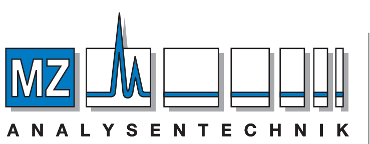

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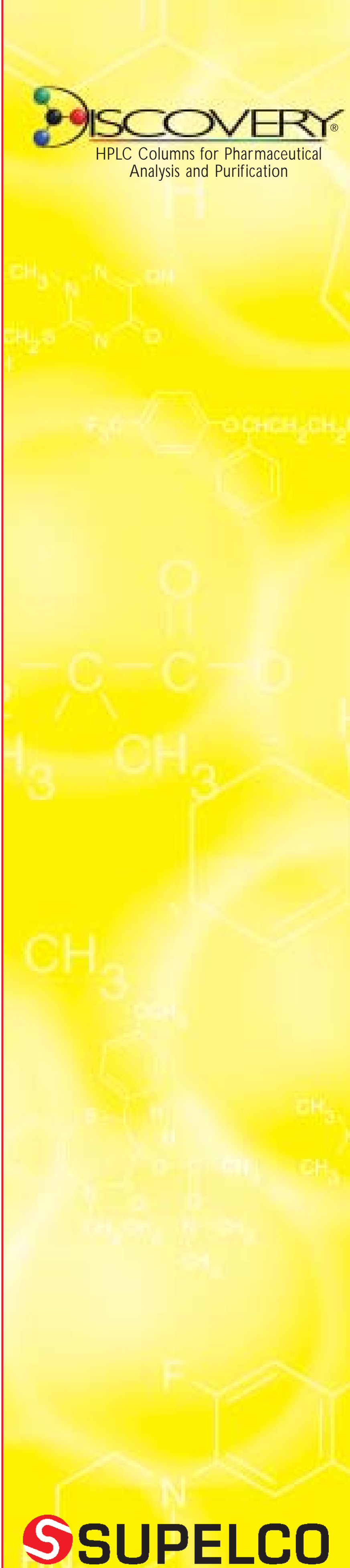

The Discovery Suite of Reversed-Phase HPLC Columns Gives Better Separations in Less Time.

Rediscover Method Development

Cyano
C8
Zr-CarbonC18
HS F5
HS PEG
C18
Zr-PBD
RP-Amide C16
Zr-PS

sigma-aldrich.com/supelco

Rediscover HPLC Method Development

Whether you are developing a new HPLC method or troubleshooting an existing method…

Turn to Discovery

Discovery is a suite of HPLC columns featuring functionalized reversed-phases designed to provide differentiated separations vs. C18 based on unique combinations of polar and hydrophobic retention mechanisms.

The Discovery suite of reversed-phases enables you to optimize your separation with respect to:

Retention	***Resolution***
Selectivity	***Analysis Time***

while minimizing method development time.

Ideal for all "small molecule" HPLC applications

Although designed to meet the exacting requirements of pharmaceutical analysis and purification, Discovery columns are also ideal for all application segments requiring reversed-phase HPLC, including:

Agriculture	***Clinical***	***Consumer Products***	***Environmental***
Food and Beverage	***Industrial / Chemical***	***Petrochemical***	***Pharmaceutical and more…***

The continually growing Discovery family currently comprises:

Discovery Silica-Based Columns

Allow the development of better HPLC separations in less time

- Discovery C18 and HS C18
- Discovery C8
- Discovery RP-AmideC16
- Discovery Cyano
- Discovery HS F5
- Discovery HS PEG

Discovery Zirconia-Based Columns

Permit HPLC method development at pH and temperature extremes

- Discovery Zr-Carbon
- Discovery Zr-CarbonC18
- Discovery Zr-PBD
- Discovery Zr-PS

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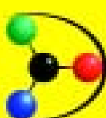


Discovery Column Quick Look-Up Guide

Use the following table to choose a Discovery column based on the physical and chemical properties of the particles. For more detailed recommendations on choosing a Discovery column, go to the Column Selection or Problem-Solution sections of this brochure.

Discovery Silica-Based Phases

Discovery Phase	Discovery C18	Discovery HS C18	Discovery C8	Discovery Cyano	Discovery RP-AmideC16	Discovery HS F5	Discovery HS PEG
USP Code	L1	L1	L7	L10	(Pending L57)	L43	
Bonded Phase	Octadecylsilane	Octadecylsilane	Octylsilane	Cyanopropyl	Palmitamido-propylsilane	Pentafluoro-phenylpropyl	Polyethylene-glycol
Endcap	Yes	Yes	Yes	Yes	Yes	Yes	No
Particle Platform	Silica	Silica	Silica	Silica	Silica	Silica	Silica
Particle Shape	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical	Spherical
Particle Purity	<10ppm metals	<10ppm metals	<10ppm metals	<10ppm metals	<10ppm metals	<10ppm metals	<10ppm metals
Particle Sizes (µm)	5	3, 5, and 10	5	5	5	3, 5, and 10	3, 5, and 10
Pore Size (Å)	180	120	180	180	180	120	120
Surface Area (m^2/g)	200	300	200	200	200	300	300
Packing Density (g/mL)	0.58	0.58	0.58	0.58	0.58	0.58	0.58
%C	12	20	7.5	4.5	11	12	12
Coverage (µmoles/m^2)	3	3.8	3.4	3.5	2.6	4	3.8
pH Range	2 to 8	2 to 8	2 to 8	2 to 8	2 to 8	2 to 8	2 to 8
Temperature Range	≤70°C	≤70°C	≤70°C	≤70°C	≤70°C	≤70°C	≤70°C

Discovery Zirconia-Based Phases

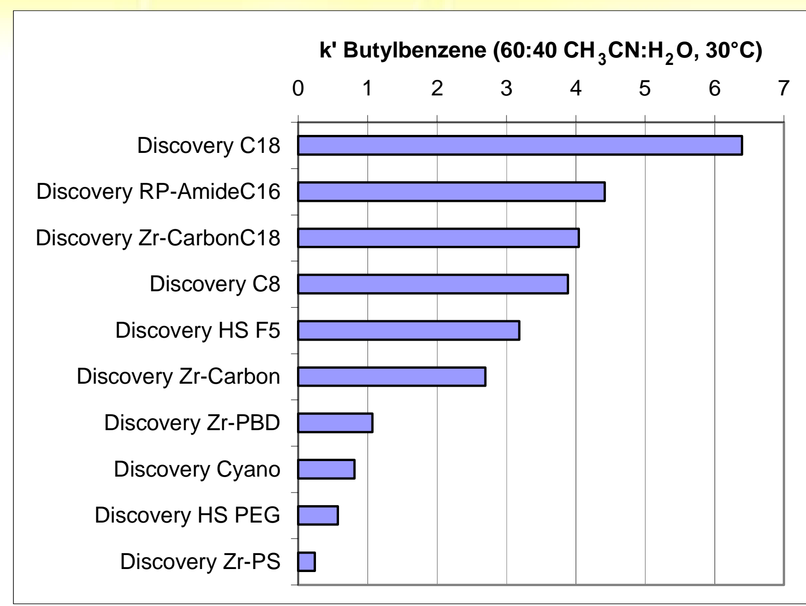
Discovery Phase	Discovery Zr-PS	Discovery Zr-PBD	Discovery Zr-Carbon	Discovery Zr-CarbonC18
USP Code		L49		
Bonded Phase	Cross-linked polystyrene	Cross-linked polybutadiene	Graphitic-like carbon	Octadecylphenyl modified carbon
Endcap	No	No	No	No
Particle Platform	Zirconia	Zirconia	Zirconia	Zirconia
Particle Shape	Spherical	Spherical	Spherical	Spherical
Particle Sizes (µm)	3 and 5	3 and 5	3 and 5	3 and 5
Pore Size (Å)	300	300	300	300
Surface Area (m^2/g)	30	30	30	30
Packing Density (g/mL)	2.21	2.21	2.21	2.21
%C	2	2	1	3
Coverage (µmoles/m^2)	n/a	n/a	n/a	2.8
pH Range	1 to 13	1 to 13	1 to 14	1 to 14
Temperature Range	≤100°C (b)	≤100°C (b)	≤100°C (c)	≤100°C (c)

(b) special hardware for operation between 100°C and 150°C is available
(c) special hardware for operation between 100°C and 200°C is available

Rediscover Method Development

Method development scientists often choose a single stationary phase for development. If the chosen phase is not the best chemistry to affect a given separation, many hours may be spent studying mobile phase compositions that may or may not yield a suitable separation. Screening several stationary phase chemistries upfront during method development and choosing the best phase for further optimization can save many precious hours. In addition, the use of a more effective stationary phase chemistry often eliminates the need for mobile phase additives that can greatly complicate separation conditions.

Figure 1: Hydrophobic Retention Ranking of Discovery Reversed-Phases



On the following pages, begin to **Rediscover Method Development...**

Take Advantage of the Discovery Suite of Reversed-Phases

Valuable, Different Separations Compared to Traditional C18 Columns

While C18 columns from different manufacturers can provide differences in retention and selectivity, these differences are frequently small and not sufficient to produce really valuable, improved separations. The Discovery suite of reversed-phases is designed to be complimentary to C18 by combining polar functionality with the standard alkyl/hydrophobic functionality. The result: You are much more likely to achieve an improved, valuable separation with a polar functionalized reversed-phase than by simply switching to another brand of C18.

Tips for Getting Started: Good Method Development Practices

Tip One: Use a column selector valve

Automated HPLC + Column Selector Valve

- While screening of functionalized reversed-phases can be done with a simple, manual HPLC system, an automated, multisolvent system with programmable, temperature controlled column selector valve is highly recommended.

Tip Two: Use a simple screening protocol of Discovery columns

Guidelines for Rapid Screening of Functionalized Reversed-Phases

Step 1: Scout for “best” mobile phase on C18

Step 2: Initial screening runs

- Chromatograph sample on Discovery HS F5 and RP-AmideC16 using “best” C18 mobile phase
- Chromatograph sample on Discovery HS PEG and Discovery Cyano using 20% lower organic than “best” C18 mobile phase

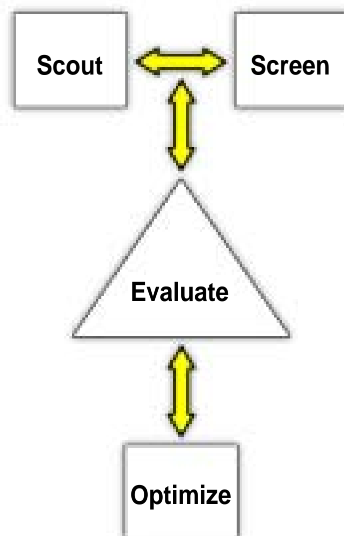
Step 3: Evaluate screening runs

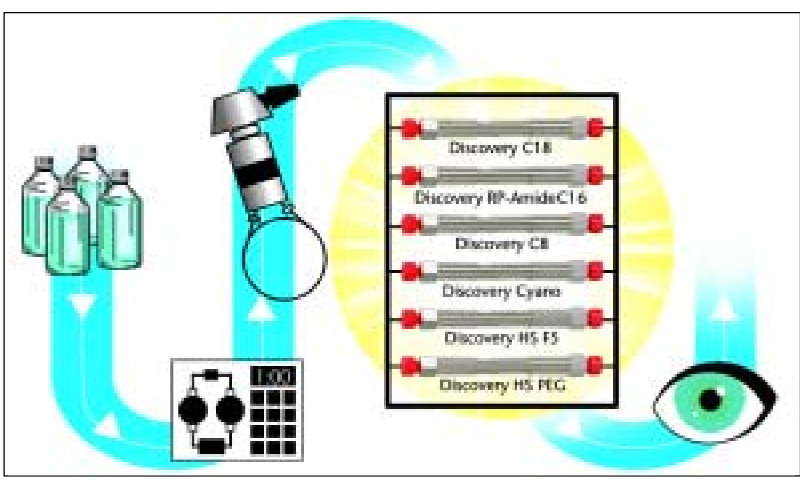
- Retention OK? If no, adjust % organic and rerun (Note: HS F5 sometimes requires stronger mobile phase than C18)

Step 4: Optimize separation on most promising 1 or 2 columns using standard reversed-phase mobile phase adjustment techniques

Tip Three: Always screen several Discovery functionalized reversed-phases along with a Discovery C18

Tip Four: Optimize your separation on the 1 or 2 most promising Discovery phases





Note: We highly recommend using Aldrich brand HPLC-grade solvents and solvent blends. These high-purity solvents can be found by visiting **sigma-aldrich.com/aldrich**

Rediscover Method Development

Deliver Better Separations in Less Time

Case Study 1

Unique retention and selectivity of Discovery HS F5 enables rapid development of simple impurity assay where C18 fails.

Impurity methods requiring retention and resolution of vastly differing analytes may not be suitably obtained using simple C18-based systems. By changing the stationary phase the method development scientist can avoid:

- complicated or forbidden gradients
- complex mobile phases
- long, drawn-out method development

On Discovery HS F5, it took just a few hours to develop an excellent separation.

2-Aminopyridine

H_2N — N

Piroxicam

OH, CONH, N, N, S, CH_3, O, O

Figure 1: 2-Aminopyridine (2-AMP) is Unretained on C18 Under Mobile Phase Conditions Used to Assay Piroxicam

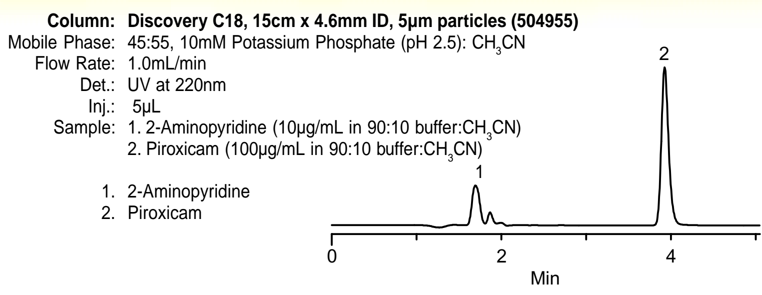


Figure 2: Decreasing the % Acetonitrile Results in Excessive Piroxicam Retention and 2-AMP is Still Unretained

Same buffer but with lower organic: 85:15, 10mM Potassium Phosphate (pH 2.5): CH_3CN

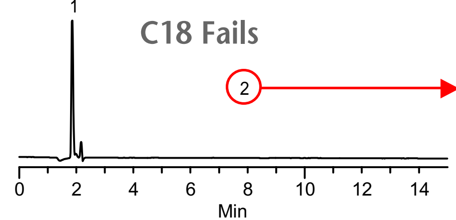


Figure 3: Increasing pH to 6.8 Retains the 2-AMP but Piroxicam Retention is Still Excessive

Same %organic, but changing the pH to 6.8: 85:15, 10mM Potassium Phosphate (pH 6.8): CH_3CN

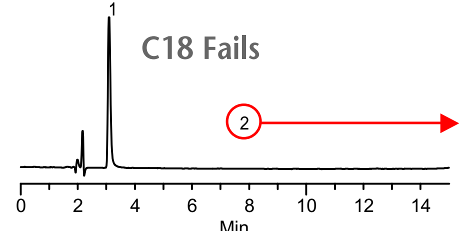
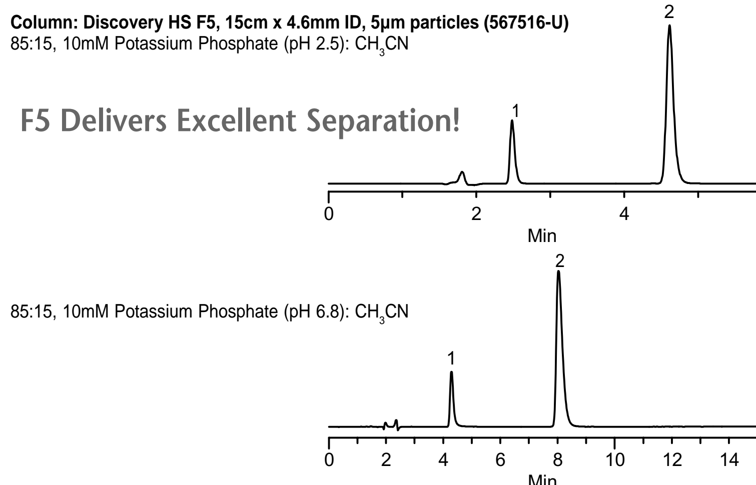


Figure 4: The Unique Retention and Selectivity of Discovery HS F5 Produces Excellent Separation at Both pH values



Deliver Better Separations in Less Time

Case Study 2

Upfront column screening facilitates development of method to separate corticosteroids.

The goal of the study was to develop HPLC conditions suitable for the separation of five corticosteroids (hydrocortisone, prednisolone, prednisone, corticosterone and hydrocortisone acetate).

Method development scientists often choose a single stationary phase for development.

However, screening several stationary phase chemistries upfront during method development and choosing the best phase for further optimization can save many precious hours.

Columns: 5cm x 4.6mm ID, 5μm particles
Mobile Phase: Water:MeOH
Flow Rate: 1.5mL/min
Temp.: 60°C
Det.: UV at 240nm
Inj.: 1μL, each compound 10mg/mL

1. Hydrocortisone
2. Prednisolone
3. Prednisone
4. Corticosterone
5. Hydrocortisone acetate

Figure 1: Scouting run on the C18 column gave good retention, but insufficient resolution. This is the “best” mobile phase on the C18.

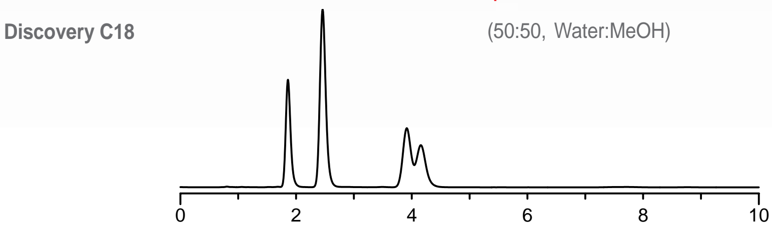


Figure 2: Screening Discovery Cyano column gave adequate retention, but peaks are broad. Decreasing %organic may improve resolution, but efficiency will suffer.

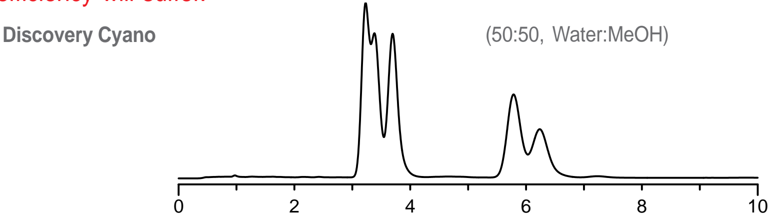


Figure 3: Screening Discovery HS PEG column showed inadequate retention, even at 80% aqueous.

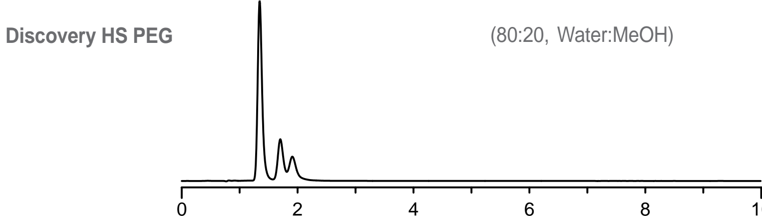


Figure 4: Screening Discovery HS F5 column gave promising results. Peak shape and band spacing (selectivity) were good. HS F5 chosen to further optimize method.

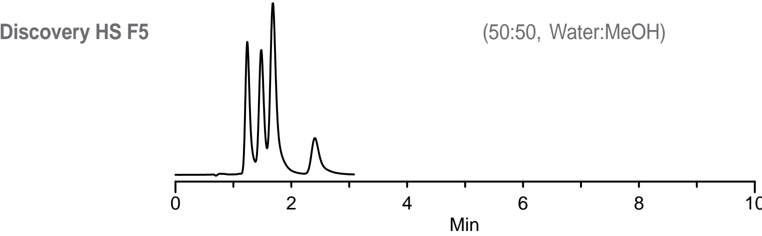
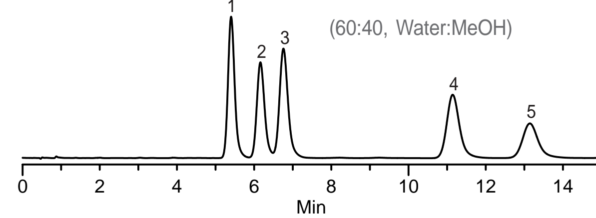


Figure 5: Optimized analysis: Discovery HS F5 column gave best resolution and analysis time. Mobile phase optimized to fine-tune the separation.



Automated Column Switching Facilitates Method Development

SupelPRO™ Automated Fluidics Instruments Complement Method Development on Discovery HPLC Columns

Supelco's SupelPRO series are precision, electronically-controlled, motorized valve instruments for repetitive fluid switching operations. Each SupelPRO instrument is self-contained and incorporates a 2-position or multi-position port valve. Standard multi-position models include a 4-line BCD (binary coded decimal) port, and the 2-position models include the Level Logic (type of electrical signal). Power requirements: 100-240VAC, 50-60Hz (auto switching). All units shipped with standard US power cord. Other power cords are available on a custom basis.

All SupelPRO units are CE approved.

SupelPRO 3-Column or 6-Column Selector

Select from among up to 3 columns or up to 6 columns. Useful for column selectivity comparisons, other column selection applications. Includes mounting clips and cover.

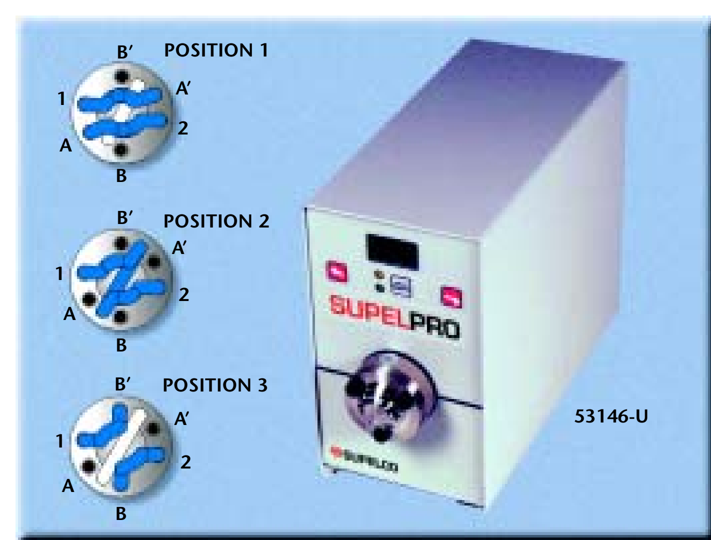
SupelPRO 2-Channel Selector with Bypass Valve

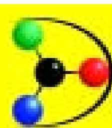
This 6-port, 3-position motorized valve is useful for selecting 1 of 2 connected columns, or flushing.

See page 75 for ordering information.

Figure 1: SupelPRO 3-Column or 6-Column Selector

Figure 2: SupelPRO 2-Channel Selector with Bypass Valve





SupelPRO 11-Port, 10-Position Valve

Use this 11-port, 10-position valve to select from up to 10 inputs to 1 output, or select 10 outputs from 1 input.

SupelPRO 2-Position Valves

Available with 6 or 10 ports. Useful for a wide variety of applications, including sample clean-up and back-flushing.

SupelPRO Solvent Selector Valve

Allows automation of mobile phase selection from 6 inlets. Comes with factory installed 1/16" or 1/8" OD tubing and 1/4-28 fittings. Rated to 300psi (20 bar).

See page 75 for ordering information.

Figure 1: SupelPRO 11-Port, 10-Position Valve

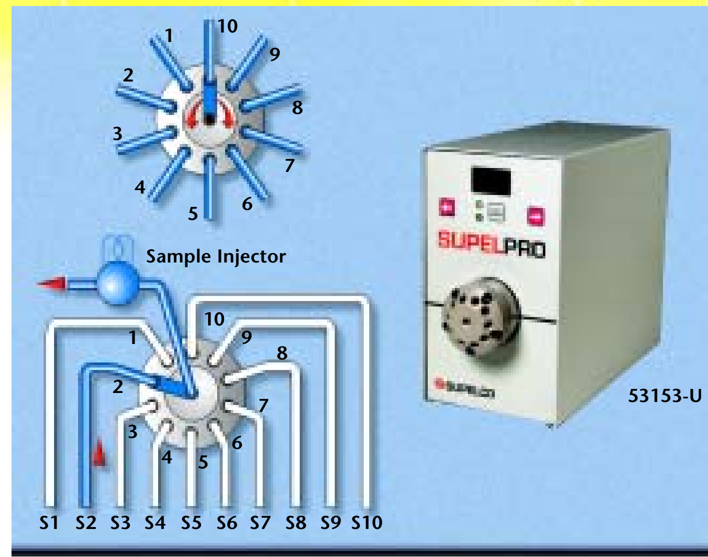


Figure 2: SupelPRO 2-Position Valves

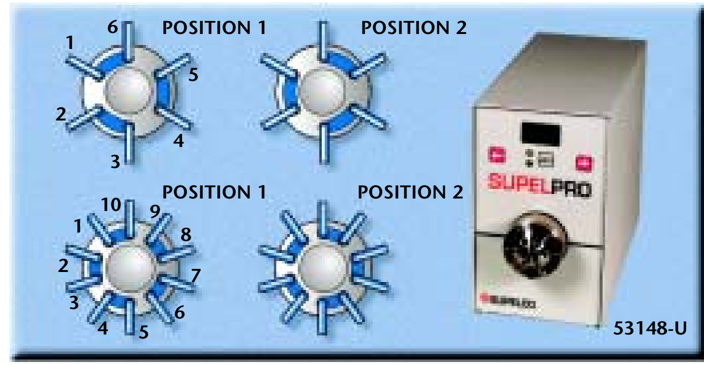
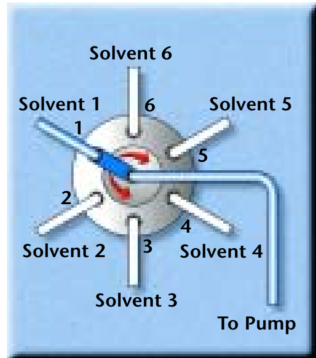


Figure 3: SupelPRO Solvent Selector Valve



Discovery Silica-Based Phases

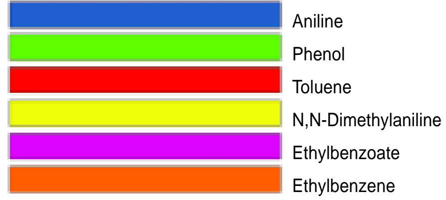
Different Selectivity is a General and Valuable Characteristic of Functionalized Reversed-Phases

Unique Retention vs. C18

As a visual representation of how the different phase chemistries give different selectivity, these charts show the k' of various analytes relative to toluene on Discovery columns.

Key to interpreting results

When a color aligns, the selectivity is similar. When a color does not align, the selectivity is different.



Mobile Phase: 45:55, 25 mM Potassium Phosphate (pH 7.0):MeOH (All columns except HS PEG which was run at 75:25, 25 mM Potassium Phosphate (pH 7.0):MeOH).
Flow Rate: 1.0mL/min

Figure 1: Similar Phases (C18 and C8) – Similar Selectivity

The nearly perfect alignment of colors in Figure 1 clearly illustrates that all bonded phases that consist of non-functionalized alkyl chains give similar selectivity, even the competitive C18 that has been bonded to a different silica particle than the Discovery phases.

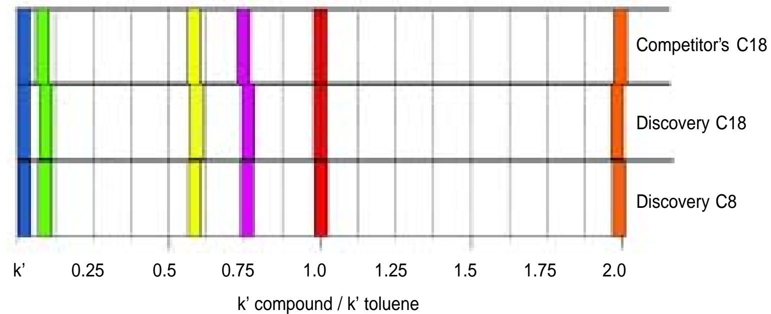
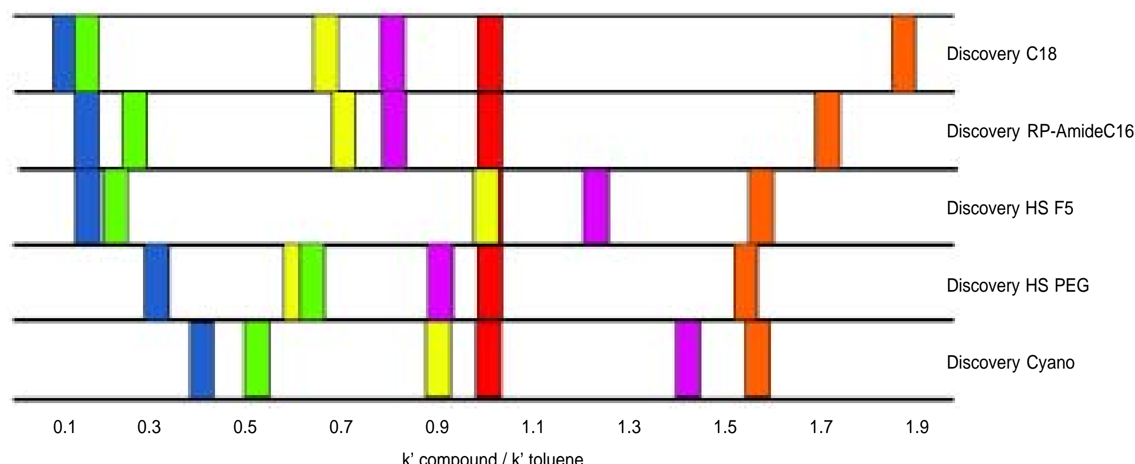


Figure 2: Functionalized Phases – Unique and Different Selectivity

The polar functional group-containing solutes - aniline, phenol, N,N-dimethylaniline (N,N-DMA) and ethylbenzoate - clearly illustrates the very different selectivities of the functionalized reversed-phases vs. C18. Observe in Figure 2 the colors representing solutes containing polar groups dramatically change positions from phase to phase. Also observe the changing hydrophobic selectivity by looking at the ethylbenzene bar. Both polar and hydrophobic selectivities are different on the different phases.

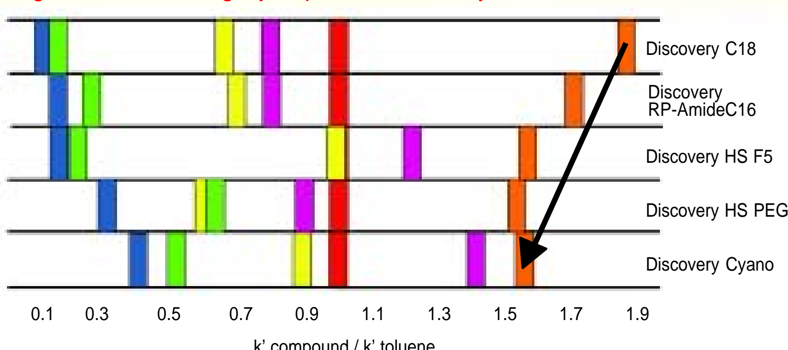


Observed Trends Demonstrate Selectivity Differences Between Discovery Functionalized Reversed-Phases

Hydrophobic Selectivity

Generally, the more polar the phase the less hydrophobic selectivity it has. The differences between retention of toluene and ethylbenzene, both of which have no polar groups, is greatest on Discovery C18.

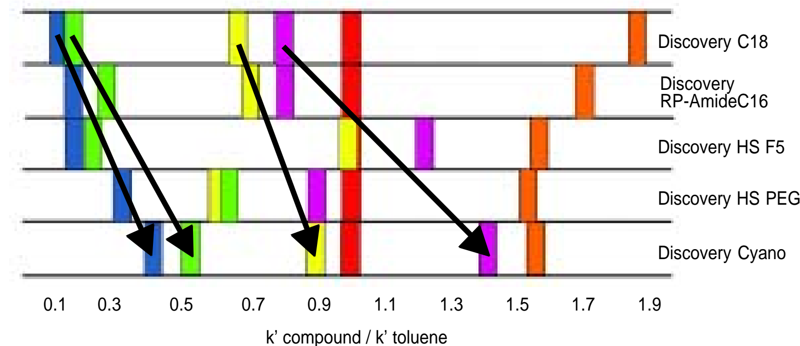
Figure 1: Decreasing Hydrophobic Selectivity



Polar Group Selectivity

When the analyte has polar groups, polar bonded phases give generally better selectivity than a C18. Here, the polar compounds N,N-dimethylaniline, ethylbenzoate, and phenol all exhibit enhanced retention relative to toluene on the polar Discovery phases over Discovery C18.

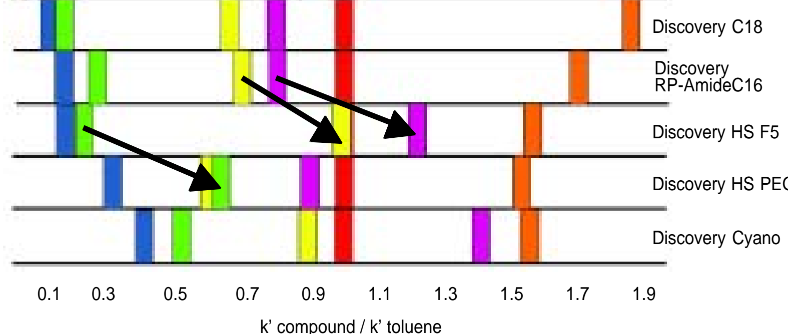
Figure 2: Greater Relative Retention of Polar Compounds Over C18



Differences between Polar Group Selectivity on Discovery Functionalized Reversed-Phases

Not only are separations of polar compounds on Discovery functionalized reversed-phases different than C18, the phases also are different from each other. This is why we recommend you screen all of the Discovery phases to find the one that is best for your separation.

Figure 3: Polar Phases Have Different Selectivities From Each Other



Polar-Embedded Phases Can Exhibit “U-Shape” Retention Profile

Under certain mobile phase conditions and with certain analytes, polar-embedded phases, like Discovery HS F5 and HS PEG, can exhibit both reversed-phase and normal-phase behavior. At low percent organic, retention decreases with increasing percent organic following reversed-phase behavior. However, at higher percent organic, retention increases with increasing percent organic following normal-phase behavior. The result is a “U-shape” retention profile for these compounds.

