HPLC Column Selection: Small Molecules (<2000 mw)

This chart is meant to be a starting point for choosing an HPLC column. In some cases, more than one column can be used for an analysis. Although general rules apply, you should first consult the technical literature, official methodology, or our Technical Service chemists for definitive recommendations.

For column choices for peptides, proteins, and other biopolymers, see pages 54-55.

Analyte Characteristics		Technique	Analyte Class	Column
water soluble	nonionic	reversed phase	GENERAL (pH 2-7.5)	Discovery C18, C8, RP-AmideC16; Discovery Cyano, Discovery HS C18, HS F5, HS PEG
water soluble	nonionic	reversed phase	GENERAL (pH 2-13)	SUPELCOGEL ODP-50, TPR-100
water soluble	nonionic	reversed phase	acids, organic	Discovery C18, C8, RP-AmideC16
water soluble	nonionic	reversed phase	acids, amino	Discovery C18, RP-AmideC16; SUPELCOSIL LC-DABS
water soluble	nonionic	reversed phase	amines, organic	Discovery C18, RP-AmideC16; Discovery Cyano, Discovery HS F5; SUPELCOGEL TPR-100
water soluble	nonionic	reversed phase	amines, quaternary (pH≥7.5)	SUPELCOGEL TPR-100, ODP-50
water soluble	nonionic	reversed phase	chelating compounds	Discovery C18, C8, RP-AmideC16; SUPELCOGEL TPR-100, ODP-50
water soluble	nonionic	reversed phase	drugs, basic	Discovery C18, C8, RP-AmideC16; Discovery Cyano, Discovery HS F5
water soluble	nonionic	reversed phase	drugs in serum	Hisep; Discovery C18, RP-AmideC16, C8; Discovery HS F5
water soluble	nonionic	reversed phase	explosives	Discovery C18, C8, RP-AmideC16; Discovery Cyano
water soluble	nonionic	reversed phase	nucleosides	SUPELCOSIL LC-18-S; Discovery C18, RP-AmideC16
water soluble	nonionic	reversed phase	nucleotides	SUPELCOSIL LC-18-T; Discovery C18, RP-AmideC16
water soluble	nonionic	reversed phase	peptides	Discovery C18, C8, RP-AmideC16
water soluble	nonionic	reversed phase	sugars	SUPELCOSIL LC-NH_2
water soluble	nonionic	reversed phase	taxol, taxanes	SUPELCOSIL LC-F
water soluble	nonionic	reversed phase	tricyclic antidepressants	SUPELCOSIL LC-PCN; Discovery C8; Discovery Cyano
water soluble	nonionic	reversed phase	vitamins, water soluble	Discovery C18, C8, RP-AmideC16
water soluble	nonionic	hydrophobic interaction	peptides	TSK-GEL
water soluble	nonionic	size exclusion	oligosaccharides	SUPELCOGEL Ag1, Ag2; TSK-GEL Oligo-PW
water soluble	nonionic	size exclusion	polymers/oligomers, hydrophilic	TSK-GEL PW_{XL}
water soluble	ionic	ion exchange	anions, organic (- charge)	SUPELCOSIL SAX1
water soluble	ionic	ion exchange	cations, organic (+ charge)	SUPELCOSIL LC-SCX
water soluble	ionic	ion pairing	acids, organic	Discovery C18, Discovery HS C18
water soluble	ionic	ion pairing	amines, organic	Discovery C18, Discovery HS C18
water soluble	ionic	ion exclusion	acids, organic	SUPELCOGEL H, C610H
water soluble	ionic	ion exclusion	carbohydrates, sugars	SUPELCOGEL C611, K, Pb, Ag1, Ag2, Ca
organic soluble		normal phase	GENERAL	Discovery Cyano, LC-NH_2-NP, LC-Si, Discovery HS PEG
organic soluble		normal phase	aflatoxins	SUPELCOSIL LC-Si
organic soluble		normal phase	steroids	SUPELCOSIL LC-Diol
organic soluble		normal phase	vitamins, fat soluble	SUPELCOSIL LC-NH_2-NP
organic soluble		reversed phase	fatty acids	SUPELCOSIL LC-18
organic soluble		reversed phase	polyaromatic hydrocarbons	SUPELCOSIL LC-PAH
organic soluble		reversed phase	polymers, hydrophobic	SUPELCOSIL LC-18
organic soluble		reversed phase	triglycerides	SUPELCOSIL LC-18
organic soluble		reversed phase	vitamins, fat soluble	Discovery C18, C8, RP-AmideC16, SUPELCOSIL LC-8
organic soluble		size exclusion	polymers/oligomers, hydrophilic	TSK-GEL H_{HR}

Comprehensive Suite of Alkyl and Functionalized Phases

HS C18

—O—Si(CH_3)(CH_3)—$(CH_2)_{17}$—CH_3

C18

—O—Si(CH_3)(CH_3)—$(CH_2)_{17}$—CH_3

RP-AmideC16

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

C8

—O—Si(CH_3)(CH_3)—$(CH_2)_7$—CH_3

HS F5

—O—Si(CH_3)(CH_3)—$(CH_2)_3$—C_6F_5

Cyano

—O—Si(CH_3)(CH_3)—$(CH_2)_3$—CN

HS PEG

Valuable, Different Separations versus C18

While C18's from different sources can provide significant 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 phase. The result – It's much more likely to achieve an improved, valuable separation with a polar functionalized reversed-phase than with "another" C18.

Ideal for all "small molecule" HPLC applications

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

- food and beverage
- environmental
- clinical
- petrochemical
- agriculture
- consumer products
- industrial chemical
- and more ....

Characteristics of Discovery Reversed-Phases

Phase	Coverage µmole/m²	%C	Endcapped	Pore Diameter Angstroms	Surface Area m²/g
HS C18	3.8	20%	yes	120	300
C18	3.0	12%	yes	180	200
RP-AmideC16	2.6	11%	yes	180	200
C8	3.4	7.5%	yes	180	200
HS F5	4.0	12%	yes	120	300
Cyano	3.5	4.5%	yes	180	200
HS PEG	3.8	12%	no	120	300

On the next page, begin to rediscover method development...

Take Advantage of the Discovery Suite of Reversed-Phases

Whether you're developing a separation that must be perfect, or you're just not satisfied with your reversed-phase separation...

Turn to Discovery!

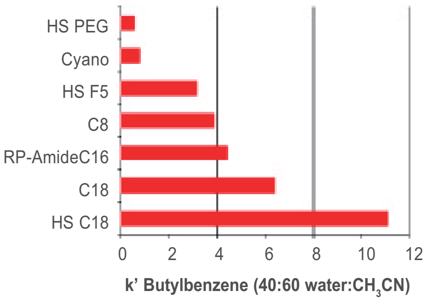
a suite of HPLC columns featuring functionalized reversed-phases designed to provide differentiated separations vs. C18 based on unique combinations of polar and hydrophobic retention.

The Discovery suite of reversed-phases enables you to optimize your separation..., e.g.

- retention
- selectivity
- resolution
- analysis time

...while minimizing method development time.

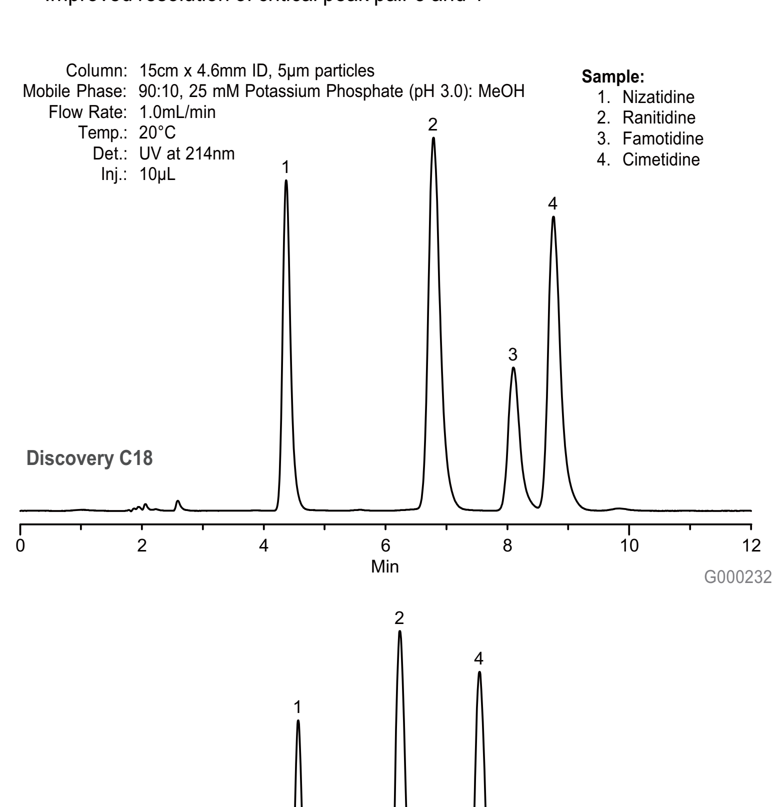
Hydrophobicity:
Discovery Reversed-Phases

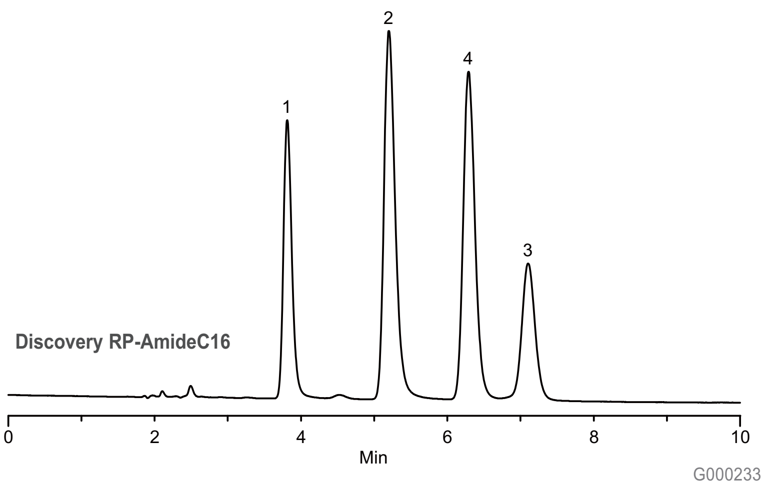


Discovery RP-AmideC16 Delivers Better Peak Spacing and Faster Analysis!

Scientists have long known polar embedded phases such as Discovery RP-AmideC16 provide unique retention and selectivity compared to C18's. Taking advantage of Discovery RP-AmideC16's unique retention and selectivity results in a better separation vs. C18.

- Unique selectivity - peak reversal of compounds 3 and 4
- Better peak spacing - less dead space between peaks 1 and 2
- Higher assay throughput - 20% reduction in analysis time
- Improved resolution of critical peak pair 3 and 4





Deliver Better Separations in Less Time

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 simply changing the stationary phase the method development scientist can avoid:

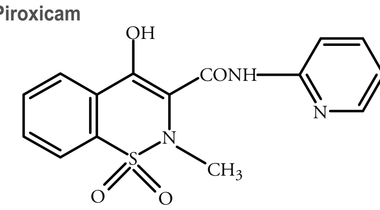
- complicated or forbidden gradients
- complex mobile phases
- long, drawn-out method development

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

2-Aminopyridine

H_2N–(pyridin-2-yl)

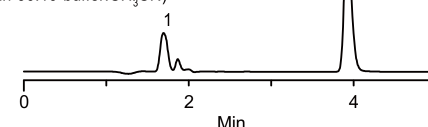
Piroxicam



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

Column: Discovery C18 15cm x 4.6mm ID, 5µm particles
Mobile Phase: 45:55, 10 mM Potassium Phosphate (pH 2.5): CH_3CN (v/v)
Flow Rate: 1.0mL/min
Det.: UV at 220nm
Inj.: 5µL
Sample: 1. 2-Aminopyridine (10µg/ml in 90:10 buffer:CH_3CN)
2. Piroxicam (100µg/ml in 90:10 buffer:CH_3CN)

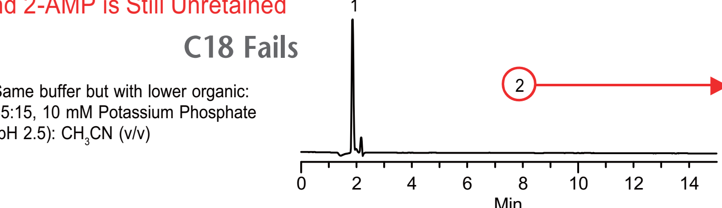
1. 2-Aminopyridine
2. Piroxicam



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

C18 Fails

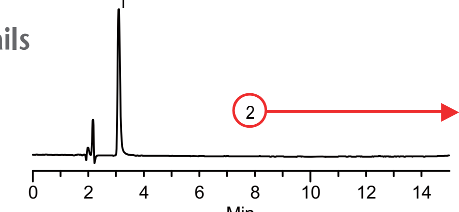
Same buffer but with lower organic: 85:15, 10 mM Potassium Phosphate (pH 2.5): CH_3CN (v/v)



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

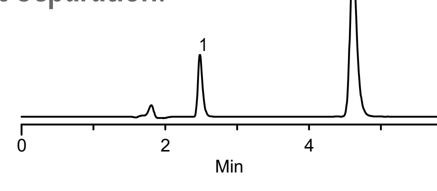
C18 Fails

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

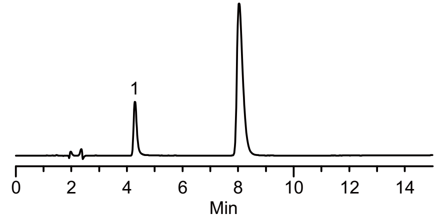


The Unique Retention and Selectivity of Discovery HS F5 Produces Excellent Separation at Both pH's

85:15, 10 mM Potassium Phosphate (pH 2.5): CH_3CN (v/v)



85:15, 10 mM Potassium Phosphate (pH 6.8): CH_3CN (v/v)



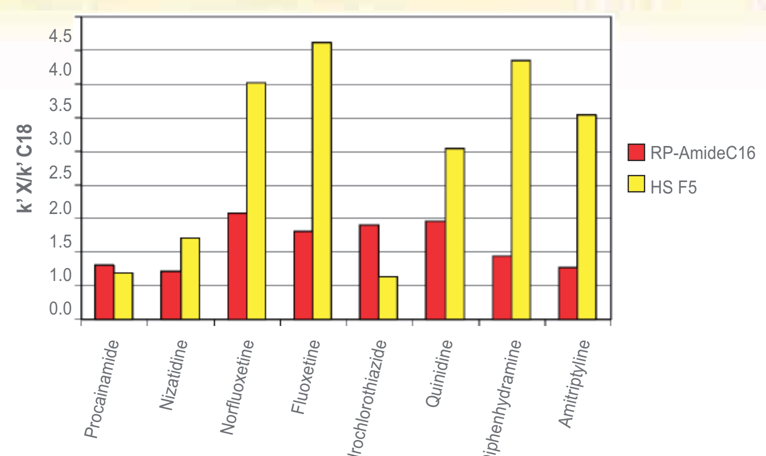
Different Retention is a General and Valuable Characteristic of Functionalized Reversed-Phases

Unique retention vs. C18

Retention of several basic drugs is compared on C18, RP-AmideC16 and F5. (F5 mobile phase %organic is +30% vs. C18 and RP-AmideC16.)

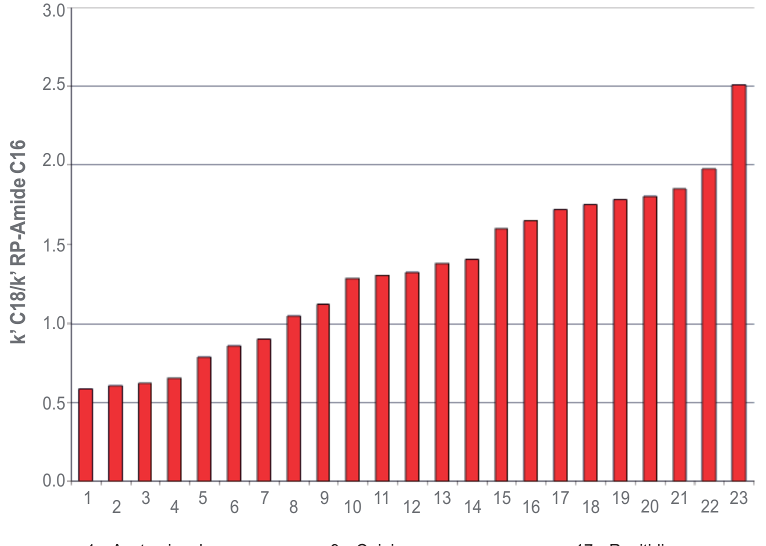
- Both F5 and RP-AmideC16 deliver valuable, different retention compared to C18.
- F5 is significantly more retentive for most compounds tested vs. both C18 and RP-AmideC16
- Retention on functionalized reversed-phases is almost always different than retention on C18!

Retention on RP-AmideC16 and F5 Relative to C18



NOTE: k' ratios not equal to 1.0 mean different retention.

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

HPLC: Small Molecules

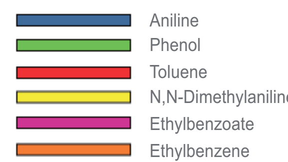
Rediscover Method Development

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

Key to interpreting results

When a color aligns, the selectivity is similar.

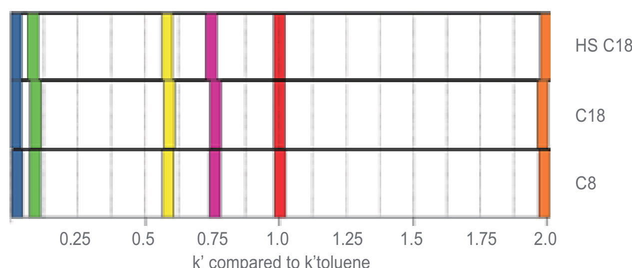
When a color does not align, the selectivity is different.



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

Similar Phases (C18's and C8's) - Similar Selectivities

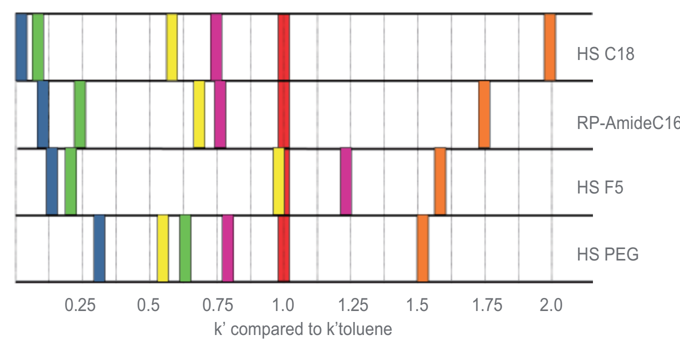
The near-perfect alignment of the colors clearly illustrates the similar selectivities of the C18 and C8 phases tested.



Unique, Functionalized Phases - Different Selectivities

The 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 the colors representing solutes containing polar groups dramatically change positions from phase to phase. Also observe the changing hydropbobic selectivity by looking at the Ethylbenzene bar.

Both polar and hydrophobic selectivities are different on the different phases.

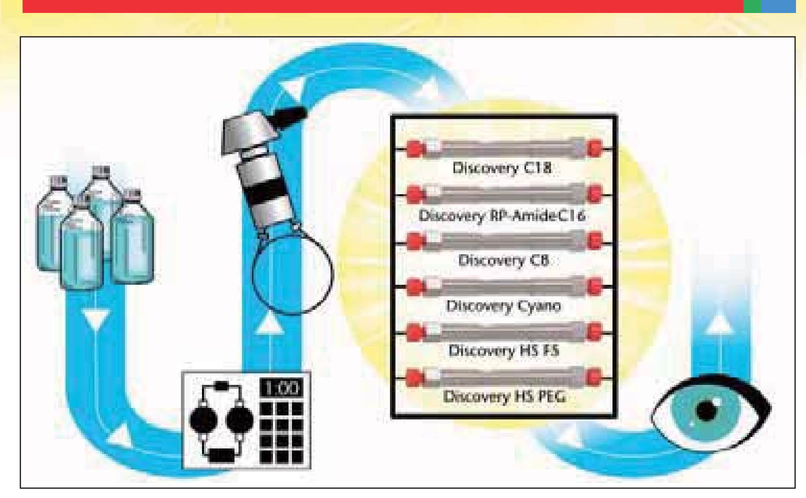


Tips for Getting Started: Good Method Development Practices

Tip One: Column Selector Valve

Automated HPLC + Column Selector Valve

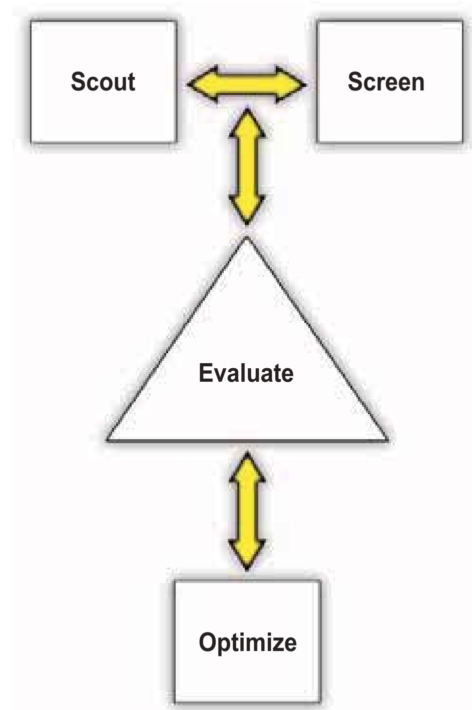
- While screening of functionalized reversed-phases can be done with a simple, manual HPLC system, an automated, multi-solvent system with programmable, temperature controlled column selector valve is highly recommended.



Tip Two: Simple Column Screening

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 RP-AmideC16 and F5 using "best" C18 mobile phase
  - Chromatograph sample on PEG using 20% lower organic than "best" C18 mobile phase
- Step 3: Evaluate screening runs
  - Retention OK? If no, adjust % organic and rerun (Note: 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 functionalized reversed-phases and a C18.

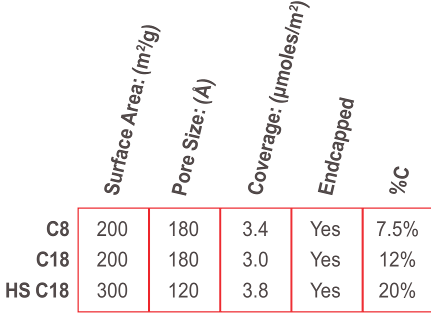
Tip Four: Optimize your separation on the 1 or 2 most promising phases!

HPLC: Small Molecules
Alkyl Reversed-Phases

Discovery C18, C8, HS C18....

C18 and C8

- Excellent performance
- Similar selectivities
- C18 generally more retentive than C8

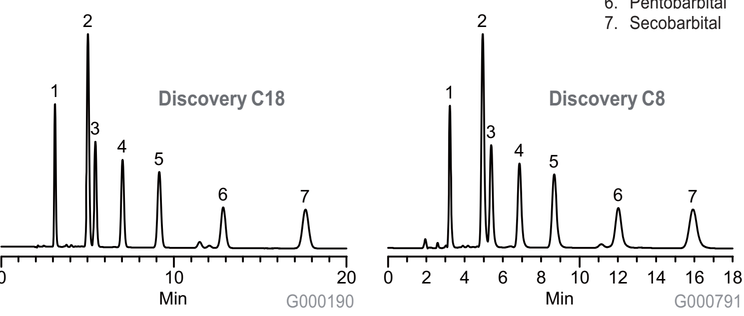


	Surface Area: (m^2/g)	Pore Size: (Å)	Coverage: ($\mu moles/m^2$)	Endcapped	%C
C8	200	180	3.4	Yes	7.5%
C18	200	180	3.0	Yes	12%
HS C18	300	120	3.8	Yes	20%

Barbiturates

Column: 15cm x 4.6mm columns, 5µm particles
Mobile Phase: 55:45 Water:MeOH
Flow Rate: 1.0mL/min
Det.: UV at 214nm
Temp.: ambient
Inj.: 5µL (Discovery C8) or 10µL (Discovery C18)

Sample: 1µg/mL of each
1. Barbital
2. Aprobarbital
3. Phenobarbital
4. Butabarbital
5. Mephobarbital
6. Pentobarbital
7. Secobarbital



Anticonvulsants

Column: 15cm x 4.6mm columns, 5µm particles
Mobile Phase: 70:30 Water:CH_3CN
Flow Rate: 2.0mL/min
Det.: UV at 254nm
Temp.: 20°C
Inj.: 10µL

Sample:
1. Clonazepam
2. Clorazepate
3. Diazepam

Discovery C18

Min G000746

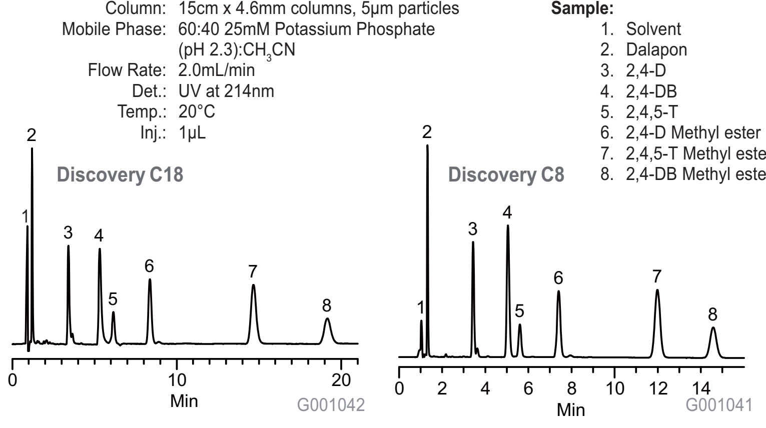
Discovery C8

Min G000748

Alhanoic / Aryloxyalhanoic Acid Using Isocratic Elution

Column: 15cm x 4.6mm columns, 5µm particles
Mobile Phase: 60:40 25mM Potassium Phosphate (pH 2.3):CH_3CN
Flow Rate: 2.0mL/min
Det.: UV at 214nm
Temp.: 20°C
Inj.: 1µL

Sample:
1. Solvent
2. Dalapon
3. 2,4-D
4. 2,4-DB
5. 2,4,5-T
6. 2,4-D Methyl ester
7. 2,4,5-T Methyl ester
8. 2,4-DB Methyl ester



...Excellent Chromatography for a Wide Range of Compounds

HS C18 and C18

- C18 most general, 1st choice
- Similar selectivities
- HS C18 generally more retentive than C18
- HS C18 is more acidic

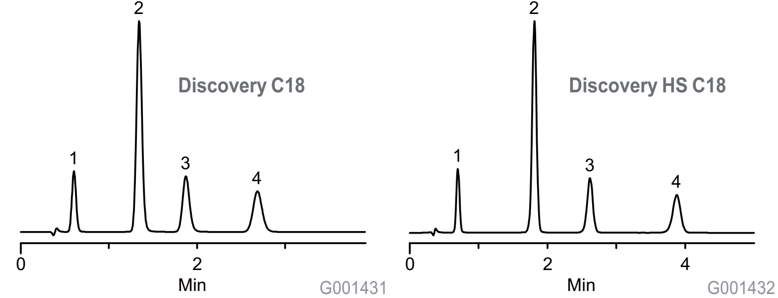
  Mobile phase additive can improve chromatography of bases

Organic Acids

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

Sample:

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)



Antibiotics (Fluoroquinolones from Tablets)

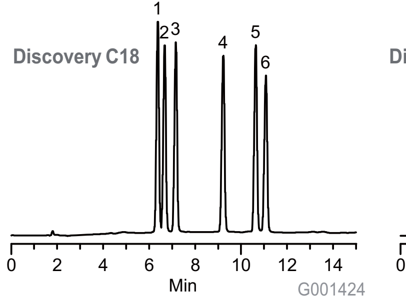
Column: 15cm x 4.6mm columns
Mobile Phase: (A) 25 mM Potassium Phosphate (pH 3.0)
(B) CH_3CN
Flow Rate: 1.0mL/min
Temp.: 35°C
Det.: UV at 220nm
Inj.: 10µL

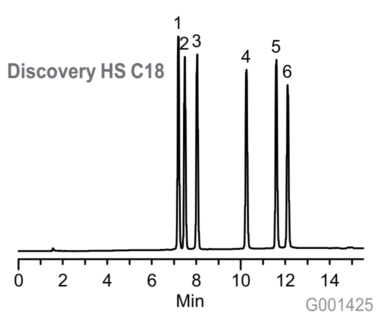
Gradient:

Min	%A	%B
0	90	10
15	65	35

Sample:

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





...HS C18 is More Acidic...

Sample:

1. Nordoxepin
2. Protriptyline/Desipramine
3. Nortriptyline
4. Doxepin
5. Imipramine
6. Amitriptyline
7. Trimipramine

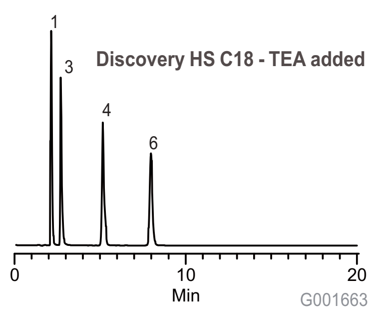
Discovery C18 – no additive

1 2 3 4 5 6 7

0 2 4 6 8 10 12 14

Min

G001662



Conditions for Discovery C18 - no additive

Column: 15cm x 4.6mm columns
5µm particles
Mobile Phase: 45:55 25mM Ammonium Phosphate (pH7.0):CH_3CN
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV at 254nm
Inj.: 10µL
Sample: 100µg/mL of each

Conditions for Discovery HS C18 - TEA added

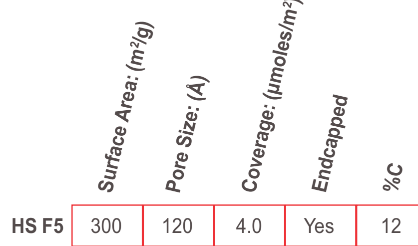
Column: 15cm x 4.6mm columns
5µm particles
Mobile Phase: 45:55 25mM TEA phosphate (pH7):CH_3CN
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV at 254nm
Inj.: 10µL
Sample: 100µg/mL of each

HPLC: Small Molecules

Discovery HS F5

Unique Retention and Selectivity Enables Better Separations

- Excellent performance
- Unique selectivity
- Similar retention to C18 (sometimes requires stronger mobile phase strength than C18)



	Surface Area: (m^2/g)	Pore Size: (Å)	Coverage: (μmoles/m^2)	Endcapped	%C
HS F5	300	120	4.0	Yes	12

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.

Column: Discovery HS F5 and Conventional C18, 15cm x 4.6mm, 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

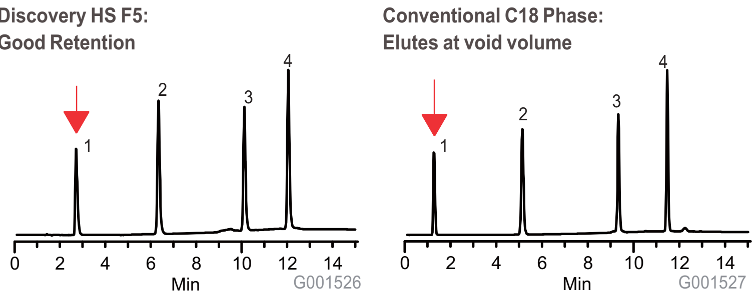
Gradient:

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

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

Discovery HS F5: Good Retention

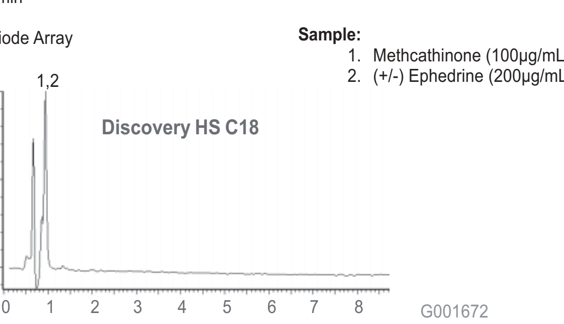
Conventional C18 Phase: Elutes at void volume



F5 Provides Excellent Separation - Solutes Are Not Retained on C18

Column: Discovery HS C18, 15cm x 4.6mm, 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

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



Column: Discovery HS F5, 15cm x 4.6mm
Conditions same as Discovery HS C18 above

Discovery HS F5

1

2

0 1 2 3 4 5 6 7 8

G001673

F5, a unique, functionalized reversed-phase uncovers a trace impurity missed by C18. "Pure" Quinidine was assayed on C18 under a variety of mobile phase conditions. Conditions C and D produced a single peak suggesting the Quinidine was pure. The peak resulting from condition B might be showing a partially resolved front shoulder. A quick screen of % organic was unable to resolve the possible impurity.

On F5 (chromatogram A) the impurity is clearly resolved. During method development a quick screen using unique, functionalized reversed-phases such as F5 greatly increases the chances of finding trace impurities early, before they can cause big problems.

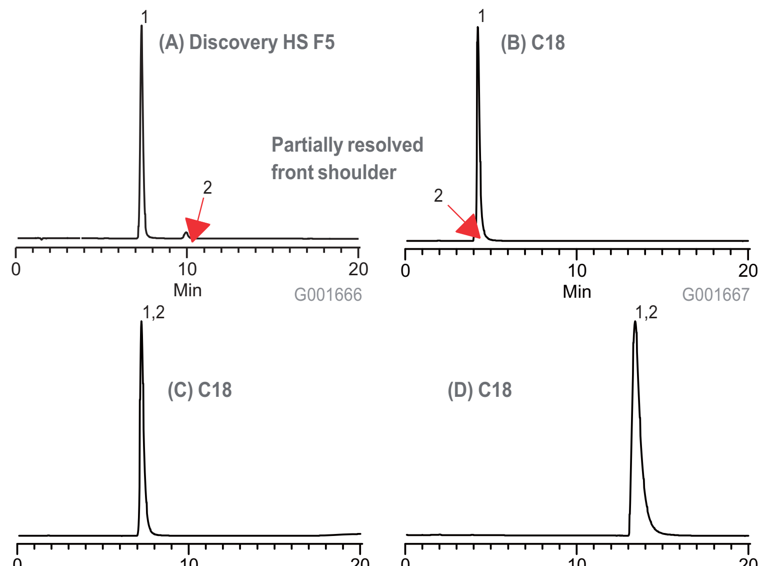
Helpful Hint

Routinely screen separations on several, complimentary functionalized reversed-phases (e.g. F5, PEG, RP-AmideC16) early in method development.

F5 Resolves Trace Impurity in Quinidine – C18 Does Not!

Column: Discovery HS F5 and Conventional C18, 15cm x 4.6mm, 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

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

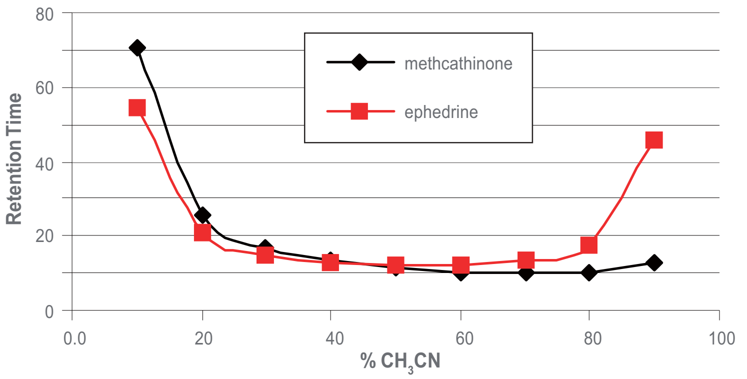


At lower organic, Discovery HS F5 exhibits reversed-phase behavior. At higher organic it exhibits normal phase behavior. At high % organic retention actually increases as % organic increases for some analytes. Benefits include:

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

F5 Exhibits "U" Shaped Retention Creating Interesting Possibilities

F5 - Retention vs. % Organic



Liquid Chromatography

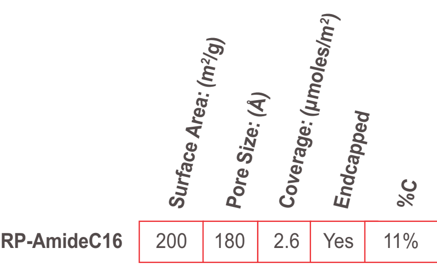
Order: 1.800.325.3010 Technical Service: 1.800.359.3041 Web: www.sigma-aldrich.com/supelco

HPLC: Small Molecules

Discovery RP-Amide C16

Unique Retention and Selectivity Enables Better Separations

- Excellent performance
- Unique selectivity
- Similar retention to C18 (typically requires similar mobile phase strength as C18)



	Surface Area: (m²/g)	Pore Size: (Å)	Coverage: (µmoles/m²)	Endcapped	%C
RP-AmideC16	200	180	2.6	Yes	11%

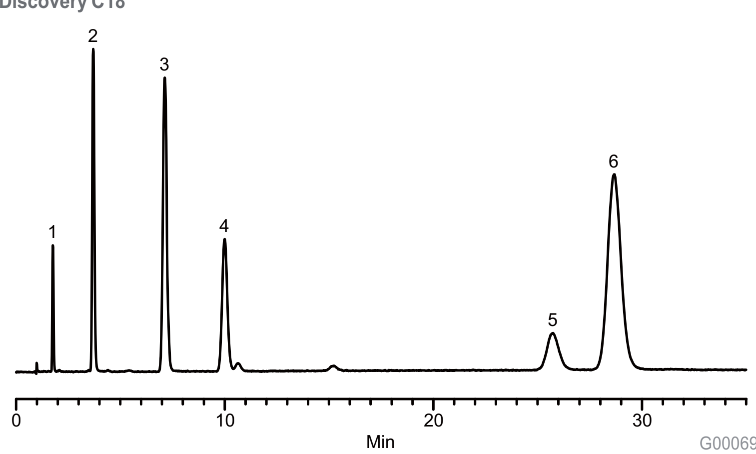
Discovery RP-AmideC16 Gives Better Resolution and Faster Analysis

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

Column: 15cm x 4.6mm, 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

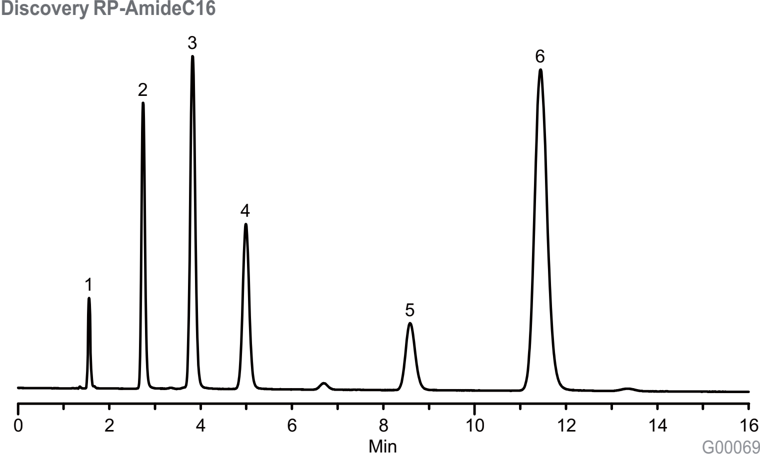
Sample:
1. Codeine
2. Strychnine
3. Quinidine
4. Quinine
5. Noscapine
6. Papaverine

Discovery C18



G000692

Discovery RP-AmideC16



G000691

Faster analysis and different selectivity.

Antitussive/Antihistamine/Antipyretic Mix

Dramatic differences in peak order and run time are demonstrated by different Discovery reversed-phase stationary phases.

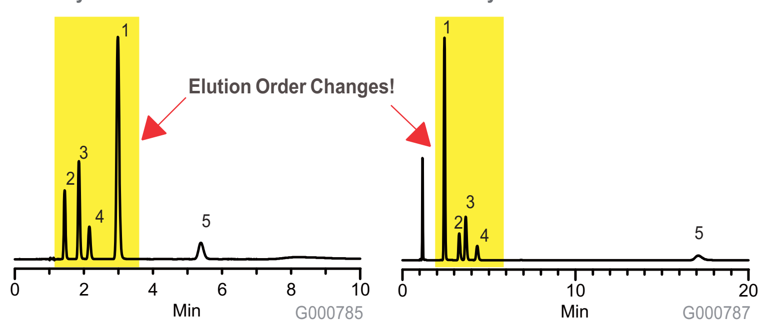
Column: 15cm x 4.6mm, 5µm particles
Mobile Phase: (A) 25 mM Potassium Phosphate (pH 2.3)
(B) CH_3CN
Flow Rate: 2.0mL/min
Temp.: ambient
Det.: UV at 214nM
Inj.: 10µL

Gradient:

Min	%A	%B
0	90	10
2	90	10
4	70	30
8	70	30
10	50	50

Sample: (1µg/mL of each)
1. Acetaminophen
2. Doxylamine
3. Pseudoephedrine
4. Codeine
5. Chlorpheniramine

Discovery RP-AmideC16



But... different selectivity is not always better!

Here C18 provides a better separation.

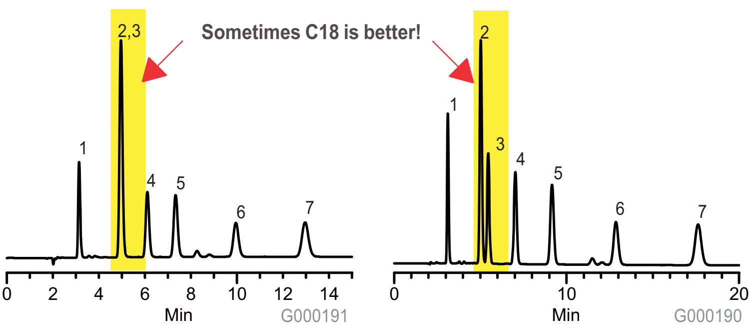
Barbiturates

Different Discovery column chemistries show different separations of barbiturates under identical conditions.

Column: 15cm x 4.6mm columns, 5µm particles
Mobile Phase: 55:45 Water:MeOH
Flow Rate: 1.0mL/min
Temp.: ambient
Det.: UV at 214nM
Inj.: 10µL

Sample: (1µg/mL of each)
1. Barbital
2. Aprobarbital
3. Phenobarbital
4. Butabarbital
5. Mephobarbital
6. Pentobarbital
7. Secobarbital

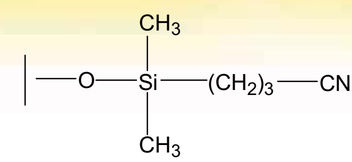
Discovery RP-AmideC16



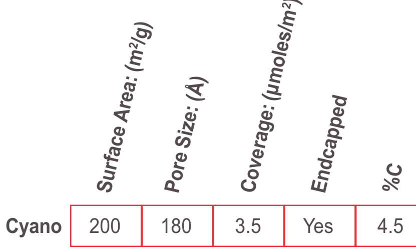
HPLC: Small Molecules

Discovery Cyano

Unique Retention and Selectivity Enables Better Separations



- Excellent performance
- Unique selectivity
- Significantly less retentive than C18 (therefore typically requires greater % H_2O mobile phase)



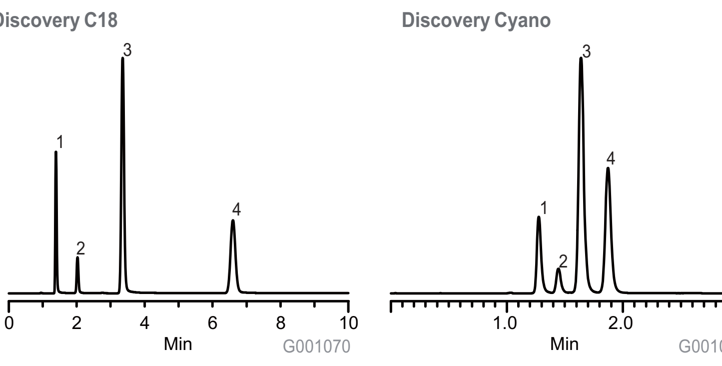
	Surface Area: (m^2/g)	*Pore Size: (Å)*	*Coverage: (µmoles/m^2)*	*Endcapped*	*%C*
Cyano	200	180	3.5	Yes	4.5

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



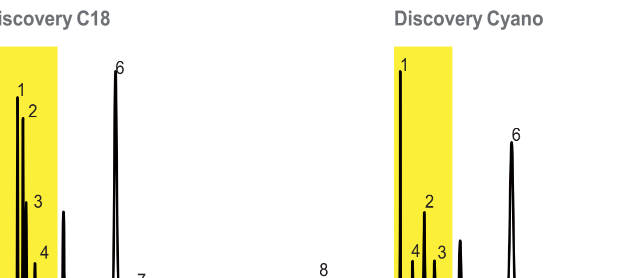
Faster Analysis - Different Selectivity

Organophosphorous Pesticides Using Isocratic Elution

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

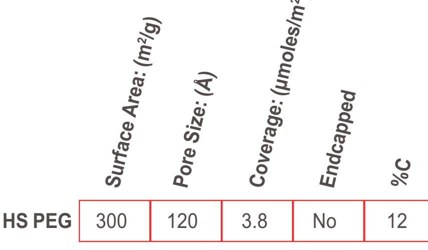
Column: Discovery Cyano, 15cm x 4.6mm, 5µm particles
Mobile Phase: 75:25 Water:CH_3CN
Flow Rate: 2.0mL/min
Temp.: 20°C
Det.: UV at 214nm
Inj.: 1µL

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



Unique Retention and Selectivity Enables Better Separations

- Excellent performance
- Unique selectivity
- Significantly less retentive than C18 (therefore typically requires greater % H_2O mobile phase)



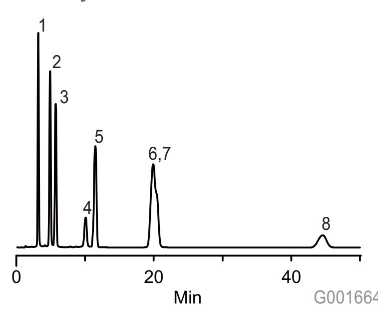
	Surface Area: (m^2/g)	Pore Size: (Å)	Coverage: (µmoles/m^2)	Endcapped	%C
HS PEG	300	120	3.8	No	12

Flavones Demonstrate Dramatic Selectivity Differences Between HS PEG and C18

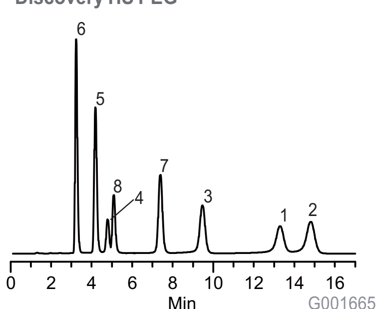
Column: 15cm x 4.6mm columns, 5µm particles
Mobile Phase: 45:55 0.1% Formic Acid in Water : 0.1% Formic Acid in MeOH
Flow Rate: 1.0mL/min
Temp.: 30°C,
Det.: UV at 254nm
Inj.: 10µL

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

Discovery HS C18



Discovery HS PEG



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

Column: 15cm x 4.6mm columns, 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

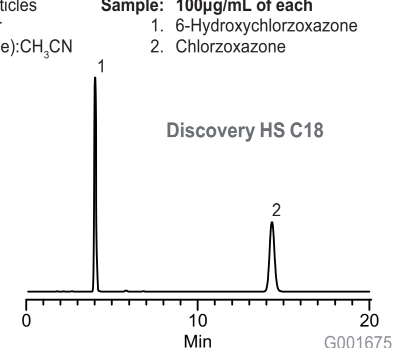
Sample: 100µg/mL of each
1. 6-Hydroxychlorzoxazone
2. Chlorzoxazone

Discovery HS PEG

1 2
0 10 20
Min
G001674

Column: 15cm x 4.6mm columns, 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

Sample: 100µg/mL of each
1. 6-Hydroxychlorzoxazone
2. Chlorzoxazone



HPLC: Small Molecules

Discovery C18 and Discovery HS C18 Columns

Discovery C18

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY C18 (180Å, 12% CARBON)			
5µm	5 x 2.1	50494721	
5µm	10 x 2.1	569220-U	
5µm	12.5 x 2.1	569229-U	
5µm	15 x 2.1	50495521	
5µm	5 x 3.0	504947-30	
5µm	10 x 3.0	569221-U	
5µm	12.5 x 3.0	569230-U	
5µm	15 x 3.0	504955-30	
5µm	25 x 3.0	504971-30	
5µm	5 x 4.0	504947-40	
5µm	10 x 4.0	569222-U	
5µm	12.5 x 4.0	569231-U	
5µm	15 x 4.0	504955-40	
5µm	25 x 4.0	504971-40	
5µm	5 x 4.6	504947	
5µm	10 x 4.6	569223-U	
5µm	12.5 x 4.6	569232-U	
5µm	15 x 4.6	504955	
5µm	25 x 4.6	504971	
5µm	25 x 10.0	569224-U	
5µm	25 x 21.2	569226-U	
DISCOVERY C18 VALIDATION PACKS*			
5µm	5 x 2.1	55700-U21	
5µm	10 x 2.1	569800-U	
5µm	15 x 2.1	55702-U21	
5µm	5 x 4.6	55700-U	
5µm	10 x 4.6	569801-U	
5µm	15 x 4.6	55702-U	
5µm	25 x 4.6	55704-U	
DISCOVERY C18 SUPELGUARD CARTRIDGES			
5µm	2 x 2.1 (2/pk)	505188	
5µm	2 x 2.1 kit **	505161	
5µm	2 x 3.0 (2/pk)	59576-U	
5µm	2 x 3.0 kit **	59575-U	
5µm	2 x 4.0 (2/pk)	505137	
5µm	2 x 4.0 kit **	505129	

* Packs include 3 columns, each from a different lot of bonded phase.

** Kits include one cartridge, a stand-alone holder, a piece of tubing, 2 nuts and ferrules.

DISCOVERY C18 PROPERTIES:	
Bonded Phase:	Octadecylsilane, endcapped
Silica:	Spherical, high purity (Fe <20; NA <7; Ca <7; Ti <1; Al <1; Mg <1ppm)
Particle Size:	5µm
Pore Size:	180Å
Surface Area:	200m²/g
%C:	~12%
Coverage:	~3µmoles/m²

Discovery HS C18 New!

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY HS C18 (120Å, 20% CARBON)			
3µm	5 x 2.1	569253-U	
3µm	7.5 x 2.1	569254-U	
3µm	15 x 2.1	569255-U	
3µm	5 x 4.6	569250-U	
3µm	7.5 x 4.6	569251-U	
3µm	15 x 4.6	569252-U	
5µm	5 x 2.1	568500-U	
5µm	10 x 2.1	568501-U	
5µm	15 x 2.1	568502-U	
5µm	25 x 2.1	568503-U	
5µm	5 x 4.0	568510-U	
5µm	10 x 4.0	568511-U	
5µm	15 x 4.0	568512-U	
5µm	25 x 4.0	568513-U	
5µm	5 x 4.6	568520-U	
5µm	10 x 4.6	568521-U	
5µm	15 x 4.6	568522-U	
5µm	25 x 4.6	568523-U	
5µm	5 x 10.0	568530-U	
5µm	10 x 10.0	568531-U	
5µm	15 x 10.0	568532-U	
5µm	25 x 10.0	568533-U	
5µm	5 x 21.2	568540-U	
5µm	10 x 21.2	568541-U	
5µm	15 x 21.2	568542-U	
5µm	25 x 21.2	568543-U	
10µm	5 x 10.0	568630-U	
10µm	10 x 10.0	568631-U	
10µm	15 x 10.0	568632-U	
10µm	25 x 10.0	568633-U	
10µm	5 x 21.2	568640-U	
10µm	10 x 21.2	568641-U	
10µm	15 x 21.2	568642-U	
10µm	25 x 21.2	568643-U	
DISCOVERY HS C18 SUPELGUARD CARTRIDGES			
3µm	2 x 2.1 (2/pk)	569276-U	
3µm	2 x 2.1 kit **	569277-U	
3µm	2 x 4.0 (2/pk)	569274-U	
3µm	2 x 4.0 kit **	569275-U	
5µm	2 x 2.1 (2/pk)	568570-U	
5µm	2 x 2.1 kit **	568571-U	
5µm	2 x 4.0 (2/pk)	568572-U	
5µm	2 x 4.0 kit **	568573-U	
5µm	1 x 10.0	568574-U	
10µm	1 x 10.0	568674-U	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

DISCOVERY HS C18 PROPERTIES:	
Bonded Phase:	Octadecylsilane, endcapped
Silica:	Spherical, high purity (<10 ppm metals)
Particle Size:	3, 5, 10µm
Pore Size:	120Å
Surface Area:	300m²/g
%C:	~20%
Coverage:	~3.8µmoles/m²

Discovery RP-AmideC16 and Discovery HS F5 Columns

Discovery RP-AmideC16

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY RP-AMIDEC16 (180Å, 11% CARBON)			
5µm	5 x 2.1	50500521	
5µm	10 x 2.1	569320-U	
5µm	12.5 x 2.1	569329-U	
5µm	15 x 2.1	50501321	
5µm	5 x 3.0	505005-30	
5µm	10 x 3.0	569321-U	
5µm	12.5 x 3.0	569330-U	
5µm	15 x 3.0	505013-30	
5µm	25 x 3.0	505064-30	
5µm	5 x 4.0	505005-40	
5µm	10 x 4.0	569322-U	
5µm	12.5 x 4.0	569331-U	
5µm	15 x 4.0	505013-40	
5µm	25 x 4.0	505064-40	
5µm	5 x 4.6	505005	
5µm	10 x 4.6	569323-U	
5µm	12.5 x 4.6	569332-U	
5µm	15 x 4.6	505013	
5µm	25 x 4.6	505064	
5µm	25 x 10.0	569324-U	
5µm	25 x 21.2	569326-U	
DISCOVERY RP-AMIDEC16 VALIDATION PACKS *			
5µm	5 x 2.1	55705-U21	
5µm	10 x 2.1	569802-U	
5µm	15 x 2.1	55707-U21	
5µm	5 x 4.6	55705-U	
5µm	10 x 4.6	569803-U	
5µm	15 x 4.6	55707-U	
5µm	25 x 4.6	55709-U	
DISCOVERY RP-AMIDEC16 SUPELGUARD CARTRIDGES			
5µm	2 x 2.1 (2/pk)	505110	
5µm	2 x 2.1 kit **	505102	
5µm	2 x 3.0 (2/pk)	59578-U	
5µm	2 x 3.0 kit **	59577-U	
5µm	2 x 4.0 (2/pk)	505099	
5µm	2 x 4.0 kit **	505080	

* Packs include 3 columns, each from a different lot of bonded phase.

** Kits include one cartridge, a stand-alone holder, a piece of tubing, 2 nuts and ferrules.

DISCOVERY RP-AMIDEC16 PROPERTIES:

Bonded Phase: Palmitamidopropylsilane, endcapped
Silica: Spherical, high purity (Fe <20; NA <7; Ca <7; Ti <1; Al <1; Mg <1ppm)
Particle Size: 5µm
Pore Size: 180Å
Surface Area: 200m²/g
%C: ~11%
Coverage: ~2.6µmoles/m²

Discovery HS F5 New!

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY HS F5 (120Å, 12% CARBON)			
3µm	5 x 2.1	567500-U	
3µm	10 x 2.1	567502-U	
3µm	15 x 2.1	567503-U	
3µm	5 x 4.0	567530-U	
3µm	10 x 4.0	567531-U	
3µm	15 x 4.0	567532-U	
3µm	5 x 4.6	567504-U	
3µm	10 x 4.6	567506-U	
3µm	15 x 4.6	567507-U	
5µm	5 x 2.1	567508-U	
5µm	10 x 2.1	567510-U	
5µm	15 x 2.1	567511-U	
5µm	25 x 2.1	567512-U	
5µm	5 x 4.0	567533-U	
5µm	10 x 4.0	567534-U	
5µm	15 x 4.0	567535-U	
5µm	25 x 4.0	567536-U	
5µm	5 x 4.6	567513-U	
5µm	10 x 4.6	567515-U	
5µm	15 x 4.6	567516-U	
5µm	25 x 4.6	567517-U	
5µm	5 x 10.0	567518-U	
5µm	10 x 10.0	567537-U	
5µm	15 x 10.0	567519-U	
5µm	25 x 10.0	567520-U	
5µm	5 x 21.2	567521-U	
5µm	10 x 21.2	567539-U	
5µm	15 x 21.2	567522-U	
5µm	25 x 21.2	567523-U	
10µm	5 x 10.0	567524-U	
10µm	10 x 10.0	567538-U	
10µm	15 x 10.0	567525-U	
10µm	25 x 10.0	567526-U	
10µm	5 x 21.2	567527-U	
10µm	10 x 21.2	567540-U	
10µm	15 x 21.2	567528-U	
10µm	25 x 21.2	567529-U	
DISCOVERY HS F5 SUPELGUARD CARTRIDGES			
3µm	2 x 2.1 (2/pk)	567570-U	
3µm	2 x 2.1 kit **	567571-U	
3µm	2 x 4.0 (2/pk)	567572-U	
3µm	2 x 4.0 kit **	567573-U	
5µm	2 x 2.1 (2/pk)	567574-U	
5µm	2 x 2.1 kit **	567575-U	
5µm	2 x 4.0 (2/pk)	567576-U	
5µm	2 x 4.0 kit **	567577-U	
5µm	1 x 10.0	567578-U	
10µm	1 x 10.0	567580-U	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

DISCOVERY HS F5 PROPERTIES:

Bonded Phase: Pentafluorophenylpropylsilane, endcapped
Silica: Spherical, high purity (<10 ppm metals)
Particle Size: 3, 5, 10µm
Pore Size: 120Å
Surface Area: 300m²/g
%C: ~12%
Coverage: ~4µmoles/m²

Liquid Chromatography

HPLC: Small Molecules

Discovery HS PEG and Discovery C8 Columns

Discovery HS PEG

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY HS PEG (120Å, 12% CARBON)			
3µm	5 x 2.1	567400-U	
3µm	10 x 2.1	567402-U	
3µm	15 x 2.1	567403-U	
3µm	5 x 4.0	567430-U	
3µm	10 x 4.0	567431-U	
3µm	15 x 4.0	567432-U	
3µm	5 x 4.6	567404-U	
3µm	10 x 4.6	567406-U	
3µm	15 x 4.6	567407-U	
5µm	5 x 2.1	567408-U	
5µm	10 x 2.1	567410-U	
5µm	15 x 2.1	567411-U	
5µm	25 x 2.1	567412-U	
5µm	5 x 4.0	567433-U	
5µm	10 x 4.0	567434-U	
5µm	15 x 4.0	567435-U	
5µm	25 x 4.0	567436-U	
5µm	5 x 4.6	567413-U	
5µm	10 x 4.6	567415-U	
5µm	15 x 4.6	567416-U	
5µm	25 x 4.6	567417-U	
5µm	5 x 10.0	567418-U	
5µm	10 x 10.0	567437-U	
5µm	15 x 10.0	567419-U	
5µm	25 x 10.0	567420-U	
5µm	5 x 21.2	567421-U	
5µm	10 x 21.2	567439-U	
5µm	15 x 21.2	567422-U	
5µm	25 x 21.2	567423-U	
10µm	5 x 10.0	567424-U	
10µm	10 x 10.0	567438-U	
10µm	15 x 10.0	567425-U	
10µm	25 x 10.0	567426-U	
10µm	5 x 21.2	567427-U	
10µm	10 x 21.2	567440-U	
10µm	15 x 21.2	567428-U	
10µm	25 x 21.2	567429-U	
DISCOVERY HS PEG SUPELGUARD CARTRIDGES			
3µm	2 x 2.1 (2/pk)	567470-U	
3µm	2 x 2.1 kit **	567471-U	
3µm	2 x 4.0 (2/pk)	567472-U	
3µm	2 x 4.0 kit **	567473-U	
5µm	2 x 2.1 (2/pk)	567474-U	
5µm	2 x 2.1 kit **	567475-U	
5µm	2 x 4.0 (2/pk)	567476-U	
5µm	2 x 4.0 kit **	567477-U	
5µm	1 x 10.0	567478-U	
10µm	1 x 10.0	567480-U	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

DISCOVERY HS PEG PROPERTIES:	
Bonded Phase:	Polyethyleneglycol, endcapped
Silica:	Spherical, high purity (<10 ppm metals)
Particle Size:	3, 5, 10µm
Pore Size:	120Å
Surface Area:	300m²/g
%C:	~12%
Coverage:	~3.8µmoles/m²

Discovery C8

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY C8 (180Å, 7.5% CARBON)			
5µm	5 x 2.1	59352-U21	
5µm	10 x 2.1	569420-U	
5µm	12.5 x 2.1	569424-U	
5µm	15 x 2.1	59353-U21	
5µm	5 x 3.0	59352-U30	
5µm	10 x 3.0	569421-U	
5µm	12.5 x 3.0	569425-U	
5µm	15 x 3.0	59353-U30	
5µm	25 x 3.0	59354-U30	
5µm	5 x 4.0	59352-U40	
5µm	10 x 4.0	569422-U	
5µm	12.5 x 4.0	569426-U	
5µm	15 x 4.0	59353-U40	
5µm	25 x 4.0	59354-U40	
5µm	5 x 4.6	59352-U	
5µm	10 x 4.6	569423-U	
5µm	12.5 x 4.6	569427-U	
5µm	15 x 4.6	59353-U	
5µm	25 x 4.6	59354-U	
DISCOVERY C8 VALIDATION PACKS *			
5µm	5 x 2.1	55710-U21	
5µm	10 x 2.1	569804-U	
5µm	15 x 2.1	55712-U21	
5µm	5 x 4.6	55710-U	
5µm	10 x 4.6	569805-U	
5µm	15 x 4.6	55712-U	
5µm	25 x 4.6	55714-U	
DISCOVERY C8 SUPELGUARD CARTRIDGES			
5µm	2 x 2.1 (2/pk)	59588-U	
5µm	2 x 2.1 kit**	59587-U	
5µm	2 x 3.0 (2/pk)	59580-U	
5µm	2 x 3.0 kit**	59579-U	
5µm	2 x 4.0 (2/pk)	59590-U	
5µm	2 x 4.0 kit**	59589-U	

* Packs include 3 columns, each from a different lot of bonded phase.

** Kits include one cartridge, a stand-alone holder, a piece of tubing, 2 nuts and ferrules.

DISCOVERY C8 PROPERTIES:	
Bonded Phase:	Octylsilane, endcapped
Silica:	Spherical, high purity (Fe <20; NA <7; Ca <7; Ti <1; Al <1; Mg <1ppm)
Particle Size:	5µm
Pore Size:	180Å
Surface Area:	200m²/g
%C:	~7.5%
Coverage:	~3.4µmoles/m²

Discovery Cyano

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY CYANO (180Å, 4.5% CARBON)			
5µm	5 x 2.1	59355-U21	
5µm	10 x 2.1	569521-U	
5µm	12.5 x 2.1	569524-U	
5µm	15 x 2.1	59356-U21	
5µm	5 x 3.0	59355-U30	
5µm	10 x 3.0	569522-U	
5µm	12.5 x 3.0	569525-U	
5µm	15 x 3.0	59356-U30	
5µm	25 x 3.0	59357-U30	
5µm	5 x 4.0	59355-U40	
5µm	10 x 4.0	569523-U	
5µm	12.5 x 4.0	569526-U	
5µm	15 x 4.0	59356-U40	
5µm	25 x 4.0	59357-U40	
5µm	5 x 4.6	59355-U	
5µm	10 x 4.6	569520-U	
5µm	12.5 x 4.6	569527-U	
5µm	15 x 4.6	59356-U	
5µm	25 x 4.6	59357-U	
DISCOVERY CYANO VALIDATION PACKS*			
5µm	5 x 2.1	55715-U21	
5µm	10 x 2.1	569806-U	
5µm	15 x 2.1	55717-U21	
5µm	5 x 4.6	55715-U	
5µm	10 x 4.6	569807-U	
5µm	15 x 4.6	55717-U	
5µm	25 x 4.6	55719-U	
DISCOVERY CYANO SUPELGUARD CARTRIDGES			
5µm	2 x 2.1 (2/pk)	59584-U	
5µm	2 x 2.1 kit**	59583-U	
5µm	2 x 3.0 (2/pk)	569571-U	
5µm	2 x 3.0 kit**	569570-U	
5µm	2 x 4.0 (2/pk)	59586-U	
5µm	2 x 4.0 kit**	59585-U	

* Packs include 3 columns, each from a different lot of bonded phase.

** Kits include one cartridge, a stand-alone holder, a piece of tubing, 2 nuts and ferrules.

DISCOVERY CYANO PROPERTIES:	
Bonded Phase:	Cyanopropylsilane, endcapped
Silica:	Spherical, high purity (Fe <20; NA <7; Ca <7; Ti <1; Al <1; Mg <1ppm)
Particle Size:	5µm
Pore Size:	180Å
Surface Area:	200m²/g
%C:	~4.5%
Coverage:	~3.5µmoles/m²

Discovery Selectivity Packs

You can conveniently order the four Discovery column chemistries – RP-AmideC16, C18 , C8, and Cyano – in your choice of column dimensions, in a single kit. Quickly evaluate mobile phases on all four columns to find the optimum combination of chemistries for your separation. The Discovery HPLC Column Selectivity Pack gives you a powerful tool for rapid, efficient, simple pharmaceutical method development.

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
5µm	5 x 2.1	55720-U21	
5µm	10 x 2.1	569853-U	
5µm	15 x 2.1	55722-U21	
5µm	5 x 3.0	55720-U30	
5µm	10 x 3.0	569852-U	
5µm	15 x 3.0	55722-U30	
5µm	25 x 3.0	55724-U30	
5µm	5 x 4.0	55720-U40	
5µm	10 x 4.0	569851-U	
5µm	15 x 4.0	55722-U40	
5µm	25 x 4.0	55724-U40	
5µm	5 x 4.6	55720-U	
5µm	10 x 4.6	569850-U	
5µm	15 x 4.6	55722-U	
5µm	25 x 4.6	55724-U	

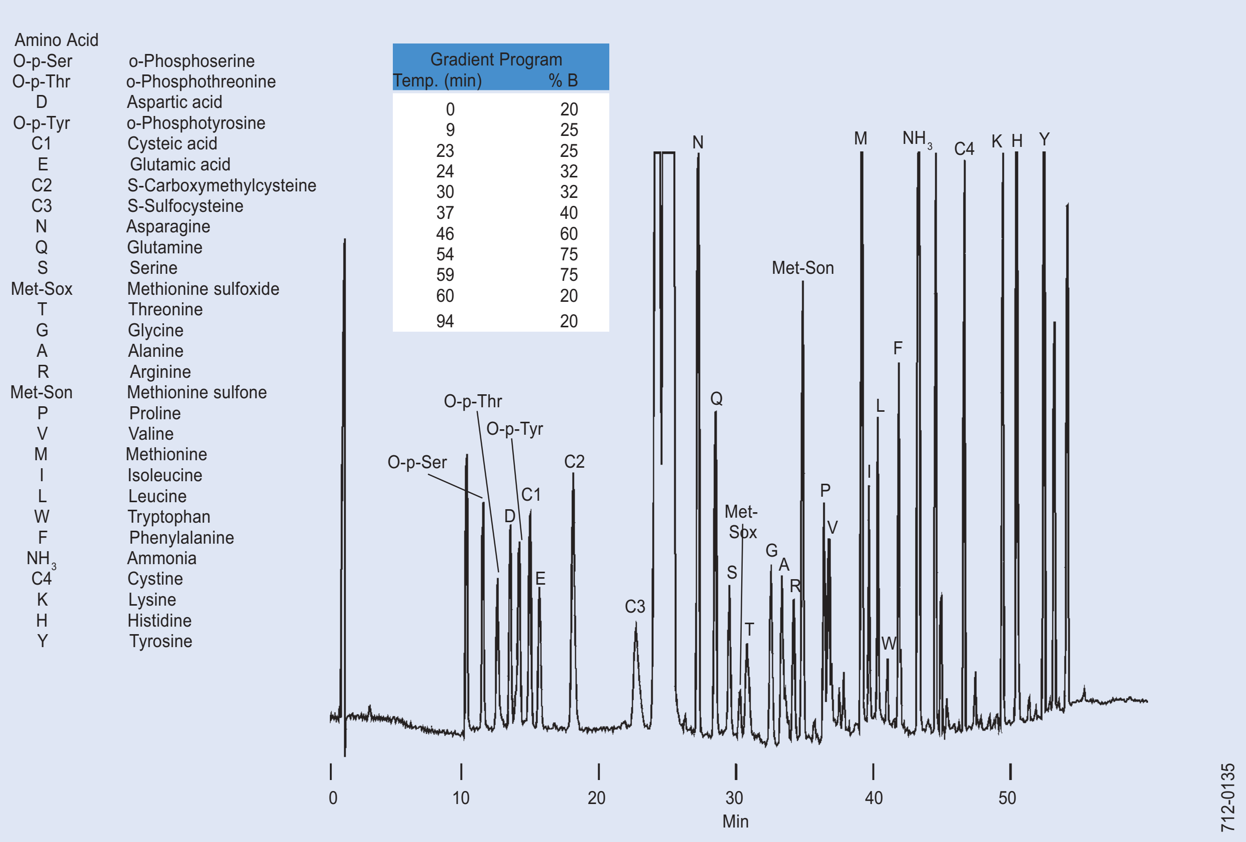
HPLC: Small Molecules

Special Purpose SUPELCOSIL Columns

Special Purpose Columns: Amino Acids

SUPELCOSIL LC-DABS columns feature a specially treated and tested octadecylsilane bonded phase, for reversed-phase separations of precolumn derivatized dabsyl amino acids. More than 30 amino acids and ammonia can be separated in less than one hour.

PROPERTIES	
Particles:	spherical silica, 3µm
Pore Size:	120Å
Bonded Phase:	octadecylsilane
Surface Area:	170m²/g
Pore Volume:	0.6mL/g
pH Range:	2 - 7.5



Dabsyl Amino Acids

Column: SUPELCOSIL LC-DABS
15cm x 4.6mm ID, 3µm particles
Cat. No.: 59137
Mobile Phase: A = 25mM KH_2PO_4, pH7.0
B = acetonitrile:methanol (70:30)
Flow Rate: 1.5mL/min
Det.: VIS, 436nm
Inj.: 5µL, approx. 50pM each derivative

RELATED INFORMATION

Literature References

Stocchi, V., et al., Anal. Biochem.178: 107-117 (1989).

Stocchi, V., et al., Amino Acids3: 303-309 (1992).

References not available from Supelco.

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-DABS (120Å, 12.3% CARBON)			
3µm	15 x 4.6	59137	
SUPELCOSIL LC-18-T Supelguard Cartridges (use for LC-DABS)			
5µm	2 x 4.0 (2/pk)	59621	
5µm	2 x 4.0 kit **	59620	

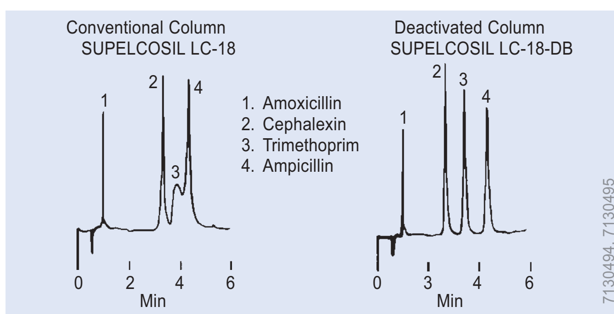
** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

Special Purpose Columns: Basic Columns

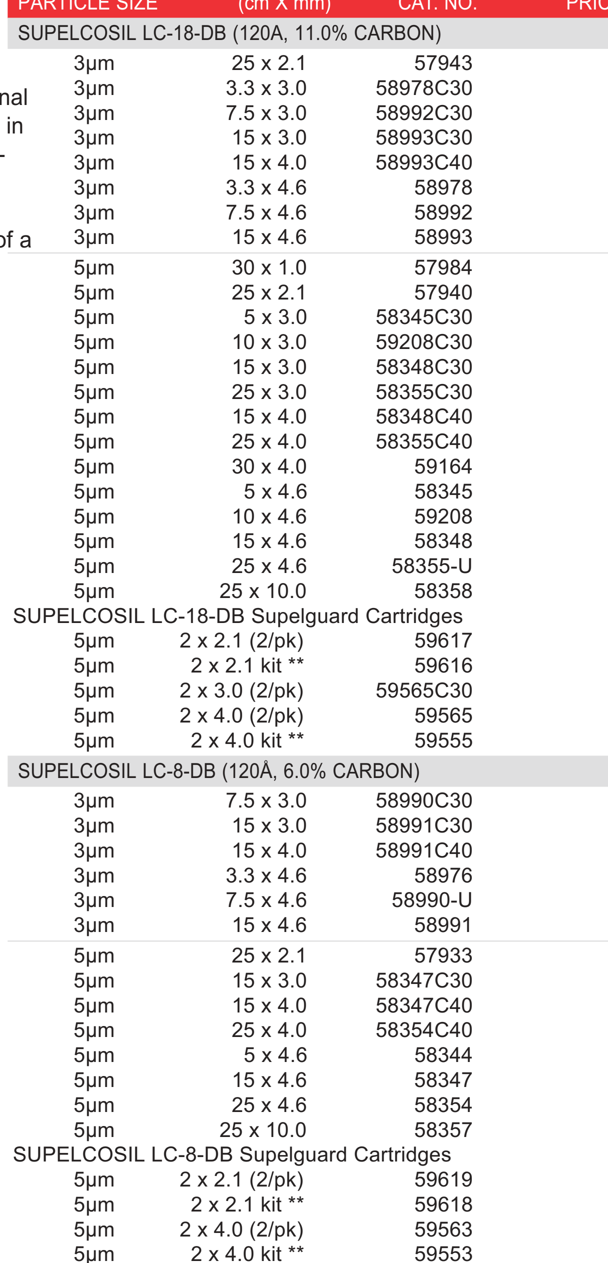
SUPELCOSIL LC-18-DB and SUPELCOSIL LC-8-DB phases are deactivated (DB) for basic compounds. These columns provide shorter retention, better peak shape, and higher efficiency for organic bases than can be obtained on conventional reversed-phase columns. DB columns have become standards in the industry for analyses of "difficult" basic compounds, particularly in the pharmaceutical industry. An example of the performance of the LC-18-DB column versus that of a conventional C18 column is shown. The deactivated column allows the use of a simpler mobile phase to elute trimethoprim with good peak shape. To obtain similar results from the LC-18 column would require the addition of competing base to the mobile phase

PROPERTIES	
Silica:	Spherical
Particle size:	3µm and 5µm
Pore size:	120Å
Pore Volume:	0.6mL/g
Surface Area:	170m^2/g
pH Range:	2-7

Improved Peak Symmetry for Antibacterials



Column: 3.3cm x 4.6mm, 3µm
Cat. No.: 58977 (LC-18), 58978 (LC-18-DB)
Mobile Phase: methanlo:50mM KH_2PO_4, pH 3.0 (23:77)
Flow Rate: 1mL/min
Det.: UV, 254nm
Inj.: 10µL, 15, 5, 5 and 106µg/mL

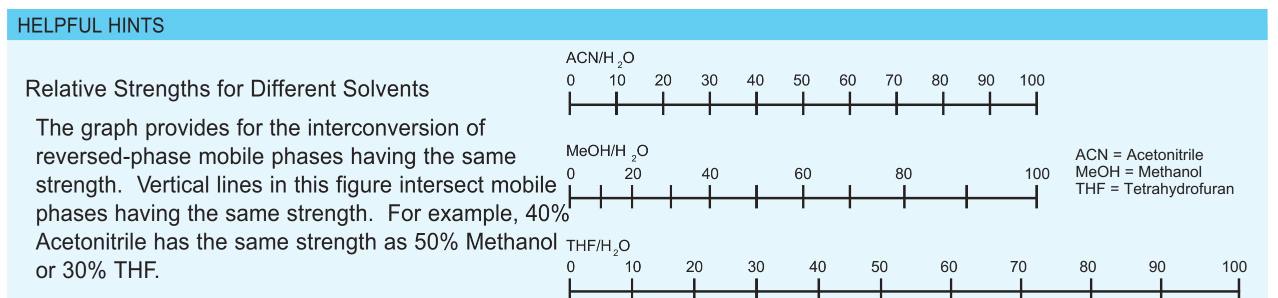


PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-18-DB (120A, 11.0% CARBON)			
3µm	25 x 2.1	57943	
3µm	3.3 x 3.0	58978C30	
3µm	7.5 x 3.0	58992C30	
3µm	15 x 3.0	58993C30	
3µm	15 x 4.0	58993C40	
3µm	3.3 x 4.6	58978	
3µm	7.5 x 4.6	58992	
3µm	15 x 4.6	58993	
5µm	30 x 1.0	57984	
5µm	25 x 2.1	57940	
5µm	5 x 3.0	58345C30	
5µm	10 x 3.0	59208C30	
5µm	15 x 3.0	58348C30	
5µm	25 x 3.0	58355C30	
5µm	15 x 4.0	58348C40	
5µm	25 x 4.0	58355C40	
5µm	30 x 4.0	59164	
5µm	5 x 4.6	58345	
5µm	10 x 4.6	59208	
5µm	15 x 4.6	58348	
5µm	25 x 4.6	58355-U	
5µm	25 x 10.0	58358	
SUPELCOSIL LC-18-DB Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59617	
5µm	2 x 2.1 kit **	59616	
5µm	2 x 3.0 (2/pk)	59565C30	
5µm	2 x 4.0 (2/pk)	59565	
5µm	2 x 4.0 kit **	59555	
SUPELCOSIL LC-8-DB (120Å, 6.0% CARBON)			
3µm	7.5 x 3.0	58990C30	
3µm	15 x 3.0	58991C30	
3µm	15 x 4.0	58991C40	
3µm	3.3 x 4.6	58976	
3µm	7.5 x 4.6	58990-U	
3µm	15 x 4.6	58991	
5µm	25 x 2.1	57933	
5µm	15 x 3.0	58347C30	
5µm	15 x 4.0	58347C40	
5µm	25 x 4.0	58354C40	
5µm	5 x 4.6	58344	
5µm	15 x 4.6	58347	
5µm	25 x 4.6	58354	
5µm	25 x 10.0	58357	
SUPELCOSIL LC-8-DB Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59619	
5µm	2 x 2.1 kit **	59618	
5µm	2 x 4.0 (2/pk)	59563	
5µm	2 x 4.0 kit **	59553	

HELPFUL HINTS

Relative Strengths for Different Solvents

The graph provides for the interconversion of reversed-phase mobile phases having the same strength. Vertical lines in this figure intersect mobile phases having the same strength. For example, 40% Acetonitrile has the same strength as 50% Methanol or 30% THF.



** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

HPLC: Small Molecules

Special Purpose SUPELCOSIL Columns

Special Purpose Columns: Carbohydrates

Within the different classes of sugars, chemical and physical properties vary only slightly. HPLC separations of carbohydrates depend on differences in conformation, configuration, and column type. Because of this complexity, no single HPLC column or method is capable of separating every carbohydrate. To choose the best column for your carbohydrate analysis, consult Table 1 (below), the Retention Time Index (next page), and the Applications section of this catalog. For more information, request Bulletin 887H (HPLC Carbohydrate Column Selection Guide).

SUPELCOGEL K columns separate raffinose, sucrose, glucose, fructose, and betaine, a trimethylammonium zwitterionic compound found in beet and cane sugars and widely distributed in other plants.

The lead-form resin in SUPELCOGEL Pb columns provides the highest resolution and best selectivity for monosaccharides. SUPELCOGEL Pb columns provide excellent separation of xylose, galactose, and mannose, which are not completely resolved on calcium-form resin columns.

SUPELCOGEL Ca columns separate monosaccharides and sugar alcohols. Di-, tri-, and oligosaccharides are separated by class. A frequent application for this column is the separation of sugars in high fructose corn syrup (HFCS).

SUPELCOGEL C-610H and H columns are ideal for separating carbohydrates, alcohols, and organic acids present in the same sample: wines and other fermentation products, fruit juices, biological samples, etc.

SUPELCOGEL C-611 columns contain a unique ion exchange resin containing two divalent cations, rather than one. This provides different selectivities for separating monosaccharides and sugar alcohols. As with resins containing a single cation, di-,tri-, and oligosaccharides are separated by class. Galactose and mannose are well separated.

SUPELCOGEL Ag columns provide rapid separations of oligosaccharides. Glycerol and ethanol are well resolved.

Silica-based SUPELCOSIL LC-NH_2 columns separate monosaccharides, disaccharides, and some trisaccharides, with elution generally in order of increasing molecular weight. Retention decreases as the proportion of water:acetonitrile in the mobile phase is increased. For more information, request T397126 (Sugars on SUPELCOSIL LC-NH_2 Columns).

SUPELCOGEL CARBOHYDRATE COLUMN CHARACTERISTICS	
Particles:	sulfonated polystyrene/divinylbenzene, spherical, 9µm
Counter Ion:	varies (see Table 1)
pH Range:	1-13
Organic Compatibility:	<10% in mobile phase
Maximum Temperature:	varies (see Table 1)
Maximum Flow Rate:	7.8mm ID columns: 1.5mL/min 4.6mm ID columns: 0.4mL/min
Maximum Pressure:	1000psi (70 bar)

SUPELCOSIL LC-NH_2 COLUMN CHARACTERISTICS	
Particles:	spherical silica, 5µm
Bonded Phase:	aminopropylsilyl
pH Range:	2-7.5
Organic Compatibility:	no limits (avoid aldehydes and ketones)
Maximum Flow Rate:	2mL/min (4.6mm ID columns)
Maximum Pressure:	6000psi (420 bar)

Table 1. Carbohydrate Column Applications and Mobile Phases

COLUMN	APPLICATION[1]	FORM	TYPICAL MOBILE PHASE	MAX. TEMP. (°C)
SUPELCOGEL K	beet sugar, cane sugar, molasses, corn syrup	potassium	10mM K_2HPO_4	90
SUPELCOGEL Pb	monosaccharides, xylose/galactose/mannose	lead	deionized water	90
SUPELCOGEL Ca	high fructose corn syrup, monosaccharides, sugar alcohols, oligosaccharides	calcium	deionized water	90
SUPELCOGEL C-610H	organic acids	hydrogen	0.1% H_2SO_4 or H_3PO_4	60
SUPELCOGEL H	organic acids	hydrogen	0.1% H_2SO_4 or H_3PO_4	90
SUPELCOGEL C-611	mono-, di-, and trisaccharides, galactose/mannose	2 divalent cations	10^{-4}N NaOH	85
SUPELCOGEL Ag1	beer, dark corn syrup	silver	deionized water	90
SUPELCOGEL Ag2	oligosaccharides, glycerol/ethanol, corn syrup, hydrolyzed starch	silver	deionized water	90
SUPELCOSIL LC-NH_2	mono-, di-, some trisaccharides	aminopropyl silica	75% CH_3CN in water	70

[1] See Applications pages.

Table 2. Retention Time Index for Carbohydrate Columns

	SUPELCOGEL COLUMNS								SUPELCOSIL
	Ca	C-610H	H	H	Pb	C-611	K	Ag2	LC-NH_2
Cat. No.:	59305-U	59320-U	59304-U	59346	59343	59310-U	59342	59315	58338
Dimensions (mm):	300 x 7.8	300 x 7.8	300 x 7.8	250 x 4.6	300 x 7.8	300 x 7.8	300 x 7.8	300 x 7.8	250 x 4.6
Temp.:	80°C	30°C	30°C	30°C	85°C	60°C	85°C	85°C	ambient
Mobile Phase:	DH_2O	0.1% H_3PO_4	0.1% H_3PO_4	0.1% H_3PO_4	DH_2O	10^{-4}N NaOH	15mM K_2HPO_4	DH_2O	ACN:DH_2O (3:1)
Flow Rate (mL/min):	0.5	0.5	0.5	0.17	0.5	0.5	0.5	0.5	1.0
Det.:	refractive index								
Compound	**Retention Times (min)**								
Arabinose	15.3	13.9	14.3	13.8	19.2	19.6	16.8	17.1	7.5
Arabitol	19.8	14.1	14.9	14.3	32.3	22.8	13.5	16.0	7.2
Betaine	ND	ND	ND	ND	NR	ND	13.0	ND	ND
Dulcitol	22.3	13.4	14.2	13.7	43.4	25.7	12.9	15.9	9.0
Erythritol	17.7	15.0	15.6	14.8	24.5	20.2	14.0	16.1	5.9
Ethanol	19.4	25.6	ND	ND	ND	21.0	ND	18.4	NR
Fructose	14.9	13.1	13.3	12.9	20.8	20.7	15.2	16.0	8.3
Galactose	13.4	12.9	13.0	12.6	17.6	17.6	15.1	15.8	10.3
Glucose	12.0	12.1	11.9	11.7	14.9	15.8	14.0	14.6	9.8
Glycerol	18.7	16.8	17.6	16.6	23.8	20.9	15.2	17.1	NR
Inositol	14.9	12.6	12.7	12.4	24.5	20.1	15.7	17.4	ND
Isomaltose	9.6	10.3	ND	ND	ND	13.8	ND	11.6	19.4
Isomaltotriose	8.5	9.5	ND	ND	ND	12.6	ND	9.8	NR
Lactitol	ND	ND	11.1	11.0	26.5	ND	10.6	ND	ND
Lactose	10.2	10.8	10.2	10.2	13.5	14.3	10.9	11.8	19.5
Maltitol	13.6	11.0	10.7	10.7	23.8	17.7	10.2	15.0	15.5
Maltoheptaose	7.5	8.8	7.6	7.9	9.2	11.6	7.2	7.3	NR
Maltohexaose	7.7	8.9	7.7	8.1	9.7	12.0	7.4	7.6	NR
Maltopentaose	7.9	9.1	7.9	8.2	10.5	12.6	7.8	8.1	NR
Maltose	9.8	10.5	9.9	9.9	13.0	14.2	10.7	11.5	17.4
Maltotetraose	8.3	9.3	8.2	8.5	11.2	13.2	8.4	8.8	NR
Maltotriose	8.8	9.7	8.8	9.0	12.0	13.6	9.2	9.8	31.0
Mannitol	19.2	13.2	13.7	13.2	32.5	22.1	12.6	15.2	9.2
Mannose	13.7	12.8	12.9	12.5	19.8	18.9	15.6	15.9	9.1
Melezitose	8.7	9.7	8.8	9.0	10.8	12.4	8.6	9.3	24.5
Psicose	22.5	13.4	14.5	13.9	36.5	32.9	15.5	17.2	6.6
Raffinose	8.7	9.7	8.7	8.9	11.2	12.6	8.7	9.6	29.7
Ribitol	16.7	13.7	14.2	13.6	25.1	19.5	13.1	15.3	ND
Ribose	24.3	14.2	15.8	15.0	40.7	34.6	17.7	19.1	6.0
Sorbitol	23.4	13.4	14.4	13.9	46.9	28.3	13.3	16.3	9.0
Stachyose	8.1	9.3	8.1	8.4	10.4	11.9	7.9	8.5	67.3
Sucrose	9.8	10.6	9.9	9.9	12.2	13.6	10.1	11.2	14.0
Xylitol	23.3	14.4	15.7	15.0	42.1	28.0	14.2	17.1	7.3
Xylose	13.2	12.8	12.8	12.6	16.1	17.2	15.3	15.6	6.8

NR - not recommended

ND - no data available

For optimal separations, allow at least 1 minute between compounds.

For resins used in processing sugars and foods, refer to the low pressure LC media section.

SUPELCOGEL and SUPELCOSIL Carbohydrate Columns and Guard Columns

COLUMN	LENGTH (cm)	ID (mm)	CAT. NO.	PRICE	SUPELGUARD GUARD COLUMN	CAT. NO.	PRICE
SUPELCOGEL K	30	7.8	59342		K*	59344	
SUPELCOGEL Pb	30	7.8	59343		Pb*	59345	
SUPELCOGEL Ca	30	7.8	59305-U		Ca*	59306-U	
SUPELCOGEL C-610H	30	7.8	59320-U		H*	59319	
SUPELCOGEL H	30	7.8	59304-U		H*	59319	
SUPELCOGEL H	25	4.6	59346		H*	59319	
SUPELCOGEL C-611	30	7.8	59310-U		Ca*	59306-U	
SUPELCOGEL Ag1	30	7.8	59318-U		Ag1*	59317-U	
SUPELCOGEL Ag2	30	7.8	59315		Ag2*	59316	
SUPELCOSIL LC-NH_2	25	4.6	58338		LC-NH_2 (kit)**	59558	
					LC-NH_2 (2 cartridges)	59568	

* 5cm x 4.6mm guard column, does not include tubing, nuts or ferrules.

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

HPLC: Small Molecules

Special Purpose SUPELCOSIL Columns

COLUMN CHARACTERISTICS	
Particles:	5µm spherical silica
Bonded Phase:	aminopropylsilyl
Pore Size:	120Å
Surface Area:	170m²/g
Pore Volume:	0.6mL/g
pH Range:	2 - 7.5

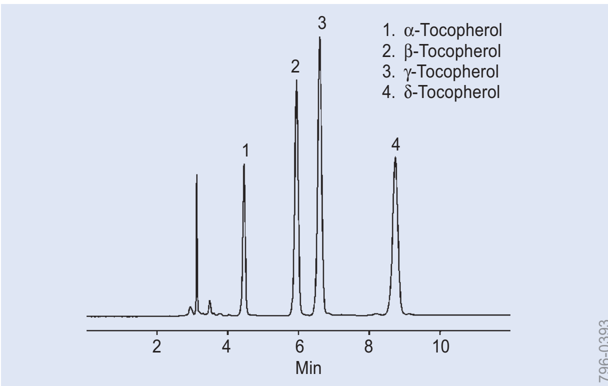


Figure A. Tocopherols

Column: SUPELCOSIL LC-NH_2-NP, 25cm x 4.6mm ID, 5µm particles
Cat. No.: 59132
Mobile Phase: hexane:ethyl acetate, 70:30
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV, 295nm
Inj.: 10µL hexane, 1.0mg/mL each analyte

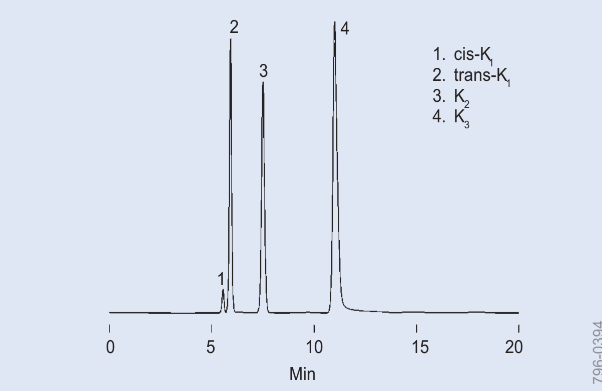


Figure B. Vitamin K Isomers

Column: SUPELCOSIL LC-NH_2-NP, 25cm x 4.6mm ID, 5µm particles
Cat. No.: 59132
Mobile Phase: hexane:ethyl acetate, 99:1
Flow Rate: 1.5mL/min
Temp.: 30°C
Det.: UV, 254nm
Inj.: 10µL hexane, 0.3mg/mL each analyte

Special Purpose Columns: Dedicated Normal Phase

SUPELCOSIL LC-NH_2-NP is an amino phase dedicated to normal phase chromatography. By employing special bonding technology, and avoiding water in manufacturing and testing the column, we have dramatically reduced the retention variation that is characteristic of normal phase chromatography.

