

ACE 300Å Columns for Biotechnology

- 300Å ultra high purity silica
- Ultimate protein and peptide application column
- C18, C8, C4, CN and Phenyl chemistries
- 3µm, 5µm and 10µm particle sizes
- Unmatched reproducibility
- Exceptional chemical stability

Excellent peak shape and reproducibility have established ACE HPLC columns as the finest available. This quality is now available for protein chemists desiring the utmost in performance and reproducibility for the separation of peptides, proteins and other high molecular weight biomolecules.

ACE 300Å columns are available in an extensive range of dimensions and particle sizes for use in micro-scale separations, LC/MS analyses, high speed preparative analyses up to process scale.

PHASE	FUNCTIONAL GROUP	ENDCAPPED	PARTICLE SIZE (µm)	PORE SIZE (Å)	SURFACE AREA (m²/g)	CARBON LOAD (%)
C18-300	Octadecyl	Yes	3, 5, 10	300	100	9.0
C8-300	Octyl	Yes	3, 5, 10	300	100	5.0
C4-300	Butyl	Yes	3, 5, 10	300	100	2.6
CN-300	Cyano	Yes	3, 5, 10	300	100	2.6
Ph-300	Phenyl	Yes	3, 5, 10	300	100	5.3

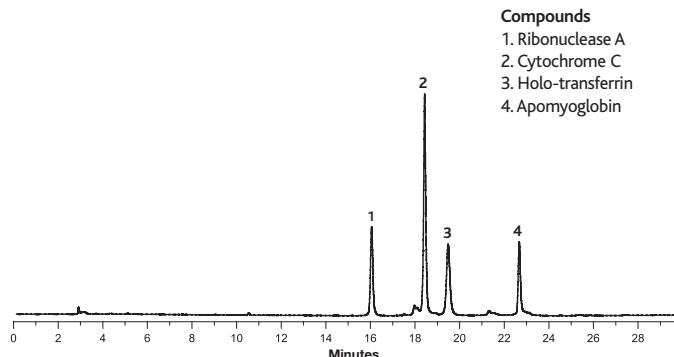
ACE 300Å Columns for Peptide and Protein Analyses

Chromatographers prefer inert stationary phases for the reversed-phase HPLC of ionic compounds because they minimize the negative effect of silanols on the separation. This results in improved peak shape and reproducibility when separating compounds that contain polar functional groups, especially amines.

A new generation of ultra-inert stationary phases, with extremely low silanol activity, has made it possible to achieve even better peak shape and reproducibility when separating these types of compounds.

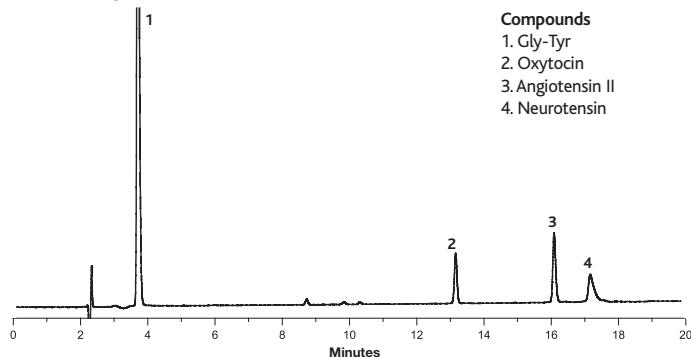
Scientists working with small molecules have been rapidly adopting this new technology and the recent introduction of wide-pore (300Å) ultra-inert phases makes the benefits of this technology available to those wanting to separate peptides and proteins by reversed-phase HPLC (see Figures 18a and 18b).

Figure 18a. Proteins

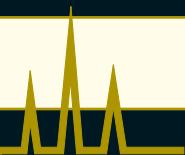


Column: ACE 5 C18-300, 250 x 4.6mm, Flow Rate: 1.0ml/min, Temperature: Ambient
Mobile Phase: A. 0.1% TFA in H₂O B. 0.1% TFA in MeCN 5% to 70% B in 30 mins, Detection: UV, 280nm

Figure 18b. Peptides



Column: ACE 5 C18-300, 250 x 4.6mm, Flow Rate: 1.0ml/min, Temperature: Ambient
Mobile Phase: A. 0.1% TFA in H₂O B. 0.1% TFA in MeCN 10% to 40% B in 25 mins, Detection: UV, 220nm



Comparison of Leading 300Å 5µm C18 Columns

- Leading 300Å 5µm, C18 Column Brands
- Neutral molecule test for packing integrity
- 2 basic molecule tests for silica inertness
- Peak efficiency and asymmetry comparison

In order to demonstrate the benefits of ultra-inert phases in biomolecule analysis, several commercially available 300Å pore size reversed-phase columns were tested using three different samples: neutral molecules to measure efficiency, pyridine/phenol to measure silanol activity and antidepressants to measure both silanol activity and

metal content. These are the same test procedures typically used to evaluate standard pore size columns (eg 100Å) used for the analysis of small molecules in the chemical and pharmaceutical industries. Columns were ranked by efficiency, N, measured at 10% peak height. In addition to measuring overall efficiency, this value also takes into consideration peak tailing usually caused by silanol interactions. The table below summarises the performance of various columns as determined by each test along with an overall ranking based on a combination of all three tests.

