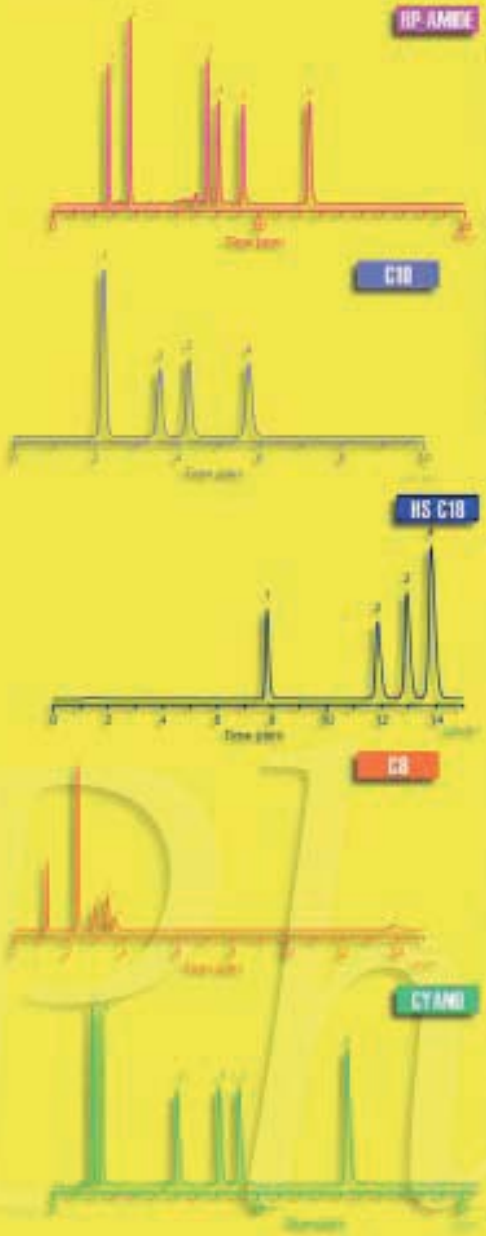
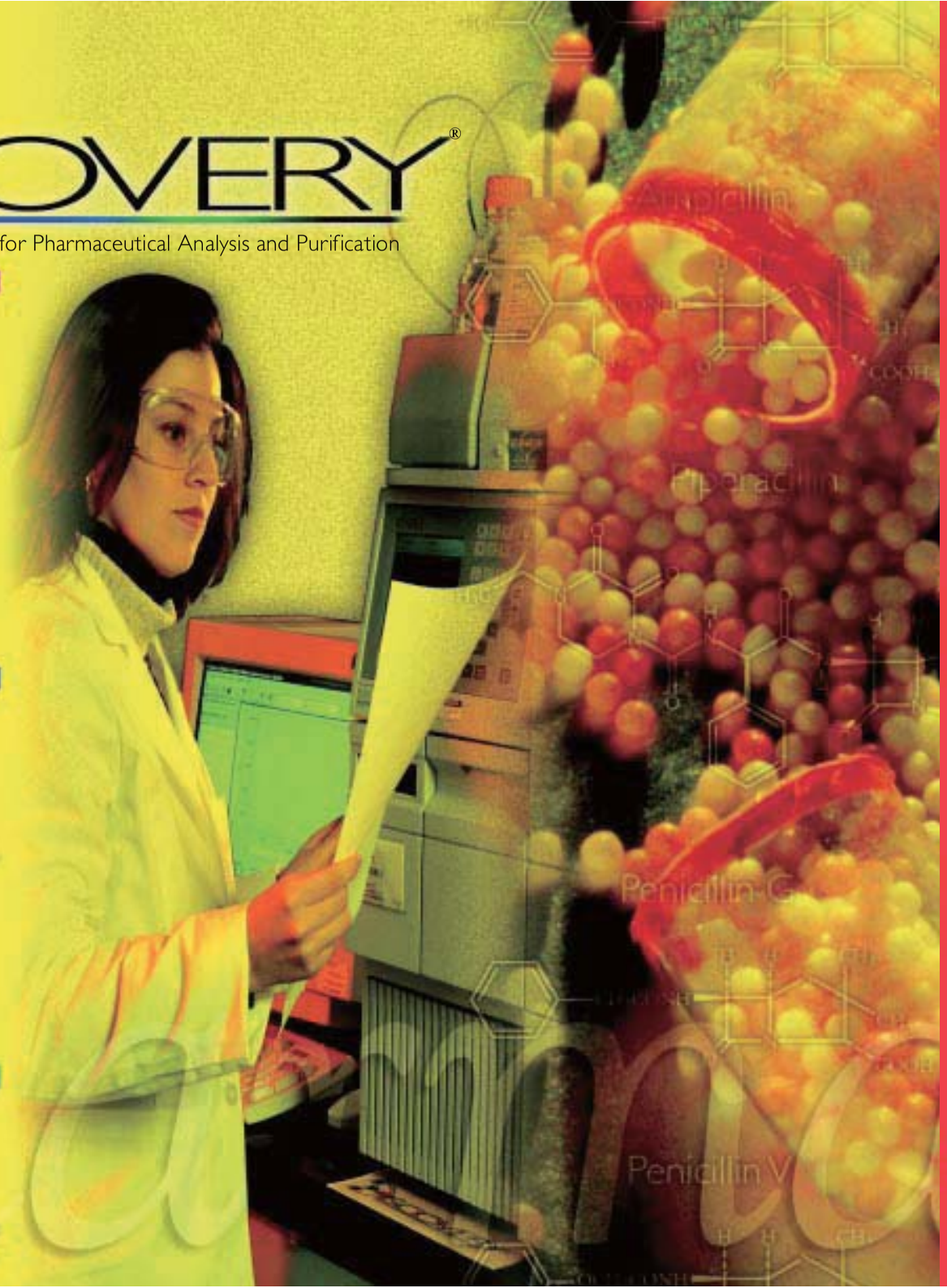
DISCOVERY®