SUPELCOSIL LC-NH_2-NP columns:

- show stable retention in normal phase separations
- are less sensitive to small or varying amounts of water in the mobile phase, relative to unmodified silica
- provide excellent separations of fat-soluble vitamins

Normal phase chromatography is especially useful when the analytes are not water soluble – for example, the fat-soluble vitamins A, D, E, and K. Figure A shows a separation of tocopherols (vitamin E) on a SUPELCOSIL LC-NH_2-NP column. The various isomers of vitamin K are separated in Figure B.

These columns should be used with non-aqueous mobile phases only.

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-NH_2-NP (120Å, 3.0% CARBON)			
5µm	25 x 4.6	59132	
SUPELCOSIL LC-NH_2-NP Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59516	
5µm	2 x 4.0 kit **	59515	

HELPFUL HINTS

Reversed-phase versus Normal Phase: Reversed-phase is characterized by strong interactions between analytes and the polar mobile phase. Interactions between analytes and the nonpolar stationary phase are weak. Mobile phases typically consist of water / organic solvent combinations. Reversed-phase columns include: Amide-C16, C18, C8, Phenyl, C5, Pentafluorinated Phenyl (F5), Cyano, C1, ODP-50, and TPR-100.

Normal phase is characterized by strong interactions between analytes and the polar stationary phase. Interactions between analytes and the nonpolar mobile phase are weak. Mobile phases consist of organic solvents. Normal phase columns include: Cyano, NH_2, and Silica.

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

Special Purpose Columns: Direct Serum Injection

The Hisep column uses a shielded hydrophobic phase (SHP) for its mechanism of separation. The silica-based material is covere with a thin polymer consisting of hydrophobic regions in a hydrophilic network. Small analytes, such as drugs, penetrate th hydrophilic network and are retained by the hydrophobic moieties. The hydrophilic network shields protein molecules fron contact with the surface and the hydrophobic groups, and thus these molecules are not retained. Direct injection of biological samples eliminates time-consuming cleanup steps and increase analytical accuracy.

Excellent column stability and an ability to exclude proteins ove a wide pH range set Hisep columns apart from other direct seru injection columns and techniques. Stability is demonstrated in Figure A – no significant change is seen in the chromatography trimethoprim after injecting 10mL of serum, in 10µL increments, onto a Hisep column. A low pH application is shown in Figure B. The profile of the excluded serum peak will change with pH due to differing protonation states of the serum proteins.

PROPERTIES	
Particles:	spherical silica, 5µm
Pore Size:	120Å
Surface Area:	170m²/g
Pore Volume:	0.6mL/g
pH range:	2-7.5

SUPELCOSIL Hisep

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL HISEP (120Å)			
5µm	25 x 2.1	57932	
5µm	5 x 4.6	59143	
5µm	15 x 4.6	58935	
5µm	25 x 4.6	58919	
SUPELCOSIL Hisep Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59640-U	
5µm	2 x 4.0 kit **	59639	

RELATED INFORMATION

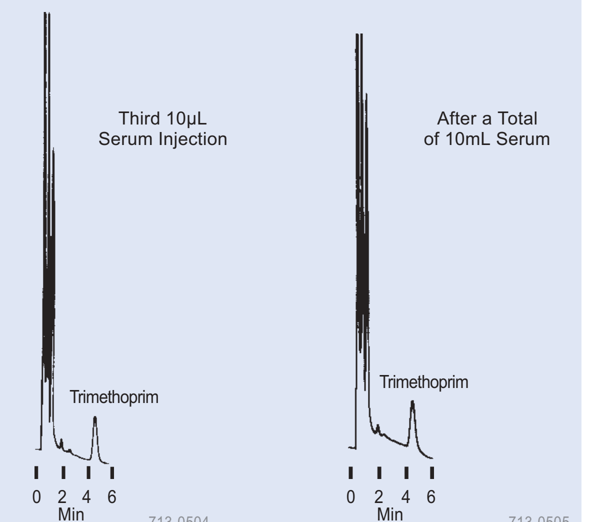
Request free literature by phone or fax, or see our website.

No.	Subject
T397145	Hisep columns for drugs

Literature References

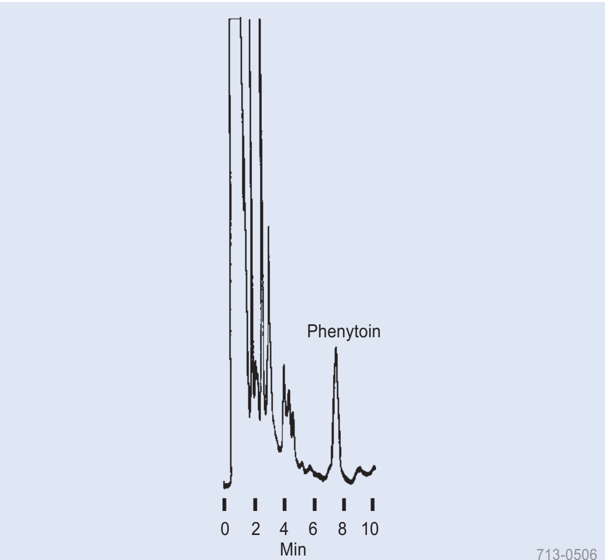
Feibush, B., C. Santasania, J. Chromatogr. 54: 441-449 (1991).
Gisch, D.J., B.T. Hunter, B. Feibush, J. Chromatogr. 433: 264-268 (1988).
Wong, S.H.Y., L.A. Bretts, A.C. Larson, J. Liq. Chromatogr. 11: 2039-2049 (1988).
References not available from Supelco.

Figure A. Hisep Columns Perform Consistently for Many Injections



Column: Hisep, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 58935
Mobile Phase: acetonitrile:180mM ammonium acetate (15:85), pH 7
Flow Rate: 2mL/min
Det.: UV, 254nm
Inj.: 10µL spiked serum (25µg/mL trimethoprim)

Figure B. Hisep Columns Are Compatible with Low pH Mobile Phases



Column: Hisep, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 58935
Mobile Phase: acetonitrile:180mM ammonium acetate (pH 4.6)
Flow Rate: 2mL/min
Det.: UV, 254nm
Inj.: 25µL phenytoin-spiked serum

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

HPLC: Small Molecules
Special Purpose SUPELCOSIL Columns

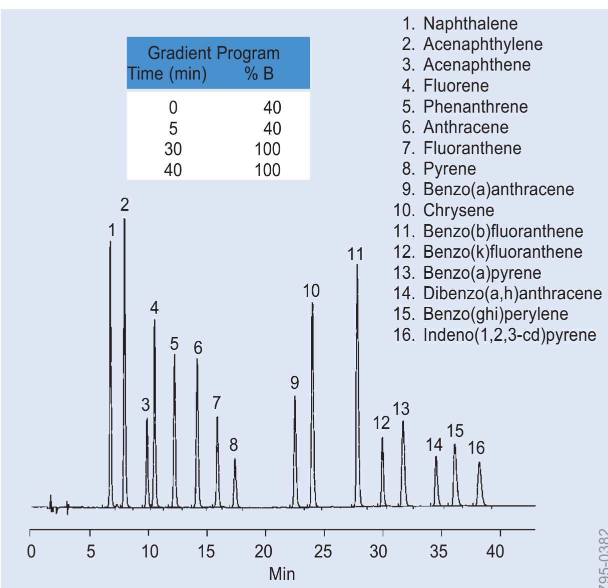


Figure A. Optimum PAH Resolution: 5µm Particles

Column: SUPELCOSIL LC-PAH, 25cm x 4.6mm ID, 5µm particles
Cat. No.: 58229
Mobile Phase: A = water, B = acetonitrile
Flow Rate: 1.5mL/min
Det.: UV, 254nm
Inj.: 3µL LC-PAH Test Mix (Cat. No. 48743), diluted 1:10 with acetonitrile

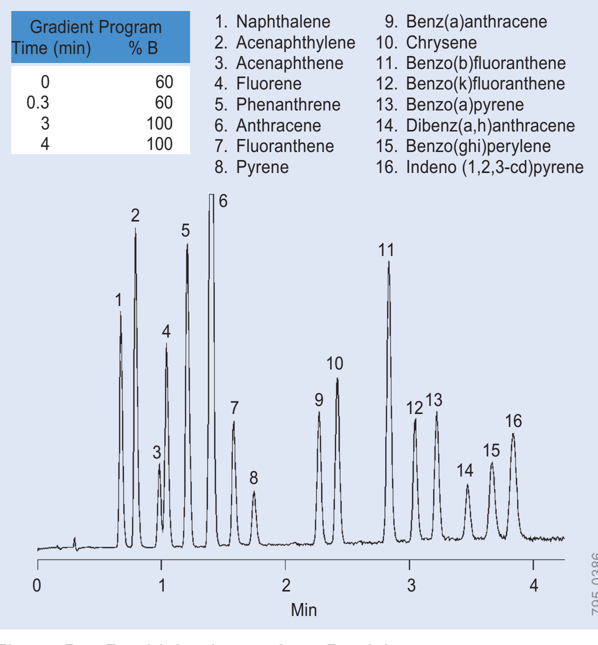


Figure B. Rapid Analyses: 3µm Particles

Column: SUPELCOSIL LC-PAH, 5cm x 4.6mm ID, 3µm particles
Cat. No.: 59133
Mobile Phase: A = water, B = acetonitrile
Flow Rate: 3.0mL/min
Det.: UV, 254nm

Special Purpose Columns: Polyaromatic Hydrocarbons

SUPELCOSIL LC-PAH columns were designed specifically for analyses of the priority pollutant PAHs listed in US EPA Method 610 (Figure A). 2.1mm and 3.0mm columns save solvent and improve sensitivity when sample mass is limited. 3µm columns provide extremely rapid, highly efficient analyses (Figure B), while retaining the durability of porous silicas. They are excellent and economical substitutes for 1.5µm nonporous silicas.

COLUMN CHARACTERISTICS

Particles: spherical silica, 3µm, 5µm
Pore Size: 120Å
Bonded Phase: octadecylsilane
Surface Area: 170m²/g
Pore Volume: 0.6mL/g
pH Range: 2 - 7.5

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-PAH (120Å)			
3µm	5 x 3.0	59133C30	
3µm	10 x 3.0	59134C30	
3µm	5 x 4.6	59133	
3µm	10 x 4.6	59134	
5µm	25 x 2.1	57945	
5µm	15 x 3.0	58318C30	
5µm	15 x 4.6	58318	
5µm	25 x 4.6	58229	
SUPELCOSIL LC-18 Supelguard Cartridges (use for LC-PAH)			
5µm	2 x 2.1 (2/pk)	59613	
5µm	2 x 2.1 kit **	59612	
5µm	2 x 3.0 (2/pk)	59564C30	
5µm	2 x 4.0 (2/pk)	59564	
5µm	2 x 4.0 kit **	59554	

HELPFUL HINTS

Reversed-phase versus Normal Phase: Reversed-phase is characterized by strong interactions between analytes and the polar mobile phase. Interactions between analytes and the nonpolar stationary phase are weak. Mobile phases typically consist of water / organic solvent combinations. Reversed-phase columns include: Amide-C16, C18, C8, Phenyl, C5, Pentafluorinated Phenyl (F5), Cyano, C1, ODP-50, and TPR-100.

Normal phase is characterized by strong interactions between analytes and the polar stationary phase. Interactions between analytes and the nonpolar mobile phase are weak. Mobile phases consist of organic solvents. Normal phase columns include: Cyano, NH_2, and Silica.

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

Special Purpose Columns: Nucleosides

SUPELCOSIL LC-18-S columnsare designed for reliable separations of deoxyribonucleosides and ribonucleosides. Each column is tested to ensure performance.

PROPERTIES	
Silica:	Spherical
Particle Size:	5µm
Pore Size:	120Å
Bonded Phase:	octadecylsilane
Surface Area:	170m²/g
Pore Volume:	0.6mL/g
pH Range:	2-7.5

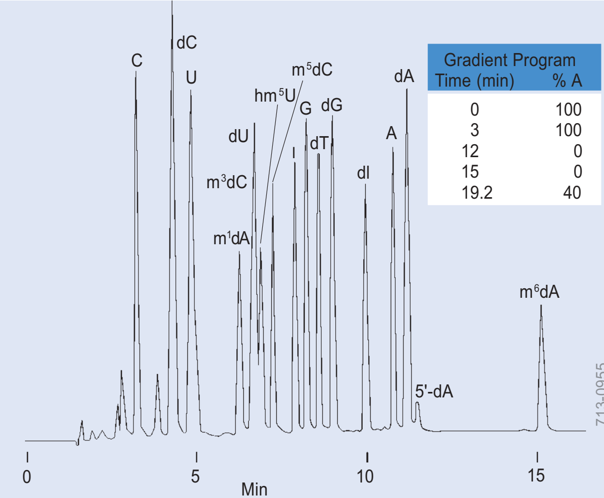


Figure provided by Dr. C. W. Gehrke and K.C. Kuo, University of Missouri-Columbia, Experimental Station Chemical Laboratories, Columbia, MO USA

Deoxyribonucleosides and Ribonucleosides on a SUPELCOSIL LC-18-S Column

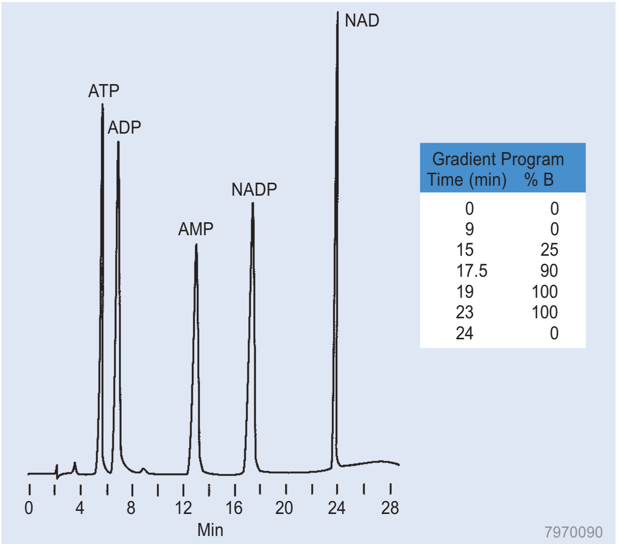
Column: SUPELCOSIL LC-18-S, 15cm x 4.6mm ID, 5µm particles
Cat. No.: 58931
Mobile Phase: 50mM K_2HPO_4/KH_2PO_4 (pH 4.0): methanol
A = 97.5:2.5, B = 80:20
Flow Rate: 1.0mL/min
Temp.: 30°C
Det.: UV, 254nm
Inj.: nucleoside standards in water

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-18-S (120A, 11.0% CARBON)			
5µm	30 x 1.0	57920	
5µm	25 x 2.1	57939	
5µm	15 x 3.0	58931C30	
5µm	15 x 4.6	58931	
5µm	25 x 4.6	58928-U	
SUPELCOSIL LC-18-S Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59630	
5µm	2 x 4.0 kit **	59629	

Special Purpose Columns: Nucleotides

SUPELCOSIL LC-18-T columns feature an octadecylsilane bonded phase and a special surface treatment, for efficient separations of nucleotides. Each batch of packing material is tested to ensure good peak shape for a representative nucleotide, adenosine diphosphate (ADP). Chromatography of other compounds that exhibit metal chelating properties also can be improved by using this phase.

PROPERTIES	
Silica:	Spherical
Particle Size:	3µm, 5µm
Pore Size:	120Å
Bonded Phase:	octadecylsilane
Surface Area:	170m²/g
Pore Volume:	0.6mL/g
pH Range:	2-7.5



Nucleotides on a SUPELCOSIL LC-18-T Column

Column: SUPELCOSIL LC-18-T, 25cm x 4.6mm ID (5µm particles)
Cat. No. 58971
Mobile Phase: A = 0.1M KH_2PO_4, pH 6
B = A:methanol, 90:10
gradient program shown on figure
Flow Rate: 1.3mL/min
Det: UV, 254nm

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-18-T (120Å, 12.3% CARBON)			
3µm	15 x 3.0	58970C30	
3µm	15 x 4.6	58970-U	
5µm	25 x 3.0	58971C30	
5µm	25 x 4.6	58971	
SUPELCOSIL LC-18-T Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59621C30	
5µm	2 x 4.0 (2/pk)	59621	
5µm	2 x 4.0 kit **	59620	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

Liquid Chromatography

HPLC: Small Molecules

Special Purpose SUPELCOGEL Columns

Special Purpose Columns: Organic Acids

SUPELCOGEL C-610H columns are prepared specifically for analyses of organic acids. Acetic, propionic, butyric, formic, malic, citric, succinic, lactic, and other acids are easily separate on these columns, using a simple isocratic mobile phase and minimal sample preparation. Retention times for many acids are shown in the table.

Separation is based on ion exclusion – the analytes selectively partition between the resin phase and the external aqueous pha. Analyses are best performed at low pH (0.1% H_3PO_4 often is used as the mobile phase); the same mobile phase conditions can be applied to a wide variety of sample matrices.

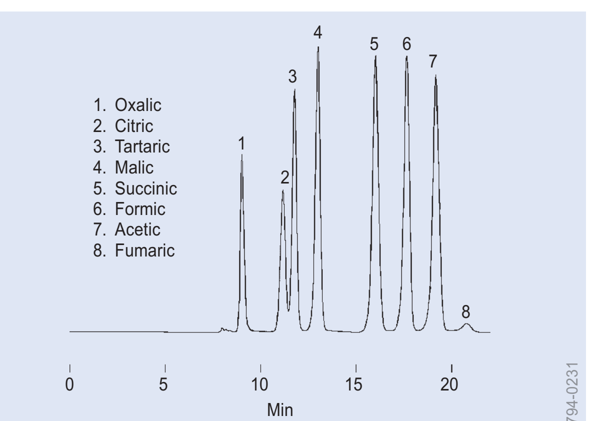
COLUMN CHARACTERISTICS	
Particles:	sulfonated polystyrene/divinylbenzene, spherical, 9µm
Counter Ion:	H^+
pH Range:	1-13
Organic Compatibility:	<20% in mobile phase
Maximum Flow Rate:	1.5mL/min
Maximum Pressure:	1000psi (70 bar)

SUPELCOGEL H columns have the same particle composition, retention mechanism, performance, sensitivity, and applications as SUPELCOGEL C-610H columns. However, particle improvements have made it possible to pack the SUPELCOGEL H packing material efficiently into conventional 4.6mm ID columns, to improve detection and reduce solvent consumption relative to 7.8mm ID columns.

COLUMN CHARACTERISTICS	
Particles:	sulfonated polystyrene/divinylbenzene, spherical, 9µm
Counter Ion:	H^+
pH Range:	1-13
Organic Compatibility:	<10% in mobile phase
Maximum Flow Rate:	1.5mL/min (7.8mm), 0.4mL/min (4.6mm)
Maximum Pressure:	1000psi (70 bar)

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOGEL C-610H			
9µm	30 x 7.8	59320-U	
SUPELCOGEL H Guard Column (use to protect C-610H)			
9µm	5 x 4.6 *	59319	
SUPELCOGEL H			
9µm	25 x 4.6	59346	
9µm	30 x 7.8	59304-U	
SUPELCOGEL H Guard Column			
9µm	5 x 4.6 *	59319	

* 5cm x 4.6mm guard column does not include tubing, nuts or ferrules.



Organic Acids

Column: SUPELCOGEL C-610H, 30cm x 7.8mm ID
Cat. No.: 59320
Mobile Phase: 0.1% H_3PO_4
Flow Rate: 0.5mL/min
Temp.: 30°C
Det.: UV, 210nm
Inj.: 10µL

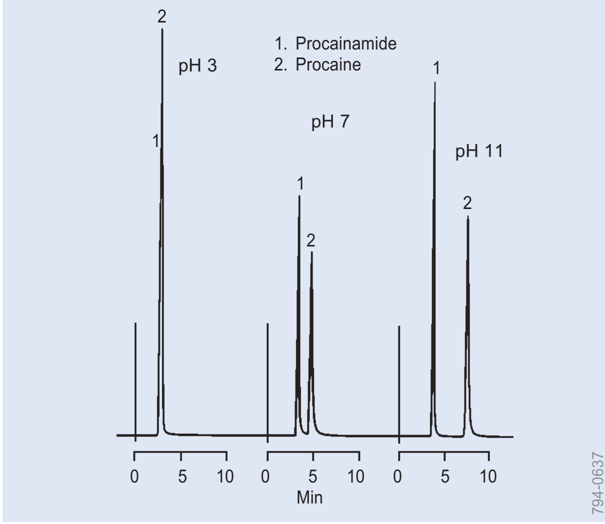
Typical Retention Times for Organic Acids on SUPELCOGEL C-610H and H Columns

COLUMN:	C-610H	H	H
LENGTH:	30cm	30cm	25cm
ID:	7.8mm	7.8mm	4.6mm
CAT. NO.:	59320	59304	59346
Acid	Typical Retention Time (min)		
Acetic	19.0	19.6	17.6
Adipic	22.5	24.0	21.3
Ascorbic	13.1	13.3	12.1
Benzoic[1]	42.4	44.3	37.9
Butyric	28.0	28.3	24.9
Citric	11.0	10.9	10.1
Formic	17.5	18.1	16.3
Fumaric	19.8	20.9	18.2
Gluconic	12.0	12.0	11.1
Isobutyric	25.6	25.9	22.9
Isocitric	11.2	11.0	10.2
Lactic	16.0	16.9	15.2
Maleic	10.4	10.1	9.0
Malic	12.9	13.2	12.0
Malonic	13.4	13.7	12.5
Oxalic	9.0	7.9	7.3
Phytic	8.3	7.0	6.8
Propionic	22.5	23.1	20.5
Quinic	13.3	14.0	12.8
Shikimic	15.5	16.5	14.9
Succinic	15.7	16.4	14.9
Tartaric	11.7	11.7	10.7

Mobile Phase: 0.1% H_3PO_4, 0.5mL/min (0.17mL/min for 25cm x 4.6mm column), Temperature: 30°C, Detection: UV, 210nm

[1] As sodium benzoate.

COLUMN CHARACTERISTICS	
Packing Composition :	polyvinylalcohol polymer, spherical
Functional Groups:	C18
Particle Size:	5µm
Pore Size:	250Å
Surface Area:	$150m^2/g$
pH:	2-13



Separation of Basic Drugs Is Improved at High pH

Column: SUPELCOGEL ODP-50, 15cm x 4.0mm ID, 5µm particles
Cat. No.: 59307
Mobile Phase: acetonitrile:25mM phosphate, 40:60
Flow Rate: 0.45mL/min (850psi/5.8MPa)
Temp.: 30°C
Det.: UV, 254nm

Special Purpose Columns: Resin Based

SUPELCOGEL ODP-50: the selectivity of a C18 column, with a wider pH range

Because silica-based bonded phase packings are not stable above pH 7, many basic compounds are analyzed on these columns as positively charged compounds. The charged compounds often interact with residual silanol groups on the packing surface, giving low efficiency and tailing peaks. Resin-based reversed-phase columns are stable over almost the entire pH range, but traditionally have shown low efficiency for most analytes, compared to silica-based columns.

Resin-based SUPELCOGEL ODP-50 columns behave like silica C18 reversed phase columns, but enable you to operate at basic pH (recommended pH range: 2-13). Octadecyl functional groups are covalently attached to the hydroxyl groups of spherical 5µm polyvinylalcohol polymer particles, giving a high density C18 coverage (17% carbon). Separation characteristics are similar to conventional C18 reversed-phase columns – even at high pH. The improved processes used to manufacture SUPELCOGEL ODP-50 particles ensure column efficiencies and mechanical stability that rival those of silica-based packings.

The figure shows the benefits of high pH separations in analyses of basic drugs – at higher pH, the compounds lose their charge and interact more strongly with the packing, prolonging their retention times. Selectivity for most compound pairs is significantly improved, and some pairs reverse their elution order.

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOGEL ODP-50 (250Å, 17.0% CARBON)			
5µm	15 x 4.0	59307-U	
SUPELCOGEL ODP-50 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59313C40	
5µm	2 x 4.0 kit**	59312-U	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

HPLC: Small Molecules

Special Purpose SUPELCOGEL Columns

Special Purpose Columns: Resin Based

SUPELCOGEL TPR-100: a high efficiency resin-based column.

- Unique combination of hydrophilic and hydrophobic (aromatic and aliphatic) monomers
- Crosslinked for mechanical stability
- Narrow pore size and particle size distributions for high efficiency and good peak symmetry
- No micropores
- No bleed from residual porogens
- Use with 100% aqueous or 100% organic mobile phases without swelling or shrinking

Relative to silica, supports composed of organic polymers or res offer superior pH stability and chemical inertness. Choose a res based column when resolution is improved by using a pH above pH 7 or below pH 2, or when silanol or metal ion interactions cause peak tailing or irreproducible retention on a silica-based column.

SUPELCOGEL TPR-100 poly(divinylbenzene/methacrylate) resin has unique selectivity – less hydrophobic than a pure divinylbenzene resin, but less hydrophilic than a methacrylate resin. The resin also offers excellent efficiency, peak symmetry, and mechanical stability. Repeated gradients do not change the bed structure, and the columns can be used with 100% aqueous 100% organic mobile phases without shrinking or swelling. We recommend using these columns at pressures below 3000 psi.

The figure demonstrates the inertness of SUPELCOGEL TPR-100 resin. Although N,N-dimethylaniline tails severely on the silica-based C18 column, its peak shape on the SUPELCOGEL column is very good, without a competing base modifier in the mobile phase.

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOGEL TPR-100 (100Å)			
5µm	15 x 3.0	59154C30	
5µm	15 x 4.0	59154C40	
5µm	15 x 4.6	59154	
SUPELCOGEL TPR-100 Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59571C30	
5µm	2 x 4.0 (2/pk)	59571	
5µm	2 x 4.0 kit **	59570-U	

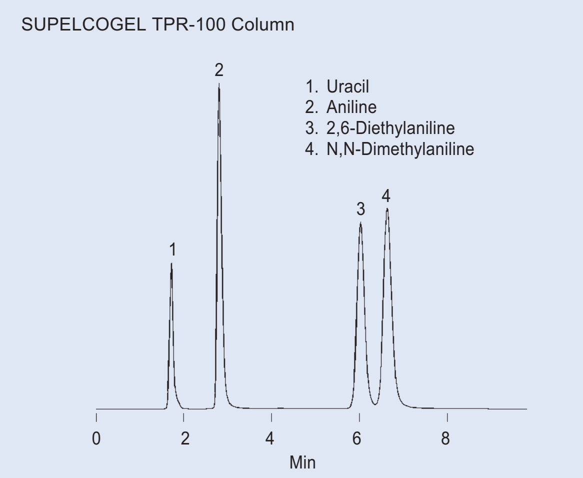
HELPFUL HINTS

Do not use SUPELCOGEL TPR-100 columns with methanol or methanol-containing mixtures.

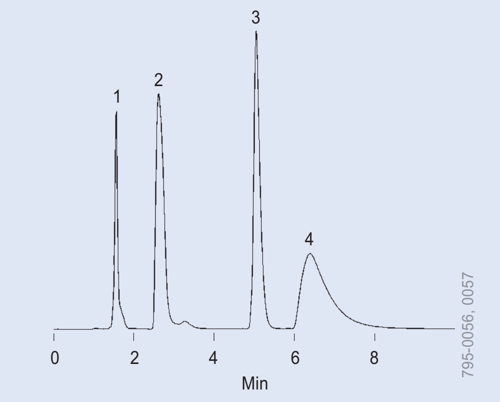
COLUMN CHARACTERISTICS

Particles: poly(divinylbenzene/methacrylate), spherical, 5µm
Pore Size: 100Å
Surface Area: 340m^2/g
pH: 2-13

SUPELCOGEL TPR-100 Column



Conventionally Deactivated C18 Column



Better Chromatography of Anilines than on a Deactivated Silica-Based Column

Columns: 15cm x 4.6mm ID, 5µm particles
Cat. No.: 59154 (SUPELCOGEL TPR-100 Column)
Mobile Phase: acetonitrile:water, 60:40
Flow Rate: 1mL/min
Temp.: ambient
Det.: UV, 254nm
Inj.: 10µL

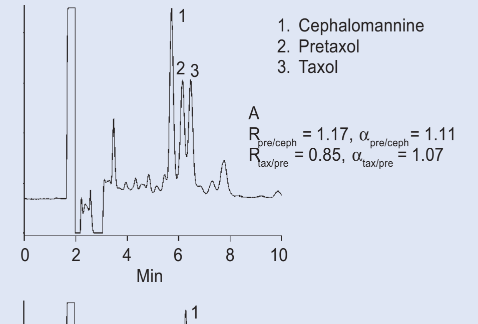
** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

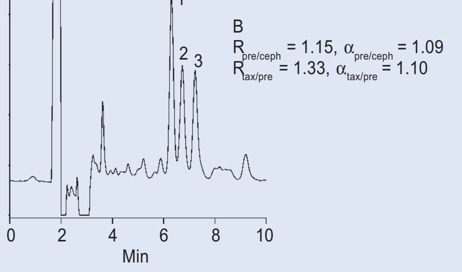
Special Purpose Columns: Taxols/Taxanes

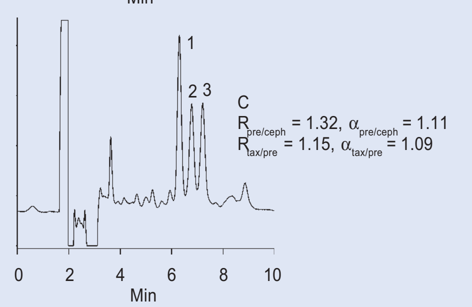
SUPELCOSIL LC-F columns contain a pentafluorophenyl functional group/endcapped packing material. These columns offer selectivities different from traditional reversed phase columns for:

- Halogenated compounds, esters, ketones, and
- Taxanes, including taxol

Taxol, a chemically and pharmacologically unique taxane diterpene amide found in the bark and needles of the Pacific ye tree, has been approved by the US Food and Drug Administrati for treatment of ovarian cancer. In isocratic analyses of taxol, th crude and complex sample matrix shortens the lifetimes of traditional reversed phase columns. Analysis of a crude taxol mixture on a SUPELCOSIL LC-F is shown. Excellent resolution of the compounds of interest is obtained by using a mobile phas of acetonitrile, tetrahydrofuran, and water. Small adjustments in the percentage of tetrahydrofuran compensate for normal variation among samples.







Sample provided by S. Richheimer, Hauser Chemical Research Company.

794-0079, 0080, 0081

Tetrahydrofuran Concentration Affects Taxol Analysis

Column: SUPELCOSIL LC-F, 25cm x 4.6mm ID, 5µm particles
Cat. No.: 59158
Mobile Phase: acetonitrile:tetrahydrofuran:water,
A: 15:30:55, B: 20:25:55, C: 17:28:55
Flow Rate: 1.5mL/min
Det.: UV, 227nm
Inj.: 10µL crude taxol mix

COLUMN CHARACTERISTICS

Particles:	spherical silica, 5µm
Pore Size:	120Å
Bonded Phase:	pentafluorophenyl
Surface Area:	170m²/g
Pore Volume:	0.6mL/g
pH Range:	2 - 7.5

PARTICLE SIZE	LENGTH (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-F (120Å, 5.0% CARBON)			
5µm	25 x 4.0	59158C40	
5µm	25 x 4.6	59158	
SUPELCOSIL LC-F Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59521	
5µm	2 x 4.0 kit **	59520	

HELPFUL HINT: PROPERTIES OF ORGANIC SOLVENTS COMMONLY USED IN HPLC

SOLVENT	POLARITY	MISCIBLE WITH WATER?	UV CUTOFF	REFRACTIVE INDEX AT 20°C	SOLVENT STRENGTH, ε_o (SILICA)	VISCOSITY AT 20°C, CP
Hexane	nonpolar	no	200	1.3750	0.00	0.33
Isooctane		no	200	1.3910	0.01	0.50
Carbon tetrachloride		no	263	1.4595	0.14	0.97
Chloroform		no	245	1.4460	0.31	0.57
Methylene chloride		no	235	1.4240	0.32	0.44
Tetrahydrofuran		yes	215	1.4070	0.35	0.55
Diethyl ether		no	215	1.3530	0.29	0.23
Acetone		yes	330	1.3590	0.43	0.32
Ethyl acetate		poorly	260	1.3720	0.45	0.45
Dioxane		yes	215	1.4220	0.49	1.54
Acetonitrile		yes	190	1.3440	0.50	0.37
2-Propanol		yes	210	1.3770	0.63	2.30
Methanol		yes	205	1.3290	0.73	0.60
Water	polar	yes	–	1.3328	>0.73	1.00

[1] Typical values.

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

HPLC: Small Molecules
SUPELCOSIL Columns

SUPELCOSIL HPLC Columns

Our SUPELCOSIL silica-based HPLC column line includes nearly 30 bonded phase chemistries in a range of particle sizes, and colu mn configurations from microbore to preparative scale. When developing a new method, see Supelco's new Discovery suite of reversed phase HPLC columns in this catalog.

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL ABZ+PLUS (120Å, 12.0% CARBON)			
3µm	3.3 x 2.1	5919121	
3µm	5 x 2.1	5919221	
3µm	10 x 2.1	57917	
3µm	3.3 x 3.0	59191C30	
3µm	7.5 x 3.0	59193C30	
3µm	15 x 3.0	59194C30	
3µm	5 x 4.0	59192C40	
3µm	3.3 x 4.6	59191	
3µm	5 x 4.6	59192-U	
3µm	7.5 x 4.6	59193	
3µm	15 x 4.6	59194	
5µm	30 x 1.0	57978	
5µm	5 x 2.1	5919521	
5µm	10 x 2.1	57925	
5µm	15 x 2.1	57926	
5µm	25 x 2.1	57927	
5µm	5 x 3.0	59195C30	
5µm	15 x 3.0	59196C30	
5µm	25 x 3.0	59197C30	
5µm	5 x 4.0	59195C40	
5µm	15 x 4.0	59196C40	
5µm	25 x 4.0	59197C40	
5µm	5 x 4.6	59195-U	
5µm	15 x 4.6	59196	
5µm	25 x 4.6	59197	
5µm	25 x 10.0	59179	
5µm	10 x 21.2	59148	
5µm	25 x 21.2	54855	
12µm	25 x 4.6	59156	
12µm	25 x 21.2	59174	
SUPELCOSIL ABZ+Plus Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59605	
5µm	2 x 2.1 kit **	59604	
5µm	2 x 3.0 (2/pk)	59535C30	
5µm	2 x 4.0 (2/pk)	59535-U	
5µm	2 x 4.0 kit **	59534-U	
SUPELCOSIL LC-ABZ (120Å, 12.0% CARBON)			
5µm	30 x 1.0	57990-U	
5µm	25 x 2.1	57936	
5µm	5 x 3.0	59141C30	
5µm	15 x 3.0	59140C30	
5µm	25 x 3.0	59142C30	
5µm	25 x 4.0	59142C40	
5µm	5 x 4.6	59141	
5µm	15 x 4.6	59140-U	
5µm	25 x 4.6	59142	
5µm	25 x 10.0	59170	
SUPELCOSIL LC-ABZ Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59611	
5µm	2 x 2.1 kit **	59610	
5µm	2 x 3.0 (2/pk)	59545C30	
5µm	2 x 4.0 (2/pk)	59545-U	
5µm	2 x 4.0 kit **	59544-U	

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL SUPLEX pKB-100 (120Å, 12.5% CARBON)			
5µm	25 x 2.1	57937	
5µm	25 x 3.0	58934C30	
5µm	25 x 4.0	58934C40	
5µm	5 x 4.6	58921-U	
5µm	15 x 4.6	58932	
5µm	25 x 4.6	58934	
5µm	25 x 10.0	59172	
SUPELCOSIL Suplex pKb-100 Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59609	
5µm	2 x 2.1 kit **	59608	
5µm	2 x 4.0 (2/pk)	59541-U	
5µm	2 x 4.0 kit **	59531-U	
SUPELCOSIL LC-18 (120Å, 11.0% CARBON)			
3µm	25 x 2.1	57942	
3µm	3.3 x 3.0	58977C30	
3µm	5 x 3.0	58973C30	
3µm	15 x 3.0	58985C30	
3µm	7.5 x 4.0	58984C40	
3µm	15 x 4.0	58985C40	
3µm	3.3 x 4.6	58977	
3µm	5 x 4.6	58973	
3µm	7.5 x 4.6	58984	
3µm	15 x 4.6	58985	
5µm	30 x 1.0	57982	
5µm	15 x 2.1	57934	
5µm	25 x 2.1	57935	
5µm	10 x 3.0	59209C30	
5µm	15 x 3.0	58230C30	
5µm	25 x 3.0	58298C30	
5µm	5 x 4.0	58239C40	
5µm	10 x 4.0	59209C40	
5µm	15 x 4.0	58230C40	
5µm	25 x 4.0	58298C40	
5µm	30 x 4.0	59165	
5µm	5 x 4.6	58239	
5µm	10 x 4.6	59209	
5µm	15 x 4.6	58230-U	
5µm	25 x 4.6	58298	
5µm	25 x 10.0	58368	
5µm	25 x 21.2	54849	
12µm	25 x 4.6	59182	
12µm	25 x 21.2	59185	
SUPELCOSIL LC-18 Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59613	
5µm	2 x 2.1 kit **	59612	
5µm	2 x 3.0 (2/pk)	59564C30	
5µm	2 x 4.0 (2/pk)	59564	
5µm	2 x 4.0 kit **	59554	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-18-DB (120Å, 11.0% CARBON)			
3µm	25 x 2.1	57943	
3µm	3.3 x 3.0	58978C30	
3µm	7.5 x 3.0	58992C30	
3µm	15 x 3.0	58993C30	
3µm	15 x 4.0	58993C40	
3µm	3.3 x 4.6	58978	
3µm	7.5 x 4.6	58992	
3µm	15 x 4.6	58993	
5µm	30 x 1.0	57984	
5µm	25 x 2.1	57940	
5µm	5 x 3.0	58345C30	
5µm	10 x 3.0	59208C30	
5µm	15 x 3.0	58348C30	
5µm	25 x 3.0	58355C30	
5µm	15 x 4.0	58348C40	
5µm	25 x 4.0	58355C40	
5µm	30 x 4.0	59164	
5µm	5 x 4.6	58345	
5µm	10 x 4.6	59208	
5µm	15 x 4.6	58348	
5µm	25 x 4.6	58355-U	
5µm	25 x 10.0	58358	
SUPELCOSIL LC-18-DB Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59617	
5µm	2 x 2.1 kit **	59616	
5µm	2 x 3.0 (2/pk)	59565C30	
5µm	2 x 4.0 (2/pk)	59565	
5µm	2 x 4.0 kit **	59555	
SUPELCOSIL LC-18-S (120Å, 11.0% CARBON)			
5µm	30 x 1.0	57920	
5µm	25 x 2.1	57939	
5µm	15 x 3.0	58931C30	
5µm	15 x 4.6	58931	
5µm	25 x 4.6	58928-U	
SUPELCOSIL LC-18-S Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59630	
5µm	2 x 4.0 kit **	59629	
SUPELCOSIL LC-18-T (120Å, 12.3% CARBON)			
3µm	15 x 3.0	58970C30	
3µm	15 x 4.6	58970-U	
5µm	25 x 3.0	58971C30	
5µm	25 x 4.6	58971	
SUPELCOSIL LC-18-T Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59621C30	
5µm	2 x 4.0 (2/pk)	59621	
5µm	2 x 4.0 kit **	59620	
SUPELCOSIL LC-DABS (120Å, 12.3% CARBON)			
3µm	15 x 4.6	59137	
SUPELCOSIL LC-18-T Supelguard Cartridges (use for LC-DABS)			
5µm	2 x 4.0 (2/pk)	59621	
5µm	2 x 4.0 kit **	59620	

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-PAH (120Å)			
3µm	5 x 3.0	59133C30	
3µm	10 x 3.0	59134C30	
3µm	5 x 4.6	59133	
3µm	10 x 4.6	59134	
5µm	25 x 2.1	57945	
5µm	15 x 3.0	58318C30	
5µm	15 x 4.6	58318	
5µm	25 x 4.6	58229	
SUPELCOSIL LC-18 Supelguard Cartridges (use for LC-PAH)			
5µm	2 x 2.1 (2/pk)	59613	
5µm	2 x 2.1 kit **	59612	
5µm	2 x 3.0 (2/pk)	59564C30	
5µm	2 x 4.0 (2/pk)	59564	
5µm	2 x 4.0 kit **	59554	
SUPELCOSIL LC-318 (300Å, 6.0% CARBON)			
5µm	5 x 4.6	58852	
5µm	25 x 4.6	58858	
SUPELCOSIL LC-318 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59512	
5µm	2 x 4.0 kit **	59502	
SUPELCOSIL LC-8 (120Å, 6.0% CARBON)			
3µm	3.3 x 3.0	58975C30	
3µm	7.5 x 3.0	58982C30	
3µm	15 x 3.0	58983C30	
3µm	15 x 4.0	58983C40	
3µm	3.3 x 4.6	58975	
3µm	7.5 x 4.6	58982	
3µm	15 x 4.6	58983	
5µm	30 x 1.0	57986	
5µm	25 x 2.1	57929	
5µm	15 x 3.0	58220C30	
5µm	25 x 3.0	58297C30	
5µm	15 x 4.0	58220C40	
5µm	25 x 4.0	58297C40	
5µm	5 x 4.6	58238	
5µm	15 x 4.6	58220-U	
5µm	25 x 4.6	58297	
5µm	25 x 10.0	58367	
5µm	25 x 21.2	54845	
12µm	25 x 4.6	59181	
12µm	25 x 21.2	59184	
SUPELCOSIL LC-8 Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59615	
5µm	2 x 2.1 kit **	59614	
5µm	2 x 3.0 (2/pk)	59562C30	
5µm	2 x 4.0 (2/pk)	59562	
5µm	2 x 4.0 kit **	59552	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

Liquid Chromatography

HPLC: Small Molecules
SUPELCOSIL Columns

SUPELCOSIL HPLC Columns (cont'd)

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-8-DB (120Å, 6.0% CARBON)			
3µm	7.5 x 3.0	58990C30	
3µm	15 x 3.0	58991C30	
3µm	15 x 4.0	58991C40	
3µm	3.3 x 4.6	58976	
3µm	7.5 x 4.6	58990-U	
3µm	15 x 4.6	58991	
5µm	25 x 2.1	57933	
5µm	15 x 3.0	58347C30	
5µm	15 x 4.0	58347C40	
5µm	25 x 4.0	58354C40	
5µm	5 x 4.6	58344	
5µm	15 x 4.6	58347	
5µm	25 x 4.6	58354	
5µm	25 x 10.0	58357	
SUPELCOSIL LC-8-DB Supelguard Cartridges			
5µm	2 x 2.1 (2/pk)	59619	
5µm	2 x 2.1 kit **	59618	
5µm	2 x 4.0 (2/pk)	59563	
5µm	2 x 4.0 kit **	59553	
SUPELCOSIL LC-308 (300Å, 3.5% CARBON)			
5µm	5 x 4.6	58851	
5µm	25 x 4.6	58857	
SUPELCOSIL LC-308 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59511-U	
5µm	2 x 4.0 kit **	59501	
SUPELCOSIL LC-DP (120Å, 6.0% CARBON)			
5µm	15 x 3.0	59150C30	
5µm	25 x 3.0	58842C30	
5µm	30 x 4.0	59167	
5µm	5 x 4.6	58841	
5µm	10 x 4.6	59211	
5µm	15 x 4.6	59150-U	
5µm	25 x 4.6	58842	
SUPELCOSIL LC-DP Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59566C30	
5µm	2 x 4.0 (2/pk)	59566	
5µm	2 x 4.0 kit **	59556	
SUPELCOSIL LC-3DP (300Å, 4.0% CARBON)			
5µm	25 x 4.6	58859	
SUPELCOSIL LC-3DP Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59513	
SUPELCOSIL LC-F (120Å, 5.0% CARBON)			
5µm	25 x 4.0	59158C40	
5µm	25 x 4.6	59158	
SUPELCOSIL LC-F Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59521	
5µm	2 x 4.0 kit **	59520	
SUPELCOSIL LC-304 (300Å, 2.7% CARBON)			
5µm	5 x 4.6	58823	
5µm	25 x 4.6	58824	
SUPELCOSIL LC-304 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59592	
5µm	2 x 4.0 kit **	59591	

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL LC-CN (120Å, 4.0% CARBON)			
3µm	3.3 x 3.0	58979C30	
3µm	7.5 x 3.0	58986C30	
3µm	3.3 x 4.6	58979	
3µm	7.5 x 4.6	58986	
5µm	5 x 3.0	58211C30	
5µm	25 x 3.0	58231C30	
5µm	15 x 4.0	58221C40	
5µm	25 x 4.0	58231C40	
5µm	5 x 4.6	58211	
5µm	15 x 4.6	58221-U	
5µm	25 x 4.6	58231	
5µm	25 x 10.0	58369	
SUPELCOSIL LC-CN Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59567C30	
5µm	2 x 4.0 (2/pk)	59567	
5µm	2 x 4.0 kit **	59557	
SUPELCOSIL LC-PCN (120Å, 4.0% CARBON)			
5µm	15 x 3.0	58377C30	
5µm	25 x 3.0	58378C30	
5µm	15 x 4.0	58377C40	
5µm	15 x 4.6	58377	
5µm	20 x 4.6	59189	
5µm	25 x 4.6	58378	
SUPELCOSIL LC-PCN Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59514	
5µm	2 x 4.0 kit **	59504	
SUPELCOSIL LC-1 (120Å, 2.0% CARBON)			
5µm	15 x 3.0	58210C30	
5µm	15 x 4.0	58210C40	
5µm	5 x 4.6	58237	
5µm	15 x 4.6	58210-U	
5µm	25 x 4.6	58296	
SUPELCOSIL LC-1 Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59561C30	
5µm	2 x 4.0 (2/pk)	59561	
SUPELCOSIL LC-NH_2 (120Å, 3.0% CARBON)			
3µm	7.5 x 3.0	58988C30	
3µm	15 x 3.0	58989C30	
3µm	7.5 x 4.6	58988	
3µm	15 x 4.6	58989	
5µm	25 x 3.0	58338C30	
5µm	25 x 4.0	58338C40	
5µm	25 x 4.6	58338	
SUPELCOSIL LC-NH_2 Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59568C30	
5µm	2 x 4.0 (2/pk)	59568	
5µm	2 x 4.0 kit **	59558	
SUPELCOSIL LC-NH_2-NP (120Å, 3.0% CARBON)			
5µm	25 x 4.6	59132	
SUPELCOSIL LC-NH_2-NP Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59516	
5µm	2 x 4.0 kit **	59515	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOSIL HISEP (120Å)			
5µm	25 x 2.1	57932	
5µm	5 x 4.6	59143	
5µm	15 x 4.6	58935	
5µm	25 x 4.6	58919	
SUPELCOSIL Hisep Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59640-U	
5µm	2 x 4.0 kit **	59639	
SUPELCOSIL LC-Si (120Å)			
3µm	7.5 x 3.0	58980C30	
3µm	15 x 3.0	58981C30	
3µm	15 x 4.0	58981C40	
3µm	3.3 x 4.6	58974	
3µm	7.5 x 4.6	58980-U	
3µm	15 x 4.6	58981	
5µm	30 x 1.0	57980-U	
5µm	25 x 2.1	57930-U	
5µm	10 x 3.0	59210C30	
5µm	15 x 3.0	58200C30	
5µm	15 x 4.0	58200C40	
5µm	25 x 4.0	58295C40	
5µm	30 x 4.0	59166	
5µm	5 x 4.6	58236	
5µm	10 x 4.6	59210-U	
5µm	15 x 4.6	58200-U	
5µm	25 x 4.6	58295	
5µm	25 x 10.0	58365	
5µm	25 x 21.2	54843	
12µm	25 x 4.6	59180-U	
12µm	25 x 21.2	59183	
SUPELCOSIL LC-Si Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59560C30	
5µm	2 x 4.0 (2/pk)	59560	
5µm	2 x 4.0 kit **	59550	
SUPELCOSIL LC-3Si (300Å)			
5µm	25 x 6.2	58965	
SUPELCOSIL LC-Si Supelguard Cartridges (use for LC-3Si)			
5µm	2 x 4.0 (2/pk)	59560	
5µm	2 x 4.0 kit **	59550	
SUPELCOSIL LC-DIOL (120Å, 3.5% CARBON)			
5µm	25 x 3.0	58201C30	
5µm	25 x 4.0	58201C40	
5µm	25 x 4.6	58201	
SUPELCOSIL LC-Diol Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59569	
5µm	2 x 4.0 kit **	59559	
SUPELCOSIL SAX1 (120Å)			
5µm	25 x 3.0	59138C30	
5µm	25 x 4.0	59138C40	
5µm	25 x 4.6	59138	
SUPELCOSIL SAX1 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59537-U	
5µm	2 x 4.0 kit **	59536-U	
SUPELCOSIL LC-SCX (120Å)			
5µm	25 x 3.0	58997C30	
5µm	25 x 4.6	58997	
SUPELCOSIL LC-SCX Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59519	
5µm	2 x 4.0 kit **	59509	

SUPELCOGEL resin-based HPLC Columns

For reversed-phase separations at high pH or low pH, we offer SUPELCOGEL TPR-100 and SUPELCOGEL ODP-50 resin-based HPLC columns. SUPELCOGEL resin-based ion exclusion HPLC columns contain sulfonated divinylbenzene resins in six cationic forms, each offering a unique selectivity for analyses of saccharides or organic acids.

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
SUPELCOGEL TPR-100 (100Å)			
5µm	15 x 3.0	59154C30	
5µm	15 x 4.0	59154C40	
5µm	15 x 4.6	59154	
SUPELCOGEL TPR-100 Supelguard Cartridges			
5µm	2 x 3.0 (2/pk)	59571C30	
5µm	2 x 4.0 (2/pk)	59571	
5µm	2 x 4.0 kit **	59570-U	
SUPELCOGEL ODP-50 (250Å, 17.0% CARBON)			
5µm	15 x 4.0	59307-U	
SUPELCOGEL ODP-50 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59313C40	
5µm	2 x 4.0 kit **	59312-U	
SUPELCOGEL AG1			
9µm	30 x 7.8	59318-U	
SUPELCOGEL AG1 Guard Column			
9µm	5 x 4.6 ****	59317-U	
SUPELCOGEL AG2			
9µm	30 x 7.8	59315	
SUPELCOGEL AG2 Guard Column			
9µm	5 x 4.6 ****	59316	
SUPELCOGEL C-610H			
9µm	30 x 7.8	59320-U	
SUPELCOGEL H Guard Column (use to protect C-610H)			
9µm	5 x 4.6 ****	59319	
SUPELCOGEL C-611			
9µm	30 x 7.8	59310-U	
SUPELCOGEL Ca Guard Column (use to protect C-611)			
9µm	5 x 4.6 ****	59306-U	
SUPELCOGEL CA			
9µm	30 x 7.8	59305-U	
SUPELCOGEL Ca Guard Column			
9µm	5 x 4.6 ****	59306-U	
SUPELCOGEL H			
9µm	25 x 4.6	59346	
9µm	30 x 7.8	59304-U	
SUPELCOGEL H Guard Column			
9µm	5 x 4.6 ****	59319	
SUPELCOGEL K			
9µm	30 x 7.8	59342	
SUPELCOGEL K Guard Column			
9µm	5 x 4.6 ****	59344	
SUPELCOGEL Pb			
9µm	30 x 7.8	59343	
SUPELCOGEL Pb Guard Column			
9µm	5 x 4.6 ****	59345	

** Kits include one cartridge, stand-alone holder, a piece of tubing, and 2 nuts and ferrules.
**** Does not include tubing, nuts or ferrules.

HPLC: Small Molecules
Other Columns

Other HPLC Columns - Small Molecules

In addition to our own product lines, we offer name brand silica-based columns: alphaBond, Hypersil, Kromasil, LiChrosorb, LiChrospher, Nucleosil, TSK-GEL, and Waters Spherisorb.

alphaBond

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
ALPHABOND (125Å)			
C18			
10µm	15 x 3.9	57488	
10µm	30 x 3.9	57489	
alphaBond Guard Cartridges			
C18			
10µm	1 x 4.6 (4/pk)	57490-U	

Hypersil

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
HYPERSIL (120Å)			
ODS (C18)			
3µm	10 x 3.2	54931	
3µm	10 x 4.6	Z226327	
5µm	15 x 3.2	54932	
5µm	25 x 3.2	54933	
5µm	15 x 4.6	Z226335	
5µm	25 x 4.6	Z226343	
BDS-C18*			
5µm	15 x 3.2	54915-U	
5µm	25 x 3.2	54916	
5µm	15 x 4.6	57485	
5µm	25 x 4.6	57486	
MOS (C8)			
3µm	10 x 3.2	54934	
3µm	10 x 4.6	Z226351	
5µm	15 x 4.6	Z226378	
5µm	25 x 4.6	Z226386	
BDS-C8*			
5µm	15 x 4.6	506109	
5µm	25 x 4.6	506095	
Phenyl			
3µm	10 x 3.2	54943	
3µm	10 x 4.6	Z226459	
5µm	25 x 3.2	54945	
5µm	15 x 4.6	Z226467	
5µm	25 x 4.6	Z226475	
CPS (Cyano)			
3µm	10 x 3.2	54940	
3µm	10 x 4.6	Z226424	
5µm	25 x 3.2	54942	
5µm	15 x 4.6	Z226432	
5µm	25 x 4.6	Z226440	
SAS (C1)			
3µm	10 x 4.6	Z226483	
5µm	15 x 3.2	54947-U	
5µm	15 x 4.6	Z226491	
5µm	25 x 4.6	Z226505	

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
HYPERSIL (120Å) CONT'D			
Silica			
3µm	10 x 4.6	Z226297	
5µm	15 x 4.6	Z226300	
5µm	25 x 4.6	Z226319	
APS (NH_2)			
3µm	10 x 3.2	54937	
3µm	10 x 4.6	Z226394	
5µm	15 x 3.2	54938	
5µm	25 x 3.2	54939	
5µm	15 x 4.6	Z226408	
5µm	25 x 4.6	Z226416	
Hypersil Guard Cartridges			
ODS (C18)			
5µm	1 x 4.6 (4/pk)	Z227064	
BDS-C18*			
5µm	1 x 4.6 (4/pk)	57487	
MOS (C8)			
5µm	1 x 4.6 (4/pk)	Z227072	
BDS-C8*			
5µm	1 x 4.6 (4/pk)	506117	
Phenyl			
5µm	1 x 4.6 (4/pk)	Z227102	
CPS (Cyano)			
5µm	1 x 4.6 (4/pk)	Z227099	
SAS (C1)			
5µm	1 x 4.6 (4/pk)	Z227110	
Silica			
5µm	1 x 4.6 (4/pk)	Z227056	
APS (NH_2)			
5µm	1 x 4.6 (4/pk)	Z227080	

Inertsil

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
INERTSIL (150Å)			
ODS-2**			
5µm	15 x 4.6	506079	
5µm	25 x 4.6	506060	
Inertsil Guard Cartridges			
ODS-2**			
5µm	1 x 4.6 (4/pk)	506087	

* BDS = Base Deactivated Silica.
** Deactivated C18.

Kromasil

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
KROMASIL (100Å)			
C18			
5µm	15 x 4.6	55148	
5µm	25 x 4.6	55147	
10µm	25 x 4.6	55149	
C8			
5µm	15 x 4.6	55151	
5µm	25 x 4.6	55150-U	
10µm	25 x 4.6	55152	
C4			
5µm	15 x 4.6	55154-U	
5µm	25 x 4.6	55153	
60-A (Silica, 60Å)			
5µm	15 x 4.6	55163	
5µm	25 x 4.6	55162	
10µm	25 x 4.6	55164	
100-A (Silica)			
5µm	25 x 4.6	55159	
10µm	25 x 4.6	55161	
NH_2			
5µm	25 x 4.6	55156-U	
10µm	25 x 4.6	55158-U	
Kromasil Guard Cartridges			
C18			
5µm	1 x 4.6 (4/pk)	55165-U	
C8			
5µm	1 x 4.6 (4/pk)	55166	
C4			
5µm	1 x 4.6 (4/pk)	55167	
60-A (Silica, 60Å)			
5µm	1 x 4.6 (4/pk)	55170-U	

Lichrosorb

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
LICHROSORB (100Å)			
RP-18 (C18)			
5µm	15 x 3.2	54952	
5µm	25 x 3.2	54950-U	
5µm	15 x 4.6	54951	
5µm	25 x 4.6	54949	
RP-8 (C8)			
5µm	15 x 4.6	54955-U	
5µm	25 x 4.6	54953-U	
Si-60 (Silica, 60Å)			
5µm	25 x 3.2	54962	
5µm	15 x 4.6	54963-U	
5µm	25 x 4.6	54961	
NH_2			
5µm	25 x 3.2	54958-U	
5µm	15 x 4.6	54959	
5µm	25 x 4.6	54957-U	

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
LiChrosorb Guard Cartridges			
RP-18 (C18)			
5µm	1 x 4.6 (4/pk)	54965-U	
RP-8 (C8)			
5µm	1 x 4.6 (4/pk)	54966	
Si-60 (Silica, 60Å)			
5µm	1 x 4.6 (4/pk)	54968	
NH_2			
5µm	1 x 4.6 (4/pk)	54967	

Licrospher

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
LICHROSPHER (100Å)			
RP-18 (C18)			
5µm	15 x 3.2	54775	
5µm	25 x 3.2	54777	
5µm	15 x 4.6	54774	
5µm	25 x 4.6	54776	
RP-8 (C8)			
5µm	15 x 3.2	54779	
5µm	15 x 4.6	54778	
5µm	25 x 4.6	54780	
CN (Cyano)			
5µm	15 x 4.6	54786	
5µm	25 x 4.6	54788	
Si-60 (Silica, 60Å)			
5µm	15 x 3.2	54791-U	
5µm	15 x 4.6	54790-U	
5µm	25 x 4.6	54792	
NH_2			
5µm	15 x 3.2	54783	
5µm	25 x 3.2	54785	
5µm	15 x 4.6	54782	
5µm	25 x 4.6	54784	
LiChrospher Guard Cartridges			
RP-18 (C18)			
5µm	1 x 4.6 (4/pk)	54794	
CN (Cyano)			
5µm	1 x 4.6 (4/pk)	54798	
Si-60 (Silica, 60Å)			
5µm	1 x 4.6 (4/pk)	54797-U	
NH_2			
5µm	1 x 4.6 (4/pk)	54796-U	

HPLC: Small Molecules
Other Columns

Nucleosil

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
NUCLEOSIL (100Å)			
C18			
3µm	10 x 3.2	54917	
3µm	10 x 4.6	Z226165	
5µm	15 x 3.2	54918	
5µm	25 x 3.2	54919	
5µm	15 x 4.6	Z226173	
5µm	25 x 4.6	Z226181	
C8			
3µm	10 x 3.2	54920-U	
3µm	10 x 4.6	Z226203	
5µm	15 x 3.2	54921	
5µm	25 x 3.2	54922	
5µm	15 x 4.6	Z226211	
5µm	25 x 4.6	Z226238	
Phenyl			
7µm	25 x 4.6	Z226246	
CN (Cyano)			
5µm	15 x 3.2	54924	
5µm	25 x 3.2	54925-U	
5µm	15 x 4.6	Z226254	
5µm	25 x 4.6	Z226262	
Silica			
5µm	25 x 3.2	54914	
5µm	15 x 4.6	Z226149	
5µm	25 x 4.6	Z226157	
NH_2			
5µm	15 x 3.2	54926	
5µm	25 x 3.2	54927	
5µm	25 x 4.6	Z226289	
Nucleosil Guard Cartridges			
C18			
5µm	1 x 4.6 (4/pk)	Z227137	
C8			
5µm	1 x 4.6 (4/pk)	Z227145	
Phenyl			
7µm	1 x 4.6 (4/pk)	Z227153	
CN (Cyano)			
5µm	1 x 4.6 (4/pk)	Z227161	
Silica			
5µm	1 x 4.6 (4/pk)	Z227129	
NH_2			
5µm	1 x 4.6 (4/pk)	Z227188	

TSK-GEL C18

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
TSK-GEL C18 COLUMNS			
ODS-80Tm (80Å)			
5µm	7.5 x 4.6	816651	
5µm	15 x 4.6	808148	
5µm	25 x 4.6	808149	
ODS-80Ts (80Å)			
5µm	7.5 x 4.6	817200	
5µm	15 x 4.6	817201	
5µm	25 x 4.6	817202	
ODS-120A (120Å)			
5µm	15 x 4.6	807636	
5µm	25 x 4.6	807124	
ODS-120T (120Å)			
5µm	15 x 4.6	807637	
5µm	25 x 4.6	807125	

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
TSK-GEL C18 COLUMNS, CONT'D			
Super-ODS (110Å)			
2µm	5 x 4.6	818154	
2µm	10 x 4.6	818197	
TSK-GEL Guard Cartridges and Filters			
ODS-80Ts (80Å)			
5µm	1.5 x 3.2 (3/pk)	817242	
ODS-120T (120Å)			
5µm	1.5 x 3.2 (3/pk)	814125	
Filter			
	(3/pk)	818207	

Waters Spherisorb

PARTICLE SIZE	LENGTH X ID (cm X mm)	CAT. NO.	PRICE
WATERS SPHERISORB (80Å)			
ODS-2 (C18)			
3µm	10 x 3.2	54903	
3µm	10 x 4.6	Z226033	
5µm	15 x 3.2	54904	
5µm	25 x 3.2	54905	
5µm	15 x 4.6	Z226041	
5µm	25 x 4.6	Z226068	
Octyl (C8)			
3µm	10 x 4.6	Z226076	
5µm	25 x 3.2	54908	
5µm	15 x 4.6	Z226084	
5µm	25 x 4.6	Z226092	
Phenyl			
5µm	25 x 4.6	Z226106	
Cyano			
5µm	25 x 3.2	54910	
5µm	25 x 4.6	Z226114	
Silica			
3µm	10 x 4.6	Z226009	
5µm	15 x 3.2	54901	
5µm	25 x 3.2	54902	
5µm	15 x 4.6	Z226017	
5µm	25 x 4.6	Z226025	
Amino (NH_2)			
5µm	25 x 3.2	54911-U	
5µm	25 x 4.6	Z226122	
SAX			
5µm	25 x 4.6	Z226130	
Waters Spherisorb Guard Cartridges			
ODS-2 (C18)			
5µm	1 x 4.6 (4/pk)	Z226971	
Octyl (C8)			
5µm	1 x 4.6 (4/pk)	Z226998	
Phenyl			
5µm	1 x 4.6 (4/pk)	Z227005	
Cyano			
5µm	1 x 4.6 (4/pk)	Z227013	
Silica			
5µm	1 x 4.6 (4/pk)	Z226963	
Amino (NH_2)			
5µm	1 x 4.6 (4/pk)	Z227021	
SAX			
5µm	1 x 4.6 (4/pk)	Z227048	

P000834

Guard Column Holders

Use these guard column holders with the guard cartridges listed on the previous pages. The Direct-Connect holders allow a guard cartridge to attach to a Supelco modular column with no dead volume. The Direct-Connect holders can only be used with Supelco modular columns. The Swivel-type holders allow the tubing to move independently of the holder, reducing the risk of leaks caused by crimped tubing. The Stand-Alone holders include the necessary tubing, nuts and ferrules for connecting to any analytical columns.

DESCRIPTION	CAT. NO.	PRICE
FOR SUPELCO SUPELGUARD CARTRIDGES		
Direct-Connect (Swivel-type) Holder*	504262	
Direct-Connect (Swivel-type) Holder**	504254	
Direct-Connect Holder**	55205	
Stand-Alone Holder***	59660-U	
New Stand-Alone Holder****	567499-U	
FOR SUPELCO PELLIGUARD CARTIDGES		
Stand-Alone Holder	500054	
FOR OTHER CARTRIDGES		
Stand-Alone Holder (includes PEEK Column Coupler)	54987	
Replacement PEEK Column Coupler	54986	
FOR TSK-GEL CARTRIDGES AND FILTERS		
Stand-Alone Holder for TSK-GEL Cartridges	814100	
Stand-Alone Holder for TSK-GEL Filters	818206	

* For 2.1mm ID Supelco columns
** For 3.0, 4.0 and 4.6mm ID Supelco columns
*** For 2.1, 3.0, 4.0 and 4.6mm ID Supelco columns
**** For 10.0mm ID Supelco columns

HELPFUL HINTS

Before flushing a reversed-phase HPLC column that contains a buffer (salt), flush with warm (60°C) DI H_2O thoroughly to remove salts. Not following this general rule may result in salt precipitation when returned to 100% organic for long-term storage. For more information, refer to literature T401012, Buffer Solubility section.

Pelliguard Guard Cartridges

For 5µm, 10µm, or 12µm SUPELCOSIL and other silica-based HPLC columns, where samples are especially dirty, and a small loss of efficiency is acceptable. Each kit contains one cartridge (2cm x 4.6mm ID) filled with 40µm Pelliguard packing, a reusable stand-alone column holder, and hardware for connecting the holder to 1/16" tubing. Replacement cartridges come in packages of four.

COLUMN TO BE PROTECTED	RECOMMENDED PELLIGUARD COLUMN	CAT. NO.	PRICE
Silica	LC-Si Kit	59641	
	Cartridges (pk. of 4)	59651	
Cyano	LC-CN Kit	59645-U	
	Cartridges (pk. of 4)	59655	
Amino	LC-NH_2 Kit	59646	
	Cartridges (pk. of 4)	59656	
C8	LC-8 Kit	59643	
	Cartridges (pk. of 4)	59653	
C18	LC-18 Kit	59644	
	Cartridges (pk. of 4)	59654	

Bulk Pellicular Packing Kits

Reusable 5cm x 4.6mm ID guard column hardware and 40µm pellicular packing, for protecting 10µm columns. Each column kit contains an empty 5cm x 4.6mm ID column, 10g of Pelliguard packing, 10 frits, and hardware for connecting the column to 1/16" tubing. About 1.3 grams of packing is needed to pack one 5cm x 4.6mm column.

COLUMN TO BE PROTECTED	RECOMMENDED PELLIGUARD COLUMN	CAT. NO.	PRICE
Silica	LC-Si Kit	58202	
	LC-Si Packing, 10g	58291	
Cyano	LC-CN Kit	58234	
	LC-CN Packing, 10g	58235	
C8	LC-8 Kit	58222-U	
	LC-8 Packing, 10g	58293	
C18	LC-18 Kit	58232	
	LC-18 Packing, 10g	58294	

Guard Column Hardware Kit, Funnel and Tubing

Kit includes 5cm x 4.6mm ID column, endfittings, 2 frits (2.0µm pores), and 2"/5cm of 0.01" ID x 1/16" OD SS tubing. Funnel connects to column with tygon tubing (included) for easier column filling.

DESCRIPTION	CAT. NO.	PRICE
Guard Column Hardware Kit	58319	
Replacement Frits (pk. of 10)	58264	
Funnel and Tubing	20390-U	

HPLC: Small Molecules

HPLC Column Test Mixes

HPLC Column Test Mixes

Performance evaluation mixes for HPLC columns.

Well defined test mixes enable you to troubleshoot chromatographic problems, optimize system efficiency, and evaluate new columns u conditions where their performance is understood. We ship these test mixes in amber ampuls to prevent photodegradation, and we include instructions for proper use and interpretation of results.

Choose from column-specific or application-specific mixes. All mixes except the amino phase test mix (Cat. No. 58424) call for UV detection; the amino phase test mix (sugars) calls for refractive index detection. We recommend our HPLC Troubleshooting Guide (Bulletin 826) for additional information about using test mixes.

1mL unless otherwise specified.

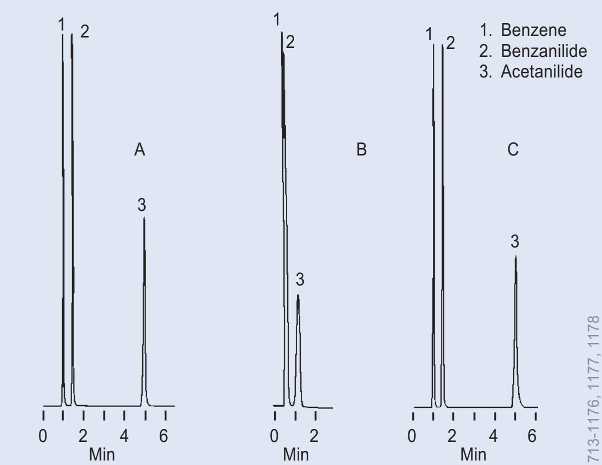
TEST MIX	USE TO TEST	SOLVENT	COMPONENTS (CONC./mL)	CAT. NO.	PRICE
Amino Phase	LC-NH_2 columns	acetonitrile:water, 25:75	D-fructose (25mg) α-D-glucose (25mg) sucrose (25mg) maltose (25mg) lactose (25mg)	58424	
Cyano Phase	LC-CN, LC-PCN, ABZ+Plus columns, any weakly hydrophobic phase	acetonitrile:water, 25:75	uracil (7µg) acetophenone (7µg) benzene (750µg) toluene (775µg)	58299	
Normal Phase Mix 1	LC-Si (silica) columns	methylene chloride	benzene (600µg) benzanilide (20µg) acetanilide (20µg)	58281	
Normal Phase Mix 2	LC-Si, LC-CN, LC-NH_2 columns	ethanol:hexane, 5:95	toluene (1mg) diethyl phthalate (1mg) dimethyl phthalate (1mg)	47640-U	
Nucleosides	LC-18-S columns	water, sodium formate (10mg/mL)	12 nucleosides (10-100µg) (see page 146)	47310-U	
LC-PAH	LC-PAH columns	methanol: methylene chloride, 50:50	16 PAHs (100-2000µg) (see page 410)	48743	
Peptide Standard	reversed phase columns used for peptide separations (e.g., 300Å phases)	none (dried film)	Gly-Tyr (~0.125mg) Val-Tyr-Val (~0.5mg) Met enkephalin (~0.5mg) Leu enkephalin (~0.5mg) angiotensin II (~0.5mg)	H2016-1VL	
Reversed Phase Mix 1	hydrophobic RP columns (e.g., LC-8, LC-18)	methanol:water, 60:40	uracil (7µg) acetophenone (7µg) benzene (750µg) toluene (775µg)	58278	
Reversed Phase Mix 2	hydrophobic RP columns (e.g., LC-8, LC-18)	acetonitrile:water, 58:42	uracil (5µg) phenol (700µg) N,N-diethyl-m-toluamide (600µg) toluene (4mg)	47641-U	
Chiral 1	chiral phases	hexane:ethyl acetate, 80:20	toluene (70µg) (+) TFAE[1] (25µg) (–) TFAE[1] (25µg)	48250-U	
Chiral 2	chiral phases	chloroform	benzene (500µg) (+) N-PDBA[2] (50µg) (–) N-PDBA[2] (50µg))	48251	

[1] 2,2,2-trifluoro-1-(9-anthryl)ethanol

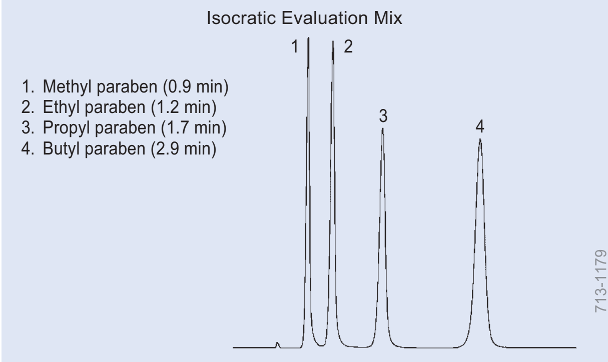
[2] N-(1-phenylethyl)-3,5-dinitrobenzamide

Custom Test Mixes

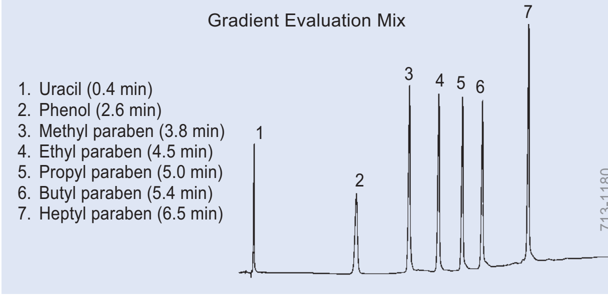
For information on made-to-order standards and test mixes, call our Technical Service chemists, or request our Custom Chemical Reference brochure (Publication No. 196905).



Column: SUPELCOSIL LC-Si, 15cm x 4.6mm, 3µm particles
Cat. No.: 58981
Mobile Phase: A = methylene chloride:methanol:water (99.4:0.5:0.1)
B = 2-propanol:water (50:50)
C = Silica Column Regeneration Solution, 4mL/min for 10 min, then methylene chloride:methanol:water (99.4:0.5:0.1), 2mL/min for 10 min
Det.: UV, 254nm
Inj.: 10µL



Flow Rate: 2mL/min
Det.: UV, 254nm
Inj.: 10µL



Flow Rate: 2mL/min methanol:water, 10:90 to 90:10 in 5 min
Det.: UV, 254nm
Inj.: 10µL

HPLC Column Test Mixes

System diagnostics: test kits and column regeneration solutions.

Silica Column Regeneration Solution -This solution effectively regenerates a silica column that has come into contact with very strongly polar solvents, such as water or alcohols. Simply flush the column with regeneration solution for 10 minutes, then reequilibrate with mobile phase for 10 minutes. Column performance usually is restored to that obtained before exposure to the polar solvent.

System Diagnostic Kit -Take a systematic approach to diagnosing problems in an HPLC system. This kit consists of:

- 5cm x 4.6mm SUPELCOSIL LC-18SD column
- 6 x 1mL Isocratic Evaluation Mix
- 6 x 1mL Gradient Evaluation Mix
- four 1.8mL screwcap vials with hole caps and septa

When you need to determine the cause of a problem, install the 5cm column, prepare a simple methanol:water mobile phase, and inject 10µL of Isocratic Evaluation Mix onto the column. Compare your chromatogram with that from a properly performing system and use the information sheet included with the kit to help isolate the source of the problem. If necessary, make injections with the gradient mix.

We recommend our HPLC Troubleshooting Guide (Bulletin 826, available free on request) to help you interpret the results you obtain.

Evaluation Test Mixes -Six 1mL ampuls of test compounds in methanol:water, 60:40.

These formulations are designed for evaluating how reliably a chromatographic system is providing such fundamentally important parameters as flow rate, proportioning, and mixing.

DESCRIPTION	QTY.	CAT. NO.	PRICE
Silica Column Regeneration Solution	200mL	33175	
LC-18SD System Diagnostic Kit		58543	
Isocratic Evaluation Mix	6 x 1mL	48270-U	
Gradient Evaluation Mix	6 x 1mL	48271	

RELATED INFORMATION

Request free literature by phone or fax, or see our website.

No.	Subject
T100826	HPLC troubleshooting guide
T196905	custom chemicals brochure

HPLC: Biopolymers

Column Selection: Nucleic Acid Separations

SAMPLE TYPE	SEPARATION MODE	MCI GEL HPLC (SEE PAGE)	AMERSHAM BIOSCIENCES FPLC (SEE PAGE)	TSK-GEL HPLC (SEE PAGE)	SUPELCO HPLC (SEE PAGE)
DNA/RNA	Gel Filtration		Superdex 200 (62)	G-DNA-PW (63)	
	Ion Exchange	ProtEx-DEAE (60)	Mono Q (60)	DEAE-NPR (61)	
PCR Fragments	Ion Exchange	ProtEx-DEAE (60)	Mono Q (60)	DEAE-NPR (61)	
Oligonucleotides	Ion Exchange	ProtEx-DEAE (60)	Mono Q (60)	DEAE-5PW (61) DEAE-NPR (61)	
	Reversed Phase			Oligo-DNA RP (64)	SUPELCOSIL LC-318 (64) Discovery C18 (28)
Nucleotides	Reversed-Phase				SUPELCOSIL LC-18-T (39) Discovery C18 (28) Discovery RP-AmideC16 (29)
	Ion Exchange			DEAE-2SW (61)	SUPELCOSIL SAX1 (47)
Nucleosides	Reversed-Phase				SUPELCOSIL LC-18-S (39) Discovery C18 (28) Discovery RP-AmideC16 (29)
Nucleic Acid Bases	Reversed-Phase				SUPELCOSIL LC-18 (44) SUPELCOSIL LC-18-DB (33) Discovery C18 (28) Discovery RP-AmideC16 (29)
	Ion Exchange				SUPELCOSIL LC-SCX (47)

RELATED INFORMATION

Request free literature by phone or fax, or see our website.

No.	Subject
T402038	Discovery BIO Wide Pore HPLC Columns and Capillaries: Solutions to Protein and Peptide Separation Challenges

McClung, G., W.T. Frankenburger, Jr. Comparison of Reversed Phase High Performance Liquid Chromatographic Methods for Precolumn Derivatized Amino Acids J. Liq. Chromatogr. 11: 613-646 (1988). Reference not available from Supelco.

HPLC: Biopolymers

Column Selection: Peptide and Protein Separations

SEPARATION MODE	MCI GEL HPLC (SEE PAGE)	AMERSHAM BIOSCIENCES FPLC (SEE PAGE)	TSK-GEL HPLC (SEE PAGE)	SUPELCO HPLC (SEE PAGE)
Gel Filtration		Superdex Peptide Superdex 75 Superdex 200 (62) Fast Desalting HR 10/10 (62)	G2000SW, G2000SW$_{XL}$ G3000SW, G3000SW$_{XL}$ G4000SW, G4000SW$_{XL}$ Super 3000 SW (62)	
Strong Anion Exchange	ProtEx-DEAE (60)	Mono Q (60)		
Weak Anion Exchange		Mono P (60)	DEAE-5PW, DEAE-NPR (61)	
Chromatofocusing		Mono P (60)		
Strong Cation Exchange	ProtEx-SP (60)	Mono S (60)	SP-5PW, SP-NPR (61)	
Weak Cation Exchange			CM-5PW (61)	
Hydrophobic Interaction			Ether-5PW Phenyl-5PW Butyl-NPR (64)	
Reversed Phase			Phenyl-5PW RP Octadecyl-4PW Octadecyl-NPR Super-ODS (64)	Discovery BIO Wide Pore C18 (57) Discovery BIO Wide Pore C8 (58) Discovery BIO Wide Pore C5 (59) Discovery RP-AmideC16 (29) Discovery C18 (28) Discovery C8 (30)
Affinity			ABA-5PW Boronate-5PW Chelate-5PW Heparin-5PW Tresyl-5PW (64)	

Note: Products in italics are used under low pressure conditions.

Columns for Amino Acid Separations

DERIVATIZED AMINO ACID	SUPELCOSIL HPLC	DIMENSIONS (cm x mm ID)	CAT. NO.	PRICE	SUPELGUARD GUARD COLUMN	CAT. NO.	PRICE
Dabsyl-AA	LC-DABS (3µm)	15 x 2.1	59137		LC-18-T	59621	
DABTH-AA	LC-18 (3µm)	15 x 4.6	58985		LC-18	59564	
OPA-AA	LC-18 (5µm)	15 x 4.6	58230-U		LC-18	59564	
PTC-AA	LC-18-DB (5µm)	25 x 4.6	58355-U		LC-18-DB	59565	
PTH-AA	LC-18-DB (3µm)	25 x 2.1	57943		LC-18-DB	59565	
	LC-18-DB (5µm)[1]	25 x 4.6	58355-U		LC-18-DB	59565	

[1] Alternative to 3µm LC-18-DB.

Liquid Chromatography

HPLC: Biopolymers

Discovery BIO Wide Pore Columns

Discovery BIO

Solutions to Protein and Peptide Separation Challenges

Discovery BIO HPLC columns and capillaries provide sensitive, stable, efficient, reproducible separations of proteins and peptides. The different phase chemistries provide unique selectivity increasing your resolution options. Separations are completely scalable from analytical to prep. The low-bleed feature and microbore and capillary dimensions make them ideal for proteomics and LC/MS applications.

Discovery BIO Wide Pore C18

Ideal for high resolution peptide mapping and synthetic and native peptide separations.

Discovery BIO Wide Pore C8

Also ideal for high resolution peptide mapping and synthetic and native peptide separations. The intermediate hydrophobicity of the C8 phase makes it very useful for method development.

Discovery BIO Wide Pore C5

The short-chain C5 phase is used for protein and hydrophobic peptide separations. Discovery BIO Wide Pore C5 is more stable than the commonly-used C4 phases.

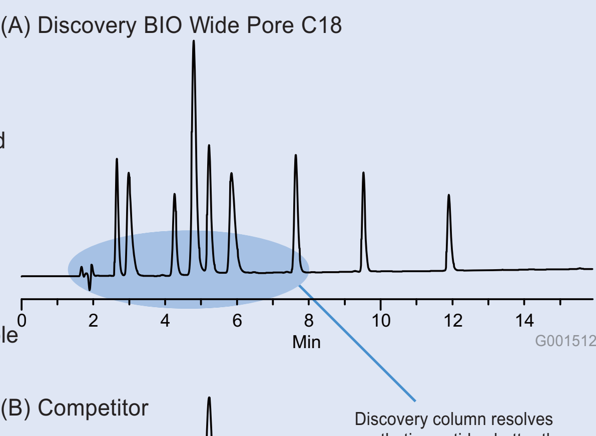
Capillary and Microbore Dimensions

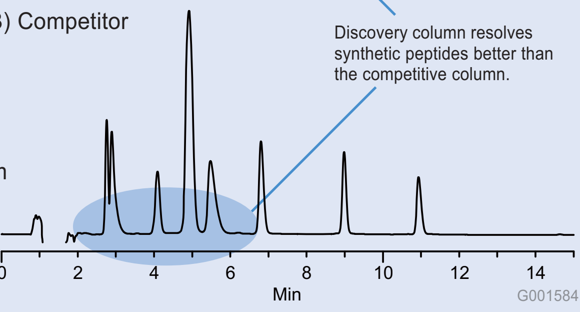
With the rapid growth of proteomics, the demand for detailed characterization on smaller sample volumes is increasing. So is the utility of smaller ID HPLC columns. Traditional column IDs of 4.6mm and even 2.1mm are being replaced by microbore (1mm ID) and capillary (< 1mm ID) dimensions. The benefits are two-fold: increased sensitivity and decreased sample consumption. Discovery BIO C18 and C5 is available in capillary and microbore dimensions to meet these criteria.

Go to www.sigma-aldrich.com/supelco-bio to see the entire Discovery BIO line, including new additions, and download the most recent literature and applications.

Mixture of Synthetic Peptides on Discovery BIO Wide Pore C18 and a Leading Competitive Column

Columns: (A) Discovery BIO Wide Pore C18, 15cm x 4.6mm, 5µm (Cat. No. 568222-U)
(B) Competitive protein and peptide C18, 15cm x 4.6mm, 300Å, 5µm
Mobile Phase: (A) 80:20, (0.1% TFA in water): (0.1% TFA in CH_3CN);
(B) 66:34, (0.1% TFA in water): (0.1% TFA in CH_3CN)
Flow Rate: 1.0mL/min
Temp: 30°C
Detection: 220nm
Injection: 10µL, ~0.25µg each peptide (Sigma Peptide Mix, Cat. No. P 2693 containing Arg[8]-vasopressin, bradykinin (fragment 1-5), oxytocin, luteinizing hormone releasing hormone, Met-enkephalin, bradykinin, Leu-enkephalin, bombesin, Substance P) in 0.1%TFA. See sequence in Figure 14.
Gradient: 0-100%B in 14 min after 1 minute delay





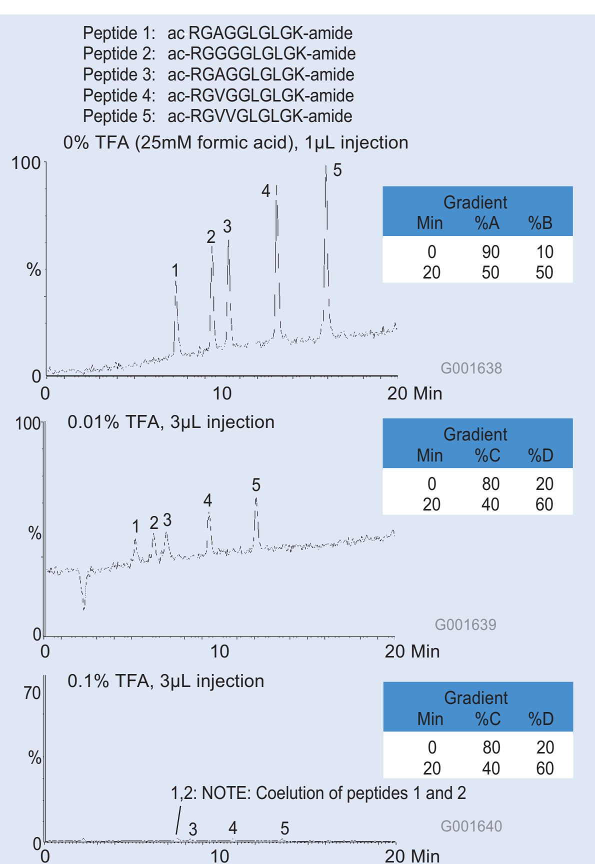
Discovery column resolves synthetic peptides better than the competitive column.

Suggestions for Choosing a Discovery BIO Wide Pore Column:

APPLICATIONS	BONDED PHASES
Proteins	BIO Wide Pore C5
Hydrophobic peptides or proteins (e.g. membrane proteins)	BIO Wide Pore C5
Peptide mapping	BIO Wide Pore C18
Proteomics	BIO Wide Pore C18
Scouting	BIO Wide Pore C8 (because of its intermediate hydrophobicity between a C18 and C5)
APPLICATION	**SILICA PARTICLE SIZES**
LC/MS	3 micron or 5 micron
Fast analysis, or highthroughput applications	3 micron
Peptide mapping	3 micron or 5 micron
Analytical HPLC	3 micron or 5 micron
Preparative	10 micron
APPLICATION	**COLUMN ID**
LC/MS	2.1mm or smaller
Peptide mapping	4.6mm, 4.0mm, 2.1mm
Analytical HPLC	4.0mm, 4.6mm
Preparative	10mm, 21.2mm
Low level detection or limited sample volume	0.32mm, 0.5mm, 1.0mm

Discovery BIO Wide Pore C18

Peptide maps generated by RP-HPLC provide valuable information about protein structure, stability, and purity. To be effective, the RP-HPLC column must be able to resolve a high percentage of the peptides in the sample. The more peptides, the better the information. Discovery BIO Wide Pore C18 gives unsurpassed RP-HPLC resolution of peptide maps from tryptic digests. The improvements in silica and bonded phase chemistry we've incorporated into the Discovery BIO Wide Pore line improve resolution by increasing efficiency and reducing the peak tailing. An added benefit to this is the ability to analyze peptides without TFA in the mobile phase thereby increasing the LC/MS signal.



Effect of Chromatographic Conditions on MS Signals of Peptides

Column: Discovery BIO Wide Pore C18, 15cm x 2.1mm, 3μm
Cat. No.: 567202-U
Mobile Phase A: 25mM formic acid in water
Mobile Phase B: 50:50 (25mM formic acid in water) : (20mM formic acid in MeCN) [1]
Mobile Phase C: 0.01 or 0.1% TFA in water
Mobile Phase D: 50:50 (0.01 or 0.1% TFA in water) : (0.01 or 0.1% TFA in MeCN)
Flow Rate: 0.208mL/min[2]
Det.: +ES
Temp.: ambient
Inj.: 1μL or 3μL
Sample: RP Peptide Performance Standard, p/n RPS-P0010 (Alberta Peptide Institute)

1. molarity of formic acid adjusted to provide minimum baseline drift.
2. linear velocity equal to 1mL/min on 4.6mm ID columns.

PARTICLE SIZE	LENGTH x ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY BIO WIDE PORE C18 (300Å, 9.2% CARBON)			
3μm	5 x 0.32	65526-U	
3μm	10 x 0.32	65527-U	
3μm	5 x 0.5	65517-U	
3μm	10 x 0.5	65518-U	
3μm	5 x 1.0	65504-U	
3μm	10 x 1.0	65506-U	
3μm	5 x 2.1	567200-U	
3μm	10 x 2.1	567201-U	
3μm	15 x 2.1	567202-U	
3μm	5 x 4.6	567203-U	
3μm	10 x 4.6	567204-U	
3μm	15 x 4.6	567205-U	
5μm	15 x 0.32	65529-U	
5μm	15 x 0.5	65519-U	
5μm	15 x 1.0	65508-U	
5μm	25 x 1.0	65509-U	
5μm	5 x 2.1	568200-U	
5μm	10 x 2.1	568201-U	
5μm	15 x 2.1	568202-U	
5μm	25 x 2.1	568203-U	
5μm	5 x 4.0	568210-U	
5μm	10 x 4.0	568211-U	
5μm	15 x 4.0	568212-U	
5μm	25 x 4.0	568213-U	
5μm	5 x 4.6	568220-U	
5μm	10 x 4.6	568221-U	
5μm	15 x 4.6	568222-U	
5μm	25 x 4.6	568223-U	
5μm	25 x 10.0	568230-U	
10μm	25 x 4.6	567206-U	
10μm	5 x 10.0	567207-U	
10μm	15 x 10.0	567208-U	
10μm	25 x 10.0	567209-U	
10μm	5 x 21.2	567210-U	
10μm	15 x 21.2	567211-U	
10μm	25 x 21.2	567212-U	
Supelguard Cartridges			
3μm	2 x 2.1 (2/pk)	567270-U	
3μm	2 x 2.1 kit **	567271-U	
3μm	2 x 4.0 (2/pk)	567272-U	
3μm	2 x 4.0 kit **	567273-U	
5μm	2 x 2.1 (2/pk)	568270-U	
5μm	2 x 2.1 kit **	568271-U	
5μm	2 x 4.0 (2/pk)	568272-U	
5μm	2 x 4.0 kit **	568273-U	
10μm	1 x 10.0	567282-U	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

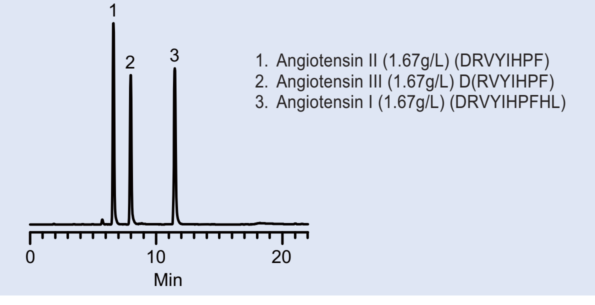
PROPERTIES:	
Bonded Phase:	Covalently-bonded octadecylsilane, endcapped
Silica:	Spherical, high purity (<10ppm metals)
Particle Size:	3, 5, and 10mm
Pore Size:	300Å
Surface Area:	100m²/g
%C:	~9%
Coverage:	~4 μmoles/m²

HPLC: Biopolymers

Discovery BIO Wide Pore C8 Columns

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY BIO WIDE PORE C8 (300Å, 5.0% CARBON)			
3µm	5 x 2.1	567213-U	
3µm	10 x 2.1	567214-U	
3µm	15 x 2.1	567215-U	
3µm	5 x 4.6	567216-U	
3µm	10 x 4.6	567217-U	
3µm	15 x 4.6	567218-U	
5µm	5 x 2.1	568300-U	
5µm	10 x 2.1	568301-U	
5µm	15 x 2.1	568302-U	
5µm	25 x 2.1	568303-U	
5µm	5 x 4.0	568310-U	
5µm	10 x 4.0	568311-U	
5µm	15 x 4.0	568312-U	
5µm	25 x 4.0	568313-U	
5µm	5 x 4.6	568320-U	
5µm	10 x 4.6	568321-U	
5µm	15 x 4.6	568322-U	
5µm	25 x 4.6	568323-U	
5µm	25 x 10.0	568330-U	
10µm	25 x 4.6	567219-U	
10µm	5 x 10.0	567220-U	
10µm	15 x 10.0	567221-U	
10µm	25 x 10.0	567222-U	
10µm	5 x 21.2	567223-U	
10µm	15 x 21.2	567224-U	
10µm	25 x 21.2	567225-U	
Supelguard Cartridges			
3µm	2 x 2.1 (2/pk)	567274-U	
3µm	2 x 2.1 kit **	567275-U	
3µm	2 x 4.0 (2/pk)	567276-U	
3µm	2 x 4.0 kit **	567277-U	
5µm	2 x 2.1 (2/pk)	568370-U	
5µm	2 x 2.1 kit **	568371-U	
5µm	2 x 4.0 (2/pk)	568372-U	
5µm	2 x 4.0 kit **	568373-U	
10µm	1 x 10.0	567284-U	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.