Results

Efficiency Measurements (N) For Leading 300Å (5µm, C18, 250 x 4.6mm) HPLC Columns

	TEST I	TEST II	TEST III	AVERAGE	
ACE C18-300	23,400	14,400	14,000	17,300	Test I: neutral molecule - toluene 80:20 MeOH/H ₂ O, 1.0ml/min
Jupiter C18	19,700	12,400	12,400	14,800	Test II: basic molecule 1 - pyridine 60:40 MeOH/H ₂ O, 1.0ml/min
Zorbax 300SB-C18	18,900	14,400	6,600	13,300	
Symmetry 300 C18	17,500	9,000	6,700	11,000	
Nucleosil 300 C18	20,300	6,700	400	9,100	
Vydac Everest C18	20,000	5,900	800	8,900	
Vydac 218MS	14,600	1,300	1,400	5,800	
Vydac 218TP	14,200	1,700	800	5,600	

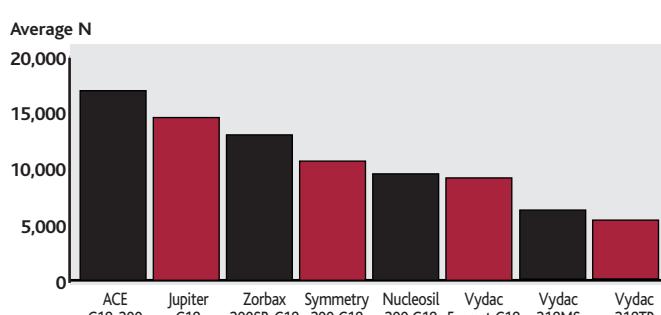
Column efficiency, as measured in Test I, is a reflection of how well a column is packed, as well as particle size and particle size distribution. Although many columns performed similarly in this test, those with lower plate counts reflect poorer physical characteristics of the silica particle. In Test II, efficiencies for pyridine are a good measure of active silanols on the silica surface. Active silanols account for most peak tailing and adsorptive losses of proteins. Since silanol activity is very hard to control in silica manufacture, columns exhibiting

low silanol activity are most likely to give consistent results column-to-column and batch-to-batch. In addition, polar and basic compounds will have better peak shapes and hence greater sensitivity on columns with low silanol activity. Since most biomolecules are polar and many are basic, columns with low silanol activity are desirable. In Test III, N values for tricyclic antidepressants measure metal content in addition to active silanol activity. Amitriptyline, chromatographed at neutral pH, is a standard test for measuring silica quality.

Conclusion

The overall ranking of the 300Å columns shown in Figure 19 reflects their performance based on how well they are packed and also the silanol and metal activity of the stationary phase. Chromatographers with experience in HPLC of basic pharmaceuticals know that columns giving good results on these tests will perform best for their samples. The benefits obtained from ultra-inert stationary phases are also important in wide-pore columns designed for the analysis of biomolecules.

Figure 19. 300Å (C18) Columns Ranked by Average Efficiency



By averaging N values obtained by each column in Tests I, II and III, columns can be ranked by overall quality, reflecting packing integrity and inertness.