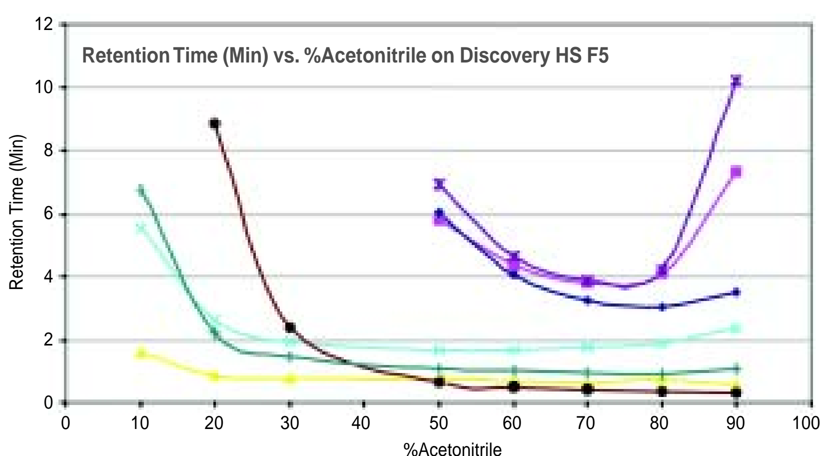
If your compounds exhibit this U-shape profile, use it to your advantage to:

- Improve LC/MS detection by using higher % organic mobile phase.
- Use mobile phase selectivity to develop valuable, different separations at high % organic.

Figure 1: U-Shape Retention Profile on Discovery HS F5

Column: **Discovery HS F5, 5cm x 4.6mm ID, 3µm particles (567504-U)**
Mobile Phase: CH_3CN in 10mM Ammonium Acetate (pH 6.8)
Flow Rate: 1mL/min
Temp.: 35°C

- Amitriptyline
- Cimetidine
- Clonidine
- Fluoxetine
- Nifedipine
- Trimethoprim
- Verapamil



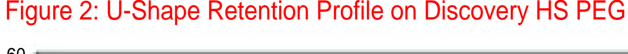
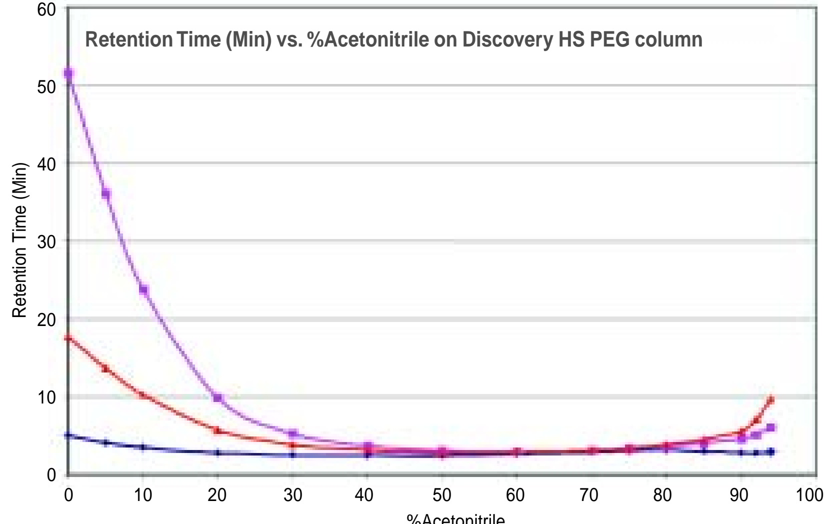


Figure 2: U-Shape Retention Profile on Discovery HS PEG

Column: **Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles (567416-U)**
Mobile Phase: CH_3CN in 5mM Ammonium Acetate (pH 4.0)
Flow Rate: 1mL/min
Temp.: 35°C

- Trimethorprim
- Amitriptyline
- Propranolol



Discovery Silica-Based Phases

Discovery C18

CH_3
$-O-Si-(CH_2)_{17}-CH_3$
CH_3

Classic Reversed-Phase Retention and Selectivity with Excellent Peak Shape

Use Discovery C18 for any method that specifies a C18. The exceptional peak shape, reproducibility, and stability make it the column of choice for all C18 methods from demanding to routine.

- Classic C18 selectivity and retention
- Excellent peak shape
- Stable, no-bleed LC/MS separations

Properties of Discovery C18

USP Code	L1
Bonded Phase	Octadecylsilane
Endcap (yes / no)	Yes
Particle Platform	Silica
Particle Shape	Spherical
Particle Purity	<10ppm metals
Particle Sizes (µm)	5
Pore Size (Å)	180
Surface Area (m²/g)	200
Packing Density (g/mL)	0.58
%C	12
Coverage (µmoles/m²)	3
pH Range	2 to 8
Temperature Range	≤70°C

Figure 1: Discovery C18 operates via a predictable reversed-phase mechanism. Compounds elute in order of increasing hydrophobicity.

Alkylparabens on Discovery C18

Mobile Phase: 60:40 Water:CH_3CN

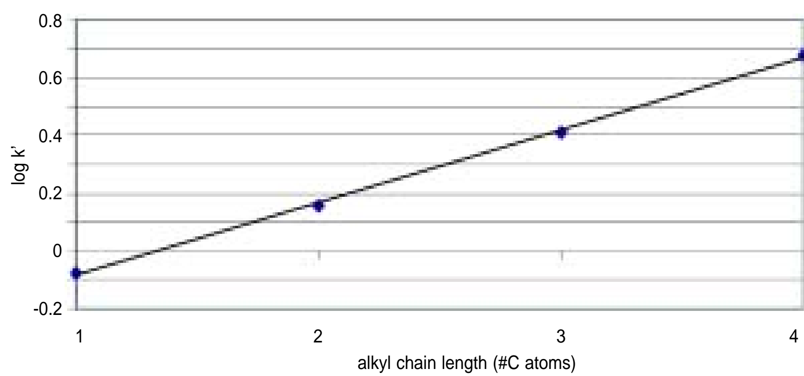


Figure 2: Organic Acids

Column: Discovery C18, 5cm x 4.6mm ID, 5µm (504947)
Mobile Phase: 60:40, 0.1%TFA in Water:MeOH
Flow Rate: 2.0mL/min
Temp.: 20°C
Det.: UV at 254nm
Inj.: 10µL

1. Homovanillic acid (0.0625µg/mL)
2. Sorbic acid (0.00625µg/mL)
3. Salicylic acid (0.0625µg/mL)
4. p-Toluic acid (0.00625µg/mL)

0 2 Min G001431

Figure 3: Antibiotics (Fluoroquinolones from Tablets)

Column: Discovery C18, 15cm x 4.6mm ID, 5µm (504955)
Mobile Phase: (A) 25 mM Potassium Phosphate (pH 3.0)
(B) CH_3CN
Flow Rate: 1.0mL/min
Temp.: 35°C
Det.: UV at 220nm
Inj.: 10µL

1. Levofloxacin
2. Ciprofloxacin
3. Lomefloxacin
4. Sparfloxacin
5. Grepafloxacin
6. Trovafloxacin

Gradient:

Min	%A	%B
0	90	10
15	65	35

0 2 4 6 8 10 12 14 Min G001424

Discovery C18

Excellent Peak Shape Compared to Competitive C18 Columns

All Discovery HPLC phases begin with pure, metal-free, high quality silica and employ advanced bonded phase technology. As a result, they give excellent peak shape in simple mobile phases.

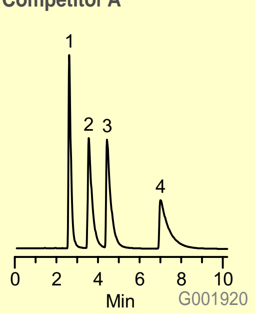
Figure 1: Tricyclic Antidepressants

Columns: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 55:45, 25mM Ammonium Phosphate (pH 7):CH_3CN
Flow Rate: 1mL/min
Temp.: 30°C
Det.: UV at 254nm
Sample: 10µL, each compound 50µg/mL

1. Nordoxepin
2. Nortriptyline
3. Doxepin
4. Amitriptyline

Discovery C18

1 2 3 4
0 2 4 6
Min G001919



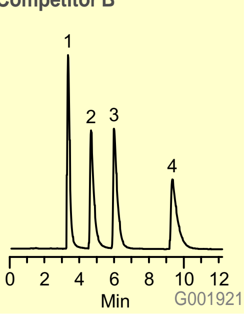
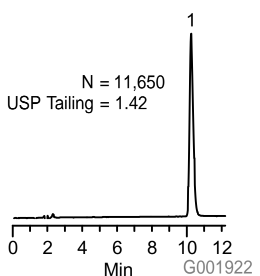
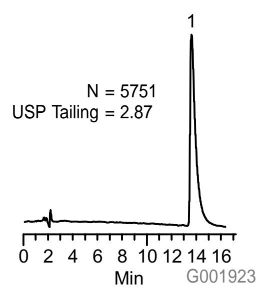


Figure 2: Phentermine

Columns: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 90:10, 25mM Ammonium Phosphate (pH 7):CH_3CN
Flow Rate: 1mL/min
Temp.: 30°C
Det.: UV at 210nm
Sample: 10µL, Phentermine, 50µg/mL





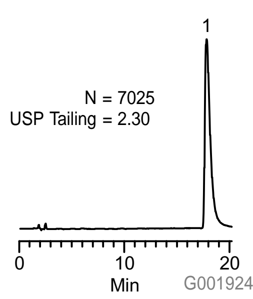
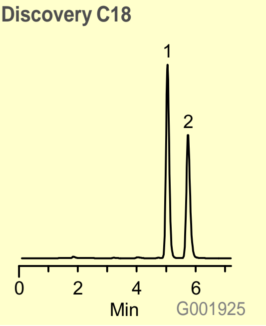
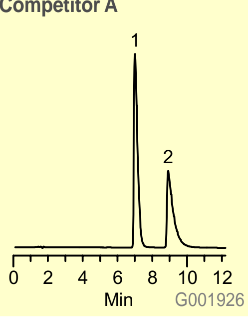


Figure 3: Fluoxetines

Columns: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 60:40, 25mM Ammonium Phosphate (pH 7):CH_3CN
Flow Rate: 1mL/min
Temp.: 30°C
Det.: UV at 227nm
Sample: 10µL, each compound 50µg/mL

1. Fluoxetine
2. Norfluoxetine





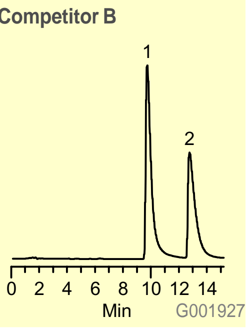
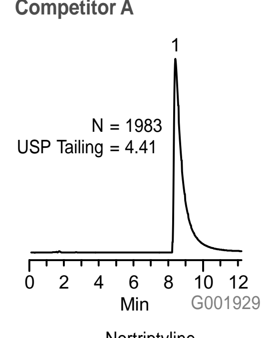


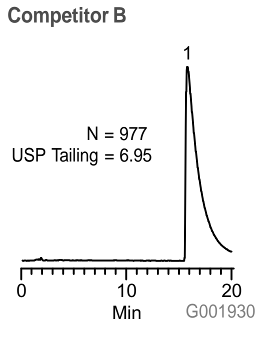
Figure 4: Quinidine

Columns: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 75:25, 25mM Ammonium Phosphate (pH 7):CH_3CN
Flow Rate: 1mL/min
Temp.: 30°C
Det.: UV at 230nm
Sample: 10µL, Quinidine, 50µg/mL

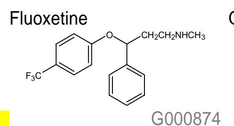
Discovery C18

1
N = 9059
USP Tailing = 1.36
0 2 4 6 8 10
Min G001928

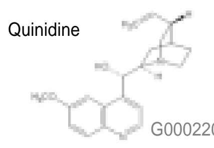




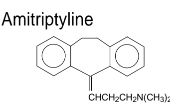
Fluoxetine



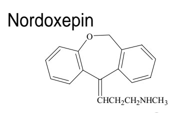
Quinidine



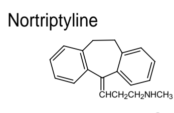
Amitriptyline



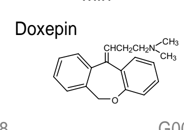
Nordoxepin

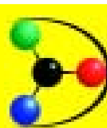


Nortriptyline



Doxepin





Discovery C18

LC/MS Compatibility

Stable Bonded Phases Suitable for LC/MS

What is column bleed and why is it important?

Column bleed manifests itself as continuous elevated background noise in a total ion chromatogram (TIC). This background, not attributable to sample, mobile phase constituents, or source contamination, may be a result of:

- Elution of non-covalently bonded reagent from the stationary phase
- Hydrolysis, under acidic conditions, of bonded phase from the column packing

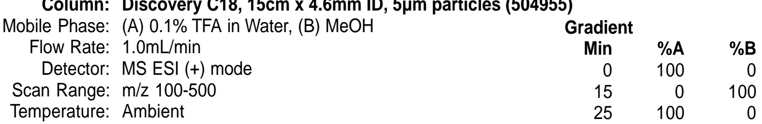
Column bleed:

- Complicates mass spectral analysis of unknowns
- Raises background noise levels which often interfere with the detection of unknowns
- Interferes with quantitation if the m/z response is close to the m/z response of the target analyte

Supelco's Discovery C18 has been extensively tested by an independent testing laboratory[1] and has been shown not to bleed under rigorous conditions. The data shows the averaged mass spectra obtained from the designated (shaded) regions of the total ion chromatogram. A blank was also run, that is, the same conditions used with the column in-line were run after removing the column and replacing it with a zero dead volume union.

The X's on each mass spectrum indicate that the mass was found both in the blank run and in the run containing the column. Note that nearly all the major masses are accounted for in the blank when comparing it when the column was installed. This indicates essentially no bleed coming from the Discovery C18 phase, but these spurious responses are coming from other origins.

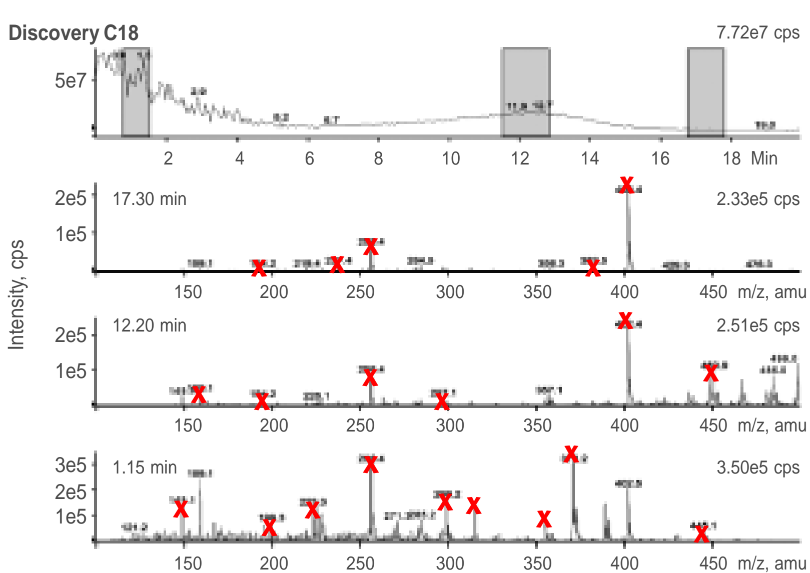
Figure 1: Discovery C18 is Low Bleed for LC/MS

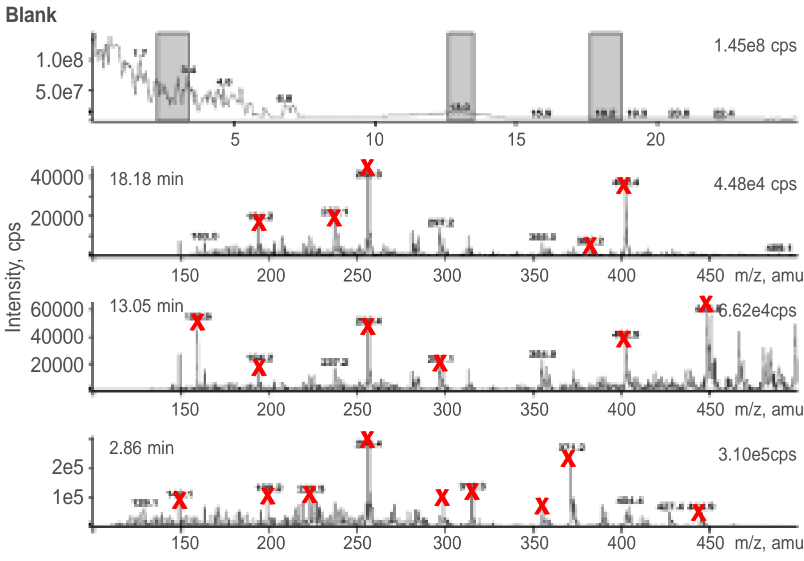


Column: Discovery C18, 15cm x 4.6mm ID, 5µm particles (504955)
Mobile Phase: (A) 0.1% TFA in Water, (B) MeOH
Flow Rate: 1.0mL/min
Detector: MS ESI (+) mode
Scan Range: m/z 100-500
Temperature: Ambient

Gradient

Min	%A	%B
0	100	0
15	0	100
25	100	0





[1] Above LC/MS results obtained from Dr. Shane Needham, Laboratory Director, Alturas Analytics, Inc. Moscow, ID, an independent testing laboratory.

Discovery C18

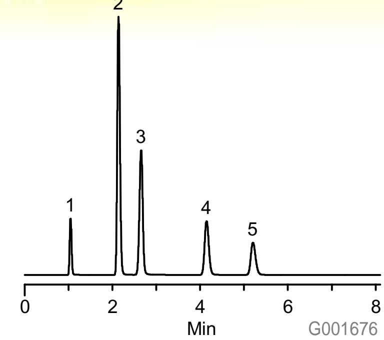
Excellent Reproducibility

Consistent Column-to-Column Reproducibility is Critical to Successful Method Development

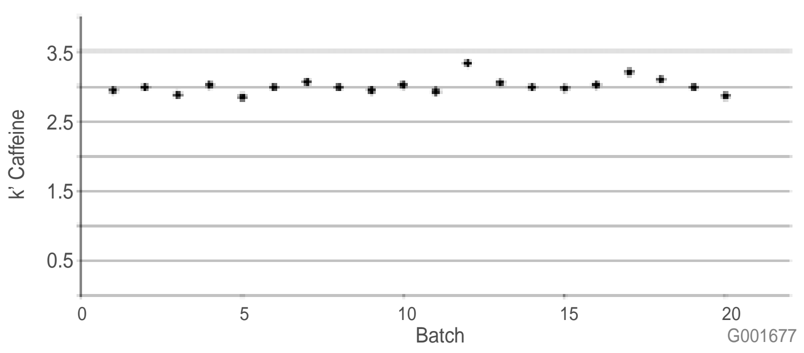
Figure 1: C18

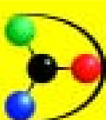
Column: **Discovery C18, 15cm x 4.6mm, 5µm particles (504955)**
Mobile Phase: 83:17, 25mM Potassium Phosphate (pH 7.5): MeOH
Flow Rate: 2mL/min
Det.: UV at 260nm
Temp.: 35°C
Inj.: 10µL
Sample: as indicated below (in 83:17 Water:MeOH)

1. Uracil (15µg/mL)
2. Sorbic Acid (30µg/mL)
3. Procainamide (150µg/mL)
4. Caffeine (100µg/mL)
5. Phenol (300µg/mL)



Reproducibility of k' Caffeine on Production Batches of Discovery C18





Discovery Silica-Based Phases

Discovery HS F5

Unique Retention and Selectivity Enables Better Separations

The Discovery HS F5 bonded phase provides reversed-phase separations that are distinctly different from C18 columns. However, compounds will generally elute within the same retention time window, making most C18 methods easily transferable.

Discovery HS F5 Delivers….

- Unique selectivity
- Similar retention to C18 (sometimes requires stronger mobile phases)
- Excellent peak shape
- Stable, low-bleed LC/MS separations
- Scalable separations from 3 to 10µm

Properties of Discovery HS F5

USP Code	L43
Bonded Phase	Pentafluorophenylpropyl
Endcap (yes / no)	Yes
Particle Platform	Silica
Particle Shape	Spherical
Particle Purity	<10ppm metals
Particle Sizes (µm)	3, 5, and 10
Pore Size (Å)	120
Surface Area (m²/g)	300
Packing Density (g/mL)	0.58
%C	12
Coverage (µmoles/m²)	4
pH Range	2 to 8
Temperature Range	≤70°C

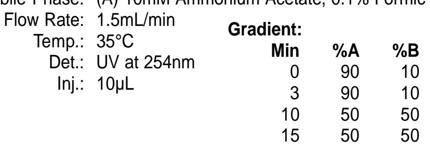
Guidelines for transferring a C18 method to Discovery HS F5:

Generally, bases are longer retained on the HS F5 than on a C18. Increasing the organic content of a C18 separation 5 to 10 percent will generally provide similar retention on a HS F5. Results with other compounds are highly variable. However, it is generally true that solutes with $\log P_{o/w}$ values less than 2.5 will be retained longer on HS F5 compared to a C18. The degree of difference is highly solute dependent.

Figure 1: Excellent Retention of Multifunctional Compounds

The Discovery HS F5 shows greater retention, versus C18, of the multifunctional compounds shown in these chromatograms. Compounds that elute too closely to the void volume (peak 1) on C18 columns are sufficiently retained by Discovery HS F5.

Columns: **(A) Discovery HS F5 and Conventional C18, (B) 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: (A) 10mM Ammonium Acetate, 0.1% Formic Acid; (B) MeOH
Flow Rate: 1.5mL/min
Temp.: 35°C
Det.: UV at 254nm
Inj.: 10µL



Gradient:

Min	%A	%B
0	90	10
3	90	10
10	50	50
15	50	50

1. p-Aminophenol (100µg/mL)
2. Acetaminophen (10µg/mL)
3. Acetanilide (10µg/mL)
4. Phenacetin (10µg/mL)

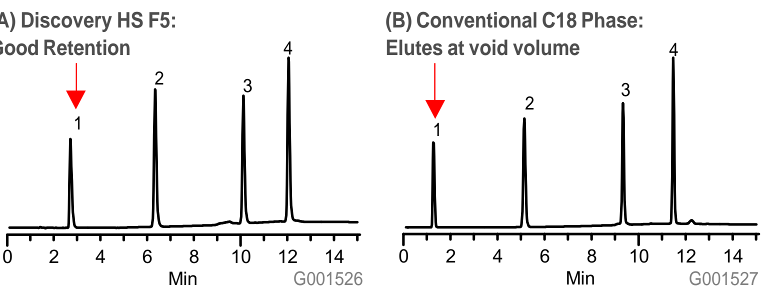


Figure 2: HS F5 Provides Excellent Separation - Solutes Are Not Retained on C18

Columns: **(A) Discovery HS F5 and (B) Conventional C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 30:70, 10mM Ammonium Acetate (pH 6.98): CH_3CN
Flow Rate: 2.0mL/min
Temp.: 35°C
Det.: Photodiode Array
Inj.: 5µL

1. Methcathinone (100µg/mL)
2. (+/-) Ephedrine (200µg/mL)

(A) Discovery HS F5

0 1 2 3 4 5 6 7 8 G001673

(B) Conventional C18 - No Retention

0 1 2 3 4 5 6 7 8 G001672

Unique Retention and Selectivity Enables Better Separations

The Discovery HS F5 bonded phase provides reversed-phase separations that are distinctly different from C18 columns. However, compounds will generally elute within the same retention time window, making most C18 methods easily transferable.

Discovery HS F5, a unique, functionalized reversed-phase uncovers a trace impurity in quinidine missed by C18. Neat quinidine was assayed on C18 under a variety of mobile phase conditions (see Figure 1). Conditions C and D produced a single peak suggesting the quinidine was pure. The peak resulting from condition B might be showing partially resolved front shoulder. A quick screen of % organic was unable to resolve the possible impurity. On the HS F5 (chromatogram A) the impurity is clearly resolved. During method development a quick screen using unique, functionalized reversed-phases such as Discovery HS F5, greatly increases the chances of finding trace impurities early, before they can cause potentially large problems.

In Figure 2, cytidine and related compounds provide another example of the power of HS F5 to provide unique and valuable separations compared to a C18. An added benefit of the HS F5 is its resistance to phase collapse under 100% aqueous conditions.

Figure 1: HS F5 Resolves Trace Impurity in Quinidine – C18 Does Not

Column: **Discovery HS F5 and Conventional C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 25 mM Ammonium Phosphate (pH 7.0):CH_3CN.
Varying Ratios: (A) 35:65, (B) 70:30, (C) 76:24, (D) 80:20
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV at 235nm
Inj.: 10µL

1. Quinidine (50µg/mL)
2. Impurity (Dihydroquinidine)

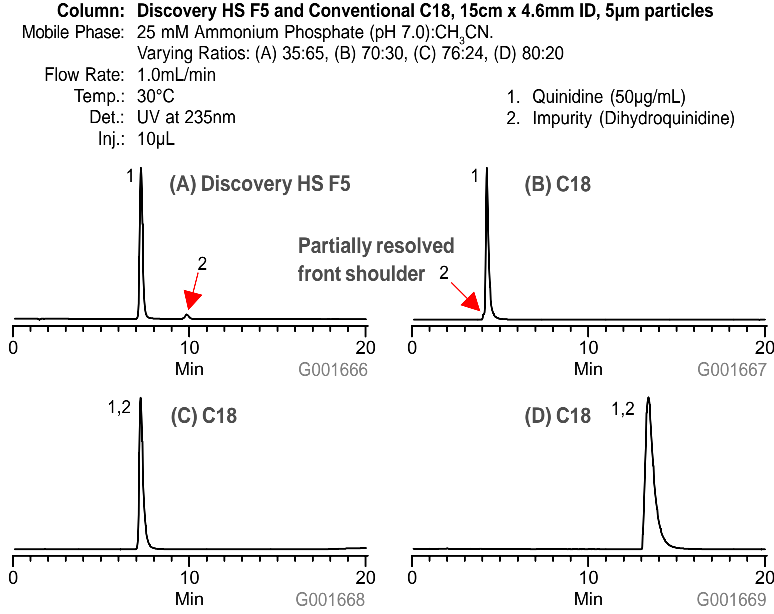
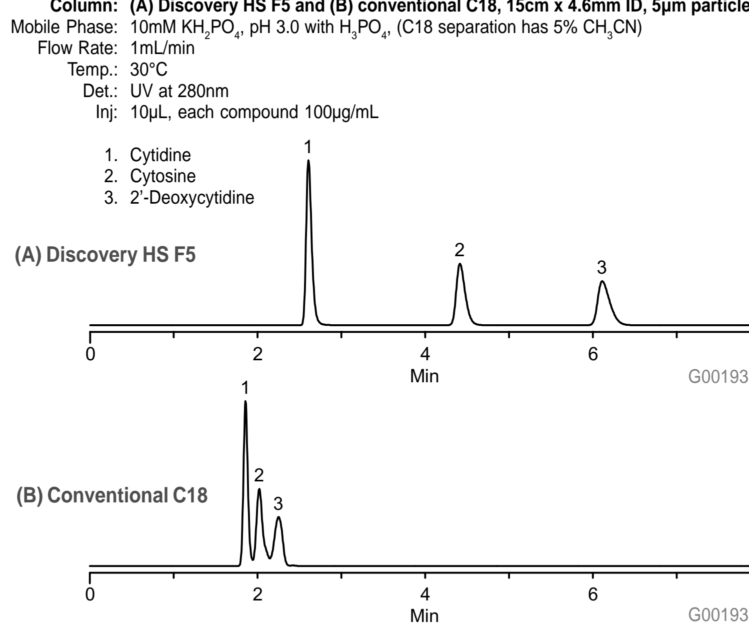
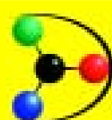


Figure 2: Unique Selectivity of HS F5 Resolves Compounds Better than C18

Column: **(A) Discovery HS F5 and (B) conventional C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 10mM KH_2PO_4, pH 3.0 with H_3PO_4, (C18 separation has 5% CH_3CN)
Flow Rate: 1mL/min
Temp.: 30°C
Det.: UV at 280nm
Inj: 10µL, each compound 100µg/mL

1. Cytidine
2. Cytosine
3. 2'-Deoxycytidine





LC/MS Compatibility

Stable Bonded Phases Suitable for LC/MS

Supelco's Discovery HS F5

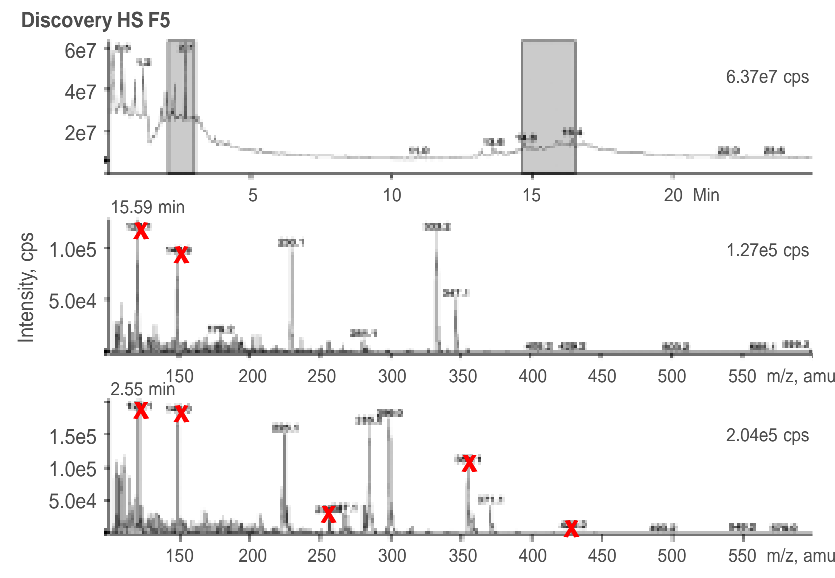
The HS F5 shows low acceptable bleed after three conditioning cycles as verified by an independent testing laboratory[1]. The data shows the averaged mass spectra obtained from the designated (shaded) regions of the total ion chromatogram. A blank was also run, that is, the same conditions used with the column in-line were run after removing the column and replacing it with a zero dead volume union.

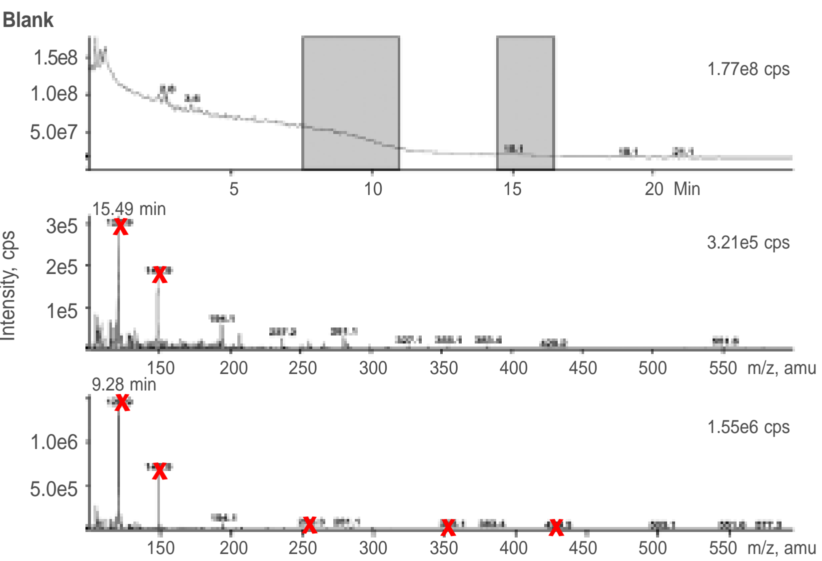
The X's on each mass spectrum indicate the mass found both in the blank run and in the run containing the column. Note that many of the major masses are accounted for in the blank when comparing it when the column was installed. This indicates low, acceptable bleed coming from the Discovery HS F5 phase.

Figure 1: HS F5 is Low Bleed for LC/MS

Column: **Discovery HS F5, 15cm x 4.6mm, 5µm particles (567516-U)**
Mobile Phase: (A) 0.1% TFA in Water, (B) MeOH
Flow Rate: 1.0mL/min
Detector: MS ESI (+) mode
Scan Range: m/z 100-500
Temperature: Ambient

HS F5 exhibits low bleed after just 3 conditioning cycles. Note also the aggressive mobile phase used for this test.





[1] Above LC/MS results obtained from Dr. Shane Needham, Laboratory Director, Alturas Analytics, Inc. Moscow, ID, an independent testing laboratory.

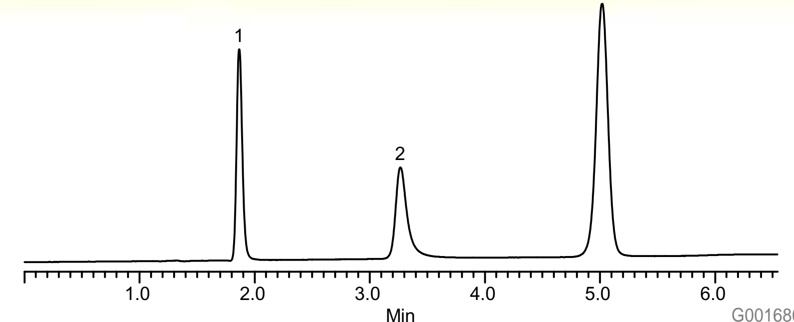
Reproducibility and Column Lifetime

Durable, Reproducible Columns Minimize Downtime for Column Replacement and Troubleshooting

Figure 1: Reproducibility

Column: **Discovery HS F5, 15cm x 4.6mm ID, 5µm particles (567516-U)**
Mobile Phase: 55:45, 10mM Potassium Phosphate (pH 7.0): MeOH
Flow Rate: 1mL/min
Det.: UV at 254nm
Temp.: 35°C
Inj.: 5µL
Sample: as indicated below (in 55:45 Water:MeOH)

1. Uracil (15µg/mL)
2. Pyridine (20µg/mL)
3. Phenol (300µg/mL)



Reproducibility of k' Pyridine on Production Batches of Discovery HS F5

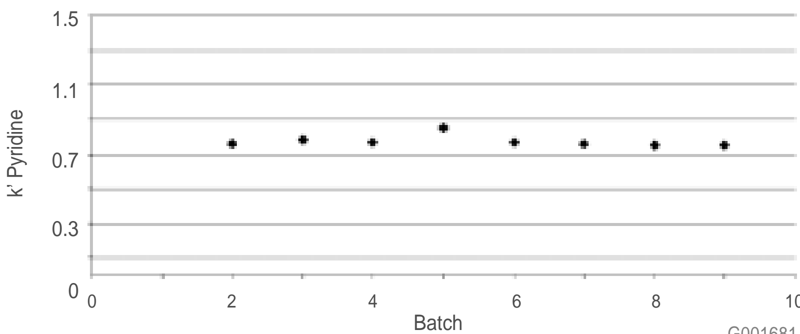
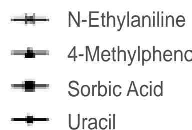


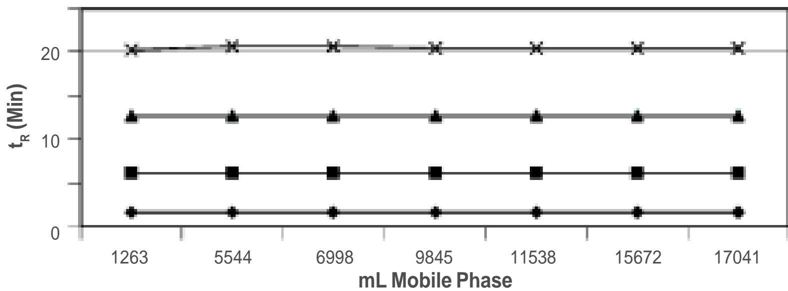
Figure 2: Column Lifetime

Good stability at pH 3

Column: **Discovery HS F5, 5cm x 4.6mm ID, 3µm particles (567504-U)**
Mobile Phase: 30:70, 0.1% Formic Acid and 10mM Ammonium Formate (pH 3.4): CH_3CN
Flow Rate: 1.0mL/min
Det.: UV at 254nm
Temp.: 35°C
Inj.: 5µL

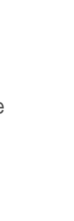


HS F5 - Stability at pH 3

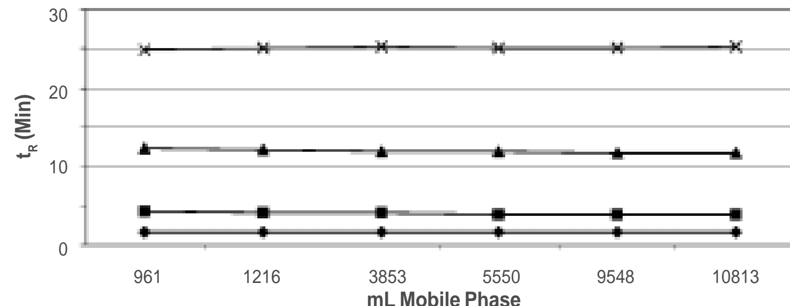


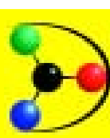
Good stability at pH 7

Column: **Discovery HS F5, 5cm x 4.6mm, 3µm particles (567504-U)**
Mobile Phase: 80:20, 10 mM Ammonium Acetate (pH 6.8): CH_3CN
Flow Rate: 1.0mL/min
Det.: UV at 254nm
Temp.: 35°C
Inj.: 5µL



HS F5 - Stability at pH 7





Discovery HS F5

Scalability

Scale-Up to Preparative; Scale-Down to High Speed or Narrowbore Separations

Bonded phase and silica chemistry are uniform across all Discovery particle sizes.

