . . 10 mM ammonium formate (pH 3.0 with formic acid)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . UV, 270 nm; 750 psi back pressure regulator on outlet of flow cell
injection . . . . . 1 µL
sample . . . . . as indicated, in mobile phase
Application No. . . . . G003710



## HPLC Analysis of Nucleotides on SUPELCOSIL™ LC-18-T

column . . . SUPELCOSIL LC-18-T, 15 cm × 4.6 mm I.D., 3 µm particles (58970-U)
mobile phase . . (A) 0.1M potassium phosphate buffer/4mM tertrabutylammonium hydrogen sulfate, pH6.0; (B) A:methanol (70:30), pH 7.2
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 254 nm
injection . . . . . 20 µL deionized water (25-530 µM each analyte)
Application No. . . . . 713-0951

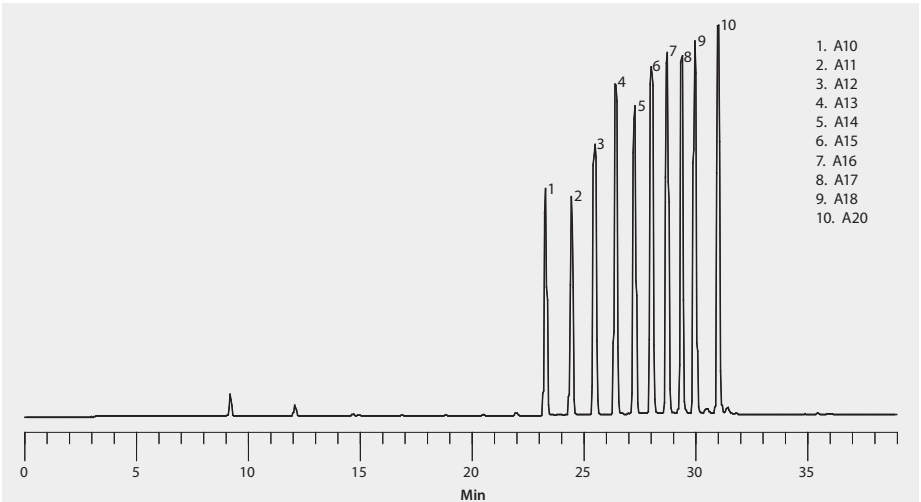
| Gradient Program | |
|---|---|
| Time (min) | %B |
| 0 | 0 |
| 2.5 | 0 |
| 5 | 30 |
| 10 | 60 |
| 13 | 100 |
| 17 | 100 |
| 18 | 0 |




1. HX
2. XAN
3. INO
4. IMP
5. $NAD^+$
6. ADO
7. AMP
8. GDP
9. $NADP^+$
10. ADP-rib
11. GTP
12. ADP
13. NADH
14. ATP
15. NADPH

## HPLC Analysis of Oligonucleotides on Proteomix® SAX

column . . . . Proteomix SAX-NP5, 25 cm x 4.6 mm I.D., 5 µm particles (Z777229)
mobile phase . . . . . A: 20 mM Tris, pH 8.0 B: A + 0.5 M NaCl
gradient . . . . . 0 - 100% B in 30 minutes
flow rate . . . . . 0.5 mL/min
pressure . . . . . 1073 psi (74 bar)
column temp. . . . . 25 °C
detector . . . . . UV 260 nm
injection . . . . . 5 µL
sample . . . . . (10 mM each oligonucleotide in water)
Application No. . . . . G005994

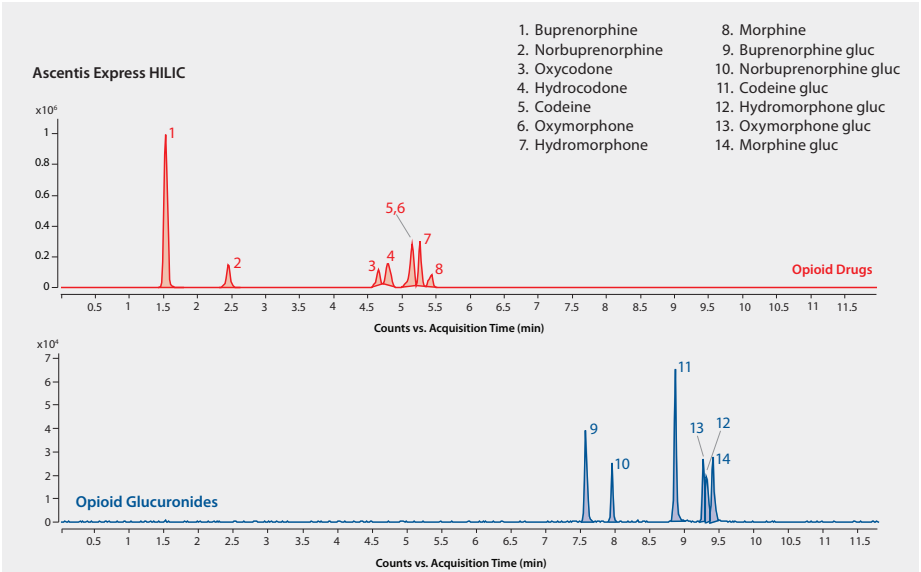





SUPELCO® Solutions within.™

## HPLC Analysis of Opioid Glucuronide Metabolites on Ascentis® Express HILIC

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . urine
SPE well plate . . . . . Supel™I-Select HLB 96-Well 60mg/Well (575662-U)
condition . . . . . 1 mL methanol, 1 mL water
sample addition . . . . . 1.0 mL urine
washing . . . . . 1 mL water, 1 mL 25% methanol
elution . . . . . 1 mL acetonitrile
column . . . Ascentis Express HILIC, 10 cm x 2.1 mm x 2.7 µm particles (53939-U)
mobile phase . . . . . (A): 5 mM ammonium formate pH 4.2
(B): 5 mM ammonium formate (95:5 acetonitrile:water) pH 5.2
gradient . . 100% B for 3 minutes, 80% B in 7 minutes, hold 80%B for 2 minutes
flow rate . . . . . 0.6 mL/min
detector . . . . . 55
detector . . . . . TOF MS
injection . . . . . 3.0 µL
sample . . . . . 300 ng/mL
Application No. . . . . G006290

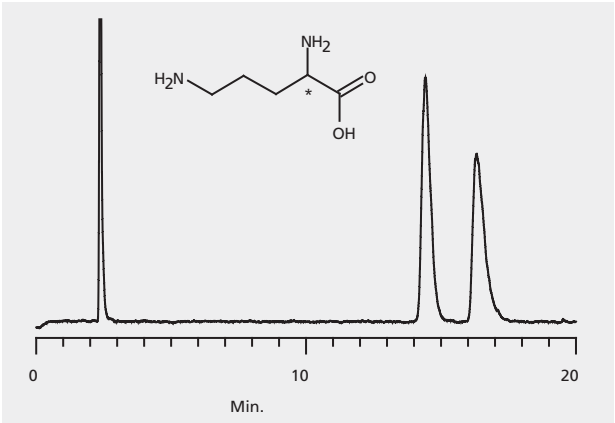





SUPELCO® Solutions within.™

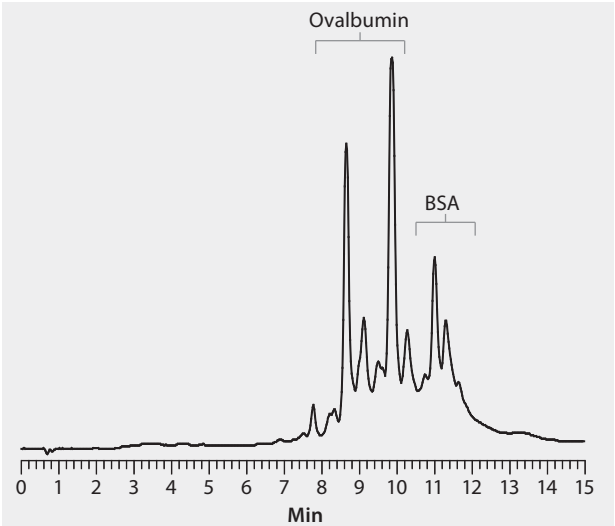
## HPLC Analysis of Ornithine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004506



## HPLC Analysis of Ovalbumin and BSA Charge Variants on Proteomix® SAX-NP3

column . . . . Proteomix SAX-NP3, 5 cm x 4.6 mm I.D., 3 µm particles (Z777219)
mobile phase . . . . . 20 mM Tris buffer, pH 8.0
gradient . . . . . 0 - 0.3 M NaCl in 15 min
flow rate . . . . . 0.5 mL/min
pressure . . . . . 1600 psi (110 bar)
detector . . . . . UV, 214 nm
Application No. . . . . G006132

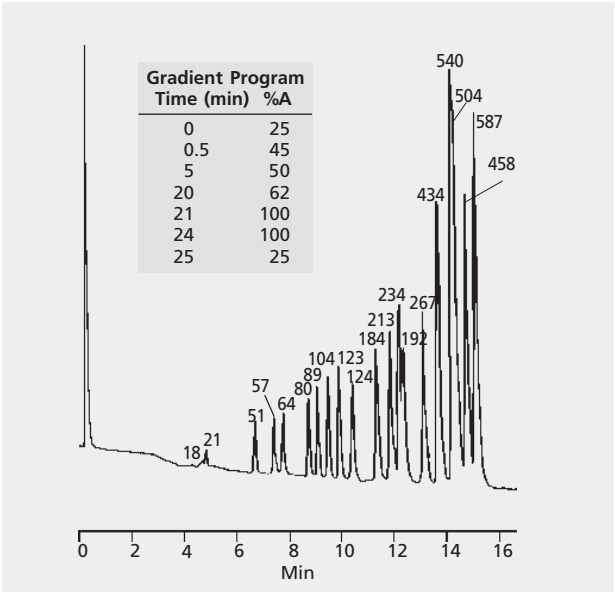





SUPELCO® Solutions within.™

## HPLC Analysis of pBr322 DNA-*Hae* III Digest on TSKgel® DEAE-NPR

column . . . . . TSKgel DEAE-NPR, 3.5 cm × 4.6 mm I.D., 2.5 µm particles (with 5 mm DEAE-NPR guard column) (813075)
mobile phase . . (A) 1 M NaCl/25 mm Tris-HCl, pH 9.0; (B) 25 mM Tris-HCl, pH 9.0
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 260 nm
injection . . . . . 20 µL of 50 µg/mL *Hae* III digest of pBR322 DNA
Application No. . . . . 794-0036



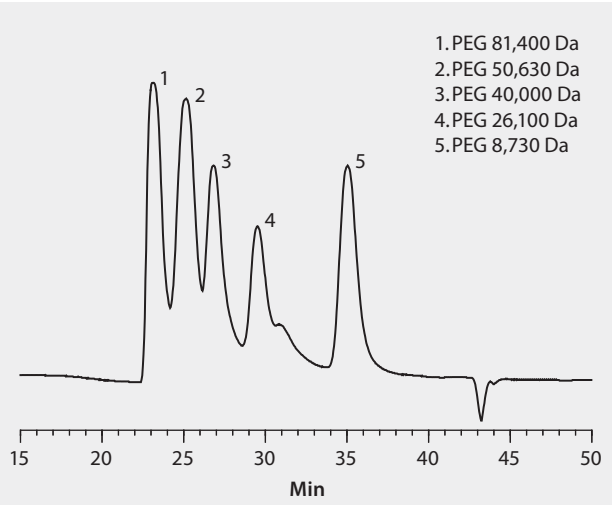

**Ref:** 1. Elena D. Katz, Lawrence A. Haff, Roy Eksteen, Rapid separation, quantitation, and purification of products of polymerase chain reaction by liquid chromatography *J. Chromatogr. A.* **512**, 433-444 (1990)

## HPLC Analysis of PEG separation on two Zenix® SEC-300 columns in Tandem

Pegylation of biotherapeutic drugs improves solubility, increases half life in the blood stream and reduces immunogenicity. Size exclusion chromatography (SEC) is often applied to characterize protein–polyethylene glycol (PEG) conjugates and establish the number of attached PEG chains, since SEC is a precise and robust technique that combines ease of set up and strainghtforward method develpopment.

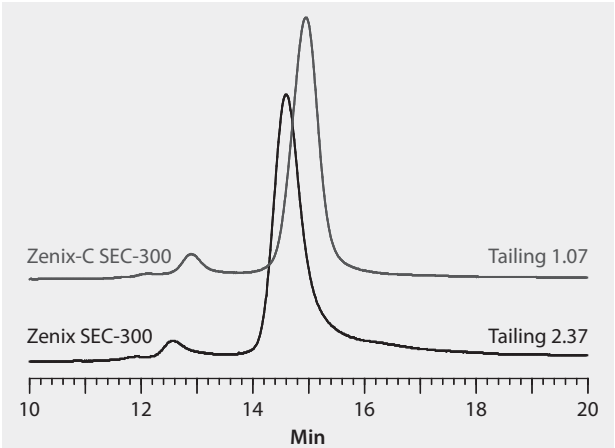
This chromatogram shows the separation of four polyethyleneglycol polymers varying in molecular mass 8730 to 81,400 Da on two Zenix® SEC-300 columns in series.

| | |
|---|---|
| column | Zenix SEC-300 (2 ea), 30 cm x 7.8 mm I.D., 3 µm partcles, 300 Å, connected in tandem (Z777033) |
| mobile phase | 150 mM Sodium Phosphate Buffer pH=7.0 |
| flow rate | 0.5 mL/min |
| column temp. | 25 °C |
| detector | Refractive Index at 30 °C |
| injection | 10 µL |
| sample | 1.PEG 81,400 Da (2mg/mL)<br>2.PEG 50,630 Da (2mg/mL)<br>3.PEG 40,000 Da (2mg/mL)<br>4.PEG 26,100 Da (2mg/mL)<br>5.PEG 8,730 Da (2mg/mL) |
| Application No. | G006200 |



## HPLC Analysis of Pegylated Exenatide on Zenix® SEC-300 and Zenix®-C SEC-300

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm, I.D., 3 µm particles (Z777092)
column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
mobile phase . . (A) 50 mM Ammonium acetate: (B) acetonitrile; (90:10, A:B, v/v)
flow rate . . . . . 0.5 mL/min
pressure . . . . . 609 psi (42 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 15 µL
sample . . . . . 3.3 mg/ml PEG-Exanatide in water (PEG 23 kD)
Application No. . . . . G005949



## HPLC Analysis of Pepides, Alberta Mix, on Discovery® BIO Wide Pore C18, No TFA

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 2.1 mm I.D., 3 µm particles (567202-U)

mobile phase . . (A) 25 mM formic acid in water, (B) (25 mM formic acid in water): (20 mM formic acid in acetonitrile, (50:50)

flow rate . . . . . 0.20 mL/min

column temp. . . . . ambient

detector . . . . . +ES

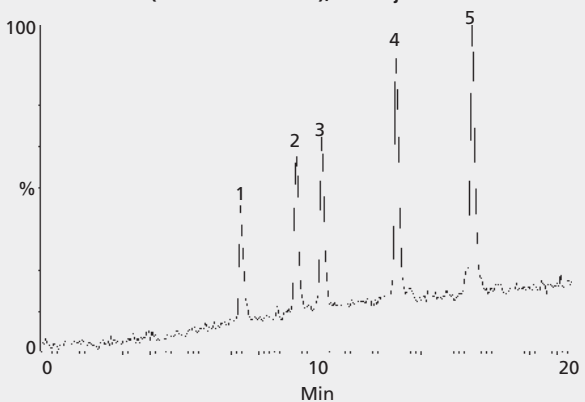
injection . . . . . 1 µL

Application No. . . . . G001638B

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 20 | 50 | 50 |

Peptide 1: ac-RGAGGLGLGK-amide
Peptide 2: ac-RGGGGLGLGK-amide
Peptide 3: ac-RGAGGLGLGK-amide
Peptide 4: ac-RGVGGLGLGK-amide
Peptide 5: ac-RGVVGLGLGK-amide



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## HPLC Analysis of Pepides, Alberta Mix on Discovery® BIO Wide Pore C5

column . . . . . Discovery BIO Wide Pore C5, 15cm × 2.1mm I.D., 5 µm particles (568402-U)

mobile phase . . (A) 25mM formic acid in water; (B) (50:50) 25mM formic acid in water, 20mM formic acid in acetonitrile

flow rate . . . . . 0.20 mL/min

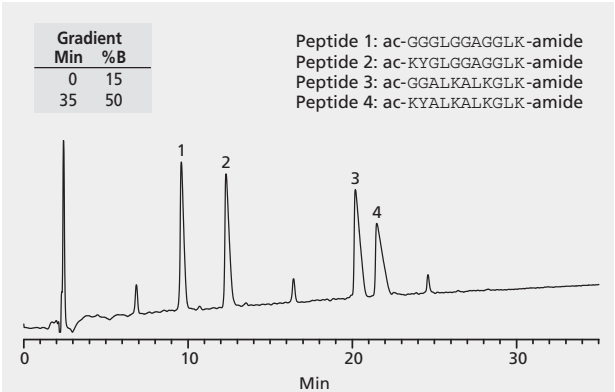
injection . . . . . 0.5 µL (~0.25 µg each peptide)

column temp. . . . . 35 °C

detector . . . . . UV, 215 nm

sample . . . . . RP Peptide Ionic Interactions Standard, p/n RPS-I0020 (Alberta Peptide Institute)

Application No. . . . . G001882



## HPLC Analysis of Pepides, Synthetic, on Discovery® BIO Wide Pore C18

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 2.1 mm I.D., 5 µm particles (568202-U)

mobile phase . . (A) 25 mM formic acid in water; (B) (50:50) 25 mM formic acid in water, 20 mM formic acid in acetonitrile

flow rate . . . . . 0.20 mL/min

column temp. . . . . 35 °C

detector . . . . . UV, 215 nm

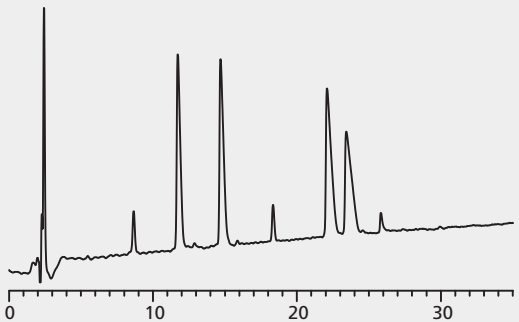
injection . . . . . 0.5 µL (~0.25 µg ea peptide)

sample . . . . . RP Peptide Ionic Interactions Standard, p/n RPS-I0020 (Alberta Peptide Institute)

Application No. . . . . G001642B

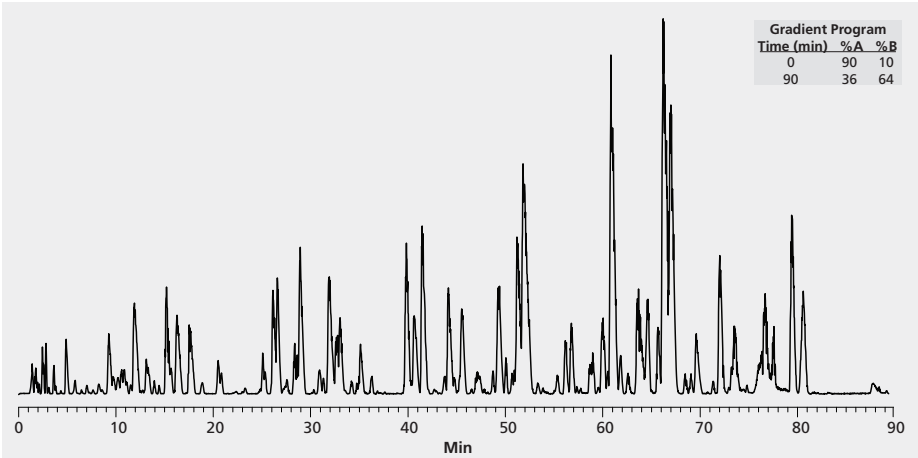
Peptide 1: ac-GGGLGGAGGLK-amide
Peptide 2: ac-KYGLGGAGGLK-amide
Peptide 3: ac-GGALKALKGLK-amide
Peptide 4: ac-KYALKALKGLK-amide

| Gradient Program Time (min) | %B |
|---|---|
| 0 | 15 |
| 45 | 60 |



## HPLC Analysis of Peptide Mix, Complex, on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 2.1 mm I.D., 5 µm particles (581304-U)
mobile phase . . (A) 25 mM formic acid in water; (B): 50:50, 25 mM formic acid in water:25 mM formic acid in acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI (+), full scan
injection . . . . . 10 µL
sample . . . . . tryptic digest of carboxymethylated protein mixture 2.4 g/L in 0.1% formic acid
Application No. . . . . G002441



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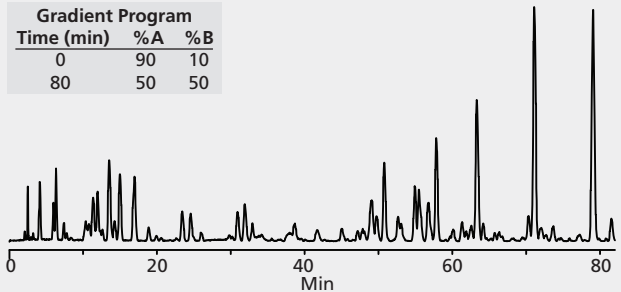
## HPLC Analysis of Peptide Mix, Complex on Ascentis® C18

column . . . . . Ascentis C18, 25 cm x 2.1 mm I.D., 5 µm particles (581305-U)
mobile phase . . (A) (50:50), 50 mM formic acid titrated with acetate (pH 3.0):water;
(B) (50:50), 50 mM formic acid titrated with acetate (pH 3.0): acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . ambient
detector . . . . . ESI (+)
injection . . . . . 4 µL
sample . . . . . tryptic digest of carboxymethylated protein mixture 1.5 g/L in 50 mM formic acid
Application No. . . . . G002669

**Gradient Program**

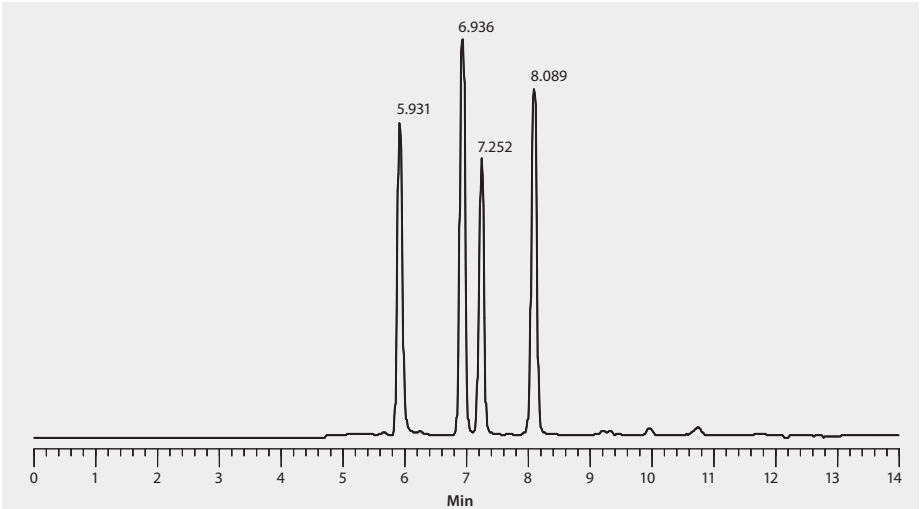
| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 80 | 50 | 50 |



## HPLC Analysis of Peptide Mixture on Zenix® SEC-100

Zenix® SEC columns can be operated in aqueous-organic mobile phases, as is shown ion this example where hydrophobic peptides and proteins are eluted with excellent peak shape in 65% acetonitrile 35% water containing 0.1% TFA.

column . . . . . Zenix-100, 30 cm x 7.8 mm I.D., 3 µm particles, 100 Å (Z777008)
mobile phase . . . . . ACN:H2O:TFA=65:35:0.1(v/v)
flow rate . . . . . 1.0 mL/min
column temp. . . . . Ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . ribonuclease A (5.931 min), human insulin (6.936 min), thymosin a1 (7.252 min), somatostatin (8.089 min)
Application No. . . . . G006196



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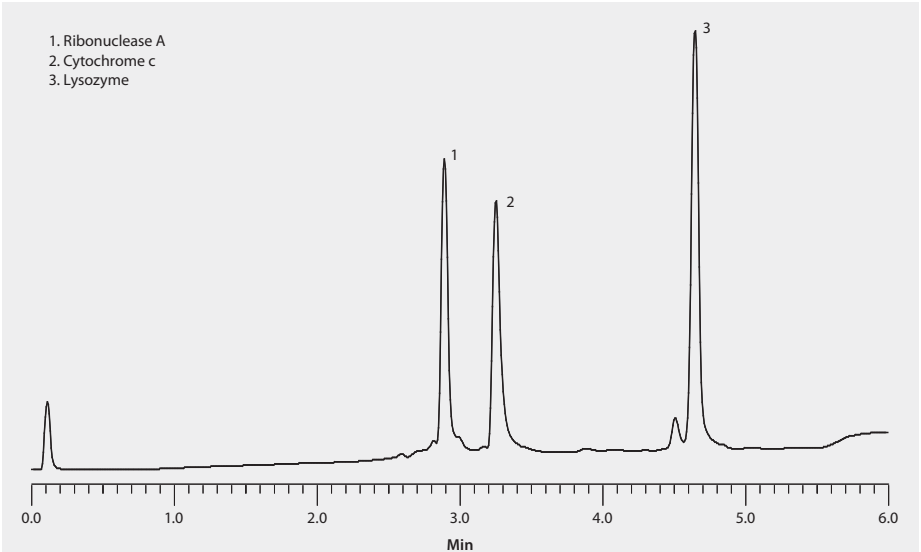
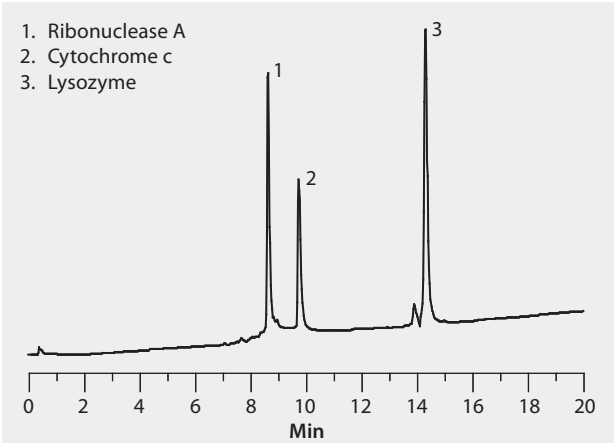
## HPLC Analysis of Peptides and Proteins on Proteomix® SCX-NP1.7, 3 cm x 4.6 mm I.D. Column

column . . Proteomix SCX-NP1.7, 3 cm x 4.6 mm I.D., 1.7 µm particles (Z777145)
mobile phase . . . . . (A) 20 mM sodium phosphate, pH 6.0; (B) A + 1.0 M NaCl
gradient . . . . . 0 to 50% B in 5 min; 15 min prewash
flow rate . . . . . 2 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL
Application No. . . . . G006144



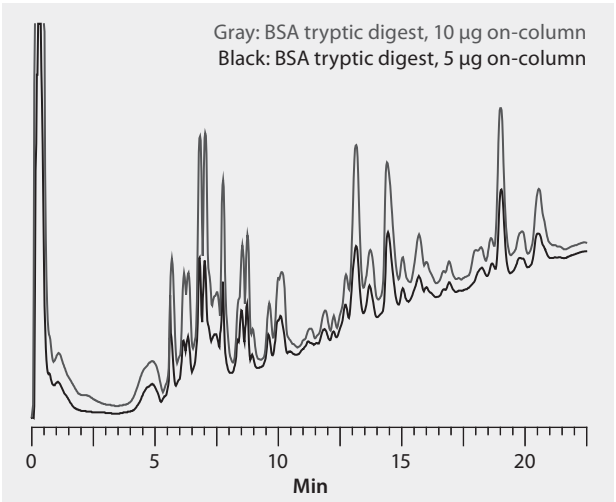
## HPLC Analysis of Peptides and Proteins on Proteomix® SCX-NP1.7, 5 cm x 4.6 mm I.D. Column

column . . Proteomix SCX-NP1.7, 5 cm x 4.6 mm I.D., 1.7 µm particles (Z777146)
mobile phase . . . . . (A) 20 mM sodium phosphate, pH 6.0; (B) A + 1.0 M NaCl
gradient . . . . . 0 to 75% B in 25 min; 15 min prewash
flow rate . . . . . 0.75 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL
Application No. . . . . G006145



## HPLC Analysis of Peptides from BSA on Proteomix® SCX-NP3, Volatile Buffer

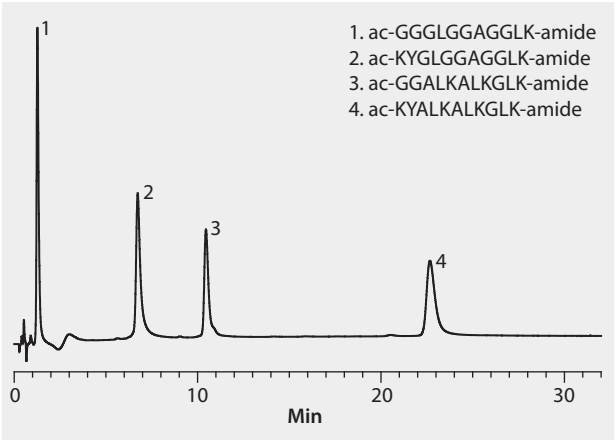
flow rate . . . . . 0.35 mL/min
column . . Proteomix SCX-NP1.7, 3 cm x 2.1 mm I.D., 1.7 µm particles (Z777142)
mobile phase . . . . . (A) 10 mM sodium phosphate, pH 2.5; (B) A + 1 M NaCl; (C) acetonitrile
gradient . . . . . 90% A, 0% B, 10% C held for 1.5 min; to 40% A, 50% B, 10% C in 18.5 min
pressure . . . . . 5511 psi (380 bar)
detector . . . . . UV, 214 nm
sample . . . . . trypsin digested bovine serum albumin (BSA)
Application No. . . . . G006022



## HPLC Analysis of Peptides on Discovery® BIO PolyMA-SCX (Ion Exchange)

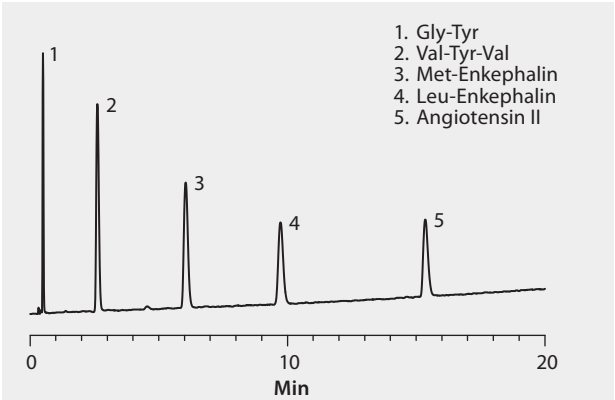
Polymer particles are frequently used in bioseparations. Their advantages over inorganic particles, however, are often offset by generally lower efficiency. Discovery BIO PolyMA-SCX and PolyMA-WAX particles offer the benefits of polymeric particles, but have higher efficiency and better resolution than competitive particles. An example of the high efficiency of Discovery BIO PolyMA-SCX is shown in this Figure. The Discovery BIO PolyMA-SCX column gives efficient, well-resolved separation of a four-component peptide mixture.

column . . Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm I.D., 5 µm particles (59601-U)
mobile phase . . ((A) 5% acetonitrile in 20 mM ammonium carbonate, pH 3.5 with phosphoric acid; (B) 5% acetonitrile in 20 mM ammonium carbonate, 480 mM ammonium phosphate, pH 3.5 with phosphoric acid)
gradient . . . . . 0 to 100% B in 24 min (linear)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . RP Peptide Ionic Interactions Standard, p/n RPS-10020 (Alberta Peptide Institute)
Application No. . . . . G001824



## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C18 (5 cm Column)

column . . . . . Discovery BIO Wide Pore C18, 5 cm × 4.6 mm I.D., 5 µm particles (568220-U)
mobile phase . . (A) (5:95), 0.5% TFA in water:0.5% TFA in acetonitrile; (B) (25:75), 0.5% TFA in water:0.5% TFA in acetonitrile
gradient . . . . . 2-24% B in 22 min, 8 min. at 100%A
flow rate . . . . . 2 mL/min
column temp. . . . . 70 °C
detector . . . . . UV, 220 nm
Application No. . . . . G001562



## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C18 (Gradient #1)

column . . . . . Discovery BIO Wide Pore C18, 15cm × 4.6mm I.D., 5µm particles (568222-U)

mobile phase . . . . . (A) (80:20), 0.1% TFA in water:0.1% TFA in acetonitrile; (B) (66:34), 0.1% TFA in water:0.1% TFA in acetonitrile

gradient . . . . . 0-100% B in 14 min after 1 minute delay

flow rate . . . . . 1 mL/min

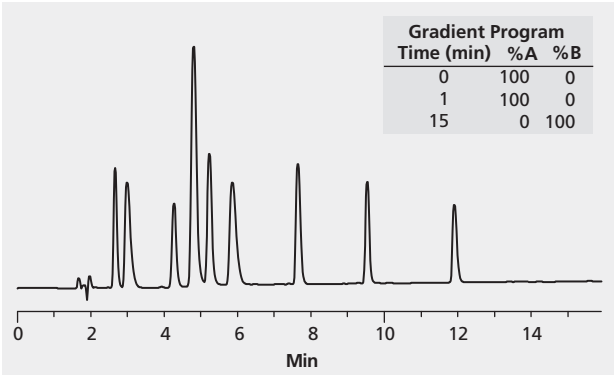
column temp. . . . . 30 °C

detector . . . . . UV, 220 nm

injection . . . . . 10 µL

sample . . . . . Sigma Peptide Mix (Cat. No. P2693)

Application No. . . . . G001512



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## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C18 (Gradient #2)

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 4.6 mm I.D., 5 µm particles (568222-U)

mobile phase . . . . . (A) (80:20), 0.1%TFA in water:0.1% TFA in acetonitrile; (B) (66:34), 0.1% TFA in water:0.1% TFA in acetonitrile

flow rate . . . . . 1 mL/min

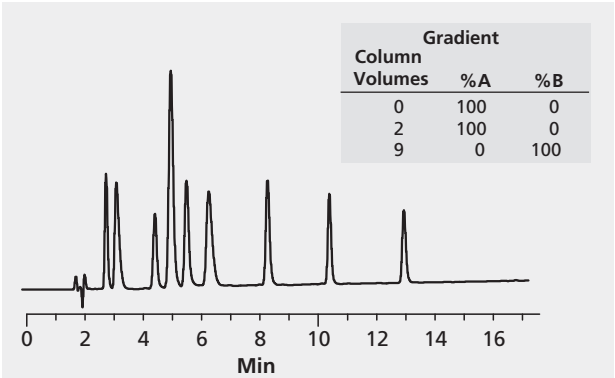
column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . 5 µL Sigma Peptide Mix P2693 (~25 µg ea. Peptide) (~25 µg ea. peptide)

sample . . . . . Sigma Peptide Mix (Cat. No. P2693)

Application No. . . . . G001593



## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C18, 0-16 Min. Window

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 4.6 mm I.D., 5 µm particles (568222-U)

mobile phase . . (A) (95:5), 0.1% TFA in water:0.1% TFA in acetonitrile; (B) (50:50), 0.1%TFA in water:0.1% TFA in acetonitrile

gradient . . . . . 0-100% B in 65 min

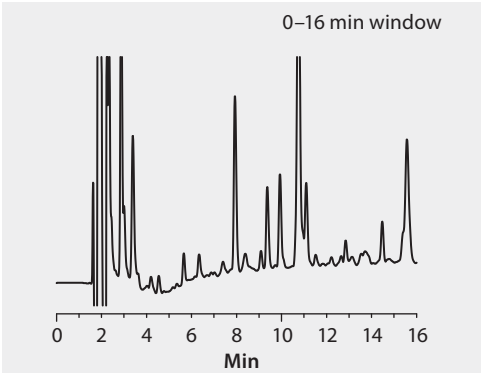
flow rate . . . . . 1 mL/min

column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 50µL carboxymethylated apohemoglobin tryptic digest in 50 mM ammonium bicarbonate

Application No. . . . . G001730



## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C18, 28-38 Min. Window

column . . . . . Discovery BIO Wide Pore C18, 15cm × 4.6mm I.D., 5 µm particles (568222-U)

mobile phase . . (A) (95:5), 0.1% TFA in water:0.1% TFA in acetonitrile; (B) (50:50), 0.1% TFA in water:0.1% TFA in acetonitrile

gradient . . . . . 0-100% B in 65 min

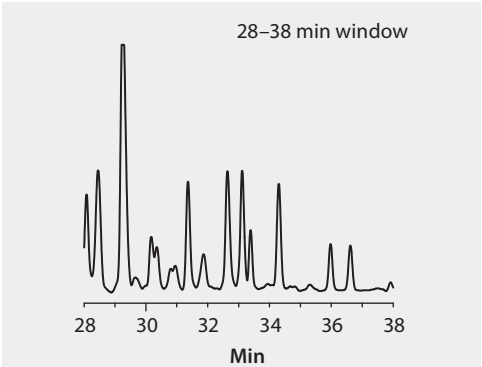
flow rate . . . . . 1 mL/min

column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 50µL carboxymethylated apohemoglobin trypic digest in 50 mM ammonium bicarbonate

Application No. . . . . G001727



## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C18 (PFPA Mobile Phase Additive)

**Sigma peptide mix (5 µm)**

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 4.6 mm I.D., 5 µm particles (568222-U)

mobile phase . . . . . (A) 81:19, (water/0.1% PFPA):(acetonitrile/0.1%PFPA); (B) 62:38, (water/0.1%PFPA):(acetonitrile/0.1%PFPA)

flow rate . . . . . 1.0 mL/min

injection . . . . . 10 µL (~25 µg each peptide)

column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

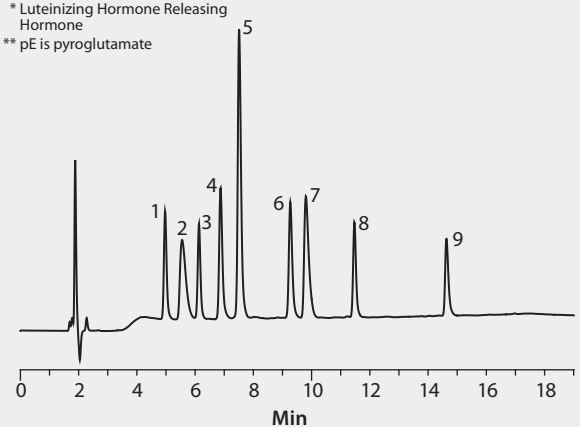
sample . . . . . Sigma Peptide Mix (Cat. No. P2693)

Application No. . . . . G001594

| Peptide | Amino Acid Sequence |
|---|---|
| 1. Arg$^8$-vassopressin | CYFQNCPRG-amide; disulfide |
| 2. Bradykinin, fragment 1-5 | RPPGF |
| 3. Oxytocin | CYIQNCPLG-amide; disulfide |
| 4. Met-Enkephalin | YGGFM |
| 5. LHRH* | pEHWSYGLRPG-amide** |
| 6. Leu-Enkephalin | YGGFL |
| 7. Bradykinin | RPPGFSPFR |
| 8. Bombesin | pEQRLGNQWAVGHLM-amide |
| 9. Substance P | RPKPQQFFGLM-amide** |

* Luteinizing Hormone Releasing Hormone

** pE is pyroglutamate



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## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C5, 0-16 Min. Window

column . . . . . Discovery BIO Wide Pore C5, 15 cm × 4.6 mm I.D., 5 µm particles (568422-U)

mobile phase . . (A) (95:5), 0.1% TFA in water:0.1% TFA acetonitrile; (B) (50:50), 0.1% TFA in water:0.1% TFA in acetonitrile

gradient . . . . . 0-100% B in 65 min

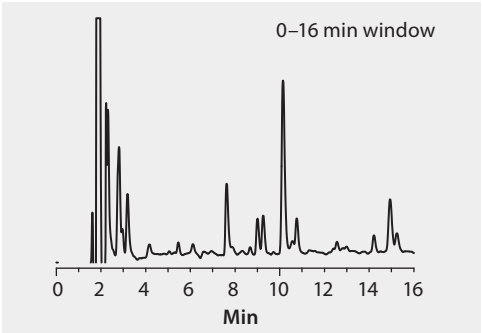
flow rate . . . . . 1 mL/min

column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 50µL carboxymethylated apohemoglobin tryptic digest in 50 mM ammonium bicarbonate (568422-U)

Application No. . . . . G001732



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## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C5, 28-38 Min. Window

column . . . . . Discovery BIO Wide Pore C5, 15 cm × 4.6 mm I.D., 5 µm particles (568422-U)

mobile phase . . (A) (95:5), 0.1% TFA in water:0.1% TFA in acetonitrile; (B) (50:50), 0.1%TFA in water:0.1% TFA in acetonitirle

gradient . . . . . 0-100% B in 65 min

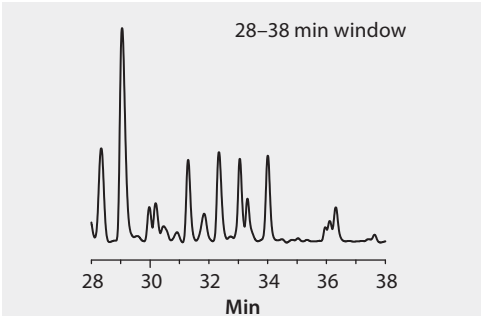
flow rate . . . . . 1 mL/min

column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

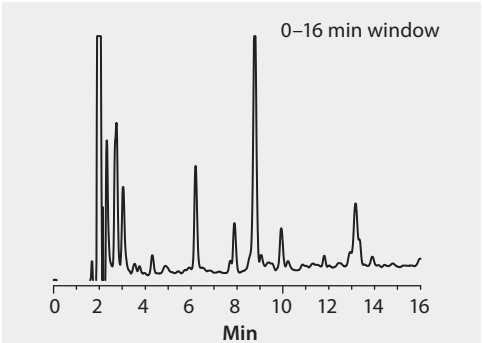
injection . . . . . 50 µL carboxymethylated apohemoglobin tryptic digest in 50 mM ammonium bicarbonate

Application No. . . . . G001729



## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C8, 0-16 Min. Window

column . . . . . Discovery BIO Wide Pore C8, 15 cm × 4.6 mm I.D., 5 µm particles (568322-U)
mobile phase . . (A) (95:5), 0.1% TFA in water:0.1% TFA in acetonitrile; (B) (50:50), 0.1% TFA in water:0.1% TFA in acetonitrile
gradient . . . . . 0-100% B in 65 min
flow rate . . . . . 1ml/min
column temp. . . . . 30 °C
detector . . . . . UV, 215 nm
injection . . . . . 50µL carboxymethylated apohemoglobin tryptic digest in 50 mM ammonium bicarbonate
Application No. . . . . G001731

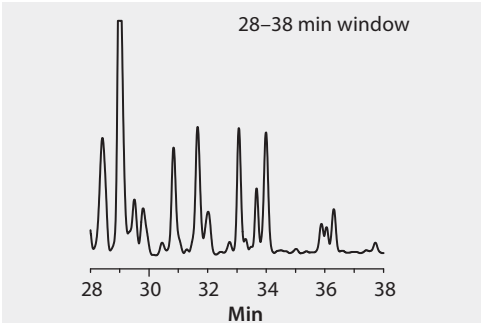





SUPELCO® Solutions within.™

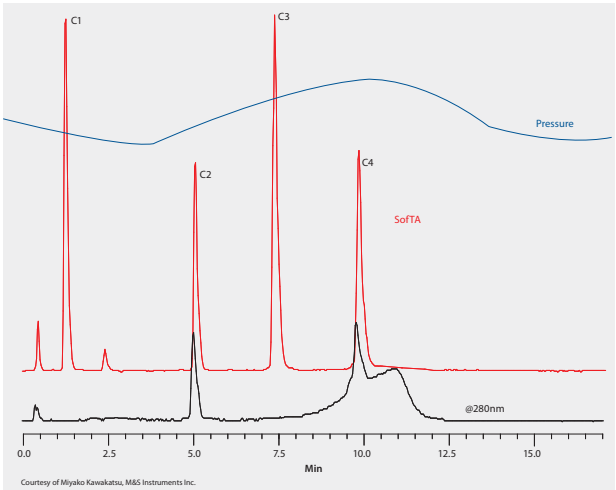
## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C8, 28-38 Min. Window

column . . . . . Discovery BIO Wide Pore C8, 15 cm × 4.6 mm I.D., 5 µm particles (568322-U)
mobile phase . . (A) (95:5), 0.1% TFA in water:0.1% TFA in acetonitrile; (B) (50:50), 0.1% TFA in water:0.1%TFA in acetonitrile
gradient . . . . . 0-100% B in 65 min
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 215 nm
injection . . . . . 50µL carboxymethylated apohemoglobin tryptic digest in 50 mM ammonium bicarbonate
Application No. . . . . G001728



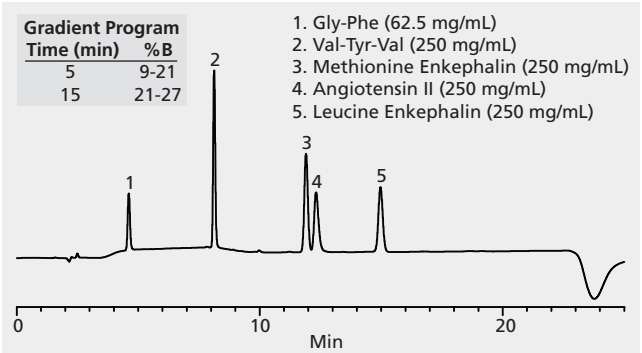
## HPLC Analysis of Peptides on Proteomix® SCX-NP3, Volatile Buffer, ELSD and UV Detection

column . . . . . Proteomix SCX-NP3, 5 cm x 4.6 mm I.D., 3 µm particles (Z777151)
mobile phase . . . . . (A) 5 mM ammonium acetate;
(B) 50 mM ammonium acetate:acetonitrile (4:1, v:v)
gradient . . . . . 0 to 50% B in 20 min
flow rate . . . . . 0.6 mL/min
detector . . . . . ELSD (SofTA) and UV, 280 nm
injection . . . . . 5 µL
sample . . . . . 0.1 mg/mL
Application No. . . . . G006021



## HPLC Analysis of Peptides on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 0.1% TFA in water; (B) 0.1% TFA in acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . as indicated in mobile phase A
Application No. . . . . G002613



## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C18, Preparative Scale

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 10 mm I.D., 10 µm particles (567208-U)

mobile phase . . . . . (A) (80:20), 0.1% TFA in water:0.1% TFA in acetonitrile; (B) (66:34), 0.1% TFA in water:0.1% TFA in acetonitrile

flow rate . . . . . 4.7 mL/min

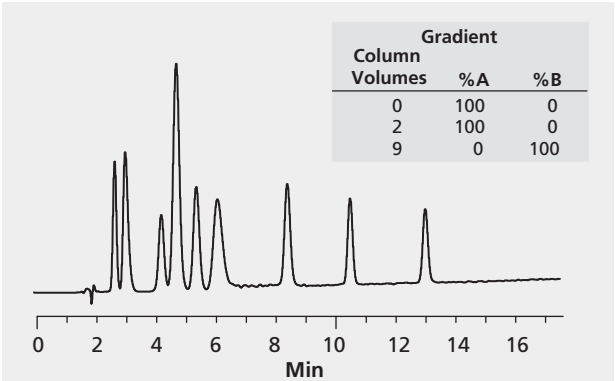
column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 24.5 µL

sample . . . . . Cat. No. P2693

Application No. . . . . G001511



## HPLC Analysis of Peptides on Discovery® BIO Wide Pore C5

column . . . . . Discovery BIO Wide Pore C5, 15 cm × 4.6 mm I.D., 5 µm particles (568422-U)

mobile phase . . . (A): 0.1% PFPA (pentafluoropropionic acid) in water:acetonitrile (81:19); (B): 0.1% PFPA in water:acetonitrile (62:38)

gradient . . . . . 0-100% B in 19 min

flow rate . . . . . 1 mL/min

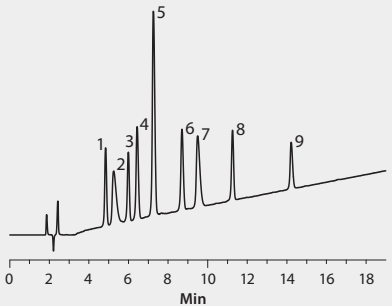
column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 10 µL ~0.25 µg each peptide

sample . . . . . Cat. No. P2693

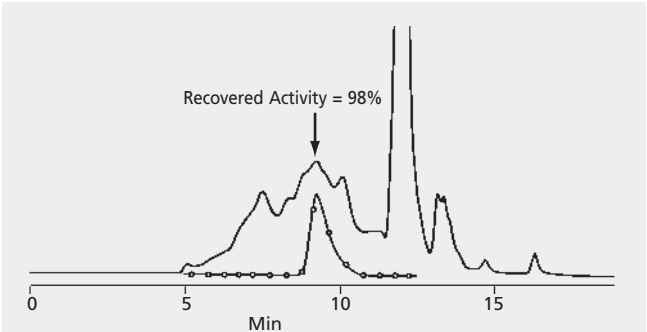
Application No. . . . . G001492




| | Peptide | Sequence |
|---|---|---|
| 1. | Arg$^8$-vasopressin | CFQNCPRG-amide; disulfide |
| 2. | Bradykinin, fragment 1–5 | RPPGF |
| 3. | Oxytocin | CYQNCPLG-amide; disulfide |
| 4. | Met-enkephalin | YGGFM |
| 5. | Luteinizing hormone releasing hormone | PEHWSYGLRPG-amide |
| 6. | Leu-enkephalin | YGGFL |
| 7. | Bradykinin | RPPGFSPFR |
| 8. | Bombesin | PEQRLGNQWAVGHLM-amide |
| 9. | Substance P | RPKPQQFFGLM-amide |

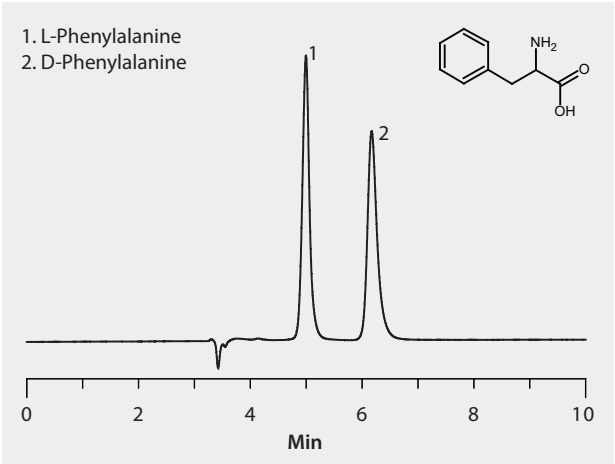
## HPLC Analysis of Peroxidase on TSKgel® G3000SWXL

column . . . . TSKgel G3000SW$_{XL}$, 30 cm × 7.8 mm I.D., 5 µm particles (808541)
mobile phase . . . . . 0.3M NaCl/0.05M phosphate buffer, pH 7.0
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . 100 µL crude peroxidase preparation from Japanese horseradish (0.15mg)
Application No. . . . . 713-0940



## HPLC Analysis of Phenylalanine Enantiomers on Astec® CHIROBIOTIC® T (Formic Acid Mobile Phase Additive)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005342



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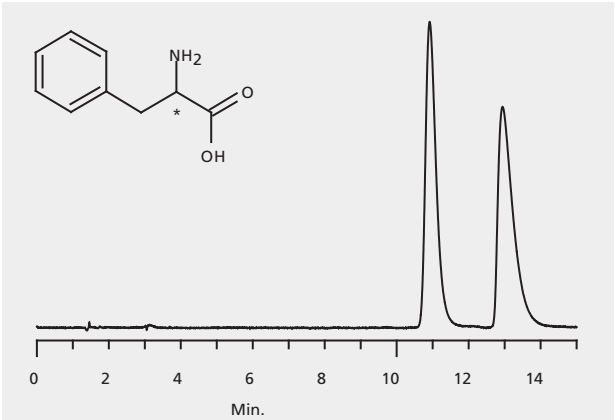
## HPLC Analysis of Phenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004508



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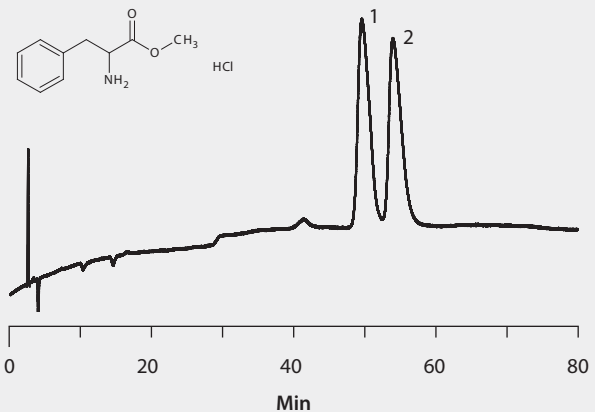
## HPLC Analysis of Phenylalanine Methyl Ester Enantiomers on LARIHC™ CF6-P

column . . LARIHC CF6-P, 25 cm x 4.6 mm I.D., 5 µm particles (AZYP Part No.L1001, available from Supelco/Sigma-Aldrich as a custom item)
mobile phase . . . . . (A) methanol: (B) acetonitrile: (C) acetic acid: (D) triethylamine, (70:30:0.3:0.2. A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . Phenylalanine methyl ester, 0.3 mg/mL in ethanol
Application No. . . . . G005259

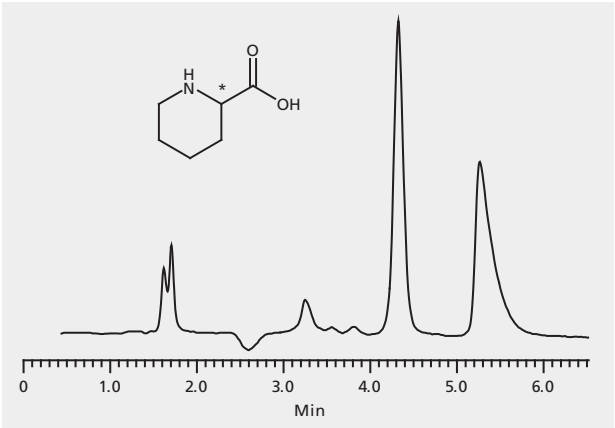
1. Phenylalanine methyl ester enantiomer
2. Phenylalanine methyl ester enantiomer




**Ref:** 1. Ping Sun, Chunlei Wang, Zachary S. Breitbach, Ying Zhang, and Daniel W. Armstrong, Development of New HPLC Chiral Stationary Phases Based on Native and Derivatized Cyclofructans *Anal. Chem.* **81**, 10215 (2009)
2. Ping Sun and Daniel W. Armstrong, Effective enantiomeric separations of racemic primary amines by the isopropyl carbamate-cyclofructan6 chiral stationary phase *J. Chromatogr. A.* **1217**, 4904 (2010)

## HPLC Analysis of Pipecolic Acid Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004554

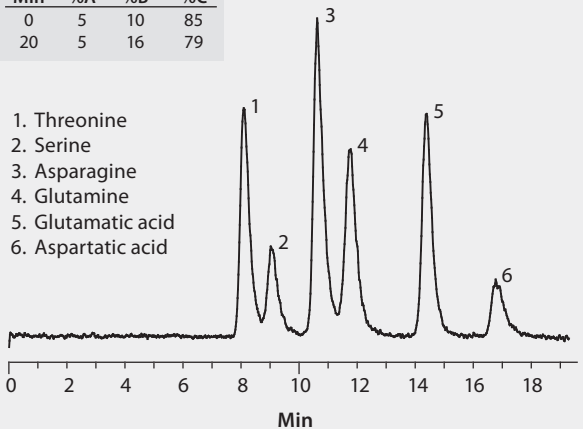


## HPLC Analysis of Polar Neutral and Acidic Amino Acids on Ascentis® Si (Silica)

column . . . . . Ascentis Si, 10 cm × 2.1 mm I.D., 5 µm particles (581508-U)
mobile phase . . . . . (A) 100 mM ammonium formate (pH 3.0, with formic acid), (B) water, (C) acetonitrile
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI (+), full scan
injection . . . . . 2 µL
sample . . . . . 100 µg/mL in 10:90, (50 mM ammonium formate / formic acid, pH 3.0):acetonitrile
Application No. . . . . G003688

**Gradient Program**

| Min | %A | %B | %C |
|---|---|---|---|
| 0 | 5 | 10 | 85 |
| 20 | 5 | 16 | 79 |



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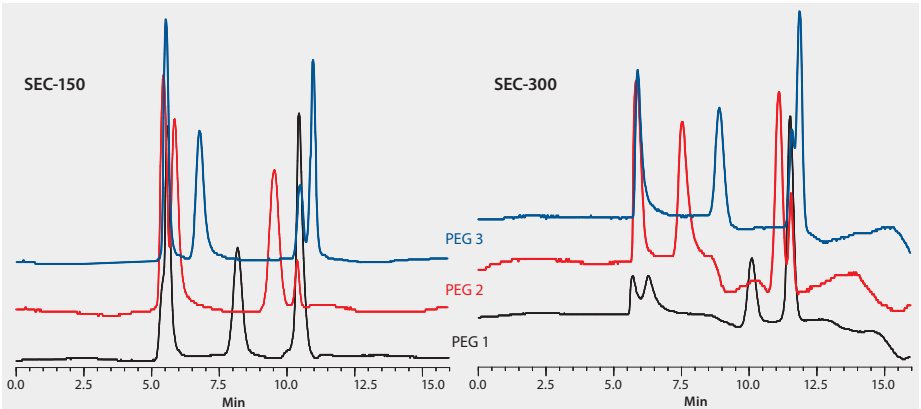
## HPLC Analysis of Poly(ethyleneglycol) polymers on SRT® SEC-150 and SRT® SEC-300

Although proteins are the most common applications of Sepax SRT and Zenix gel filtration columns, they can also be used to other water-soluble polymers such as the poly(ethyleneglycol) samples shown in these chromatograms. The conditions are very similar to those used for protein analysis, namely 150mM phosphate buffer at pH 7.0. Judging by the narrow peak widths all PEG polymers shwon here have rather narrow size distributions.

column . . . . . SRT-150, 30 cm x 7.8 mm I.D., 5 µm particles, 150 Å (Z777045)
column . . . . . SRT-300, 30 cm x 7.8 mm I.D., 5 µm particles, 150 Å (Z777051)
mobile phase . . . . . 150 mM phosphate Buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
pressure . . . . . 720 psi (49.6 bar)
detector . . . . . RI(30 C°)
injection . . . . . 20 µL
sample . . 1.5 mg/mL in mobile phase PEG 1. MW 636, 6,550, 118,000, 932,000; PEG 2. MW 2010, 42,700, 496,000 ; PEG 3. MW 232, 17,900, 271,000
Application No. . . . . G006206

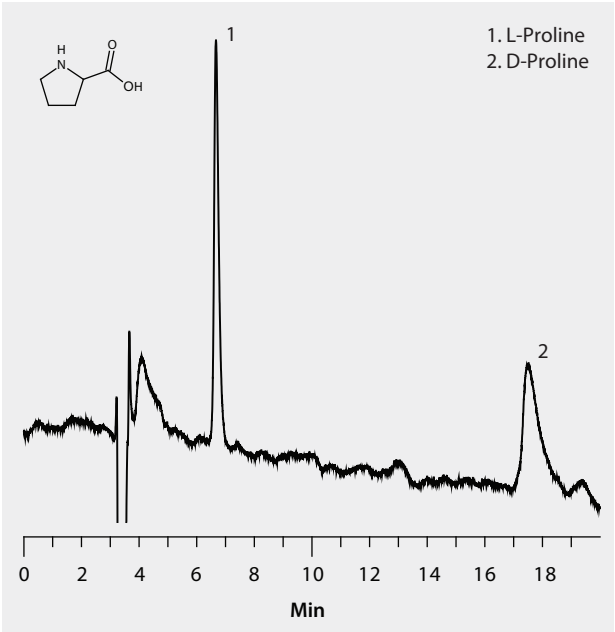



| No | Molecular Wt. | Log $M_w$ | Elution Vol (mL) |
|---|---|---|---|
| 1 | 932000 | 5.969416 | – |
| 2 | 496000 | 5.695482 | 5.517 |
| 3 | 271000 | 5.432969 | 5.559 |
| 4 | 118000 | 5.071882 | 5.622 |
| 5 | 42700 | 4.630428 | 5.923 |
| 6 | 17900 | 4.252853 | 6.803 |
| 7 | 6550 | 3.816241 | 8.201 |
| 8 | 2010 | 3.303196 | 9.603 |
| 9 | 628 | 2.79796 | 10.465 |
| 10 | 232 | 2.365488 | 10.983 |

| No | Molecular Wt. | Log $M_w$ | Elution Vol (mL) |
|---|---|---|---|
| 1 | 932000 | 5.969416 | 5.853 |
| 2 | 496000 | 5.695482 | 5.875 |
| 3 | 271000 | 5.432969 | 5.91 |
| 4 | 118000 | 5.071882 | 6.426 |
| 5 | 42700 | 4.630428 | 7.568 |
| 6 | 17900 | 4.252853 | 8.927 |
| 7 | 6550 | 3.816241 | 10.201 |
| 8 | 2010 | 3.303196 | 11.137 |
| 9 | 628 | 2.79796 | 11.604 |
| 10 | 232 | 2.365488 | 11.89 |

## HPLC Analysis of Proline Enantiomers on Astec® CHIROBIOTIC® T (Formic Acid Mobile Phase Additive)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (40:60:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 200 µg /mL in 50:50 water:methanol
Application No. . . . . G005343



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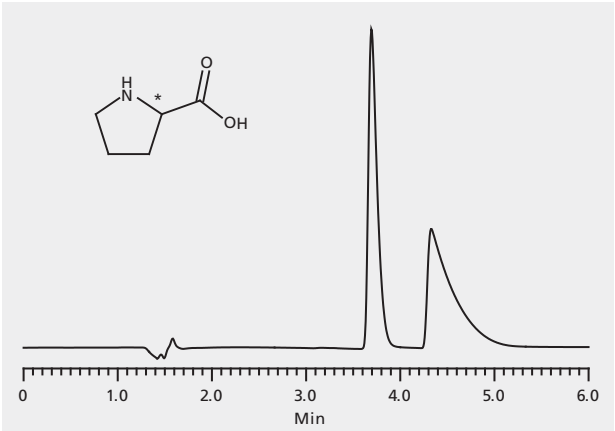
## HPLC Analysis of Proline Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 210 nm
injection . . . . . 20 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004526



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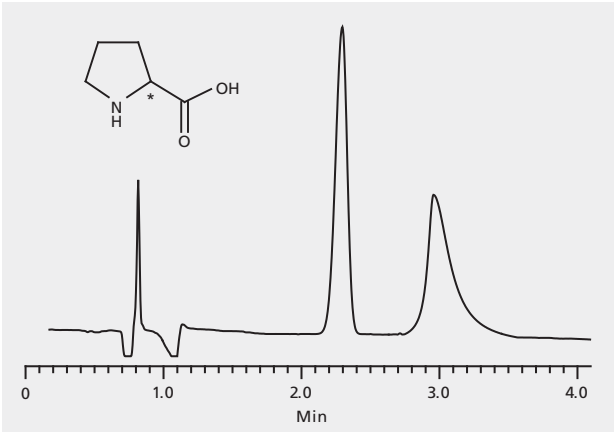
## HPLC Analysis of Proline Enantiomers on Astec® CHIROBIOTIC® T, Fast Analysis

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . water
flow rate . . . . . 0.65 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004527

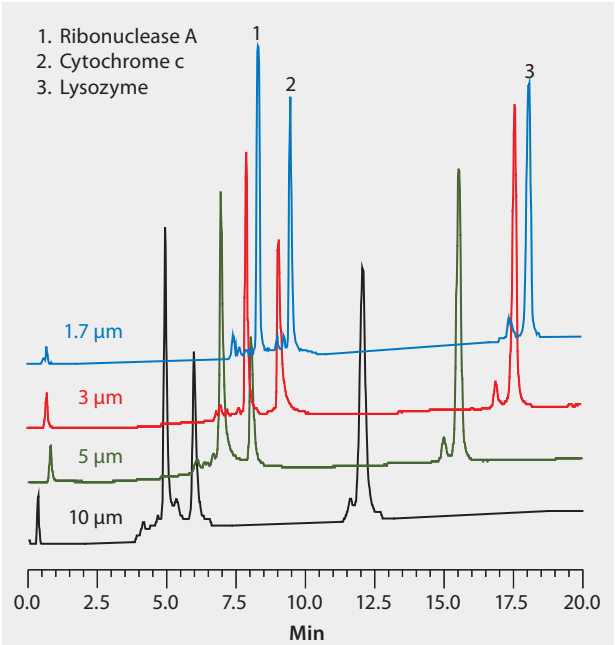





SUPELCO® Solutions within.™

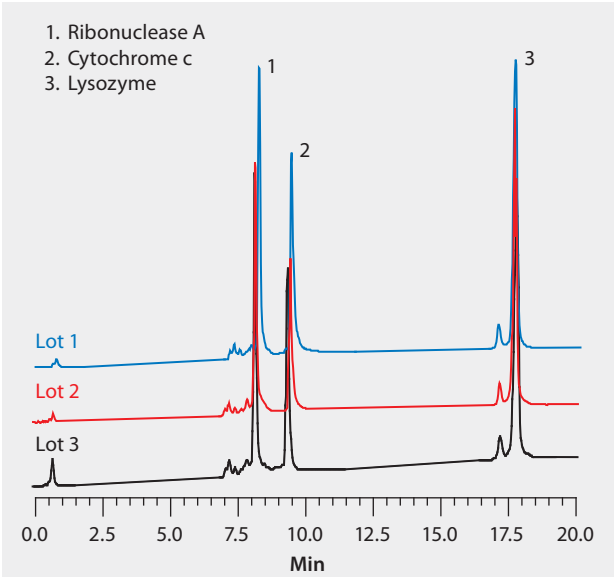
## HPLC Analysis of Proteins and Peptides on Proteomix® WCX, Comparison of Different Particle Sizes

column . . . . . Proteomix WCX-NP10, 5 cm x 4.6 mm, 10 µm particles (Z777169)
column . . . . . Proteomix WCX-NP5, 5 cm x 4.6 mm x 5 µm particles (Z777193)
column . . Proteomix WCX-NP1.7, 5 cm x 4.6 mm I.D., 1.7 µm particles (Z777180)
column . . . . Proteomix WCX NP3, 5 cm x 4.6 mm I.D., 3 µm particles (Z777185)
mobile phase . . . . . (A) 20 mM sodium phosphate, pH 6.5, (B) A + 1.0 M NaCl
gradient . . . . . 0 to 100% B in 25 min, 15 min prewash
flow rate . . . . . 0.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL) min
Application No. . . . . G006143



## HPLC Analysis of Proteins and Peptides on Proteomix® WCX-NP1.7 Demonstrating Lot-to-Lot Reproducibility

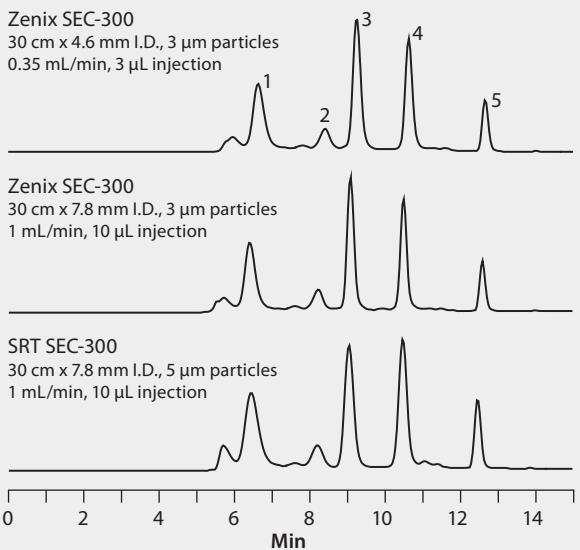
column . . Proteomix WCX-NP1.7, 5 cm x 4.6 mm I.D., 1.7 µm particles (Z777180)
mobile phase . . . . . (A) 20 mM sodium phosphate, pH 6.5; (B) A + 1.0 M NaCl
gradient . . . . . 0 to 100% B in 25 min, 15 min prewash
flow rate . . . . . 0.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL
Application No. . . . . G006142



## HPLC Analysis of Proteins by Size Exclusion on Zenix® and SRT® SEC-300, Comparison of Particle Size and Column I.D.

column . . . . . Zenix 300, 30 cm x 4.6 mm I.D., 3 µm particles, 300 Å (Z777028)
column . . . . Zenix 300, 30 cm x 7.8 mm I. D., 3 µm particles, 300 Å (Z777033)
column . . . . . SRT SEC-300, 30 cm x 7.8 mm 5 µm particles, 300 Å (Z777051)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
column temp. . . . . ambient
detector . . . . . UV, 214nm
sample . . . . . each compound 1 mg/mL except uracil, 2.5 µg/mL
Application No. . . . . G006179

1. Thyroglobulin, 670 kDa
2. BSA dimer, 132 kDa
3. Bovine serum albumin (BSA), 66 kDa
4. Ribonuclease A, 13.7 kDa
5. Uracil, 120 Da



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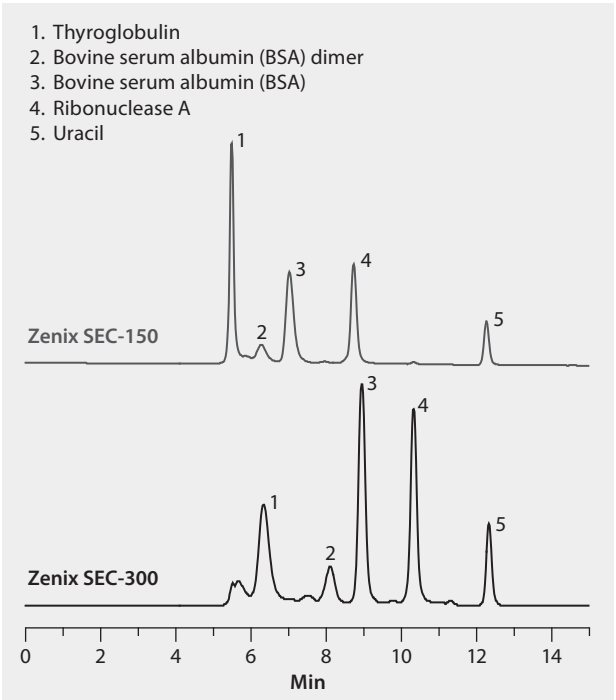
## HPLC Analysis of Proteins by Size Exclusion on Zenix® Columns, Effect of Pore Size

column . . Zenix SEC-150, 30 cm x 7.8 mm I.D., 3 µm, 150 Å particles (Z777018)
column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . each 1 mg/mL except uracil, 2.5 mg/mL
Application No. . . . . G006125

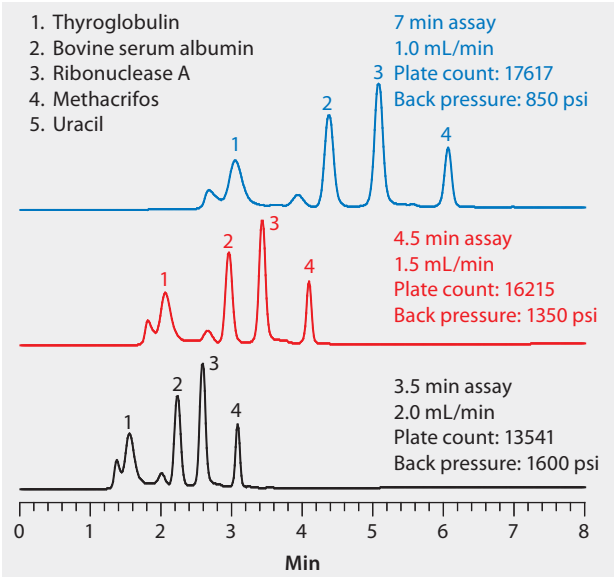





SUPELCO® Solutions within.™

## HPLC Analysis of Proteins by Size Exclusion on Zenix® SEC-300 Columns, Effect of Flow Rate (15 cm column)

column . . Zenix SEC-300, 15 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777030)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . as indicated on figure
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . each 1 mg/mL except uracil, 2.5 mg/mL
Application No. . . . . G006127

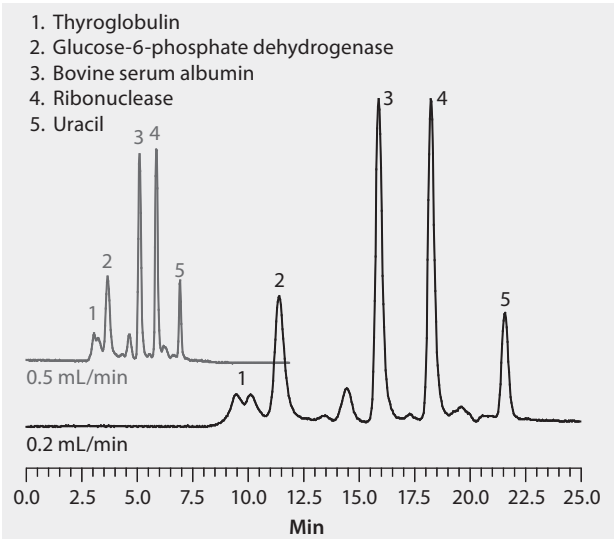





SUPELCO® Solutions within.™

## HPLC Analysis of Proteins by Size Exclusion on Zenix® SEC-300 Columns, Effect of Flow Rate (30 cm column)

column . . Zenix SEC-300, 30 cm x 4.6 mm I.D., 3 µm, 300 Å particles (Z777028)
mobile phase . . . . . 150 mM potassium phosphate, pH 6.8
flow rate . . . . . as indicated on figure
column temp. . . . . ambient
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . each 1 mg/mL except uracil, 2.5 mg/mL
Application No. . . . . G006128



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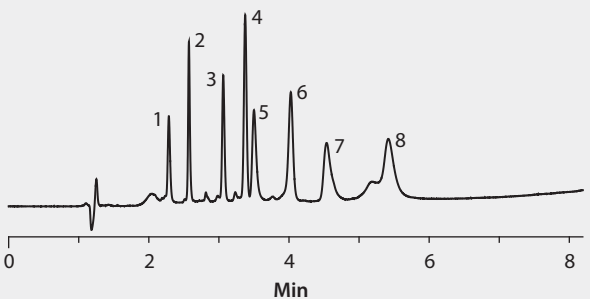
## HPLC Analysis of Proteins on Ascentis® Express Peptide ES-C18

column . . . . . Ascentis Express Peptide ES-C18, 15 cm x 2.1 mm I.D., 2.7 µm particles (53307-U)
mobile phase . . . . . (A) 75:25, 0.1% TFA in water: 0.08% TFA in acetonitrile; (B) 25:75, 0.1% TFA in water: 0.08% TFA in acetonitrile
gradient . . . . . held at 0% B for 0.5 min; 0 to 100% B in 7 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 4743 psi (327 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 1 µL
sample . . . . . 0.2 - 0.6 g/L in mobile phase A
Application No. . . . . G005724

1. Ribonuclease, 13.7 kDa
2. Adrenomedullin, 5.73 kDa
3. Cytochrome c, 12.2 kDa
4. Lysozyme, 14.3 kDa
5. Superoxide dismutase, 32.5 kDa
6. Soybean trypsin inhibitor, 20.1 kDa
7. Carbonic anhydrase, 29.0 kDa
8. Ovalbumin, 44.3 kDa






SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on Discovery® BIO PolyMA-SCX (Analyte Set #2)

**Proteins, mixture**

The large pore diameter of Discovery BIO PolyMA-SCX and PolyMA-WAX particles allows full access to small peptides and very large proteins and protein aggregates. This Figure shows the power of ion-exchange to resolve proteins with very little difference in molecular weight, an advantage over size-exclusion separations.

column . . . Discovery BIO PolyMA-SCX, 5 cm x 4.6 mm, 5 µm particles (59601-U)
mobile phase . . . . . (A) 20 mM sodium phosphate, pH 7.0; (B) 20 mM sodium phosphate, 0.5 M NaCl, pH 7.0; 5 to 100% B in 15 min. (linear)
flow rate . . . . . 0.5 mL/min
injection . . . . . 10 µg trypsin inhibitor, 5 µg conalbumin, myoglobin or 10 µg ribonuclease A, 5 µg cytochrome c, a-chymotrypsinogen A
detector . . . . . UV, 280 nm
Application No. . . . . 796-0658

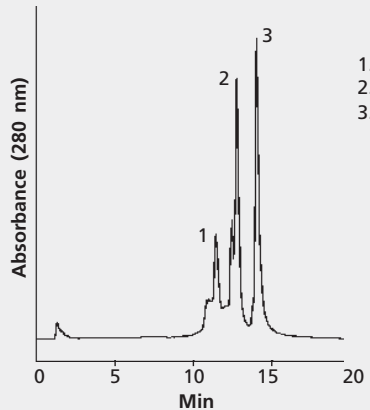



Figure used with permission of Mitsubishi Chemical Co.

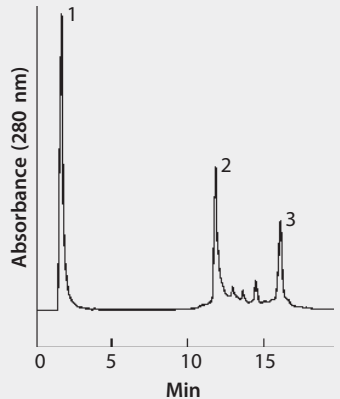


SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on Discovery® BIO PolyMA-WAX (Analyte Set #1)

The large pore diameter of Discovery BIO PolyMA-SCX and PolyMA-WAX particles allows full access to small peptides and very large proteins and protein aggregates. This Figure shows the separation of proteins that vary from 18 to 80 kDA.

column . . Discovery BIO PolyMA-WAX, 5 cm x 4.6 mm, 5 µm particles (59602-U)
mobile phase . . . . . (A) 10 mM Tris, pH 8.0 with HCl; (B) 10 mM Tris, 0.5 M NaCl, pH 8.0 with HCl; 5 to 100% B in 15 min. (linear)
flow rate . . . . . 0.5 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 10 µg trypsin inhibitor, 5 µg conalbumin, myoglobin or 10 µg ribonuclease A, 5 µg cytochrome c, a-chymotrypsinogen A
Application No. . . . . 796-0657

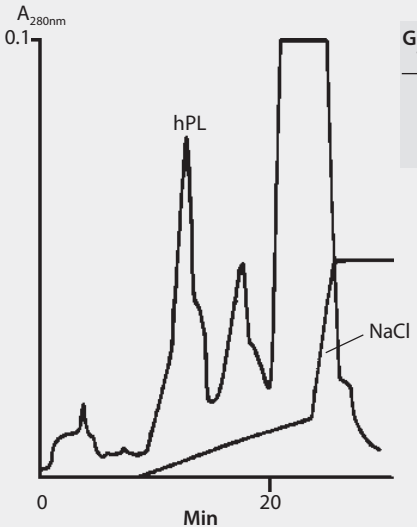



1. Myoglobin
2. Conalbumin
3. Trypsin inhibitor

Figure used with permission of Mitsubishi Chemical Co.

## HPLC Analysis of Proteins on Mono Q HR 5/5

column . . . . . Mono Q HR 5/5, 10 µm particles (54807)
mobile phase . . . . . (A): 20 mM bis-Tris, pH 6.0; (B): A + 1M NaCl
flow rate . . . . . 1.0 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 2.5 mL
sample . . . . . human placental lactogen fraction from size exclusion medium
Application No. . . . . 794-0421

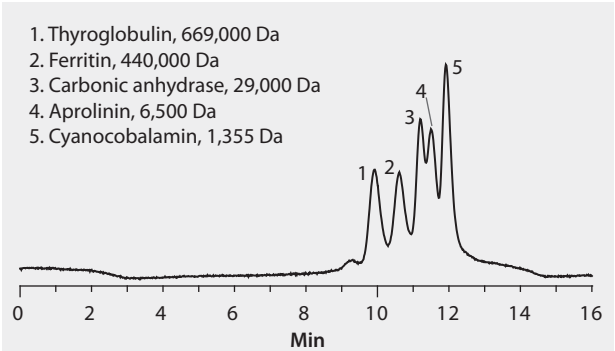



**Gradient Program**

| Time (min) | %B |
|---|---|
| 0 | 0 |
| 10 | 0 |
| 25 | 10 |
| 26 | 40 |

## HPLC Analysis of Proteins on SRT® SEC-1000, 30 cm x 7.8 mm I.D., 5 µm particles

column . . . . . SRT® SEC-1000, 30 cm x 7.8 mm I.D., 5 µm particles (Z777063)
mobile phase . . . . . 150 mM potassium phosphate monobasic, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1-10 g/L in buffer
Application No. . . . . G005726






SUPELCO® Solutions within.™

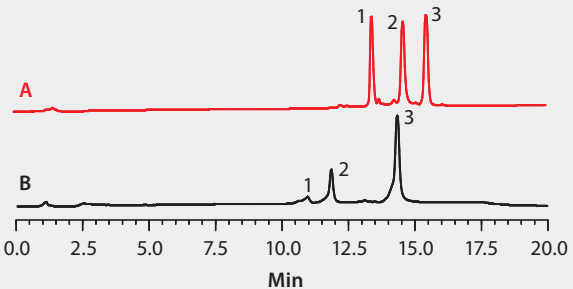
## HPLC Analysis of Proteins on Antibodix® WCX-NP10, Comparison of WCX Columns

column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 6.0; (B) A + 1.0 M NaCl
gradient . . . . . 0 to 100% B in 42 min
flow rate . . . . . 1.0 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL each
Application No. . . . . G005946

**A. Antibodix WCX-NP10**
25 cm × 4.6 mm I.D., 10 µm (non-porous)

**B. Vendor D WCX**
25 cm × 4.0 mm I.D., 10 µm (non-porous)

1. Cytochrome c (12.2 kDa)
2. Lysozyme (14.3 kDa)
3. Ribonuclease A (13.7 kDa)



## HPLC Analysis of Proteins (2) on BIOshell™ A400 Protein C4

This application demonstrates the utility of the BIOshell A400 Protein C4 column for the reversed-phase chromatography of proteins from a variety of sources and of different molecular weights.

sample . . . . . protein mixture, g/L ea: superoxide dismutase, 0.3; bovine serum albumin, 0.3; alpha-lactalbumin, 0.2; beta-lactoglobulin A, 0.3; catalase, 0.3; (carbonic anhydrase, 0.3; beta-Amylase, 1.0; glucose-6-phosphate dehydrogenase, 1.0; thioredoxin, 0.3; phosphorylase b, 2.5.)

column . . BIOshell A400 Protein C4, 10 cm X 2.1 mm I.D., 3.4 µm particles (66825-U)

mobile phase . . . . . (A) 75:25, [0.1% TFA in water]:[0.1% TFA in acetonitrile] ; (B) 25:75, [0.1% TFA in water]:[0.1% TFA in acetonitrile]

gradient . . . . . 15 to 75% B in 10 min

flow rate . . . . . 0.3 mL/min

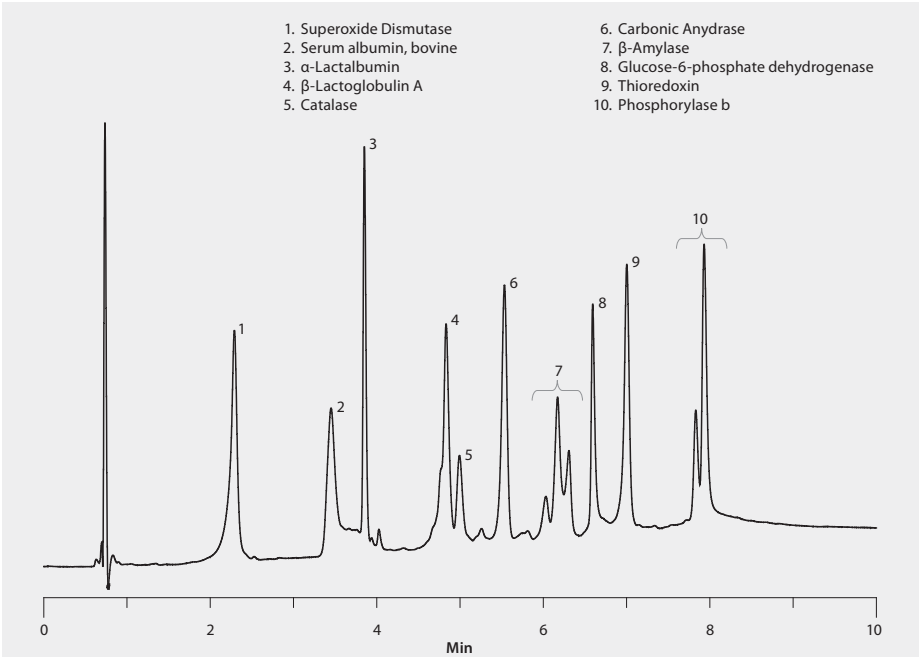
pressure . . . . . 2410 psi (166 bar)

column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

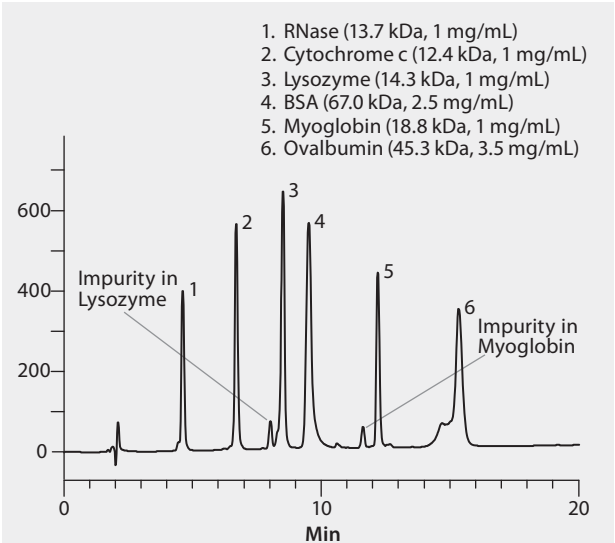
injection . . . . . 1.0 µL

Application No. . . . . G006236



## HPLC Analysis of Proteins on Discovery® BIO Wide Pore C5

column . . . . . Discovery BIO Wide Pore C5, 15 cm × 4.6 mm I.D., 5 µm particles (568422-U)
mobile phase . . (A) (0.1% TFA in water): (B) (0.1% TFA in CH3CN); (75:25, A:B)
gradient . . . . . 0-100% B in 25 min
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 220 nm
injection . . . . . 12 µL in 0.1% TFA
Application No. . . . . G001488B

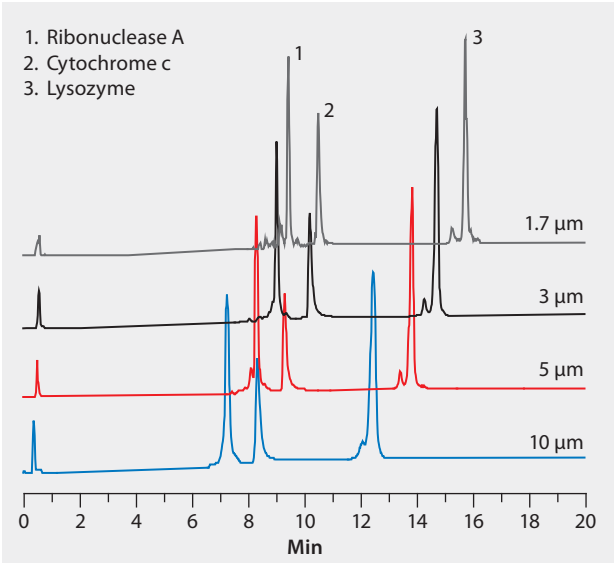





SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on Proteomix® SCX Columns, Effect of Particle Size of Resolution

column . . Proteomix SCX-NP1.7, 5 cm x 4.6 mm I.D., 1.7 µm particles (Z777146)
column . . . . . Proteomix SCX-NP3, 5 cm x 4.6 mm I.D., 3 µm particles (Z777151)
mobile phase . . (A) 20 mM sodium phosphate buffer, pH 6.0, (B) A + 1.0 M NaCl
gradient . . . . . 0-25 min from 0-75% B, 15 min prewash
flow rate . . . . . 0.75 mL/min
column temp. . . . . Ambient
detector . . . . . 214 nm
injection . . . . . 5 µL
sample . . . . . each 1 mg/mL
Application No. . . . . G006134

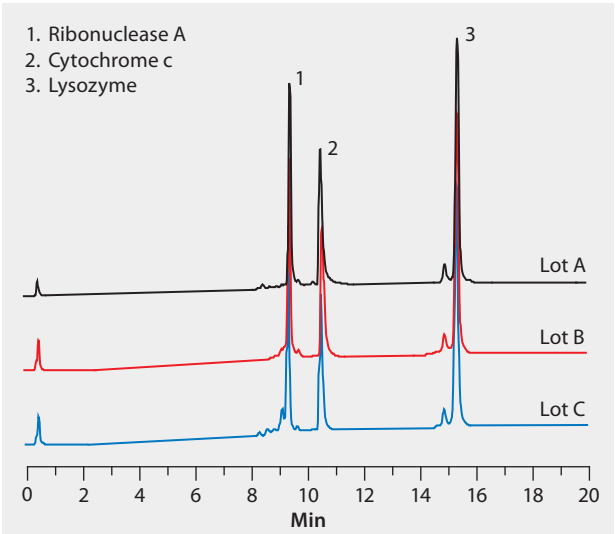





SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on Proteomix® SCX-NP1.7, Demonstration of Lot-to-Lot Reproducibility

column . . Proteomix SCX-NP1.7, 5 cm x 4.6 mm I.D., 1.7 µm particles (Z777146)
mobile phase . . (A) 20 mM sodium phosphate buffer, pH 6.0, (B) A + 1.0 M NaCl
gradient . . . . . 0 - 75% B in 25 min, 15 min prewash
flow rate . . . . . 0.75 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL each compound
Application No. . . . . G006136



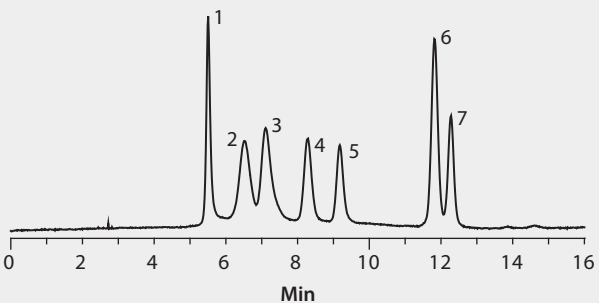



SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on SRT® SEC-150, 30 cm x 7.8 mm I.D., 5 µm particles

column . . . . . SRT® SEC-150, 30 cm x 7.8 mm I.D., 5 µm particles (Z777045)
mobile phase . . . . . 150 mM potassium phosphate, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1-10 g/L in buffer
Application No. . . . . G005728

1. Thyroglobulin, 669,000 Da
2. Alcohol dehydrogenase, 150,000 Da
3. Albumin, 66,000 Da
4. Carbonic anhydrase, 29,000 Da
5. Aprotinin, 6,500 Da
6. Cyanocobalamin, 1,355 Da
7. p-Aminobenzoic acid, 137 Da



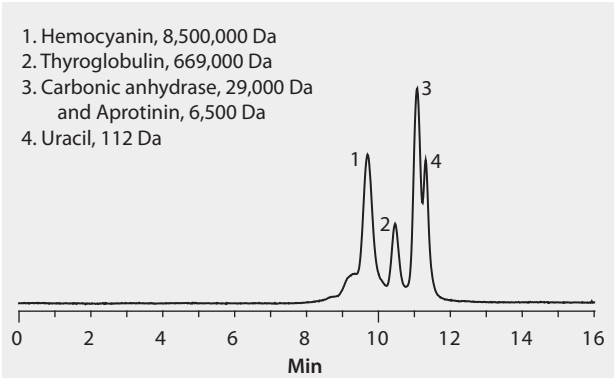



SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on SRT® SEC-2000, 30 cm x 7.8 mm I.D.

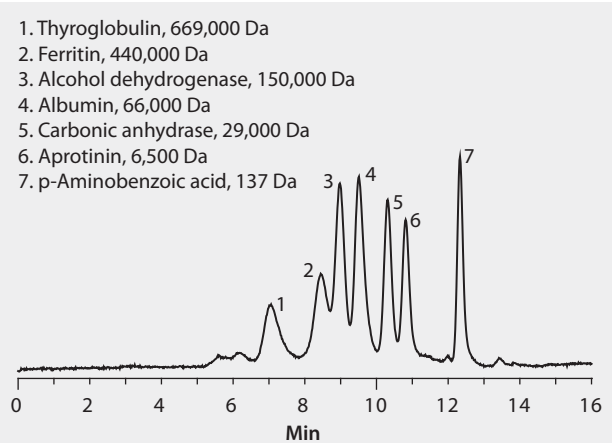
This application demonstrates the suitability of Discovery BIO GFC100 for the separation of proteins. Separation is by inclusion into the pore volume with larger molecules being excluded and therefore eluting earlier.

column . . . . . SRT® SEC-2000, 30 cm x 7.8 mm I.D., 5 µm particles (Z777069)
mobile phase . . . . . 150 mM potassium phosphate monobasic, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1- 10 g/L in buffer
Application No. . . . . G005729



## HPLC Analysis of Proteins on SRT® SEC-300, 30 cm x 7.8 mm I.D., 5 µm

column . . . . . SRT® SEC-300, 30 cm x 7.8 mm I.D., 5 µm particles (Z777059)
mobile phase . . . . . 150 mM potassium phosphate monobasic, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1-10 g/L in buffer
Application No. . . . . G005730



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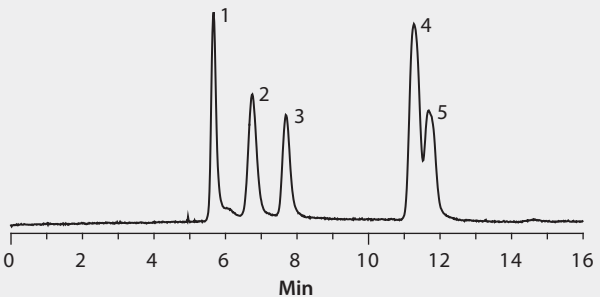
## HPLC Analysis of Proteins on SRT® SEC-300, 30 cm x 7.8 mm I.D., 5 µm particles

column . . . . . SRT® SEC-300, 30 cm x 7.8 mm I.D., 5 µm particles (Z777051)
mobile phase . . . . . 150 mM potassium phosphate, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1-10 g/L in buffer
Application No. . . . . G005725

1. Thyroglobulin, 669,000 Da
2. Carbonic anhydrase, 29,000 Da
3. Aprotinin, 6,500 Da
4. Cyanocobalamin, 1,355 Da
5. p-Aminobenzoic acid, 137 Da

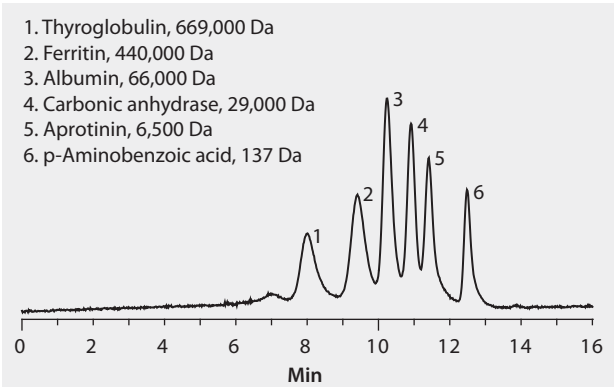





SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on SRT® SEC-500, 30 cm x 7.8 mm I.D.

column . . . . . SRT® SEC-500, 30 cm x 7.8 mm I.D., 5 µm particles (Z777057)
mobile phase . . . . . 150 mM potassium phosphate monobasic, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1-10 g/L in buffer
Application No. . . . . G005733

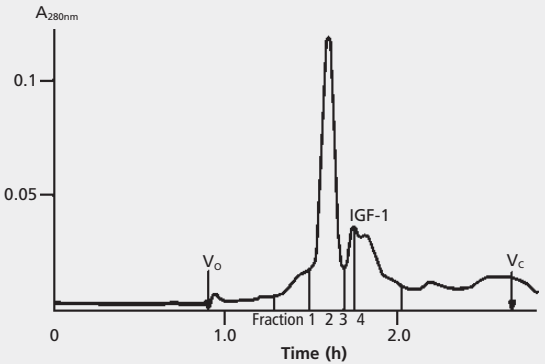





SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on Superdex® Gel Filtration column

column . . . . . HiLoad 16/60 Superdex® 75 prep grade (54802-U)
mobile phase . . . . . 0.15M ammonium acetate, pH 6.0
flow rate . . . . . 0.75 mL/min
detector . . . . . UV, 280 nm
sample . . . . . IGF-1, ZZ fusion protein and impurities
Application No. . . . . 794-0420

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SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on TSKgel® Butyl-NPR

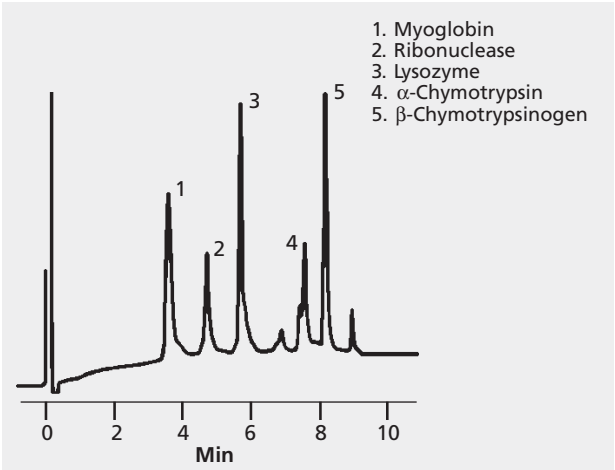
column . . . . TSKgel Butyl-NPR, 3.5 cm × 4.6 mm I.D., 2.5 µm particles (814947)

mobile phase . . . . . (A) 0.1 M phosphate buffer, pH 7.0 plus ammonium sulfate, 2.3 M to 0 M in12 min (B & C) = 0.1 M phosphate buffer, pH 7.0 plus ammonium sulfate, 1.8 M to 0 M in 60 min

flow rate . . . . . 1 mL/min

detector . . . . . UV, 280 nm

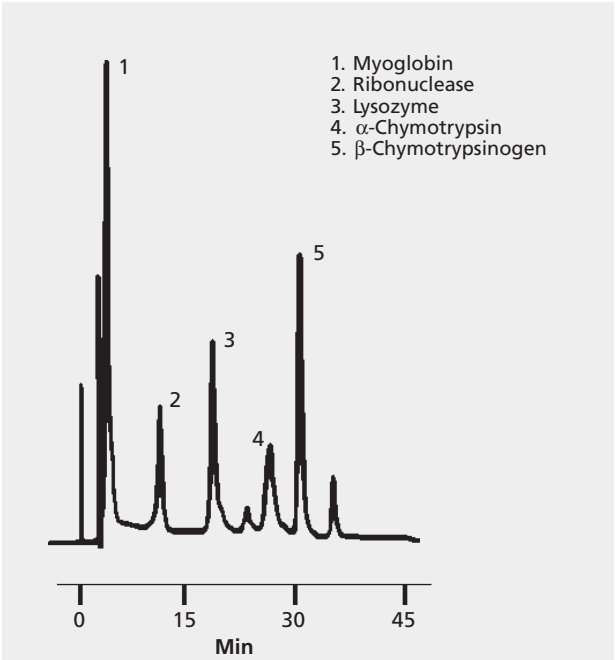
Application No. . . . . 713-1025



## HPLC Analysis of Proteins on TSKgel® Ether-5PW

**Proteins, mixture**

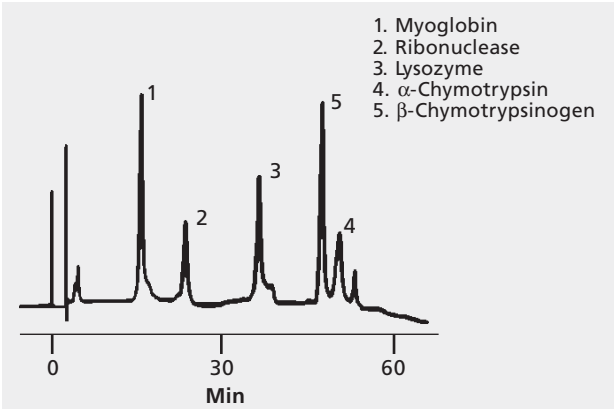
column . . . . TSKgel Ether-5PW, 7.5 cm × 7.5 mm I.D., 10 µm particles (808641)
mobile phase . . . . . 0.1 M phosphate buffer, pH 7.0 plus ammonium sulfate, 1.8 M to 0 M in 60 min
flow rate . . . . . 1 mL/min
detector . . . . . UV, 280 nm
Application No. . . . . 713-1027



## HPLC Analysis of Proteins on TSKgel® Phenyl-5PW

**Proteins, mixture**

column . . . TSKgel Phenyl-5PW, 7.5 cm × 7.5 mm I.D., 10 µm particles (807573)
mobile phase . . . . . 0.1 M phosphate buffer, pH 7.0 plus ammonium sulfate, 1.8 M to 0 M in 60 min
flow rate . . . . . 1 mL/min
detector . . . . . UV, 280 nm
Application No. . . . . 713-1026

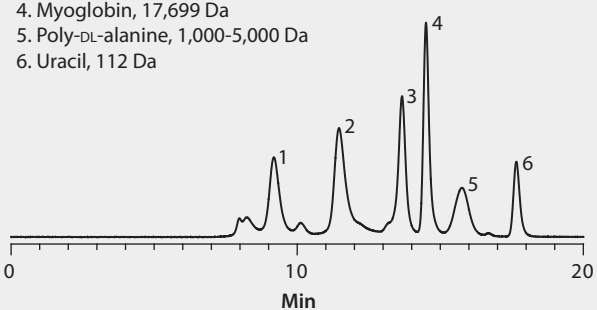


## HPLC Analysis of Proteins on Zenix® SEC-300, 30 cm x 4.6 mm I.D., 3 µm

This application demonstrates the suitability of Discovery BIO GFC300 for the separation of proteins. Separation is by inclusion into the pore volume with larger molecules being excluded and therefore eluting earlier.

column . . . . . Zenix® SEC-300, 30 cm x 4.6 mm I.D., 3 µm particles (Z777033)
mobile phase . . . . . 150 mM potassium phosphate monobasic, pH 7.0 (adjusted with potassium hydroxide)
flow rate . . . . . 0.25 mL/min
pressure . . . . . 652 psi (45 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
sample . . . . . 1 g/L each (except uracil, 0.1 g/L) in mobile phase
Application No. . . . . G005732

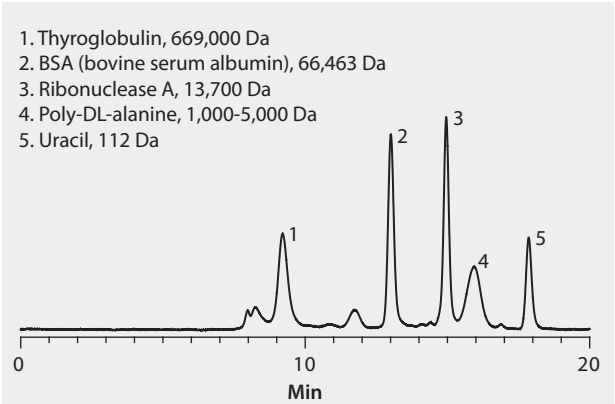
1. Thyroglobulin, 669,000 Da
2. γ-Globulins, ~300,000 Da
3. Ovalbumin, 44,281 Da
4. Myoglobin, 17,699 Da
5. Poly-DL-alanine, 1,000-5,000 Da
6. Uracil, 112 Da



## HPLC Analysis of Proteins on Zenix® SEC-300, 30 cm x 7.8 mm I.D., 3 µm

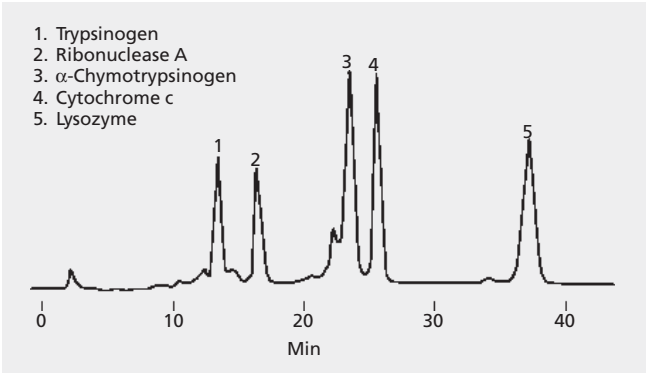
This application demonstrates the suitability of Discovery BIO GFC300 for the separation of proteins. Separation is by inclusion into the pore volume with larger molecules being excluded and therefore eluting earlier.

column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
mobile phase . . . . . 150 mM potassium phosphate monobasic, pH 7.0 (adjusted with potassium hydroxide)
flow rate . . . . . 0.7 mL/min
pressure . . . . . 812 psi (56 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
sample . . . . . 1 g/L each (except uracil, 0.1 g/L) in mobile phase
Application No. . . . . G005731



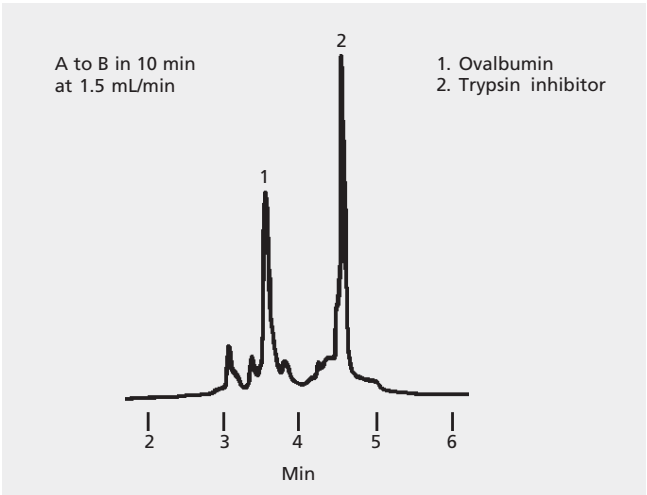
## HPLC Analysis of Proteins on TSKgel® CM-5PW

column . . . . . TSKgel CM-5PW, 7.5 cm × 7.5 mm I.D., 10 µm particles (813068)
mobile phase . . . . . (A) 0.1 M sodium phosphate, monbasic buffer, pH 7.0; (B) 0.5 M sodium chloride, A to B in 60 min
flow rate . . . . . 1 mL/min
detector . . . . . UV, 280 nm
Application No. . . . . 713-1002



## HPLC Analysis of Proteins on TSKgel® DEAE-NP

column . . . . TSKgel DEAE-NPR, 3.5 cm × 4.6 mm I.D., 2.5 µm particles (813075)
mobile phase . . (A) 20 mM Tris-HCl buffer, pH 8.0; (B) 0.5 M sodium chloride in A
detector . . . . . UV, 280 nm
Application No. . . . . 713-1020



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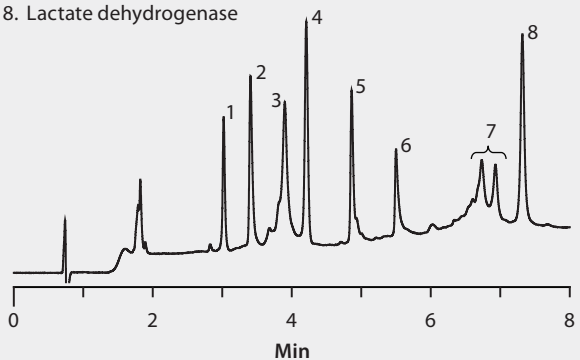
## HPLC Analysis of Protein Standards (1) on BIOshell™ A400 Protein C4

This application demonstrates the utility of the BIOshell A400 Protein C4 column for the reversed-phase chromatography of proteins from a variety of sources and of different molecular weights.

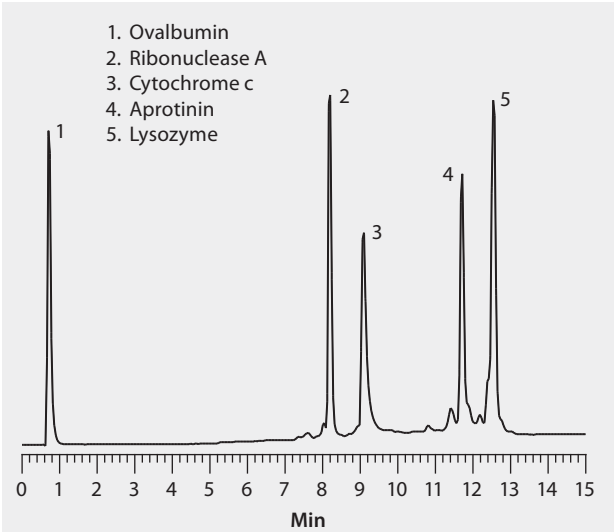
column . . BIOshell A400 Protein C4, 10 cm X 2.1 mm, 3.4 µm particles (66825-U)
mobile phase . . . . . (A) 75:25, [0.1% TFA in water]:[0.1% TFA in acetonitrile] ; (B) 25:75, [0.1% TFA in water]:[0.1% TFA in acetonitrile]
gradient . . . . . 25 to 65% B in 8 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 1430 psi (99 bar)
detector . . . . . UV, 215 nm
injection . . . . . 1.0 µL
sample . . . . . protein mixture, g/L ea: lysozyme, 0.1; haptoglobulin, 0.3; conalbumin, 0.3; trypsin inhibitor, 0.3; glutamate dehydrogenase, 0.8; beta-galactosidase, 0.7; ovalbumin, 0.8; lactate dehydrogenase, 0.4
Application No. . . . . G006201

1. Lysozyme
2. Haptoglobulin, phenotype 1-1
3. Conalbumin
4. Trypsin inhibitor, soybean
5. Glutamate dehydrogenase
6. β-Galactosidase
7. Serum albumin, chicken
8. Lactate dehydrogenase



## HPLC Analysis of Proteins with Different pI Values on Proteomix® SCX-NP3

column . . . . . Proteomix SCX-NP3, 5 cm x 4.6 mm I.D., 3 µm particles (Z777151)
mobile phase . . . . . (A) 10 mM sodium phosphate, pH 6.0; (B) A + 1 M NaCl
gradient . . . . . 0 to 100% B in 15 min
flow rate . . . . . 0.5 mL/min
detector . . . . . UV, 280 nm
Application No. . . . . G006146

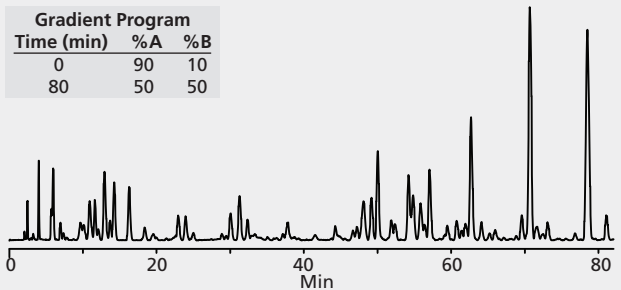


## HPLC Analysis of Protein Tryptic Digest on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 25 cm x 2.1 mm I.D., 5 µm particles (565306-U)
mobile phase . . (A) (50:50) 50 mM formic acid titrated with acetate (pH 3.0):water;
(B) (50:50) 50 mM formic acid titrated with acetate (pH 3.0):acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . ambient
detector . . . . . ESI (+)
injection . . . . . 4 µL
sample . . . . . tryptic digest of carboxymethylated protein mixture 1.5 g/L in 50 mM formic acid
Application No. . . . . G002670

**Gradient Program**

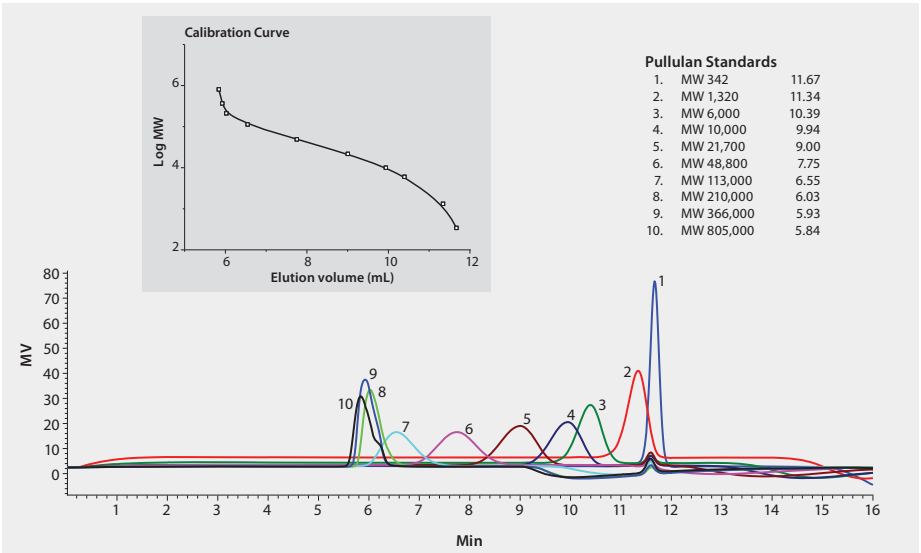
| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 80 | 50 | 50 |



## HPLC Analysis of Pullulan on an SRT® SEC-300 Gel Filtration Column

Pullulan is a water-soluble polysaccharide polymer consisting of glucan units. Pullulan is used in the manufacture of edible films that are used in breath fresheners and mints and is also used as a food additive. Another use of pullulan is as calibration standards in gel filtration chromatography.
Gel filtration chromatography (GFC) is used to determine the molecular mass composition of pullulan and many other water-soluble polymers using silica- or polymer-based particles that have been modified with a chemically bonded hydrophilic coating.
In this particular example, a SRT SEC-300 column from Sepax Technologies was used to analyze several pullulan standards. In the accompanying table shows retention volumes for the pullulan standards and the molecular mass of each; this data was then used to construct the calibration curve which allows the calculation of the molecular mass and distribution of an unknown pullulan polymer or other polymers of similar geometrical shape.

column . . . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm, 300 Å (Z777051)
mobile phase . . . . . 150 mM phosphate Buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
pressure . . . . . 600 psi (41.4 bar)
column temp. . . . . 25 °C
detector . . . . . RI (30° C)
injection . . . . . 20 µL
sample . . . 10 mg/mL in mobile phase 0.342 kD, 1.32 kD, 6 kD, 10 kD, 21.7 kD, 48.8 kD 113 kD, 210 kD, 366 kD and 805 kD
Application No. . . . . G006202

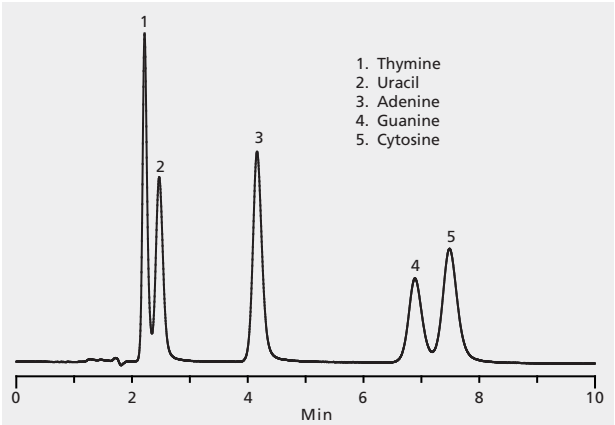





SUPELCO® Solutions within.™

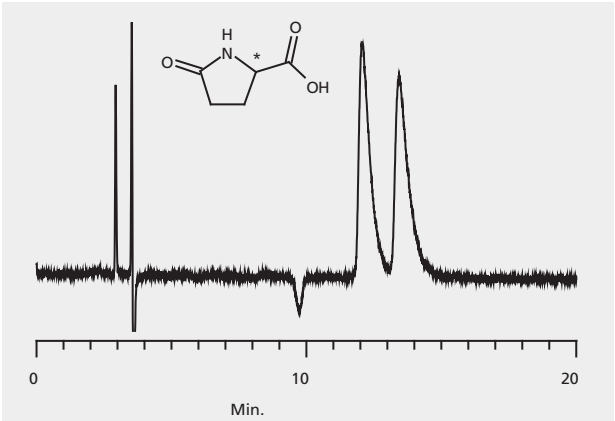
## HPLC Analysis of Purines and Pyrimidines of Nucleic Acids on Ascentis® Si (Silica)

column . . . . . Ascentis Si, 10 cm × 2.1 mm I.D., 5 µm particles (581508-U)
mobile phase . . . . . (A) 200 mM ammonium formate (pH 3.0, with formic acid): (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 270 nm
injection . . . . . 1 µL
sample . . . . . 100 µg/mL in mobile phase
Application No. . . . . G003683



## HPLC Analysis of Pyroglutamic Acid Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) triethylamine; (54.5:45:0.3:0.2, A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004511



## HPLC Analysis of Recombinant Tumor Necrosis Factor Receptor-Fc Fusion Protein (rhTNFR-Fc) on Zenix® SEC-300 versus SRT® SEC-300, Effect of Particle Size on Resolution

column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777051)

column . . . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm, 300 Å particles (Z777051) (Z777033)

mobile phase . . . . . 150 mM sodium phosphate, pH 7.0

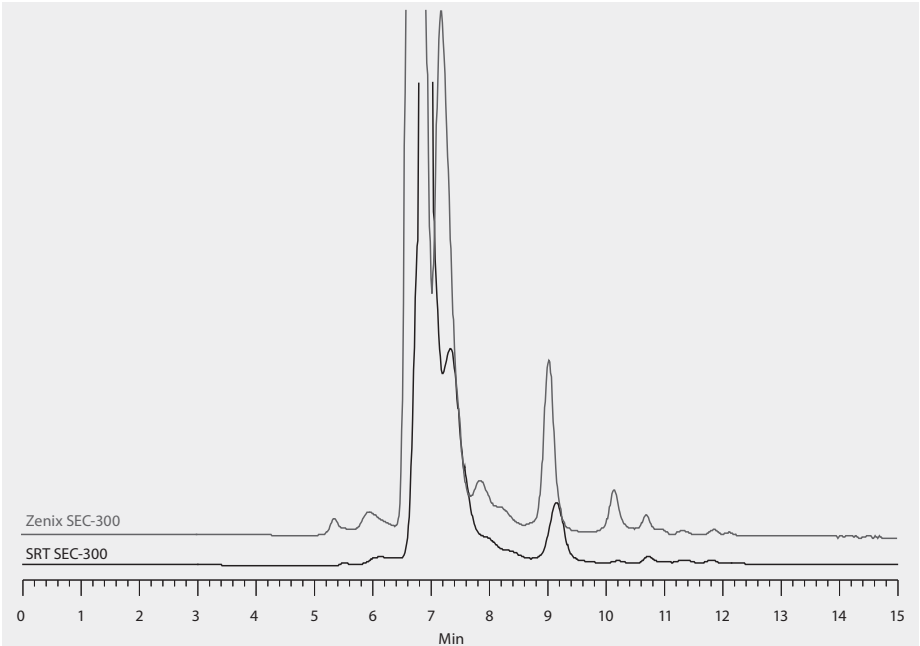
flow rate . . . . . 1 mL/min

detector . . . . . UV, 214 nm

injection . . . . . 20 µL

sample . . . . . Recombinant tumor necrosis factor receptor-Fc fusion protein (rhTNFR-Fc), including oligomers (440 kD and 360 kD), monomer (150 kD), and impurity (120 kD)

Application No. . . . . G006121



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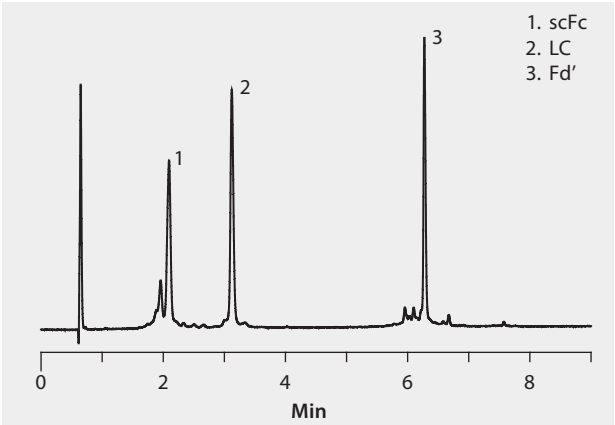
## HPLC Analysis of Reduced Monoclonal Antibody Fragments on BIOshell™ A400 Protein C4

Digesting an antibody into a few large fragments facilitates a middle-down approach for subsequent structural characterization. The IdeS enzyme, from Streptococcus pyogenes and commercialized as FabRICATOR, cleaves IgG in the hinge region resulting in two fragments: F(ab')2 and scFc. Reduction of F(ab')2 dissociates it into its component polypeptides, Fd' and LC. This application demonstrates the baseline resolution of the three fragments scFC, LC, and Fd', in reasonable time with excellent peak shape.

Monoclonal antibody SO57 (0.5 mg) was digested by IdeS protease according to Genovis (www.genovis.com) FragIT kit protocol. The digest was then exposed to 10 mM dithiobutylamine for one hour, at pH 7, before being diluted 1:5 with mobile phase A prior to injection; final protein conc. <0.3 g/L.

Genovis FragIT kit is available from Sigma, outside the USA.

column . . BIOshell A400 Protein C4, 10 cm x 2.1 mm I.D., 3.4 µm particles (66825-U)
gradient . . . . . 30 to 70%B in 12 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 1000 psi initial (69 bar)
column temp. . . . . 90 °C
detector . . . . . UV, 215 nm
injection . . . . . 1.0 µL
mobile phase . . . . . (A) 80:20, [0.1% TFA in water]:[0.1% TFA in acetonitrile] ; (B) 50:50, [0.1% TFA in water]:[0.1% TFA in acetonitrile]
Application No. . . . . G006235



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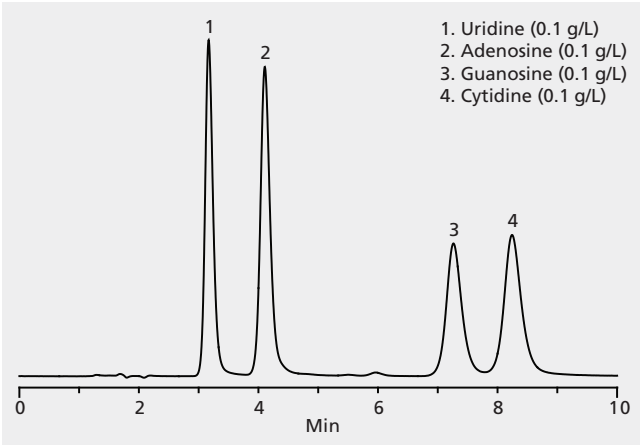
## HPLC Analysis of Ribonucleosides on Ascentis® Si

column . . . . . Ascentis Si, 10 cm x 2.1 mm I.D., 5 µm particles (581508-U)
mobile phase . . . . . (A) 150 mM ammonium formate (pH 3.0, with formic acid): (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 270 nm
injection . . . . . 1 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003725

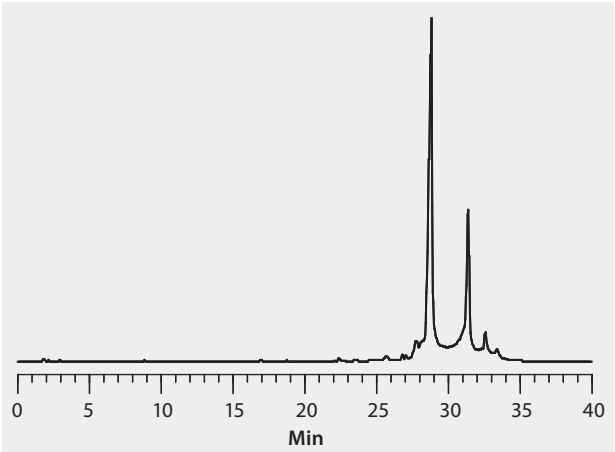





SUPELCO® Solutions within.™

## HPLC Analysis of RNA on Proteomix® WAX-NP5

column . . . Proteomix WAX-NP5, 15 cm x 4.6 mm I.D., 5 µm particles (Z777262)
mobile phase . . . . . (A) 20 mmol Tris, pH 6.0; (B): A + 1.0 M NaCl
gradient . . . . . 20%-100%B (40 min)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 1015 psi (70 bar)
column temp. . . . . Ambient
detector . . . . . UV, 260 nm
injection . . . . . 5 µL
sample . . . . . RNA
Application No. . . . . G005967



## HPLC Analysis of RP Peptide Performance Standard on Discovery® BIO Wide Pore C18, No TFA

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 2.1 mm I.D., 3 µm particles (567202-U)

mobile phase . . (A) 25 mM formic acid in water; (B) (50:50) 25 mM formic acid in water:20 mM formic acid in acetonitrile

flow rate . . . . . 0.20 mL/min

column temp. . . . . ambient

detector . . . . . +ESI

injection . . . . . 1 µL

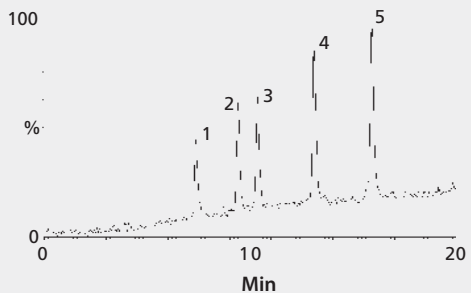
sample . . . . . RP Peptide Performance Standard, p/n RPS-P0010 (Alberta Peptide Institute)

Application No. . . . . G001638A

Peptide 1: RGAGGLGLGK-amide
Peptide 2: ac-RGGGGLGLGK-amide
Peptide 3: ac-RGAGGLGLGK-amide
Peptide 4: ac-RGVGGLGLGK-amide
Peptide 5: ac-RGVVGLGLGK-amide

| Gradient | | |
|---|---|---|
| Min | %A | %B |
| 0 | 90 | 10 |
| 20 | 50 | 50 |

0% TFA (25 mM formic acid), 1 L injection

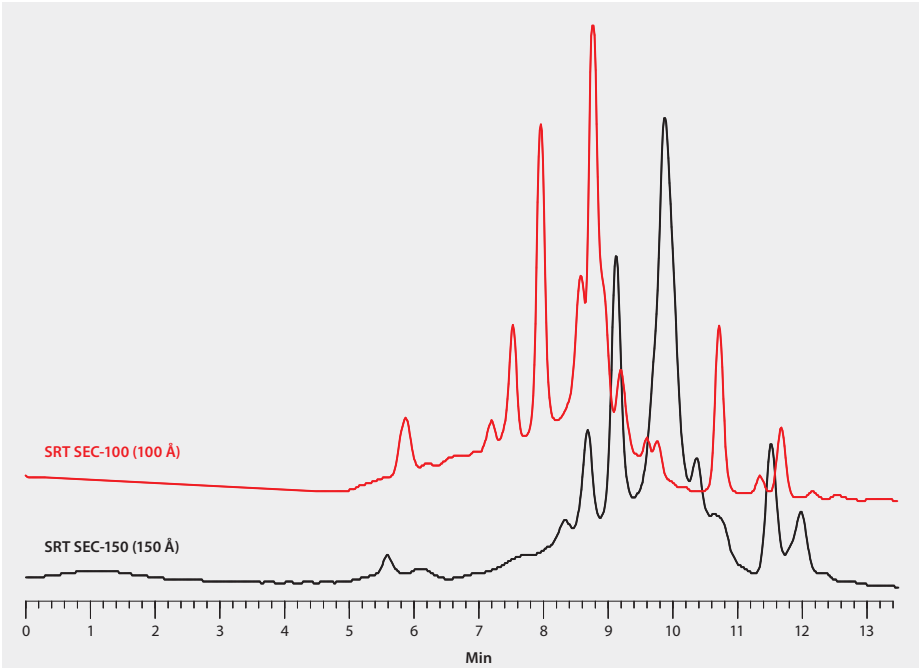


## HPLC Analysis of Separation of Low MW Polysaccharides on SRT®

Achyranthes bidentata polysaccharides (ABPS) is extracted from the root of a Chinese medicinal herb. ABPS acts as an immune modulator. It is tested for use as a replacement of antibiotics added to feedstock to protect piglets from microorganisms. ABPS contains mainly fructose and glucose units. The chromatograms show that the smallest pore size column, SRT SEC-100, provides the largest number of peaks, which is not unexpected given the low molecular mass (< 10,000 Da) of the sample.

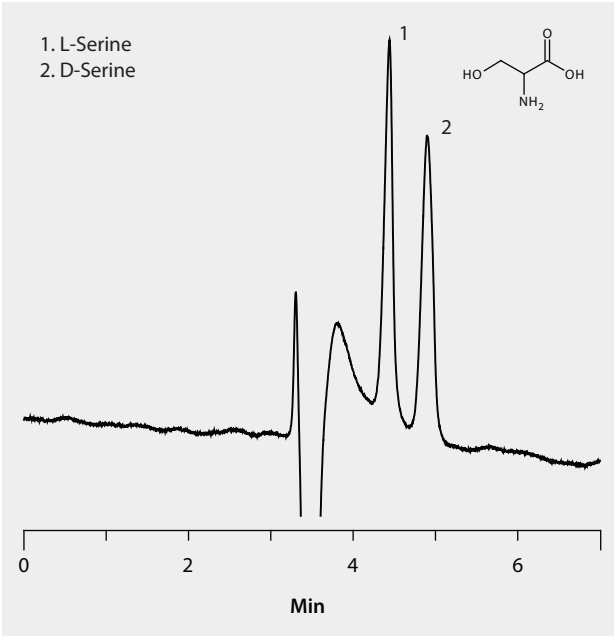
**Note** that since ABPS does not carry an electrical charge, a 15 mM buffer concentration is sufficient to prevent interaction of the sample with the underlying silica surface.

column . . . . SRT SEC-100 30 cm x 4.6 mm I.D., 5 µm particles, 100 Å (Z777037)
column . . . . SRT SEC-150 30 cm x 4.6 mm I.D., 5 µm particles, 150 Å (Z777043)
mobile phase . . . . . 0.15 M phosphate buffer, pH 7
flow rate . . . . . 0.35 mL/min
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . Achyranthes bidentata polysaccharides (ABPS) from plant root extract (MW < 10,000)
Application No. . . . . G006207



## HPLC Analysis of Serine Enantiomers on Astec® CHIROBIOTIC® T (Formic Acid Mobile Phase Additive)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005344

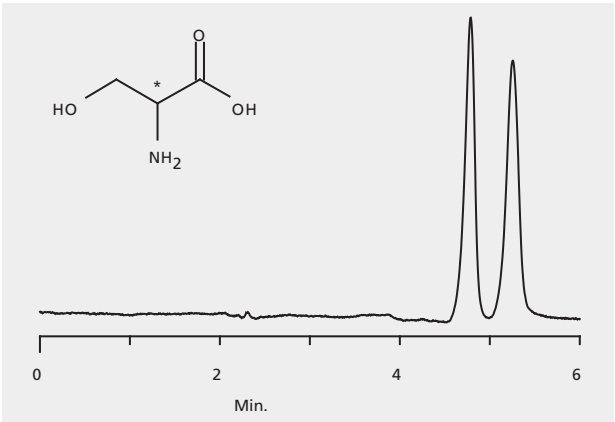





SUPELCO® Solutions within.™

## HPLC Analysis of Serine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004513

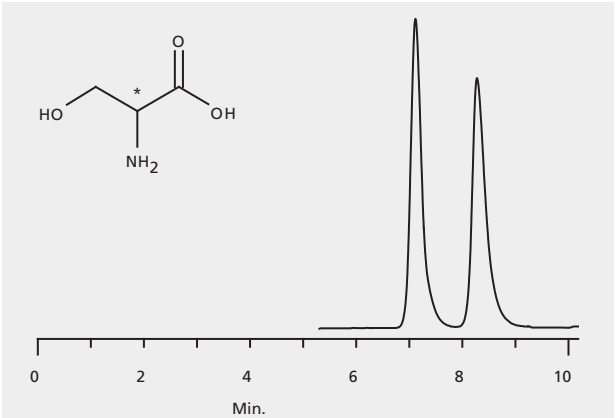





SUPELCO® Solutions within.™

## HPLC Analysis of Serine Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 10 mg/mL in methanol
Application No. . . . . G004614

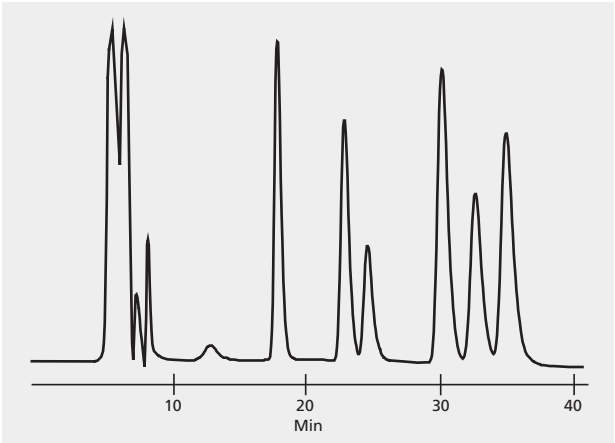





SUPELCO® Solutions within.™

## HPLC Analysis of Six Enkephalin Peptides (Chiral and Achiral) on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . (A) 5 mM ammonium formate, pH 3.3; (B) acetonitrile; (25:75, A:B)
flow rate . . . . . 0.5 mL/min
detector . . . . . UV, 230 nm
Application No. . . . . G004738

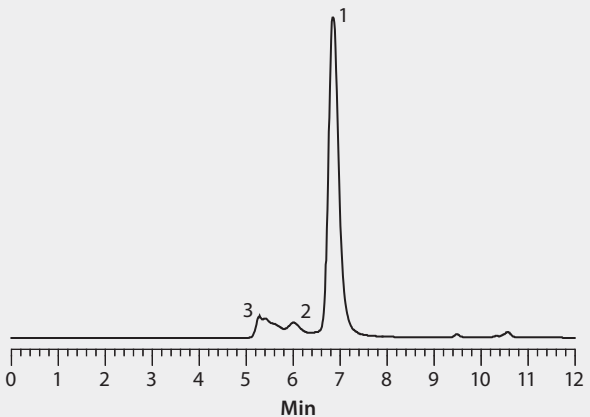


## HPLC Analysis of Size Exclusion Separation of Proteins and PEGylated Proteins

Pegylation of biotherapeutic drugs improves solubility, increases half life in the blood stream and reduces immunogenicity. Size exclusion chromatography (SEC) is often applied to characterize protein–polyethylene glycol (PEG) conjugates and establish the number of attached PEG chains, since SEC is a precise and robust technique that combines ease of set up and straighthforward method develpopment.

column . . Zenix SEC-150, 30 cm x 7.8 mm I.D., 3 µm particles, 150 Å, (Z777018)
mobile phase . . . . . 150 mM phosphate buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . Ambient
detector . . . . . UV, 214 nm
injection . . . . . 20 µL, 2.0 mg/mL
sample . . . . . 1. Protein with MW 70KD (from an undisclosed drug development company) 2. Protein 1 attached with 1 PEG (MW 40KD) molecule 3, Protein 1 attached with 2 PEG (MW 40KD) molecules
Application No. . . . . G006197

1. 70 kDa protein (undisclosed drug candidate)
2. 40 kDa PEG molecule attached to protein identified as peak 1
3. Two PEG molecules attached to protein identified as peak 1

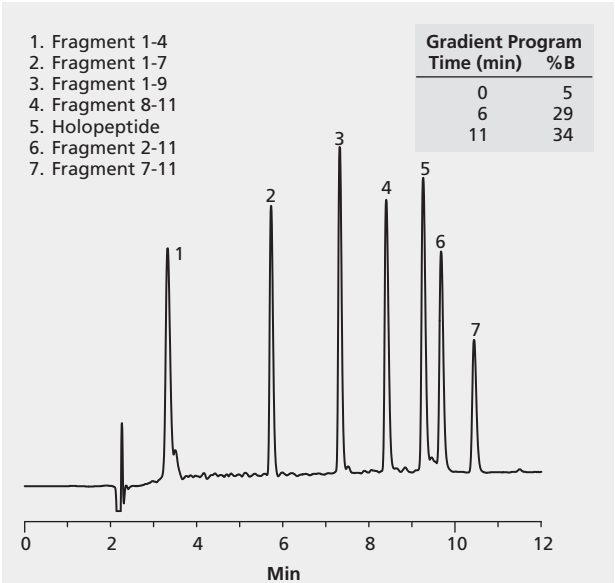





SUPELCO® Solutions within.™

## HPLC Analysis of Substance P and Its Fragments on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) water + 0.1% TFA; (B) acetonitrile + 0.1% TFA
gradient . . . . . linear; 5-29%B (6 min); 29-34B (5 min)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
sample . . . . . Substance P in initial mobile phase
Application No. . . . . G000322

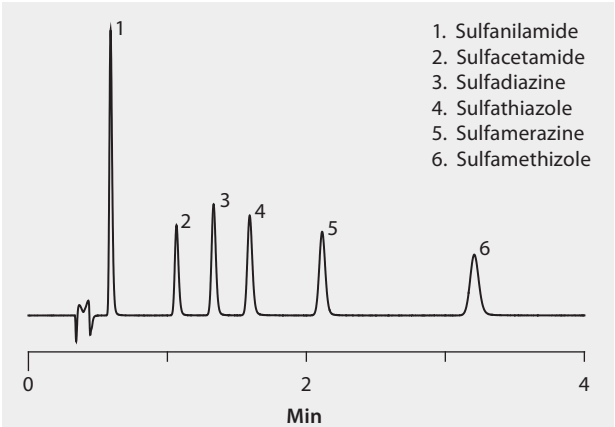





SUPELCO® Solutions within.™

## HPLC Analysis of Sulfa Drugs on Titan™ C18

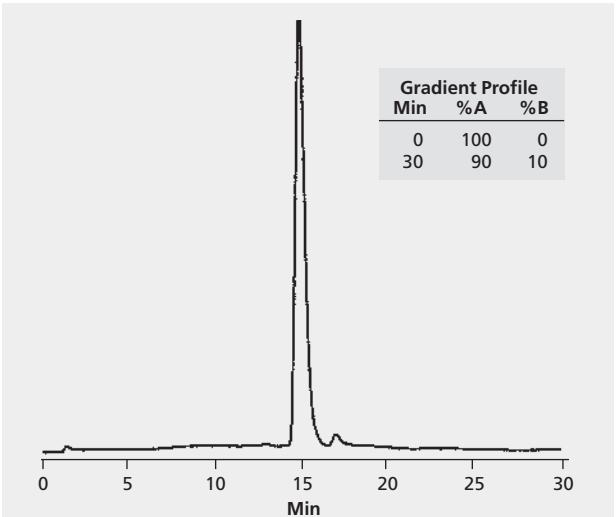
column . . . . . Titan C18 5 cm x 2.1 mm I.D., 1.9 µm particle (577122-U)
mobile phase . . . . . (A) water with 0.1% acetic acid; (B) acetonitrile with 0.1% acetic acid ; (90:10, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 2670 psi (184 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 250 nm
injection . . . . . 2 µL
sample . . . . . 25 µg/mL in 70:30, water:methanol
Application No. . . . . G006114



## HPLC Analysis of Superoxide Dismutase on Discovery® BIO PolyMA-WAX

Discovery BIO PolyMA-SCX and PolyMA-WAX columns give sharp, efficient peaks for a wide variety of proteins.

column . . Discovery BIO PolyMA-WAX, 5 cm × 4.6 mm, 5 µm particles (59602-U)
mobile phase . . . . . ((A) 20 mM Tris, pH 8.0 with hydrocloric acid; (B) 20 mM Tris, 0.5 M sodium chloride, pH 8.0 with hydrochloric acid)
gradient . . . . . 0 to 10% B in 30 min. (linear)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
Application No. . . . . G001835

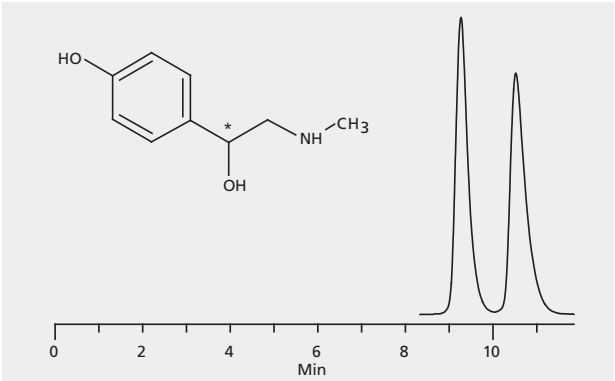





SUPELCO® Solutions within.™

## HPLC Analysis of Synephrine Enantiomers on Astec® CYCLOBOND® I 2000 AC

column . . CYCLOBOND I 2000 AC, 25 cm x 4.6 mm I.D., 5 µm particles (20124AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (95:5, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 10 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004695






SUPELCO® Solutions within.™

## HPLC Analysis of the Monoclonal Antibody (mAb) Erbitux (Cetuximab) on BIOshell™ A400 Protein C4

Antibodies can exhibit considerable hydrophobicity, and so efficient reversed-phase elution of this intact mAb requires the use of high temperature. The top chromatogram illustrates that BIOshell™ Fused-Core® columns can be operated at high flow rate while maintaining good peak shape at a reasonable back pressure.

column . . . . . BIOshell™ A400 Protein C4, 10 cm X 2.1 mm I.D., 3.4 µm particles (66825-U)

mobile phase . . . . . (A) 80:20, 0.1% TFA in water: 0.1% TFA in acetonitrile; (B) 20:80, 0.1% TFA in water: 0.1% TFA in acetonitrile

gradient . . . . . 20 to 35%B in 10 min (or 5 min)

flow rate . . . . . 0.2 mL/min

pressure . . . . . 470 psi (or 940 psi)

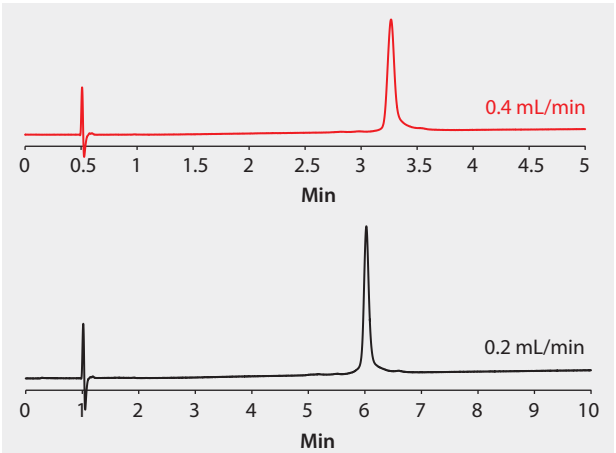
column temp. . . . . 90 °C

detector . . . . . UV, 215 nm

injection . . . . . 0.4 µL

sample . . . . . 1 g/L mAb in 20 mM Tricine/KOH, pH 7

Application No. . . . . G006254

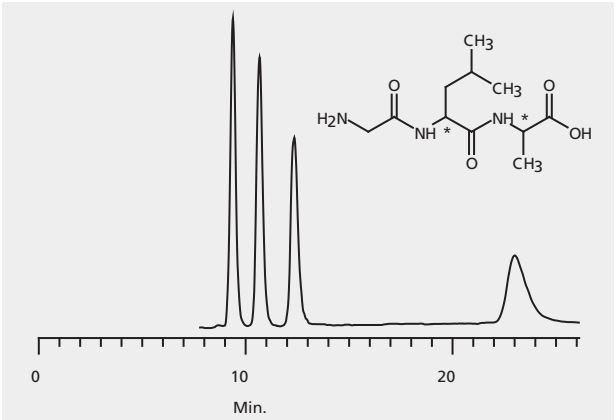





SUPELCO® Solutions within.™

## HPLC Analysis of the Peptide Glycine-Leucine-Alanine Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . (A) 5 mM ammonium acetate, pH 4.1; (B) acetonitrile; (35:65, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004617



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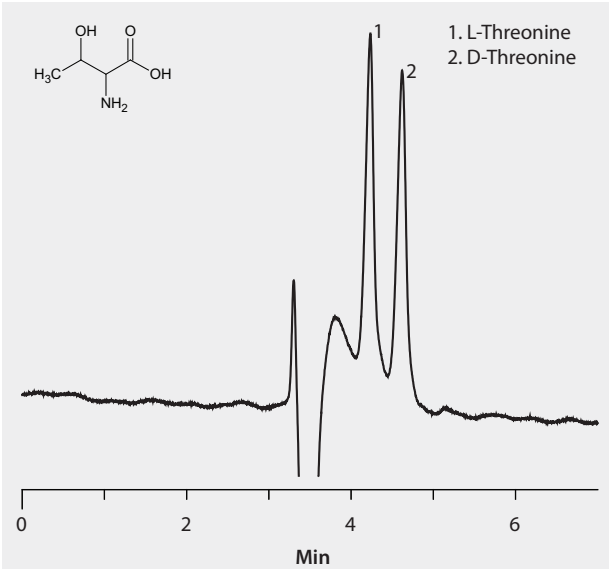
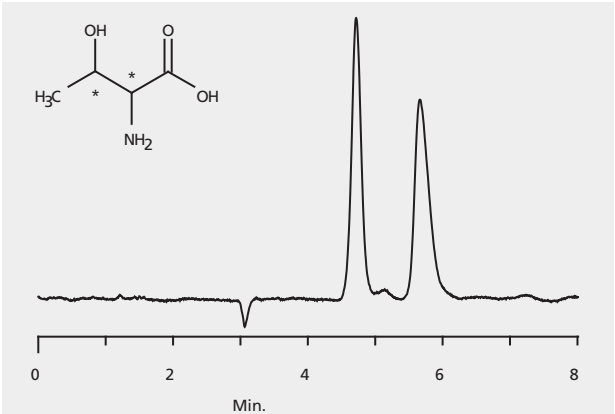
## HPLC Analysis of Threonine Enantiomers on Astec® CHIROBIOTIC® T (Formic Acid Mobile Phase Additive)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A); water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005345



## HPLC Analysis of Threonine Enantiomers on Astec® CHIROBIOTIC® T

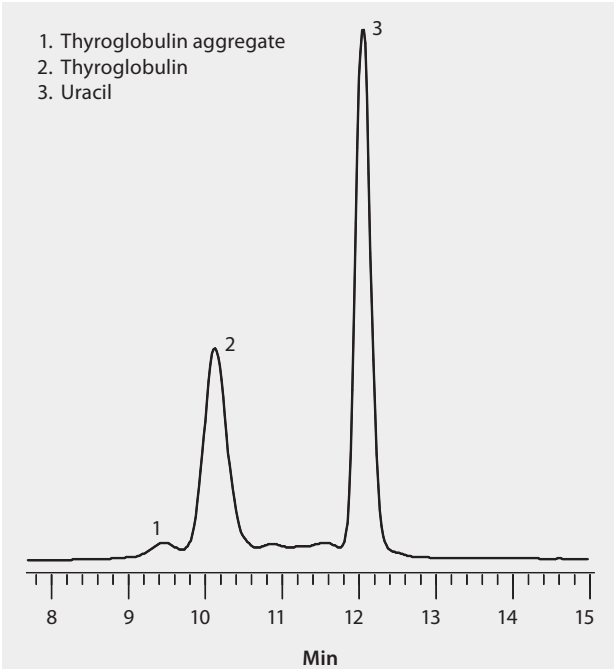
column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004517



## HPLC Analysis of Thyroglobulin on a SRT® SEC-1000 Gel Filtration Column

Thyroglobulin is a very large protein, with a molecular mass of 670 kDa. Thus,a large pore size gel filtration columns is required to prevent partial exclusion of not just the monomer, but also of the dimer and higher order aggregates. This was accomplished using the Sepax SRT SEC-1000 column, which has a nominal pore size of 1000 Angstrom. The nucleic acid base Uracil was added to the sample as a marker to indicate the inclusion volume since a small molecule like Uracil is not restricted from entering any pores in the column.

column . . SRT SEC-1000, 30 cm x 4.6 mm I.D., 5 µm particles, 1000 Å (Z777061)
mobile phase . . . . . 150 mM phosphate buffer, pH 7.0
flow rate . . . . . 0.35 mL/min
column temp. . . . . Ambient
detector . . . . . UV, 214 nm
injection . . . . . 3.0 µL
sample . . . . . 1. Thyroglobulin (670kD) 1 mg/mL;
2. Uracil (120D), 0.1 mg/mL
Application No. . . . . G006181



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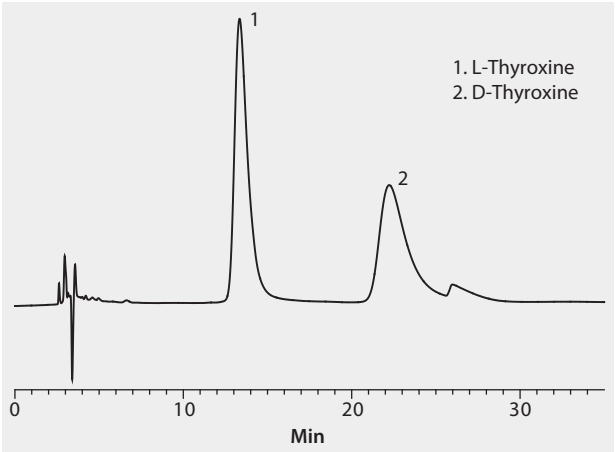
## HPLC Analysis of Thyroxine Enantiomers on Astec® CHIROBIOTIC® TAG

Baseline resolution of the chiral enantiomers of DL-Thyroxine is achieved by polar ionic mode on the Astec CHIROBIOTIC TAG with the use of an LC/MS-compatible mobile phase.