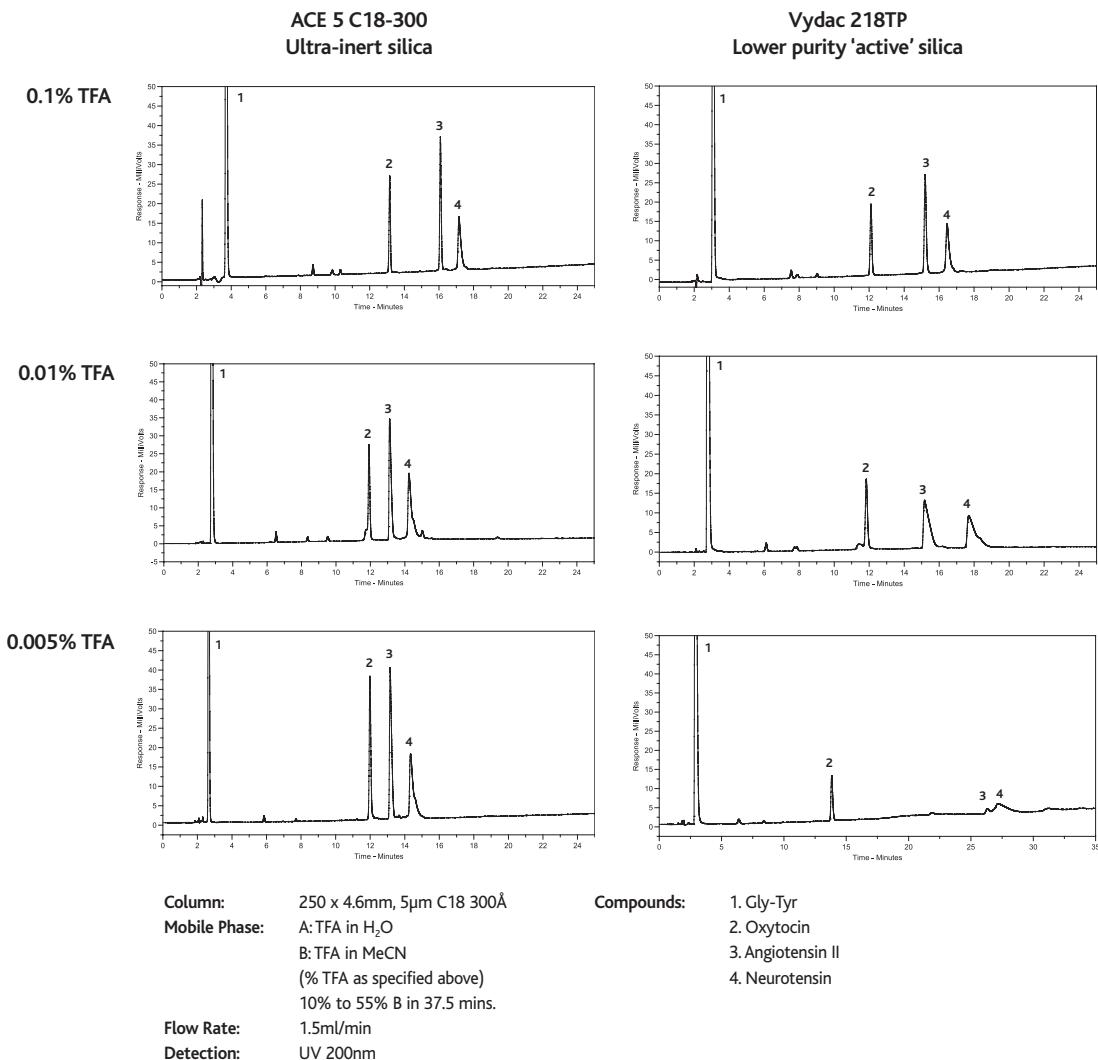
The Benefits of Ultra-Inert Stationary Phases for the Reversed-Phase HPLC of Biomolecules

Benefit #1 – Increased Sensitivity

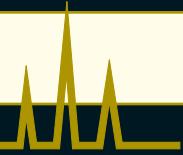
TFA or trifluoroacetic acid is used as a mobile phase additive for reversed-phase separations of peptides and proteins. This additive is typically used to improve both the peak shape and resolution of complex mixtures of peptides and proteins. As shown in Figure 20, the use of 0.1% TFA in the mobile phase enables a column packed with an active stationary phase to give peak widths comparable to those obtained from a new generation

column made from ultra-inert stationary phase. However, as the TFA concentration is lowered to 0.01% and finally 0.005%, peak widths on the ultra-inert phase stay the same, but degrade on the active stationary phase. The ability to analyse peptides and proteins using very low levels of TFA is beneficial for high sensitivity detection by mass spectrometry. TFA complexes with polypeptides and can enhance selectivity. However, this same complexation lowers sensitivity in the mass spectrometer.

Figure 20. Sensitivity and Peak Shape as a Function of TFA Concentration



Columns based on lower quality silica (chromatograms on right) show a dramatic loss in performance as TFA concentration is lowered. Columns from ultra-inert silica such as ACE maintain performance when TFA concentration is decreased.