Precious samples can be wasted during scale-up if the analytical and preparative columns do not give the same elution pattern.

Analytical separations that are developed on Discovery 3 or 5 micron particles are completely scalable to preparative separations on Discovery 10 micron particles and larger columns. Additionally, separations developed on 5 or 10 micron particles can be scaled down for fast analysis on 3 micron particles.

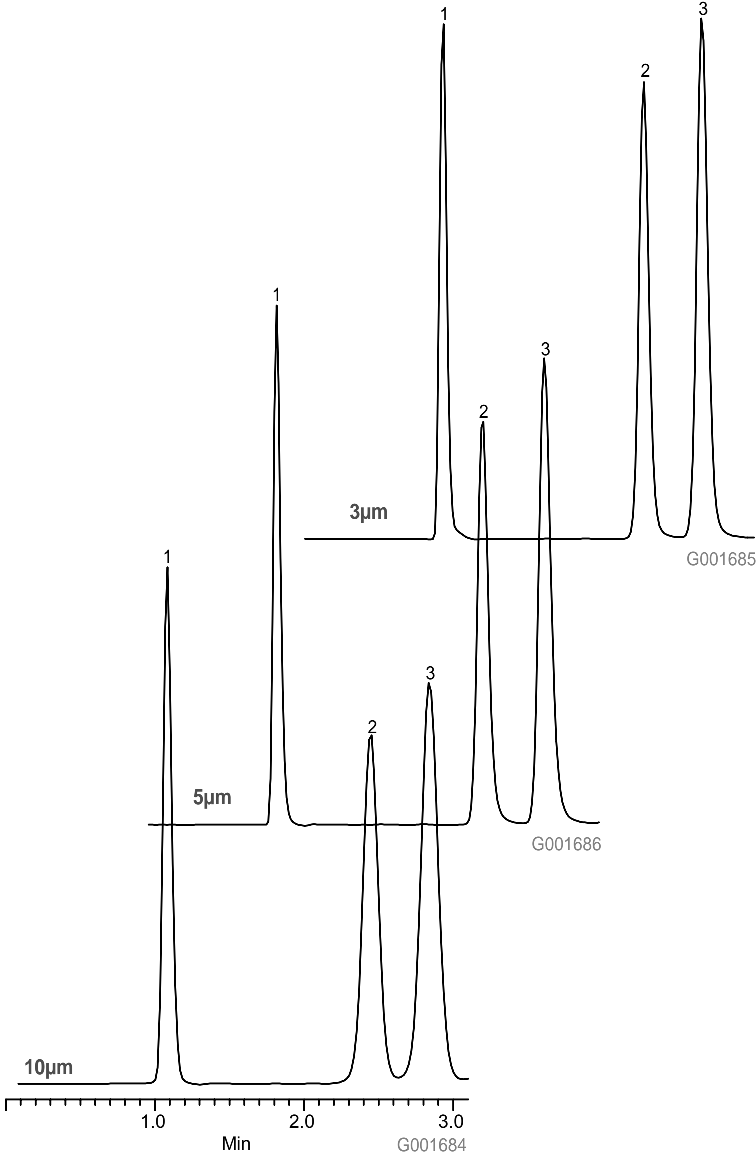
- Discovery 10 micron particles in large column dimensions are ideal for isolating and purifying mg to gram amounts of compounds for further characterization.
- Discovery 3 micron particles in short columns are ideal for rapid analysis and LC/MS applications.

The breadth of the Discovery column dimension offering can be seen in the product listing at the end of this brochure.

Figure 1: Procainamides on Three Particle Sizes of HS F5

Column: **Discovery HS F5, 10cm x 4.6mm ID, 3µm 5µm, and 10µm particles**
Mobile Phase: 65:35, 25mM Ammonium Phosphate (pH 7):CH_3CN
Flow Rate: 1.0mL/min (3.5µm); 4.73mL/min (10µm)
Temp.: 30°C
Det.: UV at 280nm
Inj.: 5µL (3.5µm); 23.7µL (10µm)
Sample: 50µg/mL of each

1. 5-Fluorocytosine (t_o)
2. N-Acetylprocainamide
3. Procainamide



Discovery HS PEG

Unique Retention and Selectivity Enables Better Separations

Discovery HS PEG bonded phase provides reversed-phase separations that are distinctly different from C18 columns. It is an ideal candidate to choose when C18 columns give too much retention, when there is too much wasted space between peaks, or when you want to convert a gradient to an isocratic separation.

Discovery HS PEG Delivers….

- Unique selectivity
- Significantly lower hydrophobic retention, requires lower % organic mobile phases
- Stable, no-bleed LC/MS separations

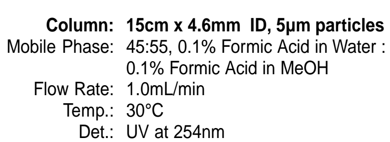
Properties of Discovery HS PEG

Bonded Phase	Polyethyleneglycol
Endcap (yes / no)	No
Particle Platform	Silica
Particle Shape	Spherical
Particle Purity	<10ppm metals
Particle Sizes (µm)	3, 5, 10
Pore Size (Å)	120
Surface Area (m²/g)	300
Packing Density (g/mL)	0.58
%C	12
Coverage (µmoles/m²)	3.8
pH Range	2 to 8
Temperature Range	≤70°C

Guidelines for transferring a C18 method to Discovery HS PEG:

When using the PEG in RP mode, reduce the % organic by at least 25% over what you would use on a C18. If retention is not obtained on C18 (except for very polar analytes capable of hydrogen bonding, like polyphenols) the likelihood of retention on PEG is small. HS PEG can also operate in a normal phase mode.

Figure 1: Flavones Demonstrate Dramatic Selectivity Differences Between HS PEG and C18



Column: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 45:55, 0.1% Formic Acid in Water : 0.1% Formic Acid in MeOH
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV at 254nm
Inj.: 10µL, each compound 50µg/mL

1. Myricetin
2. Quercetin
3. Luteolin
4. Baicalein
5. 7-Hydroxyflavone
6. Flavone
7. Chrysin
8. 5-Hydroxyflavone

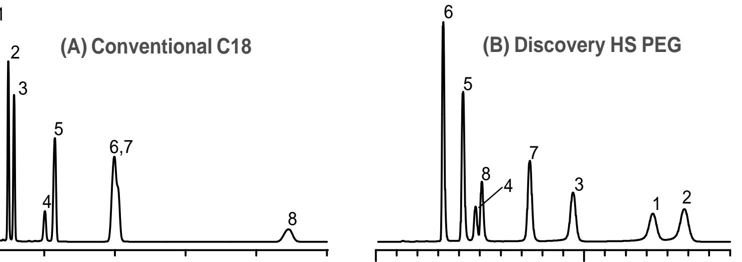
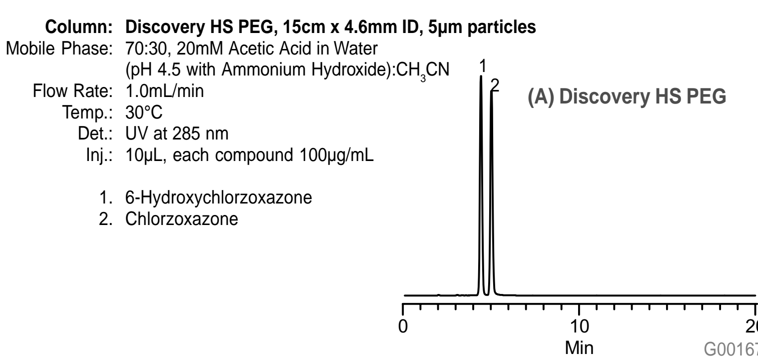


Figure 2: Chlorzoxazone - Excellent Separation on HS PEG; Excessive Retention and Resolution on Conventional C18

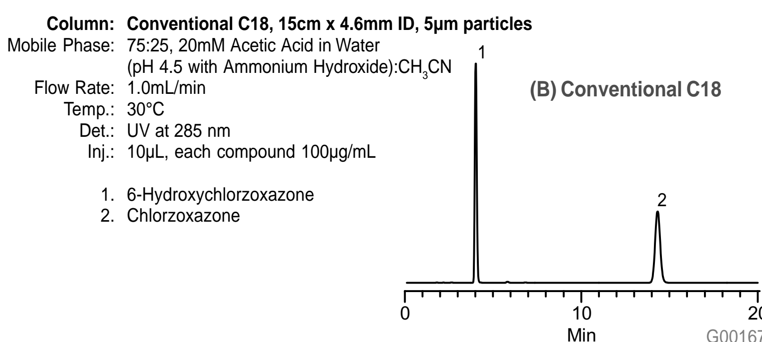
Column: **Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 70:30, 20mM Acetic Acid in Water (pH 4.5 with Ammonium Hydroxide):CH_3CN
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV at 285 nm
Inj.: 10µL, each compound 100µg/mL

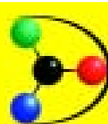
1. 6-Hydroxychlorzoxazone
2. Chlorzoxazone



Column: **Conventional C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 75:25, 20mM Acetic Acid in Water (pH 4.5 with Ammonium Hydroxide):CH_3CN
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV at 285 nm
Inj.: 10µL, each compound 100µg/mL

1. 6-Hydroxychlorzoxazone
2. Chlorzoxazone





Discovery HS PEG

Unique Retention and Selectivity Enables Better Separations

Ideal for Samples with Widely Varying Hydrophobicity. Can Eliminate the Need for Gradients.

Discovery HS PEG provides very different selectivity of polar phenolic compounds than the C18. The HS PEG column eliminates the excessive retention and wasted resolution.

Generally, as hydrophobicity of the solute increases, retention on a C18 column increases rapidly relative to retention on the HS PEG column.

Figure 1: HS PEG Compresses Analytes by Reducing the Relative Retention Difference between Polar and Non-Polar Compounds

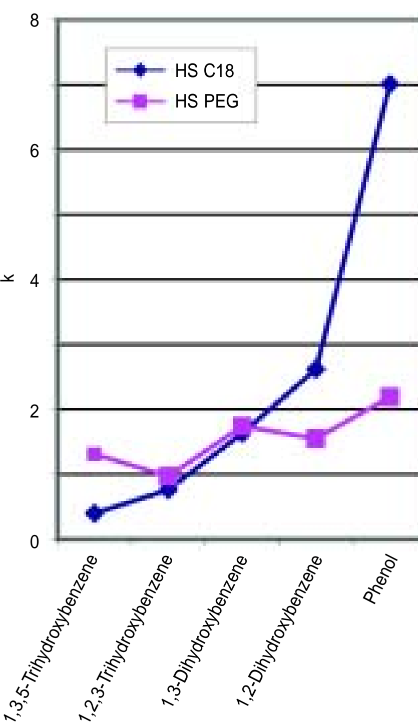


Figure 2: Resolution of Phenolic Compounds on Discovery HS PEG Compared to Standard C18

Column: **(A) Discovery HS PEG and (B) Conventional C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 85% 10mM ammonium acetate, pH 6.8:15% CH_3CN
Flow Rate: 1.0mL/min
Temp.: 20°C
Det.: Photodiode Array
Inj.: 10µL, each compound 50µg/mL

(A) Discovery HS PEG

Note the selectivity differences of the phenolic compounds between the conventional C18 and the Discovery HS PEG phase.

Especially note the improved retention of phloroglucinol (Peak 2) and phenetole (Peak 9) on the Discovery HS PEG phase

1. Uracil
2. Phloroglucinol
3. Pyrogallol
4. Resorcinol
5. Benzamide
6. Catechol
7. Phenol
8. Nitrobenzene
9. Phenetole

0 5 10 15

G001533

(B) Conventional C18 Column

Phenetole (9) is not eluted under these conditions on C18

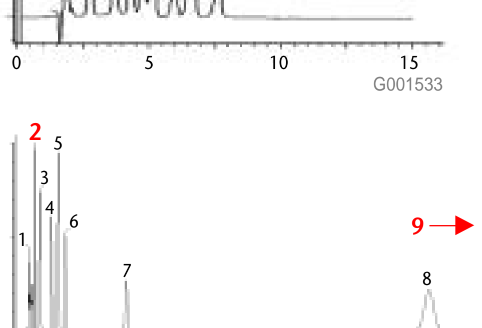
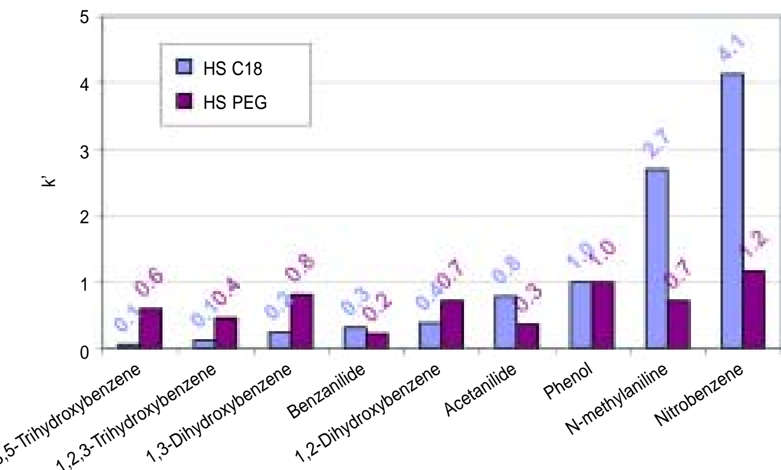


Figure 3: Selectivity Relative to Phenol for a Series of Compounds is Very Different on HS PEG Compared to C18.



Discovery HS PEG

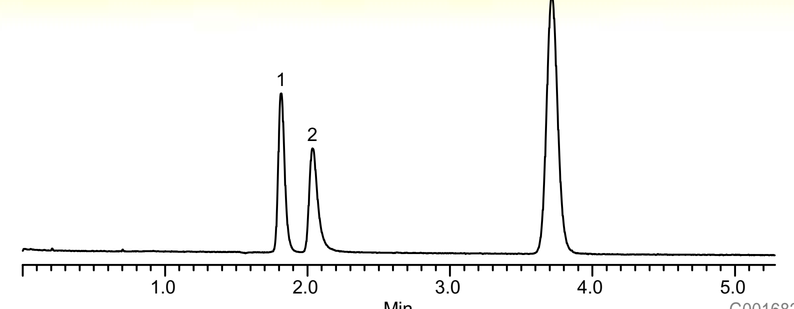
Reproducibility and Column Lifetime

Durable, Reproducible Columns Minimize Downtime for Column Replacement and Troubleshooting

Figure 1: Reproducibility

Column: **Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles (567416-U)**
Mobile Phase: 55:45, 10mM Potassium Phosphate (pH 7.0): MeOH
Flow Rate: 1mL/min
Det.: UV at 254nm
Temp.: 35°C
Inj.: 5µL
Sample: as indicated below (in 55:45 Water:MeOH)

1. Uracil (15µg/mL)
2. Pyridine (20µg/mL)
3. Phenol (300µg/mL)



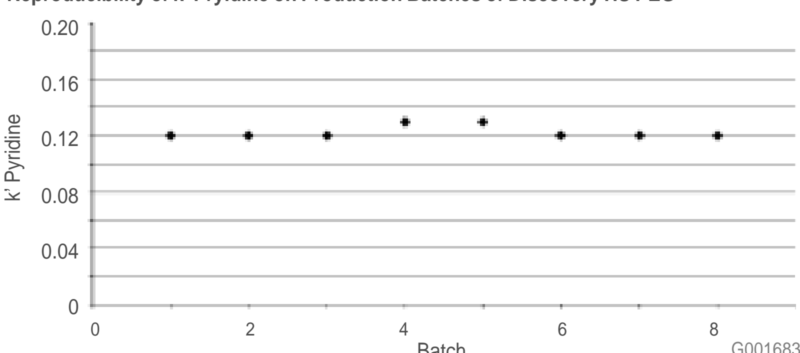
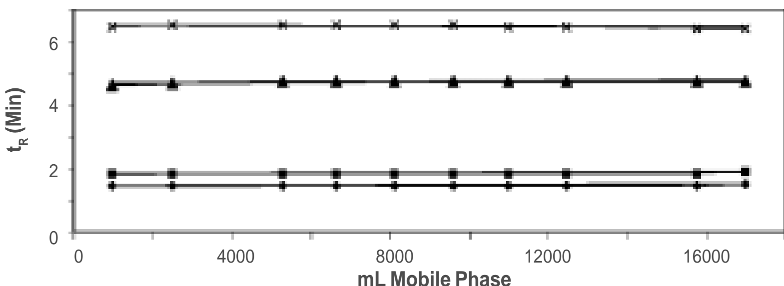


Figure 2: Column Lifetime

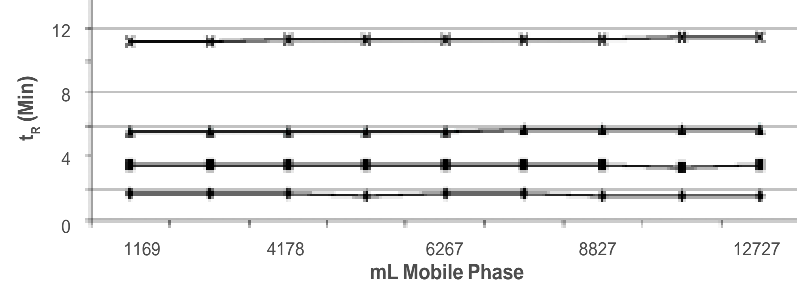
Good stability at pH 2.5

Column: **Discovery HS PEG, 5cm x 4.6mm ID, 3µm particles (567402-U)**
Mobile Phase: 90:10, 0.1% Formic Acid (pH 2.5): CH_3CN
Flow Rate: 1.0mL/min
Det.: UV at 254nm
Temp.: 35°C
Inj.: 5µL



Good stability at pH 7

Column: **Discovery HS PEG, 5cm x 4.6mm ID, 3µm particles (567402-U)**
Mobile Phase: 85:15, 10mM Ammonium Acetate (pH 6.8): CH_3CN
Flow Rate: 1.0mL/min
Det.: UV at 254nm
Temp.: 35°C
Inj.: 5µL



Discovery HS PEG

LC/MS Compatibility

Stable Bonded Phases Suitable for LC/MS

Discovery HS PEG has been extensively tested by an independent testing laboratory[1] and has been shown not to bleed under rigorous conditions. The data shows the averaged mass spectra obtained from the designated (shaded) regions of the total ion chromatogram.

A blank was also run, that is, the same conditions used with the column in-line were run after removing the column and replacing it with a zero dead volume union.

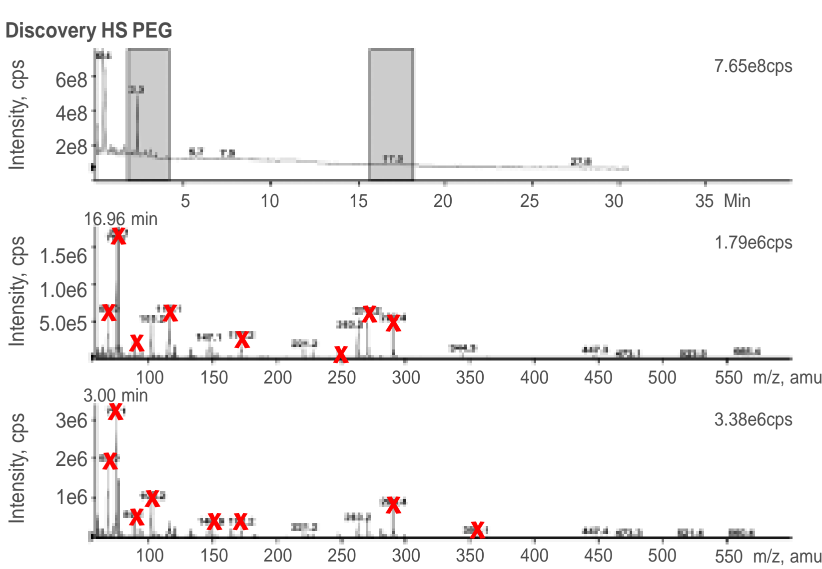
The X's on each mass spectrum indicate that the mass was found both in the blank run and in the run containing the column. Note that nearly all the major masses are accounted for in the blank when comparing it when the column was installed. This indicates essentially no bleed coming from the Discovery HS PEG phase, but these spurious responses are coming from other origins.

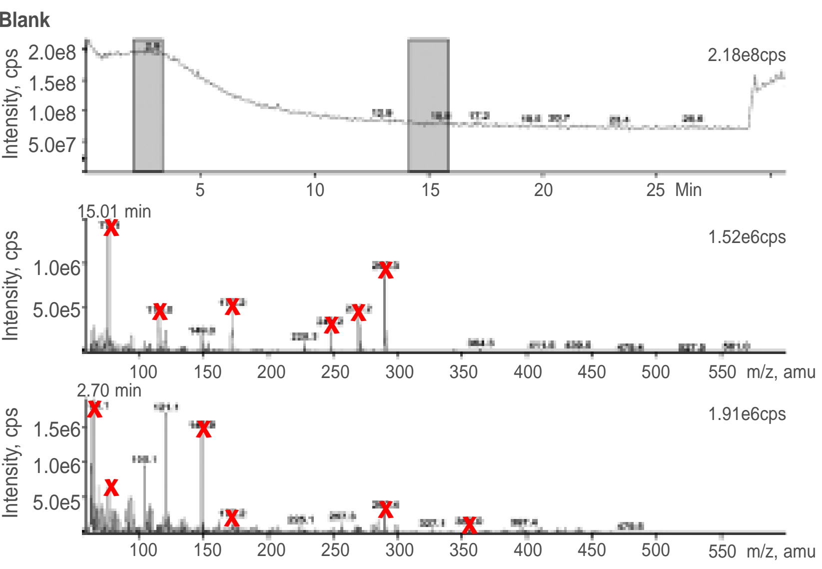
Figure 1: HS PEG is Low Bleed for LC/MS

Column: **Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: (A) 0.1% TFA in Water, (B) MeOH
Flow Rate: 1.0 mL/min
Detector: MS ESI(+) mode
Scan Range: m/z 100-500
Temperature: Ambient

Gradient

Min	%A	%B
0	100	0
15	0	100
25	100	0





[1] Above LC/MS results obtained from Dr. Shane Needham, Laboratory Director, Alturas Analytics, Inc. Moscow, ID, an independent testing laboratory.

Discovery RP-AmideC16

$-O-Si(CH_3)(CH_3)-(CH_2)_3NHCO-(CH_2)_{14}-CH_3$

Unique Retention and Selectivity Enables Better Separations

Discovery RP-AmideC16 Delivers….

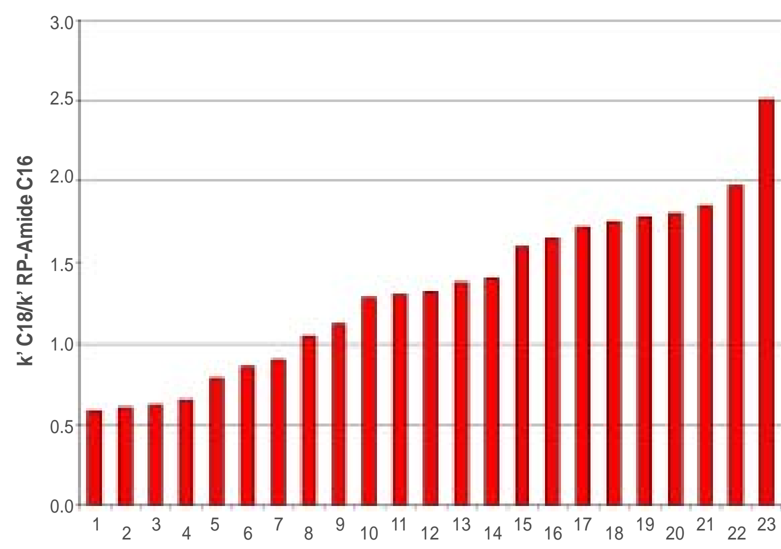
- Unique selectivity compared to C18
- Excellent peak shape and efficiency

Properties of Discovery RP-AmideC16

USP Code	(Pending L57)
Bonded Phase	Palmitamidopropylsilane
Endcap (yes / no)	Yes
Particle Platform	Silica
Particle Shape	Spherical
Particle Purity	<10ppm metals
Particle Sizes (µm)	5
Pore Size (Å)	180
Surface Area (m²/g)	200
Packing Density (g/mL)	0.58
%C	11
Coverage (µmoles/m²)	2.6
pH Range	2 to 8
Temperature Range	≤70°C

Due to the nature of the bonded phase, we do not recommend the RP-AmideC16 be used for LC/MS applications.

Figure 1: Retention on C18 Relative to RP-AmideC16



1. Acetaminophen
2. Methyl paraben
3. Ethyl paraben
4. Naproxen
5. Ketoprofen
6. Propyl paraben
7. Butyl paraben
8. Quinidine
9. Quinine
10. Desacetyldiltiazem
11. Clonazepam
12. Cimetidine
13. Diltiazem
14. Strychnine
15. Nizatidine
16. Clorazepate
17. Ranitidine
18. Desmethyldiltiazem
19. Famotodine
20. Codeine
21. Diazepam
22. Papaverine
23. Noscapine

Discovery RP-AmideC16

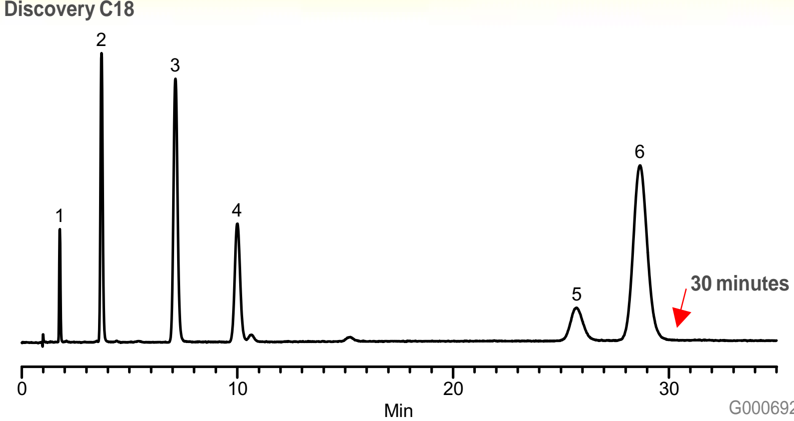
Unique Retention and Selectivity Enables Better Separations

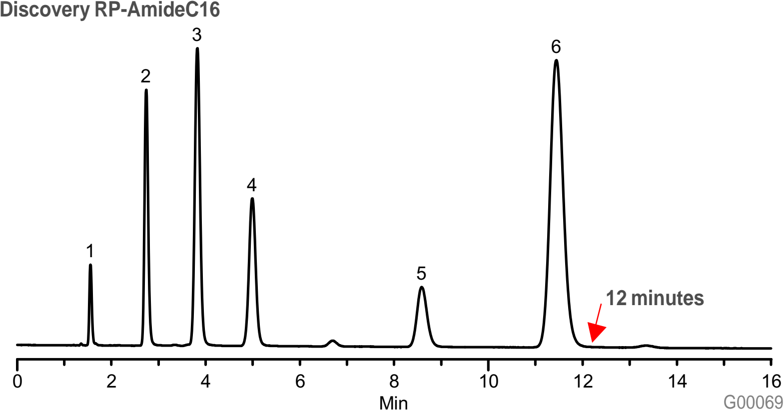
The compounds in Figure 1 show that the Discovery RP-AmideC16 can provide faster analysis (due to its lower hydrophobicity), better peak spacing, and better resolution of small impurity peaks (due to its different selectivity).

Column: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 80:20, 25mM Potassium Phosphate (pH 3.0):MeOH
Flow Rate: 2.0mL/min
Temp.: 35°C
Det.: UV at 254nm
Inj.: 10µL

1. Codeine
2. Strychnine
3. Quinidine
4. Quinine
5. Noscapine
6. Papaverine

Figure 1: Discovery RP-AmideC16 Gives Better Resolution and Faster Analysis



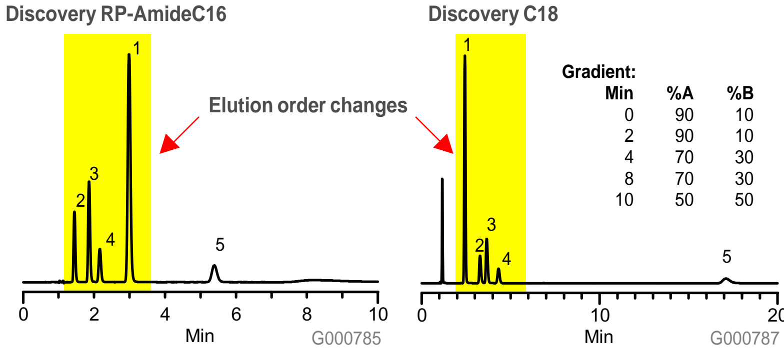


Using a Discovery RP-AmideC16 can result in dramatic differences in peak order and run time compared to a C18 as shown in Figure 2.

Column: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: (A) 25mM Potassium Phosphate (pH 2.3)
(B) CH_3CN
Flow Rate: 2.0mL/min
Temp.: ambient
Det.: UV at 214nm
Inj.: 10µL, each compound 1µg/mL

1. Acetaminophen
2. Doxylamine
3. Pseudoephedrine
4. Codeine
5. Chlorpheniramine

Figure 2: Antitussive/Antihistamine/Antipyretic Mix



Discovery Cyano

CH3
|—O—Si—(CH2)3—CN
CH3

Unique Retention and Selectivity Enables Better Separations

Discovery Cyano Delivers....

- Excellent peak shape
- Unique selectivity
- Significantly less retention than C18 (typically requires lower % organic mobile phase)
- Stable, low-bleed LC/MS separations

Properties of Discovery Cyano

USP Code	L10
Bonded Phase	Cyanopropyl
Endcap (yes / no)	Yes
Particle Platform	Silica
Particle Shape	Spherical
Particle Purity	<10ppm metals
Particle Sizes (µm)	5
Pore Size (Å)	180
Surface Area (m²/g)	200
Packing Density (g/mL)	0.58
%C	4.5
Coverage (µmoles/m²)	3.5
pH Range	2 to 8
Temperature Range	≤70°C

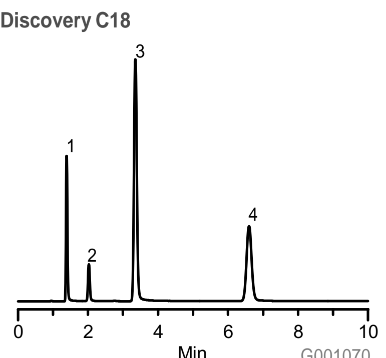
Figure 1: Faster Analysis - Eliminate Wasted Time

Urea Pesticides Using Isocratic Elution

Column: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 60:40, Water:CH_3CN
Flow Rate: 2.0mL/min
Temp.: 20°C
Det.: UV at 214nm
Inj.: 1µL

1. Fenuron
2. Monuron
3. Diuron
4. Linuron

Discovery C18



Discovery Cyano

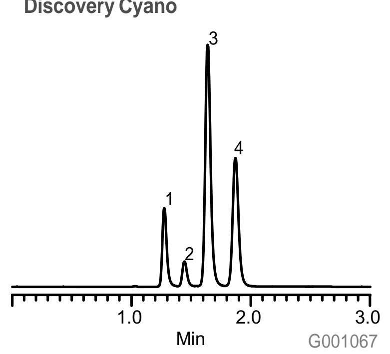


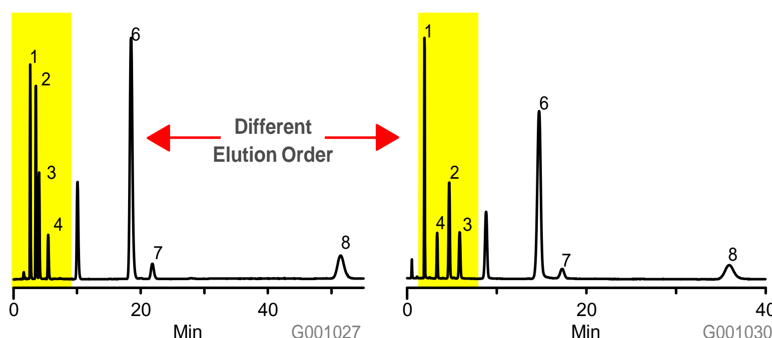
Figure 2: Faster Analysis - Different Selectivity

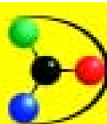
Organophosphorous Pesticides Using Isocratic Elution

Column: **Discovery C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 30:70, Water:MeOH
Flow Rate: 1.0mL/min
Temp.: 20°C
Det.: UV at 214nm
Inj.: 1µL

Column: **Discovery Cyano, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 75:25, Water:CH_3CN
Flow Rate: 2.0mL/min
Temp.: 20°C
Det.: UV at 214nm
Inj.: 1µL

1. Dichlorvos
2. Guthion
3. Methyl parathion
4. Ethoprophos
5. Disulfoton
6. Fenchlorvos
7. Chlorpyrifos
8. Prothiophos





Discovery C8

CH_3 — O — Si — $(CH_2)_7$ — CH_3, CH_3

Classic Reversed-Phase Retention and Selectivity with Excellent Peak Shape

Discovery C8 Delivers....