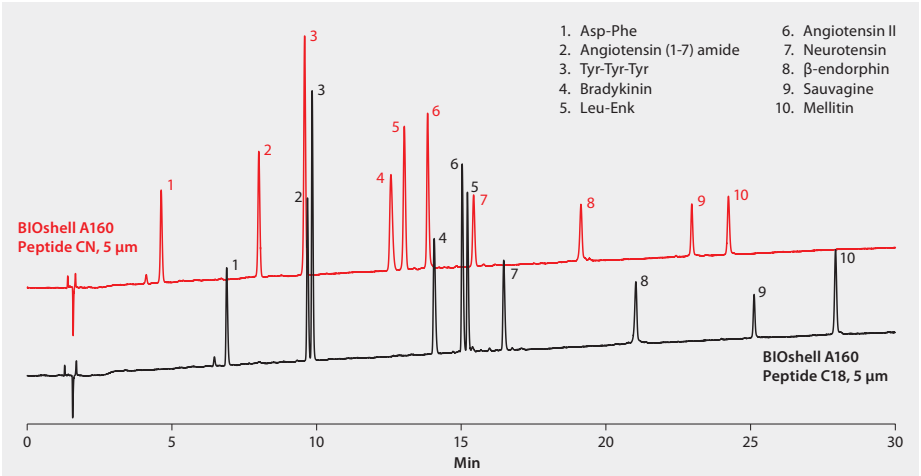
column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . 1% ammonium formate in methanol
flow rate . . . . . 1 mL/min
pressure . . . . . 826 psi (57 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . each enantiomer 0.1 mg/L in methanol
Application No. . . . . G005515



## HPLC Analysis of BIOshell™ A160 Peptide C18 and CN 5 µm Columns, Comparison of Different Phases

The availability of two distinct chemistries (C18 alkyl vs. CN) is helpful to check changes in elution order as we see for peaks # 5 and # 6 on the C18 and CN columns. As expected, peptide retention is shorter on the CN column, while peak shape is symmetrical and efficiency very high on both column types.

column . . BIOshell A160 Peptide C18, 15 cm x 4.6 mm I.D., 5 µm particles (67015-U)
column . . BIOshell A160 Peptide CN, 15 cm x 4.6 mm I.D., 5 µm particles (67081-U)
mobile phase . . . . . (A): water/0.1% TFA (B): acetonitrile/0.1% TFA
gradient . . . . . 5-50%B in 30 minutes
flow rate . . . . . 1.0 mL/min
pressure . . . . . 126 bar, 250 bar
column temp. . . . . 40 °C
detector . . . . . UV, 215 nm
injection . . . . . 10 µL
sample . . . . . 0.01 mg/mL in water/0.1% TFA
Application No. . . . . G006219



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## HPLC Analysis of tRNA Hydrolysate on SUPELCOSIL™ LC-18-S

column . . . . SUPELCOSIL LC-18-S, 15 cm × 4.6 mm I.D., 5 µm particles (58931)
mobile phase . . (A) 0.1M potassium phosphate, dibasic (pH 5.3):methanol, 97.5:2.5; (B) 0.01M potassium phosphate, dibasic (pH 5.1):methanol, 80:20; (C) 0.01M potassium phosphate, dibasic (pH 4.9):acetonitrile, 65:35
flow rate . . . . . 1 mL/min
column temp. . . . . 26 °C
detector . . . . . UV, 210 nm, 254 nm, 280 nm
injection . . . . . 50 µL hydrolysate buffer (pH 7.8) containing 5 µg bovine $tRNA^{Leu}$ hydrolysate
Application No. . . . . 713-0501

**Gradient Program**

| Time (min) | %A | %B | %C |
|---|---|---|---|
| 0 | 100 | — | — |
| 7.2 | 100 | — | — |
| 12 | 90 | 10 | — |
| 15 | 75 | 25 | — |
| 19.2 | 40 | 60 | — |
| 21.6 | 38 | 62 | — |
| 27 | — | 100 | — |
| 48 | — | — | 100 |
| 55 | — | — | 100 |

**Selective, Quantitative Analysis**

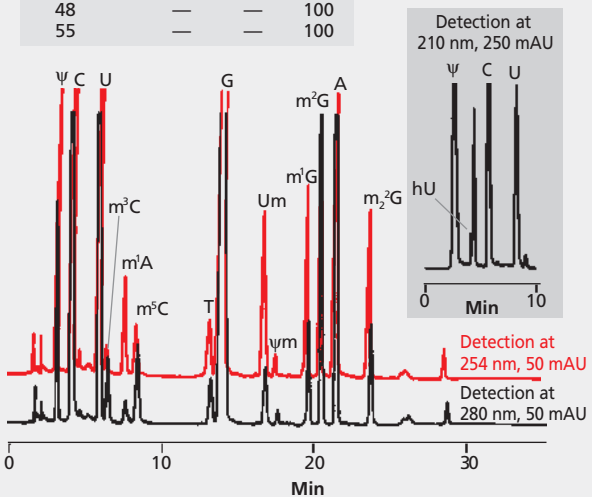
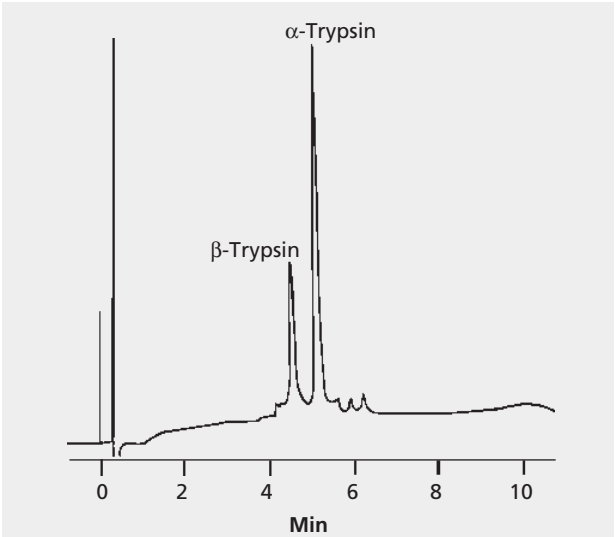



Figure provided by Dr. C.W. Gehrke and Mr. K.C. Kuo of the Interdisciplinary Chromatography-Mass Spectrometry Laboratories, University of Missouri (Columbia, MO, USA).

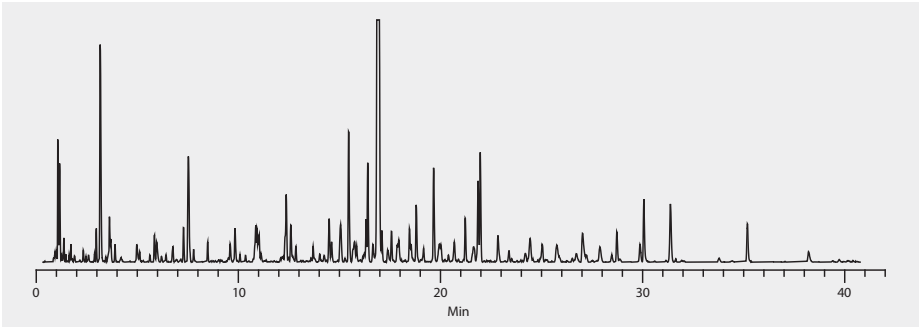
## HPLC Analysis of Trypsin on TSKgel® Butyl-NPR

column . . . . TSKgel Butyl-NPR, 3.5 cm × 4.6 mm I.D., 2.5 µm particles (814947)
mobile phase . . . . . 2 M to 0 M ammonium sulfate in 20 mM Tris-HCl buffer, pH 7.5 in 10 min
flow rate . . . . . 1 mL/min
detector . . . . . UV, 280 nm
injection . . . . . commercial trypsin sample
Application No. . . . . 713-1204



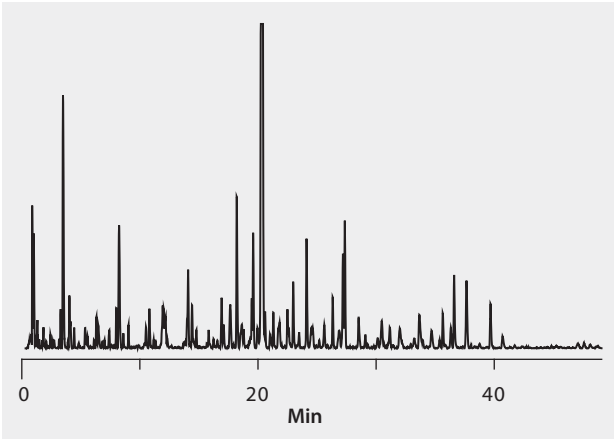
## HPLC Analysis of Tryptic Digest on Ascentis® Express Peptide ES-C18

column . . . . . Ascentis Express Peptide ES-C18, 15 cm x 2.1 mm I.D., 2.7 µm particles (53307-U)
mobile phase . . . . . (A) 0.1% formic acid in water; (B) 25:75, (0.4% formic acid in water):acetonitrile
gradient . . . . . 0 to 70% B in 40 min.
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), TOF
injection . . . . . 2 µL
sample . . . . . 10 pg/L in 0.1% formic acid
Application No. . . . . G005381



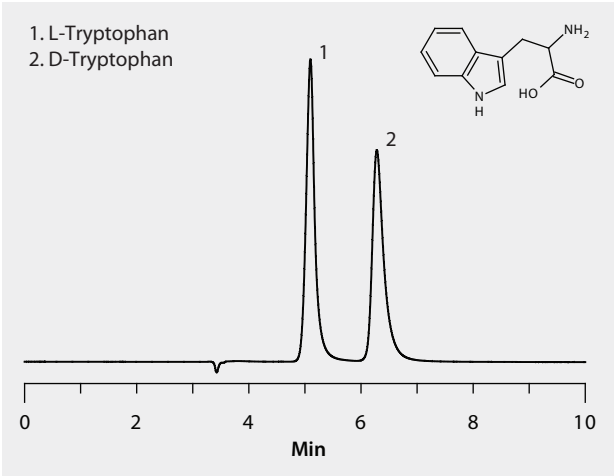
## HPLC Analysis of Tryptic Digest of Protein Mixture on Ascentis® Express Peptide ES-C18

| | |
|---|---|
| column | Ascentis Express Peptide ES-C18, 15 cm x 21 mm I.D., 2.7 µm particles (53307-U) |
| mobile phase | (A) 0.1% formic acid in water; (B) 0.4% formic acid in 25:75 (v/v) water:acetonitrile |
| gradient | 6 to 70% B in 40 min |
| flow rate | 0.3 mL/min |
| column temp. | 35 °C |
| detector | ESI(+), TOF |
| injection | 2 µL |
| sample | 10 pmol/L in 0.1% formic acid |
| Application No. | G005629 |



## HPLC Analysis of Tryptophan Enantiomers on Astec® CHIROBIOTIC® T (Formic Acid Mobile Phase Additive)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005346



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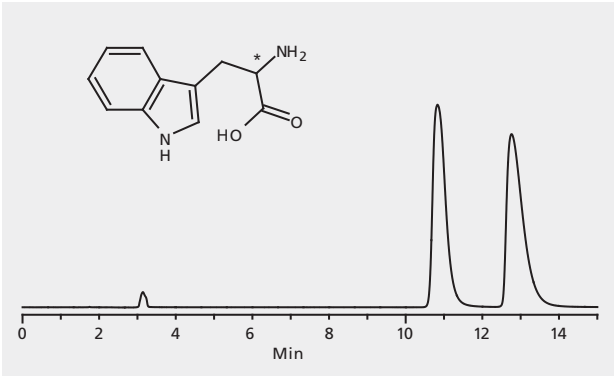
## HPLC Analysis of Tryptophan Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004521



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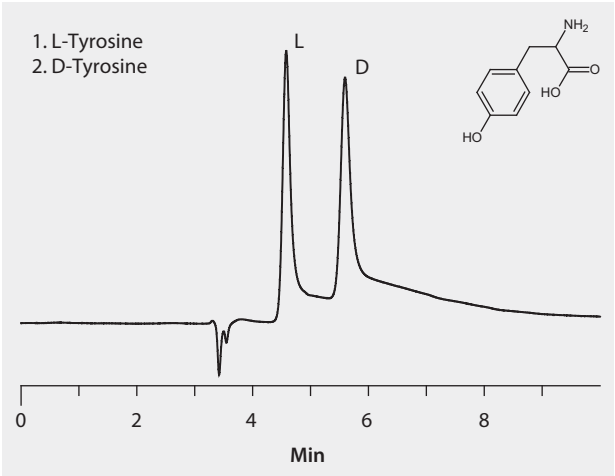
## HPLC Analysis of Tyrosine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005347



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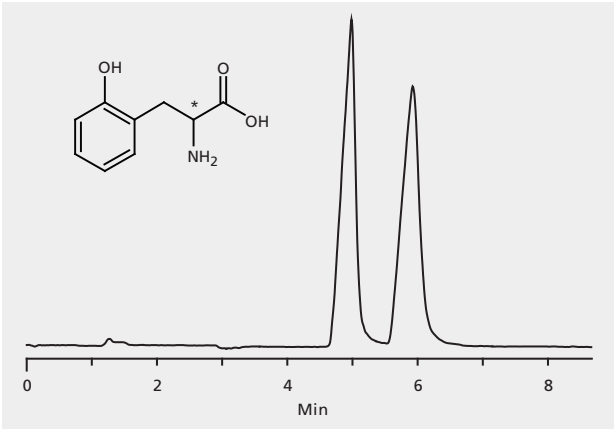
## HPLC Analysis of o-Tyrosine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 24 °C
detector . . . . . UV, 210 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004537



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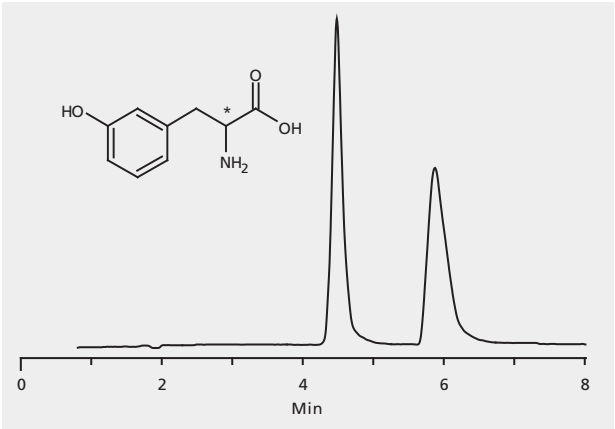
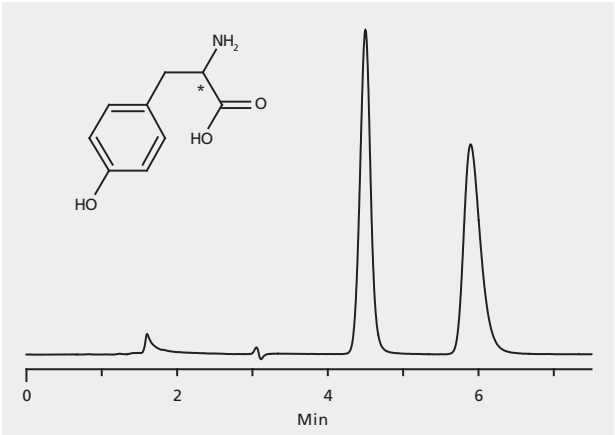
## HPLC Analysis of m-Tyrosine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 210 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004536



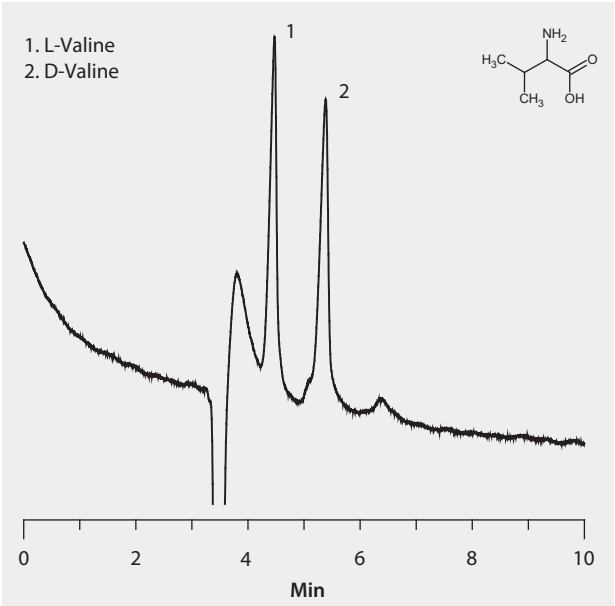
## HPLC Analysis of p-Tyrosine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004522



## HPLC Analysis of Valine Enantiomers on Astec® CHIROBIOTIC® T (Formic Acid Mobile Phase Additive)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) methanol; (C) formic acid; (30:70:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 300 µg /mL in 30:70 water:methanol
Application No. . . . . G005348



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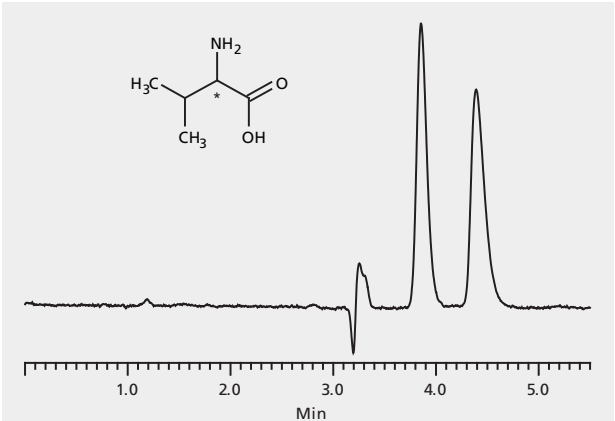
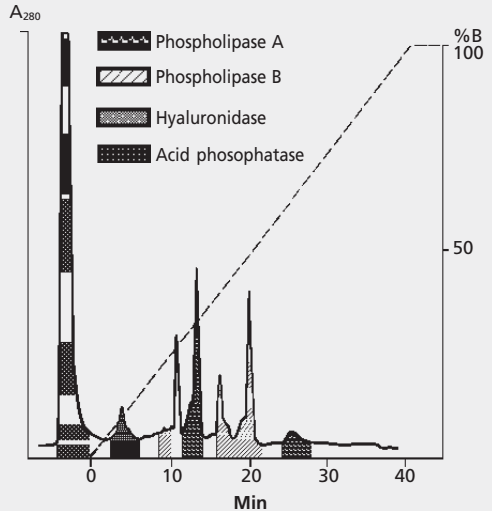
## HPLC Analysis of Valine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004523



## HPLC Analysis of White-Faced Hornet Venom on Mono S® HR5/5

column . . . . . Mono S HR5/5, 10 µm particles (54808)
mobile phase . . . . . (A) 50 mM BICINE, pH 8.4; (B) A + 0.35 M sodium chloride
flow rate . . . . . 1 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 7 mg venom in mobile phase A
Application No. . . . . 794-0411

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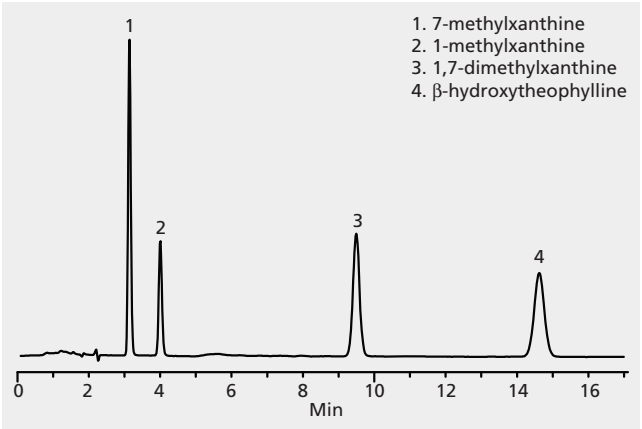
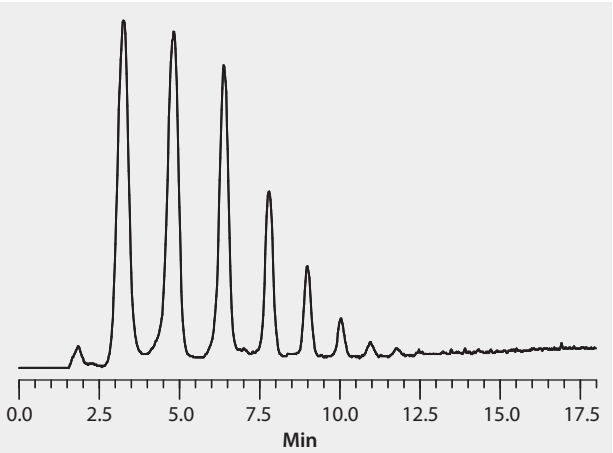
## HPLC Analysis of Xanthines on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) methanol; (94:6, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002465



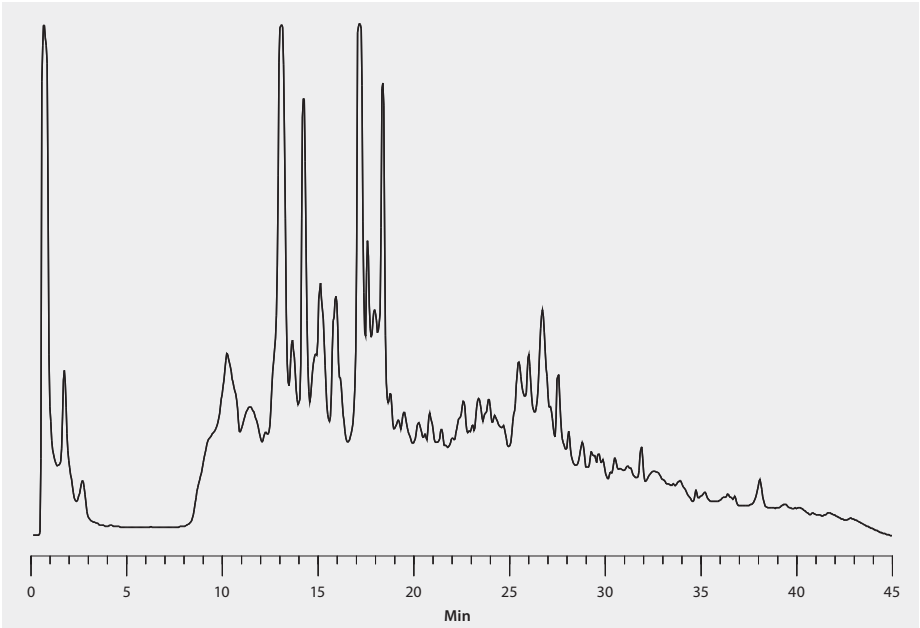
## HPLC Analysis of Xylo-oligosaccharide on Proteomix® SAX-NP5

column . . . . Proteomix SAX-NP5, 15 cm x 4.6 mm I.D., 5 µm particles (Z777228)
mobile phase . . . . . (A) 0.05% ammonium hydroxide in acetonitrile
(B) 0.05% ammonium hydroxide in water
gradient . . . . . 15-60% B (20 min)
flow rate . . . . . 0.7 mL/min
pressure . . . . . 1015 psi (70 bar)
temp. . . . . 35 °C
detector . . . . . ELSD
injection . . . . . 10 µL
sample . . . . . Xylose oligosaccharides
Application No. . . . . G005960



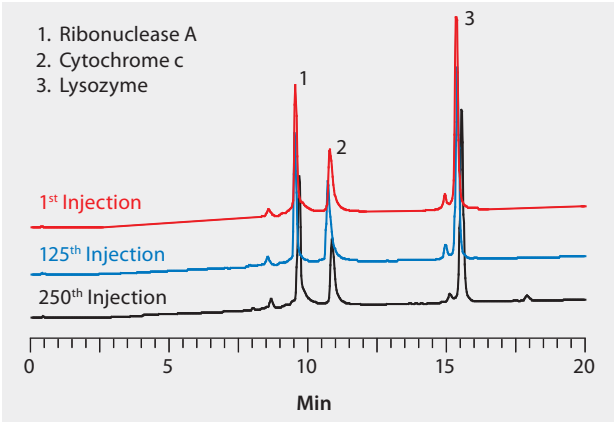
## HPLC Analysis of Yeast Lysate on Proteomix® SAX-NP3 Demonstrating High Separation Efficency

column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
column . . . . Proteomix SAX-NP3, 5 cm x 4.6 mm I.D., 3 µm particles (Z777219)
mobile phase . . . . . (A) 20 mM Tris, pH 9.0; (B) A + 0.5 M NaCl
gradient . . . . . 0 - 100% B in 30 min
flow rate . . . . . 0.5 mL/min
injection . . . . . 40 µL
sample . . . . . 2.5 mg/mL
Application No. . . . . G006131



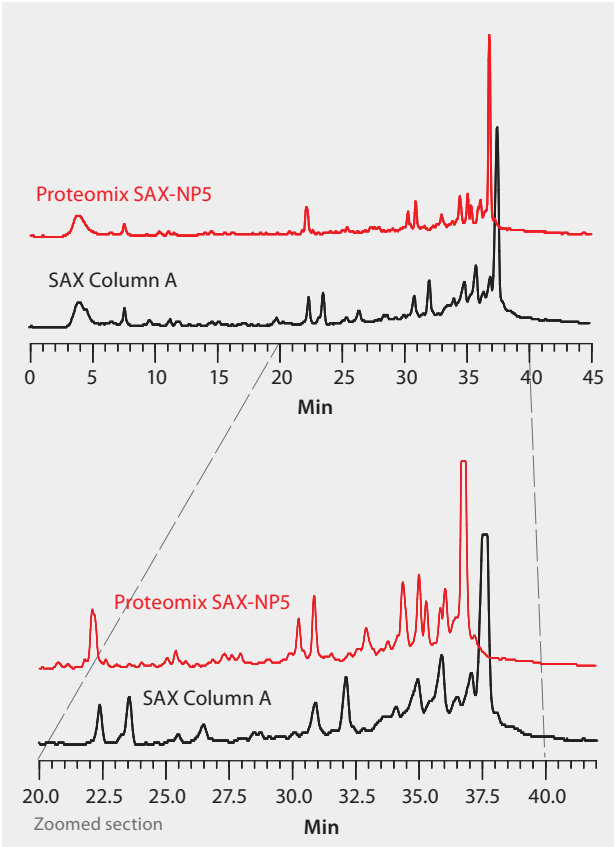
## HPLC Analysis Proteins on Proteomix® SCX-NP1.7, Demonstration of Column Stability with Repeated Injections

column . . Proteomix SCX-NP1.7, 5 cm x 4.6 mm I.D., 1.7 µm particles (Z777146)
column . . . . . Proteomix SCX-NP1.7 guard cartridge with holder, 1 cm x 4 mm I.D. (Z777144)
mobile phase . . (A) 20 mM sodium phosphate buffer, pH 6.5, (B) A + 1.0 M NaCl
gradient . . . . . 0 - 100% B in 25 min, 15 min prewash
flow rate . . . . . 0.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . each compound 1 mg/mL
Application No. . . . . G006135



## HPLC Separation of an Oligonucleotide and Impurities on Proteomix® SAX-NP5 versus Porous SAX

column . . . . Proteomix SAX-NP5, 15 cm x 4.6 mm I.D., 5 µm particles (Z777228)
mobile phase . . . . . (A) 0.025 M Tris buffer pH9; (B) A + 1.0 M LiCi
gradient . . . . . 30 to 75% B in 45 min
flow rate . . . . . 1.0 mL/min
detector . . . . . UV, 260 nm
injection . . . . . 50 µL
sample . . . . . 50-mer Oligo
Application No. . . . . G005961



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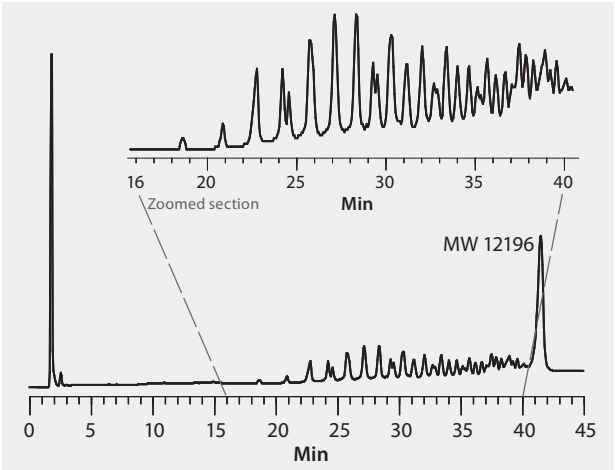
## HPLC Separation of an Oligonucleotide and Its Degradation Fragments on Proteomix® SAX-NP5

column . . . . Proteomix SAX-NP5, 15 cm x 4.6 mm I.D., 5 µm particles (Z777228)
mobile phase . . . . . (A) 25 mM Tris, 1 mM EDTA 10% acetonitrile (8.0 pH); (B) A + 1.0 M NaCl
gradient . . . . . 0 to 75% B in 50 min
flow rate . . . . . 0.5 mL/min
detector . . . . .
sample . . . . . Oligonucleotide (MW 12196) and its degradation fragments
Application No. . . . . G005962

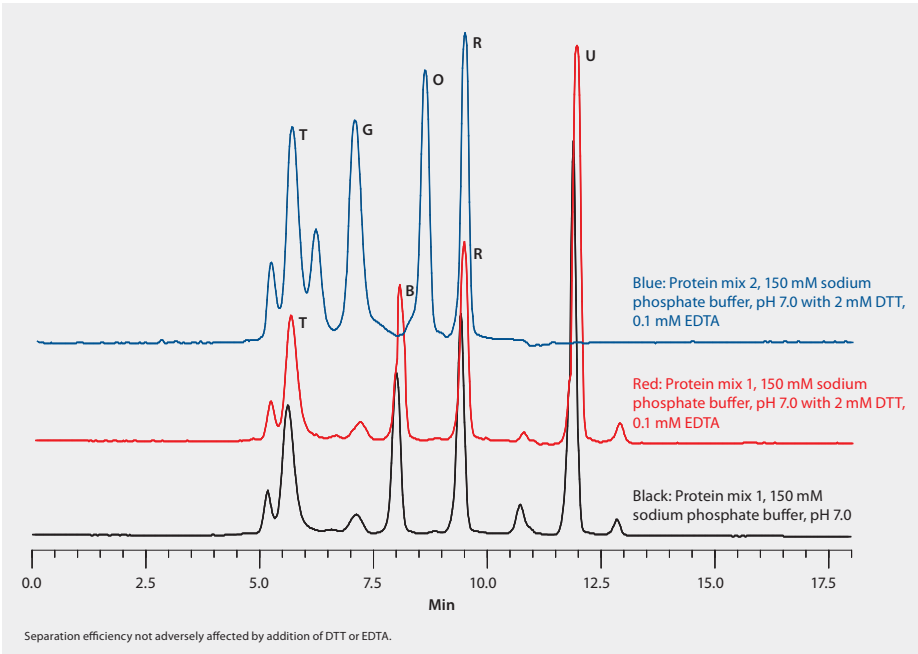





SUPELCO® Solutions within.™

## HPLC (Size Exclusion) Analysis of Proteins on Zenix®-C SEC-300, Effect of DTT and EDTA

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
mobile phase . . (A) 150 mM sodium phosphate buffer, pH 7.0 (B) 150 mM sodium phosphate buffer, pH 7.0 with 2 mM DTT, 0.1 mM EDTA (C) 150 mM sodium phosphate buffer, pH 7.0 with 2 mM DTT, 0.1 mM EDTA
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . proteins
Application No. . . . . G005954




Separation efficiency not adversely affected by addition of DTT or EDTA.

## Improved HPLC Selectivity of Discovery® BIO Wide Pore RP Phases

column . . Discovery BIO Wide Pore C18, 15 cm x 4.6 mm I.D., 5 µm particles vs. competitive wide pore C18 column of same dimensions (568222-U)

mobile phase . . . . . (A) 0.1% TFA in 80:20 water:acetonitrile; (B) 0.1% TFA in 66:34 water:acetonitrile

Gradient of 0 to 100% B in 14 min. after 1 min. delay

gradient . . . . . 0 to 100% B in 14 min. after 1 min. delay

flow rate . . . . . 1 mL/min

column temp. . . . . 30 °C

detector . . . . . UV, 220 nm

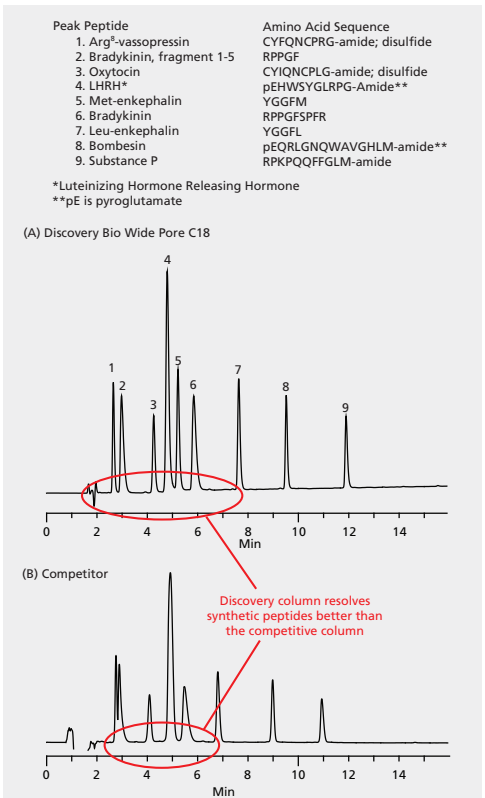
injection . . . . . 10 µL

sample . . . . peptide mixture (Sigma P2693), ~0.25 µg each peptide in 0.1% TFA

Application No. . . . . G004415

| Peak Peptide | Amino Acid Sequence |
|---|---|
| 1. Arg$^8$-vassopressin | CYFQNCPRG-amide; disulfide |
| 2. Bradykinin, fragment 1-5 | RPPGF |
| 3. Oxytocin | CYIQNCPLG-amide; disulfide |
| 4. LHRH* | pEHWSYGLRPG-Amide** |
| 5. Met-enkephalin | YGGFM |
| 6. Bradykinin | RPPGFSPFR |
| 7. Leu-enkephalin | YGGFL |
| 8. Bombesin | pEQRLGNQWAVGHLM-amide** |
| 9. Substance P | RPKPQQFFGLM-amide |

*Luteinizing Hormone Releasing Hormone
**pE is pyroglutamate

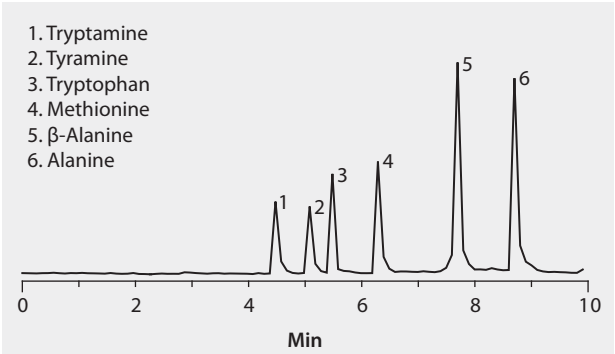





SUPELCO® Solutions within.™

## LC-MS Analysis of Amino Acids on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . (A) 13 mM ammonium acetate in 10:90 (v/v) water: acetonitrile;
(B) 13 mM ammonium acetate in water
gradient . . . . . held at 0% B for 1 min; 0 to 90% B in 19 min
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+)
injection . . . . . 10 µL
sample . . . . . 10 mg/L in Mobile Phase A
Application No. . . . . G005664



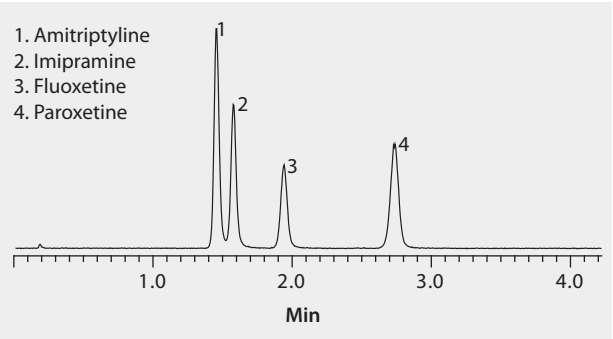



SUPELCO® Solutions within.™

## LC-MS Analysis of CYP2D6 Inhibitors on Ascentis® Express HILIC

This application demonstrates the suitability of the Ascentis Express HILIC for the analysis of CYP2D6 Inhibitors.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . 5 mM ammonium formate in 2:98 (v/v) water:acetonitrile
flow rate . . . . . 1.2 mL/min
column temp. . . . . 50 °C
detector . . . . . ESI(+), m/z 100-400
injection . . . . . 0.5 µL
Application No. . . . . G005691

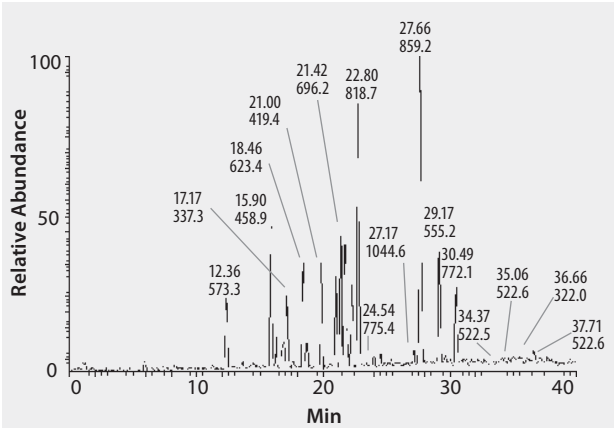





SUPELCO® Solutions within.™

## LC-MS Analysis of β-Lactoglobulin Tryptic Digest on a Discovery® BIO Wide Pore C18 Capillary Column

| | |
|---|---|
| column | Discovery BIO Wide Pore C18, 15 cm × 0.5 mm I.D., 5 µm particles (65519-U) |
| mobile phase | (A) 0.1% TFA in water; (B) 0.1% TFA in acetonitrile |
| gradient | 5-40% B in 70 min |
| flow rate | 14 µL/min |
| column temp. | 30 °C |
| detector | +ESI mode |
| injection | 500 pmol (5µL) β-Lactoglobulin tryptic digest in 50 mM ammonium bicarbonate |
| Application No. | G001591 |



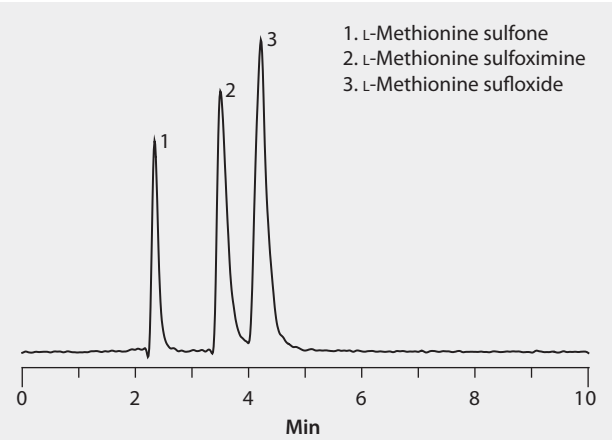



SUPELCO® Solutions within.™

## LC-MS Analysis of L-Methionine Sulfoximine, Sulfone and Sulfoxide on Ascentis® Express HILIC

L-methionine sulfoximine and L-buthionine sulfoximine are used in are used to prevent additional enzyme activity in Chinese hampster ovary (CHO) cell lines with supplemental Glutamine Synthase (GS). It is of interest to be able to monitor both purity of such molecules in order to control addition to cell media as well as for the assay of parent molecules during use. The analytes are highly polar and thus should be amenable to HILIC-LC-MS analysis. This first installment investigates the ability of Ascentis Express HILIC and Ascentis Express OH5 stationary phases for the separation of the methionine analog and related compounds as well as methionine and buthionine separations.

column . . Ascentis Express HILIC, 10 cm x 3.0 mm I.D., 2.7 µm particles (53970-U)
mobile phase . . . . . (A) acetonitrile; (B) water with 0.1% formic acid, pH 3.5 with ammonium formate; (75:25, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1350 psi 93 bar
temp. . . . . 25 °C
detector . . . . . MS, ESI (+), TIC, m/z 150-300
injection . . . . . 5 µL
sample . . . . . 10 µg/mL in 90:10, methanol:water
Application No. . . . . G005790

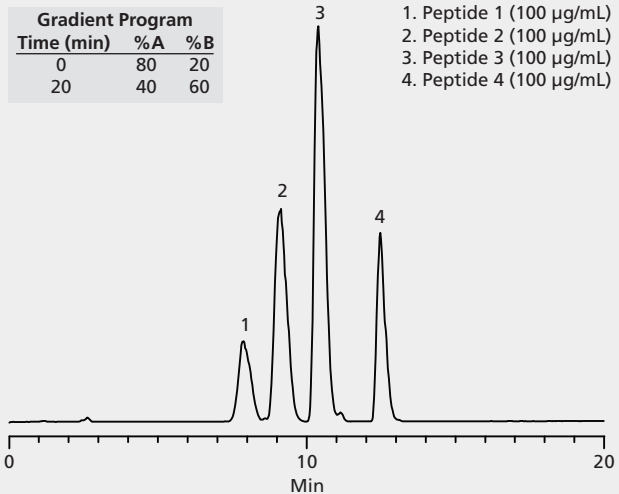


## LC-MS Analysis of Peptides, Basic, on Ascentis® C18

column . . . . . Ascentis C18, 10 cm x 2.1 mm I.D., 5 µm particles (581326-U)
mobile phase . . . . . (A) 20 mM formic acid in water; (B) (50:50), 20 mM formic acid in water:20 mM formic acid in acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 22 °C
detector . . . . . ESI (+), full scan
injection . . . . . 2 µL
Application No. . . . . G002444

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 80 | 20 |
| 20 | 40 | 60 |



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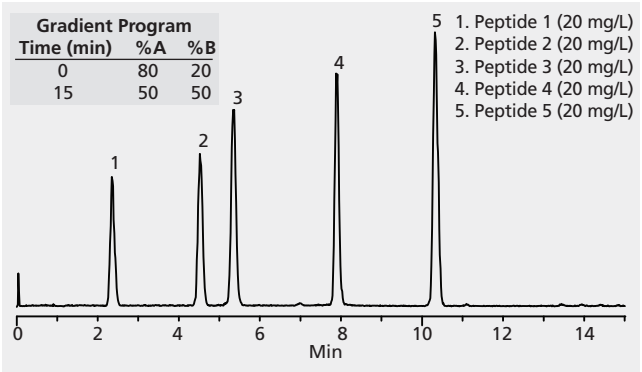
## LC-MS Analysis of Peptides, Hydrophobic, on Ascentis® C18

column . . . . . Ascentis C18, 10 cm x 2.1 mm I.D., 5 µm particles (581326-U)
mobile phase . . . . . (A) (50:50), 50 mM formic acid titrated with acetate (pH 3.0):water; (B) (50:50), 50 mM formic acid titrated with acetate (pH 3.0):acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . ambient
detector . . . . . ESI (+)
injection . . . . . 4 µL
sample . . . . . as indicated in 10 mM formic acid
Application No. . . . . G002688



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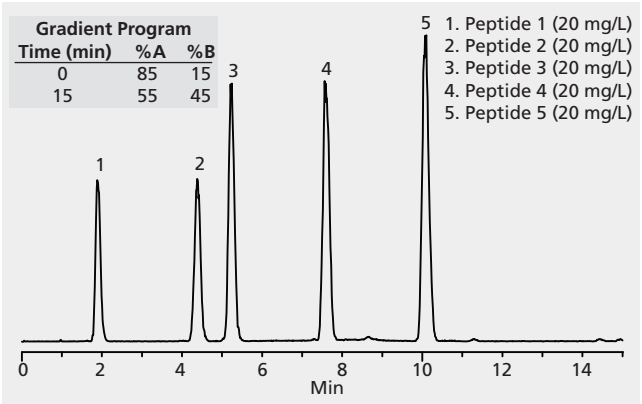
## LC-MS Analysis of Peptides, Hydrophobic, on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 10 cm x 2.1 mm I.D., 5 µm particles (565304-U)
mobile phase . . (A) (50:50), 50 mM formic acid titrated with acetate (pH 3.0):water;
(B) (50:50), 50 mM formic acid titrated with acetate (pH 3.0):acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . ambient
detector . . . . . ESI (+)
injection . . . . . 4 µL
sample . . . . . as indicated in 10 mM formic acid
Application No. . . . . G002682



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## LC-MS Analysis of Peptides on Discovery® BIO Wide Pore C18, Effect of TFA Concentration on MS Signal

column . . . . . Discovery® BIO Wide Pore C18, 15 cm x 2.1 mm I.D., 3 µm particles (567202-U)

mobile phase . . . . . (A): aqueous component (25 mM formic acid, 0.01% TFA or 0.1% TFA); (B) (50:50) water:acetonitrile containing same ionic additives as aq. component

flow rate . . . . . 0.20 mL/min

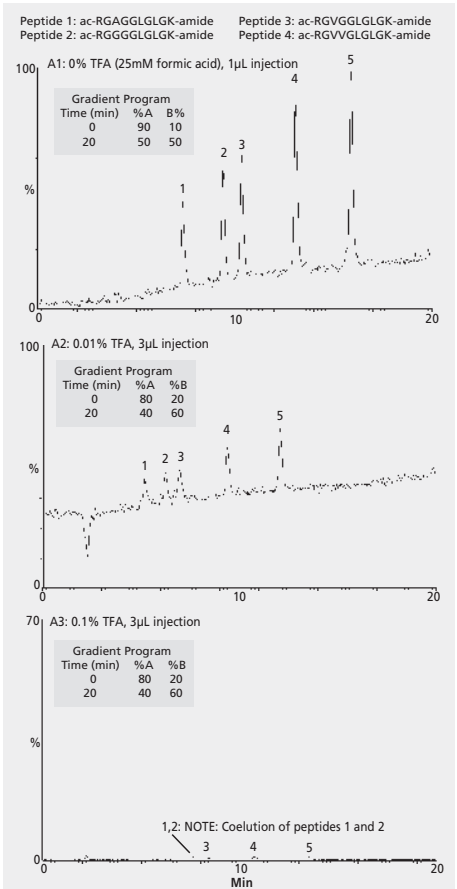
column temp. . . . . ambient

detector . . . . . (+)ESI

injection . . . . . 1-3 µL

sample . . . . . RP Peptide Performance Standard (Alberta Peptide Inst., Cat. No. RPS-P0010)

Application No. . . . . G004414



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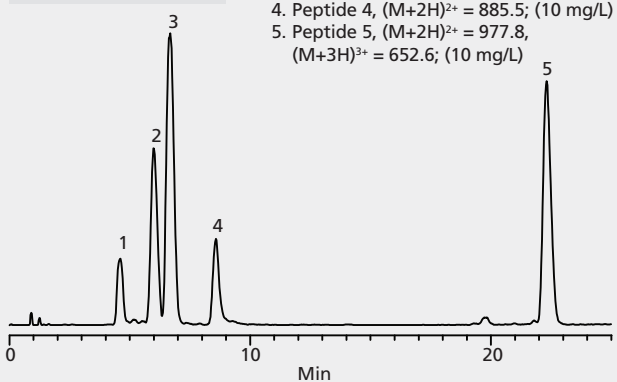
## LC-MS Analysis of Peptides, Serum Derived, on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 10 cm x 2.1 mm I.D., 5 µm particles (565304-U)
mobile phase . . (A) (50:50), 50 mM formic acid titrated with acetate (pH 3.0):water;
(B) (50:50), 50 mM formic acid titrated with acetate (pH 3.0):acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . ambient
detector . . . . . ESI (+)
injection . . . . . 4 µL
sample . . . . . as indicated in 10 mM formic acid
Application No. . . . . G002689

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 70 | 30 |
| 25 | 45 | 55 |

1. Peptide 1, $(M+H)^+$ = 1142.0, $(M+2H)^{2+}$ = 572.6; (10 mg/L)
2. Peptide 2, $(M+2H)^{2+}$ = 810.0; (10 mg/L)
3. Peptide 3, $(M+2H)^{2+}$ = 649.5; (10 mg/L)
4. Peptide 4, $(M+2H)^{2+}$ = 885.5; (10 mg/L)
5. Peptide 5, $(M+2H)^{2+}$ = 977.8, $(M+3H)^{3+}$ = 652.6; (10 mg/L)

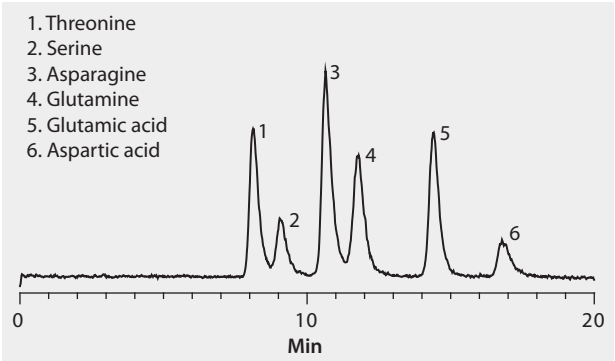





SUPELCO® Solutions within.™

## LC-MS Analysis of Polar Amino Acids on Ascentis® Si (HILIC Mode)

column . . . . . Ascentis Si, 10 cm x 2.1 mm I.D., 5 µm particles (56403AST)
mobile phase . . . . . (A) 100 mM ammonium formate, pH 3.0 with formic acid; (B) water; (C) acetonitrile
gradient . . . . . 5% A constant; 10 to 16% B, 85 to 79% C in 20 min
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+)
injection . . . . . 2 µL
sample . . . . . 0.1 g/L each in mobile phase
Application No. . . . . G005722






SUPELCO® Solutions within.™

## LC-MS Analysis of Polypeptide Drugs on Ascentis® Express RP-Amide

This application demonstrates the suitability of the Ascentis Express RP Amide for the analysis of polypeptide drugs.

column . . . . . Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U)

mobile phase . . . . . (A) 10 mM ammonium acetate in water, pH 4.2; (B) 10 mM ammonium acetate in 90:10 (v/v) acetonitrile:water, pH 4.7

gradient . . . . . held at 20% B for 0.1 min; 20 to 80% B in 2.9 min; 80 to 20% B in 0.01 min; held at 20% B for 0.99 min

flow rate . . . . . 0.3 mL/min

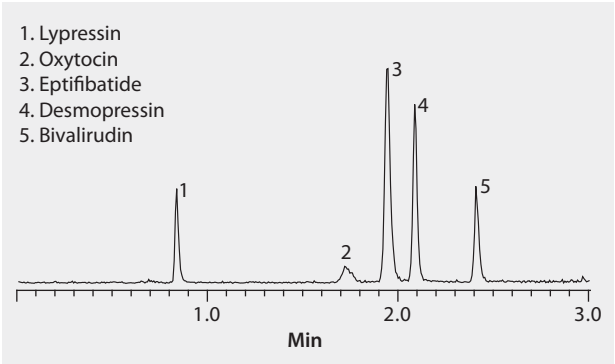
column temp. . . . . 35 °C

detector . . . . . TOF, ESI(+), m/z 100-2500

injection . . . . . 3 µL

sample . . . . . 1 µL/mL in 10mM ammonium formate, 80:20, acetonitrile:water

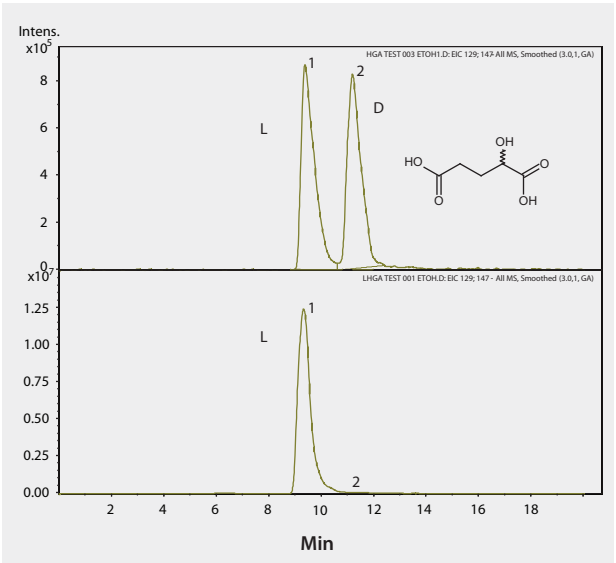
Application No. . . . . G005723



## LC-MS/MS Analysis of 2-Hydroxyglutaric Acid Enantiomers on Astec® CHIROBIOTIC® R

Chiral 2-hydroxyglutarates (2-OHG) are important molecular signatures of both healthy and diseased biological cells, their specific biochemical pathways and inborn errors of metabolism. The chiral differentiation and quantification of D-2-OHG (R(-)-2-OHG) and L-2-OHG (S(+)-2-OHG) is key for characterizing neurometabolic disorders like the 2-hydroxyglutaric acidurias that cause neurological impairment early in life. The ability to distinguish and quantify the enantiomers of 2-OHG is therefore important to be able to track its disposition throughout the metabolic pathway of normal vs. mutated cells. We show here a rapid and sensitive method to separate and measure D-2-OHG and L-2-OHG using LC-MS/MS detection. An Astec® CHIROBIOTIC R (ristocetin chiral selector) column was run under polar ionic mobile phase conditions. Using tandem MS eliminated two fundamental analytical challenges with these compounds: the lack of a UV-absorbing chromophore in the 2-OHG molecule, and the abundance of competing low molecular weight acids in urine. The upper panel in the chromatogram shows the separation of D-2-OHG and L-2-OHG obtained during method development. The lower panel shows the pure L-2-hydroxyglutaric acid. Besides providing the necessary enantioselectivity, Astec® CHIROBIOTIC columns have the advantage of operating in aqueous and polar organic mobile phases that are amenable to polar drugs and metabolites. Their ionic interactions promote analyte ionization which enhances sensitivity in ESI detection. In addition to the CHIROBIOTIC columns, Sigma-Aldrich also provides the high purity mobile phase agents, racemic and pure chiral 2-OHG enantiomers and other compounds in the isocitrate dehydrogenase (IDH) pathway.

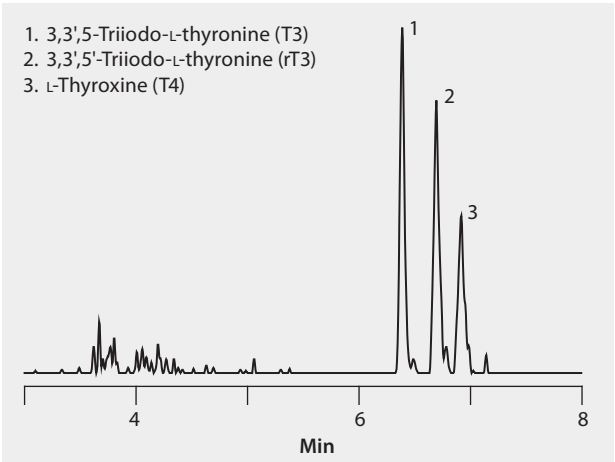
column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . (A) ethanol:methanol (3:1, v:v); (B) 0.1% v/v TEA in water, adjusted to pH 4.5 with acetic acid; 75:25 (A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 20 °C
detector . . . . . MS, ESI(-), 100-1000 m/z
injection . . . . . 5 µL
sample . . . . . each enantiomer, 100 µg/mL in water
Application No. . . . . G005848



## LC-MS with Online Solid Phase Extraction and (SPE/LC-MS) of Thyroid Hormones in Biological Fluids on Ascentis® Express Phenyl-Hexyl using RP-Amide Traps

An online SPE-LC/MS method was developed for the determination of thyroid hormones in biological fluids. The method exploits RP-Amide or C8 as the trapping column and Phenyl phase as the separation column, respectively. The preliminary experiments demonstrated that, under the optimized conditions, both RP-Amide and C8 effectively trapped the thyroid hormones extracted from spiked rabbit plasma sample which had been protein precipitated. However, the RP-Amide traps are advantageous over the commonly used C8 traps with higher signals and recoveries.
Additionally, RP-Amide traps are more flexible with washing solvent as it is compatible with 100% aqueous mobile phases and can be used with up to 20% methanol with minimal sample loss.
The online SPE/LC-MS system comprises a trapping column, a switching valve and an LC-MS instrument. The program consists of three distinct steps. The valve switches between position 1 for loading/washing (Step 1) and equilibration (Step 3), and position 2 for LC-MS analysis (Step 2). In Step 1 samples are loaded onto the trap and washed with mobile phase containing low percent organic and high flow rate to remove salts and other interferences which are directed to the waste. In Step 2 the analytes are eluted from the trap, separated and detected by the LC-MS at optimum flow for both chromatographic separation and MS signals. In Step 3, the system returns back to valve position 1 for re-equilibration under the sample loading/washing conditions.

sample preparation . . . . . SPE (Solid Phase Extraction)
column . . . . . Ascentis Express Phenyl-Hexyl, 5 cm x 2.1 mm I.D., 2.7 µm particles (53334-U)
mobile phase . . . . . (A) water; (B) methanol, both with 0.1% acetic acid
gradient . . . . . 5% B for 3 min; to 70% B in 2 min, hold 3 min
pressure . . . . . 1800 psi
detector . . MS, ESI(+), MRM mode (777.7/731.8 for T4; 651.8/605.5 for T3 or rT3)
column temp. . . . . ambient
injection . . . . . 5 µL
sample . . . . . protein precipitated rabbit plasma spiked with desired amount of T4, T3 and rT3
Application No. . . . . G006223



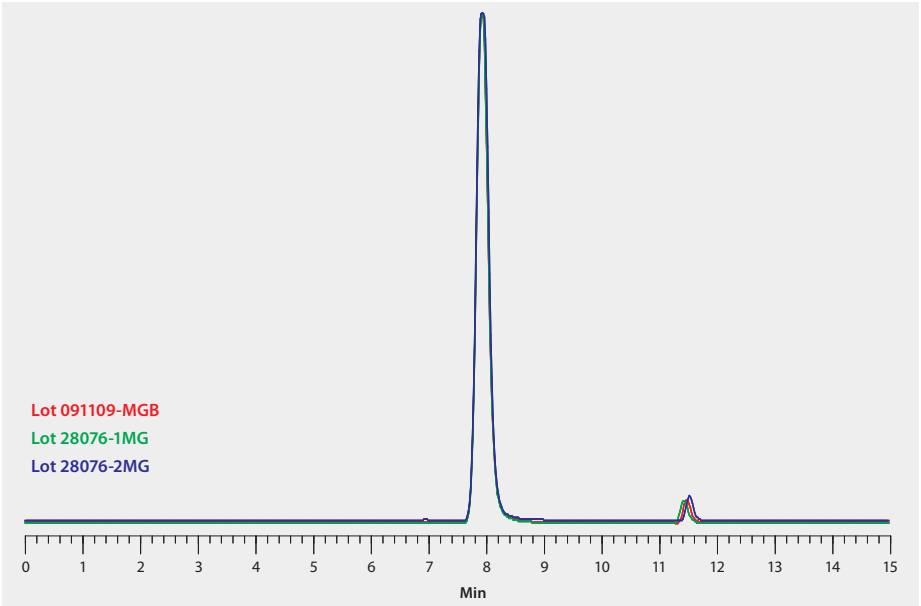



SUPELCO® Solutions within.™

## Lot-to-Lot Reproducibility of Monoclonal Antibody Analysis on Three Lots of Zenix® SEC-300 Columns

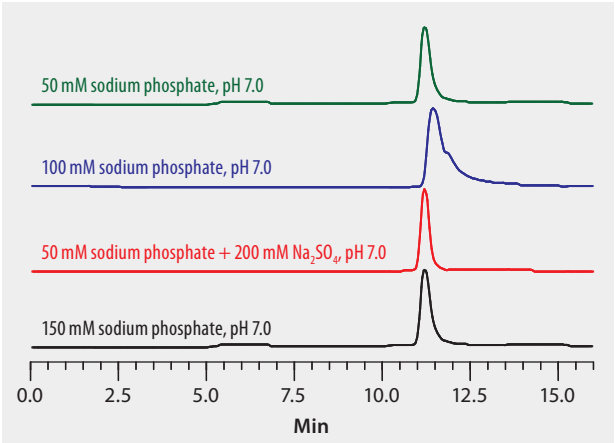
Exceptional lot-to-lot reproducibility is demonstrated for three lots of 3 micron Zenix SEC-300 columns for the release test of monoclonal antibody-based therapeutics. Monomer retention time, peak efficiency and peak height are shown to be virtually identical on the three lots.

column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å (Z777033)
mobile phase . . . . . 150 mM Phosphate Buffer, pH 7
flow rate . . . . . 1.0 mL/min
column temp. . . . . Ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . 2.5 mg/ml MAb
Application No. . . . . G006210



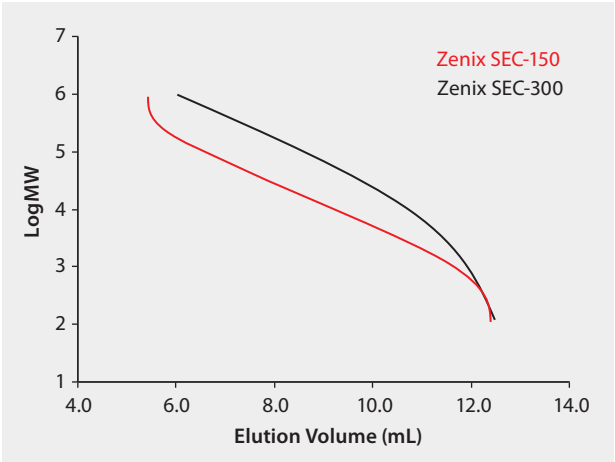
## Mobile Phase Optimization in the Analysis of Lysozyme on Zenix® SEC-300

column . . . . . Zenix SEC-300, 30 cm x 4.6 mm I.D., 3 µm particles (Z777028)
mobile phase . . . . . as indicated on figure
flow rate . . . . . 0.35 mL/min
temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . lysozyme, 1 mg/mL
Application No. . . . . G005972



## Protein Molecular Weight Calibration Curve on Zenix® Size Exclusion Columns, Effect of Pore Size

column . . Zenix SEC-150, 30 cm x 7.8 mm I.D., 3 µm, 150 Å particles (Z777018)
column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . each 1 mg/mL except uracil, 2.5 mg/mL
Application No. . . . . G006126






SUPELCO® Solutions within.™

## Protein MW Calibration Curves on SRT® SEC-100, -150 and -300, 30 cm x 7.8 mm I.D., 5 µm particles

column . . . . . SRT® SEC-100, -150 and -300, 30 cm x 7.8 mm I.D., 5 µm particles (Z777039)

mobile phase . . . . . 150 mM potassium phosphate, monobasic, pH 7 (adjusted with potassium phosphate)

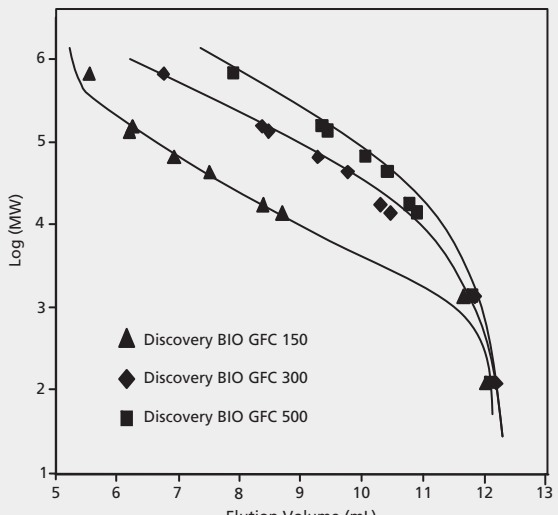
flow rate . . . . . 1 mL/min

detector . . . . . UV, 214 nm

injection . . . . . 10 µL

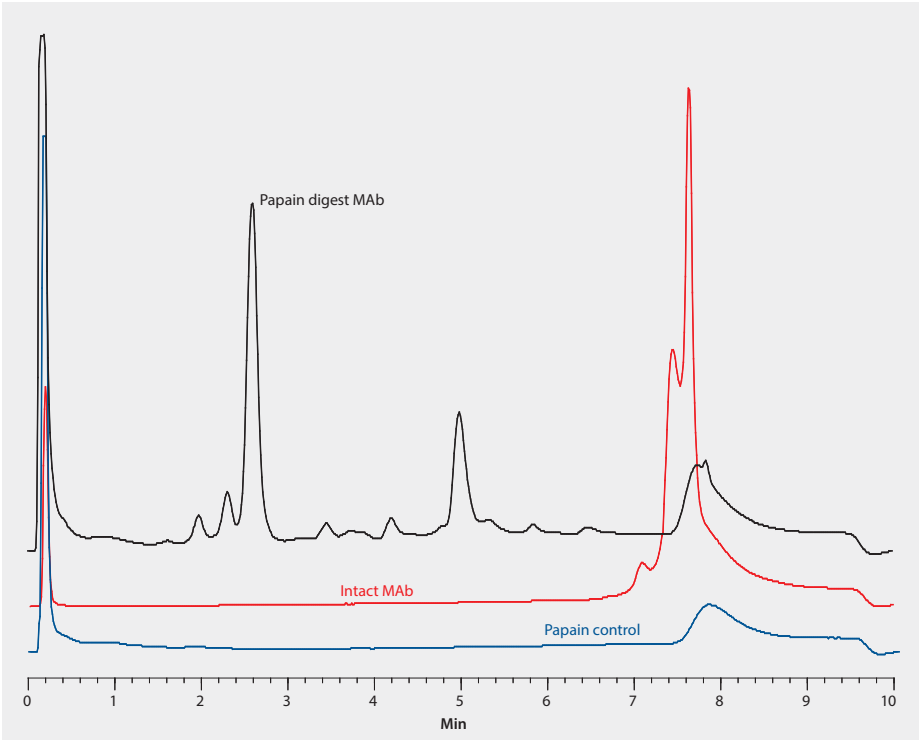
Application No. . . . . G004237

1. Thyroglobulin, 670 kDa
2. γ-Globulin, 158 kDa
3. Bovine serum albumin dimer, 132 kDa
4. Bovine serum albumin, 66 kDa
5. Ovalbumin, 44 kDa
6. Myoglobin, 17.6 kDa
7. Ribonuclease A, 13.7 kDa
8. Vitamin B12, 1.35 kDa
9. Uracil, 120 Da



## Proteomix WCX-NP1.7, 3 cm x 4.6 mm I.D., 1.7 µm particles

column . . Proteomix WCX-NP1.7, 3 cm x 4.6 mm I.D., 1.7 µm particles (Z777179)
mobile phase . . . . . (A) 20 mM sodium acetate, pH 5.15; (B) A + 1 M LiCl
gradient . . . . 12% to 40% B in 6.7 min; to 100% B in 0.1 min; held for 2 min
flow rate . . . . . (1.5 mL/min)
pressure . . . . . 3335-3916 psi (230 - 270 bar)
detector . . . . . UV 280 nm
injection . . . . . 25 µL
sample . . . . . 1 mg/mL papain digested MAb 321
Application No. . . . . G006023



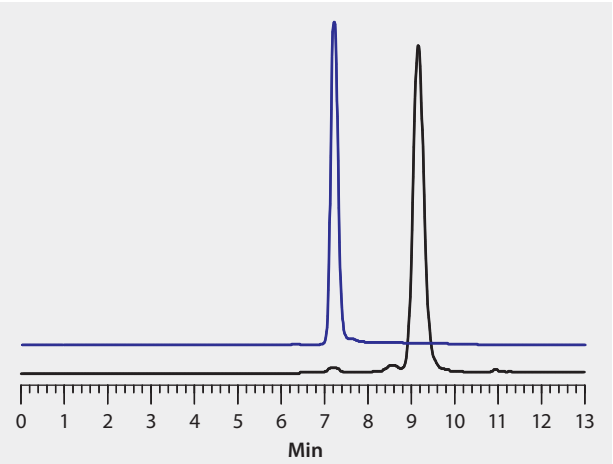



SUPELCO
Solutions within.™

## Size Exclusion Analysis of Avastin and Erbitux on Zenix® SEC-300

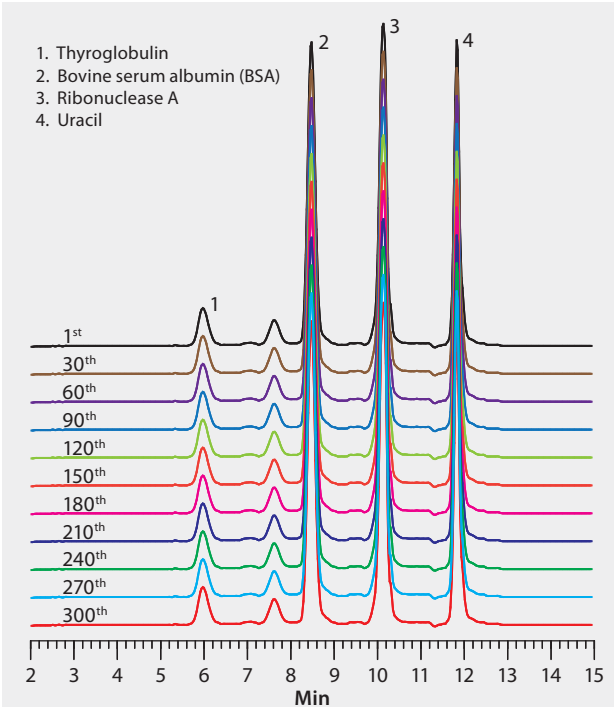
The quality control analysis of recombinant monoclonal antibody therapeutics by gel filtration chromatography is a standard release test to determine antibody purity and the level of aggregation. Bevacizumab, sold under the trade name Avastin, is a humanized monoclonal antibody used in the treatment of colorectal, breat and lung cancer. Similarly, cetuximab, sold under the trade name Erbitux, is a chimeric monoclonal antibody used in the treatment of metastatic colorectal cancer. Both mAbs have molecular masses of about 150 kD.

column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles, 300 Å (Z777033)
mobile phase . . . . . 150 mM phosphate buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . (top) Bevacizumab (trade name Avastin), a humanized monoclonal antibody
(bottom) Cetuximab (trade name Erbitux), a chimeric monoclonal antibody
Application No. . . . . G006208



## Stability and Reproducibility of Zenix® 300 under High Salt and High pH Mobile Phase Conditions

column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777018)
mobile phase . . . . . 50 mM sodium phosphate + 1 M NaCl, pH 8.5
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . each compound 1 mg/mL except uracil, 0.1 mg/mL
Application No. . . . . G005971

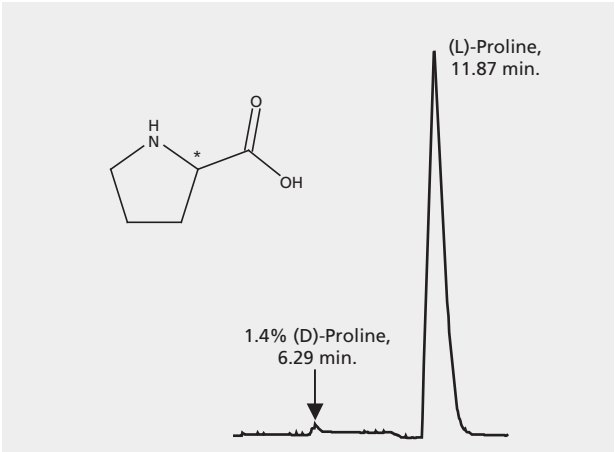





SUPELCO® Solutions within.™

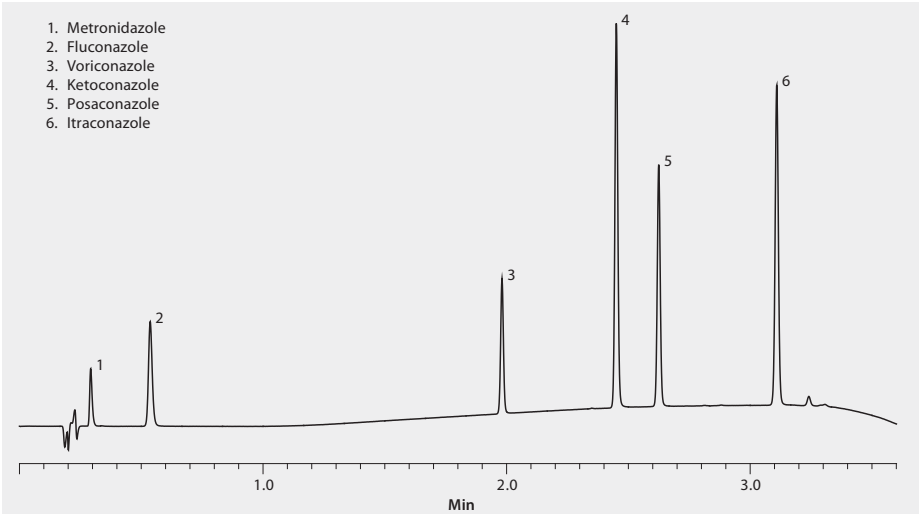
## Trace HPLC Analysis of Proline Enantiomers on Astec® CLC-L

column . . . . . Astec CLC-L, 15 cm x 4.6 mm I.D., 5 µm particles (53123AST)
mobile phase . . . . . 5 mM copper sulfate
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in water
Application No. . . . . G004662



## UHPLC Analysis of Antifungals on Ascentis® Express 2.7 µm C18

column . . . Ascentis Express C18 5 cm x 3.0 mm I.D., 2.7 µm particles (53811-U)
mobile phase . . . . . (A) 5 mM ammonium acetate pH 6.8 unadjusted in 80:20, water:acetonitrile;
(B) 5 mM ammonium acetate pH 6.8 unadjusted in 5:95, water:acetonitrile;
gradient . . . . . 0% B; to 100% B in 3 min; 100% B held for 0.6 min
flow rate . . . . . 1.0 mL/min
pressure . . . . . 2790 psi (192 bar)
column temp. . . . . 35 °C
detector . . . . .
injection . . . . . 1.0 µL
sample . . . . . 6 antifungal 50 µg/mL in 70:30, water:methanol
Application No. . . . . G006256

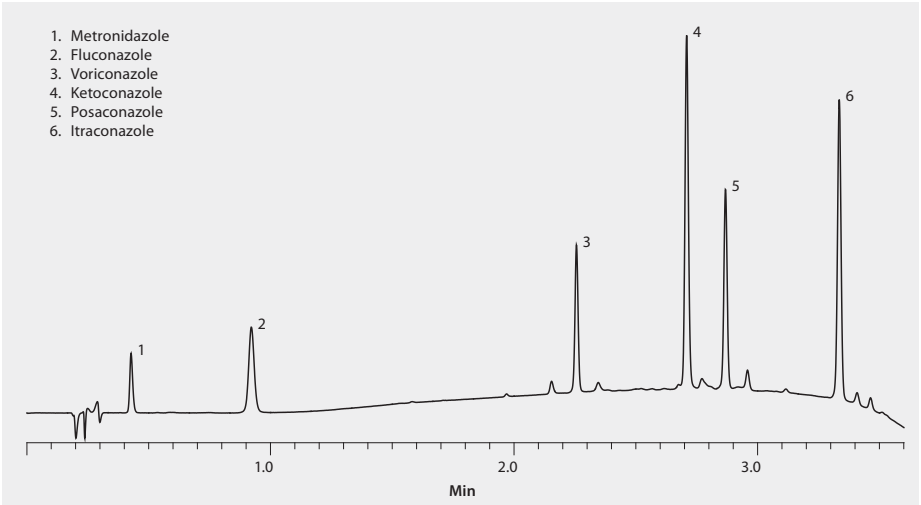





SUPELCO® Solutions within.™

## UHPLC Analysis of Antifungals on Titan™ C18

column . . . . . Titan C18, 5 cm x 3.0 mm I.D., 1.9 µm particles (577126-U)
mobile phase . . . . . (A) 5 mM ammonium acetate pH 6.8 unadjusted in 80:20, water:acetonitrile;
(B) 5 mM ammonium acetate pH 6.8 unadjusted in 5:95, water:acetonitrile;
gradient . . . . . 0% B; to 100% B in 3 min; 100% B held for 0.6 min
flow rate . . . . . 1.0 mL/min
pressure . . . . . 4350 psi (300 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 205 nm
injection . . . . . 1.0 µL
sample . . . . . 6 antifungal 50 µg/mL in 70:30, water:methanol
Application No. . . . . G006255

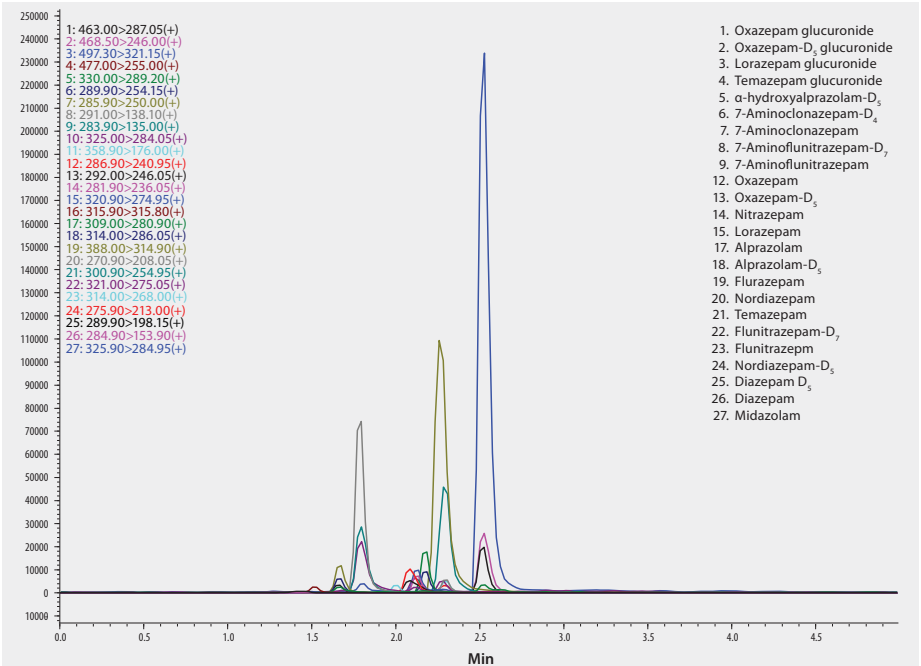





SUPELCO® Solutions within.™

## UHPLC Analysis of Benzodiazepines using Beta-Glucuronidase Enzymatic Digestion on Titan™ C18

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| SPE well plate | Supel™-Select HLB 96-Well 60 mg/well (575662-U) |
| condition | 1 mL acetonitrile, 1 mL water |
| sample addition | 1.1 mL digested urine |
| washing | 1 mL water, 1 mL 25% methanol |
| elution | 1 mL acetonitrile |
| column | TITAN C18, 5 cm x 2.1 mm, 1.9 µm particles (577122-U) |
| mobile phase | (A): 5 mM ammonium formate<br>(B): 5 mM ammmonium formate (95:5 acetonitrile:water) |
| gradient | 10% B to 90% B in 2.5 minutes, hold 90% B for 0.5 minutes |
| flow rate | 0.3 mL/min |
| column temp. | 35 °C |
| detector | MS/MS |
| injection | 2.0 µL |
| sample | 300 ng/mL in water |
| Application No. | G006291 |

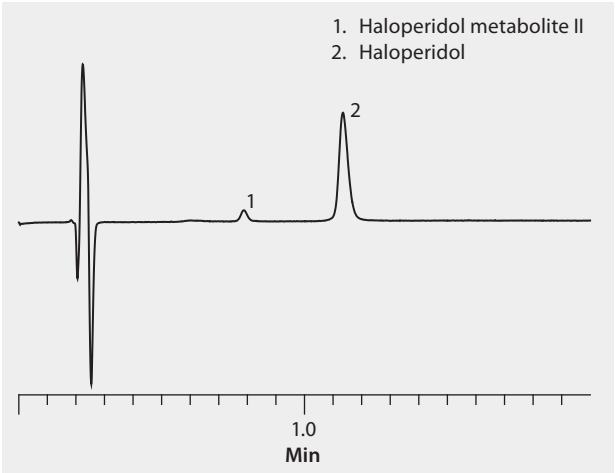





SUPELCO® Solutions within.™

## UHPLC Analysis of Haloperidol and Metabolite on Titan™ C18

column . . . . . Titan C18 5 cm x 2.1 mm, 1.9 µm particles (577122-U)
mobile phase . . . . . A) 10 mM potassium phosphate, monobasic pH 3.0; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 4310 psi (297 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 200 nm
injection . . . . . 1.0 µL
sample . . . . . 1–10 µg/mL in 70:30, water:methanol
Application No. . . . . G006282

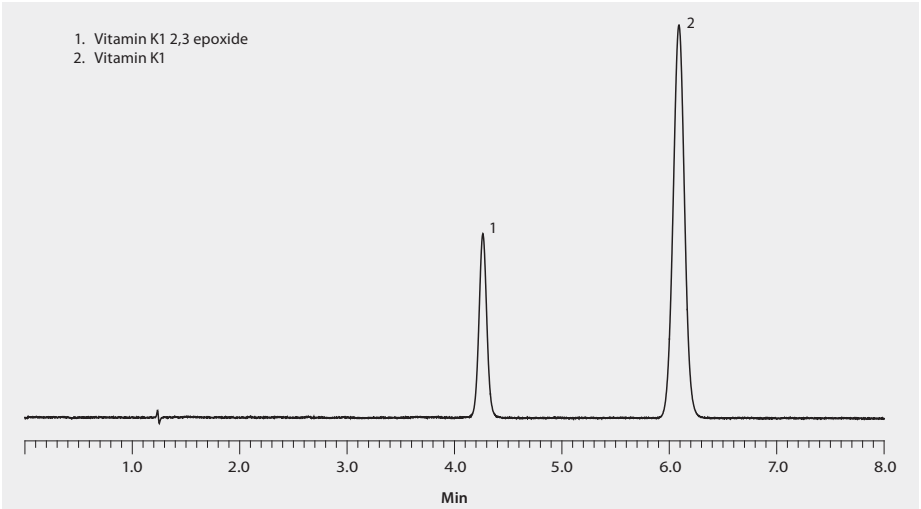





SUPELCO
Solutions within.™

## UHPLC Analysis of Vitamin K1 and Related Compound on Ascentis® Express 5 µm C18

column . . . Ascentis Express C18 15 cm x 4.6 mm I.D., 5 µm particles (50537-U)
mobile phase . . . . . (A) methanol; (B) 2-propanol; (90:10, A:B)
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1035 psi (71 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 250 nm
injection . . . . . 2.0 µL
sample . . . . . Vitamin K1 and related, 50 µg/mL 90:10, methanol:2-propanol
Application No. . . . . G006261

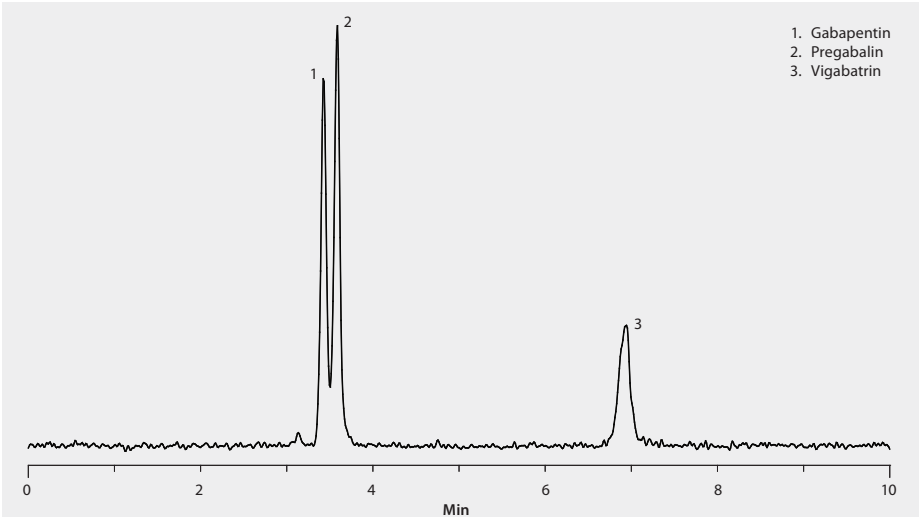





SUPELCO® Solutions within.™

## UHPLC-MS Analysis of Anticonvulsants on Ascentis® Express 2.7µm HILIC

column . . Ascentis Express HILIC, 10 cm x 3.0 mm I.D., 2.7 µm particles (53970-U)
mobile phase . . . . . (A) 5 mM ammonium formate pH unadjusted in 95:5, acetonitrile:water;
(B) 5 mM ammonium formate pH unadjusted in 75:25, acetonitrile:water; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1240 psi (85.5 bar)
column temp. . . . . 35 °C
detector . . . . . MS-ESI-, full scan 100-500 m/z
detector . . . . . 5.0 µL
sample . . . . . 3 anticonvulsants 1 µg/mL in 99:1, acetonitrile:methanol
Application No. . . . . G006302



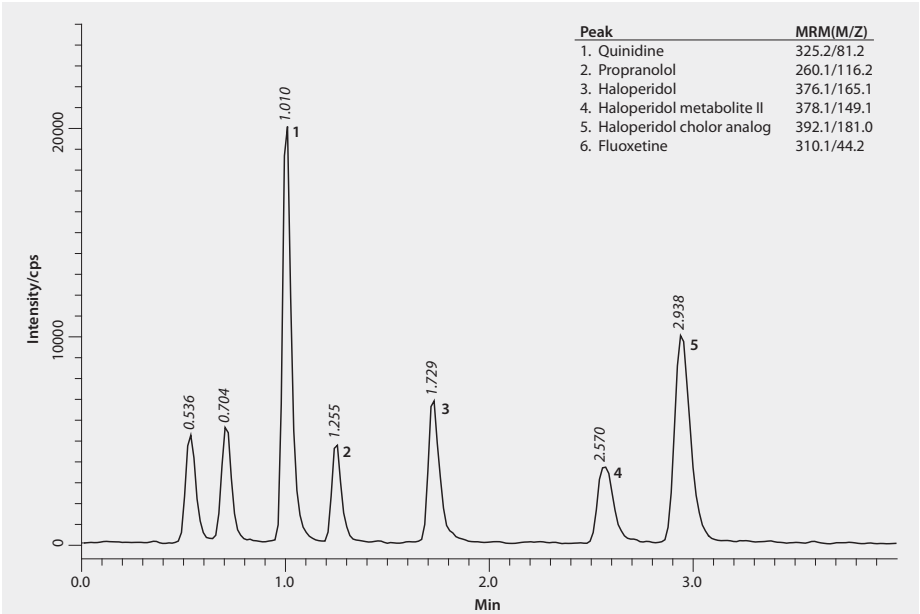



SUPELCO® Solutions within.™

## UHPLC-MS Analysis of Basic Drugs and Metabolites with Titan™ C18

MRM transitions
1. Quinidine 325.2/81.2
2. Propranolol 260.1/116.2
3. Haloperidol 376.1/165.1
4. Haloperidol metabolite II 378.1/149.1
5. Haloperidol cholor analog 392.1/181.0
6. Fluoxetine 310.1/44.2

column . . . . . Titan C18 10 cm x 2.1 mm, 1.9 µm particles (577124-U)
mobile phase . . . . . (A) Water; (B) Acetonitrile, both with 0.1% Formic acid
gradient . . . . . 35%B Isocratic
flow rate . . . . . 0.4 mL/min
pressure . . . . . 484 psi (7120)
column temp. . . . . 25 °C
detector . . . . . MS, ESI(+), MRM
injection . . . . . 1.0 µL
sample . . . . . 50 ng/mL in 50% methanol
Application No. . . . . G006280

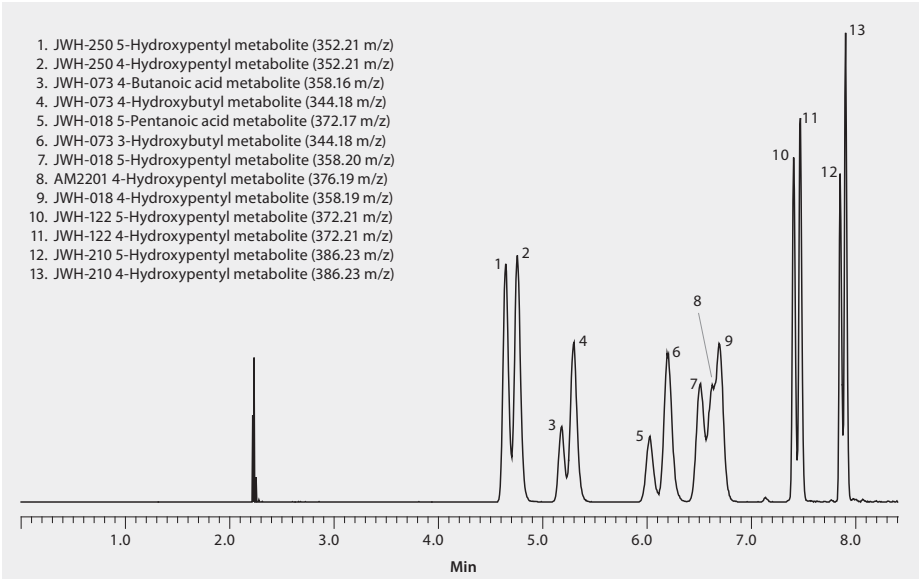





SUPELCO® Solutions within.™

## UHPLC-MS Analysis of Spice Cannabinoid Metabolites from Urine Using Titan™ C18

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| SPE well plate | SupelSelect HLB 96-Well Plate, 60mg/well (575662-U) |
| condition | 1 mL methanol, then 1 mL water |
| sample addition | 100 ng/mL in matrix |
| drying | none |
| washing | 1 mL 20% methanol in water |
| elution | 2 mL methanol |
| eluate post-treatment | none |
| column | Titan C18 10 cm x 2.1 mm, 1.9 µm particles (577124-U) |
| mobile phase | (A) 0.1% formic acid in 95:5, water:acetonitrile; (B) 0.1% formic acid in 5:95, water:acetonitrile; |
| gradient | 25% B held for 1 min; to 45% B in 0.5 min; 45% B held for 4.5 min; to 80% B in 0.5 min; 80% B held for 4 min |
| flow rate | 0.5 mL/min |
| pressure | 8756 psi (604 bar) |
| temp. | 35 °C |
| detector | MS-ESI+,100 - 1000 m/z scan, XIC |
| injection | 2.0 µL |
| sample | 100 ng/mL in methanol |
| Application No. | G006307 |

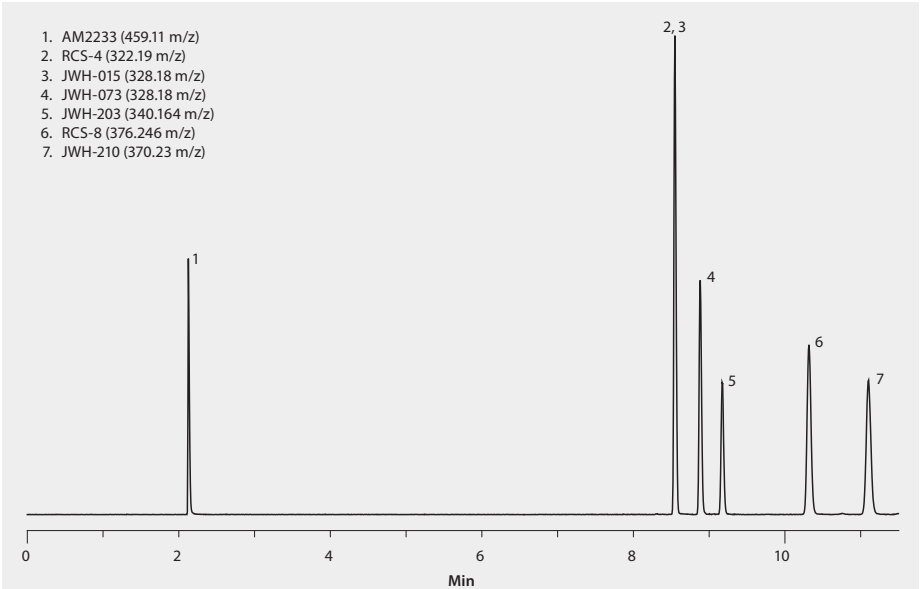





SUPELCO® Solutions within.™

## UHPLC-MS Analysis of Spice Cannabinoids Using Titan™ C18

column . . . . . TITAN C18, 100 mm x 2.1 mm x 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid in 95:5, water:acetonitrile;
(B) 0.1% formic acid in 5:95, water:acetonitrile;
gradient . . 25% B held for 1 min; to 45% B in 0.5 min; 45% B held for 4.5 min;
to 80% B in 0.5 min; 80% B held for 4 min
flow rate . . . . . 0.5 mL/min
pressure . . . . . 8756 psi (604 bar)
column temp. . . . . 35 °C
detector . . . . . MS-ESI+,100 - 1000 m/z scan, XIC
injection . . . . . 2.0 µL
sample . . . . . 100 ng/mL in 60:40, water:methanol
Application No. . . . . G006308

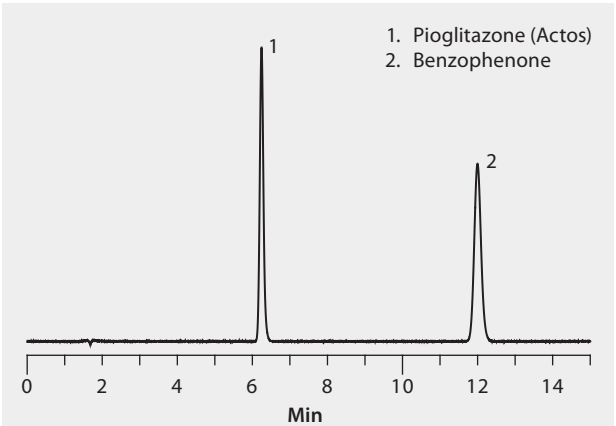





SUPELCO® Solutions within.™

## USP Analysis of Actos (Pioglitazone) using Ascentis® Express C18 5µm

column . . . . . Ascentis® Express C18, 15 cm x 4.6 mm, 5 µm particles (50537-U)
mobile phase . . . . . (A) 100 mM ammonium acetate; (B) acetonitrile; (C) acetic acid; (55:45:2, A:B:C)
flow rate . . . . . 0.7 mL/min
pressure . . . . . 1160 psi (80 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 269 nm
injection . . . . . 20 µL
sample . . . . . pioglitazone 50 µg/mL, benzophenone 13 µg/mL in mobile phase
Application No. . . . . G006203



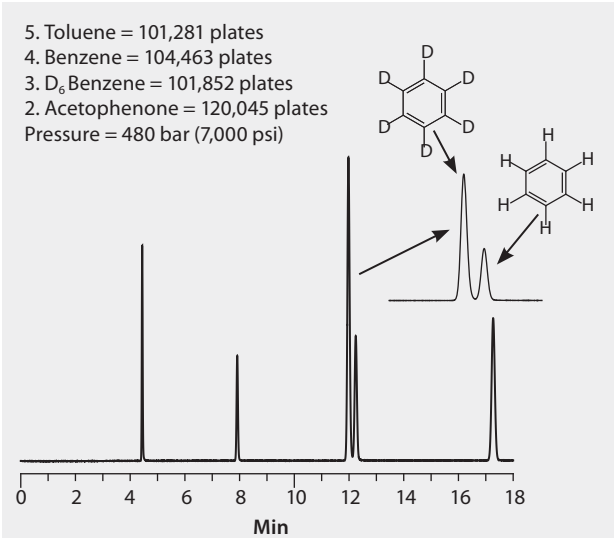



SUPELCO® Solutions within.™

# Petroleum

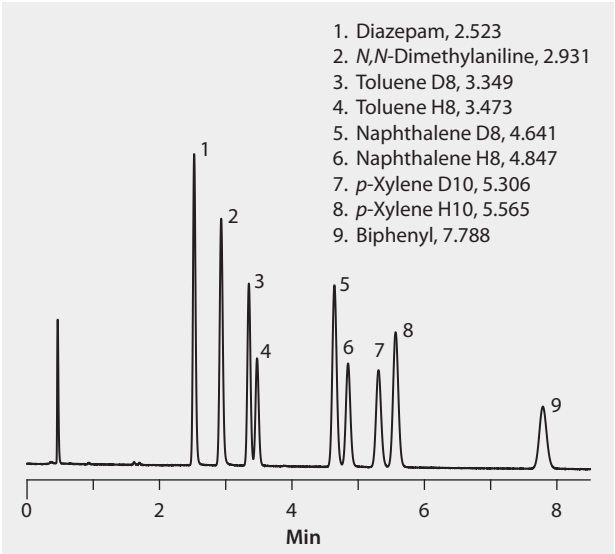
## HPLC Analysis of Benzene and Deuterated Benzene on Ascentis® Express C18

| | |
|---|---|
| column | Ascentis Express C18, 15 cm × 4.6 mm I.D., 2.7 µm particles |
| mobile phase | (A) acetonitrile; (B) water (55:45, A:B) |
| flow rate | 1.0 mL/min |
| column temp. | 50 °C |
| detector | UV, 254 nm |
| injection | 10 µL |
| Application No. | G004046 |



## UHPLC Analysis of Deuterated Isomers on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 5650 psi (390 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
Application No. . . . . G006122



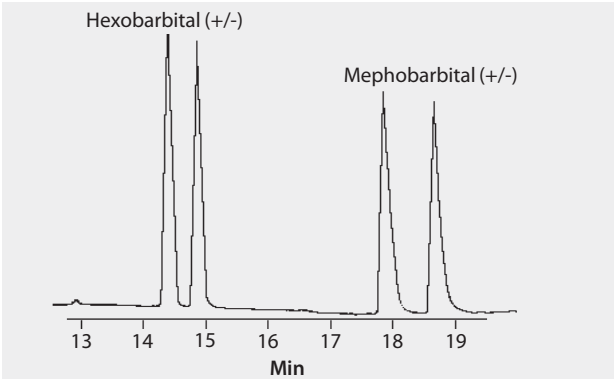



SUPELCO® Solutions within.™

# Pharmaceutical (Small Molecule)

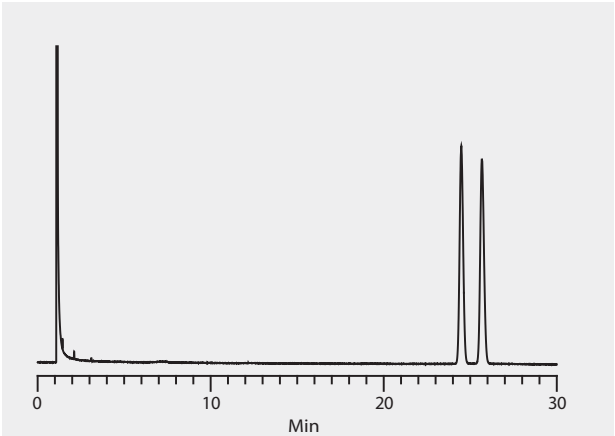
## GC Analysis of Barbiturate Enantiomers on β-DEX™ 120

| | |
|---|---|
| column | β-DEX 120, 30 m × 0.25 mm I.D., 0.25 µm (24304) |
| oven | 210 °C |
| inj. temp. | 300 °C |
| detector | FID, 300 °C |
| carrier gas | helium, 20 cm/sec @ 275 °C |
| injection | 100:1 split |
| Application No. | 92-0342 |



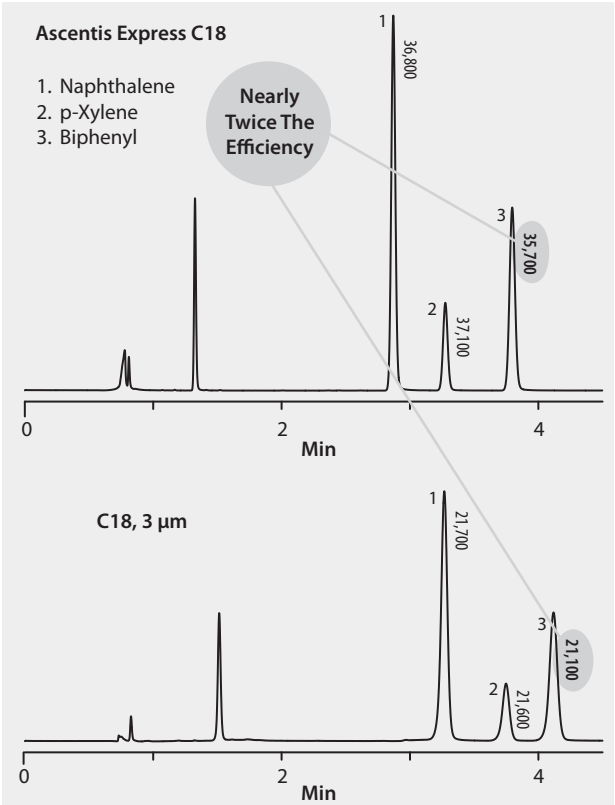
## GC Analysis of Methylphenidate (Ritalin) Enantiomers (N-Trifluoroacetyl Derivatives) on Astec® CHIRALDEX® B-DA

column . . . . Astec CHIRALDEX B-DA, 30 m x 0.25 mm I.D., 0.12 µm (72023AST)
oven . . . . . 170 °C
inj. temp. . . . . 250 °C
detector . . . . . FID, 250 °C
carrier gas . . . . . helium, 35 psi
sample . . . . . peaks 1 & 2: methylphenidate enantiomers (N-TFA derivative)
Application No. . . . . G005070



## High Efficiency Resolution on Ascentis® Express Versus 3µm Particles

column . . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles and C18, 15 cm × 4.6 mm I.D., 3 µm particles (53829-U)
mobile phase . . . . . (A) water; (B) acetontrile; (35:65 or 27.5:72.5, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
Application No. . . . . G003979






SUPELCO® Solutions within.™

## Higher Efficiency of Ascentis® Express Compared to 3 µm Particles Gives Better Sensitivity

column . . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles and C18, 5 cm x 2.1 mm I.D., 3 µm particles (53822-U)

mobile phase . . . . . (A) 25 mM dibasic ammonium phosphate (pH 7.0): (B) water: (C) acetonitrile; (35:0:65 or 35:4:61, A:B:C)

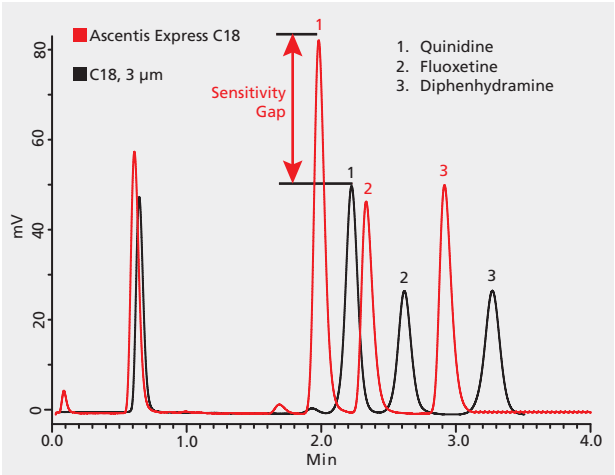
flow rate . . . . . 0.2 mL/min

column temp. . . . . 35 °C

detector . . . . . UV, 220 nm

injection . . . . . 1 µL

Application No. . . . . G003977

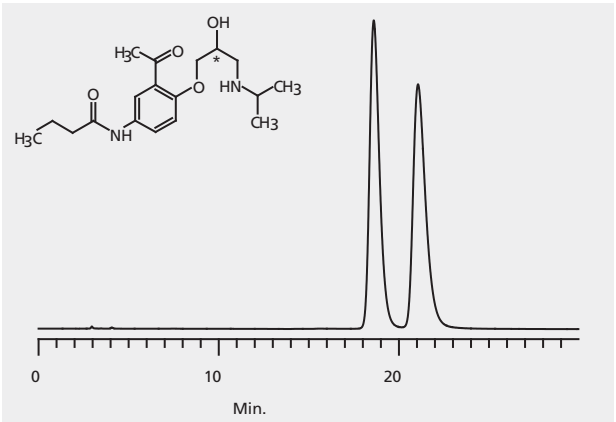





SUPELCO® Solutions within.™

## HPLC Analysis of Acebutolol Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.8:0.1:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 12 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004482

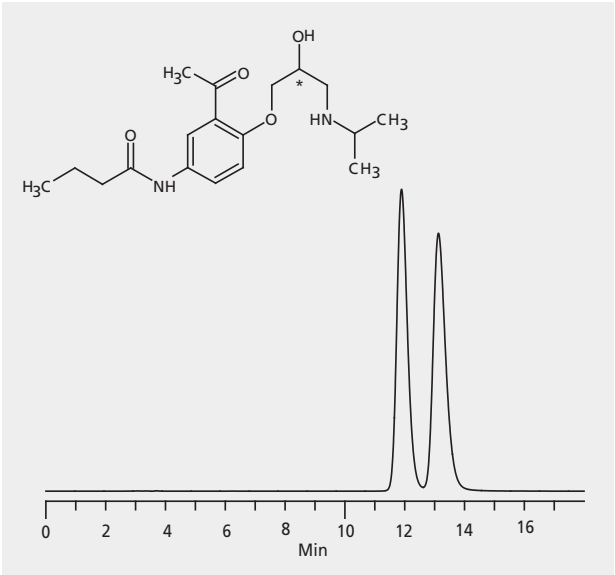





SUPELCO® Solutions within.™

## HPLC Analysis of Acebutolol Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (100:0.1:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004457

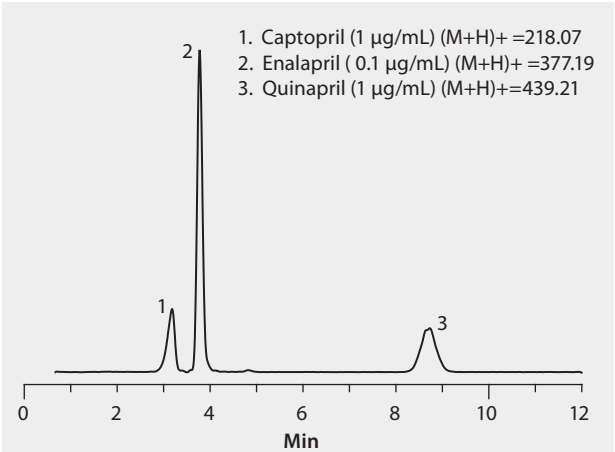





SUPELCO® Solutions within.™

## HPLC Analysis of ACE Inhibitors on Ascentis® Phenyl

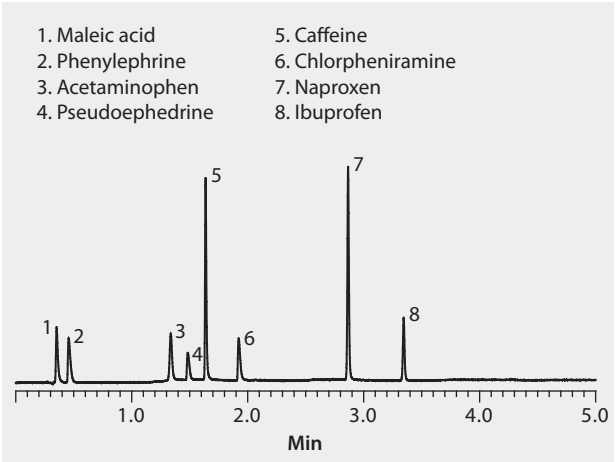
column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 13 mM ammonium formate (pH 2.0 with concentrated formic acid): (B) methanol; (40:60, A:B)
flow rate . . . . . 1 mL/min, split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 40:60, 13 mM ammonium formate (pH 2.0 with concentrated formic acid):methanol
Application No. . . . . G003702



## HPLC Analysis of Acetaminophen, Caffeine, Chlorpheniramine, Ibuprofen, Naproxen, Phenylephrine, and Pseudoephedrine on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of various over the counter drugs.

column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 5 mM ammonium phosphate monobasic, pH 2.0 with phosphoric acid: acetonitrile (98:2); (B) acetonitrile;
gradient . . . . . 0 to 68% B in 3 min; held at 68% B for 2 min
flow rate . . . . . 0.4 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 1 µL
sample . . . . . 100 µg/mL in 95:5, water: methanol
Application No. . . . . G005716

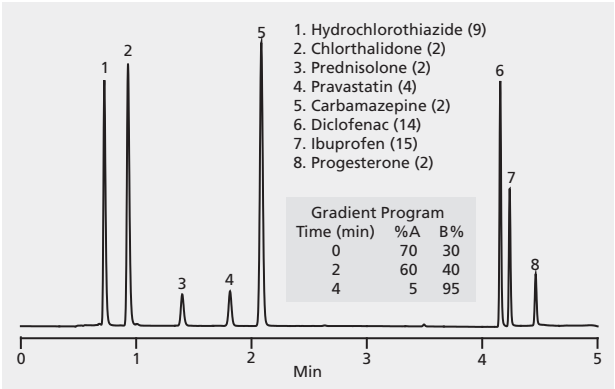





SUPELCO® Solutions within.™

## HPLC Analysis of Acidic and Neutral Drug Panel on Ascentis® Express C18

column . . Ascentis Express C18, 10 cm × 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) water with 0.1% phosphoric acid,
(B) acetonitrile with 0.1% phosphoric acid
flow rate . . . . . 1.76 mL/min
column temp. . . . . ambient
detector . . . . . UV, 215 nm
injection . . . . . 100 µL
Application No. . . . . G004212

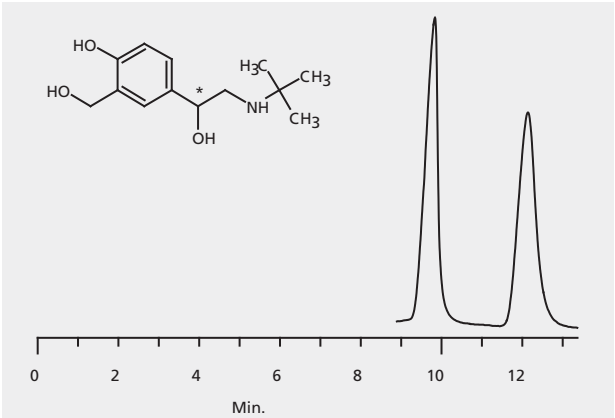





SUPELCO® Solutions within.™

## HPLC Analysis of Albuterol Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004593

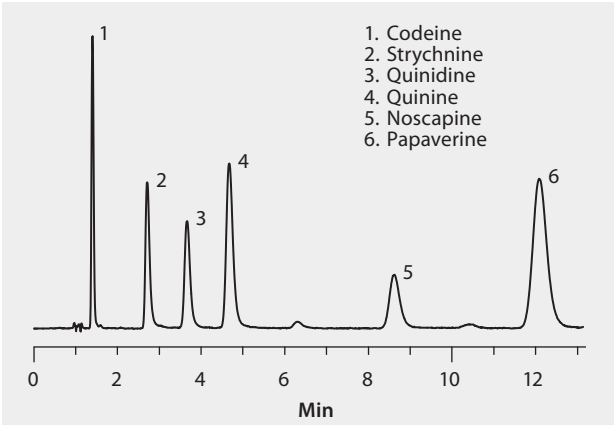





SUPELCO® Solutions within.™

## HPLC Analysis of Alkaloids on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) methanol; (B) 25 mM potassium phosphate, dibasic, pH 3.0; (20:80, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000235

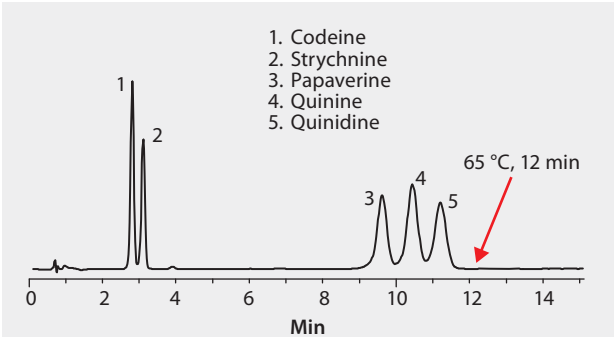





SUPELCO® Solutions within.™

## HPLC Analysis of Alkaloids on Discovery® Zr-PBD

column . . . . . Discovery Zr-PBD, 15 cm × 4.6 mm I.D., 5 µm particles (65723-U)
mobile phase . . . . . (A) 20 mM potassium phosphate (pH 12): (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 2.35 mL/min
column temp. . . . . 65 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL each compound 50 µg/mL
Application No. . . . . G001840

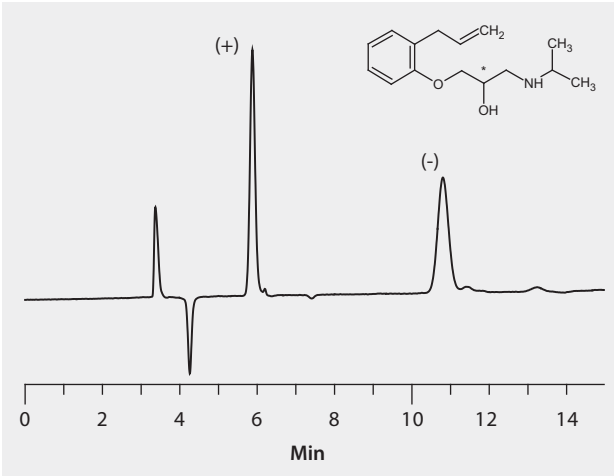





SUPELCO® Solutions within.™

## HPLC Analysis of Alprenolol Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) DEA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004984

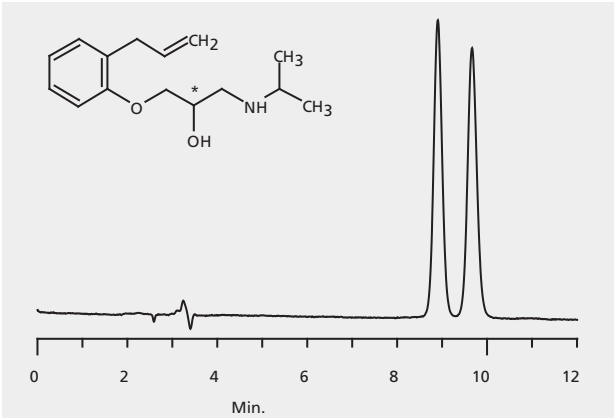





SUPELCO® Solutions within.™

## HPLC Analysis of Alprenolol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 215 nm
injection . . . . . 3 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004486

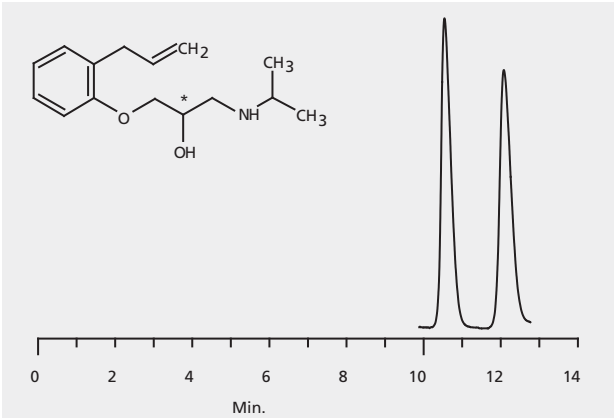





SUPELCO® Solutions within.™

## HPLC Analysis of Alprenolol Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 0.5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004592

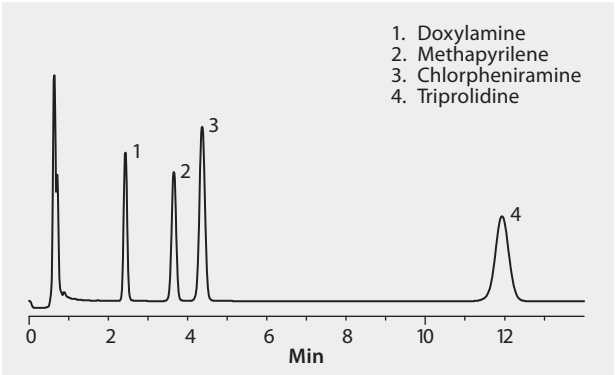





SUPELCO® Solutions within.™

## HPLC Analysis of Amines on Discovery® Zr-PBD at 20° C

column . . . . Discovery Zr-PBD, 7.5 cm × 4.6 mm I.D., 3 µm particles (65717-U)
mobile phase . . . . . (A) 50 mM Triethylammonium Hydroxide (pH 12.6): (B) acetonitrile (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
Application No. . . . . G001860

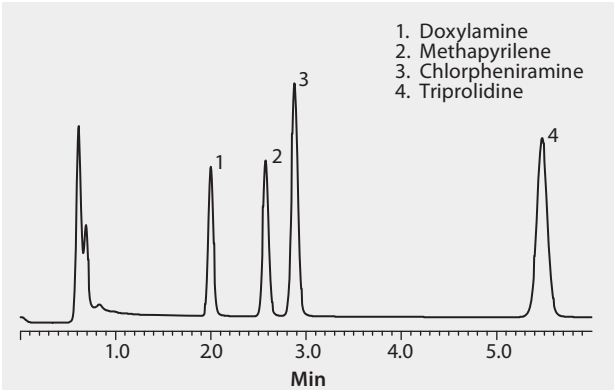





SUPELCO® Solutions within.™

## HPLC Analysis of Amines on Discovery® Zr-PBD at 80° C

column . . . . Discovery Zr-PBD, 7.5 cm × 4.6 mm I.D., 3 µm particles (65717-U)
mobile phase . . . . . (A) 50 mM Triethylammonium Hydroxide (pH 12.6): (B) acetonitrile (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 80 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
Application No. . . . . G001861

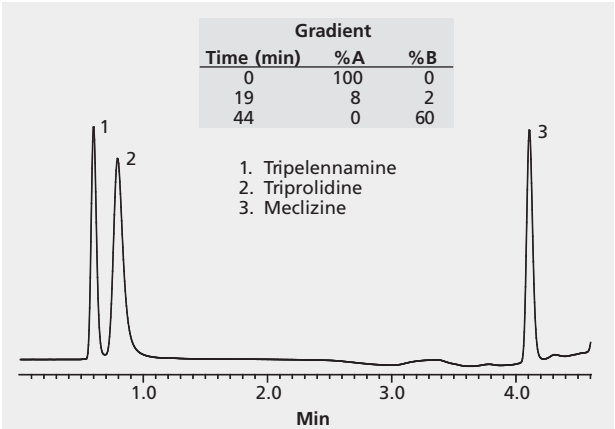





SUPELCO® Solutions within.™

## HPLC Analysis of Amines on Discovery® Zr-PS

column . . . . . Discovery Zr-PS, 5 cm × 4.6 mm I.D., 3 µm particles (65740-U)
mobile phase . . . . . (A) 25 mM HCl, pH 1.8; (B): acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . tripelennamine, triprolidine (1mg/mL), meclizine (3mg/mL)
Application No. . . . . G001870



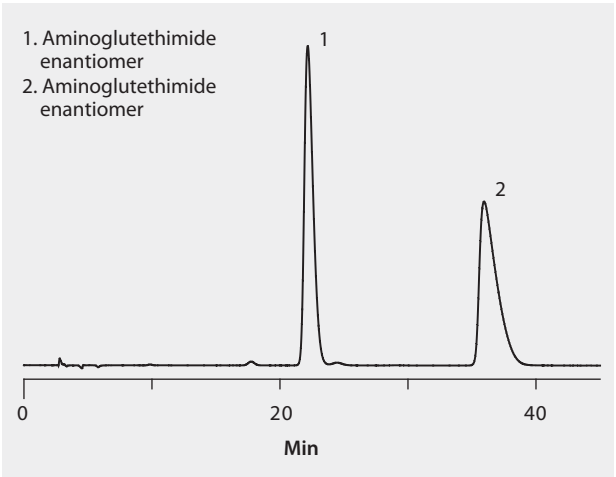



SUPELCO® Solutions within.™

## HPLC Analysis of Aminoglutethimide Enantiomers on Astec® Cellulose DMP

Aminoglutethimide enantiomers are resolved by normal phase chromatography.

column . . ASTEC Cellulose DMP; 25 cm x 4.6 mm I.D., 5 µm particles (51099AST)
mobile phase . . (A) heptane; (B) isopropanol; (C) diethylamine; (70:30:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
injection . . . . . 15 µL
sample . . . . . aminoglutethimide, 1 g/L in 50:50, heptane:isopropanol
Application No. . . . . G005466

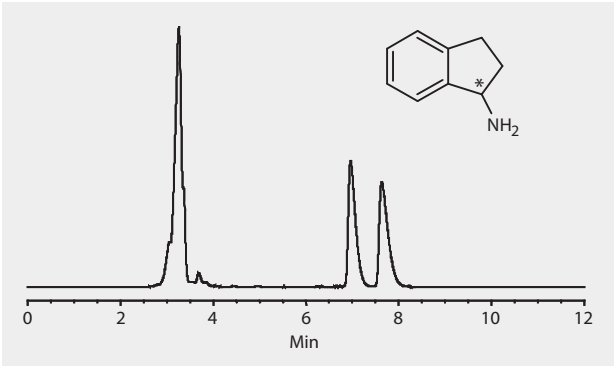





SUPELCO® Solutions within.™

## HPLC Analysis of 1-Aminoindan Enantiomers on LARIHC™ CF6-P

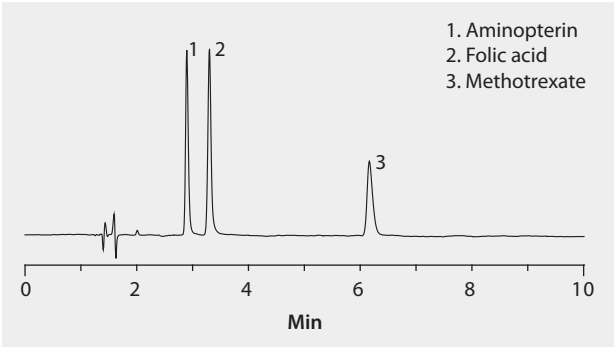
column . . LARIHC CF6-P, 25 cm x 4.6 mm I.D., 5 µm particles (AZYP Part No.L1001, available from Supelco/Sigma-Aldrich as a custom item)
mobile phase . . . . . (A) methanol: (B) acetonitrile: (C) acetic acid: (D) triethylamine, (70:30:0.3.0.2, A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1-Aminoindan, 0.3 mg/mL in ethanol
Application No. . . . . G005255



## HPLC Analysis of Aminopterin and Related Compounds on Ascentis® Express C18

This application demonstrates the suitability of the Ascentis C18 for the analysis of aminopterin, folic acid and methotrexate. Structures along with the optimized chromatogram obtained on the Ascentis Express C18 are presented

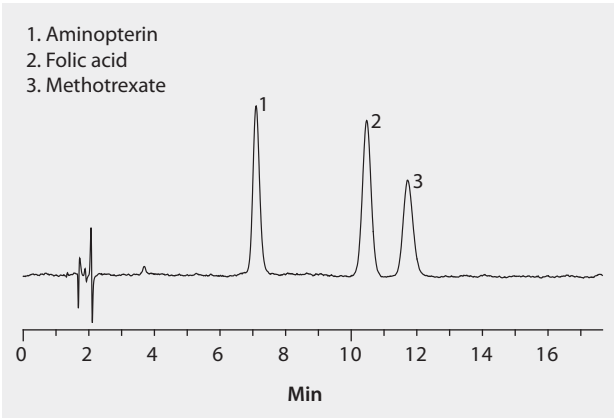
column . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)
mobile phase . . (A) 10 mM ammonium formate, pH 3.0; (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 5 µL
Application No. . . . . G005665



## HPLC Analysis of Aminopterin and Related Compounds on Ascentis® RP-Amide

This application demonstrates the suitability of the Ascentis RP-Amide for the analysis of aminopterin, folic acid and methotrexate. Structures along with the optimized chromatogram obtained on the Ascentis RP-Amide are presented.