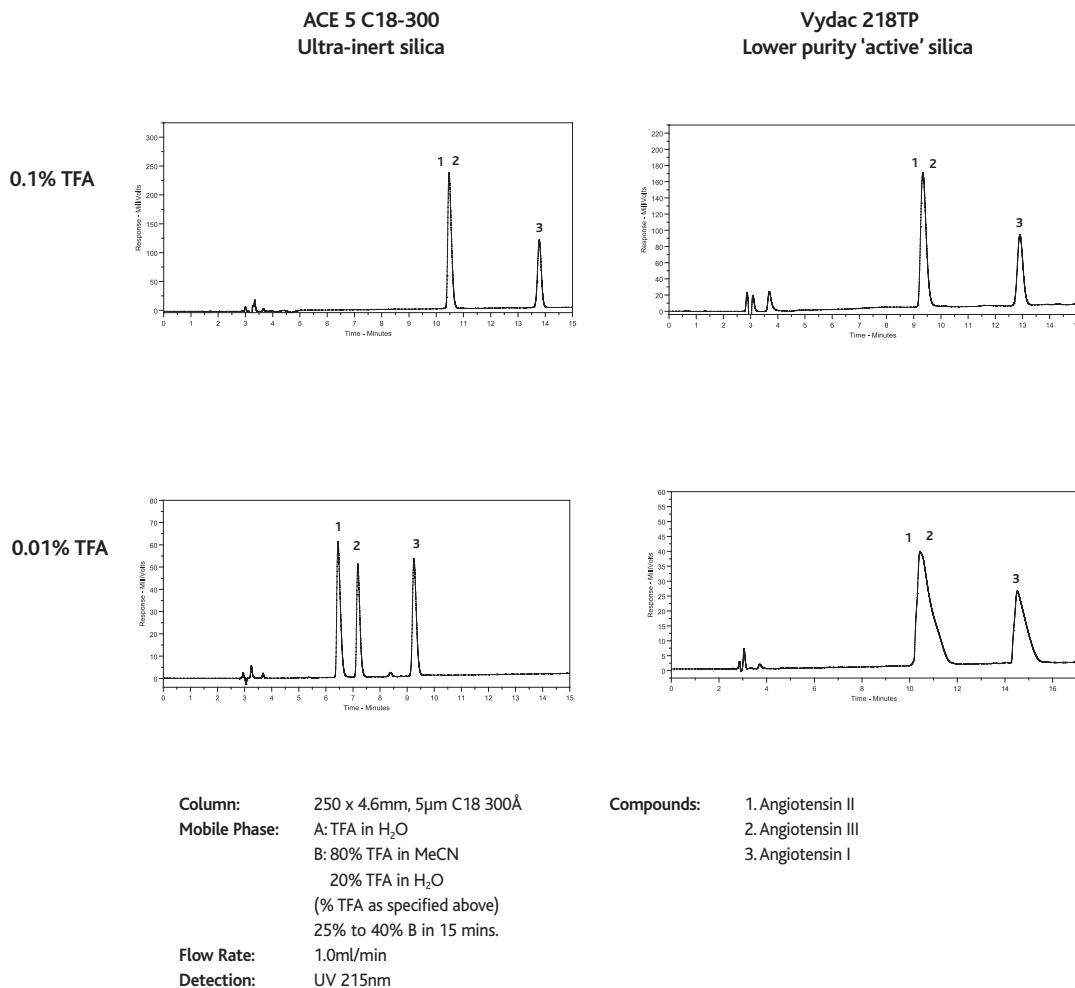
The Benefits of Ultra-Inert Stationary Phases for the Reversed-Phase HPLC of Biomolecules

Benefit #2 – Optimising Selectivity

The ability of TFA and other mobile phase additives to complex with peptides and proteins can be used to adjust selectivity and improve resolution. As shown in Figure 21, lowering TFA concentration from 0.1% to 0.01% enabled the resolution of angiotensin II and III. In the case of the

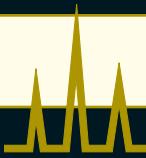
ultra-inert ACE column, peak shape and sensitivity remained constant with this change, as resolution improved dramatically. In the case of the Vydac column, packed with a more active stationary phase, peak shape was severely degraded.

Figure 21. Selectivity as a Function of TFA Concentration



Resolution has increased by lowering the TFA concentration. Columns made from lower quality silica show decreased performance.

300Å



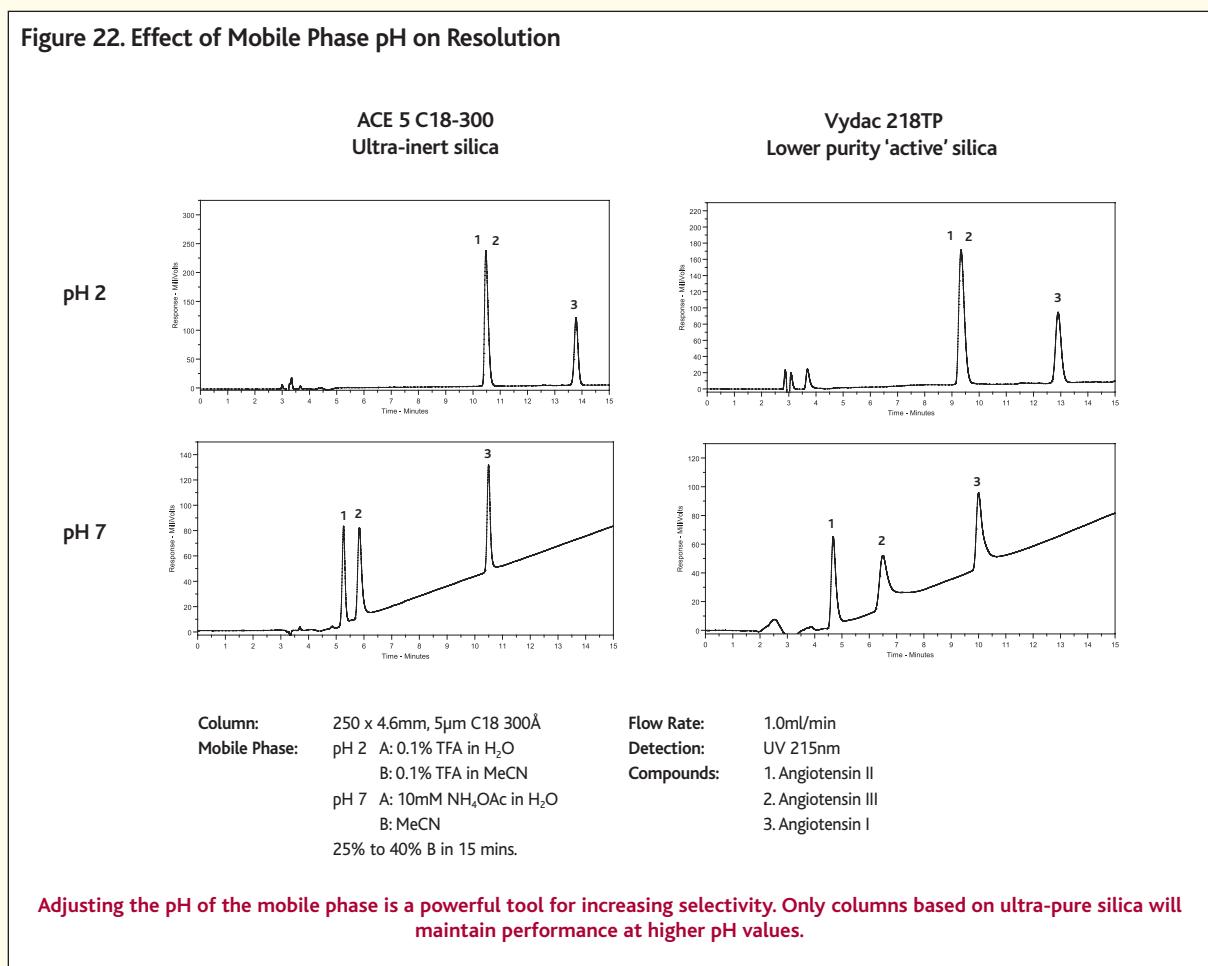
The Benefits of Ultra-Inert Stationary Phases for the Reversed-Phase HPLC of Biomolecules