HPLC and SPE Products for Pharmaceutical Analysis and Purification





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	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	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	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	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

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

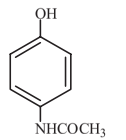
Step	Solvent/ Solution	Volume (mL)	Flow Rate (mL/min)	Comments
1. Condition	MeOH	2.0	5.0	conditions sorbent
2. Condition	H_2O	2.0	5.0	conditions sorbent
3. Load	spiked porcine serum	2.0[A]	0.75	applies serum sample
4. Rinse	5% MeOH	2.0	5.0	washes sample
5. Purge-Cannula	H_2O	4.0	30.0	cleans sample cannula
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.

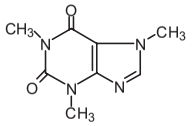
Acetaminophen and Caffeine from Serum

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

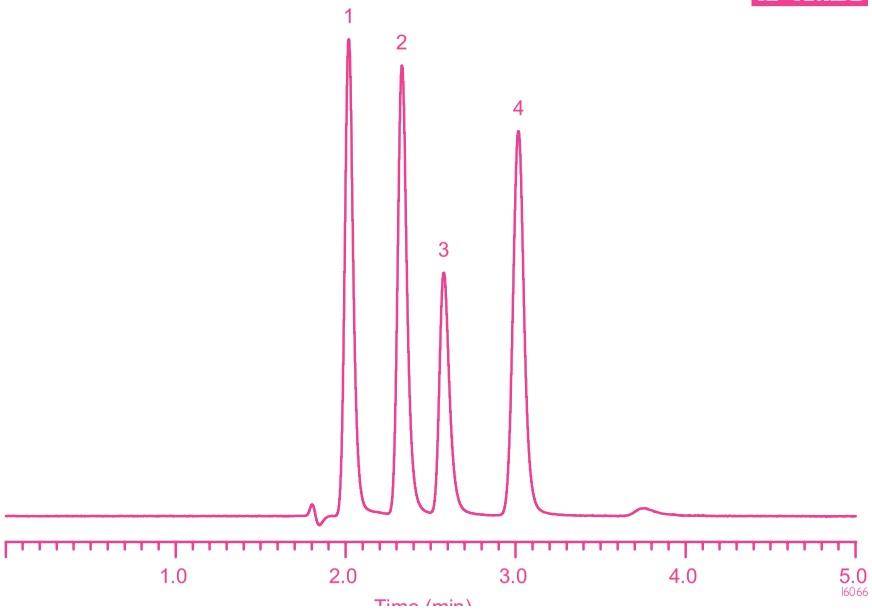
G000243, G000096

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, 1 µg/mL each analyte

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

RP-AMIDE

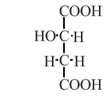


α-Hydroxy Aliphatic Acids

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.