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565352-U)
mobile phase . . (A) 10 mM ammonium formate, pH 3.0; (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
Application No. . . . . G005666



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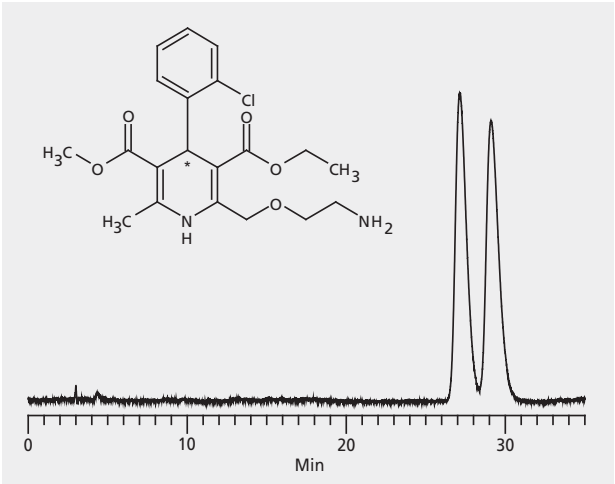
## HPLC Analysis of Amlodipine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) methanol; (B) 20 mM ammonium acetate, pH 4.1; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004463

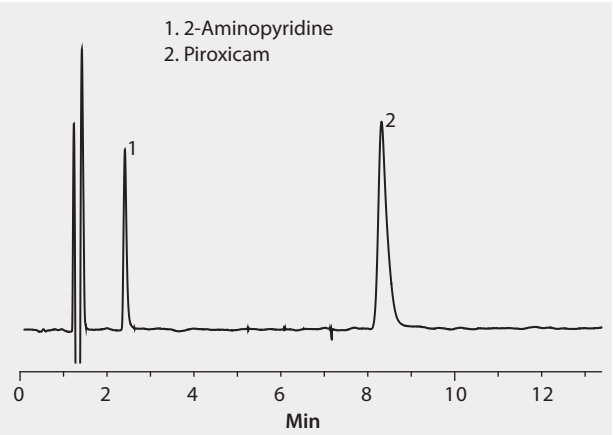





SUPELCO® Solutions within.™

## HPLC Analysis of 2-AMP and Piroxicam on Ascentis® Express F5

column . . . Ascentis Express F5, 10 cm x 4.6 mm I.D., 2.7 µm particles (53590-U)
mobile phase . . (A) 10 mM ammonium formate, pH=3; (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 0.8 mL/min
pressure . . . . . 1635 psi (113 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 100 µg/mL Piroxicam and 10 µg/mL 2-AMP in mobile phase
Application No. . . . . G005606

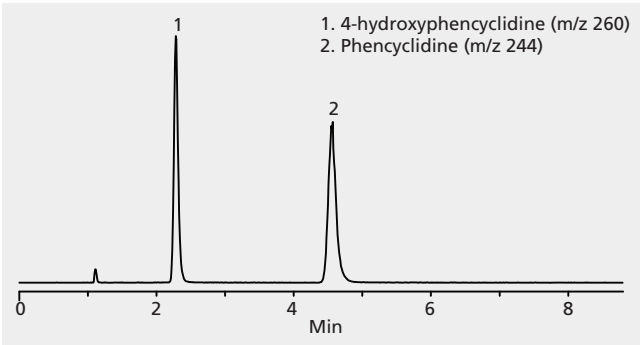





SUPELCO® Solutions within.™

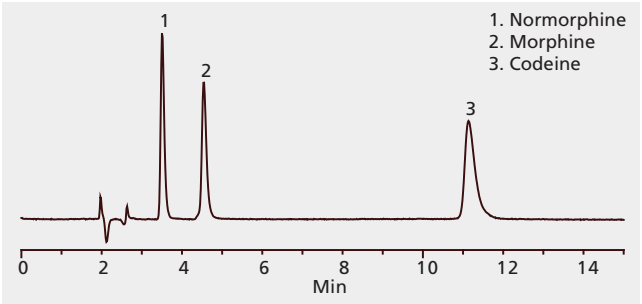
## HPLC Analysis of Anaesthetic Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 4.5 with acetic acid); (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI (+)
injection . . . . . 10 µL
sample . . . . . 1 µg/mL each in mobile phase
Application No. . . . . G002659



## HPLC Analysis of Analgesics, Narcotics on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formic acid); (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 90:10, water:methanol
Application No. . . . . G003709



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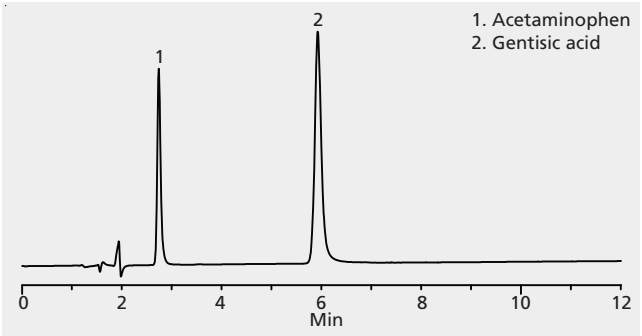
## HPLC Analysis of Analgesics on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0, unadjusted); (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002354






SUPELCO® Solutions within.™

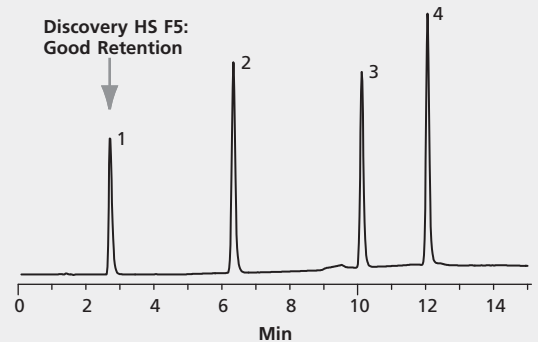
## HPLC Analysis of Analgesics on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . (A) 10 mM ammonium acetate, 0.1% formic acid; (B) methanol
flow rate . . . . . 1.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254nm
injection . . . . . 10 µL
Application No. . . . . G001526

**Gradient Program**

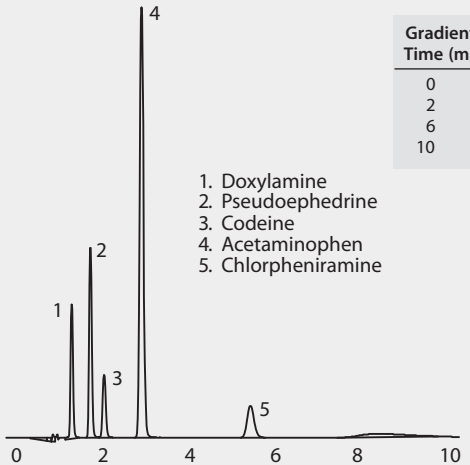
| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 3 | 90 | 10 |
| 10 | 50 | 50 |
| 15 | 50 | 50 |

1. *p*-Aminophenol (100 g/mL)
2. Acetaminophen (10 g/mL)
3. Acetanilide (10 g/mL)
4. Phenacetin (10 g/mL)



## HPLC Analysis of Analgesics on Discovery® RP-Amide C16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) 25 mM potassium phosphate (pH 2.3); (B) acetonitrile
flow rate . . . . . 2 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10µL
Application No. . . . . G000785A

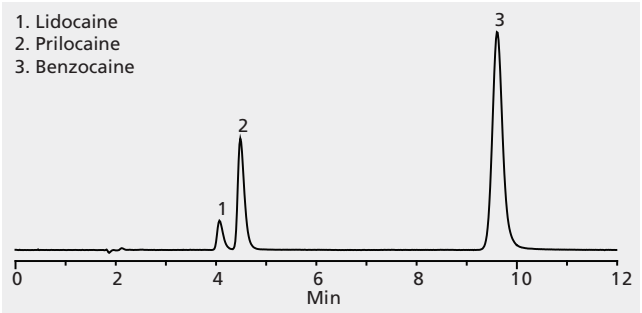



| Gradient Program | |
|---|---|
| Time (min) | %B |
| 0 | 90 |
| 2 | 90 |
| 6 | 70 |
| 10 | 70 |

1. Doxylamine
2. Pseudoephedrine
3. Codeine
4. Acetaminophen
5. Chlorpheniramine

## HPLC Analysis of Anesthetics, Local on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formic acid); (B) methanol; (55:45, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 85:15, water:methanol
Application No. . . . . G003708



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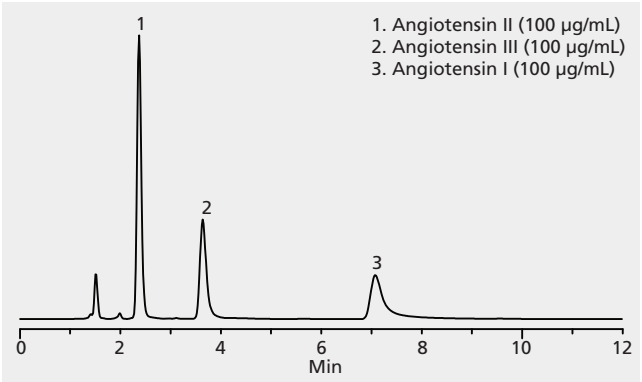
## HPLC Analysis of Angiotensins on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 5 mM ammonium phosphate (pH 7.0 with ammonium hydroxide); (B) acetonitrile; (77.5:22.5, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G002914



## HPLC Analysis of Angiotensins on Discovery® BIO Wide Pore C18

column . . . . . Discovery BIO Wide Pore C18, 15 cm × 4.6 mm I.D., 5 µm particles (568222-U)

mobile phase . . . . . (A) (10 mM $NH_4OAc$, pH 7):(50% $CH_3CN$ in 20 mM $NH_4OAc$, pH 7), 65:35 ; (B) (10 mM $NH_4OAc$, pH 7):(50% $CH_3CN$ in 20 mM $NH_4OAc$, pH 7), 25:75

gradient . . . . . 0-100% B in 12.5 min

flow rate . . . . . 1 mL/min

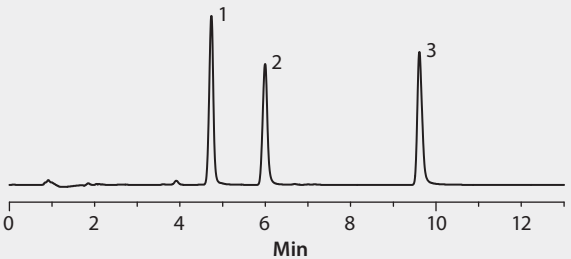
column temp. . . . . ambient

detector . . . . . UV, 254 nm

injection . . . . . 6 µL (10 µg each) in water

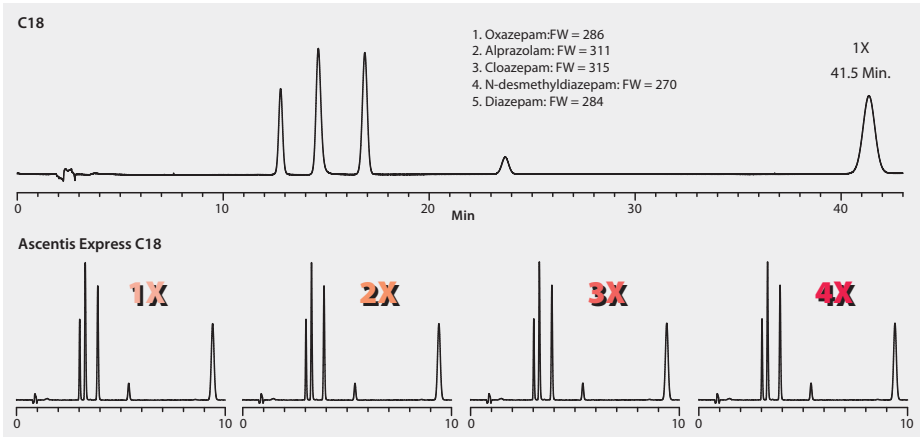
Application No. . . . . G001585

| Peptide | Sequence |
|---|---|
| 1. Angiotensin II | DRVYIHPF |
| 2. Angiotensin III | RVYIHPF |
| 3. Angiotensin I | DRVYIHPFHL |



## HPLC Analysis of Antianxiety Drugs on Ascentis® Express C18 vs. Standard C18, Demonstrating High Throughput on Ascentis Express

column . . . . . C18, 25 cm × 4.6 mm I.D., 5 µm particles and Ascentis Express C18, 10 cm × 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) water: (B) acetonitrile; (65:35, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
Application No. . . . . G004039



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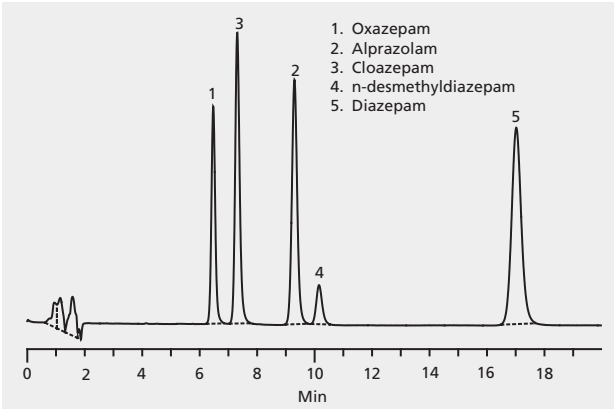
## HPLC Analysis of Antianxiety Drugs on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm × 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) acetonitrile; (B) water; (40:60, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G003664

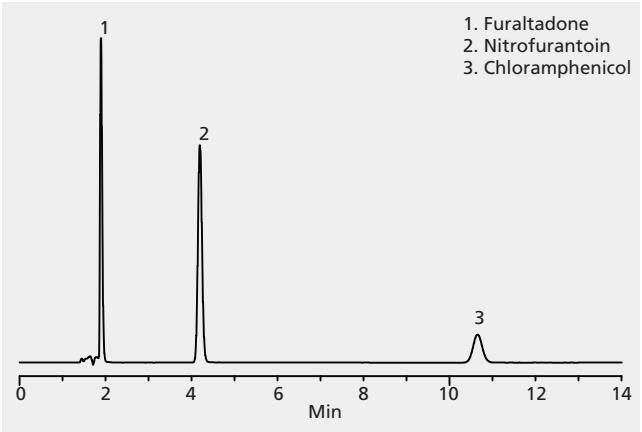





SUPELCO® Solutions within.™

## HPLC Analysis of Antibacterials/Antifungals on Ascentis® RP-Amide (Formate Mobile Phase)

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formic acid); (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002602

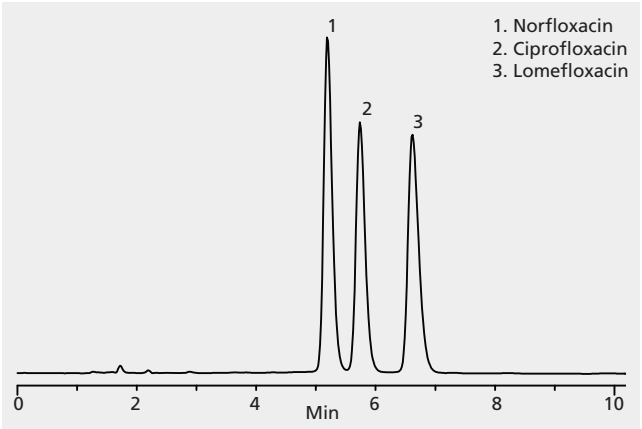





SUPELCO® Solutions within.™

## HPLC Analysis of Antibacterials/Antifungals on Ascentis® RP-Amide, Phosphate Mobile Phase

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002589

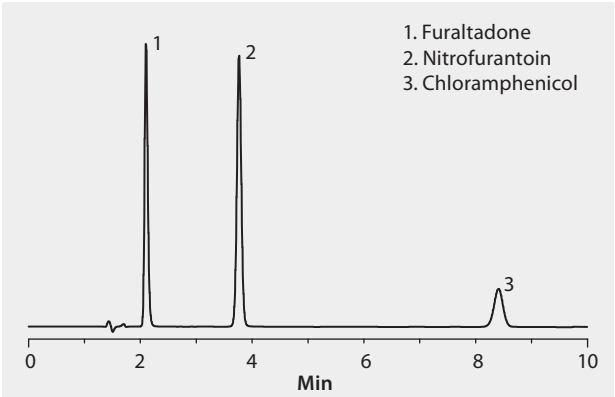





SUPELCO® Solutions within.™

## HPLC Analysis of Antibacterials/Antifungals on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formicacid); (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL each in acetonitrile
Application No. . . . . G002325



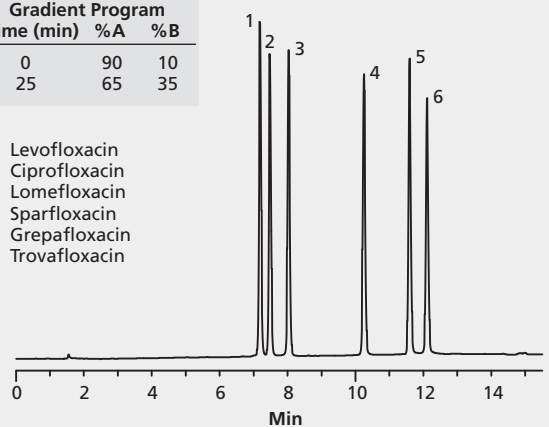
## HPLC Analysis of Antibiotics (Fluoroquinolones from Tablets) on Discovery® HS C18

column . . . . Discovery HS C18, 15 cm × 4.6 mm I.D., 5 µm particles (568522-U)
mobile phase . . . . . (A) 25 mM potassium phosphate (pH 3.0); (B) acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G001425

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 25 | 65 | 35 |

1. Levofloxacin
2. Ciprofloxacin
3. Lomefloxacin
4. Sparfloxacin
5. Grepafloxacin
6. Trovafloxacin



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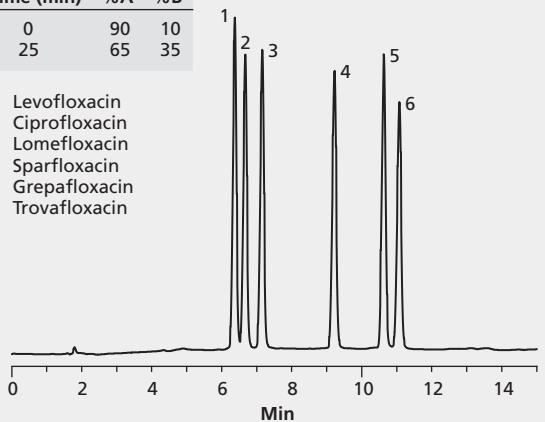
## HPLC Analysis of Antibiotics on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) 25 mM potassium phosphate (pH 3.0); (B) acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G001424

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 25 | 65 | 35 |

1. Levofloxacin
2. Ciprofloxacin
3. Lomefloxacin
4. Sparfloxacin
5. Grepafloxacin
6. Trovafloxacin

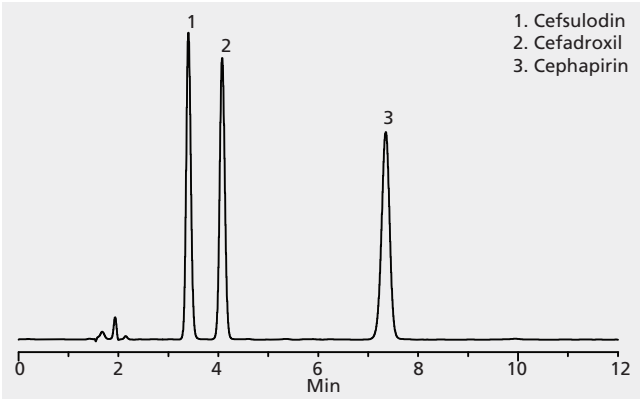





SUPELCO® Solutions within.™

## HPLC Analysis of Antibiotics on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile (93:7, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002407

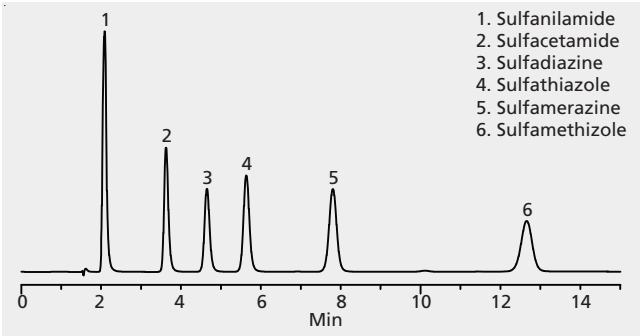





SUPELCO® Solutions within.™

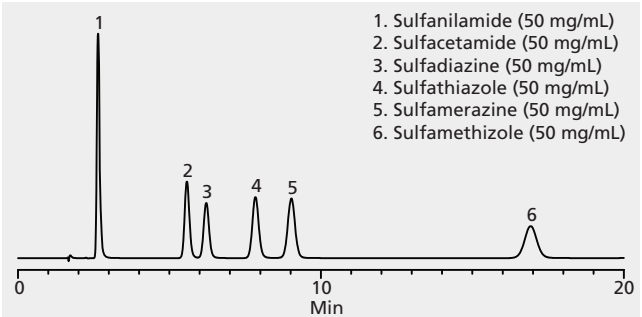
## HPLC Analysis of Antibiotic Sulfa Drugs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) water with 1% acetic acid; (B) methanol; (85:15, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002345



## HPLC Analysis of Antibiotic Sulfa Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 1% acetic acid in water; (B) methanol, (85:15, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 1% acetic acid in water
Application No. . . . . G002637

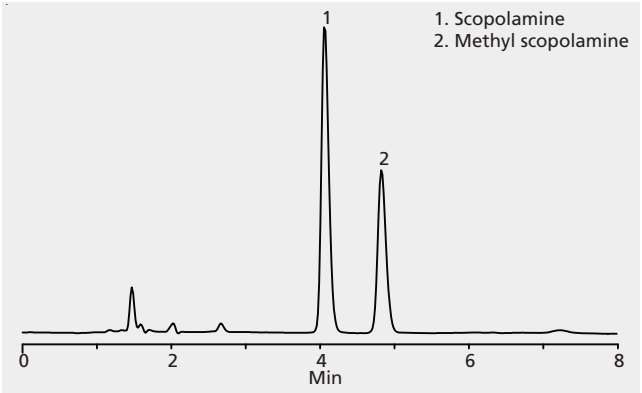





SUPELCO® Solutions within.™

## HPLC Analysis of Anticholinergic Drugs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002351



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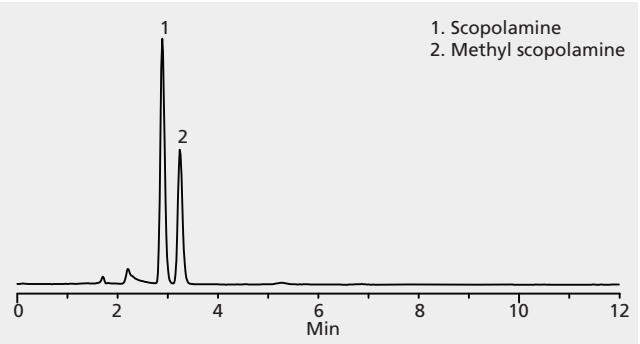
## HPLC Analysis of Anticholinergic Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002663

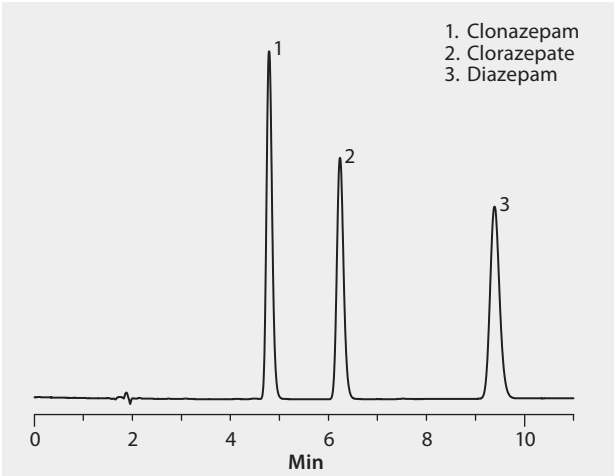





SUPELCO® Solutions within.™

## HPLC Analysis of Anticonvulsant Drugs on Discovery® RP-AmideC16

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . (A) acetonitrile; (B) 25 mM potassium phosphate, pH 7.0 (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000188

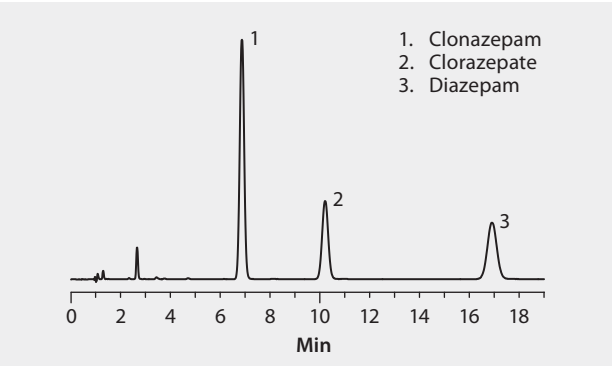





SUPELCO® Solutions within.™

## HPLC Analysis of Anticonvulsants on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) water: (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000746A

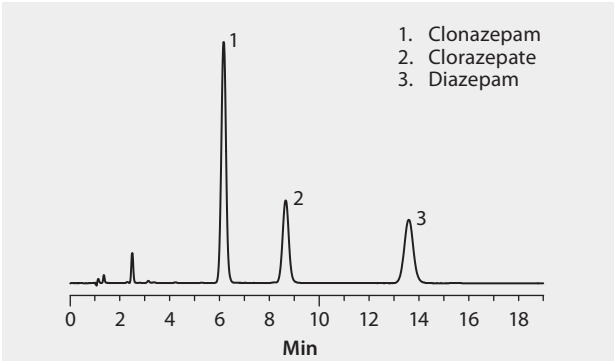





SUPELCO® Solutions within.™

## HPLC Analysis of Anticonvulsants on Discovery® C8

column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (595353-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000748

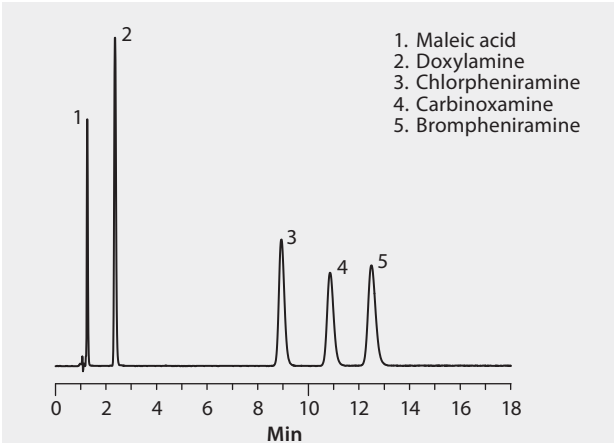





SUPELCO® Solutions within.™

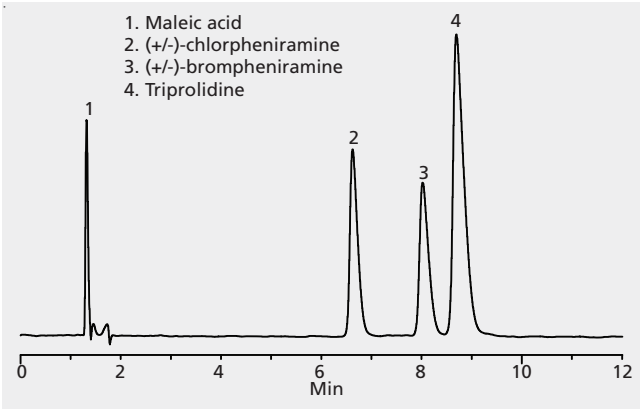
## HPLC Analysis of Antihistamines on Discovery® C8

column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . (A) acetonitrile; (B) 25 mM potassium phosphate, pH 2.3; (8:92, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
Application No. . . . . G001278



## HPLC Analysis of Antihistamines on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 10 µg/mL each in mobile phase
Application No. . . . . G002365

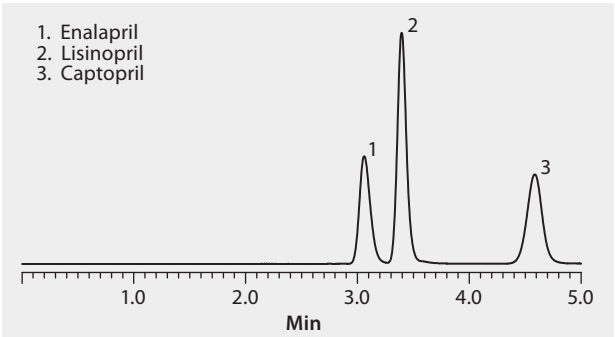





SUPELCO® Solutions within.™

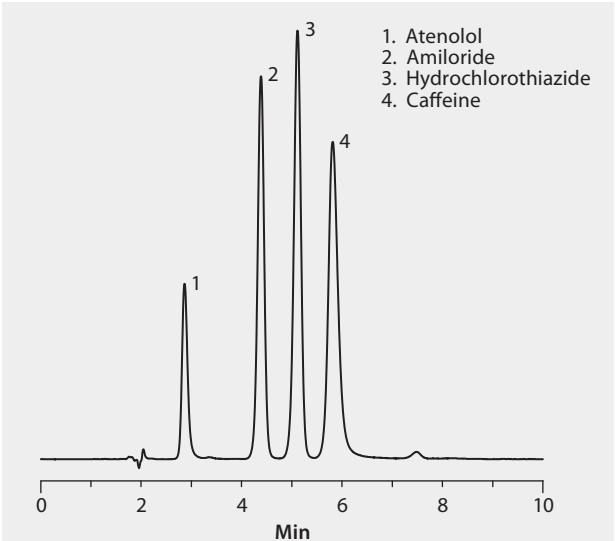
## HPLC Analysis of Antihypertensive ACE Inhibitors on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . (A) acetonitrile; (B) 25 mM potassium phosphate, pH 2.3 (33:67, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 3 µL
Application No. . . . . G000279



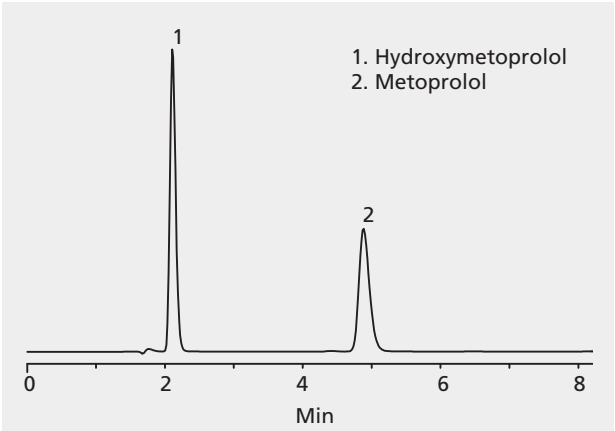
## HPLC Analysis of Antihypertensive and Diuretic Drugs on Discovery® RP-Amide C16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) methanol; (B) 25 mM potassium phosphate, dibasic, pH 7.0 (22:78, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000280



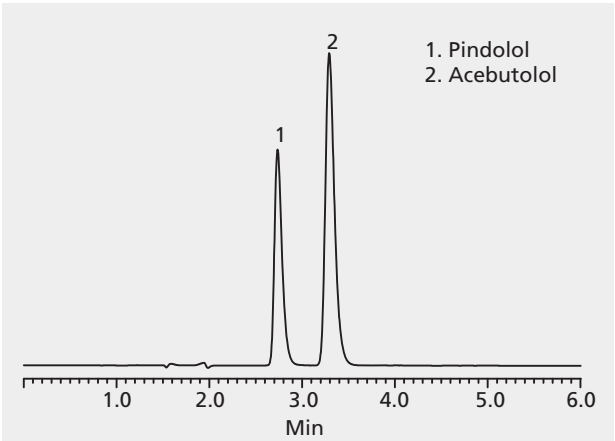
## HPLC Analysis of Antihypertensive Drugs on Ascentis® RP-Amide (UV, 220 nm)

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) methanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002598



## HPLC Analysis of Antihypertensive Drugs on Ascentis® RP-Amide (UV, 254 nm)

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002600

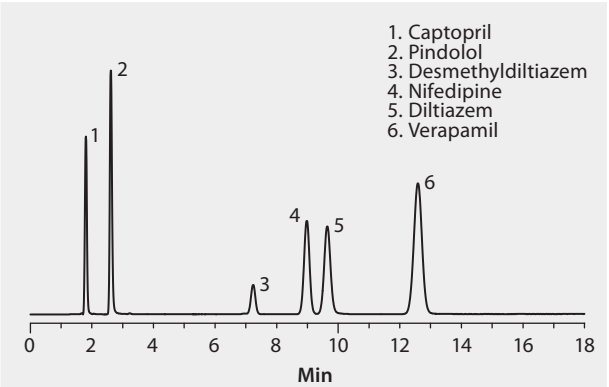





SUPELCO® Solutions within.™

## HPLC Analysis of Antihypertensive Drugs (ß-Calcium Channel Blockers and ACE Inhibitor) on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) methanol; (B) 25 mM $KH_2PO_4$, pH 7.0 (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
Application No. . . . . G000112

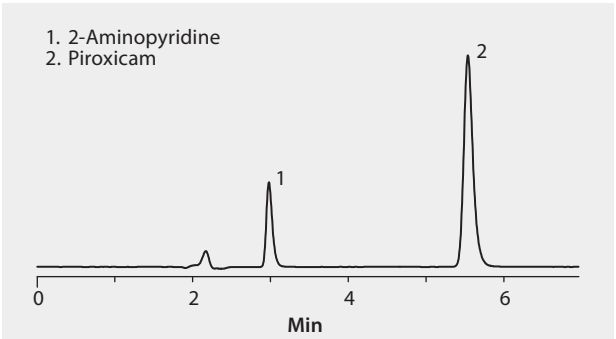





SUPELCO® Solutions within.™

## HPLC Analysis of Anti-Inflammatory Drugs (Aminopyridine and Piroxicam) on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . (A) 10 mM potassium phosphate (pH 2.5); (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
Application No. . . . . G001657

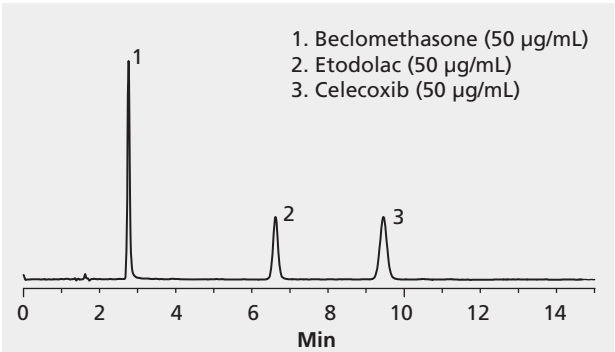





SUPELCO® Solutions within.™

## HPLC Analysis of Anti-Inflammatory Drugs on Ascentis® C18

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with formic acid); (B) acetonitrile; (45:55, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003155

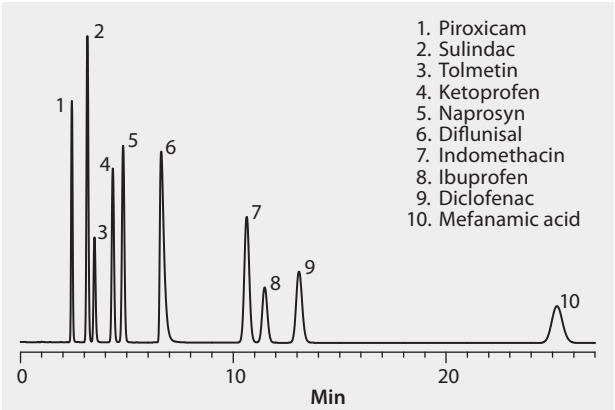





SUPELCO® Solutions within.™

## HPLC Analysis of Anti-Inflammatory Drugs on Discovery® RP-Amide C16

column . . Discovery RP-Amide C16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) acetonitrile; (B) 25 mM $KH_2PO_4$, pH 3.0; (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 230 nm
injection . . . . . 10 µL, 1 µg/mL each analyte
Application No. . . . . G000061

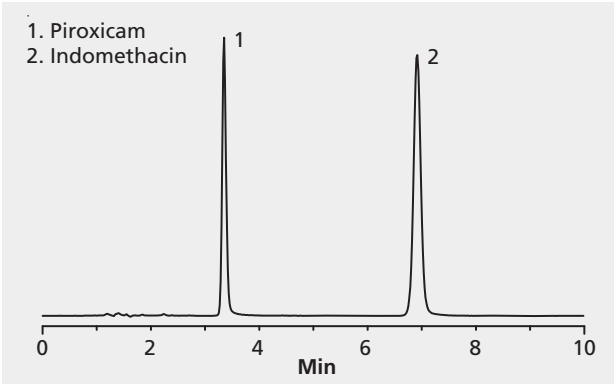





SUPELCO® Solutions within.™

## HPLC Analysis of Anti-Inflammatory Drugs on Ascentis® C8

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 25 µg/mL each in mobile phase
Application No. . . . . G002391

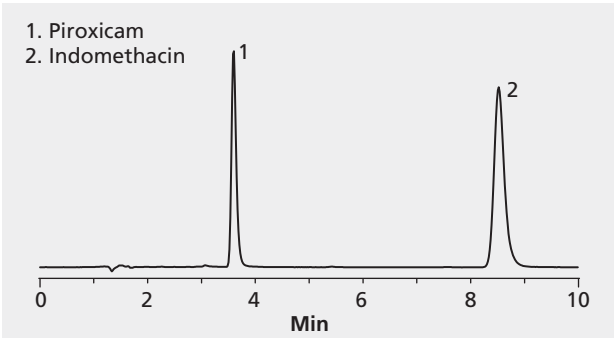





SUPELCO® Solutions within.™

## HPLC Analysis of Anti-Inflammatory Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (45:55, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 25 µg/mL each in mobile phase
Application No. . . . . G002660

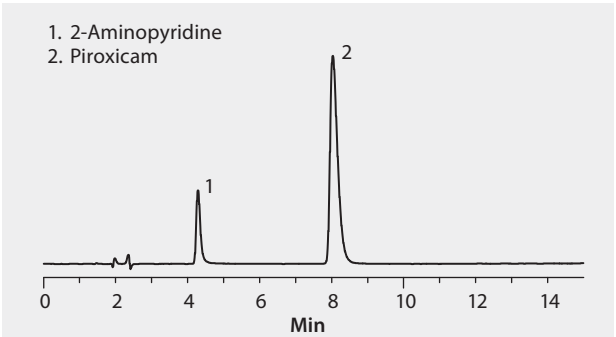





SUPELCO® Solutions within.™

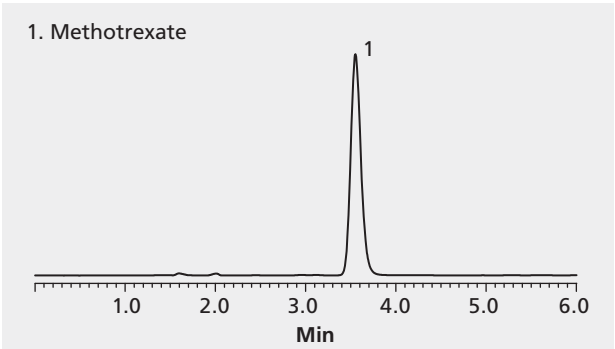
## HPLC Analysis of Anti-Inflammatory Drugs on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . (A) 10 mM potassium phosphate (pH 6.8); (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
Application No. . . . . G001658



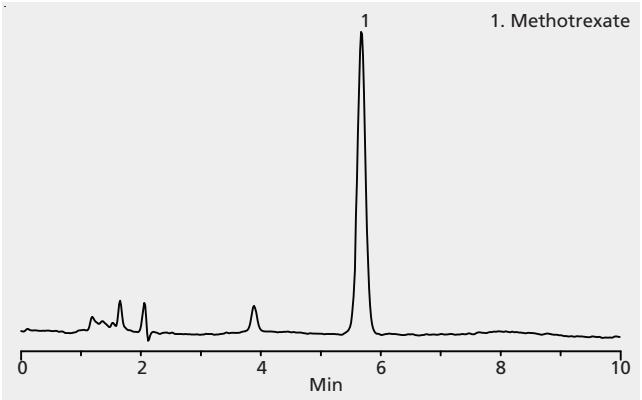
## HPLC Analysis of Antineoplastic Drugs on Ascentis® C18

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in mobile phase
Application No. . . . . G002655



## HPLC Analysis of Antineoplastic Drugs on Ascentis® RP-Amide

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (88:12, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 10 µg/mL in mobile phase
Application No. . . . . G002396



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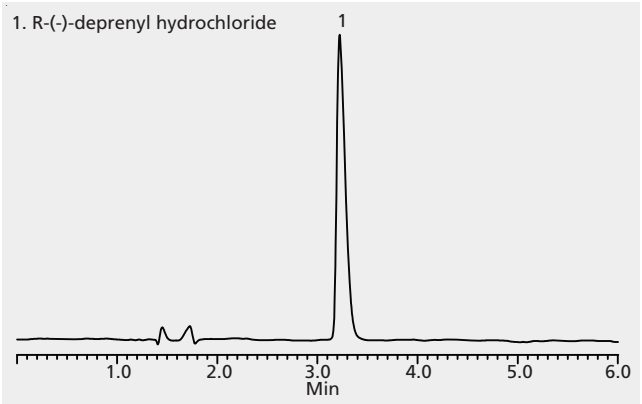
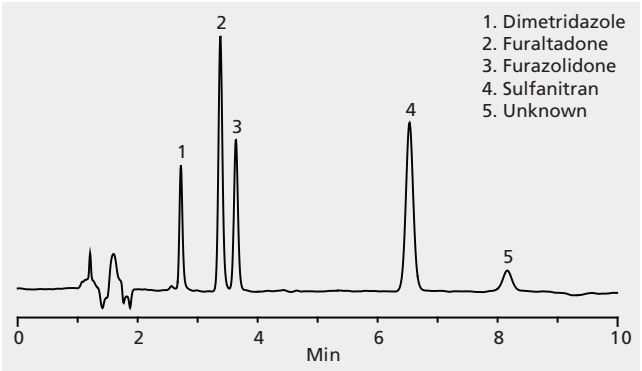
## HPLC Analysis of Antiparkinsonian Drugs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in mobile phase
Application No. . . . . G002398



## HPLC Analysis of Antiprotozoal Drugs on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 5.0 with acetic acid); (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in 85:15, water:methanol
Application No. . . . . G003703



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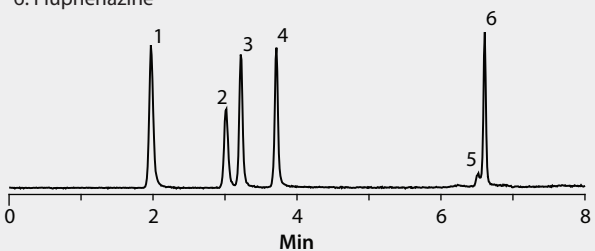
## HPLC Analysis of Antipsychotic Drugs on Ascentis® Express RP-Amide

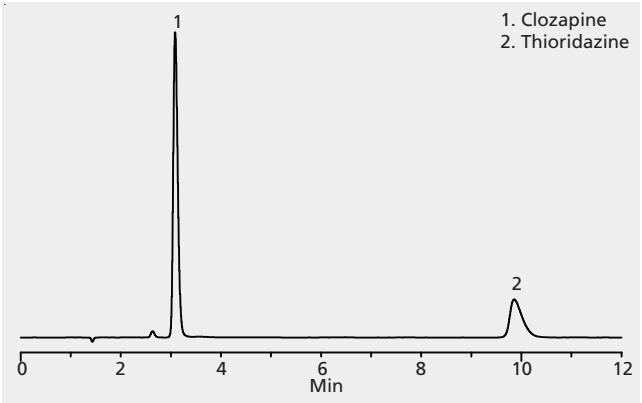
| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U) |
| mobile phase | (A) 5 mM ammonium formate (in water); (B) 5 mM ammonium formate (in 95:5 methanol:water) |
| flow rate | 3 mL/min |
| column temp. | 35 °C |
| detector | ESI(+), TIC 100 - 1000 m/z |
| injection | 1 µL |
| sample | 200 µg/L in water |
| Application No. | G005373 |

1. Desmethyl venlafaxine
2. Hydroxybupropion
3. Hydroxyrisperidon
4. Bupropion
5. Zuclopehthixol
6. Fluphenazine



## HPLC Analysis of Antipsychotic Drugs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) methanol; (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002400



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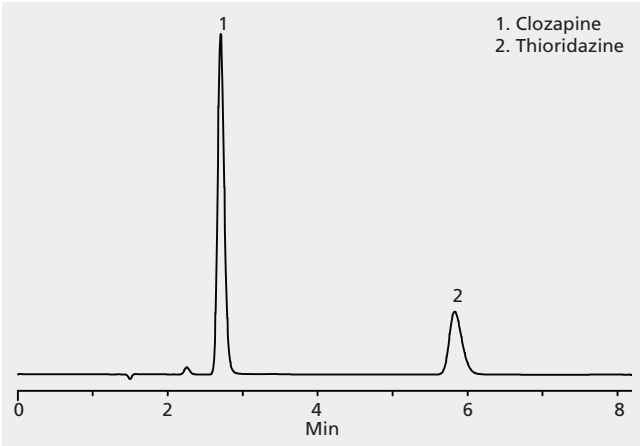
## HPLC Analysis of Antipsychotic Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) methanol; (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002603

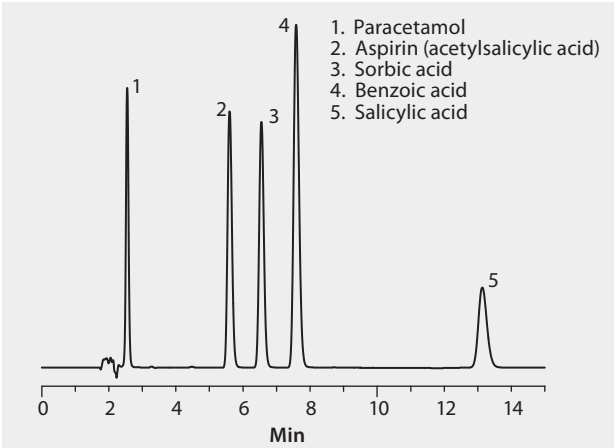





SUPELCO® Solutions within.™

## HPLC Analysis of Antipyretics/Analgesics/ Antifungals on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . 0.1% TFA in (25:75) acetonitrile:water
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G000088





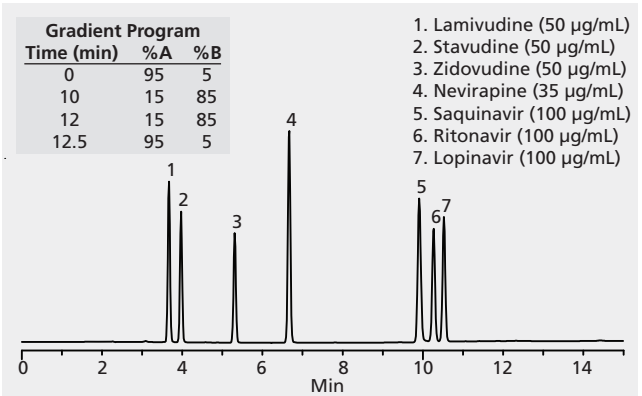

SUPELCO® Solutions within.™

## HPLC Analysis of Antiretroviral Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 25 mM ammonium phosphate (pH 5.5); (B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 25 mM ammonium phosphate (pH 5.5)
Application No. . . . . G002988

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 10 | 15 | 85 |
| 12 | 15 | 85 |
| 12.5 | 95 | 5 |






SUPELCO® Solutions within.™

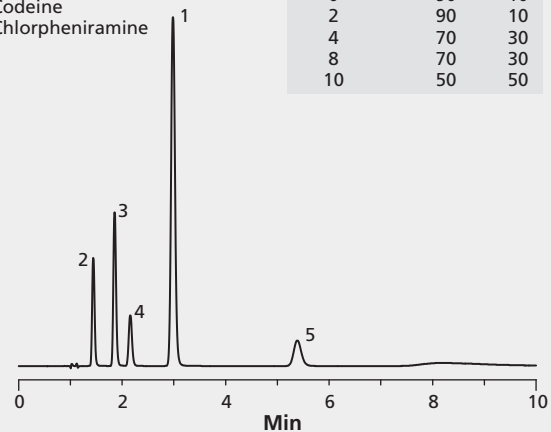
## HPLC Analysis of Antitussive/Antihistamine/ Antipyretic Mix on Discovery® RP-Amide C16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) 25 mM potassium phosphate (pH 2.3); (B) acetonitrile
flow rate . . . . . 2 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL, each compound 1 µg/mL
Application No. . . . . G000787

1. Acetaminophen
2. Doxylamine
3. Pseudoephedrine
4. Codeine
5. Chlorpheniramine

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 2 | 90 | 10 |
| 4 | 70 | 30 |
| 8 | 70 | 30 |
| 10 | 50 | 50 |



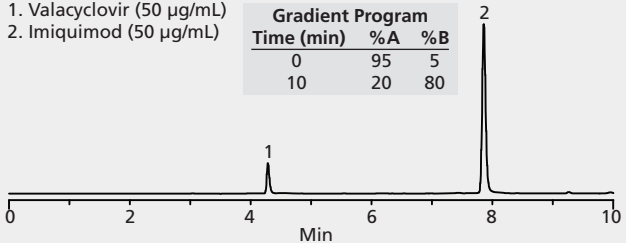
## HPLC Analysis of Antiviral Drugs on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 2.1 mm I.D., 3 µm particles (581402-U)
mobile phase . . . . . (A) 10 mM ammonium phosphate (pH 2.5 with phosphoric acid); (B) acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003201

1. Valacyclovir (50 µg/mL)
2. Imiquimod (50 µg/mL)

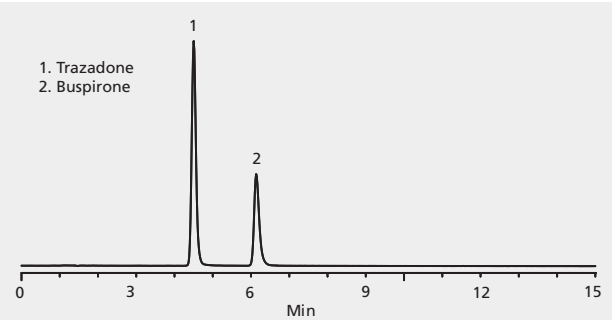
**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 10 | 20 | 80 |



## HPLC Analysis of Anxiolytic Drugs on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm × 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 5.5 with acetic acid); (B) acetonitrile; (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
Application No. . . . . G004104



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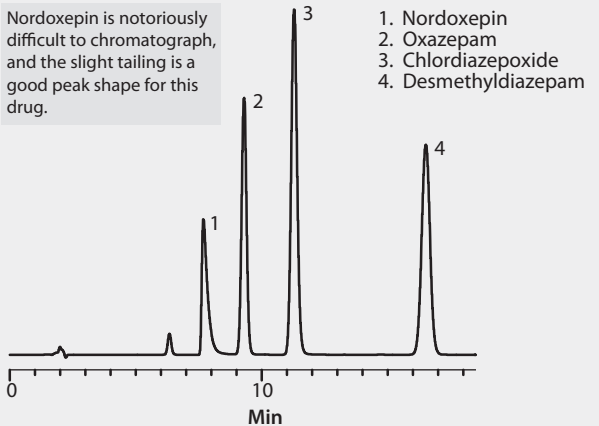
## HPLC Analysis of Anxiolytic Drugs on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) acetonitrile; (B) 25mM $KH_2PO_4$, pH 7.0 (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . 797-0662

Nordoxepin is notoriously difficult to chromatograph, and the slight tailing is a good peak shape for this drug.

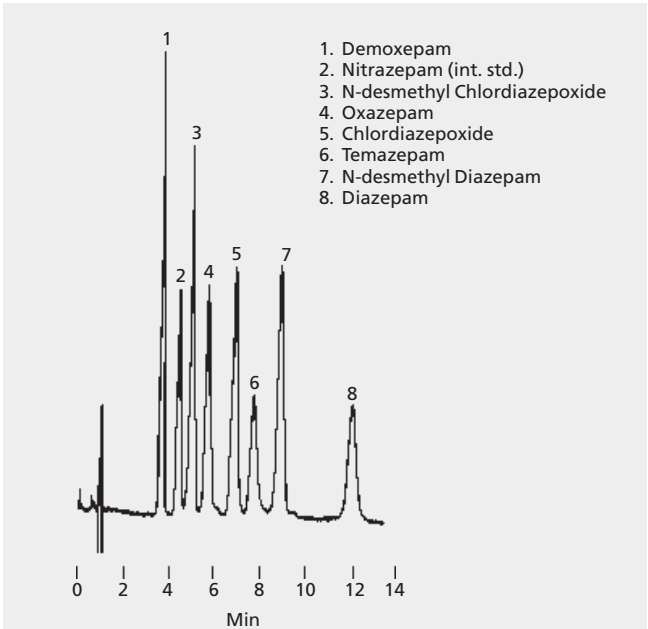





SUPELCO® Solutions within.™

## HPLC Analysis of Anxiolytic Drugs on SUPELCOSIL™ LC-8

column . . . . . SUPELCOSIL LC-8, 15 cm × 4.6 mm I.D., 5 µm particles (58220-U)
mobile phase . . methanol:acetonitrile:0.005 M $KH_2PO_4$ and 0.1 M ammonium acetate buffer (pH 6.0 with glacial acetic acid),26.5:16.5:57 (v/v/v)
flow rate . . . . . 2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 245 nm
injection . . . . . 10 µL
Application No. . . . . 713-1390

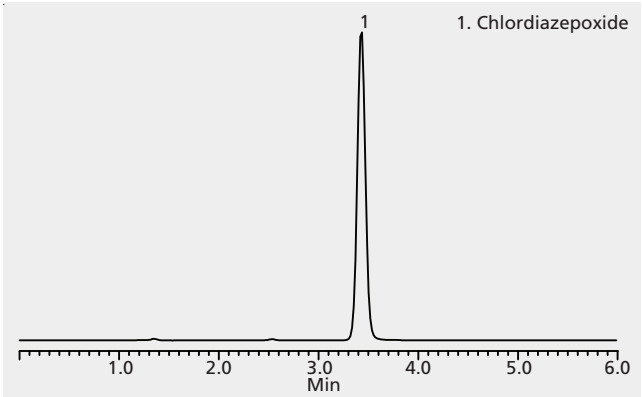





SUPELCO® Solutions within.™

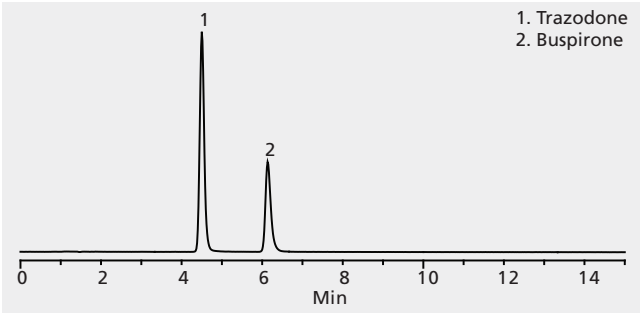
## HPLC Analysis of Anxiolytic Drugs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 7.0 with potassium hydroxide): (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002403



## HPLC Analysis of Anxiolytic Drugs on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 5.5 with acetic acid): (B) acetonitrile; (40:60, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 90:10, water:methanol
Application No. . . . . G003704

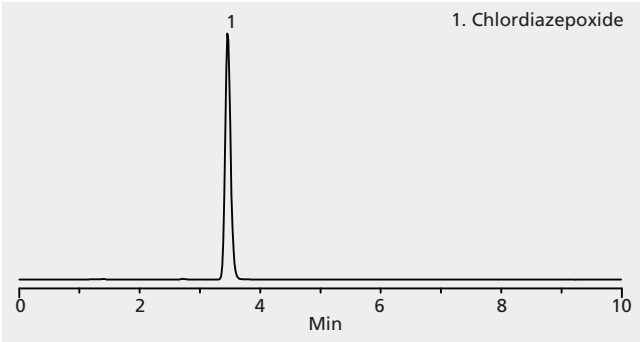





SUPELCO® Solutions within.™

## HPLC Analysis of Anxiolytic Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 7.0 with potassium hydroxide); (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002645

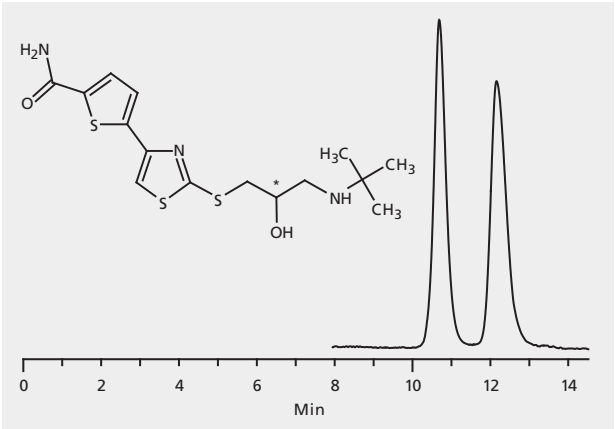





SUPELCO® Solutions within.™

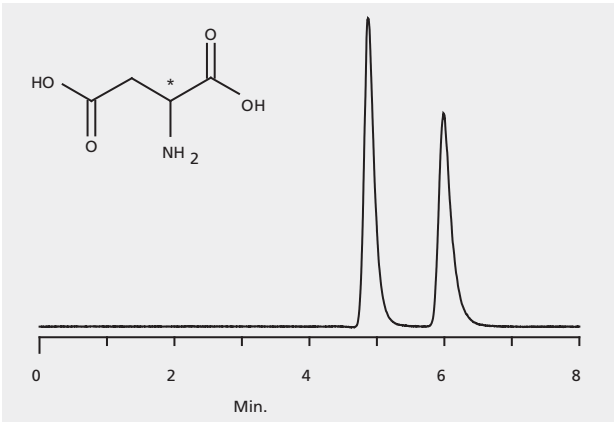
## HPLC Analysis of Arotinolol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) triethylamine; (55:45:0.3:0.2, A:B:C:D)
flow rate . . . . . 2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004539



## HPLC Analysis of Aspartic Acid Enantiomers on Astec® CHIROBIOTIC® T, Ethanol Mobile Phase

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (C) formic acid; (20:80:0.02, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004489



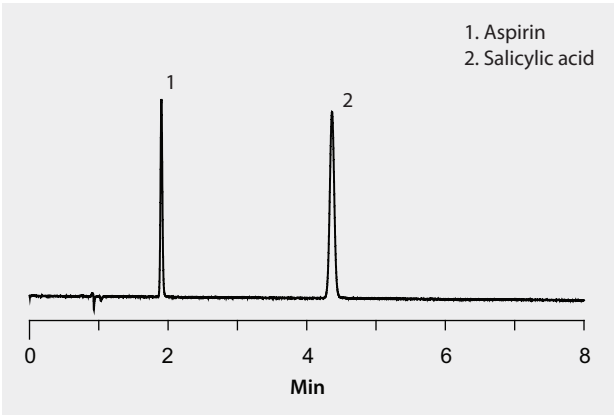



SUPELCO® Solutions within.™

## HPLC Analysis of Aspirin and Salicylic Acid on Ascentis® Express RP-Amide

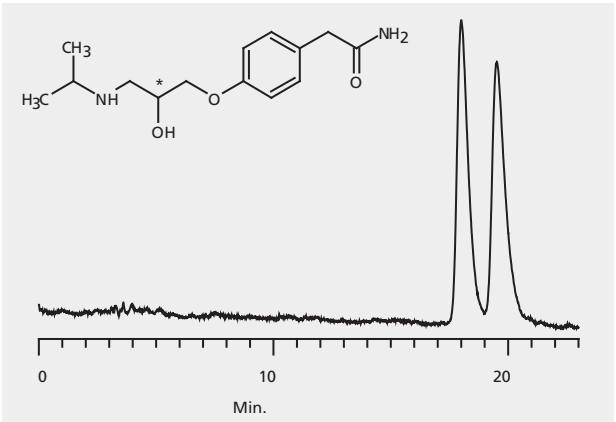
Salicylic acid is the primary metabolite of aspirin, and is likely responsible for its antiinflammatory properties via suppression of cyclooxygenase genes; it also is thought to suppress colonic carcinogenesis. Ascentis Express RP-Amide provides good retention and resolution at low pH by a combination of hydrophobic and H-bonding intereactions.

| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 4.6 mm I.D., 2.7 µm particles (53929-U) |
| mobile phase | (A) water with 0.1% TFA; (B) acetonitrile with 0.1% TFA; (65:35, A:B) |
| flow rate | 1 mL/min |
| pressure | 2200 psi (152 bar) |
| column temp. | 30 °C |
| detector | UV, 250 nm |
| injection | 5 µL |
| sample | 50 µg/mL in 75:25, water:acetonitrile |
| Application No. | G005797 |



## HPLC Analysis of Atenolol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004490

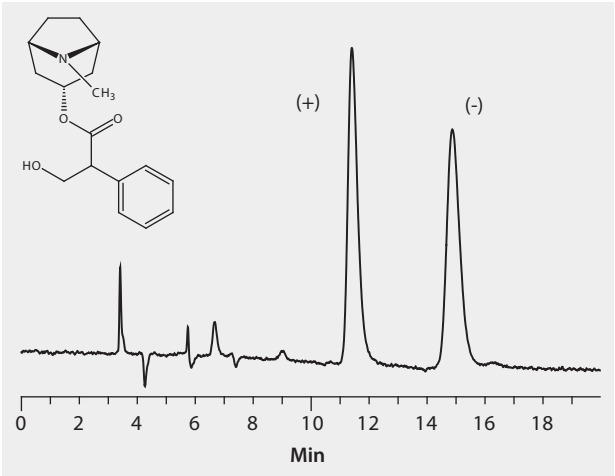





SUPELCO® Solutions within.™

## HPLC Analysis of Atropine Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) DEA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004985

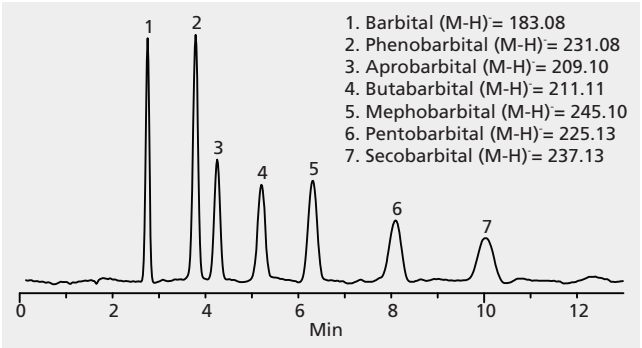





SUPELCO® Solutions within.™

## HPLC Analysis of Barbiturates on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) methanol; (B) water (45:55, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . 10 µg/mL each in 50:50 water:methanol
Application No. . . . . G003307

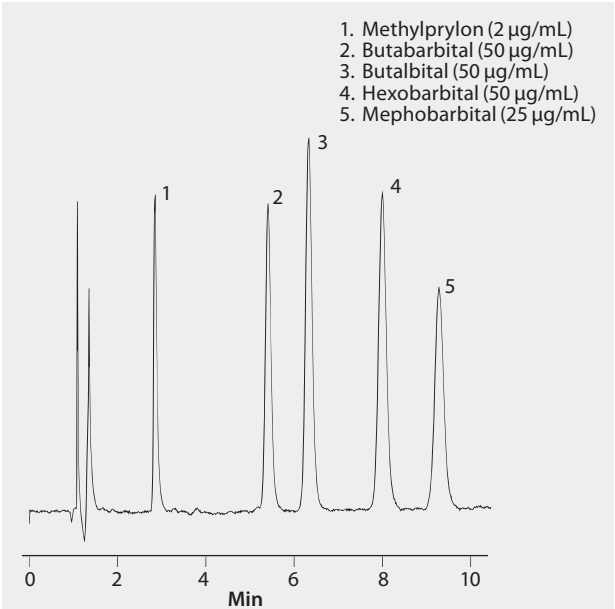





SUPELCO® Solutions within.™

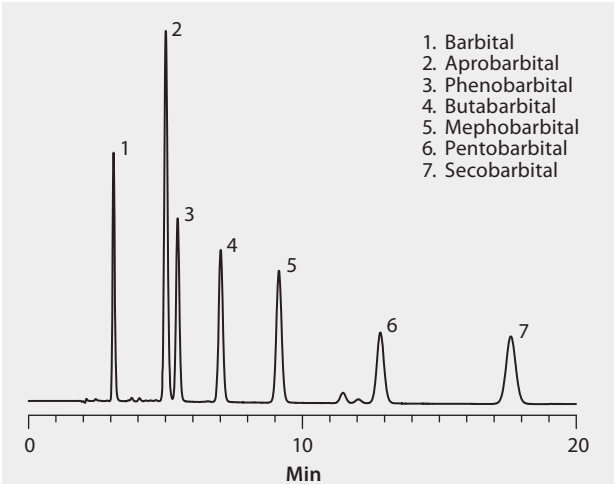
## HPLC Analysis of Barbiturates on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) acetonitrile; (B) 25 mM potassium phosphate buffer, pH 6.9 (25:75, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 25 µL
Application No. . . . . 713-0141



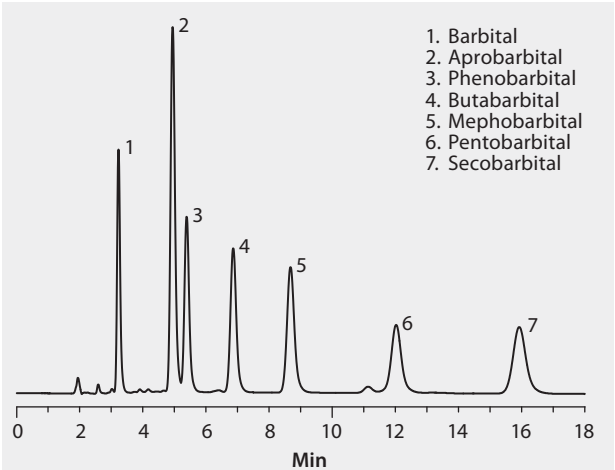
## HPLC Analysis of Barbiturates on Discovery® C18, Unbuffered Mobile Phase

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) methanol; (B) water (45:55, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
Application No. . . . . G000190



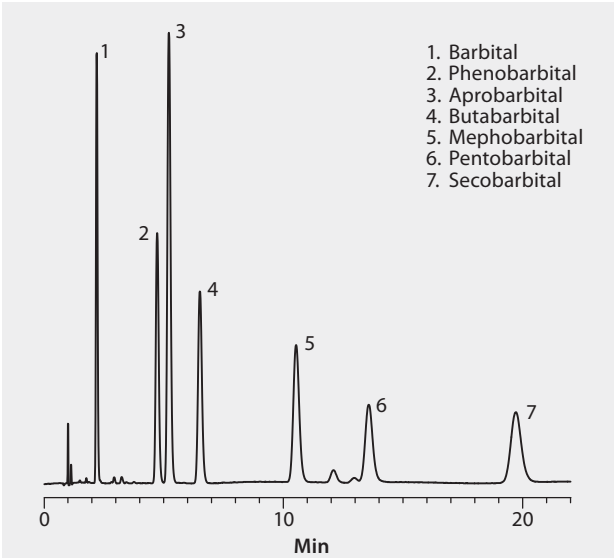
## HPLC Analysis of Barbiturates on Discovery® C8

column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . . . . (A) methanol; (B) water (45:55, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214nm
injection . . . . . 5 µL
Application No. . . . . G000791



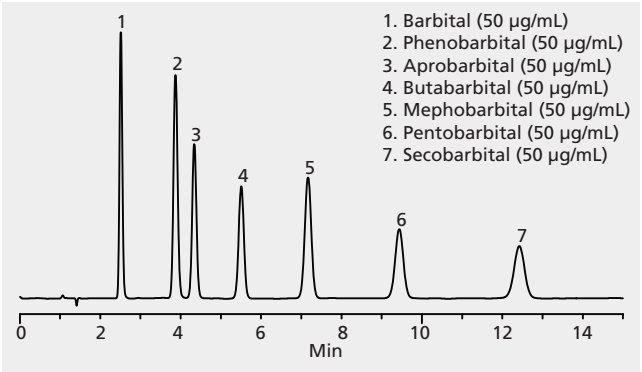
## HPLC Analysis of Barbiturates on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) methanol:acetonitrile, 60:40; (B) water; (25:75, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 214 nm
injection . . . . . 2 µL
Application No. . . . . G000193



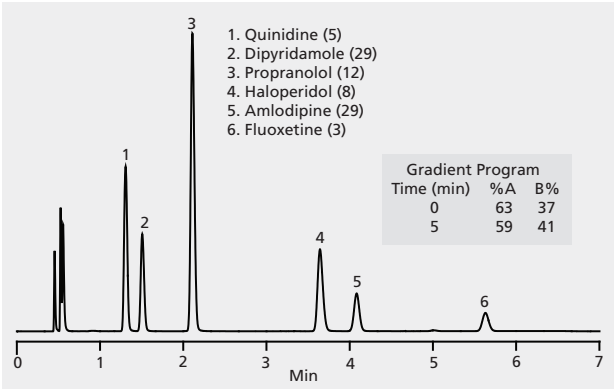
## HPLC Analysis of Barbiturates on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) methanol; (B) water (50:50, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 65:35 water:methanol
Application No. . . . . G002877



## HPLC Analysis of Basic Drug Panel on Ascentis® Express C18

column . . Ascentis Express C18, 10 cm × 4.6 mm I.D., 2.7 µm particles (53827-U)
mobile phase . . . . . (A) water with 0.05M potassium phosphate and 0.1% TEA and 0.6% OSA-Na at pH = 2.9, (B) acetonitrile
flow rate . . . . . 1.76 mL/min
column temp. . . . . ambient
detector . . . . . UV, 215 nm
injection . . . . . 100 µL
Application No. . . . . G004213



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## HPLC Analysis of Basic Peptides (Angiotensin Analogs) on Ascentis® Express Peptide ES-C18

column . . . . . Ascentis Express Peptide ES-C18, 10 cm x 3 mm I.D., 2.7 µm particles (53313-U)

mobile phase . . . . . (A) 0.1% (v/v) formic acid, pH 4.0 (titrated with ammonium hydroxide); (B) 50:50, (0.175% formic acid in water, pH 4.0) : acetonitrile

gradient . . . . . 20 to 50% B in 15 min

flow rate . . . . . 0.6 mL/min

pressure . . . . . 3002 psi (207 bar)

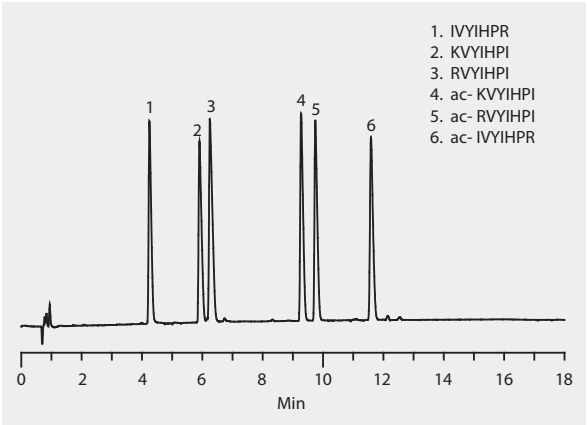
column temp. . . . . 35 °C

detector . . . . . UV, 215 nm

injection . . . . . 2.0 µL

sample . . . . . 0.5 g/L ea. peptide

Application No. . . . . G005379



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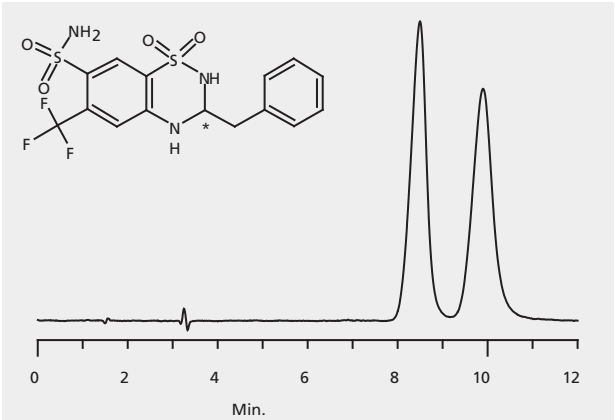
## HPLC Analysis of Bendroflumethiazide Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 215 nm
injection . . . . . 5 µg/mL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004491

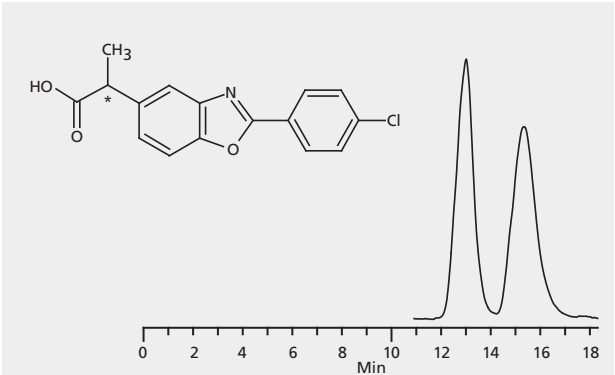





SUPELCO® Solutions within.™

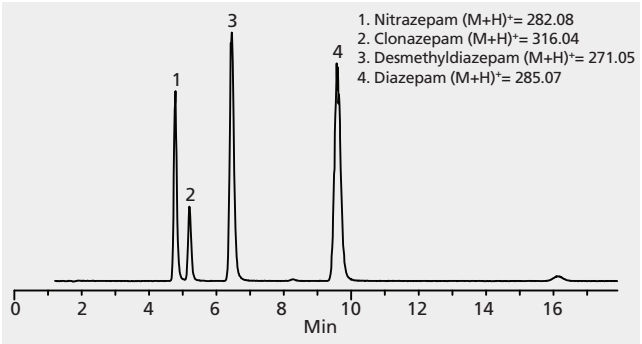
## HPLC Analysis of Benoxaprofen Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 0.1 wt% triethylamine, pH 7.0; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004637



## HPLC Analysis of Benzodiazepines on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 2.1 mm I.D., 3 µm particles (581402-U)
mobile phase . . . . . (A) 10 mM ammonium acetate (pH 6.98 unadjusted); (B) acetonitrile; (55:45, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+), Selected Ion Recording Mode
injection . . . . . 5 µL
sample . . . . . 1 µg/mL each in 10 mM ammonium acetate (pH 6.98 unadjusted)
Application No. . . . . G003309



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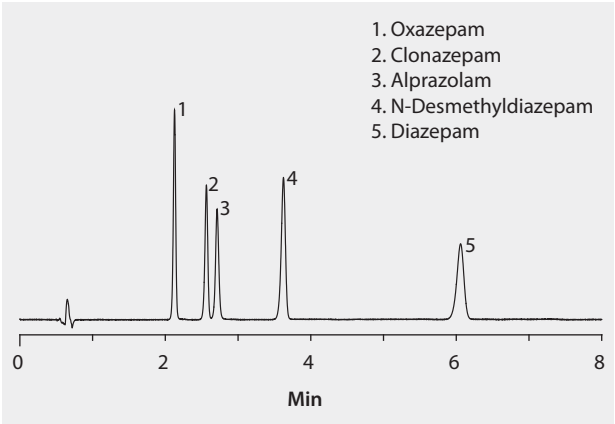
## HPLC Analysis of Benzodiazepines on Ascentis® Express C18, Acetonitrile Mobile Phase

column . . . Ascentis Express C18, 10 cm x 3 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (66:34, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 250 nm
injection . . . . . 2 µL
sample . . . . . 0.1 g/L each in 20% methanol
Application No. . . . . G005673

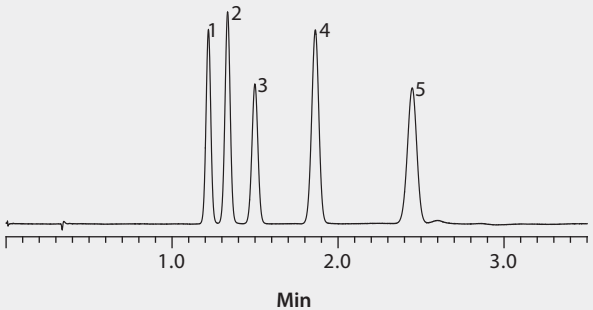


## HPLC Analysis of Benzodiazepines on Ascentis® Express Phenyl-Hexyl, Methanol Mobile Phase

The Phenyl-Hexyl phase of the Ascentis Express product line displays the best selectivity of this sample; selectivity is markedly affected by choice of organic modifier. The application also demonstrates feasibility of fast LC with Ascentis Express by virtue of low backpressure and high efficiency.

| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 5 cm x 4.6 mm I.D., 2.7 µm particles (53348-U) |
| mobile phase | (A) water; (B) methanol; (35:65, A:B) |
| flow rate | 1.4 mL/min |
| pressure | 3600 psi (248 bar) |
| column temp. | 35 °C |
| detector | UV, 250 nm |
| injection | 2 µL |
| sample | 0.1 g/L in 20% methanol |
| Application No. | G005674 |

1. Clonazepam
2. Oxazepam
3. Alprazolam
4. N-Desmethyldiazepam
5. Diazepam



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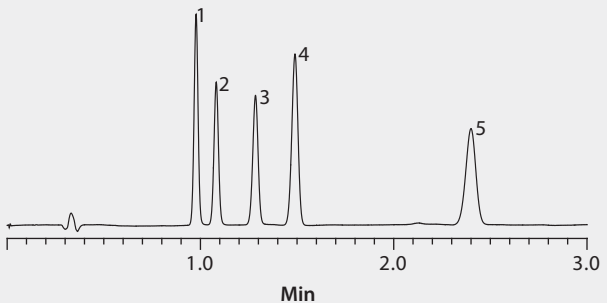
## HPLC Analysis of Benzodiazepines on Ascentis® Express Phenyl-Hexyl with Acetonitrile Mobile Phase

The Phenyl-Hexyl phase of the Ascentis Express product line displays the best selectivity of this sample; selectivity is markedly affected by choice of organic modifier. The application also demonstrates feasibility of fast LC with Ascentis Express by virtue of low backpressure and high efficiency.

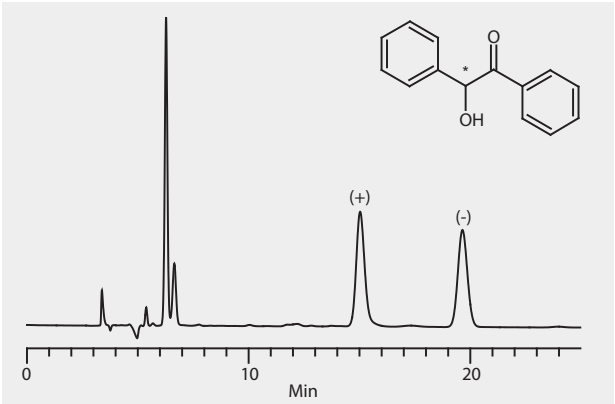
| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 5 cm x 4.6 mm I.D., 2.7 µm particles (53348-U) |
| mobile phase | (A) water; (B) acetonitrile; (62:38, A:B) |
| flow rate | 1.4 mL/min |
| pressure | 2600 psi (179 bar) |
| column temp. | 35 °C |
| detector | UV, 250 nm |
| injection | 2 µL |
| sample | 0.1 g/mL of each in 20% methanol |
| Application No. | G005675 |

1. Oxazepam
2. Alprazolam
3. Clonazepam
4. N-Desmethyldiazepam
5. Diazepam



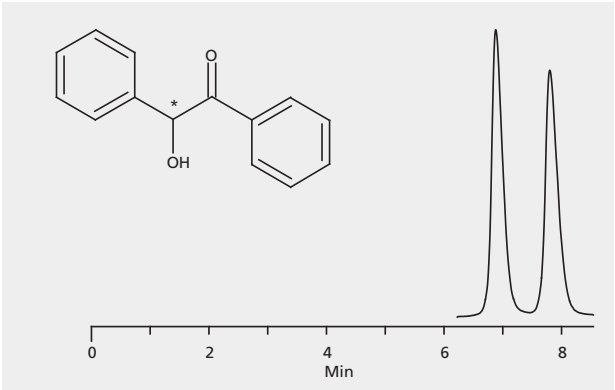
## HPLC Analysis of Benzoin Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) TFA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004986



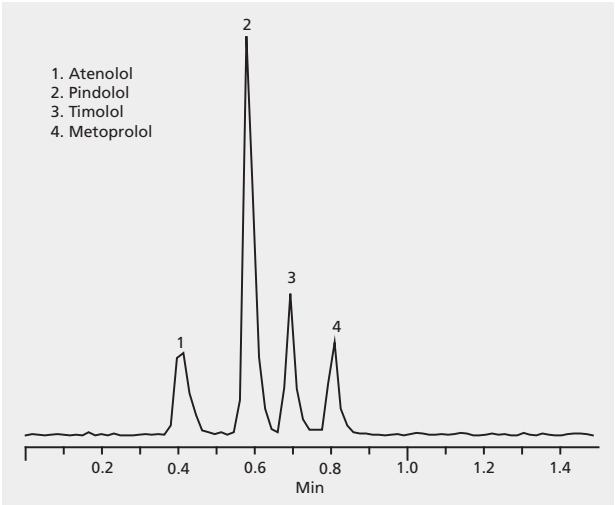
## HPLC Analysis of Benzoin Enantiomers on Astec® (R,R) P-CAP™-DP

column . . Astec (R,R) P-CAP-DP, 25 cm x 4.6 mm I.D., 5 µm particles (35024AST)
mobile phase . . . . . (A) hexane; (B) ethanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004726



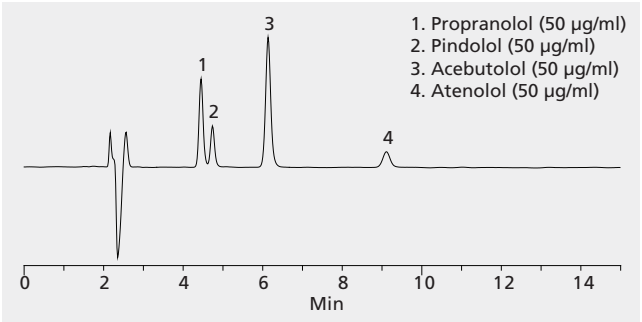
## HPLC Analysis of Beta Blockers on Ascentis® Express C18

column . . Ascentis Express C18, 5 cm × 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . (A) 0.1% acetic acid in water; (B) 0.1% acetic acid in acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . ABI 3200 QT; ESI(+), MS/MS
injection . . . . . 1 µL
instrument . . . . . Agilent 1100
Application No. . . . . G004063



## HPLC Analysis of Beta Blockers on Ascentis® Si

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted); (B) 0.1% ammonium acetate in acetontrile; (15:85, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 225 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 10:90:water:acetonitrile
Application No. . . . . G003718



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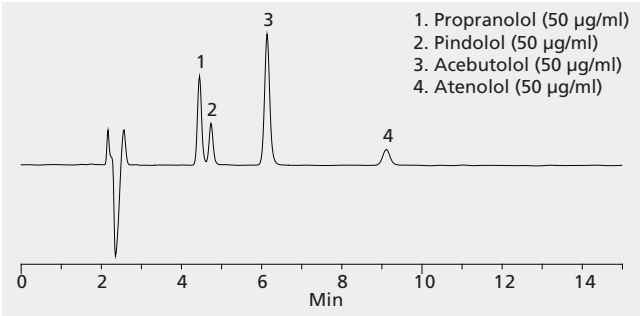
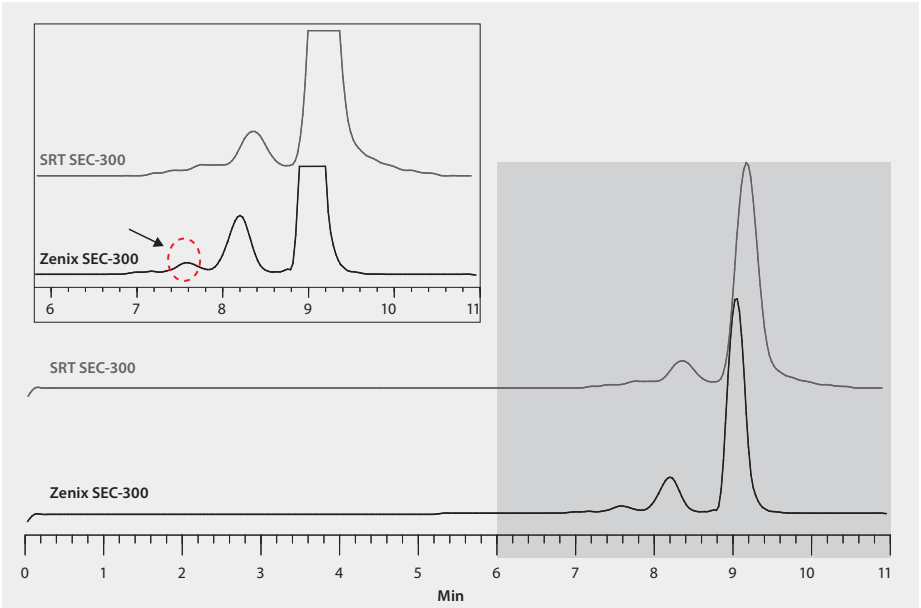
## HPLC Analysis of Beta Blockers on Ascentis® Si (Silica)

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted); (B) 0.1% ammonium acetate in acetonitrile; (15:85, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 225 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 10:90:water:acetonitrile
Application No. . . . . G003735



## HPLC Analysis of Bovine Serum Albumin (BSA) on Zenix® SEC-300 versus SRT® SEC-300, Effect of Particle Size on Resolution

column . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm, 300 Å particles (Z777051)
column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL, BSA
Application No. . . . . G006119



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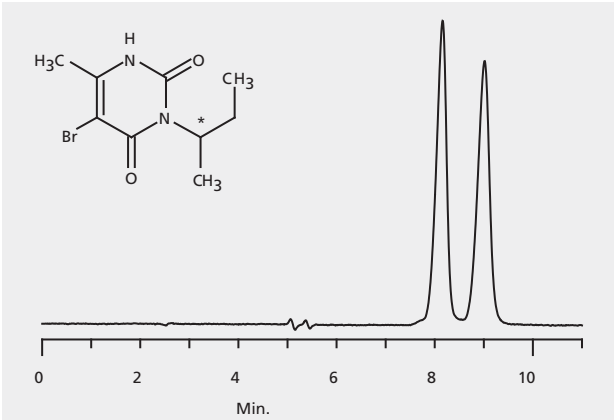
## HPLC Analysis of Bromacil Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (60:40, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 215 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004492



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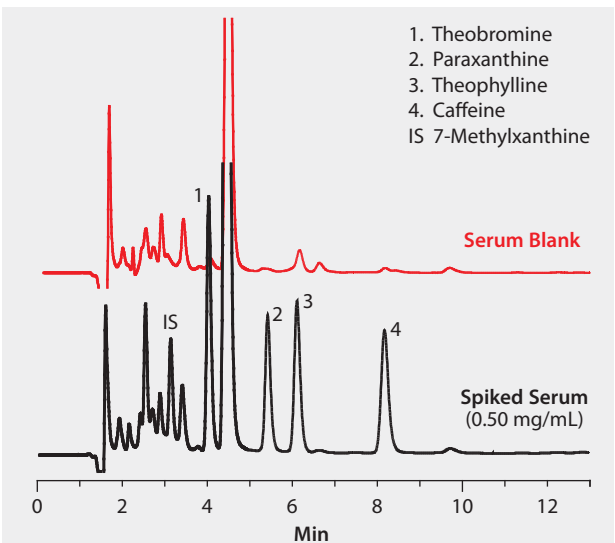
# HPLC Analysis of Bronchodilators Theophylline and Other Caffeine Metabolites from Serum on Discovery® RP-AmideC16 after SPE using Discovery® DSC-18

sample preparation . . . . . SPE (Solid Phase Extraction)
SPE tube/cartridge . . . . . Discovery® DSC-18, 500 mg/3 mL (52603-U)
condition . . . . . 2 mL methanol, then 2 mL water
sample preparation . . . . . 1 mL porcine serum spiked with 0.1 µg/mL, 0.50 µg/mL, or 1.0 µg/mL each analyte
sample preparation . . 1 mL methanol; evaporate to dryness with nitrogen stream at room temperature; reconstitute in 200 µL mobile phase containing 0.2 µg/mL or 7-methylxanthine (IS)
sample preparation . . . . . 2 mL 5% methanol in water; dry tube 10 min with nitrogen stream
column . . . . Discovery RP-AmideC16, 15 cm × 4.6 mm, 5 µm particles, preceded by a 2 cm RP-AmideC16 guard column and 0.5 µm frit filter (505013)
mobile phase . . . . . (A) Methanol; (B) 1% acetic acid (17:38, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 272 nm
injection . . . . . 20 µL reconstituted porcine serum extract
Application No. . . . . G000594

Efficiency of Recovery

| Compound | Concentration (µg/mL) | % Recovery | % RSD (n=6) |
|---|---|---|---|
| 1. Theobromine | 0.1 | 97.4 | ±6.8 |
| | 0.5 | 96.4 | ±8.5 |
| | 1.0 | 96.1 | ±5.0 |
| 2. Paraxanthine | 0.1 | 96.2 | ±8.4 |
| | 0.5 | 95.2 | ±8.7 |
| | 1.0 | 95.0 | ±8.7 |
| 3. Theophylline | 0.1 | 97.8 | ±8.5 |
| | 0.5 | 97.8 | ±8.8 |
| | 1.0 | 98.5 | ±5.7 |
| 4. Caffeine | 0.1 | 98.8 | ±3.9 |
| | 0.5 | 95.6 | ±6.7 |
| | 1.0 | 97.6 | ±5.8 |



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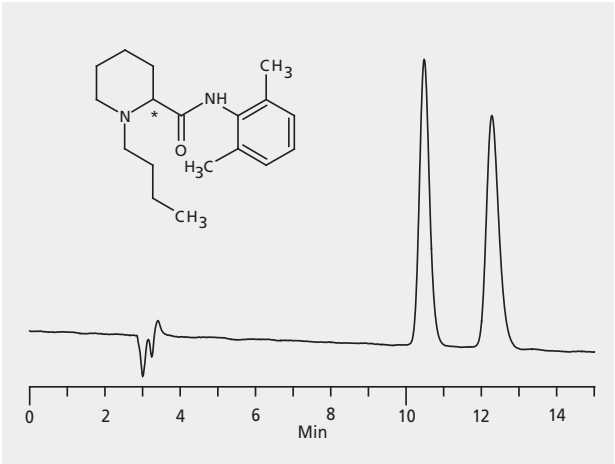
## HPLC Analysis of Bupivacaine Enantiomers on Astec® CHIROBIOTIC® V2, Acetate Mobile Phase

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) methanol; (B) 20 mM ammonium acetate, pH 4.1; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004464

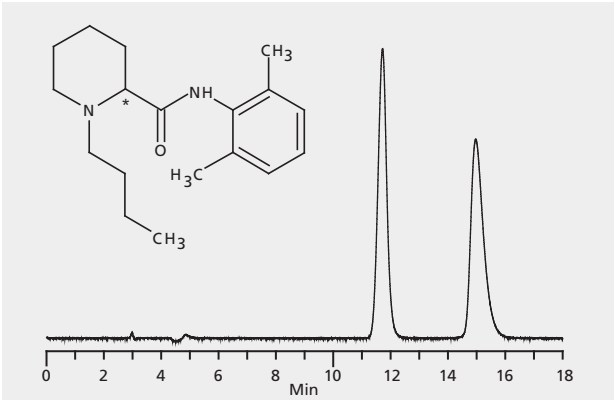





SUPELCO® Solutions within.™

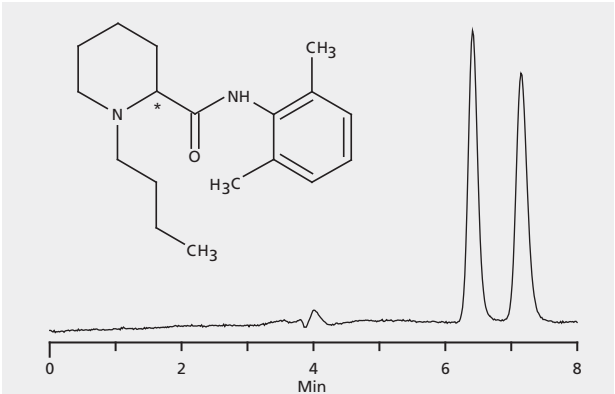
## HPLC Analysis of Bupivacaine Enantiomers on Astec® CHIROBIOTIC® V2, Formate Mobile Phase

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) 20 mM ammonium formate, pH 4.1; (B) methanol; (5:95, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004619



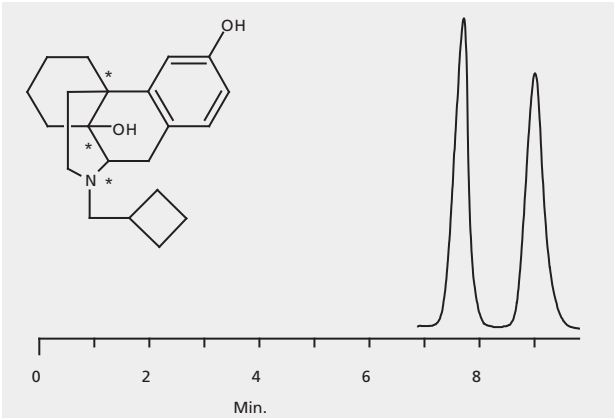
## HPLC Analysis of Bupivacaine Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 1% triethylamine, pH 4.1; (B) methanol; (5:95, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 202 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004623



## HPLC Analysis of Butorphanol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 0.1 wt% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004574



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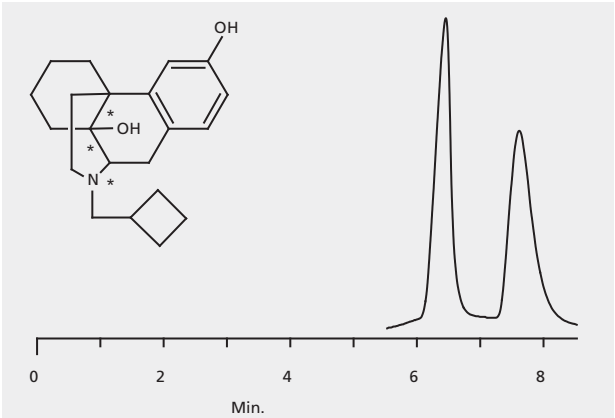
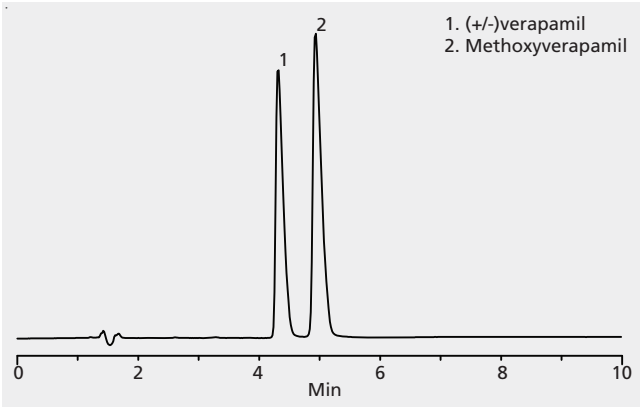
## HPLC Analysis of Butorphanol Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 0.1 wt% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004591



## HPLC Analysis of Calcium Channel Blockers on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; ( 60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002437

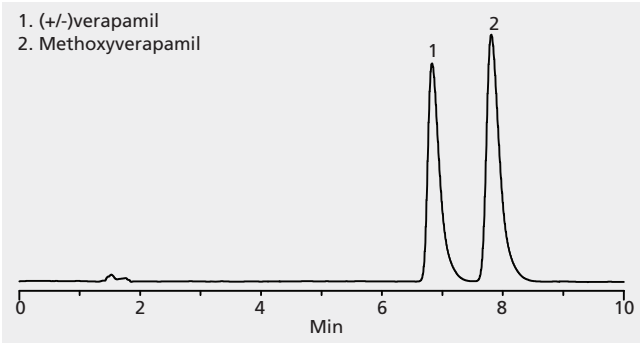





SUPELCO® Solutions within.™

## HPLC Analysis of Calcium Channel Blockers on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002665

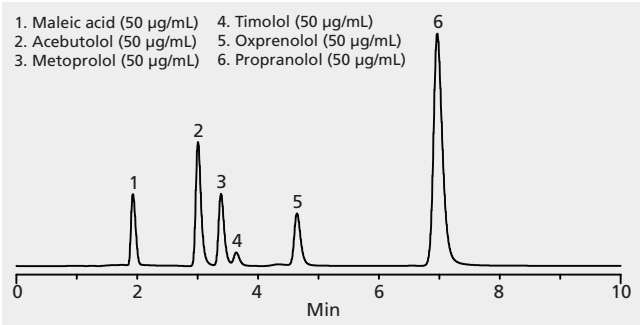





SUPELCO® Solutions within.™

## HPLC Analysis of Cardiac Drugs on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 2.1 mm I.D., 3 µm particles (581402-U)
mobile phase . . . . . 0.1% ammonium acetate:acetonitrile with 0.1% ammonium acetate, (47:53)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003172

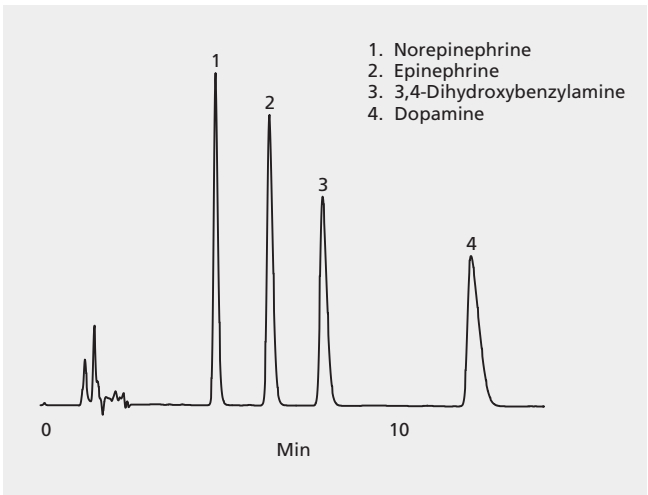





SUPELCO® Solutions within.™

## HPLC Analysis of Catecholamines on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . (A) acetonitrile: (B) 50 mM $KH_2PO_4$, pH 3.0 with phosphoric acid, 100 mg/L EDTA, 200 mg/L 1-octane-sulfonic acid (5:95, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . 797-0659

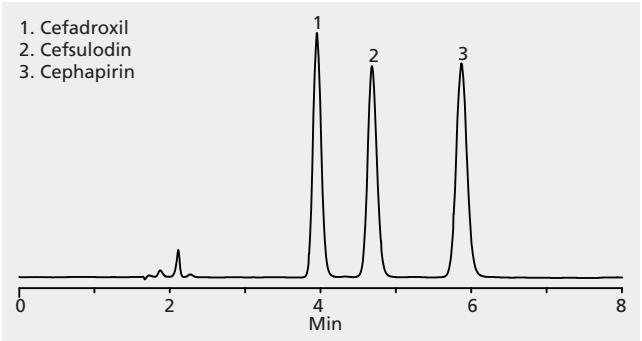





SUPELCO® Solutions within.™

## HPLC Analysis of Cephalsporin Antibiotics on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid): (B) acetonitrile; (93:7, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002642





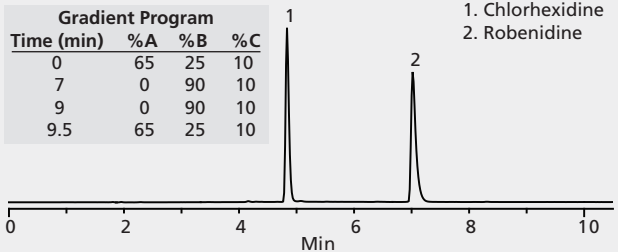

SUPELCO® Solutions within.™

## HPLC Analysis of Chlorhexidine and Robenidine on Ascentis® Phenyl

column . . . . . Ascentis Phenyl 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (C) 500 mM ammonium formate, pH 3.0, with formic acid
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 273 nm
injection . . . . . 10 µL
sample . . 100 µg/mL each in 70:30, 25 mM ammonium formate pH 3.0:acetonitrile
Application No. . . . . G003711

**Gradient Program**

| Time (min) | %A | %B | %C |
|---|---|---|---|
| 0 | 65 | 25 | 10 |
| 7 | 0 | 90 | 10 |
| 9 | 0 | 90 | 10 |
| 9.5 | 65 | 25 | 10 |

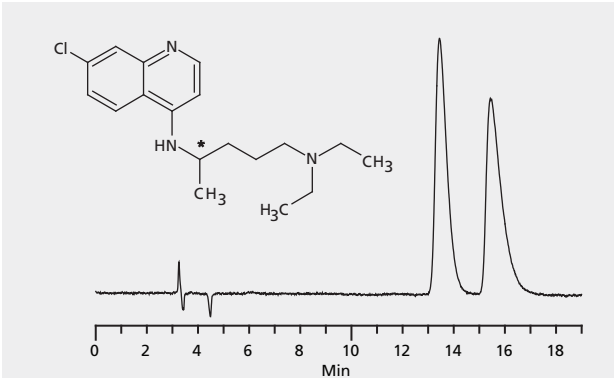





SUPELCO® Solutions within.™

## HPLC Analysis of Chloroquine Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . (A) methanol; (B) acetic acid; (C) triethylamine; (98:1:1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004620

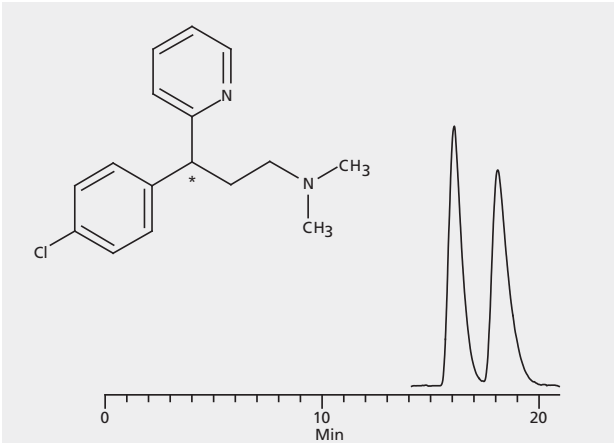





SUPELCO® Solutions within.™

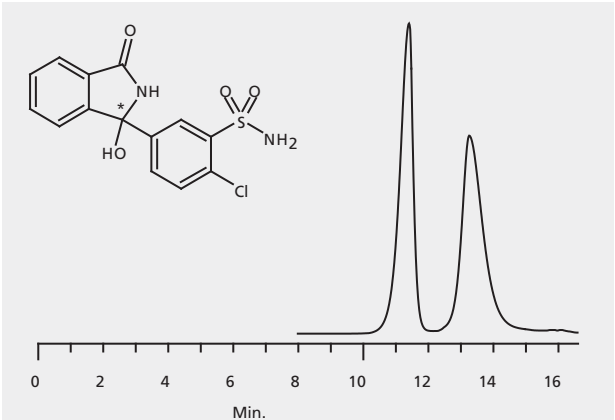
## HPLC Analysis of Chlorpheniramine Enantiomers on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . (A) 1% triethylamine acetate, pH 4.1; (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004688



## HPLC Analysis of Chlorthalidone Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) methanol; (80:20, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004584

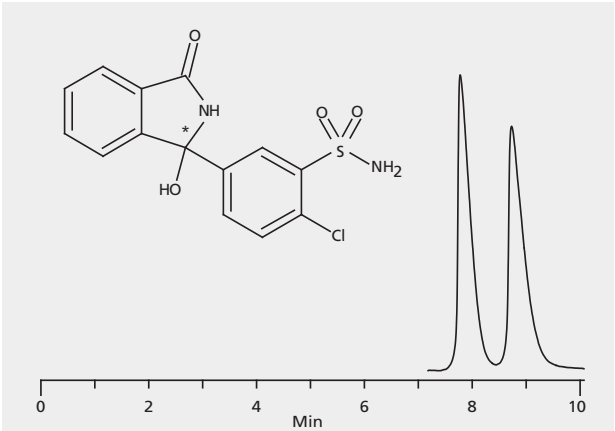





SUPELCO® Solutions within.™

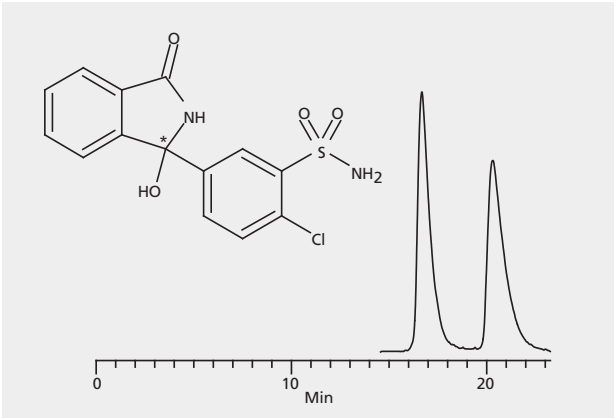
## HPLC Analysis of Chlorthalidone Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.0; (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 235 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004712



## HPLC Analysis of Chlorthalidone Enantiomers on Astec® CYCLOBOND® I 2000 RSP

column . . CYCLOBOND I 2000 RSP, 25 cm x 4.6 mm I.D., 5 µm particles (20324AST)
mobile phase . . (A) 1% triethylamine acetate, pH 4.1; (B) acetonitrile; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004704

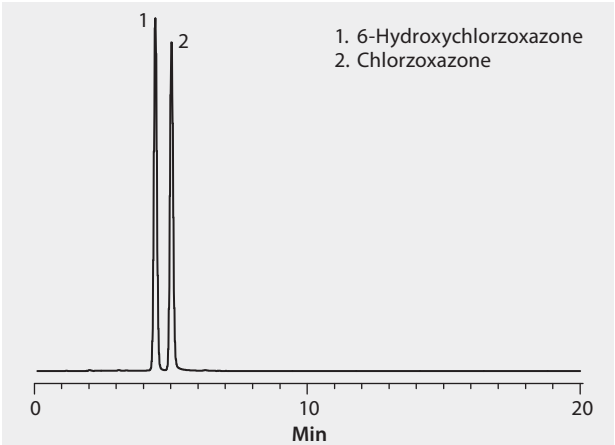





SUPELCO® Solutions within.™

## HPLC Analysis of Chlorzoxazone on Discovery® HS-PEG

column . . . . Discovery HS PEG, 15 cm × 4.6 mm I.D., 5 µm particles (567416-U)
mobile phase . . (A) 20 mM acetic acid in water (pH 4.5 with ammonium hydroxide): (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 285 nm
injection . . . . . 10 µL
Application No. . . . . G001674

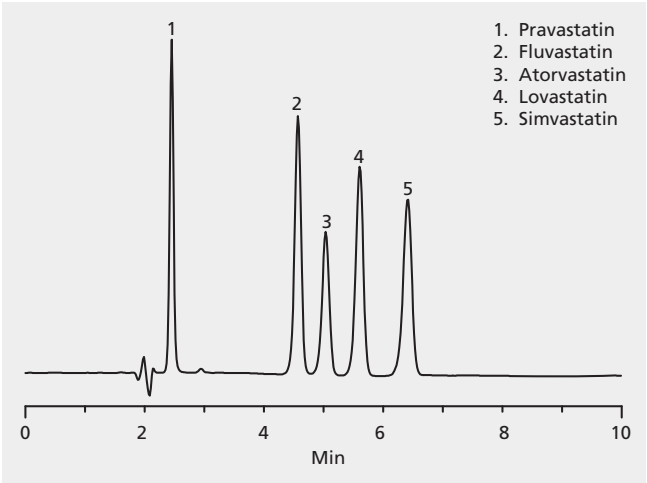





SUPELCO® Solutions within.™

## HPLC Analysis of Cholesterol-Lowering Drugs (Statins) on Discovery® Cyano

column . . . . . Discovery Cyano, 15 cm × 4.6mm I.D., 5 µm particles (59356-U)
mobile phase . . . . . 60:40 water (10 mM ammonium acetate, pH 4.0 with acetic acid):acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL each in 70:30 water:acetonitrile
Application No. . . . . G002552

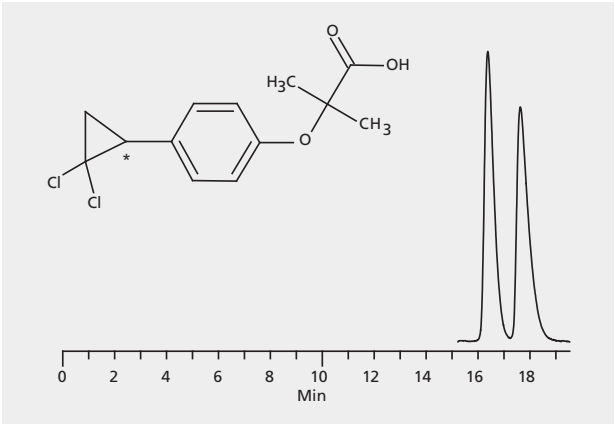





SUPELCO® Solutions within.™

## HPLC Analysis of Ciprofibrate Enantiomers on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) triethylamine; (90:10:0.3:0.2, A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004689

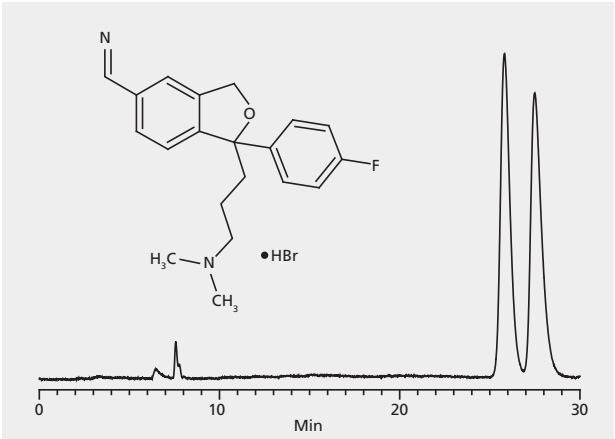





SUPELCO® Solutions within.™

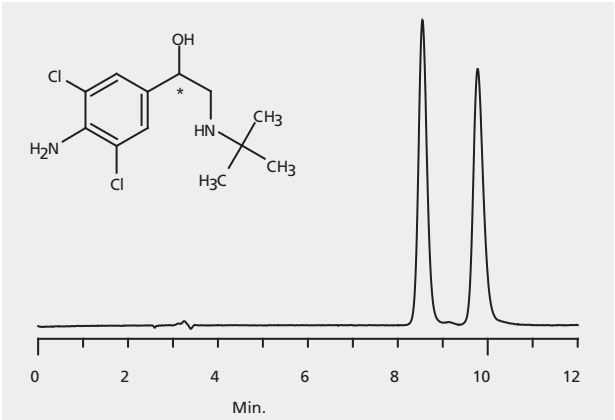
## HPLC Analysis of Citalopram Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.8:0.1:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 213 nm
injection . . . . . 5 µL
sample . . . . . citalopram, 1 mg/mL in mobile phase
Application No. . . . . G004408



## HPLC Analysis of Clenbuterol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 3 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004493



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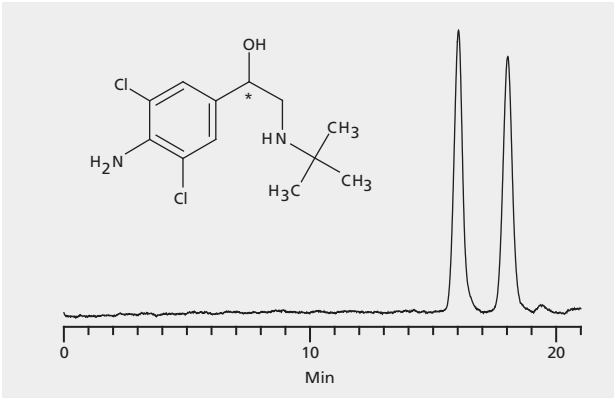
## HPLC Analysis of Clenbuterol Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.98:0.01:0.01, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004621



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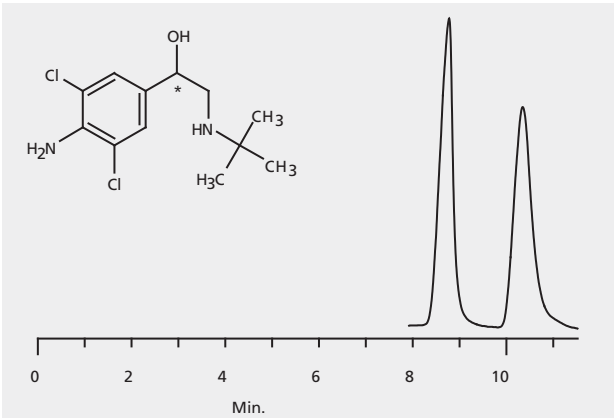
## HPLC Analysis of Clenbuterol Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004596

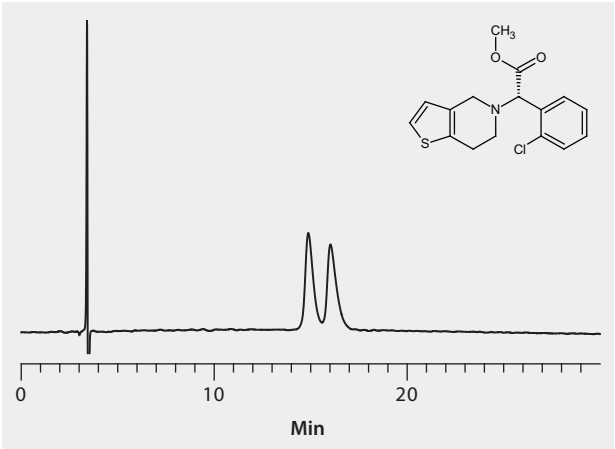





SUPELCO® Solutions within.™

## HPLC Analysis of Clopidogrel Enatiomers on Astec® CYCLOBOND® I 2000 DNP

column . . CYCLOBOND® I 2000 DNP, 25 cm x 4.6 mm I.D., 5 µm particles (25024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.0; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1 mL/min
pressure . . . . . 1100 psi (76 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
sample . . . . . 0.2 mg/mL in methanol
Application No. . . . . G004774



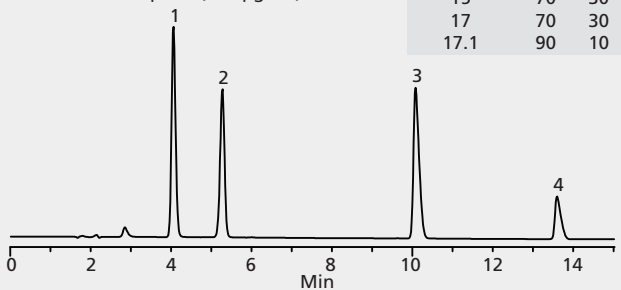
## HPLC Analysis of Cold Remedy Ingredients on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 5 mM ammonium formate (pH 2.75 with formic acid); (B) acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 270 nm
injection . . . . . 10 µL
sample . . . as indicated in 5 mM ammonium formate (pH 2.75 with formic acid)
Application No. . . . . G003006

1. Doxylamine (100 µg/mL)
2. Acetaminophen (75 µg/mL)
3. Chlorpheniramine (125 µg/mL)
4. Dextromethorphan (150 µg/mL)

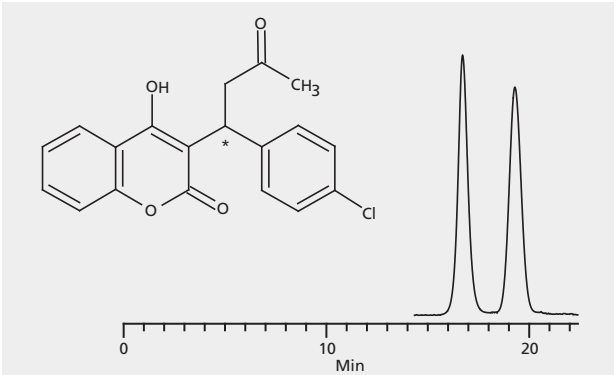
**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 15 | 70 | 30 |
| 17 | 70 | 30 |
| 17.1 | 90 | 10 |



## HPLC Analysis of Coumachlor Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM ammonium nitrate, pH 5.5; (B) THF; (75:25, A:B)
flow rate . . . . . 0.75 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2.5 mg/mL in methanol
Application No. . . . . G004638

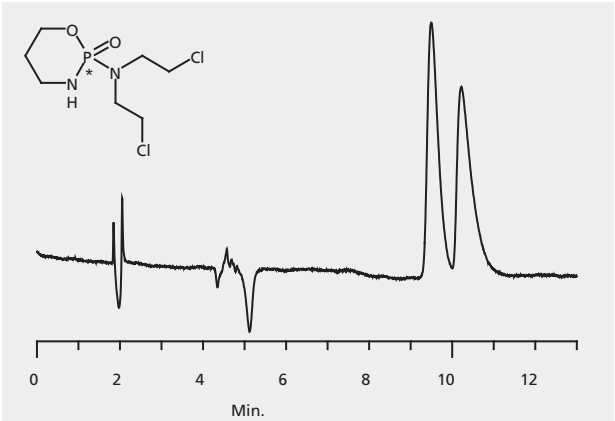





SUPELCO® Solutions within.™

## HPLC Analysis of Cyclophosphamide Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) ethanol; (C) THF; (90:5:5, A:B:C)
flow rate . . . . . 0.75 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004494

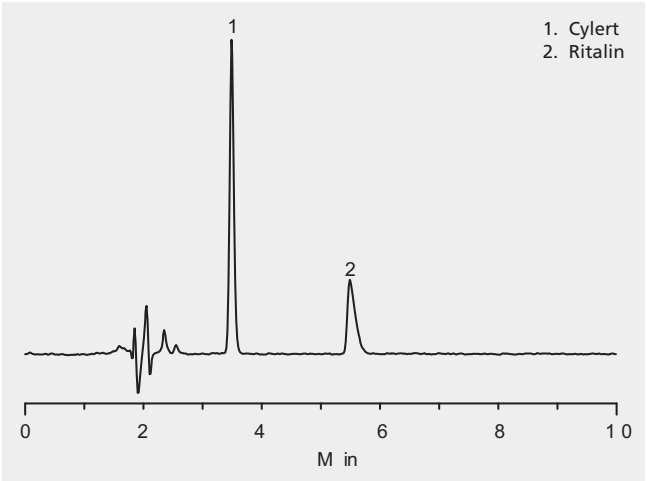





SUPELCO® Solutions within.™

## HPLC Analysis of Cylert (Pemoline) and Ritalin (Methylphenidate) on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . 80:20 water 10 mM ammonium acetate, pH 3.0 with formic acid):acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . Cylert (50 µg/mL) and Ritalin (100 µg/mL) in 85:15 water:$CH_3OH$
Application No. . . . . G002558

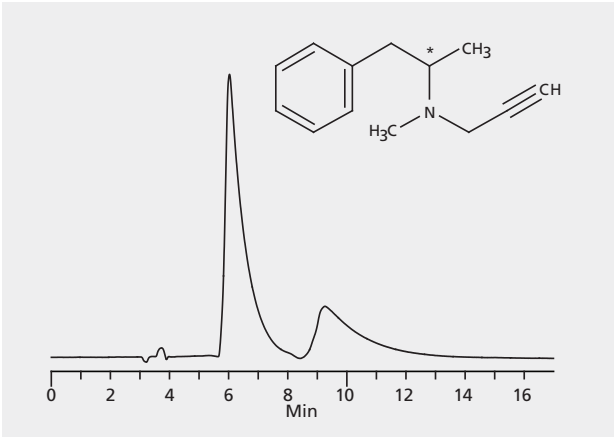





SUPELCO® Solutions within.™

## HPLC Analysis of Deprenyl (Selegiline) Enantiomers on Astec® CYCLOBOND® I 2000 DMP

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.0; (B) methanol; (65:35, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004681

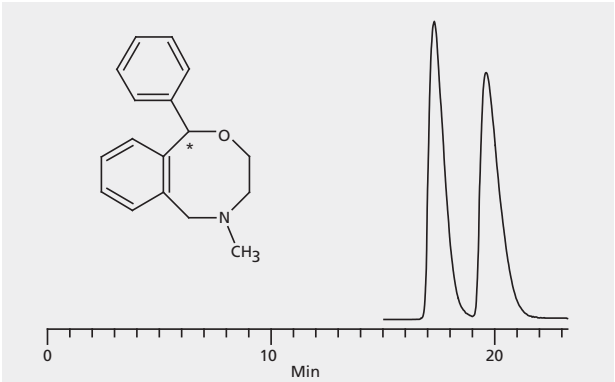





SUPELCO® Solutions within.™

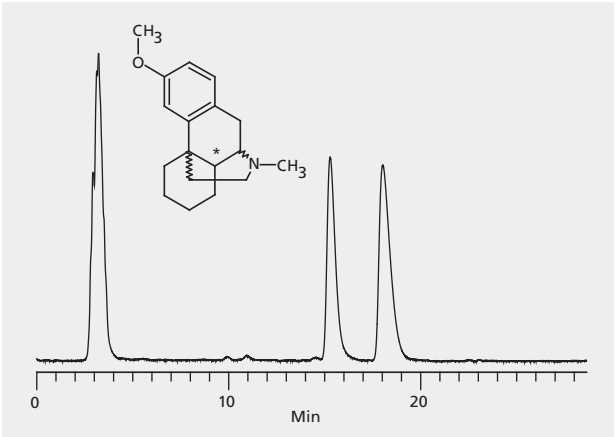
## HPLC Analysis of N-Desmethylnefopam Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) ammonium hydroxide; (100:0.1:0.02, A:B:C)
flow rate . . . . . 1.2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004655



## HPLC Analysis of Dextromethorphan Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) methanol; (B) 20 mM ammonium acetate, pH 4.1; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004468

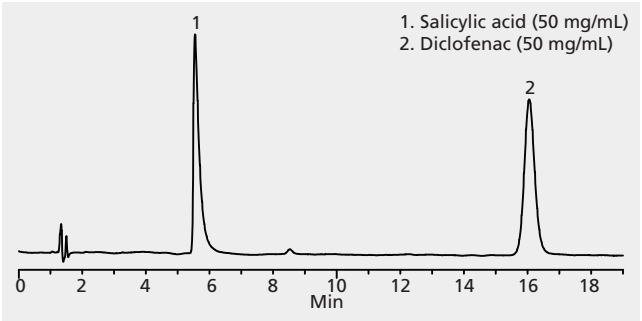





SUPELCO® Solutions within.™

## HPLC Analysis of Diclofenac and Salicylic Acid on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0 unadjusted); (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 20 mM phosphoric acid (pH 2.0)
Application No. . . . . G002617

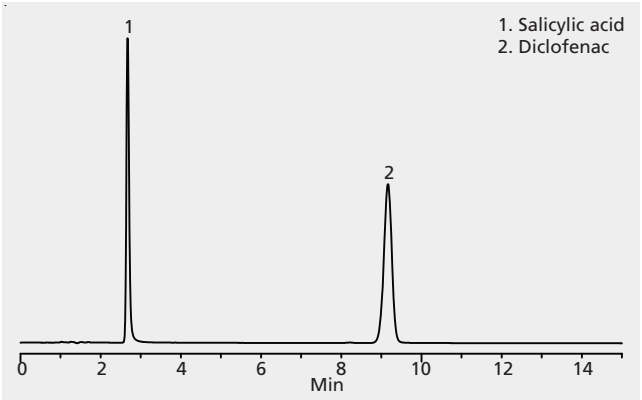





SUPELCO® Solutions within.™

## HPLC Analysis of Diclofenac and Salicylic Acid on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0 unadjusted); (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002424



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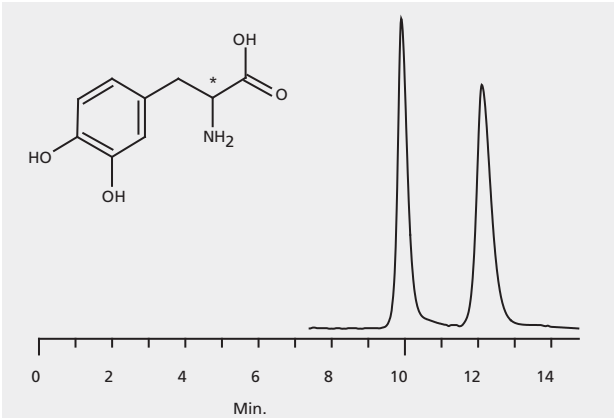
## HPLC Analysis of 3,4-Dihydroxyphenylalanine (DOPA) Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (25:75, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004586

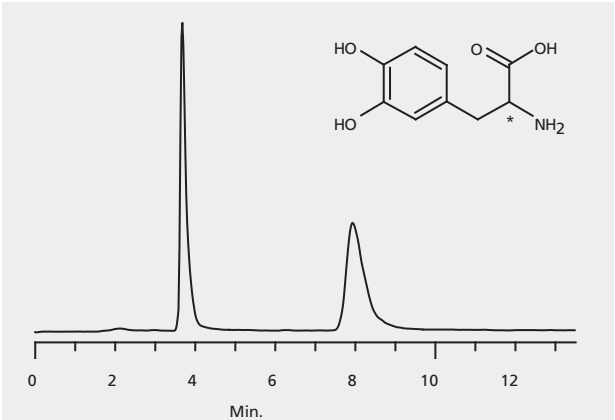





SUPELCO® Solutions within.™

## HPLC Analysis of 3,4-Dihydroxyphenylalanine (DOPA) Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004608

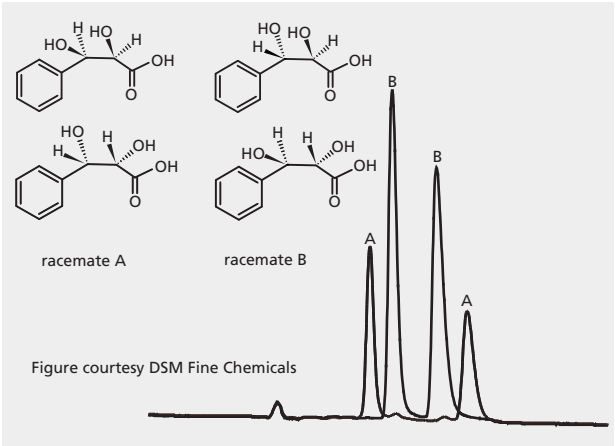





SUPELCO® Solutions within.™

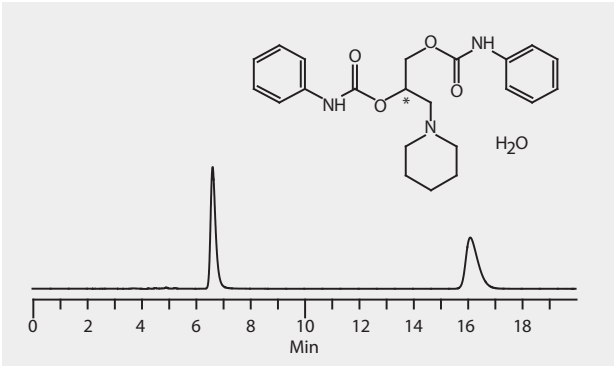
## HPLC Analysis of 2,3-Dihydroxy-3-Phenylpropionic Acid Enantiomers on CHIROBIOTIC R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . (A) 0.1% ammonia, pH 4.1 with formic acid;
(B) methanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 258 nm
injection . . . . . 10 µL
sample . . . . . 2,3-dihydroxy-3-phenyl-propionic acid isomers:
(A) racemate A (4.85 and 6.95 min)
(B) racemate B (5.33 and 6.29 min)
Application No. . . . . G004409



## HPLC Analysis of Diperodon Enantiomers on Astec® Cellulose DMP, LC/MS Conditions

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) ammonium formate; (100:0.1, A:B)
(Note: 0.1 w/v ammonium formate, 0.1 g/100 mL)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004987



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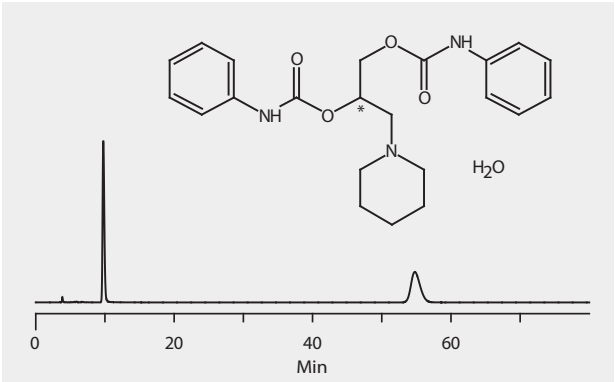
## HPLC Analysis of Diperodon Enantiomers on Astec® Cellulose DMP (no additives)

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) acetonitrile; (100, A)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005004



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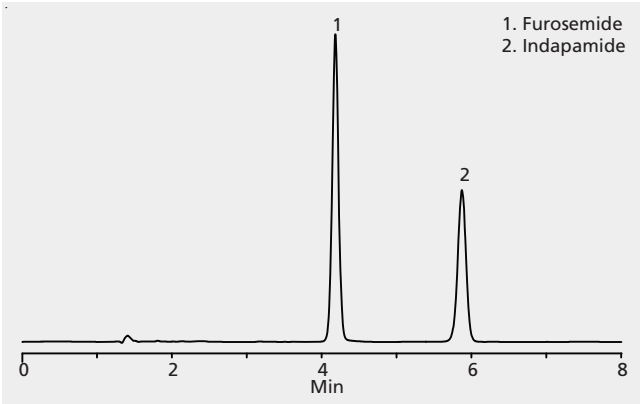
## HPLC Analysis of Diuretic Drugs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetonitrile; (A:B, 40:60)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 234 nm
injection . . . . . 10 µL
sample . . . . . 10 µg/mL each in mobile phase
Application No. . . . . G002423

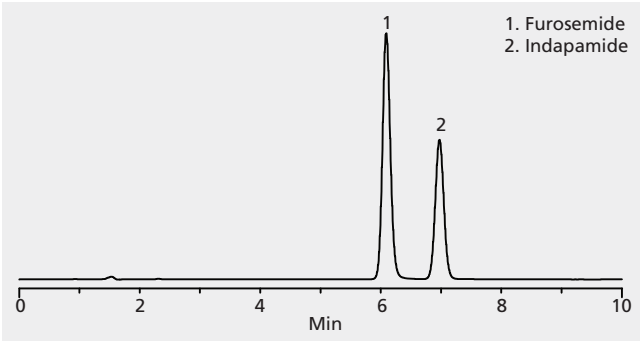





SUPELCO® Solutions within.™

## HPLC Analysis of Diuretic Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) acetronitrile; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 234 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002650



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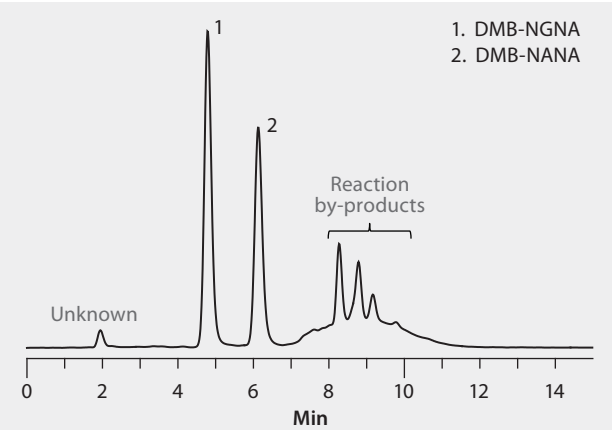
## HPLC Analysis of DMB-Labeled Sialic Acids on Ascentis® Express RP-Amide: Comparison of Biosimilars to Reference Materials

Sialic acids affect the bioavailability, function, stability, and metabolism of glycoproteins. Two forms of sialic acid are commonly present in therapeutic glycoproteins: N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid (NGNA). One of the most common quantification methods involves releasing sialic acids from the glycoprotein, derivatizing NANA and NGNA with 1,2-diamino-4, 5-methylenedioxybenzene (DMB), and analyzing by C18-HPLC with fluorescence detection. This procedure is subject to interference from peaks originating from excess reagent and other derivatized impurities, limiting sensitivity and reproducibility. The objectives of this study were to develop a significantly improved HPLC-fluorescence method for DMB-NANA and DMB-NGNA, and to apply this method to compare two candidate biosimilar therapeutic proteins to their respective reference materials.

column . . . . . Ascentis® Express RP-Amide, 10 cm x 2.1 mm I,D., 2.7 µm particles (53913-U)
mobile phase . . . . . (A) Water, 0.1% formic acid, (B) ACN, 0.1% formic acid
gradient . . . . . 0-1 min 6% B; 1.01-4 min 20% B; 4.01-12 min 6% B, total run time 15 min
flow rate . . . . . 0.2 mL/min
pressure . . . . . 1300 psi (89.6 bar)
column temp. . . . . 30 °C
detector . . . . . fluorescence, λ excitation = 373 nm, λ emission = 448 nm
injection . . . . . 0.5 µL
sample . . . . . Mix of DMB-labeled NGNA and NANAsialic acid, 5 mg/ml each
Application No. . . . . G006199



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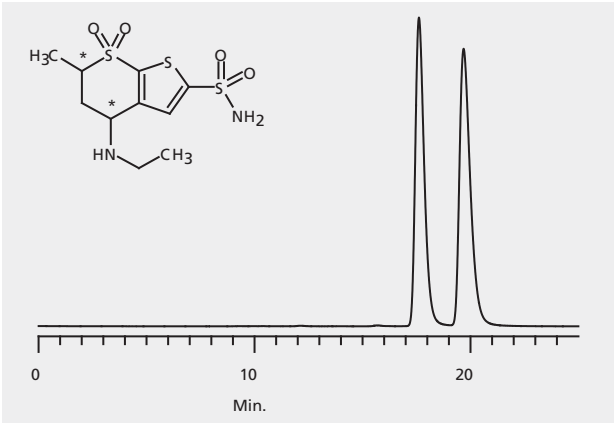
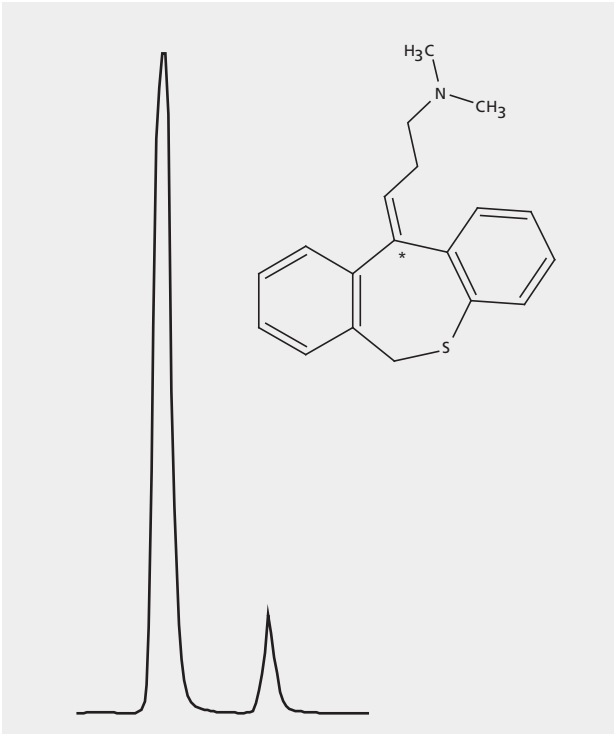
## HPLC Analysis of Dorzolamide Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . (A) 3.81 mM ammonium trifluoroacetic acid; (B) methanol; (5:95, A:B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004478



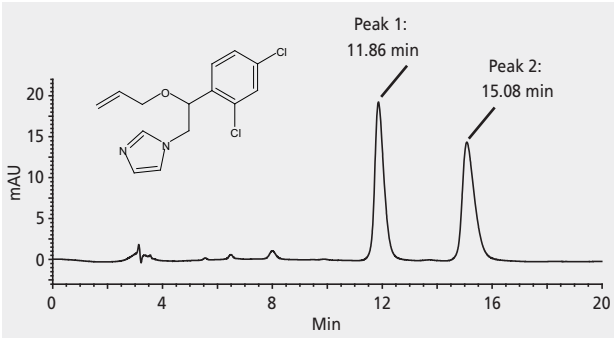
## HPLC Analysis of cis/trans-Dothiepin Enantiomers on Astec® CYCLOBOND® I 2000 AC

column . . CYCLOBOND I 2000 AC, 25 cm x 4.6 mm I.D., 5 µm particles (20124AST)
mobile phase . . (A) 1.0% triethylamine acetate, pH 4.1; (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
peak 1: 9.48 min.
peak 2: 12.48 min.
Application No. . . . . G004732



## HPLC Analysis of Enilconazole (Imazalil) Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

| | |
|---|---|
| column | CYCLOBOND I 2000 HP-RSP, 25 cm × 4.6 mm I.D., 5 µm particles (24024AST) |
| mobile phase | (A) 10 mM ammonium acetate, pH 4.0: (B) acetonitrile; (80:20, A:B) |
| flow rate | 1 mL/min |
| column temp. | 23 °C |
| detector | UV, 235 nm |
| injection | 5 µL |
| Application No. | G004029 |



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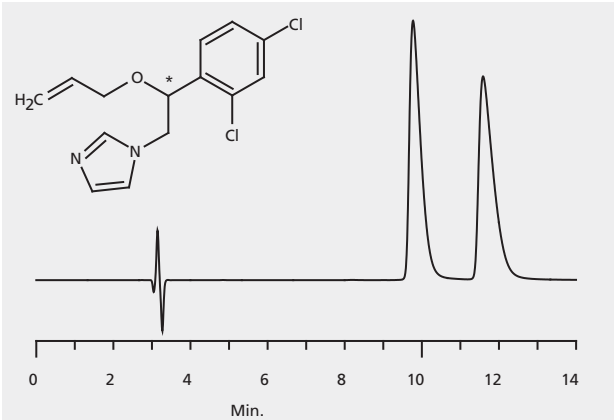
## HPLC Analysis of Enilconazol (Imazalil) Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004588



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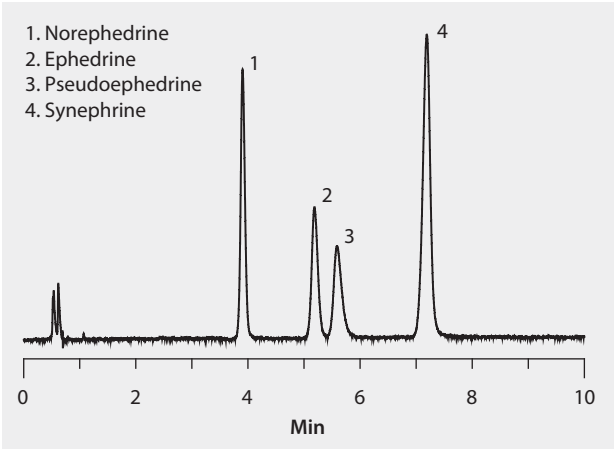
## HPLC Analysis of Ephedrine Compounds on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . (A) acetonitrile; (B) 40 mM ammonium acetate in water; (90:10, A:B)
flow rate . . . . . 0.4 mL
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 150 µg/mL each in mobile phase
Application No. . . . . G005352



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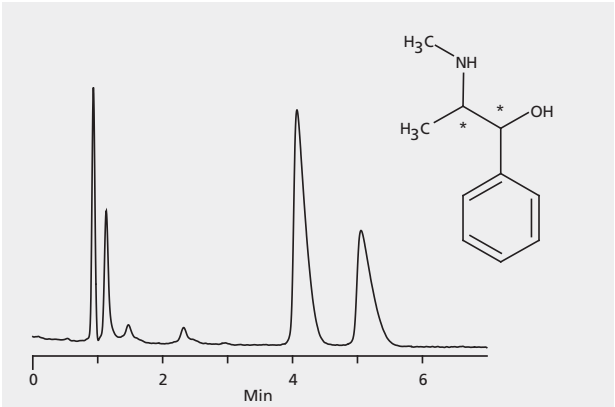
## HPLC Analysis of Ephedrine Enantiomers on CHIRALPAK® AGP

column . . . . . CHIRAL-AGP, 10 cm x 4 mm I.D., 5 µm particles (58170AST)
mobile phase . . . . . 1 mM octanoic acid in 10 mM $(NH_4)_2HPO_4$, pH 6.9
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 225 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004730



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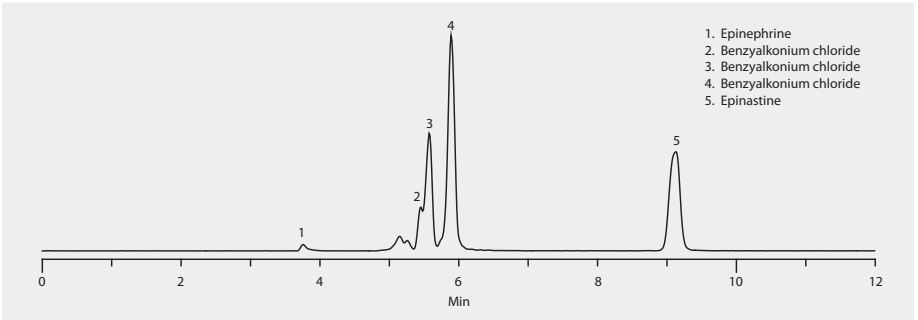
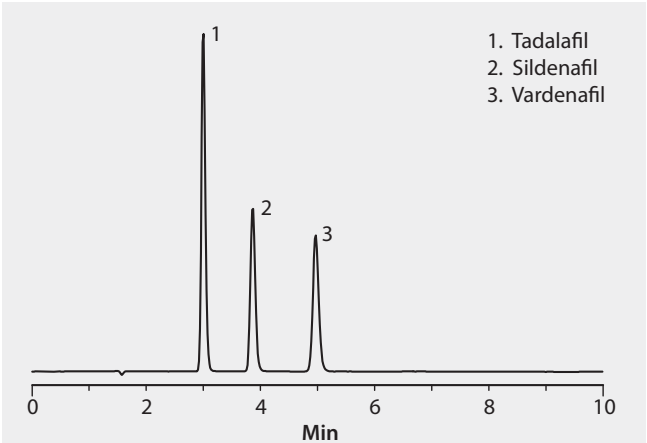
## HPLC Analysis of Epinephrine, Epinastine, and Benzalkonium Chlorides on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . . . . (A) 50 mM ammonium acetate; (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . ESI(+), XIC m/z 304 & 332 (BAC), 250 (epinastine), 180 (epinephrine)
injection . . . . . 10 µL
sample . . . . . 10 mg/L ea. in acetonitrile
Application No. . . . . G005411



## HPLC Analysis of Erectile Dysfunction Drugs on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) water (10 mM $NH_4COOH$); (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL each in 50:50 water:acetonitrile
Application No. . . . . G002546

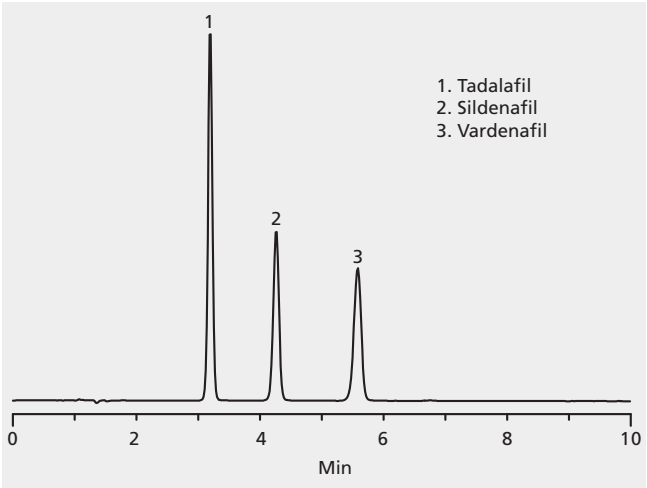





SUPELCO® Solutions within.™

## HPLC Analysis of Erectile Dysfunction Drugs on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . 50:50 water (10 mM $NH_4COOH$):$CH_3CN$
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL each in 50:50 water:acetonitrile
Application No. . . . . G002542

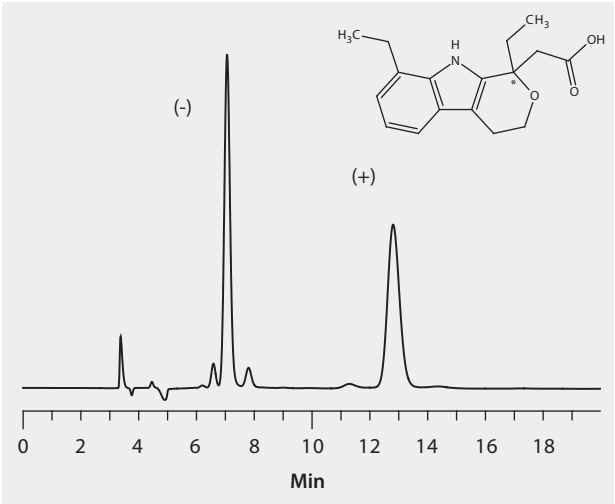





SUPELCO® Solutions within.™

## HPLC Analysis of Etodolac Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) TFA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004988

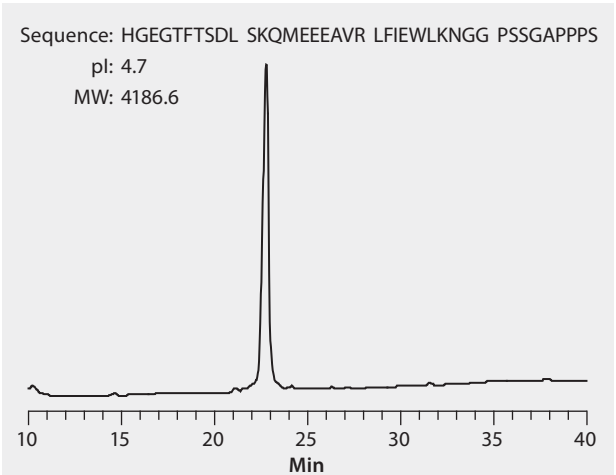





SUPELCO® Solutions within.™

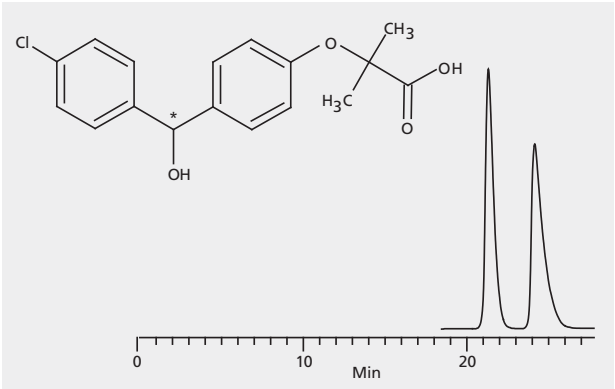
## HPLC Analysis of Exenatide Acetate on Proteomix® SCX-NP5

column . . . . Proteomix SCX NP5, 25 cm x 4.6mm I.D., 5 µm particles (Z777161)
mobile phase . . (A) 10 mM monosodium phosphate:acetonitrile (56:44, v/v), adjusted to pH to 2.0 with phosphoric acid; ((B) 0.5 M NaCl/10 mM monosodium phosphate:acetonitrile (56:44, v/v), adjusted to pH to 2.0 with phosphoric acid)
gradient . . . . . 0 to 65% B in 35 min; held for 5 min; to 0% B in 10 min
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1653 psi (114 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 235 nm
injection . . . . . 20 µL
sample . . . . . 0.325 mg/mL exenatide acetate in mobile phase A
Application No. . . . . G006163



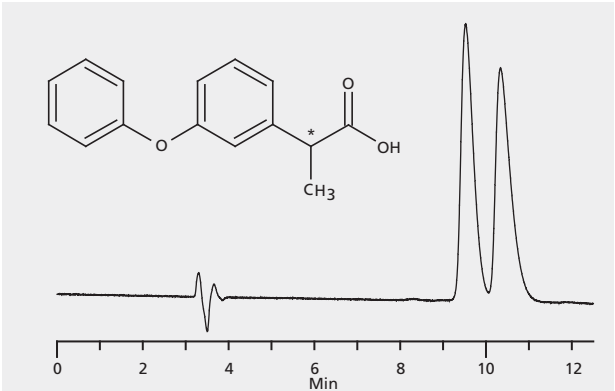
## HPLC Analysis of Fenofibric Acid Enantiomers on Astec® CYCLOBOND® I 2000

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . (A) 0.1% triethylamine acetate, pH 4.0; (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 3 mg/mL in methanol
Application No. . . . . G004691



## HPLC Analysis of Fenoprofen Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 1% triethylamine, pH 7.0; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 215 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004624

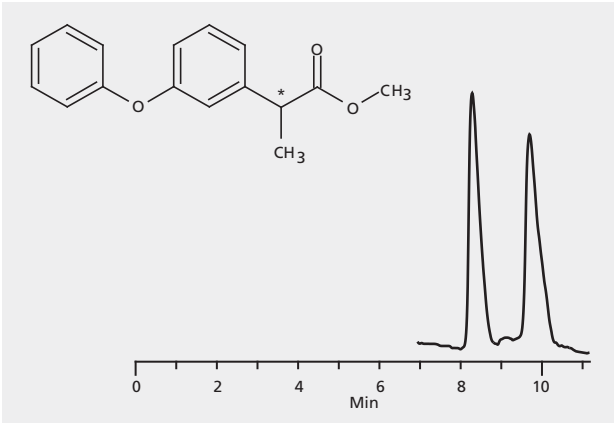





SUPELCO® Solutions within.™

## HPLC Analysis of Fenoprofen Methyl Ester Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM sodium citrate, pH 6.3; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004651



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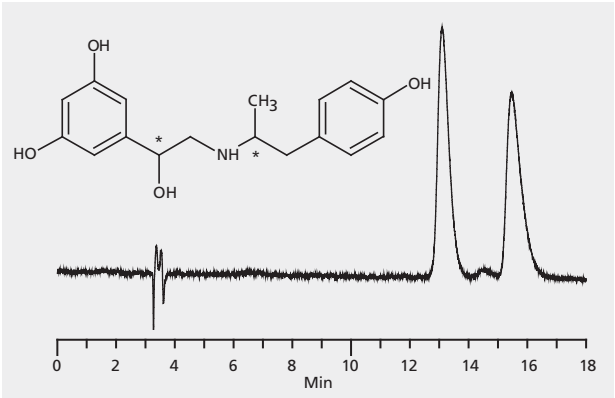
## HPLC Analysis of Fenoterol Enantiomers on Astec® CHIROBIOTIC® V

**chiral**

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM ammonium nitrate, pH 5.5; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 275 nm
injection . . . . . 15 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004625

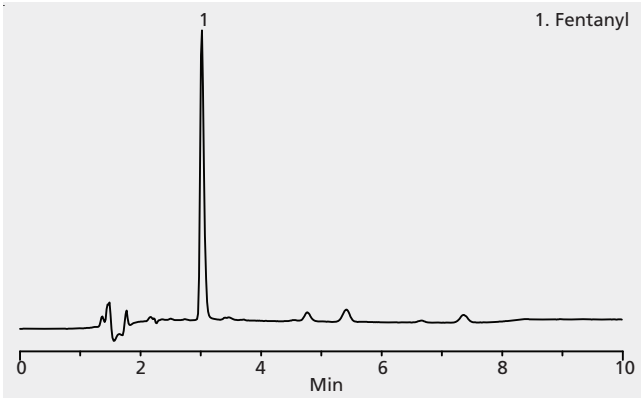





SUPELCO® Solutions within.™

## HPLC Analysis of Fentanyl Tranquilizer on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid): (B) acetonitrile; (67:33, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 10 µL
sample . . . . . 20 µg/mL in mobile phase
Application No. . . . . G002435