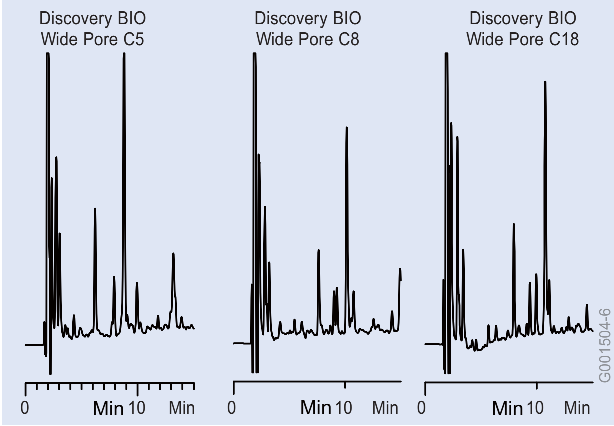
Resolution of Angiotensins at Neutral pH

Column: Discovery BIO Wide Pore C8, 15cm x 4.6mm, 5µm
Cat. No.: 568322-U
Mobile Phase: (A) 10mM $NH_4H_2PO_4$/ NH_4OH, pH 7;
(B) 50:50, (20mM $NH_4H_2PO_4$/ NH_4OH, pH 7):MeCN
Flow Rate: 1mL/min
Temp: 30°C
Det.: 215nm
Inj.: 6µL in water
Gradient: 30-60% B in 15 min

Discovery BIO Wide Pore C8

Discovery BIO Wide Pore C8 is an RP-HPLC phase for proteins and peptides that has hydrophobicity intermediate between the Discovery BIO Wide Pore C5 and the Discovery BIO Wide Pore C18. The difference in hydrophobicity gives it unique selectivity relative to these other phases. It is ideal for peptide mapping because it provides complementary information compared to a C18 separation. Because of its intermediate hydrophobicity we also recommend it for method development or scouting work. As with all Discovery BIO Wide Pore phases, the C8 phase gives efficient, symmetrical peaks, exceptional stability, long column lifetime, and LC/MS compatibility.

PROPERTIES:	
Bonded Phase:	Covalently-bonded octylsilane, endcapped
Silica:	Spherical, high purity (<10ppm metals)
Particle Size:	3, 5, and 10mm
Pore Size:	300Å
Surface Area:	100m²/g
%C:	~5
Coverage:	~4 µmoles/m²



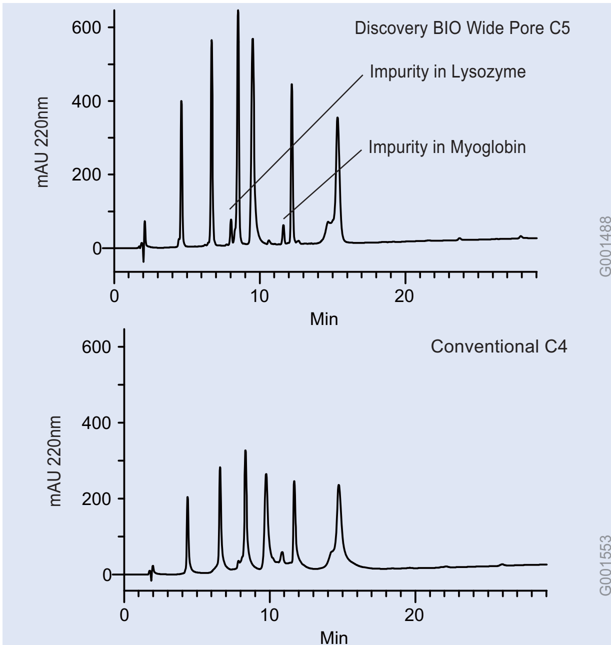
Each Discovery BIO Wide Pore Phase Gives Unique Elution Profiles of Carboxymethylated Apohemoglobin Peptide Fragments

Columns: (A) Discovery BIO Wide Pore C5 (Cat. No. 568422-U),
(B) Discovery BIO Wide Pore C8 (Cat. No. 568322-U),
or (C) Discovery BIO Wide Pore C18 (568222-U),
each 15cm x 4.6mm, 5µm
Mobile Phase: (A) 95:5, (0.1% TFA in water):(0.1% TFA in CH_3CN);
(B) 50:50, (0.1% TFA in water):(0.1% TFA in CH_3CN)
Flow Rate: 1.0mL/min
Temp: 30°C
Detection: 215nm
Injection: 50µL carboxymethylated apohemoglobin tryptic digest in 50mM NH_4HCO_3
Gradient: 0-100%B in 65 min

Discovery BIO Wide Pore C5

Discovery BIO Wide Pore C5 was designed for the efficient and reliable separation of proteins and peptides, especially hydrophobic peptides, by RP- HPLC. Long-chain phases, like C8 or C18, are often too hydrophobic for proteins and can cause excessively long retention time or even irreversible binding to the column. For this reason short-chain phases, typically C3 or C4, are often used for RP-HPLC of proteins. However, these short-chain phases are susceptible to hydrolysis resulting in short column lifetime, especially at low pH. The Discovery BIO Wide Pore C5 gives elution order similar to a conventional C4, yet has enhanced pH stability for longer column lifetime. Generally, higher efficiency separations are achievable on the Discovery BIO Wide Pore C5 because of the improvements we have made to the silica and bonded phase chemistry.

PROPERTIES:	
Bonded Phase:	Covalently-bonded pentylsilane, endcapped
Silica:	Spherical, high purity (<10ppm metals)
Particle Size:	3, 5, and 10mm
Pore Size:	300Å
Surface Area:	100m²/g
%C:	~3%
Coverage:	~4 μmoles/m²



Excellent Performance for Proteins

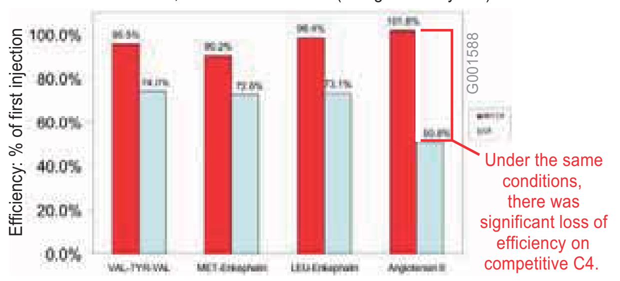
Column: Discovery BIO Wide Pore C5, 15cm x 4.6mm, 5μm
Cat. No.: 568422-U
Mobile Phase: (A) 0.1% (v/v) TFA in H_2O : MeCN (75:25), (B) 0.1% (v/v) TFA in H_2O : MeCN (25:75)
Flow Rate: 1mL/min
Temp: 30°C
Det.: 220nm
Inj.: 10μL
Sample: Protein Test Mix in 0.1% TFA
Gradient: 0-100% B in 25 min.

PARTICLE SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
DISCOVERY BIO WIDE PORE C5 (300Å, 3.5% CARBON)			
3μm	5 x 0.32	65531-U	
3μm	10 x 0.32	65532-U	
3μm	5 x 0.5	65520-U	
3μm	10 x 0.5	65521-U	
3μm	5 x 1.0	65511-U	
3μm	10 x 1.0	65512-U	
3μm	5 x 2.1	567226-U	
3μm	10 x 2.1	567227-U	
3μm	15 x 2.1	567228-U	
3μm	5 x 4.6	567229-U	
3μm	10 x 4.6	567230-U	
3μm	15 x 4.6	567231-U	
5μm	15 x 0.32	65533-U	
5μm	15 x 0.5	65522-U	
5μm	15 x 1.0	65513-U	
5μm	5 x 2.1	568400-U	
5μm	10 x 2.1	568401-U	
5μm	15 x 2.1	568402-U	
5μm	25 x 2.1	568403-U	
5μm	5 x 4.0	568410-U	
5μm	10 x 4.0	568411-U	
5μm	15 x 4.0	568412-U	
5μm	25 x 4.0	568413-U	
5μm	5 x 4.6	568420-U	
5μm	10 x 4.6	568421-U	
5μm	15 x 4.6	568422-U	
5μm	25 x 4.6	568423-U	
5μm	25 x 10.0	568430-U	
10μm	25 x 4.6	567232-U	
10μm	5 x 10.0	567233-U	
10μm	15 x 10.0	567234-U	
10μm	25 x 10.0	567235-U	
10μm	5 x 21.2	567236-U	
10μm	15 x 21.2	567237-U	
10μm	25 x 21.2	567238-U	
Supelguard Cartridges			
3μm	2 x 2.1 (2/pk)	567278-U	
3μm	2 x 2.1 kit **	567279-U	
3μm	2 x 4.0 (2/pk)	567280-U	
3μm	2 x 4.0 kit **	567281-U	
5μm	2 x 2.1 (2/pk)	568470-U	
5μm	2 x 2.1 kit **	568471-U	
5μm	2 x 4.0 (2/pk)	568472-U	
5μm	2 x 4.0 kit **	568473-U	
10μm	1 x 10.0	567286-U	

** Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

Increased Stability of Discovery BIO Wide Pore C5 over Conventional C4

Efficiency on Discovery BIO Wide Pore C5 is stable even after 25,000 column volumes (222 gradient cycles).



Liquid Chromatography

HPLC: Biopolymers
Ion Exchange Chromatography

MCI GEL Ion Exchange Columns

In ion exchange purifications and analyses of biopolymers, ProtEx-DEAE columns (diethylaminoethyl functionality) and ProtEx-SP columns (sulfopropyl functionality) offer significant benefits:

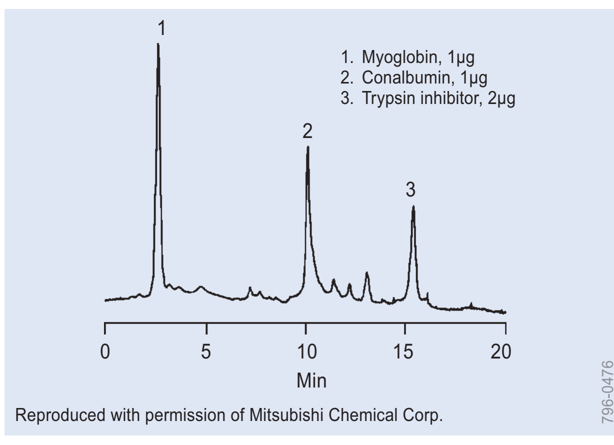
- Excellent separations of protein isoforms
- High resolution at low sample load
- Quantitative recovery – a hydrophilic surface eliminates protein adsorption
- High efficiency (plate number)

ProtEx columns are 5.0cm x 4.6mm ID polyetheretherketone (PEEK) hardware packed with high performance, monodisperse, 5µm, 1000Å macroporous polymethacrylate beads with a chemically bonded, crosslinked hydrophilic surface. Physical characteristics of these columns are summarized below.

We especially recommend ProtEx columns for applications that require high resolution, such as separating protein isoforms, isozymes, or DNA.

COLUMN CHARACTERISTICS	
Dimensions:	5.0cm x 4.6mm ID
Bed Volume:	0.83mL
Flow Rate:	0.5-1.5mL/min
Maximum Backpressure:	735psi (4.9MPa)
Temperature:	4°C - 50°C
pH:	2 - 11
Maximum Loading Capacity:	10mg/injection

Proteins at Low Loading Concentrations



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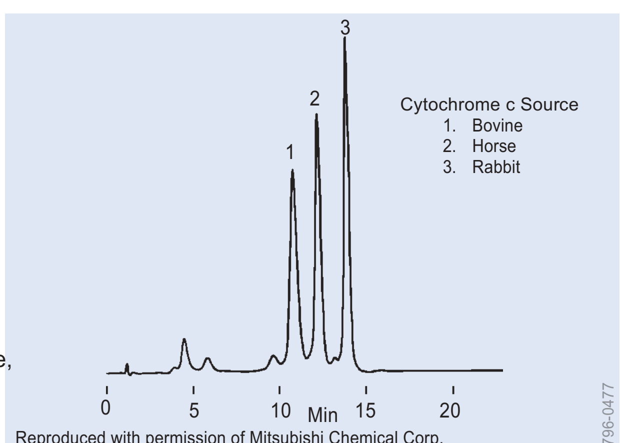
Column: ProtEx-DEAE, 5.0cm x 4.6mm ID, 5µm particles
Cat. No.: 54742
Mobile Phase: A = 20mM Tris HCl, pH 8.2
B = A + 0.5M NaCl
0% B – 100% B in 20 min
Flow Rate: 0.5mL/min
Det.: UV, 280nm

Cytochrome c Species Variants by Ion Exchange HPLC



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Column: ProtEx-SP, 5.0cm x 4.6mm ID, 5µm particles
Cat. No.: 54740-U
Mobile Phase: A = 20mM Bis-Tris HCl, pH 7.0; B = A + 0.5M NaCl
24% B – 69% B in 20 min
Flow Rate: 0.5mL/min
Det.: UV, 280nm
Inj.: 10µg each isoform

DESCRIPTION	PART. SIZE	LENGTH x ID (cm x mm)	CAT. NO.	PRICE
ProtEx-SP	5µm	5 x 4.6	54740-U	
ProtEx-DEAE	5µm	5 x 4.6	54742	

Amersham Biosciences Ion Exchange Columns

Mono columns are highly efficient, pH-stable columns designed for high performance ion exchange separations of proteins, peptides, and polynucleotides, in applications including peptide mapping and monoclonal antibody purification. The unique properties of these columns are based on MonoBeads support – a beaded hydrophilic material with the narrowest particle size distribution of any chromatographic support. This monodispersity permits high flow rates at relatively low backpressures.

DESCRIPTION	QTY.	CAT. NO.	PRICE
AMERSHAM BIOSCIENCES ION EXCHANGE COLUMNS			
Mono Q HR5/5	1	54807	
Mono S HR5/5	1	54808	
Mono P HR5/5	1	54809	
CHROMATOFOCUSING REAGENTS			
Pharmalyte 8-10.5	25mL	P2147-25ML	
Polybuffer 74	100mL	P9652-100ML	
Polybuffer 74	250mL	P9652-250ML	
Polybuffer 96	25mL	P9777-25ML	
Polybuffer 96	100mL	P9777-100ML	
Polybuffer 96	250mL	P9777-250ML	

RELATED INFORMATION	
Request free literature by phone or fax, or see our website.	
No.	Subject
T496177	ProtEx ion exchange HPLC columns
T194882	Mobile phases for ion exchange and chromatofocusing
T495047	Mono Q and Mono S columns

TSK-GEL Ion Exchange Columns

TSK-GEL ion exchange columns are highly efficient and combine sample purification with excellent recovery rates. Anion- and cat exchangers are available on porous polymer-based and silica-based matrices, and in nonporous resin (NPR) columns. The various column types are described in the table below.

TSK-GEL 5PW and NPR ion exchange columns are stable between pH 2-12. TSK-GEL SW columns can be used from pH 2-7.5. TSK-GEL 2SW ion exchange columns (125Å pores) are best suited for small molecular weight solutes, such as nucleotides. Larger biomolecules, including peptides and small proteins, can be analyzed on TSK-GEL 3SW ion exchange columns (250Å pores). The wide-pore (1000Å), polymer-based 5PW columns are suitable for analyses and purifications of large proteins and nucleic acids. Sample capacity for a 7.5cm x 7.5mm 5PW ion exchange column is approximately 1mg.

Proteins and nucleic acids can be analyzed 3-5 times faster on a nonporous TSK-GEL NPR column. The sample capacity of these columns for proteins is, however, 50-100 times smaller. TSK-GEL DEAE-NPR columns are commonly used to separate DNA fragments, particularly those obtained from the polymerase chain reaction (PCR). We strongly recommend using a DEAE-NPR guard column to protect the analytical column when analyzing PCR fragments. SP-NPR columns can provide fast results in hemoglobin A1c screening. Due to their small particle size (2.5µm), packings in TSK-GEL NPR columns must be protected by using a precolumn filter containing a 0.5µm frit (Rheodyne in-line filter or Sigma-Aldrich precolumn filter).

CHARACTERISTIC	DEAE-5PW	DEAE-3SW	DEAE-2SW	DEAE-NPR
ANION EXCHANGE COLUMNS				
Matrix	hydrophilic resin	silica	silica	hydrophilic resin
Particle Size (µm)	10	10	5	2.5
Pore Size (Å)	1000	250	125	nonporous
Functional Group	$-CH_2CH_2N^+(C_2H_5)_2$	$-CH_2CH_2N^+(C_2H_5)_2$	$-CH_2CH_2N^+(C_2H_5)_2$	$-CH_2CH_2N^+(C_2H_5)_2$
Counter Ion	Cl^-	Cl^-	Cl^-	Cl^-
pH Range	2 - 12	2 - 7.5	2 - 7.5	2 - 12
Exclusion Limit (PEG, Dalton)	1,000,000	30,000	10,000	500
Capacity (mg BSA/mL)	30	120	not available	5
Small Ion Capacity	>0.1meq/mL	>0.3meq/g	>0.3meq/mL	>0.15meq/mL
pKa	11.2	11.2	11.2	11.2

CHARACTERISTIC	SP-5PW	SP-NPR	CM-5PW	CM-2SW	CM-3SW
CATION EXCHANGE COLUMNS					
Matrix	hydrophilic resin	hydrophilic resin	hydrophilic resin	silica	silica
Particle Size (µm)	10	2.5	10	5	10
Pore Size (Å)	1000	nonporous	1000	125	250
Functional Group	$-CH_2CH_2CH_2SO_3$	$-CH_2CH_2CH_2SO_3$	$-CH_2COO^-_3$	$-CH_2COO^-_3$	$-CH_2COO^-_3$
Counter Ion	Na^+	Na^+	Na^+	Na^+	Na^+
pH Range	2 - 12	2 - 12	2 - 12	2 - 7.5	2 - 7.5
Exclusion Limit (PEG, Dalton)	1,000,000	500	1,000,000	10,000	30,000
Capacity (mg Hb/mL)	40	5	45	—	110
Small Ion Capacity	>0.1meq/mL	>0.1meq/g	>0.1meq/mL	>0.3meq/mL	>0.3meq/mL
pKa	2.3	2.3	4.2	4.2	4.2

TSK-GEL Ion Exchange (Anion) Columns

DESCRIPTION	PART. SIZE	LENGTH x ID (cm x mm)	CAT. NO.	PRICE
TSK-GEL ION EXCHANGE (ANION) COLUMNS				
DEAE-NPR	2.5µm	3.5 x 4.6	813075	
DEAE-5PW	10µm	7.5 x 7.5	807164	
DEAE-2SW	5µm	25 x 4.6	807168	
Guard Columns and Kits				
DEAE-NPR	5µm	0.5 x 4.6	817088	
DEAE-5PW[1]	20µm	1 x 6.0 kit	807210	
DEAE-SW[1]	10µm	1 x 6.0 kit	807648	

TSK-GEL Ion Exchange (Cation) Columns

DESCRIPTION	PART. SIZE	LENGTH x ID (cm x mm)	CAT. NO.	PRICE
TSK-GEL ION EXCHANGE (CATION) COLUMNS				
CM-5PW	10µm	7.5 x 7.5	813068	
SP-NPR	2.5µm	3.5 x 4.6	813076	
SP-5PW	10µm	7.5 x 7.5	807161	
CM-2SW	5µm	25 x 4.6	807167	
CM-3SW	10µm	7.5 x 7.5	807162	
Guard Column Kits				
CM-5PW[1]	20µm	1 x 6.0 kit	813069	
SP-5PW[1]	20µm	1 x 6.0 kit	807211	
CM-SW[1]	10µm	1 x 6.0 kit	807650	

[1] Kits include one cartridge, a stand-alone holder, 5mL packing, 5cm of 1/16" tubing, and 2 nuts and ferrules.

RELATED INFORMATION

Request free literature by phone or fax, or see our website.

No.	Subject
T494077	TSK-GEL Ion Exchange Columns
T109862	TSK-GEL NPR Columns

HPLC: Biopolymers
Gel Filtration Chromatography

Amersham Biosciences Gel Filtration Columns

For use in an HPLC system, use unions described under Accessories for HR and HiLoad Columns

Fast Desalting Column - Ready for use in an FPLC system.

Superdex HR Column - Filter Kit HR 10, a filter tool, wrench, and instructions are included with Superdex HR columns.

Hi-Load Superdex Column - A dismantling tool, support screen, 10mm net ring, O-ring, and domed nut (in an accessory bag) are included.

DESCRIPTION	EXCLUSION LIMIT	SEPARATION RANGE (PROTEINS)
Fast Desalting Column	>5,000 Da	1,000-4,000
Superdex Peptide	>20,000 Da	100-7,000
Superdex 75	>100,000 Da	3,000-70,000
Superdex 200	>300,000 Da	10,000-600,000

DESCRIPTION	QTY.	CAT. NO.	PRICE
FAST DESALTING COLUMNS			
Fast Desalting HR10/10		54804-U	
PD-10 Columns[1]	30	54805	
Empty PD-10[1]	50	54806	
GEL FILTRATION COLUMNS			
Superdex Peptide HR10/30		504165	
Superdex 75 HR10/30		54800-U	
Superdex 200 HR10/30		54801-U	
HiLoad 16/60 Superdex 75 PG		54802-U	
ACCESSORIES FOR HR AND HILOAD COLUMNS[2]			
Filters, HR 5	10	54860-U	
Filter Tool	1	54863	
Unions, 1/16", female to M6 female			
Waters	2	54866	
Valco Adapter, male 10-32/female M6	2	54868	
Column End Plugs (Domed Nut) for M6 male	4	54865	
Capillary Tubing, PTFE 2m x 1.8mm OD x 0.5mm ID		54870	

[1] Syringe barrel sample preparation columns; cannot be connected to any system.

[2] Amersham Biosciences columns are supplied ready for installation into an FPLC system. To connect these columns to an HPLC system, use one of the three listed unions.

RELATED INFORMATION

Request free literature by phone or fax, or see our website.

No.	Subject
T494076	TSK-GEL SW and SW_{XL} Columns

TSK-GEL Gel Filtration Columns (Silica-Based)

TSK-GEL SW and TSK-GEL SW_{XL} columns contain silica-based, hydrophilic bonded phase packings that minimize interaction with proteins. Properties of these popular columns are summarized in the table below. A 30cm TSK-GEL SW_{XL} column and a 60cm TSK-GEL SW column provide similar resolution, but the SW_{XL} column requires half the time. Sample capacity increases in proportion with column length.

Because TSK-GEL SW_{XL} and TSK-GEL SW columns are silica-based, they must be operated within the recommended pH range of 2.5-7.5. Detailed operating conditions are described in the information accompanying the columns. We recommend protecting these columns with the appropriate SW_{XL} or SW guard column.

TSK-GEL QC-PAK columns provide fast, high resolution analyses, especially in quality control applications. The 15cm glass or stainless steel columns are packed with 5µm SW_{XL} SW materials.

TSK-GEL COLUMN	PARTICLE SIZE (µm)	PORE SIZE (Å)	SAMPLE MW (GLOBULAR PROTEINS)
Super SW2000	4	125	$5\text{-}150 \times 10^3$
G2000SW_{XL}	5	125	$5\text{-}150 \times 10^3$
G2000SW	10	125	$5\text{-}100 \times 10^3$
Super SW3000	4	250	$10\text{-}500 \times 10^3$
G3000SW_{XL}	5	250	$10\text{-}500 \times 10^3$
G3000SW	10	250	$10\text{-}500 \times 10^3$
G4000SW_{XL}	8	450	$20\text{-}10{,}000 \times 10^3$
G4000SW	13	450	$20\text{-}10{,}000 \times 10^3$

Mobile Phase: 0.03M NaCl in 0.1M phosphate buffer, pH 7.0

DESCRIPTION	PART. SIZE	LENGTH x ID (cm x mm)	CAT. NO.	PRICE
TSK-GEL GEL FILTRATION COLUMNS				
G2000SW_{XL}	5µm	30 x 7.8	808540	
G3000SW_{XL}	5µm	30 x 7.8	808541	
G4000SW_{XL}	8µm	30 x 7.8	808542	
Super SW2000	4µm	30 x 4.6	818674	
Super SW3000	4µm	30 x 4.6	818675	
G2000SW	10µm	30 x 7.5	805788	
G2000SW	10µm	60 x 7.5	805102	
G3000SW	10µm	30 x 7.5	805789	
G3000SW	10µm	60 x 7.5	805103	
G4000SW	13µm	30 x 7.5	805790	
G4000SW	13µm	60 x 7.5	805104	
TSK-GEL QC-PAK GEL FILTRATION COLUMNS				
GFC 200	5µm	15 x 7.8	816215	
GFC 300	5µm	15 x 7.8	816049	
GFC 300GL[3]	5µm	15 x 8.0	816216	
GUARD COLUMNS				
SW_{XL}	7µm	4 x 6.0	808543	
SW	10µm	7.5 x 7.5	805371	

[3] Glass.

TSK-GEL Gel Filtration Columns (Polymer-Based)

TSK-GEL PW and TSK-GEL PW$_{XL}$ columns are used in high performance gel filtration separations of water-soluble polymers and oligosaccharides. The hydrophilic polymer matrix has excellent chemical and mechanical stability. Although commonly used with aqueous solvents, the polymer is compatible with up to 50% organic solvent.

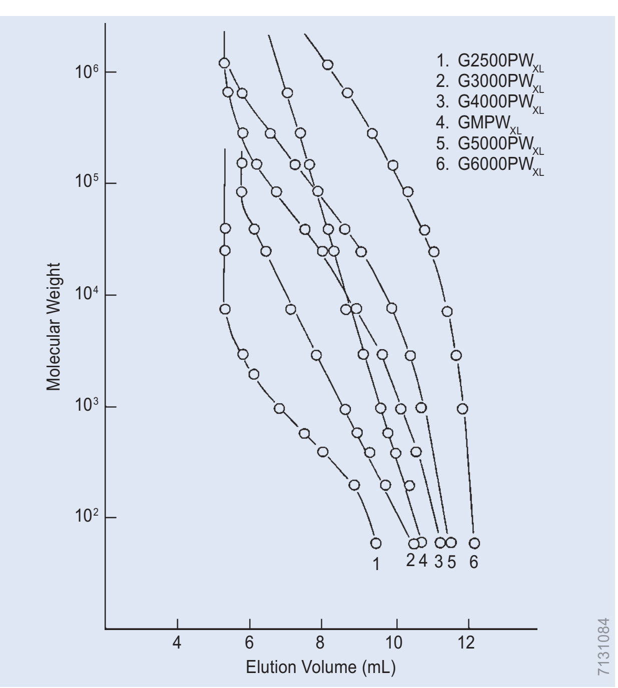
TSK-GEL GMPW and TSK-GEL GMPW$_{XL}$ columns are mixed bed columns with calibration curves that are linear over a wide range of molecular weights. Because the pore volume of a mixed bed column is the same as that for a narrow pore size column, the slope of the calibration curve is much steeper, which limits resolution. Mixed bed columns are ideal for preliminary investigations, when the molecular weight composition of a sample is unknown. Then, unless the molecular weight distribution of the sample is very broad, one selects a second column (or series of columns) with a pore size (or range of pore sizes) that can provide optimum resolution.

TSK-GEL G-Oligo-PW columnsare specially prepared for separating noncharged or cationic oligomers. A small residual positive charge makes G-Oligo-PW and G2000PW columns unsuitable for analyses of anionic oligomers.

COLUMN	PARTICLE SIZE (µm)	PORE SIZE (Å)	SAMPLE MW PEGs/PEOs	SAMPLE MW DEXTRANS
G-Oligo-PW	6	125	<2000	–
G2000PW	10	125	<2000	–
G2500PW$_{XL}$	6	<200	<3000	–
G2500PW	10	<200	<3000	–
G3000PW$_{XL}$	6	200	<50,000	<60,000
G3000PW	10	200	<50,000	<60,000
G4000PW$_{XL}$	10	500	2000-300,000	1000-700,000
G4000PW	17	500	2000-300,000	1000-700,000
G5000PW$_{XL}$	10	1000	4000-1,000,000	50,000-7,000,000
G5000PW	17	1000	4000-1,000,000	50,000-7,000,000
G6000PW$_{XL}$	13	>1000	40,000-8,000,000	500,000-50,000,000
G6000PW	17	>1000	40,000-8,000,000	500,000-50,000,000
G-DNA-PW	10	4000	40,000-8,000,000	–
GMPW$_{XL}$	13	<100-1000	500-8,000,000	<50,000,000
GMPW	17	<100-1000	500-8,000,000	<50,000,000

Mobile Phase: Polyethylene glycols/polyethylene oxides - distilled water
Mobile Phase: Dextrans - 0.2M phosphate buffer, pH 6.8

DESCRIPTION	PART. SIZE	LENGTH x ID (cm x mm)	CAT. NO.	PRICE
TSK-GEL GEL FILTRATION COLUMNS				
G-Oligo-PW	6µm	30 x 7.8	808031	
G2500PW$_{XL}$	6µm	30 x 7.8	808020	
G3000PW$_{XL}$	6µm	30 x 7.8	808021	
G4000PW$_{XL}$	10µm	30 x 7.8	808022	
G5000PW$_{XL}$	10µm	30 x 7.8	808023	
G6000PW$_{XL}$	13µm	30 x 7.8	808024	
GMPW$_{XL}$	13µm	30 x 7.8	808025	
G-DNA-PW	10µm	30 x 7.8	808032	
G2000PW	10µm	30 x 7.5	805761	
G2500PW	10µm	30 x 7.5	808028	
G3000PW	10µm	30 x 7.5	805762	
G4000PW	17µm	30 x 7.5	805763	
G5000PW	17µm	30 x 7.5	805764	
G6000PW	17µm	30 x 7.5	805765	
GMPW	17µm	30 x 7.5	808026	
GUARD COLUMNS				
G-Oligo-PW	12µm	4 x 6.0	808034	
PW$_{XL}$	12µm	4 x 6.0	808033	
PW	12µm	7.5 x 7.5	806762	



PEG/PEO Calibration Curves on TSK-GEL PW$_{XL}$ Columns

Column 1: G2500PW$_{XL}$, 30cm x 7.8mm, 6µm particles
Cat. No.: 808020
Column 2: G3000PW$_{XL}$, 30cm x 7.8mm, 6µm particles
Cat. No.: 808021
Column 3: G4000PW$_{XL}$, 30cm x 7.8mm, 10µm particles
Cat. No.: 808022
Column 4: GMPW$_{XL}$, 30cm x 7.8mm, 13µm particles
Cat. No.: 808025
Column 5: G5000PW$_{XL}$, 30cm x 7.8mm, 10µm particles
Cat. No.: 808023
Column 6: G6000PW$_{XL}$, 30cm x 7.8mm, 13µm particles
Cat. No.: 808024
Mobile Phase: DI water
Flow Rate: 1mL/min
Det.: refractive index
Inj.: polyethylene glycols and polyethylene oxides

RELATED INFORMATION

Request free literature by phone or fax, or see our website.

No.	Subject
T494075	TSK-GEL PW and PW$_{XL}$ Columns

HPLC: Biopolymers

Reversed-Phase, Affinity, Hydrophobic Interaction Chromatography

SUPELCOSIL Wide Pore (300Å) Reversed-Phase Columns

SUPELCOSIL Wide Pore reversed-phase columns continue to b available. Discovery BIO Wide Pore reversed-phase columns provide enhanced performance and are recommended as the fi choice. The Discovery BIO Wide Pore columns can be found on the preceding pages.

PARTICLE SIZE	LENGTH x ID (cm x mm)	CAT. NO.	PRICE
SUPELCOSIL LC-318 (300Å, 6.0% CARBON)			
5µm	5 x 4.6	58852	
5µm	25 x 4.6	58858	
SUPELCOSIL LC-318 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59512	
5µm	2 x 4.0 kit [1]	59502	
SUPELCOSIL LC-308 (300Å, 3.5% CARBON)			
5µm	5 x 4.6	58851	
5µm	25 x 4.6	58857	
SUPELCOSIL LC-308 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59511-U	
5µm	2 x 4.0 kit [1]	59501	
SUPELCOSIL LC-3DP (300Å, 4.0% CARBON)			
5µm	25 x 4.6	58859	
SUPELCOSIL LC-3DP Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59513	
SUPELCOSIL LC-304 (300Å, 2.7% CARBON)			
5µm	5 x 4.6	58823	
5µm	25 x 4.6	58824	
SUPELCOSIL LC-304 Supelguard Cartridges			
5µm	2 x 4.0 (2/pk)	59592	
5µm	2 x 4.0 kit [1]	59591	
SUPELCOSIL LC-3Si (300Å)			
5µm	25 x 6.2	58965	
SUPELCOSIL LC-Si Supelguard Cartridges (use for LC-3Si)			
5µm	2 x 4.0 (2/pk)	59560	
5µm	2 x 4.0 kit [1]	59550	

RELATED INFORMATION

Request free literature by phone or fax, or see our website.

No.	Subject
T100795	Separating Proteins and Peptides using SUPELCOSIL columns.
T494079	TSK-GEL Reversed-Phase Columns
T494080	TSK-GEL Affinity Columns
T494078	TSK-GEL Hydrophobic Interaction Columns

TSK-GEL Reversed-Phase Columns

DESCRIPTION	PART. SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
SILICA-BASED COLUMNS				
Oligo-DNA RP	5µm	15 x 4.6	813352	
Super ODS	2µm	5 x 4.6	818154	
Super ODS	2µm	10 x 4.6	818197	
Guard Filters				
Filter		(3/pk)	818207	
Stand-Alone Holder for Filters			818206	
RESIN-BASED COLUMNS				
C18-NPR	2.5µm	3.5 x 4.6	814005	
C18-4PW	7µm	15 x 4.6	813351	
Phenyl-5PW RP	10µm	7.5 x 4.6	808043	
Guard Filters				
Filter		(3/pk)	818207	
Stand-Alone Holder for Filters			818206	

TSK-GEL Affinity Columns

DESCRIPTION	PART. SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
ABA-5PW	10µm	7.5 x 7.5	813067	
Boronate-5PW	10µm	7.5 x 7.5	813066	
Chelate-5PW	10µm	7.5 x 7.5	808645	
Guard Column Kits [2]				
Chelate-5PW	20µm	1 x 6.0 kit	808647	
Heparin-5PW	20µm	1 x 6.0 kit	813121	

TSK-GEL Hydrophobic Interaction (HIC) Columns

DESCRIPTION	PART. SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
Butyl-NPR	2.5µm	3.5 x 4.6	814947	
Ether-5PW	10µm	7.5 x 7.5	808641	
Phenyl-5PW	10µm	7.5 x 7.5	807573	
Guard Column Kits [2]				
Phenyl-5PW	20µm	1 x 6.0 kit	807652	

[1] Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.
[2] Kits include one cartridge, a stand-alone holder, 5mL packing, 5cm of 1/16" tubing, and 2 nuts and ferrules.

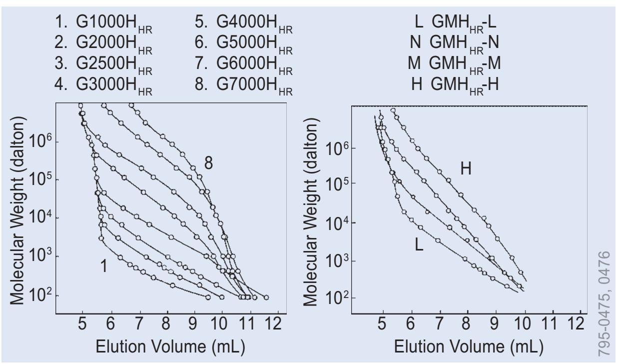
TSK-GEL Gel Permeation Columns

TSK-GEL H_{HR} gel permeation columns are stable in solvents having a wide range of polarities. The particles do not swell or shrink as the solvent is changed from toluene through methanol. However, these columns cannot be used with polar solvents, such as water or water:methanol mixtures. Spherical 5µm polystyrene/divinylbenzene particles provide a minimum of 16,000 plates per 30cm x 7.8mm ID column. Eight pore sizes are available (Figure A), ranging from an exclusion limit of about 2000 Dalton for G1000H_{HR} columns to more than 10,000,000 Dalton for G7000H_{HR} columns. The four mixed bed columns (H, L, M, N) feature extended linear molecular weight operating ranges for sample screening or more formal analyses.

Using Calibration Curves

GPC is widely used for fingerprinting molecular weights of industrial polymers. For compounds of similar molecular shape, a sigmoidal calibration curve is obtained by plotting the logarithm of molecular weight (MW) versus the elution volume (V_e) for molecules of known weight. The optimal separation range is defined by the linear portion of this curve. Once a calibration curve is prepared, the elution volume for a polymer of similar shape, but unknown weight, can be used to determine the MW. Results are most accurate when the investigator prepares the calibration curve and determines the molecular weight of the unknown molecule on the same day, with the same mobile phases, etc.

COLUMN	ANALYTE MOLECULAR WEIGHT RANGE (DALTON)
G1000H	<1500
G2000H	<4000
G2500H	<1.2 x 10^4
G3000H	<3.0 x 10^4
G4000H	<5.5 x 10^5
G5000H	<1.5 x 10^6
G6000H	<~1 x 10^7
G7000H	<~5 x 10^7
GMH-H	<~1 x 10^7
GMH-L	<1.0 x 10^4
GMH-M	<1.0 x 10^6



Sample Elution by Molecular Weight

Columns: TSK-GEL H_{HR}, 30cm x 7.8mm ID, 5µm particles
Mobile Phase: tetrahydrofuran
Temp.: ambient
Flow Rate: 1mL/min
Det.: UV, 254nm
Inj.: polystyrene standards

DESCRIPTION	PART. SIZE	LENGTH X ID (cm x mm)	CAT. NO.	PRICE
TSK-GEL H_{HR} GEL PERMEATION COLUMNS				
G1000H_{HR}	5µm	30 x 7.8	817352	
G2000H_{HR}	5µm	30 x 7.8	817353	
G2500H_{HR}	5µm	30 x 7.8	817354	
G3000H_{HR}	5µm	30 x 7.8	817355	
G4000H_{HR}	5µm	30 x 7.8	817356	
G5000H_{HR}	5µm	30 x 7.8	817357	
G7000H_{HR}	5µm	30 x 7.8	817359	
GMH_{HR}-L	5µm	30 x 7.8	817362	
GMH_{HR}-M	5µm	30 x 7.8	817392	
GMH_{HR}-H	5µm	30 x 7.8	817360	
Guard Columns				
H_{HR}-L[1]	7µm	4 x 6.0	817368	
TSK-GEL H_{XL} GEL PERMEATION COLUMNS				
G1000H_{XL}	5µm	30 x 7.8	816131	
G2000H_{XL}	5µm	30 x 7.8	816134	
G2500H_{XL}	5µm	30 x 7.8	816135	
G3000H_{XL}	6µm	30 x 7.8	816136	
G4000H_{XL}	6µm	30 x 7.8	816137	
G5000H_{XL}	9µm	30 x 7.8	816138	
G6000H_{XL}	9µm	30 x 7.8	816139	
GMH_{XL}	9µm	30 x 7.8	816141	
GMH_{XL}-HT	13µm	30 x 7.8	807112	
Guard Columns				
H_{XL}-L[2]	6µm	4 x 6.0	807113	
H_{XL}-H[3]	13µm	4 x 6.0	813727	

[1] Use to protect G1000H_{HR} – G4000H_{HR} and GMH_{HR}-L columns.
[2] Use to protect G1000H_{XL} – G4000H_{XL} columns.
[3] Use to protect G5000H_{XL} – G6000H_{XL} columns.

RELATED INFORMATION

Request free literature by phone or fax, or see our website.

No.	Subject
T496085	TSK-GEL H_{HR} Columns

HPLC

Custom, Endfittings, Connectors

Custom Prepared HPLC Columns

If the column of your choice is not listed as a stock product in our catalog, we may be able to prepare it for you as a custom. Listed below is our current listing of available packings. For the most current listing of hardware, and a price quotation, please contact Technical Service.

Delivery:
We typically ship custom prepared analytical HPLC columns within 5 to 7 business days to anywhere in the world. Larger sizes (preparative and special requests) may take longer.

Performance Testing:
Supelco's custom prepared columns are fully tested for efficiency and symmetry. Please let us know if you have special test cr

PHASE	PARTICLE SIZE (µm)
DISCOVERY BIO WIDE PORE PHASES	
RP-AmideC16	5
C18	5
C8	5
Cyano	5
DISCOVERY HS PHASES	
C18	3
C18	5
C18	10
F5	3
F5	5
F5	10
PEG	3
PEG	5
PEG	10
DISCOVERY BIO WIDE PORE PHASES	
C18	3
C18	5
C18	10
C8	3
C8	5
C8	10
C5	3
C5	5
C5	10
SUPELCOSIL PHASES	
ABZ+Plus	3
ABZ+Plus	5
ABZ+Plus	12
LC-ABZ	5
Suplex pKb-100	5
LC-18-DB	3
LC-18-DB	5
LC-18	3
LC-18	5
LC-18	12

PHASE	PARTICLE SIZE (µm)
SUPELCOSIL PHASES, CONT'D	
LC-318	5
LC-DABS	3
LC-PAH	3
LC-PAH	5
LC-18-S	5
LC-18-T	3
LC-18-T	5
LC-8-DB	3
LC-8-DB	5
LC-8	3
LC-8	5
LC-8	12
LC-308	5
LC-DP	5
LC-3DP	5
LC-F	5
LC-304	5
LC-CN	3
LC-CN	5
LC-PCN	5
LC-1	5
LC-Si	3
LC-Si	5
LC-Si	12
LC-NH_2	3
LC-NH_2	5
LC-NH_2-NP	5
LC-Diol	5
LC-3Diol	5
SAX1	5
LC-SCX	5
Hisep	5
SUPELCOGEL PHASES	
TPR-100	5
ODP-50	5
Ion Exclusion	9

Replacement Discovery/SUPELCOSIL Column Endfittings

55200-U

Use when fittings become damaged or with replacement cartridges. (pk. of 2)

DESCRIPTION	CAT. NO.	PRICE
For 2.1mm columns	55201-U	
For 3.0mm, 4.0mm, 4.6mm columns	55200-U	

Column Connector

55213

Connects two Discovery or SUPELCOSIL analytical columns. Zero dead volume design.

DESCRIPTION	CAT. NO.	PRICE
Column Connector	55213	

Pharmaceutical Applications

SUPELCO

www.sigma-aldrich.com/supelco

TABLE OF CONTENTS

Analyte	Page	HPLC Column	SPE Tube
Acetaminophen and Caffeine from Serum	4		Discovery DSC-18Lt
α-Hydroxy Aliphatic Acids	4	Discovery RP-AmideC16	
Alkaloids	5	Discovery RP-AmideC16, C18, and C8	
Aniline Homologs	5	Discovery C18	
Antibiotics (ß-Lactam)	6	Discovery RP-AmideC16, C18, C8, and Cyano	
Antibiotics (Cephalosporins)	6	Discovery RP-AmideC16, C18, and C8	
Antibiotics (Cephalosporins from Serum)	7		Discovery DSC-18Lt
Antibiotics (Fluoroquinolone from Tablets)	7	Discovery RP-AmideC16, C18, and HS C18	
Antibiotics (Peptides)	8	Discovery RP-AmideC16 and C18	
Antibiotics (Sulfa Drugs)	8	Discovery C18	
Antibiotics (Sulfa Drugs from Serum)	9		Discovery DSC-18
Anticonvulsants	9	Discovery RP-AmideC16, C18, and C8	
Anticonvulsant Compounds from Serum	10		Discovery DSC-18Lt
Anticonvulsants/Anxiolytics from Serum	10		Discovery DSC-18
Antidepressants (Tricyclic)	11	Discovery C18 and C8	
Antidepressants (Tricyclic) from Serum	11		Discovery DSC-18
Antihypertensive-Diuretic Combination	12	Discovery RP-AmideC16	
Antihypertensive ACE Inhibitors	12	Discovery RP-AmideC16	
Antihypertensive Drugs	13	Discovery RP-AmideC16	
Antihypertensive/Antiarrhythmic Compounds from Serum	13		Discovery DSC-18
Antipyretics/Analgesics/Antifungals	14	Discovery RP-AmideC16	
Antitussives/Antihistamines/Antipyretics	14	Discovery RP-AmideC16, C18, and C8	
Antiulcer Compounds	15	Discovery RP-AmideC16, C18, C8, and HS C18	
Antiulcer Compounds from Serum	15		Discovery DSC-18
Barbiturates	16	Discovery RP-AmideC16, C18, and C8	
Barbiturates from Serum	16		Discovery DSC-18Lt
Benzalkonium Chlorides	17	Discovery Cyano	
Bronchodilator (Caffeine Metabolites) from Serum	17		Discovery DSC-18
Catecholamines	18	Discovery C18	
Chlorophyll from Methanolic Plant Extracts	18		Discovery DPA-6S
Cold Remedy Ingredients	19	Discovery RP-AmideC16, C18, C8, and Cyano	
Fluoxetine (Prozac)	19	Discovery RP-AmideC16, C18, C8, and Cyano	
Humic Acids from Water	20		Discovery DPA-6S
Nonsteroidal Antiinflammatory Drugs	20	Discovery RP-AmideC16	
Nonsteroidal Antiinflammatory Drugs from Serum	21		Discovery DSC-18
Organic acids	21	Discovery C18 and HS C18	
Parabens	22	Discovery RP-AmideC16, C18, C8, and Cyano	
Peptides (Angiotensins)	22	Discovery C18	
Peptides (Carboxyamidomethylated BSA Tryptic Digest)	23	Discovery RP-AmideC16 and C18	
Peptides (Cytochrome c Tryptic Digest)	23	Discovery RP-AmideC16 and C18	
Peptides (Substance P and Fragments)	24	Discovery C18	
Phloroglucinol from Water	24		Discovery DPA-6S
Proteins (Bovine Insulin)	25	Discovery C8	
Tannic Acid from Water or Methanol	25		Discovery DPA-6S
Vitamins (Fat Soluble, A and E)	26	Discovery C18 and C8	
Vitamins (Fat Soluble, D_2 and D_3)	26	Discovery C18 and HS C18	
Vitamins (Water Soluble)	27	Discovery RP-AmideC16, C18, and C8	

HPLC COLUMNS

HPLC column	Icon	Features
RP-AmideC16 $-O-Si(CH_3)_2-(CH_2)_3NHCO-(CH_2)_{14}-CH_3$	RP-AMIDE	• excellent reproducibility • unique selectivity • excellent retention and resolution of polar compounds • less hydrophobicity than C18 phases • different elution profiles compared to C18 phases • compatible with low organic/highly aqueous mobile phases
C8 $-O-Si(CH_3)_2-(CH_2)_7-CH_3$	C8	• excellent reproducibility • faster separation of strongly hydrophobic analytes • exceptional peak shapes for basic and acidic compounds • excellent stability from pH 2 to pH 8 • suitable for LC/MS applications

HPLC column	Icon	Features
C18 $-O-Si(CH_3)_2-(CH_2)_{17}-CH_3$	C18 HS C18	• excellent reproducibility • exceptional peak shape for basic and acidic analytes • greater (Discovery HS C18) and lesser (Discovery C18) hydrophobic phases available • resolution of geometrical isomers and other structurally closely related compounds • separation of peptides and small proteins (especially Discovery C18) • suitable for LC/MS applications (especially Discovery HS C18)
Cyano $-O-Si(CH_3)_2-(CH_2)_3-CN$	CYANO	• excellent reproducibility • low hydrophobicity, for rapid elution of hydrophobic molecules • retention and separation of strongly basic analytes, including quaternary ammonium salts, with excellent peak shapes • compatible with highly aqueous mobile phases • exceptional stability and column lifetime

SOLID PHASE EXTRACTION TUBES

SPE tube	features
DSC-18	• Polymerically bonded octadecyl (C18)-bonded silica isolates, purifies and concentrates pharmaceuticals and related compounds from aqueous media, like biological fluids • 18% typical %carbon loading for high hydrophobic capacity • Careful quality-control testing ensures consistent lot-to-lot and tube-to-tube chemical and physical properties
DSC-18Lt	• A moderate carbon loading bonded silica (11% carbon) with carbon loading lower than that of its sister product, DSC-18 (18% carbon) • Lower carbon loading is useful when high hydrophobic capacity is not desired, or for extracting polar compounds that need secondary interactions with the silica backbone for efficient extraction. • Ideal for eluting hydrophobic compounds with small volumes of solvent, relative to volumes that are needed when using high-carbon loading silicas

SPE tube	features
DPA-6S	• Polyamide, used to adsorb polar compounds such as those with hydroxyl groups, especially phenolic substances • Polyamide has been used for extracting tannis, chlorophyll, humic acid, pharmacologically active terpenoids, flavonoids, gallic acid, catechol A protocatechuic acid, and phloroglucinol A from aqueous or methanolic solutions • Carboxylic acids, especially aromatic carboxylic acids and compounds with multiple carboxylic acid groups, also can bind with the polyamide sorbent, forming hydrogen bonds

Acetaminophen and Caffeine from Serum

HPLC Conditions:
Discovery C18, 15cm x 4.6mm, 5µm particles,
MeOH: 25mM K_2HPO_4, pH7.0 (3:97)
1.0mL/min
30°C
UV, 220nm
30µL

Discovery SPE Tube:
DSC-18Lt
500mg/3mL

SPE

SPE Procedure, Using Zymark® RapidTrace® SPE Workstation

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

[A] 1mL porcine serum spiked with 0.5µg/mL acetaminophen and 1.0µg/mL caffeine, then diluted with 1mL water.
[B] Eluent evaporated to dryness with nitrogen stream at 30°C, using a Zymark TurboVap® LV Workstation, then reconstituted with 200µL of water.

Acetaminophen and Caffeine from Serum

Neutral antipyretic/analgesic compounds like acetaminophen can be extracted from serum, using Discovery DSC-18Lt SPE tubes and a generic methanol and water-based SPE method. This method is applicable to many other neutral compounds. Recoveries for acetaminophen and caffeine are about 95% with this method.

Acetaminophen | Caffeine

Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Acetaminophen	0.50µg/mL	95.4	±0.9
2. Caffeine	1.0µg/mL	94.3	±1.5

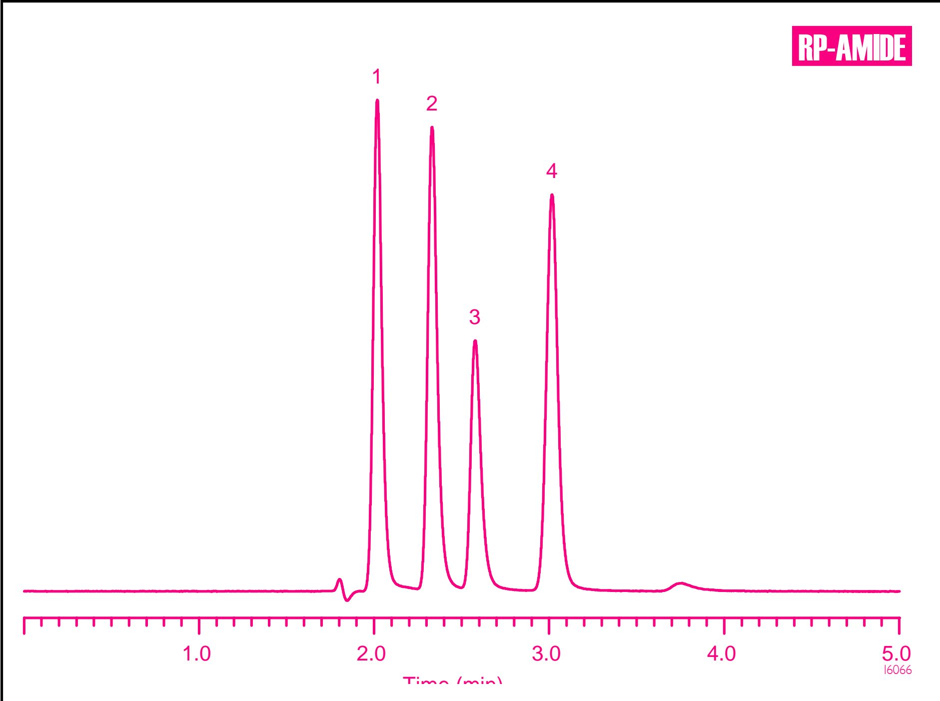
G000243, G000096

α-Hydroxy Aliphatic Acids

Discovery RP-AmideC16
15cm x 4.6mm, 5µm particles,
25mM KH_2PO_4,
pH 3.0
2mL/min
35°C
UV, 220nm
10µL, 1µg/mL each analyte

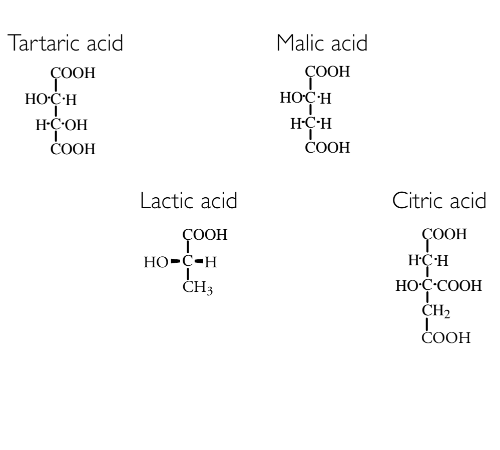
1. Tartaric acid
2. Malic acid
3. Lactic acid
4. Citric acid

RP-AMIDE



α-Hydroxy Aliphatic Acids

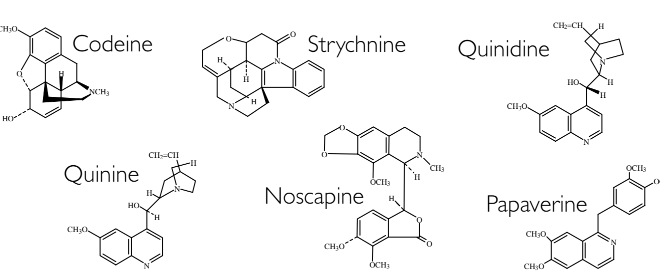
α-Hydroxy aliphatic carboxylic acids are strongly acidic compounds, with pK_a values around 3.0 and can significantly interact with residual silanols. This chromatogram shows the separation of four such hydroxy acids on a Discovery RP-AmideC16 column under buffered acidic mobile phase conditions. This example demonstrates the applicability of the amide-functionalized bonded phase for separations requiring 100% aqueous mobile phases without chain collapse as observed for C18 columns.



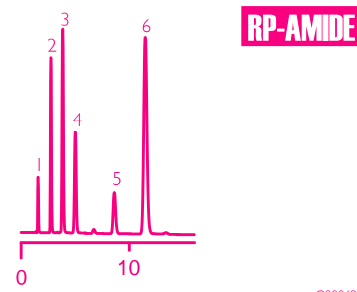
G001241, G001242
G001243, G001244

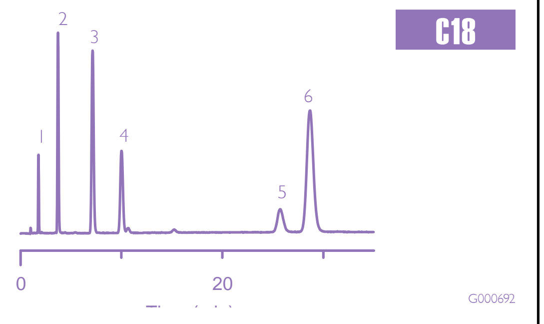
Alkaloids

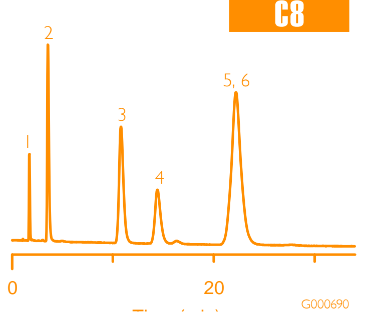
Alkaloids are naturally occurring bases with complex multicyclic ring structures. They are difficult candidates for separation based on RP-HPLC as they are amenable to hydrophobic as well as polar and ionic interactions with a silica-based reversed phase packing material. They can tail significantly due to ion exchange with residual silanols, and mobile phase additives are commonly employed to alleviate this problem. They can be analyzed on a Discovery column under isocratic conditions, without any additive, and show excellent peak symmetry. The RP-AmideC16 column shows the best separation characteristics for the test mixture, with all components eluting within 12 minutes. Under the methanol/ buffer conditions used, papaverine and noscapine coelute on the C8 column. All columns provide excellent separation for quinine and quinidine, which differ only in stereochemistry at the exocyclic carbon to the C4 carbon of the quinoline ring. Papaverine and noscapine, which differ in the number of methoxyls and the absence/presence of a lactone group, also are best resolved on the RP-AmideC16 column.



G000222, G000223, G000220, G000221, G000238, G000239







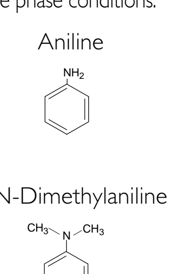
Alkaloids

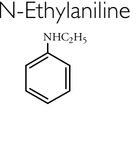
15cm x 4.6mm columns,
5µm particles,
MeOH/25mM KH_2PO_4,
pH 3.0 (20:80)
2mL/min
35°C
UV, 254nm
10µL

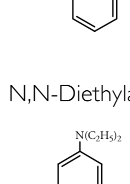
1. Codeine
2. Strychnine
3. Quinidine
4. Quinine
5. Noscapine
6. Papaverine

Aniline Homologs

Aniline derivatives are weak bases if the amine moiety is primary, but progressively become more basic with alkyl substitution on the nitrogen atom. Thus, aniline has a pK_a of 4.63, while N-ethylaniline and N,N-diethylaniline have values of 5.12 and 6.61, respectively. These bases can interact with acidic silanols through ionic interaction. The excellent peak symmetries obtained for six aniline derivatives on a Discovery C18 column indicate the absence of any such reactions under unbuffered mobile phase conditions.



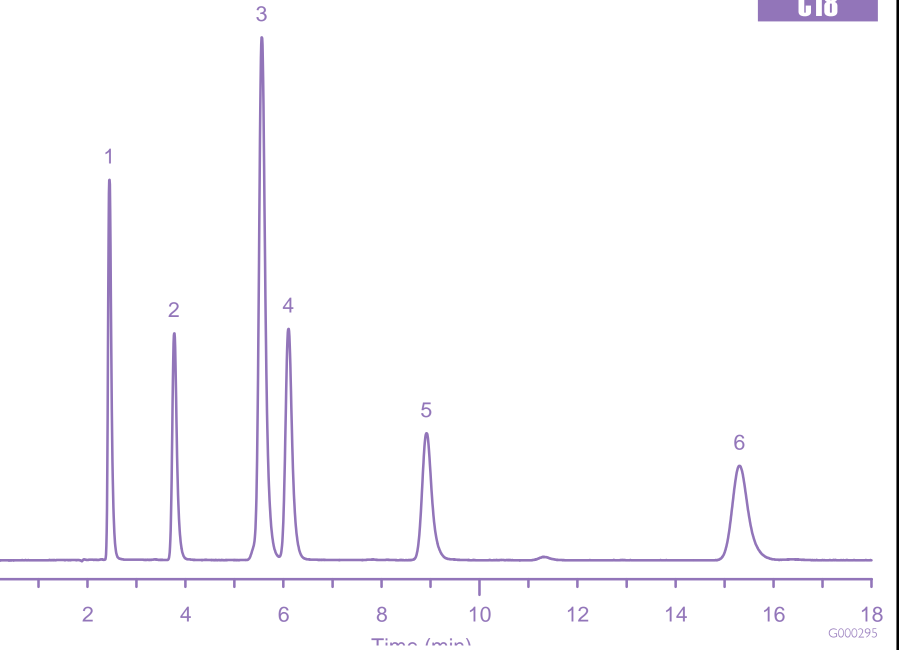




N-Propylaniline

N,N-Diethylaniline

G000739, G001245, G001246,
G000094, G001247, G001248



Aniline Homologs

Discovery C18, 15cm x 4.6mm,
5µm particles,
MeOH:H_2O (60:40)
1mL/min
30°C
UV, 254nm
10µL, 1µg/mL each analyte

1. Aniline
2. N-Ethylaniline
3. N-Propylaniline
4. N,N-Dimethylaniline
5. N-Butylaniline
6. N,N-Diethylaniline

Antibiotics (ß-Lactam):

15cm x 4.6mm columns,
5µm particles,

A: 0.01% TFA in H_2O
B: 0.01% TFA in MeCN
1.5mL/min

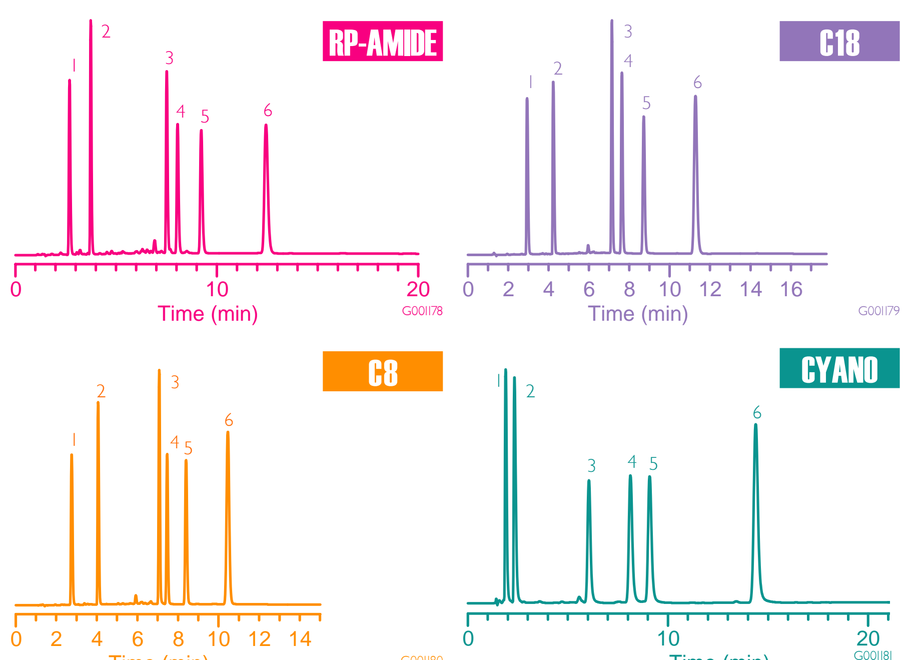
Gradient (RP-AmideC16, C18, C8)

min	%B
0	5
5	35
20	35

Gradient (Cyano)

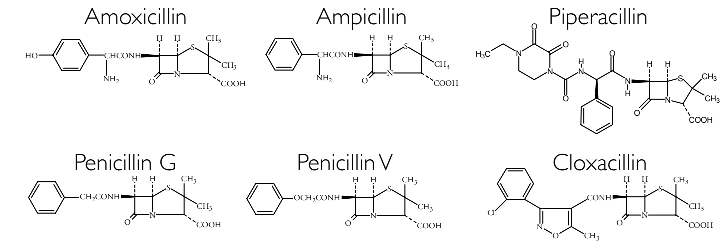
min	%B
0	5
20	25

1. Amoxicillin
2. Ampicillin
3. Piperacillin
4. Penicillin G
5. Penicillin V
6. Cloxacillin



Antibiotics (ß-Lactam):

β-Lactam antibiotics contain 6-aminopenicillanic acid as the basic skeleton and structurally vary from each other with respect to the 6-acyl substituent. These antibiotics are weakly acidic and are rapidly inactivated by strong acids or bases. Amoxicillin is the most polar of the series investigated, due to the phenolic hydroxyl and amino moieties on the acyl chain. Ampicillin, which carries an amino group on its acyl chain, is less polar than amoxicillin. Penicillin G and V are much less polar than amoxicillin or ampicillin, as they do not contain any polar functionality on the acyl chain. Cloxacillin consists of a chlorophenyl-isoxazole moiety on the acyl chain, and is the most hydrophobic due to this feature. The interaction of the stationary phase with the acyl chain appears to be the governing factor in the RP-HPLC of these antibiotics. The carboxylic group is sterically hindered by the dimethyl group on the adjoining carbon of the thiazolidine ring. Since TFA is a strong protonating agent, the functional groups on ampicillin and amoxicillin are protonated, hence these two are less retained. No significant selectivity differences among the Discovery bonded phases are observed under the gradient conditions utilized. The Discovery Cyano column requires a more gradual gradient for optimal resolution.



G001176, G001177, G000929, G001173, G001174, GG001175

Antibiotics (Cephalosporins)

conditions for RP-AmideC16:
15cm x 4.6mm column,
5µm particles,
MeOH:25mM KH_2PO_4,
pH 3.0 (10:90)
2mL/min, 20°C,
254nm, 1µL

conditions for C8, C18:
15cm x 4.6mm column,
5µm particles,
MeOH:25mM KH_2PO_4,
pH 3.0 (20:80)
2mL/min, 20°C,
254nm, 1µL

1. Cefadroxil
2. Cefaclor
3. Cephalexin

RP-AMIDE

G000747

C18

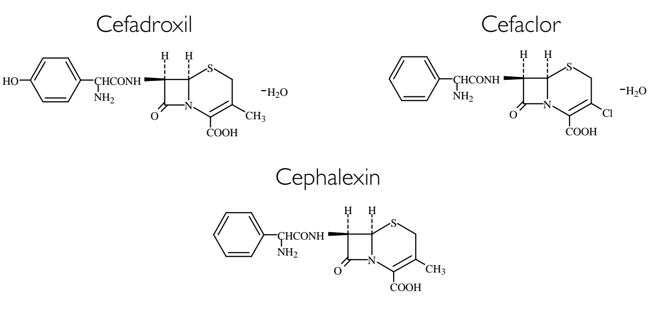
G000746

C8

G000696

Antibiotics (Cephalosporins)

Cephalosporins are weakly acidic antibiotics based on the 5-thia-1-aza bicyclo [4,2,0] oct-2-ene-2-carboxylic acid skeleton, with pK_a values of 5.2 and 7.3. Cefadroxil and cephalexin differ only by a phenolic hydroxyl on the acyl chain of the former. Cefaclor and cephalexin differ in the 3-substituent on the thiazine nucleus, with chlorine the functionality in the former and methyl in the latter. The C18 and C8 columns show similar elution patterns, with the most hydrophobic component, cephalexin, eluting last. A more aqueous mobile phase is needed for the RP-AmideC16 column to separate cefaclor from cephalexin.

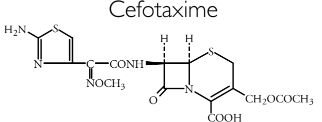


G000683, G000682, G000681

DISCOVERY

Antibiotics (Cephalosporins) from Serum

These closely related sulfur- and nitrogen-containing compounds can be extracted from serum using Discovery DSC-18Lt SPE tubes and a simple SPE method. The recoveries for all compounds are about 90%.



Cefazolin

Cefuroxime

Cefmetazole

G001206, G001207, G001208, G001209

Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Cefotaxime	1.4µg/mL	89.0	±1.2
2. Cefazolin	2.1µg/mL	91.9	±1.1
3. Cefuroxime	2.0µg/mL	89.6	±2.7
4. Cefmetazole	3.3µg/mL	91.1	±2.3

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

Step	Solvent/ Solution	Volume (mL)	Flow Rate (mL/min)	Comments
1. Condition	MeOH	2.0	12.0	conditions sorbent
2. Condition	H_2O	2.0	12.0	conditions sorbent
3. Load	sample	2.1[A]	0.75	applies serum sample
4. Rinse	H_2O	2.0	5.0	washes sample
5. Purge-Cannula	H_2O	4.0	30.0	clean sample cannula
6. Rinse	vent	0.1	2.0	positions SPE tube over waste port
7. Dry	N_2	Time = 3 min		dries sorbent
8. Purge-Cannula	MeOH	4.0	30.0	cleans sample cannula
9. Collect	MeOH	1.0	1.02	elutes analytes into collection vessel
10. Purge-Cannula	MeOH	4.0	30.0	cleans sample cannula
11. Purge-Cannula	H_2O	4.0	30.0	cleans sample cannula

[A] 1mL porcine serum spiked with each analyte, then diluted with 1mL of 0.1m K_2HPO_4 (pH 7.0).

[B] Eluent was evaporated to dryness with a nitrogen stream at 45°C, using a Zymark TurboVap LV Workstation. The residue was reconstituted with 100µL water prior to HPLC analysis.

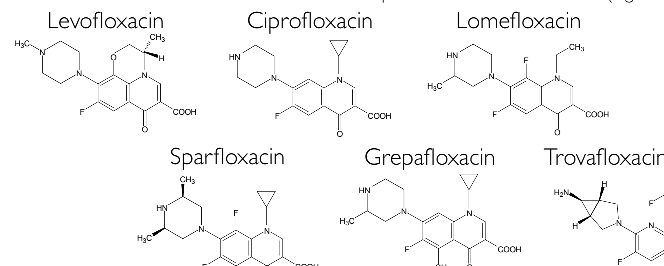
Antibiotics (Cephalosporins) from Serum

HPLC Conditions:
Discovery C18, 15cm x 4.6mm, 5µm particles, preceded by C18 guard column and 0.5µm frit filter
MeCN:20mM KH_2PO_4
pH 3.0 (adjusted with 10% phosphoric acid) (14:86)
1.2mL/min, 30°C, UV, 254nm
10µL reconstituted porcine serum

SPE Tube:
DSC-18Lt
500mg/3mL

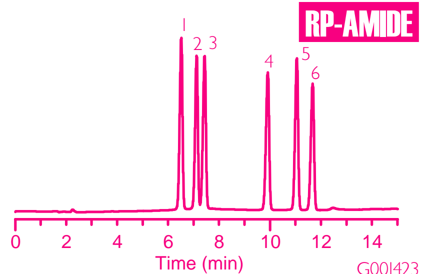
Antibiotics (Fluoroquinolones from Tablets)

Fluoroquinolone antibiotics consist of the 1,4-dihydro-4-oxo-3-quinoline carboxylic acid skeleton (nalidixic acid), along with a 6-fluoro substituent. They differ from each other in the substitution pattern on the quinolone ring nitrogen and on the piperazinyl moiety at the 7- position of the quinoline. The lone exception is trovafloxacin, which has a pyridino-pyridone skeleton. Fluoroquinolone antibiotics are amphoteric, due to the acidic carboxyl group and the basic quinoline and piperazine nitrogens. Their separation under RP-HPLC conditions is governed by the differences in substitution patterns. Thus, the elution order ciprofloxacin<lomefloxacin<sparfloxacin stems from the gradual increase in methyl substitution on the piperazine moiety. Grepafloxacin, with a 5-methyl substituent on the quinoline ring, is retained longer than lomefloxacin. Trovafloxacin, due to the difluorophenyl group on the quinoline nitrogen, is the most hydrophobic of the analytes studied and is eluted last. Under the gradient conditions employed, the only significant difference among the RP-AmideC16 and C18 columns is the longer retention of ciprofloxacin on the former, possibly due to H-bonding interactions. The HS (high surface area) C18 provides somewhat better resolution than the other C18 phase due to more retention (higher k').

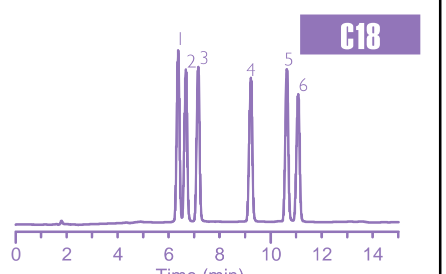


G000971, G000972, G000970, G000973, G000968, G000974

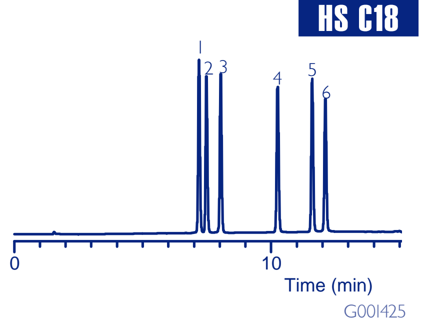
RP-AMIDE



C18



HS C18



Antibiotics (Fluoroquinolones from Tablets)

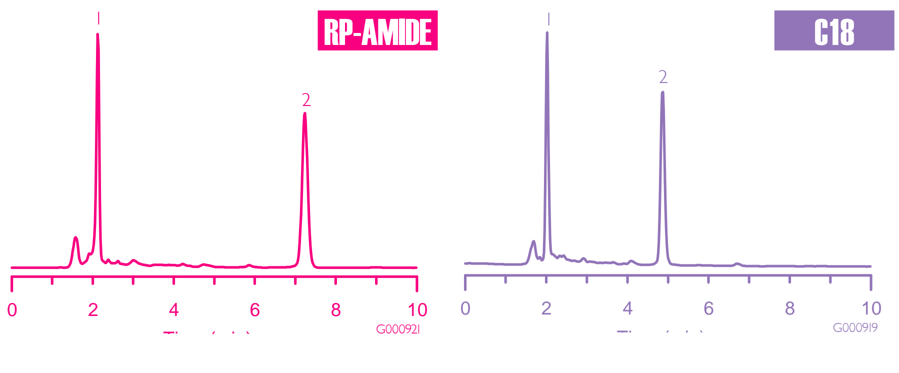
15cm x 4.6mm columns,
(A) 25mM phosphate buffer, pH 3.0
(B) MeCN
10% B to 35% B in 15 min
1mL/min
35°C
UV, 220nm
10µL

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

Antibiotics (Peptides)

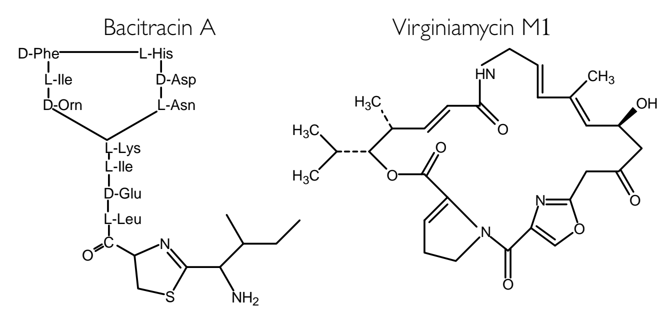
15cm x 4.6mm columns,
5µm particles,
MeCN:25mM KH_2PO_4,
pH 3.0 (40:60)
1mL/min
35°C
UV, 220nm
10µL, 50µg/mL each analyte.

1. Bacitracin A
2. Virginiamycin M1



Antibiotics (Peptides)

Bacitracin A is a cyclic decapeptide, carrying an aminoalkylthiazoline substituent on the terminal leucine moiety. Virginiamycin M1 is a macrocyclic molecule containing an oxazole and a pyrroline ring as part of the cyclic skeleton, and does not contain any amino acid in its structure. Owing to the presence of multiple amide linkages, bacitracin A is much more polar than Virginiamycin M1 and is eluted first. Virginiamycin M1 is retained longer on the RP-AmideC16 column, since it potentially undergoes dipolar interactions through the keto and ester functionalities with the amide moiety of the stationary phase.

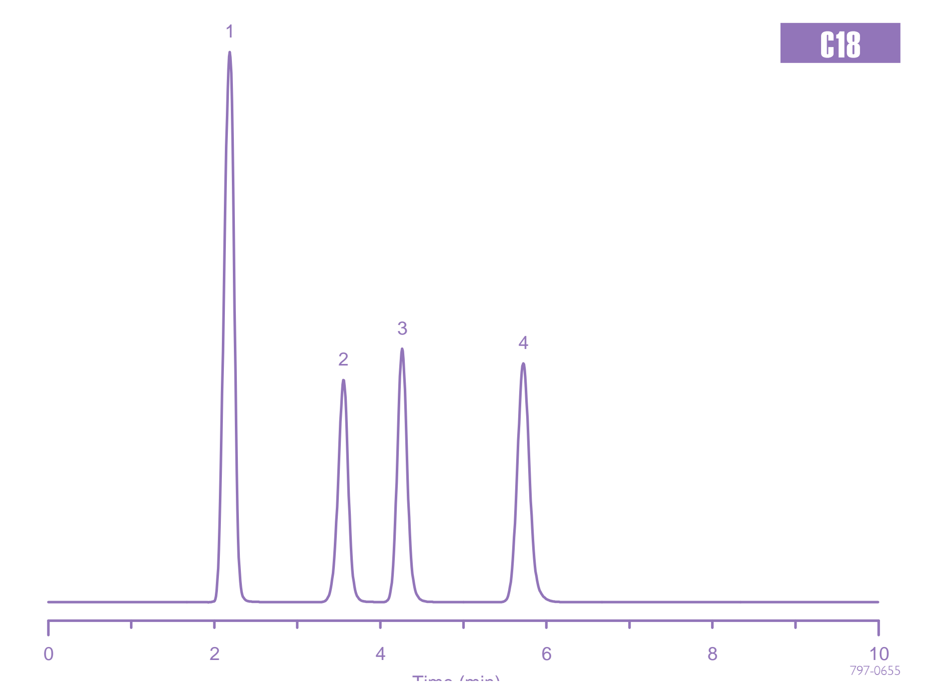


G000935, G000936

Antibiotics (Sulfa Drugs)

Discovery C18
15cm x 4.6mm column,
5µm particles,
MeOH:H_2O with 1% acetic acid
(20:80)
1mL/min
20°C
UV, 254nm
10µL, 1µg/mL each analyte

1. Sulfanilamide
2. Sulfathiazole
3. Sulfamerazine
4. Sulfamethazine



Antibiotics (Sulfa Drugs)

These drugs are based on a 4-aminobenzenesulfonamide skeleton and carry a thiazole or pyrimidine system on the sulfonamide nitrogen. The parent sulfanilamide exhibits pK_a values of 10.43 and 2.37, and the heterocyclic derivatives show values of 7.4 and 2.65. The sulfonamide moiety is acidic and forms salts readily, while the aromatic amino group imparts basic properties to these drugs. These polar functionalities make the HPLC separation of sulfa drugs difficult. The longer retention of sulfamerazine and sulfamethazine may be attributable to the hydrophobic effect of the methyl substituents.

Sulfanilamide

Sulfathiazole

Sulfamerazine

Sulfamethazine

G001249, G000597,
G000598, G000599

Antibiotics (Sulfa Drugs) from Serum

These four antibacterial agents in the sulfa drug family can be extracted from serum. After acidifying a diluted serum sample, recoveries for the compounds are greater than 90%, using a simple methanol and water-based SPE method.

Sulfathiazole

Sulfamethazine

Sulfamerazine

Sulfamethizole

G000597, G000598, G000599, G000600

Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Sulfathiazole	1.0µg/mL	90.1	±2.7
	5.0µg/mL	97.7	±2.1
2. Sulfamerazine	1.0µg/mL	91.8	±2.8
	5.0µg/mL	97.2	±2.4
3. Sulfamethazine	1.0µg/mL	91.9	±2.8
	5.0µg/mL	96.5	±2.2
4. Sulfamethizole	1.0µg/mL	88.7	±3.2
	5.0µg/mL	98.3	±2.5

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

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

[A] 1mL porcine serum spiked 1.0µg/mL or 5.0µg/mL, diluted with 1mL water, then acidified with 40µL H_3PO_4.

[B] Eluent evaporated to dryness with a nitrogen stream at 40°C, using a Zymark TurboVap LV Workstation, then reconstituted with 1mL mobile phase.

Antibiotics (Sulfa Drugs) from Serum

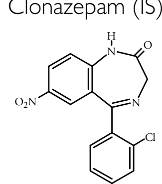
HPLC Conditions:
Discovery C18,
15cm x 4.6mm, 5µm particles, preceded by 2cm C18 guard column and 0.5µm frit filter.
MeOH: 1% acetic acid (8:92)
1.0mL/min (8 min) then 0.6mL/min
30°C
UV, 254nm
10µL reconstituted porcine serum extract

SPE Tube:
Discovery DSC-18
500mg/3mL

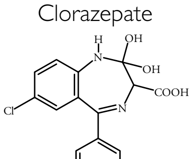
Anticonvulsants

These drugs contain a benzodiazepine nucleus. Clonazepam is the most polar, due to the presence of the nitro substituent, and shows pK_a values of 1.5 and 10.5. Clorazepate remains as the potassium salt under the neutral mobile phase conditions and is moderately acidic. Diazepam is the most hydrophobic of the three and is a weaker acid with a pK_a of 3.4. Clonazepam and clorazepate contain an NH moiety capable of H-bonding with the amide group of the RP-AmideC16 stationary phase and hence is retained longer on this phase. Diazepam is retained longer on the C18 phase, as expected from its hydrophobic interactions.

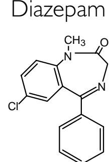
Clonazepam (IS)



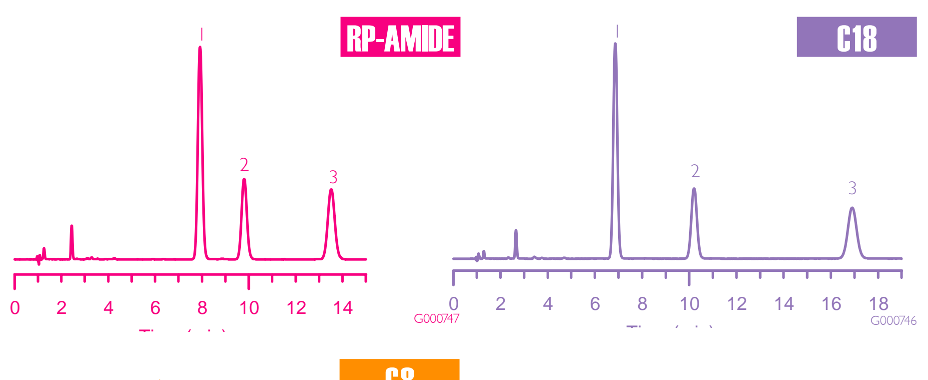
Clorazepate

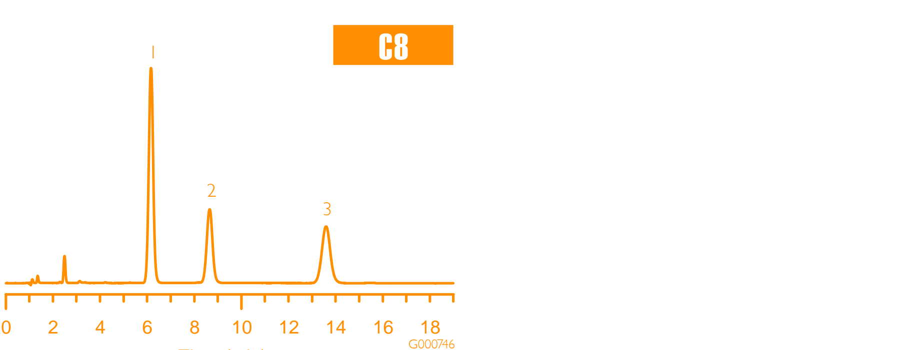


Diazepam



G001211, G000199, G000200





Anticonvulsants

15cm x 4.6mm columns, 5µm particles,
MeCN:H_2O (30:70)
2mL/min
20°C
UV, 254nm
10µL

1. Clonazepam (IS)
2. Clorazepate
3. Diazepam

Anticonvulsant Compounds from Serum

HPLC Conditions:
Discovery C18
15cm x 4.6mm, 5µm particles, preceded by a 2cm C18 guard column and 0.5µm frit filter.
MeOH: H_2O (55:45)
1.0mL/min
30°C
UV, 254nm
10µL

SPE Tube:
DSC-18Lt
500 mg/3mL

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

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

[A] 1mL porcine serum spiked with 0.2µg/mL or 0.5µg/mL each analyte and clonazepam (IS), then diluted with 1.0mL water.
[B] Eluent evaporated to dryness with a nitrogen stream at 30°C, using a Zymark TurboVap LV Workstation, then reconstituted with 200µL methanol

Anticonvulsant Compounds from Serum

These anticonvulsant compounds, similar in structure, can be extracted from serum using Discovery DSC-18Lt SPE tubes, then analyzed on a Discovery C18 HPLC column. Recoveries range from 93 to 99%.

Clorazepate   Diazepam

G001211, G000199

Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Clorazepate	0.20µg/mL	93.2	±1.0
	0.50µg/mL	99.7	±1.1
2. Diazepam	0.20µg/mL	93.8	±1.7
	0.50µg/mL	98.5	±1.0

Anticonvulsants/ Anxiolytics from Serum

HPLC Conditions:
Discovery C18,
15cm x 4.6mm, 5µm particles, preceded by 2cm C18 guard column and 0.5µm frit filter.
MeCN:MeOH: 25mM KH_2PO_4 (pH 7.0 with triethylamine) (32:23:45)
1.0mL/min, ambient temp.
UV, 254nm
20µL reconstituted porcine serum extract

SPE Tube:
Discovery DSC-18
100mg/1mL

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

Step	Solvent/ Solution	Volume (mL)	Flow Rate (mL/min)	Comments
1. Condition	MeOH	2.0	5.0	conditions sorbent
2. Condition	H_2O	2.0	5.0	conditions sorbent
3. Load	spiked porcine serum	2.0[A]	0.75	applies serum sample
4. Rinse	MeCN/MeOH/H_2O 15:15:70	2.0	5.0	washes sorbent
5. Purge-Cannula	H_2O	4.0	30.0	cleans sample cannula
6. Rinse	vent	0.1	2.0	positions SPE tube over waste port
7. Dry	N_2	Time = 10 min		dries sorbent
8. Purge-Cannula	MeOH	4.0	30.0	cleans sample cannula
9. Collect	MeOH	1.0	1.0	elutes analytes into collection vessel
10. Collect	vent	6.0	3.0	pushes residual eluent into vessel[B]
11. Purge-Cannula	H_2O	4.0	30.0	cleans sample cannula

[A] 1mL porcine serum spiked with 0.05µg/mL or 0.5µg/mL each analyte and, then diluted with 1mL water.
[B] Eluent evaporated to dryness with a nitrogen stream at room temp., using a Zymark TurboVap LV Workstation, then reconstituted with 200µL mobile phase.

Anticonvulsants/Anxiolytics from Serum

Five closely related benzodiazepine derivatives were extracted from serum using Discovery DSC-18 SPE tubes and an automated SPE method. High recoveries and low RSD's for these compounds are observed when using these SPE tubes and the Zymark RapidTrace SPE Workstation.

Oxazepam   Temazepam   Desmethyl-diazepam   Diazepam   Medazepam

G000584, G000585, G000586, G000200, G000587

Efficiency of Recovery

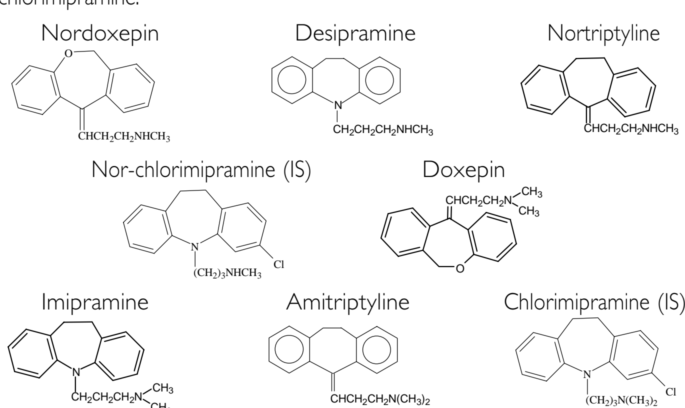
Compound	Concentration	%Recovery	%RSD (n=6)
1. Oxazepam	0.05µg/mL	98.3	±1.8
	0.50µg/mL	99.1	±0.9
2. Temazepam	0.05µg/mL	98.6	±3.3
	0.50µg/mL	97.7	±1.2
3. Desmethyldiazepam	0.05µg/mL	98.6	±1.1
	0.50µg/mL	103.7	±0.8
4. Diazepam	0.05µg/mL	101.1	±2.5
	0.50µg/mL	101.7	±0.8
5. Medazepam	0.05µg/mL	92.9	±2.0
	0.50µg/mL	97.0	±5.8

DISCOVERY

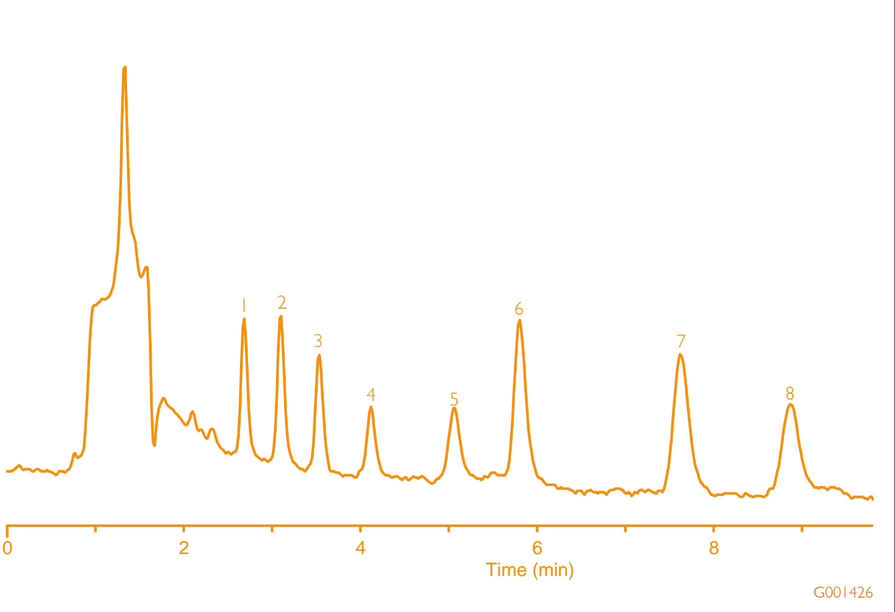
Antidepressants (Tricyclic)

C8

These drugs are comprised of a dibenzo-cycloheptene or oxepin or azepin skeleton and are strongly basic in nature. They tail significantly under RP-HPLC conditions and mobile phase additives are frequently used to overcome this problem. A Discovery C8 column can separate multi-component mixtures of these drugs without any mobile additive. This chromatogram illustrates typical therapeutic levels of these compounds, including two internal standards, Chlorimipramine and Nor-chlorimipramine.