- Classic C8 selectivity and retention
- Excellent peak shape
- Stable, no-bleed LC/MS separations
- Similar selectivity to a C18, but lower hydrophobic retention

Properties of Discovery C8

USP Code	L7
Bonded Phase	Octylsilane
Endcap (yes / no)	Yes
Particle Platform	Silica
Particle Shape	Spherical
Particle Purity	<10ppm metals
Particle Sizes (µm)	5
Pore Size (Å)	180
Surface Area (m²/g)	200
Packing Density (g/mL)	0.58
%C	7.5
Coverage (µmoles/m²)	3.4
pH Range	2 to 8
Temperature Range	≤70°C

Figure 1: Barbiturates

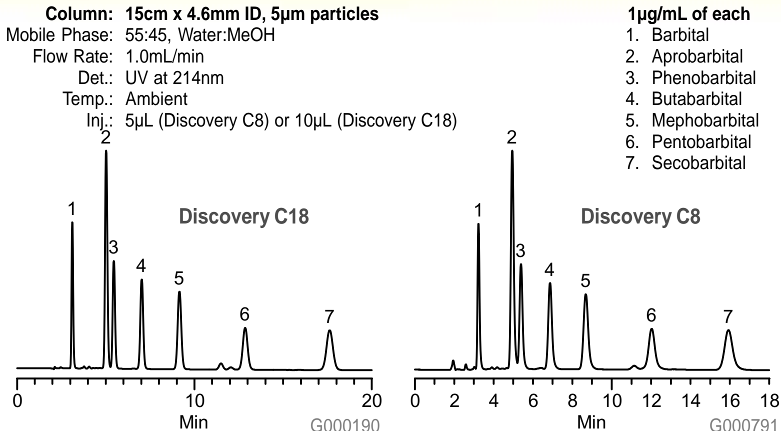


Figure 2: Anticonvulsants

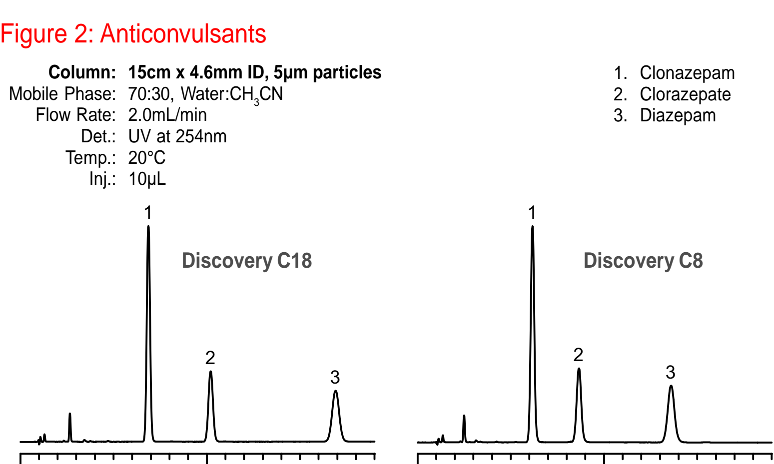
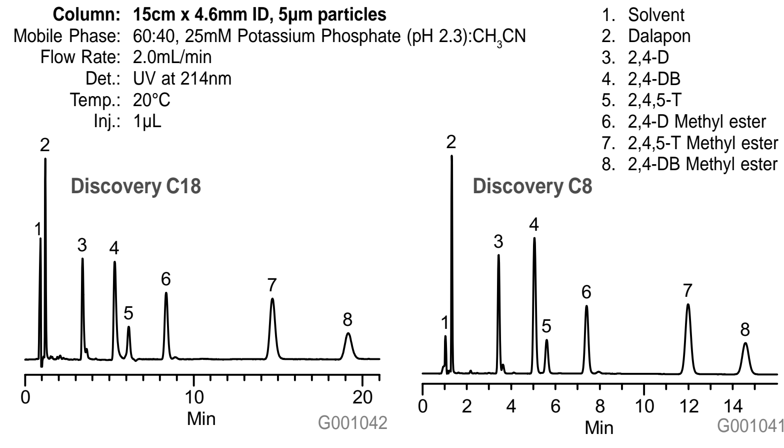


Figure 3: Alhanoic / Aryloxyalhanoic Acid Using Isocratic Elution



Discovery Zirconia-Based Phases

High pH and High Temperature HPLC

Reversed-phase, zirconia-based particles expand your HPLC method development options by leveraging the unique selectivity and retention provided by pH and temperature extremes.

Use Discovery Zr phases when:

1. Low or high pH is desirable to control the ionization state of your analyte
2. You would like a significant reduction in analysis time
3. Silica-based phases cannot give the resolution you require

Discovery Zr comprises four phase chemistries bonded to porous, spherical, 3 and 5 micron zirconia particles. Zirconia particles have exceptional pH and thermal stability compared to silica and alumina particles. Compared to polymer particles, zirconia does not shrink or swell with changes in temperature, ionic strength, or organic concentration, and has exceptional mechanical strength. The presence of controlled, predictable reversed-phase and ion-exchange retention modes combined with thermal and pH stability open up your method development options. Four different Discovery Zr bonded phase chemistries, Carbon, CarbonC18, PS, and PBD, give you choices in bonded phase selectivity.

Why use Zirconia Particles over Conventional Silica or Polymer Particles for HPLC?

Zirconia = zirconium dioxide or ZrO_2

Since the beginning of the science of chromatography, many different support particle chemistries have been employed. Inorganic oxides, including silica and alumina, and organic polymers and copolymers, including graphitic carbon, polymethacrylate, and polystyrene-divinylbenzene, comprise the vast majority of commercially-available HPLC supports. Each of these have limitations that fuel the search for the ideal HPLC particle candidate; one that has the physical attributes that give rise to efficient and stable packed column beds, can be functionalized, and are chemically immutable under a wide range of mobile phase and operating conditions. Recent developments in the science behind manufacturing spherical microparticulate zirconium dioxide (zirconia) have given rise to particles that have the physical and chemical characteristics approaching the ideal support particle for HPLC.

It all reduces to chemistry:

- The chemistry of zirconia that gives pH and thermal stability,
- Lewis acid-base chemistry that provides ion-exchange character, and allows you to adjust selectivity by the type of buffer used,
- The chemistry of our four unique bonded phases that gives diverse selectivities from each other and from silica-based phases.

The Members of the Discovery Zr Family

Discovery Zr-PBD

Polybutadiene-modified zirconia particles give separations most similar to C18-silica, but with benefits of high pH and temperature stability.

Discovery Zr-PS

Polystyrene modified zirconia particles are ideal for separations of hydrophobic compounds and amines.

Discovery Zr-CarbonC18

Octadecyl-modified carbon-clad zirconia for universal separations of acids, bases, and neutrals. Very different selectivity relative to C18-silica.

Discovery Zr-Carbon

Carbon-clad zirconia for separations of geometric isomers and diastereomers.

Discovery Zr particles are uniform spheres for high efficiency and column stability. Although they look like silica particles, they have pH stability that silica does not.

E000911

The Power of pH

Use Discovery Zr at High and Low pH

Unlike siloxane bonds (Si-O-Si), the Zr-O-Zr bonds that form the zirconia particle structure are not susceptible to chemical attack at high pH. Also unlike silica, Zr bonded phases are not susceptible to chemical attack at low pH.

Why Run an HPLC Method at pH Extremes?

pH is a powerful tool to adjust selectivity and retention in HPLC separations of ionizable compounds. The ionization state of a compound is influenced by the pH of the mobile phase until well above or below its pK_a. In purely reversed-phase separations, compounds exhibit better retention when they are not ionized. However, when working with silica-based reversed-phase packings, if the pH needed to suppress ionization for adequate retention is outside the allowable pH limits (usually pH 2 – 8), oppositely charged ion-pair agents are required to obtain adequate retention.

However, by using an HPLC material that allows for unrestricted pH, you can control the ionization state of even very basic or acidic analytes. If the HPLC material also has ion-exchange character, then you have the added dimension of an ion-exchange mechanism contributing to retention and selectivity.

Discovery Zr zirconia particles are not susceptible to acidic or basic hydrolysis and therefore do not have the pH limitation of silica. Discovery Zr particles also have ion-exchange character via the adsorbed Lewis base buffer ions. Table 1 shows the effect of pH on hydrophobicity (reversed-phase character) and ionization (ion-exchange character) of basic and acidic analytes, and the zirconia surface. Figure 1 shows the stability of Discovery Zr phases at high pH, compared to purportedly pH-stable C18-silica particles.

Table 1: Summary of Effect of pH on Ionization and Hydrophobicity of Analytes and Zr Surface

	Ionization	Hydrophobicity
Acidic Analytes	Increases with increasing pH	Decreases with increasing pH
Basic Analytes	Decreases with increasing pH	Increases with increasing pH
Zirconia (Zr) Surface	Positively charged at low pH Negatively charged at high pH	No effect

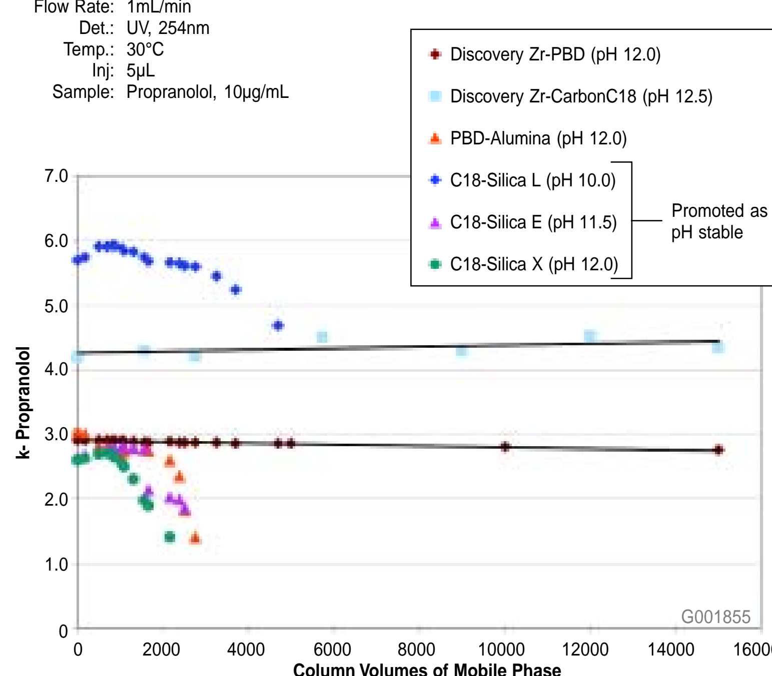
Figure 1: Effect of Exposure to High pH on "pH-Stable" Silica Particles vs. Zirconia Particles

Stress Conditions

Mobile Phase: CH_3CN in 50mM potassium phosphate (35:65) (pH as indicated in Figure)
Temp.: 30°C

Test Conditions

Mobile Phase: CH_3CN (or THF) in 50mM Potassium Phosphate (pH as indicated in Figure)
Flow Rate: 1mL/min
Det.: UV, 254nm
Temp.: 30°C
Inj: 5µL
Sample: Propranolol, 10µg/mL



Silica particles are not stable at high pH. Exposure to basic conditions will dissolve the particles and destroy the column. Discovery Zr particles do not dissolve at high pH like silica particles do.

The Power of Temperature

Use Discovery Zr up to 100°C in Conventional Hardware and 200°C in Special Hardware

The same chemistry that gives zirconia particles pH stability also gives it excellent thermal stability.

Why Run at High Temperatures?

Increasing the temperature of a separation has many desirable effects, including:

1. Sorption kinetics are increased, decreasing retention time and peak width
2. Mobile phase viscosity is reduced, allowing for higher flow rates and higher efficiency
3. Decrease in retention allows use of lower organic modifier concentration, reducing hazardous waste
4. Lower mobile phase viscosity reduces wear-and-tear on pumps

The primary requirement of utilizing elevated temperatures is the stability of the stationary phase. Typical silica-based HPLC particles will quickly deteriorate at elevated temperatures, especially at the elevated pH values necessary to be above the pK_a of most basic pharmaceutical compounds. Discovery Zr zirconia particles exhibit the necessary thermal and chemical stability to operate at elevated temperatures and extreme pH values. The most significant effect of increased temperature is decreased run time. Figure 1 shows the separation of five alkaloids on Discovery Zr-PBD columns at 30°C and 65°C at constant pressure.

An Extreme Example

The benefits of extreme pH and temperature stability of Discovery Zr are clearly demonstrated in the separation of ß-blockers in Figure 2. The high pH gives excellent resolution, and the high temperature gives short analysis time.

Figure 1: Temperature Effect on Analysis Time: Alkaloids at 30°C and 65°C

Column: **Discovery Zr-PBD, 15cm x 4.6mmID, 5µm particles (65723-U)**
Mobile Phase: (90:10) 20mM Potassium Phosphate (pH 12):CH_3CN
Flow Rate: 1.5mL/min at 30°C; 2.35mL/min at 65°C
Det.: UV, 220nm
Temp.: 30°C or 65°C
Inj: 10µL each compound 50µg/mL

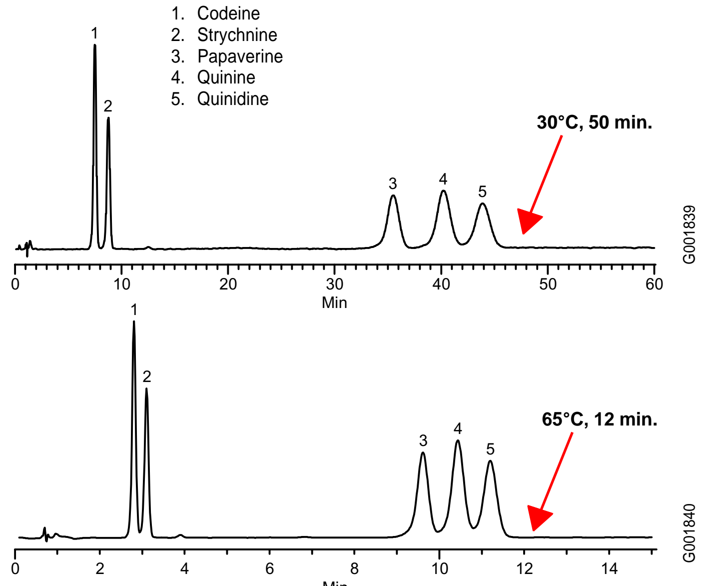
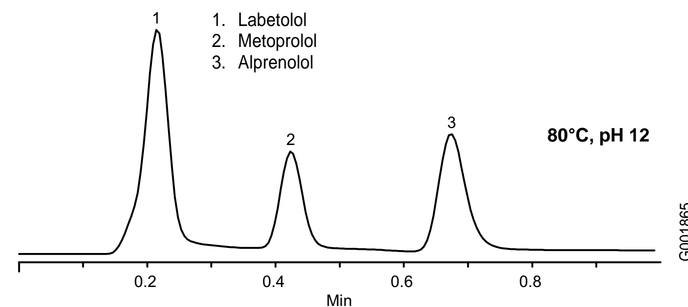
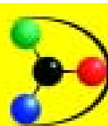


Figure 2: Extreme Temperature and pH Gives Rapid Separation of ß-Blockers on Discovery Zr-CarbonC18

Column: **Discovery Zr-CarbonC18, 5cm x 4.6mm ID, 3µm particles (65704-U)**
Mobile Phase: (55:45) 20mM Potassium Phosphate (pH 12):CH_3CN
Flow Rate: 3mL/min
Det.: UV, 210nm
Temp.: 80°C
Pressure: 99bar
Inj.: 5µL
Sample: Labetolol (500µg/mL), metoprolol (250µg/mL), alprenolol (250µg/mL)





Discovery Zirconia Based Phases

Choosing and Using Discovery Zr

Developing Methods on Discovery Zr

Discovery Zr uses all the reversed-phase method development tools you use for developing methods on silica. However, Discovery Zr gives you four new tools that silica does not allow:

1. The full power of pH: to alter the retention of acids and bases
2. The power of temperature: to decrease analysis time
3. The power of ionic strength: to alter selectivity, efficiency, and retention
4. The power of Lewis acid-base interactions: to give unique selectivity over silica for ionic compounds

Unique Lewis Acid-Base Chemistry

Although predominantly reversed-phase, Discovery Zr phases have secondary ionic interactions – called Lewis Acid-Base interactions – that give an added dimension to method development of ionic compounds.

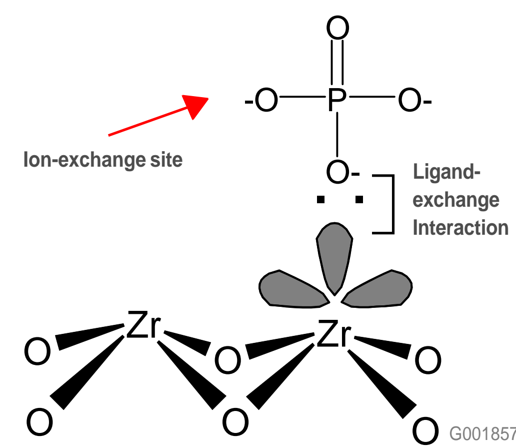
To successfully develop separations of ionic compounds on Discovery Zr, it is important to understand the role of Lewis acid-base chemistry on zirconia. The Lewis electron theory states that an acid is an electron-pair acceptor, and a base is an electron-pair donor. The zirconium atom in zirconia is a strong Lewis acid site and plays a significant role in retention of ionic analytes. The Lewis acid zirconia surface attracts Lewis base buffer ions – like phosphate – via ligand-exchange. This adsorbed buffer ion then acts as an ion-exchange site (Figure 1). If the pH is below the pK_a of the basic analyte, it will cation-exchange with the adsorbed buffer anion. The result is a significant portion of retention due to ion-exchange interactions. An added benefit is that different buffer ions give very different selectivity.

Understanding and utilizing the ion-exchange character of zirconia is important to getting the most out of your Discovery Zr column.

Table 1: Summary of Benefits of Zirconia Over Other Chromatography Particles

	Discovery Zr Particles	Silica Particles	Polymer Particles	Carbon Particles
Stability at high pH (>11)	yes	no	yes	yes
Stability at low pH (<2)	yes	no	yes	yes
Thermal stability (>60°C)	yes	no	some	yes
No limits to organic solvents	yes	yes	no	yes
High efficiency	yes	yes	no	no
Good mass transfer into and out of pores	yes	yes	no	?
Tunable selectivity for amines	yes	no	no	no
Low backpressure	yes	yes	no	yes
Predictable mixed-mode operation	yes	no	no	no

Figure 1: Discovery Zr Particles Have Strong Lewis Acid Sites That Can Undergo Ligand-Exchange Interactions with Lewis Bases



Zirconia particles possess strong Lewis acid sites that can form predictable, controllable ligand-exchange interactions. Control is via the use of strong Lewis base buffer ions, like fluoride, phosphate, and acetate.

Choosing a Discovery Zr Phase

Method development first begins by choosing the Discovery Zr phase right for the analyte and conditions. The most important things to consider:

- All Discovery Zr phases operate by reversed-phase mechanisms
- Each of the four Discovery Zr phases are different from each other and have their own unique selectivity – just like silica bonded phases are different from each other
- Ionic compounds will also interact with ion-exchange mechanism
- You are not limited by pH or temperature (up to 200°C)

Figure 2: Choosing a Discovery Zr Phase Based on Analyte and Conditions

Discovery Zr-PS high aqueous mobile phases, an alternative to ODS selectivity	**Discovery Zr-Carbon** diastereomers, geometric isomers, greatest difference from a C18-silica
Discovery Zr-PBD perfect general-purpose phase, great for bases, most similar to C18-silica for non-electrolytes	**Discovery Zr-CarbonC18** unique selectivity for acidic compounds, exhibits both RP and shape selectivity

Discovery Zirconia Based Phases

Discovery Zr-PBD

Polybutadiene-modified Zirconia Particles Give Separations Most Similar to C18-silica, but with Benefits of High pH and Temperature Stability

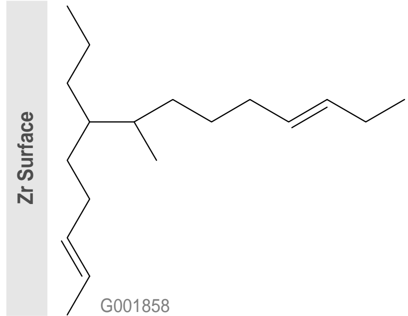
Discovery Zr-PBD comprises spherical, porous zirconia particles with a durable coating of polybutadiene. It operates via a reversed-phase mechanism, but is less hydrophobic, so less organic solvent is required for elution. Discovery Zr-PBD complements the selectivity offering of the other zirconia and silica-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 13.

Discovery Zr-PBD Characteristics

Discovery Zr-PBD - polybutadiene (PBD)-coated zirconia	
Particle Size:	3 and 5 micron
Surface Area (m^2/g):	$30m^2/g$
Pore Size:	300Å
pH Range:	1 - 13
Temperature Range*:	≤ 100°C

*Special column hardware for operations between 100°C and 150°C is available.

Structure of Discovery Zr-PBD



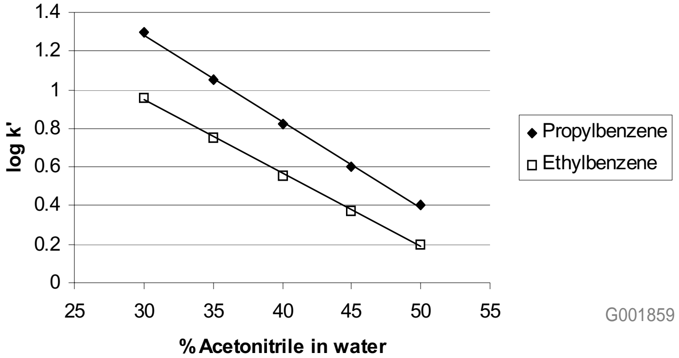
Features of Discovery Zr-PBD:

- Good for bases, amines
- Similar to ODS-silica
- pH stable from 1-13
- Thermally stable up to 100°C (up to 150°C in special hardware)

Discovery Zr-PBD is Similar to C18-silica, But with Added Selectivity and pH and Thermal Stability

Discovery Zr-PBD columns have selectivity similar to C18-silica for non-ionic compounds. Figure 1 shows that Discovery Zr-PBD operates via a predictable, reversed-phase mechanism.

Figure 1: Linear Relationship Between log k' and %CH_3CN Demonstrates a Reversed-Phase Mechanism on Discovery Zr-PBD



However, for ionic compounds, especially bases, the secondary Lewis acid-base interactions give significant added selectivity to separations on Discovery Zr-PBD. The Lewis acid zirconia surface attracts Lewis base buffer ions – like phosphate. If the pH is below the pK_a of the basic analyte, it will cation-exchange with the buffer anion. The result is a significant portion of retention due to ionic interactions. An added benefit is that different buffer ions give very different selectivity. Above the pK_a of the base, there are no ionic interactions and retention is due solely to reversed-phase interactions with the polybutadiene bonded phase.

Another significant difference between Discovery Zr-PBD and C18-silica is that it can be used with basic pH mobile phases and elevated temperatures where basic analytes have better peak shape and higher efficiency. This is demonstrated in the separation of basic antihistamine compounds in Figure 1, page 35.

Another example of the utility of Discovery Zr-PBD for basic compounds is shown in the separation of tricyclic antidepressants in Figure 2, page 35.

Discovery Zr-PBD

Figure 1: Example of Fast, High pH Separation of Amines on Discovery Zr-PBD Columns

Column: **Discovery Zr-PBD, 7.5cm x 4.6mm ID, 3µm particles (65717-U)**
Mobile Phase: (75:25) 50mM Triethylammonium Hydroxide (pH 12.6):CH_3CN
Flow Rate: 1mL/min
Det.: UV, 254nm
Temp.: 20°C or 80°C
Pressure: 130bar at 20°C
Inj.: 1µL
Sample: Doxylamine, methapyrilene, chlorpheniramine (1µg/mL), triprolidine (2µg/mL)

1. Doxylamine
2. Methapyrilene
3. Chlorpheniramine
4. Triprolidine

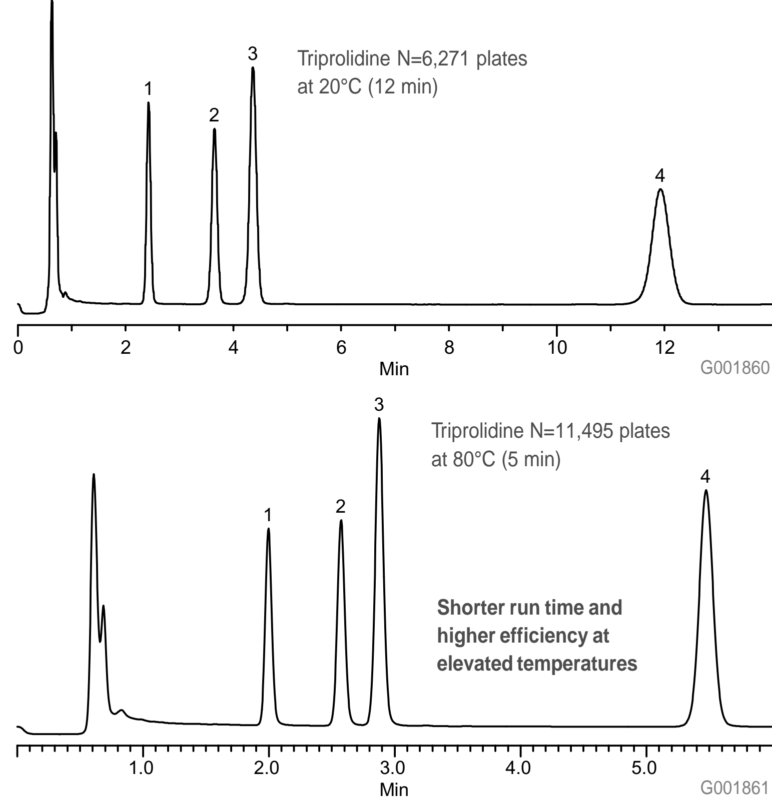
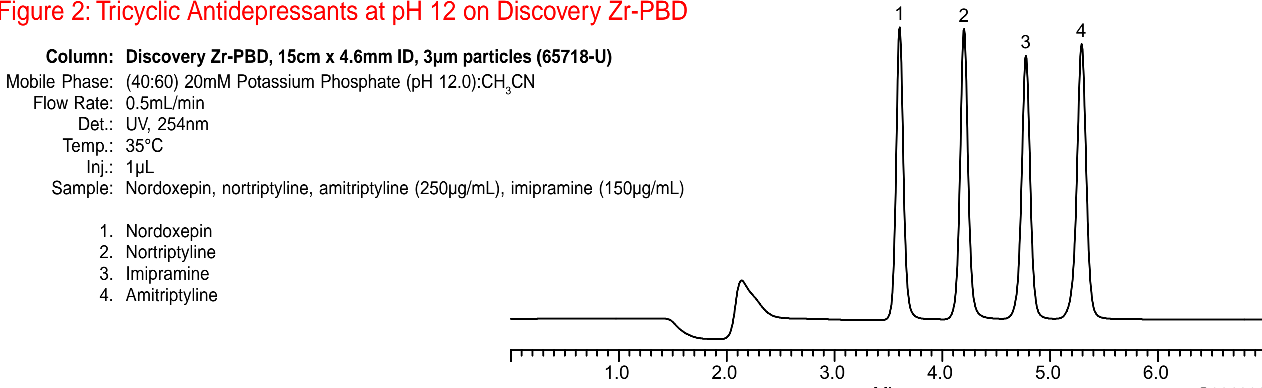


Figure 2: Tricyclic Antidepressants at pH 12 on Discovery Zr-PBD

Column: **Discovery Zr-PBD, 15cm x 4.6mm ID, 3µm particles (65718-U)**
Mobile Phase: (40:60) 20mM Potassium Phosphate (pH 12.0):CH_3CN
Flow Rate: 0.5mL/min
Det.: UV, 254nm
Temp.: 35°C
Inj.: 1µL
Sample: Nordoxepin, nortriptyline, amitriptyline (250µg/mL), imipramine (150µg/mL)

1. Nordoxepin
2. Nortriptyline
3. Imipramine
4. Amitriptyline



Discovery Zirconia Based Phases

Discovery Zr-CarbonC18

Octadecyl-modified Carbon-clad Zirconia Combines Partitioning Mechanism with Shape Selectivity

Discovery Zr-CarbonC18 comprises spherical, porous carbon-clad zirconia particles covalently modified with octadecyl (C18) groups. It complements the selectivity offering of the other zirconia- and silica-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 14.

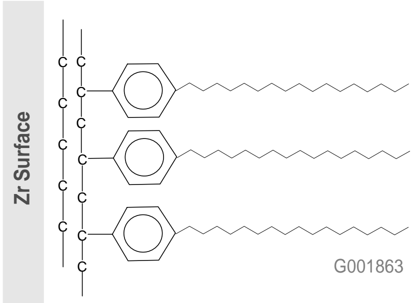
Discovery Zr-CarbonC18 Characteristics

Discovery Zr-CarbonC18 - carbon-clad zirconia with covalently-bonded octadecyl groups

Particle Size:	3 and 5 micron
Surface Area (m^2/g):	$30m^2/g$
Pore Size:	300Å
pH Range:	1 – 14
Temperature Range *:	≤ 100°C

*Special column hardware for operations between 100°C and 200°C is available.

Structure of Discovery Zr-CarbonC18:



Features of Discovery Zr-CarbonC18:

- Partitioning mechanism
- Shape selectivity
- Resistant to phase hydrolysis
- pH stable from 1-14
- Thermally stable up to 100°C (up to 200° in special hardware)

Discovery Zr-CarbonC18 Combines Partitioning Mechanism with pH and Temperature Stability.

Octadecyl (C18) is by far the most common member among the population of reversed-phased functional groups. The C18 reagent is relatively common and synthesis is straightforward and controllable. It has nearly universal application since the majority of organic compounds are hydrophobic enough to interact with C18 chains to some degree. The partitioning interactions between it and analytes are understood and therefore predictable. Indeed, the major limitations of C18 are due to the substrate it is bonded to, which is most often silica. In general, silica's limited pH range restricts the application of C18 phases bonded to it to between pH 2 and 8. Temperatures above 60°C can also damage bonded silicas. Discovery Zr-CarbonC18 overcomes the limitations of silica by covalently bonding C18 chains to a chemically and thermally inert carbon surface. The resultant phase has the partitioning mechanism of C18, but because it is bonded to a highly inert, carbonaceous support, it is immune to pH and temperature extremes. The example of the acidic non-steroidal anti-inflammatory compounds in Figure 1 run at pH 1.75 and 80°C on Discovery Zr-CarbonC18 demonstrates the extreme applicability of this phase.

Figure 1: Rapid Separation of NSAIDS on Discovery Zr-CarbonC18

Column:	**Discovery Zr-CarbonC18, 15cm x 4.6mm ID, 3µm particles (65706-U)**
Mobile Phase:	(50:50) 50mM H_3PO_4 (pH 1.75):CH_3CN
Flow Rate:	4mL/min
Det.:	UV, 254nm
Temp.:	80°C
Pressure:	260bar
Inj.:	1µL
Sample:	Ketoprofen, ibuprofen, naproxen, each 1mg/mL

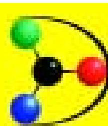
1. Ketoprofen
2. Ibuprofen
3. Naproxen

1 2 3

0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8

Min

G001864



Discovery Zr-CarbonC18

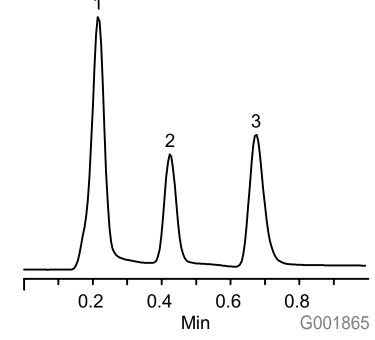
For Rapid Analysis, Consider Discovery Zr-CarbonC18 in Short Columns Run at High Temperatures

Increasing the temperature can greatly reduce the analysis time. The thermal stability of all Discovery Zr phases allows temperatures up to 100°C and higher with special hardware. The separation of ß-blockers on Discovery Zr-CarbonC18 at 80°C in less than 1 minute is shown in Figure 1.

Figure 1: Extreme Temperature and pH Give Rapid Separation of ß-blockers on Discovery Zr-CarbonC18

Column: **Discovery Zr-CarbonC18, 5cm x 4.6mm ID, 3µm particles (65704-U)**
Mobile Phase: (55:45) 20mM Potassium Phosphate (pH 12):CH_3CN
Flow Rate: 3mL/min
Det.: UV, 210nm
Temp.: 80°C
Pressure: 99bar
Inj.: 5µL
Sample: Labetolol (500µg/mL), metoprolol (250µg/mL), alprenolol (250µg/mL)

1. Labetolol
2. Metoprolol
3. Alprenolol



The Underlying Carbon Surface Confers a Degree of Shape Selectivity on Discovery Zr-CarbonC18

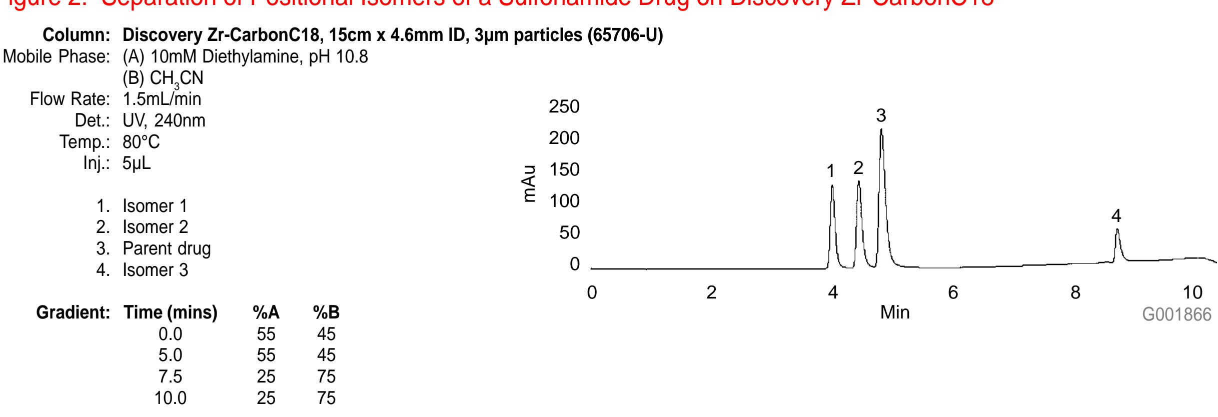
One of the benefits of carbon particles as an HPLC support is its ability to distinguish between molecular shapes. Unlike C18 chains that can conform to the shape of the molecule, the rigid carbon surface cannot. Molecules that have the same overall hydrophobicity but different shapes, like geometric isomers, are not separable on C18 phases. However, because these molecules have a different hydrophobic footprint, they can be separated on rigid supports. One of the downsides to traditional carbon supports is that they are often too hydrophobic. Discovery Zr-CarbonC18 combines a partitioning mechanism of C18 with the shape selective ability of carbon. The result is separation of positional isomers in less time with lower percent organic. The separation of positional isomers of a proprietary sulfonamide drug is shown in Figure 2. Here the parent compound is easily distinguished from its three corresponding positional isomers.