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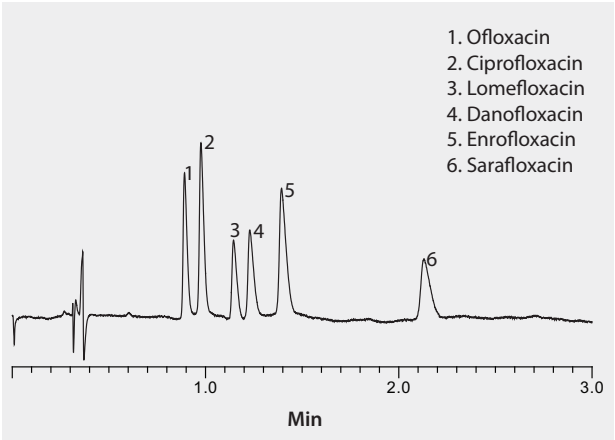
## HPLC Analysis of Fluoroquinolone Antibiotics on Ascentis® Express C18

This application demonstrates the suitability of the Ascentis Express C18 for the analysis of fluoroquinolones.

column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) 0.1% formic acid in water;
(B) 0.1% formic acid in acetonitrile; (85:15, A:B)
flow rate . . . . . 1.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 4 µL
sample . . . . . 200 ng/mL in 25:75, water: acetonitrile
Application No. . . . . G005699

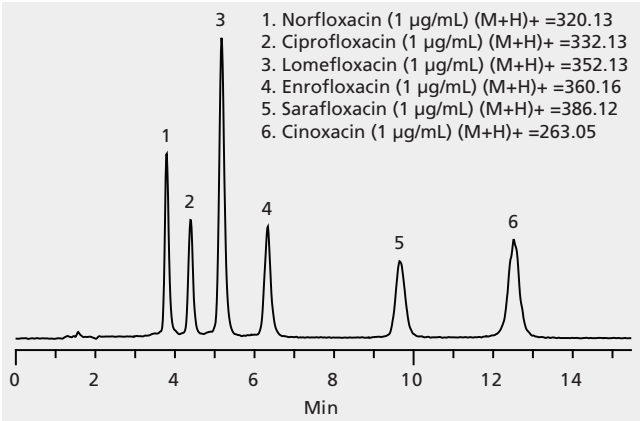





SUPELCO® Solutions within.™

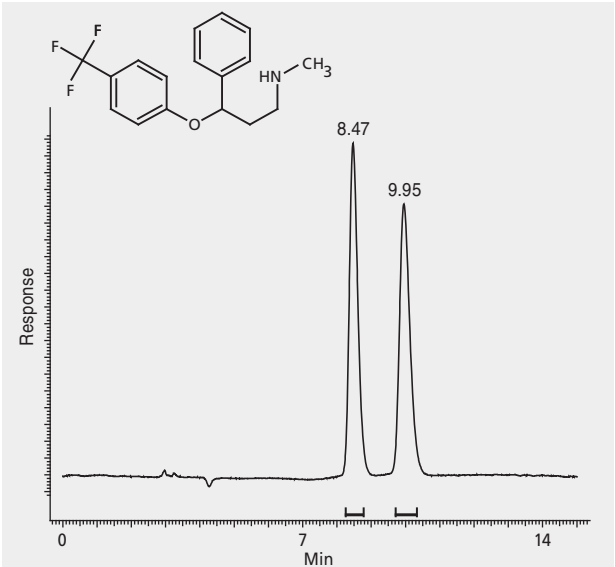
## HPLC Analysis of Fluoroquinolone Antibiotics on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 13 mM ammonium formate (pH 3.0 with concentrated formic acid): (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1 mL/min, split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 80:20, 13 mM ammonium formate (pH 3.0 with concentrated formic acid):acetonitrile
Application No. . . . . G003713



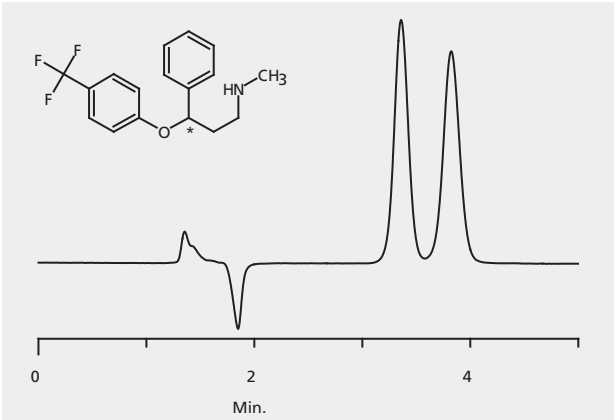
## HPLC Analysis of Fluoxetine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 15 mM ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004465



## HPLC Analysis of Fluoxetine Enantiomers on Narrowbore Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 10 cm x 2.1 mm I.D., 5 µm particles (15018AST)
mobile phase . . . . . 13 mM ammonium acetate in methanol
flow rate . . . . . 0.2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004476

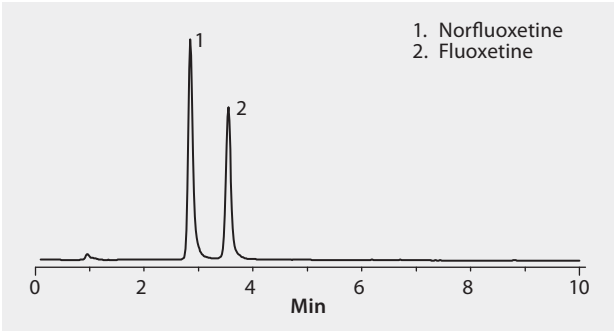





SUPELCO® Solutions within.™

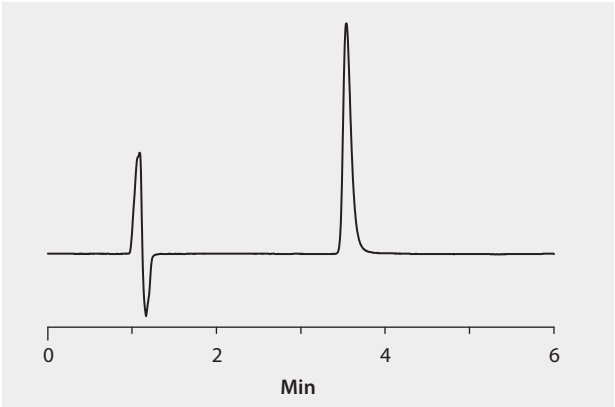
## HPLC Analysis of Fluoxetine (Prozac®) and Norfluoxetine on Discovery® Zr-PS

column . . . . . Discovery Zr-PS, 7.5 cm × 4.6 mm I.D., 3 µm particles (65741-U)
mobile phase . . . . . (A) 25 mM potassium phosphate (pH 12): (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 ml/min
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
Application No. . . . . G001852



## HPLC Analysis of Fluoxetine (Prozac®) on Discovery® C18

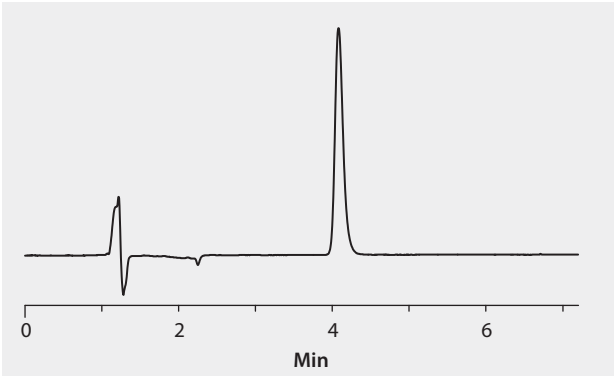
column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) acetonitrile; (B) 25 mM $KH_2PO_4$, pH 7.0 (40:60, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
Application No. . . . . G000900



## HPLC Analysis of Fluoxetine (Prozac®) on Discovery® Cyano

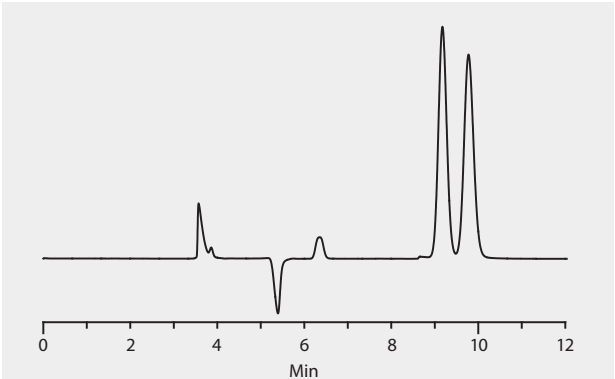
**Fluoxetine (Prozac®)**

column . . . . . Discovery Cyano, 15 cm × 4.6 mm I.D., 5 µm particles (59356-U)
mobile phase . . . . . (A) acetonitrile; (B) 25 mM $KH_2PO_4$, pH 7.0; (40:60, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 214 nm
injection . . . . . 1 µL
Application No. . . . . G000899



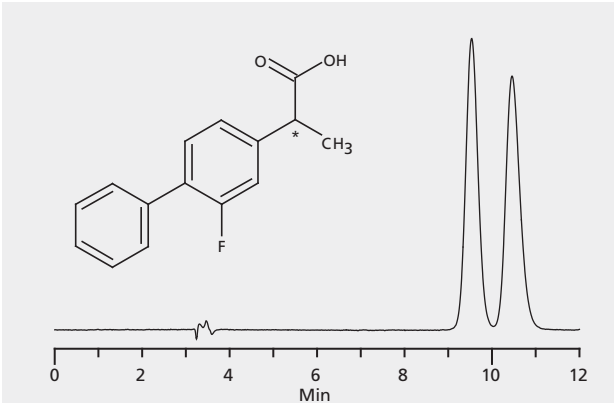
## HPLC Analysis of Flurbiprofen Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) isopropanol; (C) TFA; (95:5:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 3 µL
sample . . . . . 1 g/L in methanol
Application No. . . . . G005467



## HPLC Analysis of Flurbiprofen Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM ammonium nitrate, pH 5.5; (B) THF; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004626



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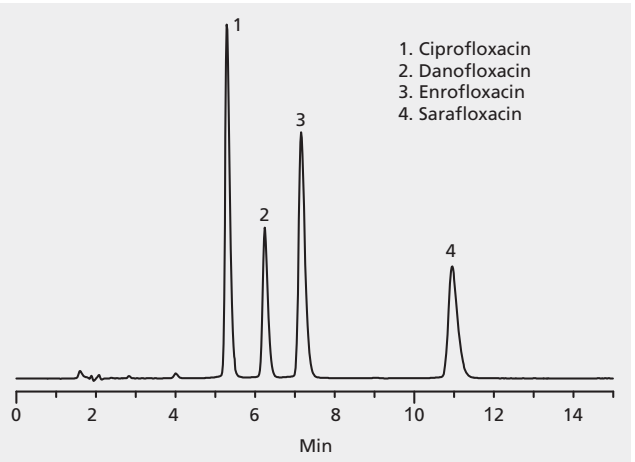
## HPLC Analysis of Fluroquinolone Antibiotics on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . (A) water (10 mM ammonium acetate, pH 3.0 with acetic acid); (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002547



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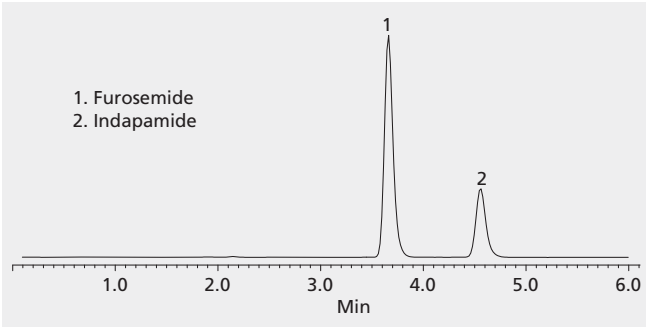
## HPLC Analysis of Furosemide and Indapamide on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm ID, 5 µm particles (504955)
mobile phase . . . . . (A) 10mM monobasic potassium phosphate, (pH 3.0 with $H_3PO_4$): (B) acetonitrile; (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 234 nm
injection . . . . . 10 µL
sample . . . . . 10 µg/mL each in mobile phase
Application No. . . . . G002044



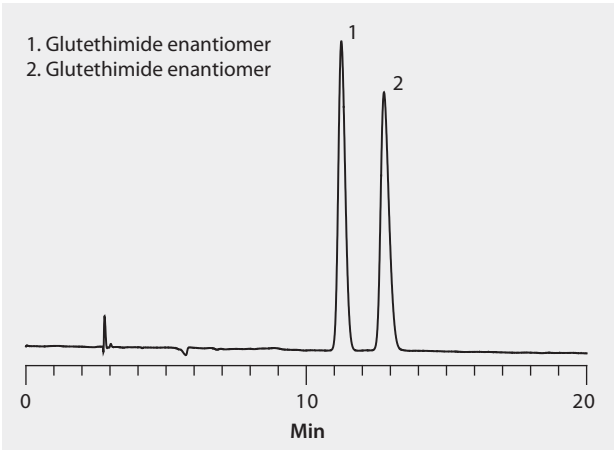



SUPELCO® Solutions within.™

## HPLC Analysis of Glutethimide Enantiomers on Astec® Cellulose DMP

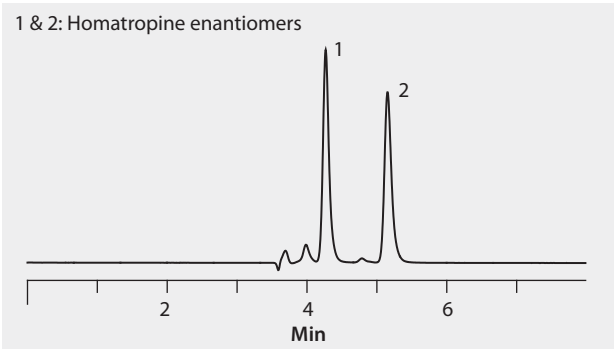
Glutethimide enantiomers are resolved by normal phase chromatography. Glutethimide is used to treat insomnia.

column . . Astec Cellulose DMP, 25 cm x 4.6 mm I.D., 5 µm particles (51099AST)
mobile phase . . . . . (A) heptane; (B) isopropanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 15 µL
sample . . . . . glutethimide, 1 g/L in heptane:isopropanol (50:50)
Application No. . . . . G005514



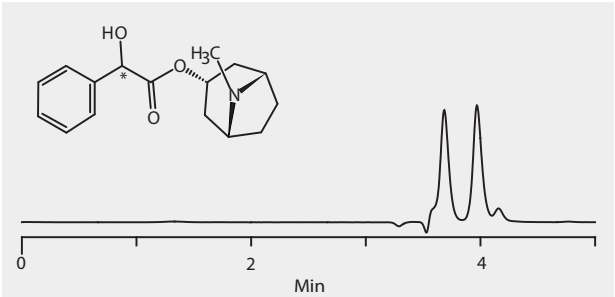
## HPLC Analysis of Homatropine Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) DEA; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004997



## HPLC Analysis of Homatropine Enantiomers on Astec® Cellulose DMP, LC/MS Conditions

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) ammonium formate; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004998



## HPLC Analysis of Hydrocortisone in Topical Cream on Discovery® HS C18 after SPE using Discovery® DSC-Si

**Sample Pre-treatment:**

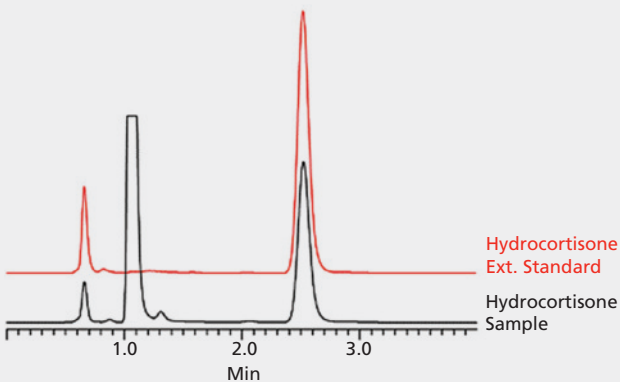
Dissove 1 g 1% hydrocortisone topical hand cream in 10 mL ethyl acetate. Dilute 2 mL of ethyl acetate-cream sample with 8 mL hexane:ethyl acetate (2:1, v/v).

sample preparation . . . . . SPE (Solid Phase Extraction)
sample addition . . . . . hydrocortisone topical hand cream in hexane:ethylacetate
SPE tube/cartridge . . . . . Discovery DSC-Si, 500 mg/3 mL (52695-U)
condition . . . . . 1 mL hexane:ethyl acetate (2:1, v/v)
sample addition . . . . . 1 mL
washing . . . . . 1 mL hexane:ethyl acetate (2:1, v/v)
elution . . . . . 1 mL methanol:DI water (1:1, v/v)
eluate post-treatment . . . dilute eluate to 2 mL with methanol:DI water (1:1, v/v)
column . . . . Discovery HS C18, 15 cm x 4.6 mm I.D., 5 µm particles (568520-U)
mobile phase . . . . . (A) methanol; (B) DI water (1:1, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G003765

**Efficiency of Recovery**

| Sample No. | Sample Weight (g) | Theoretical Hydrocortisone Weight (g)[1] | Actual Hydrocortisone weight recovered (g) | Absolute Recovery (%) | Actual % Hydrocortisone in Cream |
|---|---|---|---|---|---|
| 1 | 1.0258 | 0.0103 | 0.0092 | 89.32 | 0.9003 |
| 2 | 1.1078 | 0.0118 | 0.0111 | 94.07 | 0.9975 |
| 3 | 1.1151 | 0.0112 | 0.0119 | 106.25 | 1.0424 |
| 4 | 1.1369 | 0.0114 | 0.0119 | 104.39 | 1.0442 |
| 5 | 1.1390 | 0.0114 | 0.0113 | 99.12 | 1.0785 |
| 6 | 1.1634 | 0.0116 | 0.0123 | 106.03 | 1.0567 |
| | | | Average: | 99.86 | 1.02 |
| | | | RSD: | 6.99 | 0.06 |

[1]Based on manufacturer's label description of 1% hydrocortisone (w/w)



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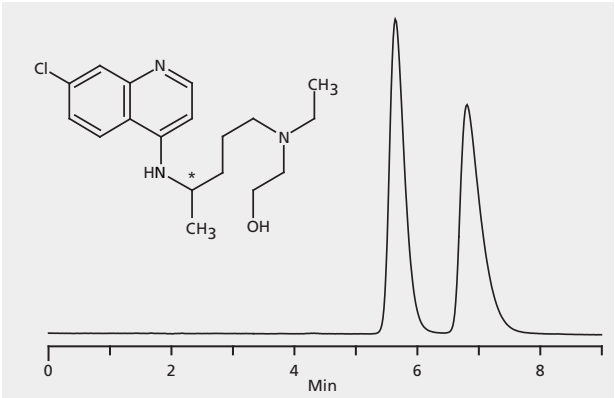
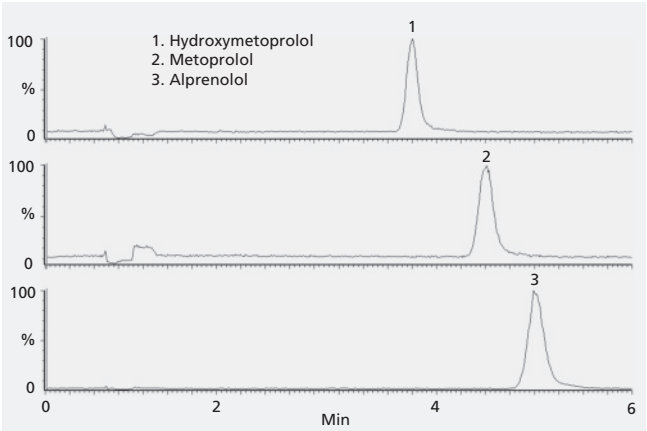
## HPLC Analysis of Hydroxychloroquine Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . (A) methanol; (B) acetic acid; (C) triethylamine; (98:1:1, A:B:C)
flow rate . . . . . 2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004627



## HPLC Analysis of Hydroxymetoprolol, Metoprolol and Alprenolol on Discovery® HS F5

column . . . . . Discovery HS F5, 5 cm × 2.1 mm I.D., 3 µm particles (567500-U)
mobile phase . . . . . 5 mM ammonium acetate in 10:90 water:acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 60 °C
detector . . . . . (+) ESI
injection . . . . . 10 µL
sample . . . . . as indicated (in mobile phase)
Application No. . . . . G002381

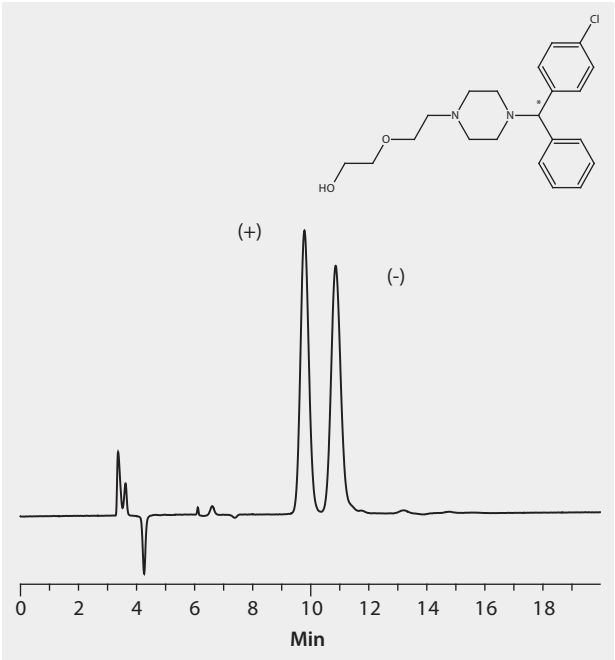





SUPELCO® Solutions within.™

## HPLC Analysis of Hydroxyzine Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) DEA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004989

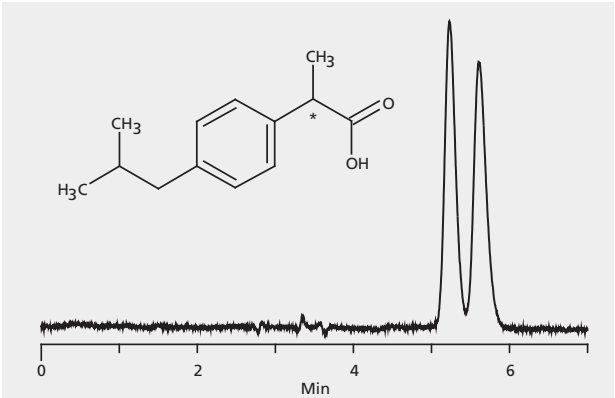





SUPELCO® Solutions within.™

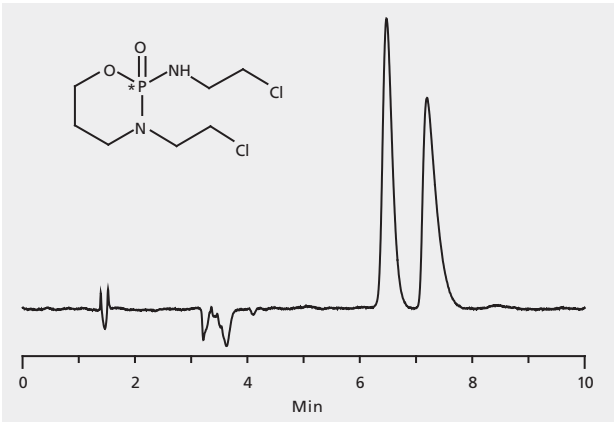
## HPLC Analysis of Ibuprofen Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM sodium citrate; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 225 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004628



## HPLC Analysis of Ifosfamide Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) water; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004499

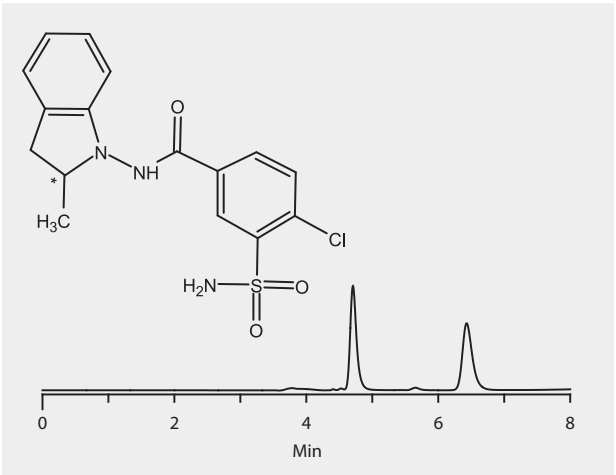





SUPELCO® Solutions within.™

## HPLC Analysis of Indapamide Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) DEA; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005000

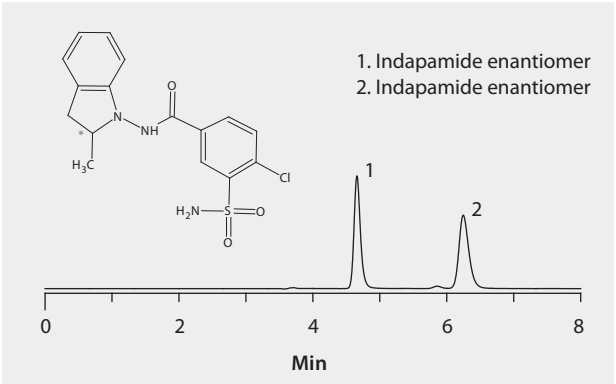





SUPELCO® Solutions within.™

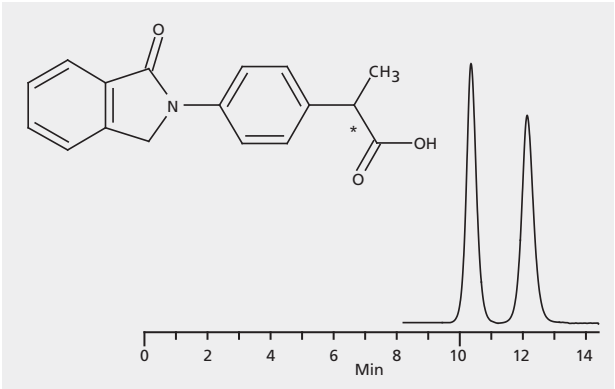
## HPLC Analysis of Indapamide Enantiomers on Astec® Cellulose DMP, LC/MS

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) ammonium formate; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µl/h
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004999



## HPLC Analysis of Indoprofen Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM sodium citrate, pH 6.3; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004652

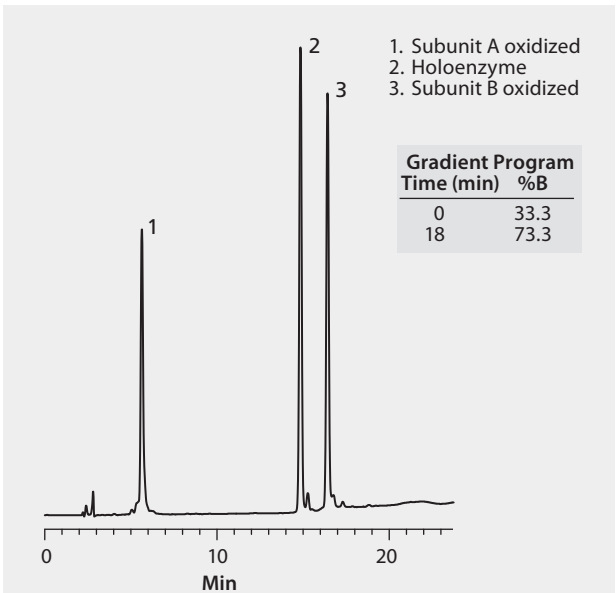





SUPELCO® Solutions within.™

## HPLC Analysis of Insulin, Bovine on Discovery® C8

column . . . . . Discovery C8, 5 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . . . 50 mM sodium phosphate, monobasic, 50 mM sodium sulfate, pH 3.0 (w/phosphoric acid), (A) +5% acetonitrile, (B) +50% acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
Application No. . . . . G000765



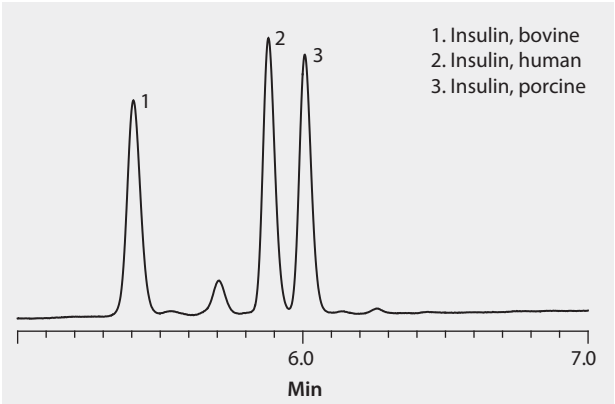

**Gradient Program**

| Time (min) | %B |
|---|---|
| 0 | 33.3 |
| 18 | 73.3 |

## HPLC Analysis of Insulin (Species Variants) on Ascentis® Express Peptide ES C18

These three insulins have more than 76% identical positions in a primary structure alignment. The human and porcine proteins are most similar with 85% identity. Baseline resolution of these insulins in achieved on Ascentis Express Peptide ES-C18, with relative band spacing of these three peaks approximating their relative sequence similarities. Ascentis Express Peptide ES-C18 is ideally suited for RP chromatography of small proteins or large peptides.

column . . . . . Ascentis Express Peptide ES C18, 15 cm x 2.1 mm I.D., 2.7 µm particles (53307-U)
mobile phase . . . . (A) 70:30, water with 0.1% TFA:acetonitrile with 0.09% TFA; (B) 55:45, water with 0.1% TFA:acetonitrile with 0.09% TFA
gradient . . . . . 0 to 100% B in 7.5 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 4040 psi (278 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 215 nm
injection . . . . . 0.5 µL
sample . . . . . 200 µg/mL insulin (bovine, human, porcine) in 0.1% formic acid
Application No. . . . . G005794






SUPELCO® Solutions within.™

## HPLC Analysis of Insulin (Species Variants) on Discovery® BIO Wide Pore C5

column . . . . . Discovery BIO Wide Pore C5, 15 cm × 4.6 mm I.D., 5 µm particles (568422-U)

mobile phase . . . . . (A): 71:29, (0.1% TFA in water):(0.1% TFA in acetonitrile); (B): 68:32, (0.1% TFA in water):(0.1% TFA in acetonitrile)

gradient . . . . . 0-100% B in 30 min

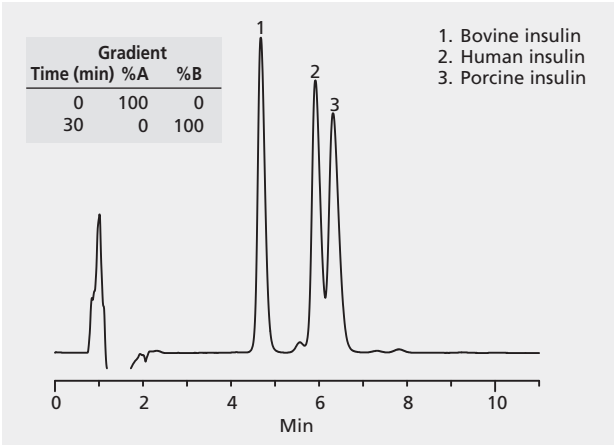
flow rate . . . . . 1 mL/min

column temp. . . . . ambient

detector . . . . . UV, 215 nm

injection . . . 5 µL 5 µL in 0.1% TFA containing 5ug each of three insulin variants

Application No. . . . . G001580






SUPELCO® Solutions within.™

## HPLC Analysis of Insulin (Species Variants) on Discovery® BIO Wide Pore C8

column . . . . . Discovery BIO Wide Pore C8, 15 cm × 4.6 mm I.D., 5 µm particles (568322-U)

mobile phase . . . . . (A) (0.1% TFA in water):(0.1% TFA in acetonitrile), 71:29 (B): (0.1% TFA in water):(0.1% TFA in acetonitrile), 68:32,

gradient . . . . . 0-100% B in 30 min

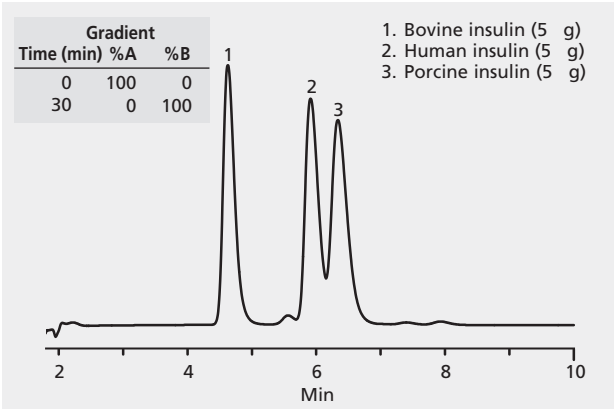
flow rate . . . . . 1 mL/min

column temp. . . . . 30 °C

detector . . . . . UV, 215 nm

injection . . . . . 5 µL in 0.1% TFA containing 5ug each of three insulin variants

Application No. . . . . G001507



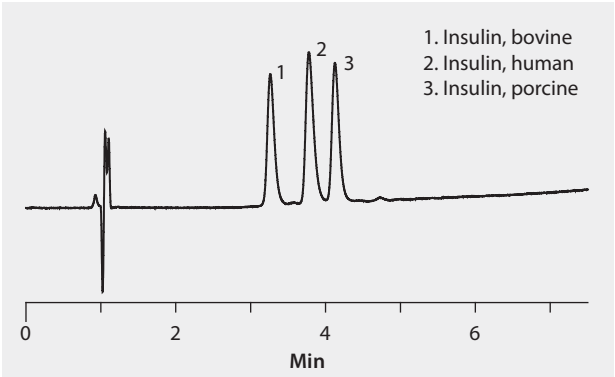



SUPELCO® Solutions within.™

## HPLC Analysis of Insulin (Specirs Variants) on Ascentis® Express Phenyl-Hexyl

These three insulins have more than 76% identical positions in a primary structure alignment. The human and porcine proteins are most similar with 85% identity. The Phenyl-Hexyl phase provides an alternate retention and selectivity to a C18 phase, particularly the C18 phase on a larger pore silica, Ascentis Express ES-C18

| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 15 cm x 2.1 mm, 2.7 µm particles (53338-U) |
| mobile phase | (A) 70:30, water with 0.1% TFA:acetonitrile with 0.09% TFA; (B) 55:45, water with 0.1% TFA:acetonitrile with 0.09% TFA |
| gradient | 0 to 100% B in 7.5 min |
| flow rate | 0.3 mL/min |
| pressure | 3960 psi (273 bar) |
| column temp. | 30 °C |
| detector | UV, 215 nm |
| injection | 0.5 µL |
| sample | 200 µg/mL in 0.1% formic acid |
| Application No. | G005795 |

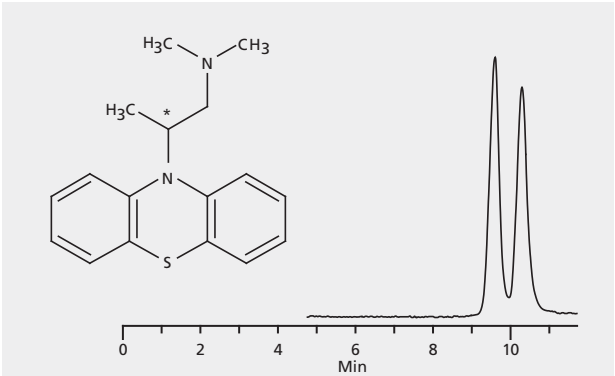





SUPELCO® Solutions within.™

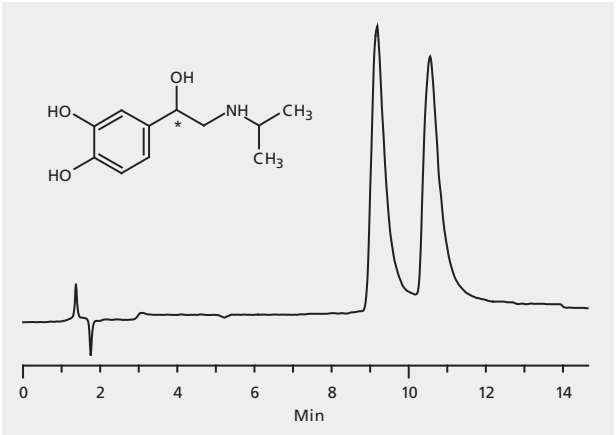
## HPLC Analysis of Isopromethazine Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (100:0.1:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004640



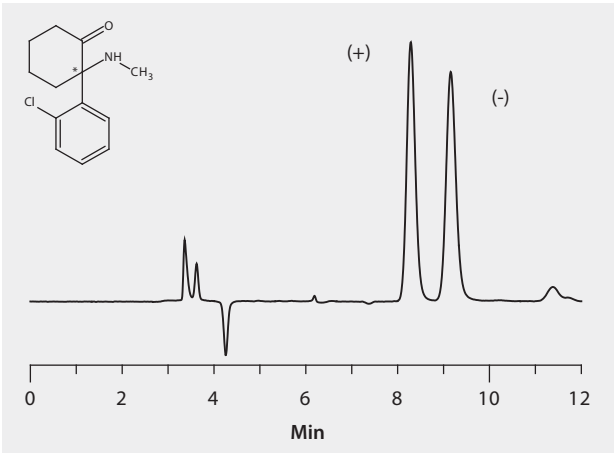
## HPLC Analysis of Isoproterenol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) triethylamine; (55:45:0.3:0.2, A:B:C:D)
flow rate . . . . . 2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004543



## HPLC Analysis of Ketamine Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) DEA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004990



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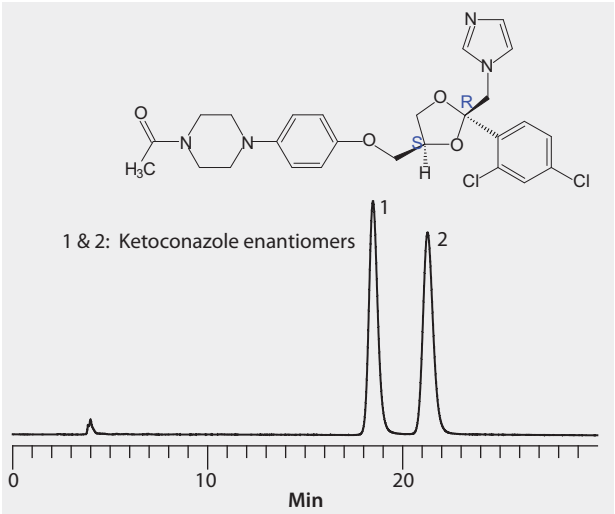
## HPLC Analysis of Ketoconazole Enantiomers on Astec® Cellulose DMP, No Additives

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) acetonitrile; (100, A)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005005

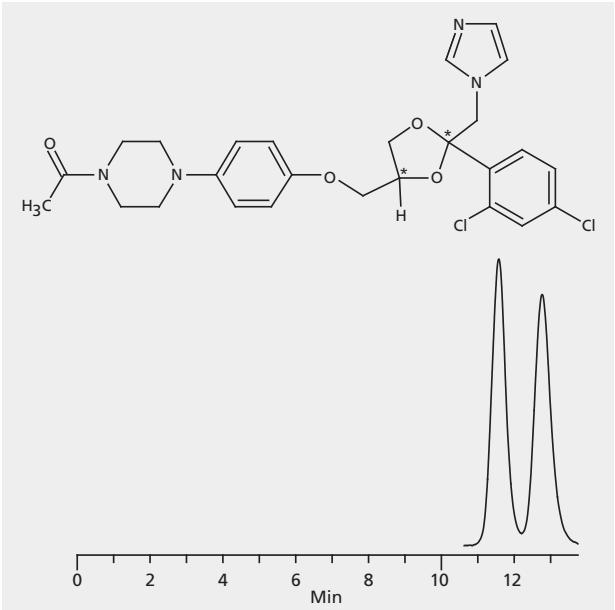





SUPELCO® Solutions within.™

## HPLC Analysis of Ketoconazole Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

| | |
|---|---|
| column | CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST) |
| mobile phase | (A) 0.1% formic acid; (B) acetonitrile; (70:30, A:B) |
| flow rate | 1 mL/min |
| column temp. | 25 °C |
| injection | 1 µL |
| detector | UV, 230 nm |
| sample | 1 mg/mL in methanol |
| Application No. | G004716 |

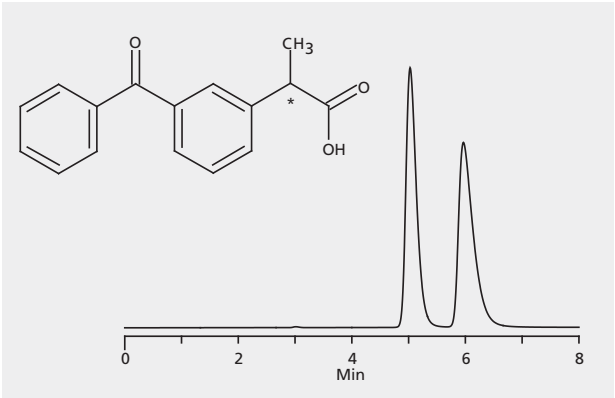





SUPELCO® Solutions within.™

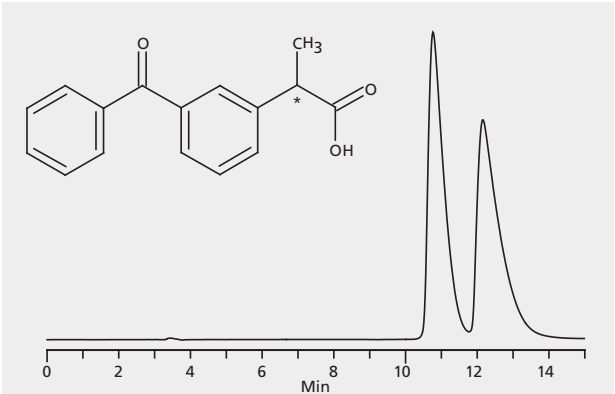
## HPLC Analysis of Ketoprofen Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 15 cm x 4.6 mm I.D., 5 µm particles (13023AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 5.6; (B) methanol; (50:50, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004666



## HPLC Analysis of Ketoprofen Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM sodium citrate; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004629

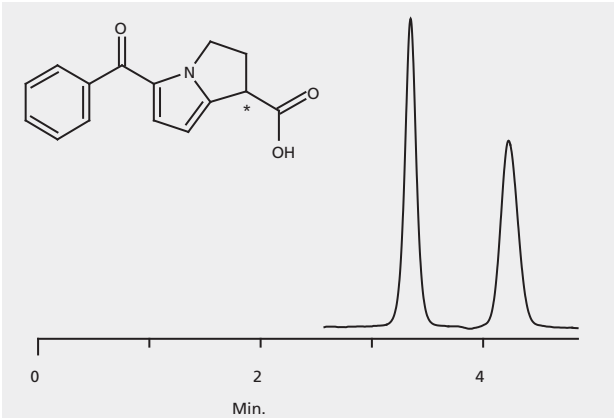





SUPELCO® Solutions within.™

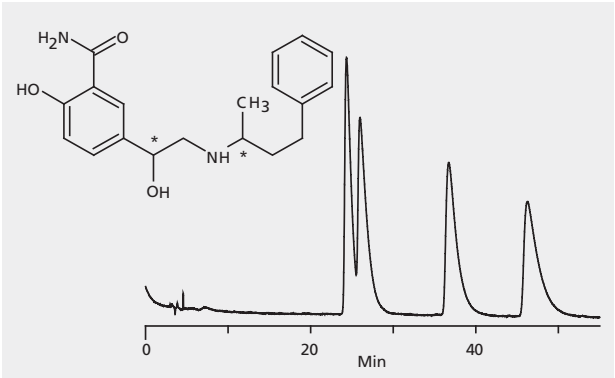
## HPLC Analysis of Ketorolac Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 0.1 wt% mM ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004581



## HPLC Analysis of Labetalol Enantiomers on Astec® CHIROBIOTIC® V

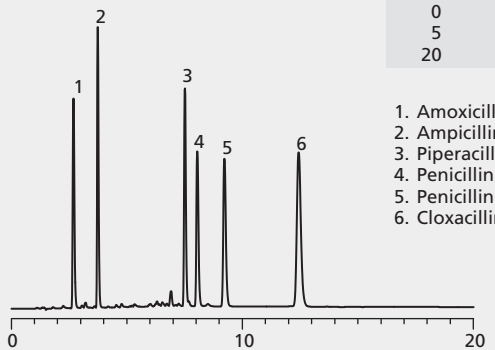
column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.8:0.15:0.05, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 206 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004630



## HPLC Analysis of β-Lactam Antibiotics on Discovery® RP-Amide C16

column . . Discovery RP-Amide C16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) 0.01% TFA in water; (B) 0.01% TFA in acetonitrile
flow rate . . . . . 1.5 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
Application No. . . . . G001178

**Discovery RP-AmideC16**

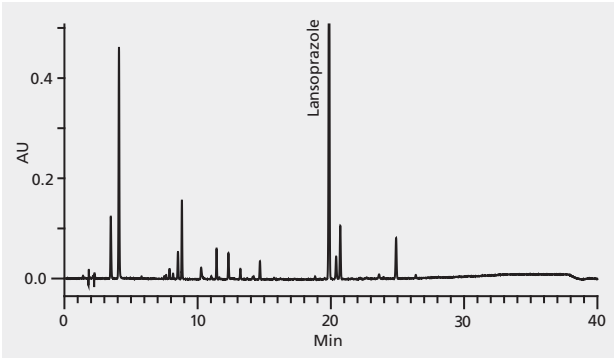



| Gradient Program | |
|---|---|
| Time (min) | %B |
| 0 | 5 |
| 5 | 35 |
| 20 | 35 |

1. Amoxicillin
2. Ampicillin
3. Piperacillin
4. Penicillin G
5. Penicillin V
6. Cloxacillin

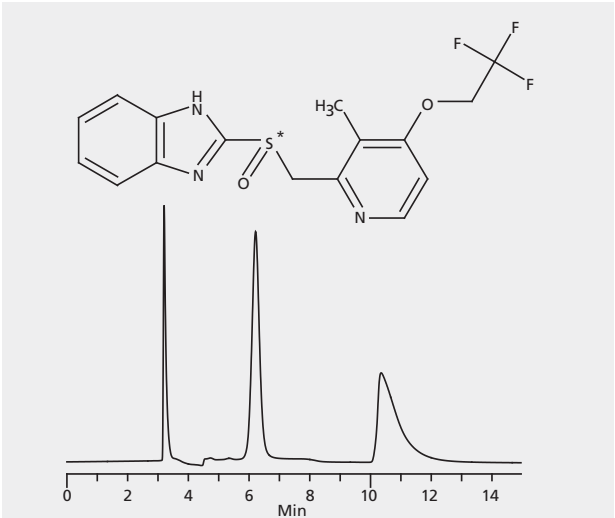
## HPLC Analysis of Lansoprazole (Degradation Profile) on Ascentis® Express C18

column . . . . Ascentis Express C18, 15 cm × 4.6mm, 2.7 µm particles (53829-U)
mobile phase . . . . . (A) water, (B) acetonitrile:0.5% triethylamine in water, pH=7.0 (80:20)
flow rate . . . . . 0.8 mL/min
column temp. . . . . ambient
detector . . . . . UV, 285 nm
injection . . . . . 15 µL
Application No. . . . . G004160



## HPLC Analysis of Lansoprazole Enantiomers on Astec® CYCLOBOND® I 2000 DMP

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) ammonium hydroxide; (95:5:0.4:0.1, A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004687

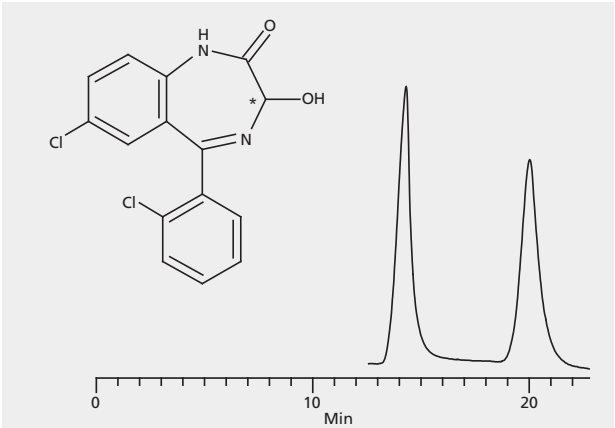





SUPELCO® Solutions within.™

## HPLC Analysis of Lorazepam Enantiomers on Astec® (R,R) P-CAP™

column . . . . Astec (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
mobile phase . . . . . (A) ethanol; (B) heptane; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004721

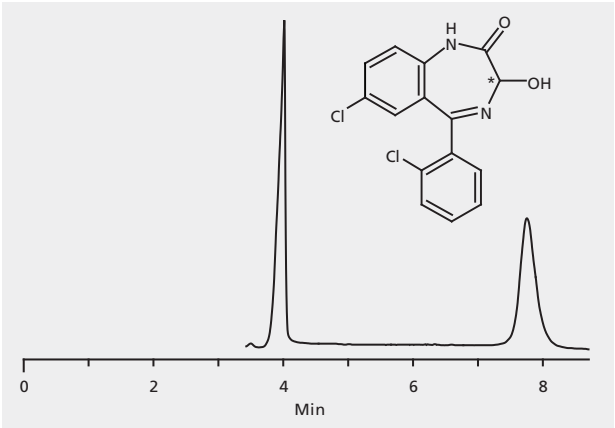





SUPELCO® Solutions within.™

## HPLC Analysis of Lorazepam Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004551

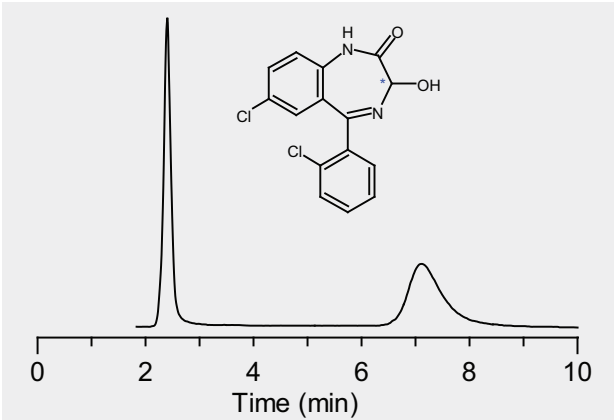





SUPELCO® Solutions within.™

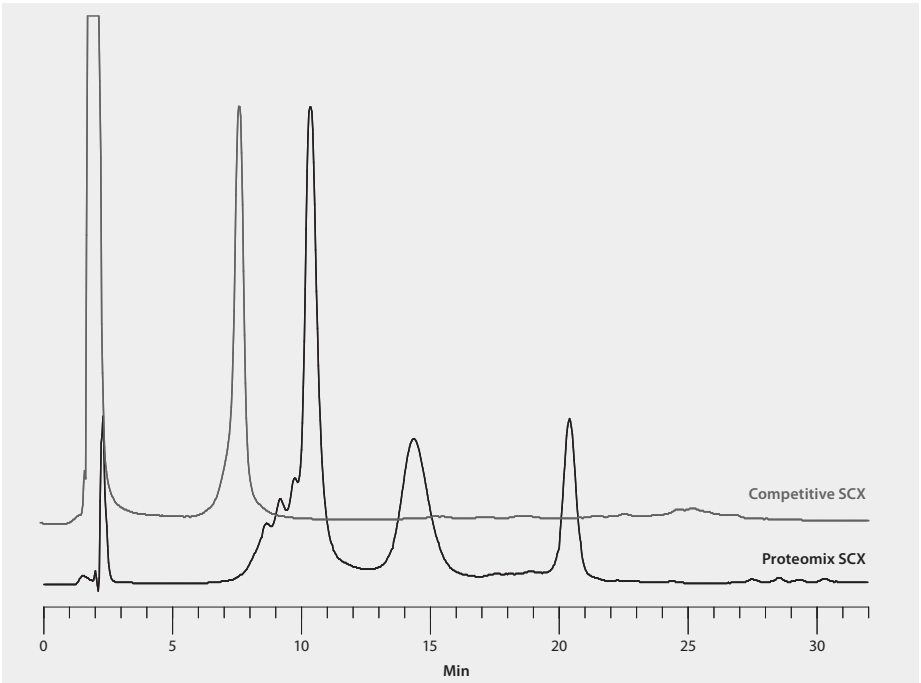
## HPLC Analysis of Lorazepam Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . methanol
flow rate . . . . . 2 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004609



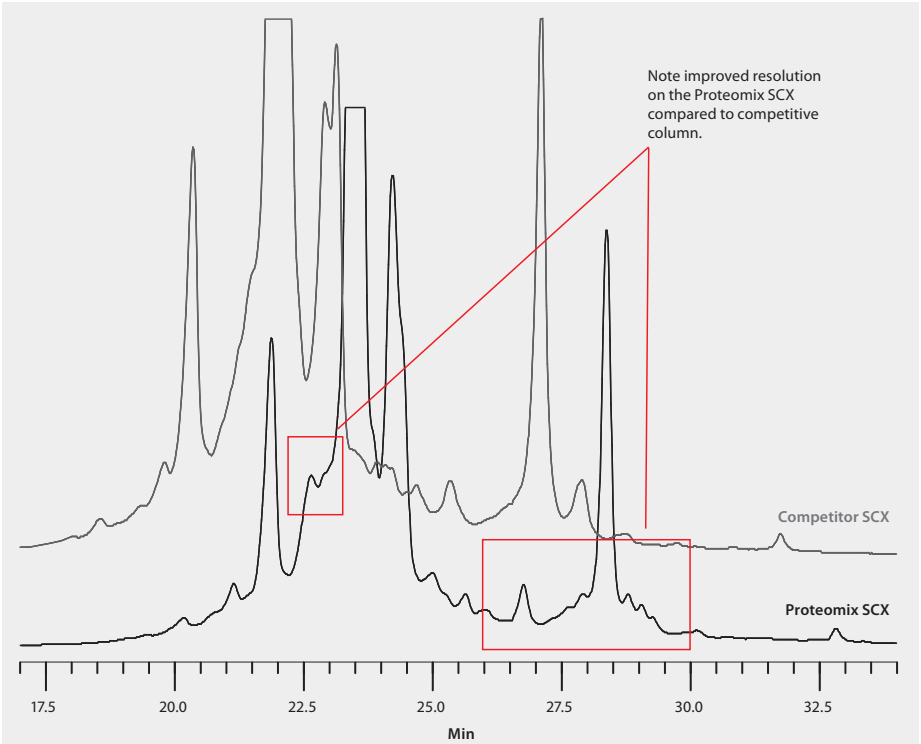
## HPLC Analysis of MAb 016 on Proteomix® SCX NP5 versus Competitive SCX using a Salt Gradient

column . . . . . Proteomix SCX-NP5, 25 cm x 4.6 mm I.D. (PEEK hardware), 5 µm particles (Z777164)
mobile phase . . . . . (A) 20 mM phosphate buffer, pH 7.5; (B) A + 1 M NaCl
gradient . . . . . 0 to 6% B in 30 min
flow rate . . . . . 0.8 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 4 µL
sample . . . . . MAb 016 (11.8 mg/mL)
Application No. . . . . G006107



## HPLC Analysis of MAb 321 on Proteomix® SCX NP5 versus Competitive SCX using pH and Salt Gradients

column . . . . . Proteomix SCX-NP5, 25 cm x 4.6 mm I.D. (PEEK hardware), 5 µm particles (Z777164)
mobile phase . . (A) 2.4 mM Tris, 1.5 mM imidazole, 11.6 mM piperazine, pH 6.0; (B) A + 0.5 M NaCl, pH 10.5
flow rate . . . . . 0.8 mL/min
detector . . . . . UV, 280 nm
injection . . . . . 20 µL
sample . . . . . MAb 321 (5 mg/mL)
Application No. . . . . G006098

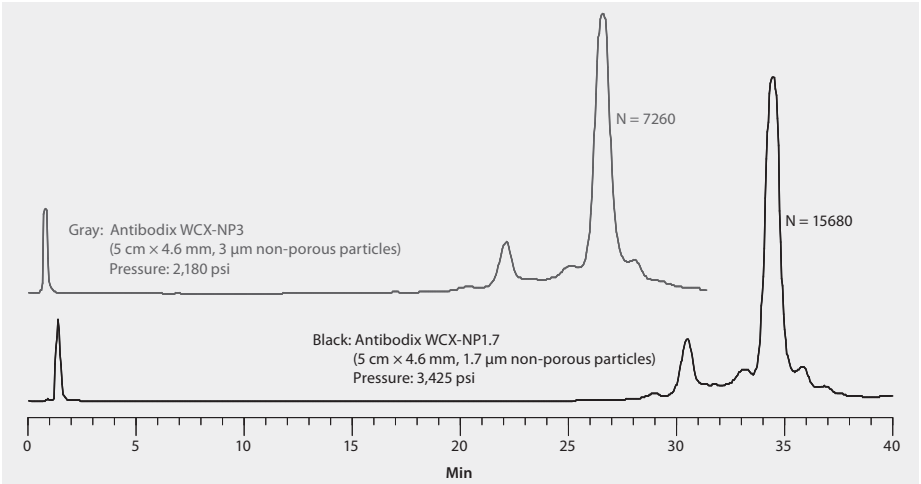





SUPELCO® Solutions within.™

## HPLC Analysis of mAb on Antibodix® WCX, Effect of Particle Size (5 cm columns)

column . . Antibodix WCX-NP3 PEEK, 5 cm x 4.6 mm I.D., 3 µm particles (Z777288)
column . . . . . Z777280 (Antibodix WCX-NP1.7, 5 cm x 4.6 mm, 1.7 µm particles)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 7.5; (B) A + 100 mM NaCl
gradient . . . . . NP3 column: 15 to 55% B in 30 min, held for 3 min;
NP1.7 column: 15 to 100% B in 60 min, held for 1.7 min
flow rate . . . . . 0.5 mL/min for 3 min; 0.3 mL/min for 1.7 min
pressure . . . . . 2180 psi (3 min), 3425 psi (1.7 min)
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . Monoclonal antibody, 5 mg/mL
Application No. . . . . G005941



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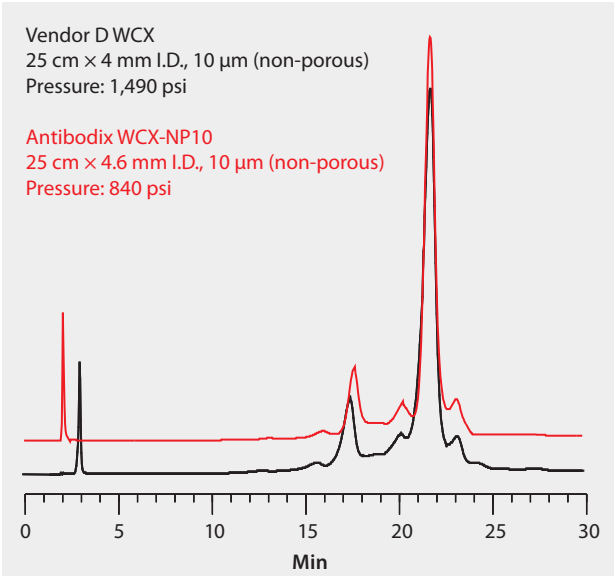
## HPLC Analysis of mAb on Antibodix® WCX-NP10, Competitive Comparison

column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . . . (A) 10 mM phosphate buffer, pH 7.5; (B) A + 100 mM NaCl
gradient . . . . . 15 to 55% B in 30 min
flow rate . . . . . 0.8 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . monoclonal antibody
Application No. . . . . G005939



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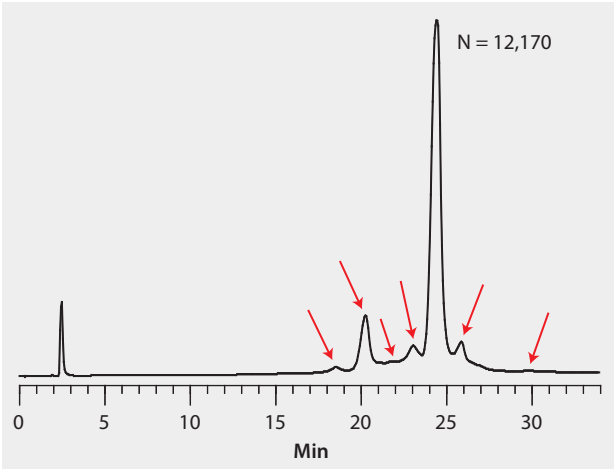
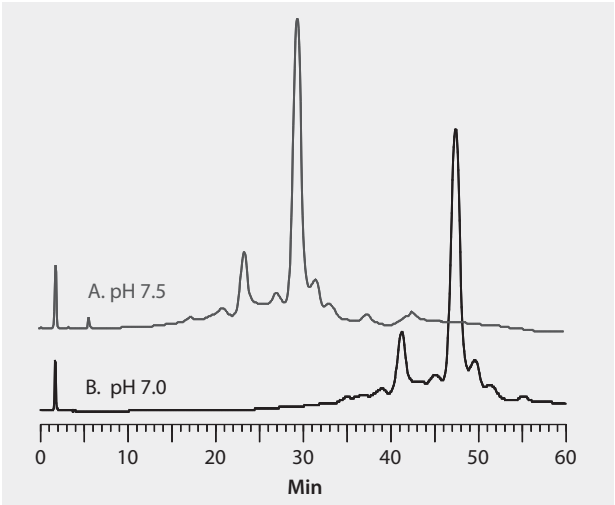
## HPLC Analysis of mAb on Antibodix® WCX-NP5, 25 cm Columns

column . . . . Antibodix WCX-NP5, 25 cm x 4.6 mm I.D., 5 µm particles (Z777294)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 7.5; (B) A + 100 mM NaCl
gradient . . . . . 15 to 55% B in 30 min
flow rate . . . . . 0.8 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . monoclonal antibody
Application No. . . . . G005942



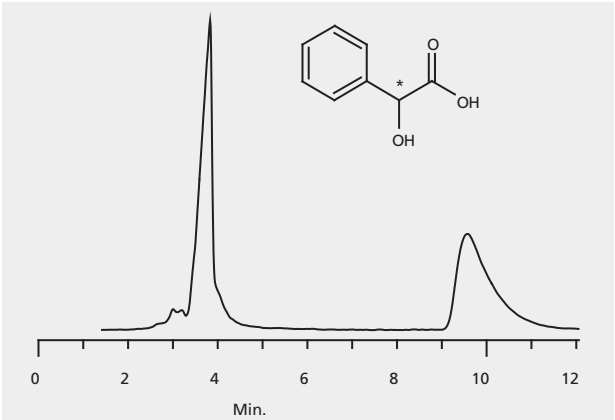
## HPLC Analysis of mAb-X22 on Antibodix® WCX-NP10, Impact of Mobile Phase pH

column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . . . . (A) 10 mM sodium phosphate buffer at 7.5 pH; (B), A + 100 mM NaCl at 7.0 pH
gradient . . . . . 15 to 65% B in 60 min
flow rate . . . . . 0.8 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . MAb-X22, 1.5 mg/mL
Application No. . . . . G005945



## HPLC Analysis of Mandelic Acid Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . 0.1 wt% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004610



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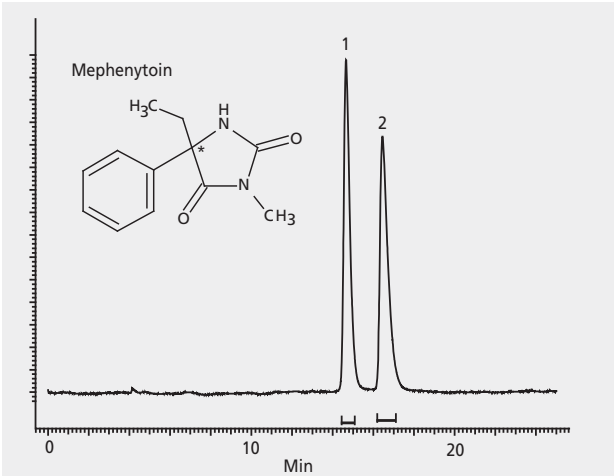
## HPLC Analysis of Mephenytoin Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) hexane; (B) ethanol; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in mobile phase
Application No. . . . . G004471



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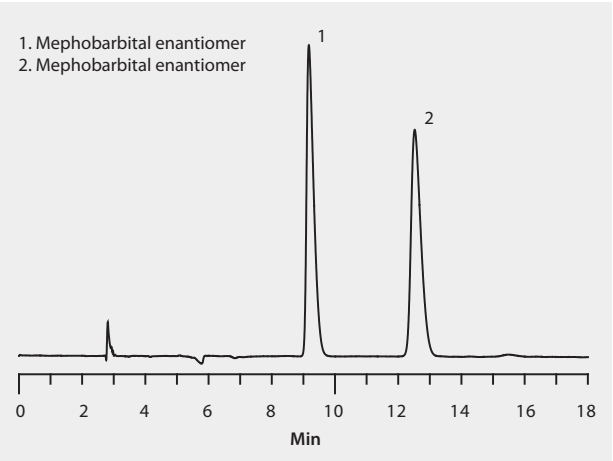
## HPLC Analysis of Mephobarbital Enantiomers on Astec® Cellulose DMP

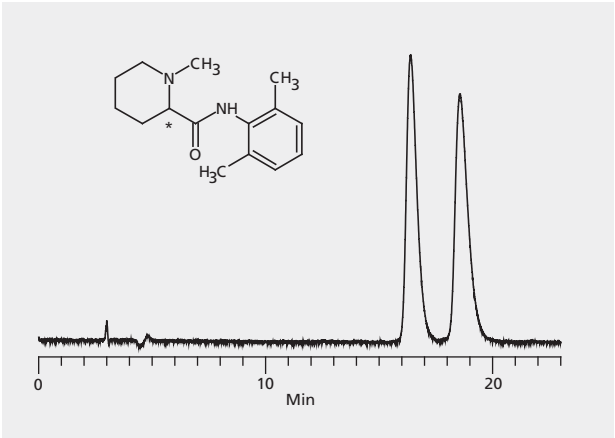
Mephobarbital enantiomers are resolved by normal phase chromatography. Mephobarbital (aka methyphenobarbital) is used as a sedative, anxiolytic and anticonvulsant. It is the N-methylated analogue of phenobarbital, and has similiar indications, therapeutic value, and tolerability.

column . . Astec Cellulose DMP; 25 cm x 4.6 mm I.D., 5 µm particles (51099AST)
mobile phase . . . . . (A) heptane; (B) isopropanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 15 µL
sample . . . . . mephobarbital, 1 g/L in 50:50, heptane:isopropanol
Application No. . . . . G005519



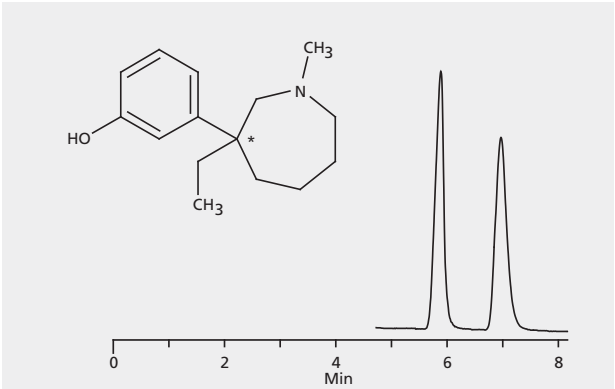
## HPLC Analysis of Mepivacaine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) methanol; (B) 20 mM ammonium acetate, pH 4.1; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004466



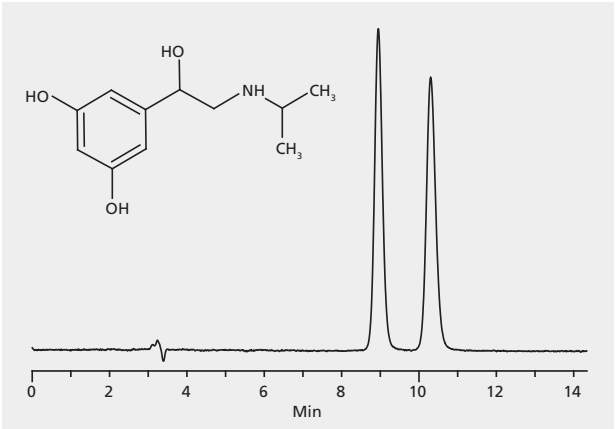
## HPLC Analysis of Meptazinol Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 0.1 wt% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004660



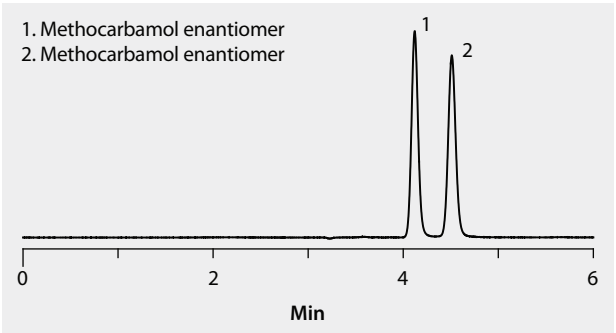
## HPLC Analysis of Metaproterenol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
sample . . . . . 1 mg/mL in mobile phase
Application No. . . . . G004406



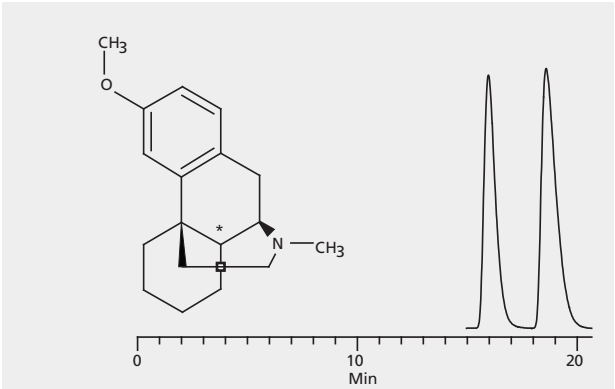
## HPLC Analysis of Methocarbamol (Guaiacol Glyceryl Ether Carbamate) Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) ammonium formate; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005372



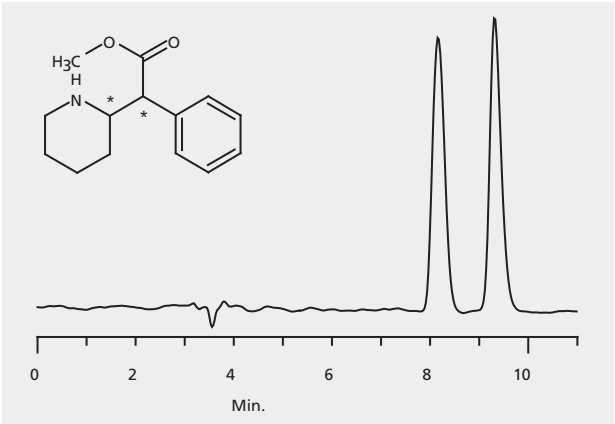
## HPLC Analysis of Methorphan Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 0.05 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004659



## HPLC Analysis of Methylphenidate Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) methanol; (B) 1% triethylamine, pH 4/1; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004505



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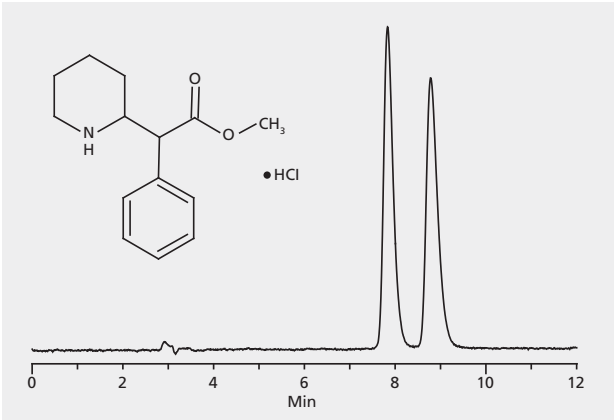
## HPLC Analysis of Methylphenidate (Ritalin) Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . methylphenidate, 1 mg/mL in mobile phase
Application No. . . . . G004405



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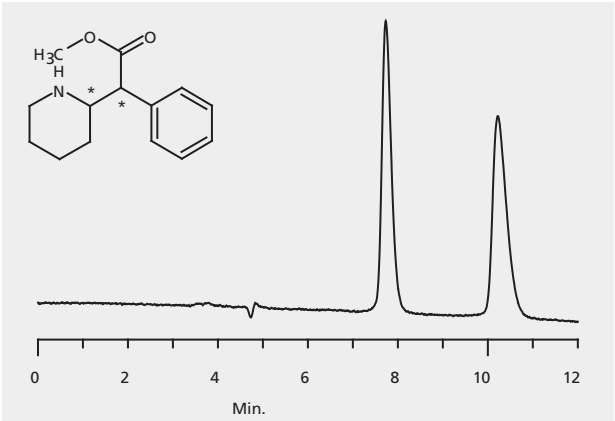
## HPLC Analysis of Methylphenidate (Ritalin) Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 15 mM ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 215 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004504



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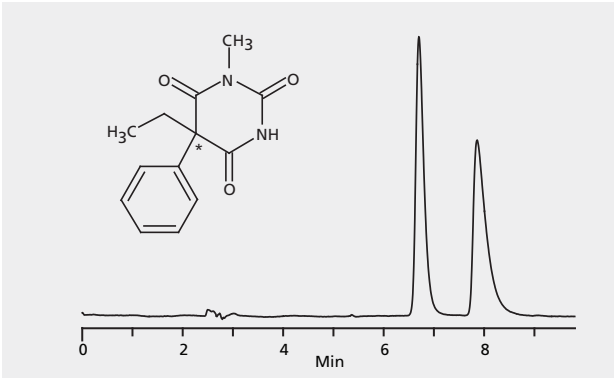
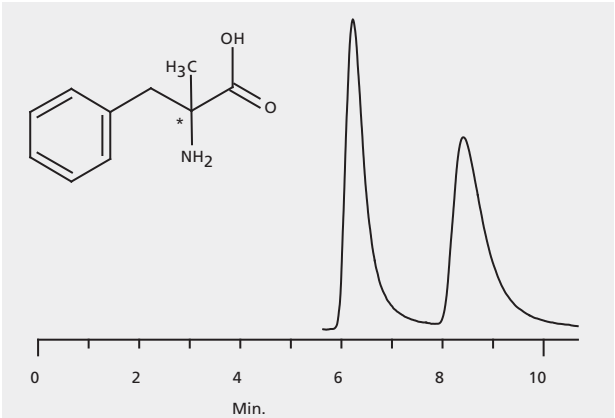
## HPLC Analysis of Methylphenobarbital (Mephobarbital) Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) ethanol; (B) hexane; (25:75, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 205 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004631



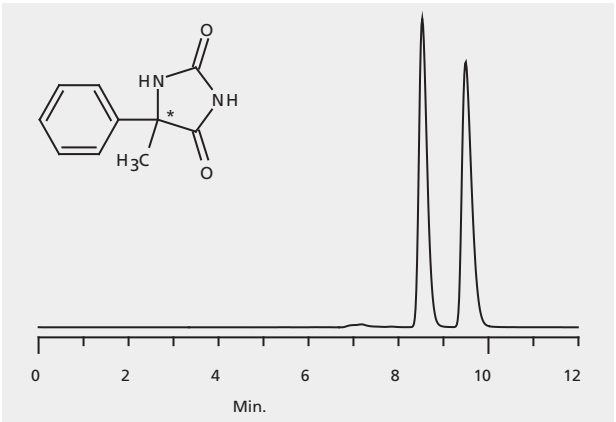
## HPLC Analysis of α-Methyl Phenylalanine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004590



## HPLC Analysis of 5-Methyl-5-Phenylhydantoin Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . methanol
flow rate . . . . . 0.48 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004477



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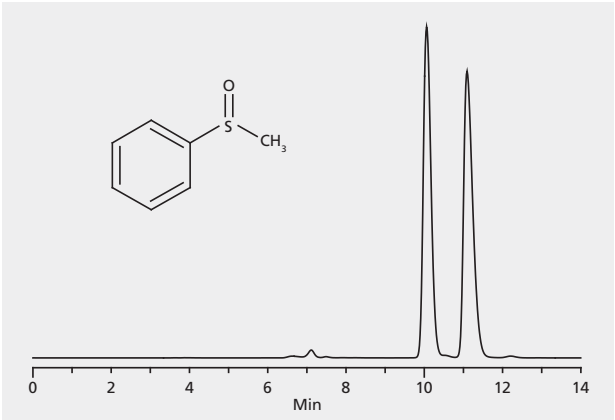
## HPLC Analysis of Methyl Phenyl Sulfoxide Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . methanol
flow rate . . . . . 0.48 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in mobile phase
Application No. . . . . G004407



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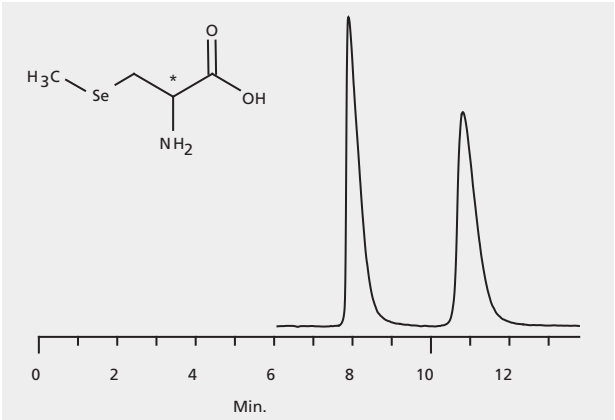
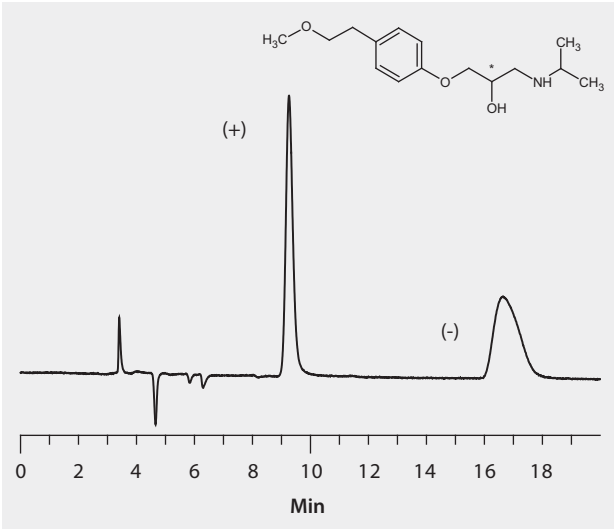
## HPLC Analysis of Methylselenocysteine Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . (A) water; (B) acetonitrile; (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV at 230 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004615



## HPLC Analysis of Metoprolol Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) DEA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004991



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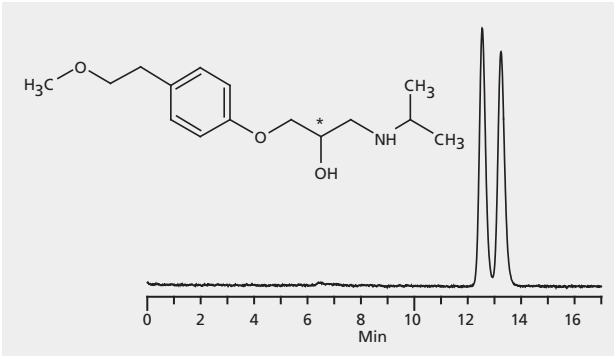
## HPLC Analysis of Metoprolol Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.8:0.1:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 225 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004632



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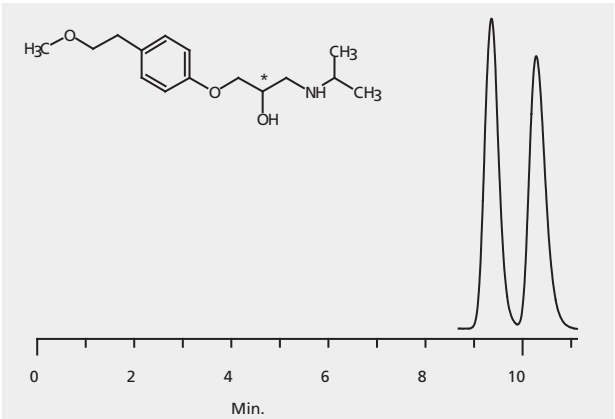
## HPLC Analysis of Metoprolol Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 0.1 wt% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004597



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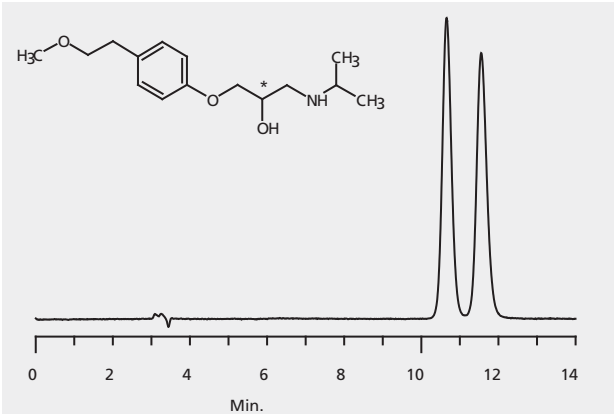
## HPLC Analysis of Metoprolol Enantiomers on Astec® CHIROBIOTIC® T2 (MS Mobile Phase)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample gas . . . . . 1 mg/mL in methanol
Application No. . . . . G004484

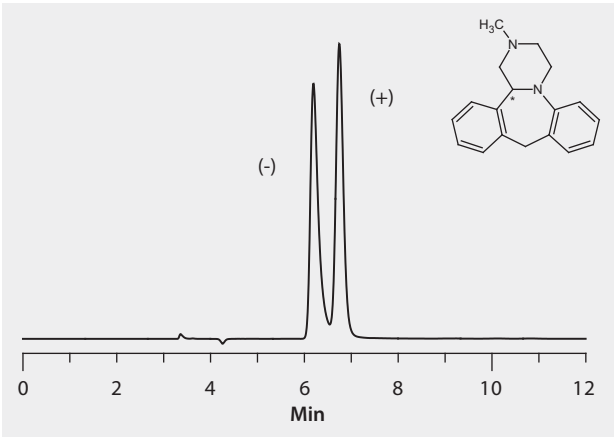





SUPELCO® Solutions within.™

## HPLC Analysis of Mianserin Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) DEA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004993



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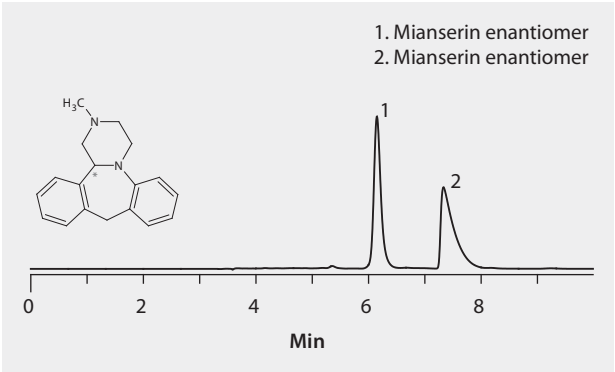
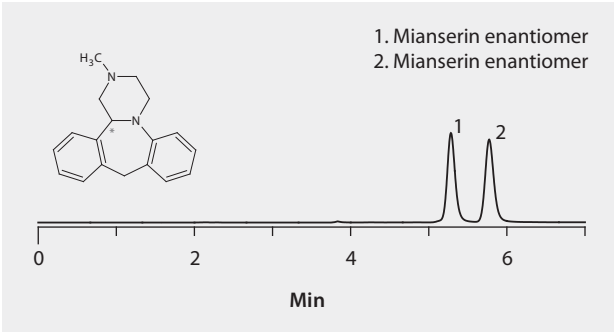
## HPLC Analysis of Mianserin Enantiomers on Astec® Cellulose DMP, LC/MS Conditions

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) ammonium formate; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005003



## HPLC Analysis of Mianserin Enantiomers on Astec® Cellulose DMP (No Additives)

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) acetonitrile; (100, A)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005006



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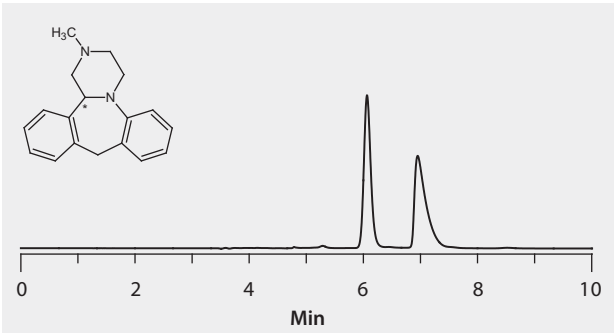
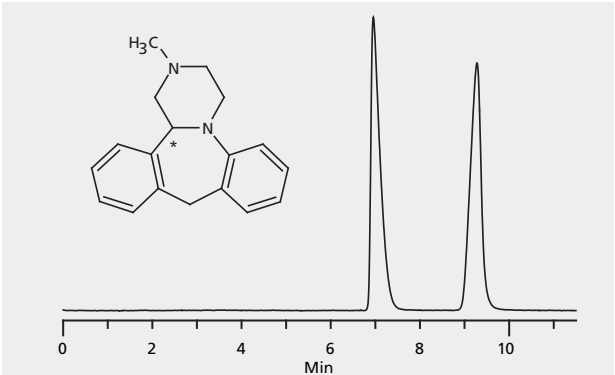
## HPLC Analysis of Mianserin Enantiomers on Astec® Cellulose DMP, POM Conditions

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) DEA; (100:0.1, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
inj. temp. . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004992



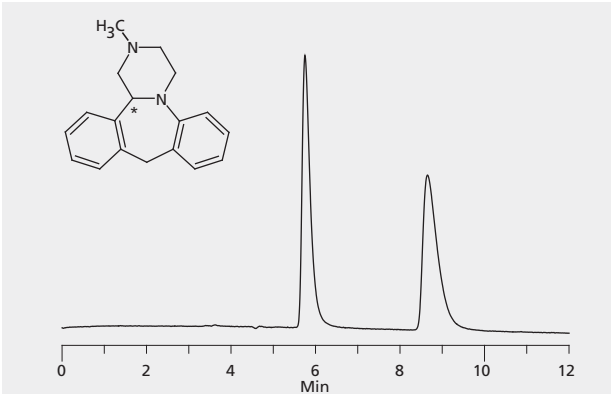
## HPLC Analysis of Mianserin Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.8:0.1:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 29 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004633



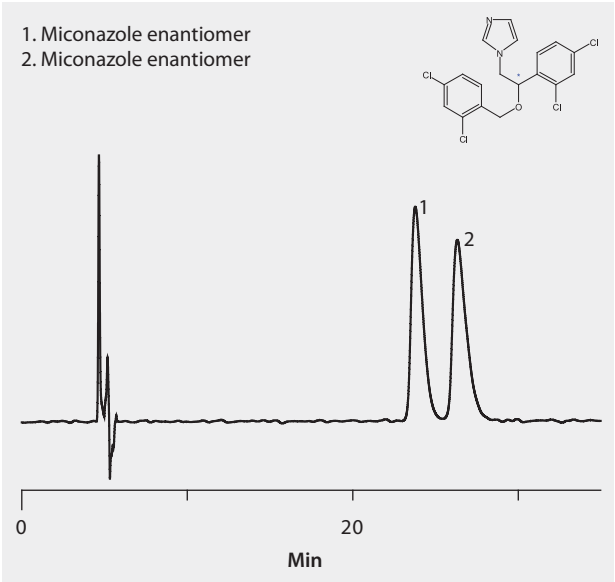
## HPLC Analysis of Mianserin Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 15 mM ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004469



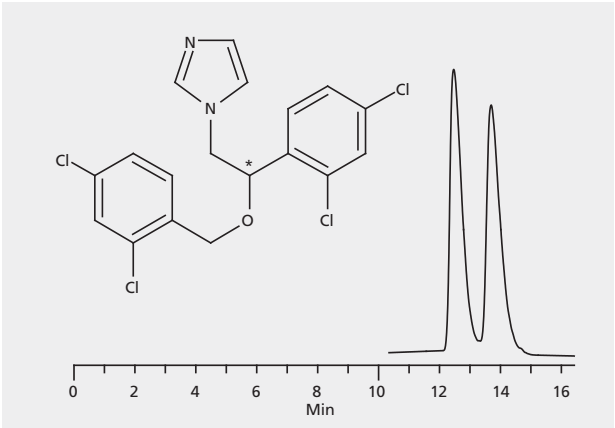
## HPLC Analysis of Miconazole Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

| | |
|---|---|
| column | CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST) |
| mobile phase | (A) acetonitrile; (B) 20 mM ammonium acetate, pH 4.0; (25:75, A:B) |
| flow rate | 0.6 mL/min |
| column temp. | 25 °C |
| detector | UV, 230 nm |
| injection | 5 µL |
| sample | miconazole, 1 mg/mL in acetonitrile:water (50:50) |
| Application No. | G005164 |



## HPLC Analysis of Miconazole Enantiomers on Astec® CYCLOBOND® I 2000 RSP

column . . CYCLOBOND I 2000 RSP, 25 cm x 4.6 mm I.D., 5 µm particles (20324AST)
mobile phase . . (A) 1% triethylamine acetate, pH 4.1; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . ambient
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004706



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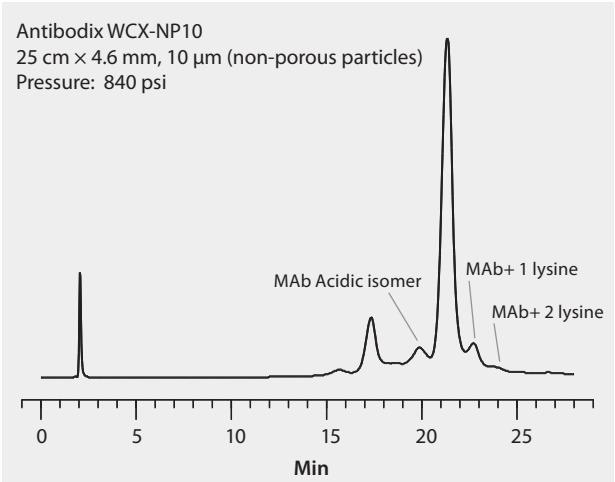
## HPLC Analysis of Monoclonal Antibodies (mAb) with Charge Variance on Antibodix® WCX-NP10

column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . (A) 10 mM sodium phosphate buffer, pH 7.5; (B) A + 100 mM NaCl
gradient . . . . . 15 to 55% B in 30 min
flow rate . . . . . 0.8 mL/min
pressure . . . . . 840 psi (58 bar)
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . monoclonal antibody
Application No. . . . . G005940

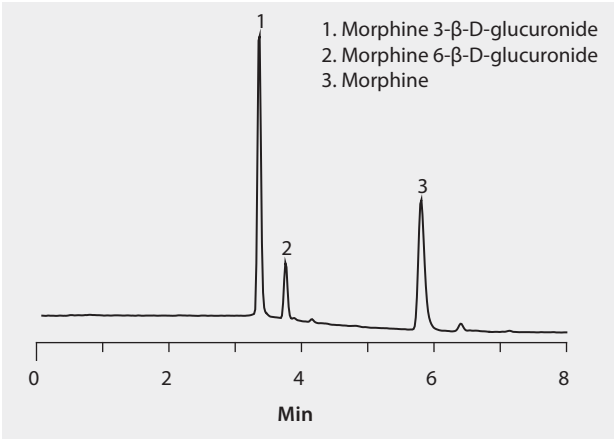





SUPELCO® Solutions within.™

## HPLC Analysis of Morphine and Glucuronide Metabolites on Discovery® HS F5

column . . . . . Discovery HS F5, 5 cm x 4.6 mm I.D., 5 µm particles (567513-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 6.8 (unadjusted); (B) acetonitrile
gradient . . . . . 0% B for 1 min, to 30% B in 4 min, held 3 min
flow rate . . . . . 1 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in final mobile phase
Application No. . . . . G006123

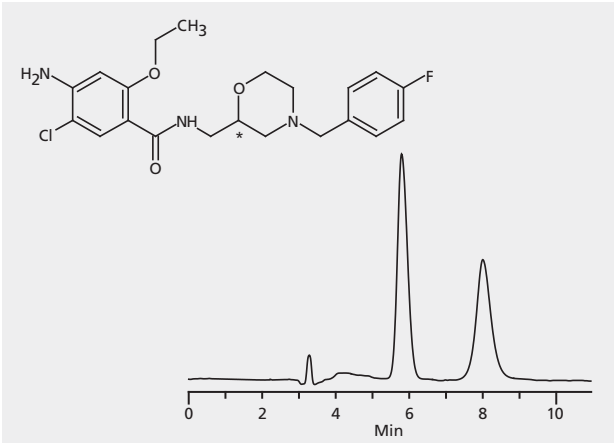





SUPELCO® Solutions within.™

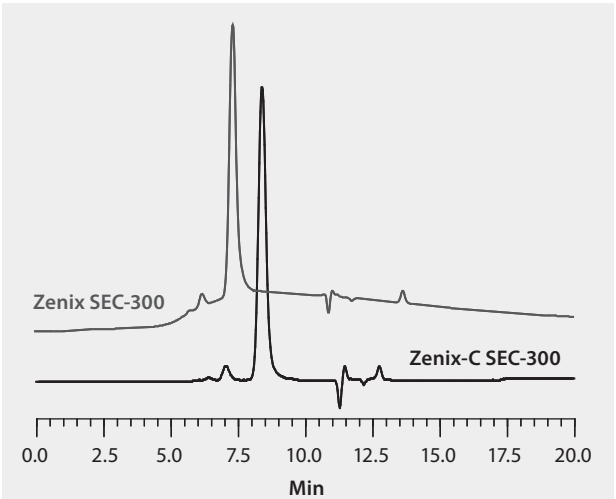
## HPLC Analysis of Mosapride Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 20 mM ammonium nitrate, pH 5.5; (B) THF; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 3 mg/mL in methanol
Application No. . . . . G004650



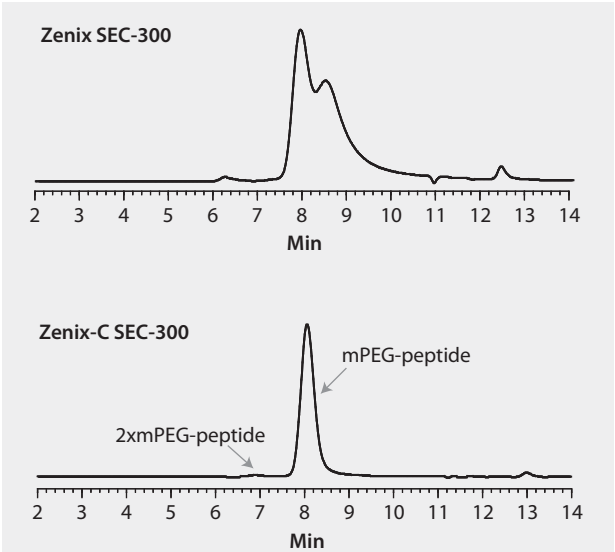
## HPLC Analysis of mPEG and mPEG-Peptide (Methoxy-PEG-Maleimide) on Zenix® and Zenix®-C SEC

column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm, I.D., 3 µm particles (Z777092)
mobile phase . . . . . 150 mM sodium phosphate buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 20 µL
sample . . . . . 20kD peptide mPEG-MAL (methoxy-PEG-Maleimide), 1 mg/mL
Application No. . . . . G005948



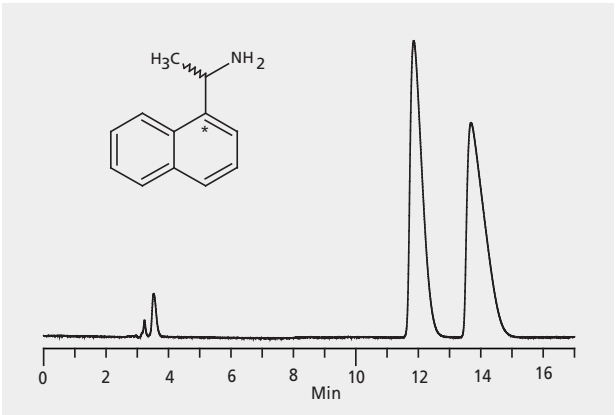
## HPLC Analysis of mPEG and mPEG-Peptide on Zenix® and Zenix®-C SEC

column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm, I.D., 3 µm particles (Z777092)
mobile phase . . . . . 150 mM sodium phosphate buffer, pH 7.0
flow rate . . . . . 1.0 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 20 µL
sample . . 4kD peptide and 20kD mPEG-MAL(methoxy-PEG-Maleimide) (6 mg/mL)
Application No. . . . . G005947



## HPLC Analysis of 1-(1-Naphthyl)ethylamine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . (A) methanol; (B) 20 mM ammonium acetate; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 1 mgl/mL in methanol
Application No. . . . . G004458



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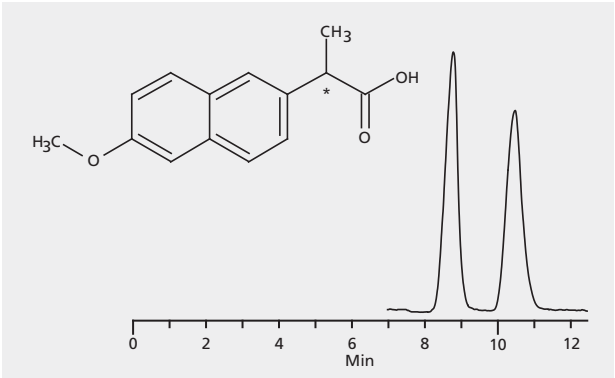
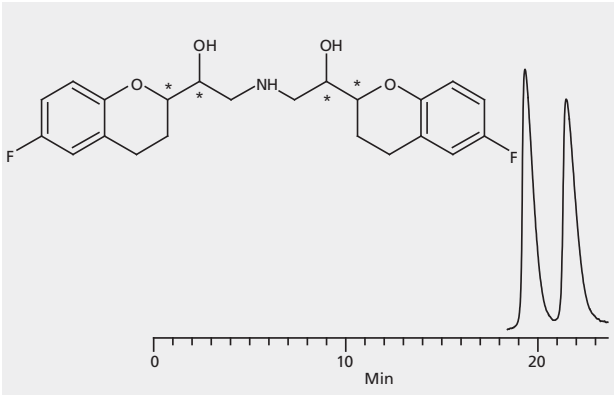
## HPLC Analysis of Naproxen Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 0.1% triethylamine, pH 7.0; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004644



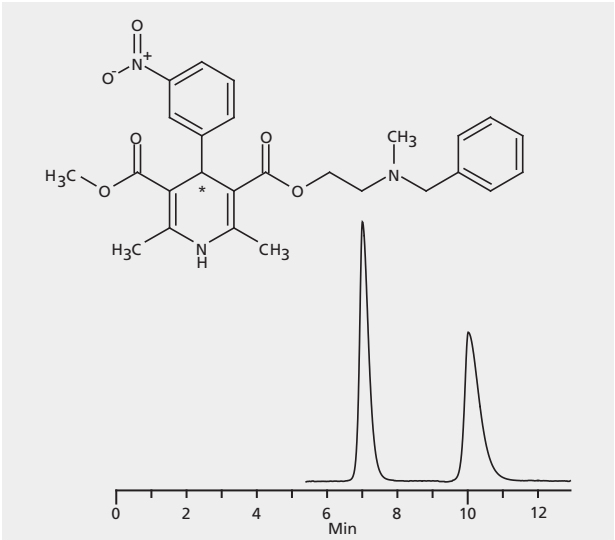
## HPLC Analysis of Nebivolol Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 0.05% ammonium trifluoroacetic acid in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 0.5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004658



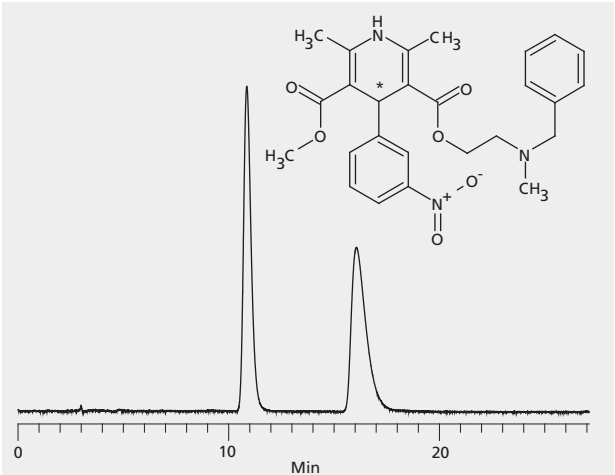
## HPLC Analysis of Nicardipine Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) triethylamine; (100:0.02:0.01, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004654



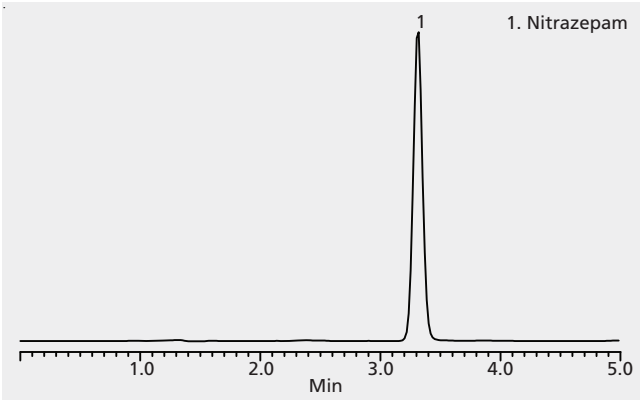
## HPLC Analysis of Nicardipine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) methanol; (B) 20 mM ammonium acetate, pH 4.1; (95:5, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004470



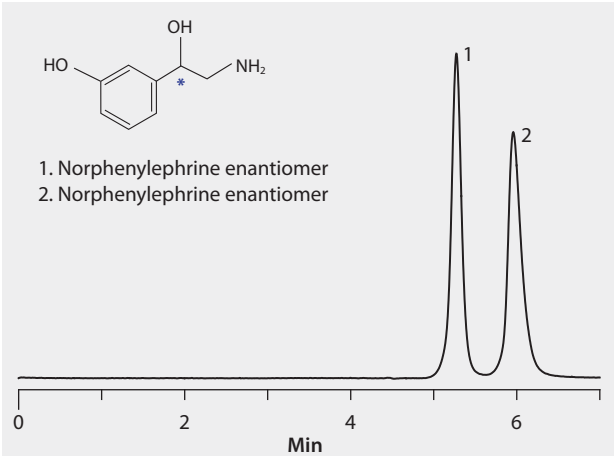
## HPLC Analysis of Nitrazepam Anticonvulsant Drugs on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 7.0 with potassium hydroxide): (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in mobile phase
Application No. . . . . G002356



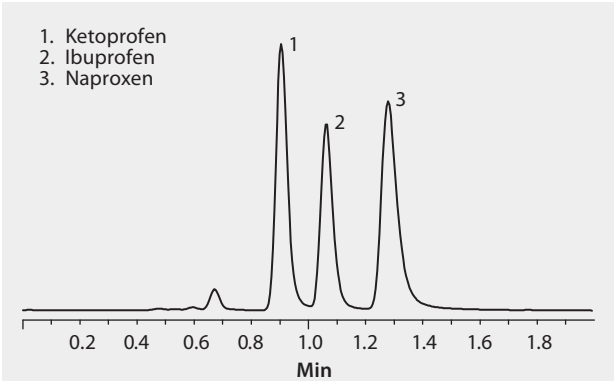
## HPLC Analysis of Norphenylephrine Enantiomers on Astec® CYCLOBOND® I 2000 AC (pH 5.0)

column . . CYCLOBOND I 2000 AC, 25 cm x 4.6 mm I.D., 5 µm particles (20124AST)
mobile phase . . (A) methanol; (B) 20 mM ammonium acetate, pH 5.0; (5:95, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . norphenylephrine, 1 mg/mL in acetonitrile:water (50:50)
Application No. . . . . G005159



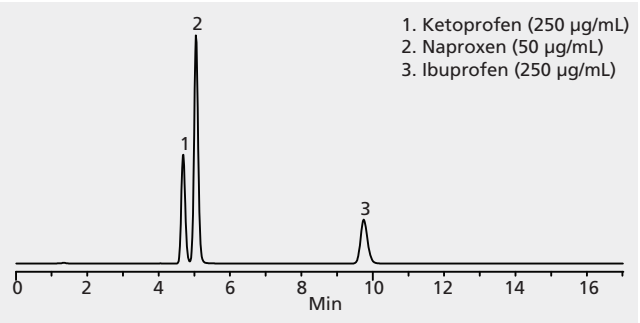
## HPLC Analysis of NSAIDs on Discovery® Zr-Carbon C18

column . . Discovery Zr-Carbon C18, 15 cm × 4.6 mm I.D., 3 µm particles (65706-U)
mobile phase . . (A) 50 mM phosphoric acid (pH 1.75): (B) acetonitrile; (50:50, A:B)
flow rate . . . . . 4 mL/min
column temp. . . . . 80 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . ketoprofen, ibuprofen, naproxen, each 1 mg/mL
Application No. . . . . G001864



## HPLC Analysis of NSAIDs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM dibasic potassium phosphate (pH 3.0): (B) acetonitrile; (45:55, A:B)
flow rate . . . . . 1.0 mL/min.
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . as indicated in 10 mM dibasic potassium phosphate (pH 3.0)
Application No. . . . . G002621



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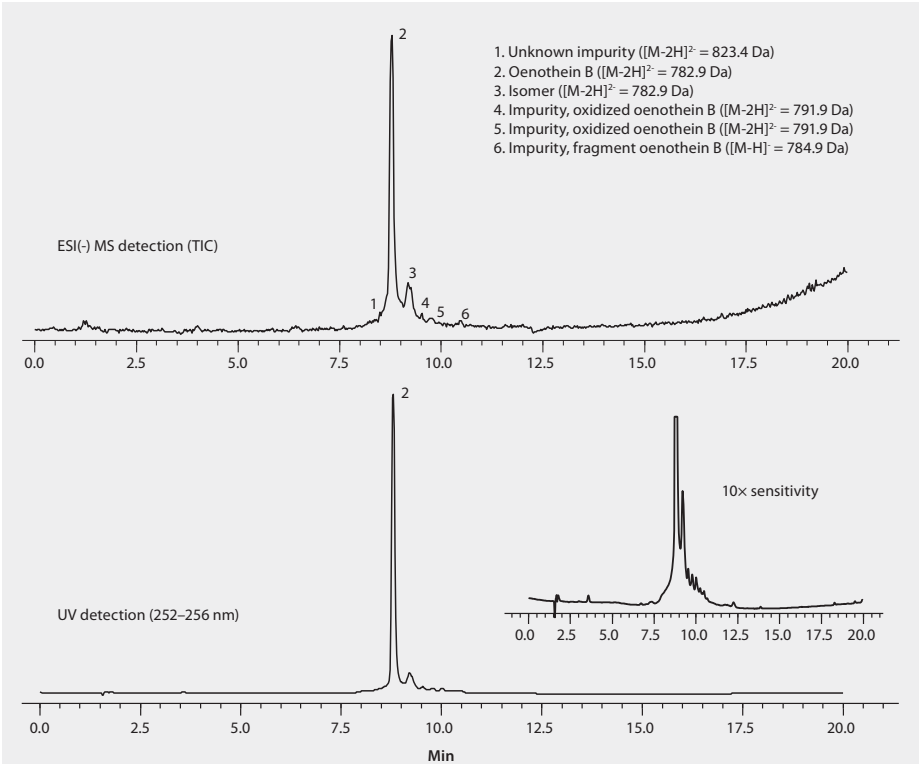
## HPLC Analysis of Oenothein B on Ascentis® Express RP-Amide, UV and MS Detection

Optimization of a published method showing much better retention, peak shape and resolution on Supelco Ascentis Express RP-Amide 2.7 µm compared to a C18 phase. Isomers and small amounts of homologues are resolved and detectable with higher sensitivity.

| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 4.6 mm I.D., 2.7 µm particles (53929-U) |
| mobile phase | (A) 0.2% formic acid; (B) methanol |
| gradient | 5% B for 2 min; to 90% B over 18 min; held at 90% B for 10 min |
| flow rate | 0.6 mL/min |
| column temp. | 35 °C |
| detector | UV, 254 nm |
| detector | MS (ion trap), m/z 100-1500 |
| injection | 2 µL |
| sample | 1 mg/mL in methanol |
| Application No. | G006164 |



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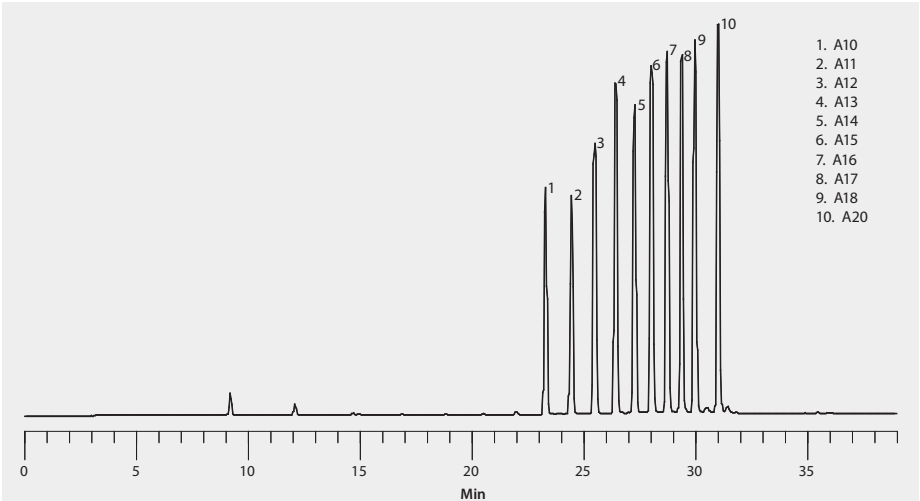
## HPLC Analysis of Oligonucleotides on Proteomix® SAX

column . . . . Proteomix SAX-NP5, 25 cm x 4.6 mm I.D., 5 µm particles (Z777229)
mobile phase . . . . . A: 20 mM Tris, pH 8.0 B: A + 0.5 M NaCl
gradient . . . . . 0 - 100% B in 30 minutes
flow rate . . . . . 0.5 mL/min
pressure . . . . . 1073 psi (74 bar)
column temp. . . . . 25 °C
detector . . . . . UV 260 nm
injection . . . . . 5 µL
sample . . . . . (10 mM each oligonucleotide in water)
Application No. . . . . G005994



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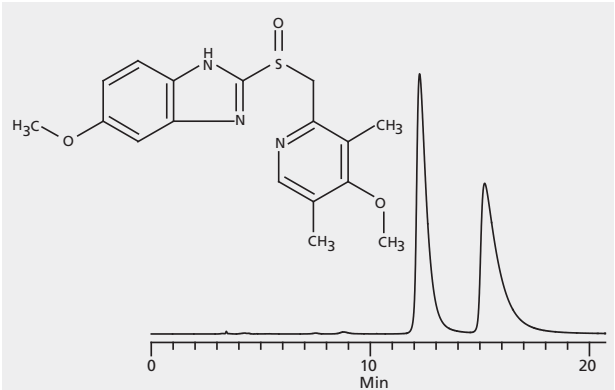
## HPLC Analysis of Omeprazole Enantiomers on Astec® CYCLOBOND® I 2000 DMP

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . . . . (A) acetonitrile; (B) acetic acid; (C) ammonium hydroxide; (100:0.4:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 302 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004685



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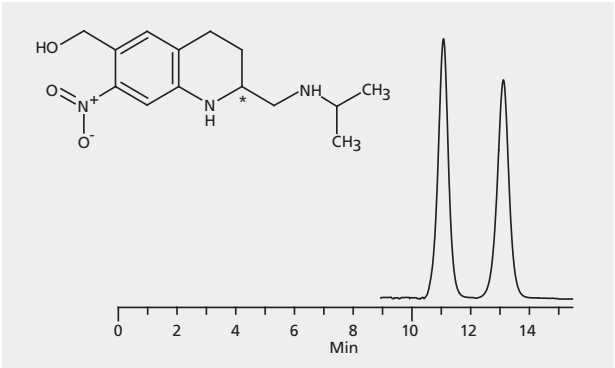
## HPLC Analysis of Oxamniquine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 0.1 wt% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004656



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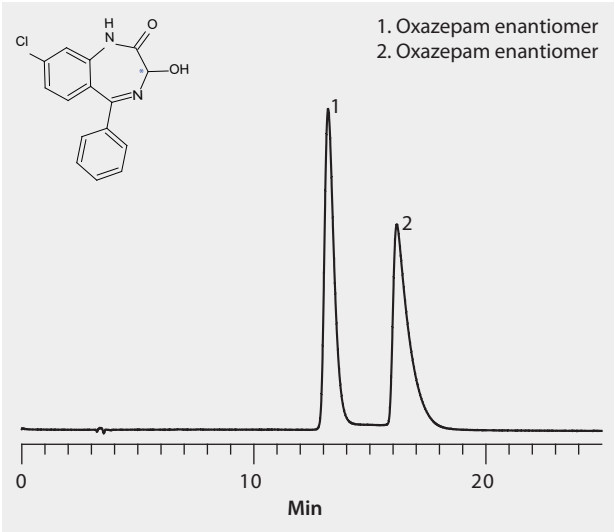
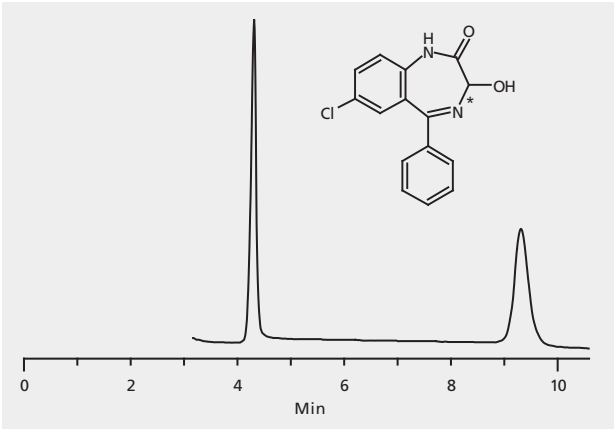
## HPLC Analysis of Oxazepam Enantiomers on Astec® CYCLOBOND® I 2000 DNP

column . . CYCLOBOND I 2000 DNP, 25 cm x 4.6 mm I.D., 5 µm particles (25024AST)
mobile phase . . . . . (A) acetonitrile; (B) 20 mM ammonium phosphate, pH 2.9; (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 5 °C
detector . . . . . UV, 254 nm
injection . . . . . 3 µL
sample . . . . . oxazepam, 1 mg/mL in acetonitrile:water (50:50)
Application No. . . . . G005162



## HPLC Analysis of Oxazepam Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 1 µL
sample gas . . . . . 1 mg/mL in methanol
Application No. . . . . G004545



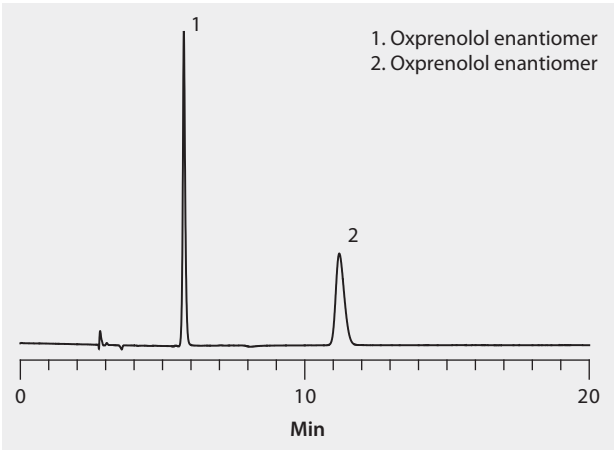



SUPELCO® Solutions within.™

## HPLC Analysis of Oxprenolol Enantiomers on Astec® Cellulose DMP

Oxprenolol enantiomers are resolved by normal phase chromatography. Oxprenolol is a nonselective beta-blocker and is used to treat angina and hypertension.

column . . Astec Cellulose DMP, 25 cm x 4.6 mm I.D., 5 µm particles (51099AST)
mobile phase . . (A) heptane; (B) isopropanol; (C) diethylamine; (80:20:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 15 µL
sample . . . . . oxprenolol, 1 g/L in 50:50 heptane:isopropanol
Application No. . . . . G005522



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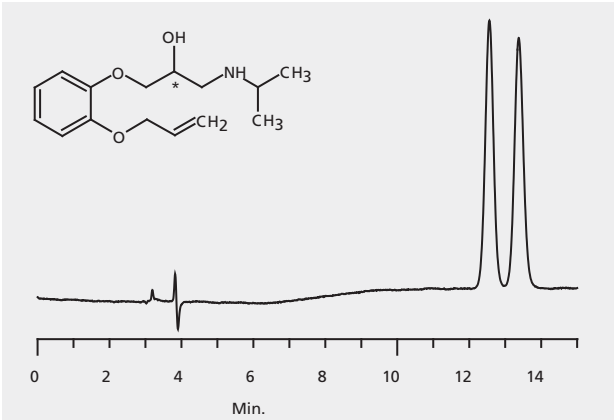
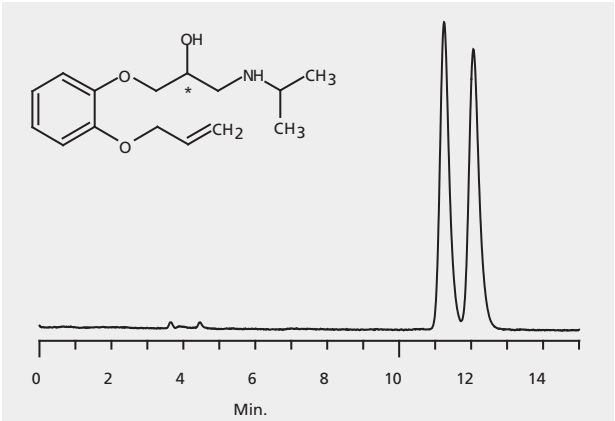
## HPLC Analysis of Oxprenolol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) triethylamine; (54.5:45:0.3:0.2, A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004507



## HPLC Analysis of Oxprenolol Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . . . . 5 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004480





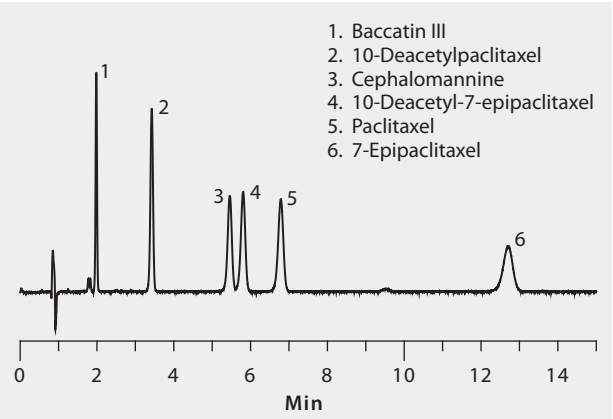

SUPELCO® Solutions within.™

## HPLC Analysis of Paclitaxel and Related Compounds on Ascentis® Express F5 5 µm

An efficient reverse phase HPLC method for the HPLC analysis of of Paclitaxel (Taxol) and related compounds is developed using the Ascentis® Express F5 5µm Fused Core HPLC column. Paclitaxel is a natural product with antitumor activity. TAXOL (paclitaxel) is obtained via a semi-synthetic process from Taxus baccata.

The developed method can be easily used for the routine quality control of bulk and Parenterals dosage form of Paclitaxel within a short analysis time. This method offers the advantages of higher efficies, reduced backpressure and shorter run times over a traditionally run methods over 5 µm totally porous particle columns.

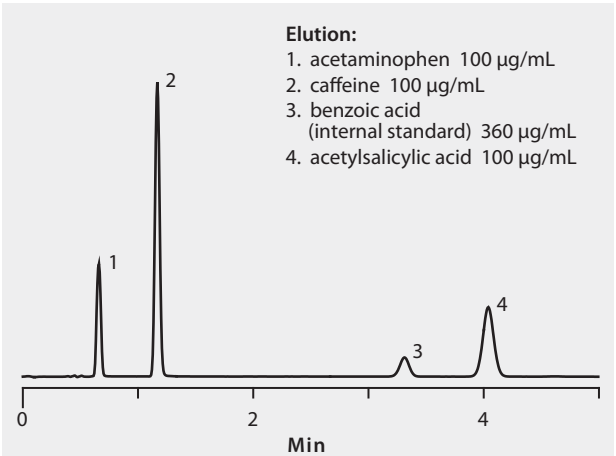
column . . . . Ascentis Express F5, 15 cm x 4.6 mm I.D., 5 µm particles (50631-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 1.5 mL/min
pressure . . . . . 2727 psi (188 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 227 nm
injection . . . . . 5 µL
sample . . . . . taxols 25 µg/mL in 70:15:15, water:methanol:acetonitrile
Application No. . . . . G005890



## HPLC Analysis of Pain Relievers on Ascentis® Express C18 5µm

This application shows the improvements in efficiency that may be obtained for USP methods using modern Fused-Core technology.

column . . . Ascentis Express C18, 10 cm x 4.6 mm I.D., 5 µm particles (50536-U)
mobile phase . . . . . (A) water with 3% acetic acid;
(B) methanol with 3% acetic acid; (79:21, A:B)
flow rate . . . . . 2.0 mL/min
pressure . . . . . 3290 psi (227 bar)
column temp. . . . . 45 °C
detector . . . . . UV,275 nm
injection . . . . . 10 µL
sample . . . . . 100 - 360 µg/mLin mobile phase
Application No. . . . . G005894



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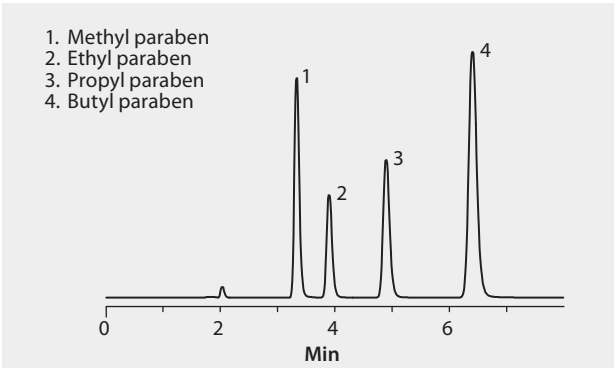
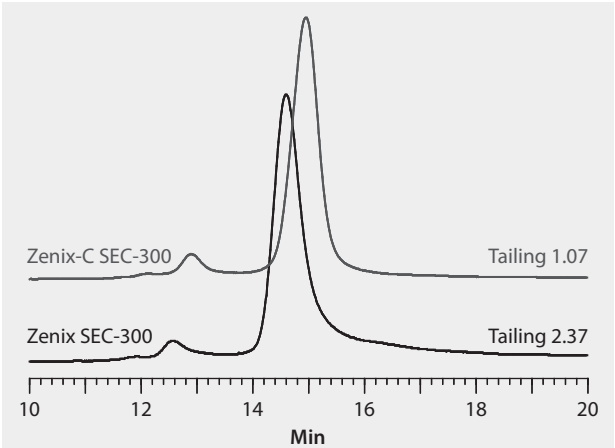
## HPLC Analysis of Paraben Preservatives on Discovery® Cyano

column . . . . . Discovery Cyano, 15 cm × 4.6 mm I.D., 5 µm particles (59356-U)
mobile phase . . . . . (A) acetonitrile; (B) water; (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . G001237



## HPLC Analysis of Pegylated Exenatide on Zenix® SEC-300 and Zenix®-C SEC-300

column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm, I.D., 3 µm particles (Z777092)
column . . . . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777033)
mobile phase . . (A) 50 mM Ammonium acetate: (B) acetonitrile; (90:10, A:B, v/v)
flow rate . . . . . 0.5 mL/min
pressure . . . . . 609 psi (42 bar)
column temp. . . . . 25 °C
detector . . . . . UV, 214 nm
injection . . . . . 15 µL
sample . . . . . 3.3 mg/ml PEG-Exanatide in water (PEG 23 kD)
Application No. . . . . G005949



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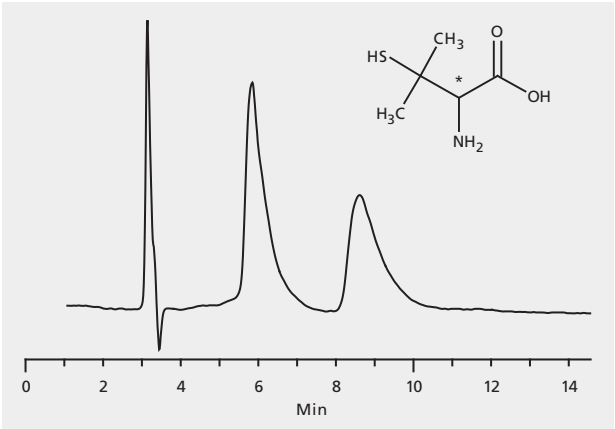
## HPLC Analysis of Penicillamine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) 0.1% triethylamine acetate, pH 3.8; (B) ethanol; (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
injection . . . . . 5 µL
detector . . . . . UV, 225 nm
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004549



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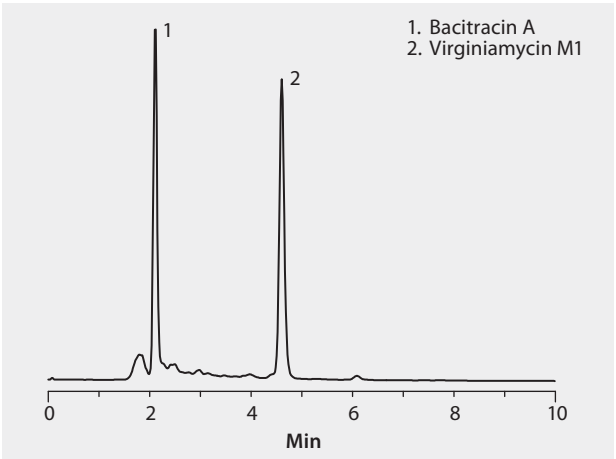
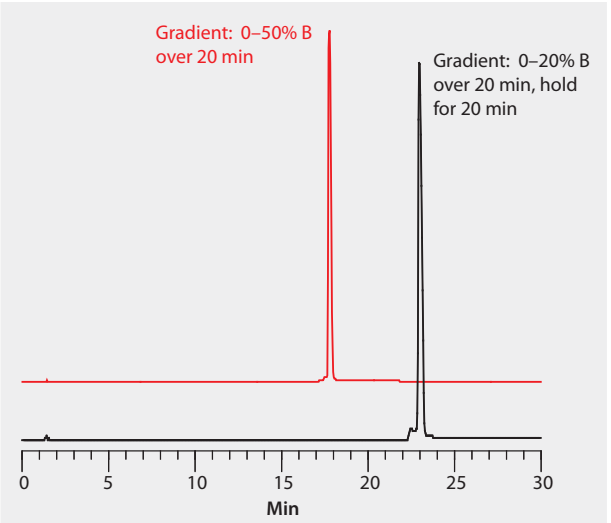
## HPLC Analysis of Peptide Antibiotics on Discovery® C8

column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (59353)
mobile phase . . (A) 25mM potassium phosphate, pH 3; (B) acetonitrile, (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL 10 µL
Application No. . . . . G000911B



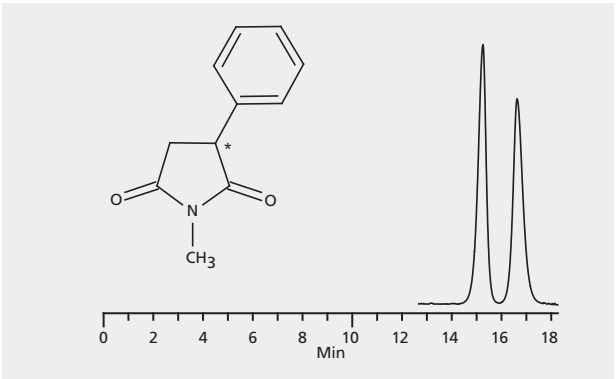
## HPLC Analysis of a Peptide Drug on Antibodix® WCX-NP10, Gradient Optimization to Separate Impurity

column . . Antibodix WCX-NP10, 25 cm x 4.6 mm I.D., 10 µm particles (Z777272)
mobile phase . . . . . (A) 3 mM potassium phosphate, pH 5.0; (B) 20 mM potassium phosphate, pH 7.5
gradient . . (A) 0% - 50% B (20 min); (B) 0% - 20% B (20 min), 20% B after 20 min
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . .
injection . . . . . 5 µL
sample . . . . . 2 mg/mL, proprietary peptide drug, pI 7-8
Application No. . . . . G005932



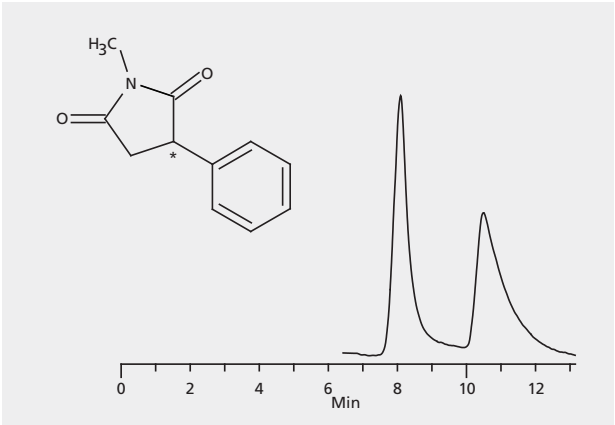
## HPLC Analysis of Phensuximide Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) hexane; (B) ethanol; (70:30, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 4 mg/mL in methanol
Application No. . . . . G004645



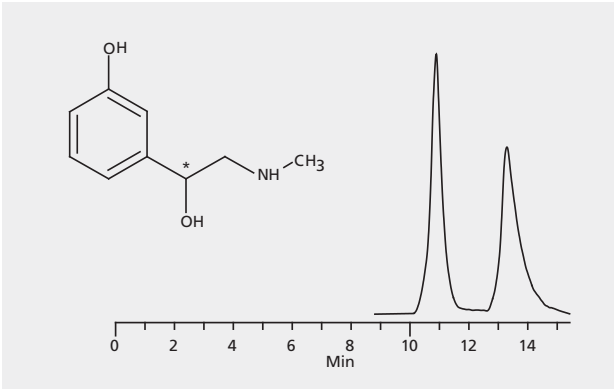
## HPLC Analysis of Phensuximide Enantiomers on Astec® CYCLOBOND® I 2000 DMP

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . . . . (A) isopropanol; (B) hexane; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 4 mg/mL in methanol
Application No. . . . . G004699



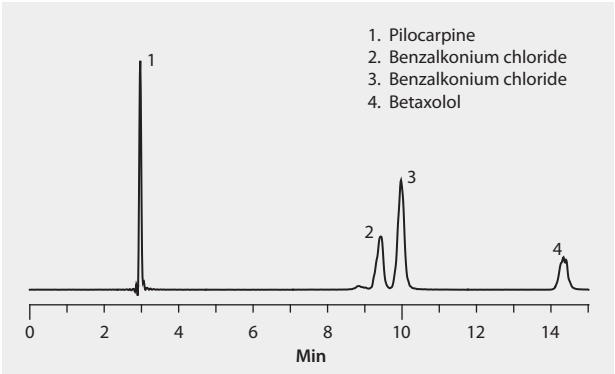
## HPLC Analysis of Phenylephrine Enantiomers on Astec® CYCLOBOND® I 2000 AC

column . . CYCLOBOND I 2000 AC, 25 cm x 4.6 mm I.D., 5 µm particles (20124AST)
mobile phase . . (A) 0.5% w/v sodium acetate, pH 5.5; (B) methanol; (90:10, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004694



## HPLC Analysis of Pilocarpine, Betaxolol, and Benzalkonium Chloride on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . . . . (A) 20 mM ammonium acetate; (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . ESI (+), XIC m/z 304, 332 (BAC), 209 (pilocarpine), 308 (betaxolol)
injection . . . . . 2 µL
sample . . . . . 10 mg/L in acetonitrile
Application No. . . . . G005412



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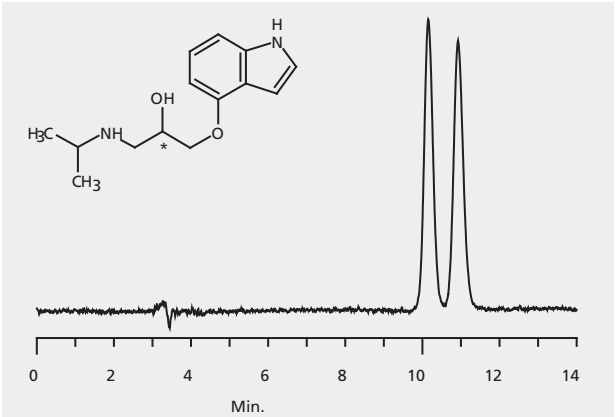
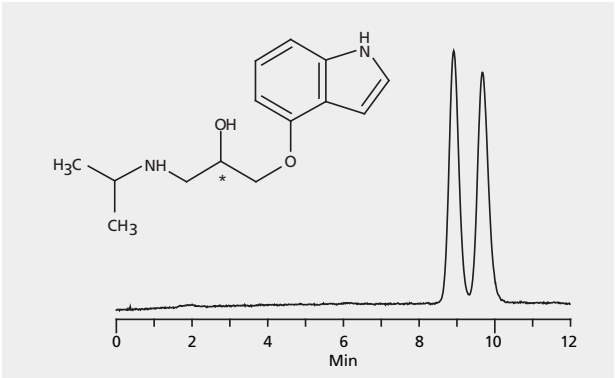
## HPLC Analysis of Pindolol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample gas . . . . . 1 mg/mL in methanol
Application No. . . . . G004509



## HPLC Analysis of Pindolol Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.98:0.01:0.01, A:B:C)
flow rate . . . . . 2 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004634



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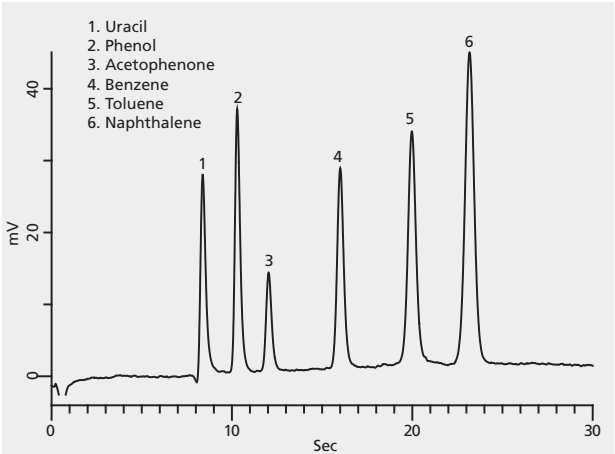
## HPLC Analysis of Polar and Non-Polar Analytes on Ascentis® Express C18, Column Test Mixture

column . . Ascentis Express C18, 5 cm × 3.0 mm I.D. 2.7 µm particles (53811-U)
mobile phase . . . . . 80% acetonitrile
flow rate . . . . . 1.2 mL/min
pressure . . . . . 3700 psi (255 bar)
column temp. . . . . 35 ° C
Application No. . . . . G004158



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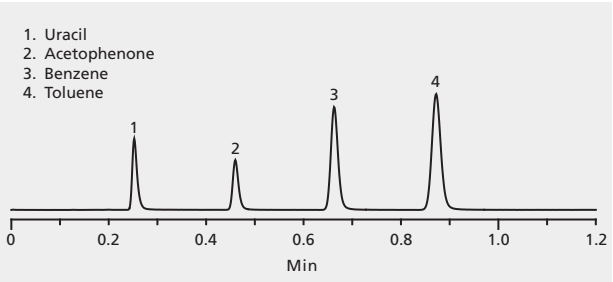
## HPLC Analysis of Polar and Non-Polar Analytes on Ascentis® Express C8, Column Test Mixture

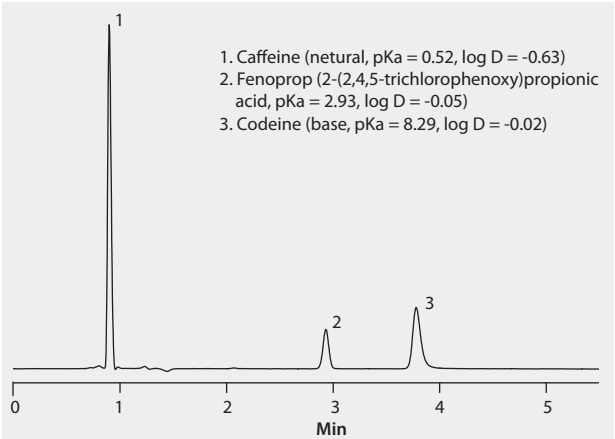
column . . . Ascentis Express C8, 5 cm × 4.6 mm I.D., 2.7 µm particles (58336-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (45:55, A:B)
flow rate . . . . . 1.76 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
Application No. . . . . G003954



## HPLC Analysis of Polar Compounds on Ascentis® Express OH5 (HILIC Mode)

The impact of partitioning and ion exchange in HILIC separations is demonstrated using a mixture of acidic, neutral, and basic polar compounds in this Figure. All three analytes have similar log D values, both acid and base are ionized at mobile phase pH.

column . . . Ascentis Express OH5, 10 cm x 3 mm I.D., 2.7 µm particles (53769-U)
mobile phase . . . . . 5 mM ammonium formate (95:5 acetonitrile:water) pH 6.8
flow rate . . . . . 0.6 mL/min
pressure . . . . . 965 psi (66.5 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 100 µg/mL in 25:75, water:methanol
Application No. . . . . G005802



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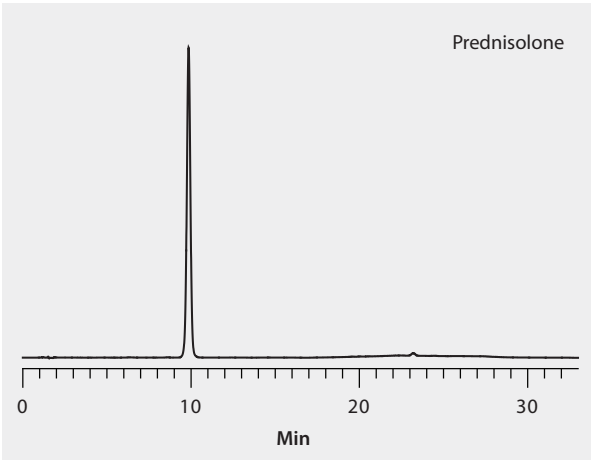
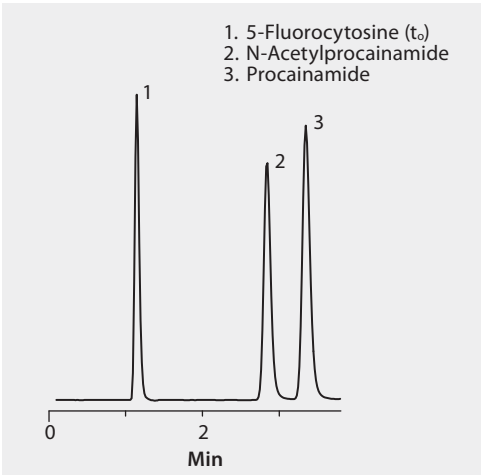
## HPLC Analysis of Prednisolone (Fluka Secondary Pharma Standard) on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) water; (B) methanol:acetonitrile (50:50, A:B)
gradient . . . . . 40% B for 14 min; to 80% B in 6 min; held at 80% B for 5 min
flow rate . . . . . 1 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 125 µg/mL in water:acetonitrile (60:40)
Application No. . . . . G005589



## HPLC Analysis of Procainamide and N-Acetylprocainamide on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . (A) 25 mM ammonium phosphate (pH 7): (B) acetonitrile; (65:35, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 280 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL
Application No. . . . . G001686



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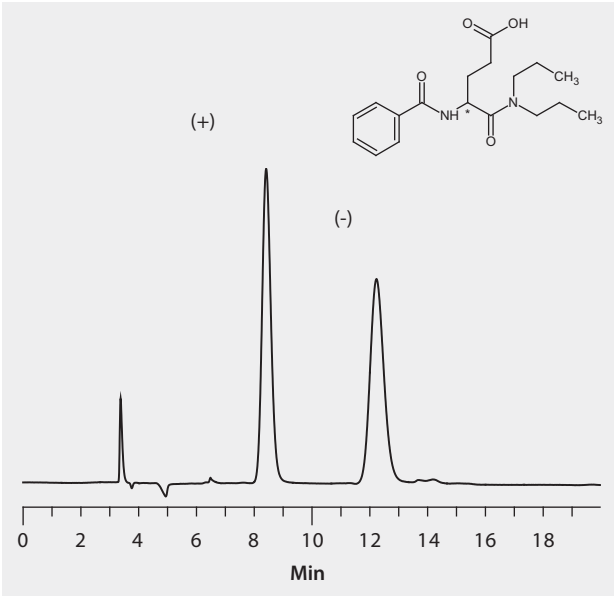
## HPLC Analysis of Proglumide Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) IPA; (C) TFA; (90:10:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G004994



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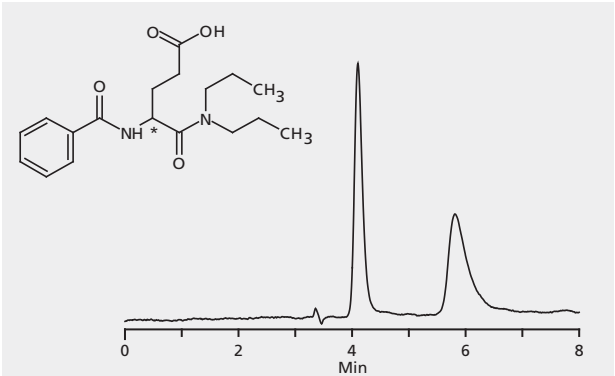
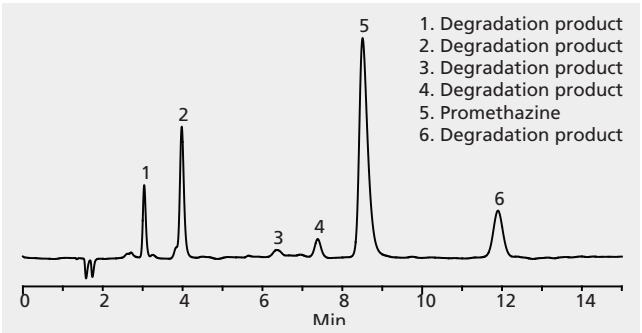
## HPLC Analysis of Proglumide Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 13 mM ammonium acetate; (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 225 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004635