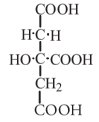
Tartaric acid

Malic acid



Lactic acid

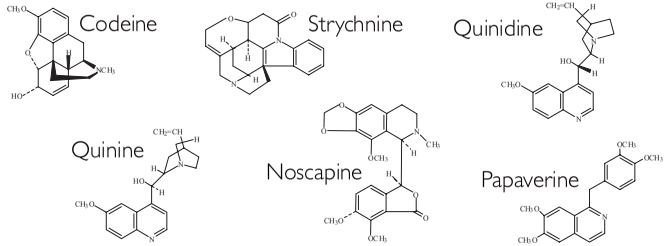
Citric acid



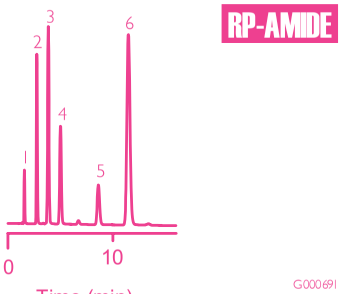
G001241, G001242
G001243, G001244

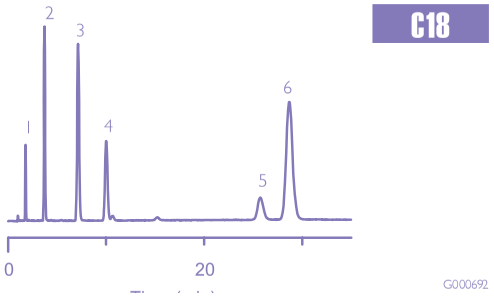
Alkaloids

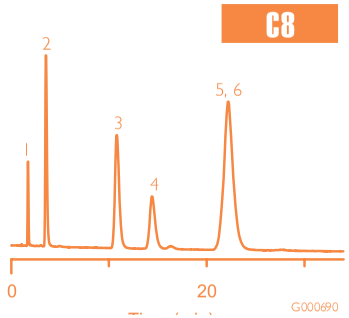
Alkaloids are naturally occurring bases with complex multicyclic ring structures. They are difficult candidates for separation based on RP-HPLC as they are amenable to hydrophobic as well as polar and ionic interactions with a silica-based reversed phase packing material. They can tail significantly 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







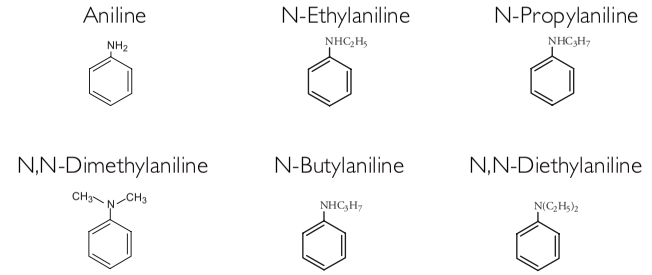
Alkaloids

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

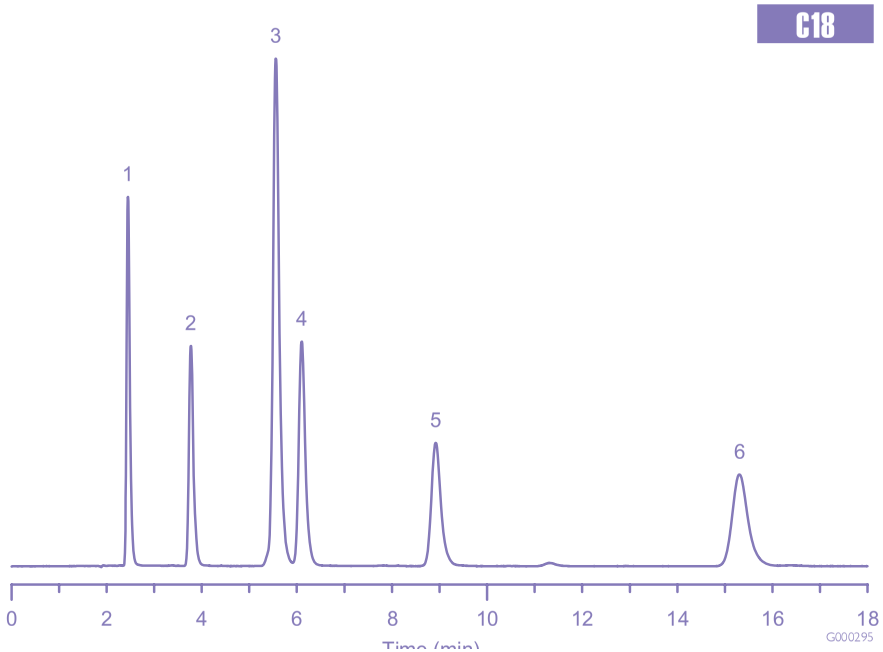
1. Codeine
2. Strychnine
3. Quinidine
4. Quinine
5. Noscapine
6. Papaverine

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.