Benefit #3 – Increased pH Range

Most biomolecules are charged. Peptides and proteins have numerous charges. From experience with small molecules, it is known that mobile phase pH can be a powerful tool for changing retention and thus optimizing the resolution of charged compounds. The same is true for peptides. Again using angiotensin II and III as an example, Figure 22 shows no resolution of these two peptides at pH 2 on either the ACE ultra-inert column or a column

packed with a more active stationary phase. By increasing the pH to 7, both columns now give good resolution. However, whereas the ACE ultra-inert column maintained good peak shape, the more active column showed poorer peak shape and a loss in performance. This phenomenon is observed in most reversed-phase applications with polar compounds. At high pH, silanol interactions are more prevalent and hence peak tailing becomes more apparent on active stationary phases.

Figure 22. Effect of Mobile Phase pH on Resolution



Summary

The chromatography of biomolecules, in particular peptides and proteins, can be improved by using HPLC columns packed with ultra-inert stationary phases. These columns will have reduced levels of silanol and metal activity to interfere with the separation. In addition, ultra-inert stationary phases perform well even when using low levels of TFA in the mobile phase. Using reduced levels of

TFA improves mass spectral detection, in addition to providing a means of increasing selectivity and resolution. Mobile phase pH is another powerful means for improving selectivity and resolution. Ultra-inert columns, such as ACE, show no loss in performance at higher pH. Methods developed on ultra-inert columns will be more rugged over time as these columns are more reproducible column-to-column and lot-to-lot.

Independent Comparison of HPLC Columns #5

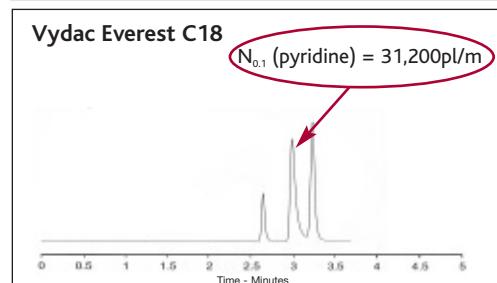
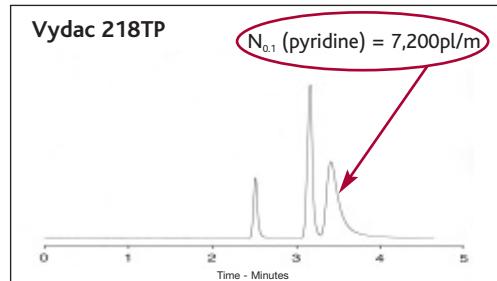
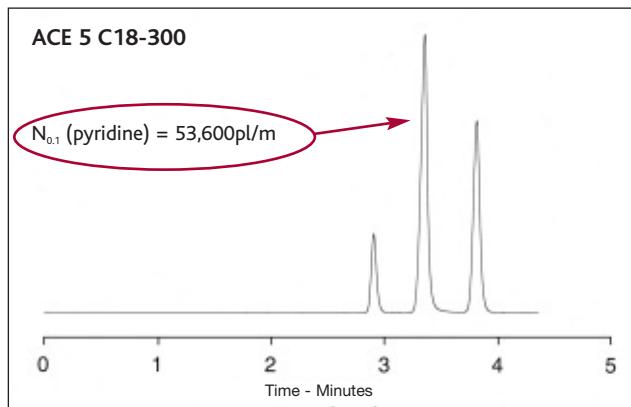