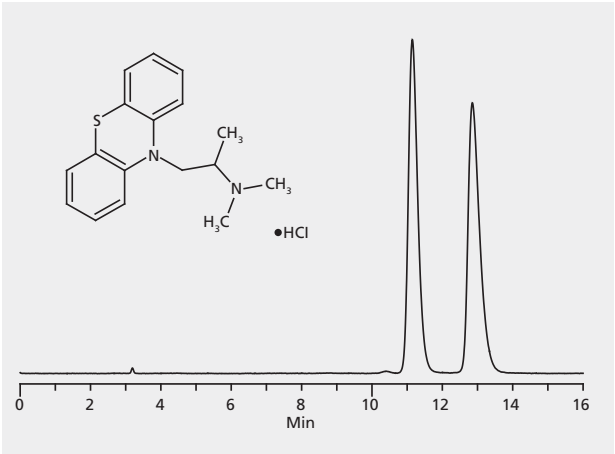
## HPLC Analysis of Promethazine and Degradants on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 0.1% ammonium acetate: (B) methanol; (25:75, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 20:80, water:methanol
Application No. . . . . G003700



## HPLC Analysis of Promethazine Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.8:0.1:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in mobile phase
Application No. . . . . G004404



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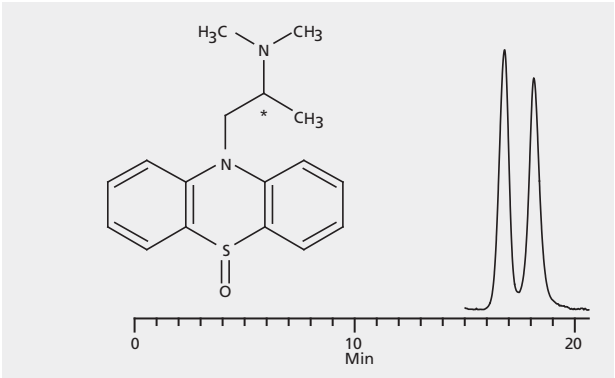
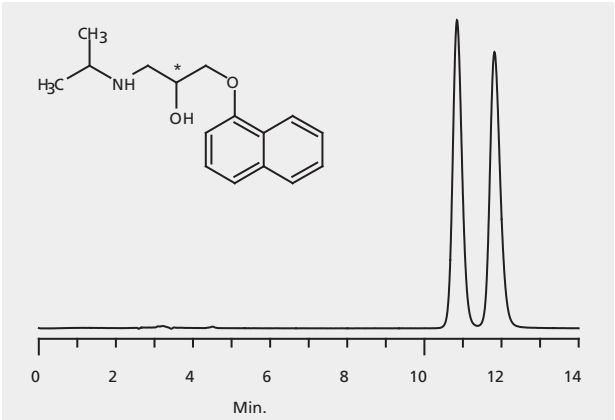
## HPLC Analysis of Promethazine Sulfoxide Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (100:0.1:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 240 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004646



## HPLC Analysis of Propranolol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004510

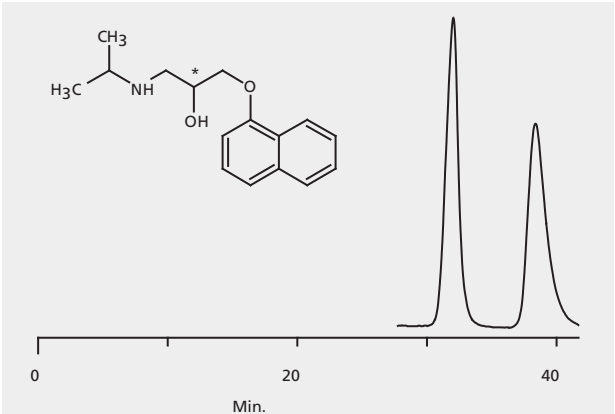





SUPELCO® Solutions within.™

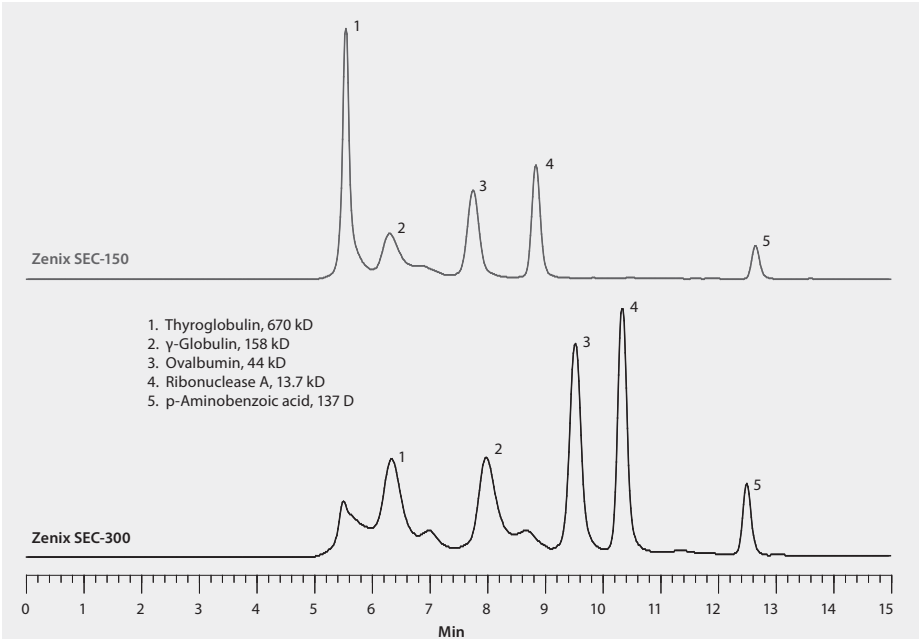
## HPLC Analysis of Propranolol Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . . . . 0.1 wt% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 0.5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004616



## HPLC Analysis of a Protein Mixture on Zenix® SEC-150 versus Zenix® SEC-300, Effect of Pore Size on Resolution

column . . Zenix SEC-150, 30 cm x 7.8 mm I.D., 3 µm, 150 Å particles (Z777018)
column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777033)
mobile phase . . . . . 150 mM sodium phosphate, pH 7
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 10 µL
sample . . . . . protein mixture
Application No. . . . . G006118

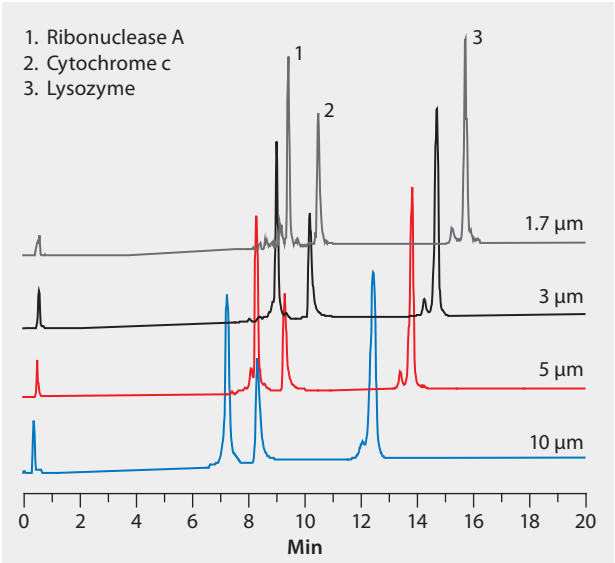





SUPELCO® Solutions within.™

## HPLC Analysis of Proteins on Proteomix® SCX Columns, Effect of Particle Size of Resolution

column . . Proteomix SCX-NP1.7, 5 cm x 4.6 mm I.D., 1.7 µm particles (Z777146)
column . . . . . Proteomix SCX-NP3, 5 cm x 4.6 mm I.D., 3 µm particles (Z777151)
mobile phase . . (A) 20 mM sodium phosphate buffer, pH 6.0, (B) A + 1.0 M NaCl
gradient . . . . . 0-25 min from 0-75% B, 15 min prewash
flow rate . . . . . 0.75 mL/min
column temp. . . . . Ambient
detector . . . . . 214 nm
injection . . . . . 5 µL
sample . . . . . each 1 mg/mL
Application No. . . . . G006134

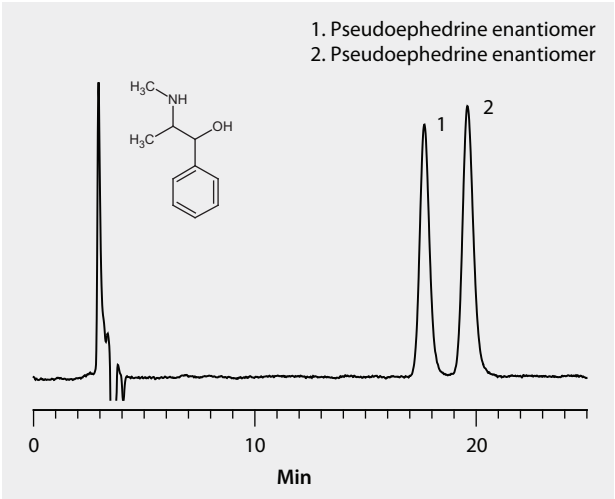





SUPELCO® Solutions within.™

## HPLC Analysis of Pseudoephedrine Enantiomers on Astec® CHIROBIOTIC® TAG

column . . . . CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles (14024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine;(99.8:0.1:0.1, A:B:C)
flow rate . . . . . 1 mL/min
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 200 µg/mL in methanol
column temp. . . . . 25 °C
Application No. . . . . G005350

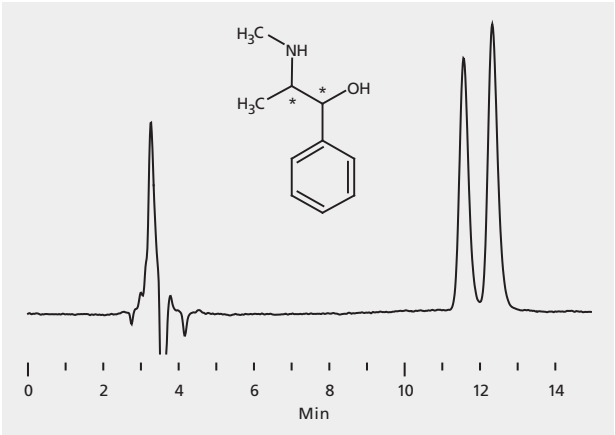





SUPELCO® Solutions within.™

## HPLC Analysis of Pseudoephedrine Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (100:0.1:0.1, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004530

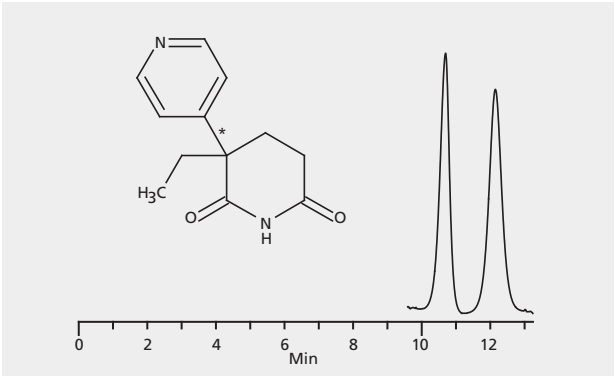





SUPELCO® Solutions within.™

## HPLC Analysis of Pyroglutethimide Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) 0.1% triethylamine acetate, pH 7.0; (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 3 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G004647

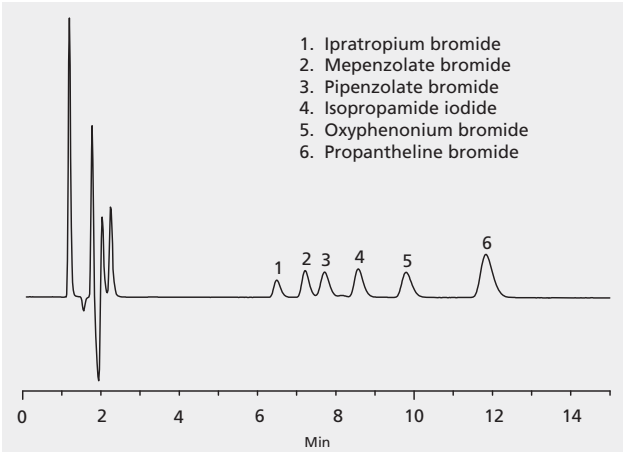





SUPELCO® Solutions within.™

## HPLC Analysis of Quaternary Ammonium Drugs on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . 10 mM ammonium acetate in 80% acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 60 °C
detector . . . . . UV, 225 nm
injection . . . . . 10 µL
sample . . . . . 50 mg/mL in 70:30, 10 mM ammonium acetate in 80% acetonitrile:methanol
Application No. . . . . G002221

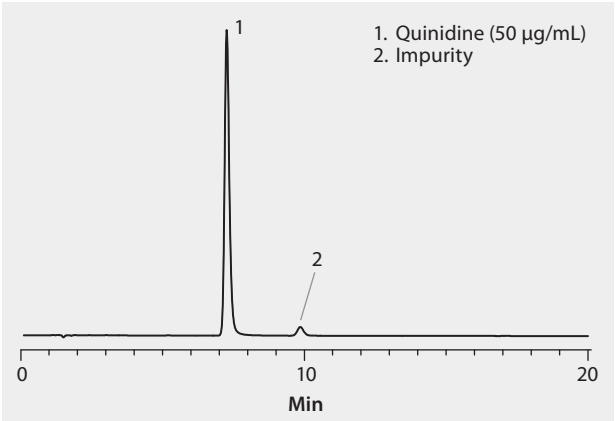





SUPELCO® Solutions within.™

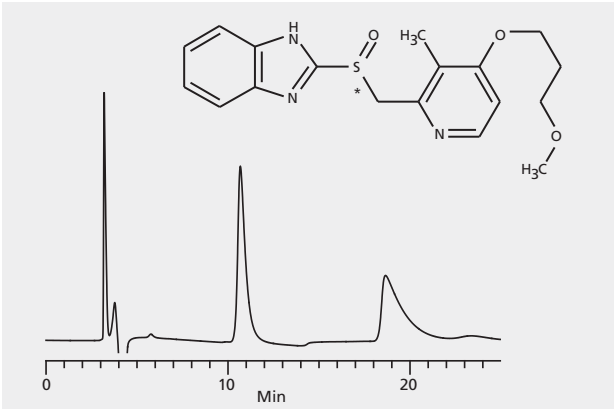
## HPLC Analysis of Quinidine and Trace Impurity on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . . . . (A) 25 mM ammonium phosphate (pH 7.0): (B) acetonitrile, (35:65, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 235 nm
injection . . . . . 10 µL
Application No. . . . . G001666



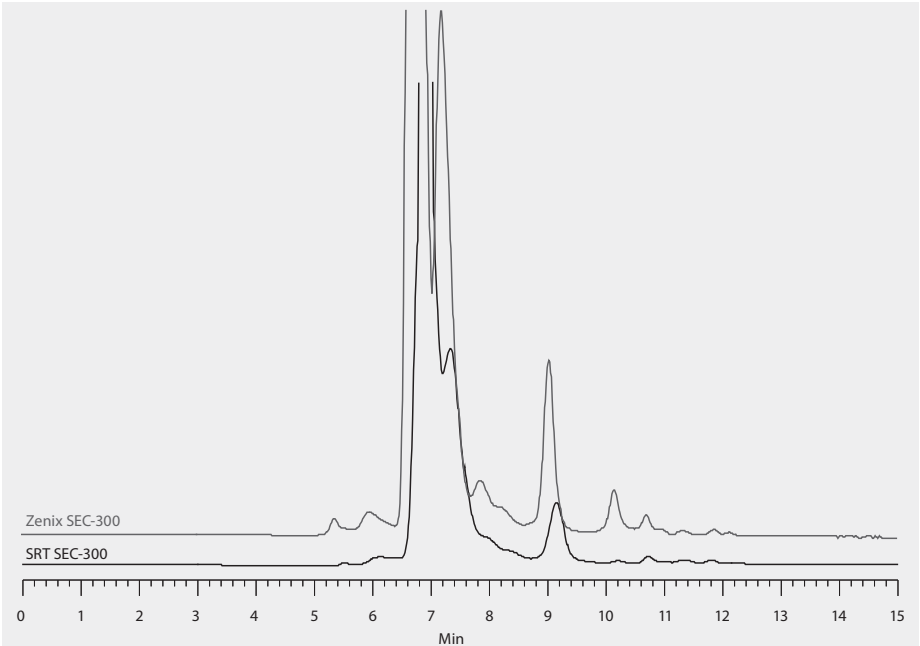
## HPLC Analysis of Rabeprazole Enantiomers on Astec® CYCLOBOND® I 2000 DMP

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (C) acetic acid; (D) ammonium hydroxide; (95:5:0.4:0.1, A:B:C:D)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004686



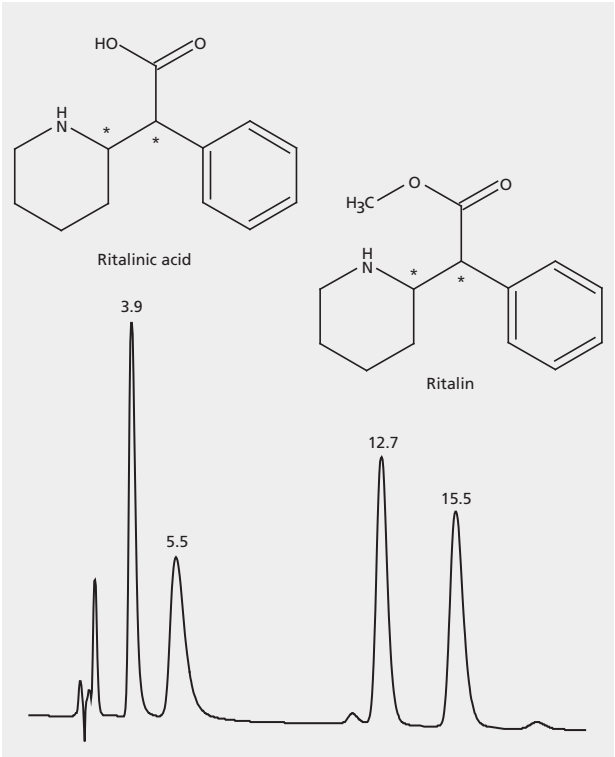
## HPLC Analysis of Recombinant Tumor Necrosis Factor Receptor-Fc Fusion Protein (rhTNFR-Fc) on Zenix® SEC-300 versus SRT® SEC-300, Effect of Particle Size on Resolution

column . . Zenix SEC-300, 30 cm x 7.8 mm I.D., 3 µm, 300 Å particles (Z777051)
column . . . . . SRT SEC-300, 30 cm x 7.8 mm I.D., 5 µm, 300 Å particles (Z777051) (Z777033)
mobile phase . . . . . 150 mM sodium phosphate, pH 7.0
flow rate . . . . . 1 mL/min
detector . . . . . UV, 214 nm
injection . . . . . 20 µL
sample . . . . . Recombinant tumor necrosis factor receptor-Fc fusion protein (rhTNFR-Fc), including oligomers (440 kD and 360 kD), monomer (150 kD), and impurity (120 kD)
Application No. . . . . G006121



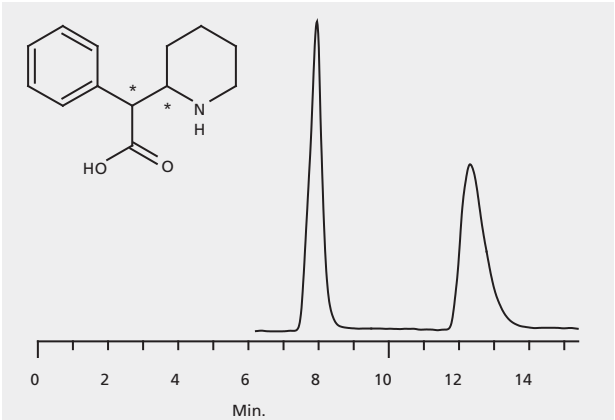
## HPLC Analysis of Ritalinic Acid and Ritalin (Methylphenidate) Enantiomers on Astec® CHIROBIOTIC® V2 with T2 Guard Column

column . . . . . CHIROBIOTIC V2, 15 cm x 4.6 mm I.D., 5 µm particles (15023AST)
column . . . . . CHIROBIOTIC T2 guard column, 2 cm x 4 mm I.D., 5 µm particles (16100AST (requires holder 21150AST))
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) methanol; (7:93, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004733



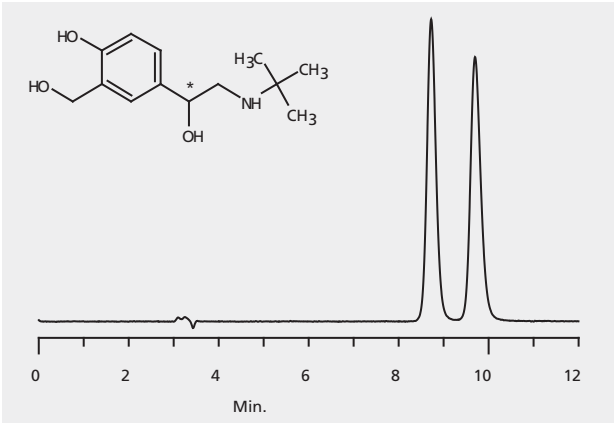
## HPLC Analysis of Ritalinic Acid Enantiomers on Astec® CHIROBIOTIC® T2

column . . . . . CHIROBIOTIC T2, 25 cm x 4.6 mm I.D., 5 µm particles (16024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 5.5; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.9 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004600



## HPLC Analysis of Salbutamol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004512

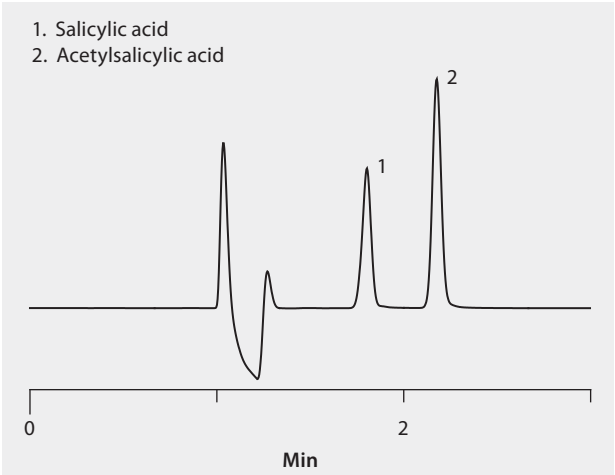





SUPELCO® Solutions within.™

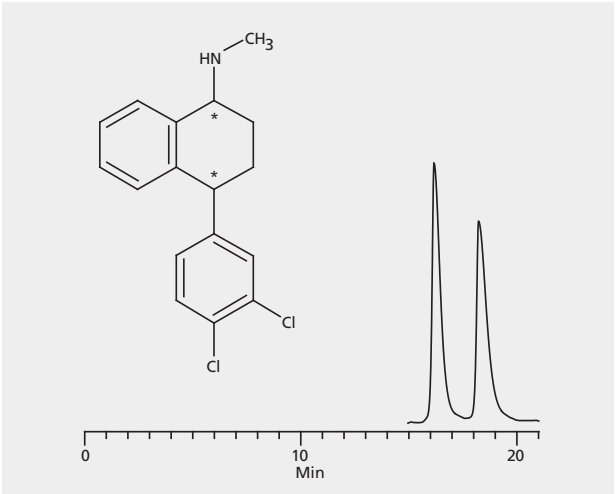
## HPLC Analysis of Salicylic Acid and Acetylsalicylic Acid (Aspirin) on Ascentis® Express OH5

column . . Ascentis Express OH5, 10 cm x 3.0 mm I.D., 2.7 µm particles (53769-U)
mobile phase . . . . . 2 mM ammonium acetate in 10:90 water:acetonitrile, pH to 7.0 with acetic acid, glacial
flow rate . . . . . 0.5 mL/min
pressure . . . . . 850 psi (59 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 235 nm
injection . . . . . 2 µL
sample . . . . . 100 µg/mL salicylic acid, 200 µg/mL acetylsalicylic acid in methanol:acetonitrile (3:7)
Application No. . . . . G006124



## HPLC Analysis of Sertraline Enantiomers on Astec® CYCLOBOND® I 2000 RSP

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.1; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004705



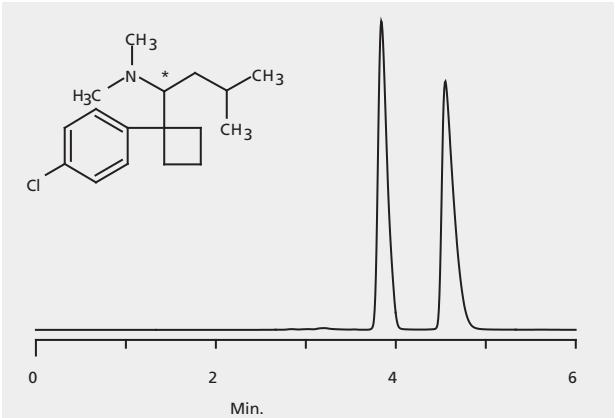



SUPELCO® Solutions within.™

## HPLC Analysis of Sibutramine Enantiomers on Astec® CHIROBIOTIC® V

Sibutramine, one of a few drugs approved for long-term treatment of obesity, is a chiral compound. The (R) enantiomer has the desired effect of reducing body weight and food intake, but the (S) enantiomer has the opposite effect: A perfect example of why chiral HPLC is important to pharmaceutical and clinical analysts. Astec® CHIROBIOTIC® V successfully separates sibutramine enantiomers, using a simple, MS-friendly mobile phase.

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004479



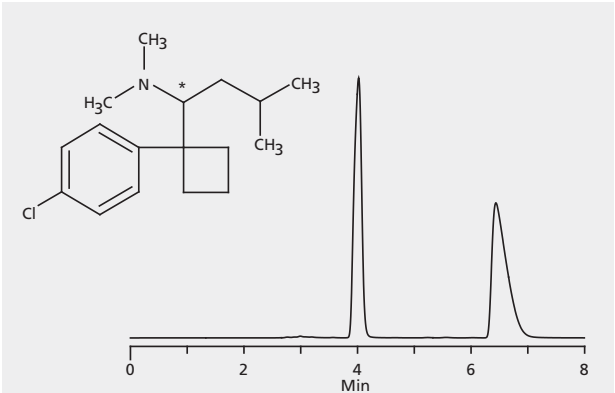



SUPELCO® Solutions within.™

## HPLC Analysis of Sibutramine Enantiomers on Astec® CHIROBIOTIC® V2

Sibutramine, one of a few drugs approved for long-term treatment of obesity, is a chiral compound. The (R) enantiomer has the desired effect of reducing body weight and food intake, but the (S) enantiomer has the opposite effect: A perfect example of why chiral HPLC is important to pharmaceutical and clinical analysts. Astec® CHIROBIOTIC® V2 successfully separates sibutramine enantiomers, using a simple, MS-friendly mobile phase.

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004661

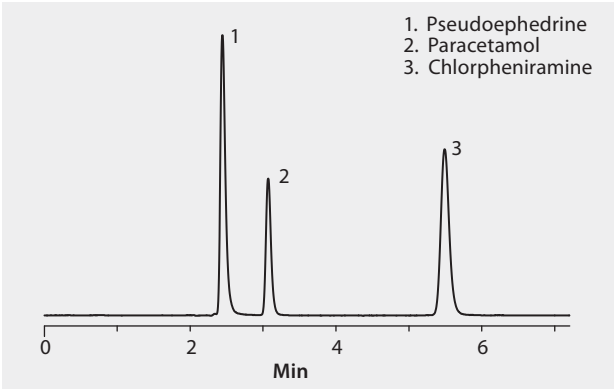





SUPELCO® Solutions within.™

## HPLC Analysis of Sinus Medications on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) acetonitrile: (B) 50 mM $KH_2PO_4$, pH 3.0 (25:75, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G000087

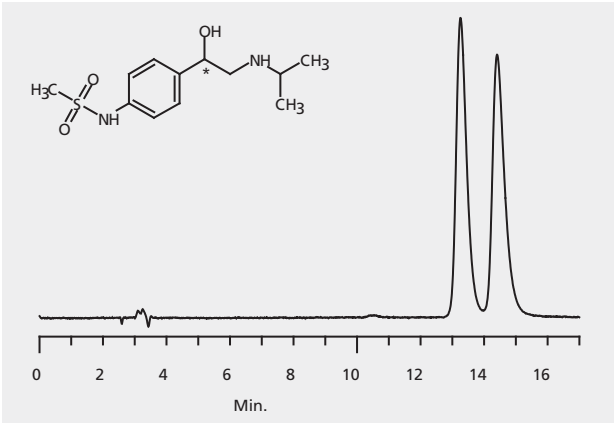





SUPELCO® Solutions within.™

## HPLC Analysis of Sotalol Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004514

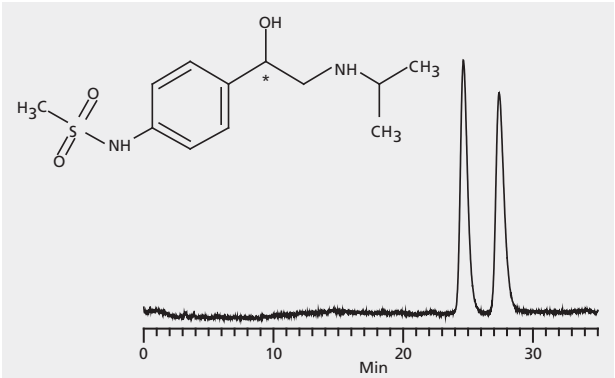





SUPELCO® Solutions within.™

## HPLC Analysis of Sotalol Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) triethylamine; (99.98:0.01:0.01, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004636

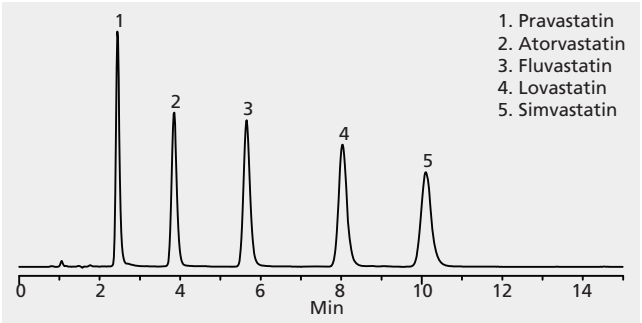





SUPELCO® Solutions within.™

## HPLC Analysis of Statins on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . (A) water with 0.1% formic acid (34673): (B) methanol; (25:75, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in mobile phase
Application No. . . . . G003270

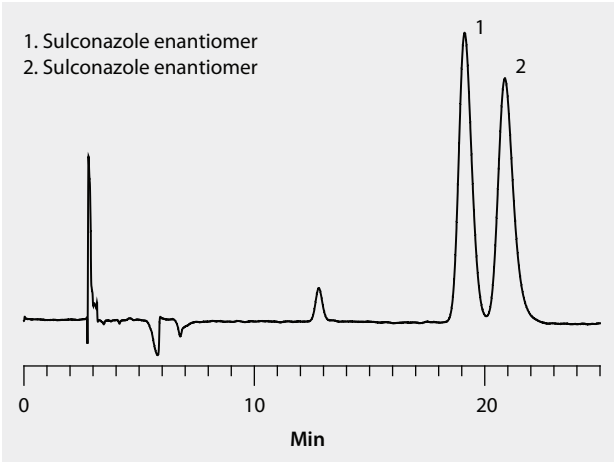





SUPELCO® Solutions within.™

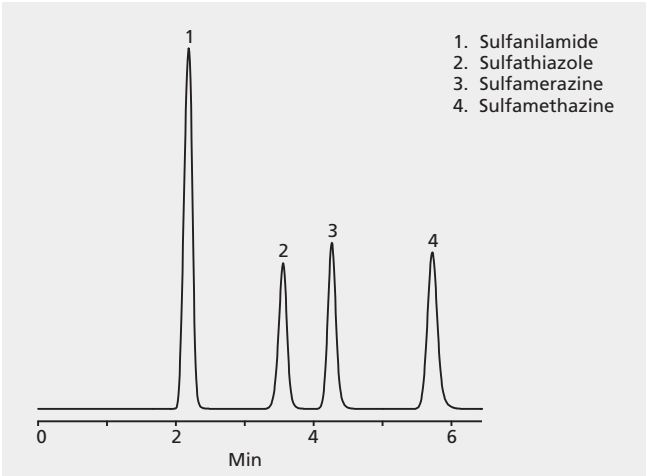
## HPLC Analysis of Sulconazole Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 25 cm x 4.6 mm I.D., 5 µm particles (51099AST)
mobile phase . . . . . (A) heptane; (B) isopropanol; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 15 µL
sample . . . . . 1 g/L in 50:50, heptane:isopropanol
Application No. . . . . G005468



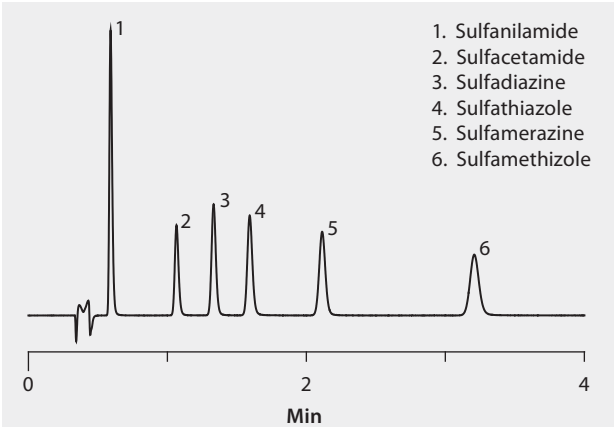
## HPLC Analysis of Sulfa Drugs on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) methanol; (B) water with 1% acetic acid (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
Application No. . . . . 797-0655



## HPLC Analysis of Sulfa Drugs on Titan™ C18

column . . . . . Titan C18 5 cm x 2.1 mm I.D., 1.9 µm particle (577122-U)
mobile phase . . . . . (A) water with 0.1% acetic acid; (B) acetonitrile with 0.1% acetic acid ; (90:10, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 2670 psi (184 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 250 nm
injection . . . . . 2 µL
sample . . . . . 25 µg/mL in 70:30, water:methanol
Application No. . . . . G006114

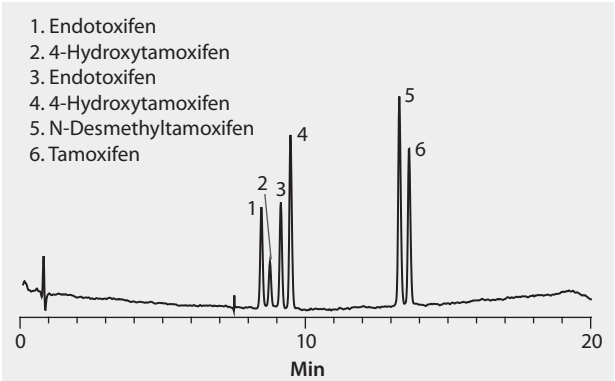





SUPELCO® Solutions within.™

## HPLC Analysis of Tamoxifen E/Z isomers and Related Compounds on Ascentis® Express RP-Amide

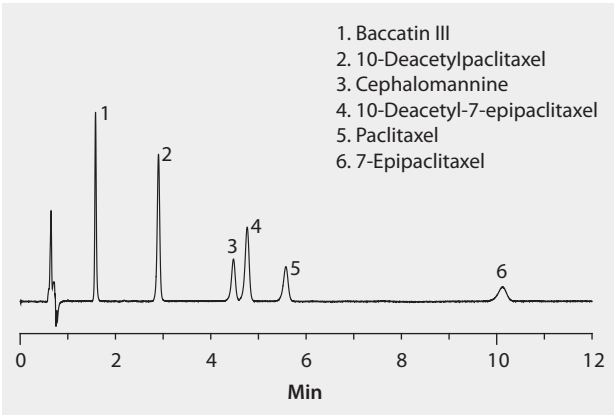
column . . Ascentis Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile
gradient . . . . . held at 25% B for 2 min; 25 to 40% B in 15 min; 40 to 25% B in 0.1 min; held at 25% B for 2.9 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 2292 psi (158 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 260 nm
injection . . . . . 2 µL
sample . . . . . 10 mg/L in 10:90, water: methanol
Application No. . . . . G005748



## HPLC Analysis of Taxols on Ascentis® Express F5

This application demonstrates the suitability of Ascentis Express F5 for the efficient separation of taxols.

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 227 nm
injection . . . . . 2 µL
sample . . . . . 25 mg/L in 70:30, water:methanol
Application No. . . . . G005749



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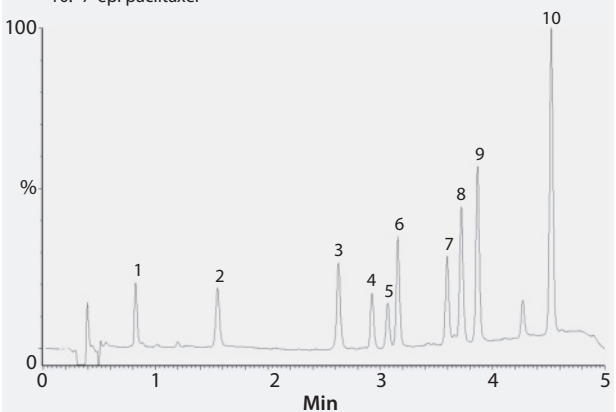
## HPLC Analysis of Taxols on Discovery® HS F5

column . . . . Discovery HS F5, 15 cm × 2.1 mm I.D., 3 µm particles (567503-U)
mobile phase . . (A) 10 mM ammonium acetate (pH 6.8 unadjusted); (B) acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . ambient
detector . . . . . UV, 227 nm
injection . . . . . 10 µL
sample . . . . . 10 µg/mL (in 50:50, water:acetonitrile)
Application No. . . . . G002150

1. 10-deacetyl baccatin III
2. Baccatin III
3. 10-deacetyl-7-xylosyl-β-paclitaxel
4. 10-deacetyl-7-β-xylosyl paclitaxel C
5. 10-deacetyl paclitaxel
6. 7-β-xylosyl paclitaxel
7. Cephalomannine
8. 10-deacetyl-7-epi paclitaxel
9. Paclitaxel
10. 7-epi paclitaxel

| Gradient Program | | |
|---|---|---|
| Time (min) | %A | %B |
| 0 | 65 | 35 |
| 5 | 65 | 35 |
| 20 | 55 | 45 |
| 23 | 55 | 45 |
| 25 | 65 | 35 |



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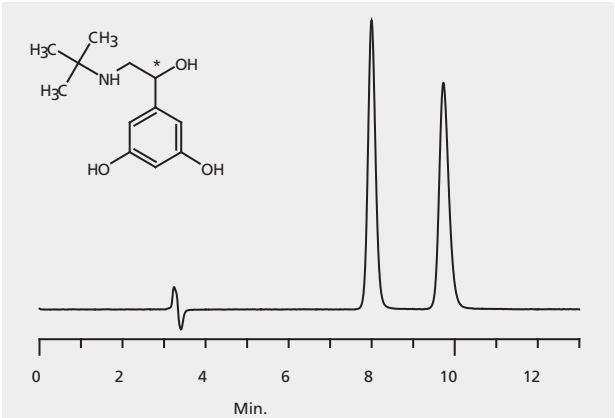
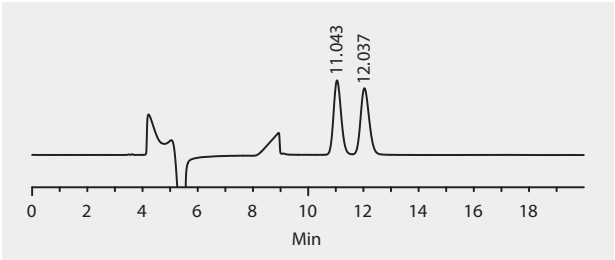
## HPLC Analysis of Terbutaline Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . . . 15 mM ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004515



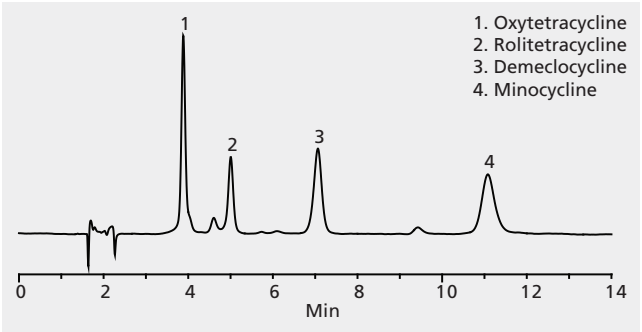
## HPLC Analysis of Terfenadine Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) heptane; (B) ethanol; (C) diethylamine; (98:2:0.1, A:B:C)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 230 nm
injection . . . . . 3 µL
sample . . . . . 1 g/L in methanol
Application No. . . . . G005465



## HPLC Analysis of Tetracycline Antibiotics on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 0.1% ammonium acetate; (B) acetonitrile with 0.1% ammonium acetate; (80:20, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 353 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in 80:20, water:methanol
Application No. . . . . G003715



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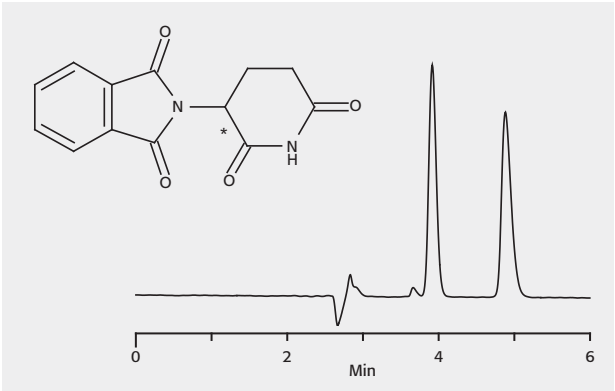
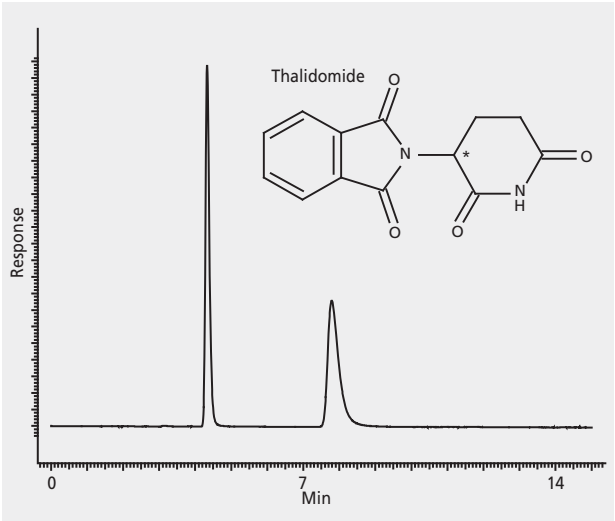
## HPLC Analysis of Thalidomide Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) 18 mM ammonium nitrate, pH 5.5; (B) acetonitrile; (65:35, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 22 °C
detector . . . . . UV, 240 nm
injection . . . . . 20 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004653



## HPLC Analysis of Thalidomide Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 µL
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004473



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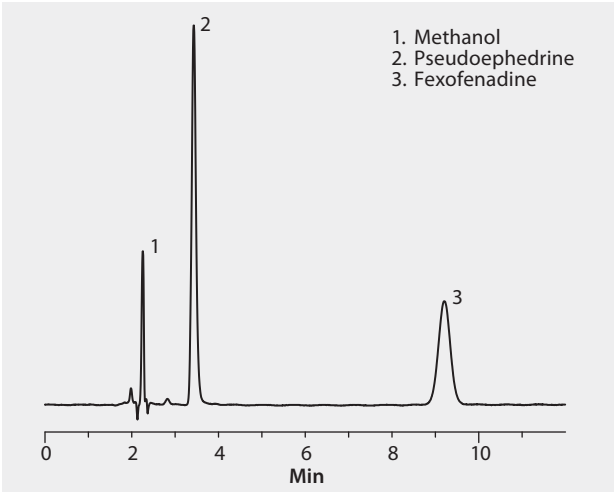
## HPLC Analysis of Allegra® on Discovery® Cyano

column . . . . . Discovery Cyano, 15 cm × 4.6 mm I.D., 5 µm particles (59356-U)
mobile phase . . . . . acetonitrile:25 mM $KH_2PO_4$, pH 7.0 (15:85)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
Application No. . . . . G001279



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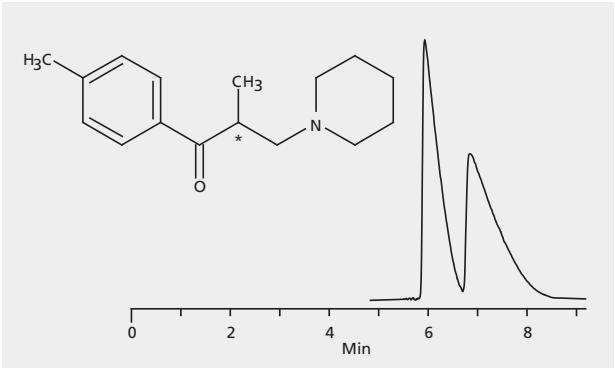
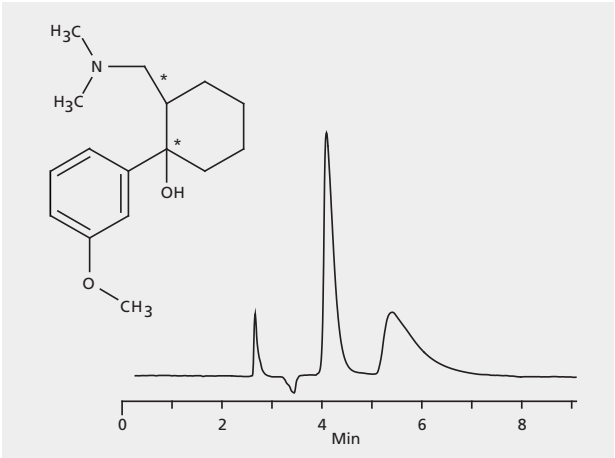
## HPLC Analysis of Tolperisone Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 0.1% ammonium formate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004657



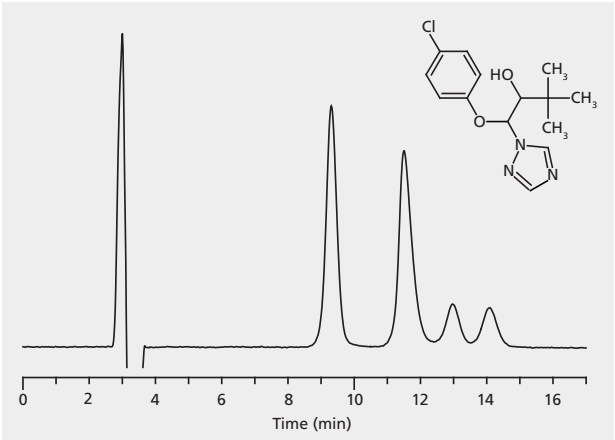
## HPLC Analysis of Tramadol Enantiomers on Astec® CYCLOBOND® I 2000 DMP

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . (A) 0.1% triethylamine acetate, pH 5.0; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1.2 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004700



## HPLC Analysis of Triadimenol Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)
mobile phase . . (A) 20 mM ammonium acetate, pH 4.0; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004396



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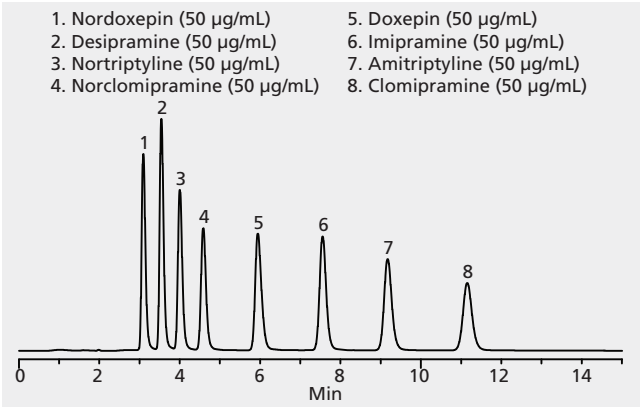
## HPLC Analysis of Tricyclic Antidepressants on Ascentis® C8

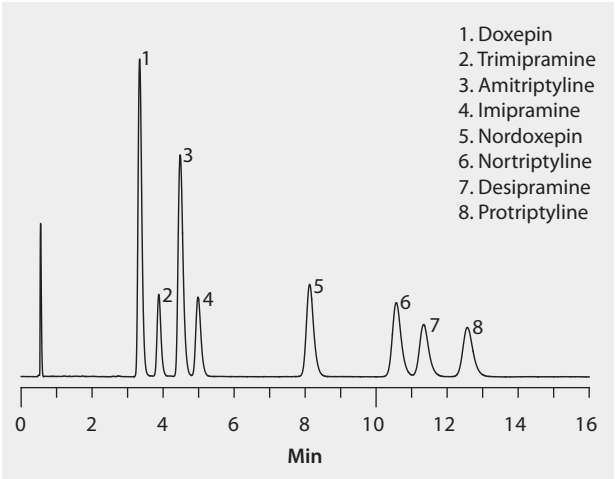
column . . . . . Ascentis C8, 15 cm x 2.1 mm I.D., 3 µm particles (581402-U)
mobile phase . . . . . (A) 25 mM ammonium phosphate dibasic (pH 7.0 with phosphoric acid): (B) methanol; (25:75, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 60:40 water:methanol
Application No. . . . . G003156



## HPLC Analysis of Tricyclic Antidepressants on Ascentis® ES Cyano

This application demonstrates the suitability of Ascentis ES Cyano for the efficient separation of tricyclic antidepressants.

column . . . . Ascentis ES Cyano, 15 cm x 4.6 mm I.D., 5 µm particles (577306-U)
mobile phase . . (A) 10 mM potassium phosphate dibasic, pH 7 with phosphoric acid; (B) acetonitrile; (C) methanol; (25:60:15, A:B:C)
flow rate . . . . . 2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 215 nm
injection . . . . . 5 µL
sample . . . . . 100 µg/mL in water: methanol (70:30)
Application No. . . . . G005630



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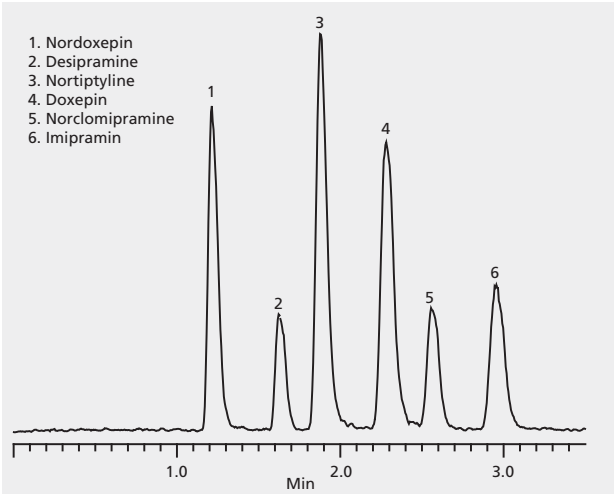
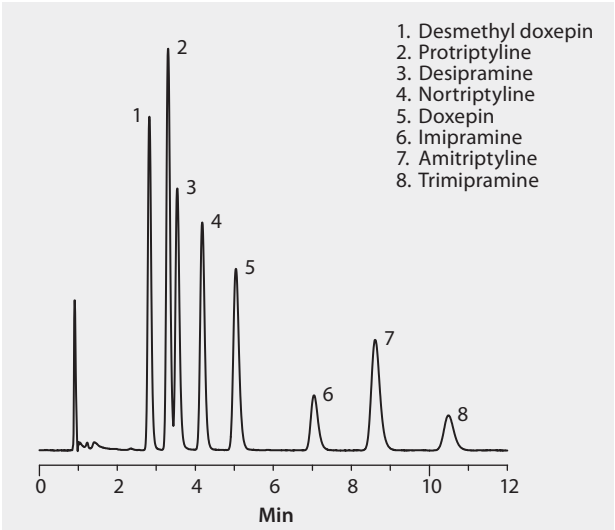
## HPLC Analysis of Tricyclic Antidepressants on Ascentis® Express C18

column . . Ascentis Express C18, 10 cm × 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) 100 mM ammonium acetate (pH 7.0; titrated with ammonium hydroxide), (B) water, (C) methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 55 °C
detector . . . . . Thermo LCQ Advantage; ESI(+), m/z 250-320
injection . . . . . 1 µL
instrument . . . . . Jasco X-LC
Application No. . . . . G004062



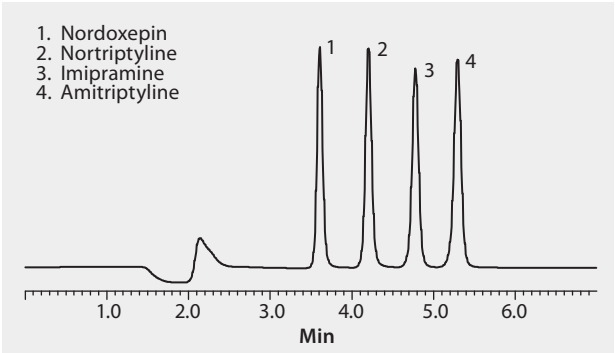
## HPLC Analysis of Tricyclic Antidepressants on Discovery® RP-AmideC16

column . . Discovery RP-AmideC16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . (A) water/acetonitrile (40:60 v/v); (B) 10 mM potassium phosphate
flow rate . . . . . 2 mL/min
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
Application No. . . . . G000163



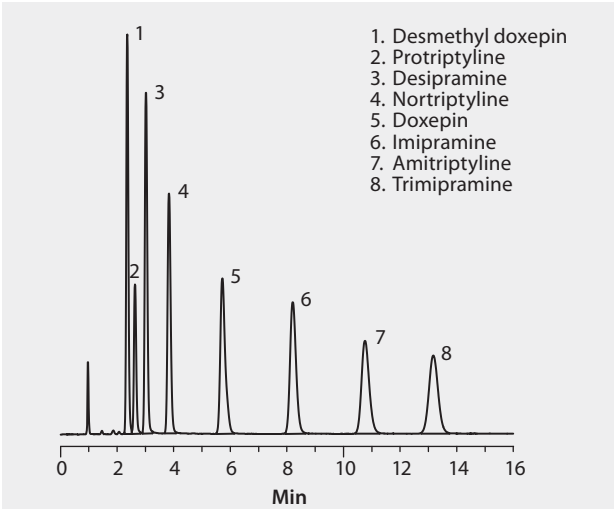
## HPLC Analysis of Tricyclic Antidepressants on Discovery® Zr-PBD

column . . . . . Discovery Zr-PBD, 15 cm × 4.6 mm I.D., 3 µm particles (65718-U)
mobile phase . . . . . (A) 20 mM potassium phosphate (pH 12.0): (B) acetonitrile, (40:60, A:B)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
Application No. . . . . G001862



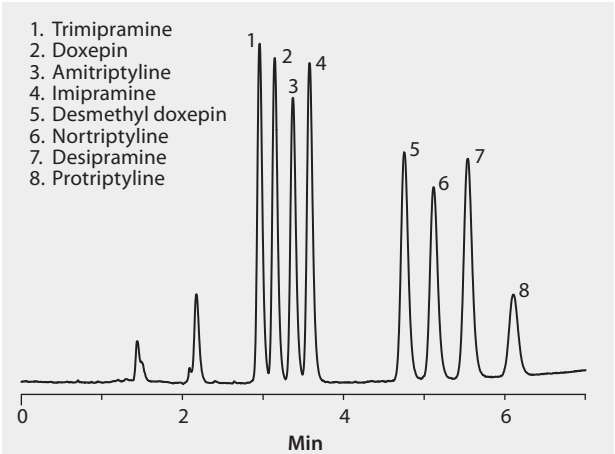
## HPLC Analysis of Tricyclic Antidepressants on Discovery® C8

column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . . (A) methanol: (B) 10 mM potassium phosphate, dibasic, pH 7.0
flow rate . . . . . 2 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 254 nm
Application No. . . . . G000541



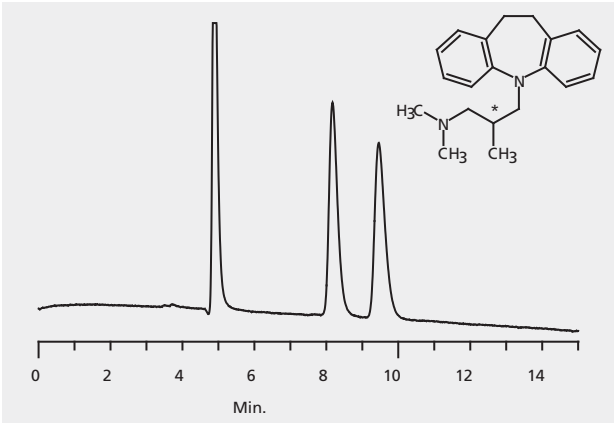
## HPLC Analysis of Tricyclic Antidepressants on Discovery® Cyano

column . . . . . Discovery Cyano, 15 cm × 4.6 mm I.D., 5 µm particles (59356-U)
mobile phase . . . . . (A) acetonitrile: (B) methanol: (C) 10 mM potassium phosphate, dibasic, pH 7.0, (60:20:20, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
Application No. . . . . G000727



## HPLC Analysis of Trimipramine Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . . . . 15 mM ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004474



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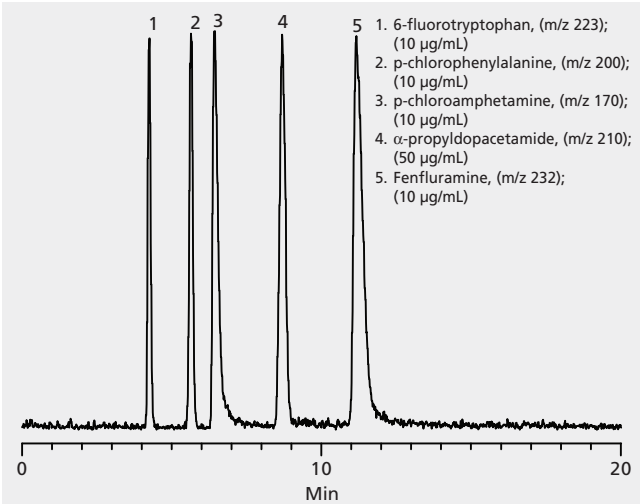
## HPLC Analysis of Tryptophan Hydroxylase Inhibitors on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . (A) 40 mM formic acid, (pH ~2.5): (B) methanol; (65:35, A:B)
flow rate . . . . . 0.7 mL/min
column temp. . . . . 35 °C
detector . . . . . full scan MS; overlay of extracted ion chromatograms of individual (M+H)+ species
injection . . . . . 10 µL
sample . . . . . as indicated in 90:10, 10 mM formic acid:methanol
Application No. . . . . G002453



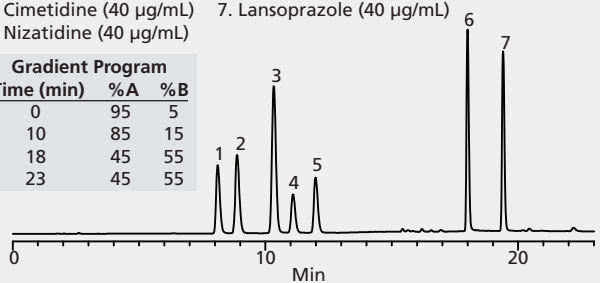
## HPLC Analysis of Ulcer Treatment Drugs on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 2.1 mm I.D., 5 µm particles (565305-U)
mobile phase . . . . . (A) 10 mM ammonium phosphate (pH 6.2 with phosphoric acid); (B) acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . as indicated in 95:5 water:methanol
Application No. . . . . G002992

1. Ranitidine (40 µg/mL)
2. Famotidine (40 µg/mL)
3. Cimetidine (40 µg/mL)
4. Nizatidine (40 µg/mL)
5. Pirenzepine (40 µg/mL)
6. Omeprazole (40 µg/mL)
7. Lansoprazole (40 µg/mL)

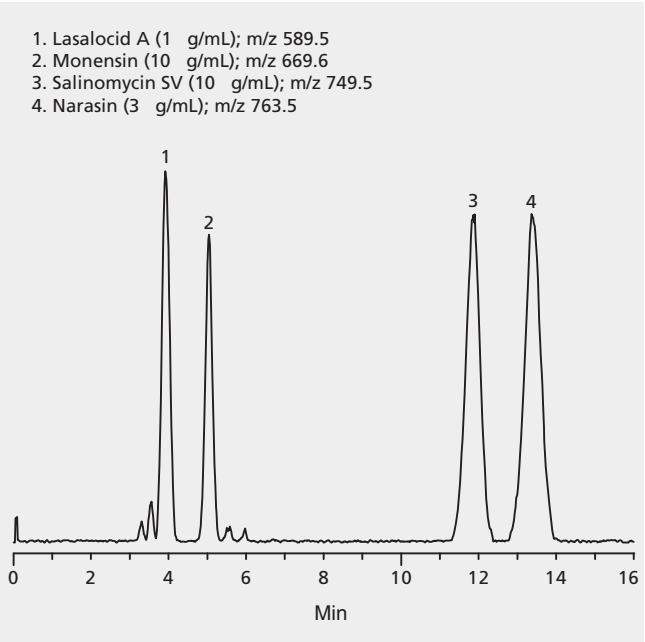
**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 10 | 85 | 15 |
| 18 | 45 | 55 |
| 23 | 45 | 55 |



## HPLC Analysis of Veterinary Antibiotics on Discovery® HS C18

column . . . . Discovery HS C18, 5 cm × 2.1 mm I.D., 3 µm particles (569253-U)
mobile phase . . . . . (A) (50 mM acetic acid/ammonium hydroxide, pH 6.0]: (B) methanol; (20:80, A:B):
flow rate . . . . . 0.2 mL/min
column temp. . . . . 22 °C
detector . . . . . ESI (+), full scan
injection . . . . . 2 µL
sample . . . . . as indicated in 50:50 water:methanol
Application No. . . . . G002575



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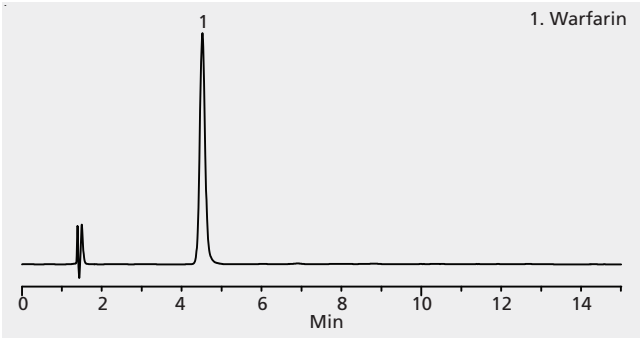
## HPLC Analysis of Warfarin™ Anticoagulant on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0, unadjusted): (B) methanol; (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002340



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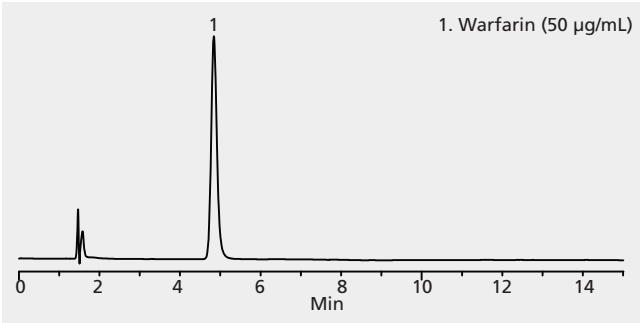
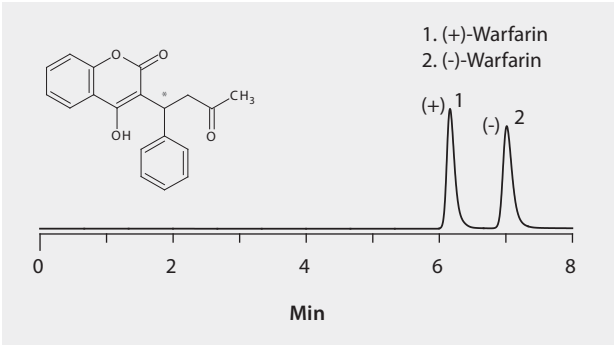
## HPLC Analysis of Warfarin™ Anticoagulant on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 20 mM phosphoric acid (pH 2.0 unadjusted): (B) methanol; (30:70, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 20 mM phosphoric acid (pH 2.0)
Application No. . . . . G002638



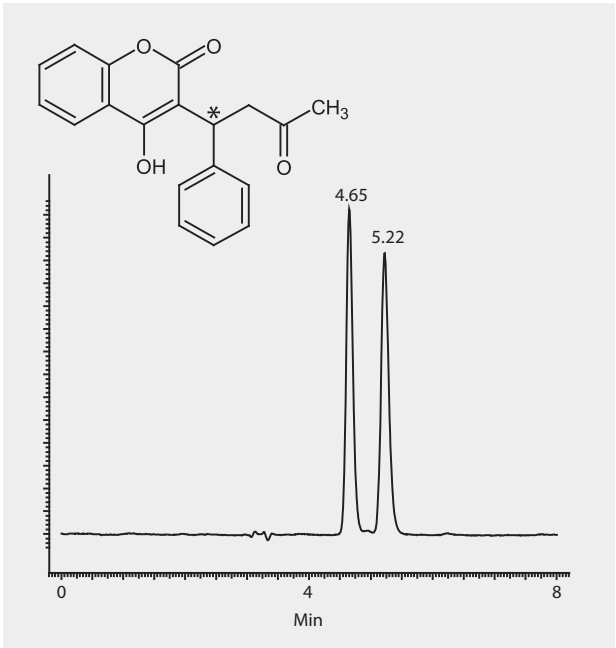
## HPLC Analysis of Warfarin™ Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) methanol; (B) acetic acid; (C) TEA; (100:0.2:0.1, A:B:C)
column temp. . . . . 25 °C
flow rate . . . . . 0.5 mL/min
detector . . . . . UV, 278 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005007



## HPLC Analysis of Warfarin™ Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) 5 mM ammonium acetate, pH 4.1; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004349



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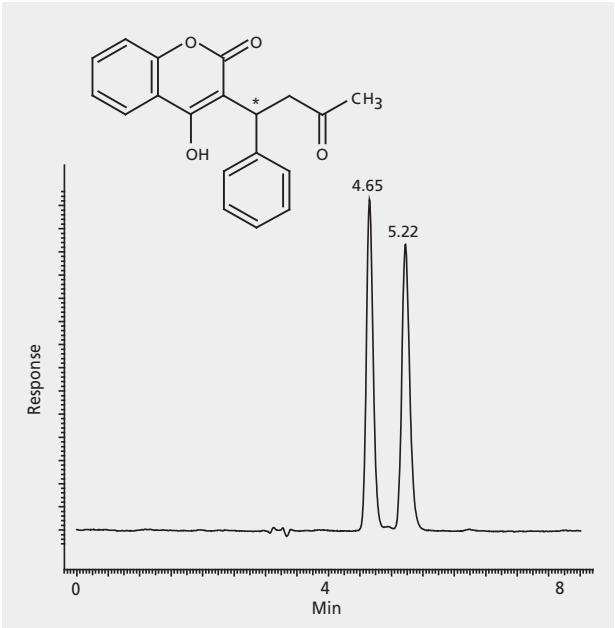
## HPLC Analysis of Warfarin™ Enantiomers on Astec® CHIROBIOTIC® V (Method 1)

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . (A) acetonitrile; (B) 5 mM ammonium acetate, pH 4.1; (30:70, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1 mg/mL in mobile phase
Application No. . . . . G004472



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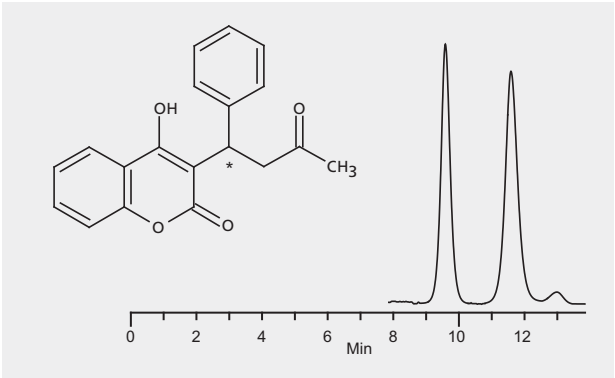
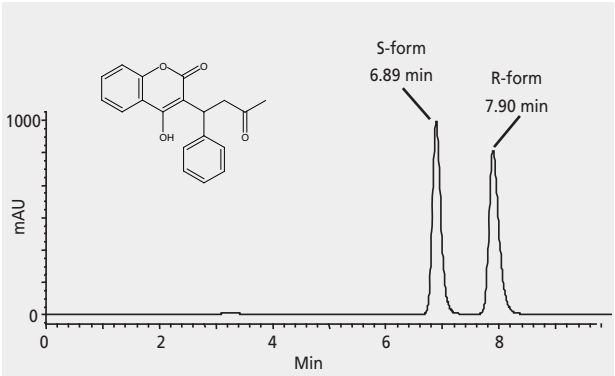
## HPLC Analysis of Warfarin™ Enantiomers on Astec® CHIROBIOTIC® V (Method 2)

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . (A) 0.1% triethylamine acetate, pH 5.0; (B) THF; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 4 mg/mL in methanol
Application No. . . . . G004649



## HPLC Analysis of Warfarin™ Enantiomers on Astec® CYCLOBOND® I 2000 (UV at 254 nm)

column . . CYCLOBOND I 2000, 25 cm × 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . . . . (A) acetonitrile: (B) acetic acid: (C) triethylamine; (100:0.3:0.25, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
Application No. . . . . G004027



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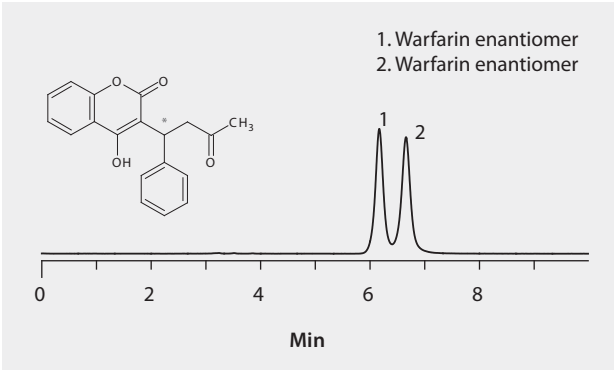
## HPLC Analysis of Warfarin™ Enantiomers on Astec® CYCLOBOND® I 2000 (UV at 278 nm)

column . . . CYCLOBOND I 2000, 25 cm x 4.6 mm I.D., 5 µm particles (20024AST)
mobile phase . . . . . (A) acetonitrile; (B) acetic acid; (C) TEA; (100:0.3:0.2, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 278 nm
injection . . . . . 2 µL
sample . . . . . 2 mg/mL in mobile phase
Application No. . . . . G005008



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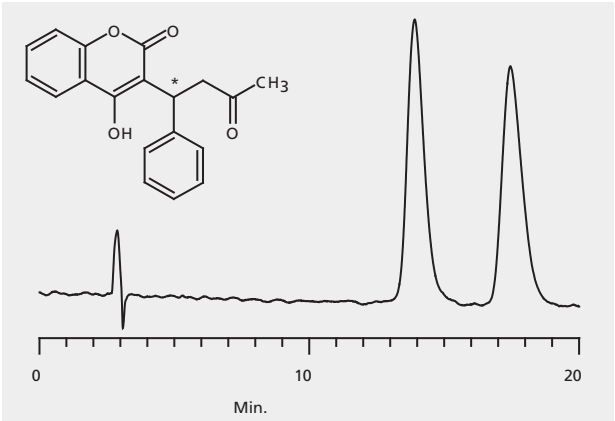
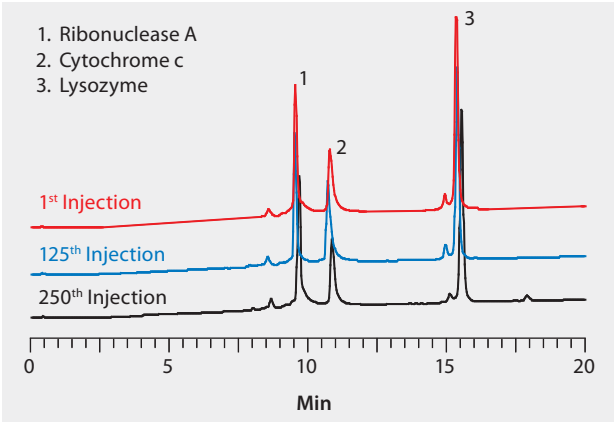
## HPLC Analysis of Warfarin™ Enantiomers on Astec® CHIROBIOTIC® V2

column . . . . . CHIROBIOTIC V2, 25 cm x 4.6 mm I.D., 5 µm particles (15024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.1; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004475



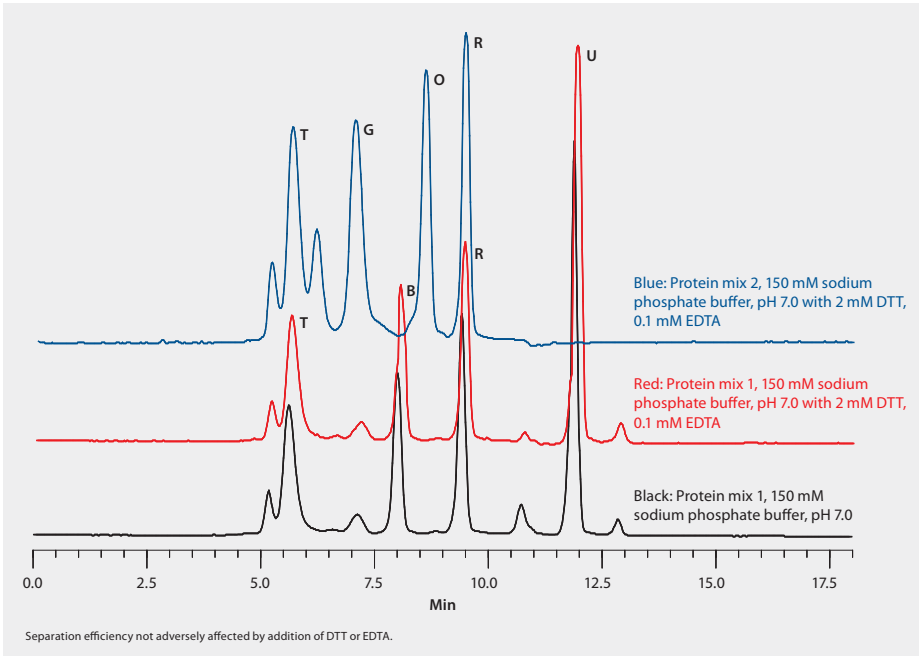
## HPLC Analysis Proteins on Proteomix® SCX-NP1.7, Demonstration of Column Stability with Repeated Injections

column . . Proteomix SCX-NP1.7, 5 cm x 4.6 mm I.D., 1.7 µm particles (Z777146)
column . . . . . Proteomix SCX-NP1.7 guard cartridge with holder, 1 cm x 4 mm I.D. (Z777144)
mobile phase . . (A) 20 mM sodium phosphate buffer, pH 6.5, (B) A + 1.0 M NaCl
gradient . . . . . 0 - 100% B in 25 min, 15 min prewash
flow rate . . . . . 0.5 mL/min
column temp. . . . . ambient
detector . . . . . UV, 214 nm
injection . . . . . 5 µL
sample . . . . . each compound 1 mg/mL
Application No. . . . . G006135



## HPLC (Size Exclusion) Analysis of Proteins on Zenix®-C SEC-300, Effect of DTT and EDTA

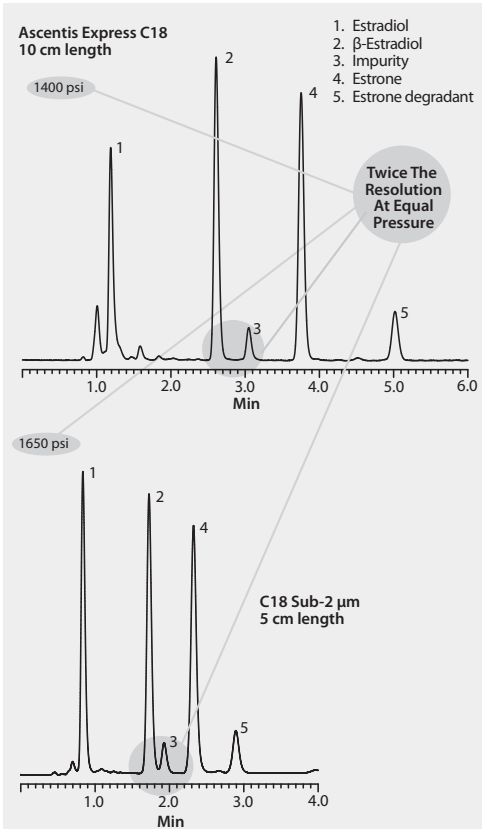
column . . . . . Zenix-C SEC-300, 30 cm x 7.8 mm I.D., 3 µm particles (Z777092)
mobile phase . . . . . (A) 150 mM sodium phosphate buffer, pH 7.0
(B) 150 mM sodium phosphate buffer, pH 7.0 with 2 mM DTT, 0.1 mM EDTA
(C) 150 mM sodium phosphate buffer, pH 7.0 with 2 mM DTT, 0.1 mM EDTA
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . proteins
Application No. . . . . G005954




Separation efficiency not adversely affected by addition of DTT or EDTA.

## Hyper-Fast Separations on Ascentis® Express at Half the Pressures of sub-2 µm Columns

column . . . . . Ascentis Express C18, 10 cm × 2.1 mm I.D., 2.7 µm particles and sub-2 µm particle column, 5 cm × 2.1 mm I.D. (53823-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (55:45 or 54:46, A:B)
flow rate . . . . . 0.2 mL/min
column temp. . . . . ambient
detector . . . . . UV, 200 nm
injection . . . . . 1 µL
Application No. . . . . G003973



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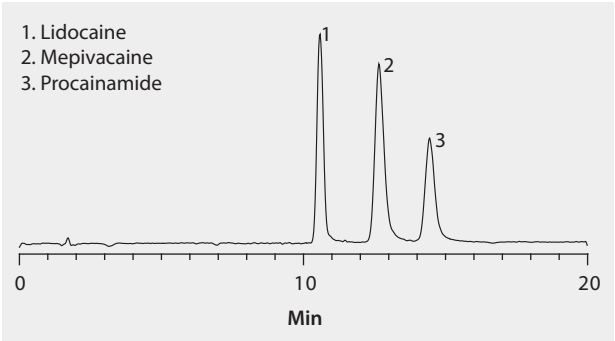
## LC-MS Analysis of Anesthetics on Ascentis® Express F5

column . . . Ascentis Express F5, 10 cm x 4.6 mm I.D., 2.7 µm particles (53590-U)
mobile phase . . . . . (A) 10 mM ammonium acetate, pH 4.0 with acetic acid; (B) acetonitrile; (20:80, A:B)
flow rate . . . . . 1.0 mL/min
pressure . . . . . 2320 psi (160 bar)
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+), SIR, m/z 235, 236, 247
injection . . . . . 2 µL
sample . . . . . 10 µg/mL in methanol
Application No. . . . . G005661



## LC-MS Analysis of Angiotensins on Discovery® BIO Wide Pore C18

column . . . . . Discovery BIO Wide Pore C18, 10 cm × 0.32 mm I.D., 3 µm particles (65527-U)

mobile phase . . (A) 65:35, (10mM ammonium acetate, pH 7):(50% acetonitrile in 20mM ammonium acetate, pH 7); (B) 25:75, (10mM ammonium acetate, pH 7):(50% acetonitrile in 20mM ammonium acetate, pH 7)

gradient . . . . . 0-100% B in 12.5 min

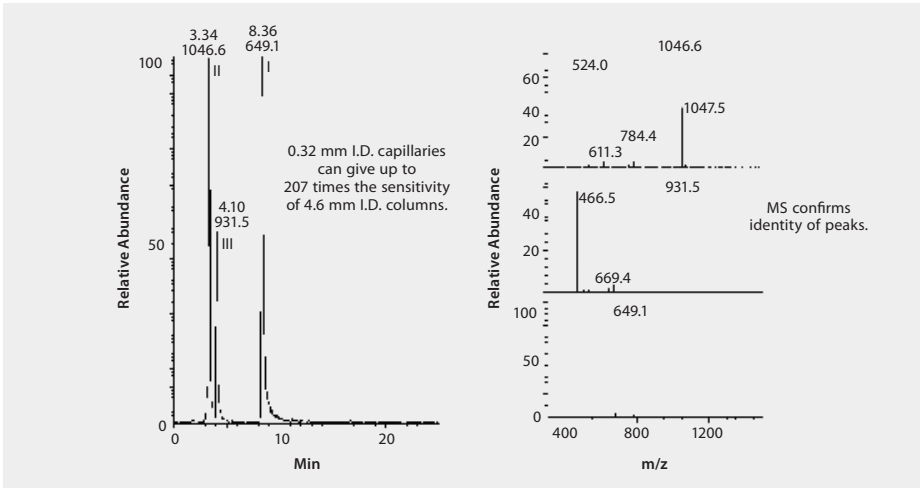
flow rate . . . . . 6 µL/min

column temp. . . . . ambient

detector . . . . . +ESI mode

injection . . . . . 50 pmol angiotensin I, II and III in water

Application No. . . . . G001592



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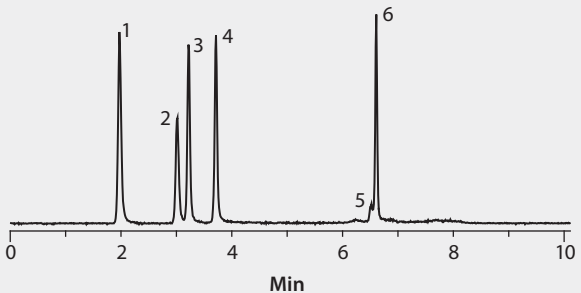
## LC-MS Analysis of Antipsychotic Drugs on Ascentis® Express RP Amide

| | |
|---|---|
| column | Ascentis Express RP Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U) |
| mobile phase | (A) 5 mM ammonium formate water; (B) 5mM ammonium formate in 95:5 (v/v) methanol:water |
| gradient | held at 35% B for 0.5 min; 35 to 85% B in 5.5 min; held at 85% B for 1 min |
| flow rate | 0.3 mL/min |
| pressure | 4641 psi (320 bar) |
| column temp. | 35 °C |
| detector | ESI(+), m/z 100-1000 |
| injection | 1 µL |
| sample | 200 ng/mL in waler |
| Application No. | G005668 |

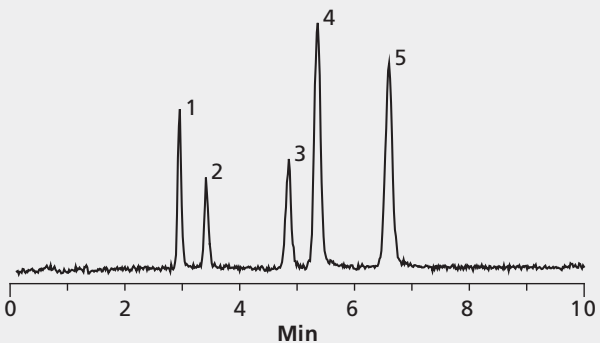
1. Desmethyl venlafaxine
2. Hydroxybupropion
3. Hydroxyrisperidon
4. Bupropion
5. Zuclopenthixol
6. Fluphenazine



## LC-MS Analysis of Benzodiazepines on Ascentis® Phenyl (Analyte Mix 1)

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted), (B): 0.1% ammonium acetate in acetonitrile, (50:50, A:B)
flow rate . . . . . 1 mL/min., split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 90:10:water:acetonitrile
Application No. . . . . G003705

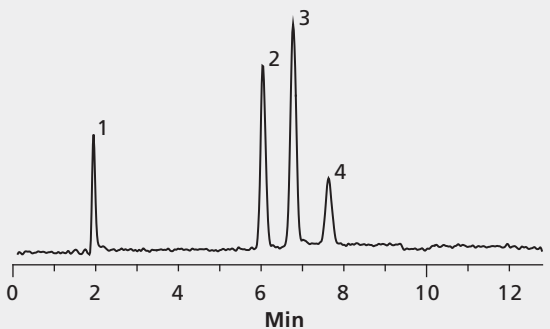
1. Demoxepam (1 µg/mL) (M+H)+ = 287.05
2. Oxazepam (1 µg/mL) (M+H)+ = 287.05
3. Temazepam (1 µg/mL) (M+H)+ = 301.06
4. Clobazam (1 µg/mL) (M+H)+ = 301.06
5. Diazepam (1 µg/mL) (M+H)+ = 285.07



## LC-MS Analysis of Benzodiazepines on Ascentis® Phenyl (Analyte Mix 2)

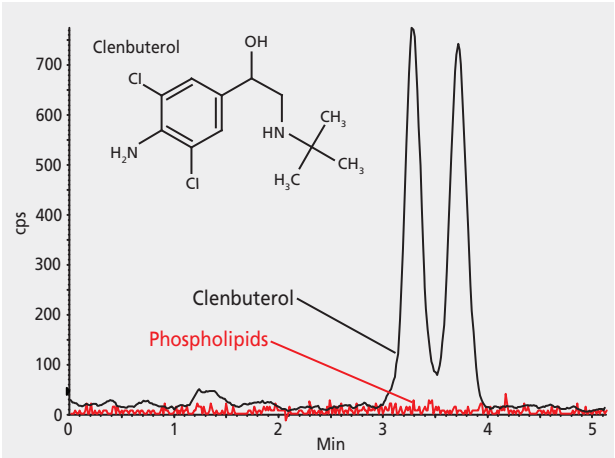
column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 0.1% ammonium acetate in water (pH unadjusted); (B) 0.1% ammonium acetate in acetonitrile; (60:40, A:B)
flow rate . . . . . 1 mL/min., split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (+) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . as indicated in 0.1% ammonium acetate in 90:10:water:acetonitrile
Application No. . . . . G003706

1. Fenoldopam (10 µg/mL) (M+H)+ = 306.08
2. Lorazepam (10 µg/mL) (M+H)+ = 321.01
3. Nitrazepam (10 µg/mL) (M+H)+ = 282.08
4. Clonazepam (10 µg/mL) (M+H)+ = 316.04



## LC-MS Analysis of Clenbuterol Enantiomers in Plasma on Astec® CHIROBIOTIC® T after SPE using HybridSPE®-Phospholipid

column . . . . . CHIROBIOTIC T, 10 cm x 2.1 mm I.D., 5 µm particles (12018AST)
mobile phase . . . . . 10 mM ammonium formate in methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . ESI(+)
sample . . . . . clenbuterol, 10 ng/mL in rat plasma (phospholipids removed by extraction with HybridSPE-Phospholipid)
Application No. . . . . G004245



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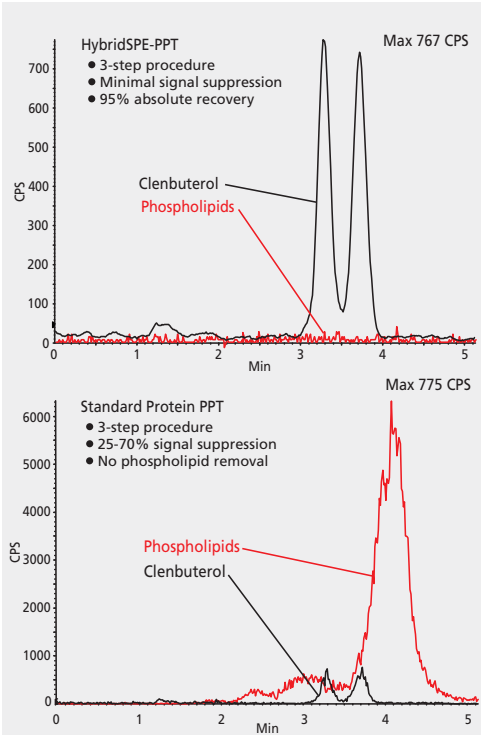
## LC-MS Analysis of Clenbuterol In Plasma on Astec® CHIROBIOTIC® T with Phospholipid Removal Using HybridSPE® Phospholipid

The HybridSPE method provides significant improvement in LC-MS baseline.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . rat plasma spiked with clenbuterol enantiomers at 10 ng/mL
SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample addition . . 100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile. Mix by vortexing the HybridSPE-PPT plate briefly.
elution . . . . . apply vacuum
column . . . . . CHIROBIOTIC T, 10 cm x 2.1 mm I.D., 5 µm particles (12018AST)
mobile phase . . . . . 10 mM ammonium formate in methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . ABI 3200 QT; ESI(+), MRM: 184/104 m/z (phospholipids); 277.2/203.1 m/z (clenbuterol)
injection . . . . . 10 µL
Application No. . . . . G004431



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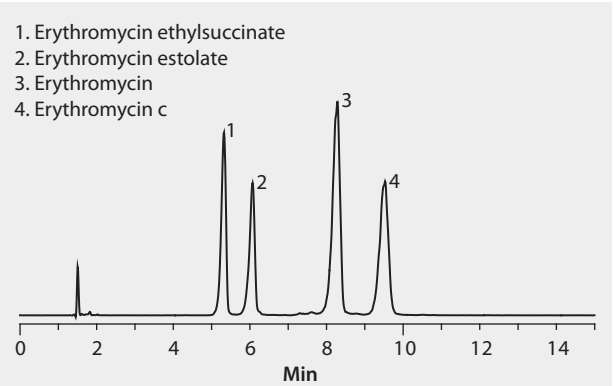
## LC-MS Analysis of Erythromycin Analogs on Ascentis® Express HILIC

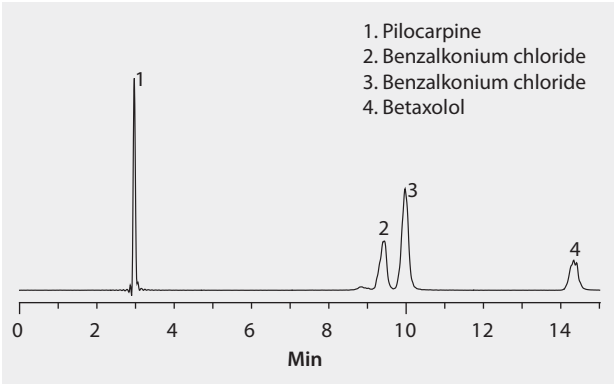
This application shows the suitability of Ascentis Express Silica columns for the analysis of erythromycin analogs in aqueous normal phase/HILIC mode of chromatography. Other relatively hydrophobic aminoglycosides have also been shown to separate under these conditions.

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . (A) 100 mM ammonium formate, pH 3.0 (titrated with formic acid); (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1247 psi (86 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+); SIR
injection . . . . . 5 µL
Application No. . . . . G005696



## LC-MS Analysis of Pilocarpine and Betaxolol in the Presence of Benzalkonium Chlorides on Ascentis® Express HILIC

column . . Ascentis Express HILIC, 15 cm x 4.6 mm I.D., 2.7 µm particles (53981-U)
mobile phase . . . . . (A) 2 mM arrmonium acetate, pH 6.7 in (B) 10:90 (v/v) water:acetonitrile
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1160 psi (80 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+), XIC, m/z 304, 332 (BACs), m/z 209 (pilocarpine), m/z 308 (betaxolol)
injection . . . . . 2 µL
sample . . . . . 10 µg/mL each in acetonitrile
Application No. . . . . G005721



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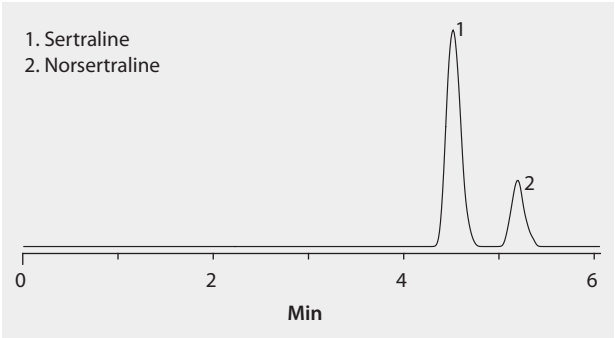
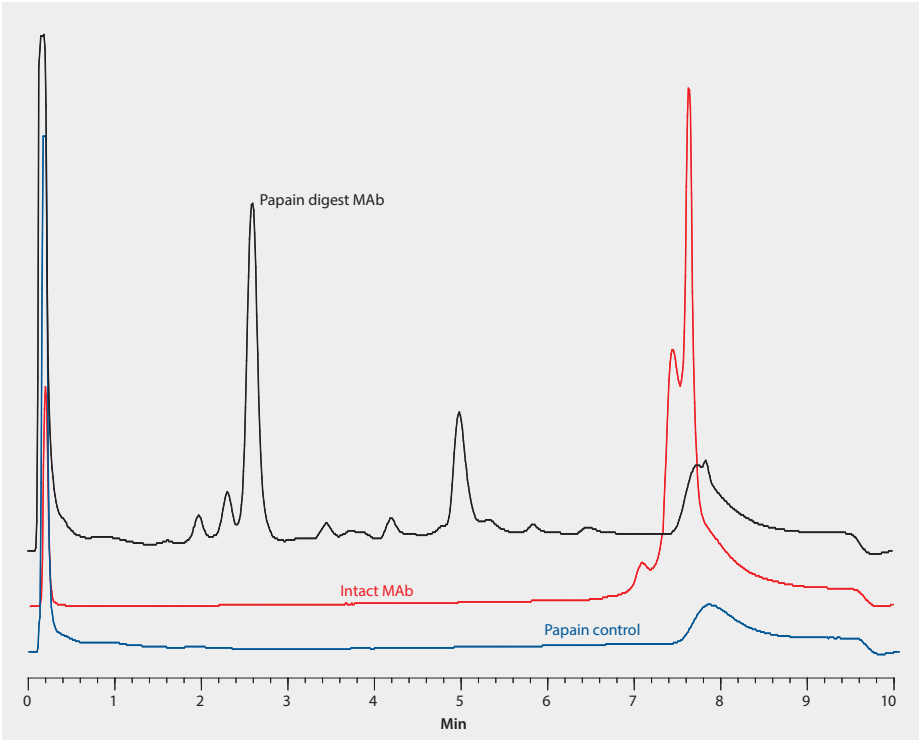
## LC-MS Analysis of Sertraline and Norsertraline on Ascentis® Express RP-Amide

column . . . . . Ascentis Express RP Amide, 10 cm x 3.0 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . . . . (A) 10 mM ammonium formate, water, pH 3.6; (B) 10 mM ammonium formate, acetonitrile, pH 3.6; (65:35, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 55 °C
detector . . . . . ESI(+), 100-1000 m/z
injection . . . . . 2 µL
sample . . . . . 200 ng/mL in 25:75, water: acetonitrile
Application No. . . . . G005736



## Proteomix WCX-NP1.7, 3 cm x 4.6 mm I.D., 1.7 µm particles

column . . Proteomix WCX-NP1.7, 3 cm x 4.6 mm I.D., 1.7 µm particles (Z777179)
mobile phase . . . . . (A) 20 mM sodium acetate, pH 5.15; (B) A + 1 M LiCl
gradient . . . . 12% to 40% B in 6.7 min; to 100% B in 0.1 min; held for 2 min
flow rate . . . . . (1.5 mL/min)
pressure . . . . . 3335-3916 psi (230 - 270 bar)
detector . . . . . UV 280 nm
injection . . . . . 25 µL
sample . . . . . 1 mg/mL papain digested MAb 321
Application No. . . . . G006023



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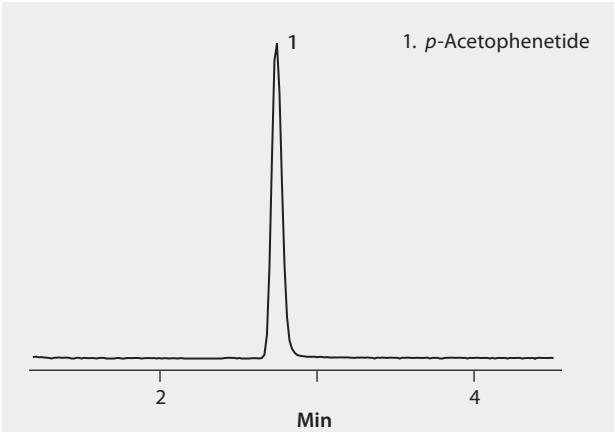
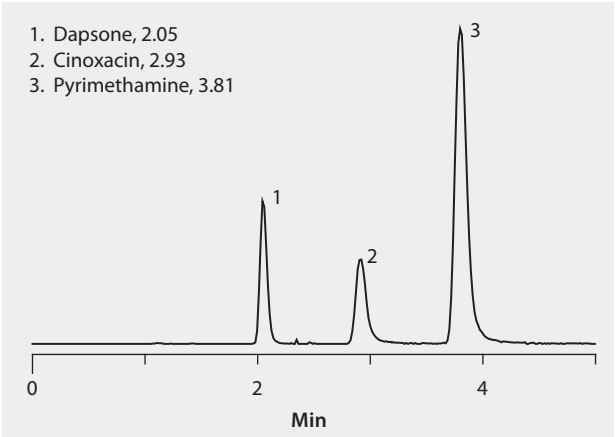
## UHPLC Analysis of p-Acetophenetide (Phenacetin) Titan™ C18

column . . . . . TITAN C18, 10 cm x 2. 1 mm I.D., 1.9 um particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 180.10
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006101



## UHPLC Analysis of Antibiotics/Antimalarials on Titan™ C18

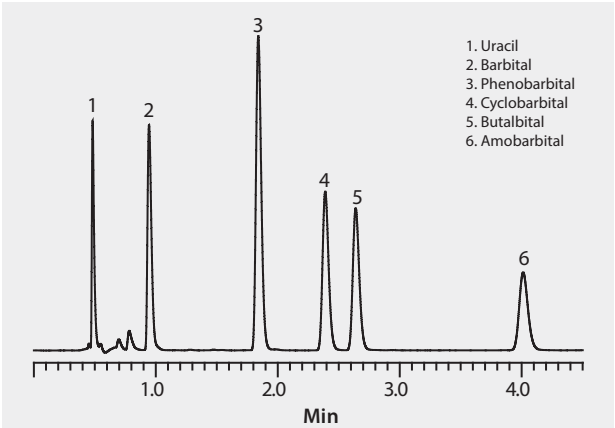
column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 249.09, 249.07, 263.14
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006088



## UHPLC Analysis of Barbituates on Titan™ C18

Analysis of a Barbituate Mix using a MS compatible mobile phase.

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% ammonium acetate (not adjusted); (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 7540 psi (520 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
Application No. . . . . G006073



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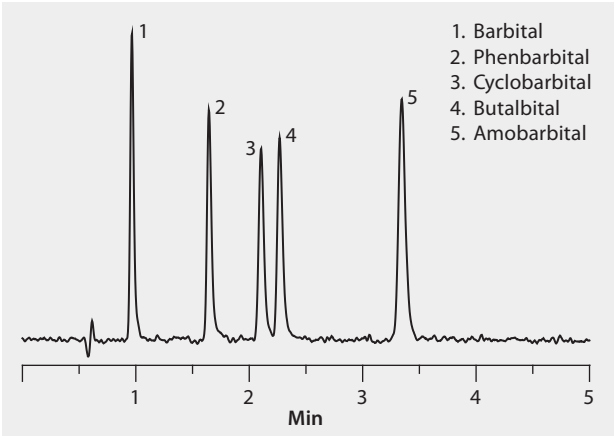
## UHPLC Analysis of Barbituates on Titan™ C18 with MS Detection

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% ammonium acetate (not adjusted); (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 6796 psi (468 bar)
column temp. . . . . 35 °C
detector . . . . . ESI (-) MS TIC MRM
injection . . . . . 1 µL
sample . . . . . 1000 ng/ml in mobile phase
Application No. . . . . G006137



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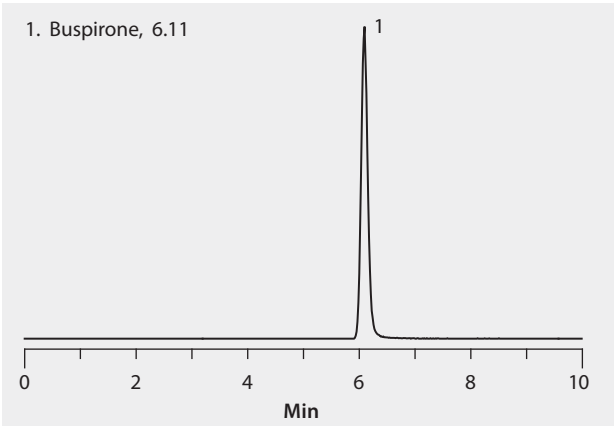
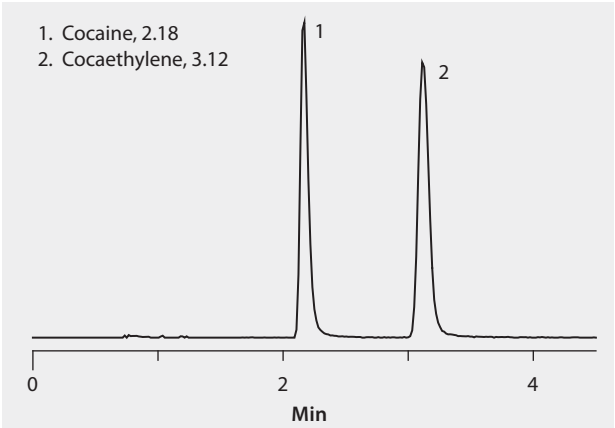
## UHPLC Analysis of Buspirone on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 386.26
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006090



## UHPLC Analysis of Cocaine and Cocaethylene on Titan™ C18

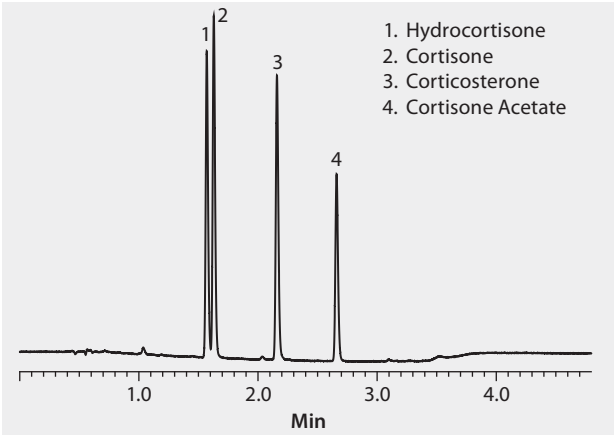
column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 304.15 and 318.17
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006091



## UHPLC Analysis of Corticosteroids on Titan™ C18

Analysis of Corticosteroids on a Titan C18 UPLC column with MS detection in ESI (+) Mode

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (70:30, A:B)
gradient . . . . . 30% to 60% B in 3 minutes, hold for 0.2 min
flow rate . . . . . 0.4 mL/min
pressure . . . . . 7423 psi (512 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 1 µL
sample . . . . . 1000 ng/ml in mobile phase
detector . . . . . ESI (+) MS TIC SIR
Application No. . . . . G006147



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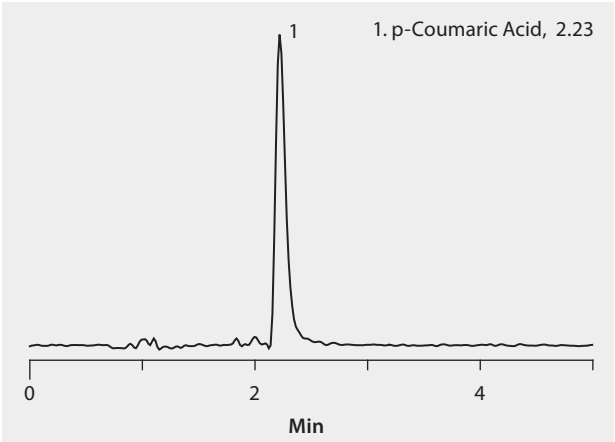
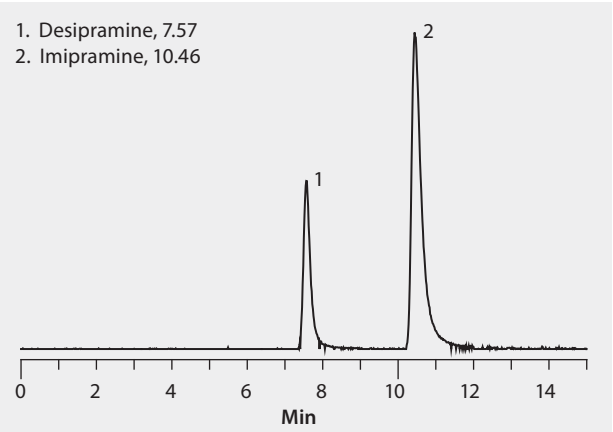
## UHPLC Analysis of p-Coumaric Acid on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 163.15
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006103



## UHPLC Analysis of Desipramine and Imipramine on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 267.19 and 281.20
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006105



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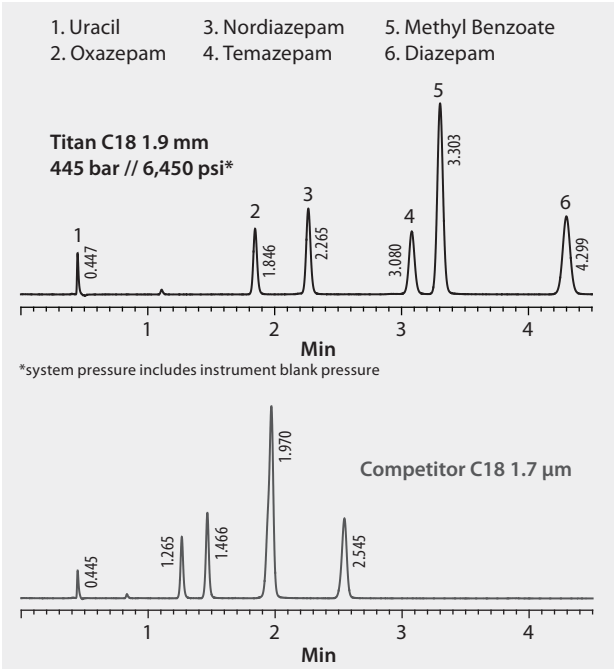
## UHPLC Analysis of Diazepam and Metabolites on Titan™ C18, Competitive Comparison

This application shows a comparison between Titan™ C18 and a competitive column for diazepam and its metabolites using a MS compatible mobile phase.

Used 75 µm I.D. x 250 mm L tubing in system.

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid; (B) acetonitrile; (60:40, A:B)
pressure . . . . . 6450 psi (445 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
Application No. . . . . G006001




*system pressure includes instrument blank pressure



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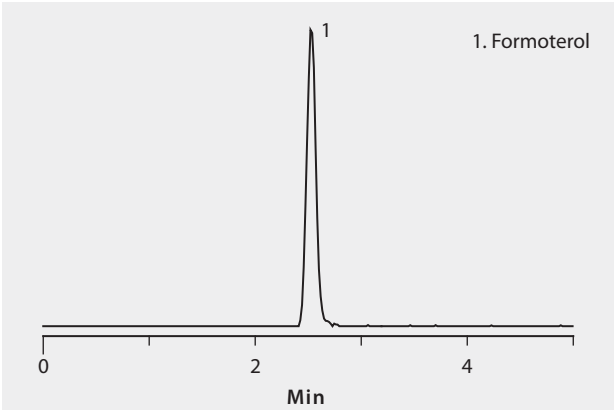
## UHPLC Analysis of Formoterol on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 345.20
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006092



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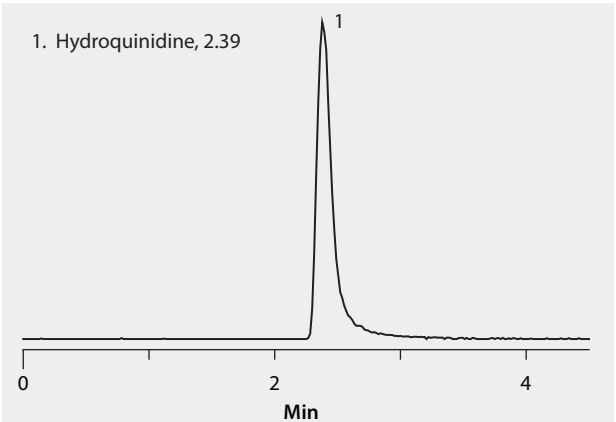
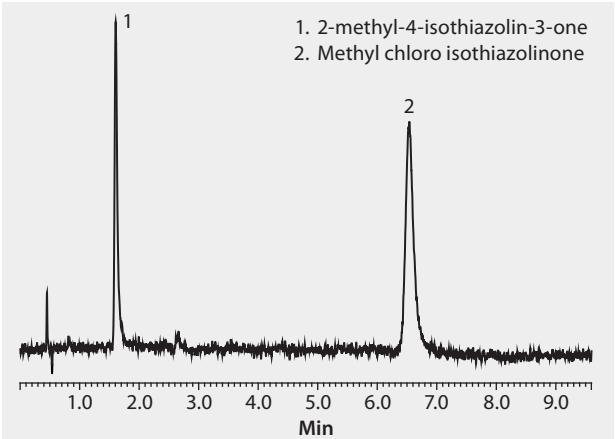
## UHPLC Analysis of Hydroquinidine on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 327.21
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006094



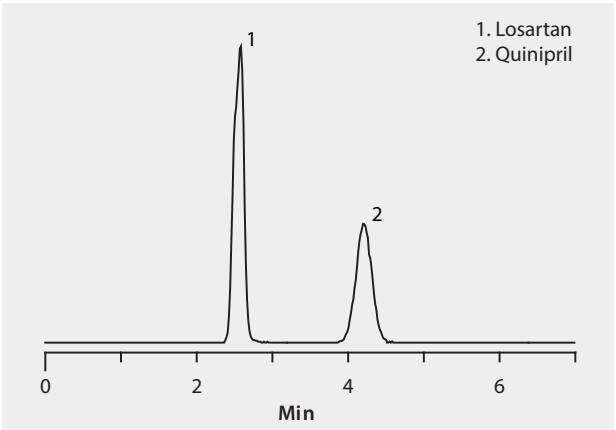
## UHPLC Analysis of Kathon on Titan™ C18 Using MS Detection

column . . . . . Titan C18 50 mm x 2.1 mm, I.D, 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 0.1% formic acid in water (B):methanol
flow rate . . . . . 500 µL/min
detector . . . . . MS, ESI (+) MRM TIC
detector . . . . . Diode Array at 275 nm
injection . . . . . 1.0 µL
sample . . Diluted Kathon at 1 ug/ml in 95:5 0.1% formic acid in water::methanol
Application No. . . . . G006148



## UHPLC Analysis of Losartan and Quinapril on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 423.17 and 439.22
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006095



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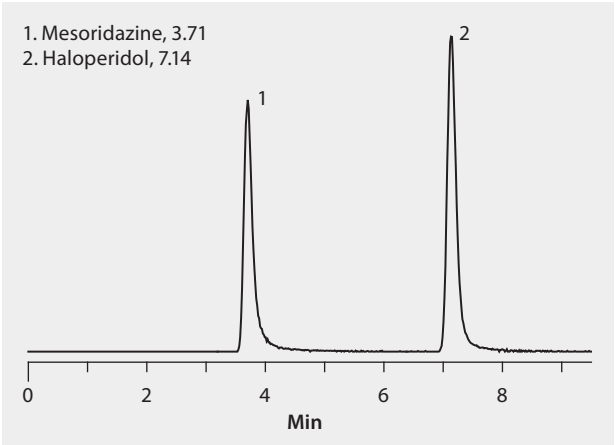
## UHPLC Analysis of Mesoridazine and Haloperidol on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 387.16 and 376.15
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006099



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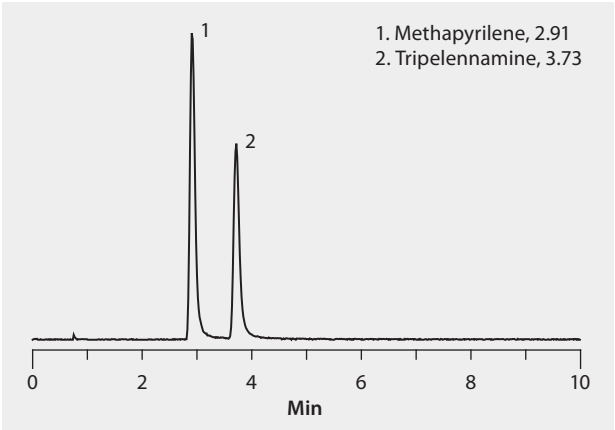
## UHPLC Analysis of Methapyrilene and Tripelennamine on Titan™ C18

column . . . . . Titan C18, 10 cm x 2. 1 mm I.D., 1.9 um particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 262.14 and 256.18
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006106



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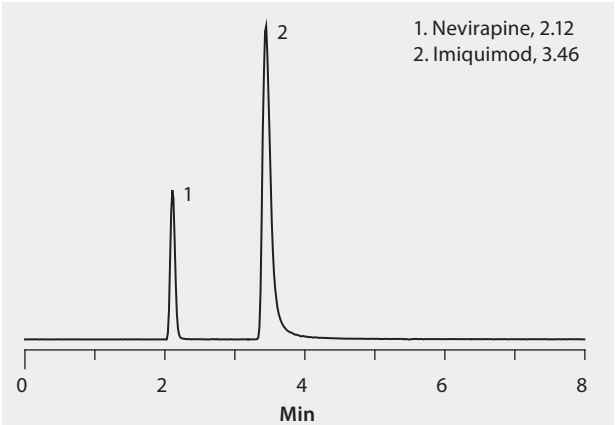
## UHPLC Analysis of Nevirapine and Imiquimod on Titan™ C18

column . . . . . TITAN C18, 10 cm x 2. 1 mm I.D., 1.9 um particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 267.12 and 241.15
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006113



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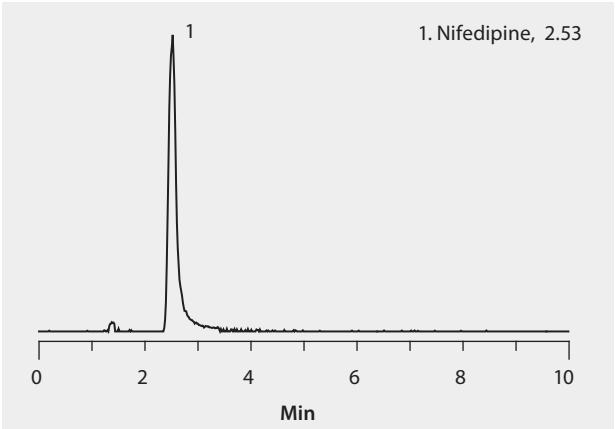
## UHPLC Analysis of Nifedipine on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 347.18
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006100



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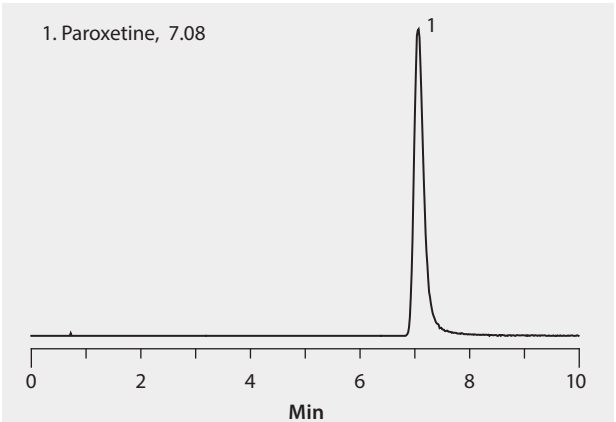
## UHPLC Analysis of Paroxetine Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 330.15
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006102



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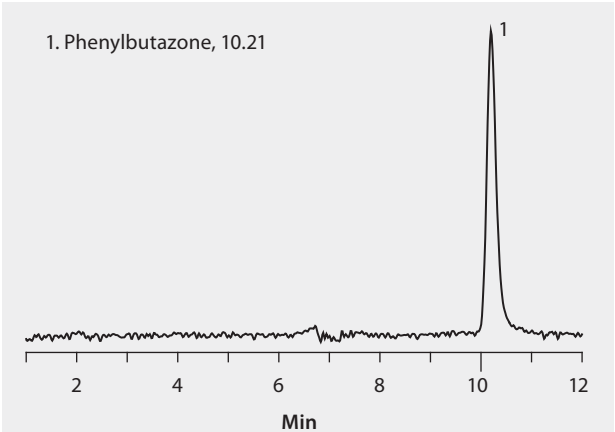
## UHPLC Analysis of Phenylbutazone on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 309.20
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006104



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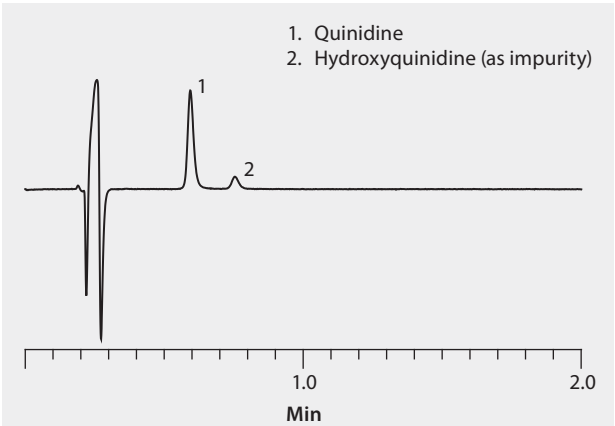
## UHPLC Analysis of Quinidine and Metabolite on Titan™ C18

column . . . . . Titan C18, 5 cm x 2.1 mm I.D., 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 10 mM potassium phosphate, monobasic pH 3.0; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 4310 psi (297 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 200 nm
injection . . . . . 1.0 µL
sample . . . . . Quinidine 5 µg/mL in 80:20, water:methanol
Application No. . . . . G006218



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## UHPLC Analysis of Riboflavin and Impurities on Titan™ C18 with MS Detection

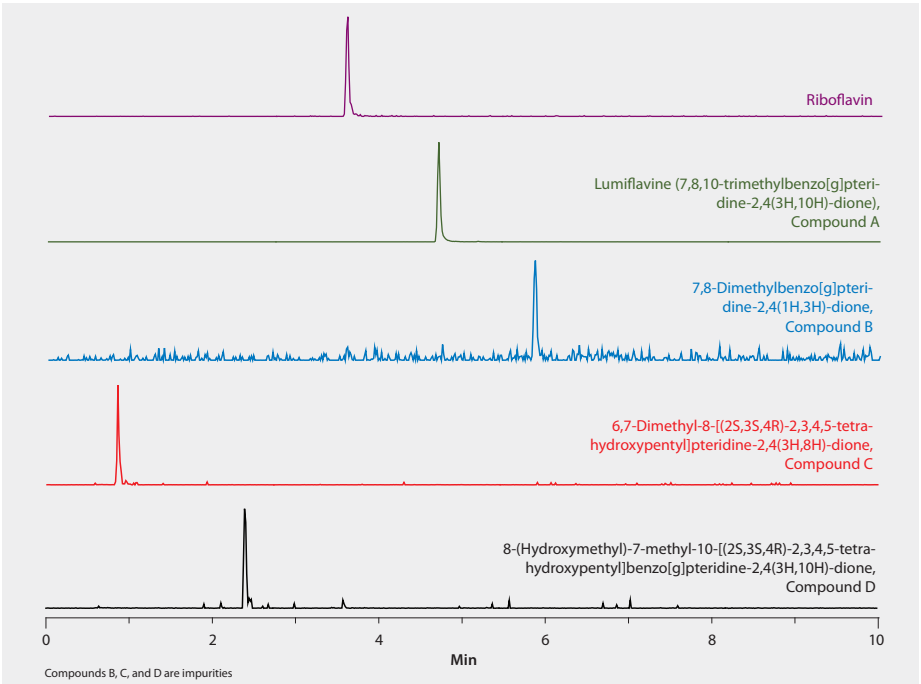
Riboflavin (vitamin B2) is a water-soluble vitamin. It is synthesized by all plants and many microorganisms, but it is not produced by higher animals. It is a precursor of coenzymes that are required for the enzymatic oxidation of carbohydrates, so it is essential to basic metabolism. Riboflavin is used as an additive to food and feed and is also used in fortification of baby food and cereal. The current USP method for impurity analysis is non-quantitative, and uses an ion pair reagent in the mobile phase. An alternative to USP and EP methods using LC-MS/MS to identify and quantify the riboflavin and impurities is presented. This new proposed method is also compatible with UV-Vis detection.

A riboflavin certified reference material (CRM) was used in this study. MRM transitions for the determination of ions for the parent and four impurites were made. Three transitions were used for each ion. Structures for each of these major impurities were confirmed by MS. In addition to improving the specificity of the method with MS/MS detection, two additional goals were to decrease analysis time and eliminate the need for ion pairing reagents that are not compatible with MS.

### Other MS Conditions

Capillary (kV) 2.29
Cone (V) 54.95
Extractor (V) 5.13
Source Temperature (°C) 151
Desolvation Temperature (°C) 349
Cone Gas Flow (L/Hr) 2
Desolvation Gas Flow (L/Hr) 646
Collision Gas Flow (mL/Min) ON

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile (95:5)
gradient . . . . . 5% to 25% in 6 min, return to 5% b in 0.1 min, and re-equilibrate for 4 min
flow rate . . . . . 500 µL/min
pressure . . . . . 5000 psi (345 bar)
column temp. . . . . 35 °C
detector . . . . . ESI (+), MRM and TIC
detector . . . . . Diode Array at 276 nm
injection . . . . . 2.0 µL
Application No. . . . . G006138



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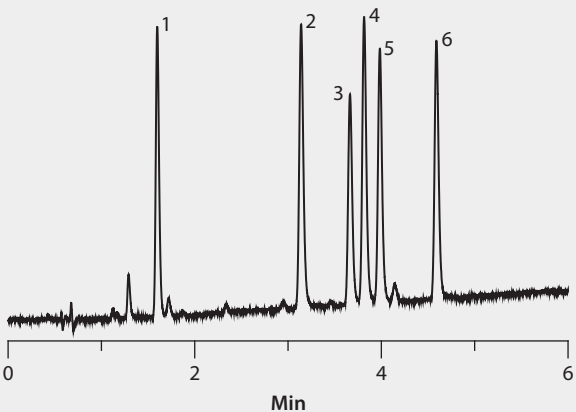
## UHPLC analysis of Taxanes on Titan C18 Column

| | |
|---|---|
| column | Titan C18 10 cm x 2.1 mm, 1.9 µm (577124-U) |
| mobile phase | (A) water; (B) (50:50, methanol:acetonitrile) |
| gradient | 60 to 80% B in 4.5 min; 80% B held for 1.5 min |
| flow rate | 0.4 mL/min |
| pressure | 6090 psi (420 bar) |
| column temp. | 40 °C |
| detector | UV, 227 nm |
| injection | 2 µL |
| sample | 25 µg/mL in 70:30, water:methanol |
| Application No. | G006139 |

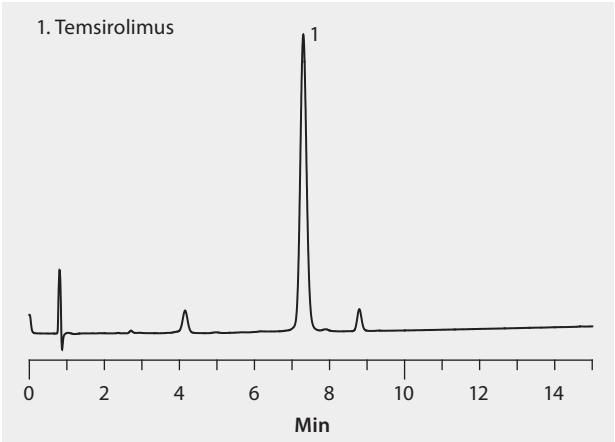
1. baccatin III
2. 10-deacetyl paclitaxel
3. cephalomannine
4. paclitaxel
5. 10-deacetyl-7-epipaclitaxel
6. 7-epipaclitaxel



## UHPLC Analysis of Temsirolimus on Titan™ C18

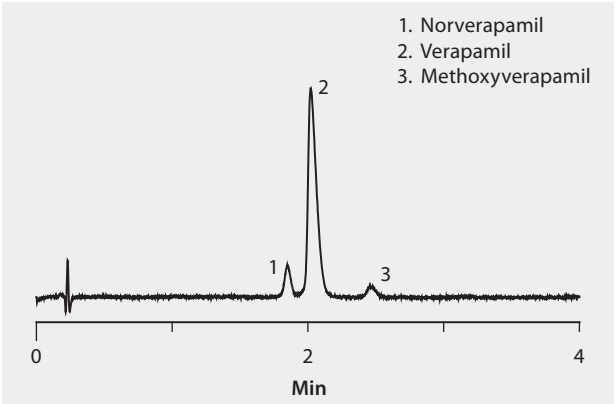
Intravenous drug for the treatment of renal cell carcinoma

column . . . . . Titan C18 10 cm x 2.1 mm I.D., 1.9 um particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid in water, (B) methanol
gradient . . . . . 75% B for 2 minutes, 75% B to 90% B from 2 to 12 minutes, 90% B from 12 to 14 minutes.
flow rate . . . . . 0.3 mL/min
pressure . . . . . 3300 psi (228 bar)
column temp. . . . . 55 °C
detector . . . . . UV, 276 nm
injection . . . . . 2.0 µL
sample . . . . . 100 ug/mL in 10% methanol
Application No. . . . . G006283



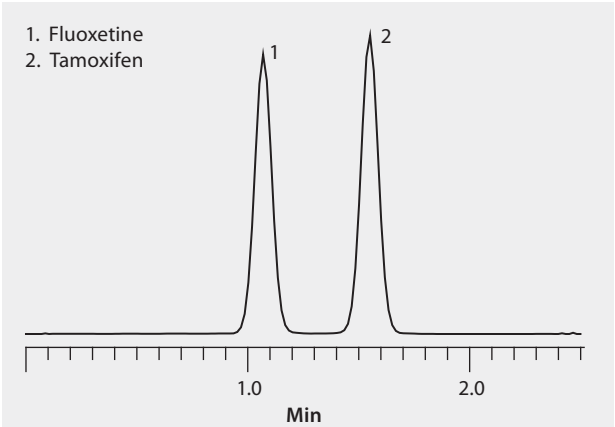
## UHPLC Analysis of Verapamil and Metabolites on Titan™ C18

column . . . . . Titan C18 5 cm x 2.1 mm, 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 10 mM potassium phosphate, monobasic pH 3.0; (B) acetonitrile; (70:30, A:B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 4290 psi (296 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 200 nm
injection . . . . . 1.0 µL
sample . . . . . 10 µg/mL in 70:30, water:methanol
Application No. . . . . G006284



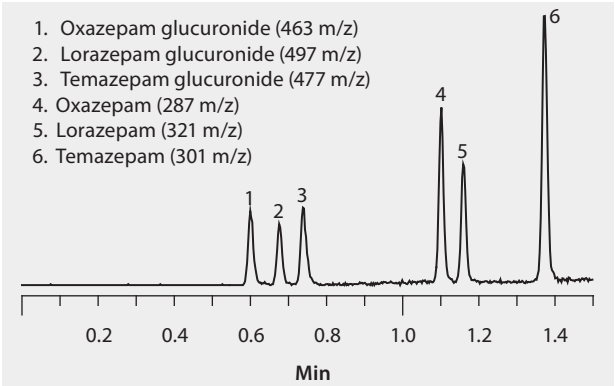
## UHPLC-MS Analysis of Antineoplastic and Antidepressants in Plasma on Titan™ C18 1.9 µm after SPE using HybridSPE®-PLus

| | |
|---|---|
| sample/matrix | rat plasma |
| SPE tube/cartridge | HybridSPE-PLus 96-Well Plate, 50 mg/2 mL per well (575659-U) |
| sample addition | 400 µL of 1% formic acid acetonitile:spiked plasma (3:1) per well |
| sample preparation | final analyte concentration 50 ng/mL |
| column | Titan C18, 5 cm x 2.1 mm I.D., 1.9 µm particles (577122-U) |
| mobile phase | (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in 90:10 (v:v) acetonitrile:water |
| gradient | 35 to 90% B in 1.5 min, held at 90% B for 0.4 min |
| flow rate | 0.4 mL/min |
| pressure | 3582 psi (247 bar) |
| column temp. | 35 °C |
| detector | TOF/MS |
| injection | 1 µL |
| Application No. | G006151 |



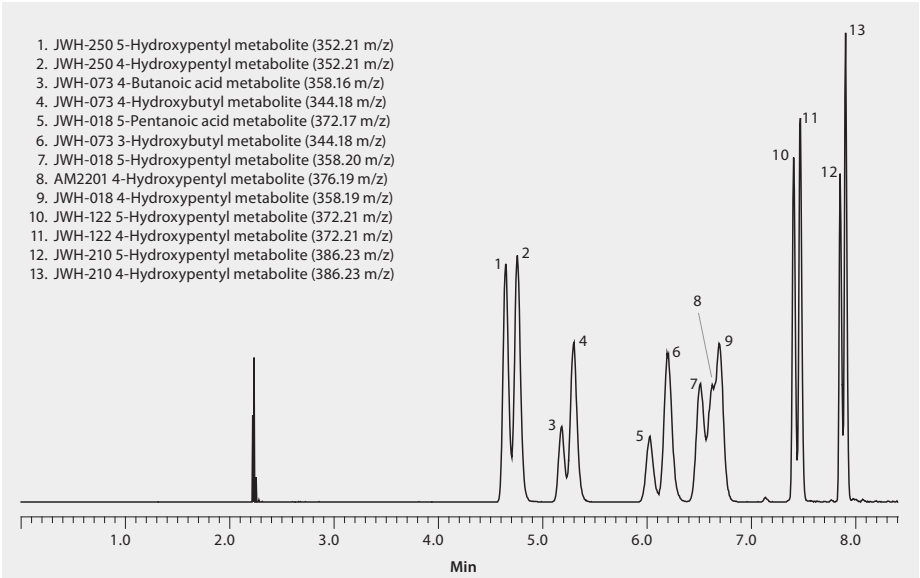
## UHPLC-MS Analysis of Benzodiazepines and Glucuronide Metabolites on Titan™ C18

column . . . . . Titan C18 5 cm x 3.0 mm, 1.9 µm particles (577126-U)
mobile phase . . . . . (A) 0.1% formic acid in 95:5, water:acetonitrile;
(B) 0.1% formic acid in 5:95, water:acetonitrile;
gradient . . . . . 35% B to 60% B in 1 min; 60% B held for 0.5 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 5800 psi (400 bar)
column temp. . . . . 35 °C
detector . . . . . MS-TOF, ESI+, XIC, 100 -1000 m/z scan
injection . . . . . 2 µL
sample . . . . . 300 ng/mL in 97:3, water:methanol
Application No. . . . . G006150



## UHPLC-MS Analysis of Spice Cannabinoid Metabolites from Urine Using Titan™ C18

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| SPE well plate | SupelSelect HLB 96-Well Plate, 60mg/well (575662-U) |
| condition | 1 mL methanol, then 1 mL water |
| sample addition | 100 ng/mL in matrix |
| drying | none |
| washing | 1 mL 20% methanol in water |
| elution | 2 mL methanol |
| eluate post-treatment | none |
| column | Titan C18 10 cm x 2.1 mm, 1.9 µm particles (577124-U) |
| mobile phase | (A) 0.1% formic acid in 95:5, water:acetonitrile; (B) 0.1% formic acid in 5:95, water:acetonitrile; |
| gradient | 25% B held for 1 min; to 45% B in 0.5 min; 45% B held for 4.5 min; to 80% B in 0.5 min; 80% B held for 4 min |
| flow rate | 0.5 mL/min |
| pressure | 8756 psi (604 bar) |
| temp. | 35 °C |
| detector | MS-ESI+,100 - 1000 m/z scan, XIC |
| injection | 2.0 µL |
| sample | 100 ng/mL in methanol |
| Application No. | G006307 |



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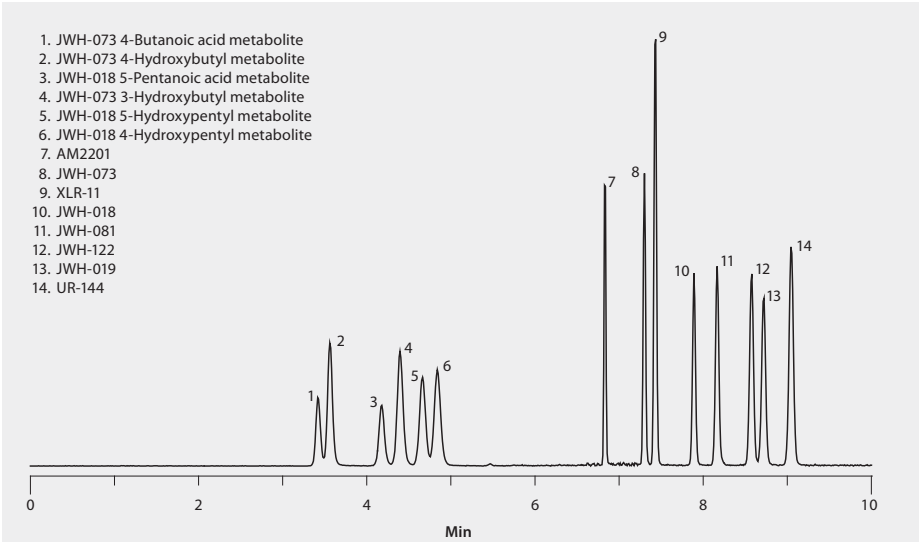
## UHPLC-MS Analysis of Spice Cannabinoids and Metabolites Using Titan™ C18

column . . . . . Titan C18 10 cm x 2.1 mm, 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid in 95:5, water:acetonitrile;
(B) 0.1% formic acid in 5:95, water:acetonitrile;
gradient . . 45% B held for 4.5 min; to 80% B in 1.5 min; 80% B held for 4 min
flow rate . . . . . 0.5 mL/min
pressure . . . . . 7208 psi (497 bar)
column temp. . . . . 35 °C
detector . . . . . MS-ESI+,100 - 1000 m/z scan, XIC
injection . . . . . 2 µL
sample . . . . . 14 Spice cannabinoids 300 ng/mL in 80:20, water:methanol
Application No. . . . . G006185



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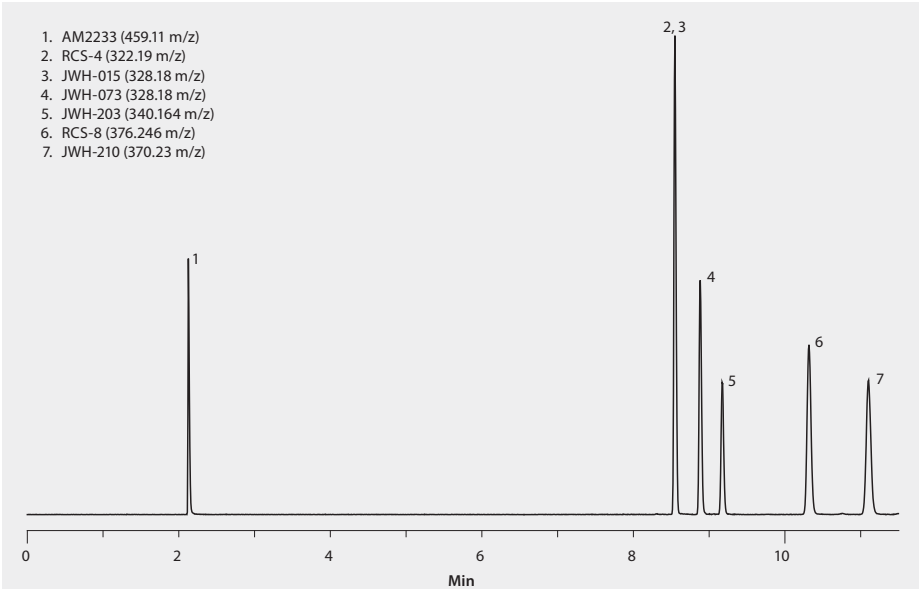
## UHPLC-MS Analysis of Spice Cannabinoids Using Titan™ C18

column . . . . . TITAN C18, 100 mm x 2.1 mm x 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid in 95:5, water:acetonitrile;
(B) 0.1% formic acid in 5:95, water:acetonitrile;
gradient . . 25% B held for 1 min; to 45% B in 0.5 min; 45% B held for 4.5 min;
to 80% B in 0.5 min; 80% B held for 4 min
flow rate . . . . . 0.5 mL/min
pressure . . . . . 8756 psi (604 bar)
column temp. . . . . 35 °C
detector . . . . . MS-ESI+,100 - 1000 m/z scan, XIC
injection . . . . . 2.0 µL
sample . . . . . 100 ng/mL in 60:40, water:methanol
Application No. . . . . G006308

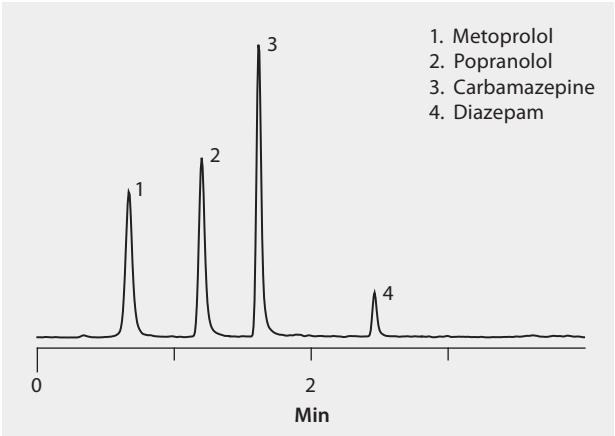





SUPELCO® Solutions within.™

## UHPLC-MS/MS Analysis of Various Drug Substances on Titan™ C18 1.9 µm

column . . . . . Titan C18, 5 cm x 2.1 mm I.D., 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 5 mM ammonium formate;
(B) 5 mM ammonium formate in 90:10 (v:v) acetonitrile:water
gradient . . . . . 30 to 70% B in 3.0 min
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS/MS
injection . . . . . 3.0 µL
sample . . . . . 50 ng/mL in methanol
Application No. . . . . G006149



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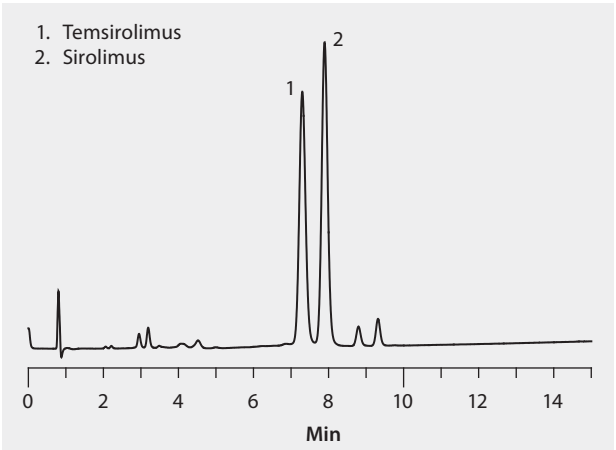
## UHPLC Separation of Temsirolimus and Sirolimus on Titan™ C18

column . . . . . Titan C18 10 cm x 2.1 mm, 1.9 um particles (577124-U)
mobile phase . . . . . (A) 0.1% formic acid in water, (B) methanol
gradient . . . . . 75% B for 2 minutes, 75% B to 90% B from 2 to 12 minutes, 90% B from 12 to 14 minutes.
flow rate . . . . . 0.3 mL/min
pressure . . . . . 3300 psi (228 bar)
column temp. . . . . 55 °C
detector . . . . . UV, 276 nm
injection . . . . . 2.0 µL
sample . . . . . 100 ug/mL each in 20% methanol
Application No. . . . . G006270



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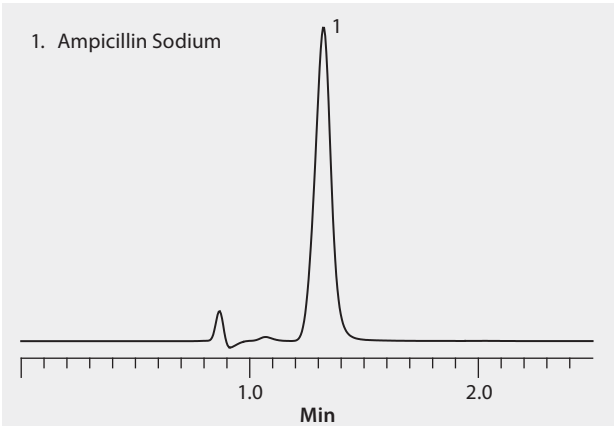
## USP HPLC Analysis of Ampicillin Sodium on Ascentis® Express 5µm C18

This application was generated by RTC on an Ascentis Express C18.