G001422, G000160, G000618, G001421, G000619, G000620, G000159, G000420



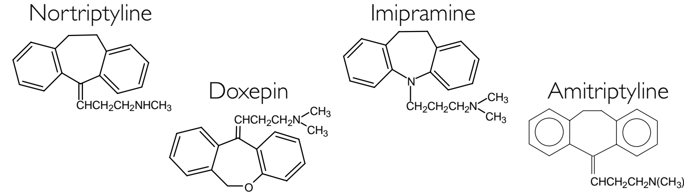
Antidepressants (Tricyclic)

15cm x 4.6mm column, with C8 guard column,
5µm particles,
MeCN:25mM K_2HPO_4,
pH 7.0 (45:55)
1.4mL/min
25°C
UV, 248nm
10µL, 40ng/mL of each analyte

1. Nordoxepin
2. Desimipramine
3. Nortriptyline
4. Nor-chlorimipramine (IS)
5. Doxepin
6. Imipramine
7. Amitriptyline
8. Chlorimipramine (IS)

Antidepressants (Tricyclic) from Serum

This application shows the recoveries for four common tricyclic antidepressant compounds. These basic compounds can be extracted and analyzed with a simple SPE method, using Discovery DSC-18 SPE tubes. Recoveries for all are 92 to 100%.



G000618, G000619, G000620, G000159

Efficiency of Recovery

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

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

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

[A] 1mL porcine serum spiked with 0.1µg/mL or 0.5µg/mL each analyte basified with 3µL 10N KOH, then diluted with 1mL water
[B] 350µL water added per mL methanolic eluent before analysis.

Antidepressants (Tricyclic) from Serum

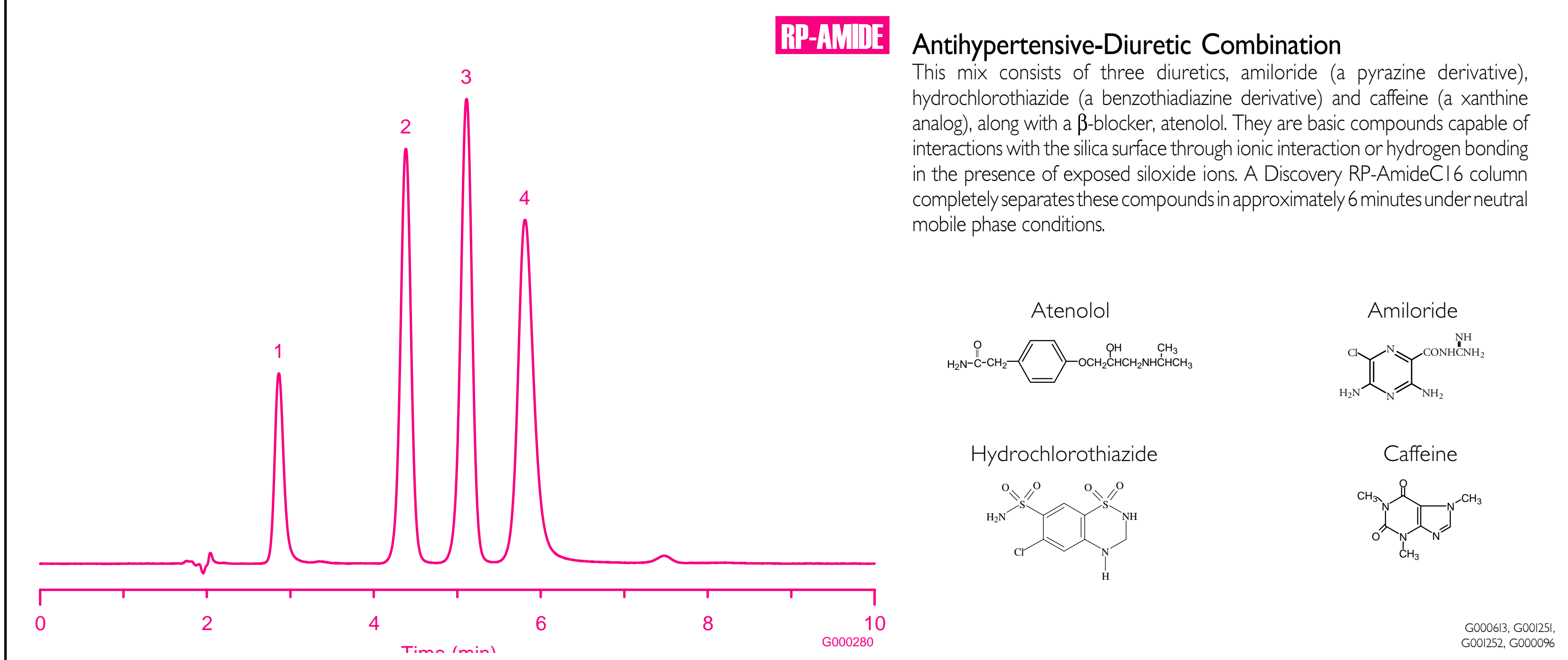
HPLC Conditions:
Discovery C18 column,
15cm x 4.6mm, 5µm particles, preceded by 2cm C18 guard column and 0.5µm frit filter
MeCN:MeOH: 25mM KH_2PO_4 (pH 7.0 with triethylamine) (45:25:30)
1.0mL/min, ambient temp.
UV, 254nm
50µL diluted porcine serum extract

SPE Tube:
Discovery DSC-18
100mg/1mL

Antihypertensive-Diuretic Combination

Discovery RP-AmideC16
15cm x 4.6mm column,
5µm particles,
MeOH: 25mM K_2HPO_4,
pH 7.0 (22:78)
1mL/min
30°C
UV, 254nm
10µL, 1µg/mL of each analyte

1. Atenolol
2. Amiloride
3. Hydrochlorothiazide
4. Caffeine



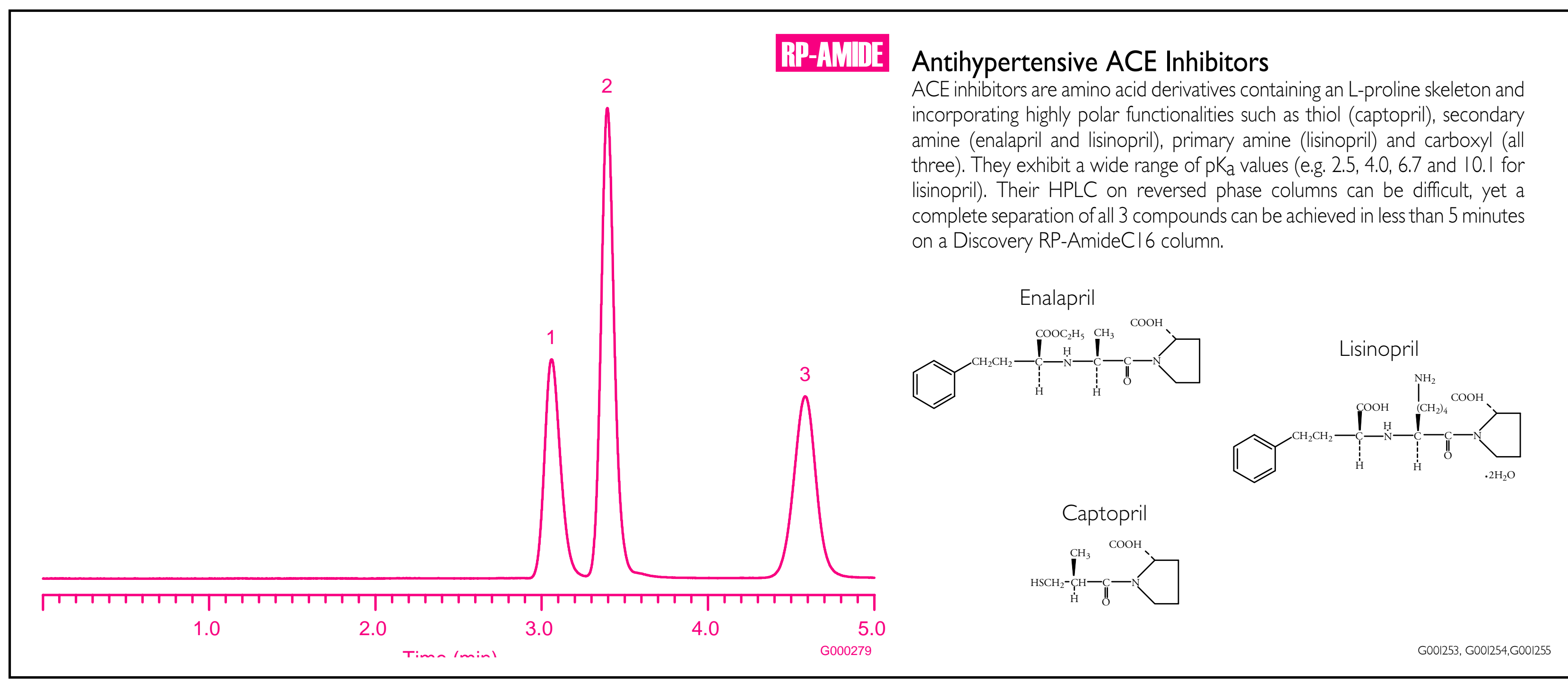
Antihypertensive-Diuretic Combination

This mix consists of three diuretics, amiloride (a pyrazine derivative), hydrochlorothiazide (a benzothiadiazine derivative) and caffeine (a xanthine analog), along with a β-blocker, atenolol. They are basic compounds capable of interactions with the silica surface through ionic interaction or hydrogen bonding in the presence of exposed siloxide ions. A Discovery RP-AmideC16 column completely separates these compounds in approximately 6 minutes under neutral mobile phase conditions.

Antihypertensive ACE Inhibitors

Discovery RP-AmideC16
15cm x 4.6mm column,
5µm particles,
MeCN:25mM KH_2PO_4,
pH 2.3 (33:67)
0.6mL/min
35°C
UV, 214nm
3µL, 1µg/mL of each analyte

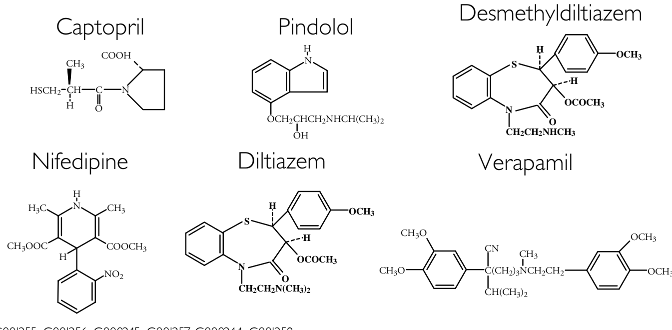
1. Enalapril
2. Lisinopril
3. Captopril



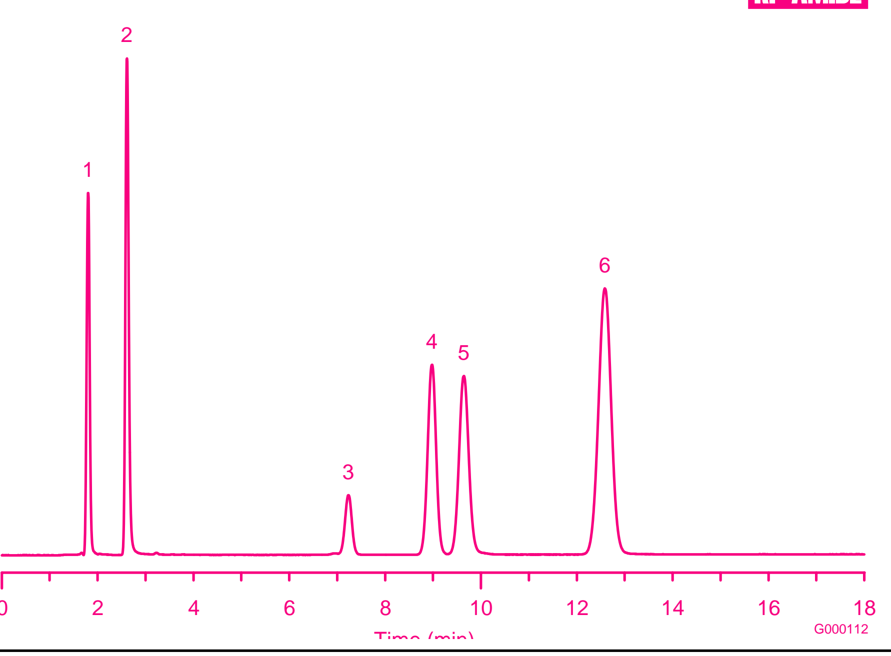
Antihypertensive ACE Inhibitors

ACE inhibitors are amino acid derivatives containing an L-proline skeleton and incorporating highly polar functionalities such as thiol (captopril), secondary amine (enalapril and lisinopril), primary amine (lisinopril) and carboxyl (all three). They exhibit a wide range of pK_a values (e.g. 2.5, 4.0, 6.7 and 10.1 for lisinopril). Their HPLC on reversed phase columns can be difficult, yet a complete separation of all 3 compounds can be achieved in less than 5 minutes on a Discovery RP-AmideC16 column.

Five antihypertensive drugs of different classes are resolved in a single separation on a Discovery RP-AmideC16 column. They include the calcium channel blockers diltiazem, verapamil and nifedipine, with a benzothiazepinone, valeronitrile, and dihydropyridine skeleton, respectively, pindolol, a β-blocker with an indole skeleton, and captopril, an ACE inhibitor with an L-proline skeleton. Desmethyldiltiazem is a metabolite of diltiazem and is commonly encountered in fluids of patients taking diltiazem. These strongly basic molecules are difficult candidates for HPLC. A Discovery RP-AmideC16 column demonstrates excellent separation of these compounds at neutral pH, and provides good peak characteristics.



RP-AMIDE



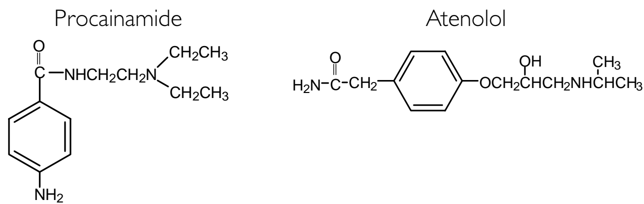
Antihypertensive Drugs (calcium channel blockers, β-blocker and ACE inhibitor)

Discovery RP-AmideC16
15cm x 4.6mm column,
5µm particles,
MeOH:25mM KH_2PO_4,
pH 7.0 (40:60)
1mL/min
35°C
UV, 214nm
5µL, 0.5µg/mL of each analyte

1. Captopril
2. Pindolol
3. Desmethyldiltiazem
4. Nifedipine
5. Diltiazem
6. Verapamil

Antihypertensive/Antiarrhythmic Compounds from Serum

Basic drugs like procainamide and atenolol can be extracted from serum, using Discovery DSC-18 SPE tubes. The results shown here are approximately 100%, recovery.



G000095, G000613

Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Procainamide	0.25µg/mL	98.6	±3.5
	0.50µg/mL	99.7	±2.3
2. Atenolol	0.25µg/mL	101.7	±3.7
	0.50µg/mL	100.3	±2.3

SPE

SPE Procedure, Using Visiprep™ SPE Vacuum Manifold

Condition: 2mL MeOH, then 2mL 25mM K_2HPO_4 (pH 9.0)

Apply Sample: 1 mL porcine serum spiked with 0.25µg/mL or 0.50µg/mL each analyte, basified with 2.5µL 10M KOH

Wash and Dry: 2mL 15% MeOH in 25mM K_2HPO_4 (pH 9.0): dry tube 10 min with nitrogen stream

Elute: 1mL MeOH; evaporate to dryness with nitrogen stream at room temperature; reconstitute in 200µL H_2O

Antihypertensive/ Antiarrhythmic Compounds from Serum

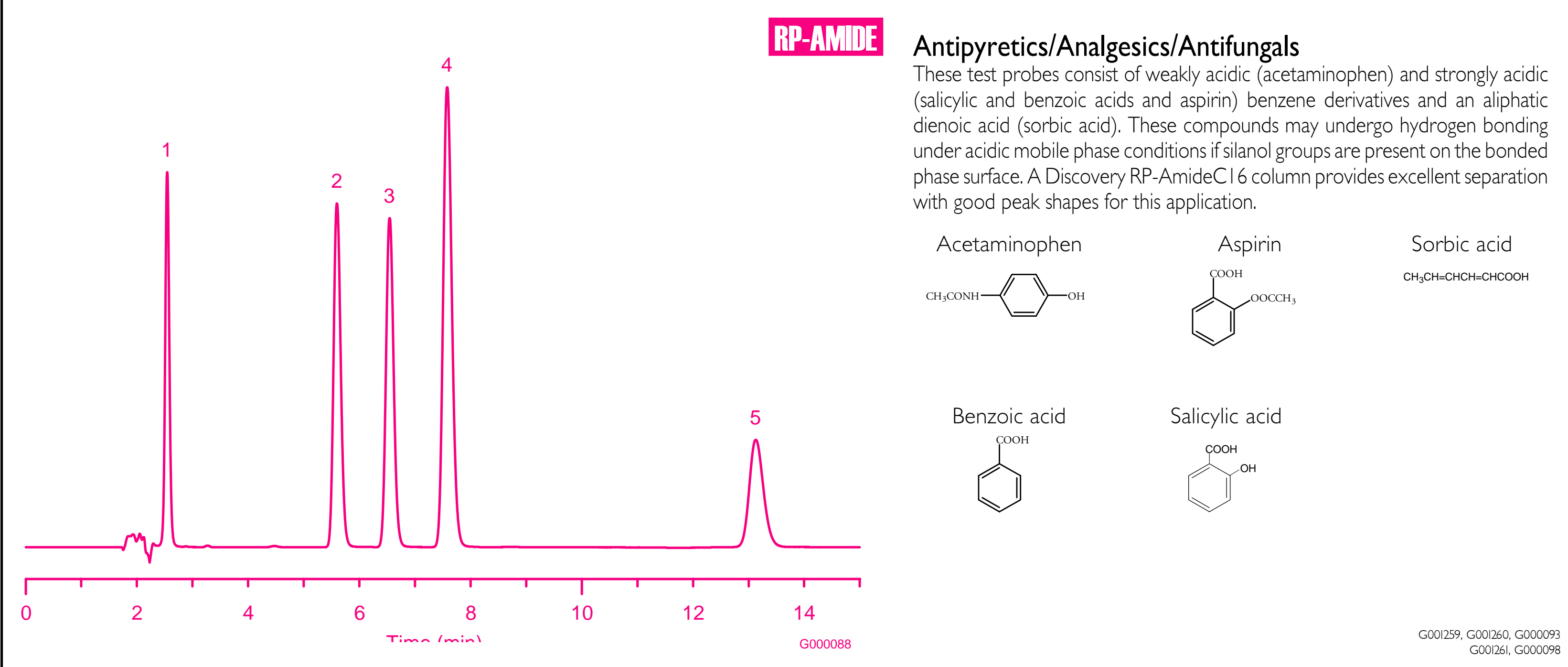
HPLC Conditions:
Discovery C18,
15cm x 4.6mm column, 5µm particles, preceded by a 2cm C18 guard column and 0.5µm frit filter.
MeOH:25mM K_2HPO_4
pH 7.0 (10:90)
1mL/min
30°C
UV, 220nm
50µL reconstituted porcine serum extract

SPE Tube:
Discovery DSC-18
500mg/3mL

Antipyretics/Analgesics/Antifungals

Discovery RP-AmideC16
15cm x 4.6mm column,
5µm particles,
MeCN:H_2O, 0.1% TFA (25:75)
1mL/min
30°C
UV, 254nm
10µL, 1µg/mL of each analyte

1. Acetaminophen
2. Aspirin
3. Sorbic acid
4. Benzoic acid
5. Salicylic acid



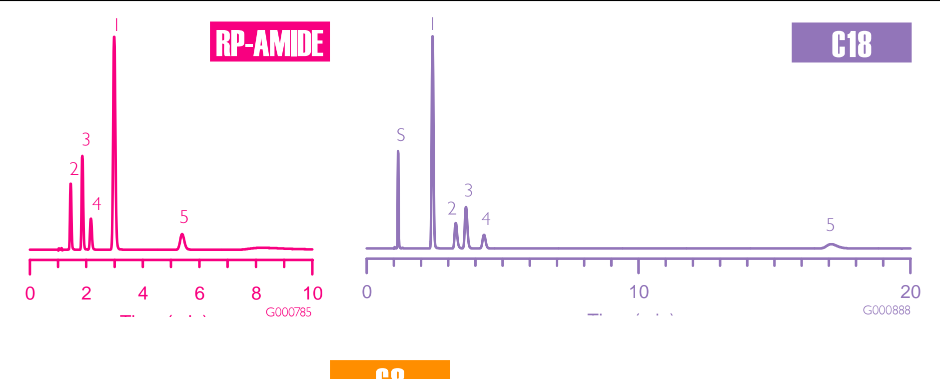
Antipyretics/Analgesics/Antifungals

These test probes consist of weakly acidic (acetaminophen) and strongly acidic (salicylic and benzoic acids and aspirin) benzene derivatives and an aliphatic dienoic acid (sorbic acid). These compounds may undergo hydrogen bonding under acidic mobile phase conditions if silanol groups are present on the bonded phase surface. A Discovery RP-AmideC16 column provides excellent separation with good peak shapes for this application.

Antitussives/ Antihistamines/ Antipyretics

15cm x 4.6mm columns,
5µm particles,
A) 25mM KH_2PO_4, pH 2.3
B) MeCN
10% (2min) to 30% B in 4 min, hold 4 min, to 50% B in 10 min
2mL/min, ambient temp.
UV, 214nm
10µL, 1µg/mL of each analyte

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



C8

S 1 2 3 4 5

0 2 4 6 8 10 12 14

G000786

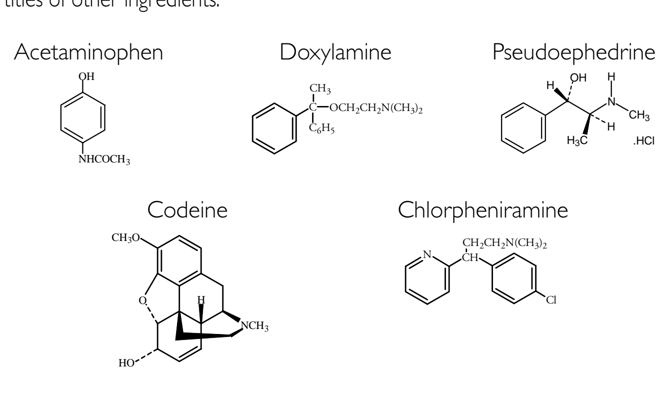
Antitussives/Antihistamines/Antipyretics

Many cold remedies contain various combinations of acetaminophen, pseudoephedrine, and an antihistamine as ingredients. The separation of a five component mix on Discovery columns is shown in this figure. It is striking that the RP-AmideC16 column elutes doxylamine, pseudoephedrine and codeine before acetaminophen, while the C18 and C8 columns display the opposite behavior. This selectivity difference may be exploited for method development of those cold remedies which contain large amounts of acetaminophen with minor quantities of other ingredients.

Acetaminophen

Doxylamine

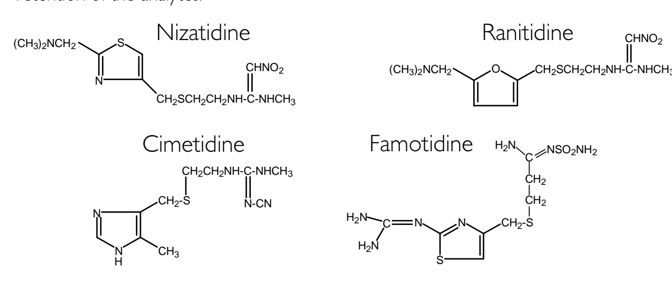
Pseudoephedrine



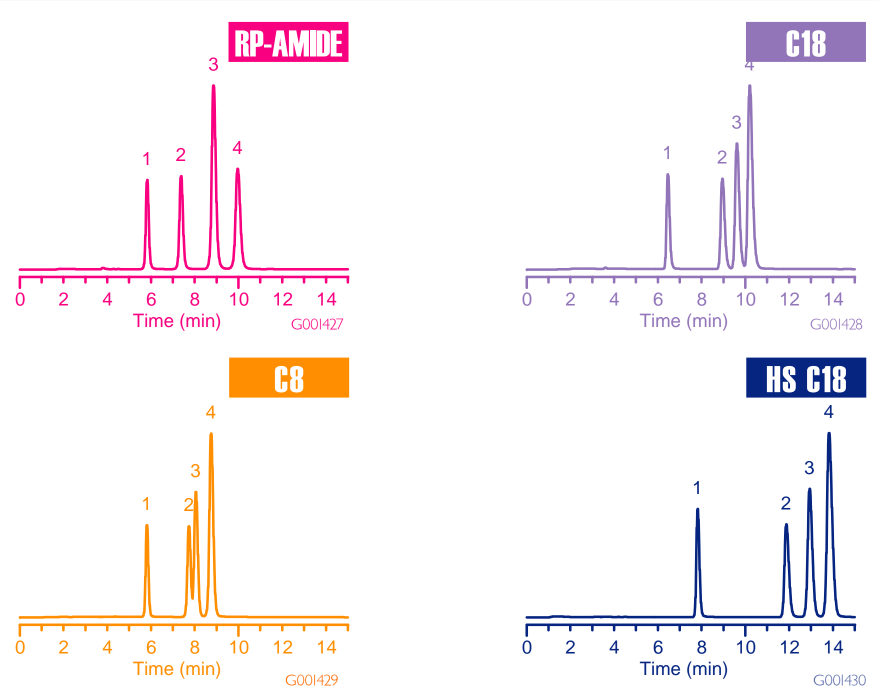
G000243, G001212, G000873, G000222, G001213

Antiulcer Compounds

These molecules are complex in structure, with a core heterocyclic ring (furan, imidazole or thiazole) and a side chain carrying a sulfide and secondary amine functionalities. Other polar groups such as nitro, cyano or iminosulfonamide also are present on the side chain. The hetero ring carries a tertiary amine or guanidine moiety. These compounds are strongly basic and are not only difficult to separate, but also produce severe tailing from silanol interactions. They require highly aqueous mobile phases for sufficient retention needed for achieving good separation. A notable selectivity difference between the RP-AmideC16 and C18 columns can be seen in the reversal of the elution order of cimetidine and famotidine. This reversal may be attributable to the H-bonding interactions of the guanidine and/or sulfonamide groups on famotidine with the stationary phase amide moiety of RP-AmideC16, leading to its longer retention. Note the better resolution of the HS (high surface area) C18 compared to the other C18 column, attributable to the former column's greater retention of the analytes.



G000247, G000248, G000249, G000250



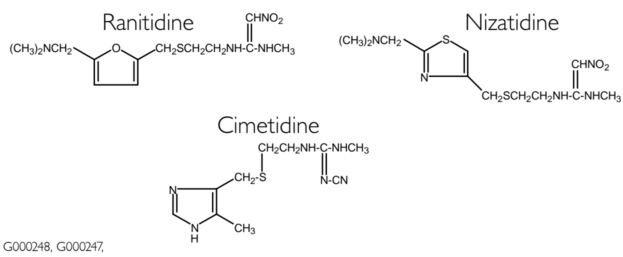
Antiulcer Compounds

15cm x 4.6mm columns,
MeOH:25mM H_3PO_4/KOH,
pH 3.0 (15:85)
0.5mL/min
ambient temp.
215nm
3µL, 1.6µg/mL of each analyte

1. Nizatidine
2. Ranitidine
3. Famotidine
4. Cimetidine

Antiulcer Compounds from Serum

These basic, sulfur-containing compounds can be extracted from serum using Discovery DSC-18 SPE tubes under basic conditions. The compounds can be analyzed subsequently on a Discovery C18 HPLC column. This method provides recoveries in the range of 93-98% for these compounds.



G000248, G000247, G000249

Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Ranitidine	0.25µg/mL	92.5	±5.4
	0.50µg/mL	95.5	±5.1
2. Cimetidine	0.25µg/mL	94.5	±5.2
	0.50µg/mL	98.2	±3.2
3. Nizatidine	0.25µg/mL	97.0	±7.0
	0.50µg/mL	94.8	±3.4

SPE

SPE Procedure, Using Visiprep SPE Vacuum Manifold

Condition: 2mL MeOH, then 2mL 25mM K_2HPO_4 (pH 9.0)

Apply Sample: 1mL porcine serum spiked with 0.25µg/mL or 0.50µg/mL each analyte, basified with 2.5µL 10M KOH

Wash and Dry: 2mL 5% MeOH in 25mM K_2HPO_4 (pH 9.0): dry tube 10 min with nitrogen stream

Elute: 1mL MeOH; evaporate to dryness with nitrogen stream at room temperature; reconstitute in 200µL mobile phase

Antiulcer Compounds from Serum

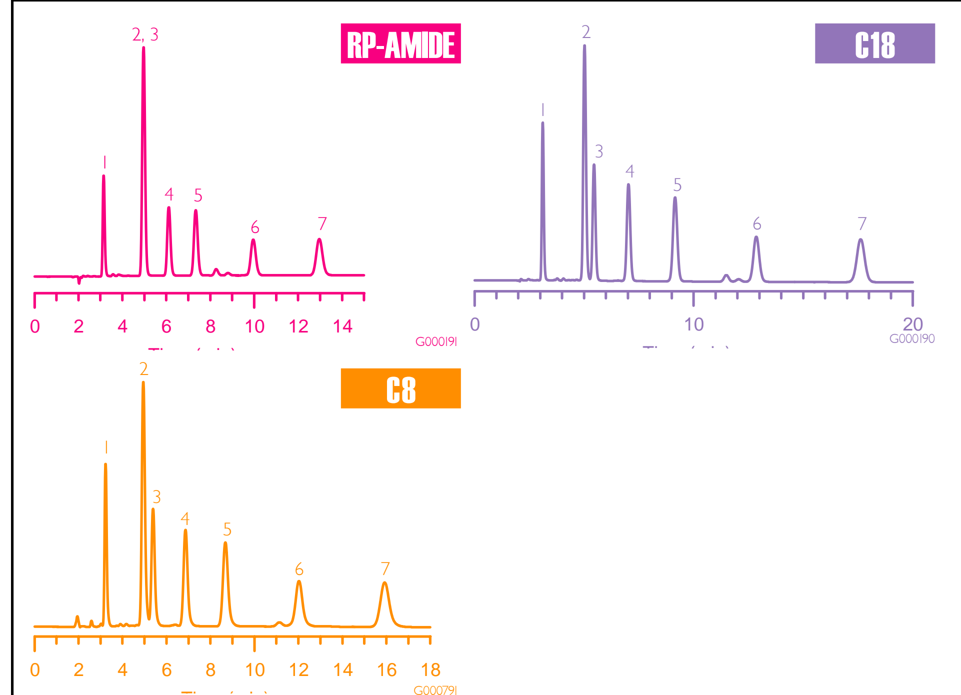
HPLC Conditions:
Discovery C18,
15cm x 4.6mm, 5µm particles, preceded by 2cm C18 guard column and 0.5µm frit filter.
MeOH: 25mM K_2HPO_4,
pH 7.0 (20:80)
1.0mL/min, ambient temp.
UV, 235nm
60µL reconstituted porcine serum extract

SPE Tube:
Discovery DSC-18
500mg/3mL

Barbiturates

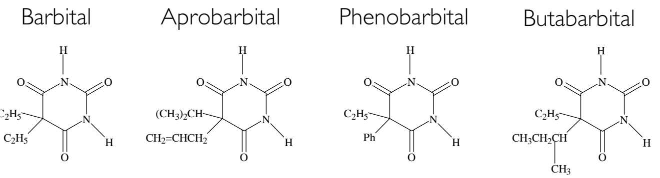
15cm x 4.6mm columns,
5µm particles,
MeOH:H_2O, (45:55)
1mL/min
ambient temp.
UV, 214nm
5µL (Discovery C8) or 10µL
(Discovery RP-AmideC16,
Discovery C18)
1µg/mL each of analyte

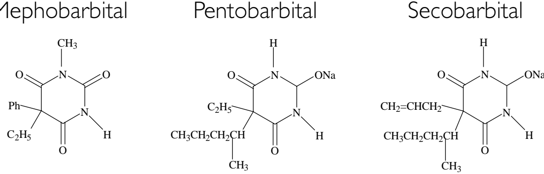
1. Barbital
2. Aprobarbital
3. Phenobarbital
4. Butabarbital
5. Mephobarbital
6. Pentobarbital
7. Secobarbital



Barbiturates

These sedative drugs are good candidates for comparing the hydrophobic selectivity of reversed phase columns. A mixture of seven barbiturates differing from each other in the extent of alkyl substitution were screened. The RP-AmideC16 column coelutes phenobarbital and aprobarbital under these conditions, while the C8 and C18 show good baseline separation of these two.





G000201, G000202, G000203, G000204
G000205, G000206, G000207

Barbiturates from Serum

HPLC Conditions:
Discovery C18
15cm x 4.6mm, 5µm particles,
preceded by 2cm C18 guard
column and 0.5µm frit filter.
MeOH:H_2O (40:60)
1.0mL/min
30°C
UV, 214nm
30µL

SPE Tube:
DSC-18Lt
500mg/3mL

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

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

[A] 0.5mL porcine serum spiked with 0.5µg/mL or 1.0µg/mL each analyte, then diluted with 0.5mL water.
[B] Eluent evaporated to dryness with a nitrogen stream at 30°C, using a Zymark TurboVap LV Workstation, then reconstituted with 200µL water.

Barbiturates from Serum

Six drugs from this class of compounds were extracted from serum, using DSC-18Lt SPE tubes, and were analyzed subsequently on a Discovery C18 HPLC column. Recoveries for these compounds are greater than 95% using this simple methanol and water-based SPE method.

For chemical structures, see above.

Efficiency of Recovery

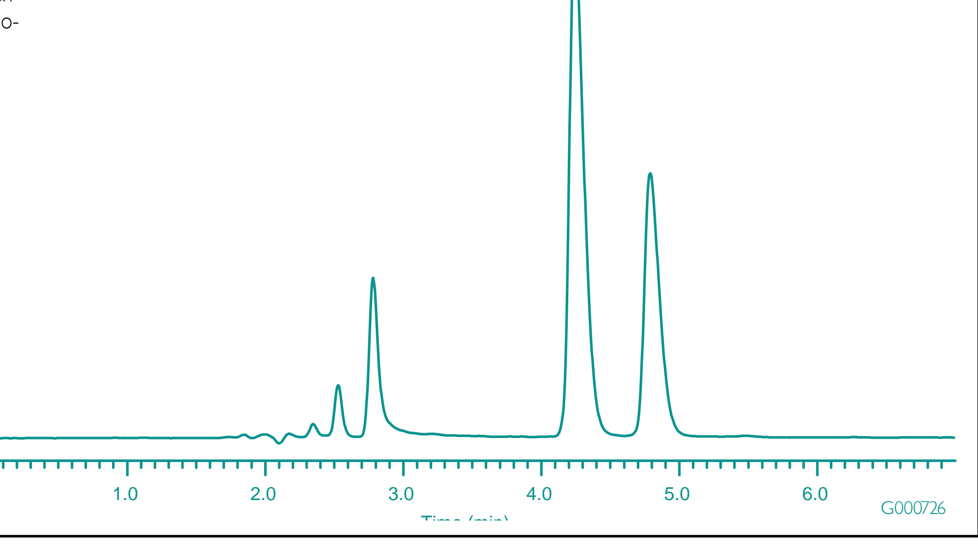
Compound	Concentration	%Recovery	%RSD (n=6)
1. Aprobarbital	0.5µg/mL	98.5	±2.1
	1.0µg/mL	100.8	±0.8
2. Phenobarbital	0.5µg/mL	96.2	±1.6
	1.0µg/mL	94.9	±1.7
3. Butabarbital	0.5µg/mL	97.2	±1.9
	1.0µg/mL	98.7	±1.8
4. Mephobarbital	0.5µg/mL	99.7	±2.4
	1.0µg/mL	101.0	±2.0
5. Pentobarbital	0.5µg/mL	96.4	±1.7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

Benzalkonium Chlorides

These compounds are mixtures of alkyl benzyldimethylammonium salts with the alkyl moiety ranging from C_8 to C_{18}. They are used as topical antiseptives and preservatives. These are retained for long periods on conventional reversed phase columns, and are not well resolved. Because of its low hydrophobic retention, the Cyano column is best suited for this separation and elutes all components within 6 minutes.
The sample contained components of undefined alkyl chain length.

Benzalkonium Chlorides

CYANO



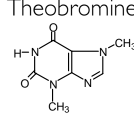
Benzalkonium Chlorides

Discovery Cyano
15cm x 4.6mm column,
5µm particles,
MeCN:acetate buffer,
pH 4.5, (60:40)
1mL/min
ambient temp.
UV, 254nm

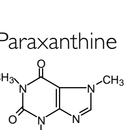
Bronchodilator (Caffeine Metabolites) from Serum

Theophylline and other caffeine metabolites can be extracted from serum with Discovery DSC-18 SPE tubes, using the method shown here. Recoveries are greater than 95% using a Visiprep SPE vacuum manifold.

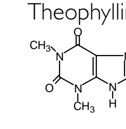
Theobromine



Paraxanthine



Theophylline



Caffeine

G000588, G000589, G000590, G000096

Efficiency of Recovery

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

SPE

SPE Procedure, Using Visiprep SPE Vacuum Manifold

Condition: 2mL MeOH, then 2mL H_2O

Apply Sample: 1 mL porcine serum spiked with 0.1µg/mL, 0.5µg/mL, or 1.0µg/mL each analyte

Wash and Dry: 2mL 5% MeOH in H_2O: dry tube 10 min with nitrogen stream

Elute: 1mL MeOH; evaporate to dryness with nitrogen stream at room temperature; reconstitute in 200µL mobile phase

Bronchodilator (Caffeine Metabolites) from Serum

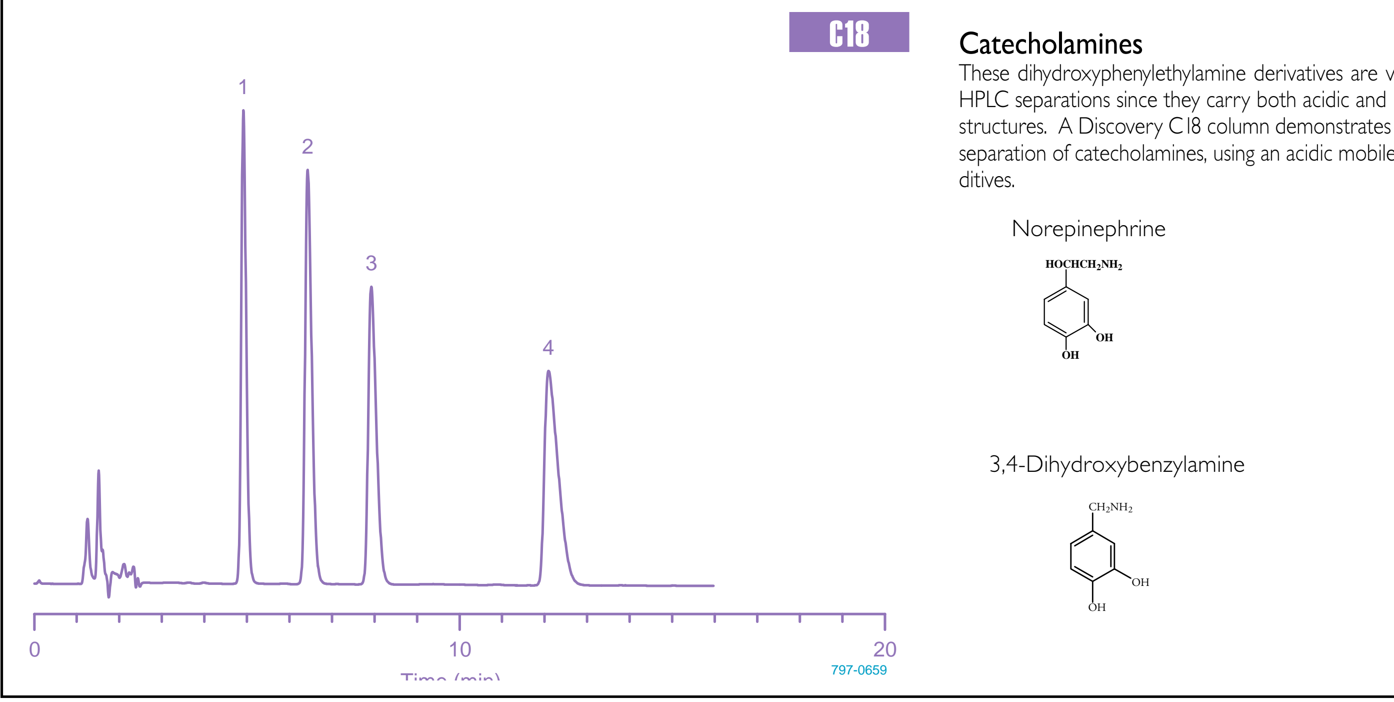
HPLC Conditions:
Discovery RP-AmideC16 column, 15cm x 4.6mm, 5µm particles, preceded by 2cm RP-AmideC16 guard column and 0.5µm frit filter.
MeOH:1% acetic acid (17:83)
1.0mL/min
30°C
UV, 272nm
20µL reconstituted porcine serum extract

SPE Tube:
DSC-18
500mg/3mL

Catecholamines

Discovery C18
15cm x 4.6mm column,
5µm particles,
MeCN:50mM KH_2PO_4/H_3PO_4,
pH 3.0
100mg/L EDTA, 200mg/L
1-octane-sulfonic acid (5:95)
1mL/min
20°C
UV, 254nm
10µL, 1µg/mL of each analyte

1. Norepinephrine
2. Epinephrine
3. 3,4-Dihydroxybenzylamine
4. Dopamine



Catecholamines

These dihydroxyphenylethylamine derivatives are very difficult candidates for HPLC separations since they carry both acidic and basic functionalities in their structures. A Discovery C18 column demonstrates excellent selectivity for the separation of catecholamines, using an acidic mobile phase with ion-pairing additives.

G000559, G001262, G001263, G001264

Chlorophyll from Methanolic Plant Extracts

SPE Tube:
DPA-6S
300mg/3mL

Breakthrough Analysis
The eluents that were collected during sample application were tested by UV absorption at 660nm using a 1cm cell. Breakthrough was defined as the point at which the absorbance of the fraction was greater than that of a standard 2% solution of chlorophyll in methanol.

SPE

SPE Procedure, Using Visiprep SPE Vacuum Manifold

Sample Prep.: 10g of freshly cut green leaves of *Philodendron* were blended for 5 min with 80mL MeOH. After centrifugation for 5min at 2000rpm, the green supernatant was decanted and used as the sample.

Condition: 2mL MeOH

Apply Sample: 1mL aliquots of the methanolic plant extract were applied using a flow rate of 0.75mL/min; fractions were collected after each 1mL of sample was applied.

Breakthrough Results

Fraction	Absorbance at 660nm (AU)	Appearance
1	0.081	Clear
2	0.178	Clear
3	0.255	Clear
4	0.286	Clear
5	0.330	Clear
6	0.402	Slightly green
7	0.503	Slightly green
2% chlorophyll standard	0.399	Slightly green

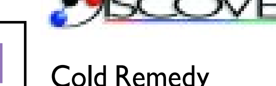
Breakthrough of chlorophyll occurred while fraction 6 was applied to the DPA-6S SPE tube. This result indicates that Discovery DPA-6S products may be used to remove chlorophyll from plant extracts, while allowing unretained species to pass through, free of chlorophyll.

Chlorophyll from Methanolic Plant Extracts

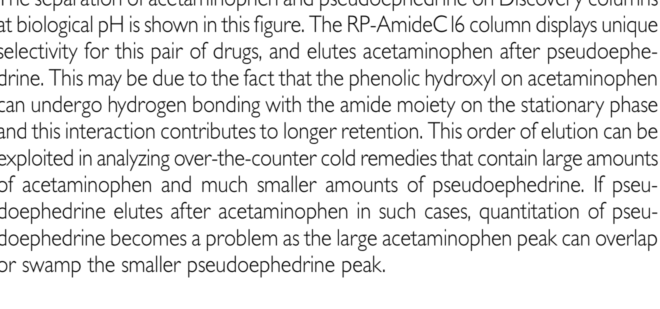
Chlorophyll is a green pigment that is present in all photosynthetic plants, as well as in some bacteria. It occurs in three forms, all of which are magnesium-centered porphyrins containing a hydrophilic carbocyclic ring with a lipophilic phytyl tail. Chlorophyll is a photoreceptor up to a wavelength of 700nm; it is sparingly soluble in alcohols; and its solutions are blue-green in color. Chlorophyll often interferes with the analysis of bioactives in natural product research or in pesticide analysis. Discovery DPA-6S polyamide SPE products can be used to remove chlorophyll from aqueous or methanolic extracts in these applications. In this experiment, 300mg of bulk DPA-6S sorbent was packed into 3mL SPE tubes and plant extracts were processed through the tubes.

Chlorophyll A

G001273



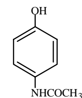
Cold Remedy Ingredients



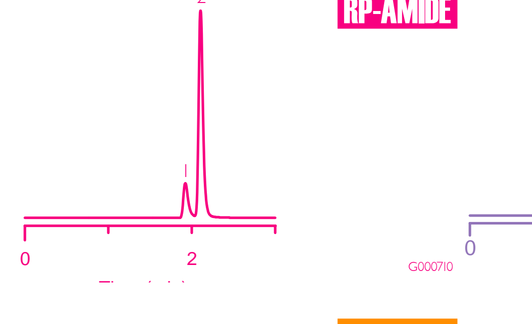
The separation of acetaminophen and pseudoephedrine on Discovery columns at biological pH is shown in this figure. The RP-AmideC16 column displays unique selectivity for this pair of drugs, and elutes acetaminophen after pseudoephedrine. This may be due to the fact that the phenolic hydroxyl on acetaminophen can undergo hydrogen bonding with the amide moiety on the stationary phase and this interaction contributes to longer retention. This order of elution can be exploited in analyzing over-the-counter cold remedies that contain large amounts of acetaminophen and much smaller amounts of pseudoephedrine. If pseudoephedrine elutes after acetaminophen in such cases, quantitation of pseudoephedrine becomes a problem as the large acetaminophen peak can overlap or swamp the smaller pseudoephedrine peak.

Pseudoephedrine

Acetaminophen



G000873, G000243



Cold Remedy Ingredients

15cm x 4.6mm columns
5µm particles,
MeCN:25mM KH_2PO_4
pH 7.0 (15:85)
1mL/min
20°C
UV, 214nm
1µL

1. Pseudoephedrine
2. Acetaminophen

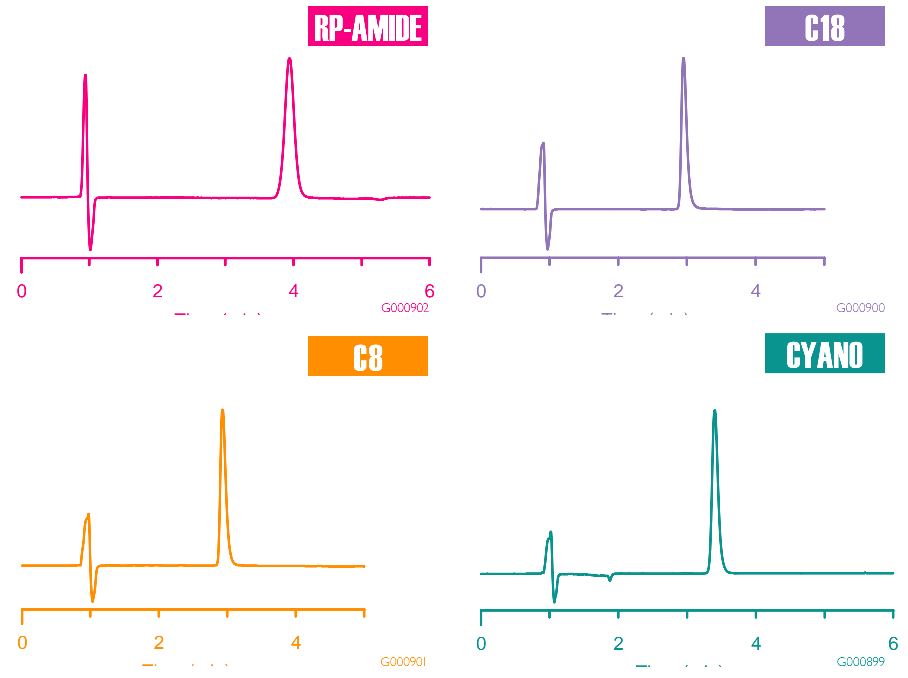
Fluoxetine (Prozac)

This drug is a phenyl benzyl ether with a methylaminoethyl chain attached to the benzylic carbon. It shows longer retention on the RP-AmideC16 column than on C8 or C18, possibly due to the H-bonding interactions of the secondary amine functionality on the drug with the amide of the stationary phase. In fact, the RP-AmideC16 forms a stronger interaction than the Cyano phase, as evidenced by the longer retention of fluoxetine on the former.

Fluoxetine (Prozac®)

F_3C — O — $CH_2CH_2NHCH_3$

G000874



Fluoxetine (Prozac)

15cm x 4.6mm columns,
5µm particles
MeCN:25mM KH_2PO_4
pH 7.0 (40:60)
2mL/min
30°C
UV, 214nm
1µL

Humic Acids from Water

SPE Tube:
DPA-6S
300mg/3mL and 600mg/3mL

Breakthrough Analysis
The eluents that were collected during sample application were tested by UV absorption over the range of 220nm to 700nm, in 2nm intervals, using a 1cm cell. Breakthrough was defined as the point at which the total absorbance of the fraction was greater than that of a standard 2% solution of humic acids in water.

SPE

SPE Procedure, Using Visiprep SPE Vacuum Manifold

Condition: 2mL MeOH, then 2mL H_2O

Apply Sample: 1mL aliquots of 0.2mg/mL and 1.0mg/mL humic acid in H_2O were applied using a flow rate of 0.75mL/min; fractions were collected after each 1mL of sample was applied.

Breakthrough Results

Humic Acids Conc. (mg/mL)	DPA-6S Sorbent Mass (mg)	Fraction	Absorbance of Fraction Versus 2% Standard
0.2	300	1-3 4	Lower Higher (breakthrough)
0.2	600	1-6 7	Lower Higher (breakthrough)
1.0	300	1 2	Lower Higher (breakthrough)
1.0	600	1-3 4	Lower Higher (breakthrough)

These results indicate that Discovery DPA-6S products can be used to remove humic acids from aqueous solutions, while allowing unretained species to pass through, free of humic acids. The capacity of the sorbent for humic acids depends on the concentration of the sample, and these studies indicate that the relationship may be linear. Perform breakthrough studies for your sample, to select the best SPE tube size.

Humic Acids from Water

Humic acids are found in soils, coals, and peat. They are brown-colored, mixtures of not-well-defined macromolecules with polymeric phenolic and heterocyclic structures containing carboxyl groups and nitrogen functionalities. They are soluble in water and bases, but insoluble in mineral acids and alcohols. Humic acids may interfere with the analysis of bioactives in natural product research or in pesticide analysis. Discovery DPA-6S polyamide SPE products can be used to remove humic acids from aqueous solutions in these applications. In this experiment, 300mg and 600mg of bulk DPA-6S sorbent was packed into 3mL SPE tubes and aqueous humic acid solutions were processed through the tubes.

Nonsteroidal Antiinflammatory Drugs

15cm x 4.6mm column, 5µm particles
MeCN:25mM KH_2PO_4, pH 3.0 (40:60)
1mL/min
30°C
UV, 230nm
10µL, 1µg/mL of each analyte

1. Piroxicam
2. Sulindac
3. Tolmetin
4. Ketoprofen
5. Naproxen
6. Diflunisal
7. Indomethacin
8. Ibuprofen
9. Diclofenac
10. Mefanamic acid

RP-AMIDE

1 2 3 4 5 6 7 8 9 10

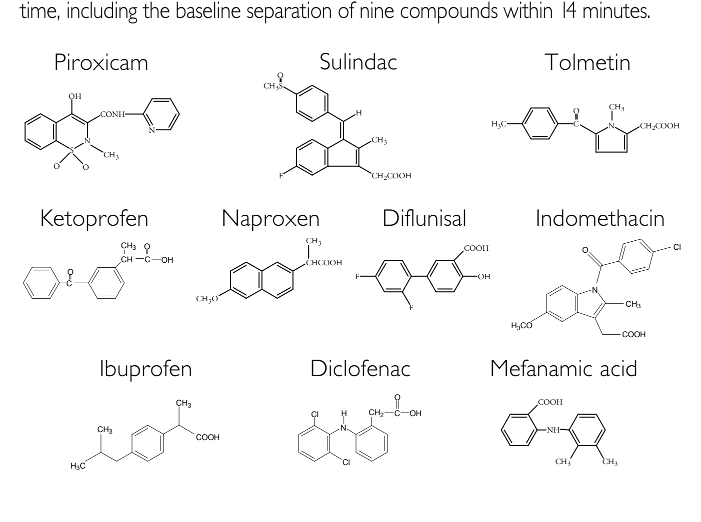
0 10 20

Time (min)

G000061

Nonsteroidal Antiinflamatory Drugs

NSAIDs are strongly acidic molecules possessing diverse structural features. They typically present problems during RP-HPLC separations/analyses. A Discovery RP-AmideC16 column provides an isocratic separation of these drugs within a short time, including the baseline separation of nine compounds within 14 minutes.



G001265, G001266, G001267, G000240, G001268, G001269, G001137, G000603, G000604, G001270

Nonsteroidal Antiinflammatory Drugs from Serum

Ketoprofen, ibuprofen, and diclofenac are commonly used antiinflammatory drugs. Serum levels for these acidic compounds can be monitored using this SPE method, after acidification of the serum sample. Recoveries of the compounds are greater than 90% using this method.

Ketoprofen Diclofenac Ibuprofen

G000240, G000603, G000604

Efficiency of Recovery

Compound	Concentration	%Recovery	%RSD (n=6)
1. Ketoprofen	0.5µg/mL	95.0	±7.7
	3.0µg/mL	90.8	±4.7
2. Ibuprofen	0.5µg/mL	94.3	±8.3
	3.0µg/mL	100.7	±5.9
3. Diclofenac	0.5µg/mL	101.2	±7.1
	3.0µg/mL	97.2	±3.0

SPE

SPE Procedure, Using Visiprep SPE Vacuum Manifold

Condition: 2mL MeOH, then 2mL H_2O

Apply Sample: 1 mL porcine serum spiked with 0.5µg/mL or 3.0µg/mL each NSAID, acidified with 20µL H_3PO_4

Wash and Dry: 2mL 5% MeOH in H_2O: dry tube 10 min with nitrogen stream

Elute: 1mL MeOH; evaporate to dryness with nitrogen stream at room temperature; reconstitute in 200µL mobile phase

Nonsteroidal Antiinflammatory Drugs from Serum

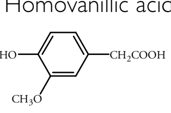
HPLC Conditions:
Discovery RP-AmideC16 column, 15cm x 4.6mm, 5µm particles, preceded by 2cm RP-AmideC16 guard column and 0.5µm frit filter.
MeCN: 25mM KH_2PO_4 pH 3.0 (50:50)
1.0mL/min
30°C
UV, 230nm
20µL reconstituted porcine serum extract

SPE Tube:
DSC-18
500mg/3mL

Organic Acids

Aromatic carboxylic acids have pK_a values ranging from 2.5 to 4.5. They can interact with residual silanols on a reversed phase packing material through hydrogen bonding under acidic mobile phase conditions. In addition, chelating molecules like salicylic acid can bind to metallic impurities, if present, on the silica surface. This chromatogram of four organic acids on the C18 columns indicates the absence of silanol and metal chelating interactions. The longer retention of Discovery HS (high surface area) C18 may be desirable for serum or urine samples, where matrix peaks appear just after the void volume (not shown).

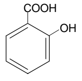
Homovanillic acid



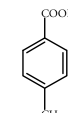
Sorbic acid

$CH_3CH{=}CHCH{=}CHCOOH$

Salicylic acid

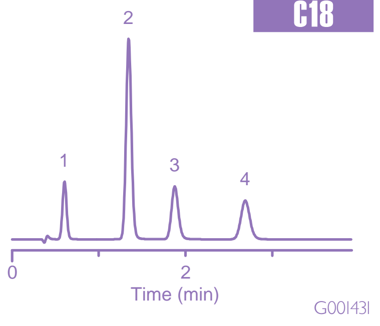


p-Toluic acid



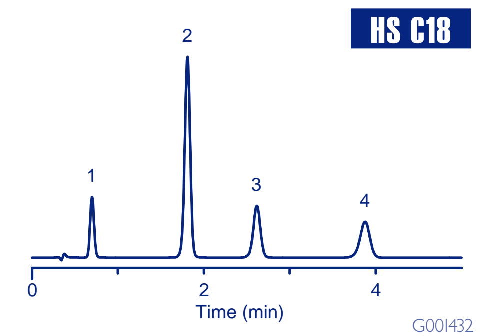
G001271, G000093, G000098, G001272

C18



G001431

HS C18



G001432

Organic Acids

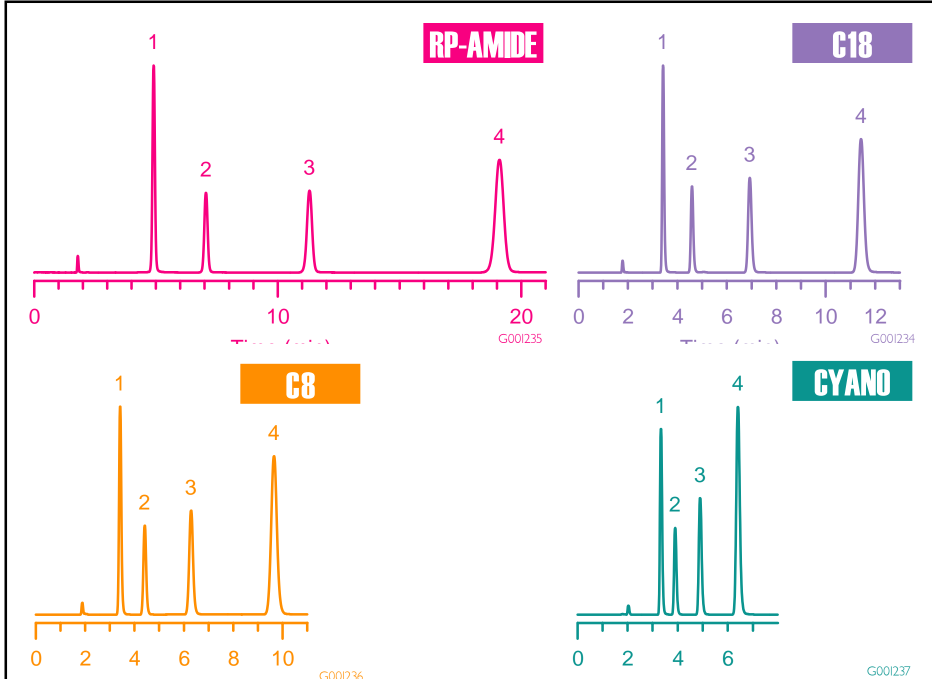
5cm x 4.6mm column,
MeOH:H_2O, 0.1% TFA (40:60)
2.0mL/min
20°C
UV, 254nm
10µL

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

Parabens

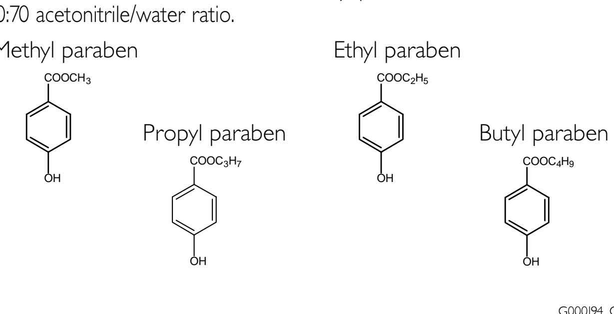
conditions for RP-AmideC16, C18, and C8
MeCN:H_2O, (40:60),
1mL/min, 20°C, UV, 254nm,
10µL
conditions for Cyano
MeCN:H_2O, (30:70),
1mL/min, 20°C, UV, 254nm,
10µL

1. Methyl paraben
2. Ethyl paraben
3. Propyl paraben
4. Butyl paraben



Parabens

Parabens are alkyl esters of 4-hydroxybenzoic acid and are commonly used in formulations of a variety of drugs. The lower members of the sequence are very polar, and hydrophobicity increases in the order methyl<ethyl<propyl<butyl. A significant feature in the RP-HPLC of these molecules under unbuffered mobile phase conditions is that all four parabens are retained longer on the RP-AmideC16 column with either acetonitrile or methanol as organic component, in comparison with C8 or C18 columns. This behavior may be attributable to the strong H-bonding capability of the phenolic hydroxyl with the amide moiety of the RP-AmideC16 stationary phase. The Cyano column requires more aqueous mobile phase conditions for resolution of the four parabens and its low hydrophobic retention is reflected in the elution of butyl paraben in about 6min even at this 30:70 acetonitrile/water ratio.

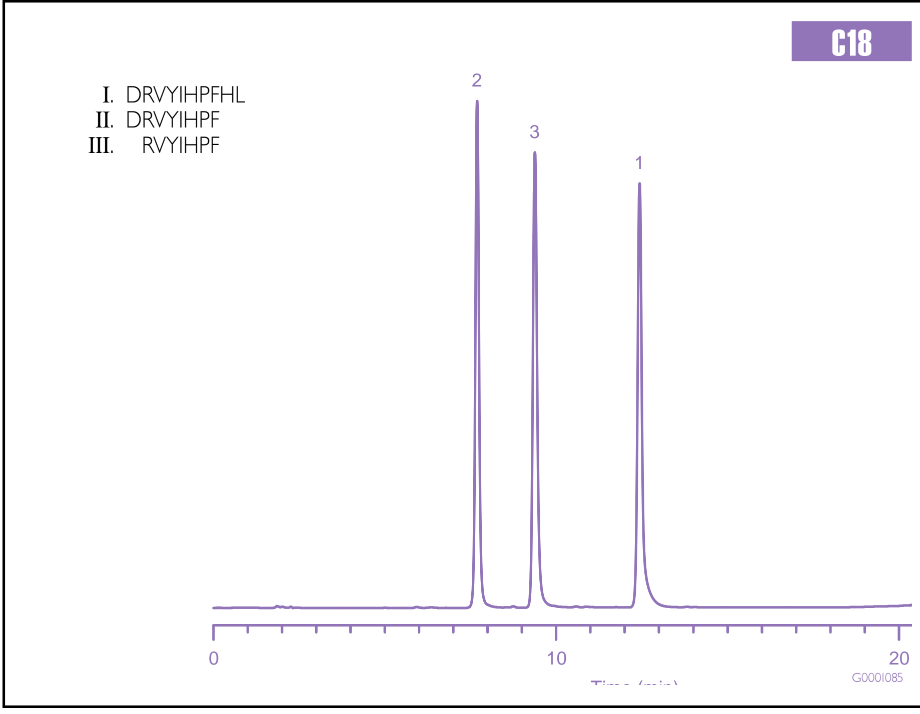


G000194, G000195, G000196, G000197

Peptides (Angiotensins)

Discovery C18
15cm x 4.6mm column,
5µm particles,
(A) 5mM $(NH_4)H_2PO_4$/ NH_4OH, pH 7.0
(B) 5mM $(NH_4)H_2PO_4$/ NH_4OH, pH 7.0: MeCN (50:50)
30-60% B in 15 min
1mL/min
35°C

1. Angiotensin I
2. Angiotensin II
3. Angiotensin III



Peptides (Angiotensins)

Angiotensins are peptide hormones that effect blood pressure and aldosterone release from adrenal glands. Human angiotensins I, II, & III contain a conserved seven amino acid sequence exemplified by angiotensin III. Two additional *C*-terminal amino acids, histidine and leucine, of angiotensin I confer greater retention on RPC columns compared to angiotensins II & III. Angiotensin II differs from angiotensin III only by the addition of an *N*-terminal aspartate. Under conditions typical for peptide chromatography (pH ≤ 2.0), angiotensins II & III are not resolved since the aspartate contains no charge and the retention coefficient (pH 2.0) is only 0.2. Usually, resolution of these two angiotensins is performed at alkaline pH, but with Discovery C18, baseline resolution is achieved at neutral pH. At pH 7.0 the retention coefficient of aspartate is -2.6 and thus significantly diminishes retention. Consequently, separating the angiotensins at neutral pH on Discovery C18 provides baseline resolution of all three components.

Peptides (Carboxyamidomethylated BSA Tryptic Digest)

Discovery columns provide high resolution of very complex peptide mixtures. Selectivity can be conveniently altered by use of different bonded phases. RP-AmideC16, with its embedded polar group, provides a unique selectivity compared to conventional alkyl bonded phases typically employed for peptide mapping.

RP-AMIDE

0 20 40 60 80 100 120

Time (min)

G001087

C18

0 20 40 60 80 100 120

Time (min)

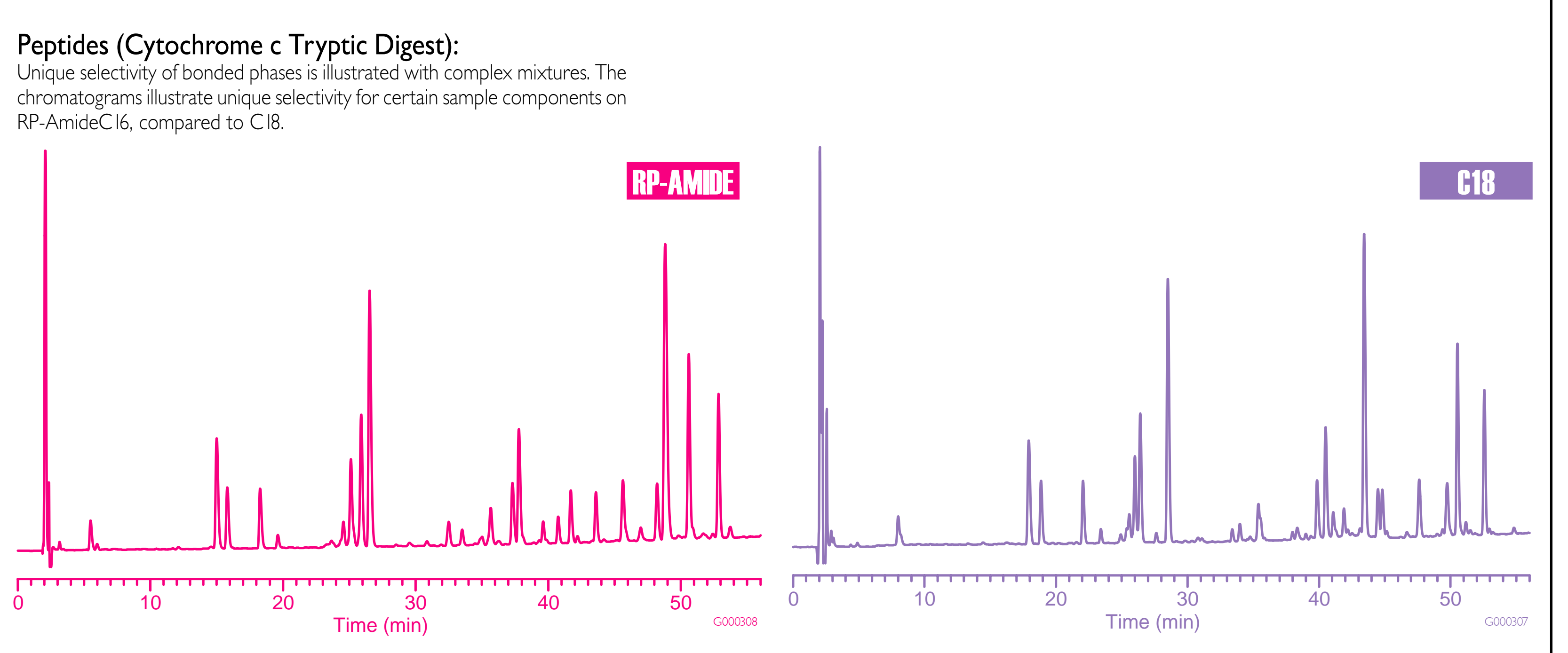
G001086

Peptides (Carboxyamido-methylated BSA Tryptic Digest)

25cm x 4.6mm columns,
5µm particles,
(A) 0.1% TFA, 1% MeCN in H_2O
(B) 0.1% TFA, 40% MeCN
1 mL/min
35°C
0-100% B in 130 min

Peptides (Cytochrome c Tryptic Digest):

Unique selectivity of bonded phases is illustrated with complex mixtures. The chromatograms illustrate unique selectivity for certain sample components on RP-AmideC16, compared to C18.



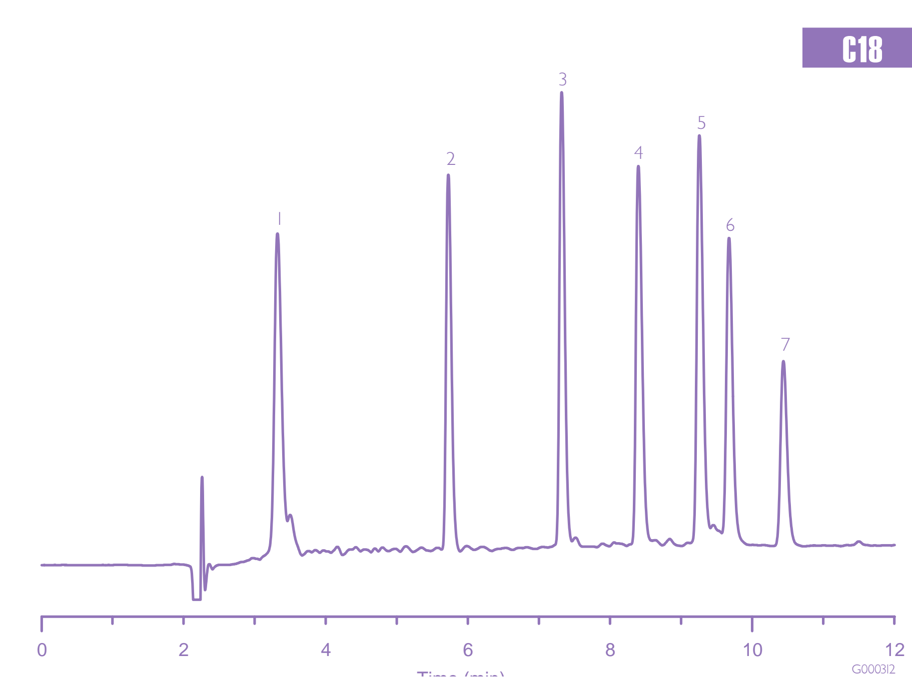
Peptides (Cytochrome c Tryptic Digest)

15cm x 4.6mm columns,
5µm particles,
(A) 0.1% TFA, 3% n-propanol in H_2O
(B) 0.1% TFA, 3% n-propanol in MeCN
1 - 37% B in 72 min
1.0mL/min
35°C

Peptides (Substance P and Fragments)

Discovery C18
15cm x 4.6mm column
5µm particles,
(A) 0.1% TFA in H_2O
(B) 0.1% TFA in MeCN
5 - 29% B in 6 min, then to 34% B in 5 min
1mL/min
35°C

Substance P:
RPKPQQFFGLM
Fragments
1. 1-4
2. 1-7
3. 1-9
4. 8-11
5. Holopeptide
6. 2-11
7. 7-11



Peptides (Substance P and Fragments)

Substance P is a vasoactive peptide which among other things, induces vasodilation and increases capillary permeability. Several features can be discerned from the chromatogram of this undecapeptide. Presence of an additional phenylalanyl residue in fragment 7 makes it substantially more hydrophobic than fragment 4, which lacks this additional residue. Presence of an additional phenylalanyl residue in fragment 3 also imparts significant hydrophobicity relative to fragment 2. The difference between fragment 3 and the holopeptide, the lack of the *C*-terminal amino acids leucine and methionine in the former, make the former much less hydrophobic, thereby resulting in earlier elution. This example demonstrates that in general, at acidic pH (2.0), *N*-terminal variants impact retention less than *C*-terminal variants, because of the α-amino group's positive charge, while the *C*-terminal carboxylate is neutral.

Phloroglucinol from Water

Discovery C18 column,
15cm x 4.6mm, 5µm particles,
MeOH:H_2O (60:40)
1mL/min
ambient temp.
UV, 254nm
10µL of each fraction

SPE Tube:
DPA6-S
600mg/3mL

Breakthrough Analysis
Breakthrough was defined as the point at which the concentration of phloroglucinol in the eluent from the tube was greater than that of a 2% solution of phloroglucinol in water. Concentrations were determined via HPLC-UV and by comparing peak heights of the phloroglucinol peak from each fraction to peak heights of phloroglucinol in standards.

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

Condition: 2mL MeOH, then 2mL H_2O

Apply Sample: 1 mL aliquots of 1mg/mL phloroglucinol in water were applied using a flow rate of 0.75mL/min; fractions were collected after each 1mL of sample was applied.

Breakthrough Results

Fraction	Peak Height (mAU)
1	4.2
2	4.0
3	4.3
4	4.4
5	4.7
6	6.2
7	28.5
2% phloroglucinol standard	9.3

Breakthrough of phloroglucinol occurred while fraction 7 was applied to the DPA-6S SPE tube. This result corresponds to a loading capacity of about 12mg of phloroglucinol in water per gram of DPA-6S sorbent in this tube configuration.

Phloroglucinol from Water

Phloroglucinol (1,3,5-trihydroxybenzene or phloroglucine) was used as a representative compound for polyhydroxybenzene adsorption on Discovery DPA-6S polyamide sorbent. 600mg of bulk DPA-6S sorbent was packed into 3mL SPE tubes and samples were processed through the tubes.

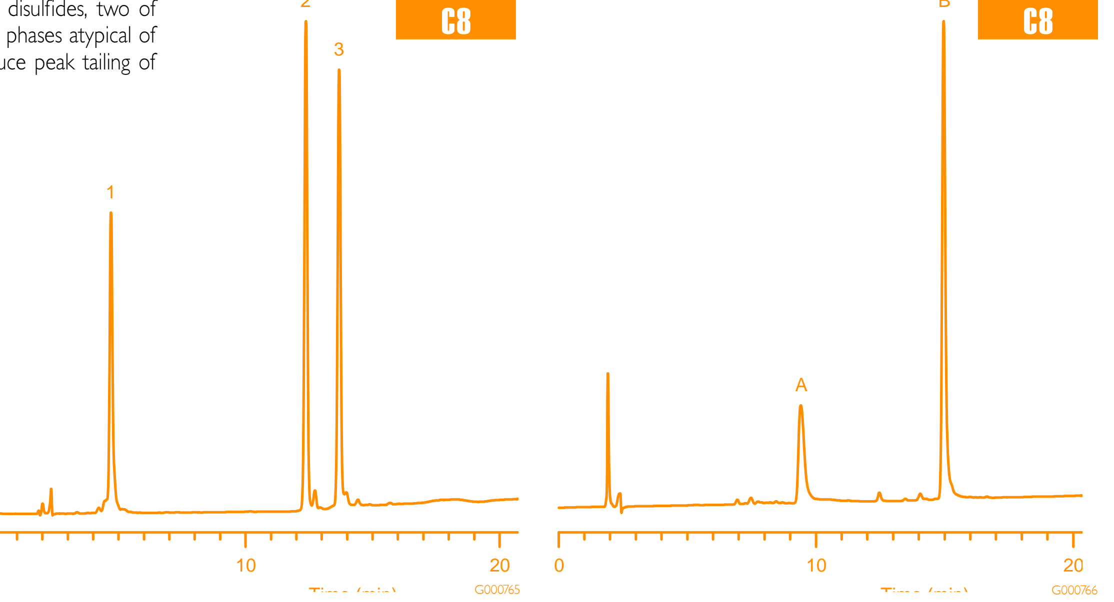
Phloroglucinol

OH
HO OH

G001274

Proteins (Bovine Insulin)

Insulin is a heterodimeric protein hormone that directly affects uptake and metabolism of glucose. The native protein contains three disulfides, two of which serve to crosslink the two subunits. Buffered mobile phases atypical of peptide chromatography are required to dramatically reduce peak tailing of subunit A.



Proteins (Bovine Insulin)

15cm x 4.6mm columns,
5µm particles,
(A) 50mM H_2NaPO_4, 50mM Na_2SO_4, pH 3.0 (w/H_3PO_4): MeCN (95:5)
(B) 50mM H_2NaPO_4, 50mM Na_2SO_4, pH 3.0 (w/H_3PO_4): MeCN (50:50)
33.3% to 73.3% B (20% to 38% MeCN) in 18 min
1mL/min, 35°C, UV, 220nM

1. Subunit A, oxidized (sulfonic acids; Sigma® I1633)
2. Holoenzyme (Sigma I5500)
3. Subunit B, oxidized (sulfonic acids; Sigma I6383)

Holoenzyme (Sigma I5500) reduced and carboxy-methylated
A. Subunit A
B. Subunit B

Tannic Acid from Water or Methanol

Tannic acid (gallotannic acid) is a naturally occurring substance found in tree barks, fruits, and other plant parts. Tannins are derivatives of gallic acid or flavanols, and comprise a broad group of plant-derived phenolic compounds which have the ability to precipitate proteins. Some tannins may be more toxic than others, depending upon the source. Tannins derived from nutgalls are believed to be carcinogens, while those found in tea and coffee are considered non-toxic. These compounds are soluble in water and alcohols and can be extracted from plant matter. Tannins may interfere with the analysis of bioactives in natural product research. Discovery DPA-6S polyamide SPE products can be used to remove tannins from aqueous and methanolic solutions in these applications. In this experiment, 300mg or 600mg of bulk DPA-6S sorbent was packed into 3mL SPE tubes and tannic acid solutions were processed through the tubes.

SPE

SPE Procedure, Using Zymark RapidTrace SPE Workstation

Condition: 2mL MeOH for methanolic samples
2mL MeOH followed by 2mL H_2O for aqueous samples.

Apply Sample: 1mL aliquots of 10mg/mL tannic acid in MeOH or H_2O were applied using a flow rate of 0.75mL/min; fractions were collected after each 1mL of sample was applied.

Breakthrough Results

DPA-6S Sorbent Mass (mg)	Fraction	Methanolic sample Peak Height (mAU)	Aqueous Sample Peak Height (mAU)
	1	3.7	3.0
	2	8.8	3.5
	3	77.1	6.3
300	4	466.5=breakthrough	21.2
	5	> 3000	26.2
	6	> 3000	28.7
	7	> 3000	201.1=breakthrough
600	7	breakthrough	-
	14	-	breakthrough
	2% acid standard	170.5	145.2

Equivalent results were obtained when using a slower flow rate (0.36mL/min) during sample application, indicating that capacity of DPA-6S for tannic acid does not depend heavily on flow rate. Capacity also seems to be linear with respect to sorbent mass. These results indicate that Discovery DPA-6S products can be used to remove tannic acid and other forms of the compound from aqueous or methanolic solutions, while allowing unretained species to pass through, free of these interfering species.

Tannic Acid from Water or Methanol

HPLC Analysis Conditions
Discovery C18 column,
15cm x 4.6mm, 5µm particles,
MeOH:H_2O (60:40)
1mL/min, ambient
UV, 254nm
10mL of each fraction

SPE Tube:
DPA-6S
300mg/3mL and 600mg/3mL

Breakthrough Analysis
Breakthrough was defined as the point at which the concentration of tannic acid in the eluent from the tube was greater than that of a 2% solution of tannic acid in methanol or water. Concentrations were determined via HPLC-UV and by comparing peak heights of the tannic acid peak from each fraction to peak heights of tannic acid in the appropriate standard.

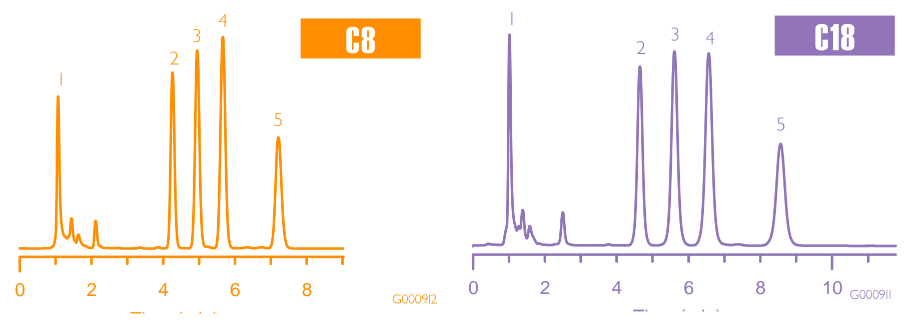
Fat Soluble Vitamins (A and E)

15cm x 4.6mm columns,
5µm particles,
2mL/min
30°C
UV, 290nm
10µL

Discovery C18
MeOH:H_2O (95:5)

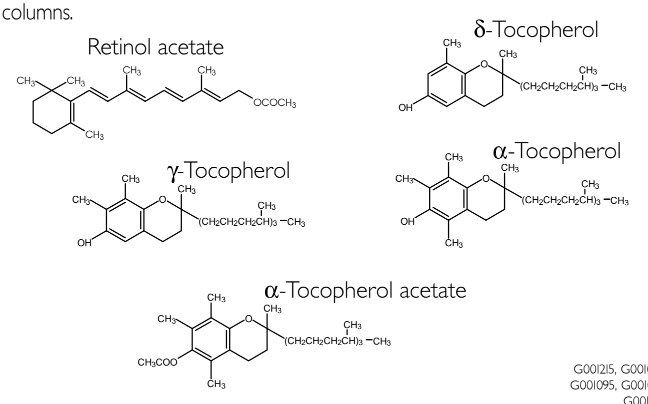
Discovery C8
MeCN:H_2O (90:10)

1. Retinol acetate (Vitamin A acetate), 50µg/mL
2. δ-Tocopherol, 165µg/mL
3. γ-Tocopherol, 200µg/mL
4. α-Tocopherol (Vitamin E), 292µg/mL
5. α-Tocopherol acetate (Vitamin E acetate), 405µg/mL



Fat Soluble Vitamins (A and E)

The three isomeric tocopherols (alpha, gamma, and delta) differ in the substitution pattern on the aromatic ring of the 2,2-dimethylchroman skeleton. The delta isomer has one methyl, the gamma analog has two, in the 7, 8 positions, and the alpha isomer has three, in the 5, 7, 8 positions. The hydrophobicity-based elution of the three tocopherols follows the same order. Alpha tocopheryl acetate, being much less polar than the tocopherols, is eluted last. Vitamin A acetate (retinol acetate) contains a series of five conjugated double bonds and is evidently the most polar of all the components, resulting in its fast elution on both C8 and C18 columns.

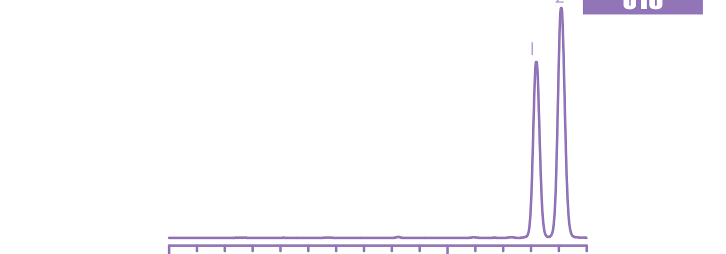


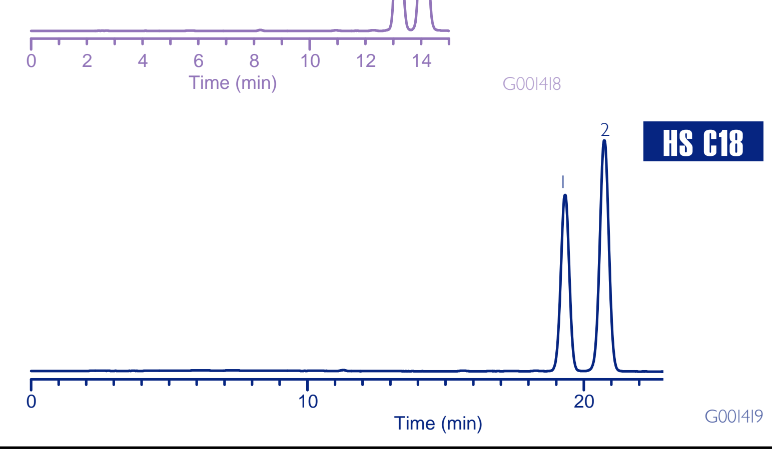
G001215, G001094, G001095, G001096, G001097

Fat Soluble Vitamins (D_2 and D_3)

Discovery C18
15cm x 4.6mm column,
100% MeCN
0.8mL/min
30°C
UV, 290nm
10µL, 50µg/mL of each analyte

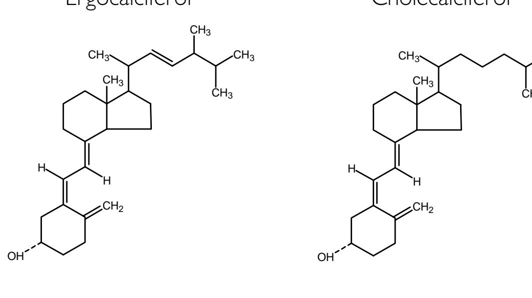
1. Ergocalciferol (Vitamin D_2)
2. Cholecalciferol (Vitamin D_3)





Fat Soluble Vitamins (D_2 and D_3)

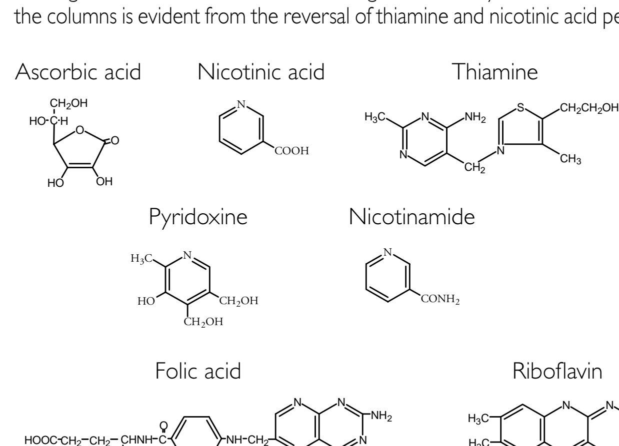
Vitamin D_2 (ergocalciferol) is 9,10-seco-ergosta-5,7,10(19),22-tetraen-3-ol and the D_3 analog is 9,10-secocholesta-5,7,10-trien-3-ol. They differ in the number of double bonds (one more in D_2) and methyl groups (one more in D_2 in the form of a branched chain). Thus, they are closely related structures. The versatility of the C18 column in separating this pair in less than 15 minutes is evident. The D_2 analog, being more polar, is eluted first. The HS C18 column provides better resolution with slightly longer run times.



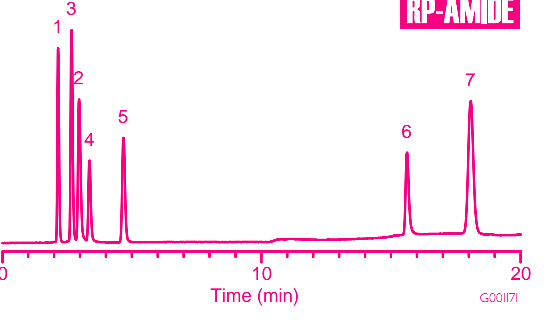
G001098, G001099

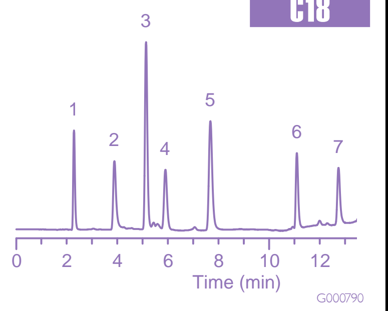
Water Soluble Vitamins

The separation of seven water soluble vitamins on three Discovery columns under gradient conditions is shown in this figure. Selectivity differences among the columns is evident from the reversal of thiamine and nicotinic acid peaks.



G001122, G001216, G001124, G001217, G001218, G001127, G001130





C8

1 2 3 4 5 6 7

0 2 4 6 8 10 12

Time (min)

G000789

Water Soluble Vitamins

RP-AmideC16
15cm x 4.6mm column,
5µm particles,
MeOH:50mM KH_2PO_4 in H_2O,
pH 4.5 (30:70), 35°C, UV, 220nm,
10µL

C18 and C8
15cm x 4.6mm columns,
5µm particles,
50mM KH_2PO_4, pH 4.5 to
MeOH:50mM KH_2PO_4, pH 4.5
(30:70)
12 min (Discovery C8) or
8 min (Discovery C18)
1mL/min, ambient temp., UV,
254nm, 1µL, 1µg/mL of each analyte

1. Ascorbic acid
2. Nicotinic acid
3. Thiamine
4. Pyridoxine
5. Nicotinamide
6. Folic acid
7. Riboflavin

Discovery SPE-96 Well Plates & Accessories

96-Well Plates

Discovery DSC-18 SPE-96 Plate, 25mg/well	575601-U
Discovery DSC-18 SPE-96 Plate, 50mg/well	575602-U
Discovery DSC-18 SPE-96 Plate, 100mg/well	575603-U
Discovery DSC-18Lt SPE-96 Plate, 25mg/well	575604-U
Discovery DSC-18Lt SPE-96 Plate, 50mg/well	575605-U
Discovery DSC-18Lt SPE-96 Plate, 100mg/well	575606-U
Discovery DSC-Si SPE-96 Plate, 25mg/well	575607-U
Discovery DSC-Si SPE-96 Plate, 50mg/well	575608-U
Discovery DSC-Si SPE-96 Plate, 100mg/well	575609-U
Discovery DSC-PS/DVB SPE-96 Plate, 25mg/well	575610-U
Discovery DSC-PS/DVB SPE-96 Plate, 50mg/well	575611-U

96-Well Plate Accessories

96 Sq. Well Collection Plates, 0.35mL, PP, 50/pk	575651-U
96 Sq. Well Collection Plates, 1mL, PP, 50/pk	575652-U
96 Sq. Well Collection Plates, 2mL, PP, 50/pk	575653-U
Disposable Reservoir/Waste Tray, PVC, 25/pk	575654-U
96 Sq. Well Piercable Cap Mats, 50/pk	575655-U
Reagent Reservoir	R9259 - 100ea.
Cluster Tube Rack	Z372226 - 1pak

PlatePrep Manifold and Manifold Replacement Parts

96 Well Plate Starter Kit with Manifold	575650-U

Contents of kit: 1 Plate Prep Manifold; 1 96 Sq. Well Collection Plates, 2mL, PP; 2 Disposable Reservoir/Waste Trays, PVC; 1 96 Sq. Well Piercable Cap Mats; 5 Reagent Reservoirs; 1 Cluster Tube Rack

PlatePrep Vacuum Manifold	57192-U
Acrylic Clear Top for Manifold	57193-U
Polypropylene Base for Manifold	57194-U
Gasket Kit for Manifold	57195-U
Vacuum Gauge/Bleed Valve for Manifold	57161-U

Improve your sample preparation with Discovery SPE-96 Well Plates!