Figure 2: Separation of Positional Isomers of a Sulfonamide Drug on Discovery Zr-CarbonC18

Column: **Discovery Zr-CarbonC18, 15cm x 4.6mm ID, 3µm particles (65706-U)**
Mobile Phase: (A) 10mM Diethylamine, pH 10.8
(B) CH_3CN
Flow Rate: 1.5mL/min
Det.: UV, 240nm
Temp.: 80°C
Inj.: 5µL

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

Gradient:	Time (mins)	%A	%B
	0.0	55	45
	5.0	55	45
	7.5	25	75
	10.0	25	75



Discovery Zirconia Based Phases

Discovery Zr-PS

Polystyrene-modified Zirconia Particles are Ideal for Separations of Hydrophobic Compounds and Amines

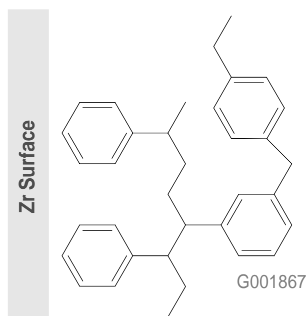
Discovery Zr-PS comprises spherical, porous zirconia particles modified with cross-linked polystyrene. It operates via a reversed-phase mechanism, but is less retentive. It has unique selectivity, especially for aromatic compounds. Discovery Zr-PS complements the selectivity offering of the other zirconia- and silica-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 13.

Discovery Zr-PS Characteristics

Discovery Zr-PS - cross-linked polystyrene on zirconia	
Particle Size:	3 and 5 micron
Surface Area (m^2/g):	$30m^2/g$
Pore Size:	300Å
pH Range:	1 – 13
Temperature Range*:	≤ 100°C

*Special column hardware for operations between 100°C and 150°C is available.

Structure of Discovery Zr-PS:



Features of Discovery Zr-PS:

- Good for very hydrophobic compounds
- Good for basic compounds and amines
- pH stable from 1-13
- Thermally stable up to 100°C (up to 150°C in special hardware)

Discovery Zr-PS Gives Short Retention of Hydrophobic Amines with Excellent Peak Shape.

The relatively polar surface of Discovery Zr-PS permits rapid analysis of hydrophobic compounds. Because of the stability of the underlying zirconia surface, analyses can be run at low and high pH, and temperatures up to 150°C. Figure 1 shows a rapid gradient of acetonitrile in pH 1.8 buffer that effectively resolved three aromatic, hydrophobic amine drugs.

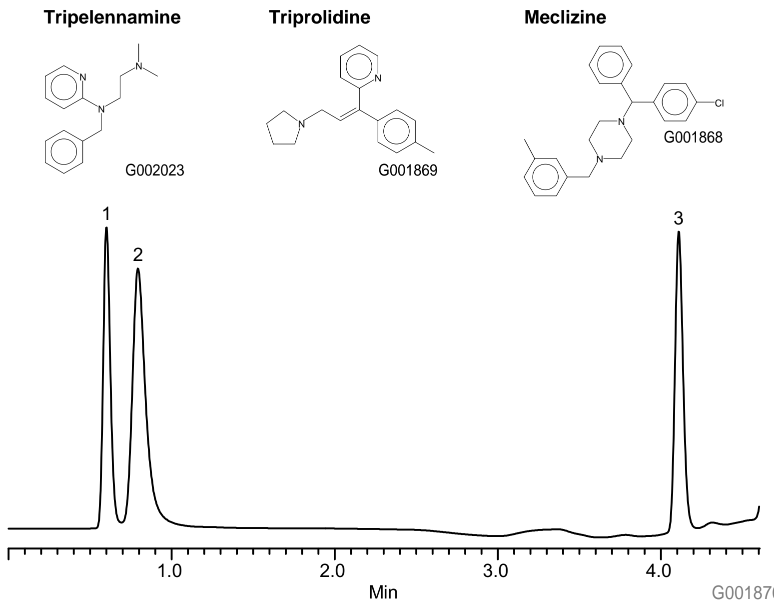
Figure 1: Rapid Gradient Resolution of Hydrophobic Amines at Low pH on Discovery Zr-PS

Column: **Discovery Zr-PS, 5cm x 4.6mm ID, 3µm particles (65740-U)**
Mobile Phase: (A) 25mM HCl, pH 1.8
(B) CH_3CN
Flow Rate: 1mL/min
Det.: UV, 254nm
Temp.: 40°C
Inj.: 1µL
Sample: Tripelennamine, triprolidine (1mg/mL), meclizine (3mg/mL)

1. Tripelennamine
2. Triprolidine
3. Meclizine

Gradient:

Time (mins)	**%A**	**%B**
0	100	0
1	98	2
4	40	60

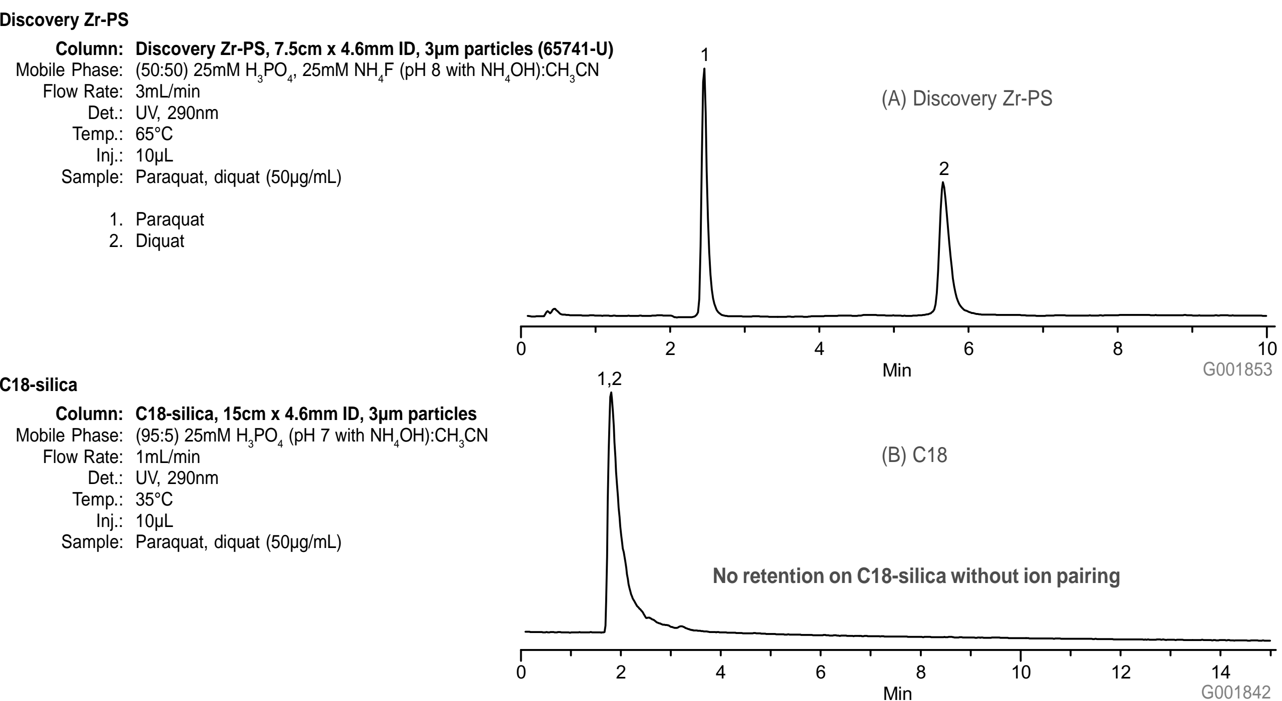


Discovery Zr-PS

Quaternary Amines can be Analyzed on Discovery Zr-PS at High pH without Ion-pairing

Basic compounds, especially quaternary amines, often suffer from lack of hydrophobic retention on C18-silica phases. To remedy this, ion-pairing is employed. However, ion-pair agents have well-known disadvantages. By running at high pH, the hydrophobicity of the amine is increased and ion-pair agents are not required. Discovery Zr-PS is stable at high pH. Figure 1 shows the separation of paraquat and diquat, two quaternary amines, on Discovery Zr-PS and C18-silica. Note that ion-pairing is not needed to have retention on the Discovery Zr-PS. Retention is due to both hydrophobicity and the presence of ion-exchange with the adsorbed Lewis base mobile phase buffer ion (phosphate).

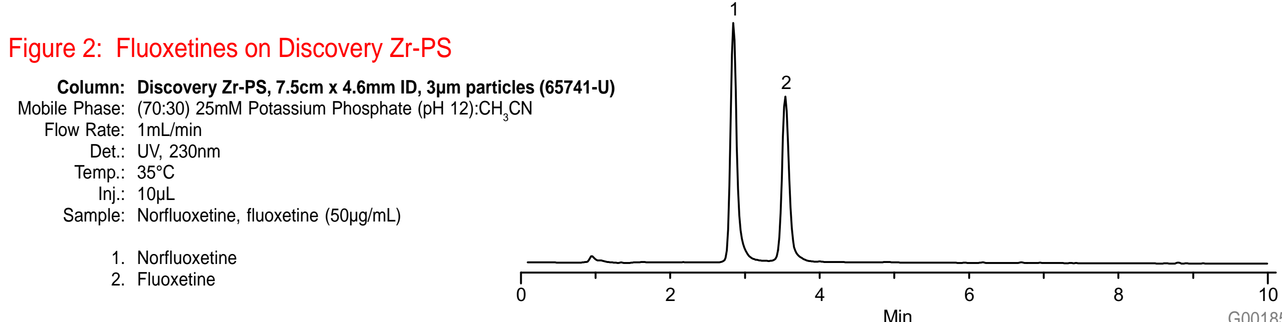
Figure 1: Paraquat and Diquat on Discovery Zr-PS vs. C18-silica



Difficult Basic Compounds Exhibit Symmetrical Peaks on Discovery Zr-PS at High pH

Another problem with basic compounds on silica is their tendency to tail because of silanol interactions. This can be avoided by running at high pH where the charge on the base is neutralized. However, silica is typically limited to below pH 8. Figure 2 shows a difficult pair of bases on Discovery Zr-PS at pH 12. The symmetrical peaks are testimony to the lack of undesirable secondary interactions.

Figure 2: Fluoxetines on Discovery Zr-PS



Discovery Zr-Carbon

Carbon-clad Zirconia is Ideal for Separations of Geometric Isomers and Diastereomers and Enhanced Retention of Polar Compounds

Discovery Zr-Carbon comprises spherical, porous carbon-coated zirconia particles. It is ideal for the reversed-phase separation of positional isomers and diastereomers. It complements the selectivity offering of the other zirconia- and silica-based Discovery phases, and allows the use of the full range of mobile phase pH from pH 1 to 14. It is a great alternative when C18 does not work.

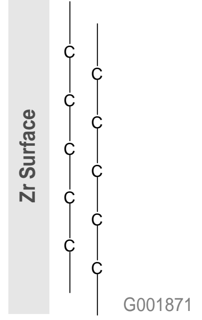
Discovery Zr-Carbon Characteristics

Discovery Zr-Carbon - zirconia coated with permanent layer of carbon

Particle Size:	3 and 5 micron
Surface Area (m^2/g):	$30m^2/g$
Pore Size:	300Å
pH Range:	1 – 14
Temperature Range *:	≤ 100°C

*Special column hardware for operations between 100°C and 200°C is available.

Structure of Discovery Zr-Carbon:



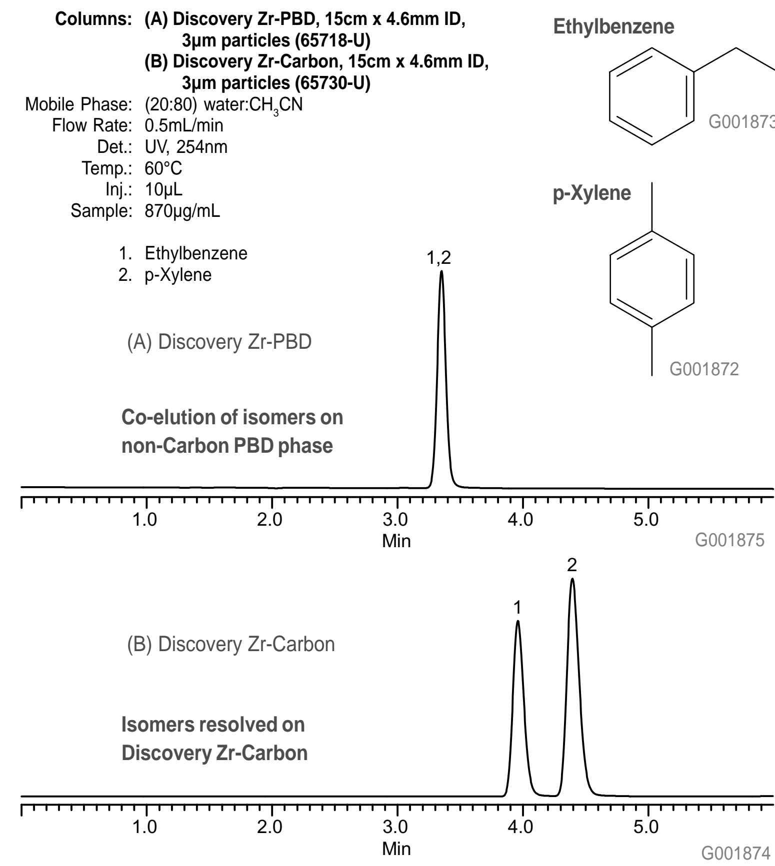
Features of Discovery Zr-Carbon:

- Excellent separation of geometric isomers and diastereomers
- Very hydrophobic surface
- Most different retention compared to other Discovery Zr phases for non-ionic compounds
- Similar to porous graphitic carbon, but with added ion-exchange interactions
- pH stable from 1-14
- Thermally stable up to 100°C (up to 150°C in special hardware)
- Avoid fused-ring aromatics as they are too strongly retained by Discovery Zr-Carbon

The Rigid Surface of Discovery Zr-Carbon Permits the Separation of Structurally Similar Compounds.

Carbon-based packings have found a niche within the population of HPLC supports. The main benefits of carbon over silica are enhanced chemical and thermal stability, and the ability to separate positional isomers. Compounds that have the same hydrophobicity, but different molecular shape, can be separated on the rigid carbon surface but not on phases that comprise flexible ligands. In Figure 1, the isomers ethylbenzene and p-xylene co-elute on the non-carbon Discovery Zr-PBD phase, but are resolved on Discovery Zr-Carbon.

Figure 1: Separation of Structurally Similar Compounds on Discovery Zr-Carbon vs. Non-Carbon Phase



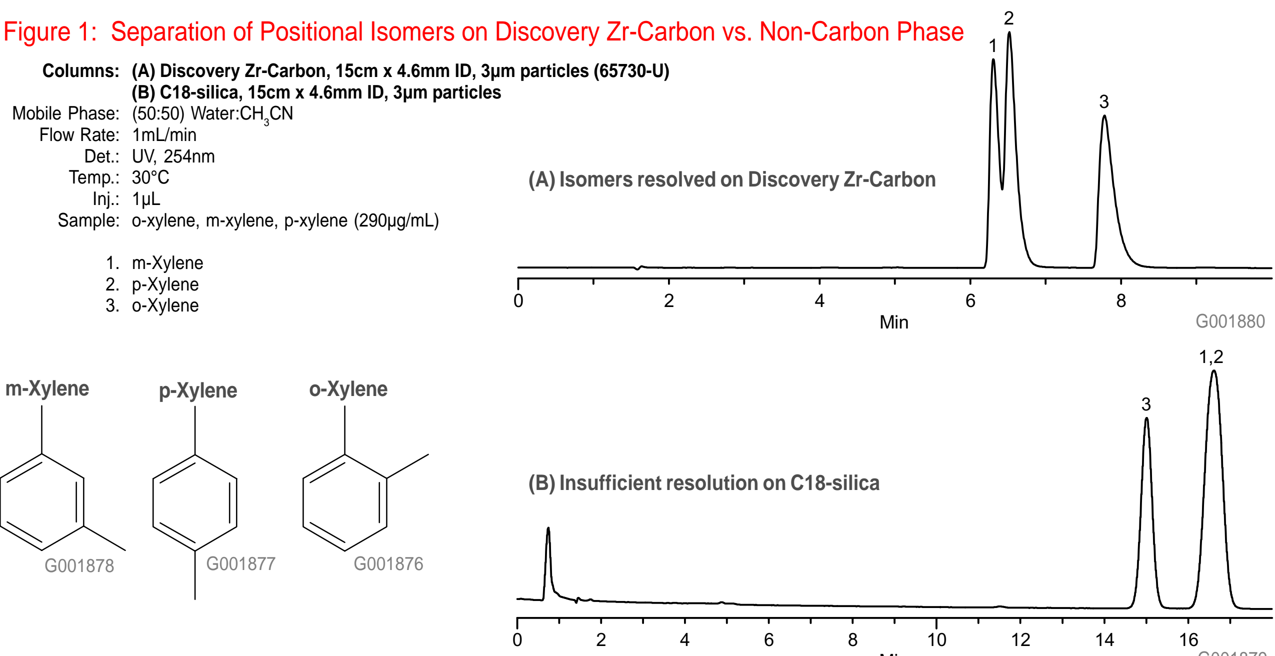
Discovery Zirconia Based Phases

Discovery Zr-Carbon

Positional Isomers are Easily Resolved on Discovery Zr-Carbon

The ability of Discovery Zr to distinguish positional isomers is demonstrated in Figure 1 below. The isomers co-elute on a C18-silica column, but are resolved on the Discovery Zr-Carbon column.

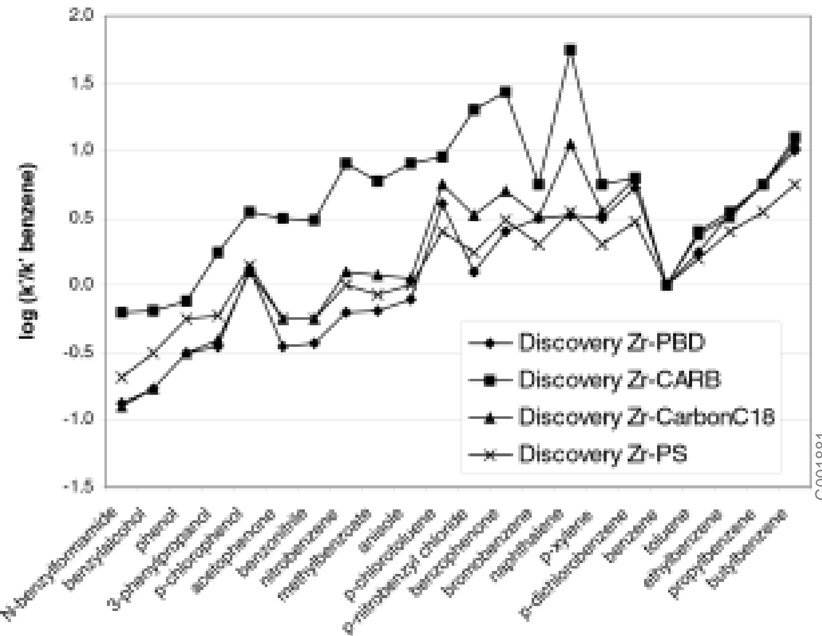
Figure 1: Separation of Positional Isomers on Discovery Zr-Carbon vs. Non-Carbon Phase



Discovery Zr-Carbon Has the Most Unique Selectivity Within the Discovery Zr Family

Figure 2 shows a selection of twenty three different non-ionic probes. Each was run on the four Discovery Zr phases. Retention relative to benzene was plotted. For these compounds, the Discovery Zr-Carbon has the most unique selectivity.

Figure 2: Comparison of Selectivity Among Discovery Zr Phases



Discovery Column Selection by Compound

Guidelines for Narrowing Down the Candidate Columns Based on:

- Your compound
- Your preferred mobile phase conditions

Column screening data tables appear on pages 44 to 45

Which Discovery column should you choose when developing a new method?

The current Discovery family comprises seven silica-based phases, and four zirconia-based phases; and it is growing. Each phase is unique, and each gives different, valuable separations. If time does not allow you to test every Discovery phase, use the column screening data we've provided on the following pages to point out the most likely candidates.

How was the column screening data generated?

Compounds: We chose compounds that represented the basic structure or functional groups you are most likely to encounter in small molecule HPLC separations.

Conditions: For non-ionic compounds, we used a simple acetonitrile-water mobile phase. For ionizable compounds, we chose simple, low ionic strength phosphate buffers at pH 2 and pH 7. These pH values represent the typical range of HPLC operation. Two pH values were necessary to show the power of pH to alter selectivity. The concentration of acetonitrile was varied to give a k' between 1 and 5 for most compounds.

Calculations: We reported retention in k' (capacity factor). The equation for k' is: $\mathbf{k' = (T_r - T_0)/T_0}$, where T_r is the retention time of the analyte, and T_0 is the void volume (the elution time of an unretained peak).

How should you use the column screening data?

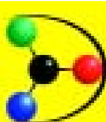
Your conditions: Choose either the pH 2 or the pH 7 table if you have a pH preference. Non-ionic compounds that were screened without buffers in the mobile phase appear in both tables.

Your compound: Look up your compound in the pH 2 or pH 7 table. If your exact compound does not appear in the table, chances are there will be one of similar structure or functionality in the tables. Choose the column that gave the right amount of retention for your compound or representative compound.

Multiple compounds: If you are looking at resolving two or more compounds, find the Discovery phase that gives the best separation (usually a minute or more) between your compounds or representative compounds.

Considering the acetonitrile concentration: If you want to run under isocratic conditions and the compounds you are interested in were screened at different percentages of acetonitrile, simply use the very general rule-of-thumb for reversed-phase HPLC that an increase of 5% (v/v) of the organic modifier results in a 2-fold decrease in k'. For example, a compound with a k' of 10 at 30% acetonitrile would have a k' of 5 at 35% acetonitrile.

Consider elution order: Many samples contain a large excess of one compound over another. The best quantitation is obtained when the smaller peak (peak that is in lower abundance or has a lower signal) elutes before the large peak. When you look at the screening data, chose the column or columns that give you the right elution order.



Guidelines for Narrowing Down the Candidate Columns

Here's an example:

Compounds of interest: Your sample contains phenacetin and a compound that closely resembles codeine in structure.

Elution order: In your sample, codeine is about 100X less concentrated than the phenacetin, so you want codeine to elute first or at least be far enough away that the phenacetin peak doesn't interfere with the quantitation of codeine.

Preferred pH: We'll assume you can run at either pH 2 or pH 7. On the pH 2 chart, the compounds elute at very widely different % acetonitrile (10% and 25%) making an isocratic separation potentially difficult. At pH 7, however, codeine was run at 15% acetonitrile, and phenacetin at 20% acetonitrile. Choose the pH 7 condition.

Choosing the Discovery column – first pass: The pH 7 screening data shows the compounds have the right elution order (codeine then phenacetin) on all but the Discovery HS F5 column if the preferred elution order was reversed, the HS F5 would be the best choice.

Adjusting the % organic: Estimate the k' for the two compounds at the same % acetonitrile. A concentration of 15% would be a good start. Following the rule-of-thumb, decreasing the % acetonitrile to 15% would double the k' of phenacetin.

Choosing the Discovery column – second pass: Double the k' for phenacetin, and look at the resulting estimated k' on the remaining Discovery phases.

Discovery Column	k' Codeine at 15% CH_3CN	Estimated k' Phenacetin at 15% CH_3CN	alpha (k' phenacetin / k' codeine)
C18	4.4	4.7 x 2 = 9.4	2.1
RP-AmideC16	3.3	4.8 x 2 = 9.6	2.9
C8	3.6	4.1 x 2 = 8.2	2.3
Cyano	1.1	1.3 x 2 = 2.6	2.4

It looks like all four phases gives similar selectivity. If a low % organic mobile phase is desired, the Discovery Cyano would be the best choice. The Discovery RP-AmideC16 gave the largest alpha value. The Discovery C18 and C8 selectivity and retention were very similar. Here we would recommend doing the actual screening on three Discovery columns: Discovery C18 (or C8), Discovery RP-AmideC16, and Discovery Cyano.

High pH, high temperature operation

When to use Discovery Zr?

If you want to work at pH values above 8 or below 2, or at temperatures above 70°C, we recommend using Discovery Zr. Just like the silica-based Discovery phases, the four Discovery Zr phases each give unique selectivity and retention. Consult pages 47 through 70 for guidelines on choosing a Discovery Zr based on your analyte, conditions, or separation challenge.

pH 2 Operation

Guidelines for Narrowing Down the Candidate Discovery Functionalized Reversed-Phase Column for Operation at pH 2

Use this chart as a starting point to choose one, two, three or more Discovery silica-based functionalized reversed-phase columns.

See page 43 for instructions.

Screening Conditions:

Columns: 15cm x 4.6mm ID, 5µm particles
Mobile Phase Buffer: 25mM Phosphoric Acid, adjusted to pH 2.0 with Ammonium Hydroxide (buffer was not used in the mobile phase when non-ionic compounds were screened)
Mobile Phase Organic Modifier: CH_3CN
Flow Rate: 1mL/min
Temperature: 30°C

Note: A k' of 5 is approximately 10 minutes retention time on a 15cm x 4.6mm ID column with a flow rate of 1mL/min.

Note: For most RP-HPLC separations, assume a 2-fold decrease in k' for every 5% increase in % organic.

Compound Name	% Organic	pH	C18 k'	RP-AmideC16 k'	C8 k'	Cyano k'	HS F5 k'
5% CH_3CN							
aniline	5	pH 2	0.7	0.5	0.7	0.4	1.5
benzyl amine	5	pH 2	1.4	0.8	1.3	0.5	3.1
nizatidine	5	pH 2	1.6	1.0	1.3	0.7	2.4
o-aminobenzoic acid	5	pH 2	6.2	4.6	5.8	1.0	8.3
procainamide	5	pH 2	0.7	0.5	0.6	0.4	3.0
pyridine	5	pH 2	0.2	0.2	0.2	0.3	0.5
10% CH_3CN							
codeine	10	pH 2	2.0	1.2	1.7	0.7	2.8
hydrochlorothiazide	10	pH 2	3.0	4.3	2.7	3.1	2.3
lidocaine	10	pH 2	5.9	3.0	5.1	1.0	3.0
phentermine	10	pH 2	4.8	2.6	4.3	0.8	3.5
quinidine	10	pH 2	2.1	1.4	1.9	1.0	8.7
20% CH_3CN							
benzoic acid	20	pH 2	4.1	5.2	4.0	1.3	5.4
m-nitrobenzoic acid	20	pH 2	5.4	8.1	5.1	2.0	12.4
o-nitrobenzoic acid	20	pH 2	2.8	3.9	2.8	1.3	6.2
o-toluic acid	20	pH 2	8.4	10.3	7.8	1.8	9.7
phthalic acid	20	pH 2	1.1	1.4	1.2	0.7	2.3
p-nitrobenzoic acid	20	pH 2	6.1	9.0	5.7	2.2	15.1
sorbic acid	20	pH 2	4.1	4.3	3.8	1.1	4.5
25% CH_3CN							
acetamide	25	no buffer	0.1	0.1	0.2	0.3	0.1
anisole	25	no buffer	10.1	8.1	8.0	1.8	*
benzaldehyde	25	no buffer	3.6	3.2	3.2	1.2	4.8
benzamide	25	no buffer	0.6	0.7	0.7	0.6	1.0
benzyl alcohol	25	no buffer	1.4	1.5	1.5	0.8	1.8
methyl benzoate	25	no buffer	9.4	7.8	7.7	1.7	10.4
o-cresol	25	no buffer	4.4	6.1	4.2	1.5	5.6
phenol	25	no buffer	2.0	2.9	2.0	1.0	2.8
papaverine	25	pH 2	1.7	1.1	1.5	0.8	4.5
phenacetin	25	pH 2	2.7	3.0	2.4	1.0	1.2
30% CH_3CN							
diphenhydramine	30	pH 2	2.7	1.5	2.5	1.2	11.0
furosemide	30	pH 2	5.7	6.3	3.5	2.0	5.7
salicylic acid	30	pH 2	2.4	4.4	2.2	1.1	5.0
35% CH_3CN							
nordoxepin	35	pH 2	1.5	1.0	1.4	*	10.1
doxepin	35	pH 2	1.7	1.0	1.5	*	*
protriptyline	35	pH 2	2.5	1.6	2.1	*	*
desipramine	35	pH 2	2.5	1.5	2.1	*	*
imipramine	35	pH 2	2.8	1.5	2.4	*	13.4
nortriptyline	35	pH 2	3.0	1.8	2.6	*	12.2
amitriptyline	35	pH 2	3.4	1.9	2.9	*	14.2
trimipramine	35	pH 2	3.9	2.0	3.3	*	15.2
40% CH_3CN							
butyl paraben	40	no buffer	4.8	7.9	4.0	1.3	4.4
ethyl paraben	40	no buffer	1.4	2.5	1.4	0.8	1.9
methyl paraben	40	no buffer	0.8	1.5	0.9	0.7	1.3
propyl paraben	40	no buffer	2.6	4.4	2.4	1.0	2.9
50% CH_3CN							
bromobenzene	50	no buffer	3.8	3.2	2.8	1.0	3.2
chlorobenzene	50	no buffer	3.3	2.8	2.5	1.0	3.0
fluorobenzene	50	no buffer	2.0	1.8	1.7	0.8	2.3
nitrobenzene	50	no buffer	1.4	1.4	1.3	0.8	1.9
nitrosobenzene	50	no buffer	1.6	1.6	1.5	0.8	2.1
fluoxetine	50	pH 2	2.1	1.2	0.8	0.6	13.4
ibuprofen	50	pH 2	4.3	4.9	3.4	1.0	2.9
norfluoxetine	50	pH 2	1.8	1.2	0.7	0.6	11.1
55% CH_3CN							
1,3,5-tribromobenzene	55	no buffer	13.0	9.4	6.0	1.1	5.0
1,3-dinitrobenzene	55	no buffer	1.0	1.0	1.0	0.7	1.5
1-chloro-2-fluorobenzene	55	no buffer	2.3	2.1	1.9	0.7	2.3
2-chloronitrobenzene	55	no buffer	1.4	1.4	1.3	0.7	1.9
4-bromochlorobenzene	55	no buffer	4.5	3.8	2.9	0.9	3.1
4-nitrophenol	55	no buffer	0.5	1.0	0.6	0.5	0.8
hexafluorobenzene	55	no buffer	2.6	2.1	2.2	0.7	3.1
pentachlorobenzene	55	no buffer	18.1	12.4	8.0	1.3	7.5
60% CH_3CN							
benzene	60	no buffer	1.2	1.0	1.1	0.6	1.2
butyl benzene	60	no buffer	6.4	4.4	3.9	0.8	3.2
ethyl benzene	60	no buffer	2.6	2.1	1.9	0.7	1.9
propyl benzene	60	no buffer	4.1	3.0	2.7	0.7	2.5
toluene	60	no buffer	1.8	1.5	1.4	0.6	1.6

* meaningful data could not be obtained due to coelution or other problem

Choosing a Discovery Phase

pH 7 Operation

Guidelines for Narrowing Down the Candidate Discovery Functionalized Reversed-Phase Column for Operation at pH 7

Use this chart as a starting point to choose one, two, three or more Discovery silica-based functionalized reversed-phase columns.

See page 43 for instructions.

Screening Conditions:

Columns: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase Buffer: 25mM Phosphoric Acid, adjusted to pH 7 with Ammonium Hydroxide (buffer was not used in the mobile phase when non-ionic compounds were screened)
Mobile Phase Organic Modifier: CH_3CN
Flow Rate: 1mL/min
Temperature: 30°C

Note: A k' of 5 is approximately 10 minutes retention time on a 15cm x 4.6mm ID column with a flow rate of 1mL/min.

Note: For most RP-HPLC separations, assume a 2-fold decrease in k' for every 5% increase in % organic.

Compound Name	% Organic	pH	C18 k'	RP-AmideC16 k'	C8 k'	Cyano k'	HS F5 k'
5% CH_3CN							
aniline	5	pH 7	7.1	4.4	6.6	1.3	8.6
benzoic acid	5	pH 7	1.4	1.1	1.5	*	2.4
benzyl amine	5	pH 7	1.5	1.2	1.4	0.7	6.7
m-nitrobenzoic acid	5	pH 7	3.5	3.0	*	1.0	10.2
o-aminobenzoic acid	5	pH 7	1.2	1.0	1.2	0.5	0.4
o-nitrobenzoic acid	5	pH 7	1.0	0.7	1.0	*	0.9
o-toluic acid	5	pH 7	1.7	1.2	1.8	*	2.0
phthalic acid	5	pH 7	0.1	0.2	0.3	0.2	0.0
p-nitrobenzoic acid	5	pH 7	3.2	3.1	*	1.1	*
procainamide	5	pH 7	3.0	2.4	2.4	1.0	2.4
pyridine	5	pH 7	3.5	2.3	3.5	0.9	5.6
sorbic acid	5	pH 7	1.8	1.3	1.9	*	2.6
10% CH_3CN							
hydrochlorothiazide	10	pH 7	3.0	4.2	2.7	3.0	1.9
nizatidine	10	pH 7	6.1	4.3	4.9	1.2	7.4
phentermine	10	pH 7	5.3	4.0	4.8	1.3	3.8
15% CH_3CN							
codeine	15	pH 7	4.4	3.3	3.6	1.1	3.0
20% CH_3CN							
phenacetin	20	pH 7	4.7	4.8	4.1	1.3	2.2
25% CH_3CN							
acetamide	25	no buffer	0.1	0.1	0.2	0.3	0.1
anisole	25	no buffer	10.1	8.1	8.0	1.8	*
benzaldehyde	25	no buffer	3.6	3.2	3.2	1.2	4.8
benzamide	25	no buffer	0.6	0.7	0.7	0.6	1.0
benzyl alcohol	25	no buffer	1.4	1.5	1.5	0.8	1.8
methyl benzoate	25	no buffer	9.4	7.8	7.7	1.7	10.4
o-cresol	25	no buffer	4.4	6.1	4.2	1.5	5.6
phenol	25	no buffer	2.0	2.9	2.0	1.0	2.8
furosemide	25	pH 7	1.8	1.7	1.7	1.0	1.3
salicylic acid	25	pH 7	0.4	0.4	0.5	0.5	1.0
30% CH_3CN							
papaverine	30	pH 7	5.9	5.8	4.9	1.7	2.9
quinidine	30	pH 7	1.5	2.2	1.4	1.3	5.0
40% CH_3CN							
butyl paraben	40	no buffer	4.8	7.9	4.0	1.3	4.4
ethyl paraben	40	no buffer	1.4	2.5	1.4	0.8	1.9
methyl paraben	40	no buffer	0.8	1.5	0.9	0.7	1.3
propyl paraben	40	no buffer	2.6	4.4	2.4	1.0	2.9
diphenhydramine	40	pH 7	2.0	1.9	1.9	1.6	6.8
fluoxetine	40	pH 7	2.6	3.4	2.6	2.4	9.0
ibuprofen	40	pH 7	0.8	0.8	0.9	0.5	1.7
lidocaine	40	pH 7	4.4	3.6	3.3	1.1	3.0
norfluoxetine	40	pH 7	2.1	3.3	2.1	2.0	6.4
50% CH_3CN							
bromobenzene	50	no buffer	3.8	3.2	2.8	1.0	3.2
chlorobenzene	50	no buffer	3.3	2.8	2.5	1.0	3.0
fluorobenzene	50	no buffer	2.0	1.8	1.7	0.8	2.3
nitrobenzene	50	no buffer	1.4	1.4	1.3	0.8	1.9
nitrosobenzene	50	no buffer	1.6	1.6	1.5	0.8	2.1
55% CH_3CN							
1,3,5-tribromobenzene	55	no buffer	13.0	9.4	6.0	1.1	5.0
1,3-dinitrobenzene	55	no buffer	1.0	1.0	1.0	0.7	1.5
1-chloro-2-fluorobenzene	55	no buffer	2.3	2.1	1.9	0.7	2.3
2-chloronitrobenzene	55	no buffer	1.4	1.4	1.3	0.7	1.9
4-bromochlorobenzene	55	no buffer	4.5	3.8	2.9	0.9	3.1
4-nitrophenol	55	no buffer	0.5	1.0	0.6	0.5	0.8
hexafluorobenzene	55	no buffer	2.6	2.1	2.2	0.7	3.1
pentachlorobenzene	55	no buffer	18.1	12.4	8.0	1.3	7.5
amitriptyline	55	pH 7	2.0	1.7	1.8	*	8.4
doxepin	55	pH 7	1.2	1.1	1.2	*	7.8
imipramine	55	pH 7	1.4	1.3	1.4	*	8.4
nordoxepin	55	pH 7	0.4	0.6	0.5	*	6.3
nortriptyline	55	pH 7	0.6	1.0	0.7	*	7.6
protriptyline, desipramine	55	pH 7	0.5	0.8	0.6	*	6.3
trimipramine	55	pH 7	3.0	2.3	2.2	*	9.1
60% CH_3CN							
benzene	60	no buffer	1.2	1.0	1.1	0.6	1.2
butyl benzene	60	no buffer	6.4	4.4	3.9	0.8	3.2
ethyl benzene	60	no buffer	2.6	2.1	1.9	0.7	1.9
propyl benzene	60	no buffer	4.1	3.0	2.7	0.7	2.5
toluene	60	no buffer	1.8	1.5	1.4	0.6	1.6

* meaningful data could not be obtained due to coelution or other problem

Discovery Column Selection by Separation Problem

Guidelines for narrowing down the candidate columns based on your separation problem or challenge

Problem-solution data appears on pages 47 to 70.