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

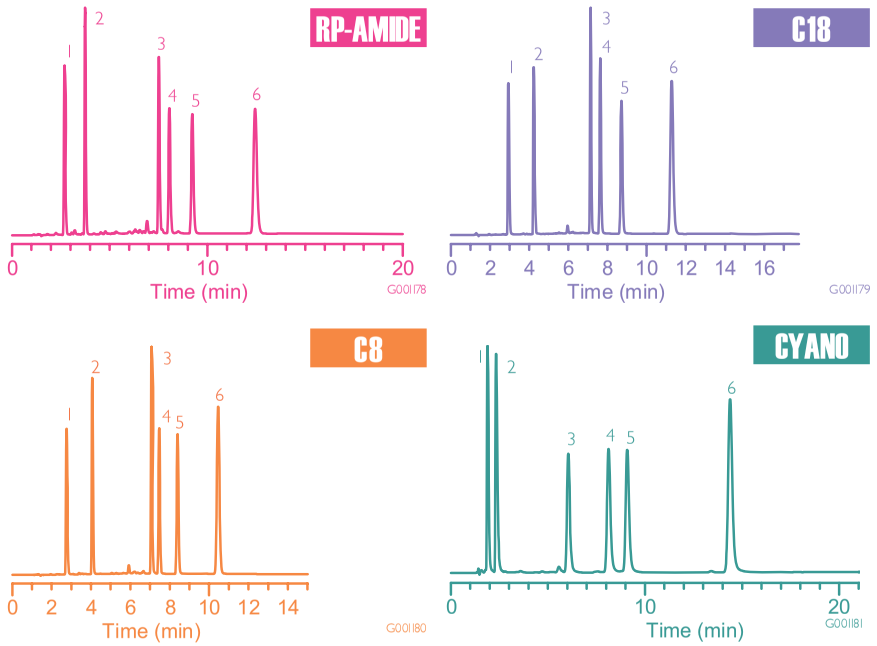
Gradient (RP-AmideC16, C18, C8)

min	%B
0	5
5	35
20	35

Gradient (Cyano)

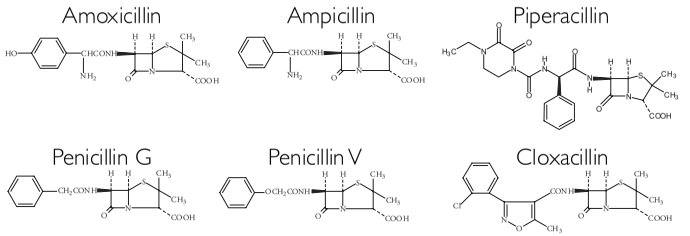
min	%B
0	5
20	25

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



Antibiotics (ß-Lactam):

ß-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.



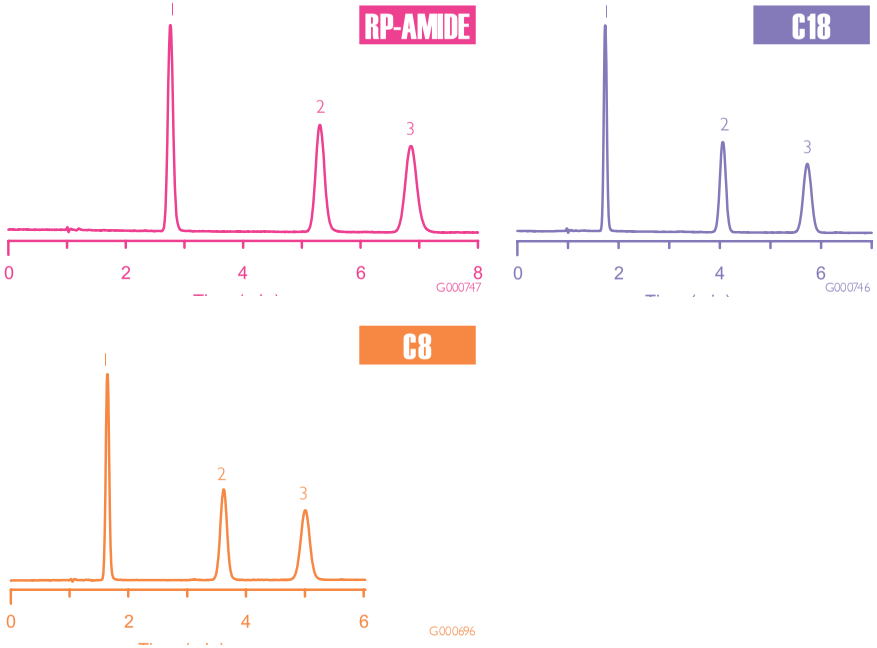
G001176, G001177, G000929, G001173, G001174, GG001175

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

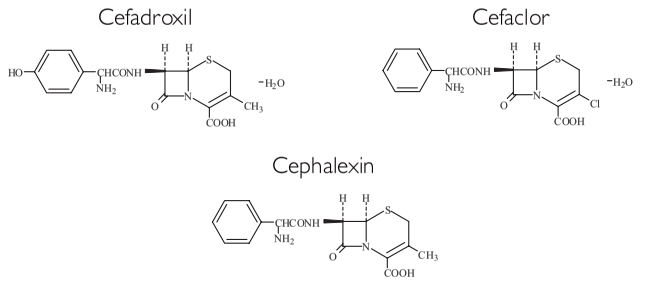
conditions for C8, C18:
15cm x 4.6mm column,
5µm particles,
MeOH:25mM KH_2PO_4,
pH 3.0 (20:80)
2mL/min, 20°C,
254nm, 1µL

1. Cefadroxil
2. Cefaclor
3. Cephalexin



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.