Higher throughput and more...

consistent bedweight and flow rates for automated or robotic processing reduced height for larger volumes without leak-prone extensions standard dimensions that are common to most square well extraction plate designs ideal for fulfilling ISO, GMP, GLP requirements compatible with most vacuum manifolds and collection plates works with most robotic and automated sample processing systems reproducible from lot to lot, plate to plate, and well to well consistent capacities, recoveries and flow rates certificate of analysis included with each plate

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RapidTrace, TurboVap, Zymark - Zymark Corp.

HPLC and SPE Products for Pharmaceutical Analysis and Purification

Discovery HPLC Special Application Kits

DESCRIPTION	CAT. NO.
DISCOVERY SELECTIVITY PACKS[1]	
5cm x 2.1mm ID Columns	55720-U21
10cm x 2.1mm ID Columns	569853-U
15cm x 2.1mm ID Columns	55722-U21
5cm x 3.0mm ID Columns	55720-U30
10cm x 3.0mm ID Columns	569852-U
15cm x 3.0mm ID Columns	55722-U30
25cm x 3.0mm ID Columns	55724-U30
5cm x 4.0mm ID Columns	55720-U40
10cm x 4.0mm ID Columns	569851-U
15cm x 4.0mm ID Columns	55722-U40
25cm x 4.0mm ID Columns	55724-U40
5cm x 4.6mm ID Columns	55720-U
10cm x 4.6mm ID Columns	569850-U
15cm x 4.6mm ID Columns	55722-U
25cm x 4.6mm ID Columns	55724-U
DISCOVERY RP-AMIDEC16 VALIDATION PACKS[2]	
5cm x 2.1mm ID Columns	55705-U21
10cm x 2.1mm ID Columns	569802-U
15cm x 2.1mm ID Columns	55707-U21
5cm x 4.6mm ID Columns	55705-U
10cm x 4.6mm ID Columns	569803-U
15cm x 4.6mm ID Columns	55707-U
25cm x 4.6mm ID Columns	55709-U

DESCRIPTION	CAT. NO.
DISCOVERY C18 VALIDATION PACKS[2]	
5cm x 2.1mm ID Columns	55700-U21
10cm x 2.1mm ID Columns	569800-U
15cm x 2.1mm ID Columns	55702-U21
5cm x 4.6mm ID Columns	55700-U
10cm x 4.6mm ID Columns	569801-U
15cm x 4.6mm ID Columns	55702-U
25cm x 4.6mm ID Columns	55704-U
DISCOVERY C8 VALIDATION PACKS[2]	
5cm x 2.1mm ID Columns	55710-U21
10cm x 2.1mm ID Columns	569804-U
15cm x 2.1mm ID Columns	55712-U21
5cm x 4.6mm ID Columns	55710-U
10cm x 4.6mm ID Columns	569805-U
15cm x 4.6mm ID Columns	55712-U
25cm x 4.6mm ID Columns	55714-U
DISCOVERY CYANO VALIDATION PACKS[2]	
5cm x 2.1mm ID Columns	55715-U21
10cm x 2.1mm ID Columns	569806-U
15cm x 2.1mm ID Columns	55717-U21
5cm x 4.6mm ID Columns	55715-U
10cm x 4.6mm ID Columns	569807-U
15cm x 4.6mm ID Columns	55717-U
25cm x 4.6mm ID Columns	55719-U

Discovery SPE Tubes

DESCRIPTION	QTY.PKG.	CAT. NO.
DSC-18		
50mg/1mL	108	52601-U
100mg/1mL	108	52602-U
500mg/3mL	54	52603-U
500mg/6mL	30	52604-U
1g/6mL	30	52606-U
2g/12mL	20	52607-U
5g/20mL	20	52608-U
10g/60mL	16	52609-U
bulk packing	100g	52600-U
DSC-18Lt		
50mg/1mL	108	52610-U
100mg/1mL	108	52611-U
500mg/3mL	54	52613-U
500mg/6mL	30	52615-U
1g/6mL	30	52616-U
2g/12mL	20	52618-U
5g/20mL	20	52621-U
10g/60mL	16	52622-U
bulk packing	100g	52623-U
DPA-6S		
50mg/1mL	108	52624-U
250mg/3mL	54	52625-U
250mg/6mL	30	52626-U
500mg/6mL	30	52627-U
1g/12mL	20	52629-U
2g/20mL	20	52631-U
5g/60mL	16	52632-U
50g/in 800mL Büchner funnel	1	52634-U
bulk packing	50g	52633-U

Discovery HPLC Columns

ID (mm)	LENGTH (cm)	CAT. NO.
DISCOVERY RP-AMIDEC16		
2.1	5	505005-21
2.1	10	569320-U
2.1	12.5	569329-U
2.1	15	505013-21
3.0	5	505005-30
3.0	10	569321-U
3.0	12.5	569330-U
3.0	15	505013-30
3.0	25	505064-30
4.0	5	505005-40
4.0	10	569322-U
4.0	12.5	569331-U
4.0	15	505013-40
4.0	25	505064-40
4.6	5	505005
4.6	10	569323-U
4.6	12.5	569332-U
4.6	15	505013
4.6	25	505064
DISCOVERY C18		
2.1	5	504947-21
2.1	10	569220-U
2.1	12.5	569229-U
2.1	15	504955-21
3.0	5	504947-30
3.0	10	569221-U
3.0	12.5	569230-U
3.0	15	504955-30
3.0	25	504971-30
4.0	5	504947-40
4.0	10	569222-U
4.0	12.5	569231-U
4.0	15	504955-40
4.0	25	504971-40
4.6	5	504947
4.6	10	569223-U
4.6	12.5	569232-U
4.6	15	504955
4.6	25	504971
DISCOVERY HS C18		
2.1	5	569253-U
2.1	7.5	569254-U
2.1	15	569255-U
4.6	5	569250-U
4.6	7.5	569251-U
4.6	15	569252-U

ID (mm)	LENGTH (cm)	CAT. NO.
DISCOVERY C8		
2.1	5	59352-U21
2.1	10	569420-U
2.1	12.5	569424-U
2.1	15	59353-U21
3.0	5	59352-U30
3.0	10	569421-U
3.0	12.5	569425-U
3.0	15	59353-U30
3.0	25	59354-U30
4.0	5	59352-U40
4.0	10	569422-U
4.0	12.5	569426-U
4.0	15	59353-U40
4.0	25	59354-U40
4.6	5	59352-U
4.6	10	569423-U
4.6	12.5	569427-U
4.6	15	59353-U
4.6	25	59354-U
DISCOVERY CYANO		
2.1	5	59355-U21
2.1	10	569521-U
2.1	12.5	569524-U
2.1	15	59356-U21
3.0	5	59355-U30
3.0	10	569522-U
3.0	12.5	569525-U
3.0	15	59356-U30
3.0	25	59357-U30
4.0	5	59355-U40
4.0	10	569523-U
4.0	12.5	569526-U
4.0	15	59356-U40
4.0	25	59357-U40
4.6	5	59355-U
4.6	10	569520-U
4.6	12.5	569527-U
4.6	15	59356-U
4.6	25	59357-U

Call for availability on prep columns.

2cm Supelguard Cartridges with 5µm Discovery Packings

Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

	4.0mm ID CARTRIDGE[3]		3.0mm ID CARTRIDGE		2.1mm ID CARTRIDGE	
DISCOVERY PHASE	KIT CAT. NO.	PK. OF 2 CAT. NO.	KIT CAT. NO.	PK. OF 2 CAT. NO.	KIT CAT. NO.	PK. OF 2 CAT. NO.
Discovery C18	505129	505137	59575-U	59576-U	505161	505188
Discovery RP-AmideC16	505080	505099	59577-U	59578-U	505102	505110
Discovery C8	59589-U	59590-U	59579-U	59580-U	59587-U	59588-U
Discovery HS C18	569275-U	569274-U	-	-	569277-U	569276-U
Discovery Cyano	59585-U	59586-U	569570-U	569571-U	59583-U	59584-U

1 Four columns of equal dimensions, one of each Discovery phase (C18, RP-AmideC16, C8, Cyano).
2 Identical columns made from three different bonded phase lots.
3 For 4.0mm ID or 4.6mm ID analytical columns.

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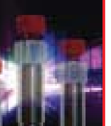
CJW

Ascentis® HPLC Columns

Elevated HPLC Performance from a Wide Range of Phases

- Wide range of chromatographic selectivities
- Enhanced polar compound retention
- Ideal for LC-MS and all modern applications

SIGMA-ALDRICH®



Ascentis: Elevated HPLC Performance

We have placed heavy emphasis on optimizing Ascentis phases with relation to the three terms of the resolution equation: efficiency, retention and selectivity. However, our strongest emphasis has been on the most powerful term, selectivity. Together, Ascentis bonded phases have a wide range of selectivities. It is likely that one or more Ascentis phase will accomplish any small molecule HPLC separation.

Packed in micro- to preparative hardware dimensions, Ascentis products cover all HPLC application areas, including the most sensitive trace-level analyses.

The General Features of the Ascentis Family Include:

- High purity, type B silica for inertness, reproducibility and stability
- Modern bonding processes that optimize bonded phase coverage and maximize stability, while minimizing bleed and unwanted secondary interactions
- Wide selection of bonded phase chemistries and bare silica
- Phases with enhanced polar compound retention
- Compatible with LC-MS and all of today's sensitive instruments and methods
- Scalable selectivity from analytical to preparative
- High surface area silica for high preparative loading capacity

Ascentis Characteristics

Phase	USP Code	Key Competitive Feature	Modes	Primary Uses	Page
Ascentis C18	L1	High surface area, inert surface	**Reversed-phase**	Small molecules and peptides	**9**
Ascentis RP-Amide	L60	Phase stability, low bleed	**Reversed-phase**	Excellent "first choice" alternative to C18 for routine RP method development. Polar molecules, especially phenolics and other H-bond donors, acids, bases (uncharged), anilines	**10**
Ascentis Phenyl	L11	Phase stability, low bleed	**Reversed-phase, HILIC**	Ring systems and strong dipoles, π-acids, π-electron acceptors, heteroaromatics, nitroaromatics	**12**
Ascentis ES Cyano	L10	Phase stability	**Reversed-phase, HILIC, 100% Aqueous**	Polar compounds, strong dipoles, tricyclic antidepressants	**14**
Ascentis Silica	L3	High loading capacity, controlled and uniform surface activity	**Normal phase (non-aqueous), HILIC**	Non-polar compounds (in NP mode), highly-polar compounds (in HILIC mode), nucleosides, amino acids	**16**
Ascentis C8	L7	High surface area, inert surface	**Reversed-phase**	Small molecules and peptides	**18**
Discovery® HS F5*	L43	Orthogonal selectivity to C18, ours is well-characterized	**Reversed-phase, HILIC, ion-exchange**	All electron and π-electron donors, bases (charged), positional isomers	**20**

* We have chosen to include Discovery HS F5 in this brochure because of its complementary selectivity to the Ascentis phases and its benefits for certain analytes.

Ascentis HPLC columns represent a continuum of improvement through innovations in HPLC technology.

Developing HPLC Methods on Ascentis

Selecting An Ascentis Column

Column Screening: The Ascentis & Discovery Method Development "Tool Kit"

We recommend every HPLC method developer have these five columns in their arsenal.

Ascentis C18 – Classic C18 selectivity will achieve most reversed-phase separations

Ascentis RP-Amide – For enhanced retention and performance of polar compounds, especially bases (uncharged) and compounds with H-bond potential

Ascentis Phenyl – For enhanced retention and performance of polar compounds, especially ring systems, dipoles, and nitroaromatics

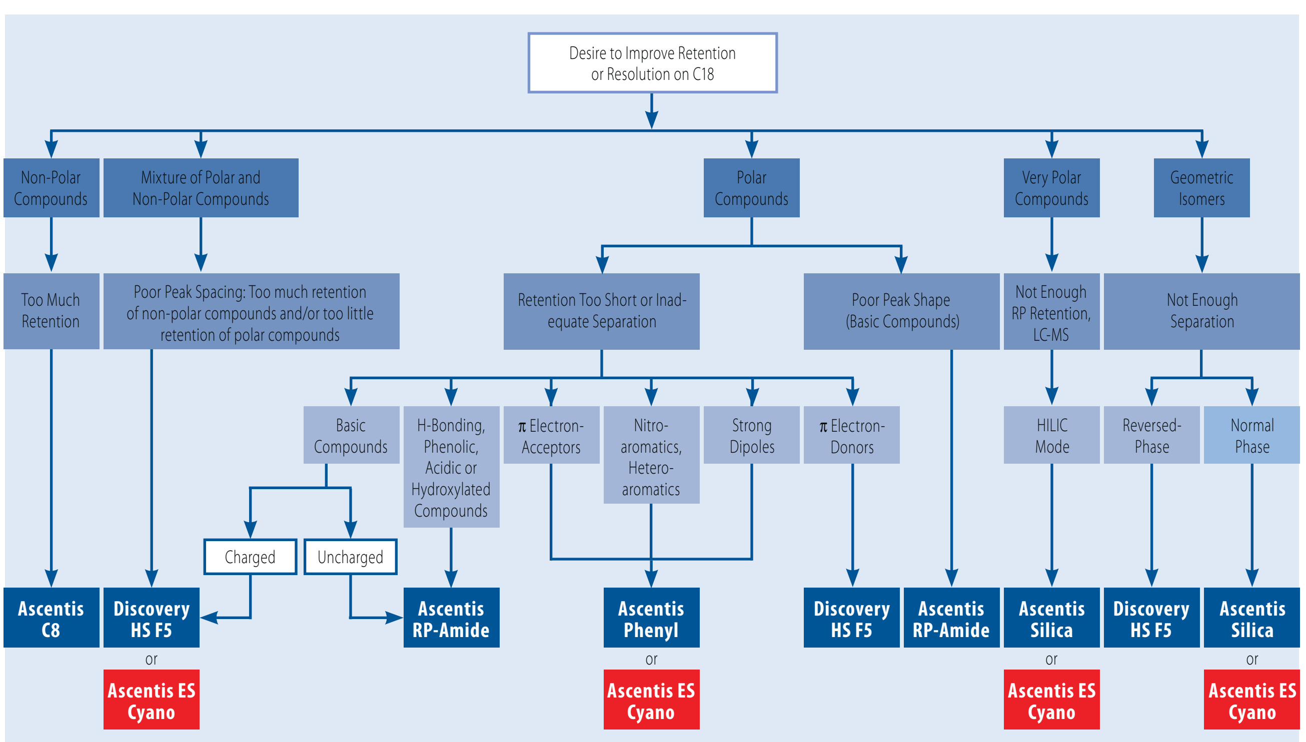
Ascentis ES Cyano – Extra stable for low pH mobile phases due to sterically protected phase. Useful for polar selectivity in reversed-phase mode. Also useful as an alternative to silica in HILIC mode

Discovery HS F5 – For enhanced retention and performance of polar compounds, especially bases (charged) and when the sample contains a mixture of non-polar and polar compounds

Simply screen these five columns in your desired mobile phase, using your preferred column.

Choosing an Ascentis or Discovery Phase Based on Compound Class and Separation Challenge or Objective

Typically, Ascentis C18 is the first choice for starting a new method. However, when a C18 doesn't give the desired separation or your sample contains compounds that are known to be difficult to retain or resolve on a C18, consider changing the stationary phase. The range of selectivity provided by Ascentis and Discovery phases makes this easy. The flow chart below helps guide the selection of Ascentis or Discovery phase based on the particular compound type or separation challenge. For more information about each phase, and the other Ascentis phases, please refer to their dedicated pages in this brochure.



Column selection guidelines for Ascentis and Discovery phases based on compound class and separation challenge or objective.

TRADEMARKS: ACE - **Advanced Chromatography Technologies;** Ascentis, Discovery, CHIROBIOTIC, CYCLOBOND, CHIRALDEX, CHROMASOLV - **Sigma-Aldrich Biotechnology LP**; Fused-Core - **Advanced Materials Technology, Inc.;** Nucleosil - **Machery-Nagel;** Prodigy - **Luna;** Symmetry, XTerra - **Waters Corporation;** Zorbax - **Agilent Technologies**

Harnessing the Power of Chromatographic Selectivity

Chromatographic resolution is a function of column efficiency (***N***), retention (***k***) and selectivity (α). It is usually written in the form of the resolution equation:

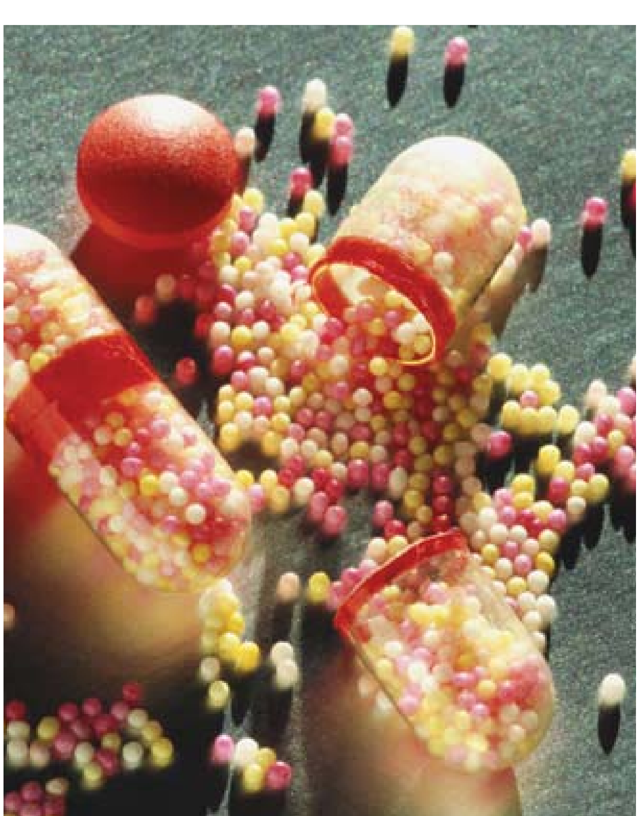
$$R = \frac{\sqrt{N}}{4} \times \frac{k}{k+1} \times \frac{\alpha - 1}{\alpha}$$

When resolution is plotted vs. these three parameters in Figure 1, it becomes apparent that selectivity has the greatest affect on resolution.

Figure 1. Affect of Selectivity on Improving Resolution

The Power to Accomplish Difficult Separations

One of the most important reasons why selectivity is leveraged in HPLC is to resolve closely-eluting compounds. A good example of this is the need to quantify a compound that elutes in the tail of a more abundant compound, perhaps a low-level impurity in the presence of the parent compound, like shown in the Figure 2. By altering the stationary phase, in this case going from a C18 to an RP-Amide, the impurity can be eluted before the main peak, thereby allowing more sensitive and reliable quantification.



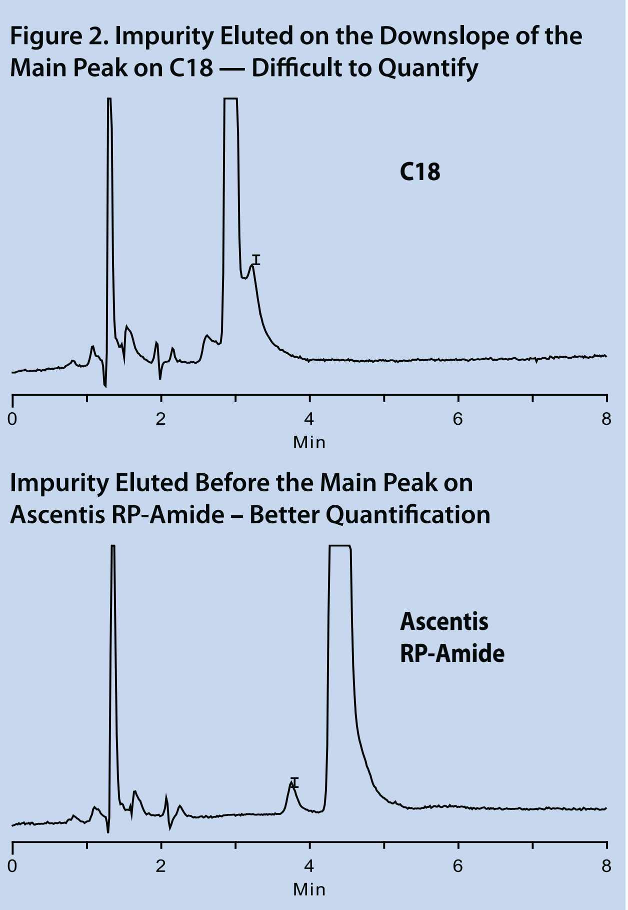


Figure 2. Impurity Eluted on the Downslope of the Main Peak on C18 — Difficult to Quantify

Impurity Eluted Before the Main Peak on Ascentis RP-Amide – Better Quantification

The power of chromatographic selectivity is demonstrated in this example. The C18 column elutes the impurity in the downslope of the major peak, limiting the ability to detect and quantify the impurity. By using a column with different selectivity, in this case an Ascentis RP-Amide, the impurity peak is eluted before the main peak.

Key Ascentis Application Areas

Polar Analytes: Enhanced Retention, Selectivity, and Compatibility with Highly-Aqueous Mobile Phases

Polar compounds are difficult to analyze by traditional reversed-phase because they lack the high proportion of hydrophobic character necessary for retention. Since most pharmaceutically- and biologically-active compounds are highly polar, this has presented a continual problem in HPLC. From the beginning of our commitment to HPLC innovation, we have focused on bonded phase to enhance polar compound retention.

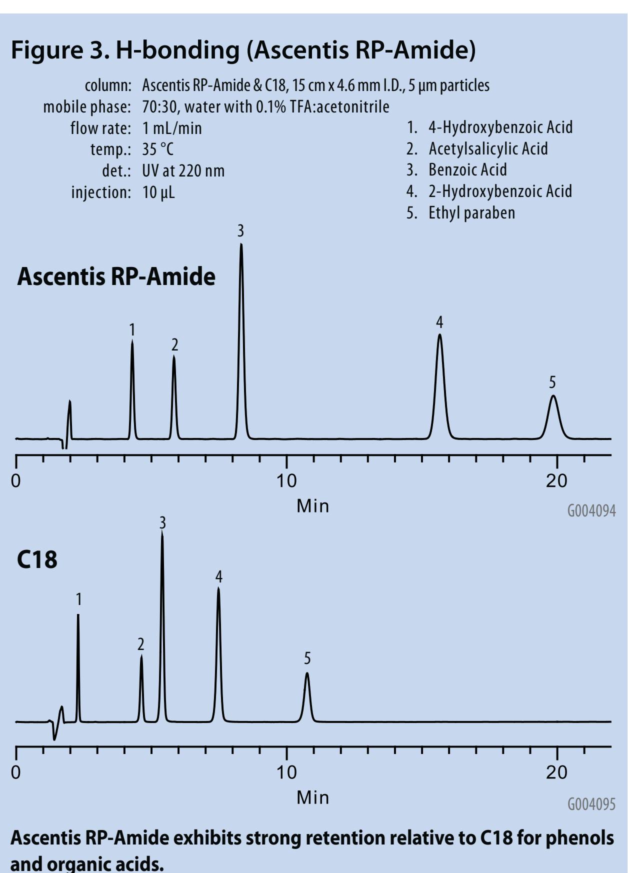
Stationary Phases with Enhanced Polar Compound Retention Compared to C18

Rather than relying solely on dispersive forces to achieve retention, our column portfolio contains bonded phases that lend additional retentive character toward analytes with specific polar functional groups.

Figure 3. H-bonding (Ascentis RP-Amide)

column: Ascentis RP-Amide & C18, 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 70:30, water with 0.1% TFA:acetonitrile
flow rate: 1 mL/min
temp.: 35 °C
det.: UV at 220 nm
injection: 10 µL

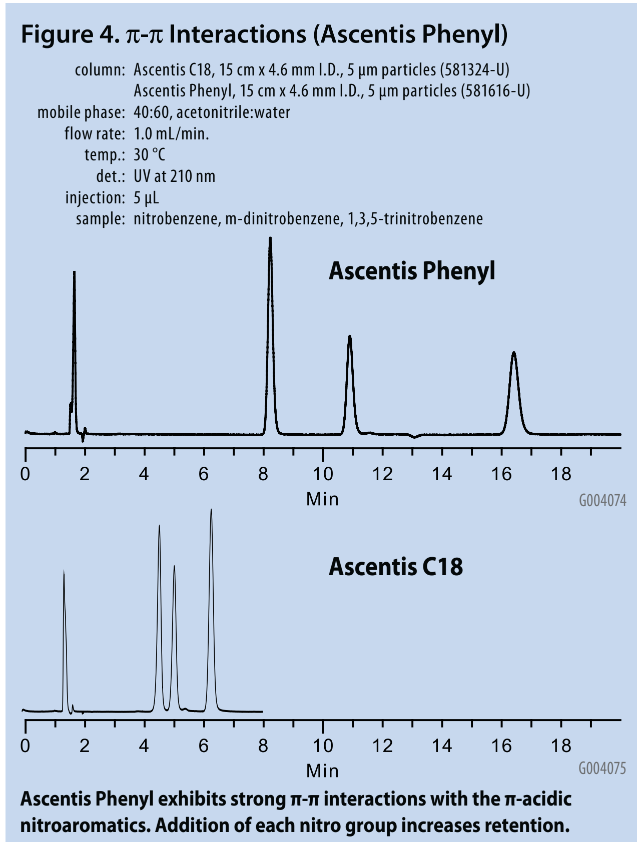
1. 4-Hydroxybenzoic Acid
2. Acetylsalicylic Acid
3. Benzoic Acid
4. 2-Hydroxybenzoic Acid
5. Ethyl paraben



Ascentis RP-Amide exhibits strong retention relative to C18 for phenols and organic acids.

Figure 4. π-π Interactions (Ascentis Phenyl)

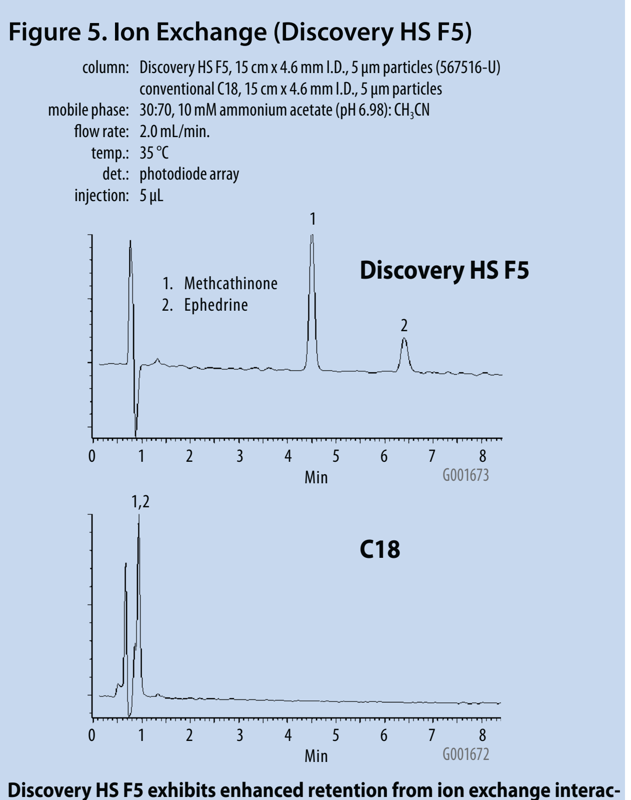
column: Ascentis C18, 15 cm x 4.6 mm I.D., 5 µm particles (581324-U)
Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase: 40:60, acetonitrile:water
flow rate: 1.0 mL/min.
temp.: 30 °C
det.: UV at 210 nm
injection: 5 µL
sample: nitrobenzene, m-dinitrobenzene, 1,3,5-trinitrobenzene



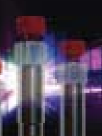
Ascentis Phenyl exhibits strong π-π interactions with the π-acidic nitroaromatics. Addition of each nitro group increases retention.

Figure 5. Ion Exchange (Discovery HS F5)

column: Discovery HS F5, 15 cm x 4.6 mm I.D., 5 µm particles (567516-U)
conventional C18, 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase: 30:70, 10 mM ammonium acetate (pH 6.98): CH_3CN
flow rate: 2.0 mL/min.
temp.: 35 °C
det.: photodiode array
injection: 5 µL



Discovery HS F5 exhibits enhanced retention from ion exchange interactions with ionized analytes.



HILIC Mode: Enhanced Retention and High MS-Suitability

Highly-polar compounds, like underivatized amino acids, nucleosides and nucleotides, are not well-retained by reversed-phase HPLC. However, under HILIC (hydrophilic interaction chromatography) conditions, they can be retained. HILIC is a variation of normal phase HPLC where the mobile phase contains high percentages of organic modifier. It is also called "aqueous normal phase" or ANP. Under high organic conditions, polar interactions become prominent which can lead to increased retention. Ascentis Phenyl, Ascentis ES Cyano, Ascentis Silica and Discovery HS F5 exhibit HILIC character under highly-organic mobile phases. An added benefit of HILIC mobile phases is the high organic (often >90%) is amenable to MS detection.

Stability in Highly-Aqueous Mobile Phases

Unless working in HILIC mode, mobile phases for polar compounds are often highly-aqueous, with only small percentages of organic modifiers. Under such conditions, C18 phases are not wetted, which causes two problems. First, the bonded phase molecules coalesce resulting in phase collapse and subsequent loss of hydrophobic retention. Second, analytes have an unpredictable approach to the silica surface, resulting in irreproducible and unstable retention run-to-run and column-to-column. Ascentis Phenyl, Ascentis ES Cyano, Ascentis RP-Amide, and Discovery HS F5 are completely aqueous-compatible, and will not undergo phase collapse even in mobile phases that contain 100% water.

Figure 6. Separation of Nucleosides on Ascentis Phenyl

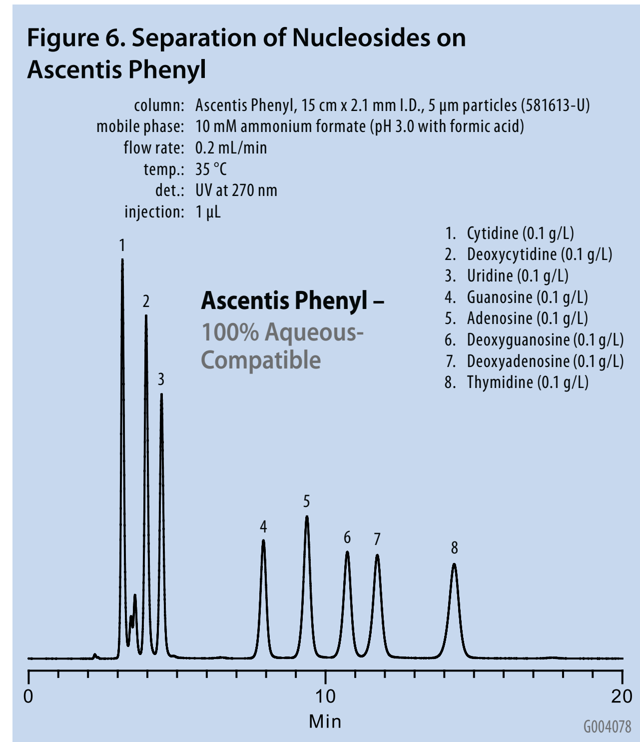
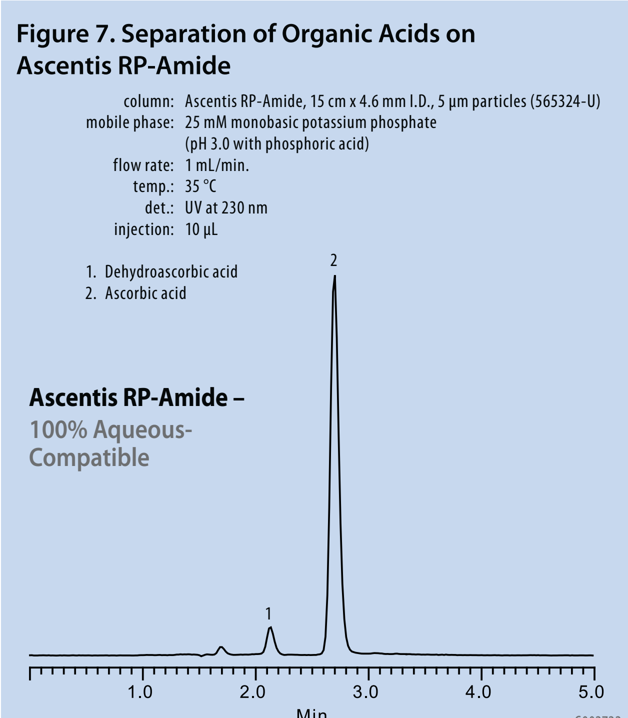


Figure 7. Separation of Organic Acids on Ascentis RP-Amide



Selecting the Right Buffer

A partial list of common buffers and their corresponding useful pH range is supplied. Perhaps the most common buffer in HPLC is the phosphate ion. Although, with the growth of LC-MS, volatile buffers such as TFA, acetate, formate, and ammonia are becoming more frequently used. Remember, the purpose of a buffer in the mobile phase is to inhibit a pH change in the mobile phase after the introduction of a sample. When developing a method, it is important to select a mobile phase with a final pH at least one pH unit away from any analytes pK value. As a rule of thumb, one should work within a ±1 pH unit of the buffer pKa. Typical buffer concentrations for HPLC tend to be 10-100 millimolar level.

Buffer	pKa @ 25 °C	Useful pH Range
Trifluoroacetic acid (TFA)	0.5	<1.5
Phosphate 1	2.1	1.1 - 3.1
Formate	3.8	2.8 - 4.8
Acetate	4.8	3.8 - 5.8
Phosphate 2	7.2	6.2 - 8.2
Ammonia	9.2	8.2 - 10.2
Phosphate 3	12.3	11.3 - 13.3

LC-MS Compatibility Through Phase Stability, Retentivity and Inertness

In today's laboratory, HPLC columns and bonded phases must be compatible with mass spectrometric detection. Complete MS compatibility is an important design input for all Ascentis phases.

Negligible Phase Bleed

Loss of stationary phase can contribute to high background interference in all forms of detection, but it is most notable in MS detection where phase bleed can also lead to fouling of the instrument and subsequent downtime for cleaning and repair. Modern bonding procedures and an intelligent selection of the Ascentis phase chemistry combine to give all Ascentis phases low detectable bleed under MS and sensitive UV detection.

Amide Chemistry Avoids the Need for TEA and TFA Additives

Silanol-suppressing mobile phase additives, like TEA and TFA, are required for good peak shape on traditional HPLC phases. However, because they suppress the MS signal they should be avoided. Ascentis RP-Amide, by virtue of the embedded amide group, does not require silanol-suppressing additives for good peak shape. Formic acid is a suitable acidic modifier for use with Ascentis RP-Amide columns.

Ascentis pH Stability: Extending the Working pH Range

Occasionally, HPLC mobile phases outside the normal pH 2-7 range are desired to control sample stability, solubility or ionization state, or for compatibility with detection methods. A limitation of most silica-based HPLC phases is their instability outside this range where hydrolysis of the bonded phase and dissolution of the underlying silica can occur. Ascentis columns have excellent stability compared to competitive silica-based columns. The high bonded phase coverage and proprietary endcapping combine to increase resistance to hydrolysis and dissolution. As a result, Ascentis columns can be used successfully between pH 1.5-10 under certain conditions. Note, however, that it is important to avoid storing Ascentis columns, and any silica-based column, in harsh mobile phases.

Ascentis Provides Scalable Separations from Microbore to Preparative

Time and precious samples are wasted during scale-up if the analytical and preparative columns do not give the same elution pattern. The high surface area of the underlying Ascentis silica provides high loading capacity to purify larger quantities of material. Additionally, bonded phase and silica chemistry are uniform across 3,5, and 10 µm particle sizes. These features combine to ensure that analytical separations that are developed on Ascentis 3 or 5 µm particles are completely scalable to preparative separations on Ascentis 10 µm particles and larger columns. Additionally, separations developed on 5 or 10 µm particles can be scaled down for fast analysis on Ascentis 3 µm particles.

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

Guidelines for Preparing Mobile Phases

It should be understood that slight variations in pH and buffer concentration could have a dramatic affect on the chromatographic process; consistent and specific techniques should be a regular practice in the preparation of mobile phases. A common practice is to place a sufficient amount of pure water into a volumetric flask and add an accurate amount of buffer. The pH of the solution should be adjusted, if necessary, and then dilute to final volume of water prior to adding or blending of organic solvents. Then, add a volumetrically measured amount of organic solvent to obtain the final mobile phase. Thorough blending, degassing, and filtering prior to use is also recommended.

To view a listing of suitable HPLC and LC-MS additives and solvents, visit ***sigma-aldrich.com/lc-ms-solvents***

Ascentis C18

The First Choice for Classic C18 Retention and Selectivity

Optimization of silica and bonded phases make Ascentis C18 a true workhorse for the vast majority of HPLC separations. High surface area and phase stability give it perfect character for demanding LC-MS and preparative separations.

Features:

- Classic C18 selectivity
- High non-polar retentivity
- Symmetric peak shape
- Highly reproducible and stable
- Ideal for LC-MS

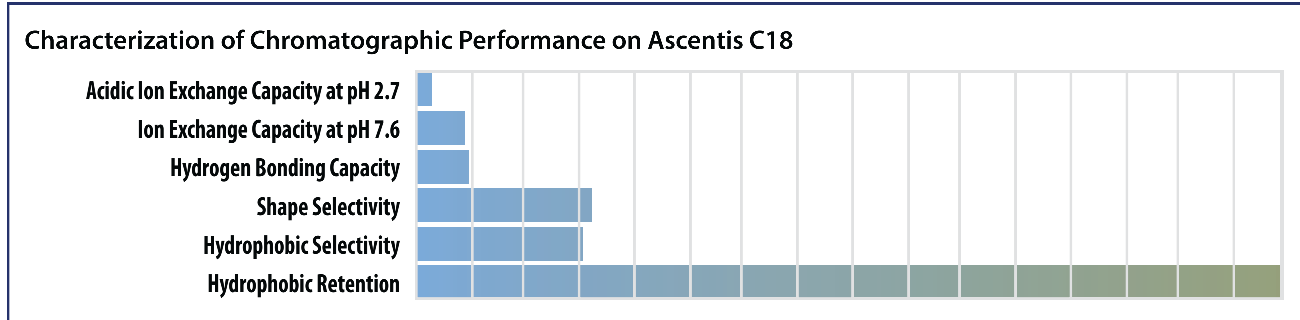
Key Applications:

General reversed phase, hydrophobic and polar compounds

Properties:

USP code:	L1
Bonded phase description:	Octadecyl
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	25%
pH range (recommended):	2-8
Extended pH range*:	1.5-10

Characterization of Chromatographic Performance on Ascentis C18



All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Use

The classic reversed-phase column, Ascentis C18 is suitable for any method that specifies a C18-type column. Its high surface area gives Ascentis C18 strong hydrophobic retention and high loading capacity for preparative applications.

LC-MS Implications

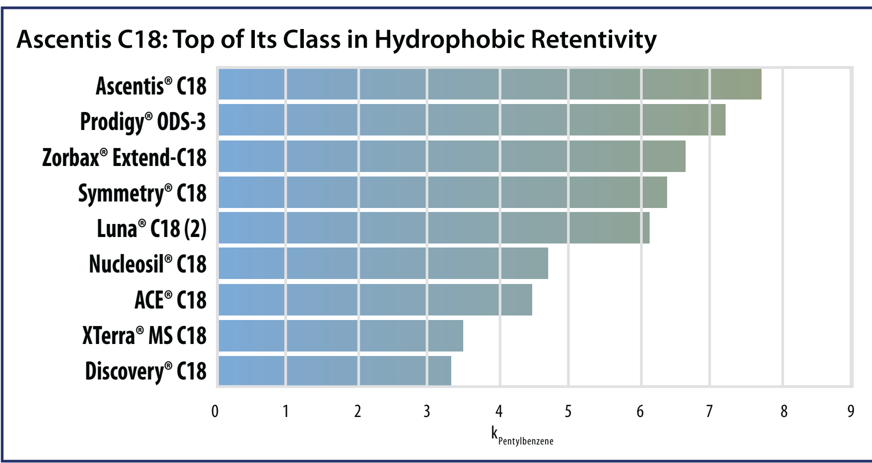
Ascentis C18 is low-bleed for clean ESI and APCI traces. The high retentivity means that the mobile phase can contain high levels of organic modifier that are more readily desolvated.

Notes

Modern C18 columns have very similar selectivity, even for basic compounds, because silica quality and bonding techniques have improved to the point that silanol effects are minimal. Unless quality with your current column is the issue, the main reasons for evaluating different brands of C18 columns are for improved peak shape and for slight changes in selectivity.

* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

Ascentis C18 is one of the most retentive reversed-phase columns available. High retentivity extends the linear range of loading capacity, making Ascentis C18 ideal for separations that are or might be used for preparative applications. High retentivity also means Ascentis C18 can accommodate the highly-organic mobile phases encountered in LC-MS.



Ascentis C18: Top of Its Class in Hydrophobic Retentivity

All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Analysis of basic compounds at neutral pH often gives longer retention compared to acidic mobile phases, but sometimes causes poor peak shape due to silanol interactions. The highly-inert surface of Ascentis C18, as with all Ascentis phases, permits analysis in neutral pH mobile phases. In this example, a mix of tricyclic antidepressants at pH 7 shows excellent peak shape on Ascentis C18.

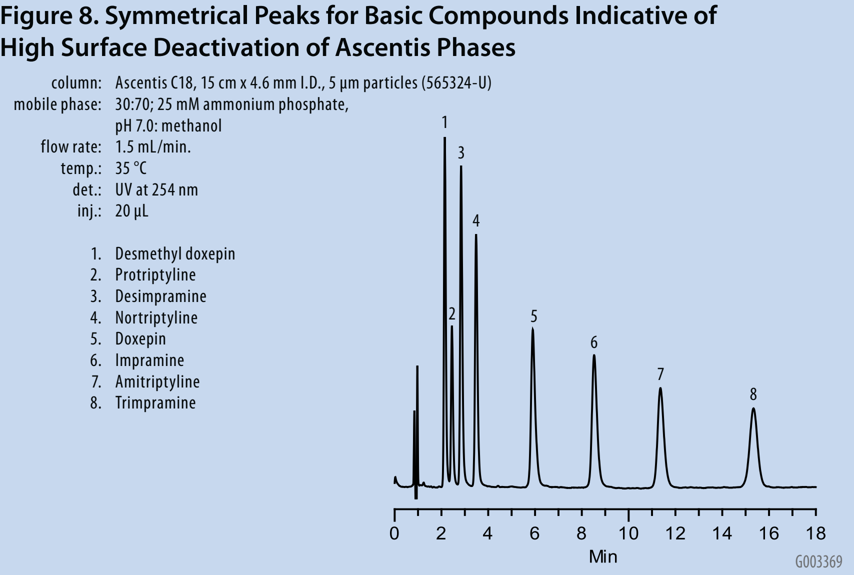


Figure 8. Symmetrical Peaks for Basic Compounds Indicative of High Surface Deactivation of Ascentis Phases

Ascentis C18 provides the selectivity and retention for a range of compounds including steroids. Ascentis C18 is a reliable first choice HPLC column that gives symmetric peak shape and excellent retention for even difficult compounds.

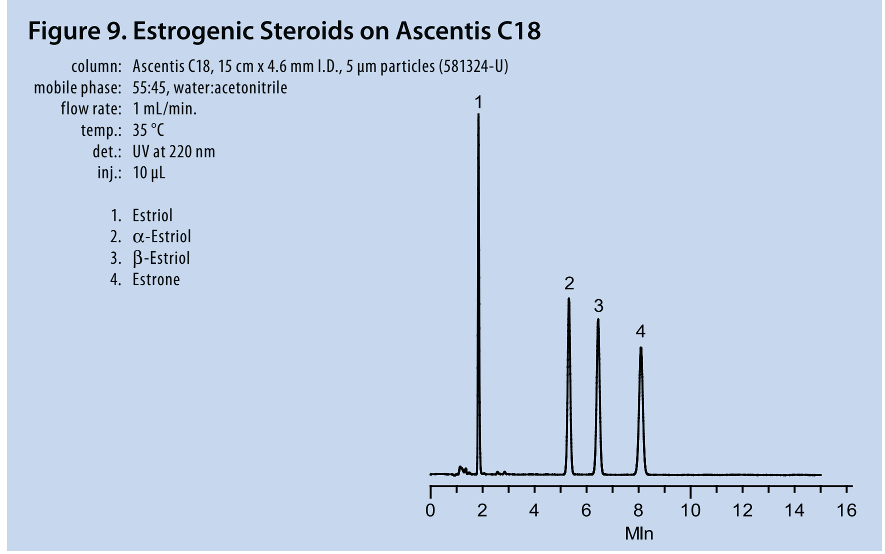
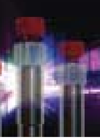


Figure 9. Estrogenic Steroids on Ascentis C18



Ascentis RP-Amide

Ultra-Low Bleed Alkyl Amide Phase that Rivals C18 as a Generic Scouting Column. Excellent Peak Shape and Resolution, Especially for Polar Compounds or Mixtures of Compound Polarity

As pioneers in embedded polar group (EPG) phases for HPLC, Supelco is pleased to offer Ascentis RP-Amide, which has all the benefits of enhanced polar compound retention and selectivity, without any of the disadvantages of competitive EPG phases.

Features

- **Improved peak shape for bases compared to C18**
- **Different selectivity than C18 or C8 for wide range of polar compounds (especially acids)**
- **Lower bleed than competitive EPG phases**
- **100% aqueous compatible**

Key Applications

Small, water soluble molecules and peptides, H-bond donors, acids, phenols, basic compounds, polar compounds

Properties

USP code:	L60
Bonded phase description:	Stable amide group embedded in an 18-carbon chain
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	19.5%
pH range (recommended):	2-8
Extended pH range*:	1.5-10

Characterization of Chromatographic Performance on Ascentis RP-Amide

All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Use

Ascentis RP-Amide can be used for many of the same separations as a C18 while avoiding some of the disadvantages of C18 such as poor wettability in high aqueous mobile phases. In addition, it is much more retentive for those molecules that can interact by hydrophobic interactions and also by H-bonding with the amide group. Compared to alkyl-only phases, Ascentis RP-Amide has enhanced retention and selectivity for phenols, organic acids and other polar solutes due to strong H-bonding between the amide carbonyl (H-bond acceptor) and H-bond donors, like phenols and acids. Compared to other EPG phases, like carbamates, ureas, sulfonamides and ethers, Ascentis RP-Amide gives retention comparable to C18 and C8 for easy column comparison without the need to change mobile phase conditions.

LC-MS Considerations

Unlike other amide-based phases, Ascentis RP-Amide uses an amidosilane reagent and a one-step bonding method similar to C18. Polymeric reagents are also employed to achieve maximum stability and low bleed with all modern HPLC detectors.

Notes

- Generally, acids are retained more and bases retained less on RP-Amide compared to C18 and C8 columns.
- Methanol can be comparable in elution strength to acetonitrile when compounds are retained by H-bonding mechanism on the RP-Amide phase.

* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

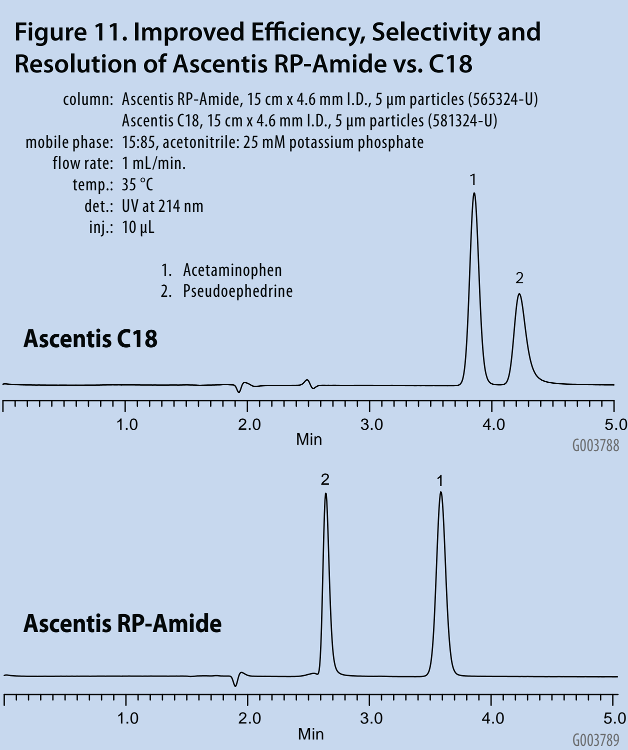
Figure 10. Structure of Ascentis RP-Amide

CH_3 $(CH_2)_{14}$ NH O Si

G003757

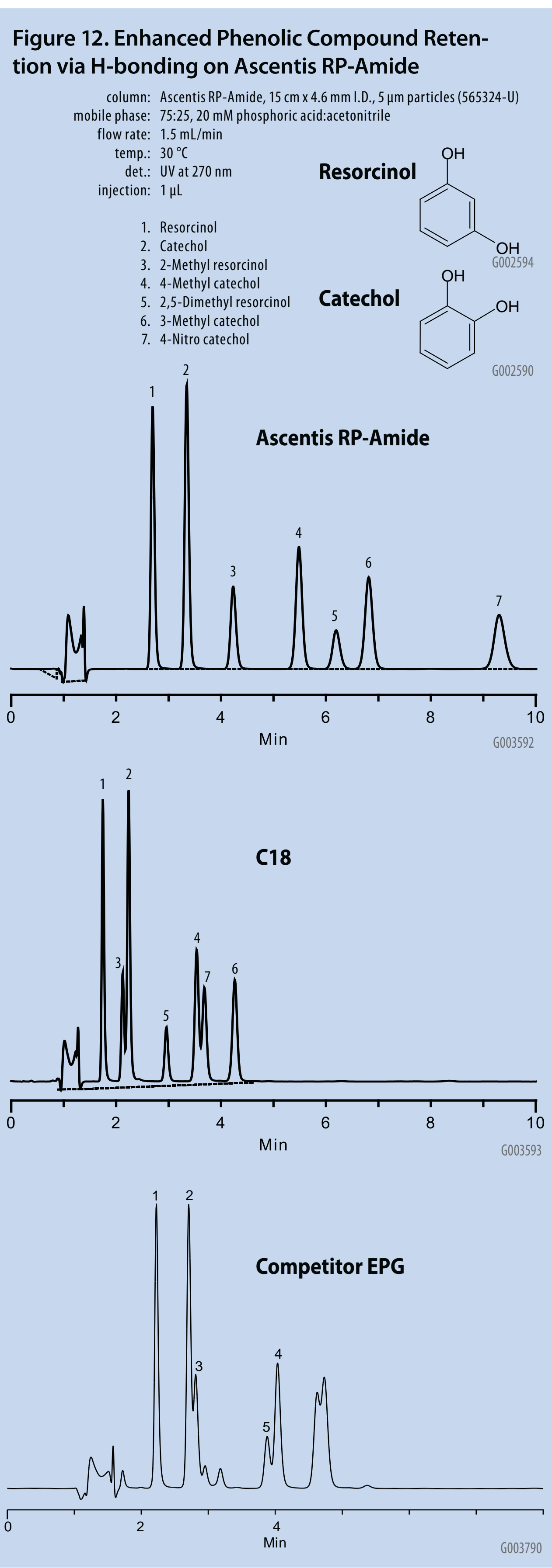
Because the amide group lies near the silica surface, it is believed to suppress tailing of basic solutes by electrostatic shielding (repulsion) or by interacting preferentially with the silanols (H-bonding between the phase and the substrate). The absence of unwanted silanol and other secondary interactions gives the Ascentis RP-Amide excellent peak shape for both acids and bases. This example of internal deactivation with acetaminophen and pseudoephedrine (Figure 11) shows the dramatic effect of changing stationary phase: not only are the peaks more symmetrical, but elution order is reversed.

Figure 11. Improved Efficiency, Selectivity and Resolution of Ascentis RP-Amide vs. C18



An Ascentis RP-Amide column is more retentive and selective for phenolic compounds, like catechols and resorcinols, compared to a C18 and an ether-type polar embedded phase (Figure 12). The ether phase does not have the ability to H-bond with phenolic groups like the amide group does. Although comparing an ether phase to a C18 phase may be useful if only slightly different selectivity is needed, the most dramatic results for acids such as phenols and carboxylic acids will be obtained with amide-based phases, like Ascentis RP-Amide.

Figure 12. Enhanced Phenolic Compound Retention via H-bonding on Ascentis RP-Amide



Ascentis Phenyl

Ultra-low Bleed Phenyl Phase with Enhanced Phenyl Selectivity

Phenyl-based reversed-phases were one of the first alternatives to C18 selectivity. Our Ascentis Phenyl has been improved to offer exceptional phase stability and enhanced phenyl retention. Ascentis Phenyl offers versatility by also operating in the HILIC mode.

Features

- **Low-bleed for MS or UV gradient applications due to the use of trifunctional bonding reagent**
- **Outstanding phenyl selectivity due to high phase loading and short butyl spacer**
- **100% aqueous-compatible for highly-polar compounds**

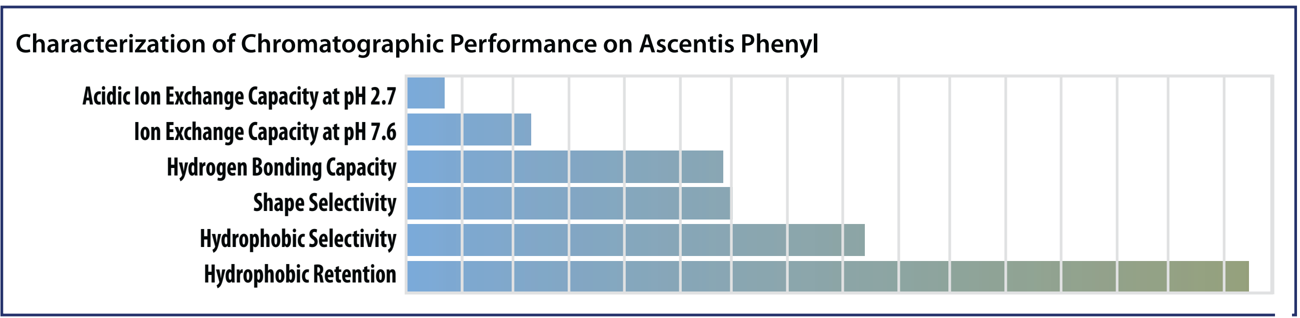
Key Applications

Small, water soluble molecules and peptides, π-acceptors, nitroaromatics, polar compounds, dipoles, heterocyclics, HILIC mode

Properties

USP code:	L11
Bonded phase description:	Phenyl ring with short butyl spacer
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	19.5%
pH range (recommended):	2-8
Extended pH range*:	1.5-10

Characterization of Chromatographic Performance on Ascentis Phenyl



All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Use

Phenyl phases are π-basic (electron donating) and are similar in overall retention to alkyl and EPG phases for easy column screening. The alternate selectivity of phenyl phases is often explained by the π-π interactions available through the phenyl ring. Compounds that appear to exhibit differential selectivity on the phenyl phase include:

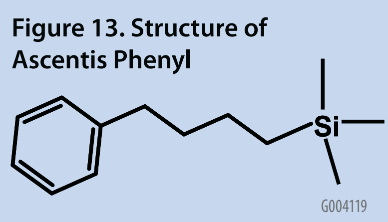
- hydrophobic bases (TCAs, tetracyclines)
- moderate bases (anesthetics and narcotic analgesics)
- benzodiazepines
- some acidic compounds such as ACE inhibitors and quinoline antibiotics
- nucleosides (e.g. cytidine)
- nitro, azide and sulfonyl compounds

Notes

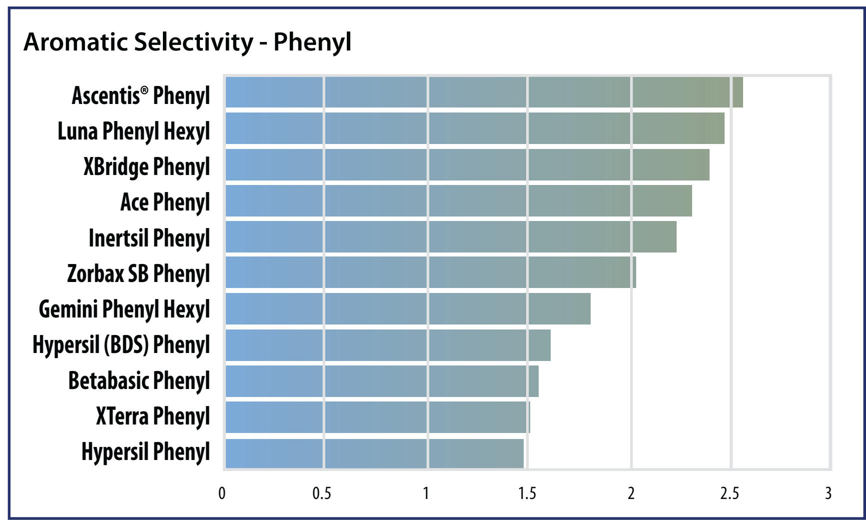
- Methanol can be a more selective mobile phase component than acetonitrile.
- Activate HILIC mode by using highly-aqueous (>90%) mobile phases.

* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

Figure 13. Structure of Ascentis Phenyl



Most commercially-available phenyl phases actually show a great deal of C18-like selectivity, negating their impact on improving the separation. With the highest level of true phenyl character among all phases tested, Ascentis Phenyl has excellent aromatic selectivity, making it a true alternative to traditional C18.



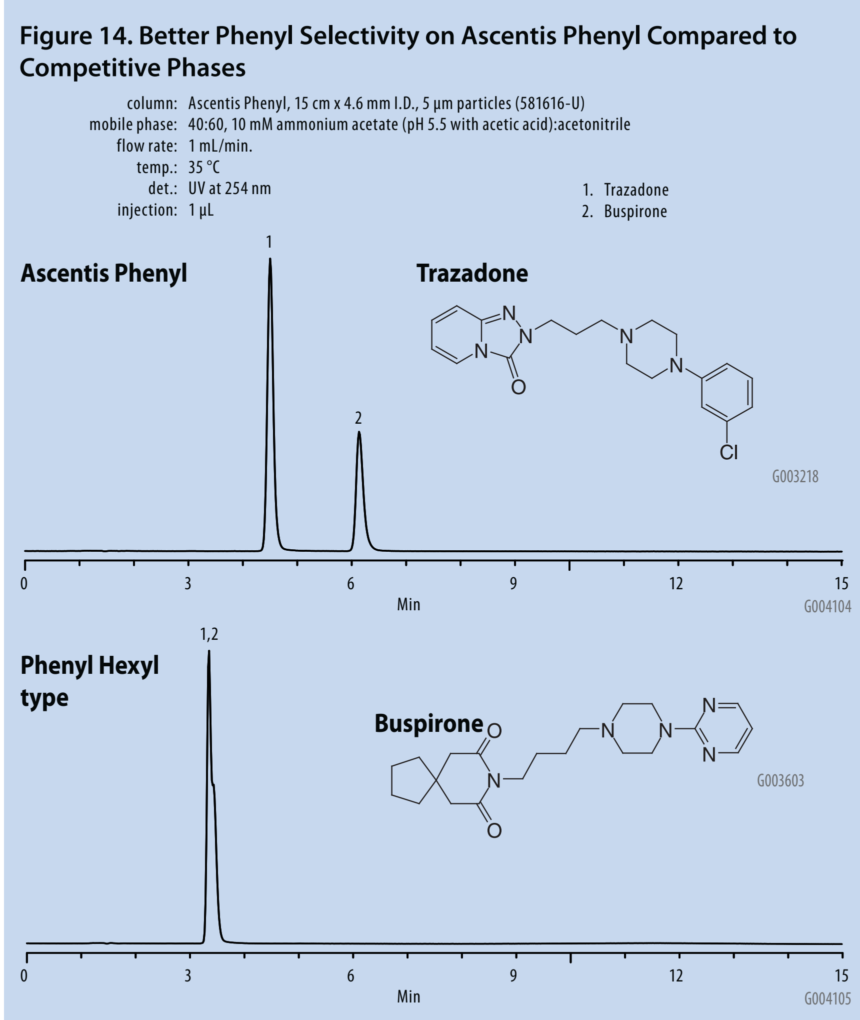
All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

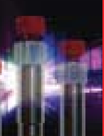
The short, butyl spacer of Ascentis Phenyl does not dilute the phenyl character as conventional hexyl spacers do. Figure 14 shows the stronger contribution of π-electron interactions from the phenyl ring on Ascentis Phenyl compared to the phenylhexyl phase allowing it to resolve buspirone and trazadone. The phenyl and alkyl selectivity tend to cancel each other out on the competitive phenylhexyl phase in this case.

Figure 14. Better Phenyl Selectivity on Ascentis Phenyl Compared to Competitive Phases

column: Ascentis Phenyl, 15 cm x 4.6 mm I.D., 5 µm particles (581616-U)
mobile phase: 40:60, 10 mM ammonium acetate (pH 5.5 with acetic acid):acetonitrile
flow rate: 1 mL/min.
temp.: 35 °C
det.: UV at 254 nm
injection: 1 µL

1. Trazadone
2. Buspirone





Ascentis ES Cyano

Extra stable for low pH mobile phases due to sterically protected phase

Useful for polar selectivity in the reversed-phase mode, including π-π and dipole-dipole interacting compounds. Can also be used in HILIC mode and normal phase chromatography.

Features:

- **Enhanced stability at low pH**
- **Operates in reversed-phase, HILIC and normal phase modes of chromatography**
- **Low MS bleed**
- **100% aqueous compatible**
- **Available as 3 µm and 5 µm particles**

Key applications:

polar compounds, nitroaromatics, tricyclic antidepressants, steroids

Properties:

USP Code:	L10
Bonded phase description:	diisopropyl cyano propyl
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	< 5 ppm metals
Particle shape:	Spherical
Particle size:	3 and 5 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	10 %
pH range recommended:	1-8

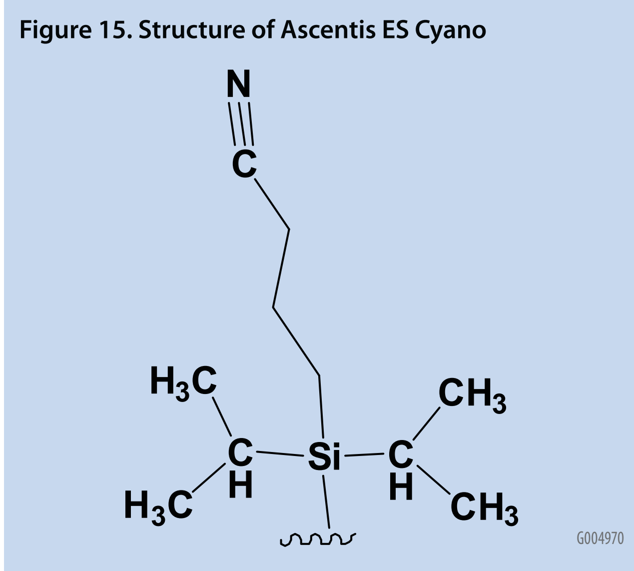
Use

Cyano phases have become very popular because of their unique selectivity for polar groups and double bonds. Their potential for dipole/dipole and dipole/induced-dipole interaction made them one of the earliest stationary phase functional groups when alternate selectivity was needed. In the past, stability of the cyano phase under reversed-phase and HILIC conditions has been poor. Exposure to aqueous organic mobile phases, acidic pH and elevated temperatures can create gradual retention and selectivity loss that is reportedly due to stationary phase hydrolysis. A new Ascentis ES Cyano column, based on 3 and 5 µm porous silica substrate, has been developed. This new phase compares very favorably in stability to C18, C8, Amide and Phenyl.

Notes

- Can be used in reversed, HILIC and normal phase modes
- It is best to dedicate a specific column to one mode of chromatography mentioned above.
- Methanol gives more selectivity than acetonitrile in the reversed phase mode.
- Cyano phases are used in EPA Method 8330 (1) for the analysis of explosives and nitroaromatics.

Figure 15. Structure of Ascentis ES Cyano



1. US EPA Method 8330A, "Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC)" Revision 1 (February 2007), obtained from the *www.epa.gov web site.*

Cyano columns have been used for the analysis of tricyclic antidepressants for some time. A comparison of the most popular competitor column and the Ascentis ES Cyano phase is shown to the right. Better resolution is seen using the ES Cyano than the competitor column for three critical peak pairs. In addition, note the selectivity change of the trimipramine and doxepin (peaks 1 and 2) between these two phases under these conditions.

Figure 16. Comparison of Tricyclic Antidepressants on Ascentis ES Cyano and a Competitor Cyano Phase

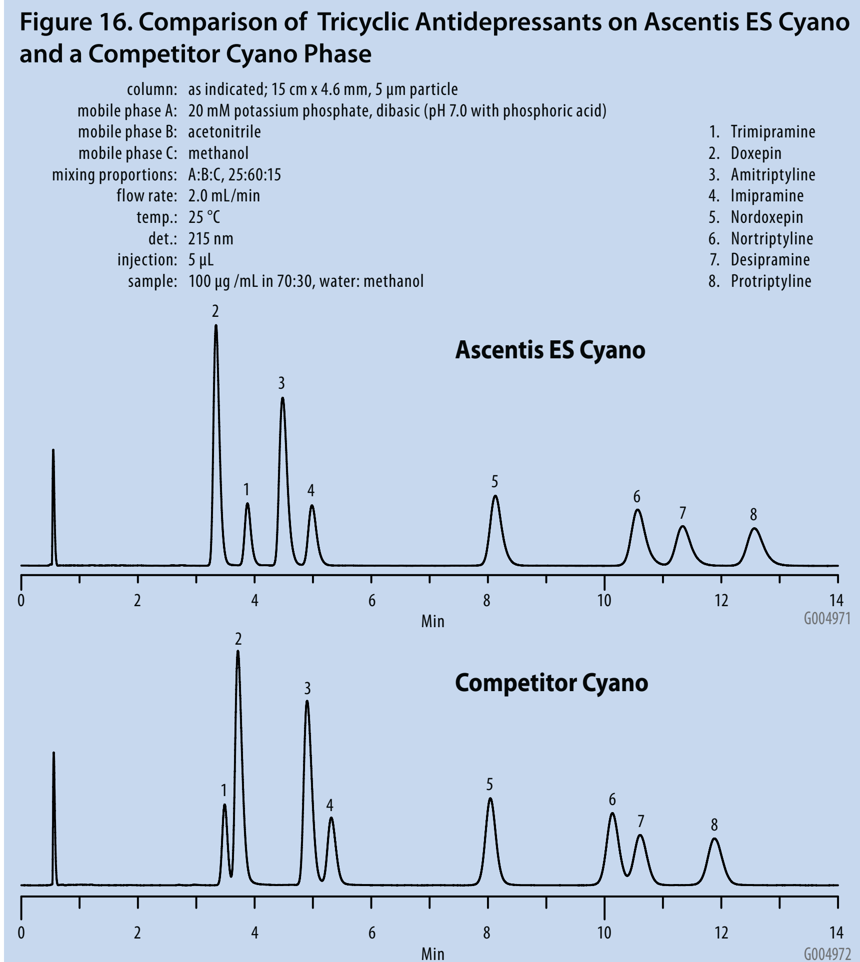
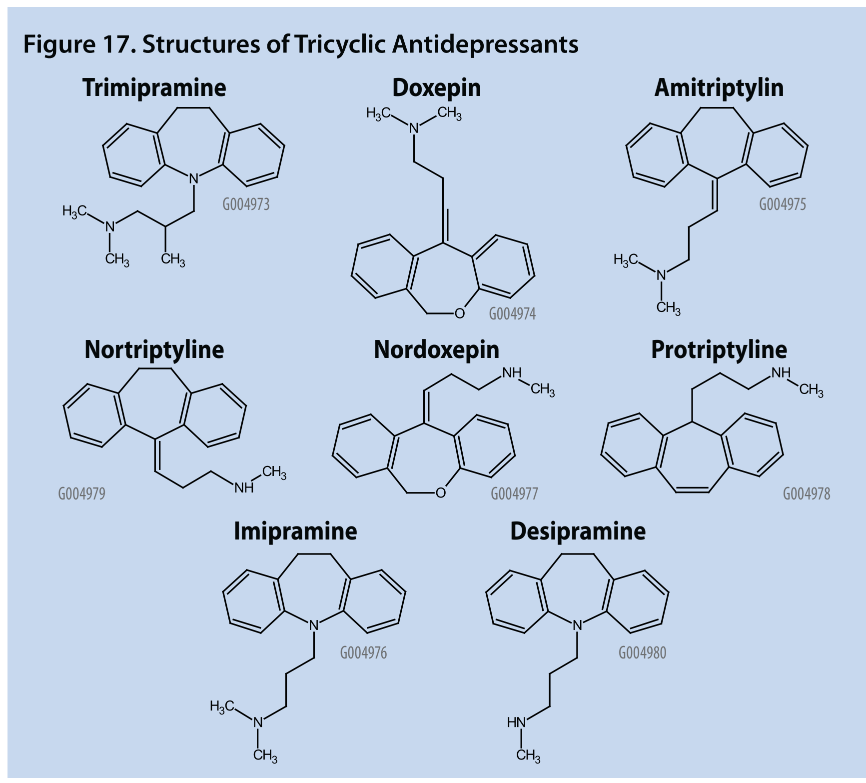
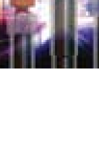
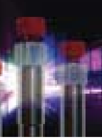


Figure 17. Structures of Tricyclic Antidepressants







Ascentis Silica

High Surface Area and High Surface Deactivation Combine to Give Ascentis Silica Exceptional Performance as a Normal Phase, HILIC and Preparative HPLC Material

Besides being the underlying support for all Ascentis phases, Ascentis Silica has applications in its own right. Silica is widely used to separate positional isomers in normal phase mode, and polar compounds in HILIC (aqueous normal phase modes). Silica is also used in organic synthesis to purify reaction mixtures. In each case, a high purity, controlled and uniform surface is necessary to impart the desirable chromatographic performance.

Features

- **High-loading capacity**
- **Operates in both normal-phase and HILIC modes**
- **Tested in both modes and shipped in ethanol, Ascentis Silica is ready to use in either mode**
- **Ultra-pure, spherical silica**
- **Available in 3, 5, and 10 µm**

Key Applications

Small molecular weight positional (geometric) isomers, non-polar compounds (in NP mode), vitamins, steroids, polar compounds (in HILIC mode)

Properties

USP code:	L3
Bonded phase description:	None (surface comprises silanol, -Si-OH, and siloxane, -Si-O-Si-, groups)
Endcapped:	No
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	0%
pH range (recommended):	2-6

Use

- Normal phase and HILIC HPLC modes
- Preparative chromatography
- Purification (organic synthesis)
- LC-MS

The classic use of silica columns is for normal phase HPLC. The rigid structure of the silica surface, as opposed to the flexible nature of bonded phases, allows it to distinguish between molecules with different footprints that may have the same hydrophobicity. Geometric isomers and closely-related substances, like the steroids shown in Figure 15, can be separated on Ascentis Silica under normal phase conditions. Normal phase is also widely used in preparative chromatography because the mobile phase is more easily removed by evaporation than the water-containing reversed-phase mobile phases.

Figure 18. Ascentis Silica: Normal Phase Separation of Geometric Isomers and Closely-Related Compounds

column:	Ascentis Si, 15 cm x 4.6 mm I.D., 5 µm particles (581512-U)
mobile phase:	88:12, hexane:ethanol
flow rate:	1.0 mL/min.
temp.:	35 °C
det.:	UV at 245 nm
injection:	10 µL
sample:	50 µg/mL in 85:15, hexane: 2-propanol

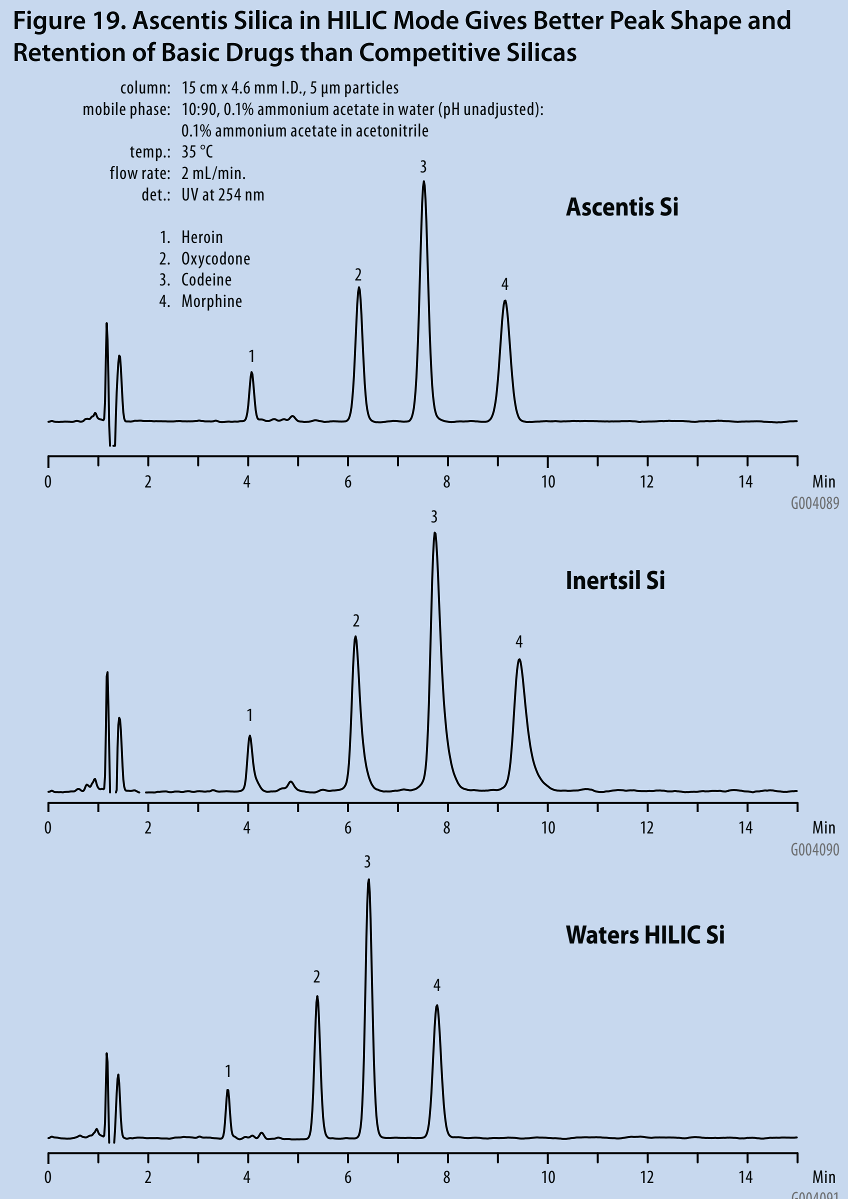
1. Cortisone
2. Prednisone
3. Prednisolone

Ascentis Silica is used successfully in the aqueous normal-phase or HILIC mode. In this mode, water is the strong modifier and the organic is the weak modifier of the mobile phase. Like reversed-phase, HILIC offers the flexibility of using pH and ionic strength to control retention.

HILIC is ideal for very polar compounds and is highly compatible with LC-MS.

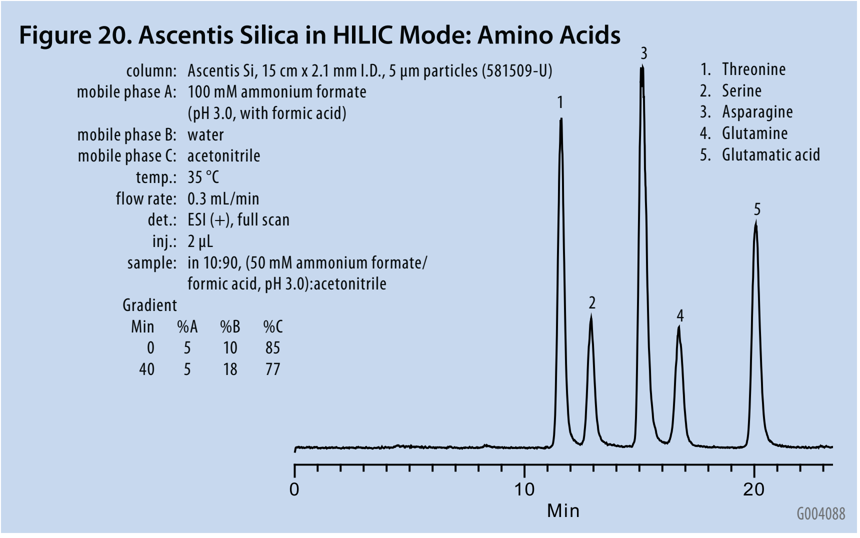
Elution order is generally opposite to that obtained under reversed-phase conditions. Figure 16 compares Ascentis Silica with two competing silicas, demonstrating better resolution by virtue of excellent peak shape and high retentivity.

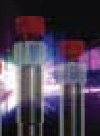
Figure 19. Ascentis Silica in HILIC Mode Gives Better Peak Shape and Retention of Basic Drugs than Competitive Silicas



Polar biomolecules, like amino acids, nucleotides and nucleosides, typically require derivatization for their analysis by reversed phase HPLC. The HILIC mode offered by Ascentis Silica permits the retention and resolution of these compounds without derivatization, eliminating a time-consuming sample preparation step (Figure 17).

Figure 20. Ascentis Silica in HILIC Mode: Amino Acids





Ascentis C8

One of The Most Hydrophobic C8 Phases Available

Leveraging the improvements to silica and bonded phase properties that made Ascentis C18 so useful, its shorter alkyl chain cousin, Ascentis C8, is also suitable for routine HPLC and LC-MS.

Features:

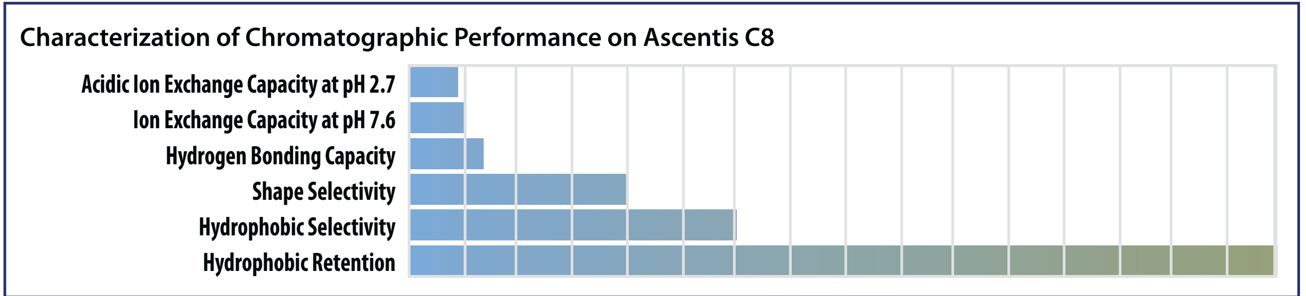
- Selectivity similar to C18 for non-polar compounds
- Different selectivity for polar compounds
- Less hydrophobic than C18, more hydrophobic than other C8 phase
- Symmetric peak shape
- Highly reproducible and stable
- Ideal for LC-MS

Key Applications:

Small, water soluble molecules and peptides, less hydrophobic retention than C18 but comparable selectivity, LC-MS

Properties:

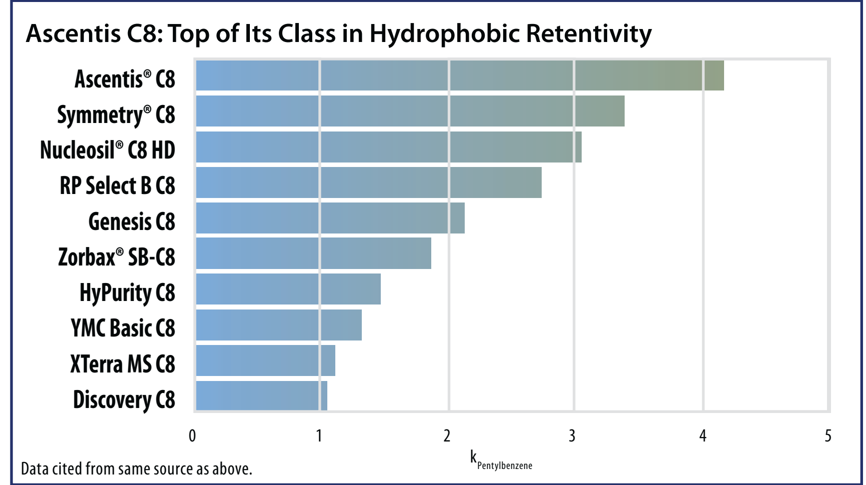
USP code:	L7
Bonded phase description:	Octyl
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<5 ppm metals
Particle shape:	Spherical
Particle size:	3 and 5 µm
Pore size:	100 Å
Surface area:	450 m^2/g
Carbon load:	15%
pH range (recommended):	2-8
Extended pH range*:	1.5-10



All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Use

Ascentis C8 is suitable for any method that specifies a C8-type column. Although C8 columns often show similar selectivity to C18 columns, shorter alkyl chains sometimes show different selectivity toward polar compounds because they can solvate differently with the mobile phase and interact differently due to the size and shape of certain molecules. Also, C8 reagents are smaller than C18 reagents and have improved primary phase coverage, thereby requiring less end-capping. Ascentis C8 has excellent peak shape and very high phase stability.



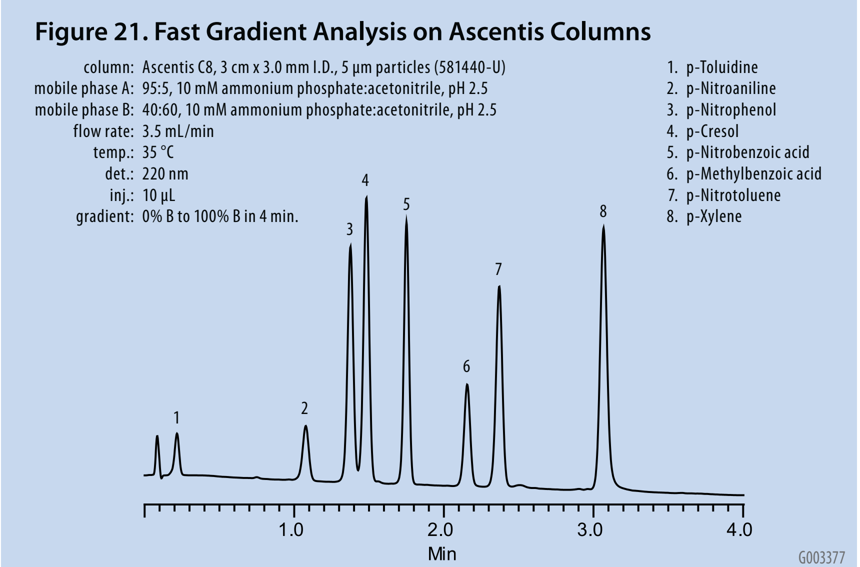
Data cited from same source as above.

Among commercially available C8 columns, Ascentis C8 has the highest degree of hydrophobic retention. This permits the use of higher percentages of organic modifier, a benefit to LC-MS users.

* Under certain conditions, the Ascentis family can be operated in the extended pH range. For more information, request an electronic file of "Acid/Base Stability of Silica Based on C8, C18, and Amide HPLC Columns" (T406018)

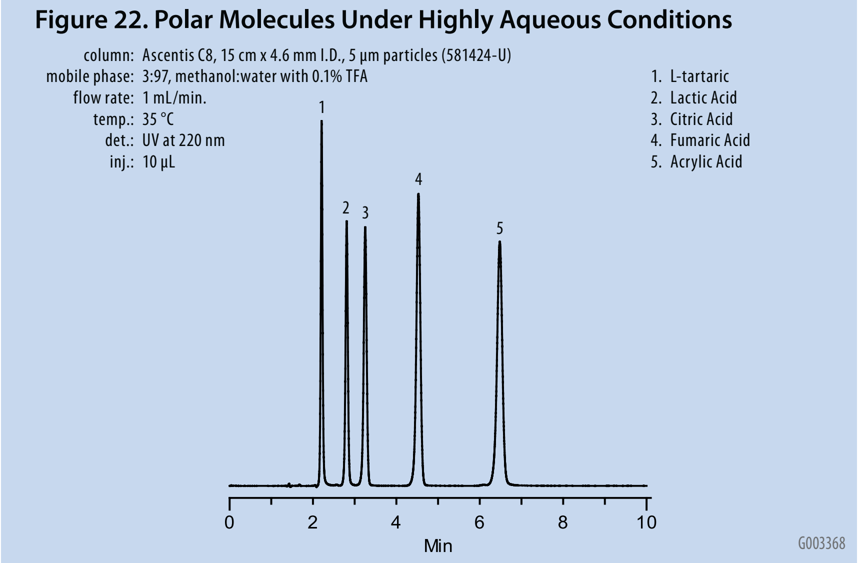
Ascentis C8 is an excellent choice for fast gradient analysis. Ascentis C8 is typically more retentive at low organic composition that C18 and less retentive at high organic composition. Furthermore, Ascentis C8 has better aqueous compatibility for gradients that start at 100% aqueous composition.

Figure 21. Fast Gradient Analysis on Ascentis Columns



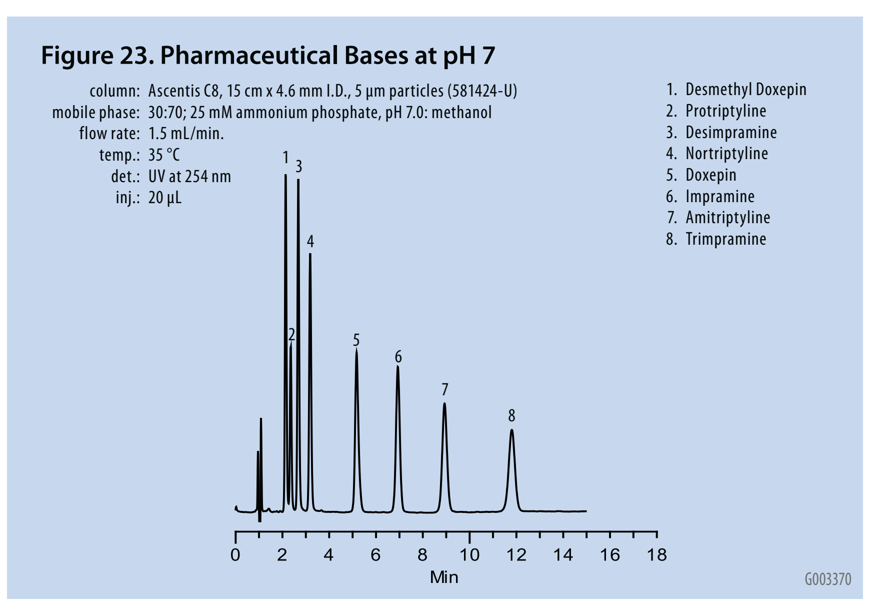
Ascentis C8 often yields enhanced retention than Ascentis C18 for small polar molecules under highly aqueous conditions. Greater retention for Ascentis C8 may be related to greater wettability of Ascentis C8 as compared to Ascentis C18.

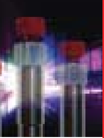
Figure 22. Polar Molecules Under Highly Aqueous Conditions



Analysis of bases at neutral pH often yields enhanced retention over acidic mobile phase but sometimes causes poor peak shape with C18 and C8 columns due to silanol interaction. In this example, a mix of tricyclic antidepressants at pH 7 shows excellent peak shape on Ascentis C8.

Figure 23. Pharmaceutical Bases at pH 7





Discovery HS F5

Unique Reversed-phase Selectivity Compared to C18 and C8

Discovery HS F5 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.

Features

- Unique (orthogonal) selectivity compared to C18 and C8
- Stable, low-bleed LC-MS separations
- Both reversed-phase and HILIC modes
- Possesses multiple types of interactions: dispersive, dipole-dipole, π-π, charge-transfer

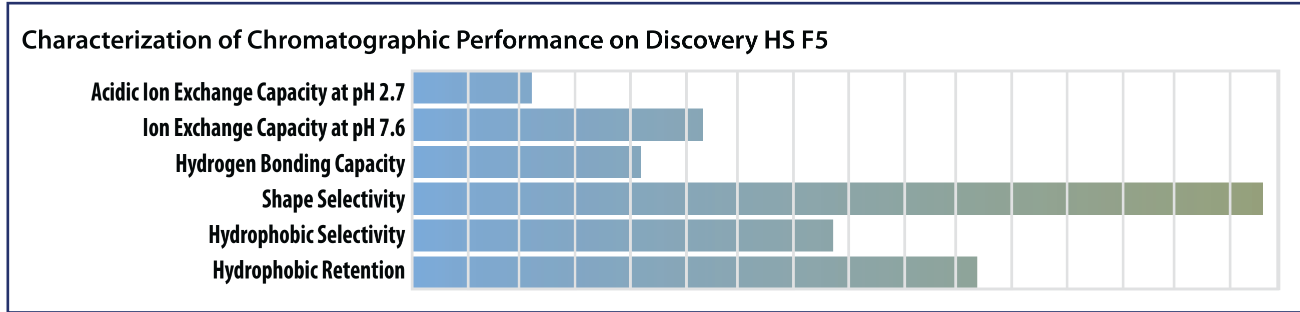
Key Applications

Small, water soluble molecules and peptides, polar compounds, basic compounds, positional isomers

Properties

USP code:	L43
Bonded phase description:	Pentafluorophenylpropyl
Endcapped:	Yes
Particle composition:	Type B silica gel
Particle purity:	<10 ppm metals
Particle shape:	Spherical
Particle size:	3, 5 and 10 µm
Pore size:	120 Å
Surface area:	300 m^2/g
Carbon load:	12%
pH range (recommended):	2-8

Characterization of Chromatographic Performance on Discovery HS F5



All competitive data obtained from M. R. Euerby, P. Perterson, J. Chromatogr. A, 994 (2003) 13-36; or M. R. Euerby, P. Perterson, W. Campbell, W. Roe, J. Chromatogr. A, 1154 (2007) 138-151. Ascentis data developed by Supelco/Sigma-Aldrich scientists using the Euerby methodology or obtained directly from cited references. Data for Aromatic selectivity is calculated as aTNB/NB/aTNB/DNT.

Notes

Compared to a C18: Generally, bases are retained longer on the HS F5 than on a C18, hydrophobic compounds are retained less. Increasing the organic content of a C18 separation 5 to 10 percent will generally provide similar retention on a HS F5. Also, as a general rule, solutes with log $P_{o/w}$ values less than 2.5 will be retained longer on HS F5 compared to a C18.

Compared to a phenyl phase: Although aromatic in nature, the pentafluorophenylpropyl (F5) phase does not resemble a phenyl phase in retention or selectivity. The F5 is a strong Lewis acid due to the electron withdrawing effects of five fluorine groups; the F5 ring is electron deficient whereas the phenyl ring is electron rich.

Figure 24. Structure of Discovery HS F5

G004120

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.