Not only does Discovery help you develop the best HPLC methods, it will also solve common HPLC problems.

The majority of HPLC separation problems fall into two categories:

Peak Shape and / or Efficiency-Related Problems

Discovery high-quality particle and bonded phase technology improve efficiency by eliminating unwanted secondary interactions. Removing these secondary interactions also removes sources of variation, making separations developed on Discovery columns reproducible column-to-column and lot-to-lot.

Retention and / or Selectivity-Related Problems

The Discovery functionalized reversed-phases have different, unique bonded phase chemistries. Analyte molecules have different affinities to the different bonded phases and interact with them to differing degrees. An increase in affinity toward the bonded phase relative to the mobile phase increases retention, while a decrease in affinity decreases retention. Discovery functionalized reversed-phases can be more sensitive to differences between analyte molecules than a C18, and can therefore distinguish between them and give greater resolution.

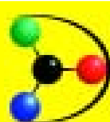
Separation Problems Addressed by Discovery Columns

The following pages show examples of how Discovery columns can solve the most common HPLC separation problems. Only examples, your compounds will vary and the solution may be a different Discovery phase than we've presented.

Use these Problem-Solution Guidelines along with the Column Screening Data to choose the right Discovery phase to meet your separation criteria.

See How Discovery Can Solve These Common HPLC Problems

1. Poor retention or not enough retention of polar compounds, need to eliminate ion-pair additives — pg. 47-49
2. Too much and too little retention on the same run — pg. 50-51
3. Too much resolution or wasted space in the chromatogram — pg. 52-55
4. Poor resolution of closely-eluting compounds — pg. 56-61
5. Switching of critical peak pair — pg. 62-64
6. Broad or tailing peaks, small peaks elute in tail of larger peak — pg. 65-67
7. Lengthy analysis time — pg. 68-70



Discovery Solves HPLC Problems

PROBLEM 1: Poor Retention of Polar Compounds

How does Discovery solve this problem?

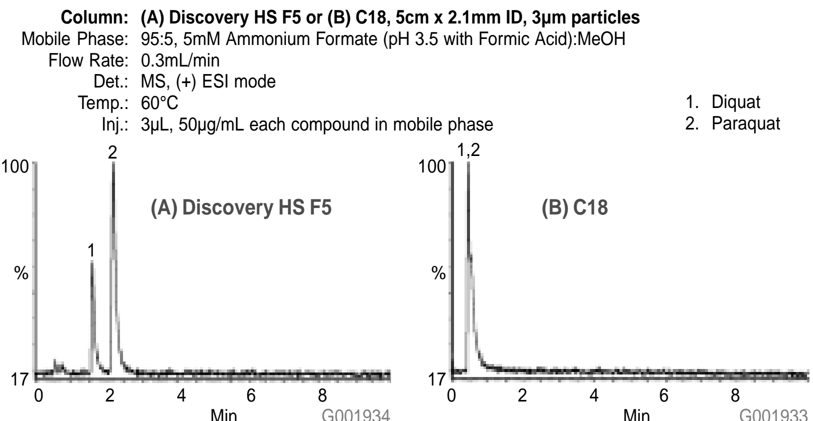
The different phase chemistries of the Discovery family give enhanced retention of polar compounds compared to a C18. By using one of the functionalized reversed-phases, you can obtain a different separation based on unique combinations of polar and hydrophobic retention.

Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

Demonstration 1: Enhanced retention of polar quaternary amines on Discovery HS F5

As shown in Figure 1, quaternary amines are not well retained on C18 without ion pairing. By changing the stationary phase to the Discovery HS F5 column, adequate retention and peak shape were obtained. Note that this separation is done with volatile, mass spec friendly mobile phases and no ion-pair reagents are used. The separation was done on a 5cm x 2.1mm ID column packed with 3µm Discovery HS F5 particles; ideal for LC/MS work.

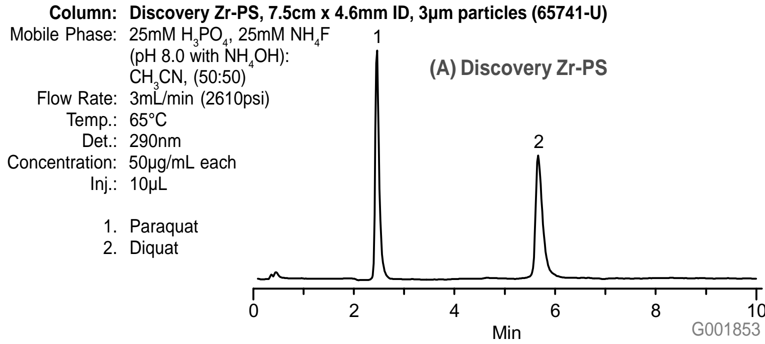
Figure 1: Longer Retention of Polar Quaternary Amines on Discovery HS F5

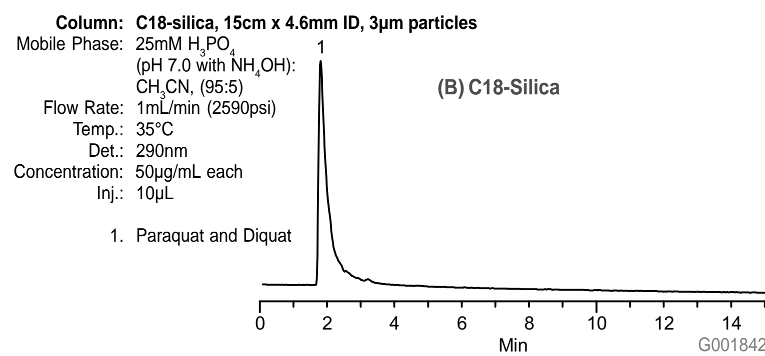


Demonstration 2: Enhanced retention of polar quaternary amines on Discovery Zr-PS

As shown in Figure 2, there are often multiple Discovery solutions to an HPLC problem. Discovery Zr-PS gives another example of enhanced quaternary amine retention compared to a C18. Here, natural ionic interactions from the Zr-PS particles enhance retention.

Figure 2. Paraquat and Diquat on Discovery Zr-PS vs. C18





Discovery Solves HPLC Problems

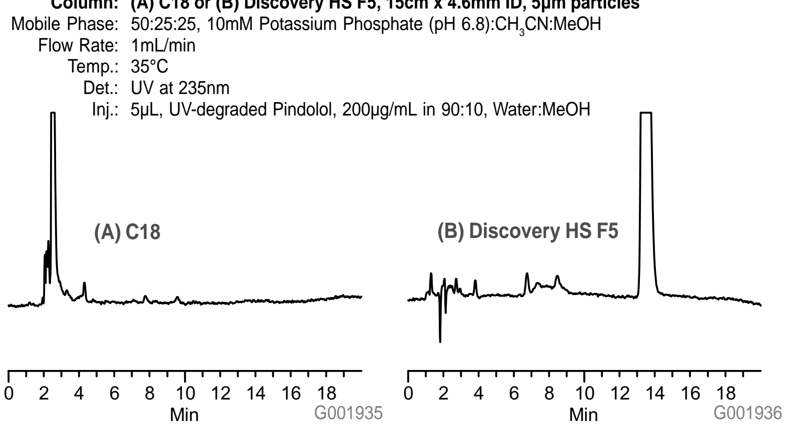
PROBLEM 1: Poor Retention of Polar Compounds

Demonstration 3: Poor retention of polar degradation products.

This example of changing the stationary phase to enhance retention shows the anti-hypertensive compound pindolol that has been degraded with UV light for 62 hours. Figure 1 shows that a C18 column gave poor retention of the parent compound. It was not able to resolve early-eluting degradants from the parent compound. In contrast, Discovery HS F5 gave adequate retention of pindolol and resolved many more degradants that eluted prior to the parent peak.

Figure 1: Discovery HS F5 Gives Enhanced Retention of Pindolol and Degradation Products

Column: **(A) C18 or (B) Discovery HS F5, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 50:25:25, 10mM Potassium Phosphate (pH 6.8):CH_3CN:MeOH
Flow Rate: 1mL/min
Temp.: 35°C
Det.: UV at 235nm
Inj.: 5µL, UV-degraded Pindolol, 200µg/mL in 90:10, Water:MeOH



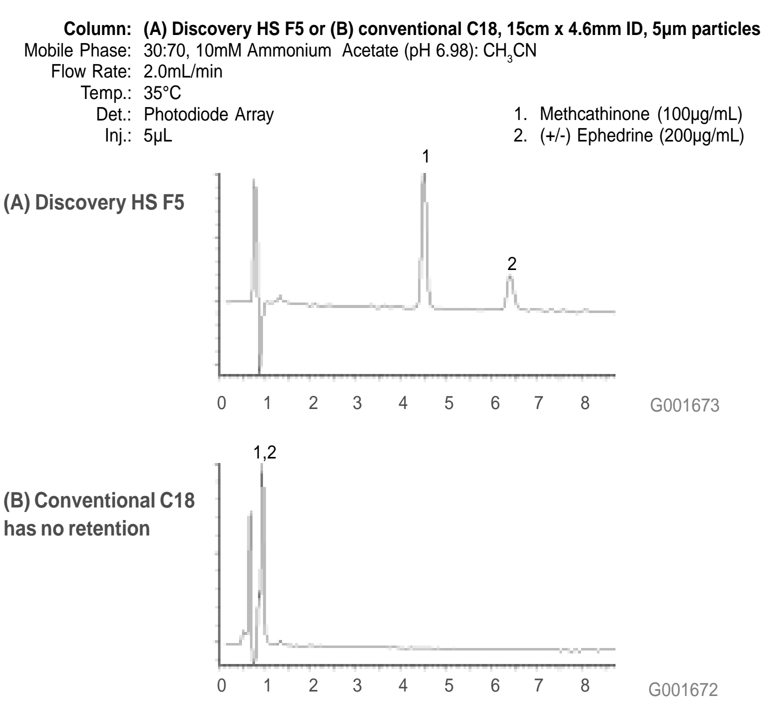
Demonstration 4: Poor retention of polar amines.

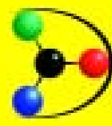
This example shows how changing the stationary phase from a standard C18 to a Discovery HS F5 column can enhance retention. Methcathinone, a psychoactive designer drug, is synthesized in clandestine labs by oxidation of ephedrine. Analysis and absolute identification are critical in criminal proceedings. A C18 column did not give adequate retention, even after much mobile phase manipulation. However, Discovery HS F5 gave adequate enhanced retention. Note also the high organic in the mobile phase for better desolvation in the MS.

Figure 2: Discovery HS F5 Provides Excellent Separation - Solutes Are Not Retained on C18

Column: **(A) Discovery HS F5 or (B) conventional C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 30:70, 10mM Ammonium Acetate (pH 6.98): CH_3CN
Flow Rate: 2.0mL/min
Temp.: 35°C
Det.: Photodiode Array
Inj.: 5µL

1. Methcathinone (100µg/mL)
2. (+/-) Ephedrine (200µg/mL)





Discovery Solves HPLC Problems

PROBLEM 1: Poor Retention of Polar Compounds

Demonstration 5:
Poor retention of polar antibiotic compounds.

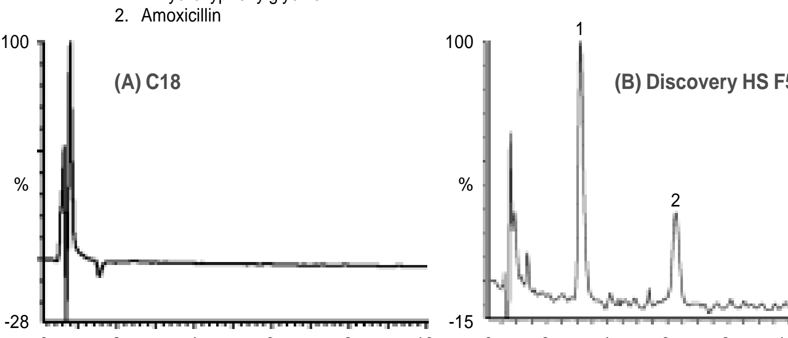
In this example, changing the stationary phase once again enhanced retention over a C18, this time for amoxicilin and an impurity. 4-Hydroxyphenylglycine is a common impurity of amoxicillin. Neither compound is retained by a C18 column. Both elute at the void volume. Conversely, on the Discovery HS F5, both compounds are retained and resolved, allowing reliable quantitation and purity profiling.

These examples show that if there is a problem with poor retention of polar compounds on a C18, a change in the stationary phase will likely give you enhanced retention and different selectivity.

Figure 1: Discovery HS F5 Gives Enhanced Retention of Antibiotic Compounds

Column: **(A) C18 or (B) Discovery HS F5, 5cm x 4.6mm ID, 5µm particles**
Mobile Phase: 20:80, 0.1% Formic Acid in Water: MeOH
Flow Rate: 1mL/min
Temp.: 35°C
Det.: UV photodiode array and MS
Inj.: 10µL, each compound 50µg/mL in 0.1% formic acid

1. 4-Hydroxyphenylglycine
2. Amoxicillin



Discovery Solves HPLC Problems

PROBLEM 2: Too Much and Too Little Retention on the Same Run

How does Discovery solve this problem?

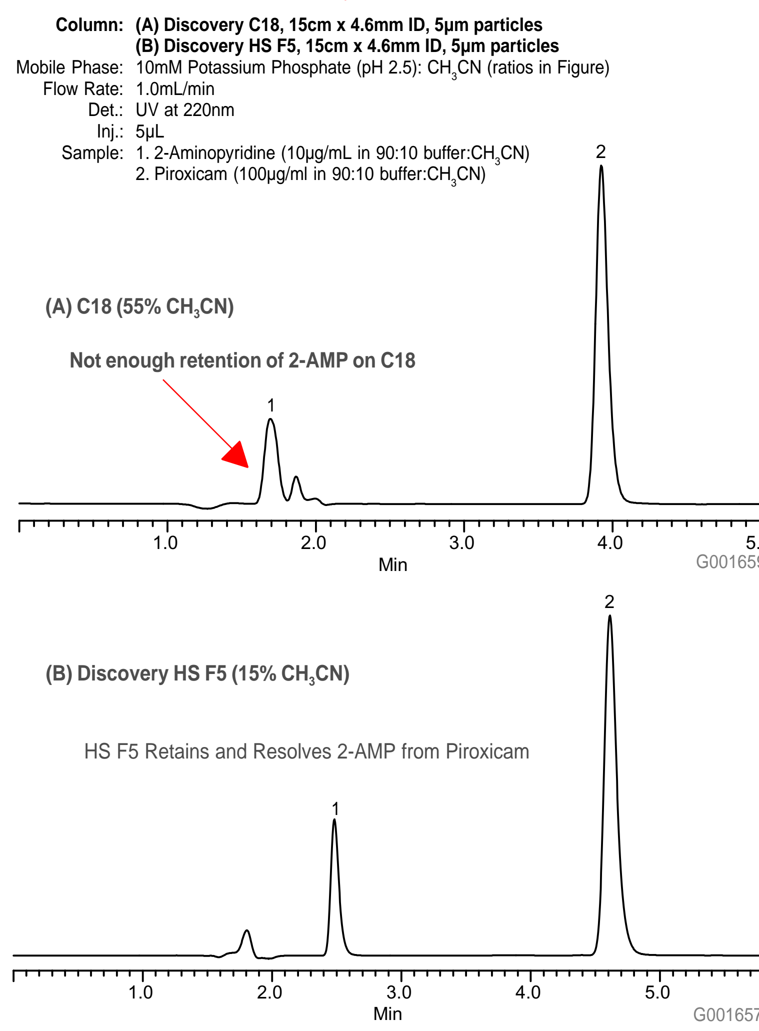
The Discovery family of functionalized RP columns offers unique selectivity compared to C18. These chemistries provide different retention that can bring peaks closer together. Generally, early eluting peaks (polar compounds) will have more retention, and later eluting peaks (non-polar compounds) will have less retention, thereby completing your separation in less time.

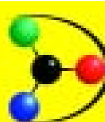
Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

Demonstration 1: Too much and too little retention of amine-containing compounds.

In the example shown in Figure 1, piroxicam and 2-aminopyridine (2-AMP) pose a problem on C18. 2-AMP elutes at the void volume while piroxicam is retained. Decreasing the % organic and changing the pH to increase retention of 2-AMP causes piroxicam to have excessive retention. By changing the reversed-phase stationary phase from a C18 to a pentafluorophenyl (the Discovery HS F5), the affinity of the two molecules toward the stationary phase changes. 2-AMP has more retention, while piroxicam has less retention. This is a prime example of the power of stationary phase chemistry in altering chromatographic selectivity.

Figure 1: 2-Aminopyridine (2-AMP) is Unretained on C18 Under Mobile Phase Conditions Used to Assay Piroxicam





Discovery Solves HPLC Problems

PROBLEM 2: Too Much and Too Little Retention on the Same Run

Demonstration 2:
Too much and too little retention of phenolic compounds.

In this second example, a series of phenolic compounds are shown on a C18 column. Note that on the C18 column the most polar compounds in the sample, such as phloroglucinol (peak #2), are essentially unretained, while the more non-polar compounds, like phenetole, do not elute from the column under isocratic conditions. By changing the reversed-phase stationary phase from a C18 to a polyethyleneglycol phase (the Discovery HS PEG), the affinity of the phenolic compounds is dramatically changed. The polar compounds elute later, the non-polar compounds elute sooner on the HS PEG column compared to the C18. This is another example of the power of stationary phase chemistry in altering chromatographic selectivity.

These examples show that if there is a problem with too much and too little retention in the same run, the different selectivity provided by Discovery functionalized reversed-phases may be the solution.

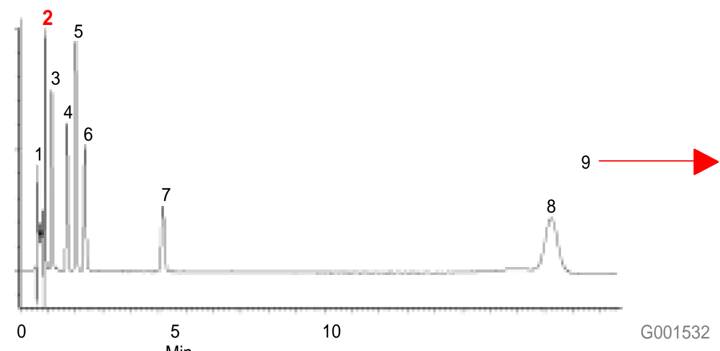
Figure 1. Resolution of Phenolic Compounds on Discovery HS PEG Compared to Standard C18

Columns: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 85:15, 10mM Ammonium Acetate (pH 6.8):CH_3CN
Flow Rate: 1.0mL/min
Temp: 20°C
Detection: UV/Photodiode Array
Injection: 10µL (50µg/mL for each analyte)

1. Uracil
2. Phloroglucinol
3. Pyrogallol
4. Resorcinol
5. Benzamide
6. Catechol
7. Phenol
8. Nitrobenzene
9. Phenetole

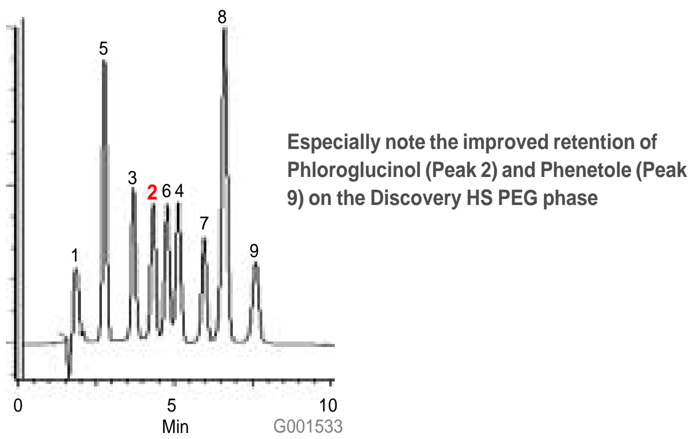
(A) Conventional C18 Column

Phenetole (9) is not eluted under these conditions on C18



(B) Discovery HS PEG

Note the selectivity differences of the phenolic compounds between the conventional C18 and the Discovery HS PEG phase.



Especially note the improved retention of Phloroglucinol (Peak 2) and Phenetole (Peak 9) on the Discovery HS PEG phase

Discovery Solves HPLC Problems

PROBLEM 3: Too Much Resolution or Wasted Space in the Chromatogram

How does Discovery solve this problem?

The Discovery family of functionalized RP columns offers unique selectivity compared to C18. These chemistries provide different retention that can bring peaks closer together.

Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

Demonstration 1: Too much resolution of alkaloids.

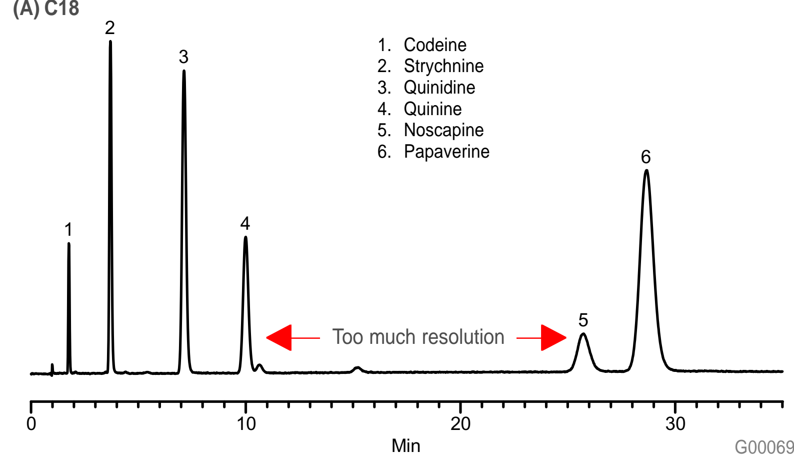
Alkaloids are naturally occurring bases with complex multicyclic ring structures. They are easily separated on the Discovery C18 column with good peak shape and adequate retention. However, there is excessive run time using C18, greater than 20 minutes. By changing to a more polar stationary phase such as the Discovery RP-AmideC16 as shown in Figure 1, a shorter analysis time is obtained with baseline resolution. If there is a requirement for shorter analysis time or you have too much resolution, consider going to a column that will provide different retention and offer unique selectivity such as the Discovery RP-AmideC16.

Figure 1: Discovery RP-AmideC16 Gives Better Resolution and Faster Analysis

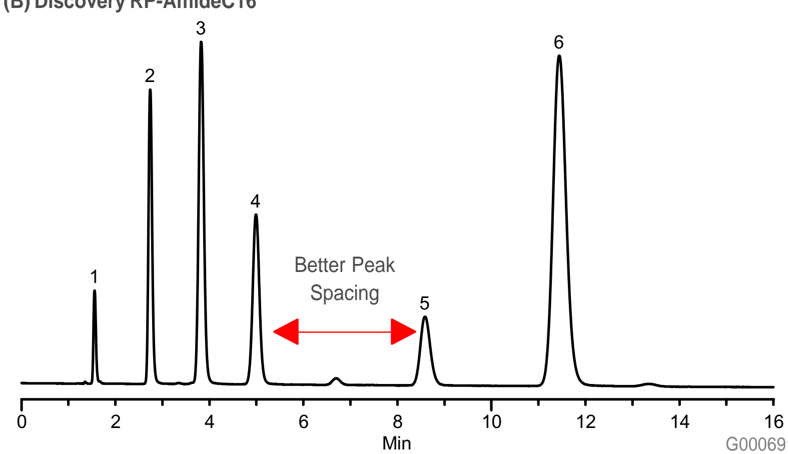
- faster analysis from lower hydrophobicity
- better peak spacing (RP-AmideC16)
- better resolution of small impurities (RP-AmideC16)

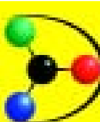
Columns: **(A) C18 and (B) Discovery RP-AmideC16, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 80:20, 25mM Potassium Phosphate (pH 3.0):MeOH
Flow Rate: 2.0mL/min
Temp.: 35°C
Det.: UV at 254nm
Inj.: 10µL

(A) C18



(B) Discovery RP-AmideC16





Discovery Solves HPLC Problems

PROBLEM 3: Too Much Resolution or Wasted Space in the Chromatogram

Demonstration 2:
High pH reduces excessive resolution of alkaloids.

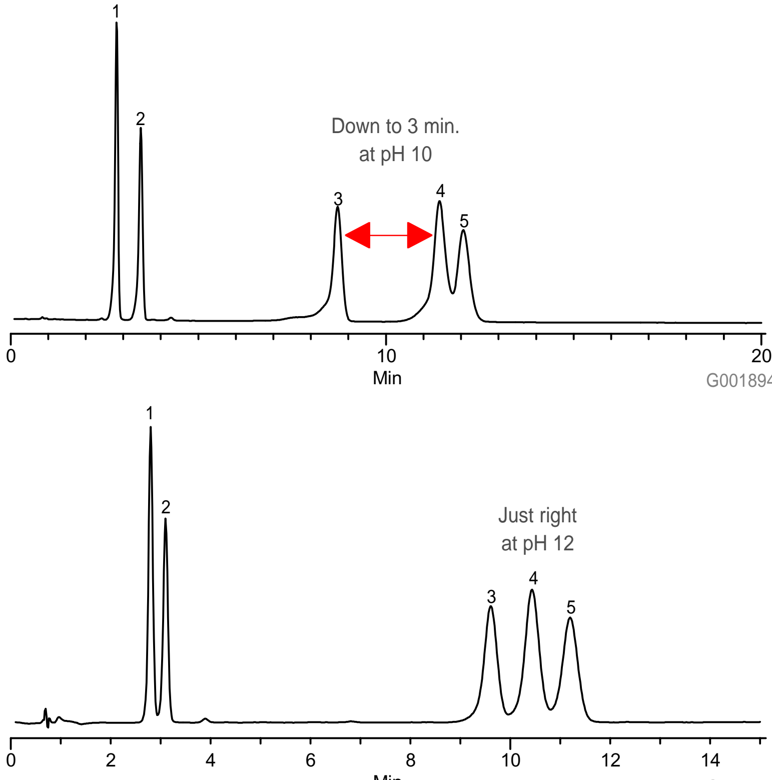
There are usually multiple Discovery solutions to every HPLC separation problem. The mobile phase pH influences retention of ionic compounds. Excessive retention may be solved by running at high or low pH. Silica-based phases are not stable above pH 8. However, Discovery Zr particles are stable from pH 1 to 14 allowing the full range of pH to alter selectivity. Here, excessive resolution of the five alkaloids is solved by using a Discovery Zr-PBD column at pH 12.

Figure 1: pH Change Can Reduce Wasted Space in Chromatogram

Column: **Discovery Zr-PBD 15cm x 4.6mm ID, 5µm particles (65718-U)**
Mobile Phase: 90:10, 20 mM Potassium Phosphate (pH 8, 10 or 12):CH_3CN
Flow Rate: 2.35mL/min
Temp.: 65°C
Det.: UV at 220nm
Inj.: 10µL, each compound 50µg/mL in mobile phase

1. Codeine
2. Strychnine
3. Papaverine
4. Quinine
5. Quinidine

1
2
3
20 min. between peaks
3 and 4 at pH 8
4,5
0 10 20 30 40 50 60
Min
G001895



Discovery Solves HPLC Problems

PROBLEM 3: Too Much Resolution or Wasted Space in the Chromatogram

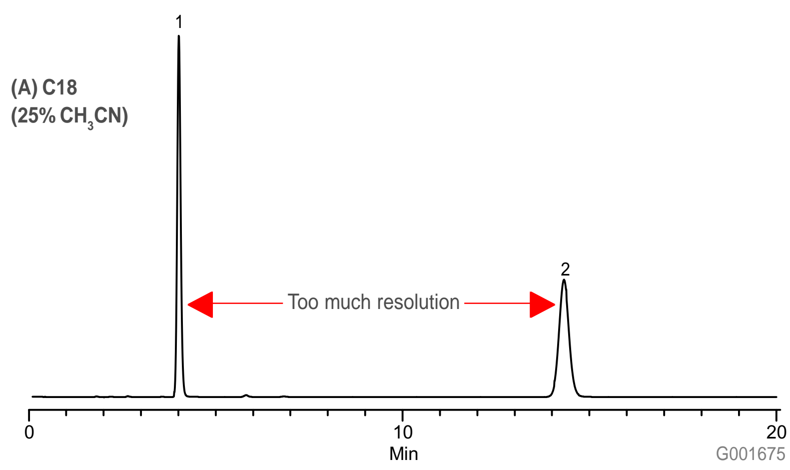
Demonstration 3:
Solving excessive retention of pharmaceutical compounds.

This example of excessive retention and resolution shows the skeletal muscle relaxant chlorzoxazone and its metabolite 6-hydroxychlorzoxazone. Analysis on a C18 column had excessive retention and resolution. The challenge was to reduce the retention of chlorzoxazone without losing retention of the more polar metabolite. By changing to a Discovery HS PEG column, run time and excessive resolution were decreased. Baseline separation was achieved in under six minutes. Many drug metabolites are more polar than the parent compound and subsequently elute before the parent compound. Discovery HS PEG is a good choice for looking at polar metabolites if there is a need for faster analysis while maintaining optimal resolution.

Figure 1: Chlorzoxazone - Excellent Separation on HS PEG; Excessive Retention and Resolution on C18

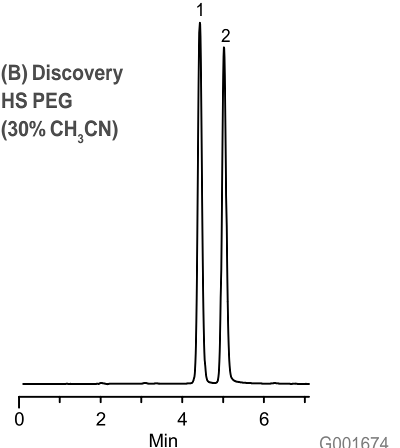
Column: **Conventional C18 , 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 20mM Acetic Acid in Water (pH 4.5 with Ammonium Hydroxide):CH_3CN
Flow Rate: 1.0mL/min
Temp.: 30°C,
Det.: UV at 285 nm
Inj.: 10µL, each compound 100µg/mL

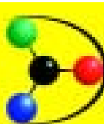
1. 6-Hydroxychlorzoxazone
2. Chlorzoxazone



Column: **Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles (567416-U**
Mobile Phase: 20mM Acetic Acid in Water (pH 4.5 with Ammonium Hydroxide):CH_3CN
Flow Rate: 1.0mL/min
Temp.: 30°C,
Det.: UV at 285 nm
Inj.: 10µL

1. 6-Hydroxychlorzoxazone
2. Chlorzoxazone





Discovery Solves HPLC Problems

PROBLEM 3: Too Much Resolution or Wasted Space in the Chromatogram

Demonstration 4:
Solving excessive retention of hydroxylated compounds.

The last example in this section shows a set of phenolic compounds run under isocratic conditions on a C18. Note the excessive time between peaks 7 and 8 on the C18. By using the Discovery HS PEG phase, the excessive resolution is compressed to an ideal isocratic separation.

These examples show that if there is a problem with excessive resolution or lengthy analysis time, the different selectivity or allowable pH range provided by Discovery functionalized reversed-phases may be the solution.

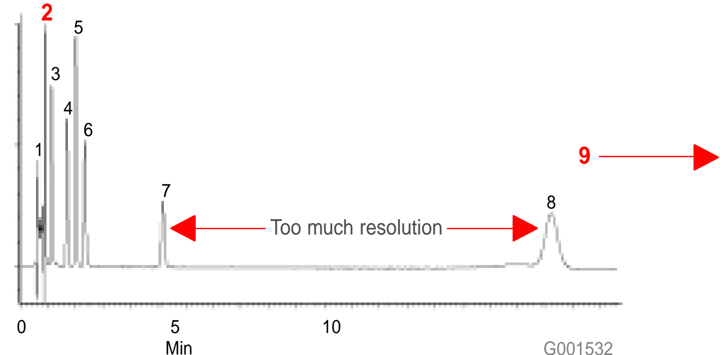
Figure 1. Resolution of Phenolic Compounds on Discovery HS PEG Compared to Standard C18

Columns: **(A) Conventional C18 and (B) Discovery HS PEG, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 85:15, 10mM Ammonium Acetate (pH 6.8):MeCN
Flow Rate: 1.0mL/min
Temp: 20°C
Det.: UV/Photodiode Array
Inj.: 10µL (50µg/mL for each analyte)

1. Uracil
2. Phloroglucinol
3. Pyrogallol
4. Resorcinol
5. Benzamide
6. Catechol
7. Phenol
8. Nitrobenzene
9. Phenetole

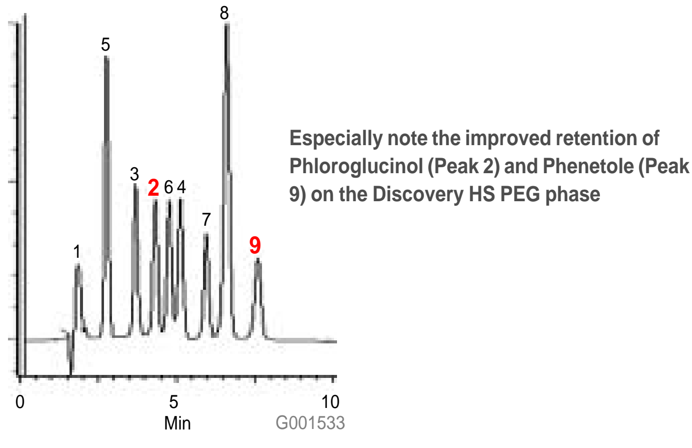
(A) C18

Phenetole (9) is not eluted under these conditions on C18



(B) Discovery HS PEG

Note the selectivity differences of the phenolic compounds between the conventional C18 and the Discovery HS PEG phase.