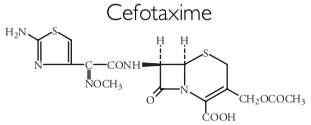


G000683, G000682, G000681

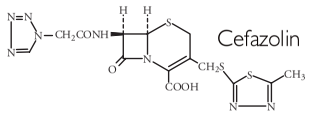
Antibiotics (Cephalosporins) from Serum

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%.

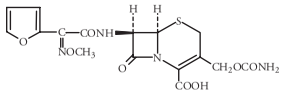
Cefotaxime



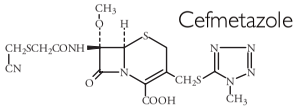
Cefazolin



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

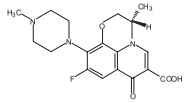
HPLC Conditions:
Discovery C18, 15cm x 4.6mm, 5µm particles, preceded by C18 guard column and 0.5µm frit filter
MeCN:20mM KH_2PO_4 pH 3.0 (adjusted with 10% phosphoric acid) (14:86)
1.2mL/min, 30°C, UV, 254nm
10µL reconstituted porcine serum

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

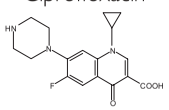
Antibiotics (Fluoroquinolones from Tablets)

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').

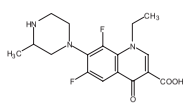
Levofloxacin



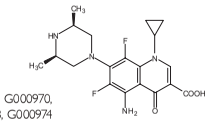
Ciprofloxacin



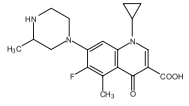
Lomefloxacin



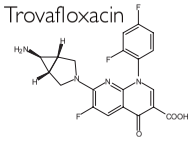
Sparfloxacin



Grepafloxacin

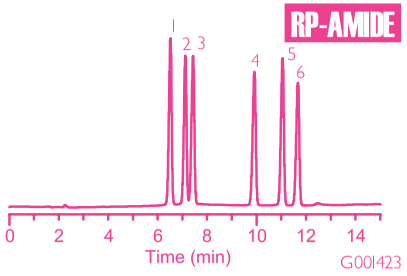


Trovafloxacin



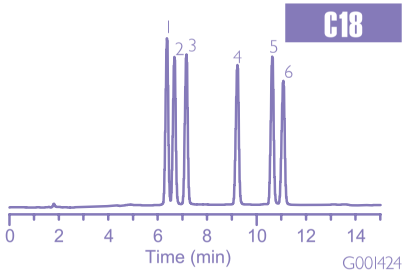
G000971, G000972, G000970, G000973, G000968, G000974

RP-AMIDE



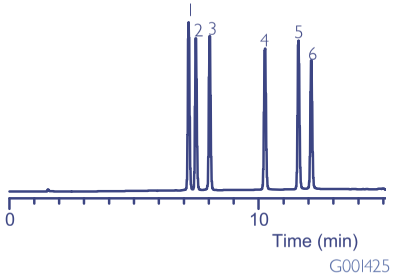
G001423

C18



G001424

HS C18



G001425

Antibiotics (Fluoroquinolones from Tablets)

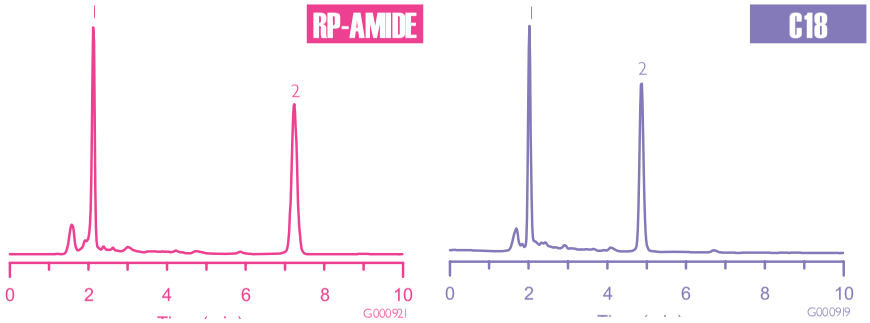
15cm x 4.6mm columns,
(A) 25mM phosphate buffer, pH 3.0
(B) MeCN
10% B to 35% B in 15 min
1mL/min
35°C
UV, 220nm
10µL

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.

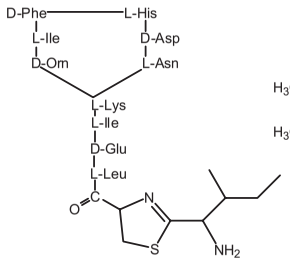
1. Bacitracin A
2. Virginiamycin M1



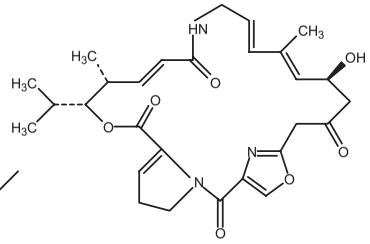
Antibiotics (Peptides)

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.