Comparison of Leading Wide Pore Columns

- Independently tested at The School of Pharmacy, University of Sunderland, UK

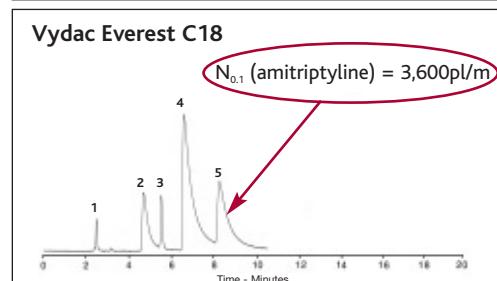
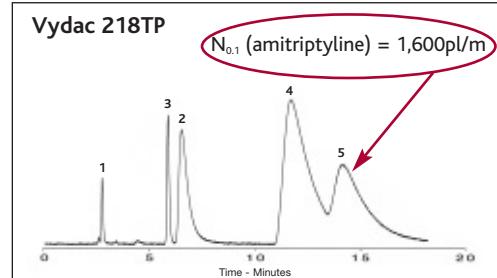
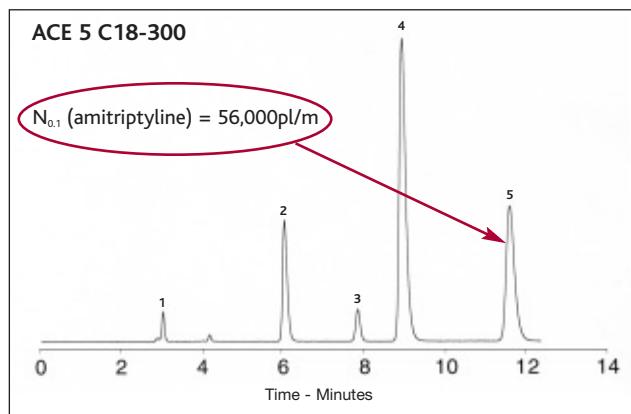
- Leading 300Å 5µm, C18 column brands - 250 x 4.6mm i.d.
- 2 Basic silica inertness tests
- Peak efficiency and asymmetry comparison

Inertness Test 1



Sample: 1) Uracil 2) Pyridine 3) Phenol Mobile Phase: 60:40 MeOH/H₂O Flow Rate: 1.0 ml/min

Inertness Test 2



Sample: 1) Norephedrine 2) Nortriptyline 3) Toluene 3) Imipramine 4) Amitriptyline Mobile Phase: 65:35 MeOH/25mM KH₂PO₄ (pH 6.0) Flow Rate: 1.0 ml/min

Conclusion

Significant differences in efficiency, peak shape and selectivity are seen with these 300Å C18 bonded phases when analyzing basic molecules. These variations are caused by undesirable secondary silanol interactions, which can also result in poor column reproducibility.

Since most biomolecules are polar, and many are basic, these inertness tests can be used to accurately predict the best column for the analysis of biomolecules, where an ultra-inert column with low silanol activity is highly desirable.

ACE 300Å columns have been repeatedly shown to be the most inert columns available.

300Å

ACE 300Å HPLC Columns - Part Numbers

ACE 3µm Columns

ACE 3µm C18-300

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	ACE-211-0201	ACE-211-0301	ACE-211-3501	ACE-211-0501	ACE-211-7501	ACE-211-1001	ACE-211-1201	ACE-211-1501	-	ACE-211-0101GD ¹
2.1mm	ACE-211-0202	ACE-211-0302	ACE-211-3502	ACE-211-0502	ACE-211-7502	ACE-211-1002	ACE-211-1202	ACE-211-1502	-	ACE-211-0102GD ²
3.0mm	ACE-211-0203	ACE-211-0303	ACE-211-3503	ACE-211-0503	ACE-211-7503	ACE-211-1003	ACE-211-1203	ACE-211-1503	-	ACE-211-0103GD ³
4.0mm	-	-	ACE-211-3504	ACE-211-0504	ACE-211-7504	ACE-211-1004	ACE-211-1204	ACE-211-1504	-	ACE-211-0103GD ³
4.6mm	ACE-211-0246	ACE-211-0346	ACE-211-3546	ACE-211-0546	ACE-211-7546	ACE-211-1046	ACE-211-1246	ACE-211-1546	-	ACE-211-0103GD ³

ACE 3µm C8-300

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	ACE-212-0201	ACE-212-0301	ACE-212-3501	ACE-212-0501	ACE-212-7501	ACE-212-1001	ACE-212-1201	ACE-212-1501	-	ACE-212-0101GD ¹
2.1mm	ACE-212-0202	ACE-212-0302	ACE-212-3502	ACE-212-0502	ACE-212-7502	ACE-212-1002	ACE-212-1202	ACE-212-1502	-	ACE-212-0102GD ²
3.0mm	ACE-212-0203	ACE-212-0303	ACE-212-3503	ACE-212-0503	ACE-212-7503	ACE-212-1003	ACE-212-1203	ACE-212-1503	-	ACE-212-0103GD ³
4.0mm	-	-	ACE-212-3504	ACE-212-0504	ACE-212-7504	ACE-212-1004	ACE-212-1204	ACE-212-1504	-	ACE-212-0103GD ³
4.6mm	ACE-212-0246	ACE-212-0346	ACE-212-3546	ACE-212-0546	ACE-212-7546	ACE-212-1046	ACE-212-1246	ACE-212-1546	-	ACE-212-0103GD ³