Especially note the improved retention of Phloroglucinol (Peak 2) and Phenetole (Peak 9) on the Discovery HS PEG phase

Discovery Solves HPLC Problems

PROBLEM 4: Poor Resolution of Closely-eluting Compounds

How does Discovery solve this problem?

The Discovery family of functionalized reversed-phase columns offer unique retention and selectivity compared to C18. These unique chemistries frequently allow you to achieve better separations compared to C18.

Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

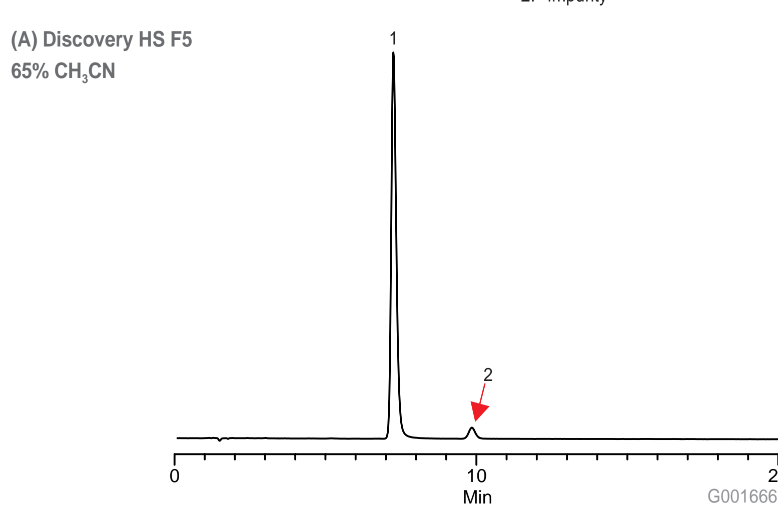
Demonstration 1: Solving co-elution of parent pharmaceutical compound and an impurity.

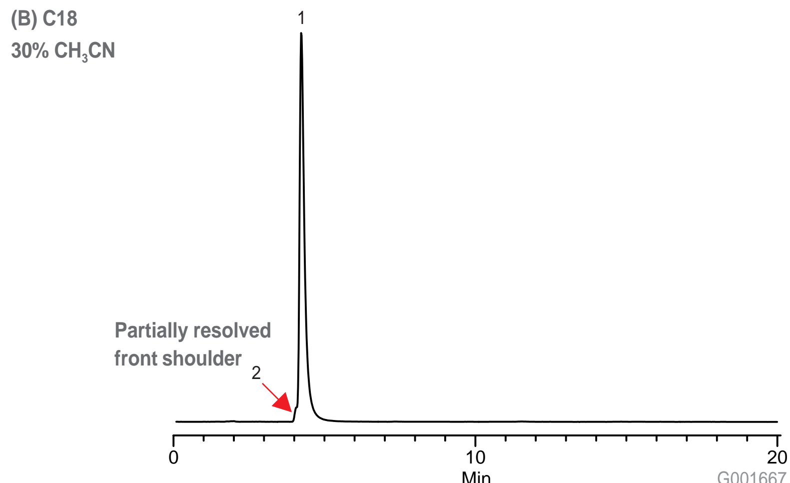
It is important to identify and quantify impurities in pharmaceutical compounds. The discovery of impurities late in the drug development process may result in substantial costs to modify the production process. The quinidine separation in Figure 1 provides an example of how Discovery functionalized reversed-phases can help researchers identify impurities. Analysis of the upslope UV spectra indicated an unknown impurity hidden under the quinidine peak. Manipulating the mobile phase and other analysis conditions did not resolve the impurity. However, by using a reversed-phase with different selectivity, in this case a Discovery HS F5 column, the impurity (identified by MS as dihydroquinidine) was fully resolved allowing quantitation.

Figure 1: F5 Resolves Trace Impurity in Quinidine – C18 Does Not

Column: **Discovery HS F5 and Conventional C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 25 mM Ammonium Phosphate (pH 7.0):CH_3CN (ratio appears in Figure)
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV at 235nm
Inj.: 10µL

Sample:
1. Quinidine (50µg/mL)
2. Impurity





Discovery Solves HPLC Problems

PROBLEM 4: Poor Resolution of Closely-eluting Compounds

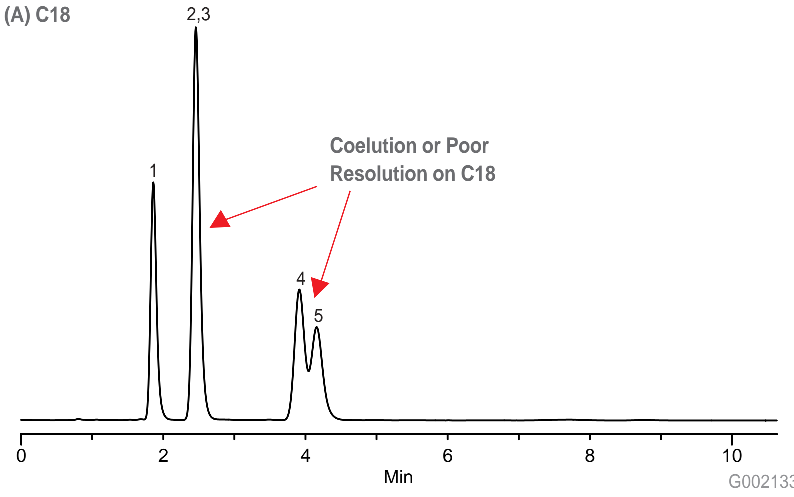
Demonstration 2: Solving co-elution of steroid compounds.

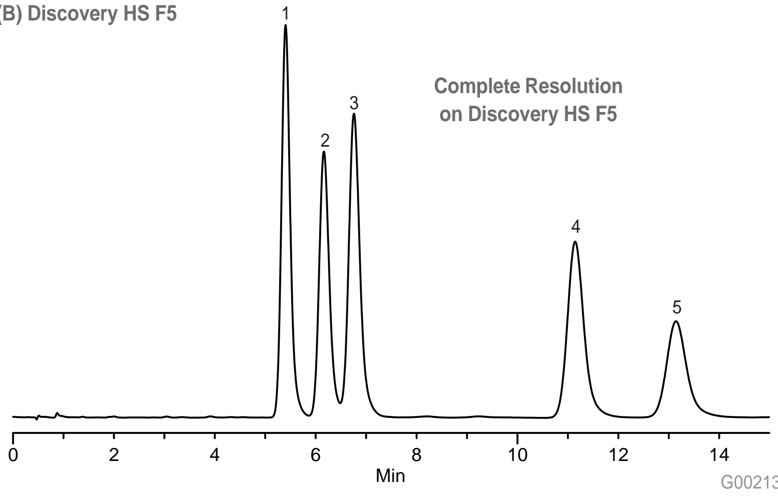
The steroidal compounds in this application are very similar in structure. A C18 column was not able to fully resolve several of the pairs. However, by using a functionalized reversed-phase column with enhanced polar-group selectivity, in this case a Discovery HS F5, resolution of all five compounds was achieved with a simple mobile phase.

Figure 1: Optimized Separation of Corticosteroids on Discovery HS F5

Column: **Discovery HS F5, 5cm x 4.6mm ID, 5µm particles**
Mobile Phase: 60:40, Water:Methanol
Flow Rate: 1.5mL/min
Temperature: 60°C
Detection: UV, 240nm
Injection Volume: 5µL
Sample: 10mg/mL mixture of corticosteroids in mobile phase

1. Hydrocortisone
2. Prednisolone
3. Prednisone
4. Corticosterone
5. Hydrocortisone acetate





Discovery Solves HPLC Problems

PROBLEM 4: Poor Resolution of Closely-eluting Compounds

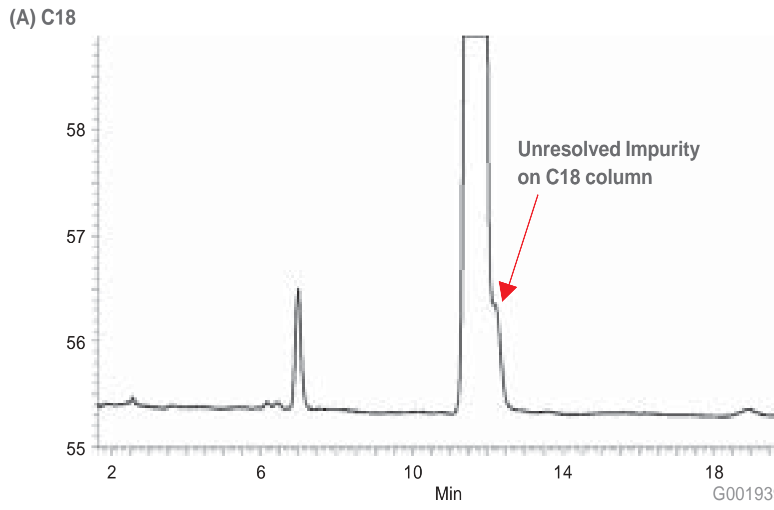
Demonstration 3:
Solving co-elution of prednisolone and an impurity.

Prednisolone is a naturally occurring steroid, chemically related to hydrocortisone. HPLC is often used to assay the purity of the synthetic form. A C18 column was not able to fully resolve a small impurity of prednisolone that appeared on the downslope of the main peak. However, by using a functionalized reversed-phase column with enhanced polar-group selectivity, in this case a Discovery HS F5, resolution of this compound was achieved.

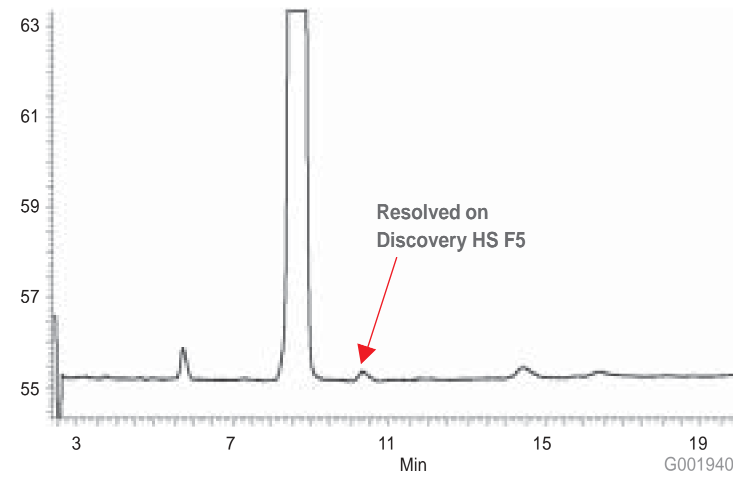
Figure 1: Discovery HS F5 Resolves Prednisolone and Impurity

Column: **(A) C18 or (B) Discovery HS F5, 25cm x 4.6mm ID, 5µm particles**
Mobile Phase: (A) Water; (B) CH_3CN; 0-26% B in 20 minutes
Flow Rate: 1.5mL/min
Det.: UV at 243nm
Temp.: Ambient
Inj.: 10µL, Prednisolone (0.25mg/mL)

(A) C18



(B) Discovery HS F5



Discovery Solves HPLC Problems

PROBLEM 4: Poor Resolution of Closely-eluting Compounds

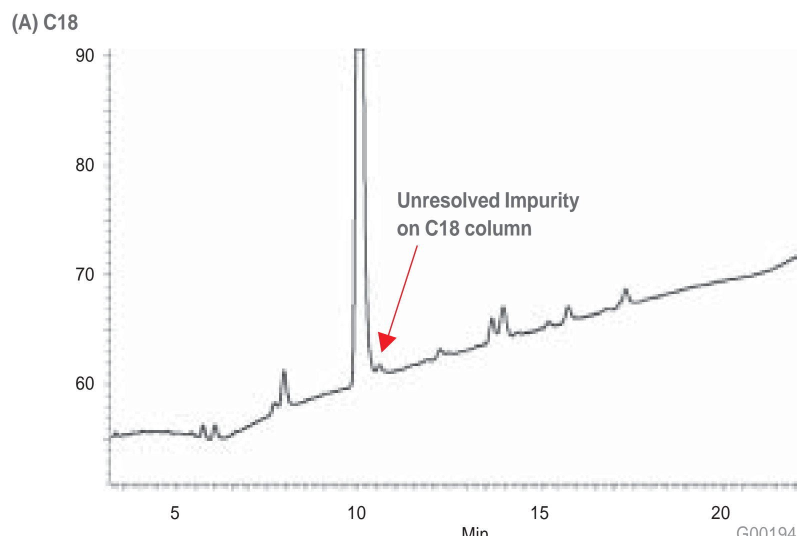
Demonstration 4:
Solving co-elution of Pepstatin A and impurity.

Pepstatin A is a pentapeptide pepsin inhibitor, isolated from cell culture broths. In this example, note that the separation on a standard C18 column shows a small peak that is barely resolved from the large pepstatin A peak. When the same gradient was run on Discovery HS F5 column, baseline resolution of the smaller impurity peak was achieved. Changing from a C18 to a functionalized reversed-phase changed selectivity, allowing an impurity peak, previously unresolved, to be separated and detected.

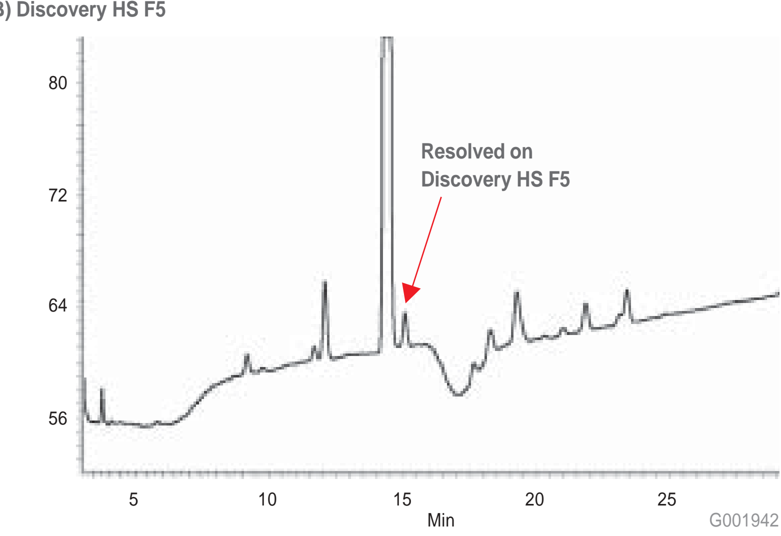
Figure 1: Discovery HS F5 Resolves Impurity from Pepstatin A

Column: (A) C18 or (B) Discovery HS F5, 25cm x 4.6mm ID, 5µm particles
Mobile Phase: 0.1% TFA in (A) Water; (B) 1:3, Water:CH_3CN; 40 – 65% B in 30 minutes
Flow Rate: 1.3mL/min
Det.: UV at 215nm
Temp.: Ambient
Inj.: 20µL, Peptstatin A (1mg/mL in CH_3OH containing 1% CH_3OOH)

(A) C18



(B) Discovery HS F5



Discovery Solves HPLC Problems

PROBLEM 4: Poor Resolution of Closely-eluting Compounds

Demonstration 5:
Solving co-elution of hydroxylated flavone compounds.

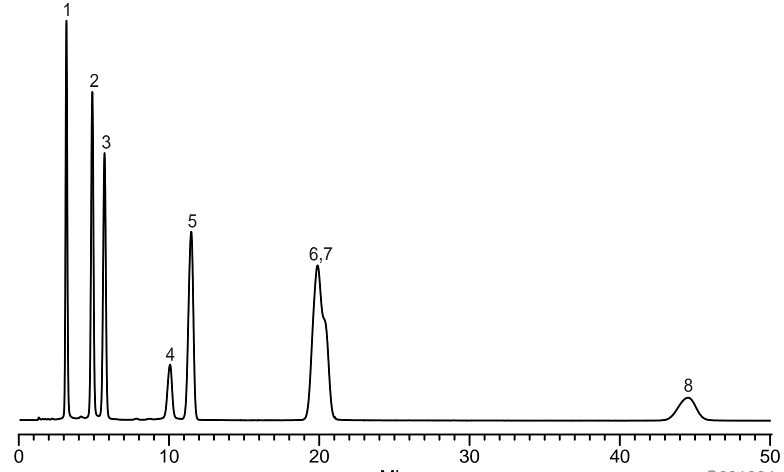
Flavones are a group of naturally-occurring, multi-ring, hydroxyl-containing compounds that are widely studied for their nutritional value and their use in preventive medicine. On a C18 column, co-elution of some flavone components typically occurs. By changing to a functionalized reversed-phase column, in this instance a Discovery HS PEG column, resolution as well as shorter run time were achieved.

Figure 1: Flavones Demonstrate Dramatic Selectivity Differences Between HS PEG and C18

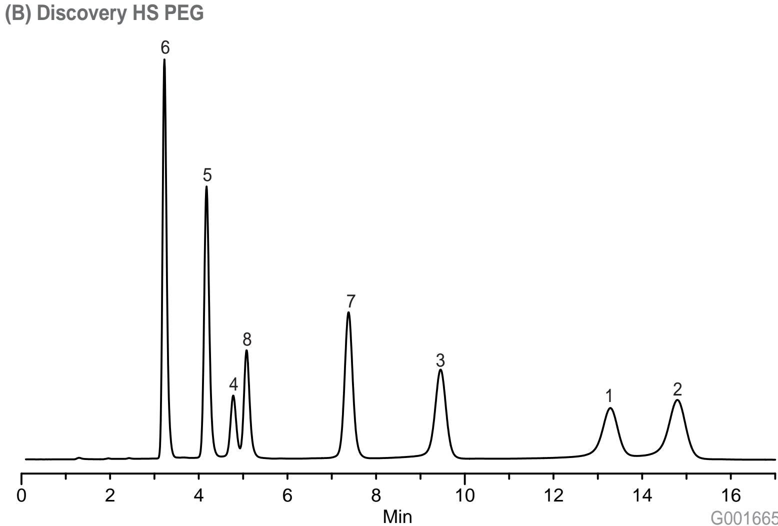
Column: 15cm x 4.6mm ID, 5µm particles
Mobile Phase: 45:55 0.1% Formic Acid in Water : 0.1% Formic Acid in MeOH
Flow Rate: 1.0mL/min
Temp.: 30°C,
Det.: UV at 254nm
Inj.: 10µL

Sample: 50µg/mL of each
1. Myricetin
2. Quercetin
3. Luteolin
4. Baicalein
5. 7-Hydroxyflavone
6. Flavone
7. Chrysin
8. 5-Hydroxyflavone

(A) Discovery HS C18



(B) Discovery HS PEG



Discovery Solves HPLC Problems

PROBLEM 4: Poor Resolution of Closely-eluting Compounds

Demonstration 6:
High pH improves resolution of alkaloids.

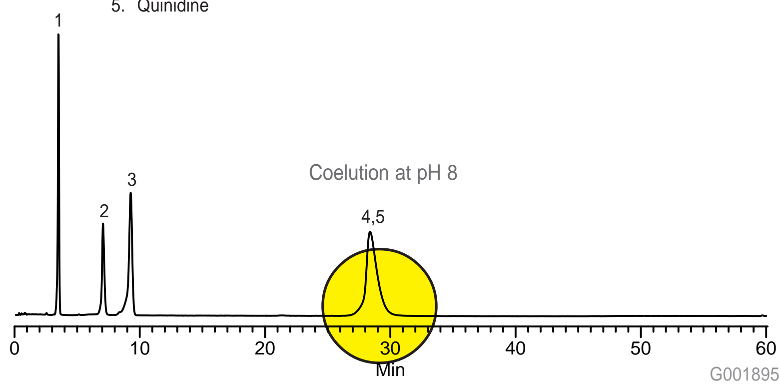
The mobile phase pH influences retention of ionic compounds. The solution to a co-elution problem may be to running at high or low pH. Silica-based phases are not stable above pH 8. However, Discovery Zr particles are stable from pH 1 to 14 allowing the full range of pH to alter selectivity. Here, two alkaloids that co-elute at pH 8 are resolved by increasing to pH 12 on a Discovery Zr-PBD column.

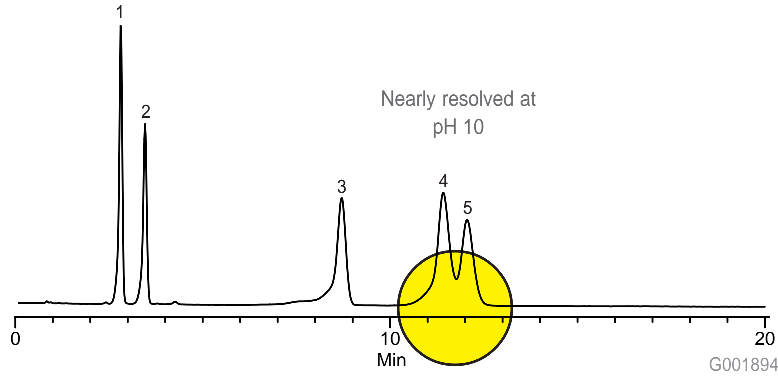
During method development, a quick screen of Discovery's unique, functionalized reversed-phases can increase the chances of finding trace impurities early in the development process, before they can become problematic. The alternate phase chemistries also are excellent choices for confirmational columns.

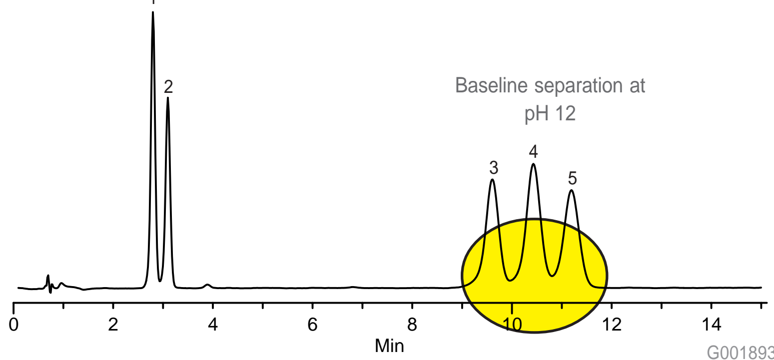
Figure 1: pH Change Can Reduce Wasted Space in Chromatogram

Column: Discovery Zr-PBD 15cm x 4.6mm ID, 5µm particles (65718-U)
Mobile Phase: 90:10, 20 mM Potassium Phosphate (pH 8, 10 or 12):CH_3CN
Flow Rate: 2.35mL/min
Temperature: 65°C
Detection: 220nm
Inj.: 10µL, each compound 50µg/mL in mobile phase

1. Codeine
2. Strychnine
3. Papaverine
4. Quinine
5. Quinidine







Discovery Solves HPLC Problems

PROBLEM 5: Switching Critical Peak Pair

How does Discovery solve this problem?

The Discovery family of functionalized reversed-phase columns offer unique retention and selectivity compared to C18. These unique chemistries frequently allow you to achieve better separations, including completely reversing the elution order compared to C18.

Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

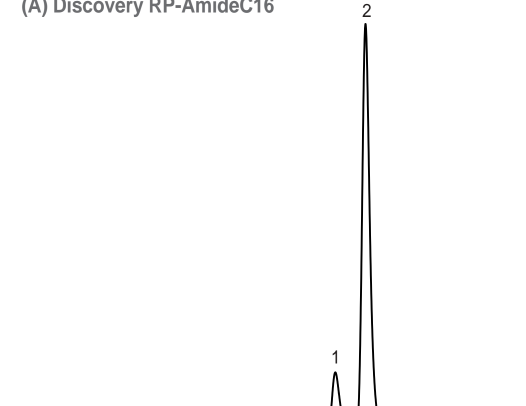
Demonstration 1: Switching peak order on two pharmaceutical compounds.

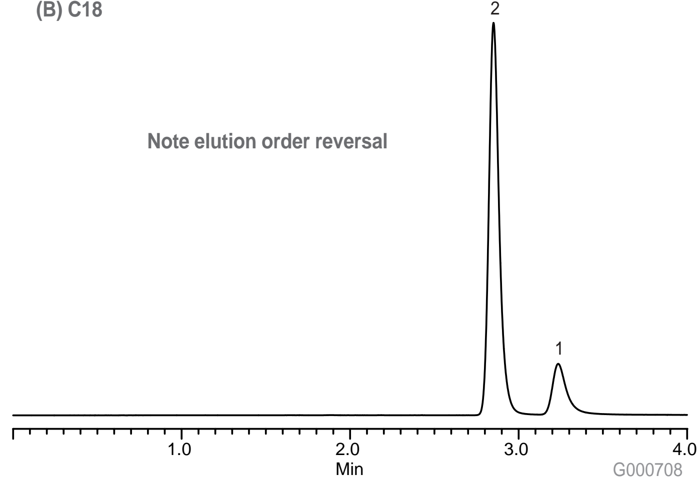
Chromatographers are likely to encounter a situation where they desire an elution order reversal. The large changes in selectivity required to accomplish an elution order reversal is easily accomplished by changing the stationary phase chemistry. An example of this is illustrated the separation of pseudoephedrine and acetaminophen shown in Figure 1. Elution order on the Discovery C18 column is reversed on a Discovery RP-AmideC16 column. The separation is perfect, with both good peak shape and good resolution in addition to a short run time. For accurate quantitation, it is best to have the peak of lower response elute before the main peak. In this demonstration, you would choose the C18 or the RP-AmideC16 depending on the desired peak order and quantitation needs.

Figure 1: Elution Order Reversal on Cold Remedy Ingredients

Column: **(A) Discovery RP-AmideC16 or (B) conventional C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 85:15, 20mM Potassium Phosphate (pH 7): CH_3CN
Flow Rate: 1mL/min
Det.: UV at 220nm
Temp.: 20°C
Inj.: 1µL, each compound 100µg/mL

1. Pseudoephedrine
2. Acetaminophen





Discovery Solves HPLC Problems

PROBLEM 5: Switching Critical Peak Pair

Demonstration 2: Switching peak order of flavone compounds.

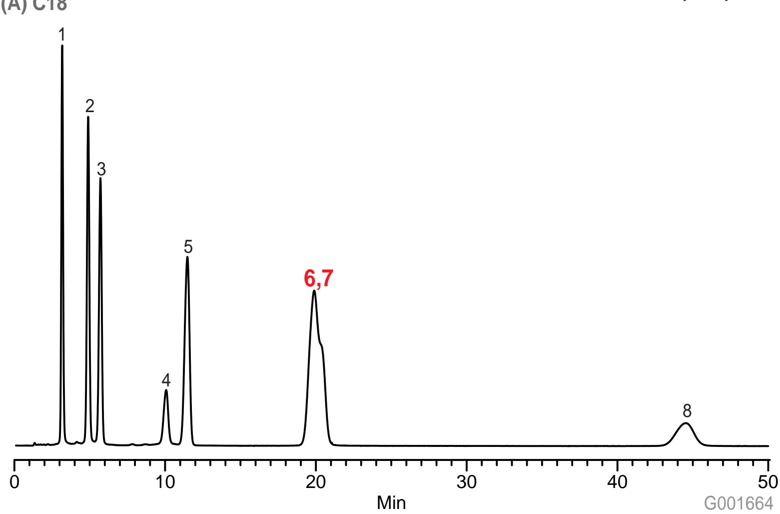
Several critical peak pairs are evident in the flavone sample shown in Figure 1. Peaks 6 and 7 (flavone and chrysin) and resolved on the Discovery HS PEG column and not on the C18. Two other pairs, 4/5 (baicalein and 7-hydroxyflavone) and 5/6 (7-hydroxyflavone and flavone) show a switching of elution order. Like the Discovery HS F5, the Discovery HS PEG functionalized reversed-phase column can have a dramatic effect on elution order and critical pair resolution.

Figure 1: Flavones Demonstrate Dramatic Selectivity Differences Between HS PEG and C18

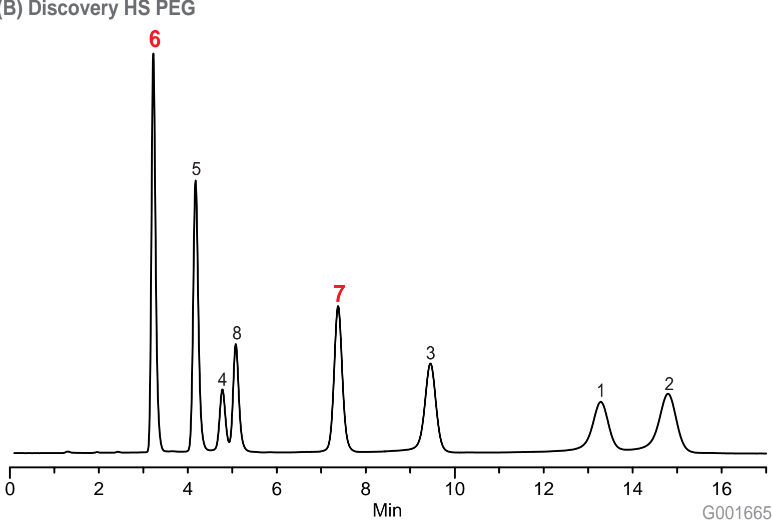
Column: **15cm x 4.6mm columns, 5µm particles**
Mobile Phase: 45:55 0.1% Formic Acid in Water : 0.1% Formic Acid in MeOH
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV at 254nm
Inj.: 10µL

Sample: **50µg/mL of each**
1. Myricetin
2. Quercetin
3. Luteolin
4. Baicalein
5. 7-Hydroxyflavone
6. Flavone
7. Chrysin
8. 5-Hydroxyflavone

(A) C18



(B) Discovery HS PEG



Discovery Solves HPLC Problems

PROBLEM 5: Switching Critical Peak Pair

Demonstration 3:
Switching peak order of organic acids.

Another example of the power of changing stationary phases is illustrated in Figure 1. Here, a mixture of organic acids is shown on a C18 and a Discovery HS F5 column under the same conditions. Take note of o-nitrobenzoic acid, benzoic acid, and sorbic acid (peaks 2, 3, and 4). The Discovery HS F5 column not only resolves the benzoic and sorbic acid pair that the C18 does not, it also provides different elution order than the C18 column. Using a functionalized reversed-phase column, like the HS F5, can have a dramatic effect on elution order and critical pair resolution.

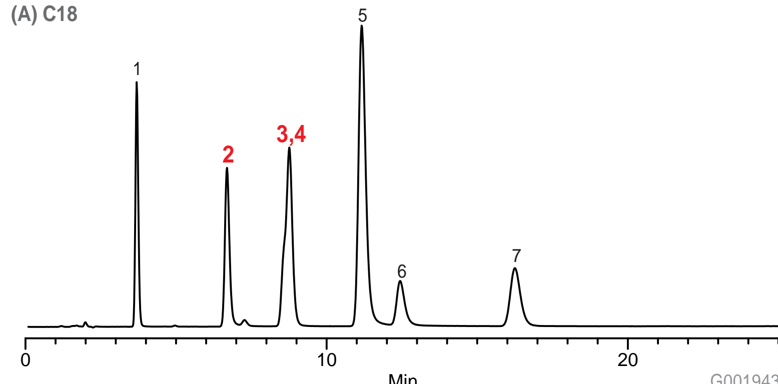
When your separation could be improved by switching the elution order of a critical peak pair, a change in the stationary phase from a C18 to a Discovery functionalized reversed-phase will likely give you the desired results.

Figure 1: Organic Acids Have Different Elution Order on C18 and HS F5 Columns

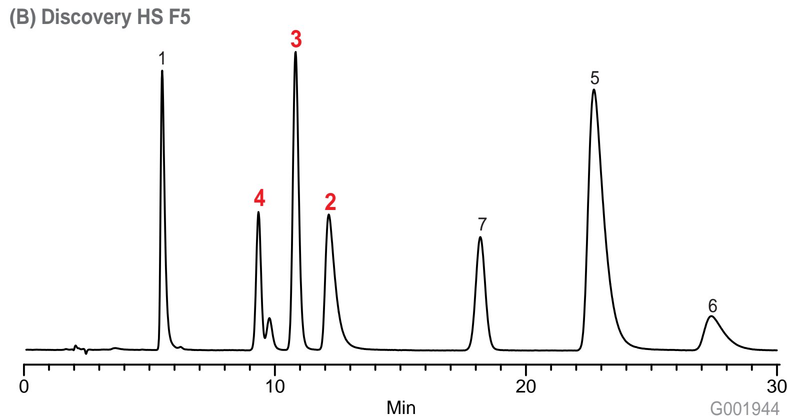
Column: **(A) C18 or (B) Discovery HS F5, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 80:20, 20mM Phosphoric Acid (pH 2.0 with NH_4OH):CH_3CN
Flow Rate: 1mL/min
Det.: UV at 220nm
Temp.: 30°C
Inj.: 10µL, each compound 25µg/mL

1. Phthalic acid
2. o-Nitrobenzoic acid
3. Benzoic acid
4. Sorbic acid
5. m-Nitrobenzoic acid
6. p-Nitrobenzoic acid
7. o-Toluic acid

(A) C18



(B) Discovery HS F5



Discovery Solves HPLC Problems

PROBLEM 6: Tailing or Broad Peaks, Small Peaks Eluting in Tail of Larger Peak

How does Discovery solve this problem?

All Discovery HPLC phases begin with pure, metal-free, high quality silica and employ advanced bonded phase technology. As a result, they give excellent peak shape in simple mobile phases.

Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

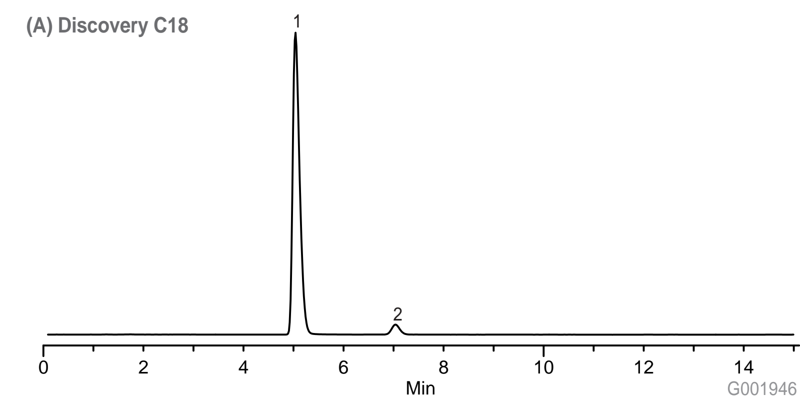
Demonstration 1: Peak tailing interferes with quantitation of quinidine impurity.