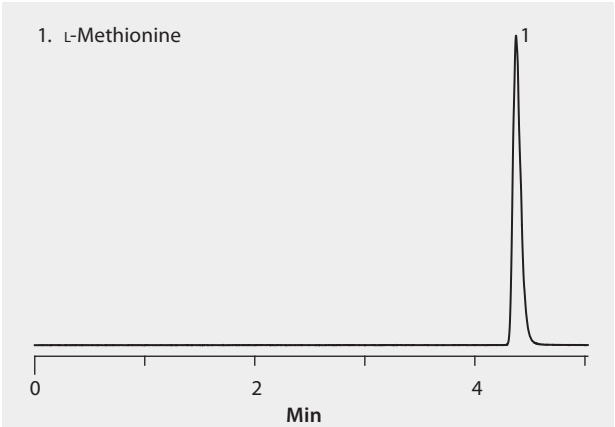
column . . Ascentis Express C18, 10 cm × 4.6 mm I.D., 5 µm particles (50536-U)
mobile phase . . . . (A:B:C:D) (A) acetonitrile,(B)water, (C)1M Potassium phosphate monobasic in water, (D)1N acetic acid in water (80:909:10:1)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL in mobile phase
Application No. . . . . G006262



## USP HPLC Analysis of L-Methionine on Ascentis® Express OH5

This USP application was generated by RTC on an Ascentis® Express OH5 column.

column . . . . . Ascentis Express OH5 15 cm x 4.6 mm, 5 µm particles (50347-U)
mobile phase . . . . . (A)0.005M Ammounium formate in Water (pH: 3.15 with Trifluoroacetic Acid) ), (B)Acetonitrile (30:70, A:B)
flow rate . . . . . 1.0 mL/min
pressure . . . . . 914 psi (63 bar)
column temp. . . . . 40 °C
detector . . . . . Agilent 380-ELSD
injection . . . . . 3.0 µL
sample . . . . . 0.8 mg/mL water:acetonitrile (1:1)
Application No. . . . . G006286



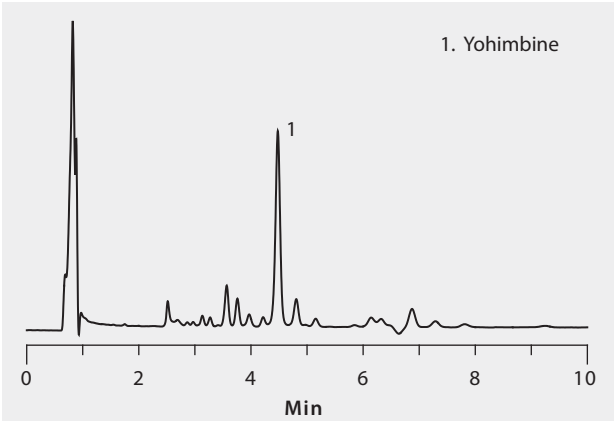



SUPELCO® Solutions within.™

# Vitamins, Nutraceuticals, and Natural Products

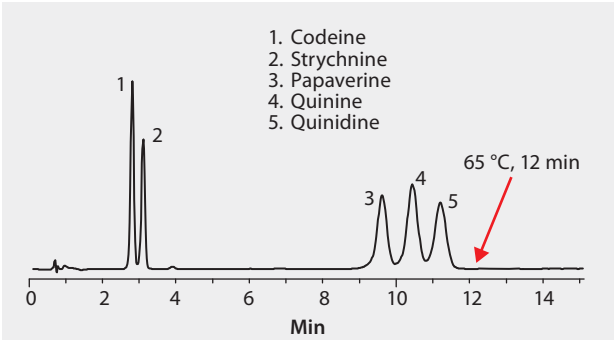
## HPLC Analysis of Alkaloids from Herbal Supplements on Ascentis® Express F5, HILIC Mode

column . . . Ascentis® Express F5, 3 cm x 3.0 mm I.D., 2.7 µm particles (53574-U)
mobile phase . . . . . 2 mM ammonium acetate in 10:90 water:acetonitrile, pH to 6.0 with acetic acid
flow rate . . . . . 0.5 mL/min
pressure . . . . . 1100 psi (76 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 275 nm
injection . . . . . 2 µL
sample . . . . . methanolic extract of Yohimbe Herbal Supplement
Application No. . . . . G005905



## HPLC Analysis of Alkaloids on Discovery® Zr-PBD

column . . . . . Discovery Zr-PBD, 15 cm × 4.6 mm I.D., 5 µm particles (65723-U)
mobile phase . . . . . (A) 20 mM potassium phosphate (pH 12): (B) acetonitrile; (90:10, A:B)
flow rate . . . . . 2.35 mL/min
column temp. . . . . 65 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL each compound 50 µg/mL
Application No. . . . . G001840



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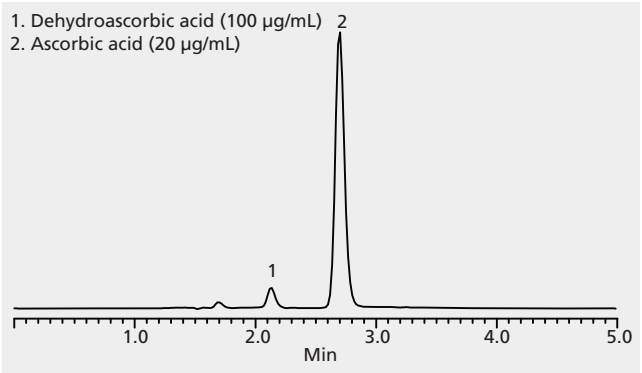
## HPLC Analysis of Ascorbic Acid on Ascentis® RP-Amide

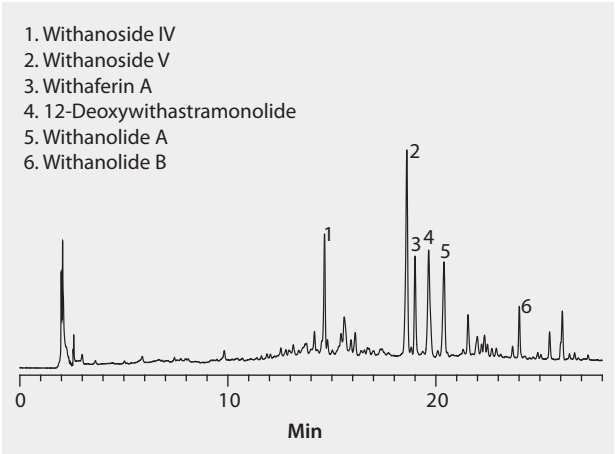
column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . 25 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
sample . . . . . as indicated each in mobile phase
Application No. . . . . G002722



## HPLC Analysis of Aswagandha Extract on Discovery® C18

This application demonstrates the suitability of Discovery C18 for the efficient separation of withania extract by the USP monograph.

column . . . . . Discovery C18, 25 cm x 4.6 mm I.D., 5 µm particles (504971)
mobile phase . . . . . (A) 1 mM potassium phosphate, monobasic; (B) acetonitrile
gradient . . 5 to 45% B in 18 min; 45 to 80% B in 7 min; held at 80% B for 3 min
flow rate . . . . . 1.5 mL/min
pressure . . . . . 3191 psi (220 bar)
column temp. . . . . 27 °C
detector . . . . . UV, 227 nm
injection . . . . . 20 µL
sample . . . . . 20 µg/mL in water: acetonitrile (86:14)
Application No. . . . . G005669



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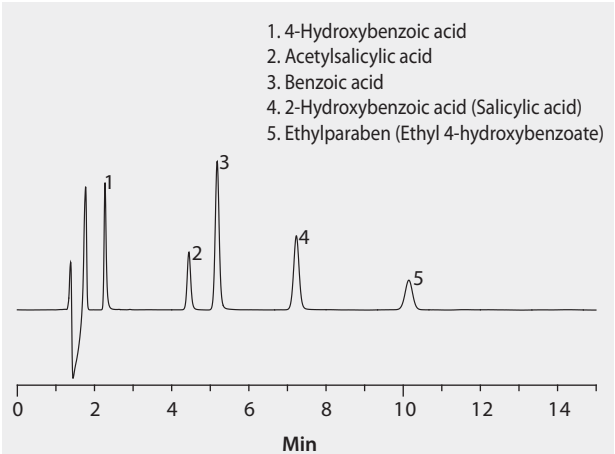
## HPLC Analysis of Benzoic Acid Derivatives on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . (A) 10mM TFA in water; (B) 10 mM TFA in acetonitrile; (70:30, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G005676

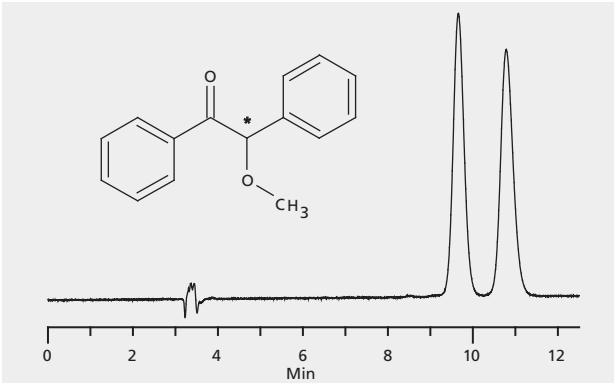





SUPELCO® Solutions within.™

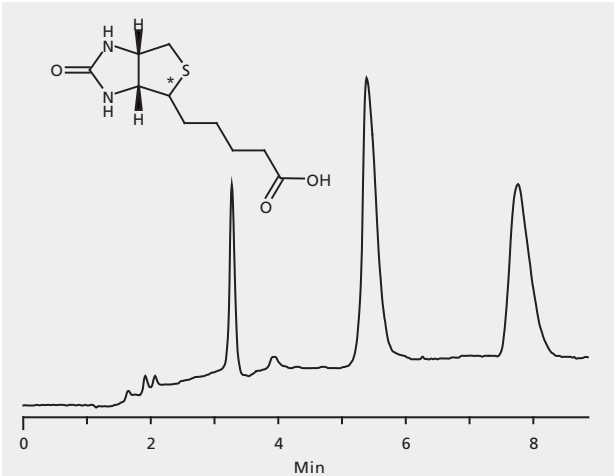
## HPLC Analysis of Benzoin Methyl Ether Enantiomers on Astec® CHIROBIOTIC® V

column . . . . . CHIROBIOTIC V, 25 cm x 4.6 mm I.D., 5 µm particles (11024AST)
mobile phase . . . . . (A) 1% triethylamine, pH 7.0; (B) THF; (90:10, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 2 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004618



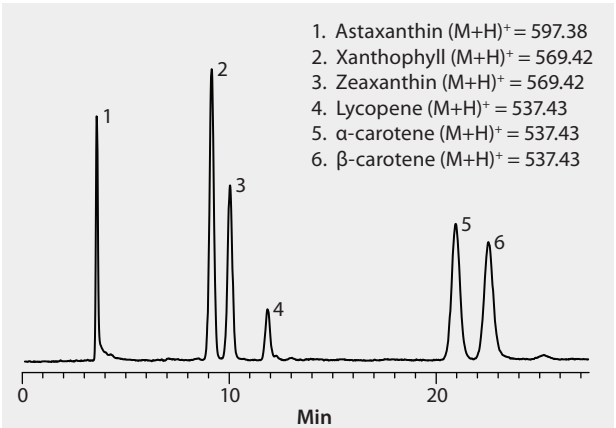
## HPLC Analysis of Biotin Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . (A) 0.1% sodium phosphate, pH 4.1; (B) methanol; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 23 °C
detector . . . . . UV, 210 nm
injection . . . . . 2 µL
sample . . . . . 4 mg/mL in methanol
Application No. . . . . G004540



## HPLC Analysis of Carotene Compounds on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . 100% acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+) in selective ion monitoring (SIR) mode
injection . . . . . 5 µL
sample . . . . . 10 µg/mL each in 100% acetonitrile
Application No. . . . . G003138



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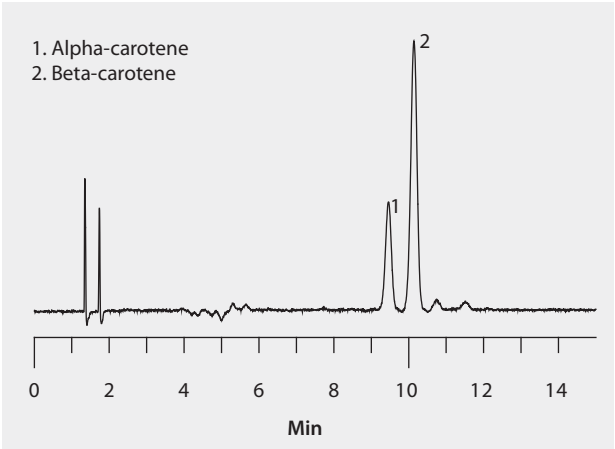
## HPLC Analysis of Carotenes on Ascentis® Express RP-Amide, Methanol Mobile Phase

Analysis was conducted using juice extract, detection via UV.

column . . . . . Ascentis Express RP-Amide, 15 cm x 4.6 mm I.D., 2.7 µm particles (53931-U)
mobile phase . . . . . methanol
flow rate . . . . . 1 mL/min
pressure . . . . . 1653 psi (114 bar)
column temp. . . . . 30 °C
detector . . . . . UV-VIS, 454 nm
injection . . . . . 5 µL
sample . . . . . V8 Splash® fruit juice was extracted with hexane, the extract dissolved 1:1 into ethanol for HPLC injection
Application No. . . . . G005859



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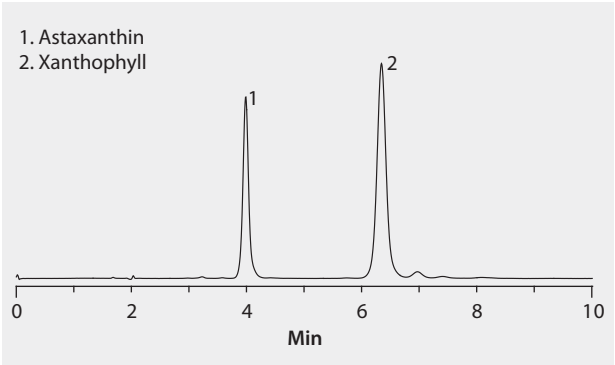
## HPLC Analysis of Carotenoids by Normal Phase Chromatography on Ascentis® Si

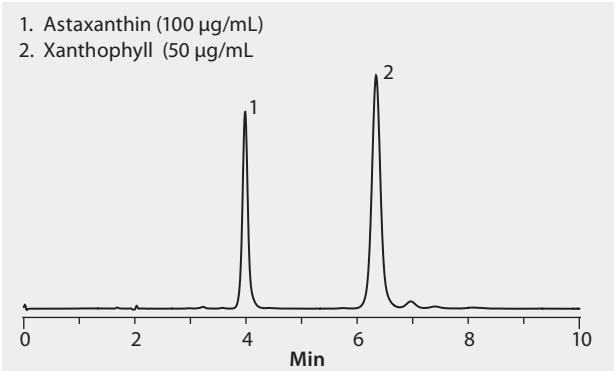
Ascentis silica is used in the analysis of naturally occurring carotenoid pigments in the form of alcohols, xanthophyll and astaxanthin.

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . (A) hexane; (B) ethyl acetate; (C) isopropanol; (85:10:5, A:B:C)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 450 nm
injection . . . . . 10 µL
sample . . . . . 50-100 µg/mL hexane
Application No. . . . . G005685



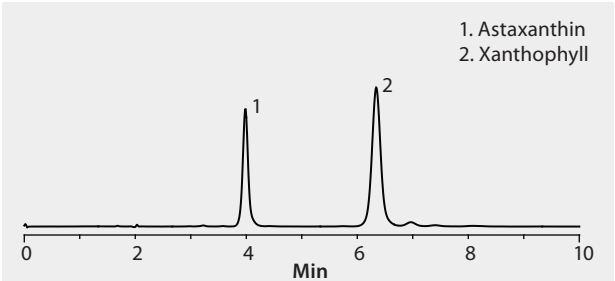
## HPLC Analysis of Carotenoids on Ascentis® Si (Silica), Normal Phase

column . . . . . Ascentis Si, 15 cm × 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . (A) hexane: (B) ethyl acetate: (C) isopropanol; (85:10:5, A:B:C)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV-VIS, 450 nm
injection . . . . . 10 µL
sample . . . . . as indicated in hexane
Application No. . . . . G003813



## HPLC Analysis of Carotenoids on Ascentis® Si

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . (A) hexane; (B) ethylacetate; (C) isopropanol; (85:10:5, A:B:C:)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV-VIS, 450 nm
injection . . . . . 10 µL
sample . . . . . as indicated in hexane
Application No. . . . . G003726



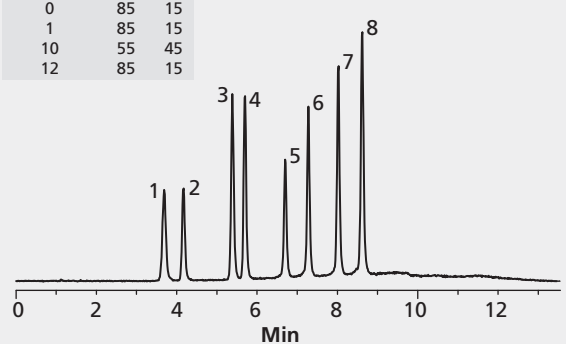
## HPLC Analysis of Catechins on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with concentrated formic acid); (B) acetonitrile
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI (-) in selected ion recording (SIR) mode
injection . . . . . 5 µL
sample . . . . . 10 µg/mL each in water:acetonitrile 1:1
Application No. . . . . G002723

1. Gallocatechin, m/z 305
2. Epigallocatechin, m/z 305
3. Catechin, m/z 289
4. Epicatechin, m/z 289
5. Epigallocatechin gallate, m/z 457
6. Gallocatechin gallate, m/z 457
7. Epicatechin gallate, m/z 441
8. Catechin gallate, m/z 441

**Gradient Program**

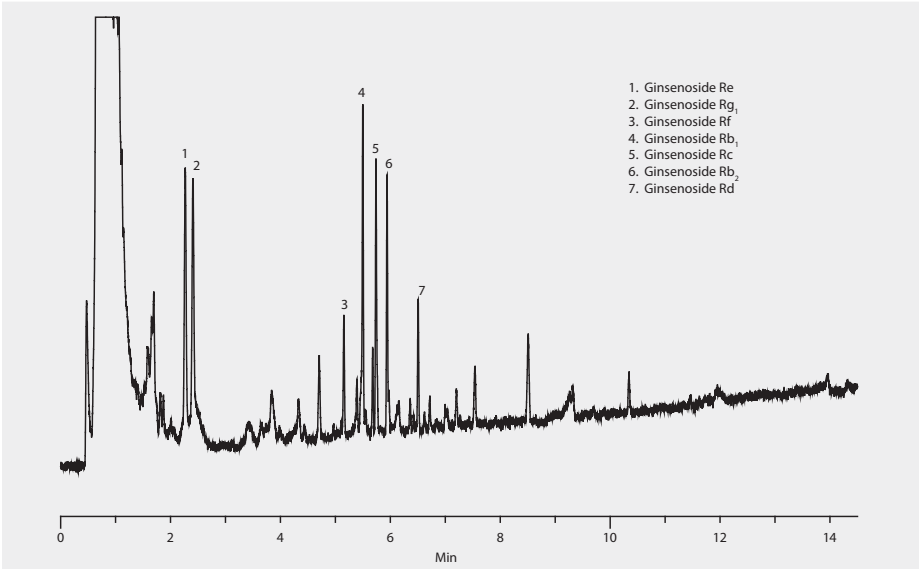
| Time (min) | %A | %B |
|---|---|---|
| 0 | 85 | 15 |
| 1 | 85 | 15 |
| 10 | 55 | 45 |
| 12 | 85 | 15 |



## HPLC Analysis of Chinese Ginseng Extract on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of Chinese ginseng root extract.

column . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . held at 25% B for 1.5 min; 25 to 85% B in 12 min; held at 85% for 1 min
flow rate . . . . . 1 mL/min
pressure . . . . . 3481 psi (240 bar)
column temp. . . . . 60 °C
detector . . . . . UV, 205 nm
injection . . . . . 25 µL
sample . . . . . Extracted from dried, ground plant material; 10 mg/mL in water:acetonitrile (85:15); sonicate 10 min at ambient
Application No. . . . . G005396



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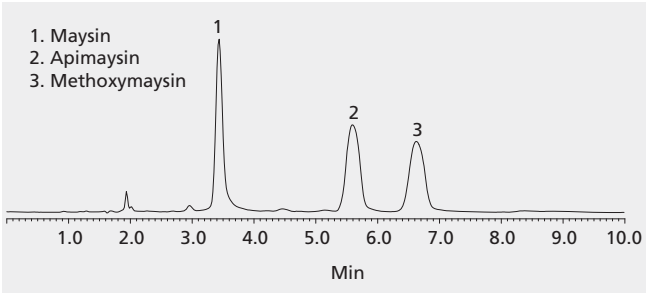
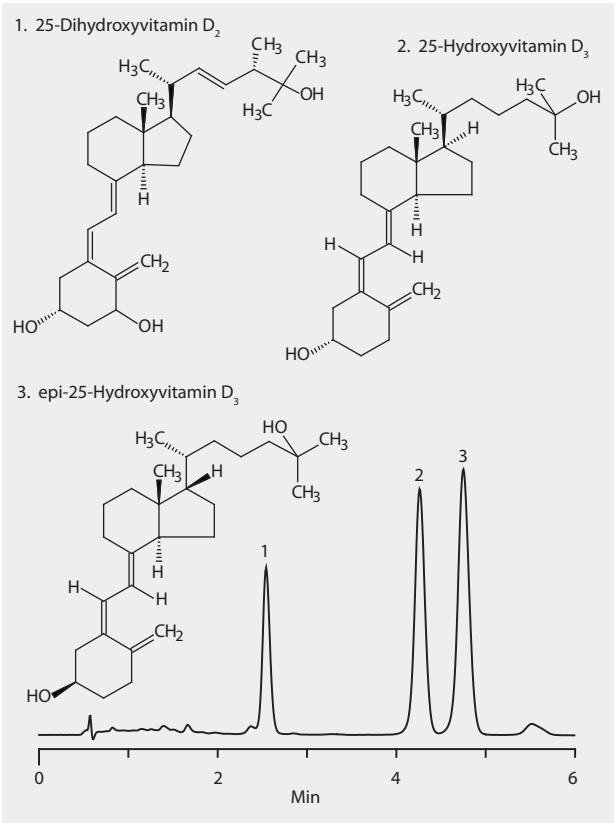
## HPLC Analysis of Conjugated Flavonoids on Discovery® HS-C18

column . . . . Discovery HS C18, 15 cm × 4.6 mm I.D., 3 µm particles (569252-U)
mobile phase . . . . . (A) 10 mM sodium citrate (pH 7.0 with 10 mM citric acid); (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 60 °C
detector . . . . . UV, 340 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each (Maysin, Apimaysin, Methoxymaysin) in 10 mM sodium citrate (pH 7.0 with 10 mM citric acid)
Application No. . . . . G002048



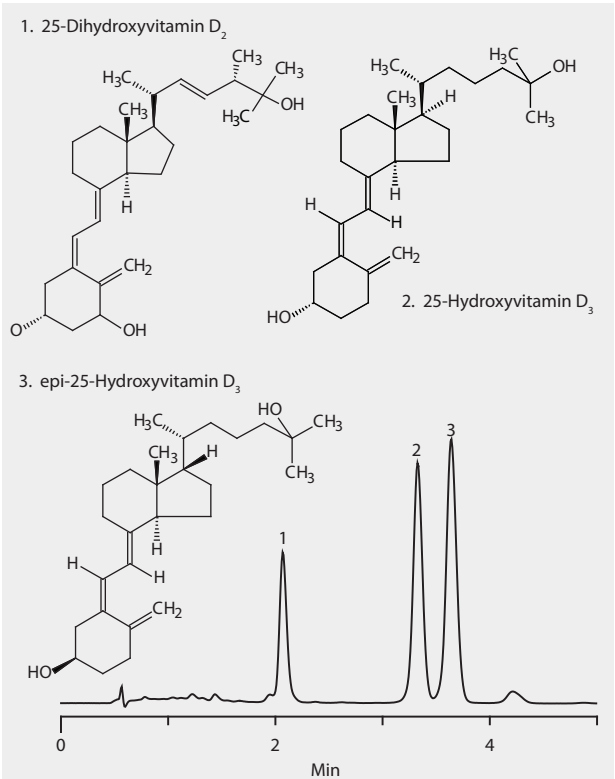
## HPLC Analysis of 25-Dihydroxyvitamin D2, 25-Hydroxyvitamin D3 and 3-epi-25-Hydroxyvitamin D3 on Ascentis® Express F5, 20 °C

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) 5 mM ammonium formate; (B) methanol:water (75:25)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 3553 psi (245 bar)
column temp. . . . . 20 °C
detector . . . . . (UV, 265 nm, ESI(+), m/z 100 - 1000)
injection . . . . . 1 µL
sample . . . . . 500 mg/L in 25:75, water:methanol
Application No. . . . . G005375



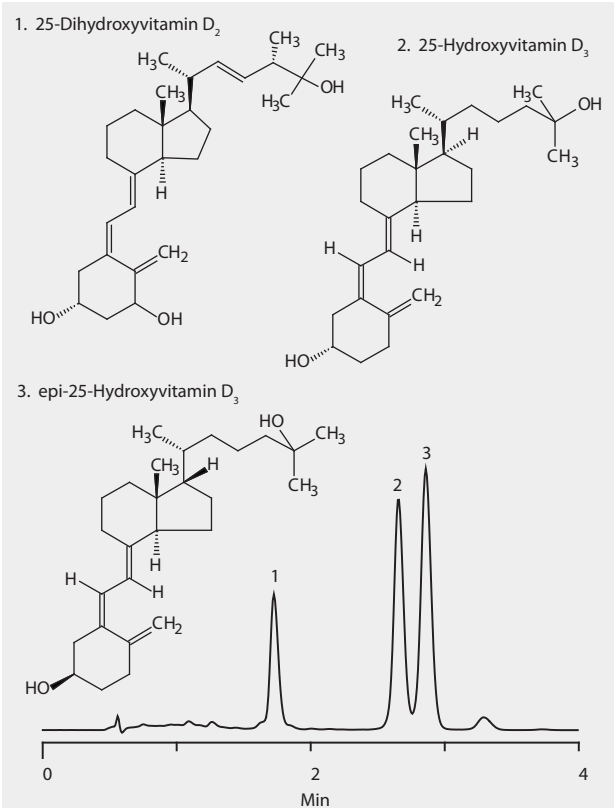
## HPLC Analysis of 25-Dihydroxyvitamin D2, 25-Hydroxyvitamin D3 and 3-epi-25-Hydroxyvitamin D3 on Ascentis® Express F5, 30 °C

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . (A) 5 mM ammonium formate in methanol; (B) water (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 4293 psi (296 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 265 nm
ESI(+), m/z 100 - 1000
injection . . . . . 1 µL
sample . . . . . 500 µg/mL in methanol
Application No. . . . . G005376



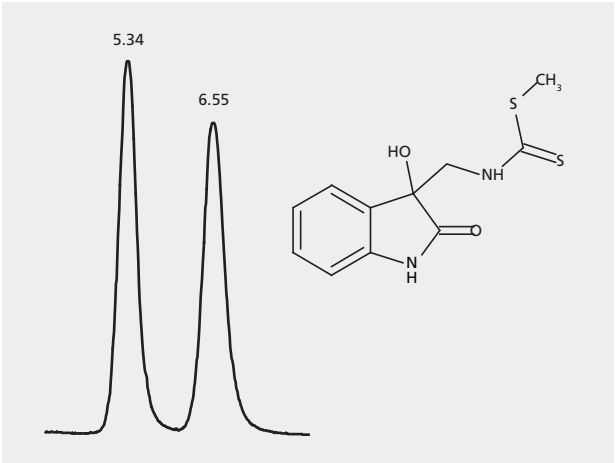
## HPLC Analysis of 25-Dihydroxyvitamin D2, 25-Hydroxyvitamin D3 and 3-epi-25-Hydroxyvitamin D3 on Ascentis® Express F5, 40 °C

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . (A) 5 mM ammonium formate in methanol; (B) water (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 5221 psi (360 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 265 nm
ESI(+), m/z 100 - 1000
injection . . . . . 1 µL
sample . . . . . 300 mg/L in 25:75, water:methanol
Application No. . . . . G005377



## HPLC Analysis of Dioxibrassinin Enantiomers on Astec® P-CAP™

column . . Astec (R,R) P-CAP-DP, 25 cm x 4.6 mm I.D., 5 µm particles (35024AST)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (97:3, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 255 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004400





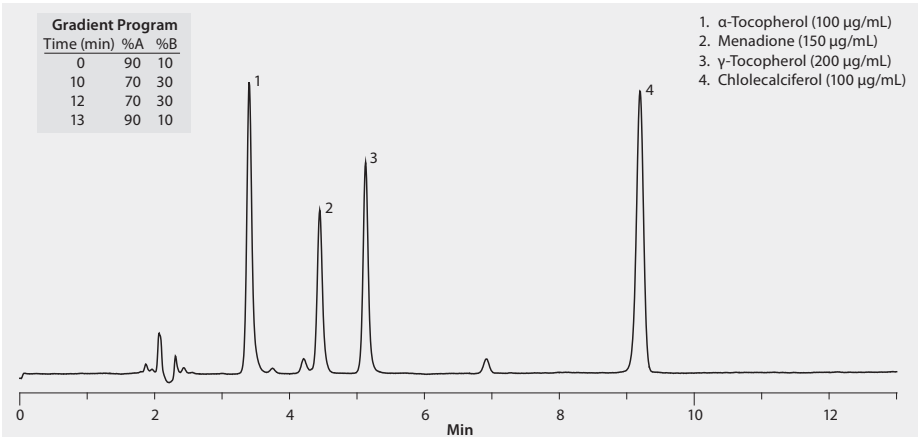

SUPELCO® Solutions within.™

## HPLC Analysis of Fat Soluble Vitamins by Normal Phase Chromatography on Ascentis® Si, 15 cm Column

column . . . . . Ascentis Si, 15 cm × 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) hexane, (B) ethyl acetate
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 96:4 (hexane:isopropanol)
Application No. . . . . G003693

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 90 | 10 |
| 10 | 70 | 30 |
| 12 | 70 | 30 |
| 13 | 90 | 10 |





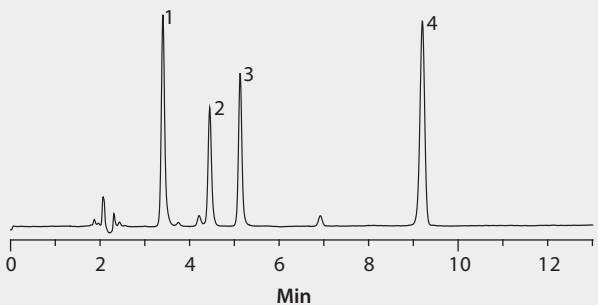

SUPELCO® Solutions within.™

## HPLC Analysis of Fat Soluble Vitamins by Normal Phase Chromatography on Ascentis® Si (25 cm Column)

Ascentis silica is used in the analysis of fat-soluble vitamins using a normal phase gradient. These include vitamin K 3 (menadione), vitamin E (alpha and gamma tocopherol), and vitamin D3 (cholecalciferol).

| | |
|---|---|
| column | Ascentis Si, 25 cm x 4.6 mm I.D., 5 µm particles (581512-U) |
| mobile phase | (A) hexane; (B) ethyl acetate |
| gradient | 10 to 30% B in 10 min; held at 30% B for 2 min |
| flow rate | 1.0 mL/min |
| column temp. | 30 °C |
| detector | UV, 290 nm |
| injection | 10 µL |
| Application No. | G005697 |

1. alpha-Tocopherol
2. Menadione
3. gamma-Tocopherol
4. Chlolecalciferol



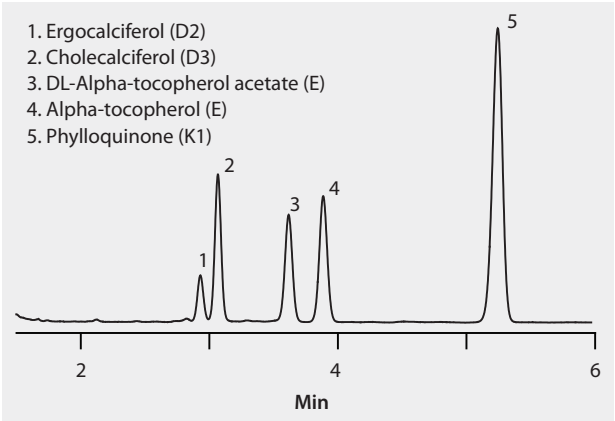



SUPELCO® Solutions within.™

## HPLC Analysis of Fat Soluble Vitamins on Ascentis® Express RP-Amide, Methanol Mobile Phase

Analysis was conducted using standard solution, detection via UV.

| | |
|---|---|
| column | Ascentis Express RP-Amide, 15 cm x 4.6 mm I.D., 2.7 µm particles (53931-U) |
| mobile phase | methanol |
| flow rate | 1.0 mL/min |
| pressure | 1653 psi (114 bar) |
| column temp. | 30 °C |
| detector | UV, 280 nm |
| injection | 5 µL |
| sample | 0.4 mg/mL tocopherol acetate, alpha-tocopherol and K1, 0.04 mg/mL D2 and D3, all in ethanol |
| Application No. | G005862 |



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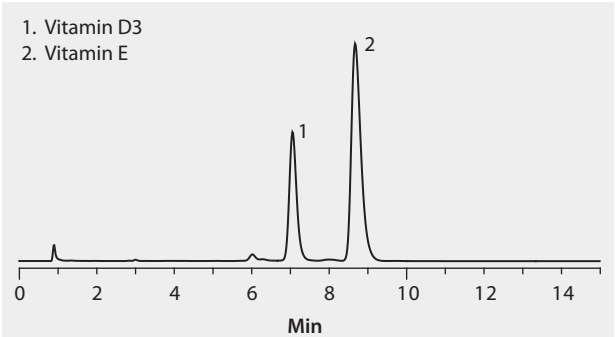
## HPLC Analysis of Fat Soluble Vitamins on Ascentis® RP-Amide

The Ascentis RP-Amide is shown to be suitable for the analysis of fat soluble vitamins, alpha tocopherol (vitamin E) and cholecalciferol (vitamin D3 ).

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . acetonitrile
flow rate . . . . . 2.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 215 nm
injection . . . . . 20 µL
Application No. . . . . G005698

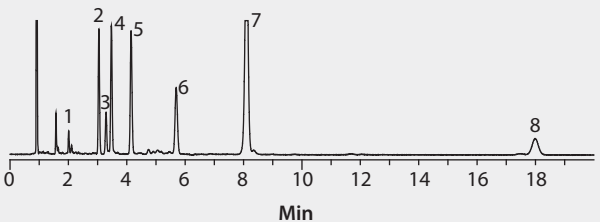


## HPLC Analysis of Fat-Soluble Vitamins on Ascentis® Express C18, Methanol Mobile Phase

Analysis was conducted using standard solution, detection via UV.

column . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)
mobile phase . . . . . methanol
flow rate . . . . . 1.5 mL/min
pressure . . . . . 3249 psi (224 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 280 nm
injection . . . . . 5 µL
sample . . . . . 0.5 mg/mL tocopherol acetate, alpha-tocopherol, delta-tocopherol and K1, 0.05 mg/mL D2, D3 and retinol acetate, 1.5 mg/mL retinol palmitate, all in ethanol.
Application No. . . . . G005861

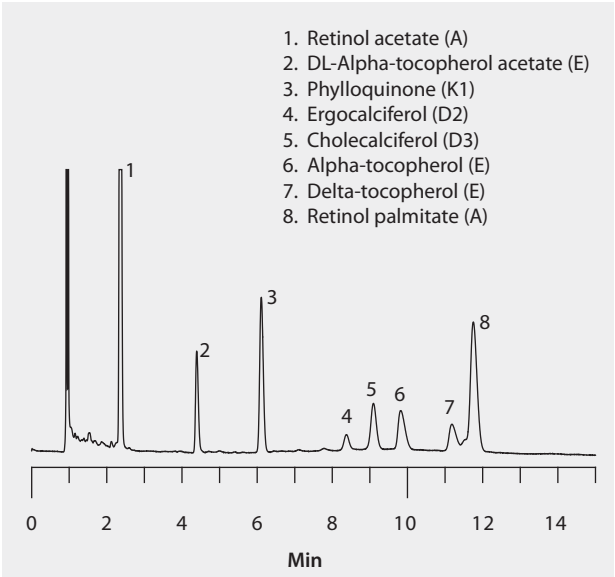
1. Retinol acetate (A)
2. Delta-tocopherol (E)
3. Ergocalciferol (D2)
4. Cholecalciferol (D3)
5. Alpha-tocopherol (E)
6. DL-alpha-tocopherol acetate (E)
7. Phylloquinone (K1)
8. Retinol palmitate (A)



## HPLC Analysis of Fat Soluble Vitamins on Ascentis® Express RP-Amide, Acetonitrile Mobile Phase

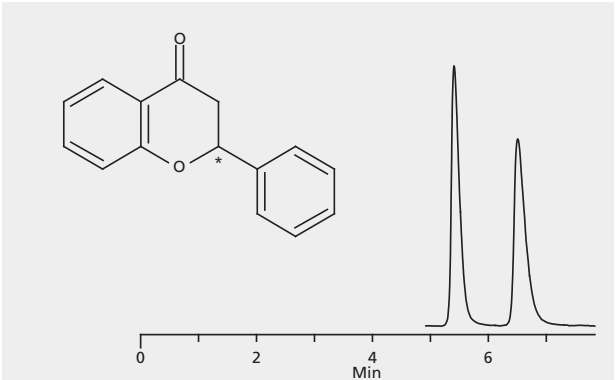
Analysis was conducted using standard solution, detection via UV.

| | |
|---|---|
| column | Ascentis Express RP-Amide, 15 cm x 4.6 mm I.D., 2.7 µm particles (53931-U) |
| mobile phase | acetonitrile |
| flow rate | 1.5 mL/min |
| pressure | 1682 psi (116 bar) |
| column temp. | 30 °C |
| detector | UV, 280 nm |
| injection | 5 µL |
| sample | 0.5 mg/mL tocopherol acetate, alpha-tocopherol, delta-tocopherol and K1, 0.05 mg/mL D2, D3 and retinol acetate, 1.5 mg/mL retinol palmitate, all in ethanol |
| Application No. | G005860 |



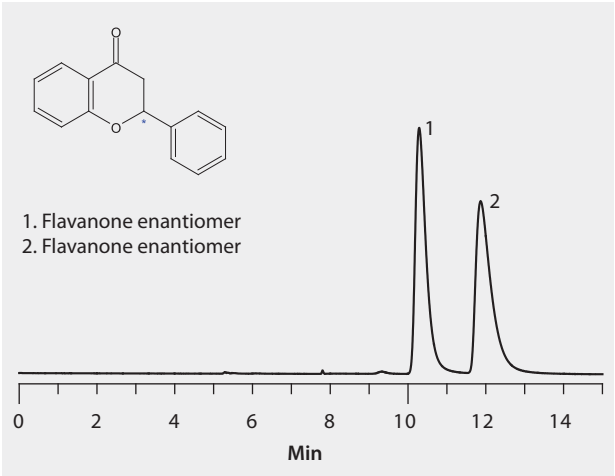
## HPLC Analysis of Flavanone Enantiomers on Astec® CYCLOBOND® I 2000 DMP, Ethanol:Hexane Mobile Phase

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . . . . (A) ethanol; (B) hexane; (20:80, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004697



## HPLC Analysis of Flavanone Enantiomers on Astec® CYCLOBOND® I 2000 DMP, IPA:Heptane Mobile Phase

column . . CYCLOBOND I 2000 DMP, 25 cm x 4.6 mm I.D., 5 µm particles (20724AST)
mobile phase . . . . . (A) isopropanol; (B) heptane; (30:70, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . flavanone, 1 mg/mL in heptane
Application No. . . . . G005161



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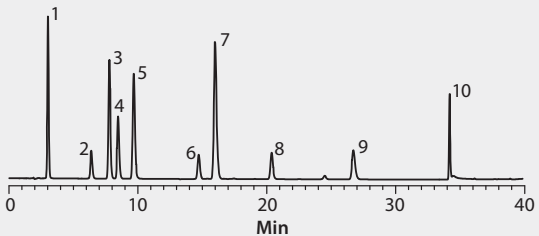
## HPLC Analysis of Flavonoids on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . (A) 0.085% orthophosphoric acid; (B) acetonitrile;
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase A
Application No. . . . . G003025

1. Gallic acid
2. (+) catechin
3. Syringic acid
4. Vanillic acid
5. Caffeic acid
6. Rutin
7. *p*-coumaric acid
8. Quercitrin
9. Myricetin
10. Quercetin

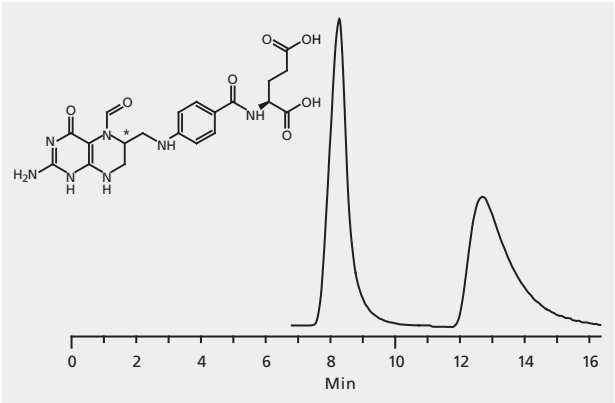
**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 85 | 15 |
| 30 | 65 | 35 |
| 35 | 15 | 85 |



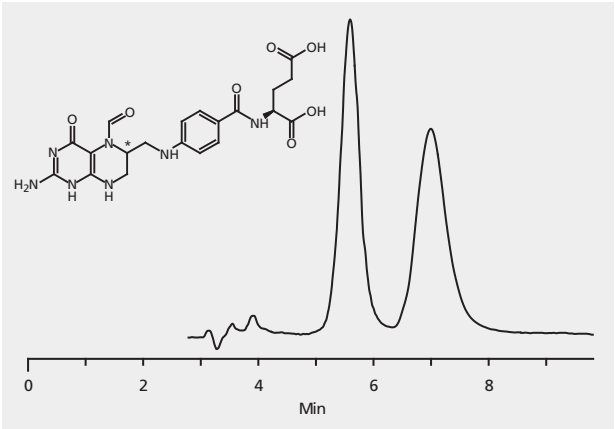
## HPLC Analysis of Folinic Acid Enantiomers on Astec® CHIROBIOTIC® T

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . (A) methanol; (B) acetic acid; (C) triethylamine; (100:1:0.5, A:B:C)
flow rate . . . . . 2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 2 µL
sample . . . . . 5 mg/mL in methanol
Application No. . . . . G004541



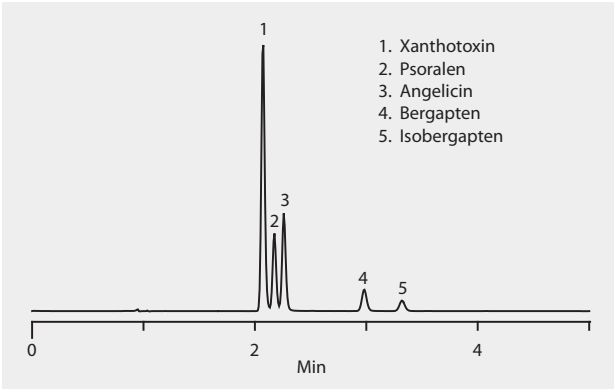
## HPLC Analysis of Folinic Acid Enantiomers on Astec® CHIROBIOTIC® T (RP mode)

column . . . . . CHIROBIOTIC T, 25 cm x 4.6 mm I.D., 5 µm particles (12024AST)
mobile phase . . . (A) 1% triethylamine acetate, pH 4.5; (B) ethanol; (50:50, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 1.25 mg/mL in methanol
Application No. . . . . G004542



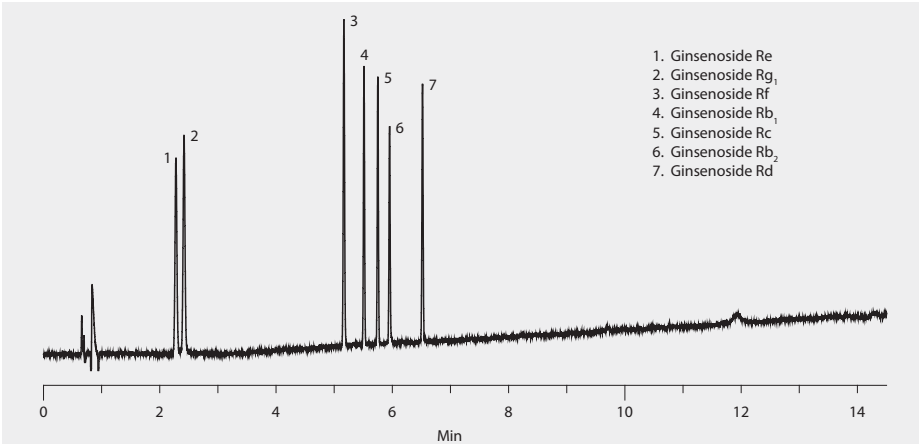
## HPLC Analysis of Furocoumarins on Ascentis® Express RP-Amide

| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 4.6 mm I.D., 2.7 µm particles (53929-U) |
| mobile phase | (A) water; (B) methanol; (40:60, A:B) |
| flow rate | 1.0 mL/min |
| pressure | 2843 psi (196 bar) |
| column temp. | 35 °C |
| detector | UV, 254 nm |
| injection | 5 µL |
| sample | 25 µg/mL in water:methanol (90:10) |
| Application No. | G005394 |



## HPLC Analysis of Ginsenosides on Ascentis® Express C18

column . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . 25% B for 1.5 min; 25 to 85% B in 12 min; hold at 85% B for 1 min
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL in 82:18, water:methanol
Application No. . . . . G005443



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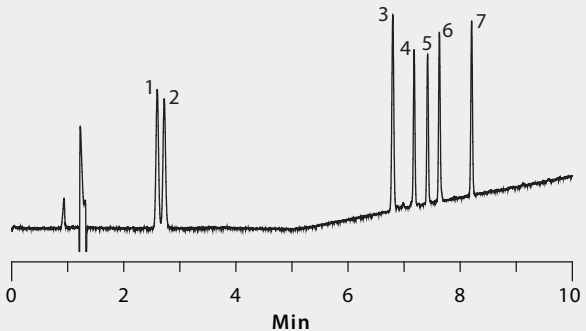
## HPLC Analysis of Ginsenosides on Ascentis® Express 5 µm

column . . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 5 µm particles (50537-U)
mobile phase . . . . . (A) water, (B) acetonitrile
gradient . . . . 25% B held for 3 min; to 85% B in 12 min;85% B held for 1 min
flow rate . . . . . 1 mL/min
pressure . . . . . 1470 psi (101 bar)
column temp. . . . . 60 °C
detector . . . . . UV, 205 nm
injection . . . . . 5 µL
sample . . . . . 50 µg/mL in 82:18, water:acetonitrile
Application No. . . . . G005888

1. Ginsenoside Re
2. Ginsenoside Rg1
3. Ginsenoside Rf
4. Ginsenoside Rb1
5. Ginsenoside Rc
6. Ginsenoside Rb2
7. Ginsenoside Rd



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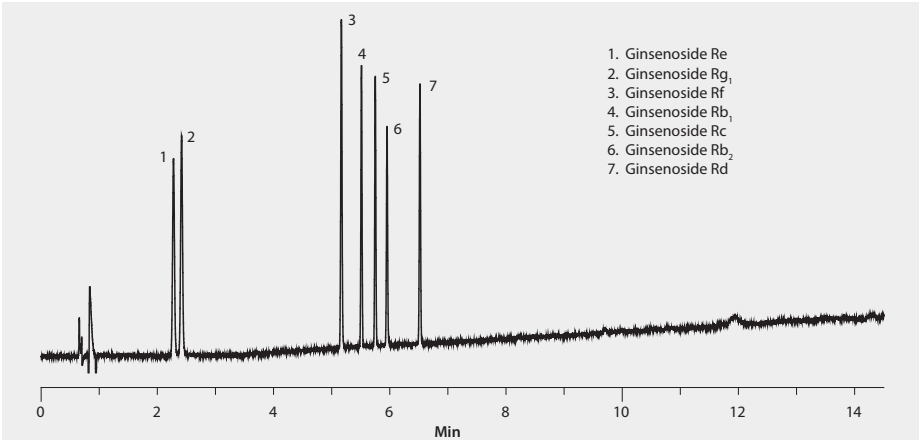
## HPLC Analysis of Ginsenoside Standards on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of ginseng.

column . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U)
mobile phase . . . . . (A): water; (B): acetonitrile
gradient . . held at 25% for 1.5 min; 25 to 85% B in 12 min; held at 85% B for 1 min
flow rate . . . . . 1.0 mL/min
pressure . . . . . 3481 psi (240 bar)
column temp. . . . . 60 °C
detector . . . . . UV, 205 nm
injection . . . . . 25 µL
sample . . . . . 50 µg/mL in water:methanol (82:18)
Application No. . . . . G005397



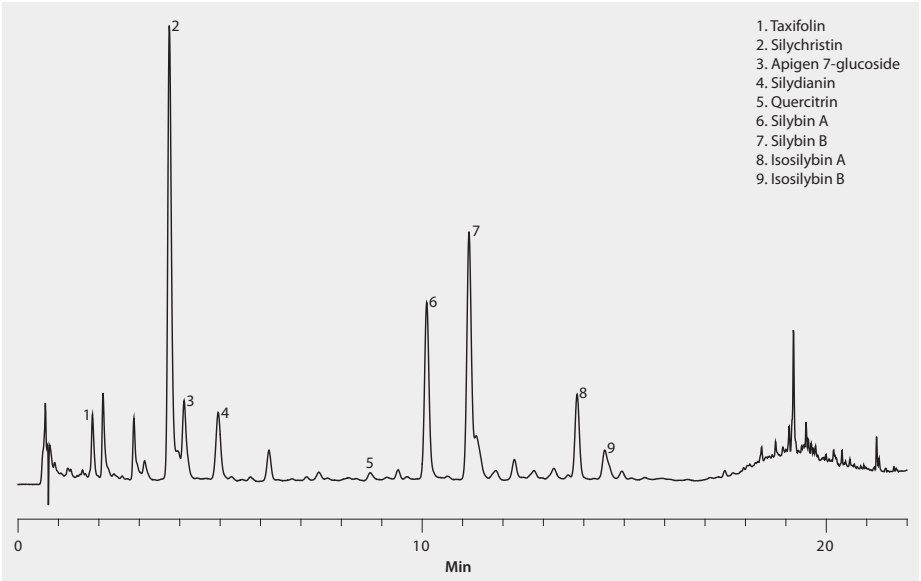



SUPELCO® Solutions within.™

## HPLC Analysis of Herbal Supplement Containing Milk Thistle on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of milk thistle containing herbal supplement.

column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) water with 0.1% formic acid; (B) methanol
gradient . . . . . held at 35% B for 3 min; 35 to 45% B in 10 min; held at 45% B for 2 min; 45 to 100% B in 5 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 4105 psi (283 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 20 µL
sample . . . . . 20 mg/mL in water:ethanol (5:95); sonicate 15 minutes; filter 0.45 µm; dilute to water:ethanol (80:20)
Application No. . . . . G005604

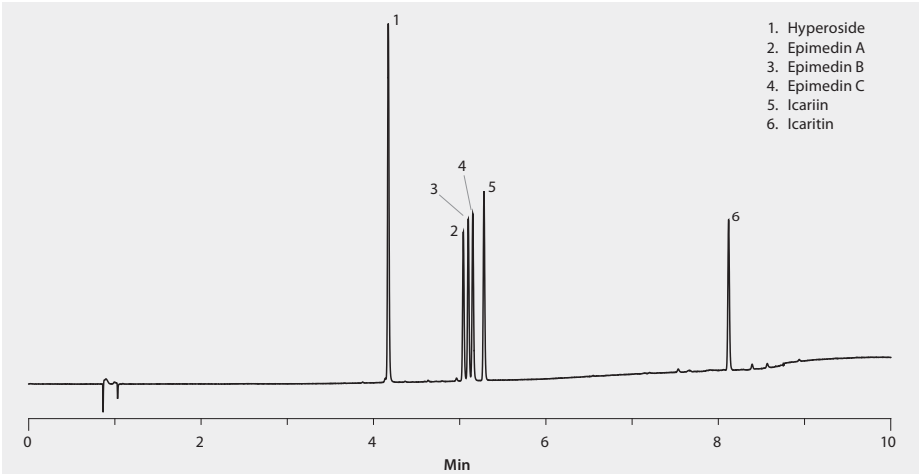





SUPELCO® Solutions within.™

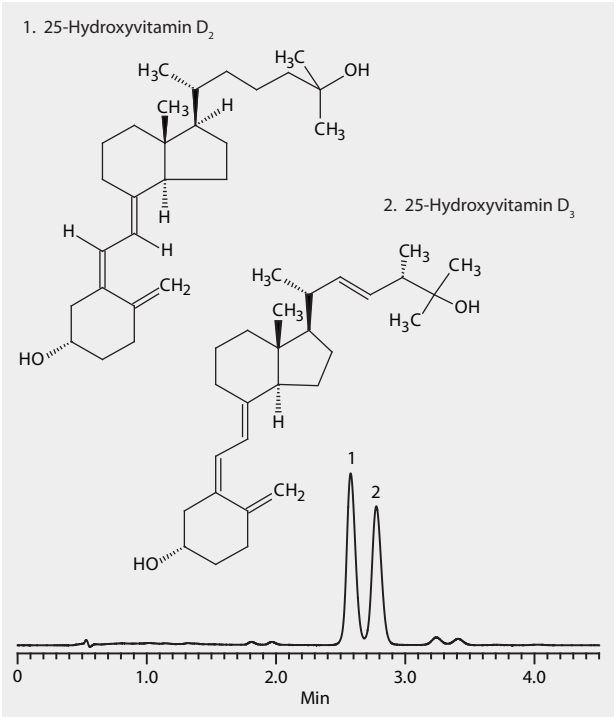
## HPLC Analysis of Horn Goat Weed Standards Using Ascentis® Express C18 5 µm

column . . . Ascentis Express C18, 15 cm x 4.6 mm I.D., 5 µm particles (50537-U)
mobile phase . . (A) 0.1% acetic acid in water; (B) 0.1% acetic acid in acetonitrile;
gradient . . . . . 5% B held for 0.75 min; to 40% B in 3.75 min; to 100% B in 3.5 min; 100% B held for 2 min
flow rate . . . . . 1.5 mL/min
pressure . . . . . 2120 psi (146 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 250 nm
injection . . . . . 5.0 µL
sample . . . . . 50 µg/mL 70:30, water:methanol
Application No. . . . . G006310



## HPLC Analysis of 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 on Ascentis® Express F5

mobile phase . . . . . (A) 7 mM ammonium formate; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 250 nm
ESI(+), TIC m/z 100 - 1000
injection . . . . . 1 µL
sample . . . . . 300 mg/L in 25:75, water:methanol
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
Application No. . . . . G005374

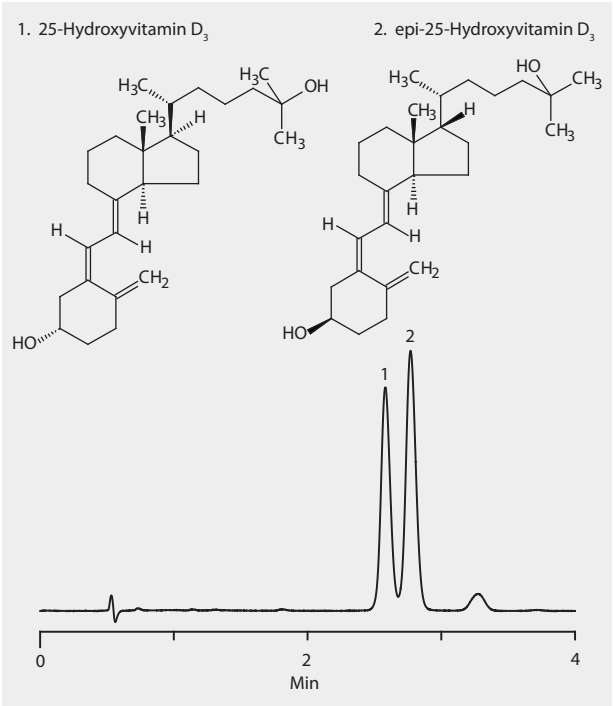





SUPELCO® Solutions within.™

## HPLC Analysis of 25-Hydroxyvitamin D3 and epi-25-Hydroxyvitamin D3 on Ascentis® Express F5

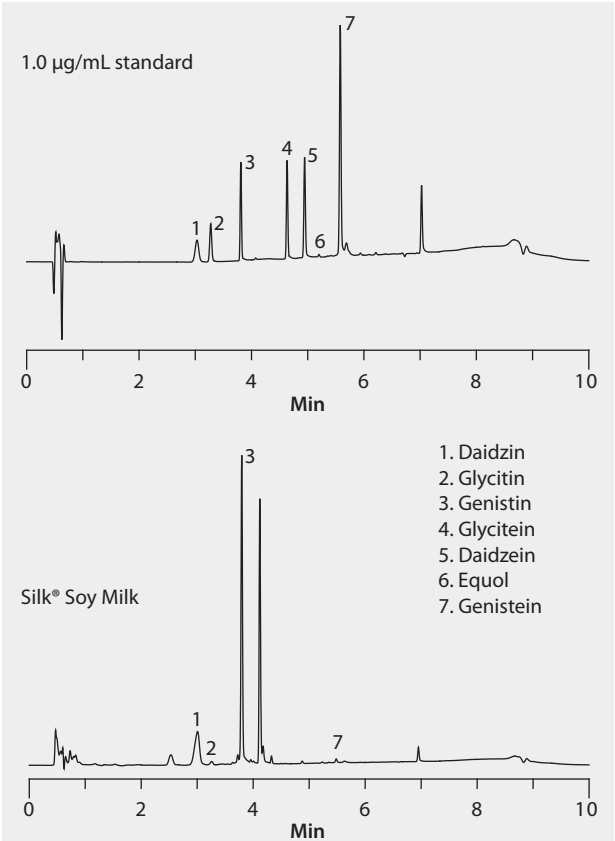
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) 7 mM ammonium formate; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 265 nm
ESI(+), m/z 100 - 1000
injection . . . . . 1 µL
sample . . . . . 300 mg/L in 25:75, water:methanol
Application No. . . . . G005378



## HPLC Analysis of Isoflavones in Soy Milk on Ascentis® Express RP Amide (Milk Brand 1)

This application demonstrates the separation of seven isoflavones on Ascentis Express RP Amide

column . . Ascentis Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in methanol
gradient . . held at 15% B for 1 min; 15 to 100% in 5 min; held at 100% B for 1 min; 100-15% B in 0.1 min; held at 15% B for 2.9 min
flow rate . . . . . 0.8 mL/min
pressure . . . . . 2900 psi (200 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 260 nm
injection . . . . . 10 µL
sample . . . . . soy milk, methanol extract (1. Measure 2 mL of each brand of soy milk (6 samples) 2. Add 8 mL of methanol and shake for 1 hour 3. Centrifuge tubes at 10000 rpm for 30 minutes 4. Transfer aliquot to autosampler vial)
Application No. . . . . G005918



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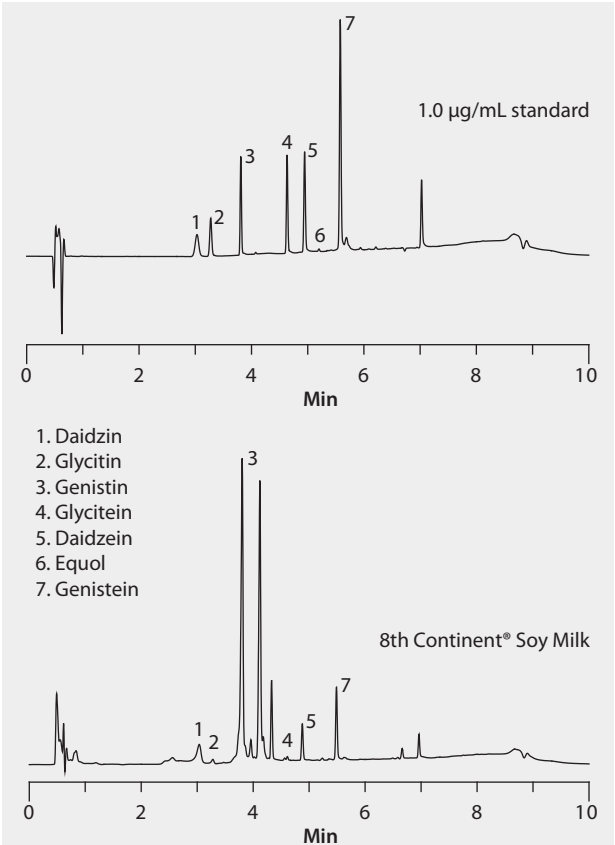
## HPLC Analysis of Isoflavones in Soy Milk on Ascentis® Express RP Amide (Milk Brand 2)

This application demonstrates the separation of seven isoflavones on Ascentis Express RP Amide

column . . Ascentis Express RP-Amide, 10 cm x 3 mm I.D., 2.7 µm particles (53918-U)
mobile phase . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in methanol
gradient . . held at 15% B for 1 min; 15 to 100% in 5 min; held at 100% B for 1 min; 100-15% B in 0.1 min; held at 15% B for 2.9 min
flow rate . . . . . 0.8 mL/min
pressure . . . . . 2900 psi (200 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 260 nm
injection . . . . . 10 µL
sample . . . . . soy milk, methanol extract (1. Measure 2 mL of each brand of soy milk (6 samples) 2. Add 8 mL of methanol and shake for 1 hour 3. Centrifuge tubes at 10000 rpm for 30 minutes 4. Transfer aliquot to autosampler vial)
Application No. . . . . G005917



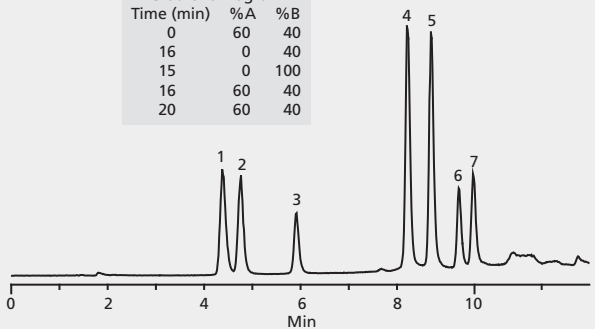
## HPLC Analysis of Isoflavones on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm × 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3.0 with concentrated formic acid), (B) methanol
flow rate . . . . . 1 mL/min split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI(+) in selected ion recording (SIR) mode
injection . . . . . 10 µL
sample . . . . . glycitein, genistein at 1 µg/mL, equol at 50 µg/mL and daidzin, glycitin, genistin at 10 µg/mL in 60:40 10 mM ammonium formate (pH 3.0 with concentrated formic acid):methanol
Application No. . . . . G003868

1. Daidzin (M+H)+ = 417.11
2. Glycitin (M+H)+ = 447.12
3. Genistin (M+H)+ = 433.10
4. Daidzein (M+H)+ = 255.05
5. Glycitein (M+H)+ = 285.06
6. Equol (M+H)+ = 243.09
7. Genistein (M+H)+ = 271.05

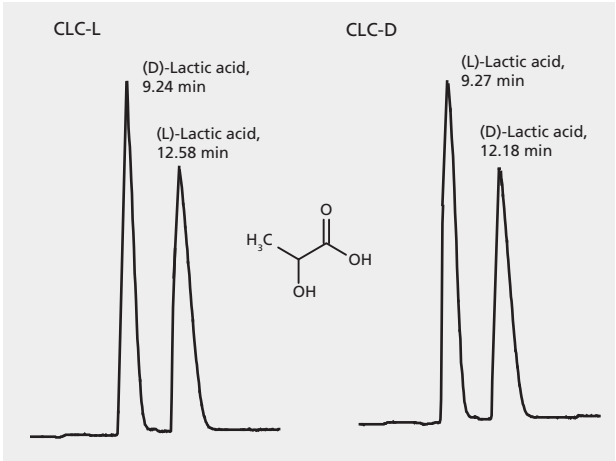
Gradient Program

| Time (min) | %A | %B |
|---|---|---|
| 0 | 60 | 40 |
| 16 | 0 | 40 |
| 15 | 0 | 100 |
| 16 | 60 | 40 |
| 20 | 60 | 40 |



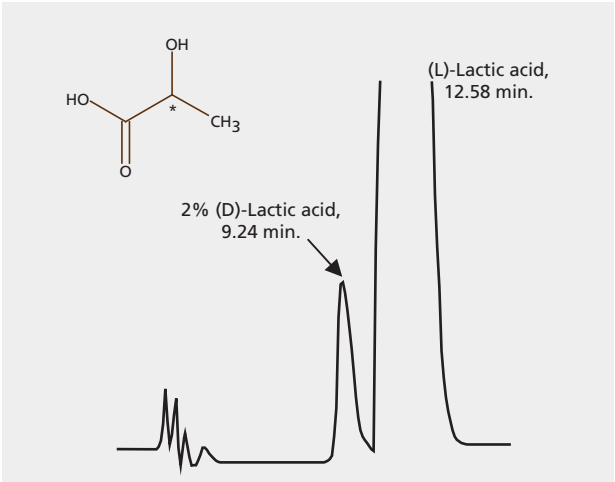
## HPLC Analysis of Lactic Acid Enantiomers on Astec® CLC-L and CLC-D

| | |
|---|---|
| column | Astec CLC-L and CLC-D, 15 cm x 4.6 mm I.D., 5 µm particles (53023AST, 53123AST) |
| mobile phase | 5 mM copper sulfate |
| flow rate | 1 mL/min |
| column temp. | 25 °C |
| detector | UV, 254 nm |
| injection | 10 µL |
| Application No. | G004399 |



## HPLC Analysis of Lactic Acid Enantiomers on Astec® CLC-L, Trace Level Detection

column . . . . . Astec CLC-L, 15 cm x 4.6 mm I.D., 5 µm particles (53123AST)
mobile phase . . . . . 5 mM copper sulfate
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL in water
Application No. . . . . G004663



## HPLC Analysis of Maté Leaves on Ascentis® Express C18

sample/matrix . . UAE (Ultrasonic Extraction) maté extract (dried and minced leaves (7.5 g) from maté were extracted with 225 mL of methanol in an ultrasonic bath, thermostated at 75 ± 0.5 °C (potency: 90 W; frequency: 40 kHz))

column . . . Ascentis Express C18, 150 x 4.6 mm I.D., 2.7 µm particles (53829-U)

mobile phase . . . . . (A) water, pH 3 with formic acid; (B) acetonitrile, pH 3 with formic acid

gradient . . . . . 0 to 5% B in 20 min; 5 to 40% B in 30 min

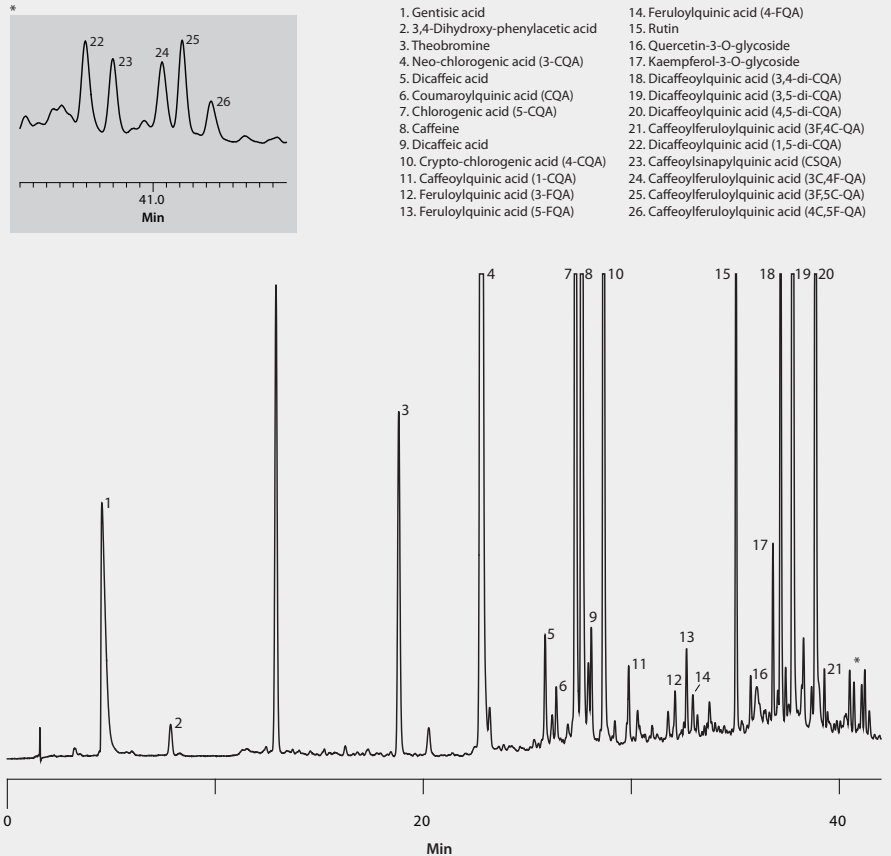
flow rate . . . . . 1 mL/min

column temp. . . . . 25 °C

detector . . . . . PDA: range 215- 420 nm (8 µL detector cell volume, cell temp. 40 °C, sampling rate 12.5 Hz, time constant 0.025 s)

injection . . . . . 10 µL

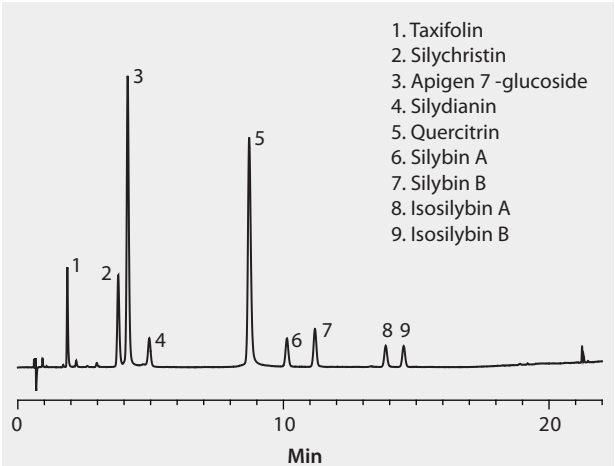
Application No. . . . . G005594



## HPLC Analysis of Milk Thistle Standards on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of milk thistle related compounds.

column . . . Ascentis Express C18, 10 cm x 3 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) water with 0.1% formic acid; (B) methanol
gradient . . . . . held at 35% B for 3 min; 35 to 45% B in 10 min; held at 45% B for 2 min; 45 to 100% B in 5 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 4105 psi (283 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 20 µL
sample . . . . . 20 µg/mL in 80:20, water:methanol
Application No. . . . . G005445



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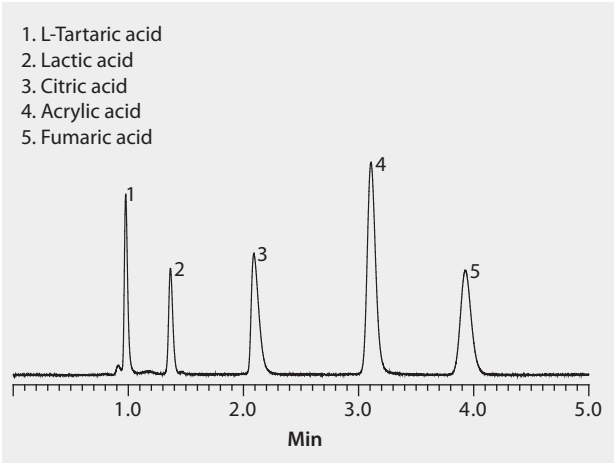
## HPLC Analysis of Organic Acids on Ascentis® Express RP-Amide

This application demonstrates the suitability of Ascentis Express RP-Amide for the analysis of organic acids under 100% aqueous conditions.

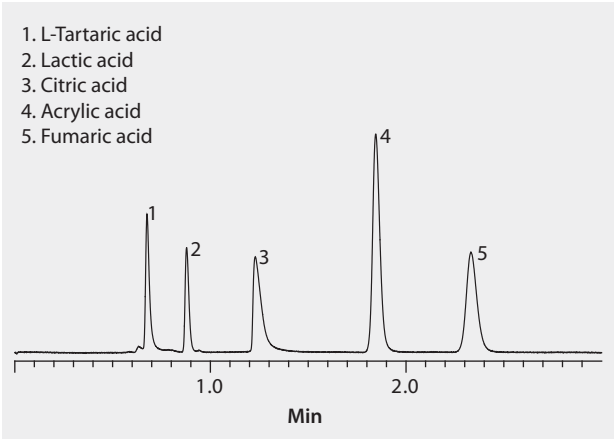
| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 2.1 mm I.D., 2.7 µm particles (53913-U) |
| mobile phase | (A) 0.1% (v/v) TFA in (B) water |
| flow rate | 0.3 mL/min |
| column temp. | 35 °C |
| detector | UV, 210 nm |
| injection | 1 µL |
| sample | 0.2 - 4 g/L in mobile phase |
| Application No. | G005714 |



## HPLC Analysis of Organic Acids on Ascentis® RP-Amide

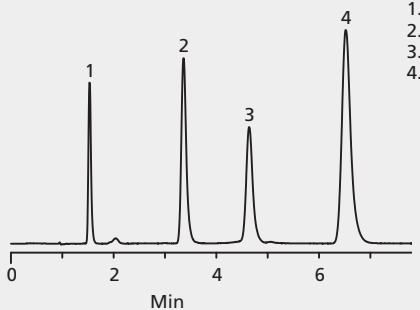
This application demonstrates the suitability of Ascentis RP-Amide for the analysis of organic acids under 100% aqueous conditions.

column . . . Ascentis RP-Amide, 10 cm x 2.1 mm I.D., 3 µm particles (565301-U)
mobile phase . . . . . (A) 0.1% (v/v) TFA in (B) water
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 1 µL
sample . . . . . 0.2 - 4 g/L in mobile phase
Application No. . . . . G005715



## HPLC Analysis of Organic Acids on Discovery® C18

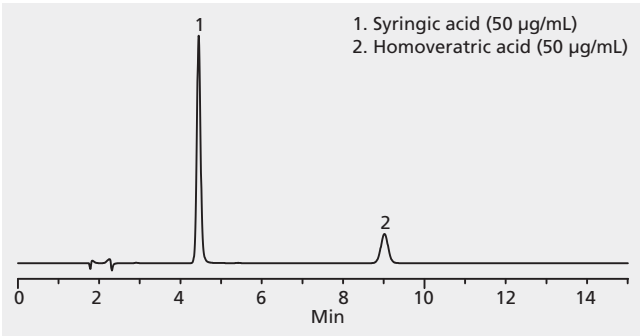
column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) methanol; (B) water, 0.1% TFA (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 20 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 1 mg/mL each analyte
Application No. . . . . 797-0657




1. Homovanillic acid
2. Sorbic acid
3. Salicylic acid
4. p-Toluic acid

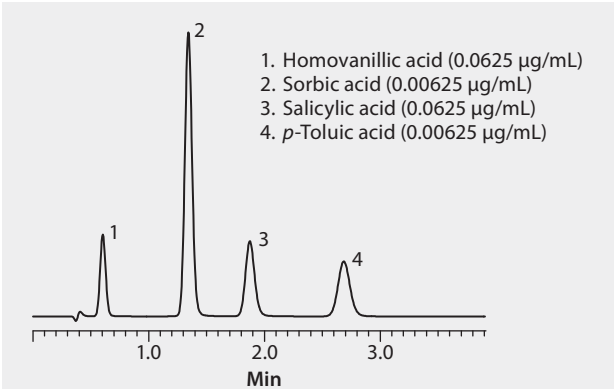
## HPLC Analysis of Organic Acids on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) 10 mM ammonium phosphate monobasic (pH 2.50 with phosphoric acid): (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . as indicated in mobile phase
Application No. . . . . G003160



## HPLC Analysis of Organic Acids on Discovery® C18

| | |
|---|---|
| column | Discovery C18, 5 cm × 4.6 mm I.D., 5 µm particles (504947) |
| mobile phase | (A) 0.1% TFA in (B) water:methanol; (60:40, A:B) |
| flow rate | 2.0 mL/min |
| column temp. | 20 °C |
| detector | UV, 254 nm |
| injection | 10 µL |
| Application No. | G001431 |



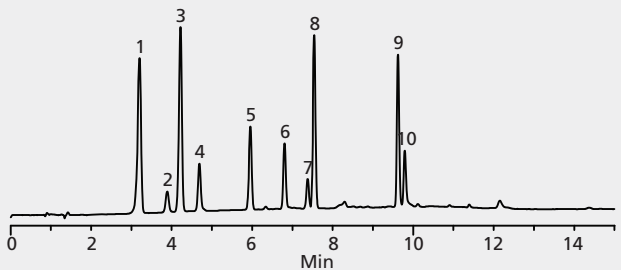
## HPLC Analysis of Phenolic Antioxidants on Ascentis® C8

column . . . . . Ascentis C8, 15 cm x 4.6 mm I.D., 5 µm particles (581424-U)
mobile phase . . . . . (A) 5% acetic acid; (B) (50:50) methanol:acetonitrile
flow rate . . . . . 2.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 280 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 50:50 acetonitrile:2-propanol
Application No. . . . . G003054

1. Propyl gallate (10 µg/mL)
2. Ethoxyquin (10 µg/mL)
3. 2,4,5-trihydroxybutyrophenone (10 µg/mL)
4. tert-butylhydroquinone (10 µg/mL)
5. Nordihydroguaiaretic acid (10 µg/mL)
6. 3-tert-butyl-4-hydroxyanisole (10 µg/mL)
7. 2,6-di-tert-butyl-4-hydroxymethylphenol (10 µg/mL)
8. Octyl gallate (10 µg/mL)
9. Lauryl gallate (10 µg/mL)
10. 3,5-di-tert-butyl-4-hydroxytoluene (10 µg/mL)

**Gradient Program**

| Time (min) | %A | %B |
|---|---|---|
| 0 | 70 | 30 |
| 10 | 0 | 100 |
| 15 | 0 | 100 |



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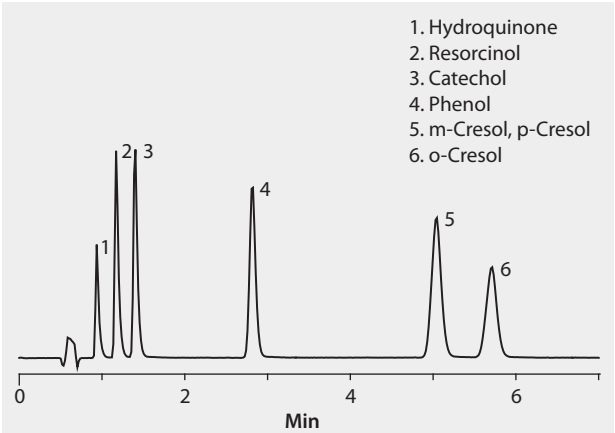
## HPLC Analysis of Phenolic Compounds on Ascentis® Express RP-Amide

Chromatography of Seven Phenol Compounds on Ascentis Express RPA

column . . . . . Ascentis Express RP-Amide, 10 cm x 4.6 mm I.D., 2.7 µm particles (53929-U)
mobile phase . . . (A) 20 mM phosphoric acid, pH 2; (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 270 nm
injection . . . . . 25 µL
sample . . . . . 50 mg/L in mobile phase
Application No. . . . . G005719



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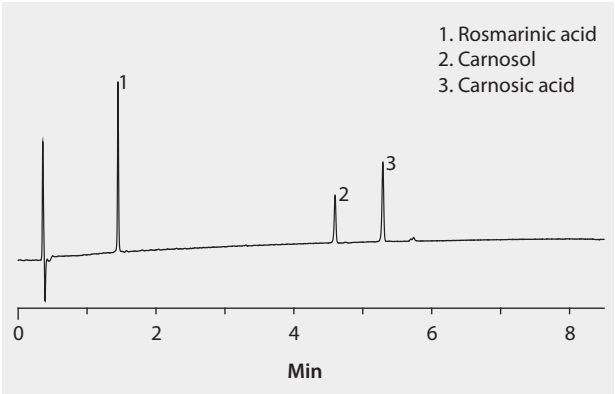
## HPLC Analysis of Phenolics from Rosemary on Ascentis® Express C8

This application demonstrates the suitability of Ascentis Express C8 for the efficient separation of phenolics from rosemary.

column . . . . Ascentis Express C8, 5 cm x 3 mm I.D., 2.7 µm particles (53848-U)
mobile phase . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile
gradient . . . . . 15 to 100% B in 8.5 min
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 214 nm
injection . . . . . 2 µL
sample . . . . . 100 mg/L in 75 µM EDTA, 1.5 mM ascorbate, 15% acetonitrile
Application No. . . . . G005717



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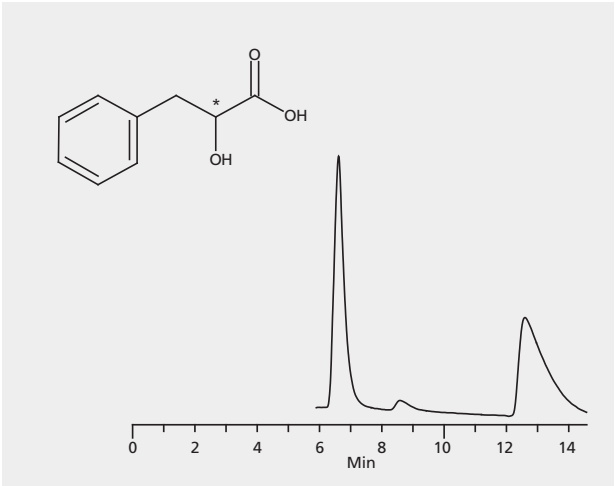
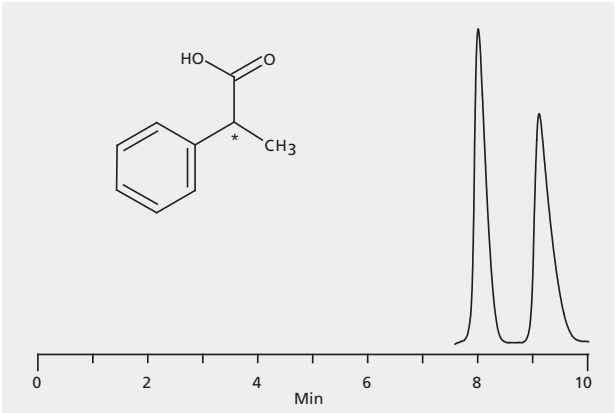
## HPLC Analysis of 3-Phenyllactic Acid Enantiomers on Astec® CHIROBIOTIC® R

column . . . . . CHIROBIOTIC R, 25 cm x 4.6 mm I.D., 5 µm particles (13024AST)
mobile phase . . . . . 0.1% ammonium acetate in methanol
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 230 nm
injection . . . . . 0.5 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004680



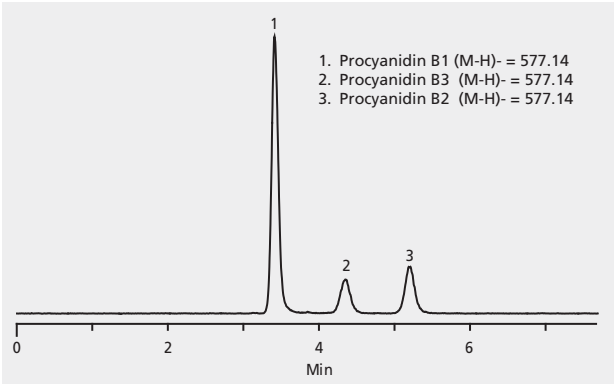
## HPLC Analysis of 2-Phenylpropionic Acid Enantiomers on Astec® CYCLOBOND® I 2000 HP-RSP

column . . . . . CYCLOBOND I 2000 HP-RSP, 25 cm x 4.6 mm I.D., 5 µm particles (24024AST)
mobile phase . . (A) 10 mM ammonium acetate, pH 4.0; (B) acetonitrile; (75:25, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 235 nm
injection . . . . . 1 µL
sample . . . . . 1 mg/mL in methanol
Application No. . . . . G004717



## HPLC Analysis of Procyanidins on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm × 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium formate, (pH 3.0 with concentrated formic acid): (B) acetonitrile; (85:15, A:B)
flow rate . . . . . 1.0 mL/min split to the MS
column temp. . . . . 35 °C
detector . . . . . MS, ESI (-) in Single Ion Recording (SIR) Mode
injection . . . . . 10 µL
sample . . . . . 10 mg/mL in 85:15, 10 mM ammonium formate, (pH 3.0 with concentrated formic acid): acetonitrile
Application No. . . . . G003879



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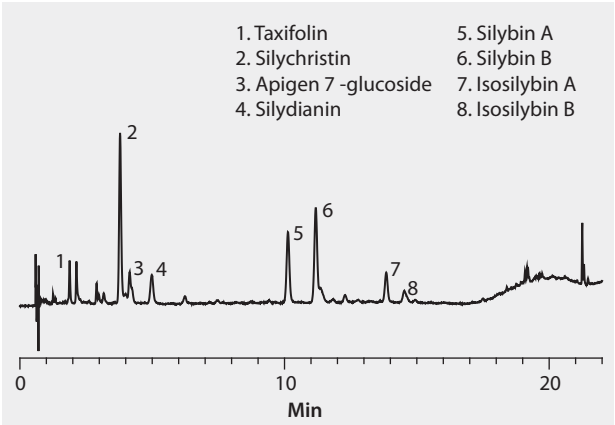
## HPLC Analysis of Silymarin on Ascentis® Express C18

This application demonstrates the suitability of Ascentis Express C18 for the efficient separation of silymarin.

column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . . . . (A) water with 0.1% formic acid; (B) methanol
gradient . . . . . held at 35% B for 3 min; 35 to 45% B in 10 min; held at 45% B for 2 min; 45 to 100% B in 5 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 4105 psi (283 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 20 µL
sample . . . . . 100 µg/mL in 90:10, water:methanol
Application No. . . . . G005739



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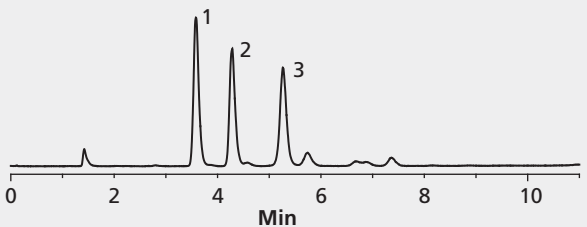
## HPLC Analysis of Steroidal Glycosides on Ascentis® Phenyl

column . . . . . Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase . . . . . (A) 10 mM ammonium formate (pH 3 with formic acid); (B) methanol
flow rate . . . . . 1.25 mL/min
column temp. . . . . 35 °C
detector . . . . . ELSD at 45 °C, gain 6, 3.5 bar N2
injection . . . . . 20 µL
sample . . . . . as indicated in mobile phase A
Application No. . . . . G003714

**Gradient Program**

| Time (min) | %B |
|---|---|
| 0 | 70 |
| 10 | 90 |
| 10.5 | 70 |

1. Digoxin (100 µg/mL)
2. Glycyrrhetinic acid (100 µg/mL)
3. Digitonenin (200 ug/mL)



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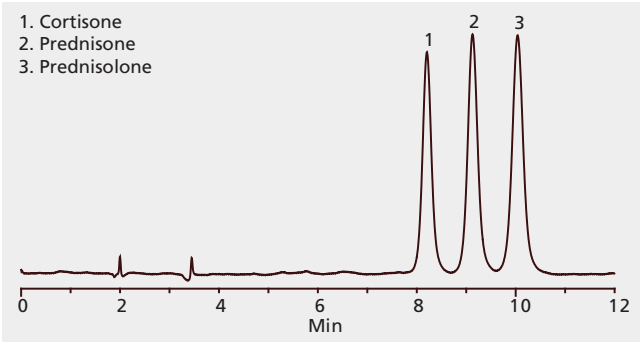
## HPLC Analysis of Steroids on Ascentis® Si

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) hexane; (B) ethanol; (88:12, A:B)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 245 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 85:15, hexane:2-propanol
Application No. . . . . G003728



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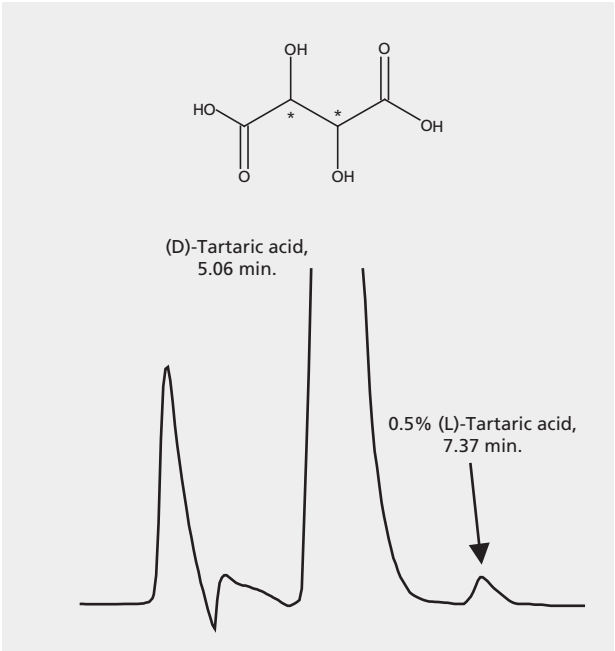
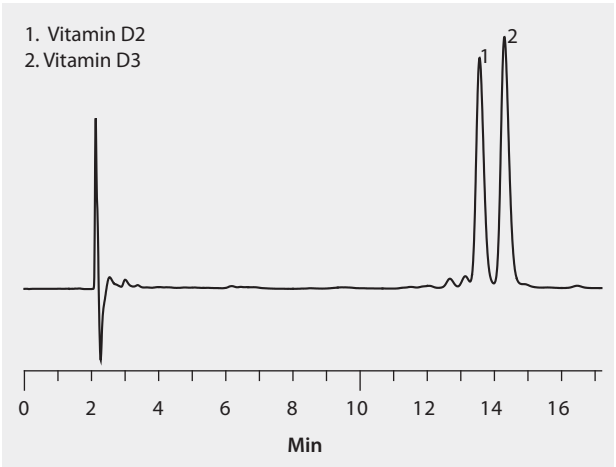
## HPLC Analysis of Tartaric Acid Enantiomers on Astec® CLC-D, Trace Level Detection

column . . . . . Astec CLC-D, 15 cm x 4.6 mm I.D., 5 µm particles (53023AST)
mobile phase . . . . . 3 mM copper sulfate, pH 3.2
flow rate . . . . . 1 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 254 nm
injection . . . . . 10 µL
sample . . . . . 5 mg/mL in water
Application No. . . . . G004665



## HPLC Analysis of Vitamin D2 and D3 on Ascentis® Express C18

column . . Ascentis Express C18, 15 cm x 2.1 mm I.D., 2.7 µm particles (53825-U)
mobile phase . . . . . acetonitrile
flow rate . . . . . 0.15 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
sample . . . . . 10 mg/L each (vitamins D2 and D3) in methanol
Application No. . . . . G005615



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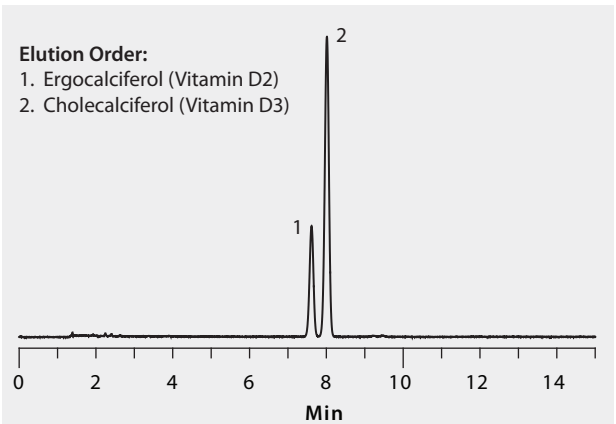
## HPLC Analysis of Vitamin D2 and D3 on Ascentis® Express C18 5 µm

This application demonstrates the suitability of the Ascentis Express C18, 5 µm, for the routine analysis of the fat-soluble vitamins D2 and D3 . Structures along with the optimized chromatogram obtained on the Ascentis Express C18, 5 µm are presented.

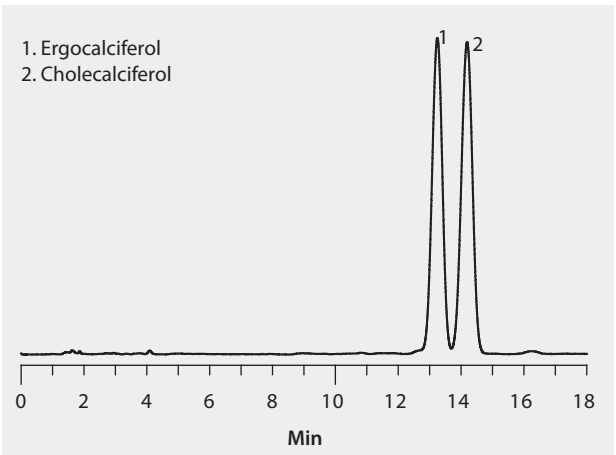
column . . Ascentis® Express C18, 25 cm x 4.6 mm I.D., 5 µm particles (50538-U)
mobile phase . . . . . (A) acetonitrile; (B) methanol; (95:5, A:B)
flow rate . . . . . 1.5 mL/min
pressure . . . . . 1726 psi (119 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 265 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL in 90:10, acetonitrile:methanol
Application No. . . . . G005891



## HPLC Analysis of Vitamin D2 and Vitamin D3 on Ascentis® C18

This application demonstrates the suitability of the Ascentis C18 for the analysis of the fat-soluble vitamins D2 and D3 . Structures along with the optimized chromatogram obtained on the Ascentis C18 are presented.

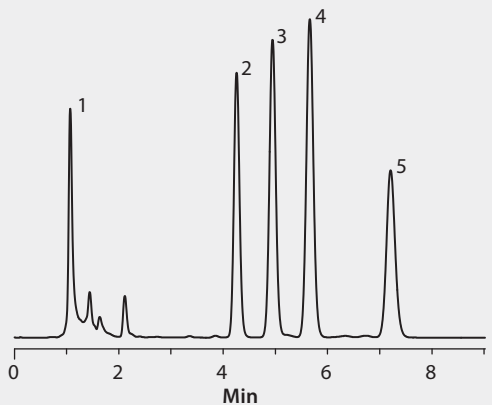
column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . acetonitrile
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 290 nm
sample . . . . . 50 µg/mL each in acetonitrile
Application No. . . . . G005614



## HPLC Analysis of Vitamins, Fat Soluble (A and E), on Discovery® C8

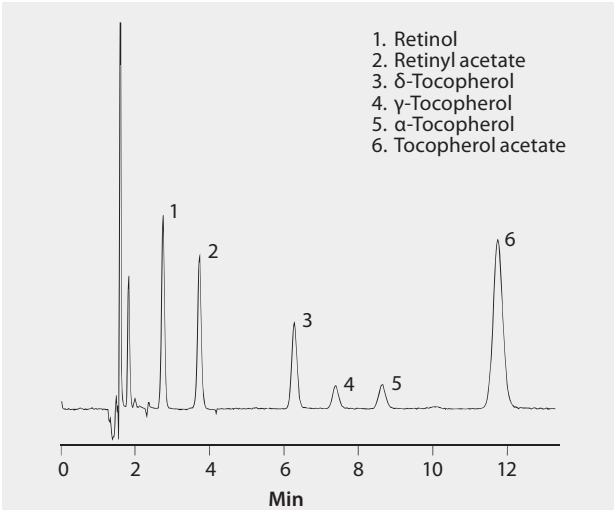
column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . . . . (A) acetonitrile: (B) water (90:10 v/v, A:B)
flow rate . . . . . 2 mL/min
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
Application No. . . . . G000912

1. Retinol acetate (Vitamin A acetate), 50 µg/mL
2. δ-Tocopherol, 165 µg/mL
3. γ-Tocopherol, 200 µg/mL
4. α-Tocopherol (Vitamin E), 292 µg/mL
5. α-Tocopherol acetate (Vitamin E acetate), 405 µg/mL



## HPLC Analysis of Vitamins, Fat Soluble (A and E), on SUPELCOSIL™ LC-18

column . . . . . SUPELCOSIL LC-18, 25 cm × 4.6 mm I.D., 5 µm particles (58298)
mobile phase . . . . . (A) methanol: (B) deionized water (98:2, A:B)
flow rate . . . . . 2 mL/min
detector . . . . . UV, 325 nm (retinol, retinyl acetate) or 290 nm (tocopherols, tocopherol acetate)
injection . . . . . 20 µL
sample . . . . . 0.2-1 mg/mL each analyte in methanol
Application No. . . . . 712-0679



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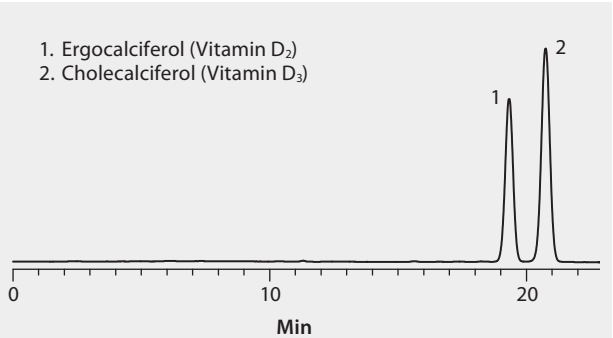
## HPLC Analysis of Vitamins, Fat Soluble (D2 and D3), on Discovery® HS C18

column . . . . Discovery HS C18, 15 cm × 4.6 mm I.D., 3 µm particles (569252-U)
mobile phase . . . . . 100% acetonitrile
flow rate . . . . . 0.8 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each analyte
Application No. . . . . G001419



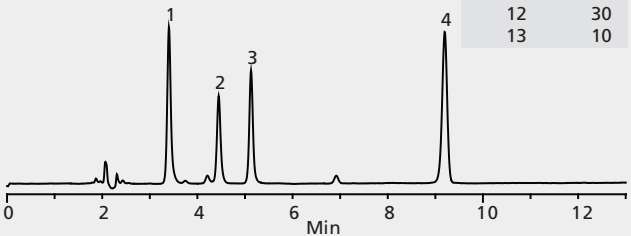
## HPLC Analysis of Vitamins, Fat Soluble, on Ascentis® Si

column . . . . . Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase . . . . . (A) hexane; (B) ethylacetate
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 290 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 96:4 (hexane:isopropanol)
Application No. . . . . G003727

1. alpha Tocopherol
2. Menadione
3. gamma Tocopherol
4. Chlolecalciferol

**Gradient Program**

| Time (min) | %B |
|---|---|
| 0 | 10 |
| 10 | 30 |
| 12 | 30 |
| 13 | 10 |






SUPELCO® Solutions within.™

## HPLC Analysis of B-vitamins in vitamin water using Ascentis® Express RP-Amide and UV detection

The vitamin water drink was filtered using 0.2 um Nylon filter and injected into UHPLC for B-vitamin analysis

column . . . . . Ascentis Express RP-Amide, 10 cm x 3.0 mm I.D., 2.7 µm particles (53918-U)

mobile phase . . . . . (A) 20 mM potassium phosphate, pH 3; (B) methanol

gradient . . . . . held at 0.5% B for 1.5 minutes, to 25% B in 1.0 minutes, held at 25% B for 1.7 minute, held at 0.5% B for 1.2 minutes

flow rate . . . . . 0.75 mL/min

pressure . . . . . 5584 psi (385 bar)

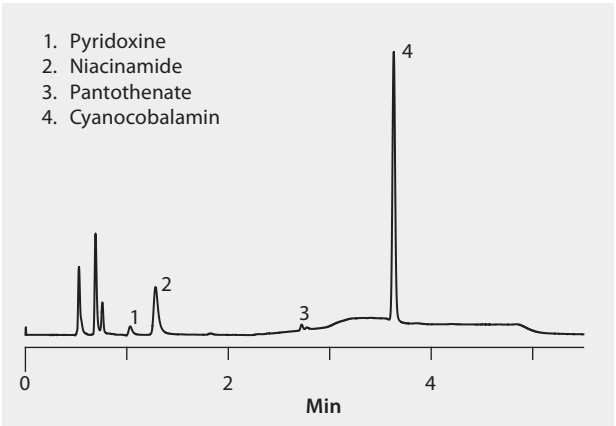
column temp. . . . . 30 °C

detector . . . . . UV, 210 nm

injection . . . . . 0.5 µL

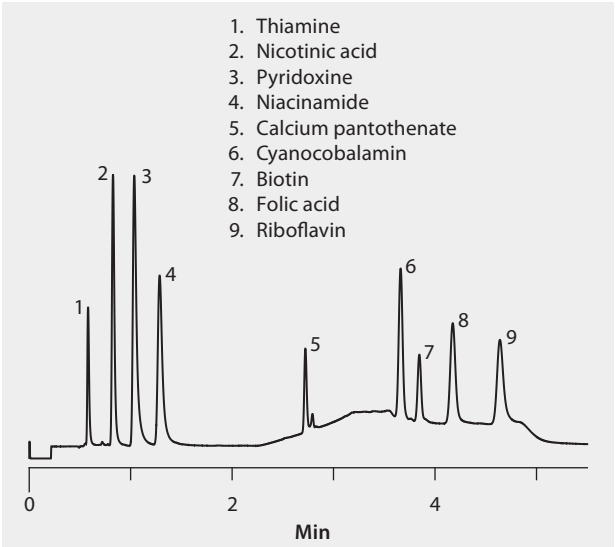
sample . . . . . vitamin water was filtered through 0.2um nylon syringe filter

Application No. . . . . G006214



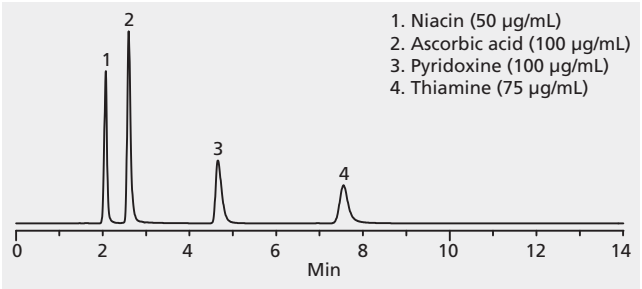
## HPLC Analysis of B-vitamins on Ascentis® Express RP-Amide using UV detection

| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 3.0 mm I.D., 2.7 µm particles (53918-U) |
| mobile phase | (A) 20 mM potassium phosphate, pH 3; (B) methanol; |
| gradient | 0.5% B held for 1.5 minutes, to 25% B in 1.0 minutes, held at 25% B for 1.7 minute, held at 0.5% B for 1.2 minutes |
| flow rate | 0.75 mL/min |
| pressure | 5584 psi (385 bar) |
| column temp. | 30 °C |
| detector | UV, 210 nm |
| injection | 0.5 µL |
| sample | 100 ug/mL of all vitamins except 250 ug/mL of calcium pantothenate, biotin and riboflavin in water |
| Application No. | G006215 |



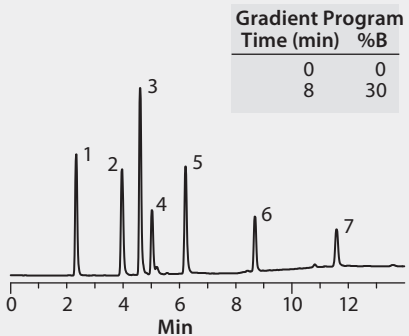
## HPLC Analysis of Vitamins, Water Soluble, on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . 25 mM potassium phosphate, dibasic (pH 3.5 with phosphoric acid)
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
sample . . . . . as indicated in 25 mM potassium phosphate, dibasic (pH 3.5 with phosphoric acid)
Application No. . . . . G003035



## HPLC Analysis of Vitamins, Water Soluble, on Discovery® C8

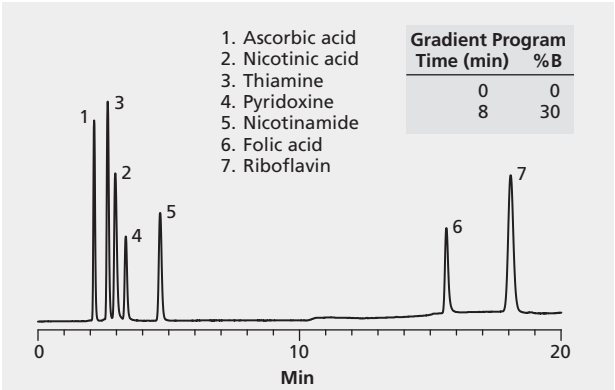
column . . . . . Discovery C8, 15 cm × 4.6 mm I.D., 5 µm particles (59353-U)
mobile phase . . (A) 50 mM potassium phosphate, monobasic, pH 4.5 to methanol:
(B) 50 mM potassium phosphate, monobasic, pH 4.(30:70) in 12 min
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
Application No. . . . . G000789




1. Ascorbic acid
2. Nicotinic acid
3. Thiamine
4. Pyridoxine
5. Nicotinamide
6. Folic acid
7. Riboflavin

## HPLC Analysis of Vitamins, Water Soluble, on Discovery® RP-Amide C16

column . . Discovery RP-Amide C16, 15 cm × 4.6 mm I.D., 5 µm particles (505013)
mobile phase . . . . . (A) methanol; (B) 50 mM potassium phosphote, monobasic, pH 4.5 (30:70, A:B)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G001171



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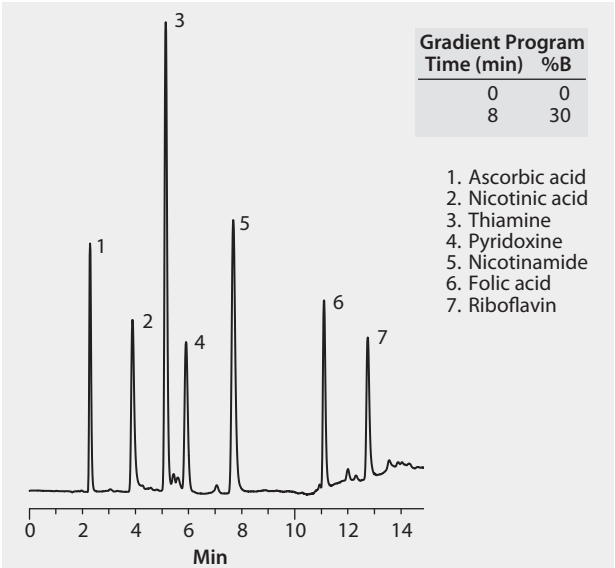
## HPLC Analysis of Vitamins, Water Soluble on Discovery® C18

column . . . . . Discovery C18, 15 cm × 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . (A) 50 mM potassium phosphate, dibasic, pH 4.5 to methanol: (B) 50mM potassium phosphate, dibasic, pH 4.0 (30:70, A:B) in 8 min
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 1 µL
Application No. . . . . G000790



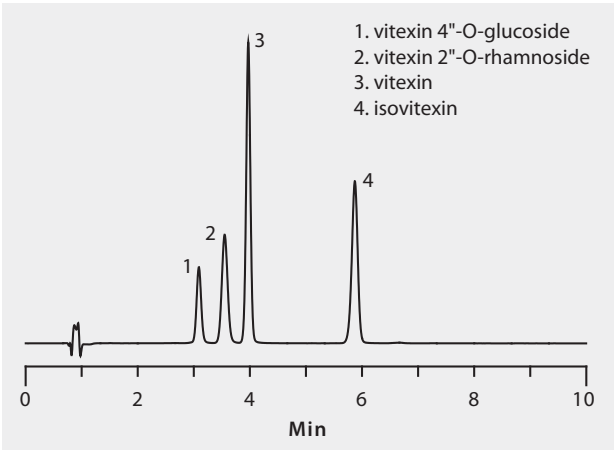

| Gradient Program | |
|---|---|
| Time (min) | %B |
| 0 | 0 |
| 8 | 30 |

1. Ascorbic acid
2. Nicotinic acid
3. Thiamine
4. Pyridoxine
5. Nicotinamide
6. Folic acid
7. Riboflavin

## HPLC Analysis of Vitexin and Related Compounds on Ascentis® Express RP-Amide

Vitexin is an apigenin flavone glucoside; a chemical coupound found in the passion flower; Vitex agnus-castus (chaste tree or chasteberry) and in the Phyllostachys nigra bamboo leaves.[1] It is also found in the pearl millet (Pennisetum millet).[2] Vitexin is also found in Hawthorn.
Improved peak shape for the conjugates was obtained at higher temperatures. Note that citric acid may not be necessary as it was added as an attempt to improve peak shape at lower temps. It is expected that phophoric acid or other acids will provide similar results.

| | |
|---|---|
| column | Ascentis Express RP-Amide, 10 cm x 3.0 mm I.D., 2.7 um particles (53918-U) |
| mobile phase | (A) 10 mM citric acid (B) acetontrile (85:15, A:B) |
| flow rate | 0.5 mL/min |
| pressure | 1770 psi (122 bar) |
| column temp. | 55 °C |
| detector | UV, 335 nm |
| injection | 2 µL |
| sample | 100 ug/mL each in 40% methanol |
| Application No. | G005903 |



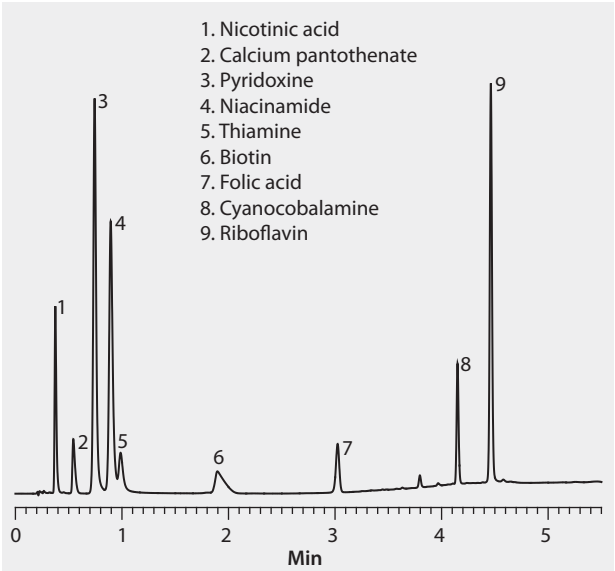



SUPELCO® Solutions within.™

## HPLC Analysis of Water-Soluble B-Vitamins on Ascentis® Express C18

A mix of 9 water-soluble B-vitamins was separated on Ascentis Express C18 column

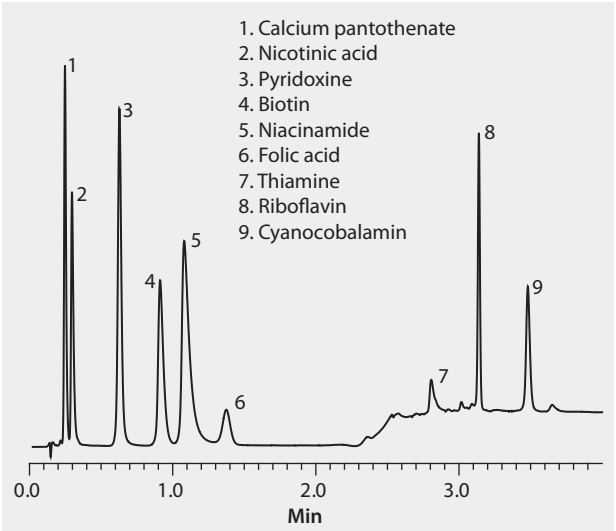
column . . Ascentis Express C18, 5 cm x 3.0 mm I.D., 2.7 µm particles (53811-U)
mobile phase . . . . (A) 20 mM potassium phosphate, dibasic, pH 7; (B) methanol
gradient . . . . . 0.5% B for 1.3 min, 0.5-30%B over 1.7 min; to 30% B in 1.4 min, 2 min equilibration at 0.5% B
flow rate . . . . . 1.0 mL/min
pressure . . . . . 4130 psi (285 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
sample . . . . . 9 vitamins in water, 10 µg/mL for nicotinic acid and folic acid, 12 µg/mL for thiamine hydrochloride and cyanocobalamin, (20 µg/mL for pyridoxine, 30 µg/mL for riboflavin, 40 µg/mL for niacineamide, 100 µg/mL for calcium pantothenate, 120 µg/mL for biotin)
Application No. . . . . G005625



## HPLC Analysis of Water-Soluble B-Vitamins on Ascentis® Express F5

A mix of 9 water-soluble B-vitamins was separated on Ascentis Express F5 column

column . . . Ascentis Express F5, 5 cm x 3.0 mm I.D., 2.7 µm particles (53576-U)
mobile phase . . (A) 20 mM potassium phosphate, dibasic, pH 6.2; (B) acetonitrile
gradient . . . . . 0% B for 1.5 min, 0-10% over 0.5 min; to 15% B in 1.5 min, 2 min re-equilibration at 0% B
flow rate . . . . . 1.5 mL/min
pressure . . . . . 4931 psi (340 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . all vitamins in water, 10 µg/mL for nicotinic acid and folic acid, 12 µg/mL for thiamine hydrochloride and cyanocobalamin, (20 µg/mL for pyridoxine, 30 µg/mL for riboflavin, 40 µg/mL for niacineamide, 100 µg/mL for calcium pantothenate, 120 µg/mL for biotin)
Application No. . . . . G005624



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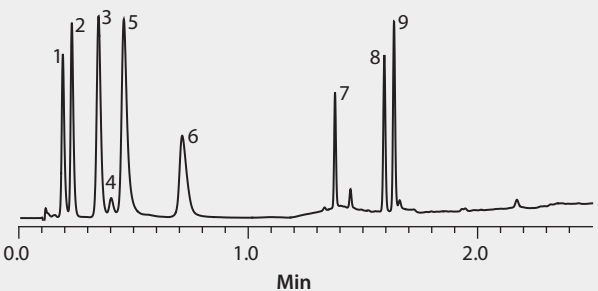
## HPLC Analysis of Water Soluble B Vitamins on Ascentis® Express RP-Amide

A mix of 9 water-soluble B-vitamins was separated on Ascentis Express RPA column

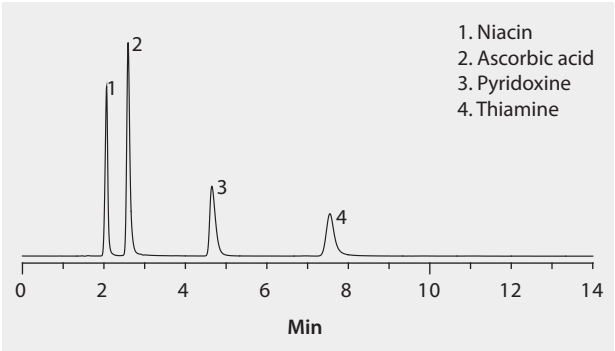
column . . Ascentis Express RP-Amide, 5 cm x 3.0 mm I.D., 2.7 µm particles (53916-U)
mobile phase . . (A) 20 mM potassium phosphate, dibasic, pH 6.2; (B) acetonitrile
gradient . . . . . 0% B for 0.5 min, to 15% B over 0.3 min; up to 35% B in 1.2 min, 1 min equilibration at 0% B
flow rate . . . . . 2.0 mL/min
pressure . . . . . 5076 psi (350 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 5 µL
sample . . . . . 9 vitamins in water, 10 µg/mL for nicotinic acid and folic acid, 12 µg/mL for thiamine hydrochloride and cyanocobalamin, (20 µg/mL for pyridoxine, 30 µg/mL for riboflavin, 40 µg/mL for niacineamide, 100 µg/mL for calcium pantothenate, 120 µg/mL for biotin)
Application No. . . . . G005623

1. Nicotinic acid
2. Calcium Pantothenate
3. Pyridoxine
4. Thiamine
5. Niacinamide
6. Biotin
7. Folic acid
8. Cyanocobalamin
9. Riboflavin



## HPLC Analysis of Water Soluble Vitamins on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 4.6 mm I.D., 5 µm particles (565324-U)
mobile phase . . . . . 25 mM potassium phosphate monobasic, pH 3.5
flow rate . . . . . 1.0 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 230 nm
injection . . . . . 10 µL
Application No. . . . . G005735



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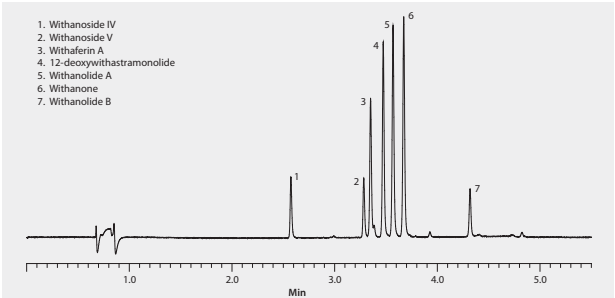
## HPLC Analysis of Withania (Aswagandha) Standard on Ascentis® Express F5

This application demonstrates the suitability of Ascentis Express F5 for the efficient separation of withania standards.

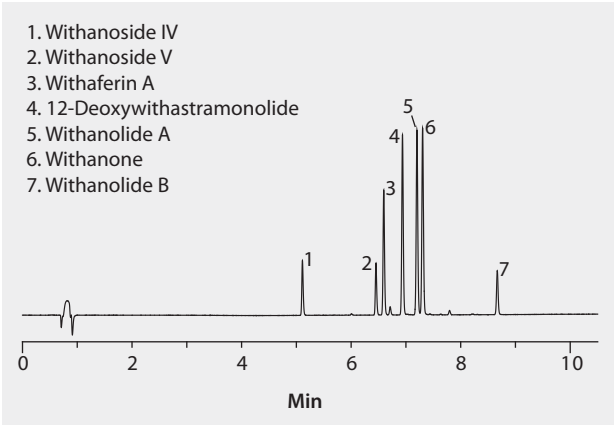
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . . . . 20 to 100% B in 10 min: held at 100% B for 0.5 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 2756 psi (190 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 227 nm
injection . . . . . 5 µL
sample . . . . . 20 µg/mL in 80:20, water:methanol
Application No. . . . . G005447



## HPLC Analysis of Withania Compounds on Ascentis® Express Phenyl-Hexyl

This application demonstrates the suitability of Ascentis Express Phenyl-Hexyl for the efficient separation of withania standards.

| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 10 cm x 2.1 mm I.D., 2.7 µm particles (53336-U) |
| mobile phase | (A) water; (B) acetonitrile |
| gradient | 20 to 100% B in 10 min; held at 100% B for 0.5 min |
| flow rate | 0.3 mL/min |
| pressure | 2321 psi (160 bar) |
| column temp. | 35 °C |
| detector | UV, 227 nm |
| injection | 5 µL |
| sample | 20 µg/mL in water: methanol (80:20) |
| Application No. | G005660 |

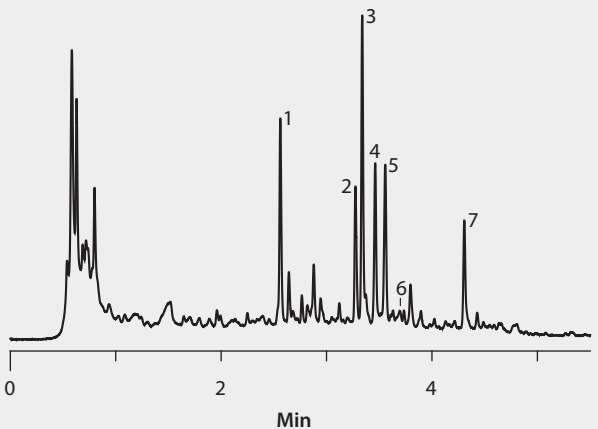


## HPLC Analysis of Withania Extract on Ascentis® Express F5

This application demonstrates the suitability of Ascentis Express F5 for the efficient separation of withania extract.

column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . . . . (A) water; (B) acetonitrile
gradient . . . . . 20 to 100% B in 5 min; held at 100% B for 0.5 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 2750 psi (190 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 227 nm
injection . . . . . 5 µL
sample . . . . . 20 mg/mL in water:acetonitrile (50:50); sonicate for 15 minutes; filter 0.45 µm; dilute to water:acetonitrile (80:20)
Application No. . . . . G005613

1. Withanoside IV
2. Withanoside V
3. Withaferin A
4. 12-Deoxywithasramonolide
5. Withanolide A
6. Withanone
7. Withanolide B



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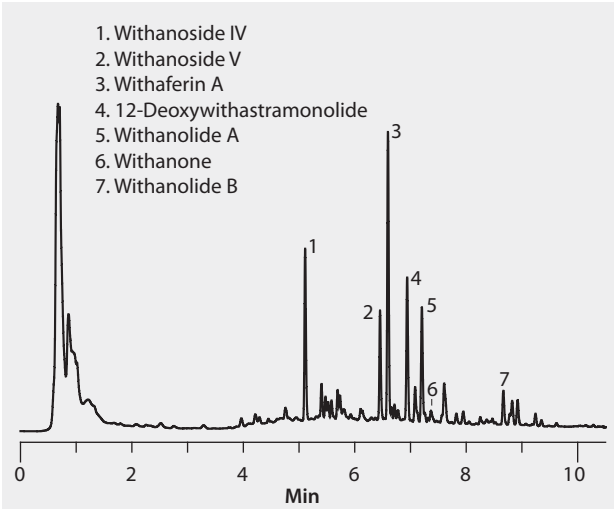
## HPLC Analysis of Withania Extract on Ascentis® Express Phenyl-Hexyl

This application demonstrates the suitability of Ascentis Express Phenyl-Hexyl for the efficient separation of withania extract.

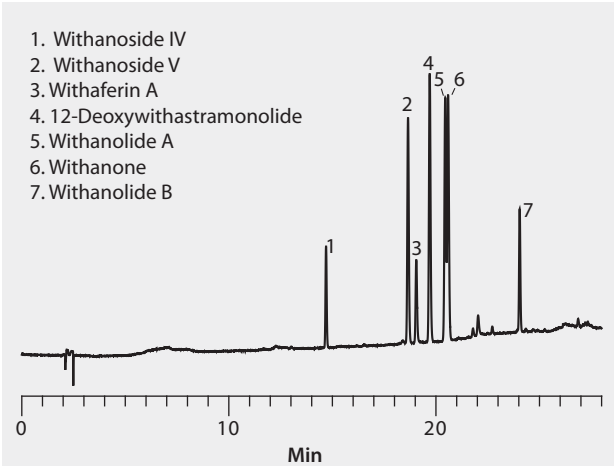
| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 10 cm x 2.1 mm I.D., 2.7 µm particles (53336-U) |
| mobile phase | (A) water; (B) acetonitrile |
| gradient | 20 to 100% B in 10 min; held at 100% B for 0.5 min |
| flow rate | 0.3 mL/min |
| pressure | 160 bar (2320 psi) |
| column temp. | 35 °C |
| detector | UV, 227 nm |
| injection | 5 µL |
| sample | 20 mg/mL in water:acetonitrile (50:50); sonicate 15 minutes; filter 0.45 µm; dilute to water:acetonitrile (80:20) |
| Application No. | G005610 |



## HPLC Analysis of Withania Standards on Discovery® C18

This application demonstrates the suitability of Discovery C18 for the efficient separation of withania standards by the USP monograph.

column . . . . . Discovery C18, 25 cm x 4.6 mm I.D., 5 µm particles (504971)
mobile phase . . . . . (A) 1 mM potassium phosphate, monobasic; (B) acetonitrile
gradient . . 5 to 45% B in 18 min; 45 to 80% B in 7 min; held at 80% B for 3 min
flow rate . . . . . 1.5 mL/min
pressure . . . . . 220 bar (3200 psi)
column temp. . . . . 27 °C
detector . . . . . UV, 227 nm
injection . . . . . 20 µL
sample . . . . . 20 µg/mL in water: acetonitrile (86:14)
Application No. . . . . G005611

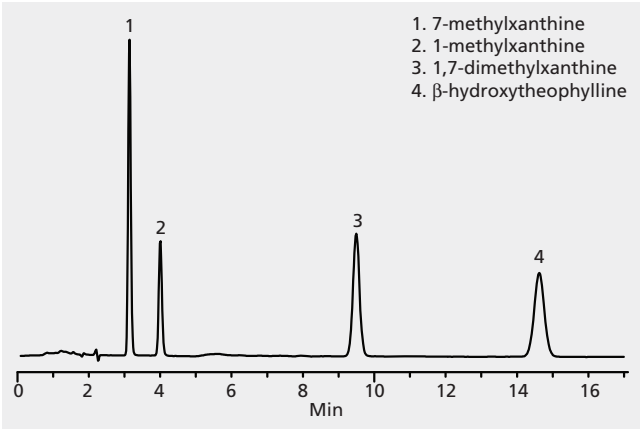





SUPELCO® Solutions within.™

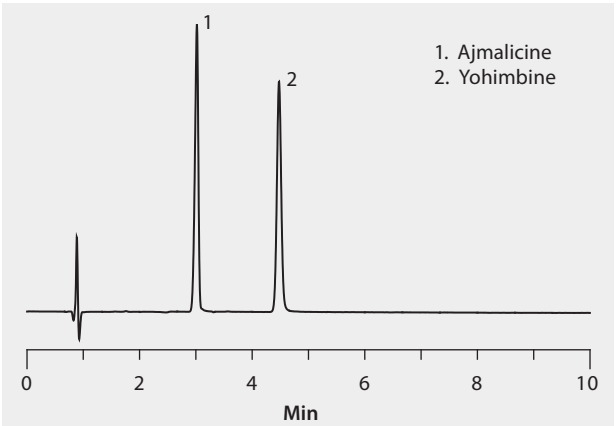
## HPLC Analysis of Xanthines on Ascentis® C18

column . . . . . Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
mobile phase . . . . . (A) 10 mM monobasic potassium phosphate (pH 3.0 with phosphoric acid); (B) methanol; (94:6, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
sample . . . . . 50 µg/mL each in mobile phase
Application No. . . . . G002465



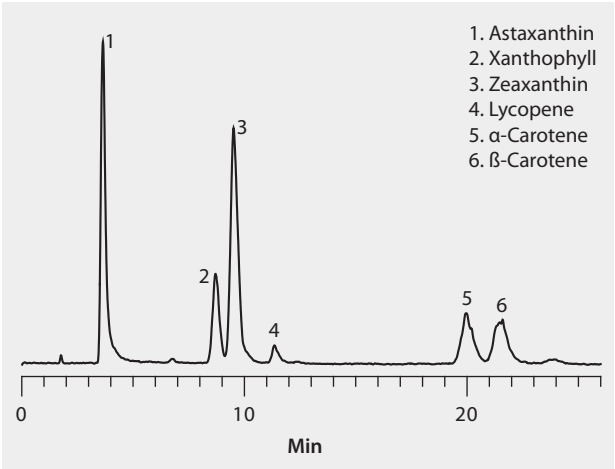
## HPLC Analysis of Yohimbe Alkaloids on Ascentis® Express F5, HILIC Mode

column . . . Ascentis Express F5, 3 cm x 3.0 mm I.D., 2.7 µm particles (53574-U)
mobile phase . . . . . 2 mM ammonium acetate in 10:90 water:acetonitrile, pH to 6.0 with acetic acid
flow rate . . . . . 0.5 mL/min
pressure . . . . . 1100 psi (76 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 275 nm
injection . . . . . 2 µL
sample . . . . . 100 ug/mL each in methanol
Application No. . . . . G005904



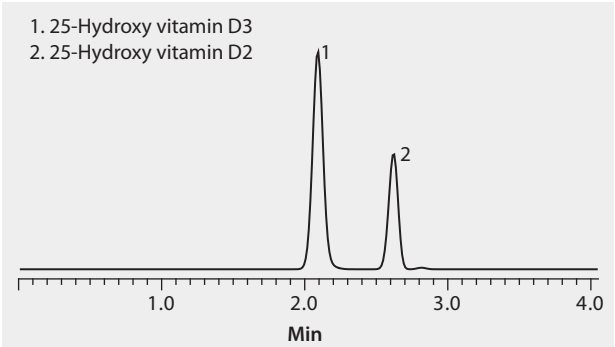
## LC-MS Analysis of Carotene Compounds on Ascentis® RP-Amide

column . . . Ascentis RP-Amide, 15 cm x 2.1 mm I.D., 5 µm particles (565305-U)
mobile phase . . . . . acetonitrile
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, APCI(+), SIM
injection . . . . . 5 µL
sample . . . . . 10 mg/L in acetonitrile
Application No. . . . . G005609



## LC-MS Analysis of 25-Hydroxyvitam D3 and 25-Hydroxyvitamin D2 on Ascentis® Express C18

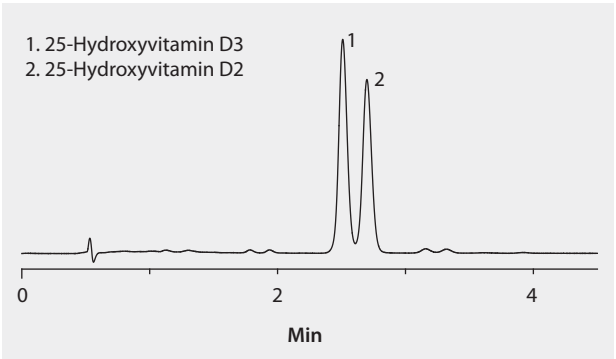
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 10mM ammonium formate in water;
(B) 10mM ammonium formate in acetonitrile; (32:68, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1711 psi (118 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+)
injection . . . . . 2 µL
sample . . . . . 10 µg/mL in 25:75, water:methanol
Application No. . . . . G005658



## LC-MS Analysis of 25-Hydroxyvitamin D2 and 25-Hydroxyvitamin D3 on Ascentis® Express F5

The 25-OH forms of the secosteroids vitamin D2 and D3 are indicators of the vitamin D status of an individual. However, because they have different biological activity and clinical interest, they should be distinguishable. The F5 phase was able to resolve the two compounds under highly LC-MS/MS compatible conditions.