In Figure 25, 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 25. HS F5 Provides Excellent Separation - Solutes Are Not Retained on C18

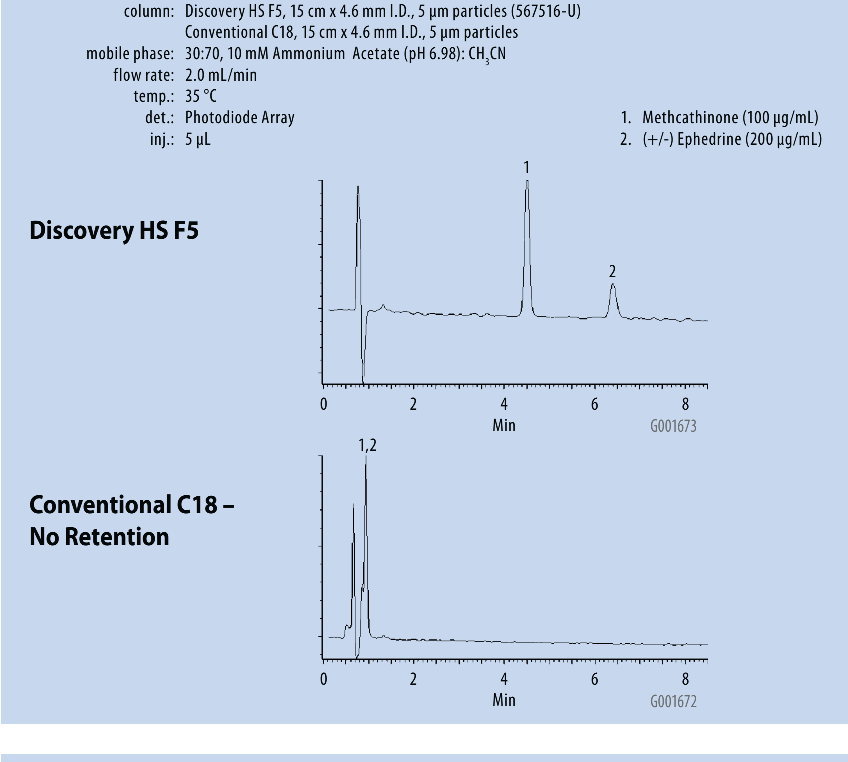
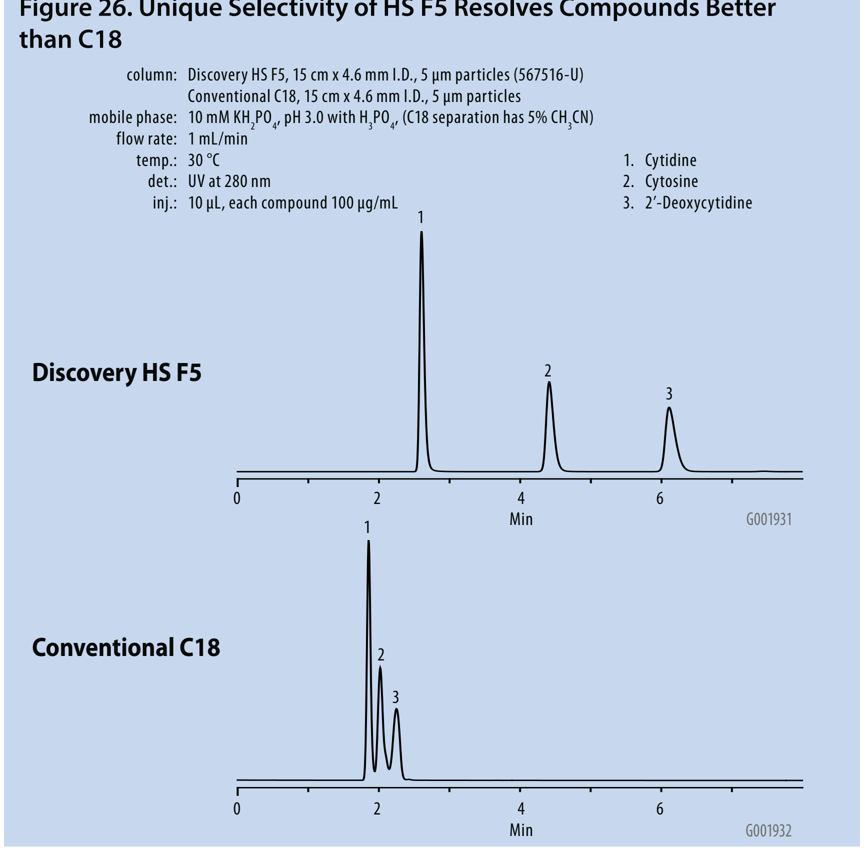
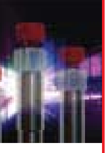


Figure 26. Unique Selectivity of HS F5 Resolves Compounds Better than C18





Ascentis® Express

Extreme Performance on Any LC System

The demand for increased sample throughput and speed of results has driven HPLC users to search for breakthroughs in HPLC instrument and column technology. Although improvements have been realized, setbacks have been encountered. Reductions in column ruggedness, costly replacements of existing instrumentation, and difficulties in transferring methods to new systems have often made these past "improvements" unappealing to analysts.

Ascentis Express has changed all of that.

Ascentis Express with Fused-Core™ Particle Technology provides the ultimate solution for today's separation demands - high speed and high efficiency with low backpressure.

By simply changing to Ascentis Express Columns, sample throughput can be improved by 400%!

No longer will you have to make changes to:

- **sample prep**
- **flow rate**
- **system pressure**

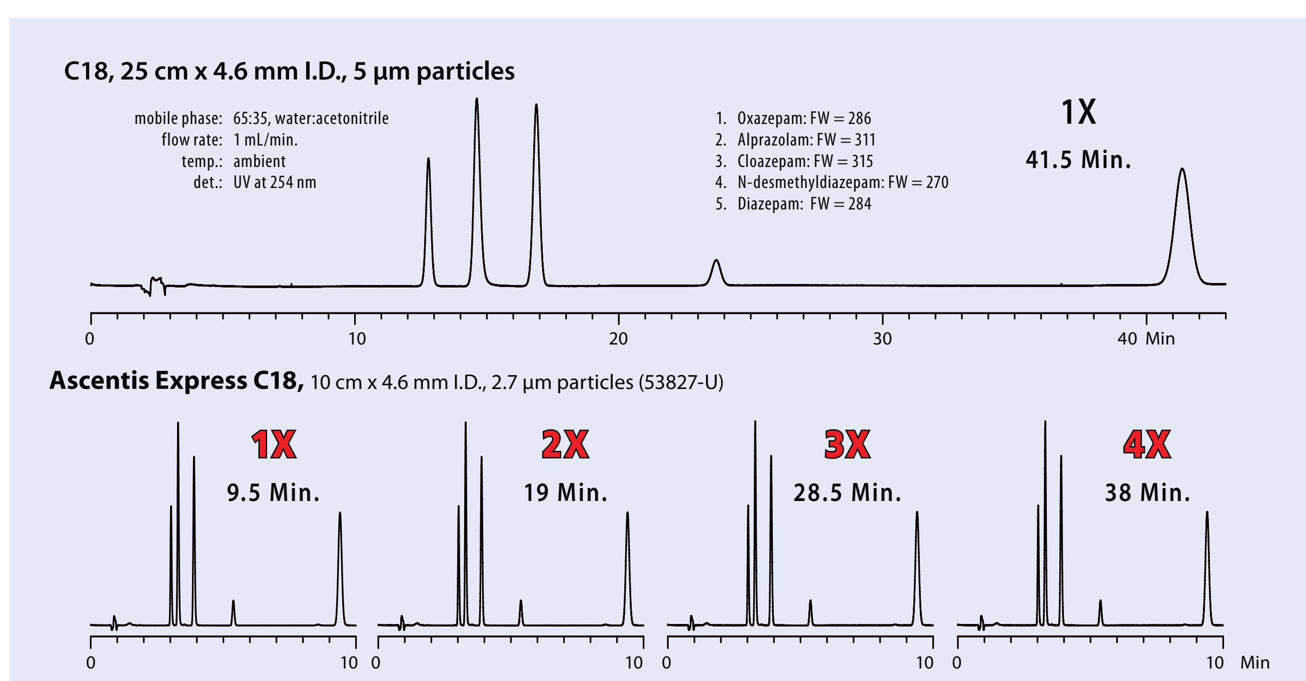
And no new instrumentation is required!

For more information on this exciting new technology, visit our website: ***sigma-aldrich.com/express***

Do More Work in Less Time without Changing your Method

With identical conditions, the Ascentis Express C18 column performs **4X** as many separations as a standard C18 column in less time.

CONDITIONS						
Sample Prep	Flow Rate	System Pressure	HPLC System	Theoretical Plates	Standard C18 Throughput	**Ascentis C18 Throughput**
SAME	SAME	SAME	SAME	SAME	1X	**4X**



Ordering Information

Particle Size	ID (mm)	Length (cm)	Ascentis C18	Ascentis RP-Amide	Ascentis Phenyl	Ascentis ES Cyano	Ascentis Silica	Ascentis C8	Discovery HS F5
3 µm									
	1	5	581311-U	565309-U	inquire	inquire	inquire	581412-U	inquire
	1	10	581364-U	565389-U	581600-U	inquire	581520-U	581435-U	inquire
	1	15	581365-U	65566-U	581601-U	inquire	581521-U	581436-U	inquire
	2.1	2	581312-U	565313-U	inquire	inquire	inquire	581413-U	inquire
	2.1	3	581313-U	565314-U	581602-U	inquire	581522-U	581414-U	567501-U
	2.1	5	581300-U	565300-U	581603-U	577308-U	581500-U	581400-U	567500-U
	2.1	10	581301-U	565301-U	581604-U	577309-U	581501-U	581401-U	567502-U
	2.1	15	581302-U	565302-U	581605-U	577310-U	581502-U	581402-U	567503-U
	3	2	581314-U	565315-U	inquire	inquire	inquire	581415-U	inquire
	3	3	581302-U	565310-U	581606-U	inquire	581523-U	581403-U	567505-U
	3	5	581307-U	565311-U	inquire	inquire	inquire	581404-U	inquire
	3	10	581308-U	565312-U	581607-U	inquire	581503-U	581405-U	567581-U
	4.6	2	581315-U	565316-U	inquire	inquire	inquire	581416-U	inquire
	4.6	3	581316-U	565317-U	inquire	inquire	inquire	581417-U	567509-U
	4.6	5	581320-U	565320-U	581608-U	577311-U	581504-U	581406-U	567504-U
	4.6	10	581321-U	565321-U	581609-U	577312-U	581505-U	581407-U	567505-U
	4.6	15	581322-U	565322-U	581610-U	inquire	581506-U	581408-U	567507-U
5 µm									
	2.1	2	581368-U	565391-U	inquire	inquire	inquire	581439-U	inquire
	2.1	3	581327-U	565331-U	inquire	inquire	inquire	581430-U	inquire
	2.1	5	581303-U	565303-U	581611-U	577300-U	581507-U	581420-U	567508-U
	2.1	10	581326-U	565304-U	581612-U	577301-U	581508-U	581419-U	567510-U
	2.1	15	581304-U	565305-U	581613-U	577303-U	581509-U	581421-U	567511-U
	2.1	25	581305-U	565306-U	581614-U	inquire	581510-U	581422-U	567512-U
	3	2	581328-U	565332-U	inquire	inquire	inquire	581431-U	inquire
	3	3	581369-U	565392-U	inquire	inquire	inquire	581440-U	inquire
	3	5	581329-U	565333-U	inquire	inquire	inquire	581432-U	inquire
	4.6	2	581330-U	565335-U	inquire	inquire	inquire	581433-U	inquire
	4.6	3	581331-U	565336-U	inquire	inquire	inquire	581434-U	inquire
	4.6	5	581323-U	565323-U	581615-U	577304-U	581511-U	581423-U	567513-U
	4.6	10	inquire	565328-U	inquire	577305-U	inquire	inquire	567515-U
	4.6	15	581324-U	565324-U	581616-U	577306-U	581512-U	581424-U	567516-U
	4.6	25	581325-U	565325-U	581617-U	577307-U	581513-U	581425-U	567517-U
	10	5	581340-U	565340-U	inquire	inquire	inquire	inquire	567518-U
	10	10	581341-U	565341-U	inquire	inquire	inquire	inquire	567537-U
	10	15	581342-U	565343-U	inquire	inquire	inquire	inquire	567519-U
	10	25	581343-U	565344-U	581618-U	inquire	581514-U	inquire	567520-U
	21.2	5	581344-U	565345-U	inquire	inquire	inquire	inquire	inquire
	21.2	25	581347-U	565348-U	581619-U	inquire	581515-U	inquire	567523-U
10 µm									
	4.6	15	581350-U	565352-U	inquire	inquire	inquire	inquire	inquire
	4.6	25	581351-U	565353-U	inquire	inquire	581524-U	inquire	inquire
	10	5	581352-U	565354-U	inquire	inquire	inquire	inquire	inquire
	10	10	581353-U	565355-U	inquire	inquire	inquire	inquire	inquire
	10	15	581354-U	565356-U	inquire	inquire	inquire	inquire	inquire
	10	25	581355-U	565357-U	inquire	inquire	581516-U	inquire	inquire
	21.2	5	581356-U	565358-U	inquire	inquire	inquire	inquire	inquire
	21.2	10	581357-U	565359-U	inquire	inquire	inquire	inquire	inquire
	21.2	15	581358-U	565360-U	inquire	inquire	inquire	inquire	567528-U
	21.2	25	581359-U	565361-U	inquire	inquire	581517-U	inquire	567529-U

	ID (mm)	Length (cm)	Particle Size (µm)	Ascentis C18	Ascentis RP-Amide	Ascentis Phenyl	Ascentis Silica	Ascentis C8	Discovery HS F5
Ascentis Supelguard Cartridges									
Kit	2.1	2	3	581376-U	inquire	inquire	inquire	inquire	567571-U
Pack of 2	2.1	2	3	581377-U	inquire	inquire	inquire	inquire	567570-U
Pack of 2	2.1	2	5	581370-U	565372-U	inquire	inquire	inquire	567574-U
Kit	2.1	2	5	581371-U	565373-U	inquire	inquire	inquire	567575-U
Pack of 2	3	2	5	581374-U	565374-U	inquire	inquire	inquire	inquire
Kit	3	2	5	581375-U	565375-U	inquire	inquire	inquire	inquire
Kit	4	2	3	581378-U	inquire	inquire	inquire	inquire	567573-U
Pack of 2	4	2	3	581379-U	inquire	inquire	inquire	inquire	567572-U
Pack of 2	4	2	5	581372-U	565370-U	581620-U	581518-U	581426-U	567576-U
Kit	4	2	5	581373-U	565371-U	581621-U	581519-U	581427-U	567577-U

Kits include one cartridge, stand alone holder, a piece of tubing, 2 nuts and 2 ferrules.

	ID (mm)	Length (cm)	Particle Size (µm)	Ascentis C18	Ascentis RP-Amide	Ascentis Phenyl	Ascentis Silica	Ascentis C8	Discovery HS F5
Ascentis Validation Packs									
	4.6	15	5	581390-U	565394-U	581695-U	inquire	inquire	inquire
	4.6	25	5	581391-U	565395-U	581696-U	inquire	inquire	inquire

Validation Packs include 3 columns, each from a different bond lot.

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SIGMA-ALDRICH®

Ascentis Express HPLC Columns with Fused-Core Technology

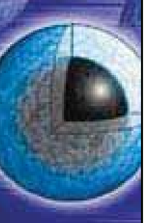
Extreme Performance on **Any** LC System

2.7 µm

1.7 µm

- Hyper-Fast Separations
- High Definition Resolution
- Super-Sensitive
- Super-Rugged

SIGMA-ALDRICH



A Breakthrough in HPLC Performance

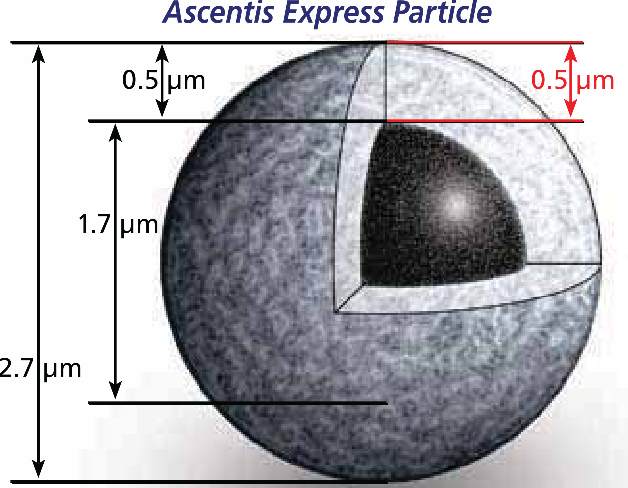
The Fused-Core Advantage

Ascentis Express provides the high speed and high efficiency of sub-2 µm particles, but at approximately half the backpressure for the same column length. This lower pressure means that Ascentis Express can be run on conventional HPLC and LC-MS systems, as well as mid-pressure, UPLC™ and other ultra-high pressure systems. Lower pressure also means longer columns can be used for additional resolving power. Ascentis Express offers these benefits over sub-2 µm particles, along with excellent column lifetime.

At the heart of Ascentis Express is the 2.7 µm Fused-Core™ particle which comprises a 1.7 µm solid core and a 0.5 µm porous shell (Figure 1). Compared to totally porous particles, the Fused-Core particles have a much shorter diffusion path because of the solid core. This partial porosity reduces axial dispersion of solutes and minimizes peak broadening. Other features, such as a very tight particle size distribution and high packing density, result in Ascentis Express columns that are capable of 240,000 N/m. This is comparable to the efficiency of sub-2 µm particle columns and nearly twice the efficiency possible with 3 µm particles.

While the Ascentis Express efficiency is as high as sub-2 µm columns, the larger particle size delivers approximately half the backpressure for the same column dimensions and conditions. This allows Ascentis Express to turn any HPLC system into an extreme performance workhorse for your lab

Figure 1. Fused-Core Structure of Ascentis Express Compared to Totally Porous Particles



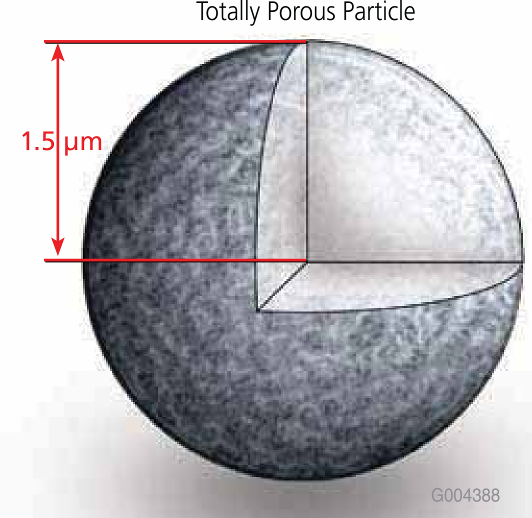


Table of Contents

Ascentis Express Topics	Page
Speed	4
Resolution	5
Sensitivity	6
Ruggedness	7
Alternate Selectivity	8-9
Throughput	10-11
Fast HPLC	12-13
Column Coupling	14
Ordering Information	15

Ascentis Express FAQs

What is unique about Ascentis Express?

Ascentis Express columns provide a breakthrough in HPLC performance. Based on Fused-Core particle technology, Ascentis Express provides the benefits of sub-2 µm particles but at much lower backpressure. These benefits include the capability of providing fast HPLC and higher resolution chromatography. The Fused-Core particle consists of a 1.7 µm solid core and a 0.5 µm porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.5 µm) compared to conventional fully porous particles. The shorter diffusion path reduces axial dispersion of solutes and minimizes peak broadening.

What phases are available in Ascentis Express?

Currently, C18, C8, RP-Amide, and HILIC (bare silica) phases are available for Ascentis Express.

When are additional phases expected?

Additional phases are being developed. The best way to track new products is to visit *sigma-aldrich.com/express* for the latest updates.

Can I use Ascentis Express on any type of HPLC system?

Ascentis Express HPLC columns are capable of use on standard HPLC systems as well as UHPLC systems. Columns are packed in high pressure hardware capable of withstanding the pressures used in UHPLC systems.

Is there anything I need to do to my HPLC system to use Ascentis Express?

Nothing special is required to use Ascentis Express HPLC columns. To obtain the full benefits of Ascentis Express, one should minimize dispersion or instrument bandwidth in the HPLC system (tubing, detector flow cell) as well as confirm the detector response system is set at a fast level. For more information, request Guidelines for Optimizing Systems for Ascentis Express Columns (T407102) or visit *sigma-aldrich.com/express* and download.

How can I measure my instrument bandwidth (IBW) and determine what columns can be used with minimal efficiency loss created by too much internal instrument volume?

For simple instructions on how to measure IBW, request *Guide to Dispersion Measurement* (T408143) or visit our website *sigma-aldrich.com/express* and download.

Do I need special fittings and tubing to connect Ascentis Express columns?

While operating pressures may not exceed the 400 bar (6,000 psi) capability of your traditional instruments, sustained pressures of about 200 bar (3,000 psi) will exceed the recommended pressure for conventional PEEK tubing and fittings at the column inlet. We recommend changing to stainless steel fittings in all high pressure locations and have designed special low-dispersion connectors (pg. 14) that will stay tight at pressures of 1,000 bar (15,000 psi) or greater, even when elevated column temperatures are employed.

Can I use Ascentis Express on a UHPLC system?

Yes. Ascentis Express columns are packed in a way making them suitable for these ultra high pressure instruments. In fact, Ascentis Express outperforms sub-2 µm columns on many applications since Ascentis Express provides the benefits of sub-2 µm particles but at much lower backpressure.

Can Ascentis Express columns be used for LC-MS?

Ascentis Express Fused-Core particles were designed with LC-MS in mind. Even extremely short column lengths exhibit sufficient plate counts to show high resolving power. The flat van Deemter plots permit resolution to be maintained at very high flow rates to maximize sample throughput. All Ascentis stationary phases have been evaluated for MS compatibility during their development, and the Express phases are no exception. A bonus of Ascentis Express columns for high throughput UHPLC and LC-MS is that they are extremely rugged and highly resistant to plugging, a very common failure mode for competitor columns.

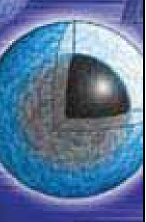
What flow rate should I use with Ascentis Express columns?

Based on the minimum in the van Deemter curves, higher flows than 5 µm particle columns are required in order to maximize Ascentis Express column efficiency.

Ascentis Express HPLC Column ID	Suggested Starting Point for Flow Rate
4.6 mm I.D.	1.6 mL/min
3.0 mm I.D.	0.8 mL/min
2.1 mm I.D.	0.4 mL/min

Are guard columns available?

Guard columns packed with Ascentis Express are currently not available. Ascentis Express columns are rugged and almost all users prefer operation of Ascentis Express columns without a guard column. If you would like to use a guard column, we recommend the Ascentis guard columns.



Hyper-Fast Separations

Double the Speed

- Designed for high flow rates
- Half the backpressure of sub-2 µm particles

Compared to sub-2 µm particles, the 2.7 µm Ascentis Express particles generate approximately half the back-pressure. This permits both longer columns and faster flow rates.

Figure 2 shows the separation of a steroid mixture on Ascentis Express (top) and a conventional sub-2 µm column (lower) of the same dimensions. Because higher flow rates on Ascentis Express – even doubled in this example – generate similar backpressure, hyper fast separations are possible that have efficiency and resolution equal to the sub-2 µm particle column.

Shown in Figure 3 is a comparison against a traditional 15 cm, 5 µm column and a 5 cm, Ascentis Express. The chromatograms further illustrate the high-speed capabilities of Ascentis Express at backpressures manageable by all HPLC systems. High flow rates are quite amenable to Ascentis Express HPLC columns due to the Fused-Core particle.

Figure 2. Hyper-Fast Separations on Ascentis Express: Twice the Speed at Equivalent Pressure

columns: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column (same dimensions)
mobile phase: 49:51 or 55:45, water:acetontrile
flow rate: 0.4 or 0.2 mL/min.
temp.: ambient
det.: UV at 200 nm
injection: 1 µL

1. Estradiol
2. β-Estradiol
3. Impurity
4. Estrone
5. Estrone degradant

Ascentis Express C18
0.4 mL/min flow rate

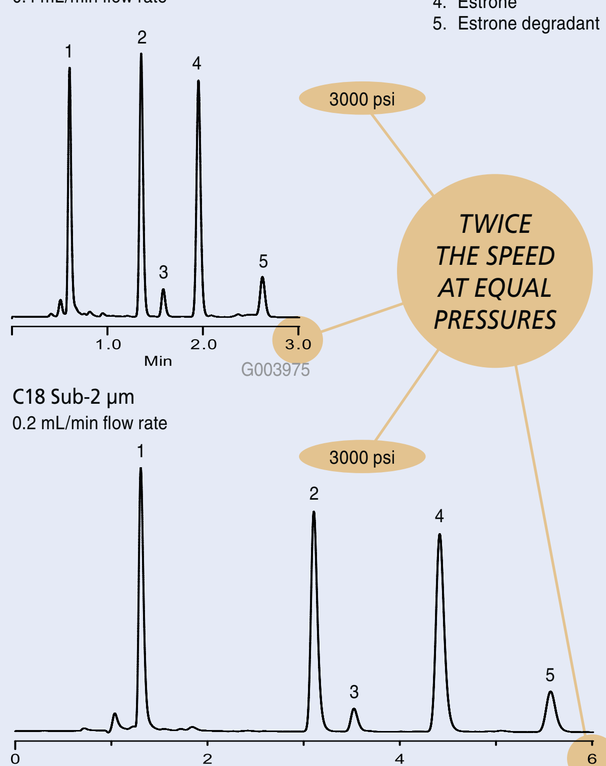
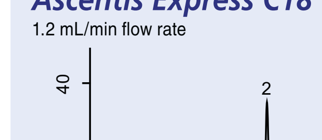
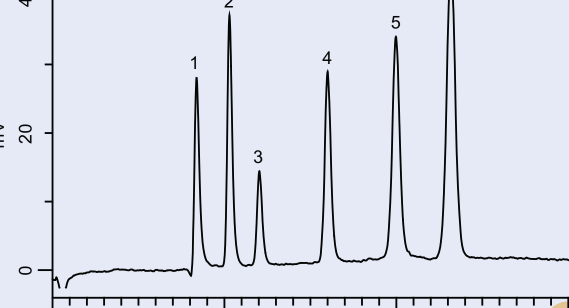


Figure 3. Hyper-Fast Separations on Ascentis Express: Eight Times the Speed of Traditional Columns

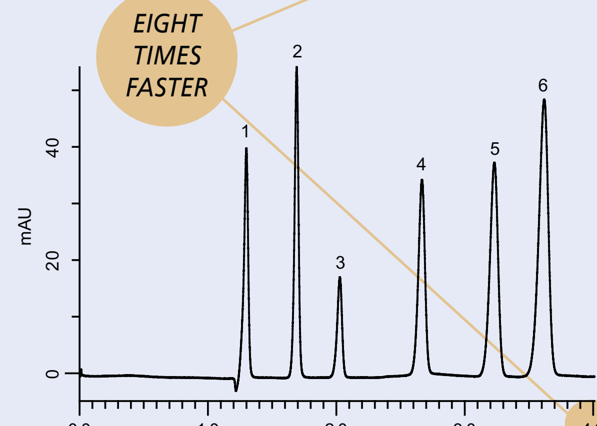
column: Ascentis Express C18, 5 cm x 3.0 mm I.D. and 5 µm particle column, 15 cm x 3.0 mm I.D.
mobile phase: 31:69, water:acetonitrile
flow rate: 1.2 mL/min or 0.4 mL/min
temp.: 35° C
inj.: 0.5 µL

1. Uracil
2. Phenol
3. Acetophenone
4. Benzene
5. Toluene
6. Naphthalene





C18 5 µm
0.4 mL/min flow rate



High Definition "HD"-Resolution

Double the Efficiency

- Short analyte diffusion path
- Twice the efficiency of 3 µm particles
- Longer columns permit doubling the plates over sub-2 µm particles

Compared to conventional 3 µm and 5 µm particles, Ascentis Express HPLC columns provide sharper peaks under the same conditions. By simply swapping in an Ascentis Express HPLC column of the equivalent dimensions to your current 3 µm and 5 µm particle HPLC columns, an improvement in resolution can be achieved. This improvement is shown in Figure 4. Note: Remember Ascentis Express HPLC column recommended flow rates are higher than that for conventional 3 µm and 5 µm particles.

Ascentis Express and sub-2 µm columns of the same dimensions give approximately the same number of theoretical plates (efficiency). However, because Ascentis Express columns are more permeable and exhibit half the backpressure, you can use longer columns for even more resolving power. The high backpressure generated by the sub-2 µm particles precludes the use of longer columns, even on ultra-high pressure systems under ambient conditions.

An example of the HD-Resolution is shown in Figure 5 where the additional theoretical plates on the 10 cm Ascentis Express column provided significantly better resolution of β-estradiol and the impurity compared to the 5 cm sub-2 µm column at comparable backpressures.

Figure 4. HD-Resolution on Ascentis Express: Sharper Peaks than Traditional Columns

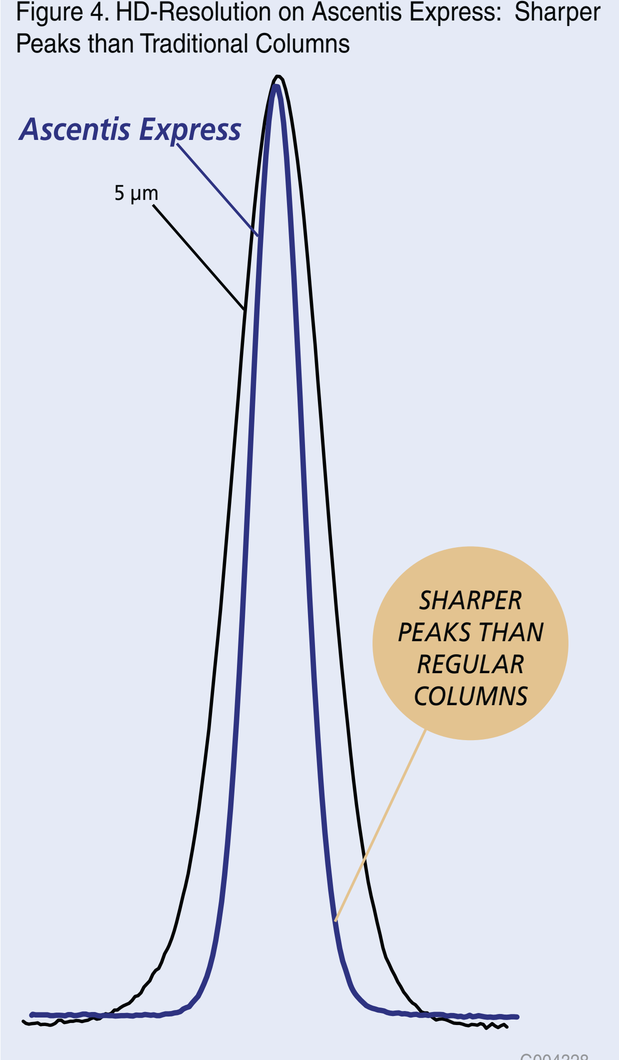
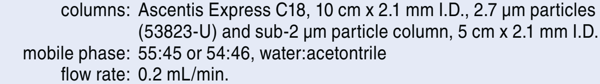
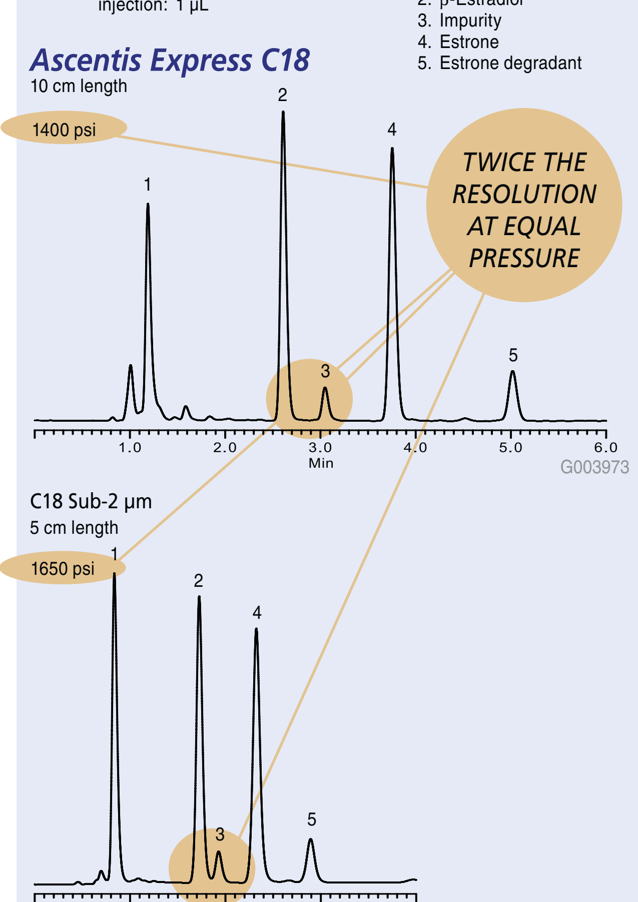


Figure 5. HD-Resolution on Ascentis Express Compared to Sub-2 µm Columns



columns: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column, 5 cm x 2.1 mm I.D.
mobile phase: 55:45 or 54:46, water:acetontrile
flow rate: 0.2 mL/min.
temp.: ambient
det.: UV at 200 nm
injection: 1 µL

1. Estradiol
2. β-Estradiol
3. Impurity
4. Estrone
5. Estrone degradant



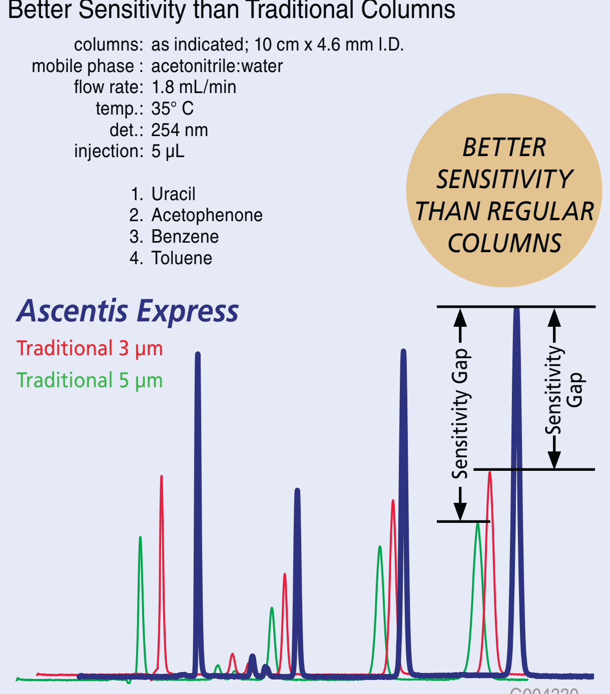
Super-Sensitive

High Sample Loading Capacity and Signal/Noise for Trace Analysis

- High column efficiency for high S/N
- High sample loading from thick, porous shell layer

Trace analysis benefits from high column efficiency. Efficient peaks are taller and provide higher S/N ratios. As discussed in earlier sections of this brochure, the Ascentis Express columns can provide higher efficiency than any traditional particle. The added sensitivity of the higher efficiency Ascentis Express particles is visualized as the "sensitivity gap" in Figure 6.

Figure 6. Higher Efficiency of Ascentis Express Provides Better Sensitivity than Traditional Columns



Although they have a solid core, the 0.5 µm-thick "shell" of the Fused-Core particles provides roughly 75% of the surface area as a totally porous particle of the same diameter. Only the pores with very long diffusion paths are fused in the Ascentis Express HPLC columns. The resulting particles have effective surface areas of ~225 m^2/g; comparable to totally porous particles. The higher surface area gives higher sample loading capacity compared to sub-2 µm particles, as evidenced by the symmetry vs. concentration relationship in Figure 7. Above 5 ppm, the sub-2 µm experiences sample overload and subsequent loss of peak shape.

Figure 7. Higher Loading Capacity of Ascentis Express Compared to Sub-2 µm Particles

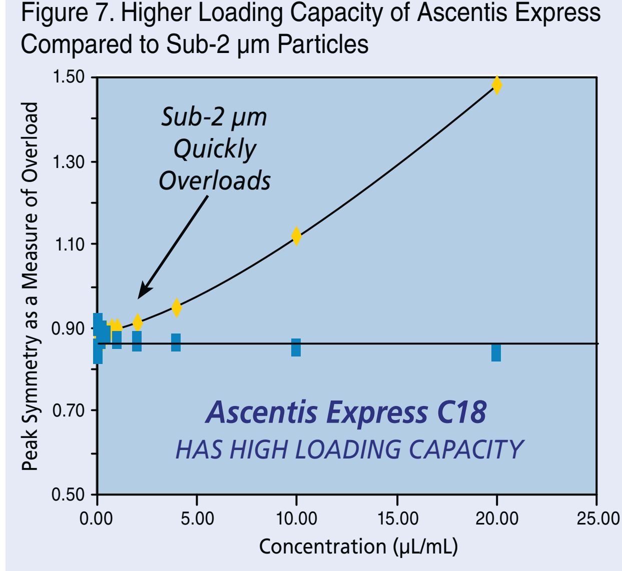
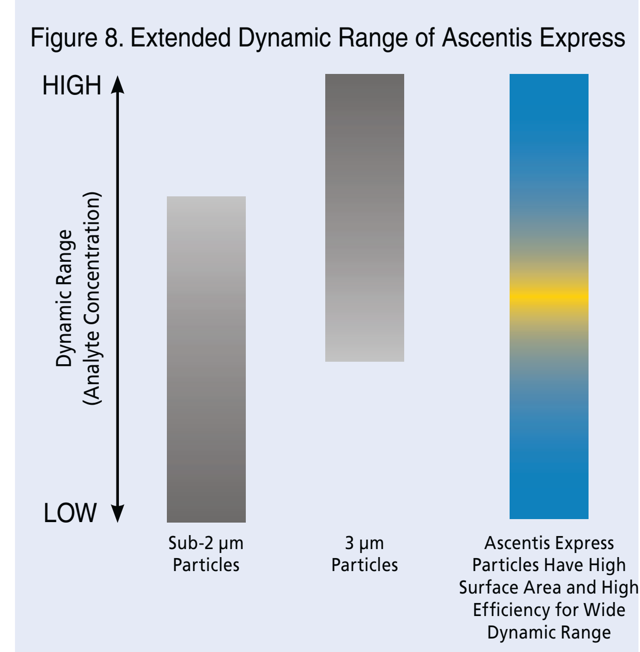


Figure 8 shows how Ascentis Express extends the dynamic range. It has the high efficiency of sub-2 µm particles needed for trace analysis, and the high surface area of totally porous particles needed for high sample capacity.

Figure 8. Extended Dynamic Range of Ascentis Express



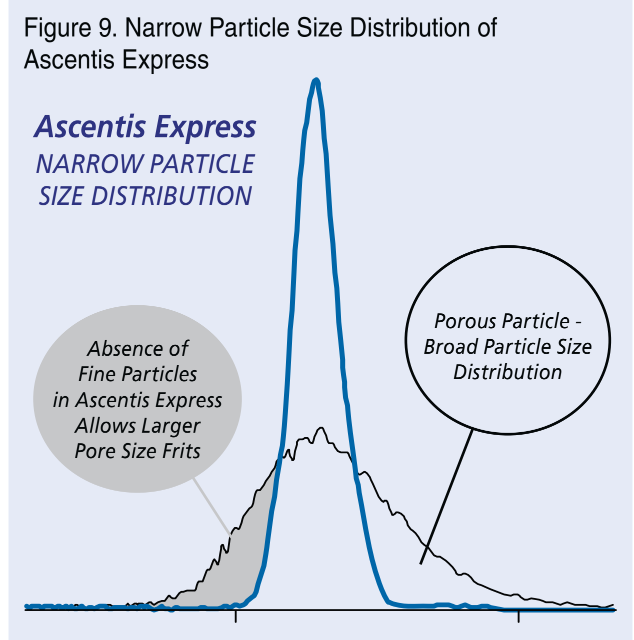
Super-Rugged Columns

Extended Column Lifetime Compared to Both 3 μm and sub-2 μm Columns

- Narrow particle size distribution allows use of 2 μm frits
- Dense particles for more stable bed

Fused-Core particles are produced in a way that yields an extremely narrow particle size distribution (Figure 9). This narrow particle size distribution permits the use of frits with nominal 2 μm pores, the same as used on most columns packed with 5 μm particles. In comparison, sub-2 μm particles require frits with much smaller pore size – 0.5 μm or smaller – that are prone to fouling, lead to peak-splitting and high backpressure, and ultimately shorten the column lifetime. Another feature of the Fused-Core particles that contributes to their ruggedness is that they are denser than totally porous particles and form highly stable beds in the packed column.

Figure 9. Narrow Particle Size Distribution of Ascentis Express



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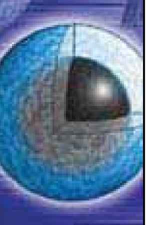


Alternative Selectivity with Ascentis Express RP-Amide

Ascentis Express RP-Amide can solve

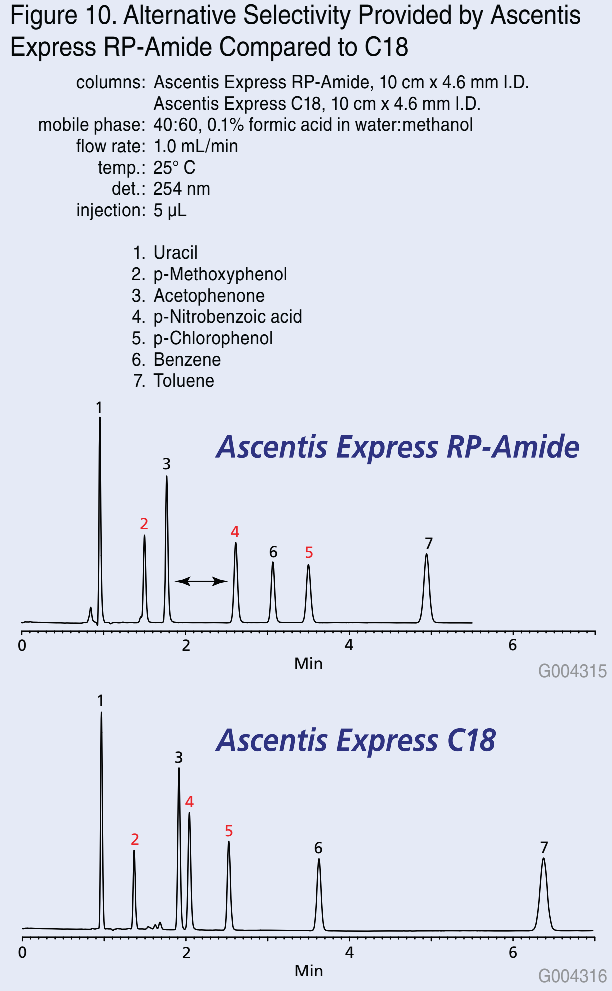
- Co-eluting peaks
- Unresolved components
- Poor retention of polar compounds
- Peak tailing of basic compounds
- Silanol interactions causing poor reproducibility

While the Ascentis Express C18 provides classic reversed-phase selectivity, the Ascentis Express RP-Amide provides increased selectivity for polar compounds, especially those that can act as a hydrogen-bond donor. Other attributes of the RP-Amide include improved peak shape for bases, 100% aqueous compatibility, and low bleed for LC-MS applications.

The amide group provides enhanced selectivity with analytes that have hydrogen bonded to a heteroatom. Phenols, carboxylic acids, amines and, to a lesser extent, alcohols show enhanced retention on the RP-Amide phase when compared to neutral non-polar analytes. An example of the power of the hydrogen bonding mechanism is shown in Figure 10. The Ascentis Express C18 and RP-Amide columns are compared. The analyte mixture contains neutral, non-polar analytes (benzene and toluene) and protic analytes (p-methoxyphenol, p-nitrobenzoic acid, and p-chlorophenol). As observed from the chromatograms in Figure 1, the neutral molecules show slightly reduced retention on the RP-Amide, but the protic molecules show greatly enhanced retention yielding a chromatogram with very different selectivities and even a change in elution order. The potential for solving separations difficulties is tremendous.

Two other points should be noted. The Ascentis Express RP-Amide has the same high efficiency as the Ascentis Express C18 with the same low back-pressure. Secondly, both separations were carried out in the same mobile phase. This is important since it simplifies method development. If a separation is not adequate on an Express C18, there is no need to change mobile phase to optimize the separation, simply switch to the Ascentis Express RP Amide and if protic moieties are present, a change in selectivity will be achieved.

Figure 10. Alternative Selectivity Provided by Ascentis Express RP-Amide Compared to C18



Ascentis Express RP-Amide Applications

- Natural products
- Phenolics
- Bases
- Metabolites
- Polar Compounds

Polar Compound Retention with Ascentis Express HILIC

Benefits of HILIC Separation

- Retention of highly polar analytes like metabolites
- Complimentary selectivity to reversed-phase chromatography
- Increased MS sensitivity
- Quick transfer from final steps of sample prep (SPE, protein precipitation, etc.)

HILIC chromatography is gaining popularity due to increased retention of polar compounds. Many classes of polar compounds can be retained in HILIC. These include polar neutrals, polar acids, and polar and non-polar basic amines. Both polar and ionic interactions can contribute to retention and selectivity in this mode of chromatography.

How HILIC works

HILIC separates compounds by using a mostly organic mobile phase across a polar stationary phase, causing solutes to elute in order of increasing polarity-the opposite of Reversed-Phase. Retention in HILIC is likely to be a combination of hydrophilic interaction, ion-exchange, and some reversed-phase retention. A typical mobile phase consists of 60-95% acetonitrile and an aqueous buffer. 10-20 mM ammonium acetate or ammonium formate are useful due to volatility and solubility. The sample solvent should be similar in type and strength as the mobile phase. The sample solvent can contain a higher amount of organic than the mobile phase, but should not contain more water than the mobile phase.

Shown in Figure 11 is a comparison of the analysis of highly polar molecules on Ascentis Express HILIC and Ascentis Express C18.

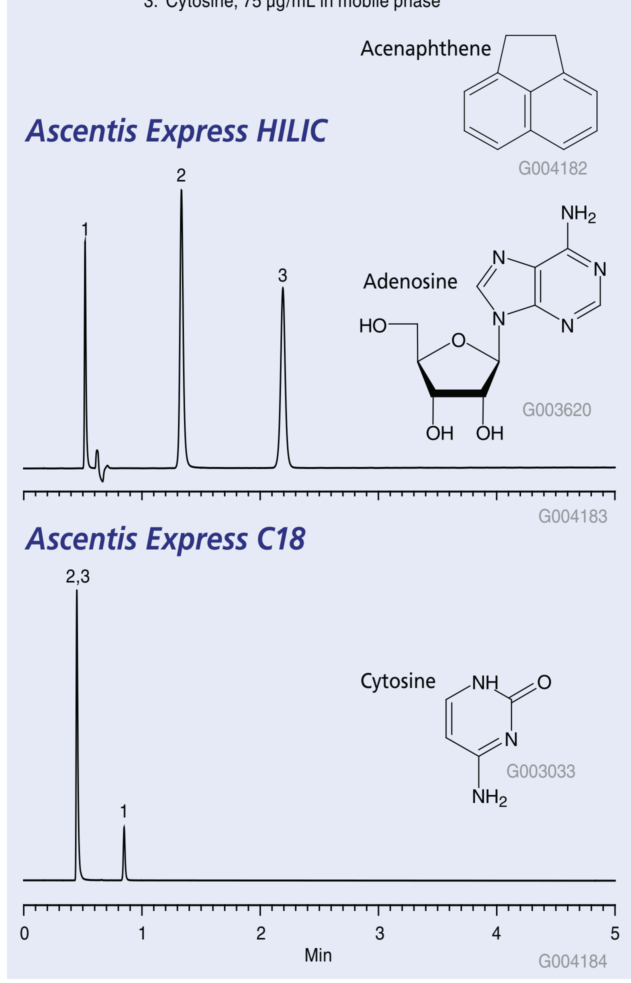
Ascentis Express HILIC Applications

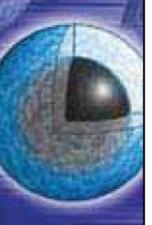
- Amino acids
- Small, polar acids – (metabolomics)
- Biogenic amines – (neurotransmitters, contaminants in food & beverage)
- Phosphates – (pesticides, herbicides)
- Sugars
- Drug metabolites and conjugates

Figure 11. Comparison of the Analysis of Polar Molecules on Ascentis Express HILIC and Ascentis Express C18

columns: Ascentis Express HILIC, 10 cm x 2.1 mm ID, 2.7 µm particles (53939-U)
Ascentis Express C18, 10 cm x 2.1 mm ID, 2.7 µm particles (53823-U)
mobile phase: 10:90; 100 mM ammonium formate, pH 3.0 with concentrated formic acid:acetonitrile
flow rate: 0.4 mL/min
temp.: 35 °C
det.: UV at 254 nm
injection: 1 µL

1. Acenaphthene, 80 µg/mL in mobile phase
2. Adenosine, 35 µg/mL in mobile phase
3. Cytosine, 75 µg/mL in mobile phase





Improving HPLC Sample Throughput

Do More Work in Less Time Without Changing Your Method

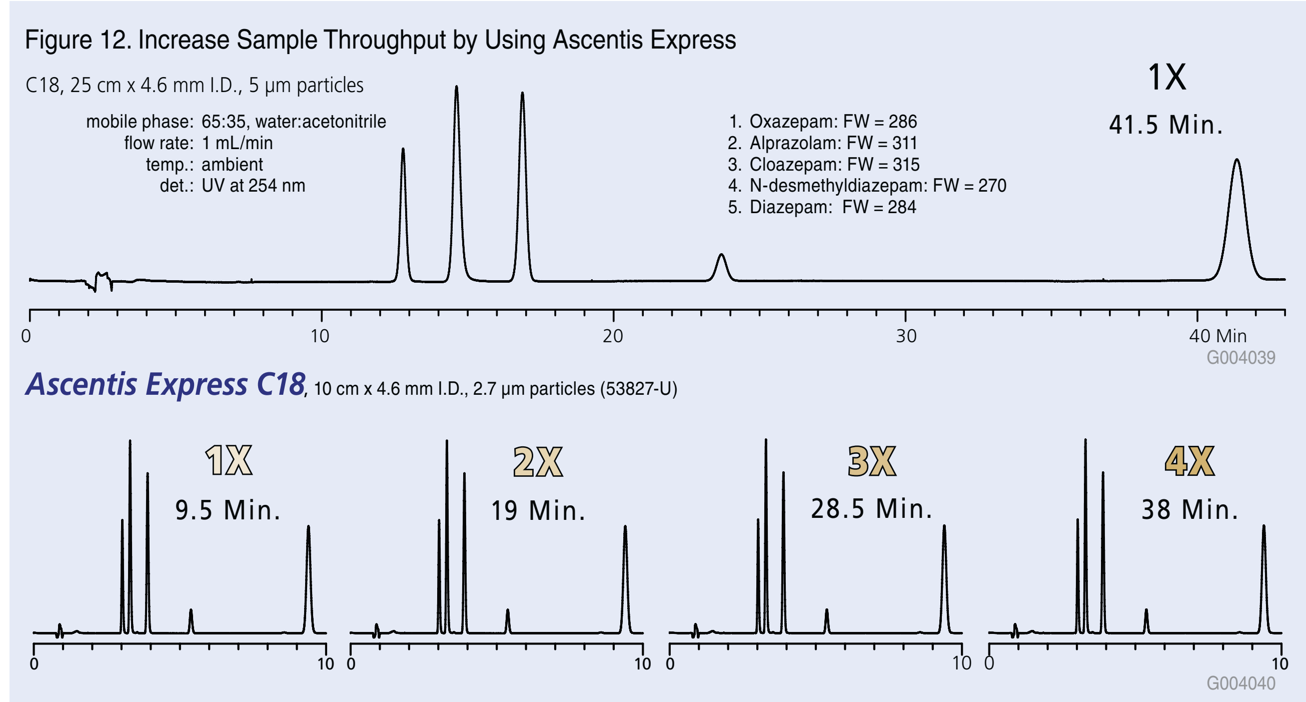
The demand for increased sample throughput and speed of results has driven HPLC users to search for breakthroughs in HPLC instruments and column technology. Although improvements have been realized, setbacks have been encountered. Reduction in column ruggedness, costly replacements of existing instrumentation, and difficulties in transferring methods to new systems have often made these past improvements unappealing to analysts.

The Fused-Core HPLC particle technology behind Ascentis Express permits 4- to 6-fold reduction in analysis time, with a subsequent increase in sample throughput compared to conventional HPLC columns, without sacrificing resolution or column ruggedness and without the need to change systems or sample prep procedures The current high resolution column for traditional HPLC methods is a 25 cm column packed with 5 µm particles.

Until now, this dimension provided the most efficiency within the pressure limit of a conventional HPLC system. With the high efficiency Ascentis Express, one can now achieve the same number of plates as a 25 cm column packed with 5 µm particles with a 10 cm column or even more efficiency and resolution with a 15 cm Ascentis Express column. Therefore, by simply changing columns and keeping all other conditions the same, you can reduce the runtime and increase the resolution of your method.

Figure 12 compares the resolution of a five-component sample on 25 cm, 5 µm C18 and 10 cm Ascentis Express C18 columns. Each column has approximately the same number of theoretical plates and hence the same resolving power. However the shorter Ascentis Express column delivers this separation in a much shorter time, in this case less than one-fourth the time as the 25 cm column.

Figure 12. Increase Sample Throughput by Using Ascentis Express



Ready to do more work in less time?
See the flow chart on the next page.

Selecting the Optimal Ascentis Express Column

Is your column a **25 cm (5 µm), 15 cm (5 µm),** or **10 cm (3 µm)**?

- YES → Do you want to **MAXIMIZE SPEED** or **IMPROVE RESOLUTION & SPEED?**
- NO → Email Supelco Technical Service to assist in selecting an optimal Ascentis Express Column

  techservice@sial.com

Convert to Ascentis Express HPLC Columns for Both!

MAXIMIZE SPEED

1A.1: Conversion Table Current Column	Ascentis Express
25 cm (5 µm)	10 cm
15 cm (5 µm)	7.5 cm
10 cm (3 µm)	5 cm

IMPROVE RESOLUTION & SPEED

1B.1: Conversion Table Current Column	Ascentis Express
25 cm (5 µm)	15 cm
15 cm (5 µm)	10 cm
10 cm (3 µm)	7.5 cm

2: Maximize flow rate within system pressure constraints *

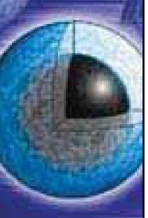
Ascentis Express HPLC Column ID	Suggested Starting Point for Flow Rate
4.6 mm I.D.	1.6 mL/min
3.0 mm I.D.	0.8 mL/min
2.1 mm I.D.	0.4 mL/min

3: Confirm method suitability parameters

3.1: Is resolution maintained?

- NO → 3.2: Select longer column (return to 3.1)
- YES → 3.3: Is additional throughput or resolution desired?
  - YES → 3.2: Select shorter column or faster flow rate (return to 3.1)
  - NO → **Improved sample throughput with Ascentis Express and your existing HPLC system**

*Read *Guidelines for Optimizing Systems for Ascentis Express Columns* (T407102) and *Guide to Dispersion Measurement* (T408143).



Fast HPLC for Rapid Screening of Pharmaceutical Compounds

Ideal for Walk-up LC-MS Systems

HPLC is critical to the discovery, development and eventual commercialization of pharmaceutical products. HPLC is the benchmark analytical method in the pharmaceutical industry due to its ability to score such high marks in analytical validation characteristics including accuracy, precision, limit of detection, specificity, linearity and range, and ruggedness. No other analytical techniques can consistently score high in all characteristics on compounds and matrices that are of interest to the pharmaceutical industry.

Furthermore, it has been generally accepted that a typical HPLC analysis takes 15-30 minutes with some as great as an hour. When multiplied by the number of samples to be analyzed either in discovery or product release, the total instrument time required is staggering. This overwhelming amount of instrument time has resulted in a growing number of instruments, around-the-clock analysis, and a push for faster methods.

Fast HPLC, using short columns (3-10 cm) packed with small particles (<3 µm) and high flow rates has recently become an effective means to reduce analysis time. This is primarily due to the improved quality of sub-3 µm particle columns and the introduction of new instrumentation to meet the requirements of higher column backpressure and low instrument dispersion. The reasons for using sub-3 µm particle columns in fast HPLC are evident by examining Van Deemter plots for various particle sizes. The smaller particles yield lower HETP or higher efficiency per unit length. Furthermore, the optimum flow rate is higher for smaller particles. Smaller particle columns have less efficiency loss at high flow rates because mass transfer is less sensitive to velocity changes as illustrated by "flatter" Van Deemter plots.

Unfortunately, column backpressure increases at a greater rate than column efficiency as you decrease particle size. This increase in backpressure is so great for sub-2 µm particle columns that they are practically unusable using standard HPLC systems.

Shown in Figures 13-15 are the chromatograms for the separation of three sets of closely related pharmaceutical compounds. These examples include both basic and neutral as well as polar and non-polar compounds. While each example utilizes 2.1 mm I.D. columns, three different flow rates and three unique mobile phase conditions are presented to demonstrate the versatility of fast HPLC with Fused-Core particle columns.

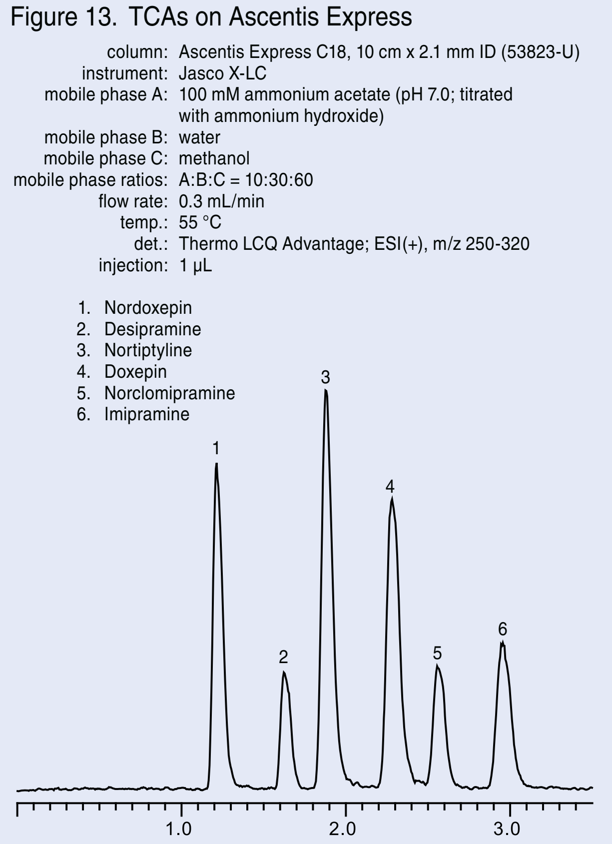


Figure 13. TCAs on Ascentis Express

column: Ascentis Express C18, 10 cm x 2.1 mm ID (53823-U)
instrument: Jasco X-LC
mobile phase A: 100 mM ammonium acetate (pH 7.0; titrated with ammonium hydroxide)
mobile phase B: water
mobile phase C: methanol
mobile phase ratios: A:B:C = 10:30:60
flow rate: 0.3 mL/min
temp.: 55 °C
det.: Thermo LCQ Advantage; ESI(+), m/z 250-320
injection: 1 µL

1. Nordoxepin
2. Desipramine
3. Nortiptyline
4. Doxepin
5. Norclomipramine
6. Imipramine

Shown in Figure 13 is the separation of six tricyclic antidepressants (TCAs). The separation of these closely related compounds was performed under isocratic mobile phase conditions with mass spectrometric (MS) detection. Baseline resolution was achieved with a total separation time of 3 minutes demonstrating not only the potential speed of the Ascentis Express columns but also the resolving power. Note the MS compatible mobile phase and flow rate. Furthermore, the use of 2.1 mm I.D. columns provides a reduction in solvent consumption compared to typical flow rates for 4.6 mm I.D. or monolithic columns.

Data in Figure 14 further illustrates the speed in which closely related compounds can be resolved using the Fused-Core particle. In this example, four β-blockers are resolved in less than one minute under isocratic conditions utilizing MS detection. While a 10 cm column was utilized for the TCAs separation, a 5 cm column was used for the β-blockers example.

The separation of three steroids as well as a related impurity and degradant is shown in Figure 15. A high mobile phase flow rate of 0.6 mL/min was utilized and is suitable for Ascentis Express columns due to the Van Deemter curve associated with these columns. Isocratic mobile phase conditions were utilized as well as UV detection at 200 nm, a common detection wavelength for impurity profiling. Again, baseline resolution was achieved for all compounds with a total runtime of less than two minutes. It should be noted that the isocratic conditions used in these examples further enhances sample throughput versus gradient conditions due to no need for column re-equilibration. With a backpressure of just 4500 psi, this analysis could be performed on almost any HPLC system. A similar separation was attempted using a sub-2 µm particle column but was not possible given the same instrument constraints put on the Ascentis Express column.

Figure 14. β-Blockers on Ascentis Express

column: Ascentis Express C18, 5 cm x 2.1 mm ID (53822-U)
instrument: Agilent 1100
mobile phase A: 0.1% acetic acid in water
mobile phase B: 0.1% acetic acid in acetonitrile
mobile phase ratios: A:B = 74:26
flow rate: 0.2 mL/min
temp.: 35 °C
det.: ABI 3200 QT; ESI(+), MS/MS
injection: 1 µL

1. Atenolol
2. Pindolol
3. Timolol
4. Metoprolol

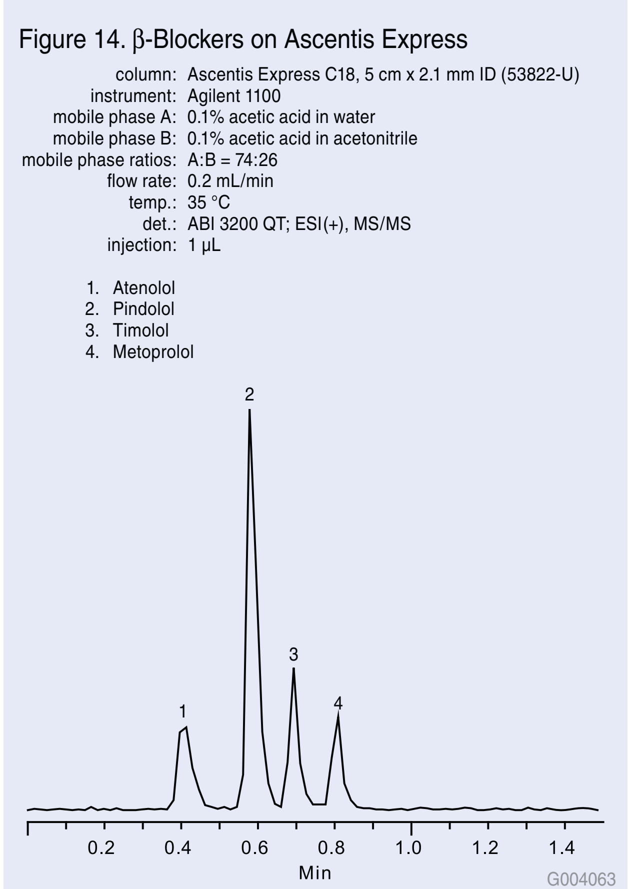
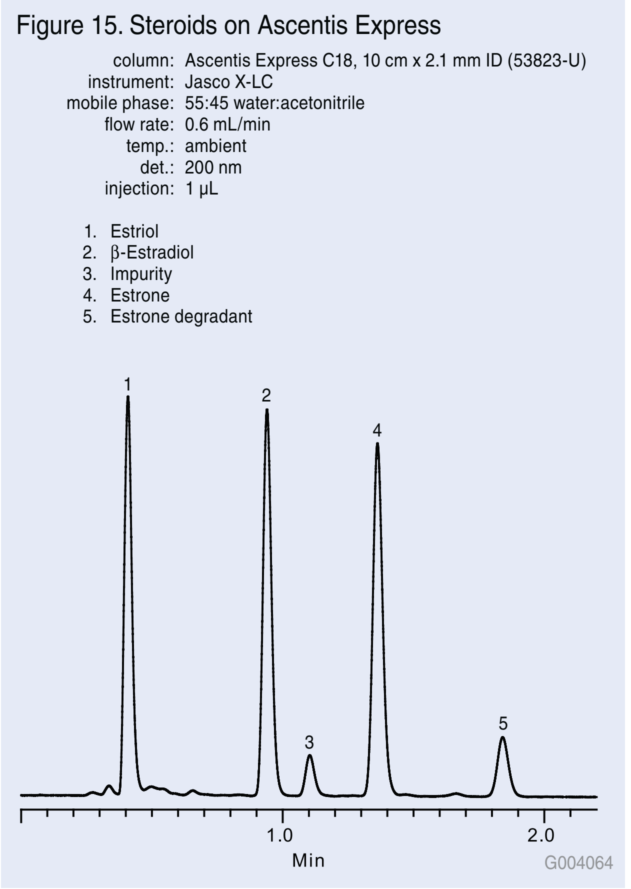
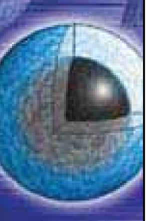


Figure 15. Steroids on Ascentis Express

column: Ascentis Express C18, 10 cm x 2.1 mm ID (53823-U)
instrument: Jasco X-LC
mobile phase: 55:45 water:acetonitrile
flow rate: 0.6 mL/min
temp.: ambient
det.: 200 nm
injection: 1 µL

1. Estriol
2. β-Estradiol
3. Impurity
4. Estrone
5. Estrone degradant





Ultra-High Resolution HPLC: Column Coupling

Maximize the Resolution of UHPLC systems

Column coupling in HPLC is gaining interest since LC systems are being designed to withstand column back pressures of up to 15,000 psi. Column coupling is a simple and practical way to increase resolution by simply increasing column length. Because Ascentis Express HPLC columns provide higher efficiencies at any pressure compared to 3 µm and sub-2 µm particles, the coupling of Ascentis Express columns enables significantly higher resolution than any other column on any commercial HPLC system.

Efficiencies greater than 150,000 plates/column are possible and demonstrated in the isocratic separation of benzene and toluene with various deuterium substitutions.

Figure 16 shows the efficiency obtained by coupling 5 Ascentis Express 15 cm columns together.

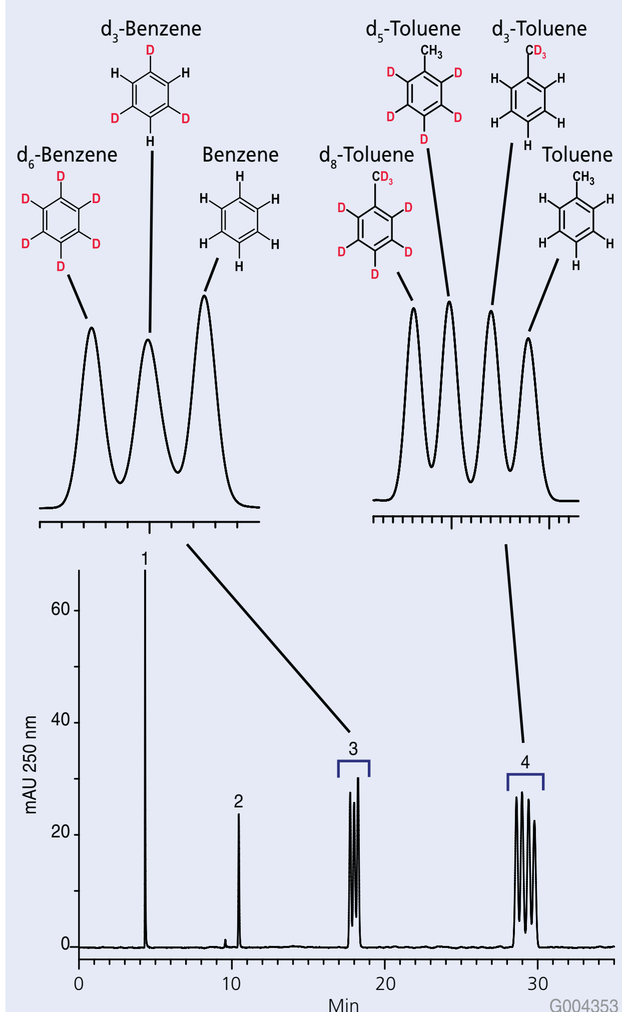
Column Coupling Applications

- Natural product chemistry
- Tryptic digests
- Synthetic peptide mapping
- Stress studies of APIs
- LC-NMR

Figure 16. Column Coupling of Ascentis Express Provides over 100,000 Plates per Separation

column: Ascentis Express C18, 15 cm x 3 mm I.D., x5
mobile phase: 56:44, water:acetonitrile
flow rate: 0.6 mL/min
temp.: 60° C
pressure: 13500 psi (930 bar)

1. Uracil
2. Acetophenone
3. Benzene
4. Toluene



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TRADEMARKS:
Ascentis, HybridSPE – Sigma-Aldrich Biotechnology LP
Fused-Core – Advanced Materials Technology
UPLC – Waters Associates, Inc.

Ordering Information

Analytical

ID (mm)	Length (cm)	Ascentis Express C18	Ascentis Express C8	Ascentis Express RP-Amide	Ascentis Express HILIC
2.1	3	53802-U	53839-U	53910-U	53933-U
2.1	5	53822-U	53831-U	53911-U	53934-U
2.1	7.5	53804-U	53843-U	53912-U	53938-U
2.1	10	53823-U	53832-U	53913-U	53939-U
2.1	15	53825-U	53834-U	53914-U	53946-U
3.0	3	53805-U	53844-U	53915-U	53964-U
3.0	5	53811-U	53848-U	53916-U	53967-U
3.0	7.5	53812-U	53849-U	53917-U	53969-U
3.0	10	53814-U	53852-U	53918-U	53970-U
3.0	15	53816-U	53853-U	53919-U	53972-U
4.6	3	53818-U	53857-U	53921-U	53974-U
4.6	5	53826-U	53836-U	53922-U	53975-U
4.6	7.5	53819-U	53858-U	53923-U	53977-U
4.6	10	53827-U	53837-U	53929-U	53979-U
4.6	15	53829-U	53838-U	53931-U	53981-U

Capillary

	Ascentis Express C18 Length		Ascentis Express C8 Length	
	5 cm	15 cm	5 cm	15 cm
75 µm I.D.	53982-U	54219-U	53983-U	54229-U
100 µm I.D.	53985-U	54256-U	53987-U	54260-U
200 µm I.D.	53989-U	54261-U	53991-U	54262-U
300 µm I.D.	53992-U	54271-U	53997-U	54272-U
500 µm I.D.	53998-U	54273-U	53999-U	54275-U

Ascentis Express Properties

Stationary Phase Support

- Ultra-pure, Type B silica
- 1.7 µm solid core particle with 0.5 µm porous silica shell (effective 2.7 µm)
- 150 m^2/gram surface area (comparable to ~225 m^2/g porous particle)
- 90 Å pore size

Bonded Phase

	Coverage µmoles/m^2	pH Range	Endcapping
C18	*3.5*	*2-9*	*Yes*
C8	*3.7*	*2-9*	*Yes*
RP-Amide	*3.0*	*2-9*	*Yes*
HILIC	*n/a*	*2-8*	*No*

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Ascentis® Express HPLC Resource Guide

Application Articles

Choosing an Alternate Phase

Bibliography of Fused-Core Publications

Practical Recommendations for Success

Listing of Available Technical Literature

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Table of Contents

The Fused-Core Advantage 2

Introducing Ascentis Express 3

Practical Recommendations for Success 3

Rapid, Sensitive, General-Purpose Cleaning Validation Using Ascentis Express HPLC Columns 6

Ascentis Express Peptide ES-C18 Expands the Fused-Core Particle Platform into Bioseparations 8

Improving the Current USP Method for the Analysis of Lansoprazole Drug Substance Using HPLC Columns Based on Fused-Core Particle Technology 9

Selecting an Ascentis Express Phase 11

Profiling of Stevia rebaudiana Extract by Accurate Mass Using HILIC and Reversed-Phase Chromatography 12

Bibliography of Fused-Core Publications 14

Colors of the World: Fast Separation of Dyes with Ascentis Express 17

Ascentis Express RP-Amide 20

Ascentis Express Phenyl-Hexyl 23

Ascentis Express FAQs 26

Available Technical Literature 26

Ordering Information 27

Available Resources

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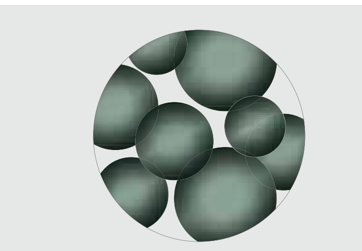
The Fused-Core™ Advantage

Extreme performance on any LC system

Half the backpressure of sub-2 µm columns
Twice the performance of 5 µm columns

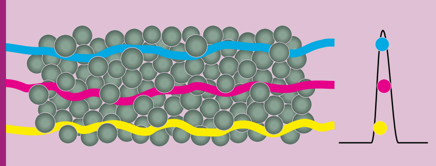
Narrow Particle Size Distribution

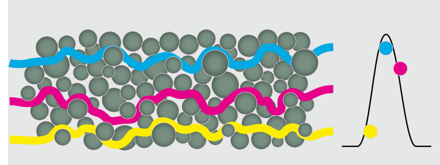
Fused-Core Particles | **Traditional Porous Particles**



The innovative manufacturing process for Fused-Core particles produces a very narrow particle size distribution. A narrow particle size distribution allows for the use of large porosity frits that resist clogging, resulting in a **more rugged column**. Traditional porous particles are not manufactured in a way to yield extremely narrow particle size distributions.

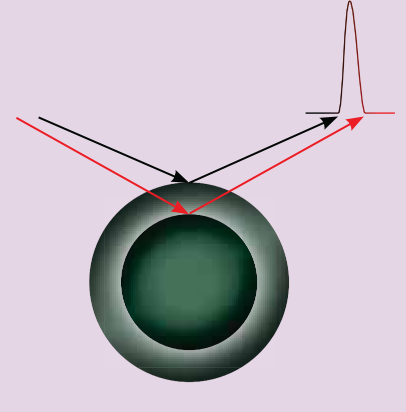
More Consistent Bed

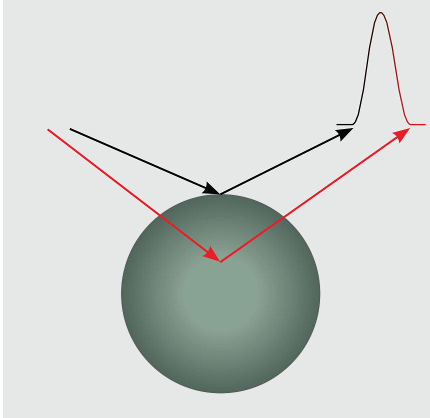




The "A" term in the van Deemter equation accounts for the effects of inhomogeneities in the packed bed of an HPLC column. Narrow particle size distributions form a more consistent packed bed and a consistent path length, **minimizing analyte diffusion** through the column. This eddy diffusion is effectively independent of mobile phase velocity.

Shorter Diffusion Path





The short diffusion path of the Fused-Core particle **yields sharper peaks** than traditional porous particle columns. The minimized resistance to mass transfer, the "C" term in the van Deemter equation, of the Fused-Core particle provides sharper peaks than traditional porous particles. The short diffusion path also **permits the use of higher flow rates** without peak broadening.

Introducing Ascentis Express…

Now, High Speed and High Efficiency HPLC Separations are Possible on Any LC System

Increasing speed and resolution of HPLC analyses are drivers for innovation in both HPLC column and hardware design. While columns packed with 5 µm particles have been the standard, reducing particle size has been the strategy of many column manufacturers and users alike. Smaller particles result in faster chromatography. The cost for the improved speed is higher column backpressures. To obtain the benefit of the small particles, instrumentation beyond conventional HPLC is required.

Ascentis Express columns provide a breakthrough in HPLC column performance. Based on Fused-Core particle technology, Ascentis Express provides the benefits of high speed of much smaller particles but at a backpressure suitable to conventional HPLC systems. Due to this fundamental performance advantage, Ascentis Express can benefit both conventional HPLC users as well as UPLC™ or other ultra pressure system users.

Exceeding the Performance of Other "Fast" HPLC Particles

Designed to deliver speed and resolution on all LC systems, Ascentis Express meets and exceeds the benefits of competitive particles, including 3 µm and sub-2 µm particles. Under the same conditions, Ascentis Express columns deliver the same efficiencies at half the backpressure of sub-2 µm particles and nearly twice the efficiency of 3 µm particles.

Compared to Sub-2 µm Particles:

Advantage: *Ascentis Express columns can be run successfully on conventional, mid-pressure and ultra high pressure HPLC and LC-MS instruments.*

Advantage: *Double the flow rate. Run Ascentis Express columns at higher flow rates for faster analyses.*

Advantage: *Double the column length. Longer Ascentis Express columns can be used, giving additional resolving power.*

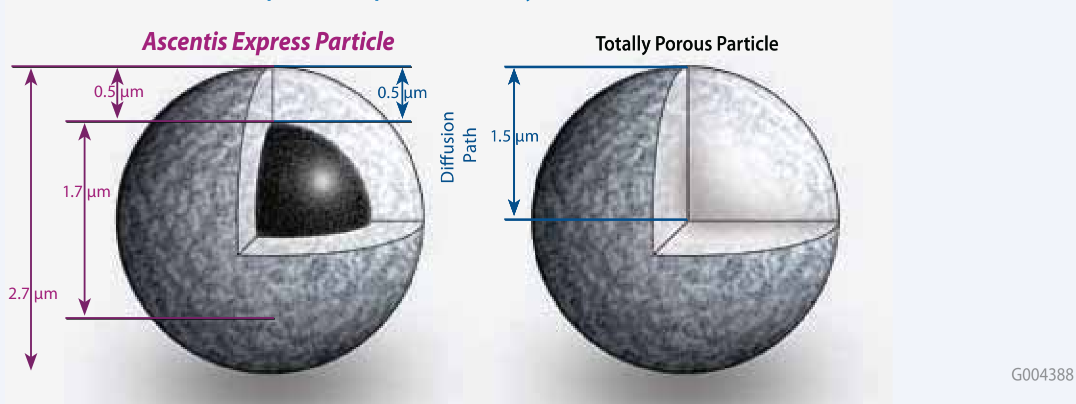
Compared to 3 µm Particles:

Advantage: *Double the efficiency. Ascentis Express columns have nearly twice the column efficiency of 3 µm particles.*

Practical Recommendations for Success

- Use 0.005 in. I.D. inlet and outlet tubes. Broadening is much less sensitive to the tube length than to the I.D. Minimize lengths of the inlet and outlet tubes for best performance, but do not worry about having a few extra centimeters of length if it makes maintenance or column installation easier.
- If high pressure becomes a problem, then use acetonitrile as modifier and elevate the column temperature whenever possible. If methanol, THF, or another more viscous modifier is required, then elevating the temperature becomes even more beneficial. Even a modest temperature increase will greatly reduce the mobile phase viscosity and the required pressure while improving mass transfer.
- Use only 4.6 mm diameter Fused-Core columns on conventional HPLC systems to minimize broadening problems from the remaining system components. Extra column broadening worsens as the column diameter is decreased.
- Keep the sample volumes small — 5 µL or less if the peaks of interest elute early ($k = 1$). Up to 20 µL is acceptable if k exceeds 10.
- Avoid sample solvents that are stronger than the mobile phase.
- Use data rates of 10 Hz or greater, and watch out for bunching factors.

Figure 1. Fused-Core Structure of Ascentis Express Compared to Totally Porous Particles



The Particle Platform Innovations Behind Ascentis Express

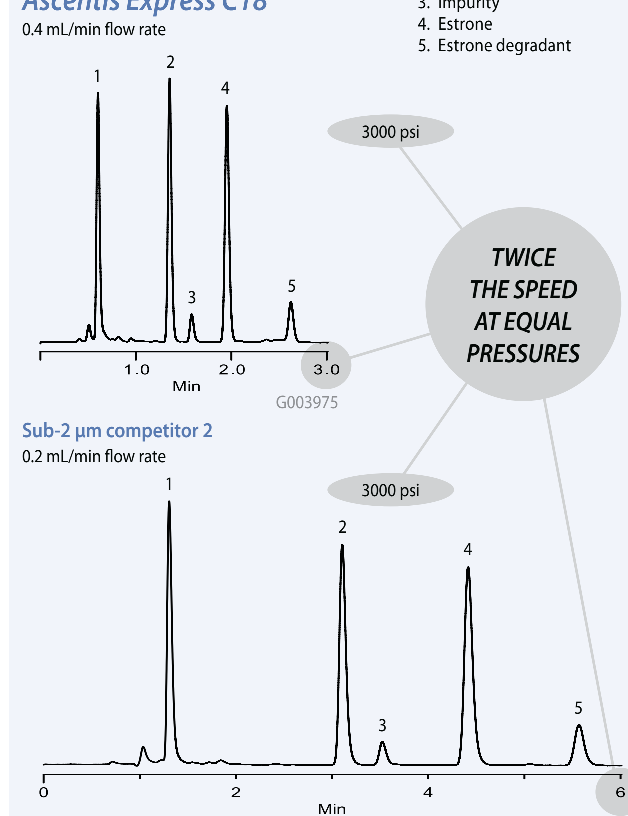
Like most modern HPLC particles, Ascentis Express particles are high surface area spheres made from high purity silica gel. The total particle diameter is 2.7 µm. However, here the comparison ends. What sets apart Ascentis Express from conventional HPLC particles is the patent pending Fused-Core technology. Ascentis Express particles comprise a solid 1.7 µm diameter silica core that is encapsulated in a 0.5 µm thick layer of porous silica gel.

There are five distinct properties of Ascentis Express particles that account for their high performance and are worth emphasizing:

1. **The solid core**
   Because of the solid core, analytes cannot diffuse as deeply into the particle, resulting in less band broadening, and hence higher efficiency and sensitivity, compared to totally porous particles of the same diameter.
2. **The 0.5 µm porous shell surrounding the solid core**
   The porous shell gives the particles a surface area comparable to totally porous particles for excellent phase loading and sample capacity.
3. **The total particle diameter (2.7 µm)**
   Compared to sub-2 µm porous particles, Ascentis Express yields half the column backpressure, allowing longer columns and faster flow rates (Figures 2 and 3).
   Compared to 3 µm porous particles, Ascentis Express yields nearly twice the efficiency (Figure 4).
4. **The narrow particle size distribution.**
   Compared to both sub-2 µm and 3 µm particles, Ascentis Express provides longer column lifetime because the narrow particle size distribution allows us to use larger pore size frits (2 µm vs. 0.5 µm) that are less susceptible to fouling.
5. **The high particle density**
   By virtue of the solid core, Ascentis Express particles yield a more densely packed bed for added stability and long column lifetime.

Figure 2. Hyper-Fast Separations on Ascentis Express

column: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column (same dimensions)
mobile phase: 49:51 or 55:45, water:acetontrile
flow rate: 0.4 or 0.2 mL/min.
temp.: ambient
det.: UV at 200 nm
injection: 1 µL



Ascentis Express: High Speed, High Efficiency Separations Adaptable Equally to R&D and Routine Analysis Settings

The recent introduction of UPLC™ and other ultra high pressure LC systems addressed the need for high throughput separations. However, speed is not the only important criteria: the need for more sensitivity, more resolution and improved ruggedness of the technique has lead to a continual stream of new LC and LC-MS instruments. Coupled with the large installed base of conventional HPLC instruments, the result is that most laboratories have a mixture of instruments, old and new. Whereas columns packed with sub-2 µm particles require ultra high pressure instruments, Ascentis Express columns can be run on any LC system. Methods developed on Ascentis Express can be readily and reliably validated and transferred from R&D to routine analysis labs, whether across the building or across the world.

We hope this article has sparked an interest in Ascentis Express and the benefits it can bring to your laboratory. Subsequent articles will develop the Ascentis Express message by focusing on specific features and application areas.

Figure 3. HD-Resolution on Ascentis Express Compared to Sub-2 µm Columns

column: Ascentis Express C18, 10 cm x 2.1 mm I.D., 2.7 µm particles (53823-U) and sub-2 µm particle column, 5 cm x 2.1 mm I.D.
mobile phase: 55:45 or 54:46, water:acetontrile
flow rate: 0.2 mL/min.
temp.: ambient
det.: UV at 200 nm
injection: 1 µL

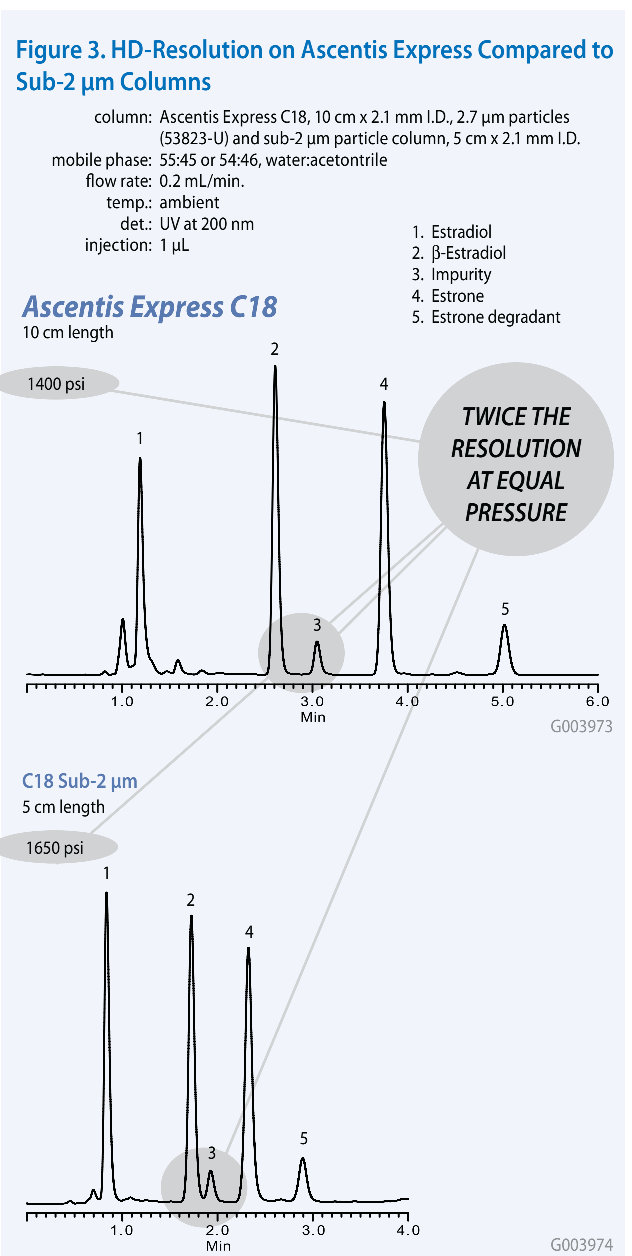
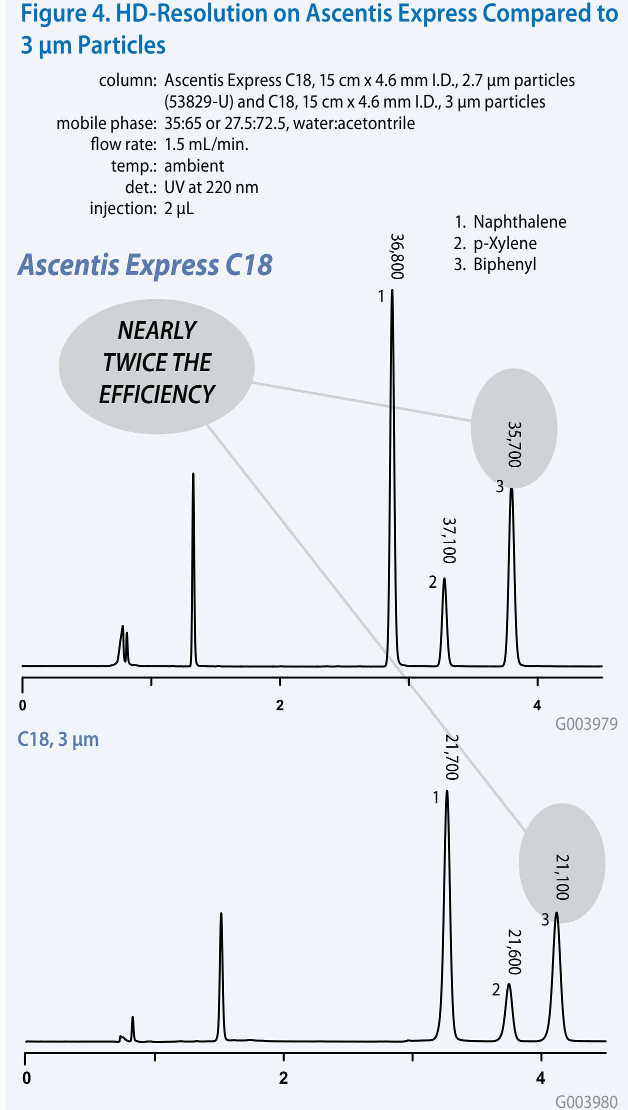


Figure 4. HD-Resolution on Ascentis Express Compared to 3 µm Particles

column: Ascentis Express C18, 15 cm x 4.6 mm I.D., 2.7 µm particles (53829-U) and C18, 15 cm x 4.6 mm I.D., 3 µm particles
mobile phase: 35:65 or 27.5:72.5, water:acetontrile
flow rate: 1.5 mL/min.
temp.: ambient
det.: UV at 220 nm
injection: 2 µL



Rapid, Sensitive, General-Purpose Cleaning Validation Using Ascentis Express HPLC Columns

Cleaning Validation

Contributed Article

The following was generated by an outside source using Sigma-Aldrich products. Technical content provided by:

S. Bannister, M. Talbott, F. Hanciles
Xcelience LLC, Tampa, FL

Verification of the removal of drug residue from multi-product manufacturing equipment is required by GMP regulations and the suitability of applied analytical methods is judged with a combination of sensitivity, selectivity, and because the release of equipment is dependent - speed. The FDA does not set quantitative acceptance specifications, but the commonly used limit is based on not more than 0.1% of a dose carried over into a single dose of the next product. Translation of this into an analytical limit combines the total product contact area, the mass (or volume) of product contacting the surface, the mass (or volume) of each dose unit, the sampled area, the rinse volume and the fraction of the rinse sample used for analysis. The requisite limits are commonly measured in ng/mL of injected sample.

The ubiquity of HPLC in drug analysis makes it an attractive choice for cleaning validation. Methods qualified for cleaning validation are often adaptations of drug-substance methods. The original methods are capable of determining the drug and its related impurities, but the ability to simultaneously measure multiple closely related analytes comes at the expense of run time and is not needed in cleaning validation.

This work was undertaken to investigate the use of rapid gradients using recently introduced FCP columns on conventional instrumentation in the development of general-purpose methods for cleaning validation. The benefits include high sensitivity and reductions in the time needed to set up and run the method.