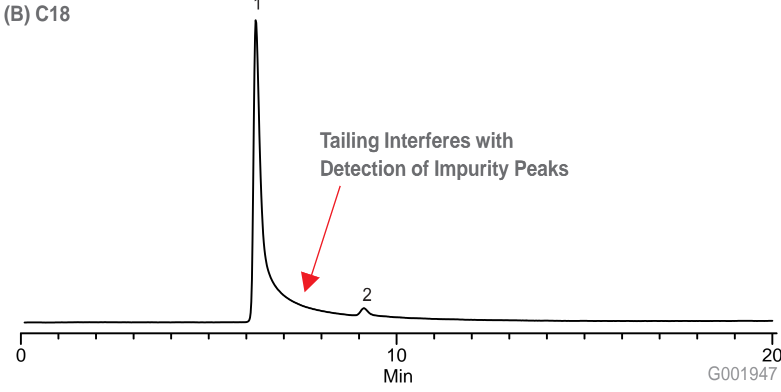
A common cause of peak tailing is adsorption (H-bonding) between of a basic analyte and silanol groups on the silica particle's surface. Tailing peaks are difficult to quantify, reduce sensitivity, and can mask small peaks that elute within the tail of a larger peak. Mobile phase additives (e.g. TEA) can reduce tailing, but they have their own set of problems and are to be avoided whenever possible. Discovery reduces tailing because of the silica particle and bonded phase synthesis procedures we apply to their production. An example of the power of Discovery particles to reduce tailing is shown in the separation of the antiarrhythmic and antimalarial drug quinidine. The sample contains an impurity peak (dihydroquinidine). On the Discovery C18 column, the impurity peak is well resolved from the main quinidine peak. However, on the competitive C18 column, tailing of the quinidine peak interferes with the impurity peak presenting potential problems in identification and quantitation.

Figure 1: Discovery C18 Provides Excellent Peak Shape of Quinidine

Columns: **Discovery C18 or competitive C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 90:10, 25mM H_3PO_4/NH_4OH pH 2:CH_3CN
Flow Rate: 1mL/min
Det.: UV at 250nm
Temp.: 30°C
Inj.: 10µL, Quinidine, 50µg/mL

1. Quinidine
2. Dihydroquinidine





Discovery Solves HPLC Problems

PROBLEM 6: Tailing or Broad Peaks, Small Peaks Eluting in Tail of Larger Peak

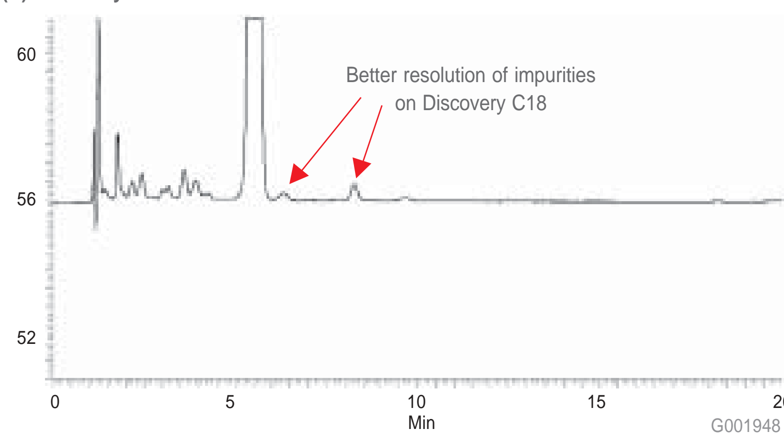
Demonstration 2:
Higher efficiency improves resolution of NHDP impurity.

The purity check of NHDP (nicotinamide hypoxanthine dinucleotide phosphate) shows several small impurity peaks eluting after the main NHDP peak. This analysis is shown on two C18 columns in Figure 1. On the Discovery C18 column the main NHDP peak elutes in an efficient, symmetrical fashion, allowing easy identification of impurity peaks that elutes after the main peak. On the competitive C18 column, lower efficiency reduces the ability to quantify the impurity peaks. Only one of the three impurity peaks can be visualized.

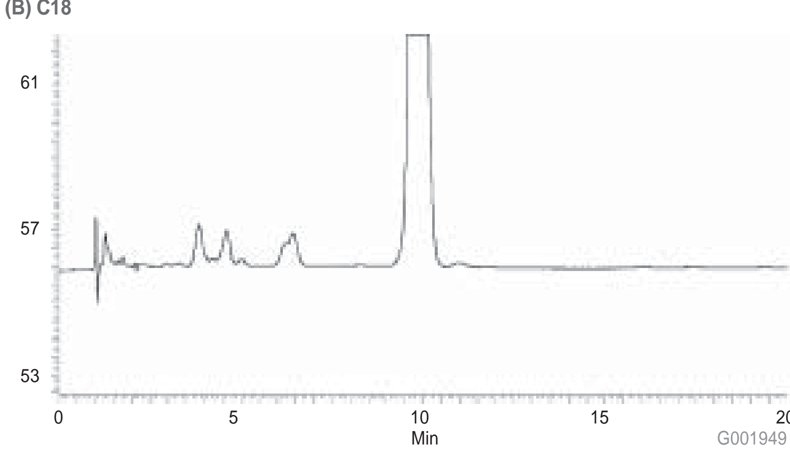
Figure 1: Discovery C18 Resolves Impurity that Competitive C18 Does Not

Columns: **(A) Discovery C18 or (B) competitive C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: (A) 100mM $NH_4H_2PO_4$ (pH 7.0); (B) 1:3 $CH_3CN:H_2O$
Gradient: 0 – 5% B in 20 minutes
Flow Rate: 1.5mL/min
Det.: UV at 260nm
Temp.: Ambient
Inj.: 10µL, NHDP, 0.5mg/mL

(A) Discovery C18



(B) C18



Discovery Solves HPLC Problems

PROBLEM 6: Tailing or Broad Peaks, Small Peaks Eluting in Tail of Larger Peak

Demonstration 3:
Improved peak symmetry of basic pharmaceutical compounds.

Discovery columns solve tailing problems on many different types of basic compounds. This example shows a mixture of four basic tricyclic antidepressants on Discovery C18 and two modern, competitive C18 columns under the same conditions.

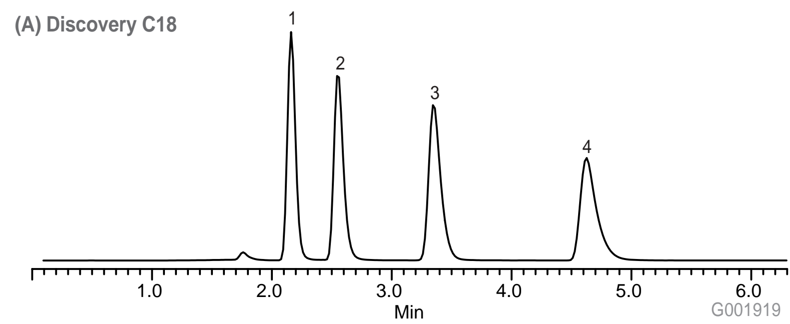
To maximize peak symmetry and ensure reliable quantitation, it is important to choose a column that uses the highest quality silica and bonded phase technology. Discovery phases give excellent peak shape for basic compounds under simple mobile phase conditions.

Figure 1: Improved Peak Shape of Tricyclics on Discovery C18

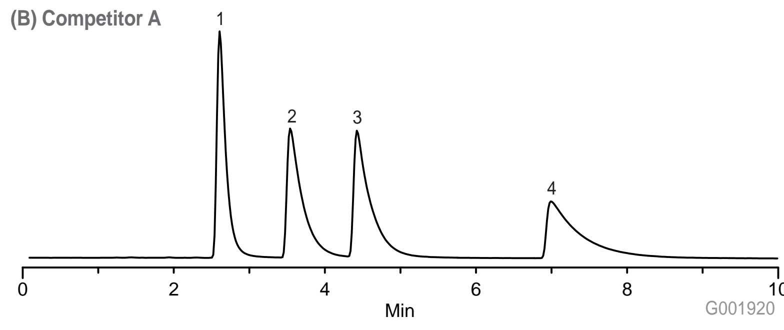
Columns: **Discovery C18 or competitive C18, 15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 55:45, 25mM Ammonium Phosphate (pH 7.0):CH_3CN
Flow Rate: 1mL/min
Det.: UV at 254nm
Temp.: 30°C
Inj.: 10µL, each compound 50µg/mL

1. Nordoxepin
2. Nortriptyline
3. Doxepin
4. Amitriptyline

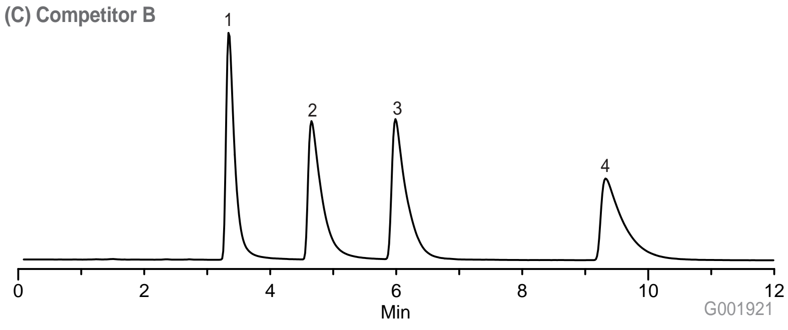
(A) Discovery C18



(B) Competitor A



(C) Competitor B



Discovery Solves HPLC Problems

PROBLEM 7: Lengthy Analysis Time

How does Discovery solve this problem?

The Discovery solution to lengthy analysis time comes in two forms. Discovery Zr uses the power of extreme pH and temperature while Discovery functionalized reversed-phases use the power of bonded phase selectivity to reduce analysis time.

Note that there are many paths to take, and different Discovery phases to choose that will solve a problem. We have just highlighted a few examples.

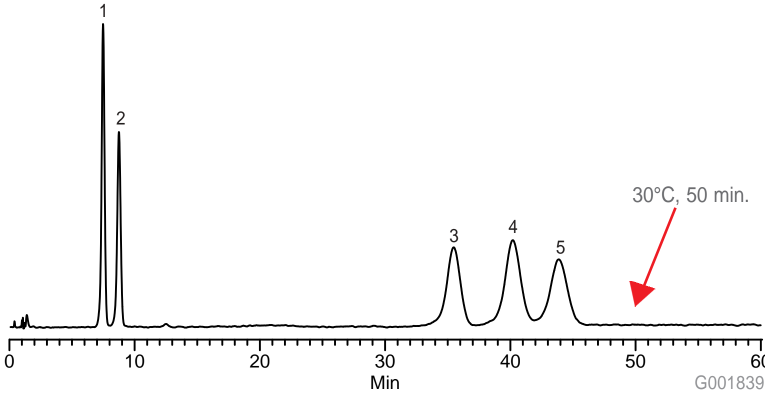
Demonstration 1: Increased temperature reduces run time of alkaloids.

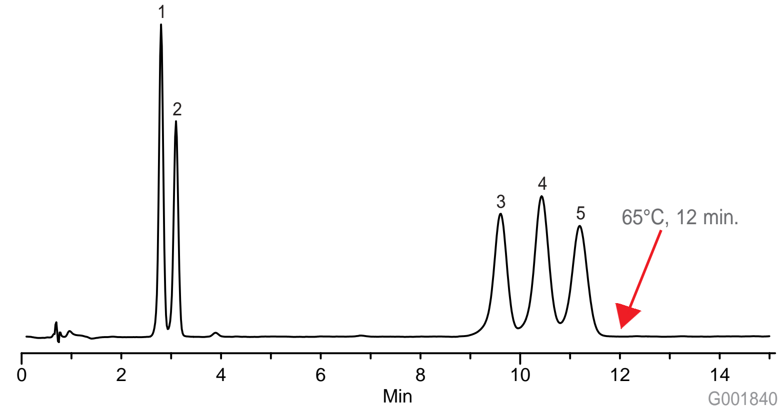
Using high temperature and high pH improves resolution and reduces analysis time in HPLC of basic alkaloid compounds. Until now, the range of permissible mobile phase pH and the temperature has been limited by the chemical or physical stability of the support particle. By using Discovery Zr zirconia-based particles, the full range of mobile phase temperature and pH can be exploited to optimize the HPLC method. In this example, increased temperature dramatically decreased the analysis time of five alkaloid compounds. Increased temperature gave lower mobile phase viscosity which in turn permitted higher flow rates at constant pressure. Choose Discovery Zr columns to take advantage of the power of temperature to give rapid separations.

Figure 1: Temperature Effect on Analysis Time: Alkaloids at 30°C and 65°C

Column: **Discovery Zr-PBD, 15cm x 4.6mm, 3µm**
Cat. No.: **65718-U**
Mobile Phase: 90:10, 20mM Potassium Phosphate (pH 12):CH_3CN
Flow Rate: 1mL/min at 30°C; 2.35mL/min at 65°C
Det.: UV, 220nm
Temp.: 30°C or 65°C
Inj: 10µL
Sample: codeine, strychnine, papaverine, quinine, quinidine, each compound 50µg/mL

1. Codeine
2. Strychnine
3. Papaverine
4. Quinine
5. Quinidine





Discovery Solves HPLC Problems

PROBLEM 7: Lengthy Analysis Time

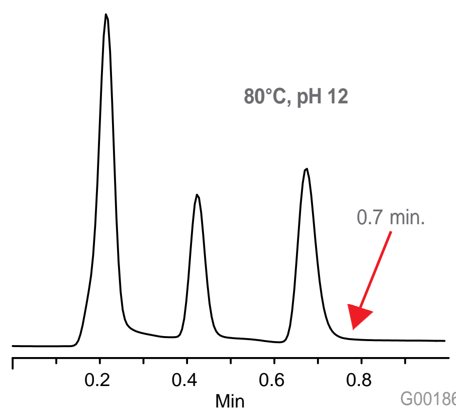
Demonstration 2:
An extreme example of the power of temperature.

An extreme example of the power of temperature to reduce analysis time is shown in the separation of β-blockers on Discovery Zr-CarbonC18 at 80°C. Analysis time is less than 0.7 minutes with baseline resolution. Note that the mobile phase was pH 12. Both temperature and pH settings are outside the permissible range for silica-based packings.

Figure 1: Extreme Temperature and pH Gives Rapid Separation of β-Blockers on Discovery Zr-CarbonC18

Column: **Discovery Zr-CarbonC18, 5cm x 4.6mm, 3µm**
Cat. No.: **65704-U**
Mobile Phase: 55:45, 20mM Potassium Phosphate (pH 12):CH_3CN
Flow Rate: 3mL/min
Det.: UV, 210nm
Temp.: 80°C
Pressure: 99bar
Inj.: 5µL
Sample: Labetolol (500µg/mL), metoprolol (250µg/mL), alprenolol (250µg/mL)

1. Labetolol
2. Metoprolol
3. Alprenolol



Discovery Solves HPLC Problems

PROBLEM 7: Lengthy Analysis Time

Demonstration 3:
Cyano stationary phase reduces hydrophobic retention and analysis time.

The most common technique to decrease retention on a C18 column is to increase the percent organic in the mobile phase. While this reduces the retention of all compounds, it often causes the early-eluting peaks to elute too close to the void volume. Switching from a C18 to a functionalized reversed-phase column can reduce the analysis time without sacrificing resolution or retention of early-eluting compounds. For a particular compound, one or more of the Discovery functionalized reversed-phases is likely to give shorter analysis time than a C18. This is due to the fact the polar functional groups reduce the overall hydrophobicity compared to a C18. (However, there are cases where the unique selectivity of the functionalized reversed-phases will cause an increase in retention.) In this example, a Discovery Cyano column gave baseline resolution of four urea pesticides in about one-fourth the run time as on a C18 under the same conditions.

Discovery functionalized reversed-phases reduce analysis time often without sacrificing resolution. When faced with a need to reduce analysis time, consider changing to one of the Discovery or Discovery Zr columns.

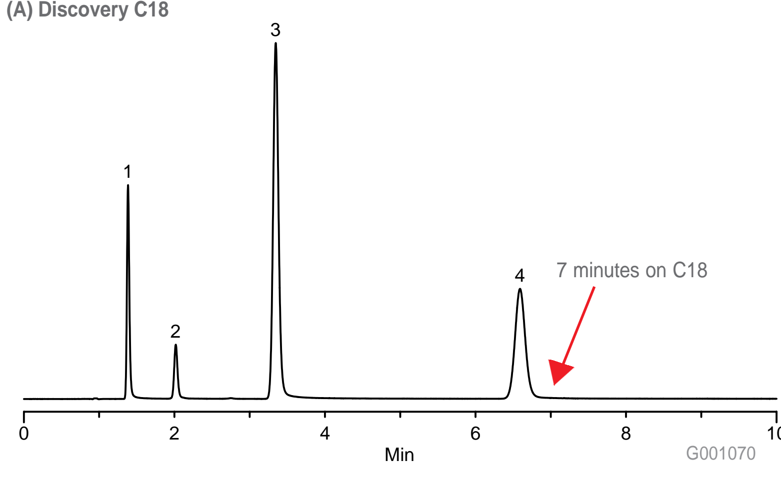
Figure 1: Faster Analysis - Eliminate Wasted Time

Urea Pesticides Using Isocratic Elution

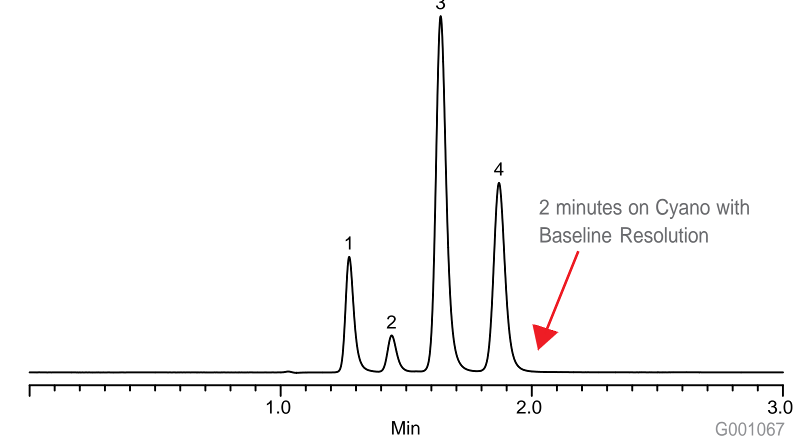
Column: 15cm x 4.6mm columns, 5µm particles
Mobile Phase: 60:40 Water:CH_3CN
Flow Rate: 2.0mL/min
Temp.: 20°C,
Det.: UV at 214nm
Inj.: 1µL

Sample:
1. Fenuron
2. Monuron
3. Diuron
4. Linuron

(A) Discovery C18



(B) Discovery Cyano



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Ordering Information

Discovery HS C18

Phase Type	ID (mm)	Length (cm)	Cat. No.
3µm Particles			
	2.1	5.0	**569253-U**
	2.1	7.5	**569254-U**
	2.1	10.0	**CUSTOM**
	2.1	15.0	**569255-U**
	4.6	5.0	**569250-U**
	4.6	7.5	**569251-U**
	4.6	10.0	**CUSTOM**
	4.6	15.0	**569252-U**
5µm Particles			
	2.1	5.0	**568500-U**
	2.1	10.0	**568501-U**
	2.1	15.0	**568502-U**
	2.1	25.0	**568503-U**
	4.0	5.0	**568510-U**
	4.0	10.0	**568511-U**
	4.0	15.0	**568512-U**
	4.0	25.0	**568513-U**
	4.6	5.0	**568520-U**
	4.6	10.0	**568521-U**
	4.6	15.0	**568522-U**
	4.6	25.0	**568523-U**
	10.0	5.0	**568530-U**
	10.0	10.0	**568531-U**
	10.0	15.0	**568532-U**
	10.0	25.0	**568533-U**
	21.2	5.0	**568540-U**
	21.2	10.0	**568541-U**
	21.2	15.0	**568542-U**
	21.2	25.0	**568543-U**
10µm Particles			
	10.0	5.0	**568630-U**
	10.0	10.0	**568631-U**
	10.0	15.0	**568632-U**
	10.0	25.0	**568633-U**
	21.2	5.0	**568640-U**
	21.2	10.0	**568641-U**
	21.2	15.0	**568642-U**
	21.2	25.0	**568643-U**

2cm Supelguard Cartridges with Discovery HS C18 Packings

Description	Cat. No.
2.1mm x 3µm	
2 Pack	**569276-U**
Kit	**569277-U**
2.1mm x 5µm	
2 Pack	**568570-U**
Kit	**568571-U**
4.0mm x 3µm	
2 Pack	**569274-U**
Kit	**569275-U**
4.0mm x5µm	
2 Pack	**568572-U**
Kit	**568573-U**

1cm Supelguard Cartridges with Discovery HS C18 Packings

Description	Cat. No.
10mm x 5µm	**568574-U**
10mm x 10µm	**568674-U**

Discovery C18

Phase Type	ID (mm)	Length (cm)	Cat. No.
5µm Particles			
	2.1	5.0	**50494721**
	2.1	10.0	**569220-U**
	2.1	12.5	**569229-U**
	2.1	15.0	**50495521**
	3.0	5.0	**504947-30**
	3.0	10.0	**569221-U**
	3.0	12.5	**569230-U**
	3.0	15.0	**504955-30**
	3.0	25.0	**504971-30**
	4.0	5.0	**504947-40**
	4.0	10.0	**569222-U**
	4.0	12.5	**569231-U**
	4.0	15.0	**504955-40**
	4.0	25.0	**504971-40**
	4.6	5.0	**504947**
	4.6	10.0	**569223-U**
	4.6	12.5	**569232-U**
	4.6	15.0	**504955**
	4.6	25.0	**504971**

2cm Supelguard Catridges with 5µm Discovery Packings

Description	Cat. No.
2.1mm ID	
2 Pack	**505188**
Kit[3]	**505161**
3.0mm ID	
2 Pack	**59576-U**
Kit[3]	**59575-U**
4.0mm ID[2]	
2 Pack	**505137**
Kit[3]	**505129**

[2] For 4.0mm ID and 4.6mm ID analytical columns.
[3] Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules. Additional sizes are available, please inquire.

Discovery RP-AmideC16

Phase Type	ID (mm)	Length (cm)	Cat. No.
5µm Particles			
	2.1	5.0	**50500521**
	2.1	10.0	**569320-U**
	2.1	12.5	**569329-U**
	2.1	15.0	**50501321**
	3.0	5.0	**505005-30**
	3.0	10.0	**569321-U**
	3.0	12.5	**569330-U**
	3.0	15.0	**505013-30**
	3.0	25.0	**505064-30**
	4.0	5.0	**505005-40**
	4.0	10.0	**569322-U**
	4.0	12.5	**569331-U**
	4.0	15.0	**505013-40**
	4.0	25.0	**505064-40**
	4.6	5.0	**505005**
	4.6	10.0	**569323-U**
	4.6	12.5	**569332-U**
	4.6	15.0	**505013**
	4.6	25.0	**505064**

2cm Supelguard Cartridges with 5µm Discovery Packings

Description	Cat. No.
2.1mm ID Cartridges	
2 Pack	**505110**
kit[3]	**505102**
3.0mm ID Cartridges	
2 Pack	**59578-U**
kit[3]	**59577-U**
4.0mm ID Cartridges[2]	
2 Pack	**505099**
kit[3]	**505080**

[2] For 4.0mm ID and 4.6mm ID analytical columns.
[3] Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

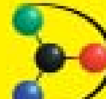
Discovery C8

Phase Type	ID (mm)	Length (cm)	Cat. No.
5µm Particles			
	2.1	5.0	**59352-U21**
	2.1	10.0	**569420-U**
	2.1	12.5	**569424-U**
	2.1	15.0	**59353-U21**
	3.0	5.0	**59352-U30**
	3.0	10.0	**569421-U**
	3.0	12.5	**569425-U**
	3.0	15.0	**59353-U30**
	3.0	25.0	**59354-U30**
	4.0	5.0	**59352-U40**
	4.0	10.0	**569422-U**
	4.0	12.5	**569426-U**
	4.0	15.0	**59353-U40**
	4.0	25.0	**59354-U40**
	4.6	5.0	**59352-U**
	4.6	10.0	**569423-U**
	4.6	12.5	**569427-U**
	4.6	15.0	**59353-U**
	4.6	25.0	**59354-U**

2cm Supelguard Cartridges with 5µm Discovery Packings

Description	Cat. No.
2.1mm ID Cartridges	
2 Pack	**59588-U**
kit[3]	**59587-U**
3.0mm ID Cartridges	
2 Pack	**59580-U**
kit[3]	**59579-U**
4.0mm ID Cartridges[2]	
2 Pack	**59590-U**
kit[3]	**59589-U**

[2] For 4.0mm ID and 4.6mm ID analytical columns.
[3] Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.



Discovery HS F5

Phase Type	ID (mm)	Length (cm)	Cat. No.
3µm Particles			
	2.1	3.3	**567501-U**
	2.1	5.0	**567500-U**
	2.1	10.0	**567502-U**
	2.1	15.0	**567503-U**
	3.0	15.0	**567542-U**
	4.0	5.0	**567530-U**
	4.0	10.0	**567531-U**
	4.0	15.0	**567532-U**
	4.6	5.0	**567504-U**
	4.6	10.0	**567506-U**
	4.6	15.0	**567507-U**
5µm Particles			
	2.1	5.0	**567508-U**
	2.1	10.0	**567510-U**
	2.1	15.0	**567511-U**
	2.1	25.0	**567512-U**
	4.0	5.0	**567533-U**
	4.0	10.0	**567534-U**
	4.0	15.0	**567535-U**
	4.0	25.0	**567536-U**
	4.6	5.0	**567513-U**
	4.6	10.0	**567515-U**
	4.6	15.0	**567516-U**
	4.6	25.0	**567517-U**
	10.0	5.0	**567518-U**
	10.0	10.0	**567537-U**
	10.0	15.0	**567519-U**
	10.0	25.0	**567520-U**
	21.2	5.0	**567521-U**
	21.2	10.0	**567539-U**
	21.2	15.0	**567522-U**
	21.2	25.0	**567523-U**
10µm Particles			
	10.0	5.0	**567524-U**
	10.0	10.0	**567538-U**
	10.0	15.0	**567525-U**
	10.0	25.0	**567526-U**
	21.2	5.0	**567527-U**
	21.2	10.0	**567540-U**
	21.2	15.0	**567528-U**
	21.2	25.0	**567529-U**

2cm Supelguard Cartridges with Discovery HS F5 Packings	Cat. No.
2.1mm x 3µm	
2 Pack	**567570-U**
Kit	**567571-U**
2.1mm x 5µm	
2 Pack	**567574-U**
Kit	**567575-U**
4.0mm x 3µm	
2 Pack	**567572-U**
Kit	**567573-U**
4.0mm x 5µm	
2 Pack	**567576-U**
Kit	**567577-U**
1cm Supelguard Cartridges with Discovery HS F5 Packings	
10mm x 5µm	**567578-U**
10mm x 10µm	**567580-U**

Discovery Cyano

Phase Type	ID (mm)	Length (cm)	Cat. No.
5µm Particles			
	2.1	5.0	**59355-U21**
	2.1	10.0	**569521-U**
	2.1	12.5	**569524-U**
	2.1	15.0	**59356-U21**
	3.0	5.0	**59355-U30**
	3.0	10.0	**569522-U**
	3.0	12.5	**569525-U**
	3.0	15.0	**59356-U30**
	3.0	25.0	**59357-U30**
	4.0	5.0	**59355-U40**
	4.0	10.0	**569523-U**
	4.0	12.5	**569526-U**
	4.0	15.0	**59356-U40**
	4.0	25.0	**59357-U40**
	4.6	5.0	**59355-U**
	4.6	10.0	**569520-U**
	4.6	12.5	**569527-U**
	4.6	15.0	**59356-U**
	4.6	25.0	**59357-U**

2cm Supelguard Cartridges with 5µm Discovery Packings	Cat. No.
4.0mm ID Cartridges[2]	
kit[3]	**59585-U**
pk of 2	**59586-U**
3.0mm ID Cartridges	
kit[3]	**569570-U**
pk of 2	**569571-U**
2.1mm ID Cartridges	
kit[3]	**59583-U**
pk of 2	**59584-U**

[2] For 4.0mm ID and 4.6mm ID analytical columns.
[3] Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

Discovery HS PEG

Phase Type	ID (mm)	Length (cm)	Cat. No.
3µm Discovery HS PEG HPLC Columns			
	2.1	5.0	**567400-U**
	2.1	10.0	**567402-U**
	2.1	15.0	**567403-U**
	4.0	5.0	**567430-U**
	4.0	10.0	**567431-U**
	4.0	15.0	**567432-U**
	4.6	5.0	**567404-U**
	4.6	10.0	**567406-U**
	4.6	15.0	**567407-U**
5µm Discovery HS PEG HPLC Columns			
	2.1	5.0	**567408-U**
	2.1	10.0	**567410-U**
	2.1	15.0	**567411-U**
	2.1	25.0	**567412-U**
	4.0	5.0	**567433-U**
	4.0	10.0	**567434-U**
	4.0	15.0	**567435-U**
	4.0	25.0	**567436-U**
	4.6	5.0	**567413-U**
	4.6	10.0	**567415-U**
	4.6	15.0	**567416-U**
	4.6	25.0	**567417-U**
	10.0	5.0	**567418-U**
	10.0	10.0	**567437-U**
	10.0	15.0	**567419-U**
	10.0	25.0	**567420-U**
	21.2	5.0	**567421-U**
	21.2	10.0	**567439-U**
	21.2	15.0	**567422-U**
	21.2	25.0	**567423-U**
10µm Discovery HS PEG HPLC Columns			
	10.0	5.0	**567424-U**
	10.0	10.0	**567438-U**
	10.0	15.0	**567425-U**
	10.0	25.0	**567426-U**
	21.2	5.0	**567427-U**
	21.2	10.0	**567440-U**
	21.2	15.0	**567428-U**
	21.2	25.0	**567429-U**

2cm Supelguard Cartridges with Discovery HS PEG Packings	Cat. No.
2.1mm x 3µm	
2 Pack	**567470-U**
Kit	**567471-U**
2.1mm x 5µm	
2 Pack	**567474-U**
Kit	**567475-U**
4.0mm x 3µm	
2 Pack	**567472-U**
Kit	**567473-U**
4.0mm x 5µm	
2 Pack	**567476-U**
Kit	**567477-U**

1cm Supelguard Cartridges with Discovery HS PEG Packings	Cat. No.
10mm x 5µm	**567478-U**
10mm x 10µm	**567480-U**

Ordering Information

Discovery Zr-PBD

Phase Type	ID (mm)	Length (cm)	Cat. No.
3µm Particles			
	2.1	5.0	**65713-U**
	2.1	7.5	**65714-U**
	2.1	15.0	**65715-U**
	4.6	5.0	**65716-U**
	4.6	7.5	**65717-U**
	4.6	15.0	**65718-U**
5µm Particles			
	2.1	5.0	**65719-U**
	2.1	15.0	**65720-U**
	4.6	5.0	**65722-U**
	4.6	15.0	**65723-U**
	4.6	25.0	**65724-U**

1cm Supelguard Cartridges with Discovery Zr-PBD Packings

	Cat. No.
2.1mm x 3µm	
2 Pack	**65812-U**
Kit[3]	**65811-U**
2.1mm x 5µm	
2 Pack	**65816-U**
Kit[3]	**65815-U**
4.0mm x 3µm[2]	
2 Pack	**65814-U**
Kit[3]	**65813-U**
4.0mm x 5µm[2]	
2 Pack	**65818-U**
Kit[3]	**65817-U**

Discovery Zr-PS

Phase Type	ID (mm)	Length (cm)	Cat. No.
3µm Particles			
	2.1	5.0	**65737-U**
	2.1	7.5	**65738-U**
	2.1	15.0	**65739-U**
	4.6	5.0	**65740-U**
	4.6	7.5	**65741-U**
	4.6	15.0	**65742-U**
5µm Particles			
	2.1	5.0	**65743-U**
	2.1	15.0	**65744-U**
	4.6	5.0	**65746-U**
	4.6	15.0	**65747-U**
	4.6	25.0	**65748-U**

1cm Supelguard Cartridges with Discovery Zr-PS Packings

	Cat. No.
2.1mm x 3µm	
2 Pack	**65842-U**
Kit[3]	**65841-U**
2.1mm x 5µm	
2 Pack	**65846-U**
Kit[3]	**65845-U**
4.0mm x 3µm[2]	
2 Pack	**65844-U**
Kit[3]	**65843-U**
4.0mm x 5µm[2]	
2 Pack	**65848-U**
Kit[3]	**65847-U**

[2] For 4.0mm ID and 4.6mm ID analytical columns.
[3] Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules. Additional sizes are available, please inquire.

Discovery Zr-CarbonC18

Phase Type	ID (mm)	Length (cm)	Cat. No.
3µm Particles			
	2.1	5.0	**65701-U**
	2.1	7.5	**65702-U**
	2.1	15.0	**65703-U**
	4.6	5.0	**65704-U**
	4.6	7.5	**65705-U**
	4.6	15.0	**65706-U**
5µm Particles			
	2.1	5.0	**65707-U**
	2.1	15.0	**65708-U**
	4.6	5.0	**65710-U**
	4.6	15.0	**65711-U**

1cm Supelguard Cartridges with Discovery Zr-CarbonC18 Packings

	Cat. No.
2.1mm x 3µm	
2 Pack	**65802-U**
Kit[3]	**65801-U**
2.1mm x 5µm	
2 Pack	**65806-U**
Kit[3]	**65805-U**
4.0mm x 3µm[2]	
2 Pack	**65804-U**
Kit[3]	**65803-U**
4.0mm x 5µm[2]	
2 Pack	**65808-U**
Kit[3]	**65807-U**

Discovery Zr-Carbon

Phase Type	ID (mm)	Length (cm)	Cat. No.
3µm Particles			
	2.1	5.0	**65725-U**
	2.1	7.5	**65726-U**
	2.1	15.0	**65727-U**
	4.6	5.0	**65728-U**
	4.6	7.5	**65729-U**
	4.6	15.0	**65730-U**
5µm Particles			
	2.1	5.0	**65731-U**
	2.1	15.0	**65732-U**
	4.6	5.0	**65734-U**
	4.6	15.0	**65735-U**

1cm Supelguard Cartridges with Discovery Zr-Carbon Packings

	Cat. No.
2.1mm x 3µm	
Kit[3]	**65821-U**
2 Pack	**65822-U**
2.1mm x 5µm	
Kit[3]	**65826-U**
2 Pack	**65828-U**
4.0mm x 3µm[2]	
Kit[3]	**65823-U**
2 Pack	**65824-U**
4.0mm x 5µm[2]	
Kit[3]	**65827-U**
2 Pack	**65829-U**

Column Switching Valves

Description	Cat. No.
SupelPRO™ 3-Column or 6-Column Selector	
3-Column	
Stainless Steel	**53140-U**
PEEK	**53142-U**
6-Column	
Stainless Steel	**53141-U**
PEEK	**53143-U**
SupelPRO 2-Channel Selector with Bypass Valve	
Stainless Steel	**53146-U**
PEEK	**53147-U**
SupelPRO 11-Port, 10-Position Valve	
Stainless Steel	**53152-U**
PEEK	**53153-U**
SupelPRO 2-Position Valves	
6-Port	
Stainless Steel	**53148-U**
PEEK	**53149-U**
10-Port	
Stainless Steel	**53150-U**
PEEK	**53151-U**
SupelPRO Solvent Selector Valve	
1/16"	**53144-U**
1/8"	**53145-U**

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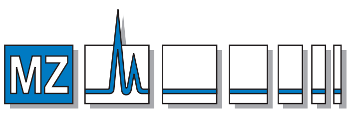

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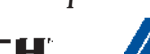

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