Bacitracin A



Virginiamycin M1

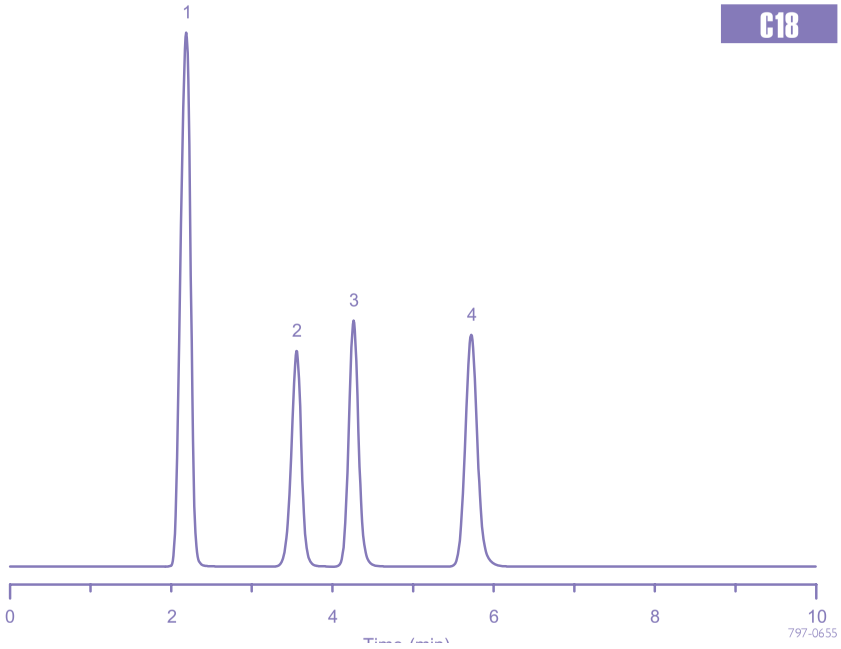


G000935, G000936

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

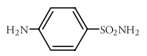
1. Sulfanilamide
2. Sulfathiazole
3. Sulfamerazine
4. Sulfamethazine



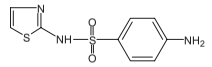
Antibiotics (Sulfa Drugs)

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 heterocylic 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.

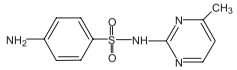
Sulfanilamide



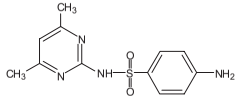
Sulfathiazole



Sulfamerazine



Sulfamethazine

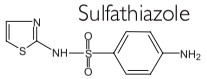


G001249, G000597,
G000598, G000599

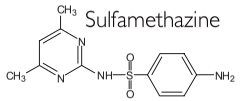
Antibiotics (Sulfa Drugs) from Serum

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.

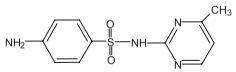
Sulfathiazole



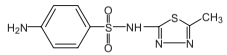
Sulfamethazine



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

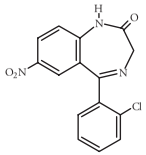
HPLC Conditions:
Discovery C18,
15cm x 4.6mm, 5µm particles,
preceded by 2cm C18 guard column and 0.5µm frit filter.
MeOH: 1% acetic acid (8:92)
1.0mL/min (8 min) then 0.6mL/min
30°C
UV, 254nm
10µL reconstituted porcine serum extract

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

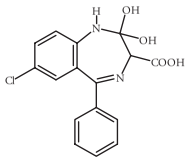
Anticonvulsants

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.

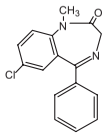
Clonazepam (IS)



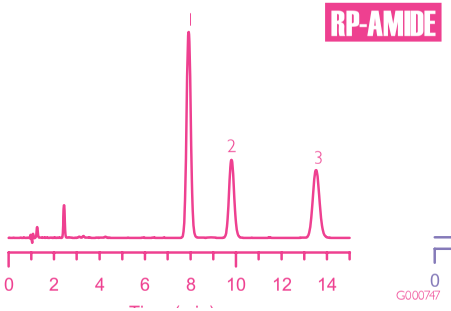
Clorazepate



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

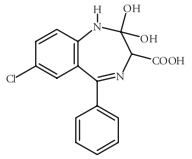
Step	Solvent/ Solution	Volume (mL)	Flow Rate (mL/min)	Comments
1. Condition	MeOH	2.0	5.0	conditions sorbent
2. Condition	H_2O	2.0	5.0	conditions sorbent
3. Load	spiked porcine serum	2.0[A]	0.75	applies serum sample
4. Rinse	20% MeOH in H_2O	2.0	5.0	washes sample
5. Purge-Cannula	H_2O	4.0	30.0	cleans 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

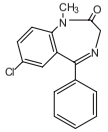
Anticonvulsant Compounds from Serum

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



G00121I, 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

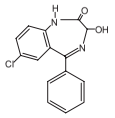
Step	Solvent/ Solution	Volume (mL)	Flow Rate (mL/min)	Comments
1. Condition	MeOH	2.0	5.0	conditions sorbent
2. Condition	H_2O	2.0	5.0	conditions sorbent
3. Load	spiked porcine serum	2.0[A]	0.75	applies serum sample
4. Rinse	MeCN/MeOH/H_2O 15:15:70	2.0	5.0	washes sorbent
5. Purge-Cannula	H_2O	4.0	30.0	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.