Resolution, limits of detection and quantitation, and run time in HPLC analyses are improved by reducing the width of eluted bands. Contributions to bandwidth include both column (particle size, packing structure and resistance to mass transfer in the stationary and mobile phases) and extracolumn volumes (injection, unswept and tubing). Columns packed with 5 µm fully porous particles have been the standard for conventional HPLC for twenty-five years. Smaller-particle packings (3 µm) have been available almost as long and offer higher efficiency (lower band dispersion)

Figure1. Acidic and Neutral Drug Panel

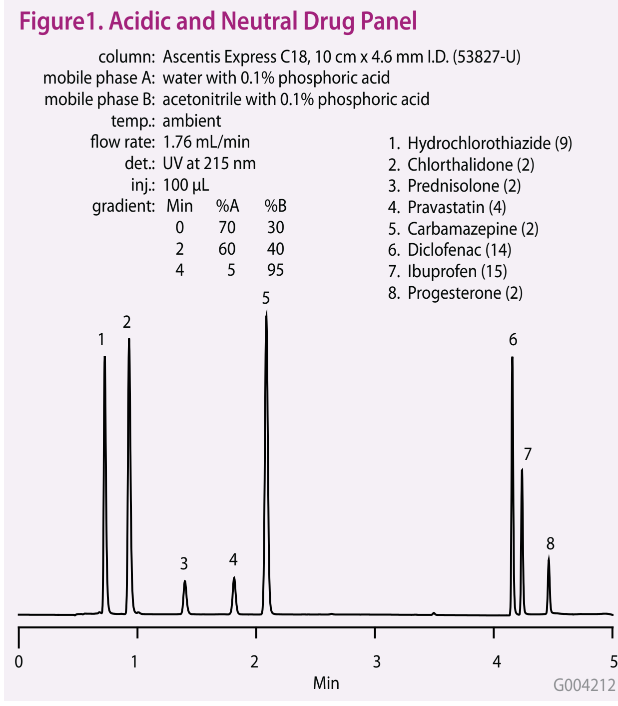
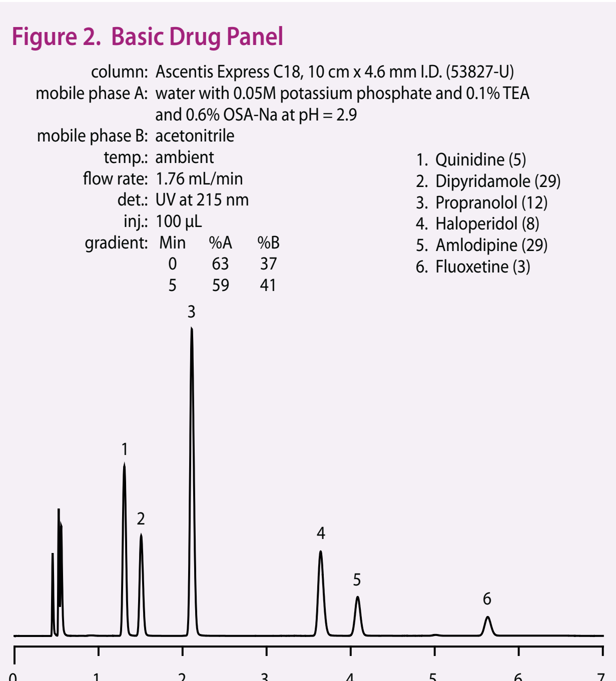


Figure 2. Basic Drug Panel



dispersion) on conventional instrumentation, but require higher pumping pressures due to lower bed permeability. Efficiency can be further increased by the use of particles smaller than 3 µm but only with the use of instrumentation optimized with respect to both pressure and extra-column effects.

Supelco has recently introduced reversed-phase packings based on 2.7 µm silica particles in which a 0.5 µm layer of 90-Å porous silica has been deposited onto a 1.7 µm solid spherical core. Advantages of columns packed with these particles include high efficiency, lower backpressure due to a very narrow particle size distribution, and smaller efficiency losses with increasing velocity due to improved mass-transfer kinetics in the shallow porous layer. The narrow particle size distribution allows the use of larger pore column frits, which combined with the greater stability of the packed bed should produce longer column lifetimes in routine use.

The high resolving power of gradient elution in the analysis of closely related substances is the result of the reduction of peak width as a band moves through the column. The back of the band is accelerated by the stronger solvent. A broad gradient will elute a wide range of substances and a steep gradient will elute them quickly.

Versatile Separations

To judge the utility of Ascentis Express columns in cleaning validation, an Agilent 1100 component system with standard components (including a 10 mm/13 µL flow cell) was used to develop a short gradient separation using Ascentis Express C18, 10 cm x 4.6 mm for each of two panels: eight acidic or neutral drugs (AN) and six basic drugs (B). For each separation, the flow rate was 1.76 mL/min, detection was at 215 nm, and 100 µL injections were made of aqueous solutions representing the final equipment rinse. The separations are shown in Figures 1 & 2. Limits of detection (ng/mL) are listed next to each analyte in Figures 1 and 2.

These separations demonstrate the capabilities of Ascentis Express columns on conventional, robust, instrumentation in rapid analyses of multiple drugs at low ppb levels suitable for development as methods for cleaning validations in multiproduct manufacturing facilities.

Selecting the Right Buffer

A partial list of common buffers and their corresponding useful pH range is supplied. Perhaps the most common buffer in HPLC is the phosphate ion. Although, with the growth of LC-MS, volatile buffers such as TFA, acetate, formate, and ammonia are becoming more frequently used. Remember, the purpose of a buffer in the mobile phase is to inhibit a pH change in the mobile phase after the introduction of a sample. When developing a method, it is important to select a mobile phase with a final pH at least one pH unit away from any analytes pK value. As a rule of thumb, one should work within a ±1 pH unit of the buffer pKa. Typical buffer concentrations for HPLC tend to be 10-100 millimolar level.

Buffer	pKa @ 25 °C	Useful pH Range
Trifluoroacetic acid (TFA)	0.5	<1.5
Phosphate 1	2.1	1.1 - 3.1
Formate	3.8	2.8 - 4.8
Acetate	4.8	3.8 - 5.8
Phosphate 2	7.2	6.2 - 8.2
Ammonia	9.2	8.2 - 10.2
Phosphate 3	12.3	11.3 - 13.3

Guidelines for Preparing Mobile Phases

It should be understood that slight variations in pH and buffer concentration could have a dramatic affect on the chromatographic process; consistent and specific techniques should be a regular practice in the preparation of mobile phases. A common practice is to place a sufficient amount of pure water into a volumetric flask and add an accurate amount of buffer. The pH of the solution should be adjusted, if necessary, and then dilute to final volume of water prior to adding or blending of organic solvents. Then, add a volumetrically measured amount of organic solvent to obtain the final mobile phase. Thorough blending, degassing, and filtering prior to use is also recommended.

To view a listing of suitable HPLC and LC-MS additives and solvents, visit *sigma-aldrich.com/lc-ms-solvents*

Ascentis Express Peptide ES-C18 Expands the Fused-Core Particle Platform into Bioseparations

Introduction

Ascentis Express Peptide ES-C18 columns were specifically engineered to separate higher molecular weight compounds such as peptides and small proteins. These columns contain advanced Fused-Core particles that have bigger pores (160 Å versus 90 Å in standard Ascentis Express), which greatly expands the application range for Ascentis Express columns.

Key Applications for Ascentis Express Peptide ES-C18:

- Pharmaceutical/therapeutic peptides
- Peptide mapping
- Natural and synthetic peptide analysis
- Oligonucleotide analysis

Key Advantages:

- Higher peak capacity providing greater resolution
- Amenable to higher flow rates for faster analysis
- Exceptional ruggedness providing long column lifetime

Ascentis Express Peptide ES-C18 columns utilize a steric-protected C18 bonded-phase with extremely high resistance to acid-catalyzed hydrolysis of the siloxane bond that attaches the C18 chain to the surface. Thus, the combination of low pH and elevated temperature operation of the column is well tolerated. Peptide separations are efficiently conducted using low pH mobile phase modifiers, often at 0.01-0.1% concentration, most popularly employing trifluoroacetic acid (TFA), and the related perfluorocarboxylic acids, pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA). These acids exhibit desirable low UV transparency, volatility, and peptide ion-pairing properties. Additional opportunities for low pH operation include the normal short chain carboxylic acids, formic acid and acetic acid, as well as mineral acids, such as phosphoric acid (0.001-0.02 M).

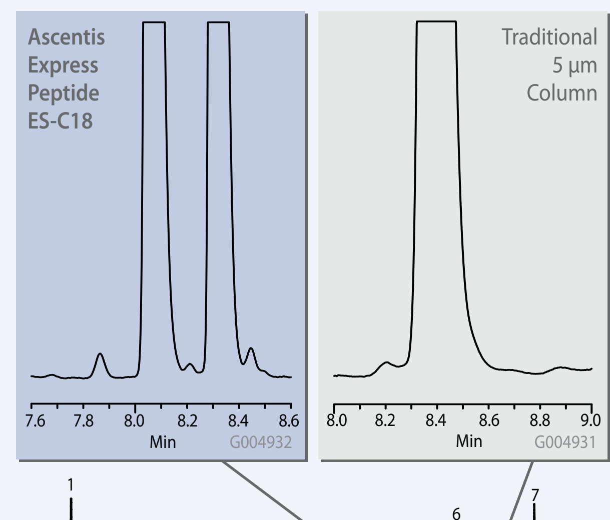
Shown in Figure 1 is the chromatographic separation of a peptide mix. The peptide mix contains a range of peptides in terms of molecular weight, basicity, and hydrophobicity. Excellent peak shape and peak width are achieved with a standard acetonitrile gradient and 0.1% TFA modifier. The resolution of small baseline impurities are shown in the inset, demonstrating the resolving power of the Ascentis Express Peptide ES-C18 column versus a traditional 5 µm column.

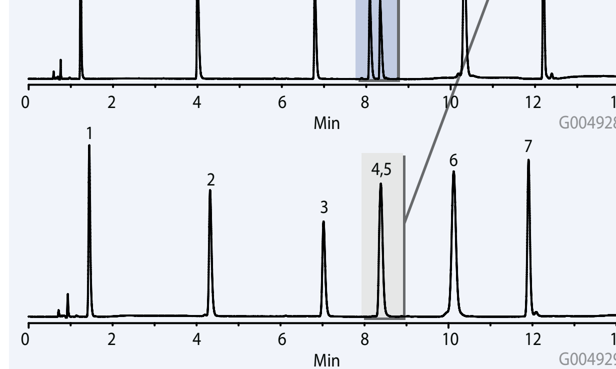
Figure 1. Comparison of Peptide Test Mix with Ascentis Express Peptide ES-C18 and Traditional Column

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 10% acetonitrile / 90% water / 0.1% trifluoroacetic acid
mobile phase B: 75% acetonitrile / 25% water / 0.1% trifluoroacetic acid
gradient: 0% to 50% B in 15 min
flow rate: 1.5 mL/min.
det: UV at 220 nm
temp: 30 °C
injection: 5 µL

Peptide Test Mix

1. Gly-Tyr MW = 252
2. Val-Tyr-Val MW = 379
3. Met Enkephalin MW = 574
4. Angiotensin II MW = 1032
5. Leu-Enkephalin MW = 555.62
6. Ribonuclease MW = 13,700
7. Bovine Insulin MW = 5733





For more information on the Ascentis Express Peptide ES-C18, request brochure T410043 (MII).

Improving the Current USP Method for the Analysis of Lansoprazole Drug Substance Using HPLC Columns Based on Fused-Core Particle Technology

Contributed Article

The following was generated by an outside source using Sigma-Aldrich products. Technical content provided by:

Kai Li, Jiajie He, and Xiaoya Ding
PPD - 8551 Research Way, Suite 90, Middleton, WI 53562

Introduction

Compendial methods from the USP (United States Pharmacopeia) are widely used in pharmaceutical drug product and raw materials testing. However, not all methods in the USP use modern technologies. In chromatographic methods, it is not uncommon that older brands of columns are specified. Therefore, the USP methods are under continuous revision to improve existing procedures or to allow the user to obtain better results.

Due to the improved resolving power of Fused-Core particles, the method was optimized with a shorter runtime without sacrificing resolution.

In an effort to improve the compliance of drug product, drug substance, and excipient monographs with current scientific/regulatory standards, USP is seeking the submission of proposals for improved methods. The intent is to replace the current procedures that may be deficient, flawed, or unsafe (e.g. *http://www.usp.org/USPNF/submitMonograph/improveMon.html)*. Requests for revision of an existing monograph are encouraged by USP in light of advances in analytical technologies. Furthermore, ease of operation, suitability for automation, and potential for high-throughput analysis can be considered in a revision. To develop the best possible analytical test method for its intended use, a fully integrated method development process such as the selection of column, mobile phase, detection technology, and LC hardware by utilizing the most advanced technologies viable should be considered to ensure the methods are robust, consistent, and easy to use.

In this study, the USP method for lansoprazole was considered for improvement. Several drawbacks in the current USP monograph for lansoprazole prompted the investigation. These drawbacks include sample solution instability, use of different columns and samples preparations for the evaluation of assay and impurity, the requirement of using internal standard for assay and a long HPLC runtime (60 min). A new HPLC column, Ascentis Express C18, based on Fused-Core particle technology was investigated for this study. The Ascentis Express HPLC column claims high efficiencies as a result of a 0.5 µm layer of porous silica on a 1.7 µm solid silica core. An additional advantage to the column is that standard HPLC instrumentation can be used as opposed to UHPLC that is required for sub-2 µm columns.

Results and Discussion

Initially, a traditional 5 µm C18 column as specified in the USP monograph was compared to the 2.7 µm Ascentis Express C18 using the standard USP conditions for chromatographic purity for lansoprazole (1, 2). Improved resolution and sensitivity were obtained using the Fused-Core column that allowed us to make several significant improvements to the method. Due to the improved resolving power of Fused-Core particles, the method was optimized with a shorter runtime without sacrificing resolution. The total run time was reduced from 60 min to 40 min (Table 1). Moreover, the improved sensitivity allowed for the reduction in concentration of the test sample for chromatographic purity from 250 mg/mL to 100 mg/mL, the level required for assay in the USP monograph. Therefore, simultaneous evaluation of assay and chromatographic purity is achieved. Finally, a change of diluent pH, was implemented to improve sample solution stability removing the requirement of injecting sample within 10 minutes after preparation.

Table 1. Method Parameters for Improved Lansoprazole Method

column:	Ascentis Express C18, 15 cm x 4.6 mm, 2.7 µm (53829-U)
mobile phase A:	Water
mobile phase B:	Acetonitrile: 0.5% Triethylamine in Water, pH=7.0 [80:20]
flow rate:	0.8 mL/minute
column temp.:	Ambient
autosampler Temp.:	5 °C
injector volume:	15 µL
detector wavelength:	285 nm
run time:	40 min
gradient:	

Time (Min)	%A	%B
0.0	90	10
30.0	20	80
35.0	20	80
35.1	90	10
40.0	90	10

Figure 1. Linearity Curve from 50 to 150% Nominal Concentration

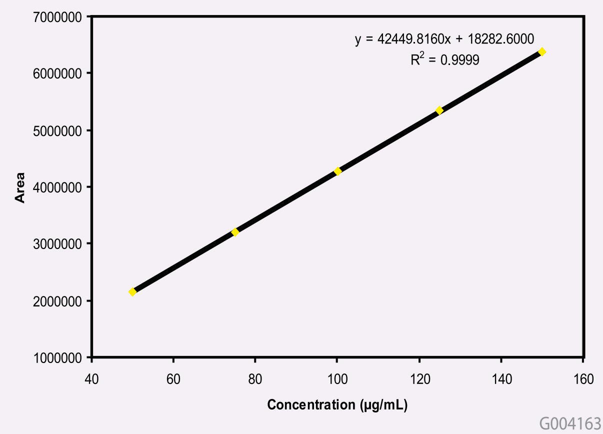
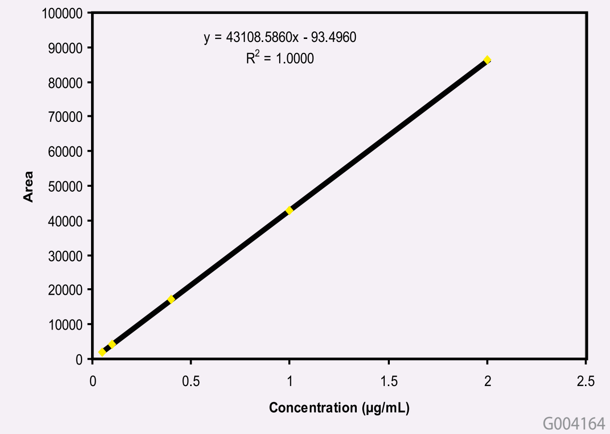
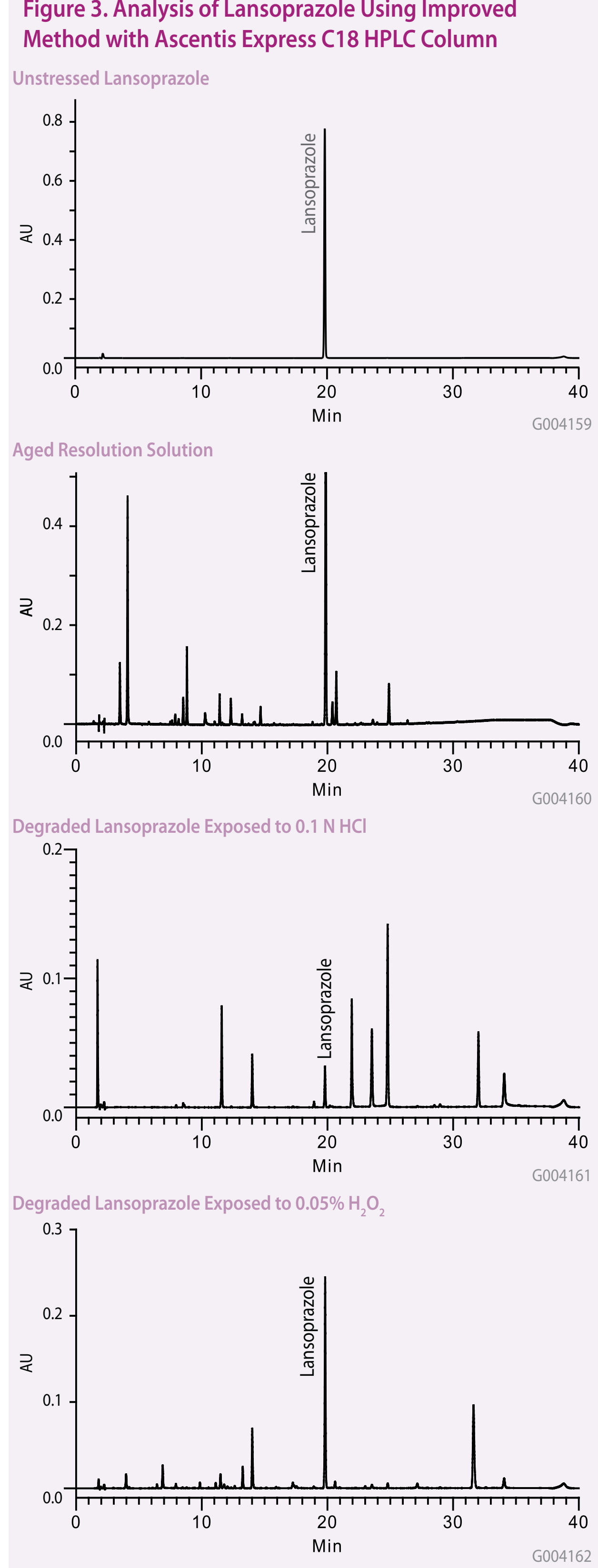


Figure 2. Linearity Curve from 0.05 to 2.0% Nominal Concentration



The new method was shown to be linear from 0.05% to 150% of nominal concentration of 100 mg/mL, with quantitation limit less than 0.05%. The broad range of linearity allows for simultaneous impurity and assay analysis. The linearity data are shown in Figures 1 and 2. The RSD of 5 replicate injections of standard solution was 0.11%. In additional experiments, the method was evaluated by analysis of degraded lansoprazole drug substance. Lansoprazole was stressed under four separate conditions by exposure to acid, base, heat and hydrogen peroxide. The chromatograms of the acid and peroxide exposed drug substance along with the unstressed drug substance are shown for reference. The resolving power of the Ascentis Express HPLC column makes it very suitable for these types of studies.

Figure 3. Analysis of Lansoprazole Using Improved Method with Ascentis Express C18 HPLC Column



Conclusion

The method developed using 2.7 µm Fused-Core C18 column provided significant improvements in comparison with the original USP method in terms of resolution, run time and sensitivity. As a result, the consolidated single method can be used for both assay and impurity quantitation. The advantages of Fused-Core columns as an alternative for sub-2 µm columns without using new UHPLC instruments could be appealing for pharmaceutical testing. Furthermore, this paper has presented one of the ways (a road map) that could be utilized by analytical scientists in the pharmaceutical field to improve USP monographs for their intended purposes using modern analytical technologies.

References

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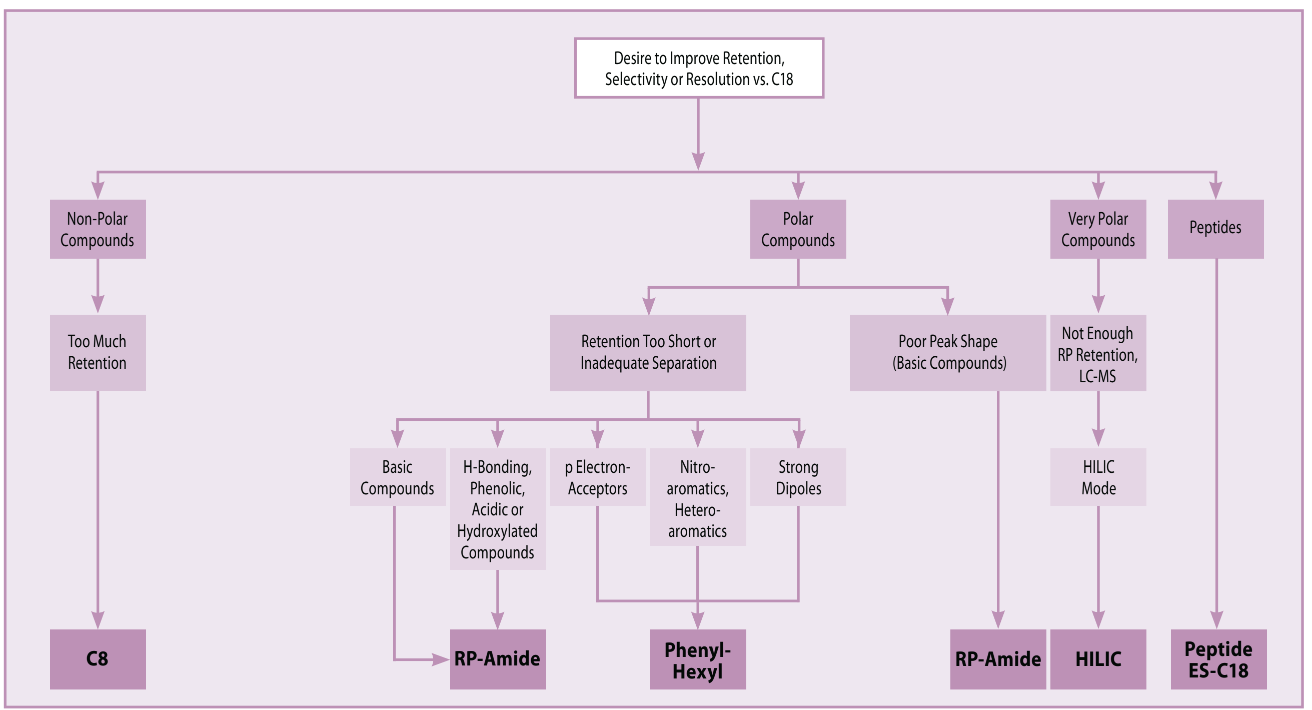
2. Pharmacopeial Forum, In-Process Revision, Lansoprazole, Vol. 32(6), 1710-1712 (2006).

Selecting an Ascentis Express Phase

Ascentis Express C18 is the first choice for starting a new method. However, when a C18 doesn't give the desired separation or your sample contains compounds that are known to be difficult to retain or resolve on a C18, consider changing stationary phases. The range of selectivity provided by Ascentis Express makes this easy. The flow chart below helps guide in the selection of an Ascentis Express phase, based on the particular compound type or separation challenge.



Profiling of Stevia rebaudiana Extract by Accurate Mass Using HILIC and Reversed-Phase Chromatography

Natural Products

Introduction

There is growing public interest in low-calorie alternatives to carbohydrate-based sweeteners. Synthetic sweeteners are often regarded as having an undesirable aftertaste. Recent publications have shown a dramatic increase in attention toward natural extracts including the *Stevia rebaudiana* plant, not only for its sweetening effect but also for additional health benefits attributed to the plant. The major sweetening components are stevioside, rebaudioside A, rebaudioside C, and dulcoside A, each of which is over 300 times sweeter than sucrose-based sweeteners. The concern with the human consumption of the stevia leaf had been attributed to the possible mutagenic properties of steviol, but more recent studies conducted by the World Health Organization have established the safety for steviol and its glycosides.

In this study, an evaluation of the *Stevia rebaudiana* plant extract was conducted using modern chromatographic and mass spectrometry techniques for the determination of extracted components. The purpose was to evaluate the utility of performing two different modes of chromatographic separation for component identification. An accurate mass time of flight (TOF) mass spectrometer was used in the detection and identification of components. A novel software package was then utilized for the determination of common components between the two chromatographic modes and to depict the impact of chromatographic selectivity.

The concept behind the study was to utilize both reversed-phase chromatography and HILIC chromatography for the determination of extract components. By using two different modes of selectivity, components that co-retain, do not retain, or do not elute under one chromatographic mode may be resolved under a separate mode. By resolving a component chromatographically, a more accurate assessment of the component can be made without relying specifically on accurate mass data.

With traditional reversed-phase chromatography, analytes are primarily retained on an alkyl based stationary phase by partitioning interaction between the non polar stationary phase and the analyte. Though this mode of chromatography is widely accepted for separation of moderately polar to non-polar compounds, highly polar analytes often have minimal or no retention on these phases. More popular polar embedded stationary phases address this issue with the addition of a polar functional group within the alkyl chain. Polar embedded phases can enhance retention of polar compounds, but it is not a solution for all applications. Often highly polar analytes require alternative modes of chromatographic retention. In particular, HILIC chromatography allows for alternative selectivity by utilizing a highly polar stationary phase with a relatively non polar mobile phase. Under HILIC conditions, the partitioning of analytes is achieved through a preferential solvation of an aqueous environment on the polar surface. More polar analytes will partition more into the surface solvent and thus be retained longer than a less polar analyte. In addition to the partitioning, the polar surface of the stationary phase allows for adsorptive interactions via hydrogen bonding, dipole, etc. When ionic samples are separated, the potential for ion-exchange interactions also exists and in many cases becomes the dominant retention mechanism. Using silica-based stationary phases, ionized surface silanol groups may interact via ion-exchange with positively charged analytes.

Experimental

In this study, both reversed-phase and HILIC separations were conducted using the Ascentis® Express RP-Amide and Ascentis Express HILIC. The polar embedded group of the Amide was chosen over traditional C18 phases to increase the retention of the polar analytes in the stevia

Stevia rebaudiana

extract. The Ascentis HILIC allowed for alternative selectivities for polar analytes. Because of the large amount of unknown components in the stevia extract, using both reversed-phase and HILIC modes enabled orthogonal selectivity to resolve co-retained components and enable better determination of components in the extract with confirmation between the two modes.

Stevia leaves were obtained from Sigma Aldrich (S5381). Sample extraction of the stevia leaves was performed by weighing 400 mg of crushed stevia leaves into a 7 mL amber vial. A total of 4 mL of 50:50 acetonitrile:water was added and the sample was vortexed and sonicated for 3 minutes. The sample was then centrifuged for 2 minutes at 15000 rpm. The supernatant was then collected and analyzed directly.

The sample extract was analyzed using a gradient elution profile for both HILIC and reversed-phase chromatographic modes. Analysis was conducted using an Agilent® 1200SL Rapid Resolution system in sequence with an Agilent 6210 TOF mass spectrometer. The TOF enabled the use of accurate mass for determination of components. The acquired data was processed using the Mass Hunter software package. The data was pushed to the Mass Profiler package for statistical comparison of the two chromatographic modes. This software package enabled the identification of common components between the two chromatographic separations of the stevia extract. By performing this type of statistical comparison, the components attributed to the stevia extract were differentiated from components attributed to chromatographic anomalies. From this comparison the major components of the stevia extract were determined. Available standards were then used to confirm the identification of several of the components.

Results and Discussion

Figure 1 and Figure 2 represent the total ion chromatogram for the stevia extract under both HILIC and reversed-phase conditions. Both of these chromatographic separations demonstrate the complexity of the stevia extract. Table 1 depicts the major components that were common in both the reversed-phase and HILIC separations of the stevia extract. More than 250 components were identified with this comparison, but only the major components were targeted in this study. The highlighted components in Table 1 depict co-retention of analytes under reversed-phase conditions. A good example of using this orthogonal approach is observed in the case of steviobioside and ducloside A. Under the reversed-phase separation, these components were co-retained. By performing the separation under HILIC conditions, steviobioside and ducloside A were well separated. Other unidentified major components that were unresolved under the reversed-phase conditions were also separated under the HILIC conditions. The data in
Table 1 also depicts the selectivity difference between the two chromatographic modes. Polar components that were poorly retained in the reversed-phase conditions were

(continued on page 16)

Figure 1. Component Chromatogram of Stevia Extract on Ascentis Express HILIC

column: Ascentis Express HILIC, 15 cm x 2.1 mm I.D., 2.7 µm with upchurch inline filter
flow: 0.2 mL/min.
mobile phase A: 2 mM ammonium formate (98:2 acetonitrile:water)
mobile phase B: 2 mM ammonium formate (80:20 acetonitrile:water)
temp.: 35 °C
inj. vol.: 1 µL
system: Agilent 1200SL 6210 TOF, ESI(+)
datafile: 1228082.d

gradient:

Time	%A	%B	Flow
0.00	100	0	0.2
10.00	100	0	0.2
40.00	0	100	0.2
45.00	0	100	0.2
45.01	100	0	0.4
57.00	100	0	0.2
60.00	100	0	0.2

0 10 20 30 40
Min
G004745

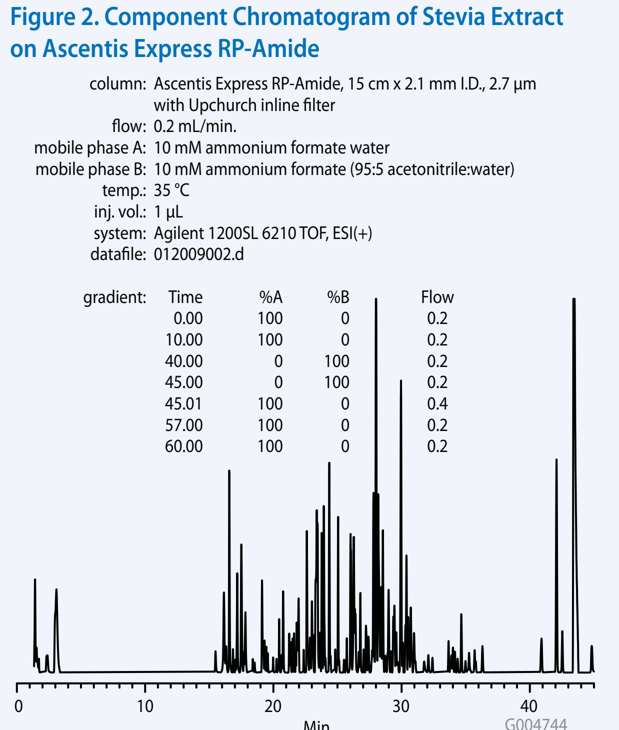


Figure 2. Component Chromatogram of Stevia Extract on Ascentis Express RP-Amide

column: Ascentis Express RP-Amide, 15 cm x 2.1 mm I.D., 2.7 µm with Upchurch inline filter
flow: 0.2 mL/min.
mobile phase A: 10 mM ammonium formate water
mobile phase B: 10 mM ammonium formate (95:5 acetonitrile:water)
temp.: 35 °C
inj. vol.: 1 µL
system: Agilent 1200SL 6210 TOF, ESI(+)
datafile: 012009002.d

gradient:

Time	%A	%B	Flow
0.00	100	0	0.2
10.00	100	0	0.2
40.00	0	100	0.2
45.00	0	100	0.2
45.01	100	0	0.4
57.00	100	0	0.2
60.00	100	0	0.2

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Title: Comprehensive two-dimensional liquid chromatography to quantify polyphenols in red wines.
Authors: Paola Dugo, Francesco Cacciola, Paola Donato, Diego Airado-Rodríguez, Miguel Herrero, Luigi Mondello
Affiliation: Dept of Food & Environment, University of Messina, Messina, Italy; Dept of Pharmaceutical Chemistry, University of Messina, Messina, Italy; Dept of Analytical Chemistry, University of Extremadura Avda, Badajoz, Spain; Campus-Biomedico, Roma, Italy
Journal: Journal of Chromatography A, 2009

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Authors: Luigi Mondello, Miguel Herrero, Francesco Cacciola, Paola Donata, Daniele Giuffrida, Giacomo, Dugo, Paola Dugo
Affliation: Dept of Pharmaceutical Chemistry; University of Messina, Messina,Italy; Campus-Biomedico, Roma, Italy; Dept of Food & Environment, University of Messina, Messina, Italy
Journal: Journal of Chromatography A, 2008, 208-215

Title: Characterization of new types of stationary phases for fast liquid chromatographic applications
Authors: Szabolcs Fekete, Jenö Fekete, Katalin Ganzler
Affliation: Formulation Development, Gedeon Richter Plc, Budapest X, Hungary; Dept of Inorganic & Analytical Chemistry; Budapest University of Technology & Economics
Journal: Journal of Pharmaceutical and Biomedical Analysis, 2009

Title: The use of partially porous particle columns for the routine, generic analysis of biological samples for pharmacokinetic studies in drug discovery by reversed-phase ultra-high performance liquid chromatography-tandem mass spectrometry
Authors: David N. Mallett, César Ramírez-Molina
Affliation: GlaxoSmithKline Medicines Research Centre; Respiratory CEDD DMPK & Immuno-Inflammation CEDD DMPK; Stevenage, Herts, United Kingdom
Journal: Journal of Pharmacuetical and Biomedical Analysis, 2009, 100-107

Title: Shell and small particles; Evaluation of new column technology
Authors: Szabolcs Fekete, Jenö Fekete, Katalin Ganzler
Affiliation: Formulation Development, Gedeion Richter Plc, Budapest X, Hungary; Dept. of Inorganic & Analytical Chemistry; Budapest University of Technology & Economics, Budapest, Hungary
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Authors: Krzysztof Kaczmarski, Georges Guiochon
Affiliation: Dept of Chemical & Process Engineering; Rzesnow University of Technology, Rzeszow, Poland; Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
Journal: Analytical Chemistry 2007, 4648-4656

Title: Mass-Transfer Kinetics in a Shell Packing Material for Chromatography
Authors: Georges Guiochon, Alberto Cavazzini, Fabrice Gritti, Krysztof Kaczmarski, Nicola Marchetti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee; Faculty of Chemistry, Rzeszow University of Technology, Rzeszow, Poland
Journal: Analytical Chemistry, 2007, 5972-5979

Title: Comprehensive Off-Line, Two-Dimensional Liquid Chromatography. Application to the Separation of Peptide Digests
Authors: Georges Guiochon, Nicola Marchetti, Jacob N Fairchild
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee, Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
Journal: Analytical Chemistry 2008, 2756-2767

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Authors: Georges Guiochon, Fabrice Gritti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
Journal: Journal of Chromatography A, 2007, 107-122

Title: Comparison between the efficiencies of columns packed with fully and partially porous C18-bonded silica materials
Authors: Georges Guiochon, Fabrice Gritti, Alberto Cavazzini, Nicola Marchetti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee; Dept of Chemistry, University of Ferrara, Ferrara, Italy
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Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee, Div of Chemical & Analytical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee, Dept of Chemistry, University of Ferrara, Ferrara, Italy
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Authors: Georges Guiochon, Fabrice Gritti
Affiliation: Dept of Chemistry, University of Tennessee, Knoxville, Tennessee; Div of Chemical Sciences, Oak Ridge National Lab, Oak Ridge, Tennessee
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Authors: David V McCalley
Affiliation: Center for Research in Biomedicine, University of the West of England, Frenchay, Bristol, United Kingdom
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Affiliation: Dept of Analytical Chemistry, University of Pardubice, Pardubice, Czech Republic
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Authors: Anthony J Alexander, Lianjia Ma
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Journal: Journal of Chromatography A, 2009, 1338-1345

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Affiliation: Dept de Química Analitica, Universitat de València, Valencia, Spain
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Authors: Francesco Gasparrini, Walter Cabri, Alessia Ciogli, Ilaria D'Acquarica, Michela Di Mattia
Affiliation: Analytical Development, R & D Dept.,Pomezia, Italy; Dept di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, Rome, Italy
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Title: Use of partially porous column as second dimension in comprehensive two-dimensional system for analysis of polyphenolic antioxidants
Authors: Paola Dugo, Francesco Cacciola, Miguel Herrero, Paola Donato, Luigi Mondello
Affiliation: Dipartimento de Scienze degli Alimenti e dell'Ambiente, Facoltà di Scienze, Messina, Italy; Dipartimento Farmacia-chimco, Facoltà di Farmacia, Università di Messina, Messina, Italy; Campus-Biomedico, Roma, Italy
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Authors: Paola Donato, Paola Dugo, Francesco Cacciola, Giovanni Dugo, Luigi Mondello
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Affiliation: Formulation Development, Gedeon Richter Plc, Budapest, Hungary; Budapest University of Technology & Econmics, Dept. of Inorganic & Analytical Chemistry, Budapest, Hungary
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Affiliation: Dept. of Medicine & Neurology & Psychiatry & Biobehavioral Sciences and the Semel Institute, UCLA, Los Angeles, CA; Greater LA Healthcare System, Geriatric Research Education Clinical Center, Sepulveda, CA; Cancer Prevention & Control Program, Moores UCSD Cancer Center, UCSD, San Diego, CA; Dept. of Post-Genomics & Diseases, Division of Psychiatry & Behavioral Proteomics, Osaka University Graduate School or Medicine, Osaka, Japan
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Authors: Tomás Hájek , Veronika Skerikova, Petr Cesla, Katerina Vynuchalova, Pavel Jandera
Affiliation: Dept of Analytical Chemistry, University of Pardubice, Pardubice, Czech Republic
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Authors: P. Yang, G.R. Litwinski, M. Pursch, T. McCabe, K. Kuppannan
Affiliation: The Dow Chemical Company, Analytical Sciences, Midland, MI
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(continued from page 13)

Table 1. Major Component Retention Comparison Between HILIC and Reversed-Phase Modes

Component	Accurate Mass	Ascentis Express HILIC	Ascentis Express RP-Amide
		RT	RT
	137.0476	44.397	1.429
	120.0574	34.695	3.075
	102.0473	29.394	3.077
	368.1698	18.414	15.502
	120.0574	31.082	17.166
	402.1518	15.471	17.196
	162.1405	8.727	19.128
	378.2242	8.736	19.13
Rebaudioside A/E	966.4281	34.439	21.755
	516.1254	20.784	23.016
	498.1154	20.714	23.016
Stevioside	804.3743	26.52	23.63
Steviolbioside	642.3234	19.213	24.356
Dulcoside A	788.3817	26.247	24.475
	338.2448	2.248	25.737
	176.1555	2.049	26.026
	284.2134	3.372	26.028
	246.1977	3.359	26.06
	380.255	2.002	26.672
	284.2131	2.811	27.881
	360.083	1.867	28.187
	444.2002	1.825	30.391
Steviol	318.2186	1.91	32.19
	592.2655	2.239	42.539

Figure 3. Stevia Extract on Ascentis Express RP-Amide, Extracted Ion Chromatogram for Steviol

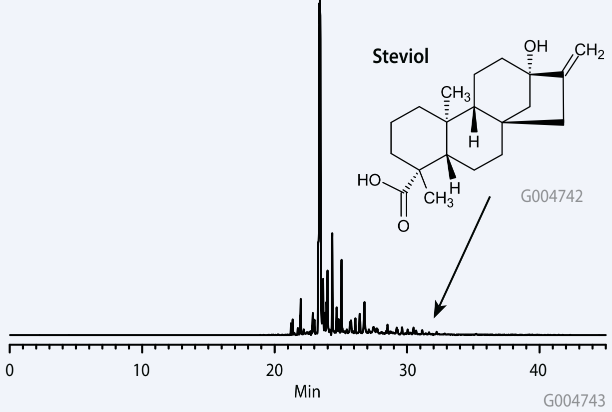
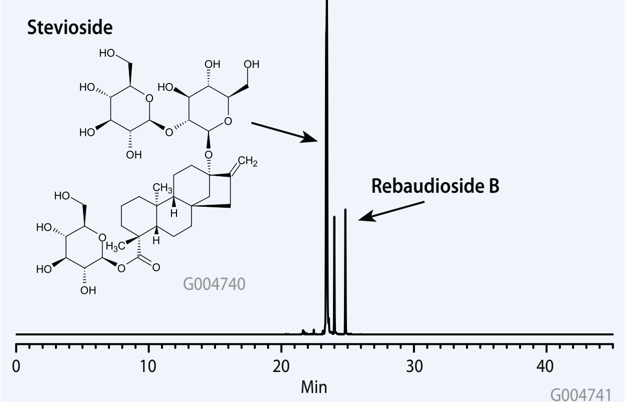


Figure 4. Stevia Extract on Ascentis Express RP-Amide, Extracted Ion Chromatogram for Stevioside



strongly retained under HILIC conditions. In two cases, where known components were identified, it was necessary to use standards to confirm their retention. Figures 3 and 4 depict the extracted ion chromatogram for the accurate mass of steviol and stevioside. As can be seen in both chromatograms, multiple peaks are observed for each of the accurate masses. In the case of stevioside, it is isobaric with rebaudioside B making identification difficult. A stevioside standard (Sigma Aldrich) was used for positive identification. In addition, the reversed-phase separation of the extract resulted in multiple peaks observed for the accurate mass of steviol. This was due to fragments from additional glycosides that resulted in a steviol fragment ion, again it was necessary to confirm the steviol retention with a standard.

Conclusions

The profiling of the *Stevia rebaudiana* extract demonstrates the utility of performing orthogonal chromatographic modes when handling complex samples. The two modes of chromatography were complimentary for the determination of major components from the stevia extract. In most cases where coelution occurred in one chromatographic mode, the components were separated under the orthogonal mode. Though component identification was made easier through the accurate mass of the TOF, it was still necessary to have good chromatographic resolution to confirm component identity. In both cases, the Fused-Core™ particle demonstrated the ability to perform complex matrix analysis in both HILIC and reversed-phase separations.

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- Introduction to Ascentis HPLC Columns
- HPLC Instrument Bandwidth and Optimizing Systems for High-Performance
- High-Performance HPLC Fittings and Interconnects

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Colors of the World: Fast Separation of Dyes with Ascentis Express

Dyes surround us everywhere every day. They can be found in common places like the printing ink in magazines or books and in plastics, textiles, and leather, but also in unusual places like diesel fuel and tattoo color. Most of these synthetic colors are based on aromatic ring structures containing heteroatoms and tend to have a high potential for causing cancer; as a result, they are not intended for use in food coloring. But since 2003, there have been several incidents of Sudan I contamination in chili powder. This situation necessitates the analysis of spice mixtures to determine if they have been adulterated (1, 2).

Further, a sensitive HPLC method is needed for quality control testing of dyes and the identification of byproducts. Supelco's Ascentis Express HPLC columns provide outstanding sensitivity and resolution for such applications.

Method Development for Dyes

Table 1 contains a list of dyes added to one sample and dissolved in a mixture of methanol and acetonitrile. The sample was injected on an Ascentis Express C8 HPLC column under varying mobile phase conditions to determine the best separation parameters. Temperature, injection volume, detector settings, and flow rate were kept constant.

The chemical and physical properties of the dyes differ strongly, so the first step in developing a suitable HPLC method was the use of a gradient run ranging from 25% acetonitrile to 100% acetonitrile (B) and 0.1% formic acid in water as an aqueous counterpart (A). The UV chromatogram of the combined wavelengths 360, 550, and 620 nm showed good chromatography of all compounds except for the poor peak shape of Sudan 410 at 17.35 minutes (Figure 1A).

To optimize that peak shape, methanol was added to the organic mobile phase (acetonitrile:methanol, 90:10); the gradient run was repeated, resulting in better peak shape for Sudan 410 (Figure 1B). In a final experiment, the gradient profile was changed and optimum conditions were attained (see Table 2). Figure 1C shows the step-by-step improvements in the chromatography.

Only three runs were needed to get the final method, showing how easy and fast it is to develop methods with Ascentis Express columns. Further, Ascentis Express columns contain Fused-Core particles that allow for faster run times; even separations performed on standard HPLC systems can be sped up by up to 30% with Ascentis Express.

Table 1. Structure and Mass of the Dyes in the Sample Mixture. Most of the Compounds are Detected as $[M+H]^+$ Ions except (5), which gives $[M]^+$ Ions

Peak No.	Structure	Name / Exact Mass
1	G004779	Parafuchsin $C_{19}H_{17}N_3$ 287.142247
2	G004780	Basic Fuchsin $C_{20}H_{19}N_3$ 301.157897
3	G004781	Methylfuchsin $C_{21}H_{21}N_3$ 315.173547
4	G004782	Newfuchsin $C_{22}H_{23}N_3$ 329.189197
5	G004783	Malachite Green $C_{23}H_{25}N_2$ 329.201773
6	G004784	Sudan III $C_{22}H_{16}N_4O$ 352.132411
7	G004785	Sudan 410 $C_{26}H_{24}N_4O$ 408.195011

Table 2. Initial and Final HPLC Method Settings for Separation of the Seven Dyes Listed in Table 1, After Optimization

Fixed Parameters

column:	Ascentis Express C8, 10 cm × 4.6 mm I.D., 2.7 µm particles
flow rate:	0.8 mL/min
temp:	55 °C
UV DAD:	200–950 nm
MS:	ESI(+), SPS target 500 m/z, stability 100%, trap lvl. 100%, optimize normal, range 100–1500 m/z, nebulizer 50 psi, dry gas 12 L/min, dry temp. 365 °C.
injection volume:	3 µL
run time:	25 min (5 min posttime)

Variable Parameters

	Initial Conditions			Final Conditions		
solvents:	(A) water with 0.1% formic acid (B) acetonitrile			(A) water with 0.1% formic acid (B) acetonitrile:methanol (90:10)		
gradients:	Time	%A	%B	Time	%A	%B
	0.0	75	25%	0.0	75	25%
	1.5	75	25%	1.5	75	25%
	15.0	0	100%	15.0	2	98%
	22.0	0	100%	22.0	2	98%
	25.0	75	25%	25.0	75	25%

Figure 1. UV Chromatograms of Sudan III and Sudan 410. (A) Initial Conditions, (B) Addition of Methanol to Mobile Phase, (C) Final Conditions after Adjusting Gradient

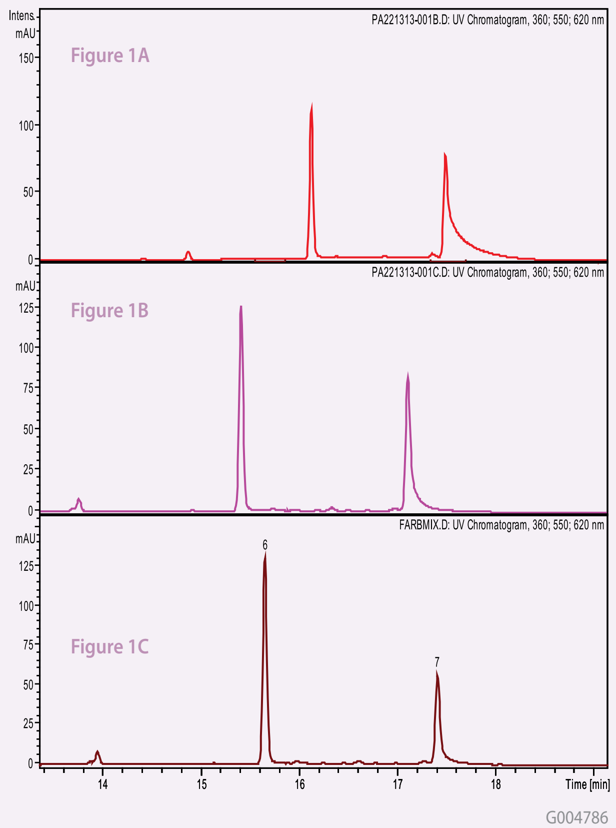
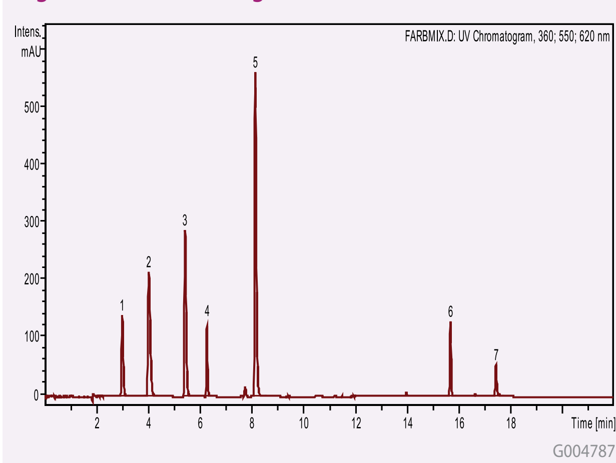


Figure 1 shows UV chromatograms of Sudan III (Peak 6) and Sudan 410 (Peak 7) after three optimization steps. An organic phase mixture of methanol:acetonitrile (10:90) and a final gradient composition of 98% organic mobile phase resulted in the best overall peak shapes with a minimum of tailing of compounds (Peak 7). Figure 3 shows the final chromatogram with very good separation of all analytes.

Figure 2 is a UV chromatogram of the final HPLC method. Resolution, sensitivity, and peak symmetry were optimal for all analytes. The total run time on a standard HPLC instrument (Agilent 1100) was 25 minutes, but the separation could easily be performed faster on ultra-performance instruments.

Figure 2. UV Chromatogram of the Final HPLC Method

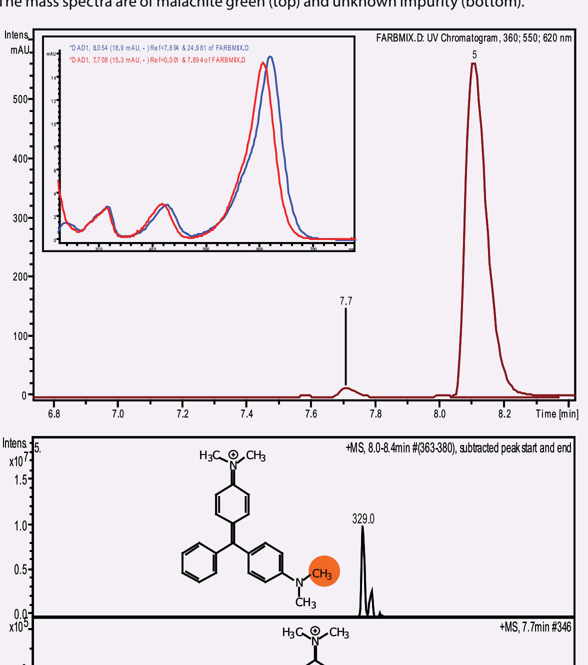


Ascentis Express HPLC Columns: High Efficiency and LC-MS Compatibility

Using Ascentis Express columns on standard HPLC, fast LC, and ultra-performance instruments can yield heightened sensitivity (Figure 2). Mass detectors in LC-MS systems are very sensitive to contaminants in solvents and column bleed, both of which are very low with Ascentis Express columns combined with the right set of Fluka® LC-MS solvents and additives. Both aspects, high efficiency and low column bleed, are basic requirements in trace analysis of small target analyte concentrations or in identification of byproducts which may influence dramatically the quality and application of dyes. Figure 3 shows an example of the identification of low concentrations of byproducts even in very complex mixtures. The unknown substance at the retention time of 7.68 minutes shows a nearly identical UV spectrum to Malachite Green (Peak 5 at 8.10 minutes), but the mass is 14 Da lower. This may correspond to the exchange of a methyl residue with a proton at a position in the Malachite Green molecule that has no influence on the chromophore. Only the displayed molecular structures fit the UV and mass spectroscopic results.

Figure 3. Expanded View of UV Chromatogram Showing Unknown Impurity at 7.7 min. and Malachite Green (5)

The inset shows the UV spectra of malachite green (blue) and the unknown impurity (red). The mass spectra are of malachite green (top) and unknown impurity (bottom).



To get optimal results from your LC-MS system and accurate UV and mass spectra of impurities with a high signal-to-noise level, it is best to use high purity LC-MS solvents from Fluka and high performance HPLC columns such as Ascentis Express from Supelco.

References

1. Commission Decision. Official Journal of the European Union. L154/114. June 10, 2003.
2. Rapid Alert System for Food and Feed (RASFF). 2004. Annual Report. European Commission of Health & Consumer Protection Directorate General.

Ascentis Express Properties

Stationary Phase Support

- Ultra-pure, Type B silica
- 1.7 µm solid core particle with 0.5 µm porous silica shell (effective 2.7 µm)
- 150 m^2/gram surface area (comparable to ~225 m^2/g porous particle)
- 90 Å pore size, 160 Å for Peptide ES C-18

Bonded Phase

	Coverage µmoles/m^2	pH Range	Endcapping
C18	*3.5*	*2-9*	*Yes*
C8	*3.7*	*2-9*	*Yes*
RP-Amide	*3.0*	*2-9*	*Yes*
HILIC	*n/a*	*2-8*	*No*
Phenyl-Hexyl	*3.4*	*2-9*	*Yes*
Peptide ES-C18	*3.5*	*1-9*	*No*

Alternate Selectivity

Ascentis Express RP-Amide:

Combining an Embedded Polar Group Stationary Phase and Fused-Core Particles

Ascentis Express RP-Amide HPLC columns are the most recent product additions to the Supelco HPLC product line. Combining an embedded polar group (EPG) stationary phase with the Fused-Core particles, Ascentis Express RP-Amide provides a host of useful benefits to the HPLC chromatographer. The benefits come from both the phase technology and the particle technology and can be summarized as:

Fused-Core Benefits

- Higher efficiency than traditional HPLC columns (3 and 5 µm)
- Half of the backpressure of sub 2 micron columns
- Capable of UHPLC performance on traditional HPLC systems

RP-Amide Benefits

- Alternative reversed-phase selectivity to C18
- Improved peak shape for bases
- 100% aqueous compatible reversed-phase column

At the heart of the Ascentis Express RP-Amide is the 2.7 µm Fused-Core particle that comprises a 1.7 µm solid core and a 0.5 µm porous shell. Compared to totally porous particles, the Fused-Core particles have a much shorter diffusion path because of the solid core. This partial porosity reduces dispersion of solutes and minimizes peak broadening. Other features, such as a very tight particle size distribution and high packing density, result in Ascentis Express columns capable of delivering extreme performance to any HPLC system. In fact, there have been many reports on the vast improvements in efficiency and speed provided by Ascentis Express HPLC columns versus traditional HPLC columns. The improvements provide UHPLC performance on traditional HPLC systems.

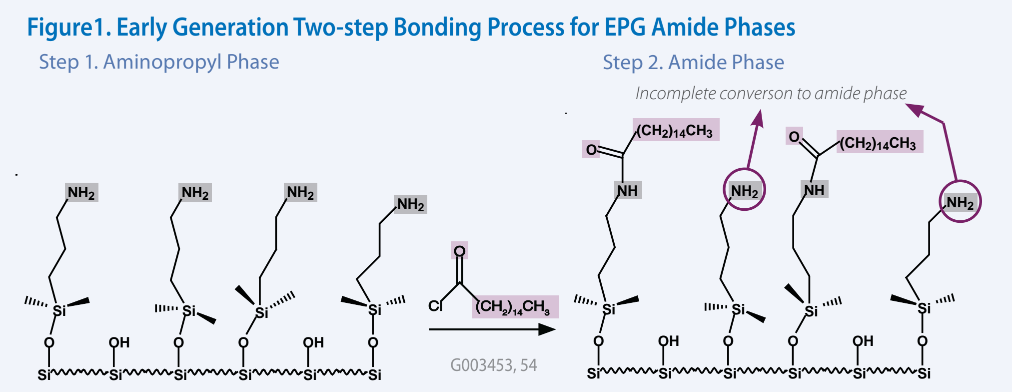
While the Ascentis Express C8 and C18 provide classic reversed-phase selectivity, the RP-Amide phase offers an alternative selectivity. Supelco first commercially introduced the EPG phase in 1988. At that time, large tailing factors for basic analytes continued to plague conventional C18 and C8 bonded phases. The EPG phase was found to improve peak shape of basic analytes. The early generation EPG phases were based on a two-step bonding process (Figure 1). The first step was the bonding of an aminopropylsilane to the bare silica surface creating a surface with amine functionality. In step two, palmitoyl chloride was reacted with the amine to create a long chain amide. Not all amines would be converted in the process, leaving a mixed system. These early generation EPG phases suffer from poor reproducibility.

Next generation phases, including Ascentis Express RP-Amide, are produced using a one-step process (Figure 2). In the single step process, no free amino ligands occur since the amide is introduced as a whole unit. This one-step bonding process yields excellent batch-to-batch reproducibility. Interestingly, not all EPG phases on the market use the modern, one-step bonding approach.

Improved Peak Shape for Basic Compounds

As previously mentioned, Ascentis Express RP-Amide phase reduces silanol interactions with basic analytes improving peak shape. A good test to demonstrate this effect is highly basic compounds using a mobile phase pH of 7. At this pH, many of the residual silanols are in the ionized form and the basic compounds are protonated.
The protonated (charged) bases interact with the charged silanols via ion exchange and result in a tailing peak. A test

Figure1. Early Generation Two-step Bonding Process for EPG Amide Phases



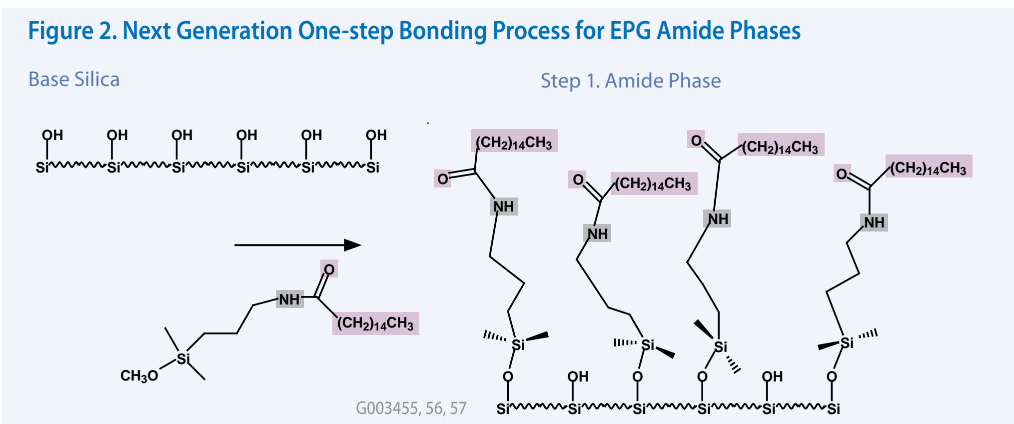


Figure 2. Next Generation One-step Bonding Process for EPG Amide Phases

mix of tricyclic antidepressants was analyzed on Ascentis Express RP-Amide and a C18 column with a mobile phase pH of 7 (Figure 3). As shown in Figure 3, the RP-Amide produces more symmetrical peaks than the C18 for these difficult test probes. Asymmetry data is summarized in Figure 4 for doxepin, imipramine, and amitriptyline.

Alternative Selectivity

Ascentis Express RP-Amide provides increased selectivity for polar compounds, especially those that can act as a hydrogen-bond donor. Phenols, carboxylic acids, amines, and to a lesser extent, alcohols show enhanced retention on the RP-Amide phase when compared to neutral, non-polar analytes. An example of the power of the hydrogen bonding mechanism is shown in Figure 5. The phenolic nature of catechols and resorcinols provides a good test for demonstrating enhanced selectivity of the RP-Amide phase. The RP-Amide phase shows complete baseline resolution of these related compounds while the C18 phase shows reduced retention, resolution, and selectivity for the phenolics. In comparing the Ascentis Express RP-Amide to the Waters™ BEH Shield RP18,
a competitive EPG phase, the selectivity is very similar. The difference in this example is the Ascentis Express RP-Amide yields a backpressure half of the 1.7 µm column. This difference in backpressure means the Ascentis Express column is suitable for traditional HPLC systems while the 1.7 µm column is not.

The selectivity differences between the RP-Amide and the C18 can be a useful tool in method development. In many cases, when peaks co-elute on a C18 phase, the RP-Amide can be substituted to achieve separation without a change in mobile phase.

Figure 3. Separation of Tricyclic Antidepressants on Ascentis Express RP-Amide and Conventional C18

columns: as indicated; 10 cm x 4.6 mm I.D.
mobile phase: 35:65, 14 mM ammonium phosphate in water, pH = 7 : methanol
flow rate: 1.5 mL/min.
temp.: 35° C
det.: 254 nm
injection: 0.5 µL

Ascentis Express RP-Amide (G004358)

C18 (G004359)

Min: 0, 2, 4, 6, 8, 10, 12

1. Maleate
2. Nordoxepin
3. Protripyline
4. Desipramine
5. Nortriptyline
6. Doxepin
7. Imipramine
8. Amitriptyline
9. Trimipramine

Figure 4. Asymmetry Factors for Difficult Bases on Ascentis Express RP-Amide and Conventional C18

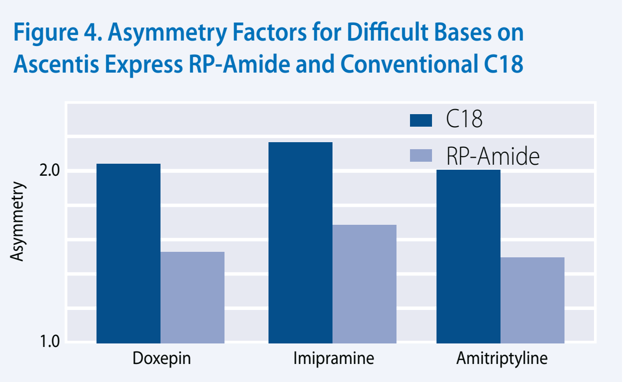


Figure 5. Separation of Phenolics on Ascentis Express RP-Amide & C18, & Waters BEH Shield RP18, 1.7 µm

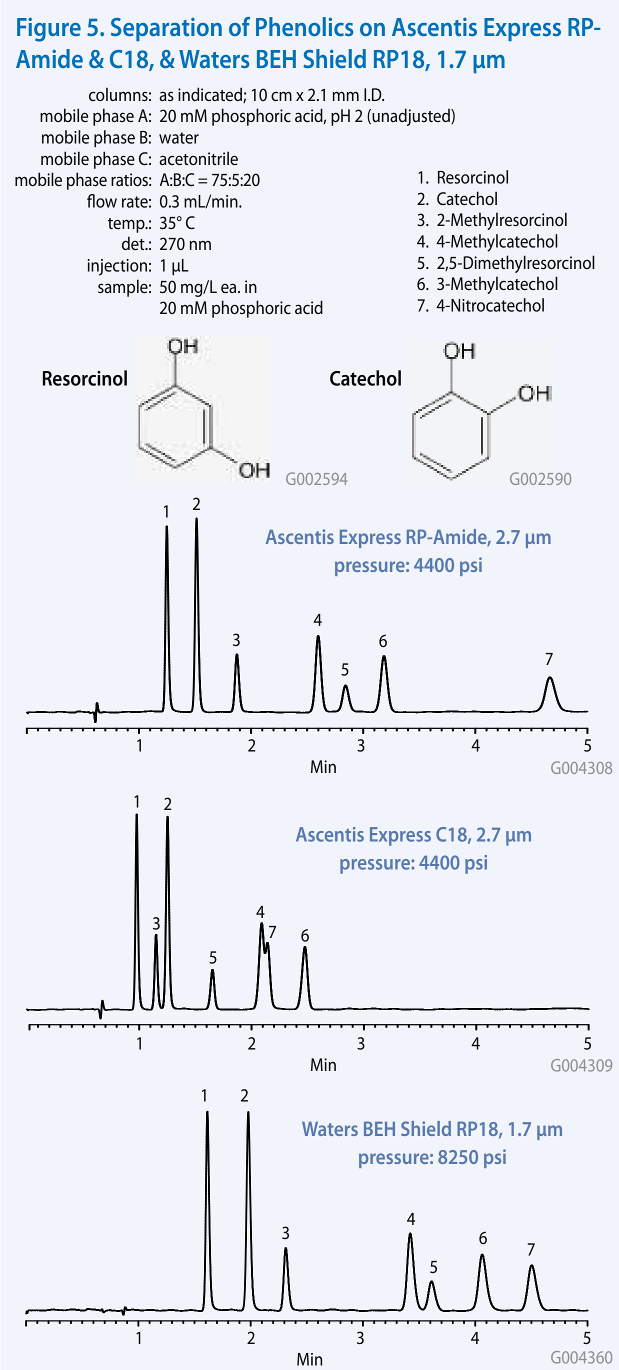
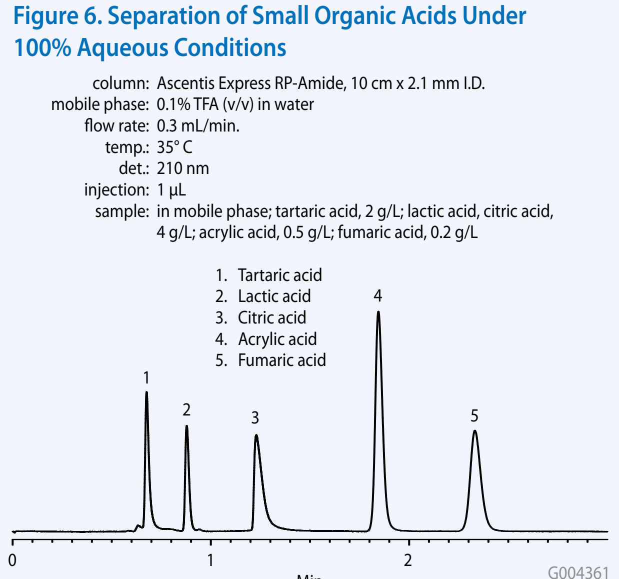


Figure 6. Separation of Small Organic Acids Under 100% Aqueous Conditions



Aqueous Compatible Reversed-Phase Column

Ascentis Express RP-Amide provides stable and reproducible analyte retention in 100% aqueous mobile phases. Many C18 phases are known to suffer from phase collapse under highly aqueous mobile phase conditions causing loss of retention. Shown in Figure 6 is a mix of organic acids analyzed under 100% aqueous conditions. Excellent selectivity and peak shape is noted for all the test probes, even citric acid, which is a notoriously difficult analyte.

Conclusion

Ascentis Express RP-Amide is a blend of modern phase technology and innovative particle technology. The Fused-Core particle provides benefits in terms of speed, resolution, sensitivity, and ruggedness. The one-step RP-Amide bonding chemistry provides benefits in terms of selectivity, aqueous stability, and improved peak shape for bases.

Ascentis Express Phenyl-Hexyl:

Combining the popular Phenyl-Hexyl Stationary Phase and Fused-Core Particles

Even though new column technologies have more than doubled the plates per meter possible with traditional 5 µm columns, resolution still cannot be routinely achieved in every case without the ability to adjust retention and selectivity by proper selection of column stationary and mobile phases. This article features Ascentis Express Phenyl-Hexyl phase, a new addition to the Fused-Core column family, and describes how column selectivity and higher efficiency can be coupled to achieve much faster separations than have previously been possible.

The vast majority of UHPLC separations have been carried out with C18 columns in the classic reversed-phase (RP) mode; however, suppliers now offer many different phases. Although no one would dispute the fact that UHPLC columns with different phases are needed, very little has been published yet on the performance that can be expected from UHPLC columns having different, complementary selectivity to C18 and C8. Two of the most popular polar-RP phases are RP-Amide, which is often categorized as an embedded polar-group phase, and Phenyl, which can interact with solutes by π-π mechanisms. A brief retention and selectivity comparison for the Ascentis Express column family is given in Table 1.

C18 and C8 phases are highly popular because they are stable, reproducible, and easy-to-use. Retention correlates closely with log P values, which have been established for many solutes. Solute ionization causes retention to decrease in a predictable manner and is relatively easy to control by adding dilute acids, bases, and buffers to the mobile phase. Changing the organic component of the mobile phase between acetonitrile and methanol (or other solvents) allows the user to tweak resolution because solvation affects phase structure and selectivity. Temperature is also a useful variable for optimizing phase selectivity. Columns with C18 and C8 phases will frequently give optimum resolution when solutes are nonpolar or slightly polar; however, columns with polar-RP phases such as RP-Amide or Phenyl-Hexyl will often show improved retention and selectivity for more polar solutes. It should be emphasized that even polar-RP phases have a significant alkyl phase character in addition to their polar character. The same mobile phase solvents and techniques may be employed with polar-RP phases, with comparable phase stability to C18.

Table 1. Brief Overview of Ascentis Express Column Retention and Selectivity

Ascentis Express Fused-Core Phase	Principle Retention Mode	Principle Solute Interaction
C18	Reversed-Phase (RP)	Hydrophobic (dispersive)
C8	Reversed-Phase (RP)	Hydrophobic (dispersive)
RP-Amide	RP with embedded polarity	Hydrophobic and H-bonding
Phenyl-Hexyl	RP with pendant aromaticity	Hydrophobic and π-π
HILIC (Silica)	HILIC (or normal phase)	Hydrophilic (dipole, H-bonding, ion exchange)

The RP-Amide phase is complementary to C18 because the amide group has several unique features: 1) strong interaction by H-bonding when solutes can donate or accept protons, 2) effective shielding of silanols by internal H-bonding between amide group and silica surface, and 3) the ability to wet and operate well, even in 100% aqueous solvents. H-bonding allows solutes with carboxyl and phenol groups to be retained much longer and separate much better on RP-Amide than on C18 or C8. Shielding prevents solutes with amino groups from interacting with silanols and can result in shorter retention and sharper peaks on amide phases. Another interesting feature of amide phases is that methanol and other alcohols become much stronger solvents when H-bonding between phase and solute occurs. Except for the special situations listed above, an RP-Amide phase often performs similar to C18 due to the long alkyl chain extending away from the surface.

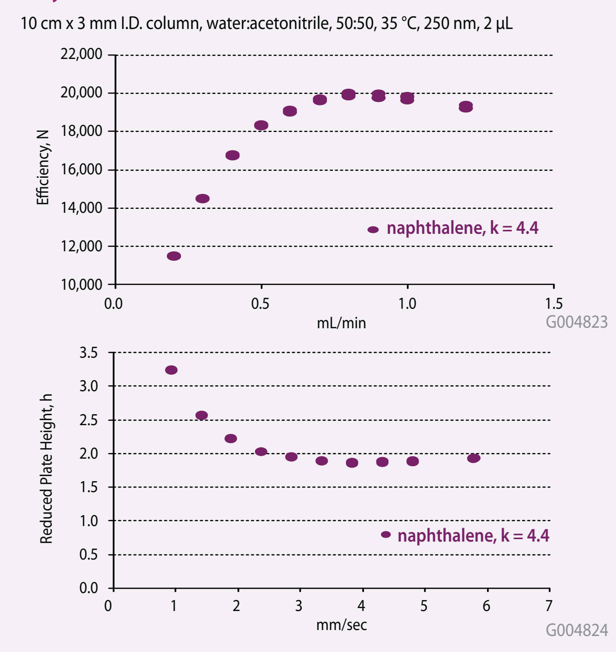
The Phenyl phase has unique selectivity arising from solute interaction with the aromatic ring and its delocalized electrons. It is complementary (orthogonal) to both C18 and RP-Amide phases because of this unique aromaticity. An unsubstituted phenyl ring is a π-donor or Lewis base, which interacts strongly with π-acceptors and any electron-deficient Lewis acid. Phenyl phases also tend to exhibit good shape selectivity, which may originate from solute multipoint interaction with the planar ring system. More retention and selectivity will often be observed for solutes with aromatic electron-withdrawing groups (fluorine, nitro, etc.) or with a delocalized heterocyclic ring system such as the benzodiazepine compounds.

UHPLC Results with Ascentis Express Phenyl-Hexyl

Low-pressure drop with high efficiency and a flat van Deemter curve have been confirmed for Phenyl-Hexyl, as shown in Figure 1. In general, more than twice the column efficiency of 5 µm particles can be expected for all Ascentis Express Fused-Core columns at pressures that are easily managed with all HPLC instruments. Note that 20,000 plates have been achieved for a 10 cm x 3 mm I.D. column operating at optimum flow. A Jasco X-LC HPLC instrument was used for the study. Figure 2 illustrates the benzodiazepine chemical structures used in this study. As shown in Figure 3, the selectivity of Ascentis Express Phenyl-Hexyl is very similar to that of other commercial Phenyl columns, so methods can be readily transferred between columns.
The difference in efficiency and pressure drop for the two porous 3 µm columns can be explained by different particle size distributions.

Figures 4-5 show comparisons of five benzodiazepines separated on the four Ascentis Express RP phases in water:acetonitrile and water:methanol mobile phases. No additives were employed in order to observe the interaction between these polar solutes and the different phases; however, a dilute buffer will normally be used for development of a validated method. The addition of 10-20 mM buffer at neutral pH typically has little or no effect upon the separation with these highly deactivated column phases.

Figure 1. Flow Performance of Ascentis Express Phenyl-Hexyl Column with Neutral Probes



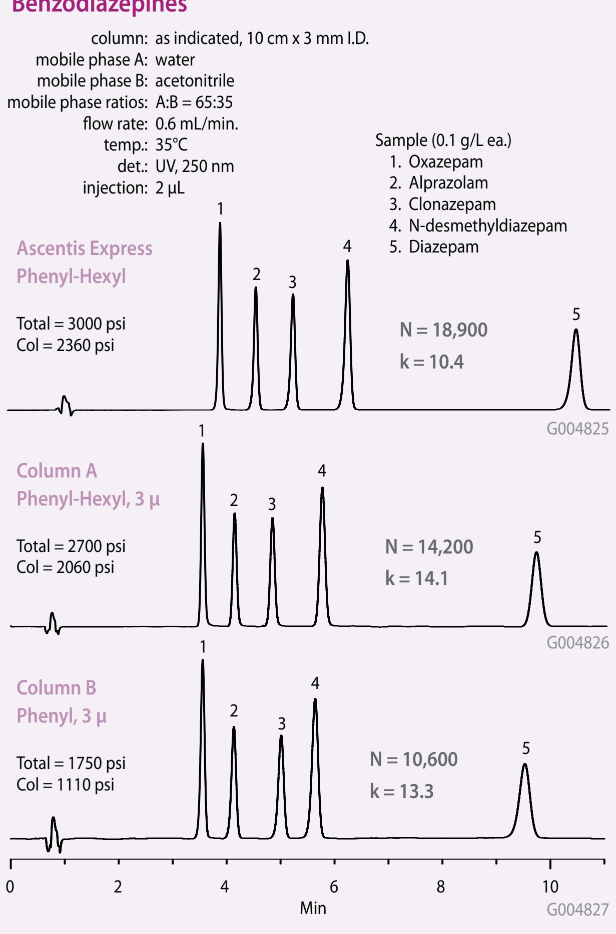
Reference

1. Kazakevitch, Y. V., et al. J. Chromatogr., A. 2005, 1082, 158–165.

Figure 2. Benzodiazepine Structures

Diazepam (G002881)
N-desmethyldiazepam (G003672)
Alprazolam (G003670)
Clonazepam (G002879)
Oxazepam (G003654)

Figure 3. Comparison of Phenyl Column Selectivity for Benzodiazepines



Note that overall retention in acetonitrile is similar for the four bonded phases, but elution order is different. The two less polar compounds, diazepam and desmethyldiazepam, elute late and show the same order for all columns due to predominance of hydrophobic interactions. The more polar solutes, however, elute earlier and interact differently with Phenyl-Hexyl and the other phases.

With this test sample and operating conditions, three of the four Ascentis Express RP columns provide good resolution with different selectivity, however, Phenyl-Hexyl shows the best retention and selectivity.

A switch to water:methanol in Figure 5 shows a dramatic change in retention for Ascentis Express Phenyl-Hexyl. In water:methanol mobile phase, the phenyl group interacts much more strongly than the other phases with the solute heterocyclic ring system, presumably by a π-π mechanism. Kazakevitch (1) has published evidence that methanol forms only monolayer coverage on aromatic phases (and also thinly solvates other phases), which allows the aromatic selectivity to shine through more strongly. Elution order for the polar compounds also changes from that of water:acetonitrile conditions. For this test sample, Ascentis Express Phenyl-Hexyl selectivity is clearly superior in water:methanol to the other phases.

Figure 4. Benzodiazepines in 35% Acetonitrile Mobile Phase with no Additive

column: Ascentis Express, as indicated, 10 cm x 3 mm I.D., 2.7 µm particles
mobile phase A: water
mobile phase B: acetonitrile
mobile phase ratios: A:B = 65:35
flow rate: 0.6 mL/min.
pressure: 3000 psi total, 2360 psi column
temp.: 35 °C
det.: UV, 250 nm
injection: 2 µL

Sample (0.1 g/L ea.)
1. Oxazepam
2. Alprazolam
3. Clonazepam
4. N-desmethyldiazepam
5. Diazepam

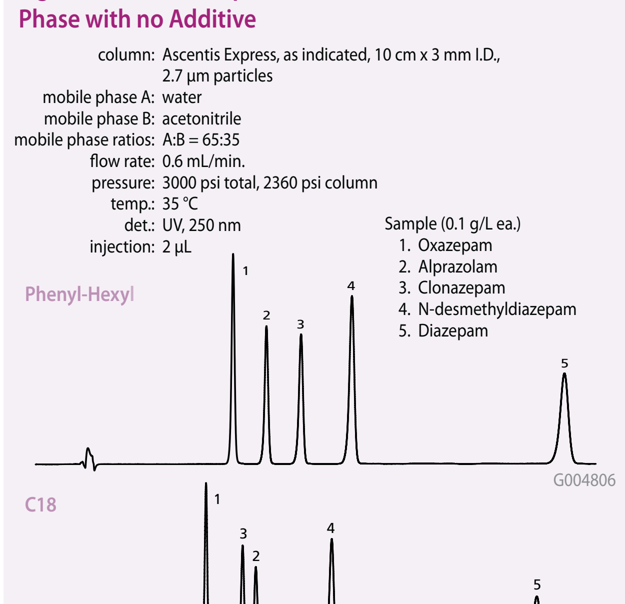
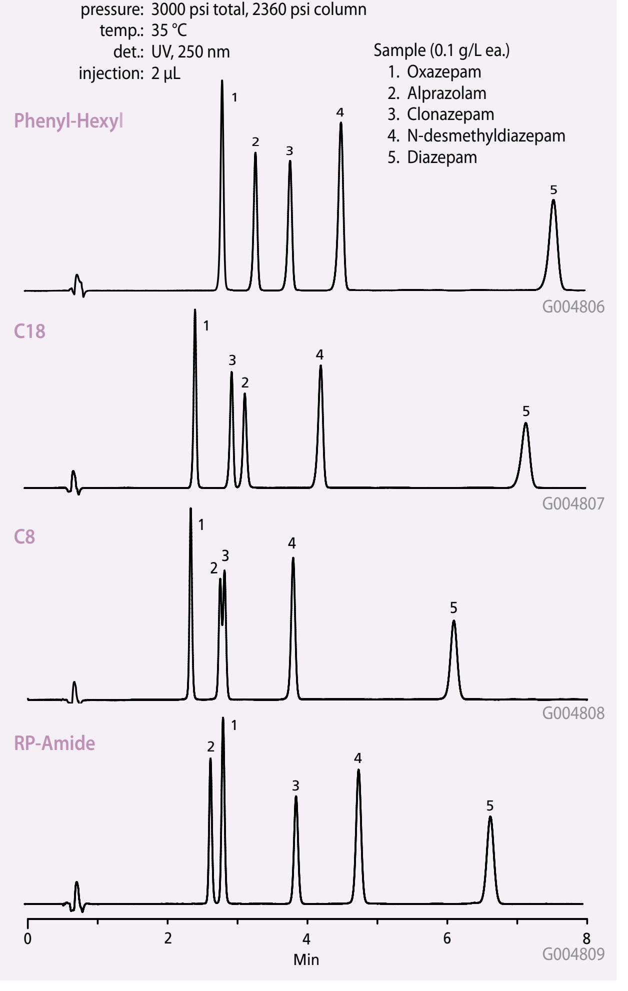
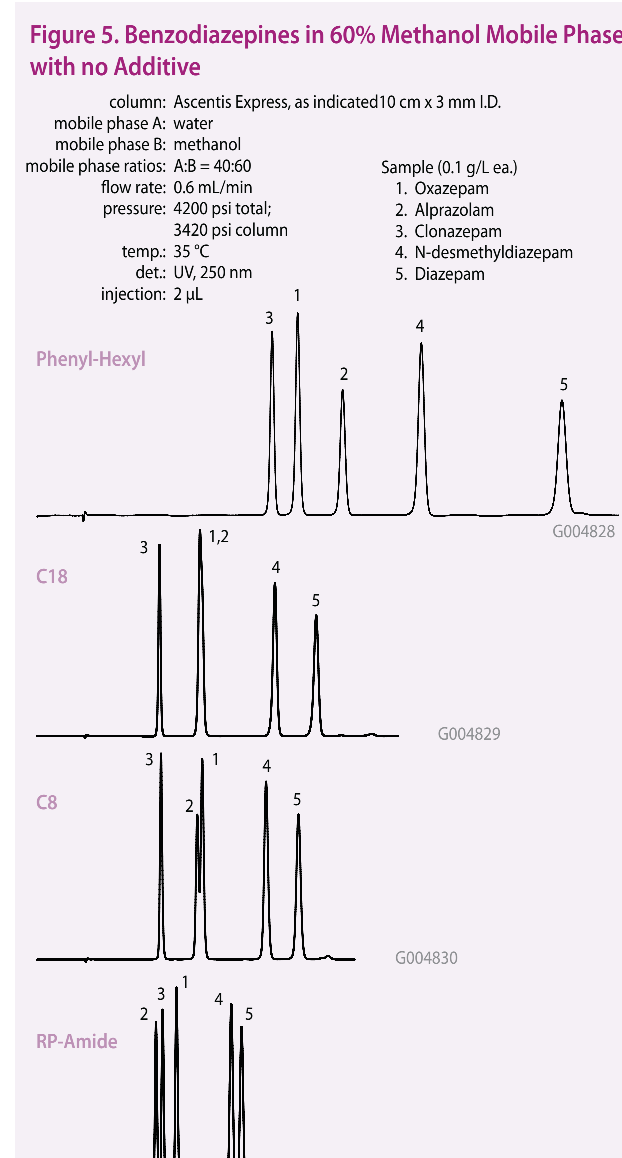



Figure 5. Benzodiazepines in 60% Methanol Mobile Phase with no Additive

column: Ascentis Express, as indicated10 cm x 3 mm I.D.
mobile phase A: water
mobile phase B: methanol
mobile phase ratios: A:B = 40:60
flow rate: 0.6 mL/min
pressure: 4200 psi total; 3420 psi column
temp.: 35 °C
det.: UV, 250 nm
injection: 2 µL

Sample (0.1 g/L ea.)
1. Oxazepam
2. Alprazolam
3. Clonazepam
4. N-desmethyldiazepam
5. Diazepam



Conclusion

A new Phenyl-Hexyl phase has been paired with Fused-Core particles to complete the primary Ascentis Express column family. High performance with lower pressure drop than other UHPLC columns has been confirmed for all Fused-Core particle phases. Ascentis Express Phenyl-Hexyl correlates well to other Phenyl phases for easy method development or method transfer. Selectivity for benzodiazepine compounds has been compared to the other Ascentis Express RP phases in water:acetonitrile and water:methanol. The extra retention possible with Phenyl phases in water:methanol has been demonstrated for these heterocyclic aromatic compounds. The potential for faster, more sensitive assays using Ascentis Express Phenyl-Hexyl and all Ascentis Express phases has been shown.

FAQs

Ascentis Express FAQs

What is unique about Ascentis Express?

Ascentis Express columns provide a breakthrough in HPLC performance. Based on Fused-Core particle technology, Ascentis Express provides the benefits of sub-2 µm particles but at much lower backpressure. These benefits include the capability of providing fast HPLC and higher resolution chromatography. The Fused-Core particle consists of a 1.7 µm solid core and a 0.5 µm porous shell. A major benefit of the Fused-Core particle is the small diffusion path (0.5 µm) compared to conventional fully porous particles. The shorter diffusion path reduces axial dispersion of solutes and minimizes peak broadening.

Can I use Ascentis Express on any type of HPLC system?

Ascentis Express HPLC columns are capable of use on standard HPLC systems as well as UHPLC systems. Columns are packed in high pressure hardware capable of withstanding the pressures used in UHPLC systems.

Is there anything I need to do to my HPLC system to use Ascentis Express?

Nothing special is required to use Ascentis Express HPLC columns. To obtain the full benefits of Ascentis Express, one should minimize dispersion or instrument bandwidth in the HPLC system (tubing, detector flow cell) as well as confirm the detector response system is set at a fast level. For more information, request Guidelines for Optimizing Systems for Ascentis Express Columns (T407102) or visit *sigma-aldrich.com/express* and download.

Can I use Ascentis Express on a UHPLC system?

Yes. Ascentis Express columns are packed in a way making them suitable for these ultra high pressure instruments. In fact, Ascentis Express outperforms sub-2 µm columns on many applications since Ascentis Express provides the benefits of sub-2 µm particles but at much lower backpressure.

Can Ascentis Express columns be used for LC-MS?

Ascentis Express Fused-Core particles were designed with LC-MS in mind. Even extremely short column lengths exhibit sufficient plate counts to show high resolving power. The flat van Deemter plots permit resolution to be maintained at very high flow rates to maximize sample throughput. All Ascentis stationary phases have been evaluated for MS compatibility during their development, and the Express phases are no exception. A bonus of Ascentis Express columns for high throughput UHPLC and LC-MS is that they are extremely rugged and highly resistant to plugging, a very common failure mode for competitor columns.

What flow rate should I use with Ascentis Express columns?

Based on the minimum in the van Deemter curves, higher flows than 5 µm particle columns are required in order to maximize Ascentis Express column efficiency.

Ascentis Express HPLC Column ID	Suggested Starting Point for Flow Rate
4.6 mm I.D.	1.6 mL/min
3.0 mm I.D.	0.8 mL/min
2.1 mm I.D.	0.4 mL/min

Key Technical Literature (available by request through technical service)

Code	Publication Title
T409113	Method Optimization using Alternative Selectivities in Fused-Core Particle HPLC Column Technology
T409110	Increased Bioanalytical Throughput Using Fused-Core HPLC with Selective Phospholipid Depletion
T409041	Extended Performance of LC Instruments with Fused-Core Particle Columns
T408141	Utilizing Fused-Core Technology for LC-MS Applications
T408088	Transfer and Speedup of Methods to Fused-Core Particle Columns
T408087	Optimization of HPLC Instrumentation for High Efficiency Separations
T408077	Achieving Sub-2 µm LC-MS Performance at Moderate Pressures using Fused-Core Particle Technology
T408035	High-Resolution HPLC Through Coupling Columns
T408034	High Resolution HPLC Performance Under Both Isocratic and Gradient Conditions
T408033	Achieving Optimum UHPLC Column Performance by Measuring and Reducing Overall System Dispersion
T408031	Achieving Ultra-HPLC Column Performance with Older Instruments
T407127	Achieving Efficient Bioanalytical Separations at Moderate Pressures using Fused-Core Particle Technology
T407078	Optimizing HPLC Particles and Column Dimensions for Fast, Efficient Separations
T407102	Guidelines for Optimizing Performance with Ascentis Express HPLC Columns
T408143	Guide to Dispersion Measurement

Ordering Information

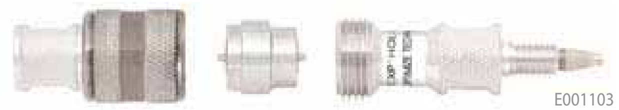
Analytical Ascentis Express Columns

ID (mm)	Length (cm)	C18	C8	Phenyl-Hexyl	HILIC	RP-Amide	Peptide ES -C18
2.1	2	53799-U	—	—	—	—	—
2.1	3	53802-U	53839-U	53332-U	53933-U	53910-U	53299-U
2.1	5	53822-U	53831-U	53334-U	53934-U	53911-U	53301-U
2.1	7.5	53804-U	53843-U	53335-U	53938-U	53912-U	53304-U
2.1	10	53823-U	53832-U	53336-U	53939-U	53913-U	53306-U
2.1	15	53825-U	53834-U	53338-U	53946-U	53914-U	53307-U
3.0	3	53805-U	53844-U	53341-U	53964-U	53915-U	53308-U
3.0	5	53811-U	53848-U	53342-U	53967-U	53916-U	53311-U
3.0	7.5	53812-U	53849-U	53343-U	53969-U	53917-U	53312-U
3.0	10	53814-U	53852-U	53345-U	53970-U	53918-U	53313-U
3.0	15	53816-U	53853-U	53346-U	53972-U	53919-U	53314-U
4.6	3	53818-U	53857-U	53347-U	53974-U	53921-U	53316-U
4.6	5	53826-U	53836-U	53348-U	53975-U	53922-U	53318-U
4.6	7.5	53819-U	53858-U	53351-U	53977-U	53923-U	53323-U
4.6	10	53827-U	53837-U	53352-U	53979-U	53929-U	53324-U
4.6	15	53829-U	53838-U	53353-U	53981-U	53931-U	53328-U

Capillary Ascentis Express Columns

Length (cm)	I.D. (µm)	Cat. No.
C18		
5	75	53982-U
15	75	54219-U
5	100	53985-U
15	100	54256-U
5	200	53989-U
15	200	54261-U
5	300	53992-U
15	300	54271-U
5	500	53998-U
15	500	54273-U
C8		
5	75	53983-U
15	75	54229-U
5	100	53987-U
15	100	54260-U
5	200	53991-U
15	200	54262-U
5	300	53997-U
15	300	54272-U
5	500	53999-U
15	500	54275-U

Ascentis Express Guard Columns



E001103

Universal Guard Holder

Description	Cat. No.
Holder w/EXP Titanium Hybrid Ferrule -- cartridge not included with holder	53500-U

Ascentis Express Guard Cartridges

Description	I.D. (mm)	Pkg. Size	Cat. No.
C18	2.1	3	53501-U
C18	3.0	3	53504-U
C18	4.6	3	53508-U
C8	2.1	3	53509-U
C8	3.0	3	53511-U
C8	4.6	3	53512-U
RP-Amide	2.1	3	53514-U
RP-Amide	3.0	3	53516-U
RP-Amide	4.6	3	53519-U
HILIC	2.1	3	53520-U
HILIC	3.0	3	53521-U
HILIC	4.6	3	53523-U
Phenyl-Hexyl	2.1	3	53524-U
Phenyl-Hexyl	3.0	3	53526-U
Phenyl-Hexyl	4.6	3	53531-U
Peptide ES-C18	2.1	3	53536-U
Peptide ES-C18	3.0	3	53537-U
Peptide ES-C18	4.6	3	53542-U

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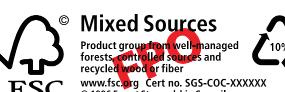

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SIGMA-ALDRICH®

Ascentis Express Peptide ES-C18 HPLC Columns

The Fused-Core Advantage for Bioseparations

- Improved Peak Shape on any HPLC System
- Extreme Stability with TFA and Other Additives
- Rugged HPLC Column Design

SIGMA-ALDRICH

A Breakthrough in Bioseparations Performance

Ascentis Express Peptide ES-C18

Ascentis® Express Peptide ES-C18 is a high-speed, high-performance liquid chromatography column based on a new 160Å Fused-Core™ particle design. The Fused-Core particle provides a thin porous shell of high-purity silica surrounding a solid silica core. This particle design exhibits very high column efficiency for high MW solutes (up to 20 kDa) due to the shallow diffusion paths in the 0.5-micron thick porous shell and the small overall particle size of 2.7-microns. The non-end-capped, sterically protected C18 bonded phase of Ascentis Express Peptide ES-C18 provides a stable, reversed phase packing with a pore structure and pore size that is optimized for reversed-phase HPLC separations of peptides and polypeptides, using typical acidic mobile phases favored for protein structure-function and proteomic applications.