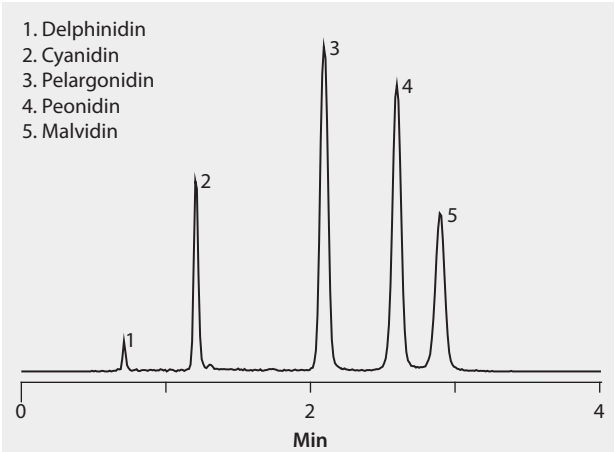
column . . . Ascentis Express F5, 10 cm x 2.1 mm I.D., 2.7 µm particles (53569-U)
mobile phase . . (A) 5 mM ammonium formate in (B) 75:25 (v/v) methanol:water
flow rate . . . . . 0.4 mL/min
column temp. . . . . 40 °C
detector . . . . . UV, 265 nm (MS, ESI(+), m/z 100-1000)
injection . . . . . 1 µL
sample . . . . . 20 µg/mL in methanol
Application No. . . . . G005858



## LC-MS Analysis of Vaccinium Anthocyanidins on Ascentis® Express C18 at Elevated Temperature and Flow

Fruits of the genus Vaccinium are popular sources for anthocyanidin antioxidants. This genus includes the commonly known blueberry, huckleberry, and bilberry.

column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) 0.1% TFA in water;
(B) 0.1% TFA in 75:25 (v/v) acetonitrile:water; (78:22, A:B)
flow rate . . . . . 0.6 mL/min
pressure . . . . . 5440 psi (375 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+), TOF
injection . . . . . 1 µL
sample . . . . . 20 µg/mL in 0.1% formic acid
Application No. . . . . G005628



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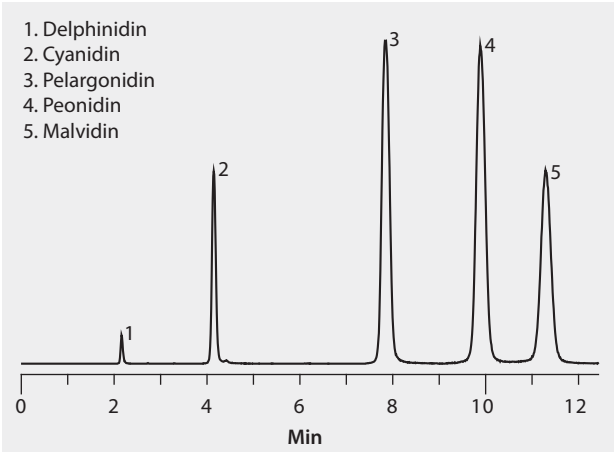
## LC-MS Analysis of Vaccinium Antioxidants on Ascentis® Express C18

Fruits of the genus Vaccinium are popular sources for anthocyanidin antioxidants. This genus includes the commonly known blueberry, huckleberry, and bilberry.

column . . Ascentis Express C18, 10 cm X 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) 0.1% TFA in water;
(B) 0.1% TFA in 75:25 (v/v) acetonitrile:water; (78:22, A:B)
flow rate . . . . . 0.3 mL/min
pressure . . . . . 3260 psi (225 bar)
column temp. . . . . 35 °C
detector . . . . . ESI(+), TOF
injection . . . . . 5 µL
sample . . . . . 20 µg/mL in 0.1% formic acid
Application No. . . . . G005627



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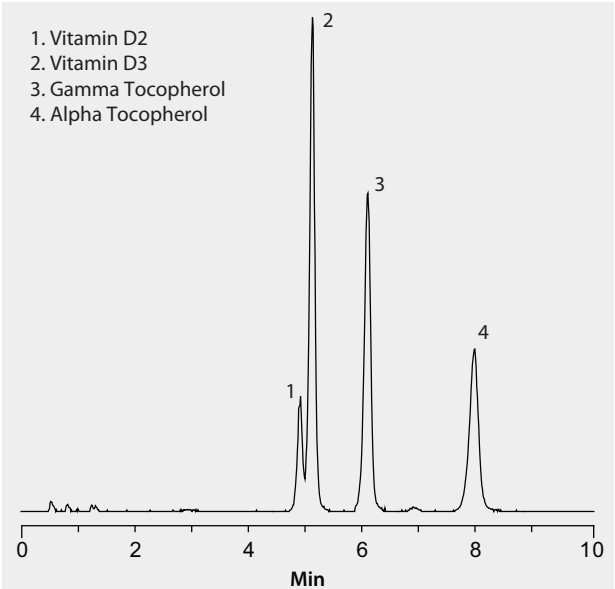
## LC-MS Analysis of Vitamin D2, D3, Gamma- and Alpha-Tocopherol on Ascentis® Express C18

Analysis was conducted using standard solution, detection on LC/MS system.

column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (5:95, A:B)
flow rate . . . . . 0.4 mL/min
column temp. . . . . 40 °C
detector . . . . . MS, ESI+
injection . . . . . 2 µL
sample . . . . . 1 µg/mL in acetonitrile
Application No. . . . . G005806



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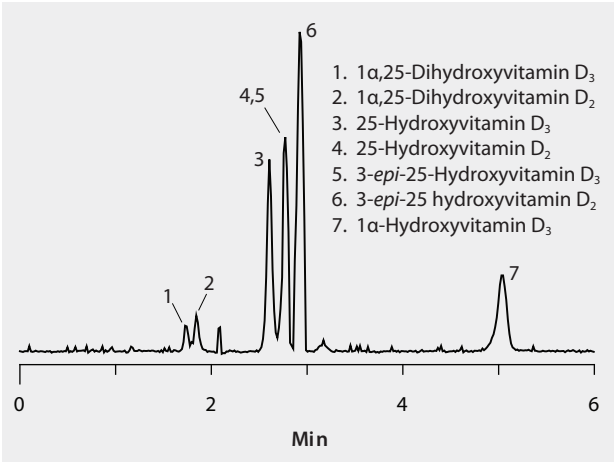
## LC-MS/MS Analysis of Vitamin D Metabolites on Ascentis® Express F5

column . . Ascentis Express F5, 15 cm x 2.1 mm I.D., 2.7 µm particles (53571-U)
mobile phase . . (A) 5 mM ammonium formate water; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 5178 psi (357 bar)
column temp. . . . . 40 °C
detector . . . . . ESI(+), 100-1000 m/z
injection . . . . . 2 µL, each compound 300 ng/mL in 40:60 (water:methanol)
Application No. . . . . G005878



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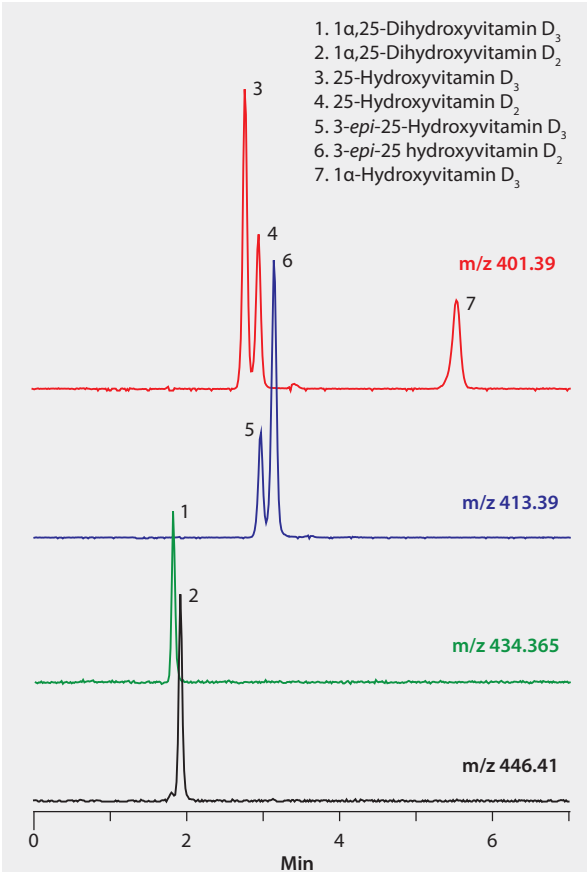
## LC-MS/MS Analysis of Vitamin D Metabolites on Ascentis® Express F5 (Monitored Ions)

column . . Ascentis Express F5, 15 cm x 2.1 mm I.D., 2.7 µm particles (53571-U)
mobile phase . . (A) 5 mM ammonium formate water; (B) methanol; (25:75, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 5178 psi (357 bar)
column temp. . . . . 40 °C
detector . . . . . ESI(+), 100-1000 m/z
injection . . . . . 2 µL, each compound 300 ng/mL in 40:60 (water:methanol)
Application No. . . . . G005876



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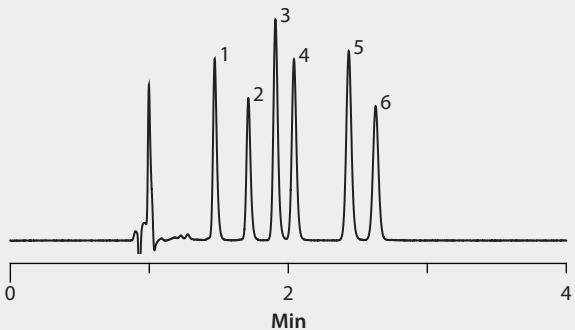
## UHPLC Analysis of Boswellic Acids on Ascentis® Express 2.7 µM Phenyl-Hexyl

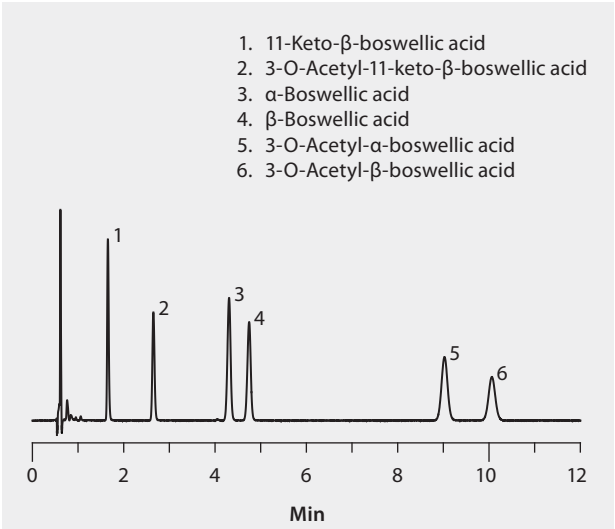
| | |
|---|---|
| column | Ascentis Express Phenyl-Hexyl, 10 cm x 3.0 mm I.D., 2.7 µm particles (53345-U) |
| mobile phase | (A) 0.1% phosphoric acid; (B) acetonitrile; (10:90, A:B) |
| flow rate | 0.4 mL/min |
| pressure | 850 psi (59 bar) |
| column temp. | 35 °C |
| detector | UV, 210 nm |
| injection | 2.0 µL |
| sample | 6 Boswellic Acids 50µg/mL in 70:30, water:methanol |
| Application No. | G006257 |

1. 11-Keto-β-boswellic acid
2. 3-O-Acetyl-11-keto-β-boswellic acid
3. α-Boswellic acid
4. β-Boswellic acid
5. 3-O-Acetyl-α-boswellic acid
6. 3-O-Acetyl-β-boswellic acid



## UHPLC Analysis of Boswellic Acids on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% phosphoric acid; (B) acetonitrile; (10:90, A:B)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 2450 psi (169 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 2.0 µL
sample . . . . . 6 Boswellic Acids 50µg/mL in 70:30, water:methanol
Application No. . . . . G006258



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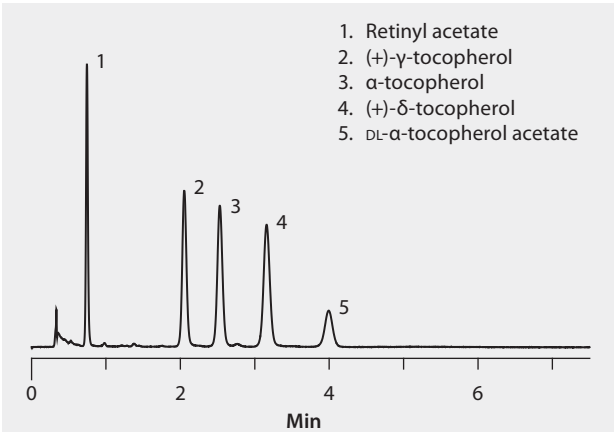
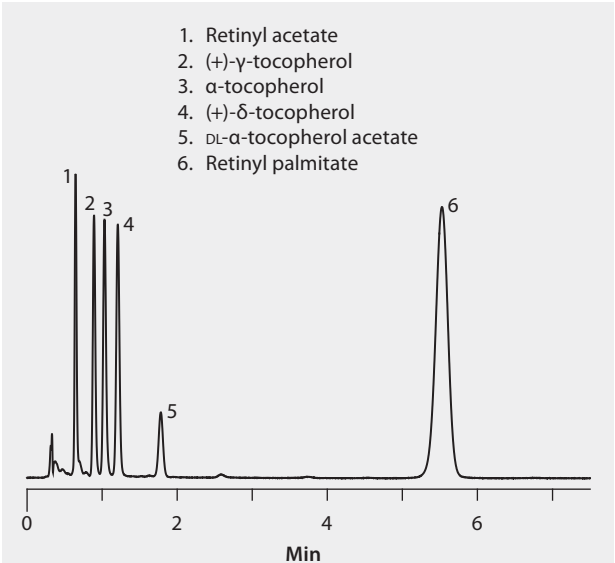
## UHPLC Analysis of Fat Soluble Vitamins on Titan™ C18, Acetonitrile Mobile Phase

column . . . . . TITAN C18, 50 mm x 2.1mm I.D. x 1.9 µm particles (577122-U)
mobile phase . . . . . 100% acetonitrile
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1755 psi (121 bar)
column temp. . . . . 30 °C
detector . . . . .
injection . . . . . 0.5 µL
sample . . . . . All compounds at 0.5 mg/mL except retinyl acetate at 0.05 mg/mL in methanol
Application No. . . . . G006221



## UHPLC Analysis of Fat Soluble Vitamins on Titan™ C18, Methanol Mobile Phase

| | |
|---|---|
| column | Titan C18, 50 mm x 2.1 mm I.D., 1.9 µm particles (577122-U) |
| mobile phase | 100% methanol |
| flow rate | 0.4 mL/min |
| pressure | 2741 psi (189 bar) |
| column temp. | 30 °C |
| injection | UV, 280 nm |
| injection | 0.5 µL |
| sample | All compounds at 0.5 mg/mL except retinyl acetate at 0.05 mg/mL in methanol |
| Application No. | G006222 |



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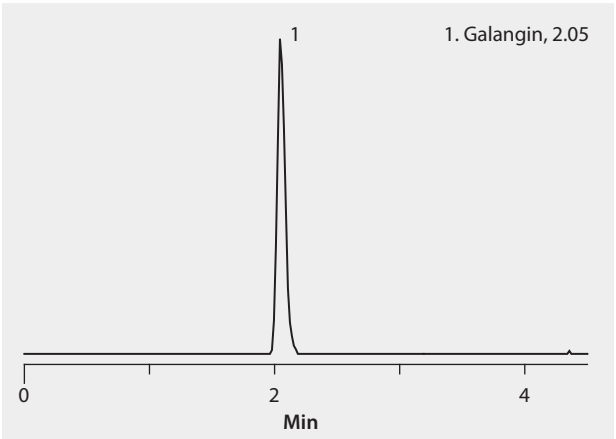
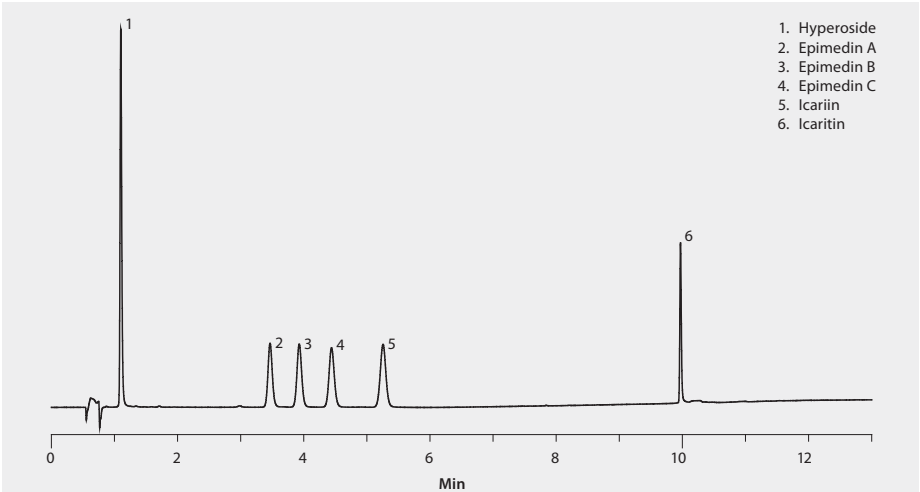
## UHPLC Analysis of Galangin on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 10 mM ammonium acetate, pH 7.0; (B) acetonitrile (70:30, A;B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, XIC m/z 271.05
injection . . . . . 2 µL
sample . . . . . 300 ng/mL each in acetonitrile:water (3:1)
Application No. . . . . G006093



## UHPLC Analysis of Horn Goat Weed Standards Using Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . (A) 0.1% acetic acid in water; (B) 0.1% acetic acid in acetonitrile;
gradient . . . 25% B held for 3 min; to 100% B in 6 min; 100% B held for 2 min
flow rate . . . . . 0.4 mL/min
pressure . . . . . 5130 psi (354 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 250 nm
injection . . . . . 5.0 µL
sample . . . . . 50 µg/mL 70:30, water:methanol
Application No. . . . . G006311

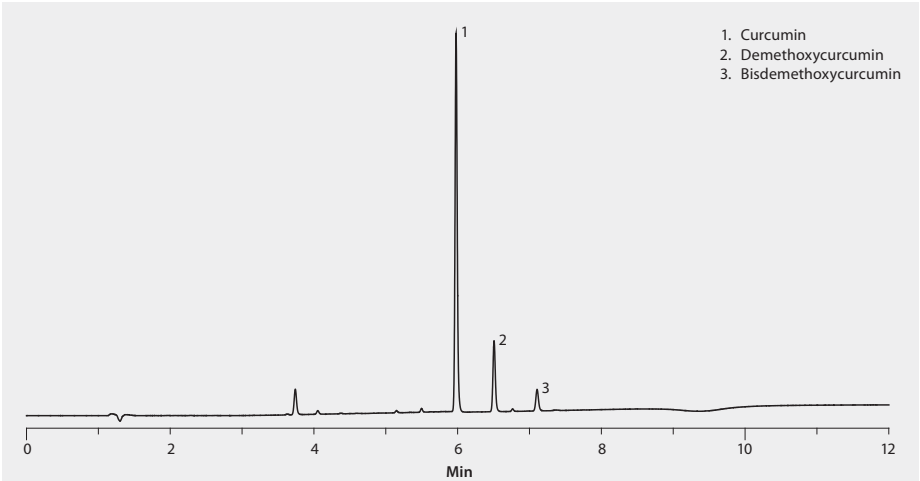





SUPELCO® Solutions within.™

## UHPLC Analysis of Turmeric Curcumin Herbal Supplement on Ascentis® Express RP-Amide 5 µm

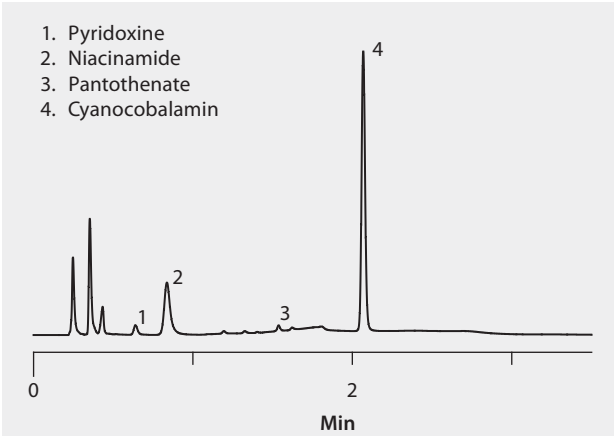
extraction . . . . . Weigh approximately 100 mg of herbal supplement ad add 100 mL ethanol
sample preparation . . . . . Sonicate 20 min
sample preparation . . . . . Filter with 0.22 µm PVDF
column . . Ascentis Express RP-Amide, 10 cm x 4.6 mm I.D., 5 µm particles (50774-U)
mobile phase . . . . . (A) water with 0.1% formic acid; (B) acetonitrile with 0.1% formic acid;
gradient . . 40% B held for 1 min; 40 to 100% B in (6 min); 100% B held for 5 min
flow rate . . . . . 1.0 mL/min
pressure . . . . . 1270 psi (88 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 250 nm
injection . . . . . 5.0 µL
sample . . . . . turmeric curcumin hernal supplement extract in ethanol dilute 1:1 with water
Application No. . . . . G006289



## UHPLC Analysis of B-vitamins in vitamin water on Titan™ C18 using UV detection

The vitamin water drink was filtered using 0.2 um Nylon filter and injected into UHPLC for B-vitamin analysis

column . . . . . TITAN C18, 50 mm x 2.1 mm I.D., 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 20 mM potassium phosphate, pH 3; (B) methanol
gradient . . . . . 0.5% B held for 0.5 minutes, to 30% B in 1.3 minutes, held at 30% B for 1 minute, held at 0.5% B for 0.8 minutes
flow rate . . . . . 0.5 mL/min
pressure . . . . . 7977 psi (550 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 210 nm
injection . . . . . 0.5 µL
sample . . . . . vitamin water was filtered through 0.2um nylon syringe filter
Application No. . . . . G006216



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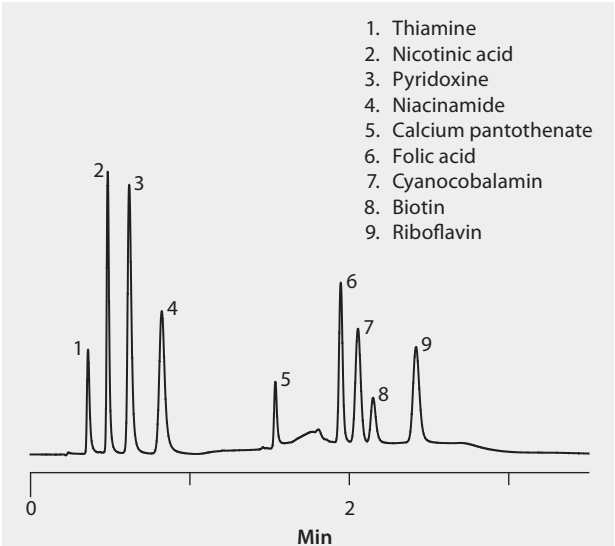
## UHPLC Analysis of B-vitamins on Titan™ C18 using UV detection

column . . . . . TITAN C18, 50 mm x 2.1 mm I.D., x 1.9 µm particles (577122-U)
mobile phase . . . . . (A) 20 mM potassium phosphate, pH 3; (B) methanol
gradient . . . . . 0.5% B held for 0.5 minutes, to 30% B in 1.3 minutes, held at 30% B for 1 minute, held at 0.5% B for 0.8 minutes
flow rate . . . . . 0.5 mL/min
pressure . . . . . 7977 psi (550 bar)
column temp. . . . . 30 °C
detector . . . . . UV, 210 nm
injection . . . . . 0.5 µL
sample . . . . . 100 ug/mL of all vitamins except 250 ug/mL of calcium pantothenate, biotin and riboflavin in water
Application No. . . . . G006217



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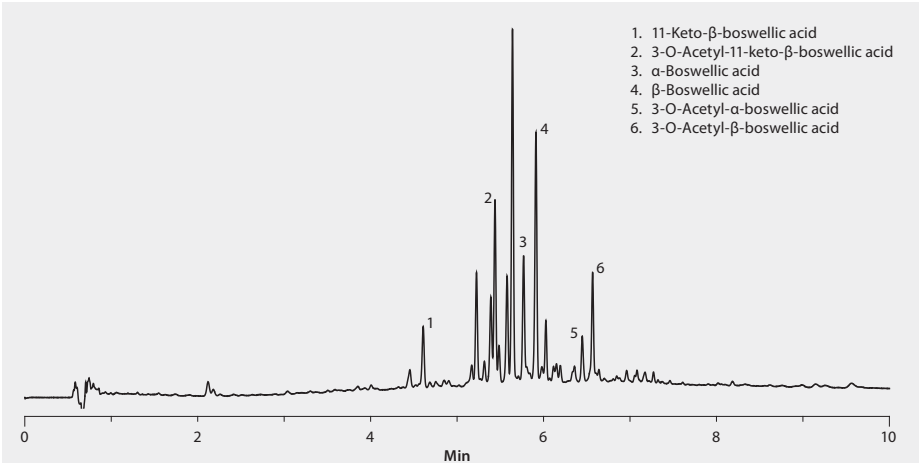
## UHPLC Gradient Analysis of Boswellia Herbal Supplement on Ascentis® Express 2.7µm Phenyl-Hexyl

**extraction:**

1. Weigh 100 mg Bosewellia Herbal Supplement
2. Add 10 mL ethanol
3. Sonicate 30 min at 40°C
4. Dilute 100 µL extract, 400 µL ethanol, 500 µL water

column . . . . . Ascentis Express Phenyl-Hexyl, 10 cm x 3.0 mm I.D., 2.7 µm particles (53345-U)
mobile phase . . . . . (A) 0.1% phosphoric acid; (B) acetonitrile
gradient . . 50% B for 0.5 min; to 100% B in 4.5 min; held at 100% B for 5 min
gradient . . . . . 0.6 mL/min
pressure . . . . . 2390 psi (165 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 2.0 µL
Application No. . . . . G006252






SUPELCO® Solutions within.™

## UHPLC Gradient Analysis of Boswellia Herbal Supplement on Titan™ C18

extraction . . . . . 1. Weigh 100 mg Bosewellia Herbal Supplement
2. Add 10 mL ethanol
3. Sonicate 30 min at 40°C
4. Dilute 100 µL extract, 400 µL ethanol, 500 µL water

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)

mobile phase . . . . . (A) 0.1% phosphoric acid; (B) acetonitrile

gradient . . 40% B for 0.5 min; to 100% B in 2 min; held at 100% B for 10 min

flow rate . . . . . 0.4 mL/min

pressure . . . . . 4050 psi (279 bar)

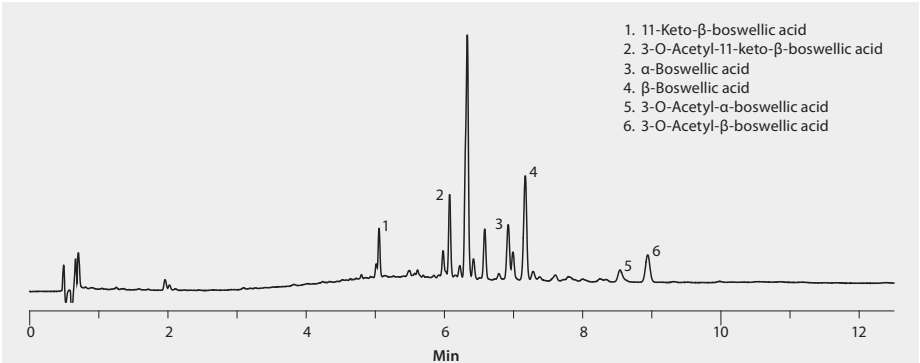
column temp. . . . . 35 °C

detector . . . . . UV, 210 nm

injection . . . . . 2.0 µL

sample . . . . . Bosewellia Extract in 50:50, water:ethanol

Application No. . . . . G006260

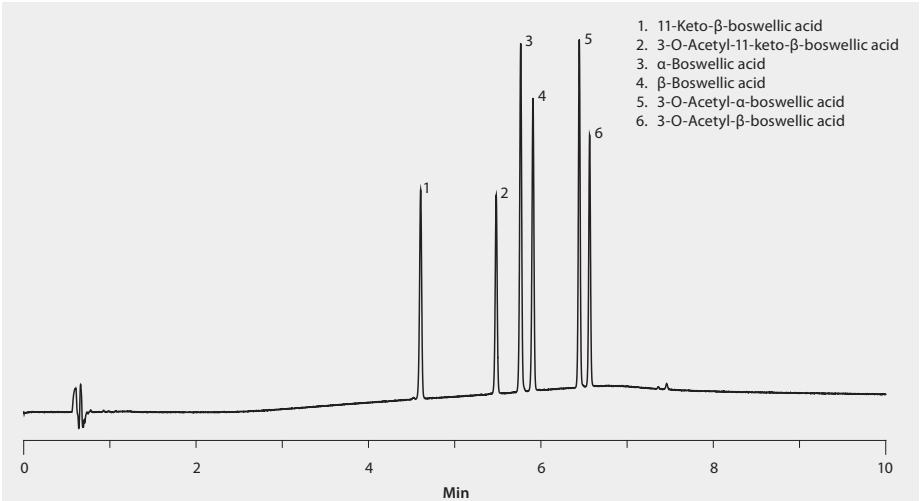





SUPELCO® Solutions within.™

## UHPLC Gradient Analysis of Boswellic Acids on Ascentis® Express Phenyl-Hexyl

column . . . . . Ascentis Express Phenyl-Hexyl, 10 cm x 3.0 mm I.D., 2.7 µm particles (53345-U)
mobile phase . . . . . (A) 0.1% phosphoric acid; (B) acetonitrile
gradient . . 50% B for 0.5 min; to 100% B in 4.5 min; held at 100% B for 5 min
flow rate . . . . . 0.6 mL/min
pressure . . . . . 2390 psi (165 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 2.0 µL
sample . . . . . 6 Boswellic Acids 50µg/mL in 70:30, water:methanol
Application No. . . . . G006285



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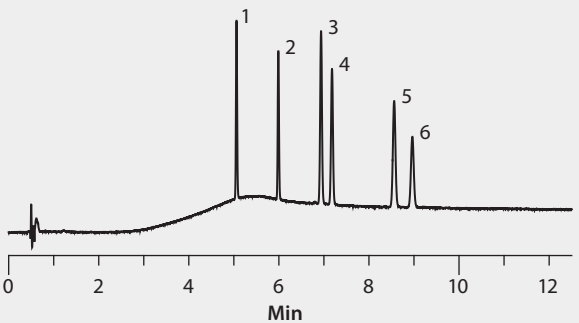
## UHPLC Gradient Analysis of Boswellic Acids on Titan™ C18

column . . . . . Titan C18, 10 cm x 2.1 mm I.D., 1.9 µm particles (577124-U)
mobile phase . . . . . (A) 0.1% phosphoric acid; (B) acetonitrile
gradient . . 40% B for 0.5 min; to 100% B in 2 min; held at 100% B for 10 min
flow rate . . . . . 0.4 mL/min
pressure . . . . . 4050 psi (279 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 210 nm
injection . . . . . 2.0 µL
sample . . . . . 6 Boswellic Acids 50µg/mL in 70:30, water:methanol
Application No. . . . . G006259

1. 11-Keto-β-boswellic acid
2. 3-O-Acetyl-11-keto-β-boswellic acid
3. α-Boswellic acid
4. β-Boswellic acid
5. 3-O-Acetyl-α-boswellic acid
6. 3-O-Acetyl-β-boswellic acid



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# SFC

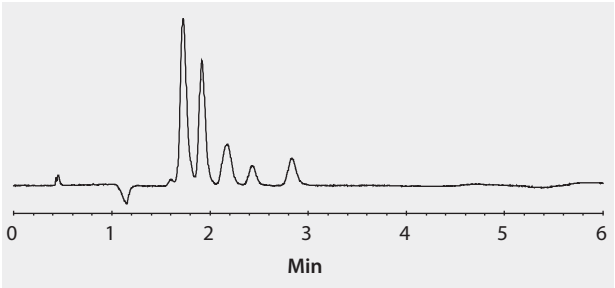
## Chemicals and Industrial Polymers

### SFC Analysis of Diastereomers on Astec® Cellulose DMP, Methanol Additive

This application shows the ability of Astec Cellulose DMP to separate a mixture of diastereomers. The analytes are proprietary pharmaceutical-type compounds.

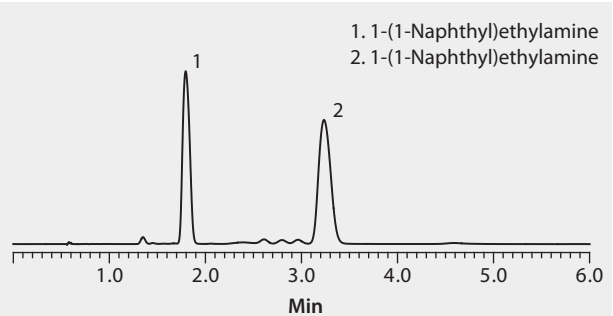
SFC data provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ. Instrument was a Berger Analytical SFC system equipped with a dual pump (FCM-1200), autosampler (ALS 3100), column oven (TCM-200) and diode-array detector (DAD-4100); from Mettler Toledo.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) $CO_2$; (B) methanol; (80:20, A:B)
flow rate . . . . . 3 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
Application No. . . . . G005176



## SFC Analysis of 1-(1-Naphthyl)ethylamine Enantiomers on Astec® Cellulose DMP

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . 20% methanol (0.1% MSA, methanesulfonic acid) in $CO_2$
flow rate . . . . . 3 mL/min
pressure . . . . . 1740 psi (120 bar)
column temp. . . . . 40 °C
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G005490

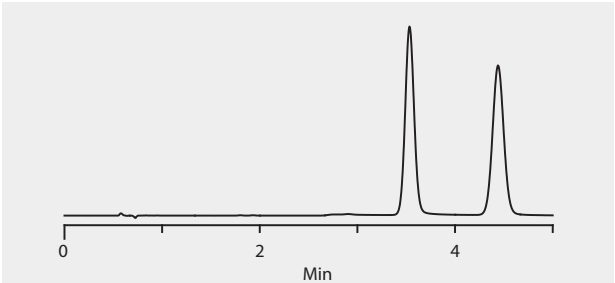


# Pharmaceutical (Small Molecule)

## SFC Analysis of Aminoglutethimide Enantiomers on Astec® Cellulose DMP

This application demonstrates the ability of the Astec® Cellulose DMP column to separate Aminoglutethimide enantiomers via Supercritical Fluid Chromatography. DMPC-derivatized cellulose is widely used for chiral separations by SFC, both analytical and prep. The Astec® Cellulose DMP works well in SFC mode, providing rapid separations with excellent selectivity.

column . . Astec Cellulose DMP, 25 cm x 4.6 mm I.D., 5 µm particles (51099AST)
mobile phase . . (A) $CO_2$, (B) methanol containing 0.1% DEA in $CO_2$, (75:25, A:B)
flow rate . . . . . 3 mL/min
pressure . . . . . 1740 psi (120 bar)
detector . . . . . UV, 230 nm
injection . . . . . 5 µL
sample . . . . . 2 mg/mL in methanol
Application No. . . . . G005454



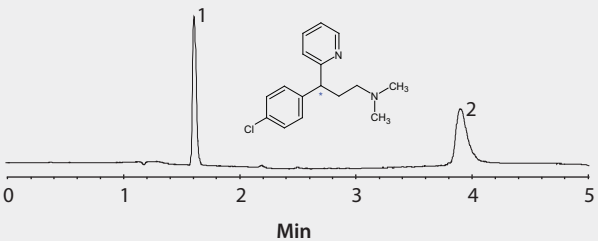
## SFC Analysis of Chlorpheniramine Enantiomers on Astec® Cellulose DMP, Methanol Gradient

SFC data provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ.

Instrument: Berger Analytical SFC system equipped with a dual pump (FCM-1200), autosampler (ALS 3100), column oven (TCM-200) and diode-array detector (DAD-4100); from Mettler Toledo.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
gradient . . . . . Gradient of 5-65% methanol in 3 min, hold for 1 min
mobile phase . . . . . (A) $CO_2$; (B) methanol;
flow rate . . . . . 4 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . chlorpheniramine, 2 mg/mL
Application No. . . . . G005175

1. Chlorpheniramine enantiomer
2. Chlorpheniramine enantiomer

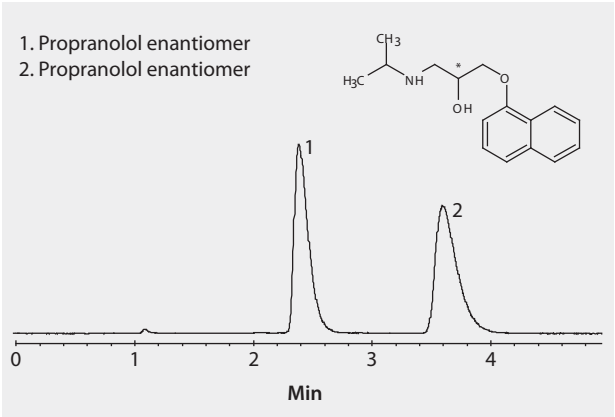


## SFC Analysis of Propranolol Enantiomers on Astec® Cellulose DMP, Methanol Additive

SFC data provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ.

Instrument: Berger Analytical SFC system equipped with a dual pump (FCM-1200), autosampler (ALS 3100), column oven (TCM-200) and diode-array detector (DAD-4100); from Mettler Toledo.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A)$CO_2$; (B) methanol; (80:20, A:B)
flow rate . . . . . 3 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . propranolol, 2 mg/mL
Application No. . . . . G005172



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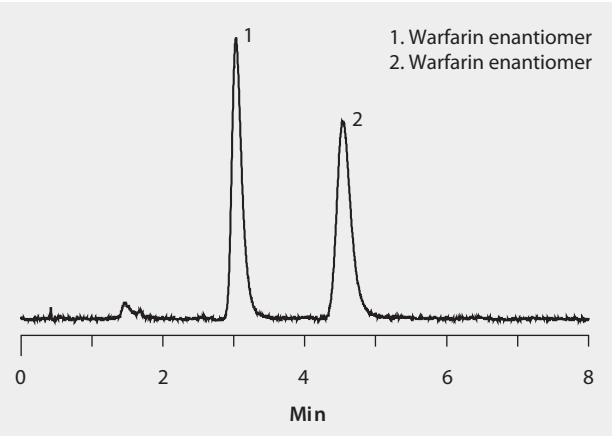
## SFC Analysis of Warfarin™ Enantiomers on Astec® Cellulose DMP, Ethanol Additive

SFC data provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ.

Instrument: Berger Analytical SFC system equipped with a dual pump (FCM-1200), autosampler (ALS 3100), column oven (TCM-200) and diode-array detector (DAD-4100); from Mettler Toledo.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) $CO_2$; (B) ethanol; (80:20, A:B)
flow rate . . . . . 3 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . warfarin enantiomers, 2 mg/mL
Application No. . . . . G005171





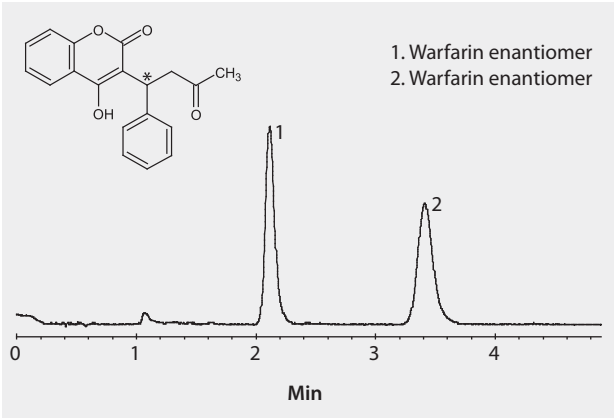

SUPELCO® Solutions within.™

## SFC Analysis of Warfarin™ Enantiomers on Astec® Cellulose DMP, Methanol Additive

SFC data provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ.

Instrument: Berger Analytical SFCsystem equipped with a dual pump (FCM-1200), autosampler (ALS 3100), column oven (TCM-200) and diode-array detector (DAD-4100); from Mettler Toledo.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) $CO_2$; (B) methanol; (80:20, A:B)
flow rate . . . . . 3 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . warfarin enantiomers, 2 mg/mL
Application No. . . . . G005170



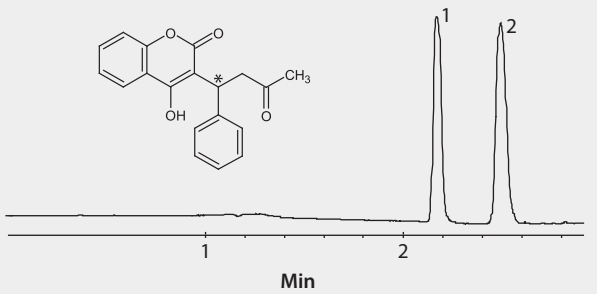
## SFC Analysis of Warfarin™ Enantiomers on Astec® Cellulose DMP, Methanol Gradient

SFC data provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ.

Instrument: Berger Analytical SFC system equipped with a dual pump (FCM-1200), autosampler (ALS 3100), column oven (TCM-200) and diode-array detector (DAD-4100); from Mettler Toledo.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) carbon dioxide; (B) methanol; Gradient of 5-65% methanol in 3 min., hold for 1 min
flow rate . . . . . 4 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . warfarin
Application No. . . . . G005174

1. Warfarin enantiomer
2. Warfarin enantiomer





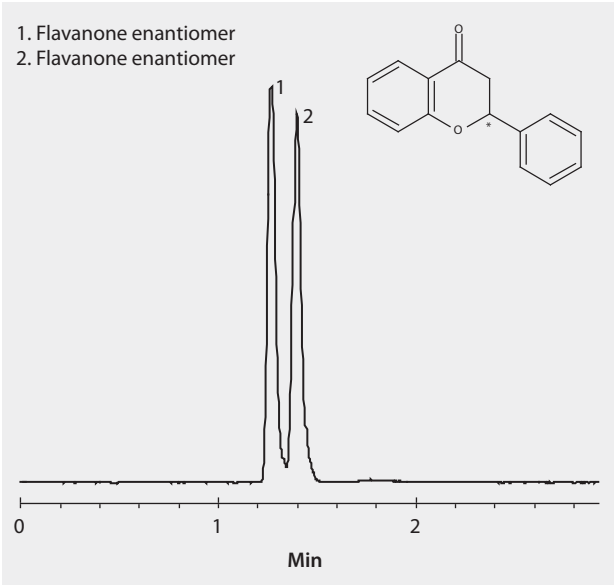

SUPELCO® Solutions within.™

# Vitamins, Nutraceuticals, and Natural Products

## SFC Analysis of Flavanone Enantiomers on Astec® Cellulose DMP, Methanol Additive

SFC data provided by Dr. Christina Kraml, Lotus Separations, LLC, Princeton, NJ.
Instrument: Berger Analytical SFC system equipped with a dual pump (FCM-1200), autosampler (ALS 3100), column oven (TCM-200) and diode-array detector (DAD-4100); from Mettler Toledo.

column . . Astec Cellulose DMP, 15 cm x 4.6 mm I.D., 5 µm particles (51098AST)
mobile phase . . . . . (A) $CO_2$; (B) isopropanol; (80:20, A:B)
flow rate . . . . . 3 mL/min
pressure . . . . . 1450 psi (100 bar)
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 5 µL
sample . . . . . flavanone, 2 mg/mL
Application No. . . . . G005173



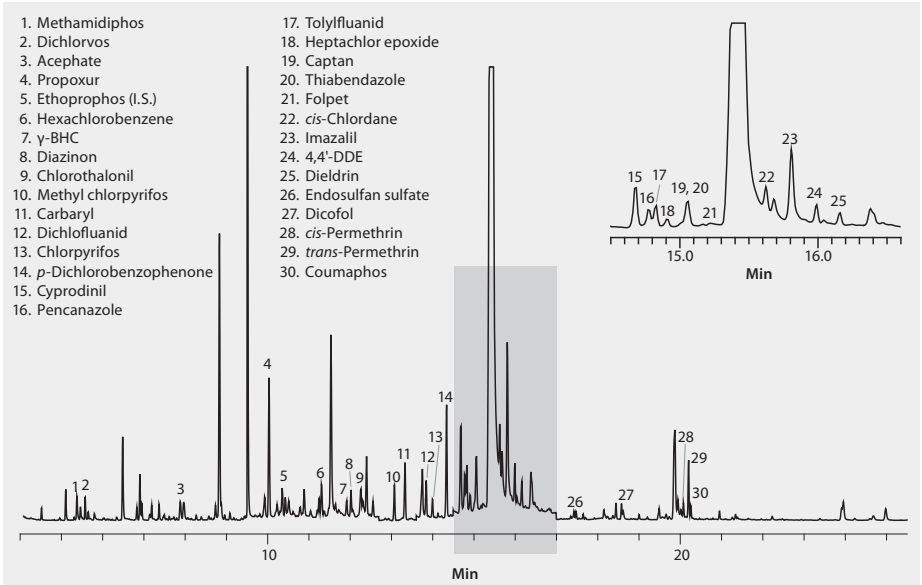
# SPE

## Agriculture

### GC Analysis of Agriculture Pesticides in Orange (Spiked) on SLB®-5ms after QuEChERS Cleanup using Primary-Secondary Amine (PSA)

Chromatograms of the spiked orange samples are presented in the Figure. Several background peaks eluting prior to nine minutes are due to impurities in the toluene. Despite extract cleanup, matrix peaks are also present in the chromatograms. Further sample cleanup may be possible by increasing SPE sorbent weight. Nevertheless, all pesticides were detected. A first order fit was used for calibration. Linearity for the five-point calibration curves was excellent, with 28 of the 29 pesticides having r2 values >0.995 at a range of 50-500 ppb. Proper calibration of imazalil was not possible due to its presence in the orange blanks.

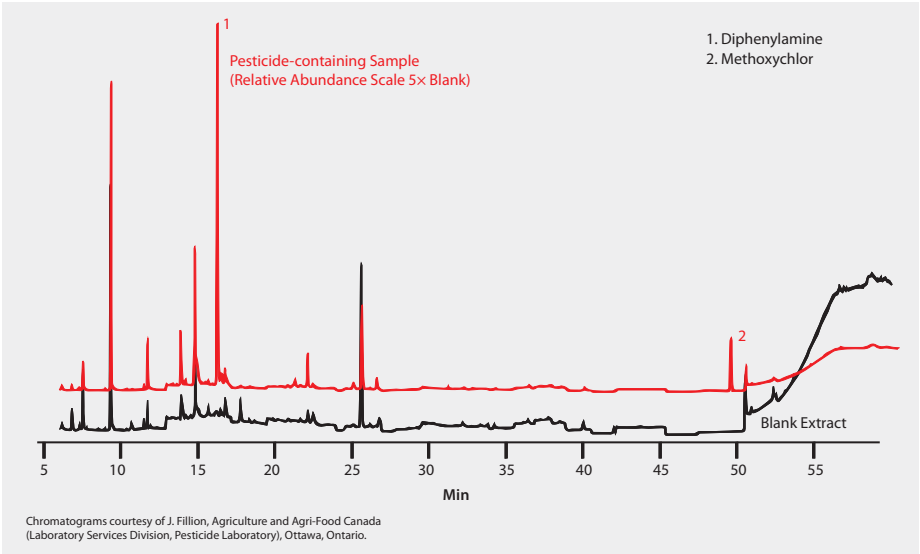
sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . 15 g of ground-up orange, 75 µL of the internal standard stock solution (ethoprophos at 20 ppm in methanol), 75 µL of the pesticide stock solution (29 pesticides, each at 20 ppm in methanol)
column . . . . . SLB-5ms, 30 m x 0.25 mm I.D, 0.25 µm (28471-U)
oven . . . . . 100 °C (1 min), 10 °C/min to 300 °C (5 min)
inj. temp. . . . . 250 °C
MSD interface . . . . . 300 °C
scan range . . . . . selected ion monitoring (SIM), 7 monitoring groups used
carrier gas . . . . . helium, 1 mL/min constant
injection . . . . . 1.0 µL pulsed (20 psi until 0.20 min), splitless (1.0 min)
liner . . . . . 4 mm I.D., single taper
sample . . . . . extract of an orange spiked with a 29-component pesticide mix plus 1 internal standard, each at 100 ppb
Application No. . . . . G003590



## GC Analysis of Diphenylamine and Methoxychlor in Apples on a 14% Cyanopropylphenyl Column after SPE using Supelclean ENVI-Carb

**Pesticides in fruits and vegetables**

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | homogenize 50 g sample in 100 mL acetonitrile then add 10 g NaCl and homogenize 5 min |
| SPE tube/cartridge | ENVI-Carb, 500 mg/6 mL |
| column | 14% cyanopropylphenyl polysiloxane, 30 m × 0.25 mm I.D., 0.15 µm (Supelco equivalent, Equity-1701, 28372-U) |
| oven | 70 °C (2 min), 25 °C/min to 130 °C, 2 °C/min to 220 °C, 10 °C/min to 280 °C (4.6 min) |
| detector | MSD, 285 °C |
| carrier gas | helium |
| injection | 2 µL splitless |
| Application No. | 796-0129 |



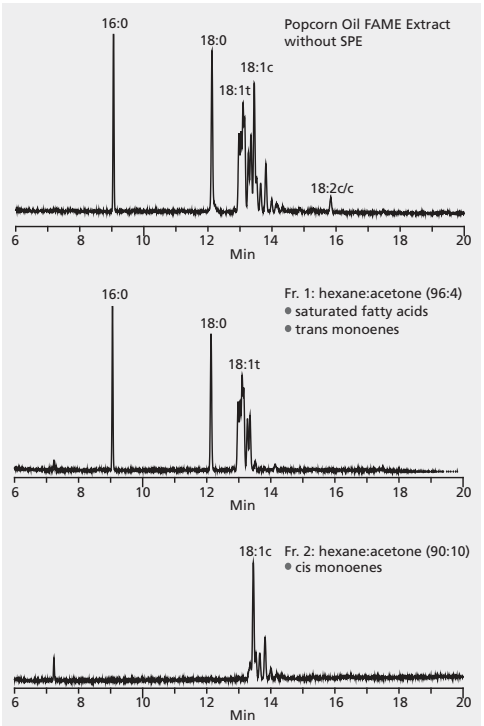

Chromatograms courtesy of J. Fillion, Agriculture and Agri-Food Canada (Laboratory Services Division, Pesticide Laboratory), Ottawa, Ontario.

## GC Analysis of FAMEs in Popcorn Oil on SP™-2560 (75 m x 0.18 mm I.D., 0.14 µm) after SPE Fractionation using Discovery® Ag-Ion, Fast GC Analysis

**Sample pretreatment**: A 1 g sample of popcorn oil was scraped from the inside of a popped microwave oven popcorn bag and mixed with 8 mL deionized water (DI) and 4 mL petroleum ether, centrifuged to remove particulates. The ether layer (upper layer) was collected. This petroleum ether extraction step was repeated four times. The upper ether layers were combined, evaporated, and reconstituted in 16 mL toluene. A 1 mL aliquot was transferred to conical reaction vial and 2 mL 7% boron triflluoride in methanol were added. The reaction was incubated at 80 °C for 15 minutes using a heating block, then cooled to room temperature. 1 mL DI water was added and FAMEs were extracted twice using 1 mL hexane each time. The upper hexane layers were combined into a fresh vial, evaporated, and reconstituted in 5 mL hexane and 50 mg anhydrous sodium sulfate.

Discovery Ag-Ion operates by argentation chromatography which is a powerful analytical and sample prep technique for the separation or fractionation of cis and trans geometric and positional fatty acid (FA), fatty acid methyl ester (FAME), diacylglycerol (DAG) and triacylglycerol (TAG) isomers. Discovery Ag-Ion SPE is shown in this application to separate FAMEs from an edible oil sample.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | 1 g of popcorn oil FAME's in Hexane |
| SPE tube/cartridge | Discovery Ag-Ion, 750 mg/6 mL (54225-U) |
| condition | 4 mL acetone and 4 mL hexane |
| sample addition | 1 mL of the hexane FAME extract |
| elution | 6 mL hexane:acetone (96:4, v/v) followed by 4 mL hexane:acetone (90:10, v/v) |
| column | SP-2560, 75 m x 0.18mm I.D., 0.14 µm (23348-U) |
| oven | 180 °C isothermal |
| inj. temp. | 220 °C |
| detector | FID, 220 °C |
| carrier gas | hydrogen, 40 cm/sec. at 180 °C |
| injection | (0.5 µL, 100:1 split) |
| liner | 0.5 µL, 100:1 split |
| Application No. | G003760 |



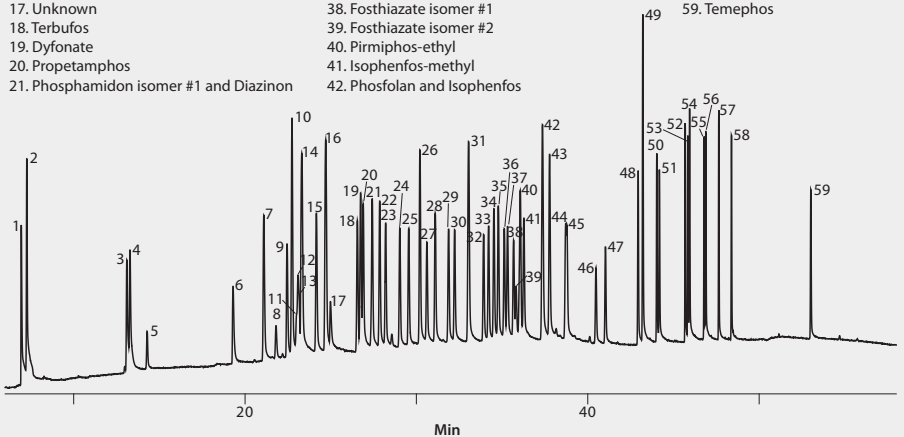
# GC Analysis of Organophosphorus Pesticides (OP-Pest) in Cabbage, Onion, and Mushrooms on SLB-5ms after SPE using Supelclean™ ENVI-Carb™ II/PSA

**Sample pretreatment**: To a 10 g sample of homogenized food (green onions, apples, Napa cabbage, button mushrooms) was added 10 mL acetonitrile and 4 g magnesium sulfate (that had been heated at 550 °C) and 1 g sodium chloride. After centrifugation, the supernatant was removed and mixed with 1 g magnesium sulfate and centrifuged. 5 mL of the supernatant was removed and evaporated to 1 mL for SPE loading.

Organophosphorous pesticide use in agriculture is widespread due to the fact that they are more amenable to environmental degradation in comparison to organochlorine or organonitrogen compounds. There are a large number of pesticides in the organophosphorous group, and because of their potential health effects, they are of particular concern on produce imported from areas in which they are commonly used. This chromatogram illustrates the separation of 63 of these pesticides by GC on the SLB-5ms using nitrogen phosphorus detection (NPD).

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | 10 g homogenized food sample (green onions, apples, Napa cabbage, button mushrooms) |
| SPE tube/cartridge | Supelclean ENVI-Carb II/PSA SPE Tube (54067-U) |
| condition | 5 mL acetone:toluene (65:35) |
| sample addition | 1 mL sample extract |
| elution | 10 mL of acetone:toluene (65:35) |
| eluate post-treatment | Evaporate fraction to 0.5 mL then adjust volume to 1 mL with ethyl acetate for GC-NPD analysis |
| column | SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U) |
| oven | 110 °C (2 min), 2.5 °C/min to 205 °C, 10 °C/min to 310 °C (10 min) |
| inj. temp. | 250 °C |
| detector | NPD, 310 °C |
| carrier gas | helium, 0.9 mL/min constant flow |
| injection | 2 µL, splitless |
| liner | 4 mm I.D. dual tapered |
| Application No. | G005815 |

1. Methamidophos
2. Dichlorvos
3. Phosdrin
4. Acephate
5. Trichlorphan
6. Omethoate
7. Ethoprophos
8. Dibromfos
9. CIPC
10. Dicrotophos
11. Sulfotepp
12. Cadusafos
13. Monocrotophos
14. Phorate
15. Thiometon
16. Dimethoate and Demeton-ethyl
17. Unknown
18. Terbufos
19. Dyfonate
20. Propetamphos
21. Phosphamidon isomer #1 and Diazinon
22. Disulfoton
23. Isazofos
24. Kitazine
25. Formothion
26. Dichlofenthion and Phosphamidon isomer #2
27. Chloropyrifos-methyl
28. Methyl parathion
29. Ronnel
30. Paraoxon-ethyl
31. Pirmiphos-methyl and Fenitrothion
32. Malathion
33. Chloropyrifos
34. Fenthion
35. Parathion
36. Isocarbophos
37. Trichloronate
38. Fosthiazate isomer #1
39. Fosthiazate isomer #2
40. Pirmiphos-ethyl
41. Isophenfos-methyl
42. Phosfolan and Isophenfos
43. Quinalphos and Phenthoate
44. Methidathion
45. Bromophos-ethyl
46. Iodophenfos
47. Profenfos
48. Fensulfothion
49. Ethion
50. Famphur
51. Edinphos
52. Pyridaphenthion
53. Phosmet
54. EPN
55. Phosalone
56. Azinphos-methyl
57. Azinphos-ethyl
58. Coumaphos
59. Temephos



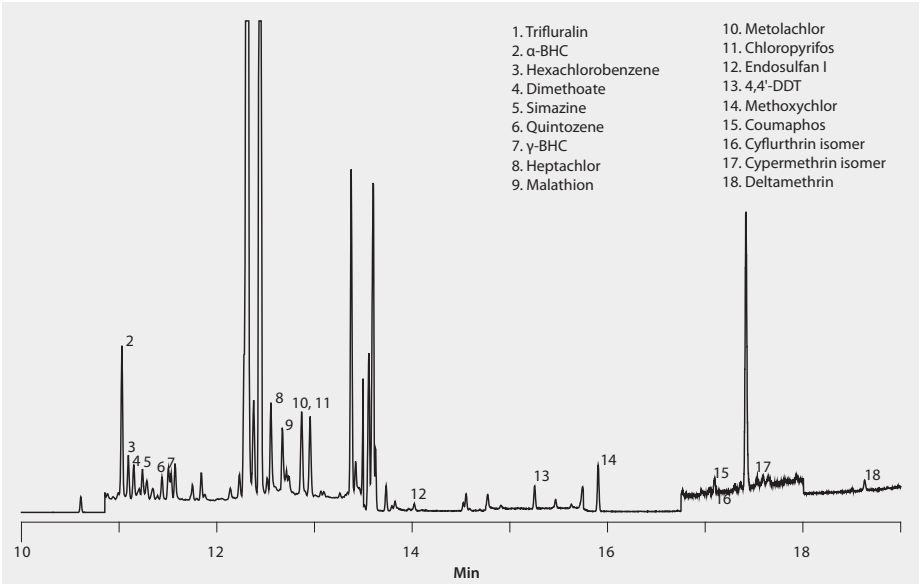



SUPELCO® Solutions within.™

# GC Analysis of Pesticides in Avocado on SLB®-5ms (20 m x 0.18 mm I.D., 0.36 µm) after QuEChERS Cleanup using Supel™ QuE Z-Sep+, Fast GC Analysis

**About this analysis:** This application demonstrates the use of dispersive SPE for the extraction of pesticides from Avocado. Cleanup is achieved using Z-Sep + for fat removal. FInal analysis of the extracts was done by LVI-GC/MS SIM on an SLB-5ms column.

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . . . . Avocado spiked with pesticides at 20 ng/g
extraction tube . . . . . Supel QuE Acetate extraction tube (55234-U)
extraction process . . add 25 mL of acetonitrile; shake for 1 minute; add contents of Supel QuE acetate extraction tube; shake immediately for 1 minute; centrifuge at 3200 rpm for 5 minutes
clean-up tube . . . . . Supel Que Z-Sep+ cleanup tube (55296-U)
clean-up process . . transfer 3 mL of the acetonitrile layer into a Supel Que Z-Sep+ cleanup tube; shake for 1 minute; centrifuge at 3400 rpm for 3 minutes; draw off supernatant for LVI-GCMS analysis
column . . . . . SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm (28576-U)
oven . . . . . 70 °C (2 min), 15 °C/min to 325 °C (6 min)
inj. temp. . . . . 60 °C (0.28 min), 600 °C/min to 325 °C (5 min)
detector . . . . . MSD
MSD interface . . . . . 330 °C
carrier gas . . . . . helium, 1 mL/min, constant
injection . . . . . PTV solvent vent; 10 µL, injection dispense speed 15000 µL/min
liner . . . . . 4 mm I.D. FocusLiner with taper
sample . . . . . pesticides spiked at 20 ng/g
Application No. . . . . G005805



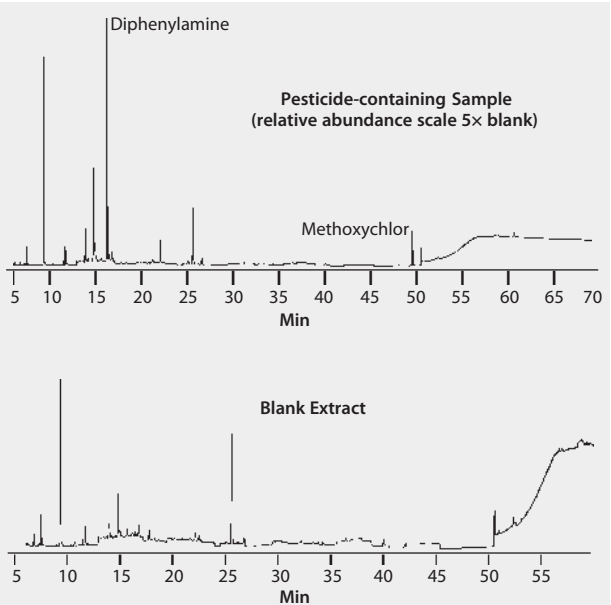



SUPELCO® Solutions within.™

## GC Analysis of Pesticides in Fruits and Vegetables on a 14% Cyanopropylphenyl Column after SPE using Supelclean ENVI-Carb™

**Sample Pre-treatment:**
Homogenize 50 g chopped fruit/vegetable sample in 100 mL acetonitrile. Add 10 g sodium chloride and homogenize 5 min. Transfer 13 mL of acetonitrile layer to clean centrifuge tube. Centrifuge at high speed for 5 min. Evaporate 10 mL aliquot of supernatant to 0.5 mL under Nitrogen at 35°C.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | extract from 50 g homogenized fruit or vegetable sample |
| SPE tube/cartridge | ENVI-Carb, 500 mg/6 mL (57094) |
| sample addition | 0.5 mL concentrated sample extract |
| elution | 20 mL acetonitrile:toluene (3:1) |
| eluate post-treatment | Concentrate to 2 mL by rotary evaporation. Add 2 x 10 mL acetone concentrating to 2 mL after each acetone addition. (Add 50 µL I.S. (500 ng/µL cis-chlordane in acetone) then dilute to 2.5 mL with acetone.) |
| column | 14% cyanopropylphenyl polysiloxane, 30m × 0.25 mm I.D., 0.15 µm (Supelco equivalent, Equity-1701, 28372-U) |
| oven | 70 °C (hold 2 min) to 130 °C at 25 °C; 130 °C to 220 °C at 2 °C/min; 220 °C to 280 °C at 10 °C/min and hold 4.6 min |
| detector | MSD, 285 °C |
| carrier gas | helium |
| injection | 2 µL, splitless |
| Application No. | 796-0128 |



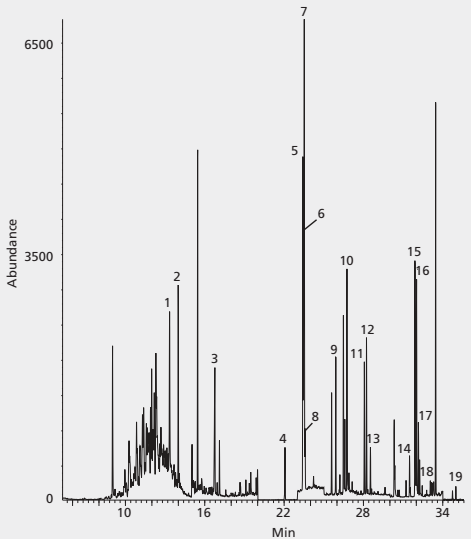

For extraction recovery data, request Bulletin T196900.
Chromatograms provided by J. Fillion, Agriculture and Agri-Food Canada (Laboratory Services Division, Pesticide Laboratory), Ottawa, Ontario.

# GC Analysis of Pesticides in Spinach on Equity®-1 after SPE using Supelclean™ ENVI-Carb-II/PSA

**Sample Pre-treatment:**

Homogenize 10 g of spinach in 10 mL of acetonitrile.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . 10 g of fresh spinach spiked at 0.2 ppm each pesticide
SPE tube/cartridge . . . Supelclean ENVI-Carb-II/PSA, 500mg/300mg/6mL (55119-U)
condition . . . . . 5 mL acetonitrile:toluene (3:1, v/v)
sample addition . . . . . 1 mL concentrated sample extract
elution . . . . . 20 mL acetonitrile:toluene (3:1, v/v)
eluate post-treatment . . . . . Evaporate eluate to dryness with nitrogen at 40 °C. Reconstitute in 1 mL acetone:hexane (1:1, v/v).
column . . . . . Equity-1, 30 m x 0.25 mm I.D., 0.25 µm (28046-U)
oven . . . . . 50 °C (5 min), 25 °C/min to 125 °C, 10 °C/min to 30 °C (8 min)
inj. temp. . . . . 200 °C, aux: 325 °C (8 min)
detector . . . . . MSD, scan range 45-450 amu
carrier gas . . . . . helium, 0.9 mL/min., constant flow mode
injection . . . . . 1 µL, splitless (splitter open at 1 min)
liner . . . . . 4 mm I.D., single taper
Application No. . . . . G003758




1. Methamidophos
2. Dichlorovos
3. Acephate
4. Quintozene
5. Methyl parathion
6. Carbaryl
7. Methyl chloropyriphos
8. Vinclozolin
9. Procymidone
10. Imazalil
11. Chlorothiophos
12. Tetrasul
13. Endosulfan sulfate
14. Acrinathrin
15. Bitertanol
16. cis-Permethrin
17. trans-Permethrin
18. Cypermethrin isomers
19. Deltamethrin

**Efficiency of Recovery**

| Peak ID | Compound | Pesticide Class | Pesticide Recovery (%) |
|---|---|---|---|
| 1 | Methamidophos | Organophosphorous | 80 |
| 2 | Dichlorovos | Organophosphorous | 70 |
| 3 | Acephate | Organophosphorous | 60 |
| 4 | Quintozene | Organochloride | 92 |
| 5 | Methyl parathion | Organophosphorous | 97 |
| 6 | Carbaryl | Carbamate | 128 |
| 7 | Methyl chloropyriphos | Organophosphorous | 99 |
| 8 | Vinclozolin | Organochloride | 83 |
| 9 | Procymidone | Dicarboximide | 84 |
| 10 | Imazalil | Imidazole | 104 |
| 11 | Chlorothiophos | Phophosulfi de | 106 |
| 12 | Tetrasul | Organochloride | 87 |
| 13 | Endosulfan sulfate | Organochloride | 124 |
| 14 | Acrinathrin | Organophosphorous | 118 |
| 15 | Bitertanol | Biphenol | 108 |
| 16 | Permethrin cis and trans | Pyrithroid | 82 |
| 17 | Cypermethrin isomers | Organochloride | 74 |
| 18 | Deltamethrin | Organobromine | 134 |

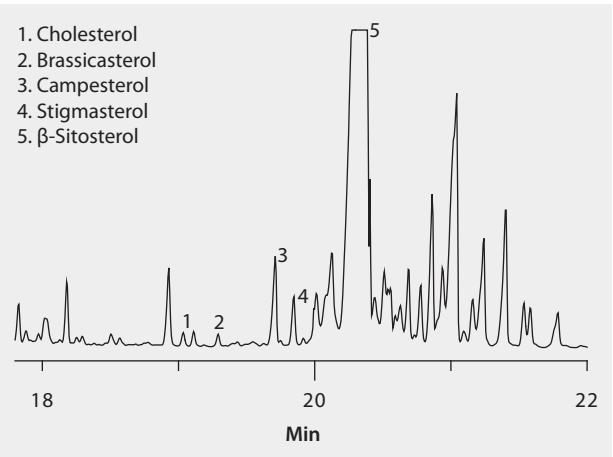
# GC Analysis of Sterols (Silylated Derivatives) in Olive Oil (Free Sterol Fraction) on SLB®-5ms after SPE using Discovery DSC-Si

**Sample pre-treatment**

Weigh 0.25 gm oil into test tube. For spiked sample add 12.5 µL of 65-100 µg/mL mixed sterol std. Add 1 ml of hexane:ethyl acetate (90:10). After SPE fractionation, evaporate solvent from each fraction, add 1 ml of 30% methanolic sodium methoxide:MeOH:MTBE solution (13:27:60), mix and let sit at room temp for 30 min. Add 1 ml of water and 2 mL of n-heptane. Withdraw aqueous phase (bottom) and replace w/ 1 mL of 1% citric acid solution. Withdraw organic phase and dry over anhydrous sodium sulfate. Silylation: Evaporate organic phase to dryness. Add 125 µL of Sylon BFT and 125 µL of pyridine. Heat at 70°C for 20 min. The sample is then ready for GC-MS analysis.

Sterol content is one in a battery of tests used to analyze the composition and determine the grade and authenticity of olive oil. Test results are compared against standards such as those established by the International Olive Oil Council (IOC) and US Dept. of Agriculture. In vegetable oils, sterols exist in the free form, or esterified to fatty acids. In this application, sterols were analyzed in olive oil separately as free and esterified. This was accomplished by taking advantage of the polarity difference between these two groups to fractionate them using silica gel SPE. In place of saponification, the fractions were transesterified to liberate the esterified sterols. The fractions were then silylated, and analyzed by GC-MS. The silylated sterols all contained a significant molecular ion in their mass spectum, which made it possible to perform peak identification free of matrix interference. The sterols isolated in the free fraction are shown here.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | olive oil spiked with a mixed sterol standard at 5 µg/g |
| SPE tube/cartridge | Discovery DSC-Si, 1 g/6 mL (52656-U) |
| condition | 2 x 5 mL hexane |
| sample addition | 1 mL |
| elution | esters fraction: 2.5 mL of hexane:ethyl acetate (90:10); free sterols fraction; 5 mL of hexane:ethyl acetate (90:10) and 3 mL x 5 of ethanol:diethyl ether:hexane (50:25:25) |
| eluate post-treatment | Evaporate to dryness and perform silylation as described in the Analysis Note. |
| column | SLB-5ms, 20 m x 0.20 mm I.D., 0.20 µm (28564-U) |
| oven | 125 °C (1 min), 10 °C/min to 325 °C (10 min) |
| inj. temp. | 250 °C |
| detector | MSD, rn/z 500 - 600 |
| MSD interface | 325 °C |
| carrier gas | helium, 0.6 mL/min, constant |
| injection | 1 µL, splitless |
| liner | 4 mm I.D. single taper |
| sample | Olive oil sample; fractionated, transesterified, and silylated |
| Application No. | G005816 |



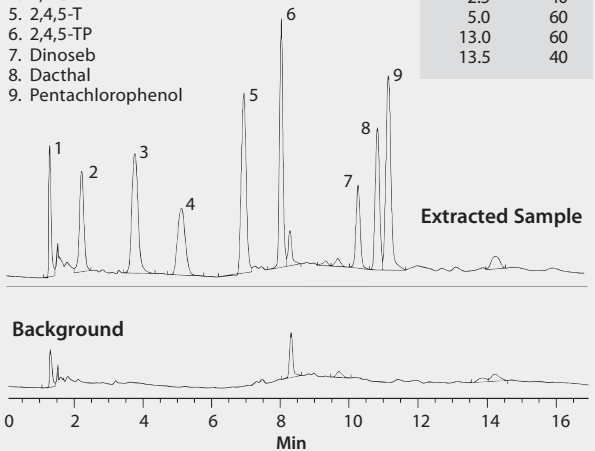
# HPLC Analysis of Acidic Herbicides in Water on a Polymeric C18 Column after SPE using Supelclean™ ENVI™-Carb

*using Zymark AutoTrace Extraction WorkStation 1.20*

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . Fresh 1 L water samples, dechlorinated with sodium thiosulfate when necessary, at ambient temperature and pH.
SPE tube/cartridge . . . . . Supelclean ENVI-Carb, 250 mg/6 mL (57092)
condition . . . . . 10 mL DI water at 20 mL/min
sample addition . . . . . 0.9 L water sample at 20 mL/min
drying . . . . . 10 min using clean nitrogen
washing . . . . . 10 mL DI water at 20 mL/min
elution . . . . . 10 mL 0.1% phosphoric acid in methylene chloride:acetonitrile (80:20) at 5 mL/min
column . . polymeric-coated silica-based PAH specialty column, 20 cm × 3 mm I.D., 5 µm (Supelco equivalent, SUPELCOSIL LC-PAH, available upon request)
mobile phase . . gradient, (A): 0.05% phosphoric acid in DI water; (B): acetonitrile
flow rate . . . . . 0.5 mL/min
column temp. . . . . 50 °C
detector . . . . . photodiode array- peak width: 0.053 min, sampling interval: 0.320 sec, monitor 210 nm & 225 nm
injection . . . . . 10 µL of extract (4-5 ppb each analyte in water)
Application No. . . . . 796-0150

1. Tetrachloroterephthalic acid
2. Picloram
3. Dicamba
4. 2,4-D
5. 2,4,5-T
6. 2,4,5-TP
7. Dinoseb
8. Dacthal
9. Pentachlorophenol

| Gradient Program | |
|---|---|
| Time (min) | %B |
| 2.5 | 40 |
| 5.0 | 60 |
| 13.0 | 60 |
| 13.5 | 40 |




Figures provided by A. Lichtman, Nassau County Dept. of Health, Hempstead, New York, USA. Request Application Note No. T196100.

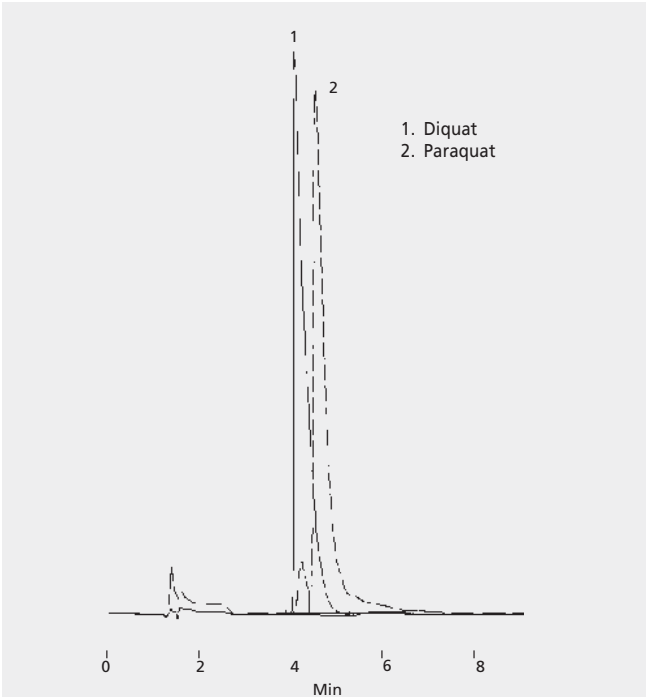
## HPLC Analysis of Paraquat and Diquat on SUPELCOSIL™ LC-18 after SPE using ENVI™-8 DSK

*Refer to US EPA Method 549.1 for full details*

**Sample Pre-treatment:**

250 mL drinking water. Adjust water sample to pH to 10.5 ± 0.2 with 10% sodium hydroxide or 10% hydrochloric acid

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . drinking water adjusted to pH to 10.
SPE tube/cartridge . . . . . ENVI-8 DSK SPE Disk, 47 mm (57172)
sample preparation . . . . . ENVI-8 DSK, 47 mm (57172)
condition . . 10 mL acetonitrile; 2 × 10 mL reagent water; 10 mL conditioning solvent A (5 g cetyl trimethyl ammonium bromide and 5 mL conc. ammonium hydroxide in 500 mL DI water, dilute to 1 L); ( 2 × 10 mL reagent water; 10 mL conditioning solvent B (10 g hexanesulfonic acid, sodium salt and 10 mL conc. ammonium hydroxide in 250 mL DI water, dilute to 500 mL))
sample addition . . . . . 250 mL water 100 mL/min
elution . . 0.5-1.0 mL acetonitrile (to cover/solvate disk); 2 × 4 mL eluting solution (13.5 mL orthophosphoric acid and 10.3 mL diethylamine in 500 mL DI water, dilute to 1 L)
column . . . . SUPELCOSIL LC-18, 15 cm × 4.6 mm I.D., 5 µm particles (58230-U)
mobile phase . . 3.5 mL triethylamine and 1.0 g 1-hexane-sulfonic acid, sodium salt to 800 mL DI $H_2O$ add orthophosphoric acid to pH 2.5, dilute to 1 L
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . photodiode array, diquat - 308 nm, paraquat - 257 nm
injection . . . . . 100 µL
Application No. . . . . 794-0715



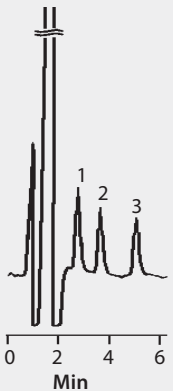



SUPELCO® Solutions within.™

# HPLC Analysis of Triazine Herbicides in Grass on SUPELCOSIL™ LC-8-DB after SPE using Supelclean™ LC-SCX

**Sample Pre-treatment:**
5 g fresh grass clippings spiked with 2 ppm each herbicide. Add 4 g anhydrous sodium sulfate and 20 mL methylene chloride:acetone (80:20). Shake 20 min and allow mixture to stand 1 min.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . fresh grass clippings spiked with herbicides at 2 ppm
SPE tube/cartridge . . . . . Supelclean LC-SCX, 500 mg/3 mL (57018)
condition . . . . . 1 mL methylene chloride
sample addition . . . . . 2 mL grass extract. Wash with 2 x 2 mL acetonitrile.
drying . . . . . Dry packing for 5 min. under nitrogen purge
washing . . Dry packing for 5 min under nitrogen purge; wash with 2 x 2 mL DI water
elution . . . . . 1.5 mL methanol
eluate post-treatment . . . . . dilute to 2 mL with DI water
column . . . . . SUPELCOSIL LC-8-DB, 15 cm × 4.6 mm I.D., 5 µm with guard column (58347)
mobile phase . . . . . (A) acetonitrile; (B) water; (45:55, A:B)
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 254 nm
injection . . . . . 100 µL
Application No. . . . . 84-109

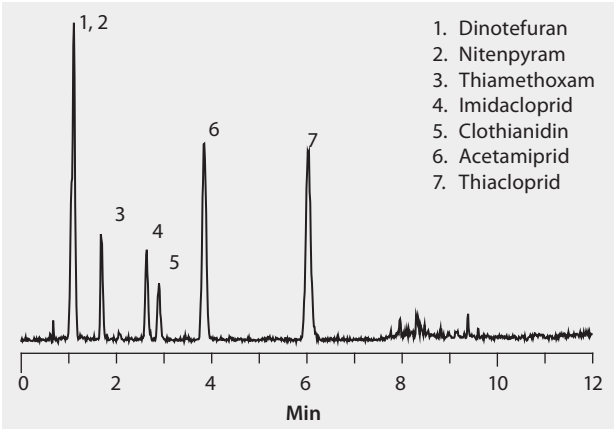



| Recovery (± SD, n = 3) | | |
|---|---|---|
| 102.0 | ± | 2.9 |
| 102.2 | ± | 1.6 |
| 100.5 | ± | 1.8 |

1. Simazine
2. Atrazine
3. Propazine

## LC-MS/MS Analysis of Neonicotinoid Pesticides in Dandelion Blossoms on Ascentis® Express C18 after Dispersive SPE (QuEChERS) using Supel™ QuE

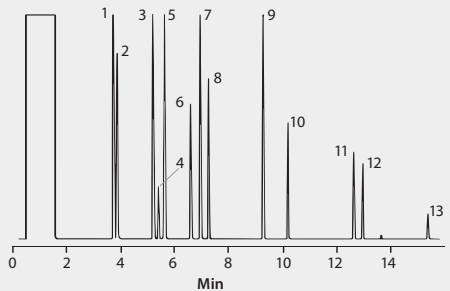
sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . 3 g pulverized dandelion blossoms, homogenized in 10 mL water
extraction tube . . . . . Supel QuE Acetate extration tube (55234-U)
extraction process . . add 25 mL of acetonitrile; add contents of Supel QuE acetate extraction tube; shake immediately for 1 minute; centrifuge at 3400 rpm for 5 minutes
clean-up tube . . . . . Supel QuE PSA/C18 tube (55288-U)
clean-up process . . Transfer 1 mL of the acetonitrile layer into a Supel QuE PSA/C18 cleanup tube; shake for 1 minute; centrifuge at 3500 rpm for 3 minutes; (draw off 700 uL of supernatant, evaporate to dryness at 50 °C under nitrogen; reconstitute in 200 µL of 50:50 0.1% formic acid:0.1% formic acid in methanol)
column . . Ascentis Express C18, 10 cm x 3.0 mm I.D., 2.7 µm particles (53814-U)
mobile phase . . (A) 0.1% formic acid in water; (B) 0.1% formic acid in methanol
gradient . . 30% B from 0 to 5 min; to 100% B in 0.2 min; held at 100% B for 5.3 min; to 30% B in 0.5 min; held at 30% B for 5 min
flow rate . . . . . 500 µL/min
pressure . . . . . 3800 psi (262 bar)
column temp. . . . . 25 °C
detector . . . . . MS, ESI(+), MRM, m/z 203.2/129.2, 271.2/225.0, 292.1/211.0, 256.0/175.2, 250.0/132.0, 223.2/126.0, 253.0/125.8
injection . . . . . 3 µL
Application No. . . . . G006046



## Chemicals and Industrial Polymers

### GC Analysis of Phenols in Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI-Chrom P

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . water spiked with phenol compounds
SPE tube/cartridge . . . . . Supelclean ENVI-Chrom P, 250 mg/6 mL (57225-U)
condition . . . . . 6 mL methyl t-butyl or ethyl acetate; 6 mL acetonitrile. Equilibrate with 6 mL DI water
sample addition . . . . . 100 mL water sample.
drying . . . . . 10 min using vacuum
elution . . . . . 5 mL methyl t-butyl ether acetate dropwise allowing initial 2 mL to soak in SPE bed
column . . . . . 5% Phenyl Polysiloxane, 15 m × 0.53 mm I.D., 0.50 µm (Supelco equivalent, Equity-5, 28252-U)
oven . . . . . 65 °C, 10 °C/min to 185 °C (1 min), 20 °C/min to 275 °C (5 min)
detector . . . . . FID, 300 °C
carrier gas . . . . . helium
injection . . . . . 1 µL, splitless (45 sec hold)
Application No. . . . . 712-0073



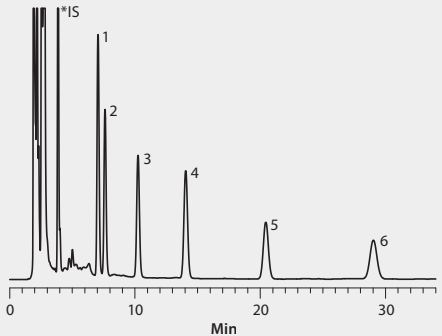

| | Analyte | %Recovery (± CV) |
|---|---|---|
| 1. | Phenol | 101.8 ± 3.7 |
| 2. | 2-Chlorophenol | 104.7 ± 1.7 |
| 3. | 2-Methylphenol | 107.0 ± 1.9 |
| 4. | 2-Bromophenol (Int. Std.) | — |
| 5. | 3-Methylphenol | 104.9 ± 1.6 |
| 6. | 2-Nitrophenol | 96.3 ± 2.8 |
| 7. | 2,4-Dimethylphenol | 105.3 ± 2.2 |
| 8. | 2,4-Dichlorophenol | 106.3 ± 2.2 |
| 9. | 4-Chloro-3-methylphenol | 104.6 ± 2.0 |
| 10. | 2,4,6-Trichlorophenol | 103.9 ± 2.1 |
| 11. | 4-Nitrophenol | 99.9 ± 4.1 |
| 12. | 2,3,4,6-Tetrachlorophenol | 104.6 ± 1.5 |
| 13. | Pentachlorophenol | 97.7 ± 4.8 |

## Clinical

### HPLC Analysis of Barbituates in Serum on Discovery® C18 after SPE using Discovery® DSC-18Lt

A Zymark® RapidTrace® SPE Workstation was used for this analysis.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . 0.5 mL porcine serum spiked with 0.5 µg/mL or 1.0 µg/mL each analyte then diluted with 0.5 mL water
SPE tube/cartridge . . . . . Discovery DSC-18Lt, 500 mg/3 mL (52613-U)
condition . . . . . 2 mL methanol; 2 mL DI water
sample addition . . . . . 1 mL at 0.75 mL/min
washing . . . . . 2 mL 5% methanol, then vacuum or air dry for 5-10 min
elution . . . . . 1-2 mL methanol
eluate post-treatment . . . . . dry eluate with nitrogen purge (40 °C; 15-20 min), reconstitute in 20 µL mobile phase
column . . . . . Discovery C18, 15 cm × 4.6 mm, 5 µm preceded by a 2 cm C18 guard column and 0.5 µm frit filter (504955)
mobile phase . . . . . (A) methanol; (B) water (40:60, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 214 nm
injection . . . . . 30 µL, diluted porcine serum extract
Application No. . . . . G001056




Barbiturates from serum using 500 mg/3 mL Discovery DSC-18Lt SPE tubes and Zymark's RapidTrace SPE Workstation.
*IS = Barbital (internal standard).

Sample Info: 0.5 mL porcine serum spike with 0.5 µg/mL or 1.0 µg/mL each analyte then diluted with 0.5 mL water.

**Efficiency of Recovery**

| Compound | Concentration (µg/mL) | %Recovery | %RSD (n=6) |
|---|---|---|---|
| 1. Phenobarbital | 0.5 | 96.2 | ±1.6 |
| | 1.0 | 94.9 | ±1.7 |
| 2. Aprobarbital | 0.5 | 98.5 | ±2.1 |
| | 1.0 | 100.8 | ±0.8 |
| 3. Butabarbital | 0.5 | 97.2 | ±1.9 |
| | 1.0 | 98.7 | ±1.8 |
| 4. Mephobarbital | 0.5 | 99.7 | ±2.4 |
| | 1.0 | 101.0 | ±2.0 |
| 5. Pentobarbital | 0.5 | 96.4 | ±1.7 |
| | 1.0 | 96.4 | ±1.9 |
| 6. Secobarbital | 0.5 | 98.2 | ±1.7 |
| | 1.0 | 97.7 | ±1.8 |

**SPE Method For RapidTrace SPE Workstation Application**

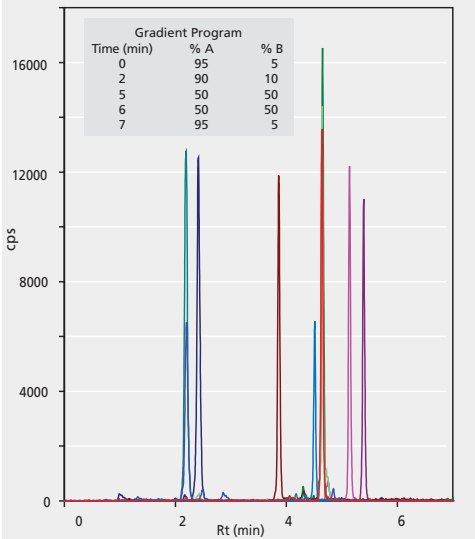
1. Condition & equilibrate each tube/well with 2 mL MeOH & 2 mL DI Water
2. Load sample
3. Wash each tube/well with 2 mL 5% MeOH
4. Vacuum or air dry for 5–10 min
   *This removes any excess water from the sorbent. The presence of water in the final eluent may prolong eluent evaporation.*
5. Elute with 1–2 mL MeOH
6. Dry eluate with nitrogen purge (40 °C; 15–20 min)
7. Reconstitute with 200 µL mobile phase
8. Quantify against internal or external standards via HPLC analyses

# HPLC Analysis of β-Blockers and β-Agonists in Urine and Wastewater on a C18 Column after SPE using SupelMIP® SPE-Beta-Receptor

**Sample Pre-treatment:**

Horse urine was centrifuged at 3000 g for 10 min, diluted 1:1 (v/v) with DI water, adjusted to pH 7. Wastewater was filtered with 1 µm filter paper and adjusted to pH 6-7. Samples were spiked with 10 beta-agonists and beta-blockers at the level of 1 ng/mL.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | horse urine or waste water spiked with 10 beta-agonists and beta-blockers at 1 ng/mL |
| SPE tube/cartridge | SupelMIP SPE - Beta-Receptor, 25 mg/10 mL (LRC) (53223-U) |
| condition | 1 mL acetonitrile; 1 mL DI water |
| sample addition | 1 mL |
| washing | 3 x 1 mL DI water; 1 mL acetonitrile; 1 mL 60% acetonitrile/40% DI water; apply 2 min of full vacuum to dry the tube after each step |
| elution | 2 x 1 mL 1% formic acid in acetonitrile |
| column | C18, 5 cm x 3 mm I.D., 3 µm particles |
| eluate post-treatment | evaporate under nitrogen and reconstitute with 150 µL 5% acetonitrile in 10 mM ammonium acetate, pH 4.6 prior to LC-MS-MS analysis |
| mobile phase | (A) 10 mM ammonium acetate, pH 4.6 (adjusted with acetic acid); and (B) acetonitrile |
| flow rate | 0.5 mL/min |
| column temp. | ambient |
| detector | MS/MS, ESI(+) |
| injection | 20 µL |
| Application No. | G004059 |




Note: Clenbuterol and Tulobuterol were spiked at the levels of 0.1 ng/mL.

| Analyte | Rt (min.) | Q1/Q3 |
|---|---|---|
| Atenolol | 3.0 | 267.2/145 |
| Carazolol | 6.2 | 299.1/194.2 |
| Metoprolol | 5.6 | 268.2/133 |
| Propranolol | 6.5 | 260.2/154.9 |
| Timolol | 5.5 | 317.2/188.1 |
| Clenbuterol | 5.6 | 277.1/202.9 |
| Ritodrine | 3.9 | 288.2/121 |
| Salbutamol | 2.6 | 240.2/147.9 |
| Terbutaline | 2.6 | 226.2/152 |
| Tulobuterol | 5.6 | 228.2/154.1 |

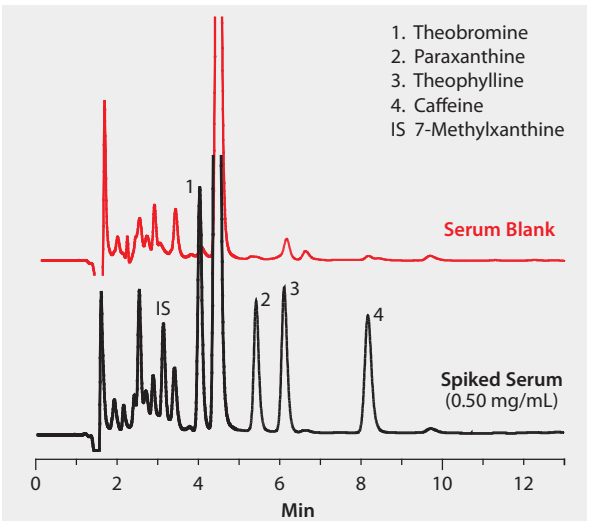
| | Lower Limit of Quantitation (ng/mL, ppb, or µg/kg) | |
|---|---|---|
| Analyte | 1 mL Horse Urine | 10 mL Wastewater |
| Atenolol | 0.1 | 0.01 |
| Carazolol | 0.1 | 0.01 |
| Metoprolol | 0.1 | 0.01 |
| Propranolol | 0.1 | 0.01 |
| Timolol | 0.1 | 0.01 |
| Clenbuterol | 0.02 | 0.002 |
| Ritodrine | 0.05 | 0.005 |
| Salbutamol | 0.1 | 0.01 |
| Terbutaline | 0.2 | 0.02 |
| Tulobuterol | 0.005 | 0.0005 |

# HPLC Analysis of Bronchodilators Theophylline and Other Caffeine Metabolites from Serum on Discovery® RP-AmideC16 after SPE using Discovery® DSC-18

sample preparation . . . . . SPE (Solid Phase Extraction)
SPE tube/cartridge . . . . . Discovery® DSC-18, 500 mg/3 mL (52603-U)
condition . . . . . 2 mL methanol, then 2 mL water
sample preparation . . . . . 1 mL porcine serum spiked with 0.1 µg/mL, 0.50 µg/mL, or 1.0 µg/mL each analyte
sample preparation . . 1 mL methanol; evaporate to dryness with nitrogen stream at room temperature; reconstitute in 200 µL mobile phase containing 0.2 µg/mL or 7-methylxanthine (IS)
sample preparation . . . . . 2 mL 5% methanol in water; dry tube 10 min with nitrogen stream
column . . Discovery RP-AmideC16, 15 cm × 4.6 mm, 5 µm particles, preceded by a 2 cm RP-AmideC16 guard column and 0.5 µm frit filter (505013)
mobile phase . . . . . (A) Methanol; (B) 1% acetic acid (17:38, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 272 nm
injection . . . . . 20 µL reconstituted porcine serum extract
Application No. . . . . G000594

Efficiency of Recovery

| Compound | Concentration (µg/mL) | % Recovery | % RSD (n=6) |
|---|---|---|---|
| 1. Theobromine | 0.1 | 97.4 | ±6.8 |
| | 0.5 | 96.4 | ±8.5 |
| | 1.0 | 96.1 | ±5.0 |
| 2. Paraxanthine | 0.1 | 96.2 | ±8.4 |
| | 0.5 | 95.2 | ±8.7 |
| | 1.0 | 95.0 | ±8.7 |
| 3. Theophylline | 0.1 | 97.8 | ±8.5 |
| | 0.5 | 97.8 | ±8.8 |
| | 1.0 | 98.5 | ±5.7 |
| 4. Caffeine | 0.1 | 98.8 | ±3.9 |
| | 0.5 | 95.6 | ±6.7 |
| | 1.0 | 97.6 | ±5.8 |



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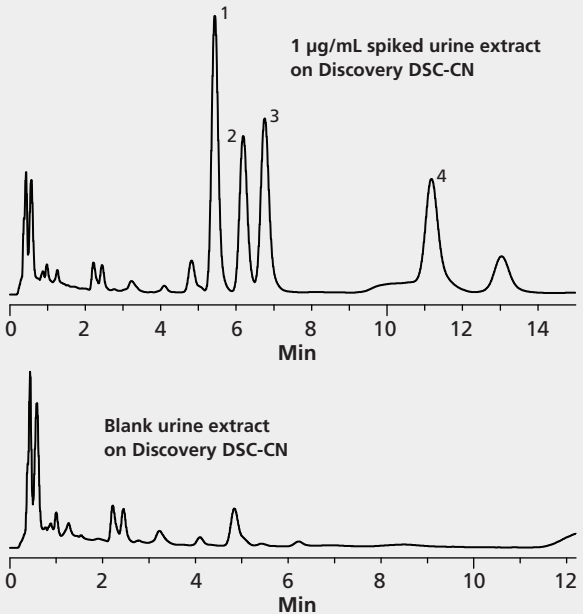
## HPLC Analysis of Cortocisteroids in Urine on Discovery® HS F5 after SPE using Discovery® DSC-CN

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | human urine spiked with corticosteroids at 0.5 and 1.0 µg/mL and diluted in DI water (1:1, v/v) |
| SPE well plate | Discovery DSC-CN 96-well SPE, 100 mg/well (575636-U) |
| condition | 1 mL methanol; 1 mL DI water. |
| sample addition | 1 mL spiked urine |
| washing | 1 mL 20% methanol |
| elution | 1 mL methanol |
| eluate post-treatment | Evaporate eluate with nitrogen at 30 °C. Reconstitute in 200 µL mobile phase. |
| column | Discovery HS F5, 5 cm x 4.6 mm I.D., 3 µm particles (567504-U) |
| mobile phase | (A) methanol; (B) DI water; (40:60, A:B) |
| flow rate | 1.5 mL/min |
| column temp. | 35 °C |
| detector | UV, 240 nm |
| injection | 5 µL |
| Application No. | G003763 |

**Efficiency of Recovery**

| Compound | %Recovery ± RSD (n=3) 0.5 µg/mL spike level | 1.0 µg/mL spike level |
|---|---|---|
| 1. Hydrocortisone | 123.3±1.4% | 95.9±1.7% |
| 2. Prednisilone | 107.2±1.1% | 91.9±1.1% |
| 3. Prednisone | 103.2±1.0% | 88.4±1.8% |

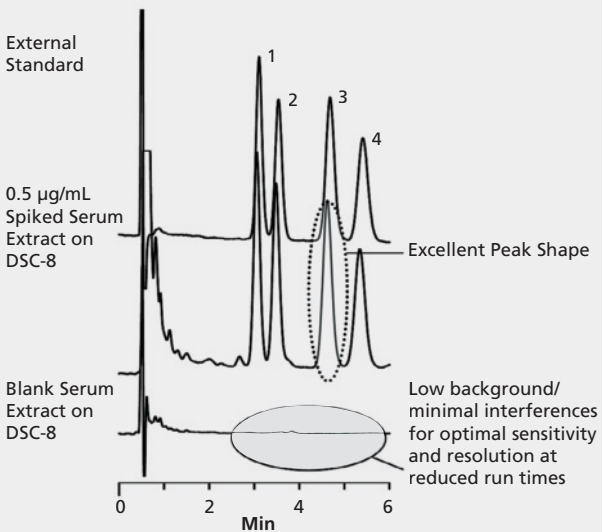


## HPLC Analysis of Diazepam and Metabolites in Serum on Discovery® C18 after SPE using Discovery® DSC-8

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . goat serum spiked with diazepam and metabolites at 0.5 µg/mL and diluted 1:1 in 25 mM ammonium formate, pH 7.1
SPE well plate . . . . Discovery DSC-8 96-well SPE Plate, 100 mg/well (575627-U)
condition . . . . . 1 mL methanol followed by 1 mL DI water.
sample addition . . . . . 1 mL spiked goat serum
washing . . . . . 1 mL 25% methanol in 25 mM ammonium formate, pH 2.75
elution . . . . . 1 mL 60% methanol in 25 mM ammonium formate, pH 2.75
elution . . . . . Evaporate eluate with nitrogen purge (30 °C; ~15 min.). Reconstitute in 200 µL mobile phase.
column . . . . . Discovery C18, 5 cm x 4.6 mm I.D., 5 µm particles (504947)
mobile phase . . (A) Methanol; (B)10 mM ammonium acetate, pH 4.5;(45:55, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 25 µL
Application No.Application No. . . . . G003766

**Efficiency of Recovery**

| Compound | %Recovery ± RSD (n=3) Systematically Developed Method on Discovery DSC-8 | %Recovery ± RSD (n=3) Generic Method On Conventional C18 |
|---|---|---|
| 1. Oxazepam | 94.7 ± 1.2% | 82.8 ± 4.0% |
| 2. Temazepam | 99.9 ± 1.1% | 89.1 ± 4.0% |
| 3. Desmethyl diazepam | 94.2 ± 1.8% | 82.4 ± 5.0% |
| 4. Diazepam | 90.0 ± 3.4% | 68.5 ± 9.1% |

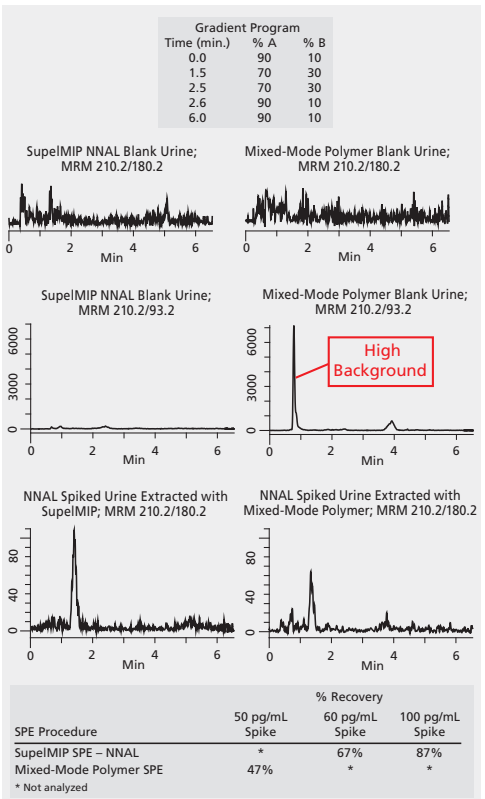


## HPLC Analysis of NNAL in Urine on Ascentis® Express C18 after SPE using SupelMIP® SPE-NNAL

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . human urine spiked with 60 or 100 pg/mL NNAL
SPE tube/cartridge . . . . . SupelMIP SPE - NNAL, 25 mg/10 mL (LRC) (53206-U)
condition . . . . . 1 mL dichloromethane; 1 mL methanol; 1 mL DI water
sample addition . . . . . 2 mL (60 pg/mL sample), 5 mL (100 pg/mL sample)
washing . . . . . 2 x 1 mL DI water, 10 min vacuum; mL toluene; 1 mL toluene: dichloromethane (9:1, v/v); 1 mL toluene:dichloromethane (4:1, v/v), 2 min. vacuum
elution . . . . . 2 x 1 mL 10% methanol in dichloromethane, apply gentle vacuum between each fraction
eluate post-treatment . . . . . evaporate under nitrogen and reconstitute in 0.15 - 0.25 mL mobile phase
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (581307-U)
mobile phase . . . . . (A) 10 mM ammonium acetate; (B) 10 mM ammonium acetate in acetonitrile
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . MS/MS, MRM Transitions, ESI(+) (210.2/180 and 210.2/93.2 m/z)
injection . . . . . 20 µL
Application No. . . . . G004434

Gradient Program

| Time (min.) | % A | % B |
|---|---|---|
| 0.0 | 90 | 10 |
| 1.5 | 70 | 30 |
| 2.5 | 70 | 30 |
| 2.6 | 90 | 10 |
| 6.0 | 90 | 10 |




| SPE Procedure | % Recovery 50 pg/mL Spike | 60 pg/mL Spike | 100 pg/mL Spike |
|---|---|---|---|
| SupelMIP SPE – NNAL | * | 67% | 87% |
| Mixed-Mode Polymer SPE | 47% | * | * |

* Not analyzed

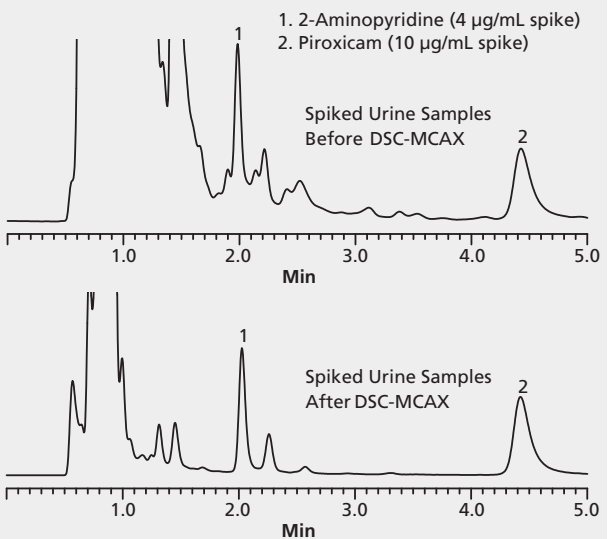
# HPLC Analysis of Piroxicam and 2-Aminopyridine in Urine on Discovery® HS F5 after SPE using Discovery® DSC-MCAX

**Sample Pre-Treatment:**
Piroxicam and 2-aminopyridine (piroxicam's polar metabolite) was spiked into human urine at the levels of 4 and 10 µg/mL, respectively. The urine sample was diluted 1:1 with 10 mM potassium phosphate, pH 3.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . human urine spiked with piroxicam (4 µg/mL) and 2-aminopyridine (10 µg/mL)
SPE tube/cartridge . . . . . Discovery DSC-MCAX, 100 mg/3 mL (52783-U)
condition . . . . . 1 mL methanol; 1 mL 10 mM potassium phosphate, pH 3
sample addition . . . . . 1 mL
washing . . . . . 1 mL 10 mM potassium phosphate, pH 3; 1 mL methanol
elution . . . . . 1 mL 5% ammonium hydroxide in methanol
eluate post-treatment . . evaporate to dryness with nitrogen at room temperature, reconstitute in 1 mL mobile phase
column . . . . . Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (567516-U)
mobile phase . . (A) 10 mM potassium phosphate, pH 6: (B) acetonitrile (85:15, A:B)
flow rate . . . . . 2 mL/min
column temp. . . . . 25 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G003762

**Efficiency of Recovery**

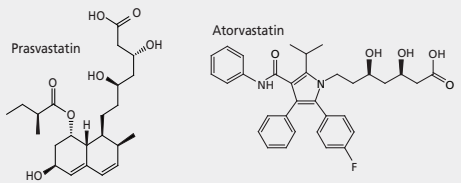
| | %Recovery ± RSD (n=4) | |
|---|---|---|
| | 2-Aminopyridine | Piroxicam |
| Discovery DSC-MCAX | 102 ± 3.5% | 101 ± 1.2% |
| Leading Competitor | A30 ± 52.5% | 98 ± 3.2% |
| Leading Competitor | B36 ± 24.2% | 83 ± 4.3% |

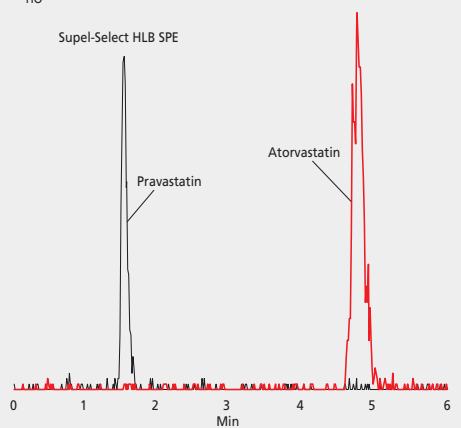


## HPLC Analysis of Statins in Rat Plasma on Ascentis® Express C18 after SPE using Supel™-Select HLB

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . rat plasma spiked with provastin and atorvastin at 5 and 100 ng/mL
SPE tube/cartridge . . . . . Supel-Select HLB SPE, 30 mg/1 mL (54181-U)
condition . . . . . 0.5 mL methanol:acetonitrile (1:1, v/v); 0.5 mL DI water
sample addition . . . . . 0.5 mL
washing . . . . . 0.5 mL 5% methanol
elution . . . . . 0.5 mL methanol:acetonitrile (1:1, v/v)
eluate post-treatment . . . . . evaporate to dryness with nitrogen at 37 °C, reconstitute in 0.5 mL DI water
column . . . . . Ascentis Express C18, 5 cm x 2.1 mm, 2.7 µm particles (53822-U)
mobile phase . . . . . 0.1% acetic acid diluted in 60% methanol
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . ABI 3200 QT; ESI(+), MRM (423.3/321.3, 423.3/101.0, 557.3/397.2 and 557.3/453.4)
injection . . . . . 5 µL
Application No. . . . . G004432

Total Ion Chromatogram (MRM, 4 pairs: 557.3/397.2)
Rat Plasma spiked with 5 ng/mL Statins Extracted by Supel-Select HLB SPE



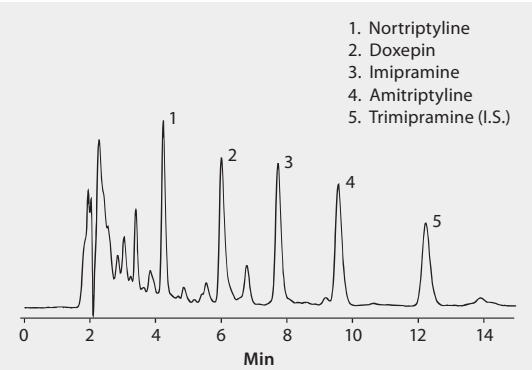

| | Absolute Recovery ± RSD (n=3) | | | |
|---|---|---|---|---|
| | 5 ng/mL spike | | 100 ng/mL spike | |
| | Pravastatin | Atorvastatin | Pravastatin | Atorvastatin |
| Supel-Select HLB | 84 ± 8% | 92 ± 5% | 103 ± 4.2% | 89 ± 3.9% |
| Competitor W | 83 ± 17% | 92 ± 2% | 104 ± 2.2% | 87 ± 1.1% |
| Competitor P | 77 ± 5% | 93 ± 2% | 102 ± 3.0% | 91 ± 1.3% |

## HPLC Analysis of Tricyclic Antidepressants in Serum on Discovery® C18 after SPE using Discovery® DSC-18

sample preparation . . . . . SPE (Solid Phase Extraction)
SPE tube/cartridge . . . . . Discovery DSC-18, 100 mg/1 mL (52602-U)
column . . . . . Discovery C18, 15 cm × 4.6 mm, 5 µm preceded by a 2 cm C18 guard column and 0.5 µm frit filter (504955)
mobile phase . . . (A) acetonitrile: (B) methanol: (C) 25 mM potassium phosphate, monobasic (pH 7 with triethlylamine) (45:25:30, A:B:C)
flow rate . . . . . 1 mL/min
column temp. . . . . ambient
detector . . . . . UV, 254 nm
injection . . . . . 50 µL, diluted porcine serum extract
Application No. . . . . G000595

### Efficiency of Recovery

| Compound | Concentration (µg/mL) | % Recovery | % RSD (n=6) |
|---|---|---|---|
| 1. Nortriptyline | 0.10<br>0.50 | 103.6<br>97.5 | ±4.5<br>±4.5 |
| 2. Doxepin | 0.10<br>0.50 | 102.2<br>100.8 | ±3.0<br>±1.8 |
| 3. Imipramine | 0.10<br>0.50 | 92.0<br>97.5 | ±1.5<br>±1.7 |
| 4. Amitriptyline | 0.10<br>0.50 | 93.6<br>95.7 | ±1.2<br>±1.4 |



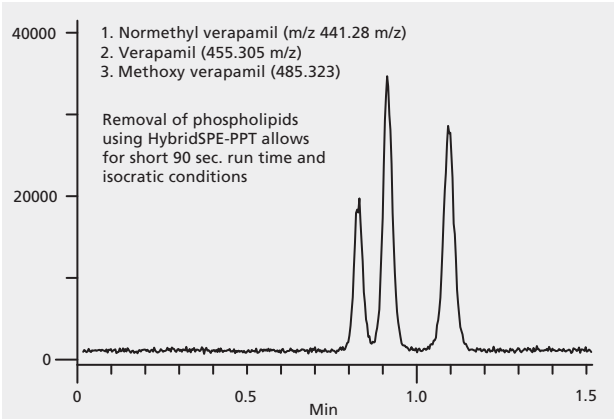

### SPE Procedure, Using Zymark RapidTrace SPE Workstation

| Step | Solvent/ Solution | Volume (mL) | Flow Rate (mL/min) | Comments |
|---|---|---|---|---|
| 1. Condition | MeOH | 2.0 | 5.0 | conditions sorbent |
| 2. Condition | $H_2O$ | 2.0 | 5.0 | conditions sorbent |
| 3. Load | spiked porcine serum | 2.0[A] | 0.75 | applies serum sample |
| 4. Rinse | 20% MeOH in $H_2O$ | 2.0 | 5.0 | washes sorbent |
| 5. Purge-Cannula | $H_2O$ | 4.0 | 30.0 | cleans sample cannula |
| 6. Rinse | vent | 0.1 | 2.0 | positions SPE tube over waste port |
| 7. Dry | $N_2$ | Time = 10 min | | dries sorbent |
| 8. Purge-Cannula | MeOH | 4.0 | 30.0 | cleans sample cannula |
| 9. Collect | MeOH | 1.0 | 1.0 | elutes analytes into collection vessel |
| 10. Collect | vent | 6.0 | 3.0 | pushes residual eluent into vessel[B] |
| 11. Purge-Cannula | $H_2O$ | 4.0 | 30.0 | cleans sample cannula |

[A]1 mL porcine serum spiked with 0.1 µg/mL each analyte basified with 3 µL 10 N KOH, then diluted with 1 mL water
[B]350 µL water added per mL methanolic eluent before analysis

## HPLC Analysis of Verapamil and Metabolites in Rat Plasma on Ascentis® Express C18 after Phospholipid Removal using HybridSPE®-Phospholipid

SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample preparation . . . . . SPE (Solid Phase Extraction)
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
sample/matrix . . . Rat plasma spiked with verapamil and metabolites at 10 ng/mL
mobile phase . . . . . (A) water; (B) acetonitrile, pH 2.55 adjust with formic acid (30:70, A:B)
flow rate . . . . . 0.6 mL/min
column temp. . . . . 35 °C
detector . . . . . TOF/MS
injection . . . . . 1 µL
sample addition . . . . . 100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile
elution . . . . . apply vacuum
Application No. . . . . G004366





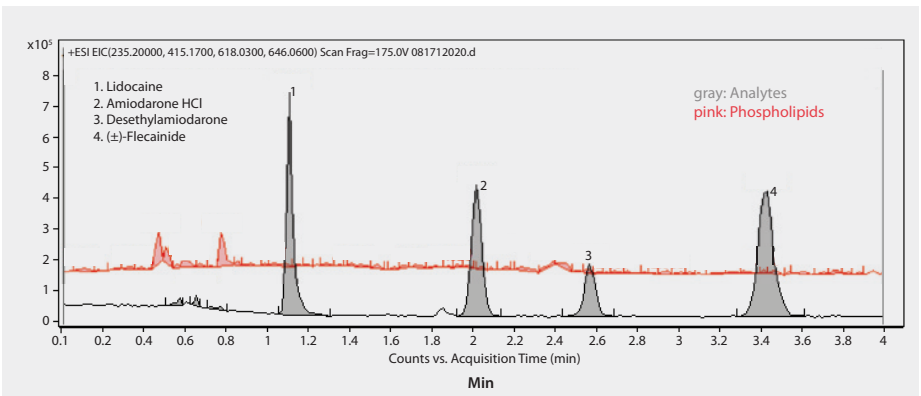

SUPELCO® Solutions within.™

## LC-MS Analysis of Antiarrhythmic Drugs and Metabolites in Plasma on Ascentis® Express HILIC following Sample Prep using HybridSPE®-Phospholipid

For efficient therapeutic drug monitoring, it is important for clinicians to have access to fast and robust analytical methods for accurate assessment of drug efficacy. Industrial trends toward highly specific LC/MS applications over traditional ELISA type immunoassay have resulted in the need for high-speed chromatographic assays along with simplified sample preparation methods. Often the limitation of a bioanalytical technique is based upon the effectiveness of the sample preparation technique. Plasma and serum samples are often susceptible to assay irregularities due to matrix-induced interferences. In this study the impact of matrix interference is investigated with respect to precision and accuracy of antiarrhythmic cardiac drugs from plasma samples. A robust bioanalytical method was developed using a combination of fast HILIC chromatography on Ascentis Express along with a selective sample preparation using HybridSPE-Phospholipid. Solvents were LC-MS Ultra CHROMASOLV® and Certified Reference Material grade standards were from Cerilliant.

Endogenous phospholipids can dramatically impact the precision and accuracy of a bioanalytical method. During development, the sample matrix impact on analyte detection should be priority in method validation. Failure to do so can result in inaccuracies in reported levels, thus impacting the assessment of patient health. Eliminating matrix effects in LC/MS is imperative to producing reliable and accurate bioanalytical methods. The targeted phospholipid selectivity of the HybridSPE-Phospholipid technique enables simplified sample processing with no detected phospholipid matrix interference, while exhibiting excellent recovery from plasma and serum samples. This application demonstrates how combining selectivity in both sample preparation and chromatographic separation allows for a simplified and efficient bioanalytical method resulting in a high precision and accurate assay.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . rabbit plasma, unfiltered K2-EDTA, spiked with each compound at 100 ng/mL (3:1, plasma:1% formic acid in acetonitrile)
SPE tube/cartridge . . . . . HybridSPE-Phospholipid, 96-well plate (575656-U)
sample addition . . . . . To each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes. Collect filtrate and analyze directly.
column . . . . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile, 5:95 (A:B), adjusted to pH 7.0 with formic acid
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1305 psi (90 bar)
column temp. . . . . 35 °C
detector . . . . . ESI+, 100-1000 m/z
injection . . . . . 0.5 µL
Application No. . . . . G005854



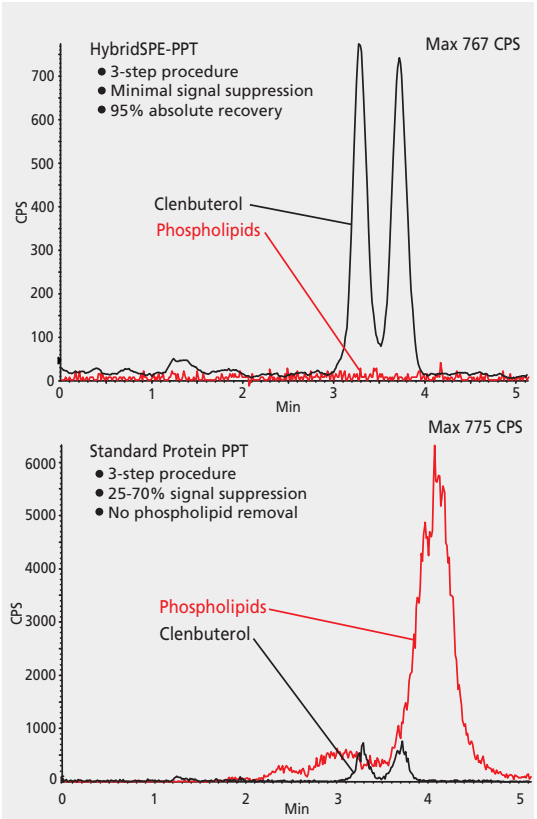



SUPELCO® Solutions within.™

## LC-MS Analysis of Clenbuterol In Plasma on Astec® CHIROBIOTIC® T with Phospholipid Removal Using HybridSPE® Phospholipid

The HybridSPE method provides significant improvement in LC-MS baseline.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . rat plasma spiked with clenbuterol enantiomers at 10 ng/mL
SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample addition . . 100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile. Mix by vortexing the HybridSPE-PPT plate briefly.
elution . . . . . apply vacuum
column . . . . . CHIROBIOTIC T, 10 cm x 2.1 mm I.D., 5 µm particles (12018AST)
mobile phase . . . . . 10 mM ammonium formate in methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . ABI 3200 QT; ESI(+), MRM: 184/104 m/z (phospholipids); 277.2/203.1 m/z (clenbuterol)
injection . . . . . 10 µL
Application No. . . . . G004431





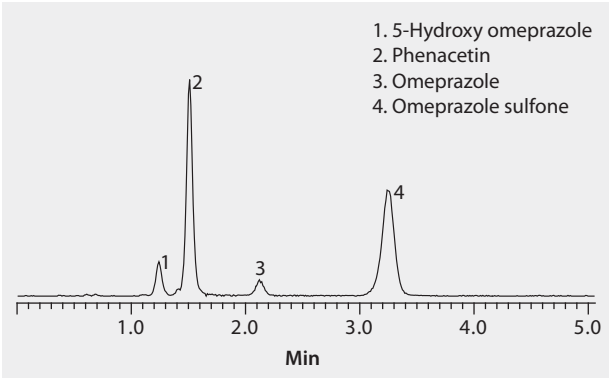

SUPELCO® Solutions within.™

## LC-MS Analysis of Omeprazole and Metabolites in Plasma on Ascentis® Express RP Amide after Sample Prep using HybridSPE®-Phospholipid

This application demonstrates the suitability of HybridSPE and the Ascentis Express RP Amide for the analysis of omeprazole metabolites.

Sample was vorlexed and centrifuged for 2 minutes at 15000 rpm. Supemate was then passed through HybridSPE 96 well plate. Elutent was collected, evaporated and reconstituted in mobile phase.

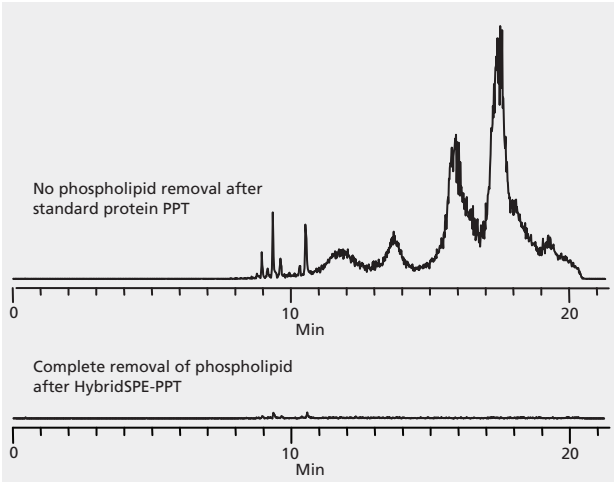
sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . rat plasma spiked with omeprazole, phenacetin and metabolites at 2 µg/mL
SPE well plate . . HybridSPE-Phospholipid, 96-well plate, 15 mg/0.8 mL (575656-U)
sample addition . . . . . 100 µL plasma to each well, followed by 300 mL of 1% formic acid in acetonitrile
elution . . . . . apply vacuum
eluate post-treatment . . . . . eluate was collected, evaporated, and reconstituted in mobile phase.
column . . Ascentis Express RP-Amide, 5 cm x 2.1 mm I.D., 2.7 µm particles (53911-U)
mobile phase . . . . . (A) water, 10 mM ammonium formate, pH 3.4; (B) methanol, 10 mM ammonium formate, pH 3.4; (65:35, A:B)
flow rate . . . . . 0.3 mL/min
column temp. . . . . 35 °C
detector . . . . . ESI(+), m/z = 50-400
injection . . . . . 1 µL
Application No. . . . . G005711



## LC-MS Analysis of Plasma Samples on Ascentis® Express C18 Showing Baseline Improvements after Phospholipid Removal using HybridSPE®-Phospholipid

**HybridSPE-Precipitation:**
**Precipitate** proteins by first adding 100 µL rat plasma to the HybridSPE-PPT plate followed by 300 µL 1% formic acid in acetonitrile. Do not apply vacuum.
**Mix** by vortexing the HybridSPE-PPT plate briefly.
**Apply vacuum** using a 96-well vacuum manifold and collect the resulting eluate for analysis.
**Standard Protein Precipitation:**
**Combine** 100 µL rat plasma with 300 µL 1% formic acid in acetonitrile.
**Mix** by vortexing and centrifuge.
**Isolate** resulting supernatant for analysis.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . rat plasma
SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample addition . . . . . 100 µL rat plasma followed by 300 µL 1% formic acid in acetonitrile
elution . . . . . apply vacuum
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 10 mM ammonium acetate; (B) 10 mM ammonium acetate in acetonitrile
gradient . . . . . 0 min - 95% (A); 10 min - 50% (A); 18 min - 50% (A); 18.1 min - 95% (A); 22 min - 95% (A)
flow rate . . . . . 0.5 mL/min
column temp. . . . . 35 °C
detector . . . . . ABI 3200QT; ESI (+), MRM (184/103 m/z)
injection . . . . . 5 µL
Application No. . . . . G004243



## LC-MS/MS Analysis of Steroid Hormones in Plasma on Ascentis® Express C18 after Sample Prep using HybridSPE®-Phospholipid

Immunoassay approaches for steroid determination are typically hindered due to the lack of specificity of antibodies for the measurement of the particular steroids. With the wide acceptance of LC-MS/MS in the clinical setting, there is a growing trend toward converting traditional IA techniques toward more specific and robust LC-MS/MS approaches. Though LC-MS/MS improves assay specificity and allows of multiplexed analyte assays to be conducted simultaneously, it is not without limitations specifically toward interferences form endogenous sample matrix. Often LC-MS/MS assays can be hindered due to ionization effects due to endogenous matrix from biological samples; this can result in random and arbitrary discrimination in analyte response. The use of HybridSPE-Phospholipid prior to LC-MS/MS analysis removes the endogenous phospholipids and precipitated proteins in one step, enabling high analyte recovery, negligable matrix background and long column lifetime.

SPE tube/cartridge . . . . . HybridSPE®-Phospholipid, 96-well plate, 50 mg/well (575656-U)

sample addition . . . . To each well add 100 µL of plasma followed by 300 µL of precipitation solvent (1% formic acid or 0.5% citric acid in acetonitrile). (Agitate via vortex for 4 minute, place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.)

column . . Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)

gradient . . 60% B for 3 min, 60% B to 95% B in 5 min, held at 95% B for 2 min

mobile phase . . (A) 5 mM ammonium formate pH 4.0 with formic acid; (B) methanol

flow rate . . . . . 0.3 mL/min

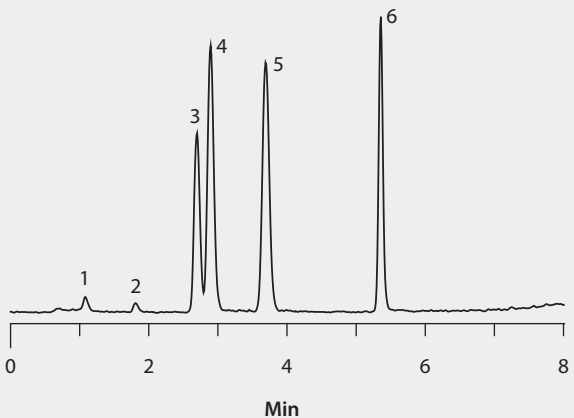
column temp. . . . . 50 °C

detector . . . . . ESI+, MRM

injection . . . . . 2 µL

Application No. . . . . G005895

| | MRM Transition |
|---|---|
| 1. Aldosterone | 361.0/343.1 |
| 2. Corticosterone | 347.6/109.0 |
| 3. 11-Deoxycorticosterone | 331.1/109.0 |
| 4. Testosterone | 289.0/109.0 |
| 5. 17α-Methyltestosterone | 303.1/97.0 |
| 6. Progesterone | 315.0/109.1 |



## LC-MS/MS Analysis of Warfarin™ in Plasma on Ascentis® Express C18 after SPE using HybridSPE®-Phospholipid

Warfarin is a widely prescribed oral anticoagulant for treatment and prevention of thrombosis and thromboembolism. The pharmacological effect of warfarin is derived from its ability to inhibit the enzyme vitamin K epoxide reductase, thereby reducing circulating levels of vitamin K which is required in the clotting process. In spite of its popularity, there are some downsides to warfarin, including a relatively narrow therapeutic index, interactions with other clinically important drugs, side effects, genetic variation in warfarin metabolism, frequent migration of blood levels outside the therapeutic range, bleeding events, and variability in time to reach therapeutic levels. These, and the fact that warfarin is the second leading cause of drug-related emergency room visits make it a commonly analyzed compound in the clinical lab. The clinical implications and importance of therapeutic drug monitoring of warfarin necessitate reliable and sensitive analytical methods, like UHPLC-MS/MS, to detect and quantify warfarin and its enantiomers in serum.

sample/matrix . . . . . rabbit plasma, unfiltered K2-EDTA, spiked with warfarin at 100 ng/mL (3:1, plasma:1% formic acid in acetonitrile)

SPE tube/cartridge . . . . . HybridSPE®-Phospholipid, 96-well plate (575656-U)

sample addition . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes

elution . . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes

column . . Ascentis® Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)

mobile phase . . . . . (A) 5mM ammonium formate, pH 4.2 with formic acid; (B) 5mM ammonium formate in 95:5 acetonitrile:water, 50:50 (A:B)

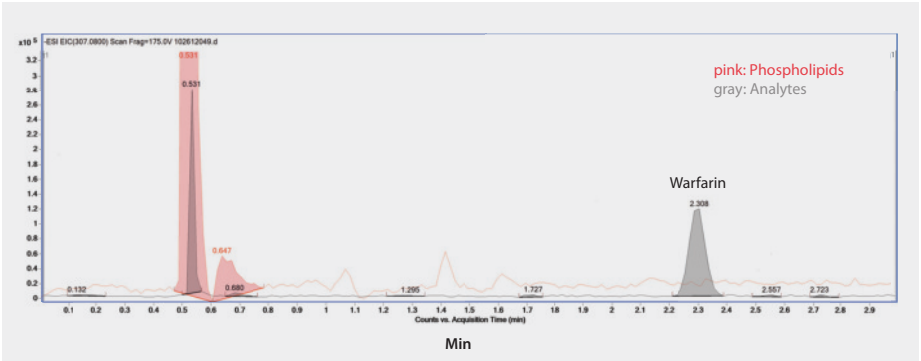
flow rate . . . . . 0.3 mL/min

column temp. . . . . 35 °C

detector . . . . . ESI+, 100-1000 m/z

injection . . . . . 2 µL

Application No. . . . . G005850



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## LC-MS/MS Analysis of Warfarin™ Enantiomers in Plasma on Astec® CHIROBIOTIC® V after SPE using HybridSPE®-Phospholipid

Warfarin is a widely prescribed oral anticoagulant for treatment and prevention of thrombosis and thromboembolism. The pharmacological effect of warfarin is derived from its ability to inhibit the enzyme vitamin K epoxide reductase, thereby reducing circulating levels of vitamin K which is required in the clotting process. In spite of its popularity, there are some downsides to warfarin, including a relatively narrow therapeutic index, interactions with other clinically important drugs, side effects, genetic variation in warfarin metabolism, frequent migration of blood levels outside the therapeutic range, bleeding events, and variability in time to reach therapeutic levels. These, and the fact that warfarin is the second leading cause of drug-related emergency room visits make it a commonly analyzed compound in the clinical lab. Warfarin is a chiral compound comprising an equal mixture of (R) and (S) enantiomers. (S)-Warfarin is considerably more potent and pharmacologically active than (R)-warfarin. Astec CHIROBIOTIC V columns resolve warfarin enantiomers under LC-MS conditions and hold up well to biological samples. The clinical implications and importance of therapeutic drug monitoring of warfarin necessitate reliable and sensitive analytical methods, like UHPLC-MS/MS, to detect and quantify warfarin and its enantiomers in serum.

sample/matrix . . . . . rabbit plasma, unfiltered K2-EDTA, spiked with warfarin at 100 ng/mL (3:1, plasma:1% formic acid in acetonitrile)

SPE tube/cartridge . . . . . HybridSPE-Phospholipid, 96-well plate (575656-U)

sample addition . . . . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes

elution . . . to each well add 100 µL plasma, followed by a 300 µL of 1% formic acid in acetonitrile, agitate on orbital shaker for 4 minutes

column . . Astec CHIROBIOTIC® V, 10 cm x 4.6 mm I.D., 5 µm particles (11022AST)

mobile phase . . (A) 0.1% formic acid (pH unadjusted); (B) acetonitrile, 75:25 (A:B)

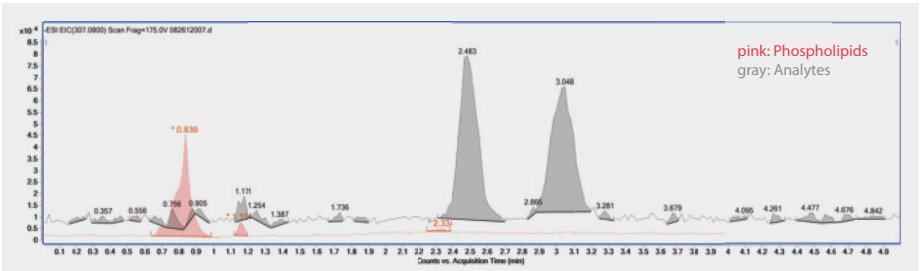
flow rate . . . . . 1 mL/min

column temp. . . . . 35 °C

detector . . . . . ESI+, 100-1000 m/z

injection . . . . . 2 µL

Application No. . . . . G005849



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## LC-MS (TOF) Analysis of Warfarin™ Enantiomers in Plasma on Astec® CHIROBIOTIC® V after SPE Using HybridSPE®-Phospholipid

This application demonstrates the benefit of using Sigma-Aldrich Analytical (Supelco, Fluka, and Cerilliant) consumables for rapid, accurate, sensitive and reliable MS detection in a clinical or bioanalytical setting.

Warfarin was originally developed as a rodenticide, but soon found medical application as an anticoagulant to prevent the formation of blood clots, especially after heart attacks. At the time of this writing, it is the most widely prescribed oral anticoagulant and is sold under Coumadin and various other brand names. Warfarin is a chiral molecule. The R- and S- forms are cleared by the body via different pathways and have different pharmacokinetic and pharmacodynamic effects. S-warfarin is more potent than the R-warfarin in producing the anticoagulant response.

The Astec® CHIROBIOTIC V column provided rapid resolution of the enantiomers under conditions that are compatible with mass spectrometric (MS) detection. Considering the rise of MS in the clinical laboratory, this is an important consideration. The CHIROBIOTIC family of chiral columns are based on the macrocyclic glycopeptide molecule and affect enantioresolution under mobile conditions that are compatible with reversed-phase and MS conditions. Their durability allows them to stand up to repeated injections of biological extracts.

By using HybridSPE-Phospholipid for the sample prep, ion-suppressing phospholipids were removed for improved sensitivity and longer column lifetime. LC-MS Ultra CHROMASOLV solvents were used to supply low background interference and particulate contaminants. Cerilliant reference material grade standards provided reliable quantitation.

system: Agilent 1290, 6210 TOF

sample/matrix . . . . . Rat plasma stabilized with K2EDTA was acquired from Lampire Biological Laboratories, (Pipersville PA). (Plasma was spiked directly from stock standard to a level of 100 ng/mL in 3:1 (plasma:1% formic acid acetonitrile).)

SPE tube/cartridge . . Pk1 50 mg/96-Well HybridSPE-Phospholipid Plate (575656-U)

condition . . Apply 100 µL of plasma to plate, followed by 300 µL of 1% formic acid acetonitrile. Agitate via vortex for 4 min.

elution . . . . . Place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

column . . . . . CHIROBIOTIC V,10 cm x 4.6 mm I.D., 5 µm particles (11022AST)

mobile phase . . . . . (A) 0.1% formic acid water; (B) acetonitrile, (75:25, A:B)

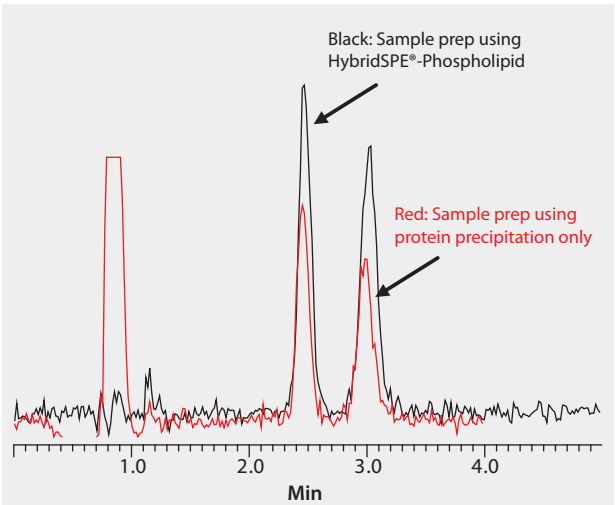
flow rate . . . . . 1 mL/min

column temp. . . . . 35 °C

detector . . . . . ESI+, 100-1000 m/z

injection . . . . . 2 µL

Application No. . . . . G005827



## LC-TOF Analysis of Antiarrhythmic Drugs and Metabolites in Plasma on Ascentis® Express HILIC after Sample Prep using HybridSPE®-Phospholipid

The basic characteristics of antiarrhythmic cardiac drugs and associated metabolites make them targets for HILIC chromatographic separation. Because HILIC mobile phases consist of a high composition of acetonitrile, this can also facilitate the direct analysis of precipitated plasma samples without the need for additional sample solvent exchange. In most cases, the high organic mobile phase also facilitates increased analyte response in ESI (+) MS detection. This application used HybridSPE-Phospholipid sample prep to remove phospholipids and precipitated proteins prior to LC-MS analysis on Ascentis Express HILIC. The resulting method was robust and sensitive.

sample/matrix . . . Rat plasma stabilized with K2EDTA was acquired from Lampire Biological Laboratories, (Pipersville PA). Plasma was spiked directly from stock standard to a level of 400 ng/mL

SPE tube/cartridge . . HybridSPE-Phospholipid 96-Well Plate, 50 mg/2 mL (575656-U)

sample addition . . . . . Apply 100 µL of plasma to plate, followed by 300 µL of 1% formic acid acetonitrile. Agitate via vortex for 4 min

elution . . . . Place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)

mobile phase . . . . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile; (5:95, A:B, pH 7.0 with formic acid) (LC-MS CHROMASOLV Ultra grade)

flow rate . . . . . 0.4 mL/min

pressure . . . . . 1305 psi (90 bar)

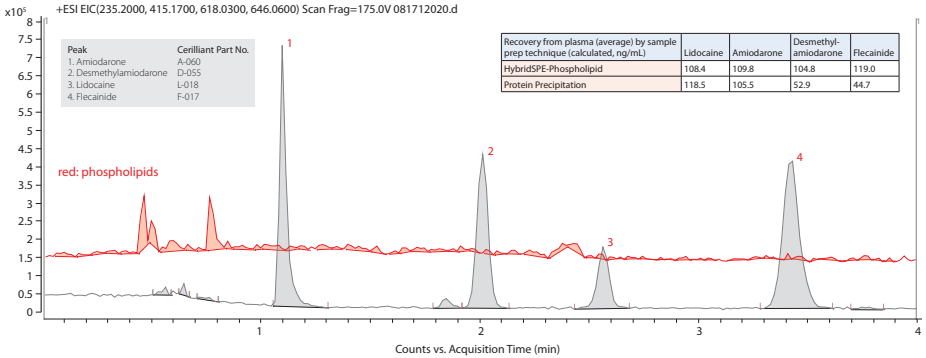
column temp. . . . . 35 °C

detector . . . . . ESI(+), full scan, m/z 100-1000

injection . . . . . 0.5 µL

sample . . . . . plasma extract, analyte concentration of final sample work up is equivalent to 100 ng/mL

Application No. . . . . G005818




| Recovery from plasma (average) by sample prep technique (calculated, ng/mL) | Lidocaine | Amiodarone | Desmethyl-amiodarone | Flecainide |
|---|---|---|---|---|
| HybridSPE-Phospholipid | 108.4 | 109.8 | 104.8 | 119.0 |
| Protein Precipitation | 118.5 | 105.5 | 52.9 | 44.7 |



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## LC-TOF Analysis of Antiarrhythmic Drugs and Metabolites on Ascentis® Express HILIC: Improvement in Analyte Response after Sample Prep using HybridSPE®-Phospholipid

The basic characteristics of antiarrhythmic cardiac drugs and associated metabolites make them targets for HILIC chromatographic separation. Because HILIC mobile phases consist of a high composition of acetonitrile, this can also facilitate the direct analysis of precipitated plasma samples without the need for additional sample solvent exchange. In most cases, the high organic mobile phase also facilitates increased analyte response in ESI (+) MS detection. This application used HybridSPE-Phospholipid sample prep to remove phospholipids and precipitated proteins prior to LC-MS analysis on Ascentis Express HILIC. These chromatograms compare the response with HybridSPE-Phospholipid vs. standard protein precipitation sample prep methods.

sample . . . . . Rat plasma stabilized with K2EDTA was acquired from Lampire Biological Laboratories, (Pipersville PA). Plasma was spiked directly from stock standard to a level of 400 ng/mL

SPE tube/cartridge . . HybridSPE-Phospholipid 96-Well Plate, 50 mg/2 mL (575656-U)

sample addition . . . . . Apply 100 µL of plasma to plate, followed by 300 µL of 1% formic acid acetonitrile. Agitate via vortex for 4 min.

elution . . . . . Place on vacuum manifold and apply 10" Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)

mobile phase . . . . . (A) 5 mM ammonium formate; (B) 5 mM ammonium formate in acetonitrile; (5:95, A:B, pH 7.0 with formic acid) (LC-MS CHROMASOLV Ultra grade)

flow rate . . . . . 0.4 mL/min

pressure . . . . . 1305 psi (90 bar)

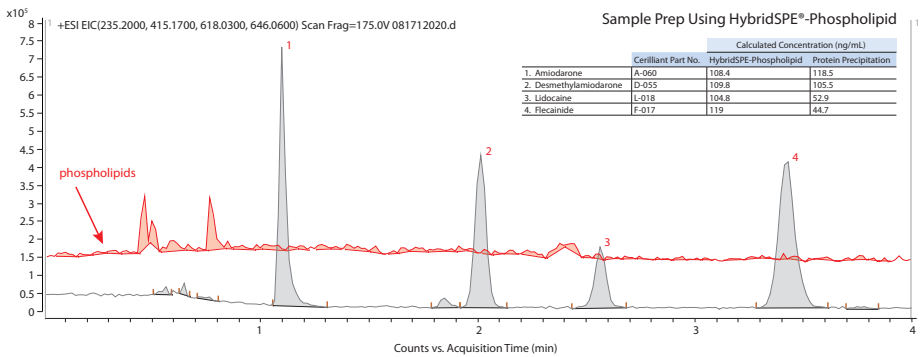
column temp. . . . . 35 °C

detector . . . . . ESI(+), full scan, m/z 100-1000

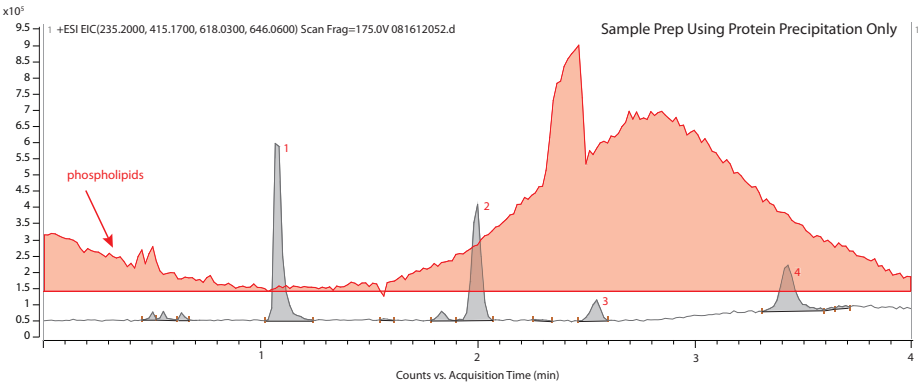
injection . . . . . 0.5 µL

sample . . . . . plasma extract, analyte concentration of final sample work up is equivalent to 100 ng/mL

Application No. . . . . G005819




| | Cerilliant Part No. | Calculated Concentration (ng/mL) HybridSPE-Phospholipid | Protein Precipitation |
|---|---|---|---|
| 1. Amiodarone | A-060 | 108.4 | 118.5 |
| 2. Desmethylamiodarone | D-055 | 109.8 | 105.5 |
| 3. Lidocaine | L-018 | 104.8 | 52.9 |
| 4. Flecainide | F-017 | 119 | 44.7 |






SUPELCO® Solutions within.™

# Cosmetics, Personal Care, and Cleaning Products

## HPLC Analysis of Hydrocortisone in Topical Cream on Discovery® HS C18 after SPE using Discovery® DSC-Si

**Sample Pre-treatment:**

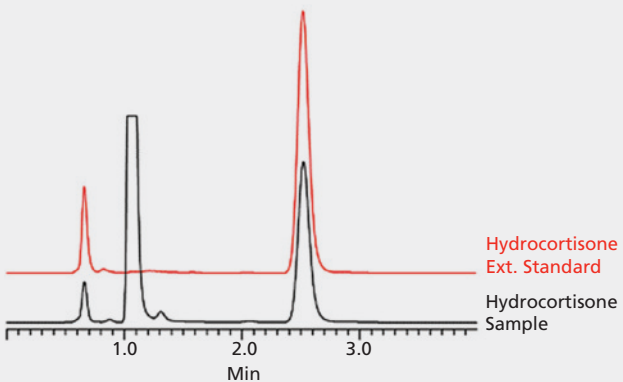
Dissove 1 g 1% hydrocortisone topical hand cream in 10 mL ethyl acetate. Dilute 2 mL of ethyl acetate-cream sample with 8 mL hexane:ethyl acetate (2:1, v/v).

sample preparation . . . . . SPE (Solid Phase Extraction)
sample addition . . . . . hydrocortisone topical hand cream in hexane:ethylacetate
SPE tube/cartridge . . . . . Discovery DSC-Si, 500 mg/3 mL (52695-U)
condition . . . . . 1 mL hexane:ethyl acetate (2:1, v/v)
sample addition . . . . . 1 mL
washing . . . . . 1 mL hexane:ethyl acetate (2:1, v/v)
elution . . . . . 1 mL methanol:DI water (1:1, v/v)
eluate post-treatment . . . dilute eluate to 2 mL with methanol:DI water (1:1, v/v)
column . . . . Discovery HS C18, 15 cm x 4.6 mm I.D., 5 µm particles (568520-U)
mobile phase . . . . . (A) methanol; (B) DI water (1:1, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G003765

**Efficiency of Recovery**

| Sample No. | Sample Weight (g) | Theoretical Hydrocortisone Weight (g)[1] | Actual Hydrocortisone weight recovered (g) | Absolute Recovery (%) | Actual % Hydrocortisone in Cream |
|---|---|---|---|---|---|
| 1 | 1.0258 | 0.0103 | 0.0092 | 89.32 | 0.9003 |
| 2 | 1.1078 | 0.0118 | 0.0111 | 94.07 | 0.9975 |
| 3 | 1.1151 | 0.0112 | 0.0119 | 106.25 | 1.0424 |
| 4 | 1.1369 | 0.0114 | 0.0119 | 104.39 | 1.0442 |
| 5 | 1.1390 | 0.0114 | 0.0113 | 99.12 | 1.0785 |
| 6 | 1.1634 | 0.0116 | 0.0123 | 106.03 | 1.0567 |
| | | | Average: | 99.86 | 1.02 |
| | | | RSD: | 6.99 | 0.06 |

[1]Based on manufacturer's label description of 1% hydrocortisone (w/w)



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Solutions within.™

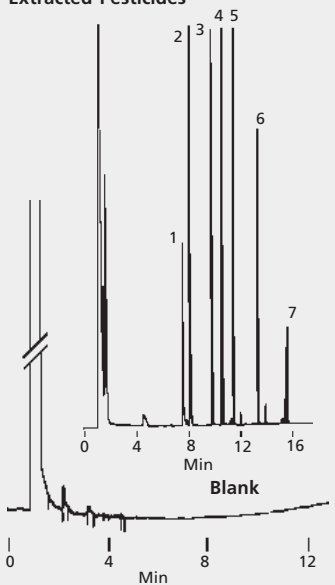
## Environmental

### GC Analysis of a 7-Component Organochlorine Pesticide Mix in Drinking Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI™-18

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . drinking water spiked with pesticides
SPE tube/cartridge . . . . . Supelclean ENVI-18, 500 mg/6 mL (57064)
condition . . . . 2 x 6 mL hexane:ethyl ether (1:1); 6 mL methanol; 6 mL DI water
sample addition . . . . . 250 mL water sample at 10 mL/min
drying . . . . . 10 min with vacuum
elution . . . . . 2 x 15 mL hexane:ethyl ether (1:1)
eluate post-treatment . . . . . concentrate to 2 mL with nitrogen stream
column . . . . . 5% Phenyl Polysiloxane, 30 m × 0.25 mm I.D., 0.25 µm (Supelco equivalent, Equity-5, 28089-U)
oven . . . . . 150 °C (2 min), 10 °C/min to 275 °C
detector . . . . . ECD, 310 °C
carrier gas . . . . . helium
injection . . . . . 1 µL
Application No. . . . . 713-1192

| Analyte | % Recovery (± CV) |
|---|---|
| 1. Hexachlorobenzene | 87 ± 11 |
| 2. γ-BHC (Lindane) | 99 ± 13 |
| 3. Heptachlor | 96 ± 12 |
| 4. Aldrin | 94 ± 13 |
| 5. Heptachlor epoxide | 98 ± 13 |
| 6. Endrin | 93 ± 11 |
| 7. Methoxychlor | 110 ± 13 |

**Extracted Pesticides**



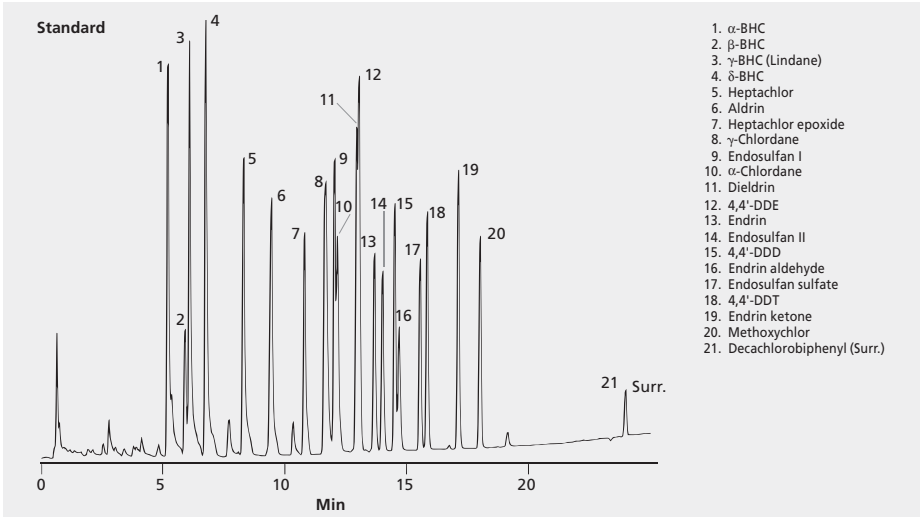
# GC Analysis of a 20-Component Organochlorine Pesticide Mix in Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI™-8

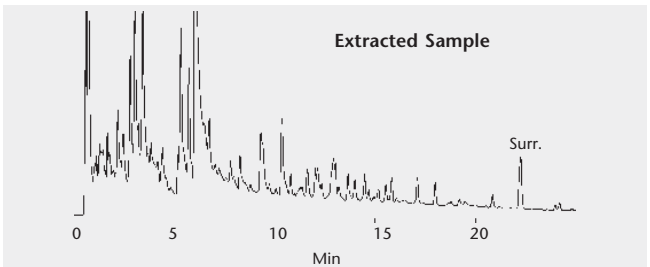
Chlorinated pesticides in hazardous waste

**Sample Pre-treatment:**

Add 5 mL methanol to 100 mL aqueous hazardous waste. Adjust to pH 5-7 if necessary.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | waste water spiked with pesticides |
| SPE tube/cartridge | Supelclean ENVI-8, glass hardware, 500 mg/6 mL (57107 (500 mg/6 mL); 57108 (1 g/6 mL)) |
| condition | 3 mL methanol; h 2 mL 5% methanol in water |
| sample addition | load sample at 5 mL/min |
| drying | 2-3 min with nitrogen stream |
| elution | 2 x 4 mL hexane:acetone (90:10), drop wise. allow to soak into bed |
| column | 5% Phenyl Polysiloxane, 15 m × 0.53 mm I.D., 0.50 µm (Supelco equivalent, Equity-5, 28252-U) |
| oven | 150 °C (0.5 min) to 275 °C (5 min) |
| inj. temp. | 200 °C |
| detector | ECD, 300 °C |
| carrier gas | helium |
| injection | 1 µL split/splitless (45 sec delay) |
| Application No. | 794-0399 |



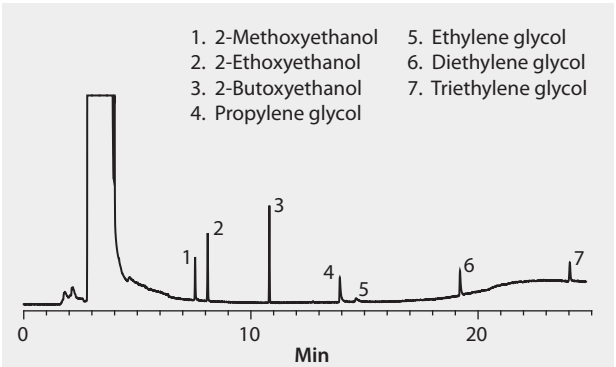






SUPELCO® Solutions within.™

## GC Analysis of Glycols in Water on SPB®-1000 after SPE using ENVI-Carb™ Plus

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . tap water spiked at 2 µg/mL with glycols
SPE tube/cartridge . . ENVI-Carb Plus reversible cartridge, 400 mg/ 1mL (54812-U)
condition . . 1 mL methylene chloride, 2 x 2 mL methanol, 3 mL deionized water (cartridge not allowed to dry during conditioning)
sample addition . . . . . 5 mL spiked tap water
drying . . . . . 10 min, 10 mm mercury
elution . . . . . 2 mL 80:20 methanol:methylene chloride
column . . . . . SPB-1000, 30 m x 0.25 mm I.D., 0.25 µm (24313)
oven . . . . . 50 °C (1 min), 8 °C/min to 200 °C (7 min)
inj. temp. . . . . 220 °C
detector . . . . . FID, 220 °C
carrier gas . . . . . helium, 1.5 mL/min. constant
injection . . . . . 1 µL, splitless (splitter open at 0.75 min.)
liner . . . . . 4 mm I.D. FocusLiner™ inlet liner with taper
Application No. . . . . G005284



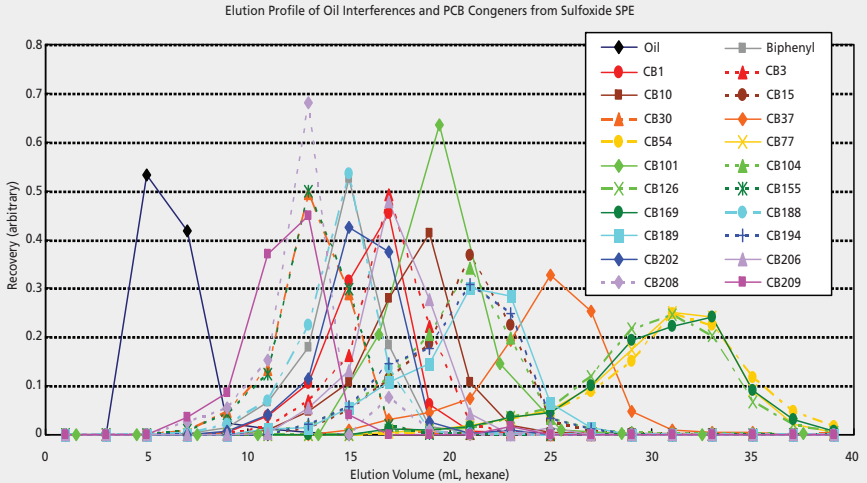



SUPELCO® Solutions within.™

## GC Analysis of PCBs in Transformer Oil on a 5% Phenyl Column after SPE using Supelclean™ Sulfoxide

**Sample Pre-treatment:**
Commercial insulation oil (Japan Industrial Standard JIS C2320-1999, insulating oil, Class 1-2/4, paraffin oil) was spiked with a Kanechlor PCB mix at the total levels of 3.7 ppm (mg/kg) and diluted with hexane (1:1 v/v).