ACE 3µm C4-300

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	ACE-213-0201	ACE-213-0301	ACE-213-3501	ACE-213-0501	ACE-213-7501	ACE-213-1001	ACE-213-1201	ACE-213-1501	-	ACE-213-0101GD ¹
2.1mm	ACE-213-0202	ACE-213-0302	ACE-213-3502	ACE-213-0502	ACE-213-7502	ACE-213-1002	ACE-213-1202	ACE-213-1502	-	ACE-213-0102GD ²
3.0mm	ACE-213-0203	ACE-213-0303	ACE-213-3503	ACE-213-0503	ACE-213-7503	ACE-213-1003	ACE-213-1203	ACE-213-1503	-	ACE-213-0103GD ³
4.0mm	-	-	ACE-213-3504	ACE-213-0504	ACE-213-7504	ACE-213-1004	ACE-213-1204	ACE-213-1504	-	ACE-213-0103GD ³
4.6mm	ACE-213-0246	ACE-213-0346	ACE-213-3546	ACE-213-0546	ACE-213-7546	ACE-213-1046	ACE-213-1246	ACE-213-1546	-	ACE-213-0103GD ³

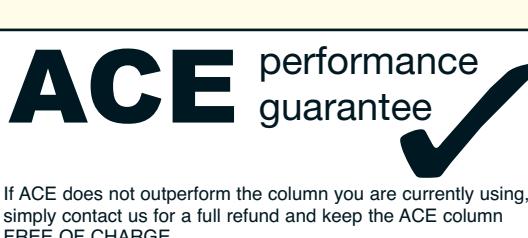
ACE 3µm CN-300

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	ACE-214-0201	ACE-214-0301	ACE-214-3501	ACE-214-0501	ACE-214-7501	ACE-214-1001	ACE-214-1201	ACE-214-1501	-	ACE-214-0101GD ¹
2.1mm	ACE-214-0202	ACE-214-0302	ACE-214-3502	ACE-214-0502	ACE-214-7502	ACE-214-1002	ACE-214-1202	ACE-214-1502	-	ACE-214-0102GD ²
3.0mm	ACE-214-0203	ACE-214-0303	ACE-214-3503	ACE-214-0503	ACE-214-7503	ACE-214-1003	ACE-214-1203	ACE-214-1503	-	ACE-214-0103GD ³
4.0mm	-	-	ACE-214-3504	ACE-214-0504	ACE-214-7504	ACE-214-1004	ACE-214-1204	ACE-214-1504	-	ACE-214-0103GD ³
4.6mm	ACE-214-0246	ACE-214-0346	ACE-214-3546	ACE-214-0546	ACE-214-7546	ACE-214-1046	ACE-214-1246	ACE-214-1546	-	ACE-214-0103GD ³

ACE 3µm Phenyl-300

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	ACE-215-0201	ACE-215-0301	ACE-215-3501	ACE-215-0501	ACE-215-7501	ACE-215-1001	ACE-215-1201	ACE-215-1501	-	ACE-215-0101GD ¹
2.1mm	ACE-215-0202	ACE-215-0302	ACE-215-3502	ACE-215-0502	ACE-215-7502	ACE-215-1002	ACE-215-1202	ACE-215-1502	-	ACE-215-0102GD ²
3.0mm	ACE-215-0203	ACE-215-0303	ACE-215-3503	ACE-215-0503	ACE-215-7503	ACE-215-1003	ACE-215-1203	ACE-215-1503	-	ACE-215-0103GD ³
4.0mm	-	-	ACE-215-3504	ACE-215-0504	ACE-215-7504	ACE-215-1004	ACE-215-1204	ACE-215-1504	-	ACE-215-0103GD ³
4.6mm	ACE-215-0246	ACE-215-0346	ACE-215-3546	ACE-215-0546	ACE-215-7546	ACE-215-1046	ACE-215-1246	ACE-215-1546	-	ACE-215-0103GD ³

¹ 5 pack - use with cartridge holder H0001 and coupler C0001
² 5 pack - use with integral microbore cartridge holder H0004
³ 5 pack - use with integral analytical cartridge holder H0005



ACE 300Å HPLC Columns - Part Numbers

ACE 300Å HPLC Columns - continued

ACE 10µm C18-300

COLUMN DIAMETER	COLUMN LENGTH										GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm		
4.6mm	ACE-231-0246	ACE-231-0346	ACE-231-3546	ACE-231-0546	ACE-231-7546	ACE-231-1046	ACE-231-1246	ACE-231-1546	ACE-231-2546	ACE-231-0103GD ³	
7.75mm	-	-	-	ACE-231-0508	ACE-231-7508	ACE-231-1008	ACE-231-1208	ACE-231-1508	ACE-231-2508	ACE-231-0110GD ⁴	
10.0mm	-	-	-	ACE-231-0510	ACE-231-7510	ACE-231-1010	ACE-231-1210	ACE-231-1510	ACE-231-2510	ACE-231-0110GD ⁴	
21.2mm	-	-	-	ACE-231-0520	ACE-231-7520	ACE-231-1020	ACE-231-1220	ACE-231-1520	ACE-231-2520	ACE-231-0110GD ⁴	
30.0mm	-	-	-	enquire	enquire	enquire	-	enquire	enquire	enquire	enquire