Table 1. Specifications for Ascentis Express Peptide ES-C18

Silica	High Purity Type B
Phase	Sterically protected C18
pH range	1 – 9
Temperature	100 °C
Average pore diameter	160 Å
Surface area, nitrogen	80 sq.m/g
Pore volume	0.30 mL/g
Particle density	1.3 g/cc

Ordering Information

Ascentis Express Peptide ES-C18 Columns

I.D. (mm)	Length (cm)				
	3	5	7.5	10	15
2.1	53299-U	53301-U	53304-U	53306-U	53307-U
3.0	53308-U	53311-U	53312-U	53313-U	53314-U
4.6	53316-U	53318-U	53323-U	53324-U	53328-U

Applications

The Ascentis Express Peptide ES-C18 columns are best utilized with mobile phases that are mixtures of acetonitrile and water or methanol and water. Higher levels of the organic solvent component will typically reduce the retention of the sample compounds. Using elevated temperatures (e.g., 40 – 100 °C) will reduce the viscosity of the mobile phase and allow the use of faster flow rates and lower column pressure for high sample throughput. Gradient-elution techniques using 5 -10% organic component as the initial mobile phase and increasing to 100% organic component as the final mobile phase often can affect separations of complex sample mixtures in minimal time.

Ionizable compounds, such as acids and bases, are generally best separated with mobile phases buffered at pH of 2 to 3. The use of 10-50 mM buffers is always recommended for optimum results and long-term stability when separating ionizable compounds.

Ascentis Express Peptide ES-C18 columns utilize a steric-protected C18 bonded phase with extremely high resistance to acid-catalyzed hydrolysis of the siloxane bond that attaches the C18 chain to the surface. Thus, the combination of low pH and elevated temperature operation of the column is well tolerated. Peptide separations are efficiently conducted using low pH mobile phase modifiers, often at 0.1% concentration. Most popularly employing trifluoroacetic acid (TFA), and the related perfluorocarboxylic acids, pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA). These acids exhibit desirable low UV transparency, volatility, and peptide ion pairing properties. Additional opportunities for UV detection at low pH operation is with mineral acids such as phosphoric acid (1-20 mM). For MS detection 0.1% formic acid is most commonly employed (sometimes acetic acid), but significant benefit to peak shape can be realized with 0.1% formic acid adjusted to pH 3.5 (with ammonium hydroxide, for instance), especially with basic peptides. This is likely due to greater availability of formate anion for ion pairing.

Pharmaceutical Peptides

Many peptides have been investigated as therapeutic pharmaceutical drugs and are active vasodilators, vasoconstrictors, hormones, and neuropeptides. Using reversed-phase HPLC, it is possible to solve the tasks of identification, purity monitoring, and quantitative analysis in many cases, including those where the application of other methods is impossible.

Synthetic Peptides

The difficulty with synthetic peptides involves the production of many "deletion variants". A deletion may occur at any point in the peptides synthesis and so several versions of the peptide are produced which are absent one, two or three amino acids from the desired product. This makes for an interesting chromatographic problem because the resultant peptide mix contains peptides that are very similar in structure.

Peptide Mapping

Protein analysis and characterization has become more crucial due to many biopharmaceutical advances. Peptide mapping via LC-MS is one such technique that is commonly used today. A typical procedure involves the preparation of a tryptic digest from the protein, with subsequent characterization using reversed-phase, gradient HPLC separation followed by mass spectral analysis and database search.

Difficult Separations on Ascentis Express Peptide ES-C18

Figure 1. Basic Peptides

column: Ascentis Express Peptide ES-C18, 10 cm x 2.1 mm I.D. (53306-U)
mobile phase A: 0.1% additive in water
mobile phase B: 25:75, (0.4 % additive):acetonitrile
additive: formic acid, pH 3.5 (adjusted with ammonium hydroxide)
gradient: initial = 15% B, slope = 2% MeCN / column volume
flow rate: 0.3 mL/min
temp.: 35 °C
det.: ESI(+)-TOF
injection: 1 µL
sample: 5 mg/L peptide 1 & 3, 1 mg/L peptide 2, 15 mg/L peptide 4

Peptide probes (listed in order of elution):

1. ac-GGGLGGAGGLKG monoisotopic mass: 941.5
2. ac-KYGLGGAGGLKG monoisotopic mass: 1118.6
3. ac-GGAVKALKGLKG monoisotopic mass: 1139.7
4. ac-KYALKALKGLKG monoisotopic mass: 1330.8

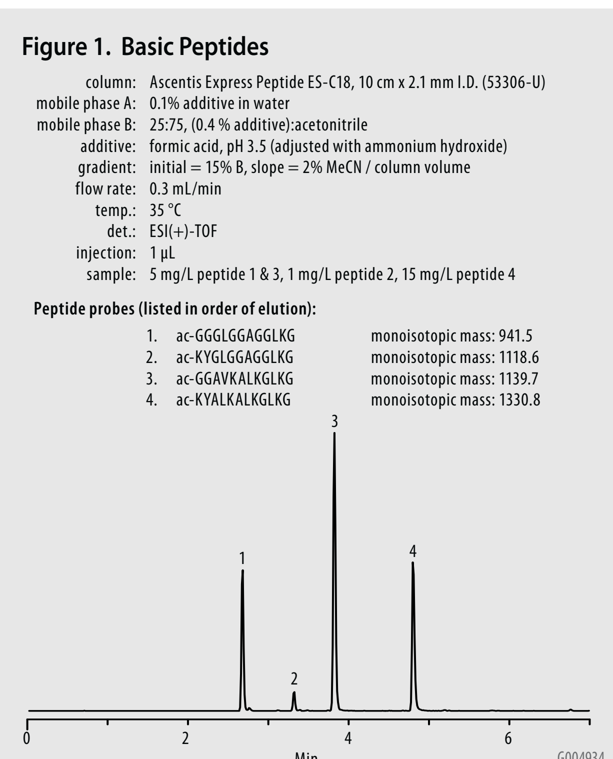


Figure 2. Peptide Test Mix

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 90:10, (0.1 % TFA):acetonitrile
mobile phase B: 25:75, (0.1% TFA):acetonitrile
gradient: initial = 0% B to 50% B in 15 min.
flow rate: 1.5 mL/min
temp.: 30 °C
det.: UV at 220 nm
injection: 5 µL

The test mix employed contains the following peptides

1. Gly-Tyr MW = 252
2. Val-Tyr-Val MW = 379
3. Met Enkephalin MW = 574
4. Angiotensin II MW = 1032
5. Leu-Enkephalin MW = 555
6. Ribonuclease MW = 13,700
7. Bovine Insulin MW = 5733

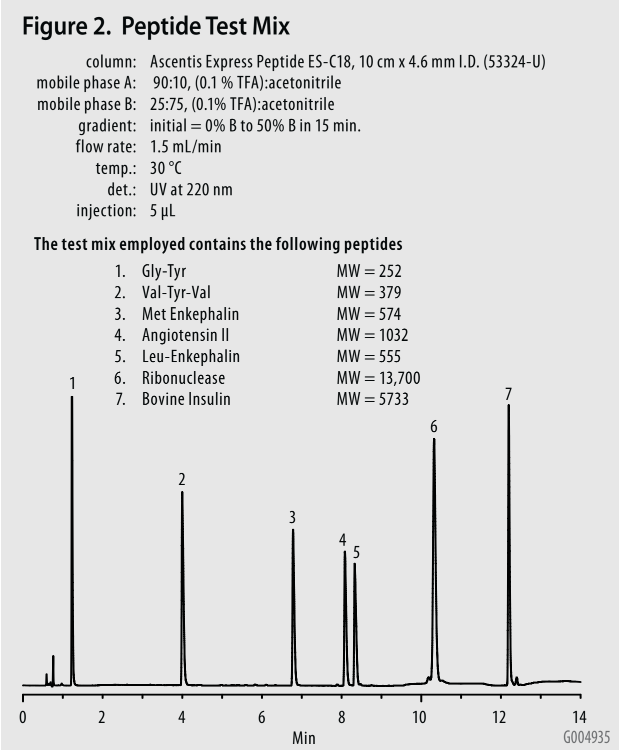


Figure 3. Carbonic Anhydrase Tryptic Digest

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 0.1 % TFA in water
mobile phase B: 40:60, (0.1% TFA):acetonitrile
gradient: initial = 3% B to 100% B in 53 min.
flow rate: 1.0 mL/min
temp.: 30 °C
det.: UV at 215 nm
injection: 20 µL

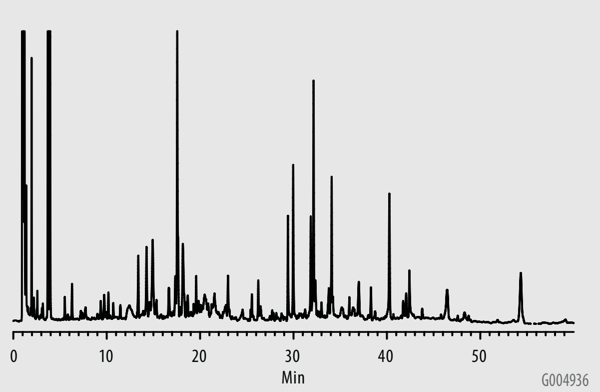
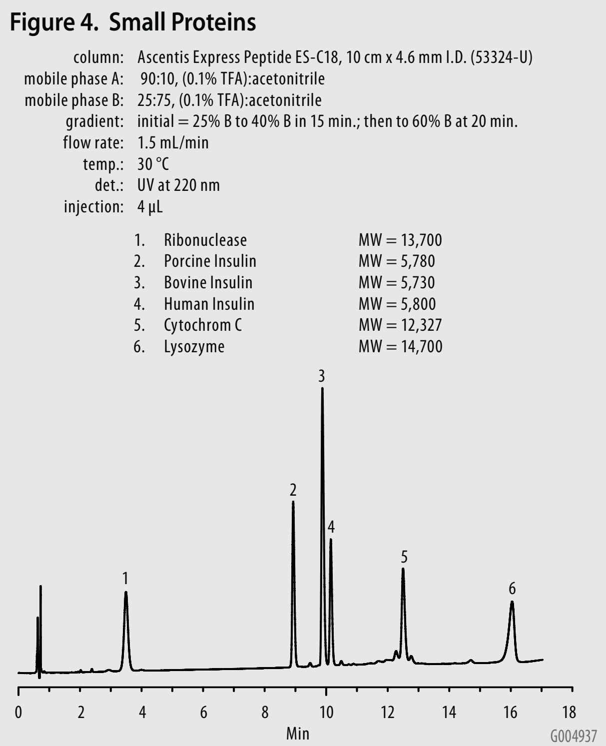


Figure 4. Small Proteins

column: Ascentis Express Peptide ES-C18, 10 cm x 4.6 mm I.D. (53324-U)
mobile phase A: 90:10, (0.1% TFA):acetonitrile
mobile phase B: 25:75, (0.1% TFA):acetonitrile
gradient: initial = 25% B to 40% B in 15 min.; then to 60% B at 20 min.
flow rate: 1.5 mL/min
temp.: 30 °C
det.: UV at 220 nm
injection: 4 µL

1. Ribonuclease MW = 13,700
2. Porcine Insulin MW = 5,780
3. Bovine Insulin MW = 5,730
4. Human Insulin MW = 5,800
5. Cytochrom C MW = 12,327
6. Lysozyme MW = 14,700



TRADEMARKS: Ascentis is a registered trademark of Sigma-Aldrich Biotechnology LP. Fused-Core is a trademark of Advanced Materials Technologies, Inc.

Competitor Comparison

Basic Peptides

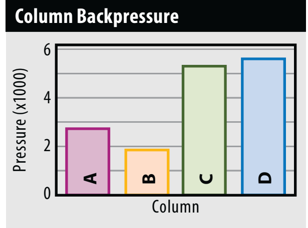
Columns run under equivalent conditions of gradient slope (Δ % MeCN per column volume).

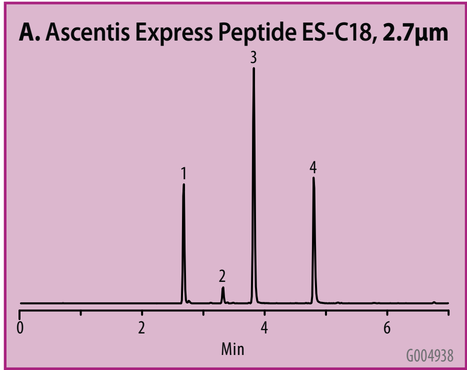
column: C18, 10 cm x 2.1 mm I.D.
mobile phase A: 0.1% additive in water
mobile phase B: 25:75, (0.4 % additive):acetonitrile
additive: formic acid, pH 3.5 (adjusted with ammonium hydroxide)
gradient: initial = 15% B, slope = 2% MeCN / column volume
flow rate: 0.3 mL/min
temp.: 35 °C
det.: ESI(+)-TOF
injection: 1 µL
sample: 5 mg/L peptide 1 & 3, 1 mg/L peptide 2, 15 mg/L peptide 4

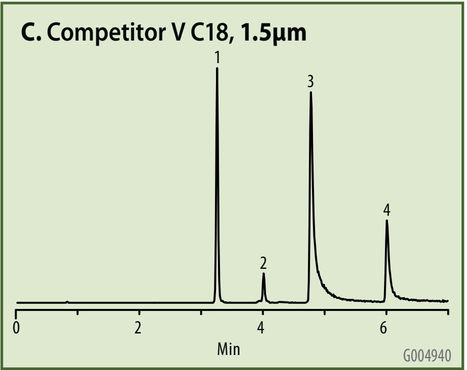
Peptide probes (listed in order of elution):

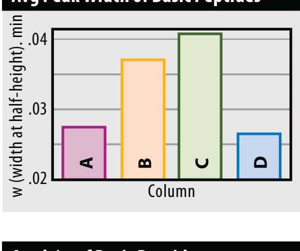
1. ac-GGGLGGAGGLKG Monoisotopic mass: 941.5
2. ac-KYGLGGAGGLKG Monoisotopic mass: 1118.6
3. ac-GGAVKALKGLKG Monoisotopic mass: 1139.7
4. ac-KYALKALKGLKG Monoisotopic mass: 1330.8

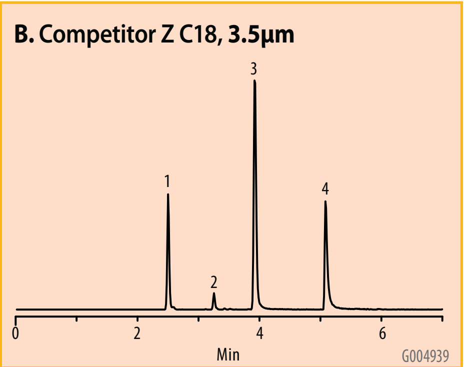
Peptide probes increase in basicity and hydrophobicity.

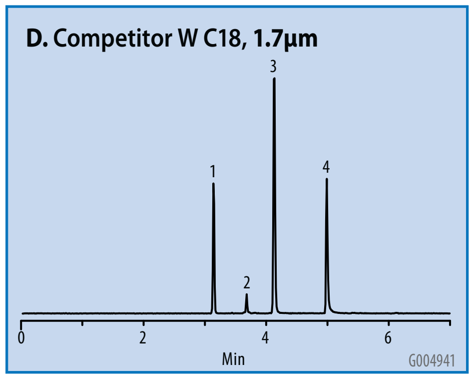






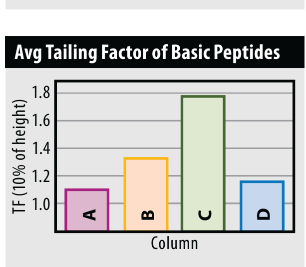






Avg h/w of Basic Peptides

h/w (x 1000): 0, 2, 4, 6, 8 — A, B, C, D — Column



Experimental Setup: Columns and Elution Gradients

Column	µm	Pore (Å)	ID (mm)	L (cm)	CV (mL)*	start %B	Grad slope [Δ % MeCN / CV]
A. Ascentis Express Peptide ES-C18	2.7	160	2.1	10	0.190	15	2
B. Competitor Z C18	3.5	300	2.1	10	0.216	15	2
C. Competitor V C18	1.5	120	2.0	10	0.205	15	2
D. Competitor W C18	1.7	130	2.1	10	0.186	15	2

* CV determined as follows: t_0 = r.t. of unretained component. d_0 = r.t. of unretained component with ZDV union in place of column. **CV** = (t_0 - d_0) x mL/min

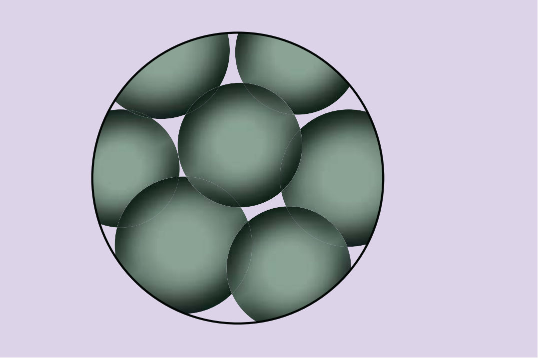
Some Quantitative Comparisons

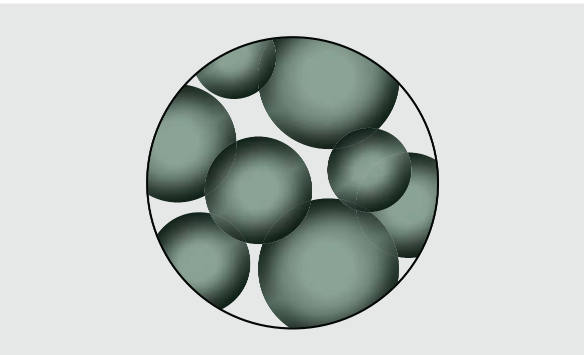
Column	Peak 1			Peak 2			Peak 3			Peak 4		
	$w_{1/2}$	$h/w_{1/2}$	$TF_{0.1}$	$w_{1/2}$	$h/w_{1/2}$	$TF_{0.1}$	$w_{1/2}$	$h/w_{1/2}$	$TF_{0.1}$	$w_{1/2}$	$h/w_{1/2}$	$TF_{0.1}$
A. Ascentis Express Peptide ES-C18, 2.7 µm	0.0258	9725	1.20	0.0224	1520	1.00	0.0344	11321	1.00	0.0267	9706	1.30
B. Competitor Z C18, 3.5 µm	0.0344	4082	1.14	0.0344	572	1.25	0.0387	8267	1.08	0.0387	3871	1.74
C. Competitor V C18, 1.5 µm	0.0344	4418	1.03	0.0344	548	1.06	0.0516	3044	2.69	0.0430	1281	2.38
D. Competitor W C18, 1.7 µm	0.0258	7568	1.10	0.0241	1203	1.20	0.0241	1942	1.00	0.0301	6684	1.30

The Fused-Core Advantage

Fused-Core Particles | Traditional Porous Particles

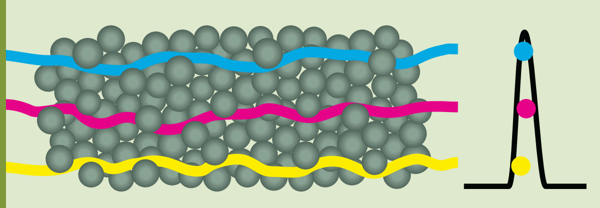
Narrow Particle Size Distribution

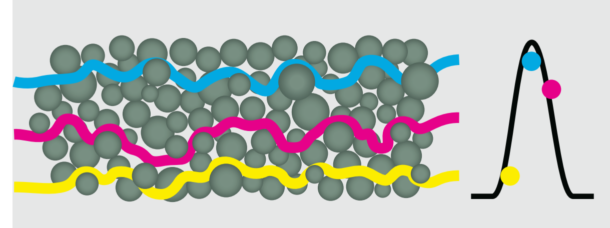




The innovative manufacturing process for Fused-Core particles produces a very narrow particle size distribution. A narrow particle size distribution allows for the use of large porosity frits that resist clogging, resulting in a **more rugged column**. Traditional porous particles are not manufactured in a way to yield extremely narrow particle size distributions.

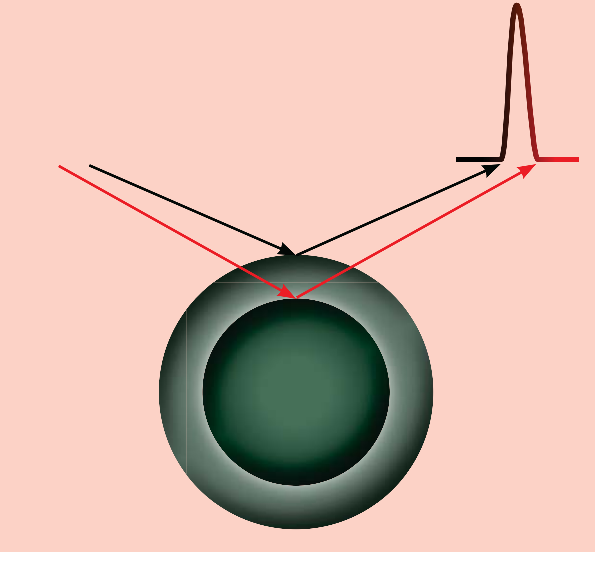
More Consistent Bed

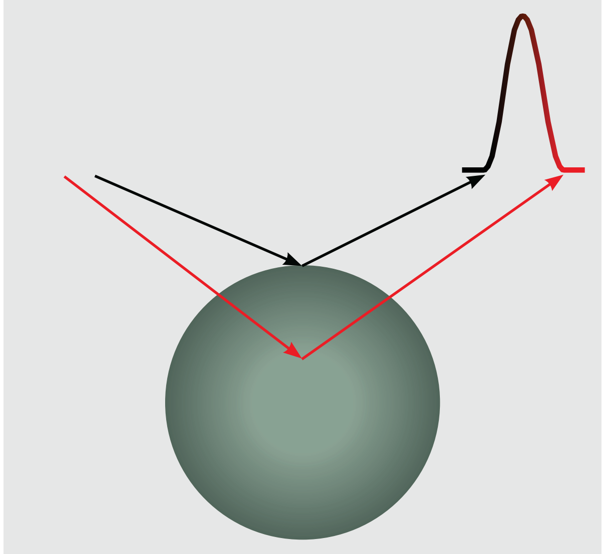




The "A" term in the van Deemter equation accounts for the effects of inhomogeneities in the packed bed of an HPLC column. Narrow particle size distributions form a more consistent packed bed and a consistent path length, **minimizing analyte diffusion** through the column. This eddy diffusion is effectively independent of mobile phase velocity.

Shorter Diffusion Path





The short diffusion path of the Fused-Core particle **yields sharper peaks** than traditional porous particle columns. The minimized resistance to mass transfer, the "C" term in the van Deemter equation, of the Fused-Core particle provides sharper peaks than traditional porous particles. The short diffusion path also **permits the use of higher flow rates** without peak broadening.

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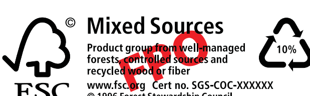

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CHIROBIOTIC

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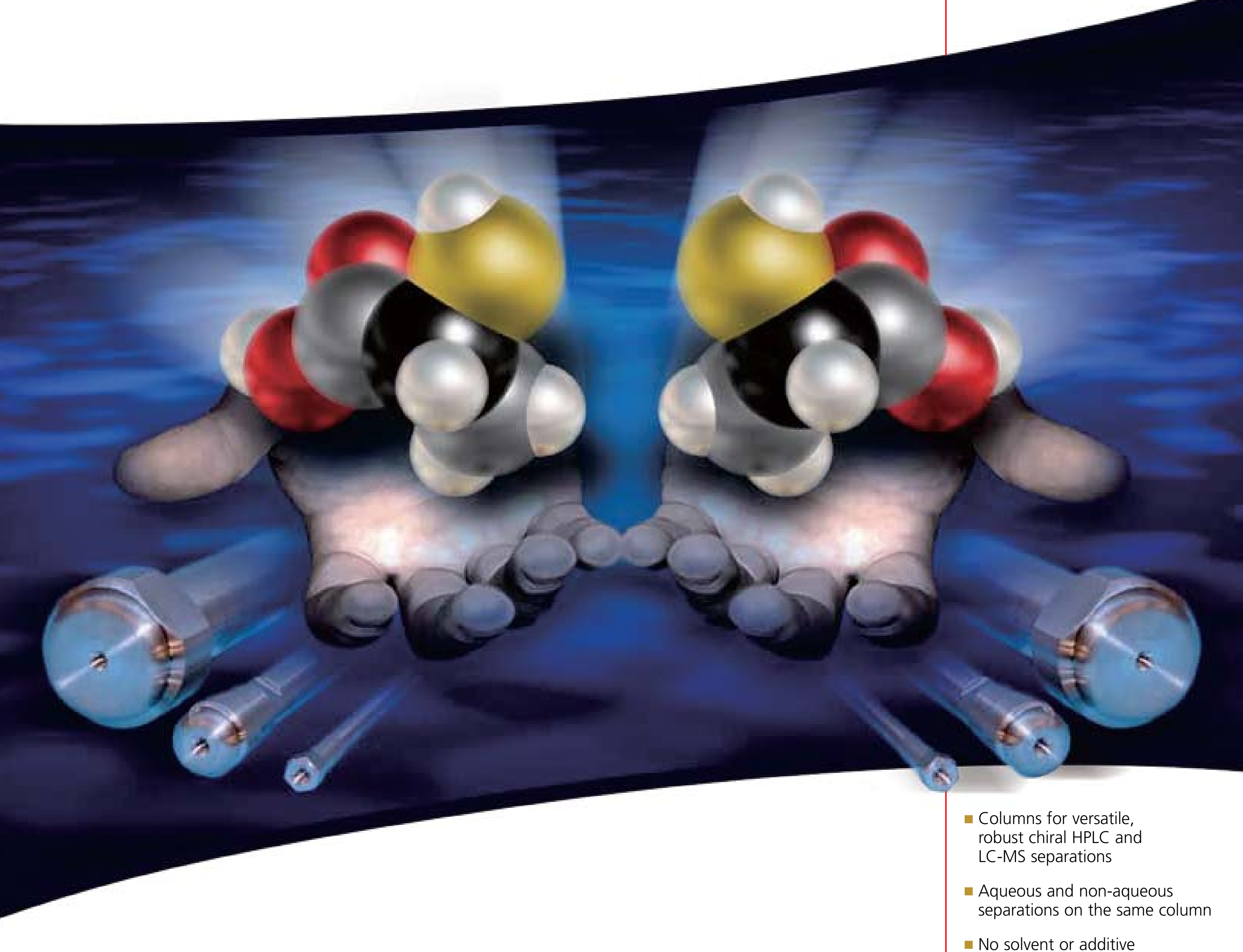
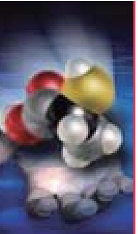
SUPELCO
Analytical



- Columns for versatile, robust chiral HPLC and LC-MS separations
- Aqueous and non-aqueous separations on the same column
- No solvent or additive memory effects
- Wide applicability, especially suited to polar and ionizable compounds
- Predictable scale-up from analytical to prep

SIGMA-ALDRICH®



CHIROBIOTIC

Versatile Chiral HPLC and LC-MS Separations of Polar, Ionizable and Neutral Compounds

CHIROBIOTIC™ CSPs (chiral stationary phases) interact with polar, ionizable and neutral analytes via multiple molecular interactions. This versatility means that the same CHIROBIOTIC column can be successfully used in a variety of mobile phases, a significant benefit over CSPs that operate only in a single mode, normal or reversed-phase, for example, and must be dedicated to those mobile phase systems. However, the most interesting feature of CHIROBIOTIC CSPs is the **presence of ionic interactions**, which allows them to be used in polar ionic and reversed-phase modes for sensitive LC-MS operation.

Key application areas

- **Drug Discovery** – High enantioselectivity, fast screening protocols, scalability to prep, reproducibility for reliable methods, polar and non-polar analytes
- **Organic Synthesis** – Compatible with all HPLC solvents to optimize sample solubility, fully scalable to prep
- **Bioanalytical, Drug Metabolism** – High throughput, MS-compatibility, aqueous samples, short run times, rugged columns
- **Amino Acid and Peptide Analysis** – Resolves underivatized natural and synthetic chiral amino acids and peptides, different selectivity and higher preparative capacity than C18 for achiral amino acids

What is the CHIROBIOTIC family?

Developed originally by Advanced Separations Technologies (Astec), the CHIROBIOTIC family comprises highly enantioselective CSPs based on macrocyclic glycopeptides that have been bonded through multiple covalent linkages to high purity silica particles. CHIROBIOTIC CSPs offer flexibility in choice of mobile phase conditions, both aqueous and non-aqueous, and are ideal for analytical and preparative separations of neutral, polar and ionic compounds.

How do CHIROBIOTIC CSPs separate enantiomers?

CHIROBIOTIC CSPs offer six different types of molecular interactions: ionic, H-bond, π-π, dipole, hydrophobic and steric. They also possess multiple inclusion sites that influence selectivity based on the molecular shape of the analyte. The optimization of enantiomer resolution is achieved by changing the mobile phase to leverage the types and relative strengths of the various interactions.

What makes CHIROBIOTIC CSPs unique?

The bonded macrocyclic glycopeptide itself (Figure 1), in terms of its morphology, molecular composition and multiple covalent linkages to the silica surface, is what makes CHIROBIOTIC CSPs unique and gives them significant and valuable benefits over other CSPs. The truly differentiating feature of CHIROBIOTIC CSPs is the presence of ionic interactions. These interactions are unique to CHIROBIOTIC CSPs and are responsible in large part for their desirable retention characteristics toward polar and ionizable analytes in aqueous and non-aqueous solvents.

How do the CHIROBIOTIC CSPs differ?

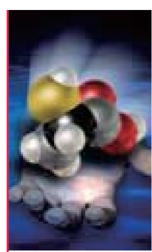
The various CHIROBIOTIC phases share the benefits of robustness, flexibility in mobile phase options, ionic interactions, compatibility with polar compounds and LC-MS and preparative scalability. However, CHIROBIOTIC CSPs differ in selectivity, primarily because of their differing number and types of interaction sites, and the number, type and accessibility of ionic sites in the bonded macrocyclic glycopeptide.

The CHIROBIOTIC CSP Family

CHIROBIOTIC CSPs are based on 5, 10 or 16 µm, high purity, porous silica gel. They differ in the nature of the bonded macrocyclic glycopeptide and resulting enantioselectivity.

- **CHIROBIOTIC V and V2*** – Vancomycin
- **CHIROBIOTIC T and T2*** – Teicoplanin
- **CHIROBIOTIC R** – Ristocetin
- **CHIROBIOTIC TAG** – Teicoplanin Aglycone

*CHIROBIOTIC V and T differ from V2 and T2, respectively, in their bonding chemistry that gives them different selectivity and preparative capacity for certain classes of analytes.

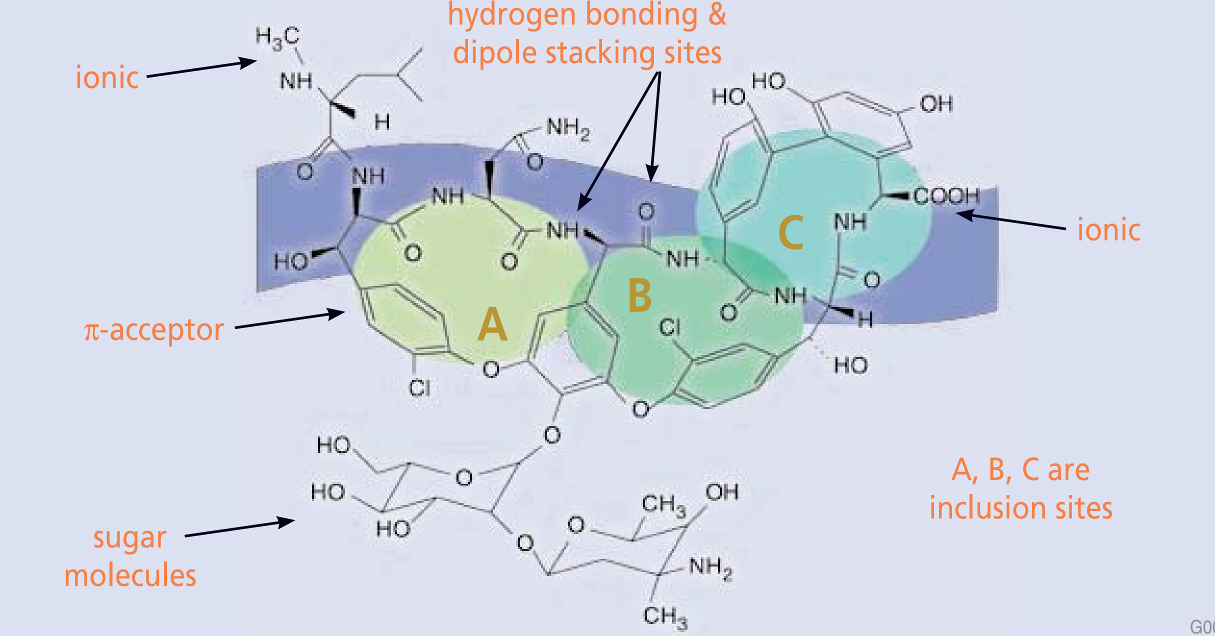


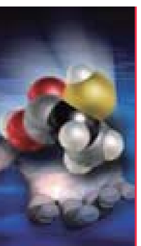
Key features of CHIROBIOTIC CSPs:

- **Aqueous and non-aqueous separations on the same column** – CHIROBIOTIC CSPs have H-bond, ionic, dispersive, π-π, dipole stacking, steric and inclusion mechanisms, usually multiple types of interactions per analyte.
- **Wide applicability** – Applications cover a very broad range of compound classes, with the different CHIROBIOTIC CSPs showing complementary selectivity.
- **LC-MS compatibility** – The wide choice of mobile phases makes CHIROBIOTIC CSPs ideal for LC-MS, where analyte ionization and detection sensitivity are of critical concern.
- **No solvent or additive memory effect** – The same CHIROBIOTIC column can be used alternately in polar, reversed-phase and normal phase solvents without damage, unlike cellulosic and amylosic phases that require dedicated operation.
- **Robust columns with long lifetimes** – Each macrocyclic glycopeptide molecule is linked to the silica surface via four or five covalent bonds for exceptional stability and long column life. They are designed to withstand high pressure and flow rates, as well as rapid changes in mobile phase conditions.
- **Solvent choices maximize sample solubility** – CHIROBIOTIC CSPs operate in highly aqueous and non-aqueous polar mobile phases for polar compound solubility. They also operate in normal phase mobile phases to maximize solubility of non-polar compounds. CHIROBIOTIC CSPs are compatible with all organic solvents.
- **Excellent preparative scalability and capacity** – From narrowbore to prep, separations on CHIROBIOTIC are fully scalable, even with polar analytes. By relying on primarily aqueous eluents, the use and disposal of toxic organic solvents are eliminated. Additionally, preparative methods in the non-aqueous polar ionic mode are just as easy to process as normal phase solvents.
- **Fast kinetics for speed and efficiency** – The kinetics of the molecular interactions between the analyte and the CHIROBIOTIC CSP are fast, providing efficient separations and relatively short retention times.
- **Orthogonal selectivity to other CSPs** – The six CHIROBIOTIC CSPs are different from each other, and from other types of CSPs to offer choices in enantioselectivity, like reversal of elution order.

Figure 1. Proposed Structure of Vancomycin-based CHIROBIOTIC V and V2

CHIROBIOTIC chiral HPLC stationary phases are macrocyclic glycopeptides attached to the surface of high purity, porous silica by four or more covalent bonds. Vancomycin is shown here, but all four CHIROBIOTIC CSPs possess multiple functional groups that can undergo different types of interactions. The CHIROBIOTIC surface is essentially ionic, allowing the use of polar ionic and aqueous mobile phases. Additionally, the glycopeptide structure has several inclusion sites that provide selectivity based on the molecular structure of the analyte.





Incorporating CHIROBIOTIC CSPs into Your Chiral Column Screening Protocol

We recommend that you incorporate CHIROBIOTIC into your routine screening protocol. Experience has shown that one or more of the CHIROBIOTIC CSPs, particularly V2, T and TAG, will perform the majority of chiral separations. Even if other CSPs give adequate resolution, a CHIROBIOTIC CSP may allow use of mobile phases that are better suited to your sample and detection method, or the CHIROBIOTIC method may be faster, more efficient or more robust. A CHIROBIOTIC method may also have advantages from a preparative standpoint in terms of solvent selection and sample capacity.

For developing a new chiral HPLC method, we have created and use routinely in our laboratories a simple and rapid chiral column screening protocol (Table 1). It is important to keep in mind that a single CHIROBIOTIC column possesses multiple types of molecular interactions that are different in each of the four distinct modes. The same column can be exposed to all of the conditions outlined in the screening protocol shown in Table 1 without any change or loss of performance. This versatility is just one advantage that CHIROBIOTIC CSPs have over other CSPs.

The four CHIROBIOTIC CSPs we recommend in the screening protocol are available in 25 cm or 10 cm column kits. Also, you can further expand the screening field by incorporating the CYCLOBOND™ bonded cyclodextrin and P-CAP™ chiral polymer CSPs into your screening protocol to accommodate other types of compounds not covered by the routine screen.

Table 1. The CHIROBIOTIC Screening Protocol

columns: CHIROBIOTIC V2, T, R and TAG
procedure: Method development follows a simple strategy that tests polar ionic, polar organic, reversed-phase and normal phase modes.

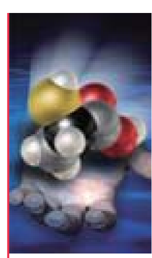
Separation Mode	Description	Types of Compound	Screening Mobile Phase	Parameters to Optimize
Polar Ionic	Polar organic solvents (CH_3OH or CH_3CN) containing small amounts of acid and base or salt	Acids, Bases, Zwitterions	(100:0.1:0.1, v:v:v) CH_3OH:Acetic Acid: Triethylamine	Change acid-base ratio, change the type of acid or base, add a volatile salt (test different ammonium salts)
Reversed-Phase	Typical RP eluents, water or buffers with CH_3OH or CH_3CN	Acids, Bases, Zwitterions, Neutrals	(30:70) CH_3CN:20mM Ammonium Acetate, pH 4.0	Change the % and type of organic modifier, adjust pH, buffer type and ionic strength
Polar Organic	Polar organic solvents without ionic additives	Neutrals	100% Ethanol	Use other polar organic solvents or blends
Normal Phase	Non-polar organic solvents with polar solvent modifiers	Neutrals	(30:70) Ethanol:Heptane	Increase % of polar modifier, change both solvents

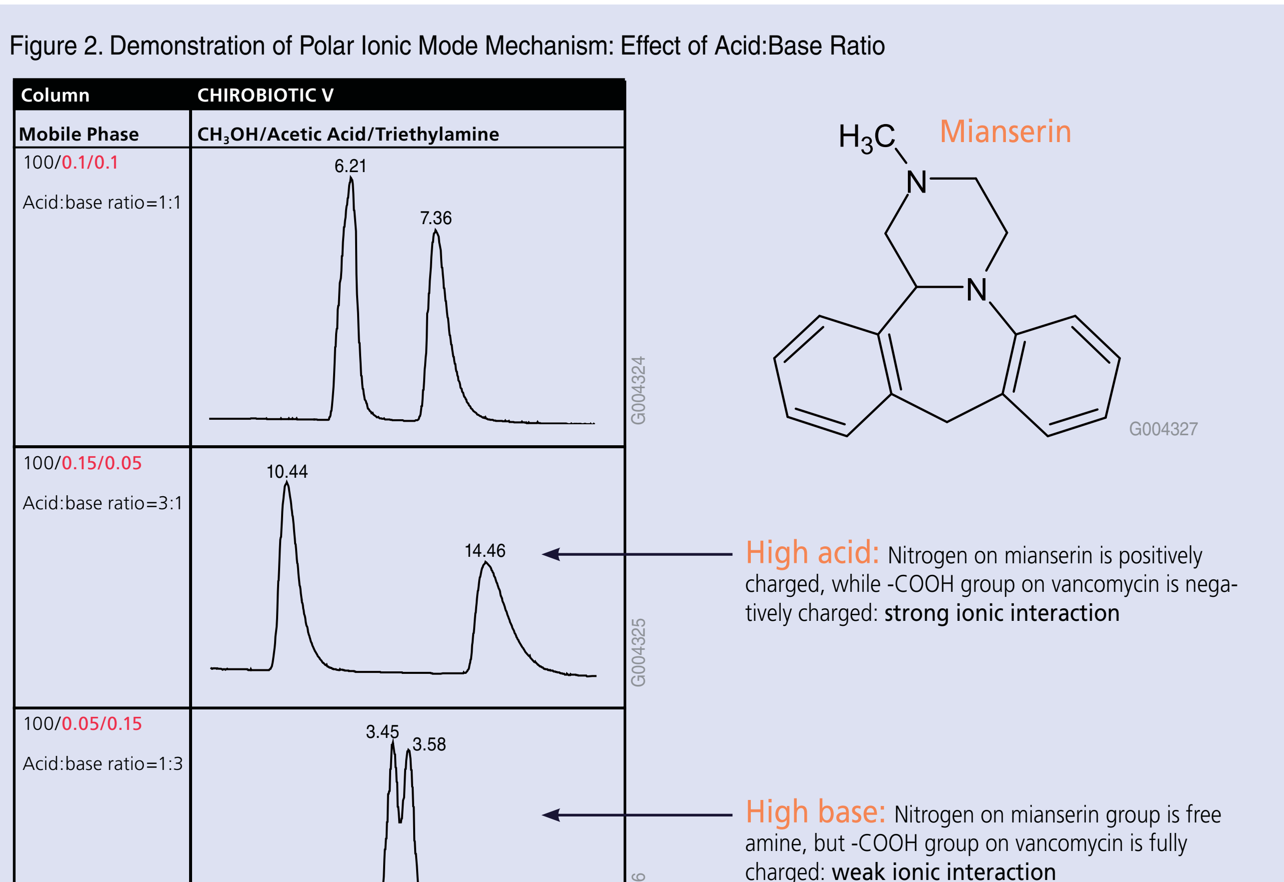
Method Optimization: Acid-Base Ratio, Temperature and Flow Rate in Polar Ionic Mode

Using CHIROBIOTIC CSPs in the polar ionic mode has the highest probability of success. Optimizing resolution usually involves changing the contribution to retention of ionic interactions between the analytes and functional groups in the macrocyclic glycopeptide structure by:

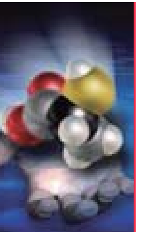
- Changing the ratio of acid to base (Figure 2)
- Adding a soluble, volatile salt (instead of the acid and base) directly to the methanol

The acid, base or salt that is ultimately selected is based on its compatibility with the detection method (e.g. LC-MS), sample solubility and whether the separation will be scaled up to preparative.





TRADEMARKS:
CHIROBIOTIC, CYCLOBOND, HybridSPE, P-CAP – **Sigma-Aldrich Biotechnology LP**



CHIROBIOTIC: Ideally Suited for LC-MS of Polar, Ionizable and Neutral Compounds

Each of the various ionization sources has an optimal set of mobile phase conditions. Outside this set, ionization may be suppressed with resulting loss in sensitivity. CHIROBIOTIC phases are uniquely able to operate across all mobile phase systems. CSPs that are limited to normal phase operation, like many cellulosic and amylosic CSPs, reduce the options in detection methods.

ESI – Operate CHIROBIOTIC CSPs in reversed-phase and unique polar ionic modes.

APCI – Operate CHIROBIOTIC CSPs in polar ionic mode.

APPI – Operate CHIROBIOTIC CSPs in normal phase mode.

Typical polar ionic mobile phases are methanol with low concentrations (0.1 – 0.001%) of volatile salts like ammonium acetate or ammonium formate. Figures 3 and 4 show examples of CHIROBIOTIC CSPs for LC-MS in reversed-phase and polar ionic mode mobile phases, respectively.

In addition to mobile phase compatibility, the allowable high flow rates and short columns make them ideally suited to fast MS applications.

CHIROBIOTIC columns can be used in conjunction with HybridSPE™-PPT plates to enhance sensitivity by completely removing endogenous proteins and phospholipids. This approach was used to resolve the enantiomers of clenbuterol on a CHIROBIOTIC T column in Figure 4.

Figure 3. ESI-MS of Ketoprofen on CHIROBIOTIC R in Reversed-phase Mode

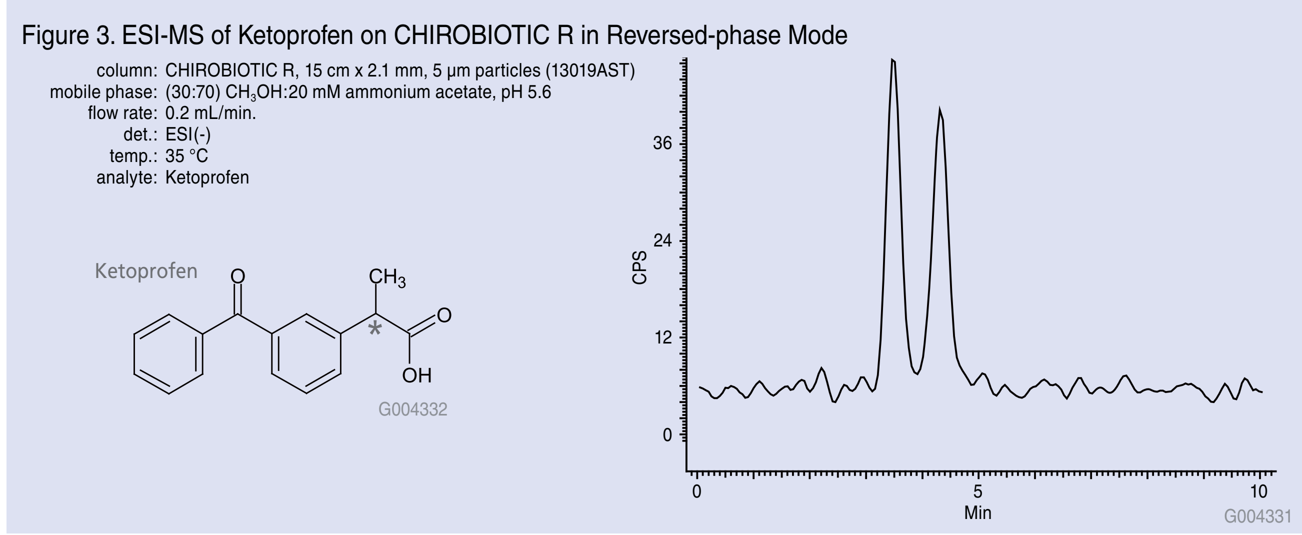
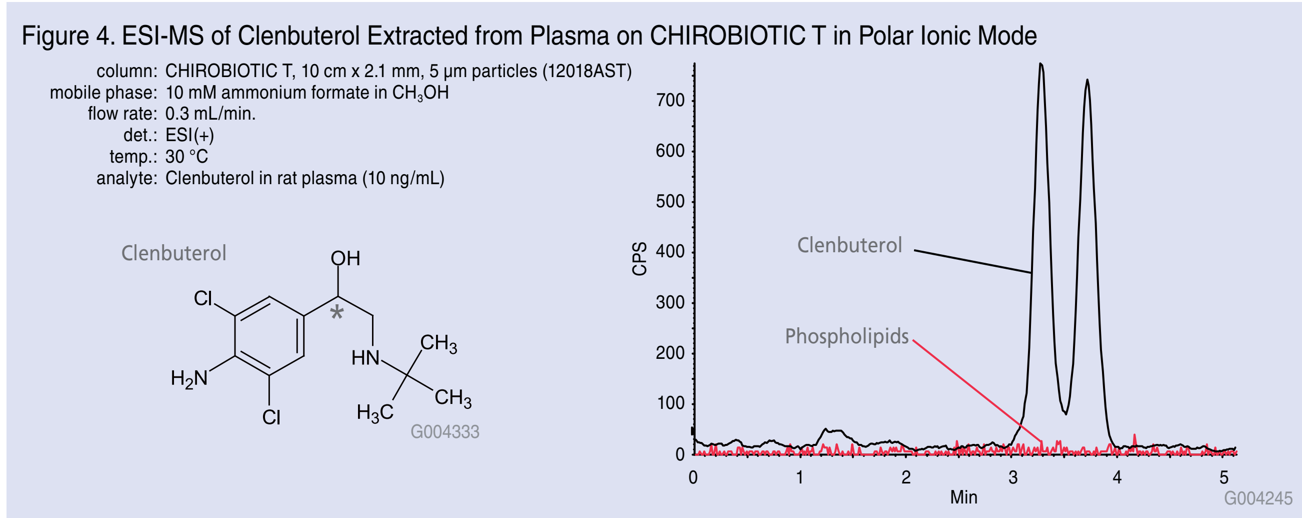


Figure 4. ESI-MS of Clenbuterol Extracted from Plasma on CHIROBIOTIC T in Polar Ionic Mode

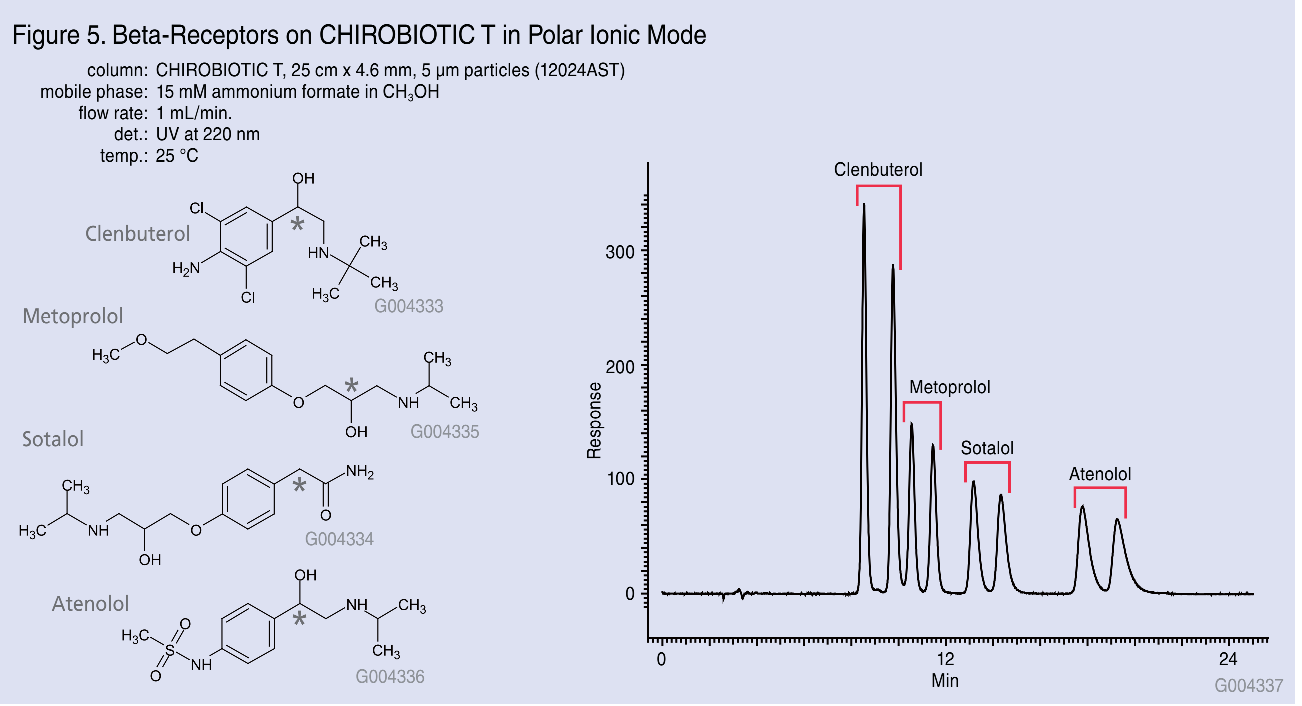


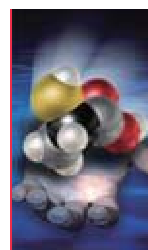
Unique Polar Ionic Mode

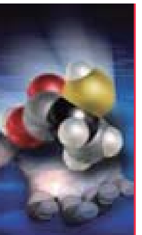
A valuable feature of CHIROBIOTIC CSPs, the novel and very versatile polar ionic mode is popular because its mobile phases are polar organic solvents containing volatile additives that are ideally suited for preparative and LC-MS applications. An example is shown in Figure 5. Additionally, compared to normal phase separations, the polar ionic mode has speed, efficiency and sensitivity advantages, all valuable assets for LC-MS.

Figure 5. Beta-Receptors on CHIROBIOTIC T in Polar Ionic Mode

column: CHIROBIOTIC T, 25 cm x 4.6 mm, 5 µm particles (12024AST)
mobile phase: 15 mM ammonium formate in CH_3OH
flow rate: 1 mL/min.
det.: UV at 220 nm
temp.: 25 °C







Multi-modal Interactions Permit Use in Aqueous and Non-aqueous Solvents

All CHIROBIOTIC CSPs possess multiple interaction sites on the same column. Changing the mobile phase affects the relative strength of specific types of interactions. The power and flexibility of multi-modal CHIROBIOTIC CSPs are demonstrated in Figures 6 through 10. The vancomycin-based CHIROBIOTIC CSPs were used successfully in four different modes.

Polar Ionic Mode

A valuable feature of CHIROBIOTIC, the novel and very versatile polar ionic mode mobile phase system is desirable because of its high volatility and beneficial ionization effect for LC-MS (Figure 6).

Reversed-phase Mode

Also highly suitable for LC-MS and polar analytes, reversed-phase (RP) is a mode familiar to all chromatographers. CHIROBIOTIC CSPs have RP character and can be used in a wide range of buffers and solvents (Figure 7).

Polar Organic Mode

Enantiomers of polar neutral analytes have been successfully separated on CHIROBIOTIC in the polar organic mode where the mobile phase is typically a polar organic solvent or solvent blend. Reaction mixtures, even in pyridine, can be run on CHIROBIOTIC in this mode (Figure 8).

Normal Phase Mode

Normal phase chiral separations are desirable to maintain solubility of hydrophobic compounds and when analyzing reaction mixtures in non-polar organic solvents. CHIROBIOTIC CSPs have the flexibility to operate in normal phase mode. The same column can be used with normal phase and polar/aqueous solvents and additives without memory effects (Figure 9).

Figure 6. Polar Ionic Mode

column: CHIROBIOTIC V2, 25 cm x 4.6 mm, 5 µm particles (15024AST)
mobile phase: 15 mM ammonium formate in CH_3OH
flow rate: 1 mL/min.
det.: UV at 230 nm
temp.: 25 °C
analyte: Fluoxetine

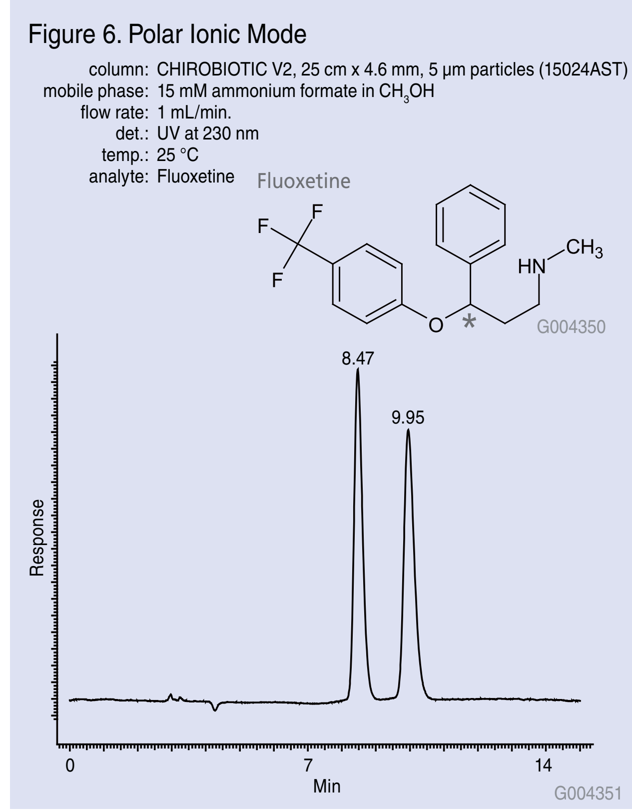
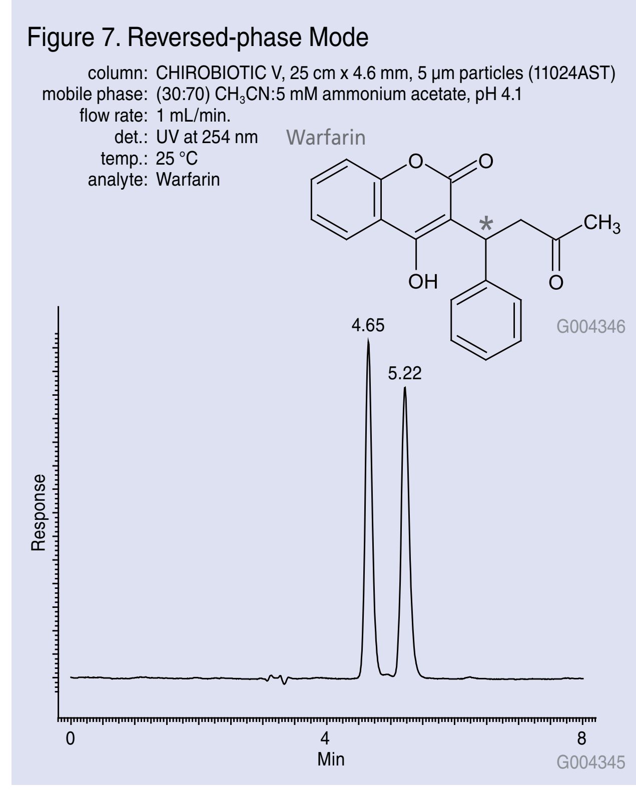


Figure 7. Reversed-phase Mode

column: CHIROBIOTIC V, 25 cm x 4.6 mm, 5 µm particles (11024AST)
mobile phase: (30:70) CH_3CN:5 mM ammonium acetate, pH 4.1
flow rate: 1 mL/min.
det.: UV at 254 nm
temp.: 25 °C
analyte: Warfarin



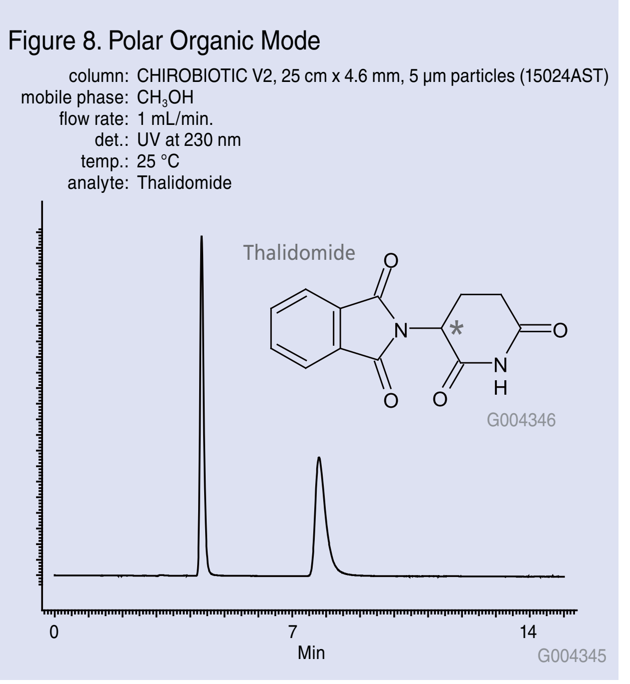


Figure 8. Polar Organic Mode

column: CHIROBIOTIC V2, 25 cm x 4.6 mm, 5 µm particles (15024AST)
mobile phase: CH_3OH
flow rate: 1 mL/min.
det.: UV at 230 nm
temp.: 25 °C
analyte: Thalidomide

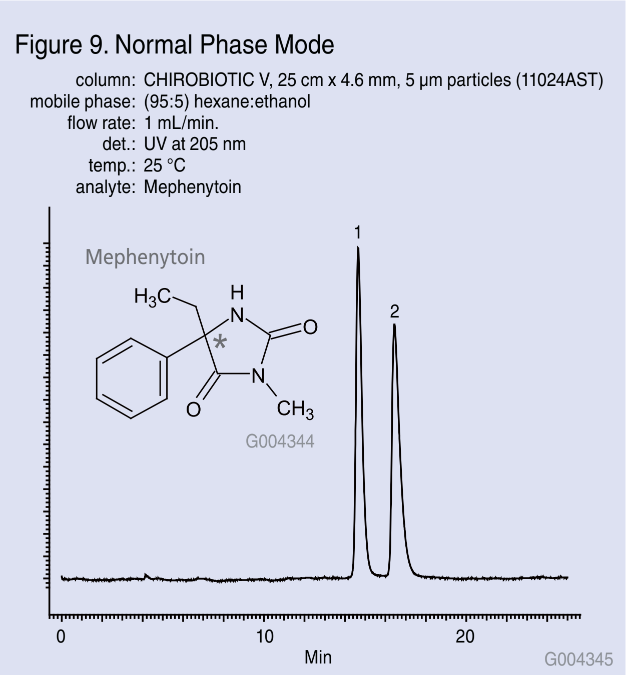


Figure 9. Normal Phase Mode

column: CHIROBIOTIC V, 25 cm x 4.6 mm, 5 µm particles (11024AST)
mobile phase: (95:5) hexane:ethanol
flow rate: 1 mL/min.
det.: UV at 205 nm
temp.: 25 °C
analyte: Mephenytoin

Preparative Applications Using CHIROBIOTIC CSPs

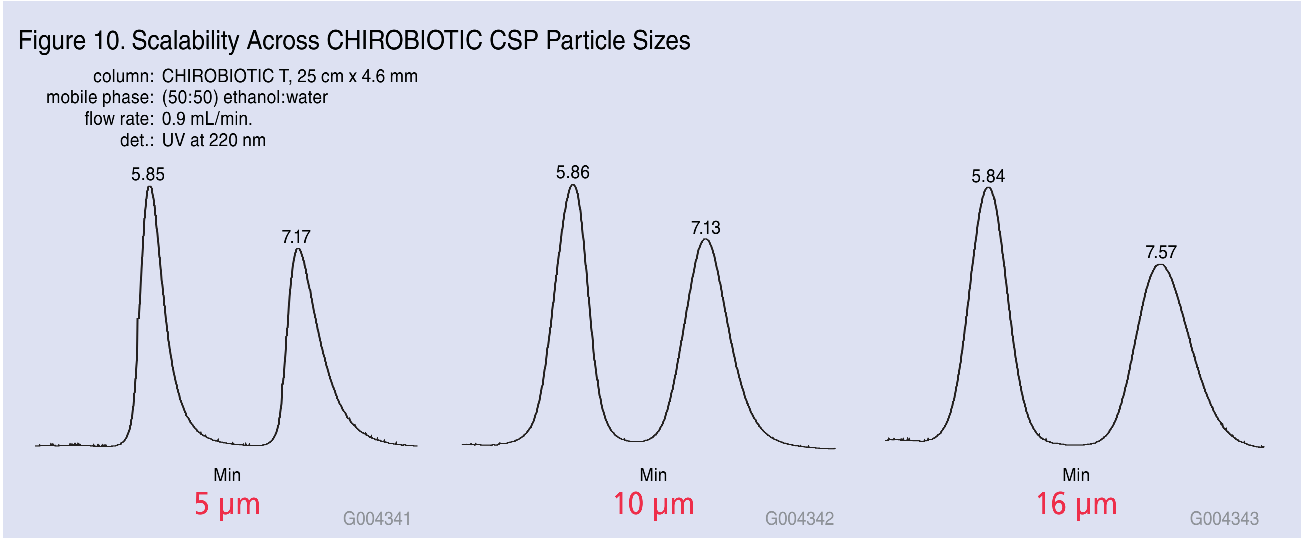
- Scalability across all CHIROBIOTIC particle sizes
- Low retention times give high throughput

CHIROBIOTIC columns can be used in all preparative HPLC techniques, including elution and recycle chromatography, mass-directed prep, SFC and simulated moving bed (SMB). Scale-up is highly predictable because the same bonded phase chemistry is employed across all particle sizes. Multiple covalent bonds attach the CHIROBIOTIC macrocyclic glycopeptides to the silica surface, meaning no CSP ligand will contaminate the product. Preparative separations on CHIROBIOTIC columns often have speed and efficiency benefits over other CSPs. In terms of loading capacity, a 25 cm x 21.2 mm column has medium to high loadings, from a few mg to over 300 mg per injection.

Prep separations on CHIROBIOTIC are reproducible and scalable. Figure 10 shows the separation of phenylalanine isomers in reversed-phase mode on columns packed with 5, 10, and 16 µm particles of CHIROBIOTIC T.

Figure 10. Scalability Across CHIROBIOTIC CSP Particle Sizes

column: CHIROBIOTIC T, 25 cm x 4.6 mm
mobile phase: (50:50) ethanol:water
flow rate: 0.9 mL/min.
det.: UV at 220 nm



Preparative Applications (contd.)

A significant advantage of CHIROBIOTIC for preparative applications is the fact that the mobile phase can be chosen to optimize sample solubility – a critical preparative consideration. The examples here show preparative CHIROBIOTIC separations in three different mobile phase systems.

Preparative Reversed-phase and Polar Ionic Modes

Preparative separations in reversed-phase and polar ionic mode solvents have benefits over normal phase preparative separations in terms of solvent safety and waste disposal costs. Figure 11 shows the use of CHIROBIOTIC TAG in a preparative separation in polar ionic mode.

Preparative Polar Organic Mode

Figure 12 shows the analytical and preparative separations of thalidomide enantiomers on CHIROBIOTIC T. The analytical scale gave an α value of 3.35 in 100% methanol and a retention time under 10 minutes. However, since thalidomide is fairly insoluble in pure methanol, it was possible to add 20% dioxane to the mobile phase to increase solubility 3.5-fold while still achieving the necessary separation.

Figure 11. Preparative Separation on CHIROBIOTIC TAG in Polar Ionic Mode

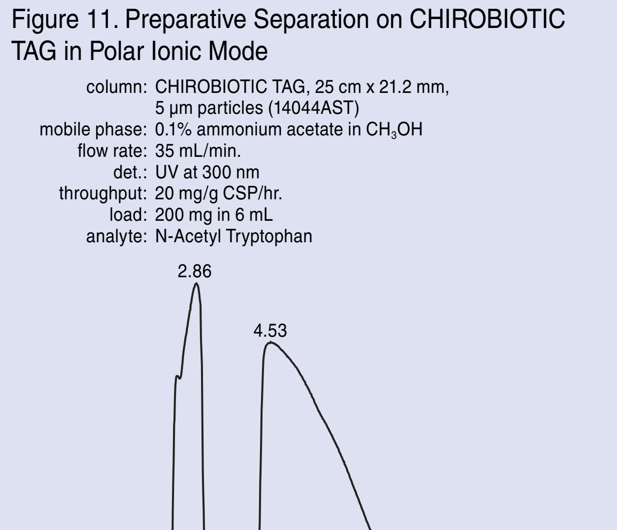
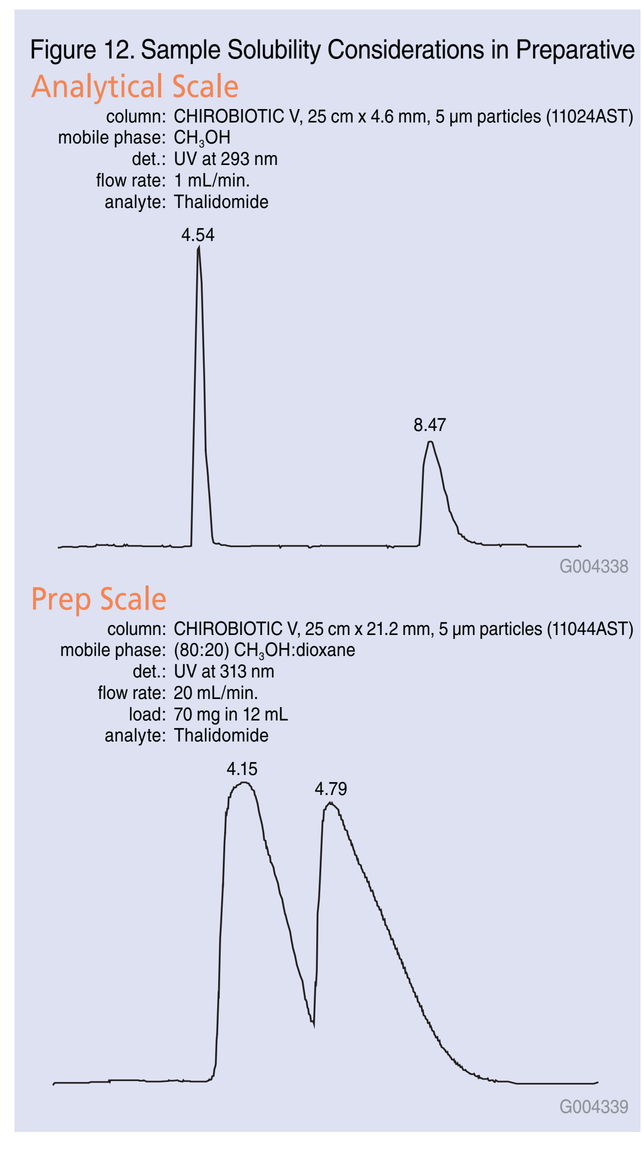


Figure 12. Sample Solubility Considerations in Preparative



CHIROBIOTIC V and V2

Bonded Macrocyclic Glycopeptide:	Vancomycin
Chiral Centers:	18
Sugar Moieties:	2
Inclusion Cavities:	3

Separates a wide variety of secondary and tertiary amines in the polar ionic mode. Have many of the separation characteristics of protein-based stationary phases but with exceptional stability and much higher sample capacity. CHIROBIOTIC V2 and V differ in the chemistry used to bond the glycopeptide to the silica, which gives them differences in selectivity.

CHIROBIOTIC T and T2

Bonded Macrocyclic Glycopeptide:	Teicoplanin
Chiral Centers:	23
Sugar Moieties:	3
Inclusion Cavities:	4

These CSPs have resolved all of the known beta-blockers and dihydrocoumarins and many other compound classes. Generally reproduces chiral crown ether or ligand-exchange for amino acid separations. CHIROBIOTIC T2 and T differ in the chemistry used to bond the glycopeptide to the silica, which gives them differences in selectivity.

CHIROBIOTIC TAG

Bonded Macrocyclic Glycopeptide:	Teicoplanin Aglycone
Chiral Centers:	8
Sugar Moieties:	0
Inclusion Cavities:	4

The removal of the three sugar moieties enhances resolution of many of the amino acids (alpha, beta, gamma and cyclic). CHIROBIOTIC TAG has shown remarkable selectivity for sulfur-containing molecules, such as sulfoxides and the amino acids methionine, histidine and cysteine. Neutral molecules, like oxazolidinones, hydantoins and diazepines, have shown enhanced resolution and, more remarkably, in single-solvent systems like methanol, ethanol and acetonitrile. Some acidic molecules have also shown increased selectivity.

CHIROBIOTIC R

Bonded Macrocyclic Glycopeptide:	Ristocetin A
Chiral Centers:	38
Sugar Moieties:	6
Inclusion Cavities:	4

The presence of amines in the ristocetin structure makes it a good choice when screening acidic compounds.

CHIROBIOTIC Product Listing

For more information and to review our complete offering of CHIROBIOTIC columns, please visit *sigma-aldrich.com/chiral*

Method Development Kits

ID (mm)	Length (cm)	Cat. No.	
4.6	10	10300AST	- One each of CHIROBIOTIC V2, T, TAG and R
4.6	25	10305AST	- One each of CHIROBIOTIC V2, T, TAG and R

CHIROBIOTIC Columns*

		V	V2	T	T2	TAG	R
ID (mm)	Length (cm)	Cat. No.	Cat. No.	Cat. No.	Cat. No.	Cat. No.	Cat. No.
5 µm							
2.1	15	11019AST	15019AST	12019AST	16019AST	14019AST	13019AST
2.1	25	11020AST	15020AST	12020AST	16020AST	14020AST	13020AST
4.6	5	11021AST	15021AST	12021AST	16021AST	14021AST	13021AST
4.6	10	11022AST	15022AST	12022AST	16022AST	14022AST	13022AST
4.6	15	11023AST	15023AST	12023AST	16023AST	14023AST	13023AST
4.6	25	11024AST	15024AST	12024AST	16024AST	14024AST	13024AST
10	25	11034AST	15034AST	12034AST	16034AST	14034AST	13034AST
10	50	11036AST	15036AST	12036AST	16036AST	14036AST	13036AST
21.2	25	11044AST	15044AST	12044AST	16044AST	14044AST	13044AST
10 µm							
4.6	25	11124AST	15124AST	12124AST	16124AST	14124AST	13124AST

*Other column dimensions, including guard columns and preparative dimensions are available on our website or by inquiring to *techservice@sial.com.*

Chiral Services: Column Screening and Small-Scale Purification

Consult Supelco to obtain a quotation for our expert services for chiral column screening (HPLC and GC), method development and optimization, as well as isolation of up to 10 grams of purified enantiomer.

The complete listing of our chiral HPLC and GC columns can be found at *sigma-aldrich.com/chiral*, our corporate chiral web portal, where you can view our other products for chiral chemistry, like chiral catalysts, building blocks, mobile phase additives, derivatization reagents and more.

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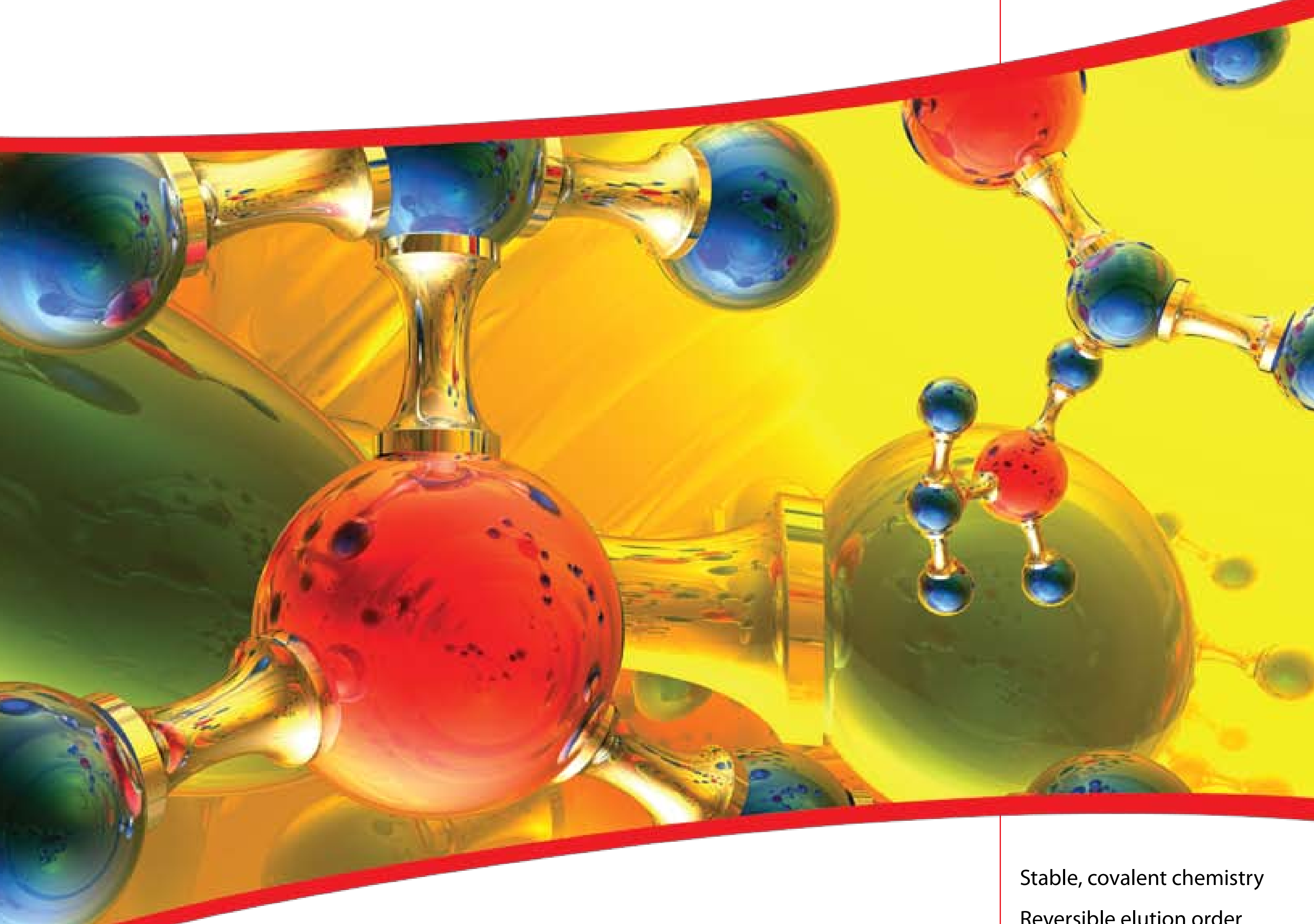
SIGMA-ALDRICH®

Astec P-CAP™ and P-CAP™-DP

SUPELCO
Analytical

Polycyclic amine polymer stationary phases for chiral HPLC



- Stable, covalent chemistry
- Reversible elution order
- No solvent limitations or memory effects
- High capacity for preparative applications
- MS and SFC-compatible

SIGMA-ALDRICH®

Chiral HPLC for Chemists: Ultimate Solvent Choice with High Capacity Using Astec's P-CAP and P-CAP-DP

Useful for chiral HPLC and SFC separations, Astec P-CAP and P-CAP-DP polymeric chiral stationary phases (CSPs) have a thin, ordered layer of chiral polymer covalently bonded to the silica surface. They offer high stability, high sample loadability, easy scale-up, and no memory effect.

Today's chiral HPLC columns too often give excellent enantioselectivity at the expense of solvent choice. Sample solubility and its link to preparative separations can mean that a compromise has to be reached between selectivity and solvent choice. Astec P-CAP and P-CAP-DP chiral HPLC columns have no solvent restrictions, so the user can select a solvent that provides optimum enantioselectivity and analyte solubility.

Astec P-CAP

- Bonded phase: Poly(trans-1,2-cyclohexanediyl-bis-acrylamide)
- Invented by Prof. Francesco Gasparrini (1), P-CAP is made from a diacryloyl -trans-1,2-diphenylethylenediamine polymerization, and utilizes hydrogen bonding and steric effects as enantiomer separation mechanisms.

Astec P-CAP-DP

- Bonded phase: Poly(diphenylethylenediamine-bis-acryloyl) or Poly-DPEDA
- Invented by Prof. Daniel Armstrong (2), Astec P-CAP-DP introduces phenyl rings to add π-π interactions, giving it one additional type of interaction compared to P-CAP. P-CAP-DP is less polar than P-CAP.

Solvent Choice and Reversal of Elution Order

The P-CAP and P-CAP-DP polyamide CSPs feature a thin, ordered polymer layer chemically bonded to 5 µm or 3.5 µm spherical silica using a patented radical polymerization. This gives the phases high permeability across the surface and, because they are synthetic, they can be identically manufactured in both R,R and S,S forms. This provides a predictable reversal of elution order in the same mobile phase (Figure 1).

The P-CAP phases have no solvent or additive memory effects, so the same column can be used in a number of different mobile phases without any detrimental effects. These phases form a new generation bridge between the traditional 'brush' type CSPs and the conventional polymeric phases.

Chiral method development is typically carried out either in normal phase (heptane/IPA or hexane/ethanol) or polar organic (acetonitrile/methanol) modes. For method optimization, a wide range of organic solvents can be used, from acetone to dichloromethane to dioxane, and many others. For acids and bases, the addition of 0.1% TFA often

Figure 1. Predictable Elution Order Reversal

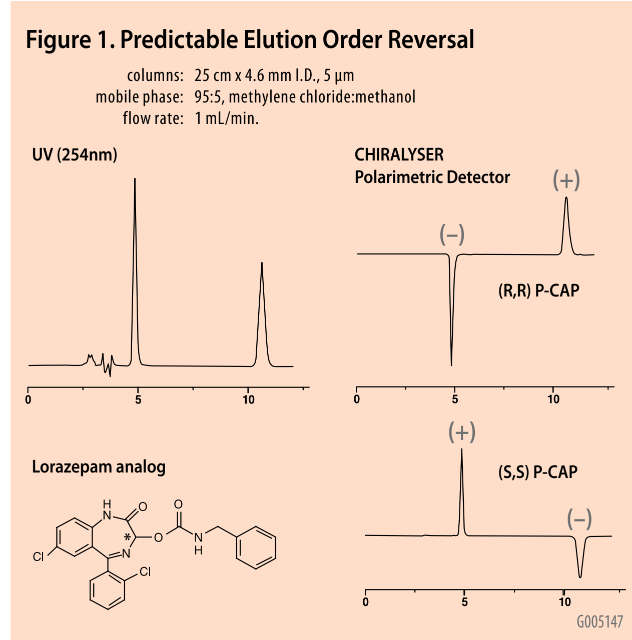
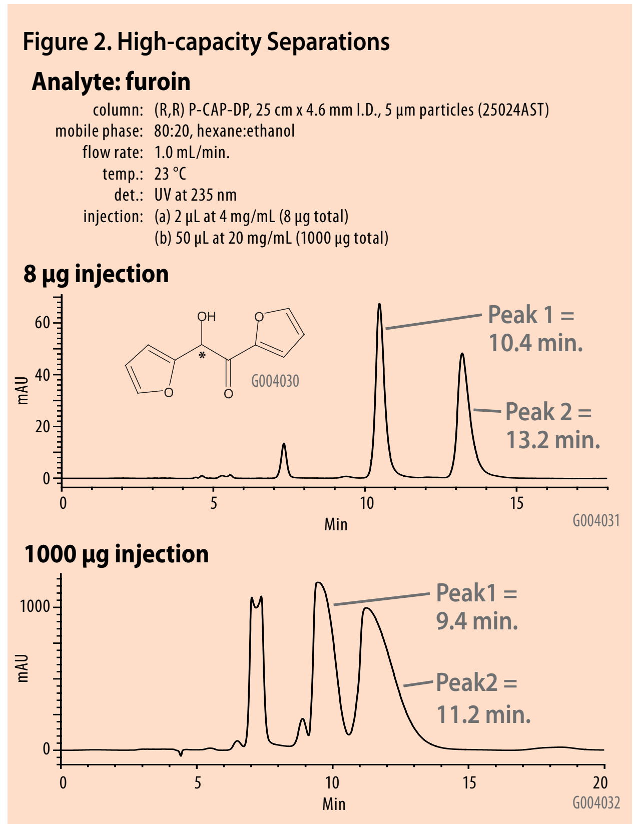


Figure 2. High-capacity Separations



increases resolution and efficiency and decreases retention times. There are no known limitations on the kind of solvents that can be used with these phases. For MS detection, volatile acids and buffers such as ammonium acetate can be added to enhance peak efficiency or to enhance ionization when needed.

High Capacity for Preparative HPLC

Solvent flexibility and high loading capacity make P-CAP and P-CAP-DP CSPs ideal for analytical, preparative and process scale separations. The separation of furoin enantiomers is shown in Figure 2 using an analytical 25 cm x 4.6 mm Astec (R,R) P-CAP-DP column and mobile phase of 80:20 hexane:ethanol. Excellent separation is demonstrated with an injection of 8 µg of the furoin racemate. Increasing the load to 1 mg on this analytical column demonstrates the phase's high loading capacity.

MS-Compatible Operation

Astec P-CAP and P-CAP-DP operate in mobile phases that are amenable to MS-detection. Salt and/or acetic acid can be added to improve efficiency or enhance ionization and detection (Figure 3).

Applications

Astec P-CAP CSPs have been used for a wide variety of molecular types and are ideal for medium to high polarity compounds. The mechanism of separation is either through hydrogen bonding for P-CAP, or through both hydrogen bonding (donor and acceptor) with additional π-π interaction for the P-CAP-DP. Both also use dipole-dipole and steric interactions. Examples of the separations completed to date are shown in Table 1.

Table 1. Examples of P-CAP and P-CAP-DP Application Areas

Hydroxycarboxylic acids	Benzene sulfonamides
Alcohols	Binaphthols
Sulfoxides	Benzodiazepines
Esters	Phosphonic acids
Amides	Bis-Sulfones
N-blocked amino acids	Chromemones

Complementary to Other Astec CSPs

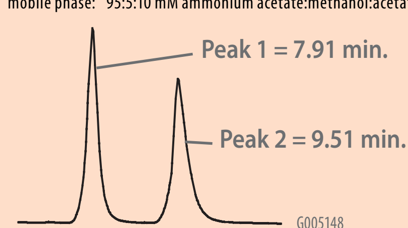
Astec P-CAP and P-CAP-DP are complementary to Astec CHIROBIOTIC, CYCLOBOND and the polysaccharide-based CSPs. We suggest you incorporate them into your chiral column screening protocol.

Figure 3. Improved Analytical Separation with MS-Compatible Mobile Phases

column: (R,R) P-CAP, 25 cm x 4.6 mm I.D., 5 µm particles (31024AST)
flow rate: 1.0 mL/min.
temp.: 25 °C
det.: UV @ 254 nm

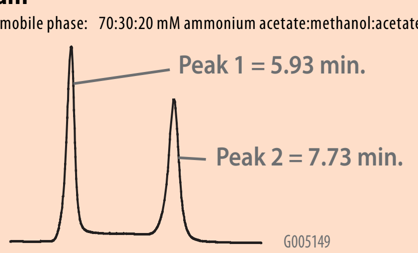
Separation of 1,1'-Bi-2-naphthol

mobile phase: 95:5:10 mM ammonium acetate:methanol:acetate



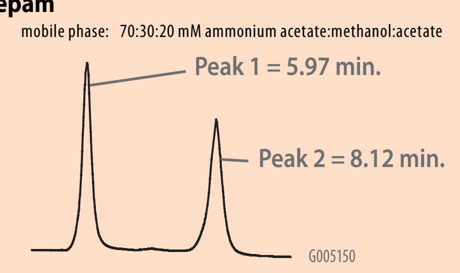
Oxazepam

mobile phase: 70:30:20 mM ammonium acetate:methanol:acetate



Lorazepam

mobile phase: 70:30:20 mM ammonium acetate:methanol:acetate



Summary

Astec P-CAP and P-CAP-DP are rugged chiral HPLC phases, experience no memory effects, and can be run in a wide variety of solvents with speed and high efficiencies. For preparative applications, a combination of wide solvent choice and high capacity make them ideal for large-scale purification.

References

1) New hybrid polymeric liquid chromatography chiral stationary phase prepared by surface initiated polymerization. Gasparrini, F.; Misiti, D.; Rompietti, R.; Villani, C. J Chromatogr, A. (2005), 1064(1), 25-38.

2) Chromatographic evaluation of poly(trans-1,2-cyclohexanediyl-bisacrylamide) as a chiral stationary phase for HPLC. Zhong, Qiqing; Han, Xinxin; He, Lingfeng; Beesley, Thomas E.; Trahanovsky, Walter S.; Armstrong, Daniel W. Department of Chemistry, Iowa State University, Ames, IA, USA. Journal of Chromatography, A (2005), 1066(1-2), 55-70.

Ordering Information

Particle Size (µm)	Length (cm)	I.D. (mm)	Cat. No.
Astec (R,R) P-CAP			
3.5	5	4.6	**30021AST**
3.5	10	4.6	**30022AST**
3.5	15	4.6	**30023AST**
5	5	4.6	**31021AST**
5	10	2.1	**31018AST**
5	10	4.6	**31022AST**
5	15	2.1	**31019AST**
5	15	4.6	**31023AST**
5	25	2.1	**31020AST**
5	25	4.6	**31024AST**
5	25	10	**31034AST**
5	25	21.2	**31044AST**
10	25	4.6	**31124AST**
Guards*			
5	2	1	**31101AST**
5	2	4	**31100AST**
Astec (S,S) P-CAP			
3.5	5	4.6	**32021AST**
3.5	10	4.6	**32022AST**
3.5	15	4.6	**32023AST**
5	5	4.6	**33021AST**
5	10	2.1	**33018AST**
5	10	4.6	**33022AST**
5	15	2.1	**33019AST**
5	15	4.6	**33023AST**
5	25	2.1	**33020AST**
5	25	4.6	**33024AST**
5	25	10	**33034AST**
5	25	21.2	**33044AST**
10	25	4.6	**33124AST**
Guards*			
5	2	1	**33101AST**
5	2	4	**33100AST**

Particle Size (µm)	Length (cm)	I.D. (mm)	Cat. No.
Astec (R,R) P-CAP-DP			
3.5	15	4.6	**34023AST**
5	15	2.1	**35019AST**
5	15	4.6	**35023AST**
5	25	4.6	**35024AST**
5	25	10	**35034AST**
5	25	21.2	**35044AST**
Guards*			
5	2	4	**35100AST**
Astec (S,S) P-CAP-DP			
3.5	15	4.6	**36023AST**
5	15	2.1	**37019AST**
5	15	4.6	**37023AST**
5	25	4.6	**37024AST**
5	25	10	**37034AST**
5	25	21.2	**37044AST**
Guards*			
5	2	4	**37100AST**

*Guard Column Holders	
Guard Holders for 4 mm I.D. cartridges (holder not required for 1 mm I.D. guards)	**21150AST**

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