SPE tube/cartridge . . . Supelclean Sulfoxide SPE Tube, Glass 6 g/20 mL (55252-U)
sample preparation . . . . . SPE (Solid Phase Extraction)
column . . . . . 5% phenyl polysiloxane (Supelco equivalent, SLB-5ms, 28471-U)
sample addition . . . . . transformer oil spiked with PCBs
detector . . . . . QMS (For full method details, please see reference (1))
extraction process . . . . . Sample Pre-treatment: paraffin oil was spiked with a Kanechlor PCB mix at the total levels of 3.7 ppm (mg/kg) and diluted with hexane (1:1 v/v). (Commercial insulation oil (Japan Industrial Standard JIS C2320-1999, insulating oil, Class 1-2/4).)
condition . . . . . 20 mL acetone; 40 mL of hexane
sample addition . . . . . 0.4 mL diluted oil sample
elution . . . . 12 mL hexane (elutes oil interferences); 25 mL hexane (elutes PCBs)
eluate post-treatment . . . . . concentrate under nitrogen
Application No. . . . . G004220




Observed Concentrations of PCB Homologues of a PCB-fortified Insulation Oil Sample (n = 3).

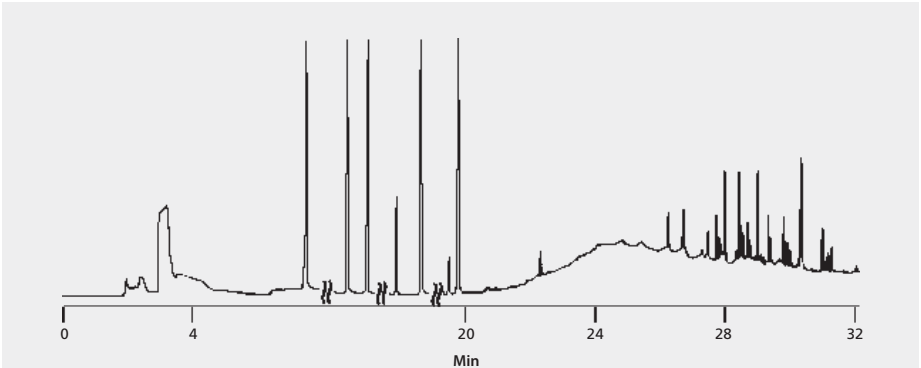
| | | mono-CBs | di-CBs | tri-CBs | tetra-CBs | penta-CBs | hexa-CBs | hepta-CBs | octa-CBs | nona-CBs | deca-CBs | total CBs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc. mg/kg | Mean | 0.045 | 0.29 | 0.80 | 0.87 | 0.90 | 0.35 | 0.55 | 0.13 | nd | nd | 3.9 |
| | SD | 0.003 | 0.05 | 0.08 | 0.06 | 0.08 | 0.002 | 0.01 | 0.004 | – | – | 0.13 |
| Recovery % | Mean | 102 | 91 | 92 | 108 | 106 | 95 | 97 | 97 | 95 | 9 | – |
| | SD | 5.2 | 2.6 | 5.1 | 5.4 | 6.8 | 2.8 | 2.7 | 3.3 | 1.2 | 3.4 | – |

**Ref:** 1. Numata et al. , *Anal. Chem.* **75**, 1450-1457 (2003)

## GC Analysis of PCBs in Transformer Oil on SPB®-5 after SPE using Supelclean™ LC-Florisil® and LC-Si

Connect Supelclean LC-Si SPE tube (1 g/6 mL) in series to male luer outlet of Supelclean LC-Florisil SPE tube (1 g/6 mL) using an SPE Tube Adapter (57020-U). Apply up to 0.2 g transformer oil or 0.1 g waste oil to upper frit of Florisil SPE tube. Pass 5 x 2 mL isooctane through both Florisil and LC-Si SPE tubes. Oil interferences will be retained on SPE tubes whereas PCBs will elute with isooctane fractions. Collect and combine isooctane fractions using 10 mL volumetric flask. Analyze isooctane extract using GC-ECD.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . used transormer oil containing 50 ppb Aroclor 1254.
SPE tube/cartridge . . . . . Supelclean LC-Florisil, 1.0 g/6 mL (57057)
SPE tube/cartridge . . . . . Supelclean LC-Si, 1.0 g/6 mL (57051)
condition . . . . . 0.5 mL isooctane (Florisil tube)
sample addition . . . . . 0.2 g transformer oil or 0.1 g waste oil
washing . . . . . 5 x 2 mL isooctane
column . . . . . SPB-5, 30 m × 0.32 mm I.D., 0.25 µm film (24048)
oven . . . . . 40 °C (4 min) to 300 °C at 10 °C/min, hold 5 min
detector . . . . . ECD
carrier gas . . . . . nitrogen
injection . . . . . 1 µL, splitless (30 sec delay), then split (50:1)
Application No. . . . . 713-0854

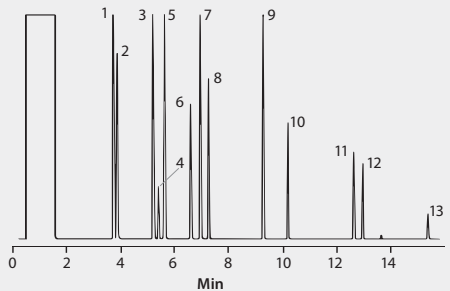





SUPELCO® Solutions within.™

## GC Analysis of Phenols in Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI-Chrom P

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . water spiked with phenol compounds
SPE tube/cartridge . . . . . Supelclean ENVI-Chrom P, 250 mg/6 mL (57225-U)
condition . . . . . 6 mL methyl t-butyl or ethyl acetate; 6 mL acetonitrile. Equilibrate with 6 mL DI water
sample addition . . . . . 100 mL water sample.
drying . . . . . 10 min using vacuum
elution . . . . . 5 mL methyl t-butyl ether acetate dropwise allowing initial 2 mL to soak in SPE bed
column . . . . . 5% Phenyl Polysiloxane, 15 m × 0.53 mm I.D., 0.50 µm (Supelco equivalent, Equity-5, 28252-U)
oven . . . . . 65 °C, 10 °C/min to 185 °C (1 min), 20 °C/min to 275 °C (5 min)
detector . . . . . FID, 300 °C
carrier gas . . . . . helium
injection . . . . . 1 µL, splitless (45 sec hold)
Application No. . . . . 712-0073

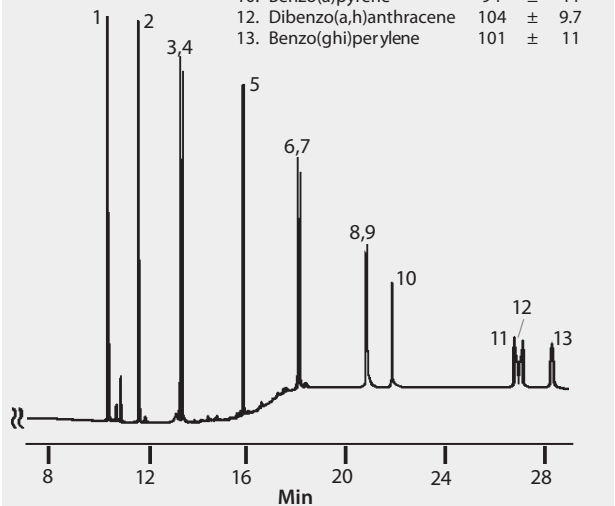



| | Analyte | %Recovery (± CV) |
|---|---|---|
| 1. | Phenol | 101.8 ± 3.7 |
| 2. | 2-Chlorophenol | 104.7 ± 1.7 |
| 3. | 2-Methylphenol | 107.0 ± 1.9 |
| 4. | 2-Bromophenol (Int. Std.) | — |
| 5. | 3-Methylphenol | 104.9 ± 1.6 |
| 6. | 2-Nitrophenol | 96.3 ± 2.8 |
| 7. | 2,4-Dimethylphenol | 105.3 ± 2.2 |
| 8. | 2,4-Dichlorophenol | 106.3 ± 2.2 |
| 9. | 4-Chloro-3-methylphenol | 104.6 ± 2.0 |
| 10. | 2,4,6-Trichlorophenol | 103.9 ± 2.1 |
| 11. | 4-Nitrophenol | 99.9 ± 4.1 |
| 12. | 2,3,4,6-Tetrachlorophenol | 104.6 ± 1.5 |
| 13. | Pentachlorophenol | 97.7 ± 4.8 |

## GC Analysis of Polynuclear Aromatic Hydrocarbons (PAHs) in Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI-18

sample preparation . . . . . SPE (Solid Phase Extraction)
sample addition . . . . . .
SPE tube/cartridge . . . . . Supelclean ENVI-18, 500 mg/6 mL (57064)
condition . . . . 2 x 6 mL toluene:methanol (10:1); 6 mL methanol; 6 mL DI water
sample addition . . . . . 250 mL water sample at 10 mL/min
drying . . . . . 10 min under vacuum
elution . . . . . 2 x 1 mL toluene:methanol (10:1)
column . . . . . 5% phenyl polysiloxane, 30m × 0.25 mm I.D., 0.25 µm film (Supelco equivalent, SLB-5ms, 28471-U)
oven . . . . . 70 °C (2 min) to 280 °C at 8 °C/min
detector . . . . . FID, 310 °C
carrier gas . . . . . helium
injection . . . . . 1 µL
Application No. . . . . 80-207

| | Analyte | % Recovery (± CV) |
|---|---|---|
| 1. | Acenaphthylene | 66 ± 28 |
| 2. | Fluorene | 95 ± 7.5 |
| 3. | Phenanthrene | 95 ± 8 |
| 4. | Anthracene | 95 ± 11 |
| 5. | Pyrene | 97 ± 7.1 |
| 6. | Benzo(a)anthracene | 94 ± 8.3 |
| 7. | Chrysene | 101 ± 8.8 |
| 8. | Benzo(b)fluoranthene | 103 ± 10 |
| 9. | Benzo(k)fluoranthene | 103 ± 9.4 |
| 11. | Indeno(1,2,3-cd)pyrene | 102 ± 10 |
| 10. | Benzo(a)pyrene | 94 ± 11 |
| 12. | Dibenzo(a,h)anthracene | 104 ± 9.7 |
| 13. | Benzo(ghi)perylene | 101 ± 11 |





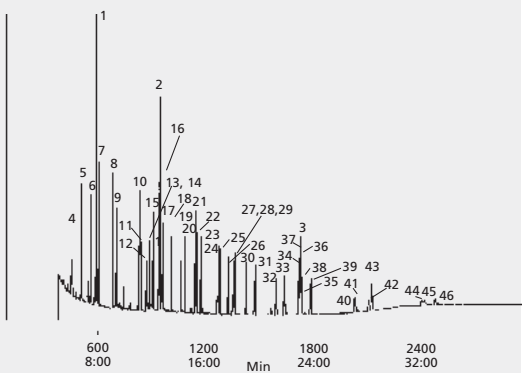

SUPELCO® Solutions within.™

# GC Analysis of Semivolatiles in Drinking Water on a 5% Phenyl Column after SPE using ENVI™-18 DSK

**Sample Pre-treatment:**
Adjust 1 L drinking water to pH <2 with 6 N HCl. Add 5 mL methanol and mix thoroughly

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . acidified drinking water
SPE tube/cartridge . . . . . ENVI-18 DSK SPE Disk, 47 mm (57171)
condition . . . . . 5 mL dichloromethane; 5 mL methanol; 5 mL DI water
sample addition . . . . . 5 mL water sample
elution . . . . . 2 x 5 mL acetonitrile rinsing internal chamber of sample reservoir thoroughly
column . . . . . 5% phenyl polysiloxane, 30 m × 0.25 mm I.D., 0.25 µm (Supelco equivalent, SLB-5ms, 28471-U)
oven . . . . . 40 °C to 160 °C (3 min), then to 300 °C (3 min) at 6 °C/min
detector . . . . . MS, scan range m/z = 45-450
carrier gas . . . . . helium, 33 cm/sec
injection . . . . . 1 µL split/splitless, 1 min delay
Application No. . . . . 749-0798

| | | µg/L* | | | g/L* |
|---|---|---|---|---|---|
| 1. | Acenapthene-$d_{10}$ | 5.0 | 24. | Heptachlor epoxide | 2.0 |
| 2. | Phenanthrene-$d_{10}$ | 5.0 | 25. | 2,2',3',4,6-Pentachlorobiphenyl | 2.0 |
| 3. | Chrysene-$d_{12}$ | 5.0 | 26. | γ-Chlordane | 2.0 |
| 4. | Hexachlorocyclopentadiene | 2.0 | 27. | Pyrene | 2.0 |
| 5. | Dimethylphthalate | 2.0 | 28. | α-Chlordane | 2.0 |
| 6. | Acenaphthylene | 2.0 | 29. | trans-Nonachlor | 2.0 |
| 7. | 2-Chlorobiphenyl | 2.0 | 30. | 2,2',4,4',5,6'-Hexachlorobiphenyl | 2.0 |
| 8. | Diethyphthalate | 2.0 | 31. | Endrin | 2.0 |
| 9. | Fluorene | 2.0 | 32. | Butylbenzylphthalate | 2.0 |
| 10. | 2,3-Dichlorobiphenyl | 2.0 | 33. | di(2-ethylhexyl)Adipate | 2.0 |
| 11. | Hexachlorobenzene | 2.0 | 34. | 2,2'3,3',4,4',6-Heptachlorobiphenyl | 2.0 |
| 12. | Simazine | 2.0 | 35. | Methoxychlor | 2.0 |
| 13. | Atrazine | 2.0 | 36. | 2,2'3,3',4,5',6,6'-Octachlorobiphenyl | 2.0 |
| 14. | Pentachlorophenol | 8.0 | 37. | Benz(a)anthracene | 2.0 |
| 15. | γ-BHC | 2.0 | 38. | Chrysene | 2.0 |
| 16. | Phenanthrene | 2.0 | 39. | Di(2-ethylhexyl)phthalate | 2.0 |
| 17. | Anthracene | 2.0 | 40. | Benzo(b)fluoranthene | 2.0 |
| 18. | 2,4,5-Trichlorobiphenyl | 2.0 | 41. | Benzo(k)fluoranthene | 2.0 |
| 19. | Alachlor | 2.0 | 42. | Benzo(a)pyrene | 2.0 |
| 20. | Heptachlor | 2.0 | 43. | Perylene-$d_{12}$ | 5.0 |
| 21. | di-n-Butylphthalate | 2.0 | 44. | Indeno(1,2,3-cd)Pyrene | 2.0 |
| 22. | 2,2',4,4'-Tetrachlorobiphenyl | 2.0 | 45. | Dibenz(a,h)anthracene | 2.0 |
| 23. | Aldrin | 2.0 | 46. | Benzo(ghi)perylene | 2.0 |



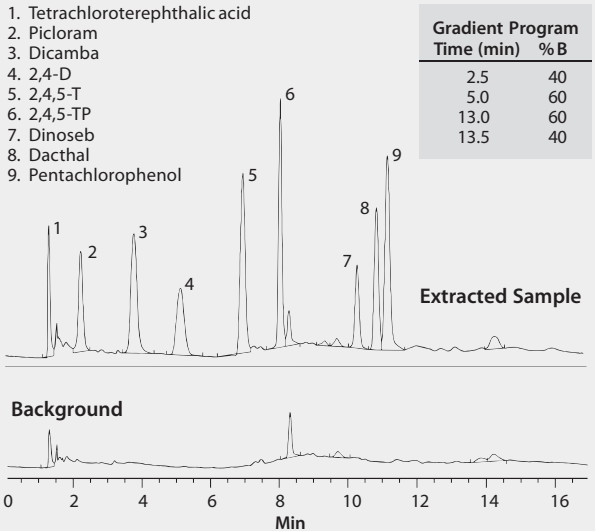



SUPELCO® Solutions within.™

## HPLC Analysis of Acidic Herbicides in Water on a Polymeric C18 Column after SPE using Supelclean™ ENVI™-Carb

*using Zymark AutoTrace Extraction WorkStation 1.20*

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . Fresh 1 L water samples, dechlorinated with sodium thiosulfate when necessary, at ambient temperature and pH.
SPE tube/cartridge . . . . . Supelclean ENVI-Carb, 250 mg/6 mL (57092)
condition . . . . . 10 mL DI water at 20 mL/min
sample addition . . . . . 0.9 L water sample at 20 mL/min
drying . . . . . 10 min using clean nitrogen
washing . . . . . 10 mL DI water at 20 mL/min
elution . . . . . 10 mL 0.1% phosphoric acid in methylene chloride:acetonitrile (80:20) at 5 mL/min
column . . polymeric-coated silica-based PAH specialty column, 20 cm × 3 mm I.D., 5 µm (Supelco equivalent, SUPELCOSIL LC-PAH, available upon request)
mobile phase . . gradient, (A): 0.05% phosphoric acid in DI water; (B): acetonitrile
flow rate . . . . . 0.5 mL/min
column temp. . . . . 50 °C
detector . . . . . photodiode array- peak width: 0.053 min, sampling interval: 0.320 sec, monitor 210 nm & 225 nm
injection . . . . . 10 µL of extract (4-5 ppb each analyte in water)
Application No. . . . . 796-0150



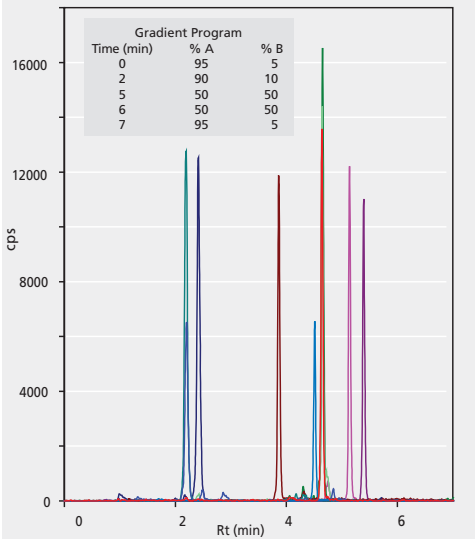

Figures provided by A. Lichtman, Nassau County Dept. of Health, Hempstead, New York, USA. Request Application Note No. T196100.

# HPLC Analysis of β-Blockers and β-Agonists in Urine and Wastewater on a C18 Column after SPE using SupelMIP® SPE-Beta-Receptor

**Sample Pre-treatment:**

Horse urine was centrifuged at 3000 g for 10 min, diluted 1:1 (v/v) with DI water, adjusted to pH 7. Wastewater was filtered with 1 µm filter paper and adjusted to pH 6-7. Samples were spiked with 10 beta-agonists and beta-blockers at the level of 1 ng/mL.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | horse urine or waste water spiked with 10 beta-agonists and beta-blockers at 1 ng/mL |
| SPE tube/cartridge | SupelMIP SPE - Beta-Receptor, 25 mg/10 mL (LRC) (53223-U) |
| condition | 1 mL acetonitrile; 1 mL DI water |
| sample addition | 1 mL |
| washing | 3 x 1 mL DI water; 1 mL acetonitrile; 1 mL 60% acetonitrile/40% DI water; apply 2 min of full vacuum to dry the tube after each step |
| elution | 2 x 1 mL 1% formic acid in acetonitrile |
| eluate post-treatment | evaporate under nitrogen and reconstitute with 150 µL 5% acetonitrile in 10 mM ammonium acetate, pH 4.6 prior to LC-MS-MS analysis |
| column | C18, 5 cm x 3 mm I.D., 3 µm particles |
| mobile phase | (A) 10 mM ammonium acetate, pH 4.6 (adjusted with acetic acid); and (B) acetonitrile |
| flow rate | 0.5 mL/min |
| column temp. | ambient |
| detector | MS/MS, ESI(+) |
| injection | 20 µL |
| Application No. | G004059 |



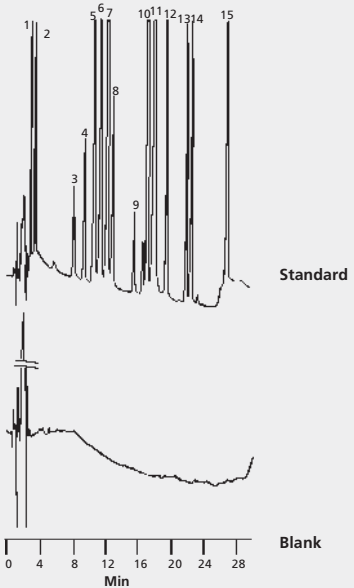

Note: Clenbuterol and Tulobuterol were spiked at the levels of 0.1 ng/mL.

| Analyte | Rt (min.) | Q1/Q3 |
|---|---|---|
| Atenolol | 3.0 | 267.2/145 |
| Carazolol | 6.2 | 299.1/194.2 |
| Metoprolol | 5.6 | 268.2/133 |
| Propranolol | 6.5 | 260.2/154.9 |
| Timolol | 5.5 | 317.2/188.1 |
| Clenbuterol | 5.6 | 277.1/202.9 |
| Ritodrine | 3.9 | 288.2/121 |
| Salbutamol | 2.6 | 240.2/147.9 |
| Terbutaline | 2.6 | 226.2/152 |
| Tulobuterol | 5.6 | 228.2/154.1 |

| | Lower Limit of Quantitation (ng/mL, ppb, or µg/kg) | |
|---|---|---|
| Analyte | 1 mL Horse Urine | 10 mL Wastewater |
| Atenolol | 0.1 | 0.01 |
| Carazolol | 0.1 | 0.01 |
| Metoprolol | 0.1 | 0.01 |
| Propranolol | 0.1 | 0.01 |
| Timolol | 0.1 | 0.01 |
| Clenbuterol | 0.02 | 0.002 |
| Ritodrine | 0.05 | 0.005 |
| Salbutamol | 0.1 | 0.01 |
| Terbutaline | 0.2 | 0.02 |
| Tulobuterol | 0.005 | 0.0005 |

## HPLC Analysis of Nonvolatile Pesticides in Water on SUPELCOSIL™ LC-18-DB after SPE using Supelclean™ ENVI-Carb™

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . water spiked with pesticides at 10–50 µg/L
SPE tube/cartridge . . . . . Supelclean ENVI-Carb, 250 mg/3 mL (57088)
condition . . . . . 5 mL methylene chloride:methanol (80:20); 1 mL methanol; 10 mL 2% acetic acid in water (keep bed moist until sample addition)
sample addition . . . . . 100 mL sample at 5 mL/min
drying . . . . . 1 minute with vacuum
elution . . . . . 0.8 mL methanol; 2 x 35 mL methylene chloride:methanol (80:20)
eluate post-treatment . . . . . dry eluate to 500 µL under gentle nitrogen stream, reconstitute to 1 mL with methanol
column . . SUPELCOSIL LC-18-DB, 25 cm × 4.6 mm I.D., 5 µm particles (58355-U)
mobile phase . . . . . (A) water:acetonitrile (90:10), (B) acetonitrile; gradient 80% A for 5 min then to 30% A over 30 min
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 220 nm
injection . . . . . 20 µL
Application No. . . . . 85-438




| Analyte | % Recovery (± RSD, n = 5) |
|---|---|
| 1. Oxamyl | 111 ± 9.6 |
| 2. Methomyl | 105 ± 5.0 |
| 3. N-1-Naphthylthiourea | — |
| 4. Aldicarb | 92 ± 0.8 |
| 5. Simazine | 91 ± 6.5 |
| 6. Monuron | 99 ± 3.2 |
| 7. Cyanazine | 90 ± 5.4 |
| 8. Metribuzin | 97 ± 3.9 |
| 9. Carbofuran | 106 ± 6.2 |
| 10. Atrazine | 89 ± 5.7 |
| 11. Carbaryl | 97 ± 3.5 |
| 12. Diuron | 88 ± 5.7 |
| 13. Propham | 95 ± 3.2 |
| 14. Propachlor | 96 ± 3.8 |
| 15. Linuron | 88 ± 5.4 |

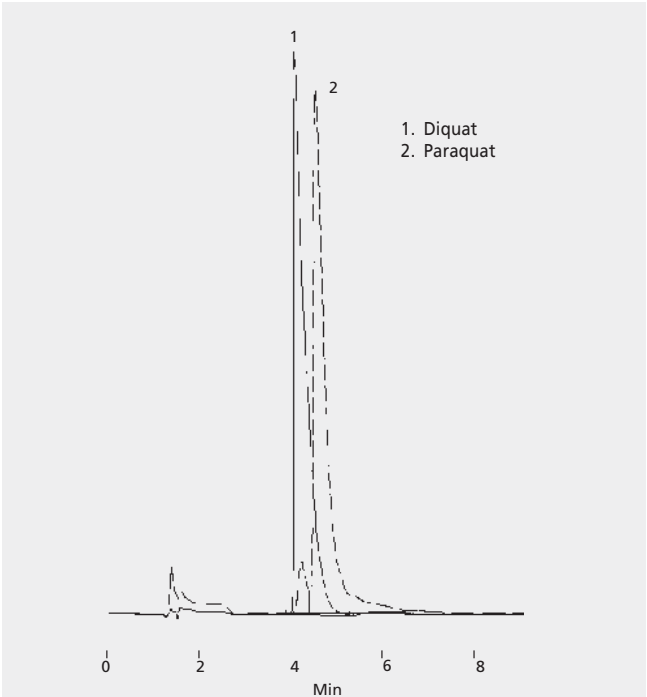
## HPLC Analysis of Paraquat and Diquat on SUPELCOSIL™ LC-18 after SPE using ENVI™-8 DSK

*Refer to US EPA Method 549.1 for full details*

**Sample Pre-treatment:**

250 mL drinking water. Adjust water sample to pH to 10.5 ± 0.2 with 10% sodium hydroxide or 10% hydrochloric acid

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . drinking water adjusted to pH to 10.
SPE tube/cartridge . . . . . ENVI-8 DSK SPE Disk, 47 mm (57172)
sample preparation . . . . . ENVI-8 DSK, 47 mm (57172)
condition . . 10 mL acetonitrile; 2 × 10 mL reagent water; 10 mL conditioning solvent A (5 g cetyl trimethyl ammonium bromide and 5 mL conc. ammonium hydroxide in 500 mL DI water, dilute to 1 L); ( 2 × 10 mL reagent water; 10 mL conditioning solvent B (10 g hexanesulfonic acid, sodium salt and 10 mL conc. ammonium hydroxide in 250 mL DI water, dilute to 500 mL))
sample addition . . . . . 250 mL water 100 mL/min
elution . . . . . 0.5-1.0 mL acetonitrile (to cover/solvate disk); 2 × 4 mL eluting solution (13.5 mL orthophosphoric acid and 10.3 mL diethylamine in 500 mL DI water, dilute to 1 L)
column . . . . SUPELCOSIL LC-18, 15 cm × 4.6 mm I.D., 5 µm particles (58230-U)
mobile phase . . 3.5 mL triethylamine and 1.0 g 1-hexane-sulfonic acid, sodium salt to 800 mL DI $H_2O$ add orthophosphoric acid to pH 2.5, dilute to 1 L
flow rate . . . . . 1.0 mL/min
column temp. . . . . 35 °C
detector . . . . . photodiode array, diquat - 308 nm, paraquat - 257 nm
injection . . . . . 100 µL
Application No. . . . . 794-0715



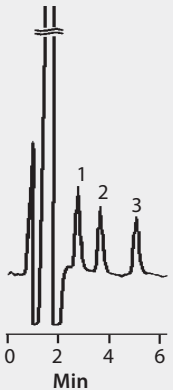



SUPELCO® Solutions within.™

## HPLC Analysis of Triazine Herbicides in Grass on SUPELCOSIL™ LC-8-DB after SPE using Supelclean™ LC-SCX

**Sample Pre-treatment:**
5 g fresh grass clippings spiked with 2 ppm each herbicide. Add 4 g anhydrous sodium sulfate and 20 mL methylene chloride:acetone (80:20). Shake 20 min and allow mixture to stand 1 min.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . fresh grass clippings spiked with herbicides at 2 ppm
SPE tube/cartridge . . . . . Supelclean LC-SCX, 500 mg/3 mL (57018)
condition . . . . . 1 mL methylene chloride
sample addition . . . . . 2 mL grass extract. Wash with 2 x 2 mL acetonitrile.
drying . . . . . Dry packing for 5 min. under nitrogen purge
washing . . Dry packing for 5 min under nitrogen purge; wash with 2 x 2 mL DI water
elution . . . . . 1.5 mL methanol
eluate post-treatment . . . . . dilute to 2 mL with DI water
column . . . . . SUPELCOSIL LC-8-DB, 15 cm × 4.6 mm I.D., 5 µm with guard column (58347)
mobile phase . . . . . (A) acetonitrile; (B) water; (45:55, A:B)
flow rate . . . . . 1.5 mL/min
detector . . . . . UV, 254 nm
injection . . . . . 100 µL
Application No. . . . . 84-109




| Recovery (± SD, n = 3) | | |
|---|---|---|
| 102.0 | ± | 2.9 |
| 102.2 | ± | 1.6 |
| 100.5 | ± | 1.8 |

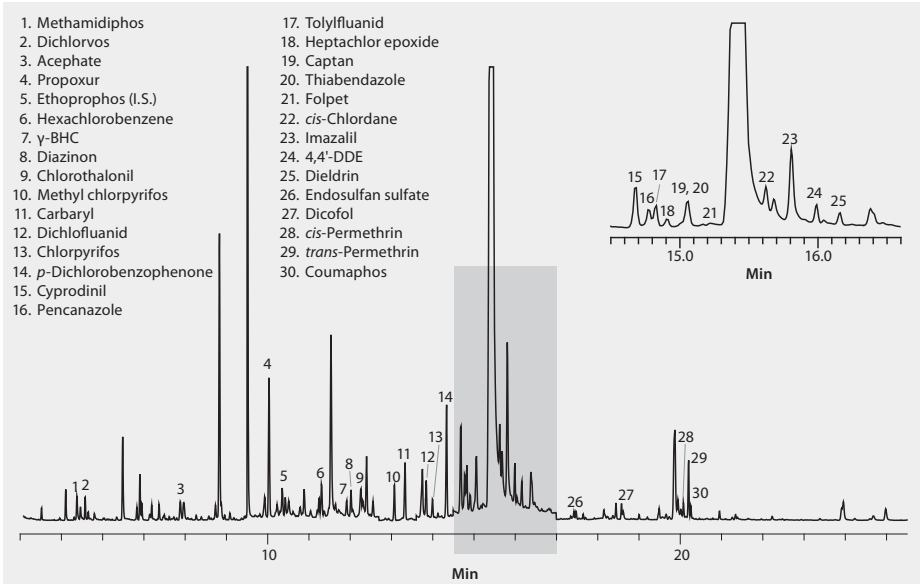
1. Simazine
2. Atrazine
3. Propazine

## Food and Beverages

### GC Analysis of Agriculture Pesticides in Orange (Spiked) on SLB®-5ms after QuEChERS Cleanup using Primary-Secondary Amine (PSA)

Chromatograms of the spiked orange samples are presented in the Figure. Several background peaks eluting prior to nine minutes are due to impurities in the toluene. Despite extract cleanup, matrix peaks are also present in the chromatograms. Further sample cleanup may be possible by increasing SPE sorbent weight. Nevertheless, all pesticides were detected. A first order fit was used for calibration. Linearity for the five-point calibration curves was excellent, with 28 of the 29 pesticides having r2 values >0.995 at a range of 50-500 ppb. Proper calibration of imazalil was not possible due to its presence in the orange blanks.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . 15 g of ground-up orange, 75 µL of the internal standard stock solution (ethoprophos at 20 ppm in methanol), 75 µL of the pesticide stock solution (29 pesticides, each at 20 ppm in methanol)
column . . . . . SLB-5ms, 30 m x 0.25 mm I.D, 0.25 µm (28471-U)
oven . . . . . 100 °C (1 min), 10 °C/min to 300 °C (5 min)
inj. temp. . . . . 250 °C
MSD interface . . . . . 300 °C
scan range . . . . . selected ion monitoring (SIM), 7 monitoring groups used
carrier gas . . . . . helium, 1 mL/min constant
injection . . . . . 1.0 µL pulsed (20 psi until 0.20 min), splitless (1.0 min)
liner . . . . . 4 mm I.D., single taper
sample . . . . . extract of an orange spiked with a 29-component pesticide mix plus 1 internal standard, each at 100 ppb
Application No. . . . . G003590



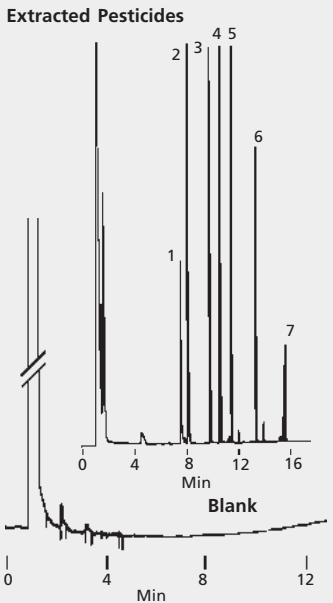



SUPELCO® Solutions within.™

## GC Analysis of a 7-Component Organochlorine Pesticide Mix in Drinking Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI™-18

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . drinking water spiked with pesticides
SPE tube/cartridge . . . . . Supelclean ENVI-18, 500 mg/6 mL (57064)
condition . . . . 2 x 6 mL hexane:ethyl ether (1:1); 6 mL methanol; 6 mL DI water
sample addition . . . . . 250 mL water sample at 10 mL/min
drying . . . . . 10 min with vacuum
elution . . . . . 2 x 15 mL hexane:ethyl ether (1:1)
eluate post-treatment . . . . . concentrate to 2 mL with nitrogen stream
column . . . . . 5% Phenyl Polysiloxane, 30 m × 0.25 mm I.D., 0.25 µm (Supelco equivalent, Equity-5, 28089-U)
oven . . . . . 150 °C (2 min), 10 °C/min to 275 °C
detector . . . . . ECD, 310 °C
carrier gas . . . . . helium
injection . . . . . 1 µL
Application No. . . . . 713-1192

| Analyte | % Recovery (± CV) |
|---|---|
| 1. Hexachlorobenzene | 87 ± 11 |
| 2. γ-BHC (Lindane) | 99 ± 13 |
| 3. Heptachlor | 96 ± 12 |
| 4. Aldrin | 94 ± 13 |
| 5. Heptachlor epoxide | 98 ± 13 |
| 6. Endrin | 93 ± 11 |
| 7. Methoxychlor | 110 ± 13 |






SUPELCO® Solutions within.™

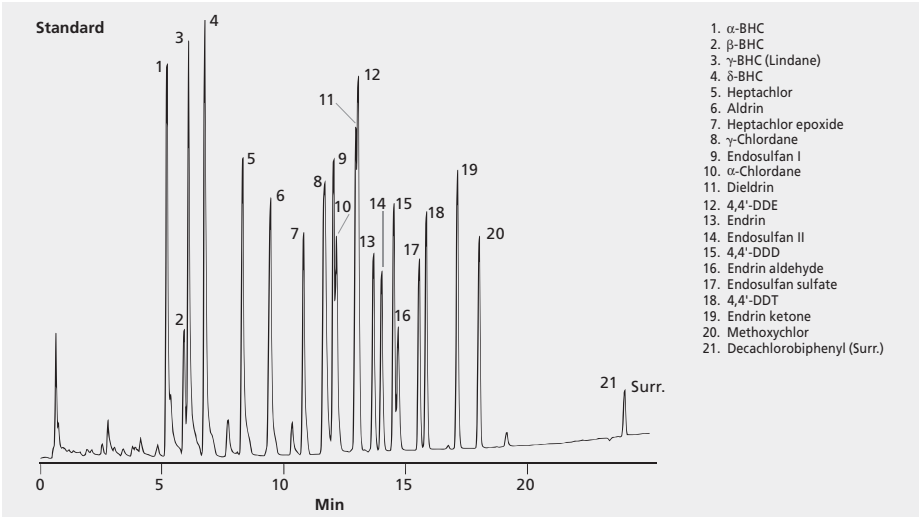
# GC Analysis of a 20-Component Organochlorine Pesticide Mix in Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI™-8

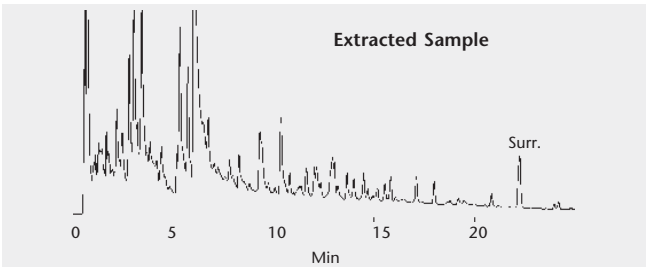
Chlorinated pesticides in hazardous waste

**Sample Pre-treatment:**

Add 5 mL methanol to 100 mL aqueous hazardous waste. Adjust to pH 5-7 if necessary.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | waste water spiked with pesticides |
| SPE tube/cartridge | Supelclean ENVI-8, glass hardware, 500 mg/6 mL (57107 (500 mg/6 mL); 57108 (1 g/6 mL)) |
| condition | 3 mL methanol; h 2 mL 5% methanol in water |
| sample addition | load sample at 5 mL/min |
| drying | 2-3 min with nitrogen stream |
| elution | 2 x 4 mL hexane:acetone (90:10), drop wise. allow to soak into bed |
| column | 5% Phenyl Polysiloxane, 15 m × 0.53 mm I.D., 0.50 µm (Supelco equivalent, Equity-5, 28252-U) |
| oven | 150 °C (0.5 min) to 275 °C (5 min) |
| inj. temp. | 200 °C |
| detector | ECD, 300 °C |
| carrier gas | helium |
| injection | 1 µL split/splitless (45 sec delay) |
| Application No. | 794-0399 |



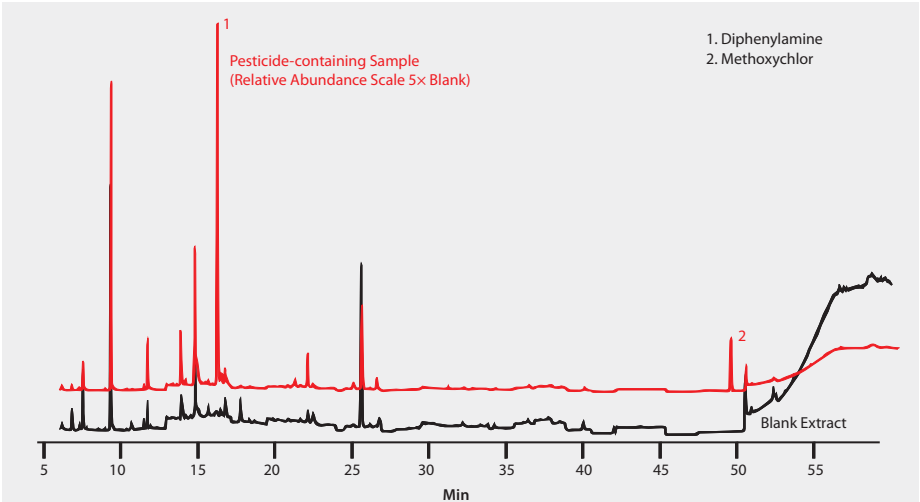



SUPELCO® Solutions within.™

## GC Analysis of Diphenylamine and Methoxychlor in Apples on a 14% Cyanopropylphenyl Column after SPE using Supelclean ENVI-Carb

**Pesticides in fruits and vegetables**

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | homogenize 50 g sample in 100 mL acetonitrile then add 10 g NaCl and homogenize 5 min |
| SPE tube/cartridge | ENVI-Carb, 500 mg/6 mL |
| column | 14% cyanopropylphenyl polysiloxane, 30 m × 0.25 mm I.D., 0.15 µm (Supelco equivalent, Equity-1701, 28372-U) |
| oven | 70 °C (2 min), 25 °C/min to 130 °C, 2 °C/min to 220 °C, 10 °C/min to 280 °C (4.6 min) |
| detector | MSD, 285 °C |
| carrier gas | helium |
| injection | 2 µL splitless |
| Application No. | 796-0129 |

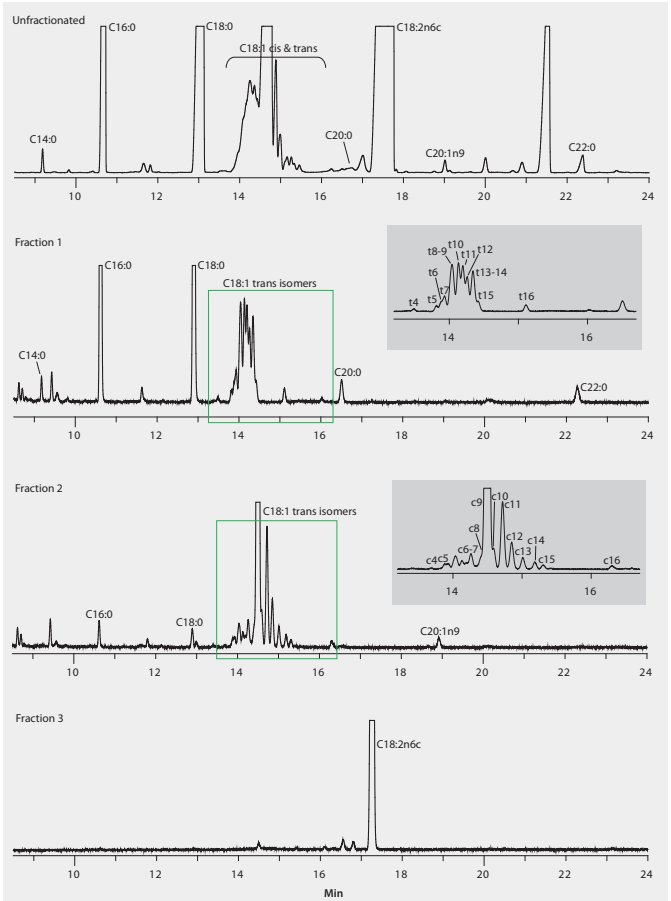



Chromatograms courtesy of J. Fillion, Agriculture and Agri-Food Canada (Laboratory Services Division, Pesticide Laboratory), Ottawa, Ontario.

## GC Analysis of FAMEs in Cookies on SLB®-IL111 after SPE Fractionation using Discovery® Ag-Ion

A sample of commercially purchased cookies were prepared using an acid digestions /alkali hydrolysis followed by methylation as described in AOCS Official Method Ce 1k-09 (1). The resulting extracts were then fractionated using Ag-ion SPE and analyzed by GC/FID on the 100 m SLB-IL111 ionic liquid capillary column.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . 1 g of commercially purchased cookies was ground and subjected to acid digestion and alkali hydrolysis followed by methylation as described in AOCS Official Method Ce 1k-09
SPE tube/cartridge . . . . . Discovery Ag-Ion SPE tubes, 750 mg/6 mL (54225-U)
condition . . 4 mL of acetone; allow solvent to gravity drip completely through tube; discard eluant; 4 mL of hexane; allow solvent to gravity drip completely through tube; discard eluant
sample addition . . . . 1 mL of extract; discard any eluant that drips through tube
elution . . . . (Fraction 1) 6 mL of hexane:acetone (96:4); collect eluant in a fresh container with slight vacuum; ((Fraction 2) 4 mL of hexane: acetone (90:10); collect eluant in a fresh container with slight vacuum; (Fraction 3) 4 mL of 100% acetone; collect eluant in a fresh container with slight vacuum)
eluate post-treatment . . evaporate each fraction at room temperature using nitrogen; reconstitute each fraction to 1 mL of hexane
column . . . . . SLB-IL111, 100 m x 0.25 mm I.D., 0.20 µm (29647-U)
oven . . . . . 168 °C
inj. temp. . . . . 250 °C
detector . . . . . FID, 250 °C
carrier gas . . . . . 1 µL, 10:1 split
injection . . . . . 1 µL, 10:1 split
liner . . . . . 4 mm I.D., split type, single taper wool packed FocusLiner™ design
Application No. . . . . G005622



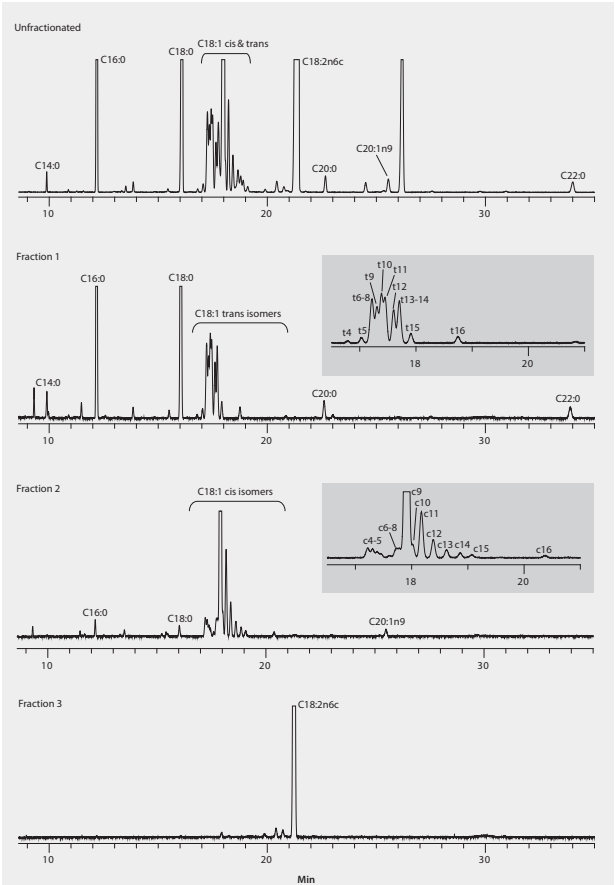



SUPELCO® Solutions within.™

## GC Analysis of FAMEs in Cookies on SP™-2560 after SPE Fractionation using Discovery® Ag-Ion

A sample of commercially purchased cookies were prepared using an acid digestions /alkali hydrolysis followed by methylation as described in AOCS Official Method Ce 1k-09 (1). The resulting extracts were then fractionated using Ag-ion SPE and analyzed by GC/FID on the 100 m SP-2560 capillary column.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . 1 g of commercially purchased cookies was ground and subjected to acid digestion and alkali hydrolysis followed by methylation as described in AOCS Official Method Ce 1k-09
SPE tube/cartridge . . . . . Discovery Ag-Ion SPE tubes, 750 mg/6 mL (54225-U)
condition . . 4 mL of acetone; allow solvent to gravity drip completely through tube; discard eluant; 4 mL of hexane; allow solvent to gravity drip completely through tube; discard eluant
sample addition . . . . 1 mL of extract; discard any eluant that drips through tube
elution . . . . (Fraction 1) 6 mL of hexane:acetone (96:4); collect eluant in a fresh container with slight vacuum; ((Fraction 2) 4 mL of hexane: acetone (90:10); collect eluant in a fresh container with slight vacuum;(Fraction 3) 4 mL of 100% acetone; collect eluant in a fresh container with slight vacuum)
eluate post-treatment . . evaporate each fraction at room temperature using nitrogen; reconstitute each fraction to 1 mL of hexane
column . . . . . SP-2560, 100 m x 0.25 mm I.D., 0.20 µm (24056)
oven . . . . . 180 °C
inj. temp. . . . . 250 °C
detector . . . . . FID, 250 °C
carrier gas . . . . . hydrogen, 1 mL/min
injection . . . . . 1 µL, 10:1 split
liner . . . . . 4 mm I.D., split type, single taper wool packed FocusLiner™ design
Application No. . . . . G005621





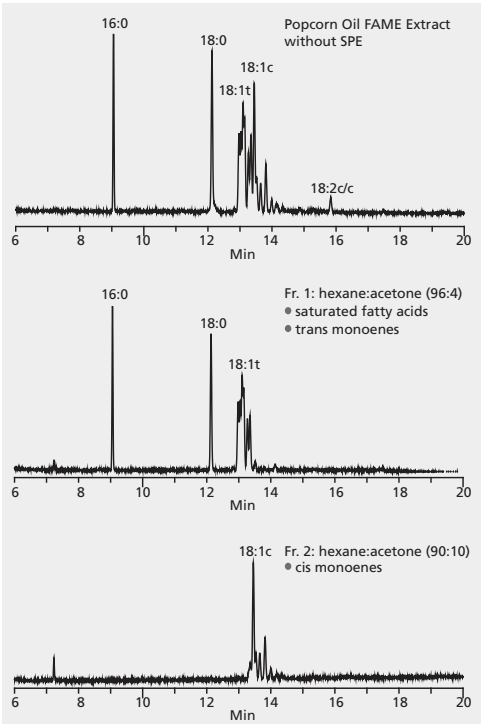

SUPELCO® Solutions within.™

## GC Analysis of FAMEs in Popcorn Oil on SP™-2560 (75 m x 0.18 mm I.D., 0.14 µm) after SPE Fractionation using Discovery® Ag-Ion, Fast GC Analysis

**Sample pretreatment**: A 1 g sample of popcorn oil was scraped from the inside of a popped microwave oven popcorn bag and mixed with 8 mL deionized water (DI) and 4 mL petroleum ether, centrifuged to remove particulates. The ether layer (upper layer) was collected. This petroleum ether extraction step was repeated four times. The upper ether layers were combined, evaporated, and reconstituted in 16 mL toluene. A 1 mL aliquot was transferred to conical reaction vial and 2 mL 7% boron triflluoride in methanol were added. The reaction was incubated at 80 °C for 15 minutes using a heating block, then cooled to room temperature. 1 mL DI water was added and FAMEs were extracted twice using 1 mL hexane each time. The upper hexane layers were combined into a fresh vial, evaporated, and reconstituted in 5 mL hexane and 50 mg anhydrous sodium sulfate.

Discovery Ag-Ion operates by argentation chromatography which is a powerful analytical and sample prep technique for the separation or fractionation of cis and trans geometric and positional fatty acid (FA), fatty acid methyl ester (FAME), diacylglycerol (DAG) and triacylglycerol (TAG) isomers. Discovery Ag-Ion SPE is shown in this application to separate FAMEs from an edible oil sample.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | 1 g of popcorn oil FAME's in Hexane |
| SPE tube/cartridge | Discovery Ag-Ion, 750 mg/6 mL (54225-U) |
| condition | 4 mL acetone and 4 mL hexane |
| sample addition | 1 mL of the hexane FAME extract |
| elution | 6 mL hexane:acetone (96:4, v/v) followed by 4 mL hexane:acetone (90:10, v/v) |
| column | SP-2560, 75 m x 0.18mm I.D., 0.14 µm (23348-U) |
| oven | 180 °C isothermal |
| inj. temp. | 220 °C |
| detector | FID, 220 °C |
| carrier gas | hydrogen, 40 cm/sec. at 180 °C |
| injection | (0.5 µL, 100:1 split) |
| liner | 0.5 µL, 100:1 split |
| Application No. | G003760 |






SUPELCO® Solutions within.™

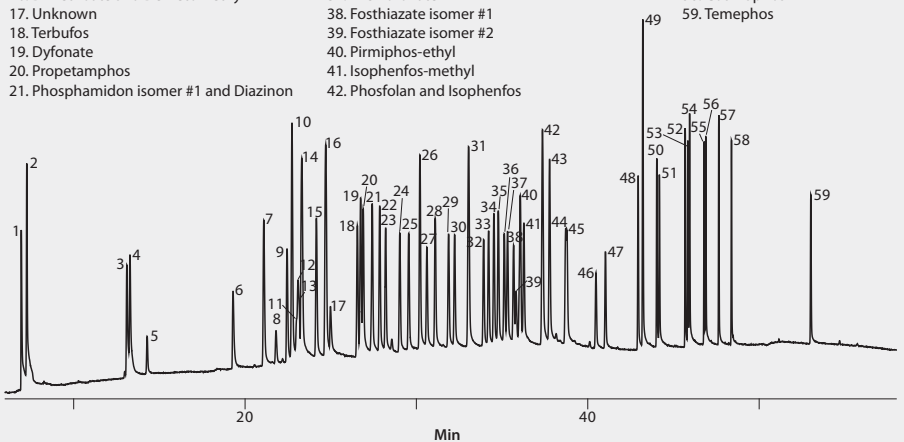
# GC Analysis of Organophosphorus Pesticides (OP-Pest) in Cabbage, Onion, and Mushrooms on SLB-5ms after SPE using Supelclean™ ENVI-Carb™ II/PSA

**Sample pretreatment**: To a 10 g sample of homogenized food (green onions, apples, Napa cabbage, button mushrooms) was added 10 mL acetonitrile and 4 g magnesium sulfate (that had been heated at 550 °C) and 1 g sodium chloride. After centrifugation, the supernatant was removed and mixed with 1 g magnesium sulfate and centrifuged. 5 mL of the supernatant was removed and evaporated to 1 mL for SPE loading.

Organophosphorous pesticide use in agriculture is widespread due to the fact that they are more amenable to environmental degradation in comparison to organochlorine or organonitrogen compounds. There are a large number of pesticides in the organophosphorous group, and because of their potential health effects, they are of particular concern on produce imported from areas in which they are commonly used. This chromatogram illustrates the separation of 63 of these pesticides by GC on the SLB-5ms using nitrogen phosphorus detection (NPD).

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | 10 g homogenized food sample (green onions, apples, Napa cabbage, button mushrooms) |
| SPE tube/cartridge | Supelclean ENVI-Carb II/PSA SPE Tube (54067-U) |
| condition | 5 mL acetone:toluene (65:35) |
| sample addition | 1 mL sample extract |
| elution | 10 mL of acetone:toluene (65:35) |
| eluate post-treatment | Evaporate fraction to 0.5 mL then adjust volume to 1 mL with ethyl acetate for GC-NPD analysis |
| column | SLB-5ms, 30 m x 0.25 mm I.D., 0.25 µm (28471-U) |
| oven | 110 °C (2 min), 2.5 °C/min to 205 °C, 10 °C/min to 310 °C (10 min) |
| inj. temp. | 250 °C |
| detector | NPD, 310 °C |
| carrier gas | helium, 0.9 mL/min constant flow |
| injection | 2 µL, splitless |
| liner | 4 mm I.D. dual tapered |
| Application No. | G005815 |

1. Methamidophos
2. Dichlorvos
3. Phosdrin
4. Acephate
5. Trichlorphan
6. Omethoate
7. Ethoprophos
8. Dibromfos
9. CIPC
10. Dicrotophos
11. Sulfotepp
12. Cadusafos
13. Monocrotophos
14. Phorate
15. Thiometon
16. Dimethoate and Demeton-ethyl
17. Unknown
18. Terbufos
19. Dyfonate
20. Propetamphos
21. Phosphamidon isomer #1 and Diazinon
22. Disulfoton
23. Isazofos
24. Kitazine
25. Formothion
26. Dichlofenthion and Phosphamidon isomer #2
27. Chloropyrifos-methyl
28. Methyl parathion
29. Ronnel
30. Paraoxon-ethyl
31. Pirmiphos-methyl and Fenitrothion
32. Malathion
33. Chloropyrifos
34. Fenthion
35. Parathion
36. Isocarbophos
37. Trichloronate
38. Fosthiazate isomer #1
39. Fosthiazate isomer #2
40. Pirmiphos-ethyl
41. Isophenfos-methyl
42. Phosfolan and Isophenfos
43. Quinalphos and Phenthoate
44. Methidathion
45. Bromophos-ethyl
46. Iodophenfos
47. Profenfos
48. Fensulfothion
49. Ethion
50. Famphur
51. Edinphos
52. Pyridaphenthion
53. Phosmet
54. EPN
55. Phosalone
56. Azinphos-methyl
57. Azinphos-ethyl
58. Coumaphos
59. Temephos






SUPELCO® Solutions within.™

## GC Analysis of PCBs in Whole Cow Milk (Spiked) on SLB®-5ms (20 m x 0.18 mm I.D., 0.18 µm) after QuEChERS Cleanup using Supel™ QuE Z-Sep, Fast GC Analysis

**Reference:** Stenerson, K.; Ye, M.; Halpenny, M., New Analytical Tools for the Determination of Persistent Organic Pollutants (POPs) in Fatty Food and Beverage Matrices Using QuEChERS Extraction/Cleanup and Gas Chromatography (GC) Analysis. Pittcon 2013. Sigma-Aldrich/Supelco publication T413034.

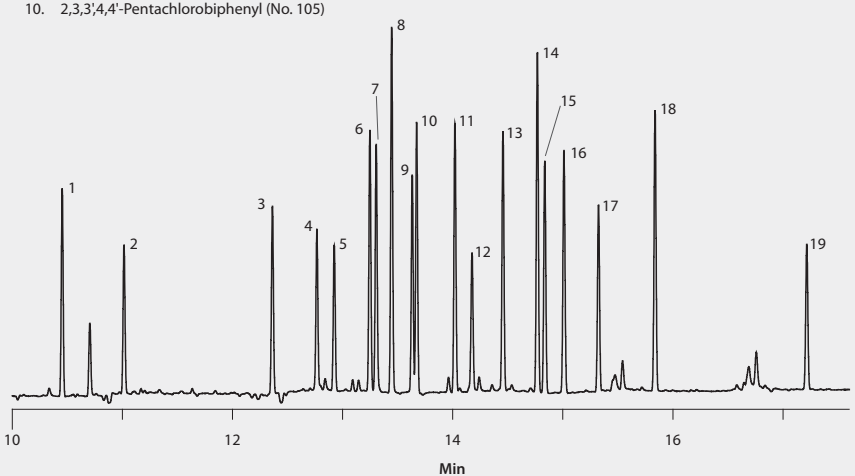
This application demonstrates the use of dispersive SPE or "QuEChERS" for the extraction and cleanup of whole cow's milk for GC-ECD analysis of polychlorinated biphenyl (PCBs). Z-Sep sorbent was used in the cleanup step and found to reduce background and yield recoveries >70% for all PCB congeners evaluated except no. 209, which was recovered at 68%.

**Extraction solvent:** acetone:hexane (1:1), 20 mL

**Clean-up volume:** 1 mL

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | 10 g whole cow's milk |
| extraction tube | Supel™ QuE Non-buffered tube (4 g magnesium sulfate, 1 g sodium chloride) (55294-U) |
| clean-up tube | Supel™ QuE Z-Sep 150 mg, magnesium sulfate (150 mg, part no. 63135) (55299-U) |
| column | SLB-5ms, 20 m x 0.18 mm I.D., 0.18 µm (28564-U) |
| oven | 75 °C (1 min), 12 °C/min to 340 °C (10 min) |
| inj. temp. | 250 °C |
| detector | µ-ECD, 340 °C |
| carrier gas | hydrogen, 1.2 mL/min, constant |
| injection | 1 µL splitless (0.75 min) |
| liner | 4 mm I.D. FocusLiner™ with taper |
| Application No. | G005921 |

1. 2,3',6-Trichlorobiphenyl (No. 28)
2. 2,2',5,5'-Tetrachlorobiphenyl (No. 52)
3. 2,2',4,5,5'-Pentachlorobiphenyl (No. 101)
4. 3,4,4',5-Tetrachlorobiphenyl (No. 81)
5. 3,3',4,4'-Tetrachlorobiphenyl (No. 77)
6. 2',3,4,4',5-Pentachlorobiphenyl (No. 123)
7. 2,3',4,4',5-Pentachlorobiphenyl (No. 118)
8. 2,3,4,4',5-Pentachlorobiphenyl (No. 114)
9. 2,2',4,4',5,5'-Hexachlorobiphenyl (No. 153)
10. 2,3,3',4,4'-Pentachlorobiphenyl (No. 105)
11. 2,2',3,4,4',5'-Hexachlorobiphenyl (No. 138)
12. 3,3',4,4',5-Pentachlorobiphenyl (No. 126)
13. 2,3',4,4',5,5'-Hexachlorbiphenyl (No. 167)
14. 2,3,3',4,4',5-Hexachlorobiphenyl (No. 156)
15. 2,3,3',4,4',5'-Hexachlorobiphenyl (No. 157)
16. 2,2',3,4,4',5,5'-Heptachlorobipheny (No. 180)
17. 3,3',4,4',5,5'-Hexachlorobiphenyl (No. 169)
18. 2,3,3',4,4',5,5'-Heptachlorobiphenyl (No. 189)
19. 2,2',3,3',4,4',5'5',6,6'-Decachlorobiphenyl (No. 209)



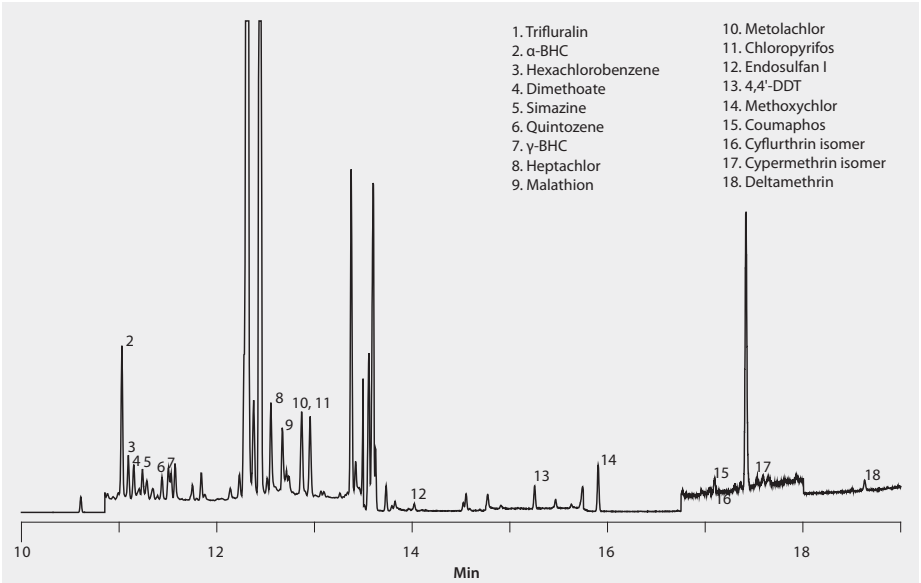



SUPELCO® Solutions within.™

# GC Analysis of Pesticides in Avocado on SLB®-5ms (20 m x 0.18 mm I.D., 0.36 µm) after QuEChERS Cleanup using Supel™ QuE Z-Sep+, Fast GC Analysis

**About this analysis:** This application demonstrates the use of dispersive SPE for the extraction of pesticides from Avocado. Cleanup is achieved using Z-Sep + for fat removal. FInal analysis of the extracts was done by LVI-GC/MS SIM on an SLB-5ms column.

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . . . . Avocado spiked with pesticides at 20 ng/g
extraction tube . . . . . Supel QuE Acetate extraction tube (55234-U)
extraction process . . . . . add 25 mL of acetonitrile; shake for 1 minute; add contents of Supel QuE acetate extraction tube; shake immediately for 1 minute; centrifuge at 3200 rpm for 5 minutes
clean-up tube . . . . . Supel Que Z-Sep+ cleanup tube (55296-U)
clean-up process . . transfer 3 mL of the acetonitrile layer into a Supel Que Z-Sep+ cleanup tube; shake for 1 minute; centrifuge at 3400 rpm for 3 minutes; draw off supernatant for LVI-GCMS analysis
column . . . . . SLB-5ms, 20 m x 0.18 mm I.D., 0.36 µm (28576-U)
oven . . . . . 70 °C (2 min), 15 °C/min to 325 °C (6 min)
inj. temp. . . . . 60 °C (0.28 min), 600 °C/min to 325 °C (5 min)
detector . . . . . MSD
MSD interface . . . . . 330 °C
carrier gas . . . . . helium, 1 mL/min, constant
injection . . . . . PTV solvent vent; 10 µL, injection dispense speed 15000 µL/min
liner . . . . . 4 mm I.D. FocusLiner with taper
sample . . . . . pesticides spiked at 20 ng/g
Application No. . . . . G005805



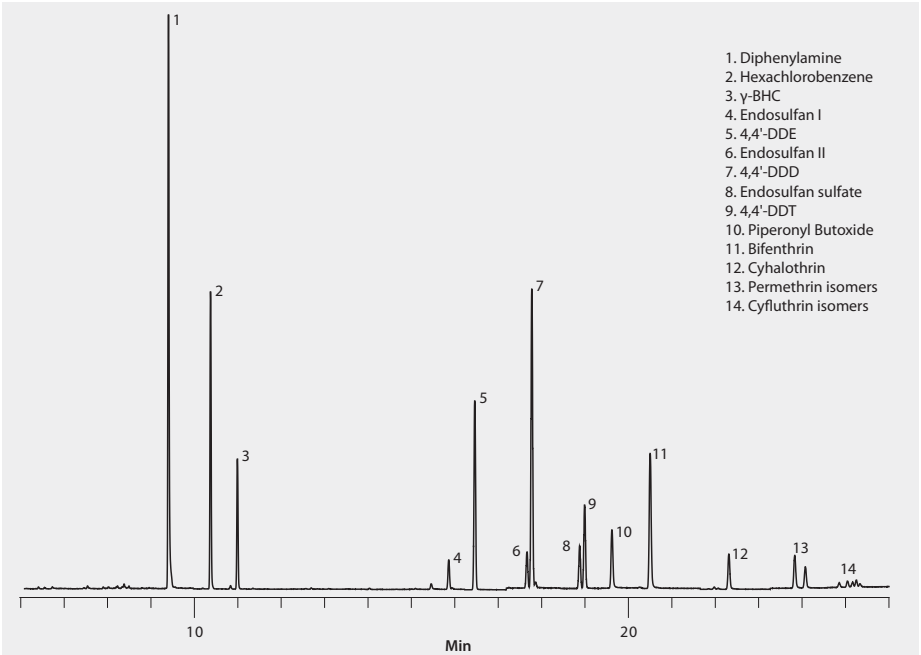



SUPELCO® Solutions within.™

## GC Analysis of Pesticides in Beef Kidney on SLB®-5ms (20 m x 0.18 mm I.D., 0.36 µm) after QuEChERS Cleanup using Supel™ Que Z-Sep+, MS in SIM Mode, Fast GC Analysis

Beef kidney was analyzed for pesticides by GC/MS-SIM after extraction and cleanup. Dispersive SPE was used for both extraction and cleanup. Since beef kideny contains approximately 5% fat, Z-Sep + was used for cleanup.

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . 10 gm homogenized beef kidney spiked at 50 ng/g with pesticides
extraction tube . . . . . Supel Que Acetate (AC) Tube (55234-U)
extraction process . . . . . add 10 mL of acetonitrile; shake for 1 minute; add contents of Supel Que acetate extraction tube (55234-U); shake immediately for 1 minute; centrifuge at 3200 rpm for 5 minutes; (Sample Pretreatment: Homogenize 10 gm beef kidney and spike with pesticides at 50 ng/g.)
clean-up tube . . . . . Supel QuE Z-Sep+ tube (55296-U)
clean-up process . . . . . transfer 3 mL of the acetonitrile layer into a Supel QuE Z-Sep+ cleanup tube (55296-U); shake for 1 minute; centrifuge at 3400 rpm for 3 minutes; draw off supernatant for LVI-GCMS analysis
column . . . . . F.S. CAP. SLB-5MS 20 m x 0.18 mm I.D., 0.36 µm (28576-U)
oven . . . . . 70 °C ( 0.5 min), 25 °C/min to 125 °C, 10 °C/min to 200 °C, 5 °C/min to 300 °C/min (1 min)
inj. temp. . . . . programmed, 60 °C (0.28 min), 600 °C/min to 325 °C (5 min)
detector . . . . . MSD
MSD interface . . . . . 325 °C
carrier gas . . . . . helium, 1 mL/min constant flow
injection . . . . . 10 µL LVI, PTV solvent vent, rapid injection speed
liner . . . . . 4 mm ID FocusLiner with taper
sample . . . . . Beef Kidney extract
Application No. . . . . G005799



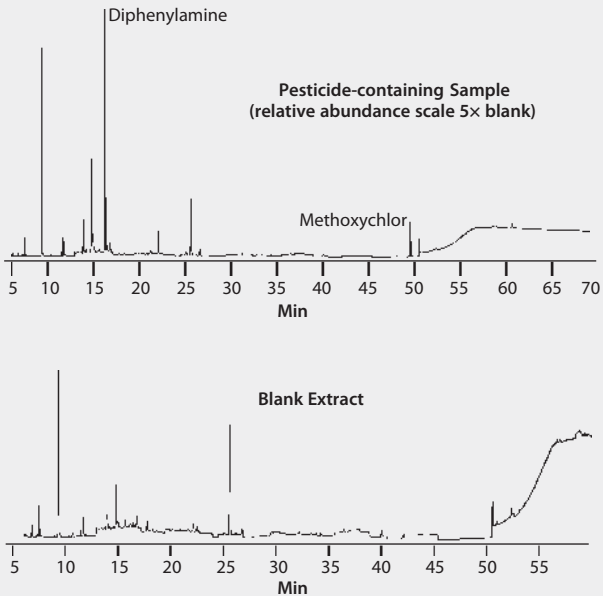



SUPELCO® Solutions within.™

## GC Analysis of Pesticides in Fruits and Vegetables on a 14% Cyanopropylphenyl Column after SPE using Supelclean ENVI-Carb™

**Sample Pre-treatment:**
Homogenize 50 g chopped fruit/vegetable sample in 100 mL acetonitrile. Add 10 g sodium chloride and homogenize 5 min. Transfer 13 mL of acetonitrile layer to clean centrifuge tube. Centrifuge at high speed for 5 min. Evaporate 10 mL aliquot of supernatant to 0.5 mL under Nitrogen at 35°C.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . extract from 50 g homogenized fruit or vegetable sample
SPE tube/cartridge . . . . . ENVI-Carb, 500 mg/6 mL (57094)
sample addition . . . . . 0.5 mL concentrated sample extract
elution . . . . . 20 mL acetonitrile:toluene (3:1)
eluate post-treatment . . . . . Concentrate to 2 mL by rotary evaporation. Add 2 x 10 mL acetone concentrating to 2 mL after each acetone addition. (Add 50 µL I.S. (500 ng/µL cis-chlordane in acetone) then dilute to 2.5 mL with acetone.)
column . . . . . 14% cyanopropylphenyl polysiloxane, 30m × 0.25 mm I.D., 0.15 µm (Supelco equivalent, Equity-1701, 28372-U)
oven . . . 70 °C (hold 2 min) to 130 °C at 25 °C; 130 °C to 220 °C at 2 °C/min; 220 °C to 280 °C at 10 °C/min and hold 4.6 min
detector . . . . . MSD, 285 °C
carrier gas . . . . . helium
injection . . . . . 2 µL, splitless
Application No. . . . . 796-0128



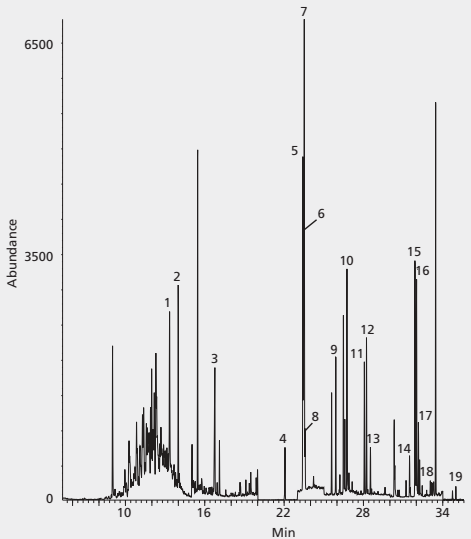

For extraction recovery data, request Bulletin T196900.
Chromatograms provided by J. Fillion, Agriculture and Agri-Food Canada (Laboratory Services Division, Pesticide Laboratory), Ottawa, Ontario.

## GC Analysis of Pesticides in Spinach on Equity®-1 after SPE using Supelclean™ ENVI-Carb-II/PSA

**Sample Pre-treatment:**

Homogenize 10 g of spinach in 10 mL of acetonitrile.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . 10 g of fresh spinach spiked at 0.2 ppm each pesticide
SPE tube/cartridge . . . Supelclean ENVI-Carb-II/PSA, 500mg/300mg/6mL (55119-U)
condition . . . . . 5 mL acetonitrile:toluene (3:1, v/v)
sample addition . . . . . 1 mL concentrated sample extract
elution . . . . . 20 mL acetonitrile:toluene (3:1, v/v)
eluate post-treatment . . . . . Evaporate eluate to dryness with nitrogen at 40 °C. Reconstitute in 1 mL acetone:hexane (1:1, v/v).
column . . . . . Equity-1, 30 m x 0.25 mm I.D., 0.25 µm (28046-U)
oven . . . . . 50 °C (5 min), 25 °C/min to 125 °C, 10 °C/min to 30 °C (8 min)
inj. temp. . . . . 200 °C, aux: 325 °C (8 min)
detector . . . . . MSD, scan range 45-450 amu
carrier gas . . . . . helium, 0.9 mL/min., constant flow mode
injection . . . . . 1 µL, splitless (splitter open at 1 min)
liner . . . . . 4 mm I.D., single taper
Application No. . . . . G003758



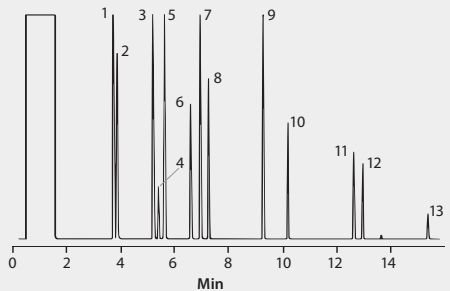

1. Methamidophos
2. Dichlorovos
3. Acephate
4. Quintozene
5. Methyl parathion
6. Carbaryl
7. Methyl chloropyriphos
8. Vinclozolin
9. Procymidone
10. Imazalil
11. Chlorothiophos
12. Tetrasul
13. Endosulfan sulfate
14. Acrinathrin
15. Bitertanol
16. cis-Permethrin
17. trans-Permethrin
18. Cypermethrin isomers
19. Deltamethrin

**Efficiency of Recovery**

| Peak ID | Compound | Pesticide Class | Pesticide Recovery (%) |
|---|---|---|---|
| 1 | Methamidophos | Organophosphorous | 80 |
| 2 | Dichlorovos | Organophosphorous | 70 |
| 3 | Acephate | Organophosphorous | 60 |
| 4 | Quintozene | Organochloride | 92 |
| 5 | Methyl parathion | Organophosphorous | 97 |
| 6 | Carbaryl | Carbamate | 128 |
| 7 | Methyl chloropyriphos | Organophosphorous | 99 |
| 8 | Vinclozolin | Organochloride | 83 |
| 9 | Procymidone | Dicarboximide | 84 |
| 10 | Imazalil | Imidazole | 104 |
| 11 | Chlorothiophos | Phophosulfi de | 106 |
| 12 | Tetrasul | Organochloride | 87 |
| 13 | Endosulfan sulfate | Organochloride | 124 |
| 14 | Acrinathrin | Organophosphorous | 118 |
| 15 | Bitertanol | Biphenol | 108 |
| 16 | Permethrin cis and trans | Pyrithroid | 82 |
| 17 | Cypermethrin isomers | Organochloride | 74 |
| 18 | Deltamethrin | Organobromine | 134 |

## GC Analysis of Phenols in Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI-Chrom P

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . water spiked with phenol compounds
SPE tube/cartridge . . . . . Supelclean ENVI-Chrom P, 250 mg/6 mL (57225-U)
condition . . . . . 6 mL methyl t-butyl or ethyl acetate; 6 mL acetonitrile. Equilibrate with 6 mL DI water
sample addition . . . . . 100 mL water sample.
drying . . . . . 10 min using vacuum
elution . . . . . 5 mL methyl t-butyl ether acetate dropwise allowing initial 2 mL to soak in SPE bed
column . . . . . 5% Phenyl Polysiloxane, 15 m × 0.53 mm I.D., 0.50 µm (Supelco equivalent, Equity-5, 28252-U)
oven . . . . . 65 °C, 10 °C/min to 185 °C (1 min), 20 °C/min to 275 °C (5 min)
detector . . . . . FID, 300 °C
carrier gas . . . . . helium
injection . . . . . 1 µL, splitless (45 sec hold)
Application No. . . . . 712-0073



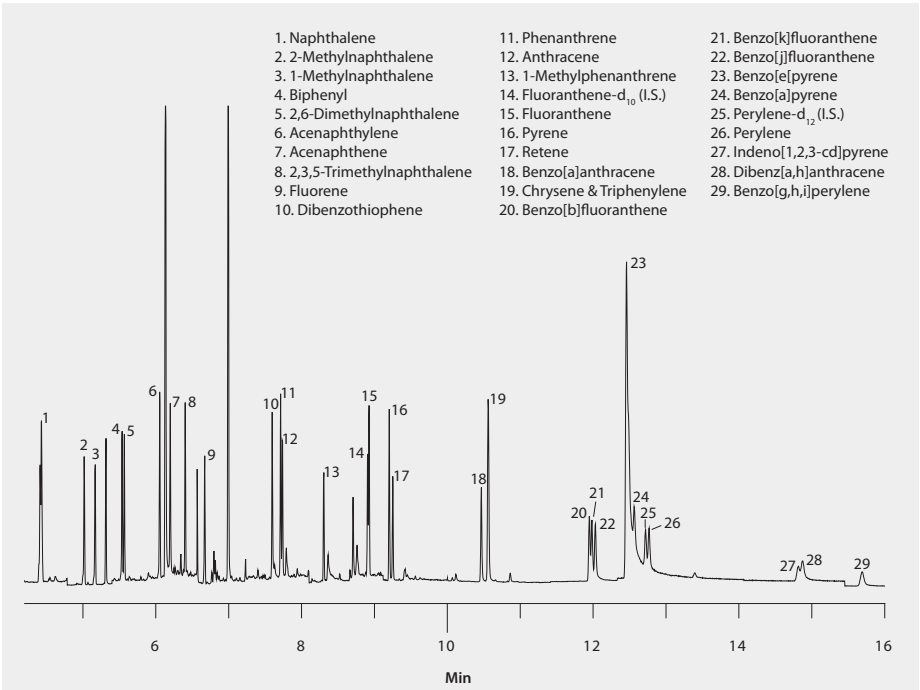

| | Analyte | %Recovery (± CV) |
|---|---|---|
| 1. | Phenol | 101.8 ± 3.7 |
| 2. | 2-Chlorophenol | 104.7 ± 1.7 |
| 3. | 2-Methylphenol | 107.0 ± 1.9 |
| 4. | 2-Bromophenol (Int. Std.) | — |
| 5. | 3-Methylphenol | 104.9 ± 1.6 |
| 6. | 2-Nitrophenol | 96.3 ± 2.8 |
| 7. | 2,4-Dimethylphenol | 105.3 ± 2.2 |
| 8. | 2,4-Dichlorophenol | 106.3 ± 2.2 |
| 9. | 4-Chloro-3-methylphenol | 104.6 ± 2.0 |
| 10. | 2,4,6-Trichlorophenol | 103.9 ± 2.1 |
| 11. | 4-Nitrophenol | 99.9 ± 4.1 |
| 12. | 2,3,4,6-Tetrachlorophenol | 104.6 ± 1.5 |
| 13. | Pentachlorophenol | 97.7 ± 4.8 |

## GC Analysis of Polynuclear Aromatic Hydrocarbons (PAHs) in Grilled Hamburger on SPB®-608 (20 m x 0.18 mm I.D., 0.18 µm) after QuEChERS Cleanup using Supel™ QuE Z-Sep, Fast GC Analysis

**Sample pretreatment:** Place 5 g ground hamburger sample into 50 mL centrifuge tube (55248-U). Spike with 100 ng/g target PAHs and internal standards. Let sit 10 min. Add 5 mL water and homogenize.

This application demonstrates the use of dispersive SPE or QuEChERS for the extraction and cleanup of grilled hamburger for GC-MS analysis of polynuclear aromatic hydrocarbons (PAHs). Z-Sep sorbent was used in the cleanup step and found to reduce background and yield recoveries >80% for a majority of the PAHs tested.

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . . . . grilled hamburger spiked with PAHs at 100 ng/g
extraction tube . . . . . Sodium chloride/magnesium sulfate (55294-U)
extraction process . . . . . add 25 mL acetonitrile, contents of unbuffered salt tube (55294-U); shake for 5 minutes; centrifuge for 5 minutes at 3200 rpm
clean-up tube . . . . . Supel QuE Z-Sep tube (55403-U)
clean-up process . . . . . transfer 3 mL of acetonitrile layer into a Supel QuE Z-Sep cleanup tube (55403-U); shake 1 minute; centrifuge for 3 minutes at 3400 rpm; remove 1 mL supernatant for GC-MS analysis
column . . . . . SPB-608, 20 m x 0.18 mm I.D. x 0.18 µm
oven . . . . . 60 °C (1 min), 25 °C/min to 275 °C, 10 °C/min to 300 °C (13 min)
inj. temp. . . . . 265 °C
detector . . . . . MS-SIM
MSD interface . . . . . 300 °C
carrier gas . . . . . helium, 1.5 mL/min constant flow
injection . . . . . 1 µL splitless (1 min.)
liner . . . . . 4 mm I.D. FocusLiner™ with taper
sample . . . . . grilled hamburger extract
Application No. . . . . G005880



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# GC Analysis of Polynuclear Aromatic Hydrocarbons (PAHs) in Salmon on SPB®-608 (20 m x 0.18 mm I.D., 0.18 µm) after QuEChERS Cleanup using Supel™ QuE Z-Sep, Fast GC Analysis

**Extraction solvent:** acetonitrile, 25 mL
**Clean-up volume:** 3 mL
**Clearn-up tube:** Supel™ QuE Z-Sep (500 mg, part no. 55299-U, magnesium sulfate (450 mg, part no. 63135)

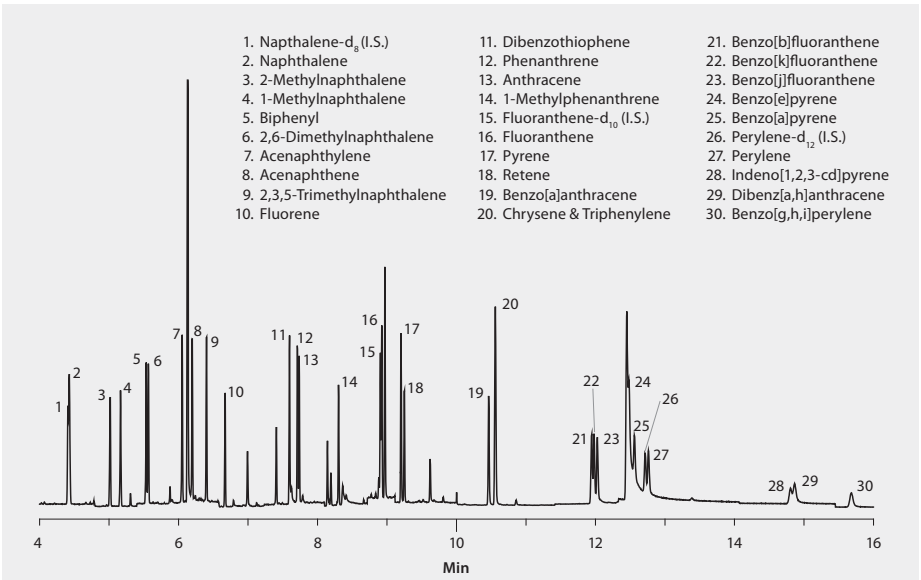
Stenerson, K.; Ye, M.; Halpenny, M., New Analytical Tools for the Determination of Persistent Organic Pollutants (POPs) in Fatty Food and Beverage Matrices Using QuEChERS Extraction/Cleanup and Gas Chromatography (GC) Analysis. Pittcon 2013. Sigma-Aldrich/Supelco publication T413034

**Sample pretreatment:** Place 5 g ground sample into 50 mL centrifuge tube. Spike with 100 ng/g target PAHs and internal standards. Let sit 30 min. Add 5 mL water and homogenize.

This application demonstrates the use of dispersive SPE or QuEChERS for the extraction and cleanup of raw salmon for GC-MS analysis of polynuclear aromatic hydrocarbons (PAHs). Supel™ QuE Z-Sep sorbent was used in the cleanup step and found to reduce background and yield recoveries >80% for a majority of the PAHs tested.

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . . . . raw salmon spiked with PAHs at 100 ng/g
extraction process . . . . . add 25 mL acetonitrile and the contents of the Supel™ QuE Non-buffered tube (55294-U); shake for 5 minutes; centrifuge for 5 minutes at 3200 rpm
extraction tube . . . . . Supel™ QuE Non-buffered tube, magnesium sulfate/sodium chloride (55294-U)
clean-up tube . . Supel™ QuE Z-Sep (500 mg, part no. 55299-U), magnesium sulfate (450 mg, part no. 63135) (55299-U, 63135)
clean-up process . . transfer 3 mL of acetonitrile layer, Supel QuE Z-Sep (500 mg) and MgSO4 (450 mg) into an extraction tube; (shake 1 minute; centrifuge for 3 minutes at 3400 rpm; remove 1 mL supernatant for GC-MS analysis)

column . . . . . SPB-608, 20 m x 0.18 mm I.D. x 0.18 µm
oven . . . . . 60 °C (1 min), 25 °C/min to 275 °C, 10 °C/min to 300 °C (13 min)
inj. temp. . . . . 265 °C
detector . . . . . MS, SIM
MSD interface . . . . . 300 °C
carrier gas . . . . . helium, 1.5 mL/min, constant
injection . . . . . 1 µL, splitless (1 min)
liner . . . . . 4 mm I.D. FocusLiner™ with taper
Application No. . . . . G005923



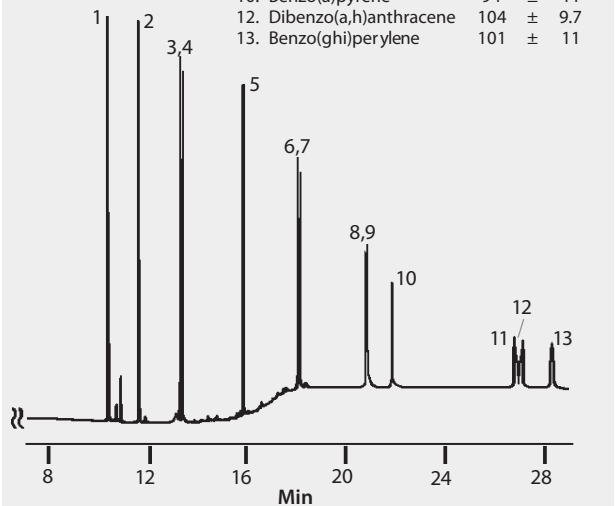



SUPELCO® Solutions within.™

## GC Analysis of Polynuclear Aromatic Hydrocarbons (PAHs) in Water on a 5% Phenyl Column after SPE using Supelclean™ ENVI-18

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample addition | |
| SPE tube/cartridge | Supelclean ENVI-18, 500 mg/6 mL (57064) |
| condition | 2 x 6 mL toluene:methanol (10:1); 6 mL methanol; 6 mL DI water |
| sample addition | 250 mL water sample at 10 mL/min |
| drying | 10 min under vacuum |
| elution | 2 x 1 mL toluene:methanol (10:1) |
| column | 5% phenyl polysiloxane, 30m × 0.25 mm I.D., 0.25 µm film (Supelco equivalent, SLB-5ms, 28471-U) |
| oven | 70 °C (2 min) to 280 °C at 8 °C/min |
| detector | FID, 310 °C |
| carrier gas | helium |
| injection | 1 µL |
| Application No. | 80-207 |

| | Analyte | % Recovery (± CV) |
|---|---|---|
| 1. | Acenaphthylene | 66 ± 28 |
| 2. | Fluorene | 95 ± 7.5 |
| 3. | Phenanthrene | 95 ± 8 |
| 4. | Anthracene | 95 ± 11 |
| 5. | Pyrene | 97 ± 7.1 |
| 6. | Benzo(a)anthracene | 94 ± 8.3 |
| 7. | Chrysene | 101 ± 8.8 |
| 8. | Benzo(b)fluoranthene | 103 ± 10 |
| 9. | Benzo(k)fluoranthene | 103 ± 9.4 |
| 11. | Indeno(1,2,3-cd)pyrene | 102 ± 10 |
| 10. | Benzo(a)pyrene | 94 ± 11 |
| 12. | Dibenzo(a,h)anthracene | 104 ± 9.7 |
| 13. | Benzo(ghi)perylene | 101 ± 11 |






SUPELCO® Solutions within.™

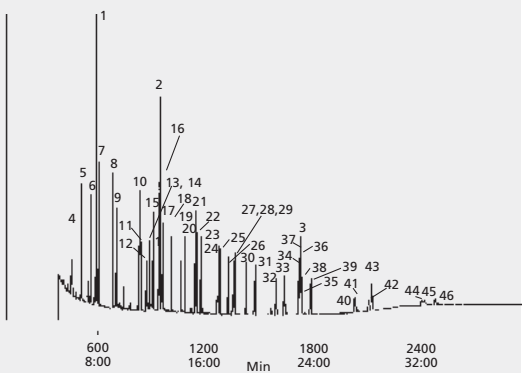
# GC Analysis of Semivolatiles in Drinking Water on a 5% Phenyl Column after SPE using ENVI™-18 DSK

**Sample Pre-treatment:**

Adjust 1 L drinking water to pH <2 with 6 N HCl. Add 5 mL methanol and mix thoroughly

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . acidified drinking water
SPE tube/cartridge . . . . . ENVI-18 DSK SPE Disk, 47 mm (57171)
condition . . . . . 5 mL dichloromethane; 5 mL methanol; 5 mL DI water
sample addition . . . . . 5 mL water sample
elution . . . . . 2 x 5 mL acetonitrile rinsing internal chamber of sample reservoir thoroughly
column . . . . . 5% phenyl polysiloxane, 30 m × 0.25 mm I.D., 0.25 µm (Supelco equivalent, SLB-5ms, 28471-U)
oven . . . . . 40 °C to 160 °C (3 min), then to 300 °C (3 min) at 6 °C/min
detector . . . . . MS, scan range m/z = 45-450
carrier gas . . . . . helium, 33 cm/sec
injection . . . . . 1 µL split/splitless, 1 min delay
Application No. . . . . 749-0798

| | | µg/L* | | | g/L* |
|---|---|---|---|---|---|
| 1. | Acenapthene-$d_{10}$ | 5.0 | 24. | Heptachlor epoxide | 2.0 |
| 2. | Phenanthrene-$d_{10}$ | 5.0 | 25. | 2,2',3',4,6-Pentachlorobiphenyl | 2.0 |
| 3. | Chrysene-$d_{12}$ | 5.0 | 26. | γ-Chlordane | 2.0 |
| 4. | Hexachlorocyclopentadiene | 2.0 | 27. | Pyrene | 2.0 |
| 5. | Dimethylphthalate | 2.0 | 28. | α-Chlordane | 2.0 |
| 6. | Acenaphthylene | 2.0 | 29. | trans-Nonachlor | 2.0 |
| 7. | 2-Chlorobiphenyl | 2.0 | 30. | 2,2',4,4',5,6'-Hexachlorobiphenyl | 2.0 |
| 8. | Diethyphthalate | 2.0 | 31. | Endrin | 2.0 |
| 9. | Fluorene | 2.0 | 32. | Butylbenzylphthalate | 2.0 |
| 10. | 2,3-Dichlorobiphenyl | 2.0 | 33. | di(2-ethylhexyl)Adipate | 2.0 |
| 11. | Hexachlorobenzene | 2.0 | 34. | 2,2'3,3',4,4',6-Heptachlorobiphenyl | 2.0 |
| 12. | Simazine | 2.0 | 35. | Methoxychlor | 2.0 |
| 13. | Atrazine | 2.0 | 36. | 2,2'3,3',4,5',6,6'-Octachlorobiphenyl | 2.0 |
| 14. | Pentachlorophenol | 8.0 | 37. | Benz(a)anthracene | 2.0 |
| 15. | γ-BHC | 2.0 | 38. | Chrysene | 2.0 |
| 16. | Phenanthrene | 2.0 | 39. | Di(2-ethylhexyl)phthalate | 2.0 |
| 17. | Anthracene | 2.0 | 40. | Benzo(b)fluoranthene | 2.0 |
| 18. | 2,4,5-Trichlorobiphenyl | 2.0 | 41. | Benzo(k)fluoranthene | 2.0 |
| 19. | Alachlor | 2.0 | 42. | Benzo(a)pyrene | 2.0 |
| 20. | Heptachlor | 2.0 | 43. | Perylene-$d_{12}$ | 5.0 |
| 21. | di-n-Butylphthalate | 2.0 | 44. | Indeno(1,2,3-cd)Pyrene | 2.0 |
| 22. | 2,2',4,4'-Tetrachlorobiphenyl | 2.0 | 45. | Dibenz(a,h)anthracene | 2.0 |
| 23. | Aldrin | 2.0 | 46. | Benzo(ghi)perylene | 2.0 |






SUPELCO® Solutions within.™

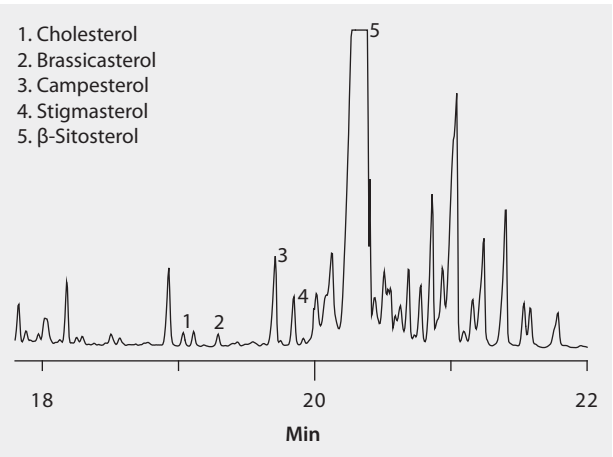
## GC Analysis of Sterols (Silylated Derivatives) in Olive Oil (Free Sterol Fraction) on SLB®-5ms after SPE using Discovery DSC-Si

### Sample pre-treatment

Weigh 0.25 gm oil into test tube. For spiked sample add 12.5 µL of 65-100 µg/mL mixed sterol std. Add 1 ml of hexane:ethyl acetate (90:10). After SPE fractionation, evaporate solvent from each fraction, add 1 ml of 30% methanolic sodium methoxide:MeOH:MTBE solution (13:27:60), mix and let sit at room temp for 30 min. Add 1 ml of water and 2 mL of n-heptane. Withdraw aqueous phase (bottom) and replace w/ 1 mL of 1% citric acid solution. Withdraw organic phase and dry over anhydrous sodium sulfate. Silylation: Evaporate organic phase to dryness. Add 125 µL of Sylon BFT and 125 µL of pyridine. Heat at 70°C for 20 min. The sample is then ready for GC-MS analysis.

Sterol content is one in a battery of tests used to analyze the composition and determine the grade and authenticity of olive oil. Test results are compared against standards such as those established by the International Olive Oil Council (IOC) and US Dept. of Agriculture. In vegetable oils, sterols exist in the free form, or esterified to fatty acids. In this application, sterols were analyzed in olive oil separately as free and esterified. This was accomplished by taking advantage of the polarity difference between these two groups to fractionate them using silica gel SPE. In place of saponification, the fractions were transesterified to liberate the esterified sterols. The fractions were then silylated, and analyzed by GC-MS. The silylated sterols all contained a significant molecular ion in their mass spectum, which made it possible to perform peak identification free of matrix interference. The sterols isolated in the free fraction are shown here.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | olive oil spiked with a mixed sterol standard at 5 µg/g |
| SPE tube/cartridge | Discovery DSC-Si, 1 g/6 mL (52656-U) |
| condition | 2 x 5 mL hexane |
| sample addition | 1 mL |
| elution | esters fraction: 2.5 mL of hexane:ethyl acetate (90:10); free sterols fraction; 5 mL of hexane:ethyl acetate (90:10) and 3 mL x 5 of ethanol:diethyl ether:hexane (50:25:25) |
| eluate post-treatment | Evaporate to dryness and perform silylation as described in the Analysis Note. |
| column | SLB-5ms, 20 m x 0.20 mm I.D., 0.20 µm (28564-U) |
| oven | 125 °C (1 min), 10 °C/min to 325 °C (10 min) |
| inj. temp. | 250 °C |
| detector | MSD, rn/z 500 - 600 |
| MSD interface | 325 °C |
| carrier gas | helium, 0.6 mL/min, constant |
| injection | 1 µL, splitless |
| liner | 4 mm I.D. single taper |
| sample | Olive oil sample; fractionated, transesterified, and silylated |
| Application No. | G005816 |



## HPLC Analysis of Acrylamide in Fried Potato Chips on Discovery® HS F5 after SPE using Discovery® DSC-MCAX and DSC-18

sample preparation . . . . . SPE (Solid Phase Extraction)

sample/matrix . . . . . Finely grind 2.0 g of potato chips, place in 20 mL vial and add 10 mL water. Mix using vortex until mixture forms a thick paste. (Place sample in centrifuge tubes and centrifuge at 16.1 rcf for 5 min. Extract aqueous portion of sample leaving oil layer and solids.)

SPE tube/cartridge . . . . . Discovery DSC-MCAX, 300 mg/3 mL (52784-U)

SPE tube/cartridge . . . . . Discovery DSC-18, 1g/6mL (52606-U)

condition . . . . . connect a Discovery DSC-MCAX SPE tube to the top of a Discovery DSC-18 SPE tube using an SPE tube adapter, pass 1 mL methanol, then 1 mL DI water through both SPE tubes until the tubes are dry

sample addition . . . . . 1 mL of aqueous extract

washing . . . . . 1 mL DI water

elution . . . . . removing upper MCAX SPE tube, pass 2 mL methanol through lower DSC-18 SPE tube

eluate post-treatment . . . . . evaporate to dryness with nitrogen at 30 °C, reconstitute in 0.5 mL DI water

column . . . . . Discovery HS F5, 15 cm x 4.6 mm I.D., 3 µm particles (567507-U)

mobile phase . . . . . 100% ultra-pure water

column temp. . . . . 35 °C

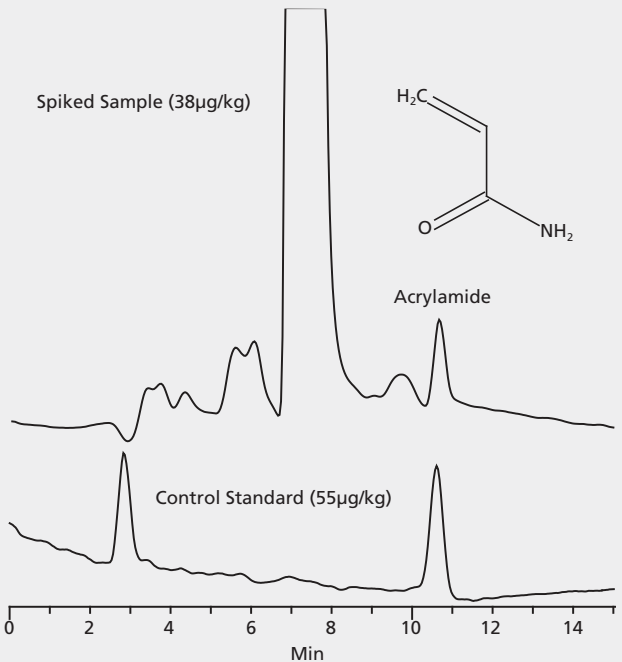
detector . . . . . ESI+

injection . . . . . 5 µL

Application No. . . . . G003759

**Efficiency of Recovery**

| Concentration (µg/mL) | %Recovery ± RSD (n=3) |
|---|---|
| 10 | 78 ± 7% |
| 55 | 93 ± 5% |



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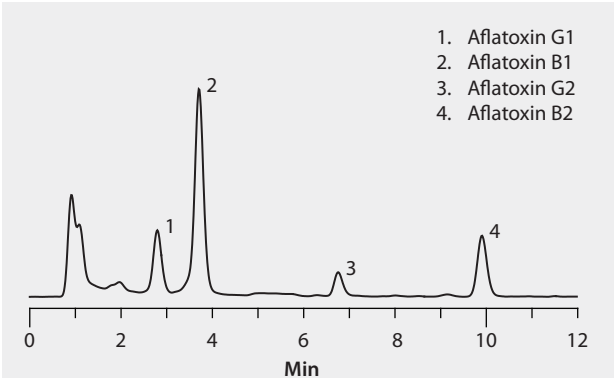
## HPLC Analysis of Aflatoxins B1, B2, G1 and G2 in Corn on Ascentis® Express C18 after SPE using Supel™ Tox AflaZea and Fluorescence Detection

Aflatoxins are closely related compounds produced as secondary metablolites of fungi. They are found in grains and nuts like corn, wheat or peanuts. Shown here is a rapid, sensitive method to extract these compounds from corn using SPE cartridges specifically designed for aflatoxins, Supel Tox AflaZea (aflatoxin zearalenone), which removes interferences from grains, feeds, TMR samples, peanuts, peanut products and aqueous solutions. Analysis was via HPLC on Ascentis Express Fused-Core C18 colums.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . 25 g corn meal in 100 mL acetonitrile:water (84:16)
SPE tube/cartridge . . . . . Supel™ Tox AflaZea, 6 mL (55314-U)
sample addition . . . . . 2 mL
elution . . . . . collect sample in 5 mL silanized glass vial
eluate post-treatment . . . . transfer 200 µL sample, add 300 µL TFA derivatization solution (70:20:10 water:TFA:acetic acid), heat in microreactor 20 min at 65 °C, cool, add 580 µL deionized water, vortex
column . . Ascentis Express C18, 15 cm x 2.1 mm I.D., 2.7 µm particles (53825-U)
mobile phase . . . . . (A) water; (B) acetonitrile; (80:20, A:B)
flow rate . . . . . 0.350 mL/min
pressure . . . . . 325 psi (22.4 bar)
column temp. . . . . 35 °C
detector . . . . . FL, Ex 360 nm, Em 440 nm
injection . . . . . 20 µL
Application No. . . . . G005922



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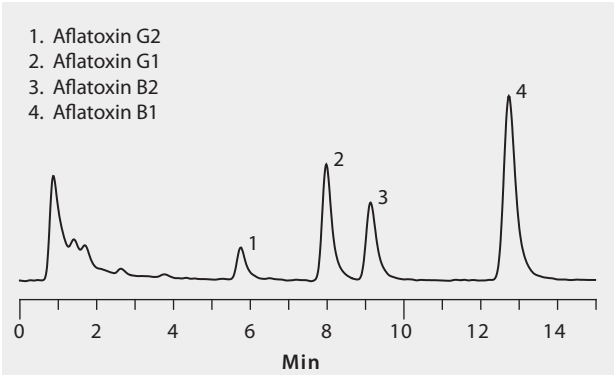
## HPLC Analysis of Aflatoxins in Wheat on Ascentis® Express C18 after SPE using Supel™ Tox AflaZea

**Sample/matrix:**
Weigh 25 grams of wheat meal into a 500 mL Erlenmeyer flask. Add 100 mL of 84:16 acetonitrile:water, seal, and place on an orbital shaker at med-high speed for 90 minutes. Filter the sample through a #2 qualitative filter. Spike sample at 2 mg/mL using analytical standards.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . 25 g wheat meal spiked with pesticide standards
SPE tube/cartridge . . . . . Supel™Tox AflaZea, 6 mL (55314-U)
sample addition . . . . . 2 mL
elution . . . . . collect eluate in 5 mL silanized culture tubes
eluate post-treatment . . . . . combine 200 µL of sample eluate and 880 µL of DI water in a 2 mL autosampler vial
column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)
mobile phase . . . . . (A) water; (B) methanol; (C) acetonitrile; (720:120:120, A:B:C) containing 0.780 g/L potassium bromide, 230 µL/L nitric acid
flow rate . . . . . 0.400 mL/min
pressure . . . . . 4568 psi (315 bar)
column temp. . . . . 35 °C
detector . . . . . FLD 360 ex; 440 em
injection . . . . . 20 µL
derivatization . . . . . via KOBRA electrochemical cell, 50 µA
Application No. . . . . G005907



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## HPLC Analysis of Aflatoxins on Ascentis® Express C18

Aflatoxins are a complex of closely related toxins produced as secondary metablolites of fungi. They are found in grains and nuts such as corn, wheat, or peanuts. They are produced by aspergillus flavus and a. Parasiticus. Members of the group include aflatoxin b1, aflatoxin b2, aflatoxin g1, aflatoxin g2, aflatoxin m1, and aflatoxin m2.

column . . Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U)

mobile phase . . . . (A) water; (B) methanol; (C) acetonitrile; (720:120:120, A:B:C) containing 0.780 g/L potassium bromide, 230 µL/L nitric acid

flow rate . . . . . 0.400 mL/min

pressure . . . . . 4568 psi (315 bar)

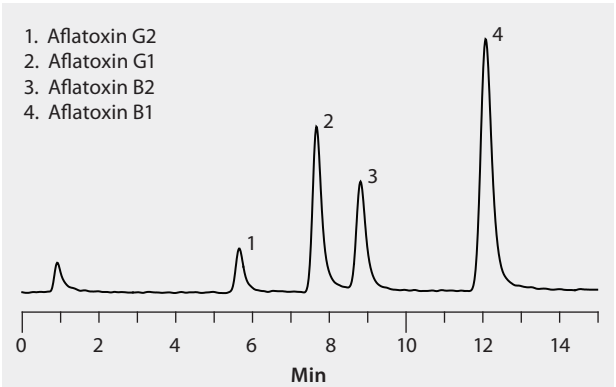
column temp. . . . . 35 °C

detector . . . . . FLD excitation = 360 nm, emission = 440 nm

injection . . . . . 20 µL

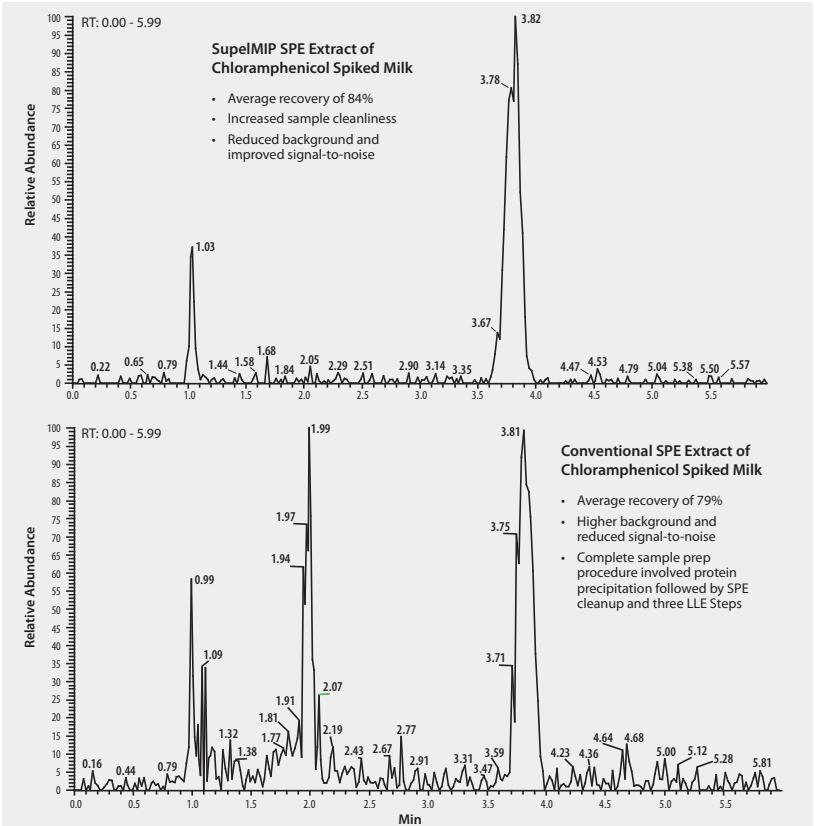
sample . . . . . 200 µL spiked 84:16 acetonitrile:di water in 880 µL di water, Spike concentrations: B1 = 16 ppb; B2 = 4 ppb; G1 = 16 ppb; B2 = 4 ppb

Application No. . . . . G005908



## HPLC Analysis of Chloramphenicol in Milk on Ascentis® C18 after SPE using SupelMIP® SPE-Chloramphenicol

sample preparation . . . . . SPE (Solid Phase Extraction)
sample addition . . . . . whole pasteurized milk spiked with chloramphenicol at 15 ng/mL and 38 ng/mL
SPE tube/cartridge . . SupelMIP SPE - Chloramphenicol, 25 mg/10mL (LRC) (53210-U)
condition . . . . . 1 mL methanol;1 mL DI water
sample addition . . . . . 1 mL
washing . . . 2 x 1 mL MS-grade water, 1 mL 5% acetonitrile in 0.5% acetic acid, 2 x 1 mL MS-grade water, 1 mL 20% acetonitrile in 1% ammonium hydroxide. (dry SPE tubes for 15 min. under gentle vacuum; 3 x 1 mL dichloromethane; dry SPE tubes for 1 min. under gentle vacuum)
elution . . . . . elute chloramphenicol with 2 x 1 mL methanol:acetic acid:MS-grade water (89:1:10, v/v/v)
eluate post-treatment . . . . evaporate combined eluate to dryness at 50 °C under nitrogen. reconstitute in 150 mL LC mobile phase prior to LC-MS analysis
column . . . . . Ascentis C18, 2.1 mm x 10 cm I.D., 3 µm particles (581301-U)
mobile phase . . . . . (A) 100 mM ammonium acetate: (B) MS-grade water: (C) acetonitrile (10:60:30, A:B:C)
flow rate . . . . . 0.2 mL/min
column temp. . . . . 35 °C
detector . . . . . MS, ESI(-), 320-323 m/z range
injection . . . . . 5 µL
Application No. . . . . G004433



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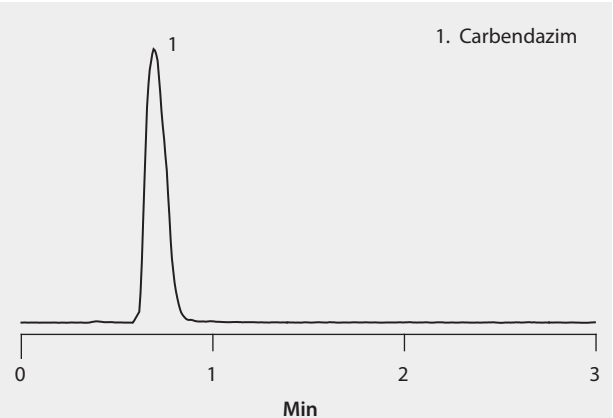
## LC-MS/MS Analysis of Carbendazim in Orange Juice (Spiked) on Ascentis® Express C18 after Supel™ QuE PSA/C18 QuEChERS Cleanup

Simple and fast extraction and analytical method were developed for analysis of fungicide carbendazim in orange juice samples. QuEChERS extraction method was used with PSA/C18 cleanup. Ascentis Express C18 column chromatography was coupled to MS/MS detection with limits of detection at 0,1 ppb.

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . . . . orange juice spiked with carbendazim at 1 ppb
extraction tube . . . . . Supel™ QuE Citrate (EN) Tube (55227-U)
extraction process . . . . . add 10 mL of orange juice with pulp to a 50 mL empty extraction tube (55248-U); add carbendazim at 1 ppb; add 10 mL acetonitrile; (shake for 1 minute; add contents of citrate extraction tube (55227-U); shake for 1 min; centrifuge at 3200 rpm for 5 minutes)
clean-up tube . . . . . Supel-Q™ PSA/C18 (55288-U)
clean-up process . . transfer 0.7 mL of the acetonitrile layer to the PSA/C18 cleanup tube (55288-U); shake 1 minute; (centrifuge for 3 minutes at 3400 rpm; remove 0.25 mL of the supernatant; mix with 0.25 mL water for LC-MS analysis)
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . A) 10 mM ammonium acetate in water; (B) 10 mM ammonium acetate in methanol
gradient . . . . . 0-1 min: 30% B; 1.5-3.5 min: 100% B; 3.5-7 min: 30% B
flow rate . . . . . 0-1 min: 0.3 mL/min; 1.5-7 min: 0.5 mL/min
column temp. . . . . 30 °C
detector . . . . . MS, ESI(+), MRM, m/z 192/160, 192/132
injection . . . . . 5 µL
Application No. . . . . G005619



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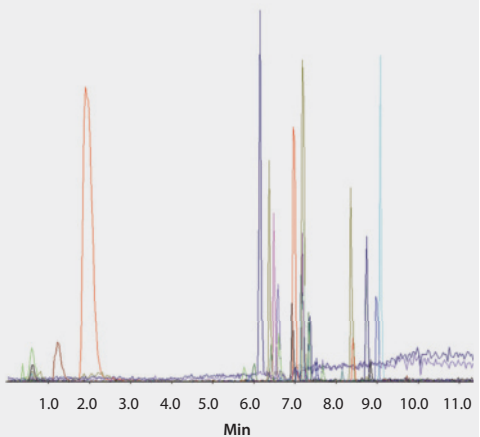
## LC-MS/MS of Pesticides in Oranges on Ascentis® Express C18 after Sample Prep using Supel™ Que Z-Sep/C18 QuEChERS sorbent

**Sample pretreatment:** 10 g of pureed oranges homogenized with rind; spike at 50 ppb by adding 16.75 µL of a pesticide mix containing each analyte at 30 µg/mL

This demonstrates the application of the Z-Sep/C18 sorbent to the cleanup of fruits in the LC-MS-MS analysis of polar pesticides including organophosphates, carbamates and benzoylureas

sample preparation . . . . . Dispersive (QuEChERS)
sample/matrix . . 10 g of pureed oranges (homogenized with rind); spike at 50 ppb (add 16.75 µL of a custom made pesticide mix, each analyte at 30 µg/mL)
extraction tube . . . . . Supel™ QuE Citrate (EN) Tube (55227-U)
extraction process . . add 10 mL acetonitrile; shake for 1 minute; add contents of a Supel QuE citrate extraction tube (55227-U); shake immediately for 1 minute; centrifuge at 3200 rpm for 5 minutes
clean-up tube . . . . . Supel-Q™ PSA/C18 (55284-U)
clean-up process . . . . . transfer 0.7 mL of the acetonitrile layer into a Supel QuE Z-Sep/C18 cleanup tube (55284-U); shake for 1 minute; centrifuge at 5000 rpm for 5 minutes; (transfer 0.2 mL of the supernatant into an empty 1.5 mL centrifuge tube; add 0.2 mL of water; centrifuge at 5000 rpm for 2 minutes.)
column . . Ascentis Express C18, 5 cm x 2.1 mm I.D., 2.7 µm particles (53822-U)
mobile phase . . . . . (A) 10 mM ammonium acetate in water; (B) 10 mM ammonium acetate in acetonitrile
gradient . . . Hold at 30% B for 1 min; 30% to 80% B in 2 min; hold at 80% B for 4 min; hold at 100% B for 3 min; hold at 30% B for 3 min
flow rate . . . . . 0.3 mL/min
pressure . . . . . 2730 psi (188 bar)
column temp. . . . . 30 °C
detector . . . . . MS/MS, ESI positive
Application No. . . . . G005645

| | $t_R$(min) | | $t_R$(min) |
|---|---|---|---|
| 1. Methomyl | 0.60 | 20. Quinalphos | 7.23 |
| 2. Trichlorfon | 1.22 | 21. Edifenphos | 7.28 |
| 3. Carbendazim | 1.94 | 22. Etrimfos | 7.33 |
| 4. Aldicarb | 2.70 | 23. Fenthion | 7.34 |
| 5. Parathion-methyl | 5.87 | 24. Fenitrothion | 7.36 |
| 6. Methabenzthiazuron | 6.16 | 25. Diazinon | 7.37 |
| 7. Naled | 6.37 | 26. Tolclofos-methyl | 7.52 |
| 8. Methidathion | 6.38 | 27. Phorate | 7.53 |
| 9. Clethodim | 6.42 | 28. Chlorpyrifos-methyl | 7.67 |
| 10. Phosmet | 6.50 | 29. EPN | 7.68 |
| 11. Ametryn | 6.58 | 30. Terbufos | 8.14 |
| 12. Sethoxydim | 6.61 | 31. Ethion | 8.36 |
| 13. Anilazine | 6.78 | 32. Lufenuron | 8.40 |
| 14. Fenxehamid | 6.94 | 33. Spiromesifen | 8.74 |
| 15. Mecarbam | 7.00 | 34. Octhilinone | 8.74 |
| 16. Oryzalin | 7.06 | 35. Pyraclostrobin | 8.75 |
| 17. Diflubenzuron | 7.18 | 36. Carbophenothion | 8.83 |
| 18. Fenoxycarb | 7.19 | 37. Flufenoxuron | 9.02 |
| 19. Iprobenfos | 7.21 | 38. Fenpyroximate | 9.10 |

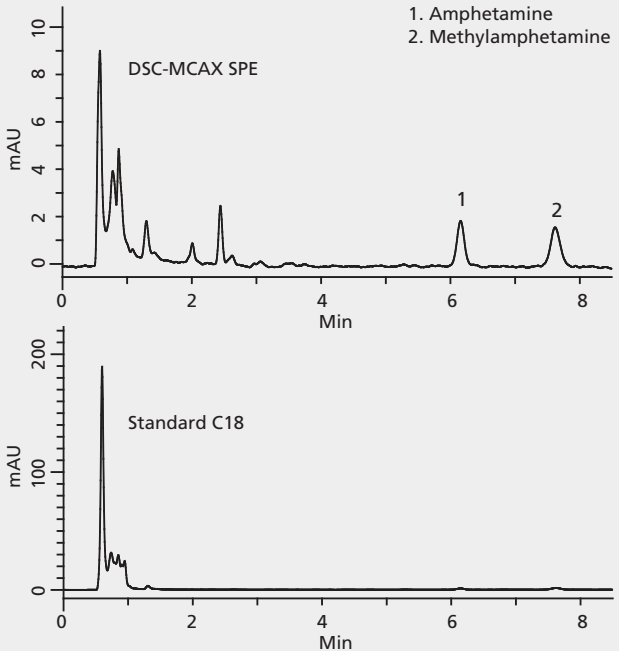


## Forensics and Toxicology

### HPLC Analysis of Amphetamines in Urine on Discovery® HS F5 after SPE using Discovery® DSC-MCAX and Standard C18

**Sample Pre-Treatment:**
1 mL human urine was spiked with 2 mg/mL amphetamine and methylamphetamine. The spiked sample was diluted 1:1 with 1:1 with 50 mM ammonium acetate, pH 6.0.

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | human urine spiked amphetamine and methylamphetamine at with 2 mg/mL |
| SPE tube/cartridge | Discovery DSC-MCAX, 100 mg/3mL (52783-U) |
| condition | DSC-MCAX SPE tube: 1 mL methanol; 1 mL 50 mM ammonium acetate, pH 6.0; C-18 SPE tube: 1 mL methanol; 1 mL DI water |
| sample addition | 1 mL |
| washing | DSC-MCAX SPE tube: 1 mL 50 mM ammonium acetate, pH 6.0; 1 mL 1M acetic acid; 1mL methanol; C-18 SPE tube: 1 mL DI water; 1 mL 20% methanol |
| elution | DSC-MCAX SPE tube: 1 mL 5% ammonium hydroxide in methanol; C-18 SPE tube: 1 mL methanol |
| eluate post-treatment | evaporate to dryness with nitrogen at room temperature, reconstitute in mobile phase |
| column | Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (567516-U) |
| mobile phase | (A) 10 mM ammonium acetate, pH 4.5: (B) methanol; (35:65, A:B) |
| flow rate | 2 mL/min |
| column temp. | 40 °C |
| detector | UV, 210 nm |
| injection | 10 µL |
| Application No. | G003761 |



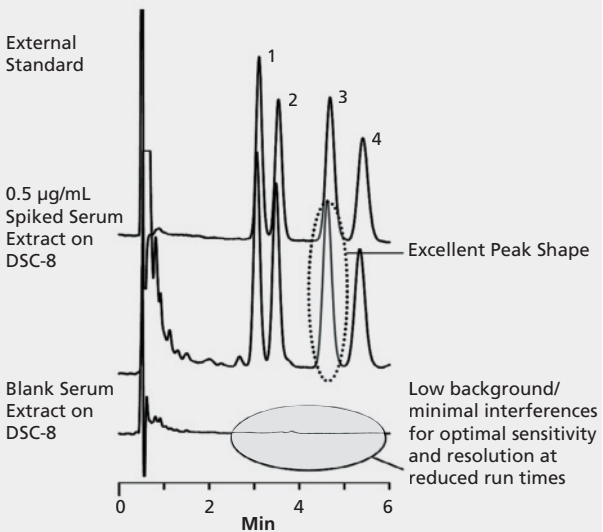

Discussion:
Note the Y-axis scale difference between DSC-MCAX and C18 SPE. DSC-MCAX SPE offered a maximum background height of ~9 mAU. In contrast, standard C18 background levels were 20 times greater than DSC-MCAX.
Also, on DSC-MCAX absolute recovery averaged at 100.3 and 101.7%, for amphetamine and methylamphetamine, respectively. On standard C18, absolute recovery averaged at 48 and 79% for the two compounds.

## HPLC Analysis of Diazepam and Metabolites in Serum on Discovery® C18 after SPE using Discovery® DSC-8

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . goat serum spiked with diazepam and metabolites at 0.5 µg/mL and diluted 1:1 in 25 mM ammonium formate, pH 7.1
SPE well plate . . . . Discovery DSC-8 96-well SPE Plate, 100 mg/well (575627-U)
condition . . . . . 1 mL methanol followed by 1 mL DI water.
sample addition . . . . . 1 mL spiked goat serum
washing . . . . . 1 mL 25% methanol in 25 mM ammonium formate, pH 2.75
elution . . . . . 1 mL 60% methanol in 25 mM ammonium formate, pH 2.75
elution . . . . . Evaporate eluate with nitrogen purge (30 °C; ~15 min.). Reconstitute in 200 µL mobile phase.
column . . . . . Discovery C18, 5 cm x 4.6 mm I.D., 5 µm particles (504947)
mobile phase . . (A) Methanol; (B)10 mM ammonium acetate, pH 4.5;(45:55, A:B)
flow rate . . . . . 1.5 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 240 nm
injection . . . . . 25 µL
Application No.Application No. . . . . G003766

**Efficiency of Recovery**

| Compound | %Recovery ± RSD (n=3) Systematically Developed Method on Discovery DSC-8 | %Recovery ± RSD (n=3) Generic Method On Conventional C18 |
|---|---|---|
| 1. Oxazepam | 94.7 ± 1.2% | 82.8 ± 4.0% |
| 2. Temazepam | 99.9 ± 1.1% | 89.1 ± 4.0% |
| 3. Desmethyl diazepam | 94.2 ± 1.8% | 82.4 ± 5.0% |
| 4. Diazepam | 90.0 ± 3.4% | 68.5 ± 9.1% |



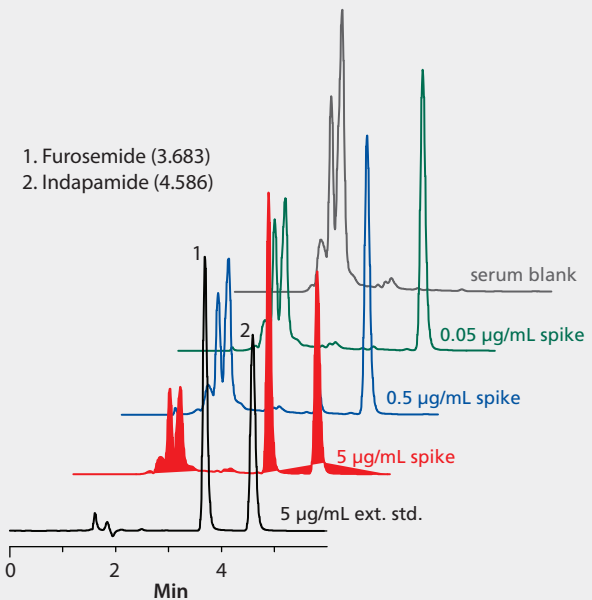
## HPLC Analysis of Furosemide (Lasix) in Horse Serum on Discovery® C18 after SPE using Discovery® DSC-18

**Sample Pre-treatment:**
Neat horse serum was acidified with 10 mL 6 M HCl/mL serum. Internal standard (I.S.) blank was prepared by spiking acidified horse serum with indapamide at 10 mg/mL. Sample A = 10 mg/mL furosemide in I.S. blank. Sample B = 5 mg/mL furosemide in I.S. blank. Sample C = 0.5 mg/mL furosemide in I.S. blank. Sample D = 0.1 mg/mL furosemide in I.S. blank. Sample E = 0.05 mg/mL furosemide in I.S. blank.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . acidified horse serum spiked with indapamide at 10 mg/mL
SPE tube/cartridge . . . . . Discovery DSC-18, 50 mg/1 mL (52601-U)
condition . . . . . 1 mL methanol; 1 mL 10 mM potassium phosphate, pH 3
sample addition . . . . . 1 mL
washing . . . . . 10 mM potassium phosphate, pH 3
elution . . . . . 1 mL 60% methanol in DI water.
column . . . . . Discovery C18, 15 cm x 4.6 mm I.D., 5 µm particles (504955)
mobile phase . . . . . (A) 10 mM $KH_2PO_4$, pH 3 (adjusted with $H_3PO_4$): (B) acetonitrile; (60:40, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 234 nm
injection . . . . . 10 µL
Application No. . . . . G003764

**Efficiency of Recovery**

| Sample | Concentration (µg/mL) | Avg. Response Factor | %Recovery ± RSD (n=3) |
|---|---|---|---|
| A | 10.00 | 2.307 | 93.1 ± 3.1 |
| B | 5.00 | 1.168 | 100.8 ± 1.4 |
| C | 1.00 | 0.107 | 97.4 ± 2.8 |
| D | 0.50 | 0.065 | 120.7 ± 1.3 |
| E | 0.10 | 0.009 | 132.8 ± 8.3 |



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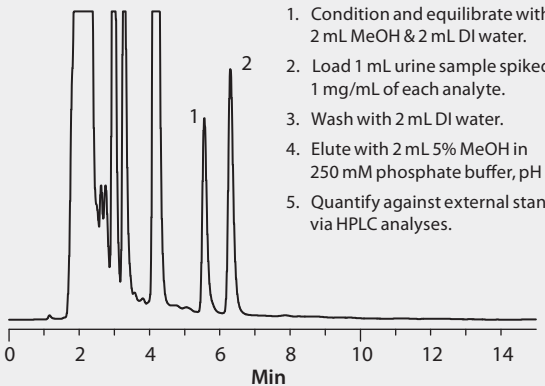
## HPLC Analysis of 3-Methylpyrazole and 4-Methylpyrazole in Urine on Discovery® C18 after SPE using Discovery® DSC-SCX

| | |
|---|---|
| sample preparation | SPE (Solid Phase Extraction) |
| sample/matrix | urine spiked with 3- and 4-methylpyrazole at 1 mg/mL |
| SPE tube/cartridge | Discovery DSC-SCX, 500 mg/3 mL (52686-U) |
| condition | 2 mL methanol; 2 mL DI water |
| sample addition | 1 mL |
| washing | 2 mL DI water |
| elution | 2 mL 5% methanol in 250 mM potassium phosphate buffer, pH 7.4 |
| column | Discovery C18, 15 cm × 4.6 mm, 5 µm preceded by a 2 cm guard column and 0.5 µm frit filter (504955) |
| mobile phase | MeOH:5 mM phosphate buffer, pH 6 (20:80) |
| flow rate | 1 mL/min |
| column temp. | 30 °C |
| detector | UV, 220 nm |
| injection | 25 µL, dilute urine extract |
| Application No. | G001635 |

**Efficiency of Recovery**

| Compound (mg/mL) | Discovery DSC-SCX (n=3) % Recovery | %RSD | Leading Competitor SCX (n=2) %Recovery | %RSD |
|---|---|---|---|---|
| 1. 3-methylpyrazole (1.0) | 89.4 | ±10.2% | 67.1 | ±20% |
| 2. 4-methylpyrazole (1.0) | 79.4 | ±6.8% | 50.5 | ±30% |



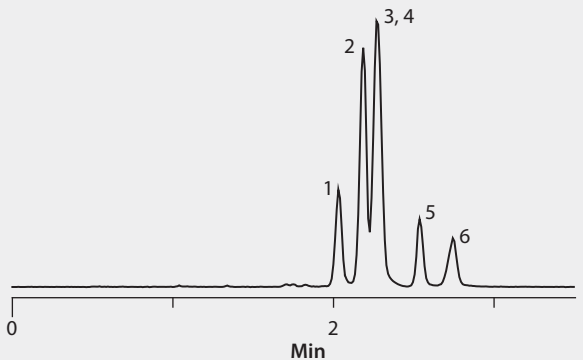

**SPE Procedure**

1. Condition and equilibrate with 2 mL MeOH & 2 mL DI water.
2. Load 1 mL urine sample spiked with 1 mg/mL of each analyte.
3. Wash with 2 mL DI water.
4. Elute with 2 mL 5% MeOH in 250 mM phosphate buffer, pH 7.4.
5. Quantify against external standards via HPLC analyses.

## LC-MS Analysis of 2C Amines Drugs of Abuse in Urine on Ascentis® Express HILIC following Sample Prep using Supel™-Select SCX SPE

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . spiked urine, 1 mL
SPE tube/cartridge . . Supel-Select SCX SPE 96-well Plate, 30 mg/well (575664-U)
condition . . . . . 1 mL methanol, then 1 mL 0.1% formic acid in water
washing . . 2 x 1 mL 10% methanol in water, 2 x 1 mL 1% formic acid in methanol
elution . . . . . 2 mL 5% ammonium hydroxide in acetonitrile
eluate post-treatment . . evaporate to 500 µL with nitrogen at room temperature, reconstitute in 250 µL acetonitrile
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . 5 mM ammonium formate in acetonitrile:water (90:10, v/v)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1570 psi (108 bar)
column temp. . . . . 35 °C
detector . . . . . TOF/MS
injection . . . . . 2.0 µL
Application No. . . . . G006193

| Peak | Compound | Rentention | m/z |
|---|---|---|---|
| 1 | Bromo Dragonfly | 2.03 | 294 |
| 2 | DOI | 2.18 | 322 |
| 3 | 2C-T-7 | 2.27 | 256.1 |
| 4 | DOB | 2.29 | 274.1 |
| 5 | 2C-B | 2.54 | 260 |
| 6 | 2C-B-Fly | 2.74 | 284 |



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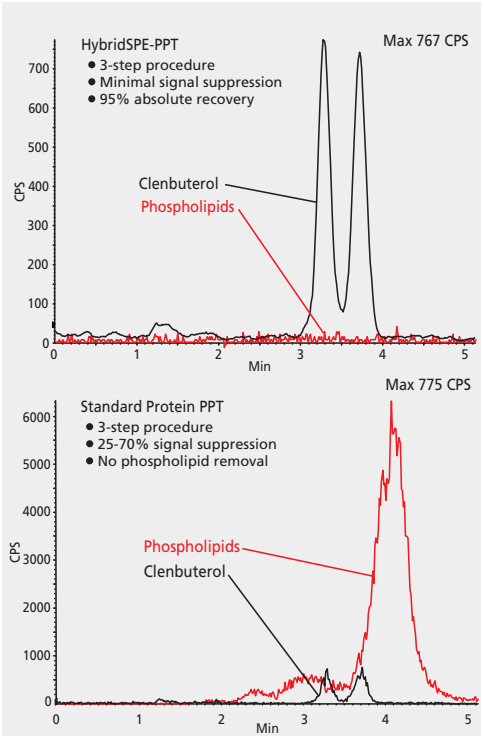
## LC-MS Analysis of Clenbuterol In Plasma on Astec® CHIROBIOTIC® T with Phospholipid Removal Using HybridSPE® Phospholipid

The HybridSPE method provides significant improvement in LC-MS baseline.

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . rat plasma spiked with clenbuterol enantiomers at 10 ng/mL
SPE well plate . . . HybridSPE-Precipitation 96-well Plate, 50 mg/well (575656-U)
sample addition . . 100 µL spiked rat plasma followed by 300 µL 1% formic acid in acetonitrile. Mix by vortexing the HybridSPE-PPT plate briefly.
elution . . . . . apply vacuum
column . . . . . CHIROBIOTIC T, 10 cm x 2.1 mm I.D., 5 µm particles (12018AST)
mobile phase . . . . . 10 mM ammonium formate in methanol
flow rate . . . . . 0.3 mL/min
column temp. . . . . 30 °C
detector . . . . . ABI 3200 QT; ESI(+), MRM: 184/104 m/z (phospholipids); 277.2/203.1 m/z (clenbuterol)
injection . . . . . 10 µL
Application No. . . . . G004431



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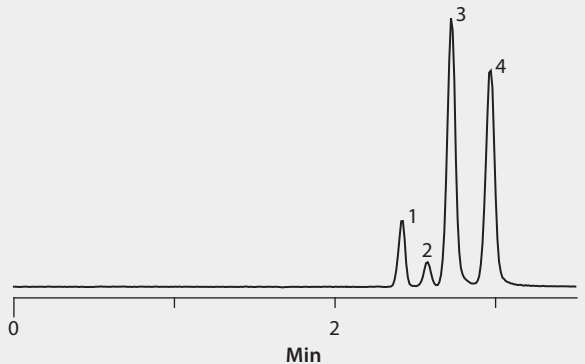
## LC-MS Analysis of Fluoroamphetamine Drugs of Abuse on Ascentis® Express HILIC after SPE using Supel™-Select SCX 96-Well Plate

sample preparation . . . . . SPE (Solid Phase Extraction)
sample/matrix . . . . . urine, 1 mL
SPE tube/cartridge . . Supel-Select SCX SPE 96-well Plate, 30 mg/well (575664-U)
condition . . . . . 1 mL methanol, then 1 mL 0.1% formic acid in water
washing . . 2 x 1 mL 10% methanol in water, 2 x 1 mL 1% formic acid in methanol
elution . . . . . 2 mL 5% ammonium hydroxide in acetonitrile
eluate post-treatment . . evaporate to 500 µL with nitrogen at room temperature, reconstitute in 250 µL acetonitrile
column . . Ascentis Express HILIC, 10 cm x 2.1 mm I.D., 2.7 µm particles (53939-U)
mobile phase . . . . . 5 mM ammonium formate in acetonitrile:water (90:10, v/v)
flow rate . . . . . 0.4 mL/min
pressure . . . . . 1570 psi (108 bar)
column temp. . . . . 35 °C
detector . . . . . TOF/MS
injection . . . . . 2.0 µL
Application No. . . . . G006194

| Peak | Compound | Rentention | m/z |
|---|---|---|---|
| 1 | 2-Fluoroamphetamine | 2.42 | 154.1 |
| 2 | 4-Fluoroamphetamine | 2.58 | 154.1 |
| 3 | 2-Fluoromethamphetamine | 2.72 | 168.1 |
| 4 | 4-Fluoromethamphetamine | 2.97 | 168.1 |



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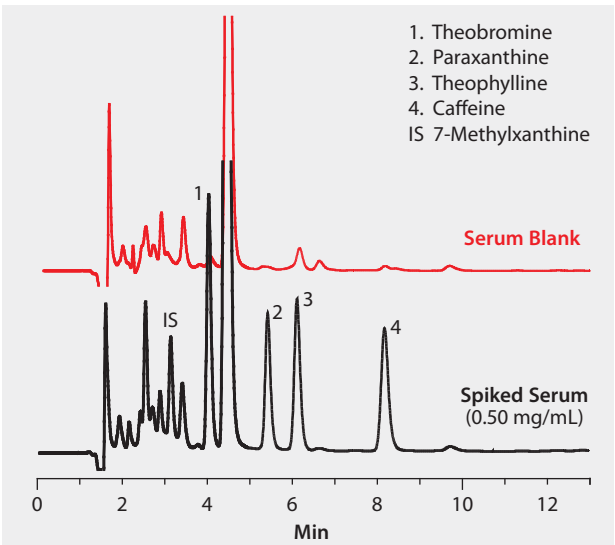
## Pharma and Biopharma

# HPLC Analysis of Bronchodilators Theophylline and Other Caffeine Metabolites from Serum on Discovery® RP-AmideC16 after SPE using Discovery® DSC-18

sample preparation . . . . . SPE (Solid Phase Extraction)
SPE tube/cartridge . . . . . Discovery® DSC-18, 500 mg/3 mL (52603-U)
condition . . . . . 2 mL methanol, then 2 mL water
sample preparation . . . . . 1 mL porcine serum spiked with 0.1 µg/mL, 0.50 µg/mL, or 1.0 µg/mL each analyte
sample preparation . . 1 mL methanol; evaporate to dryness with nitrogen stream at room temperature; reconstitute in 200 µL mobile phase containing 0.2 µg/mL or 7-methylxanthine (IS)
sample preparation . . . . . 2 mL 5% methanol in water; dry tube 10 min with nitrogen stream
column . . Discovery RP-AmideC16, 15 cm × 4.6 mm, 5 µm particles, preceded by a 2 cm RP-AmideC16 guard column and 0.5 µm frit filter (505013)
mobile phase . . . . . (A) Methanol; (B) 1% acetic acid (17:38, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 30 °C
detector . . . . . UV, 272 nm
injection . . . . . 20 µL reconstituted porcine serum extract
Application No. . . . . G000594

Efficiency of Recovery

| Compound | Concentration (µg/mL) | % Recovery | % RSD (n=6) |
|---|---|---|---|
| 1. Theobromine | 0.1 | 97.4 | ±6.8 |
| | 0.5 | 96.4 | ±8.5 |
| | 1.0 | 96.1 | ±5.0 |
| 2. Paraxanthine | 0.1 | 96.2 | ±8.4 |
| | 0.5 | 95.2 | ±8.7 |
| | 1.0 | 95.0 | ±8.7 |
| 3. Theophylline | 0.1 | 97.8 | ±8.5 |
| | 0.5 | 97.8 | ±8.8 |
| | 1.0 | 98.5 | ±5.7 |
| 4. Caffeine | 0.1 | 98.8 | ±3.9 |
| | 0.5 | 95.6 | ±6.7 |
| | 1.0 | 97.6 | ±5.8 |



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## HPLC Analysis of Hydrocortisone in Topical Cream on Discovery® HS C18 after SPE using Discovery® DSC-Si

**Sample Pre-treatment:**

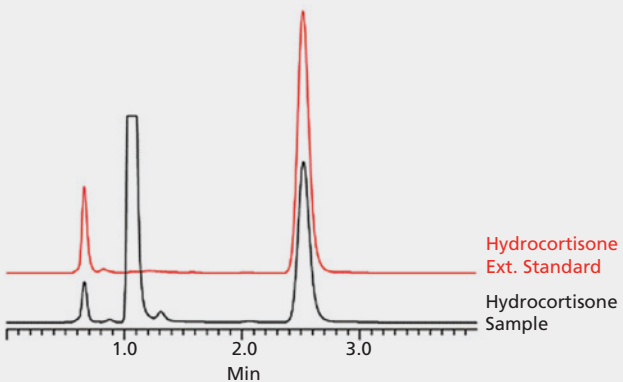
Dissove 1 g 1% hydrocortisone topical hand cream in 10 mL ethyl acetate. Dilute 2 mL of ethyl acetate-cream sample with 8 mL hexane:ethyl acetate (2:1, v/v).

sample preparation . . . . . SPE (Solid Phase Extraction)
sample addition . . . . . hydrocortisone topical hand cream in hexane:ethylacetate
SPE tube/cartridge . . . . . Discovery DSC-Si, 500 mg/3 mL (52695-U)
condition . . . . . 1 mL hexane:ethyl acetate (2:1, v/v)
sample addition . . . . . 1 mL
washing . . . . . 1 mL hexane:ethyl acetate (2:1, v/v)
elution . . . . . 1 mL methanol:DI water (1:1, v/v)
eluate post-treatment . . . dilute eluate to 2 mL with methanol:DI water (1:1, v/v)
column . . . . Discovery HS C18, 15 cm x 4.6 mm I.D., 5 µm particles (568520-U)
mobile phase . . . . . (A) methanol; (B) DI water (1:1, A:B)
flow rate . . . . . 1 mL/min
column temp. . . . . 35 °C
detector . . . . . UV, 220 nm
injection . . . . . 10 µL
Application No. . . . . G003765

**Efficiency of Recovery**

| Sample No. | Sample Weight (g) | Theoretical Hydrocortisone Weight (g)[1] | Actual Hydrocortisone weight recovered (g) | Absolute Recovery (%) | Actual % Hydrocortisone in Cream |
|---|---|---|---|---|---|
| 1 | 1.0258 | 0.0103 | 0.0092 | 89.32 | 0.9003 |
| 2 | 1.1078 | 0.0118 | 0.0111 | 94.07 | 0.9975 |
| 3 | 1.1151 | 0.0112 | 0.0119 | 106.25 | 1.0424 |
| 4 | 1.1369 | 0.0114 | 0.0119 | 104.39 | 1.0442 |
| 5 | 1.1390 | 0.0114 | 0.0113 | 99.12 | 1.0785 |
| 6 | 1.1634 | 0.0116 | 0.0123 | 106.03 | 1.0567 |
| | | | Average: | 99.86 | 1.02 |
| | | | RSD: | 6.99 | 0.06 |

[1]Based on manufacturer's label description of 1% hydrocortisone (w/w)



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# Analyte Index

Page number groupings in this index indicate the appearance of applications within multiple techniques/market focus groups. Additionally, you can activate the **Text Select** tool (bottom menu bar) and highlight the desired analyte. Right-click to copy the analyte name and paste it into the Search bar. Results for the active volume will appear on the left side of the window, allowing you to browse the results. Because this index spans all three volumes, use the footer links to open a different volume to search for additional results.

## — B —

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## — E —

## — F —

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## — U —



## — V —

## — W —



## — X —

## — Y —



## — Z —



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**AZYP, LLC** — LARIHC™
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