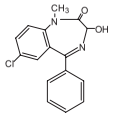
Anticonvulsants/Anxiolytics from Serum

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.

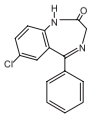
Oxazepam



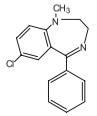
Temazepam



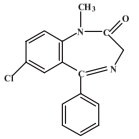
Desmethyl-diazepam



Diazepam



Medazepam



G000584, G000585, G000586, G000200, G000587

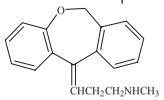
Efficiency of Recovery

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

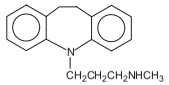
Antidepressants (Tricyclic)

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.

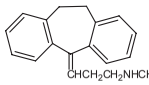
Nordoxepin



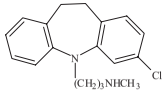
Desipramine



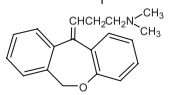
Nortriptyline



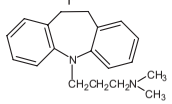
Nor-chlorimipramine (IS)



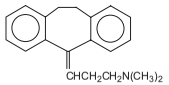
Doxepin



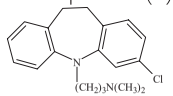
Imipramine



Amitriptyline

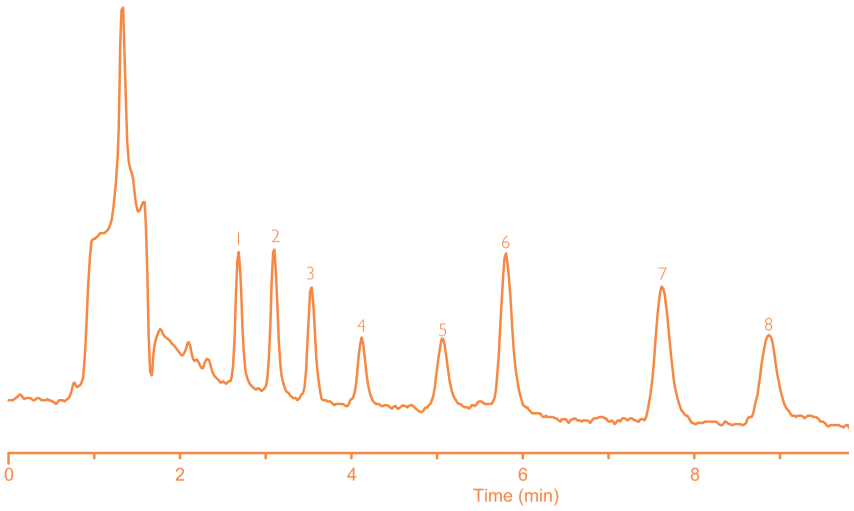


Chlorimipramine (IS)



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

C8



G001426

Antidepressants (Tricyclic)

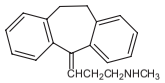
15cm x 4.6mm column, with C8 guard column,
5µm particles,
MeCN:25mM K_2HPO_4,
pH 7.0 (45:55)
1.4mL/min
25°C
UV, 248nm
10µL, 40ng/mL of each analyte

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

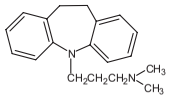
Antidepressants (Tricyclic) from Serum

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%.

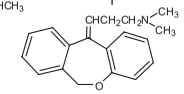
Nortriptyline



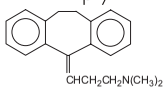
Imipramine



Doxepin



Amitriptyline



G000618, G000619, G000620, G000159

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.