ACE 10µm C8-300

COLUMN DIAMETER	COLUMN LENGTH										GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm		
4.6mm	ACE-232-0246	ACE-232-0346	ACE-232-3546	ACE-232-0546	ACE-232-7546	ACE-232-1046	ACE-232-1246	ACE-232-1546	ACE-232-2546	ACE-232-0103GD ³	
7.75mm	-	-	-	ACE-232-0508	ACE-232-7508	ACE-232-1008	ACE-232-1208	ACE-232-1508	ACE-232-2508	ACE-232-0110GD ⁴	
10.0mm	-	-	-	ACE-232-0510	ACE-232-7510	ACE-232-1010	ACE-232-1210	ACE-232-1510	ACE-232-2510	ACE-232-0110GD ⁴	
21.2mm	-	-	-	ACE-232-0520	ACE-232-7520	ACE-232-1020	ACE-232-1220	ACE-232-1520	ACE-232-2520	ACE-232-0110GD ⁴	
30.0mm	-	-	-	enquire	enquire	enquire	-	enquire	enquire	enquire	enquire

ACE 10µm C4-300

COLUMN DIAMETER	COLUMN LENGTH										GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm		
4.6mm	ACE-233-0246	ACE-233-0346	ACE-233-3546	ACE-233-0546	ACE-233-7546	ACE-233-1046	ACE-233-1246	ACE-233-1546	ACE-233-2546	ACE-233-0103GD ³	
7.75mm	-	-	-	ACE-233-0508	ACE-233-7508	ACE-233-1008	ACE-233-1208	ACE-233-1508	ACE-233-2508	ACE-233-0110GD ⁴	
10.0mm	-	-	-	ACE-233-0510	ACE-233-7510	ACE-233-1010	ACE-233-1210	ACE-233-1510	ACE-233-2510	ACE-233-0110GD ⁴	
21.2mm	-	-	-	ACE-233-0520	ACE-233-7520	ACE-233-1020	ACE-233-1220	ACE-233-1520	ACE-233-2520	ACE-233-0110GD ⁴	
30.0mm	-	-	-	enquire	enquire	enquire	-	enquire	enquire	enquire	enquire

ACE 10µm CN-300

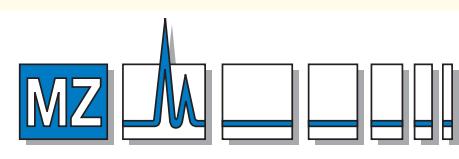
COLUMN DIAMETER	COLUMN LENGTH										GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm		
4.6mm	ACE-234-0246	ACE-234-0346	ACE-234-3546	ACE-234-0546	ACE-234-7546	ACE-234-1046	ACE-234-1246	ACE-234-1546	ACE-234-2546	ACE-234-0103GD ³	
7.75mm	-	-	-	ACE-234-0508	ACE-234-7508	ACE-234-1008	ACE-234-1208	ACE-234-1508	ACE-234-2508	ACE-234-0110GD ⁴	
10.0mm	-	-	-	ACE-234-0510	ACE-234-7510	ACE-234-1010	ACE-234-1210	ACE-234-1510	ACE-234-2510	ACE-234-0110GD ⁴	
21.2mm	-	-	-	ACE-234-0520	ACE-234-7520	ACE-234-1020	ACE-234-1220	ACE-234-1520	ACE-234-2520	ACE-234-0110GD ⁴	
30.0mm	-	-	-	enquire	enquire	enquire	-	enquire	enquire	enquire	enquire

ACE 10µm Phenyl-300

COLUMN DIAMETER	COLUMN LENGTH										GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm		
4.6mm	ACE-235-0246	ACE-235-0346	ACE-235-3546	ACE-235-0546	ACE-235-7546	ACE-235-1046	ACE-235-1246	ACE-235-1546	ACE-235-2546	ACE-235-0103GD ³	
7.75mm	-	-	-	ACE-235-0508	ACE-235-7508	ACE-235-1008	ACE-235-1208	ACE-235-1508	ACE-235-2508	ACE-235-0110GD ⁴	
10.0mm	-	-	-	ACE-235-0510	ACE-235-7510	ACE-235-1010	ACE-235-1210	ACE-235-1510	ACE-235-2510	ACE-235-0110GD ⁴	
21.2mm	-	-	-	ACE-235-0520	ACE-235-7520	ACE-235-1020	ACE-235-1220	ACE-235-1520	ACE-235-2520	ACE-235-0110GD ⁴	
30.0mm	-	-	-	enquire	enquire	enquire	-	enquire	enquire	enquire	enquire

³ 5 pack - use with integral analytical cartridge holder H0005

⁴ 3 pack - use with semi-prep cartridge holder H0002 and column coupler C0001



AUTHORIZED DISTRIBUTOR

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