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

Antidepressants (Tricyclic) from Serum

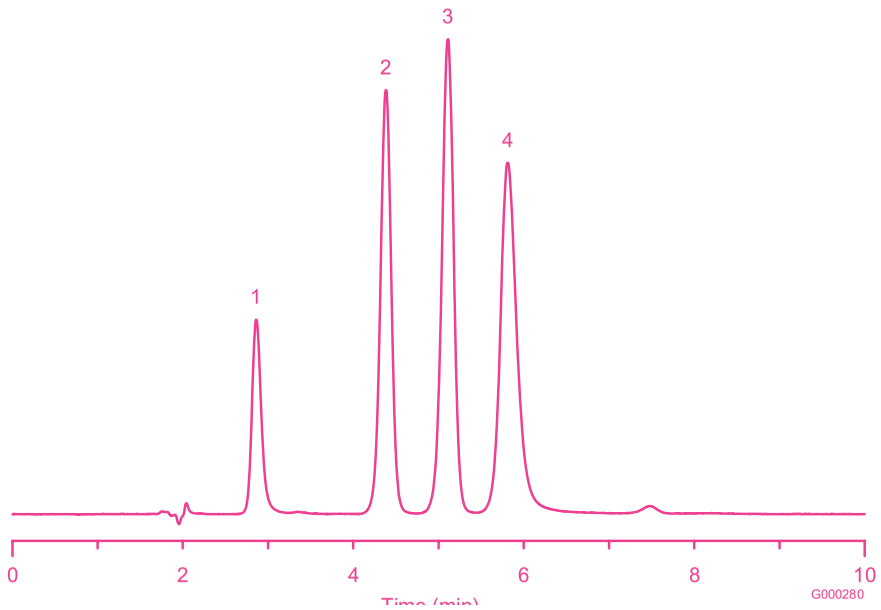
HPLC Conditions:
Discovery C18 column,
15cm x 4.6mm, 5µm particles,
preceded by 2cm C18 guard column and 0.5µm frit filter
MeCN:MeOH: 25mM KH_2PO_4 (pH 7.0 with triethylamine) (45:25:30)
1.0mL/min, ambient temp.
UV, 254nm
50µL diluted porcine serum extract

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

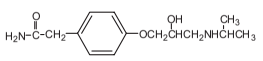


RP-AMIDE

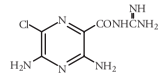
Antihypertensive-Diuretic Combination

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.

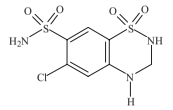
Atenolol



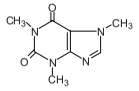
Amiloride



Hydrochlorothiazide



Caffeine

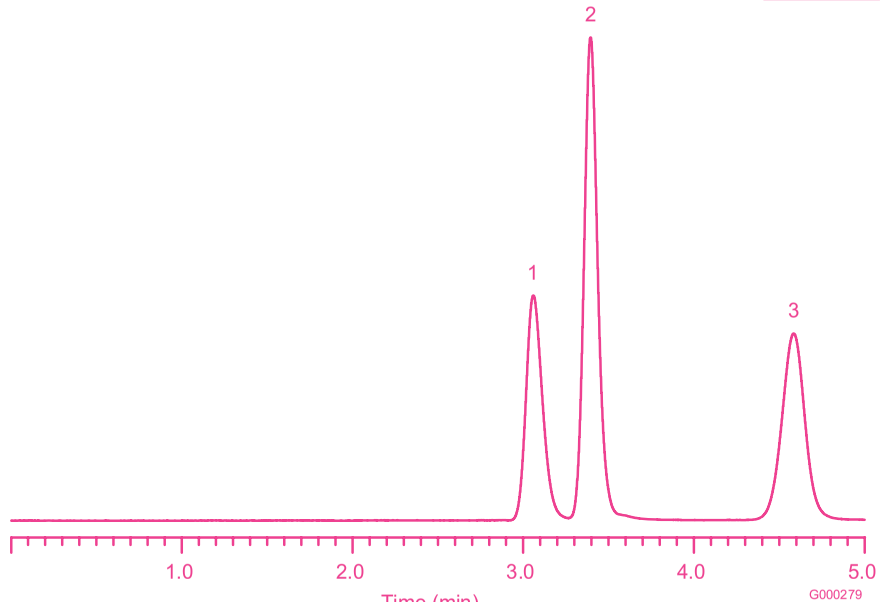


G000613, G001251, G001252, G000096

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

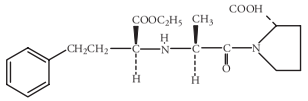


RP-AMIDE

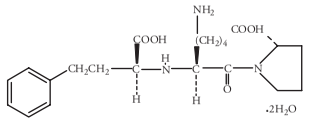
Antihypertensive ACE Inhibitors

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.

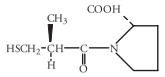
Enalapril



Lisinopril



Captopril

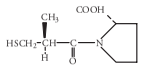


G001253, G001254, G001255

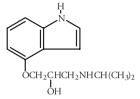
Antihypertensive Drugs (calcium channel blockers, β-blocker and ACE inhibitor)

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.

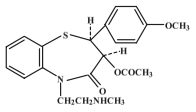
Captopril



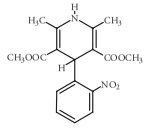
Pindolol



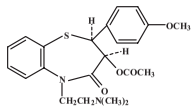
Desmethyldiltiazem



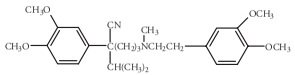
Nifedipine



Diltiazem

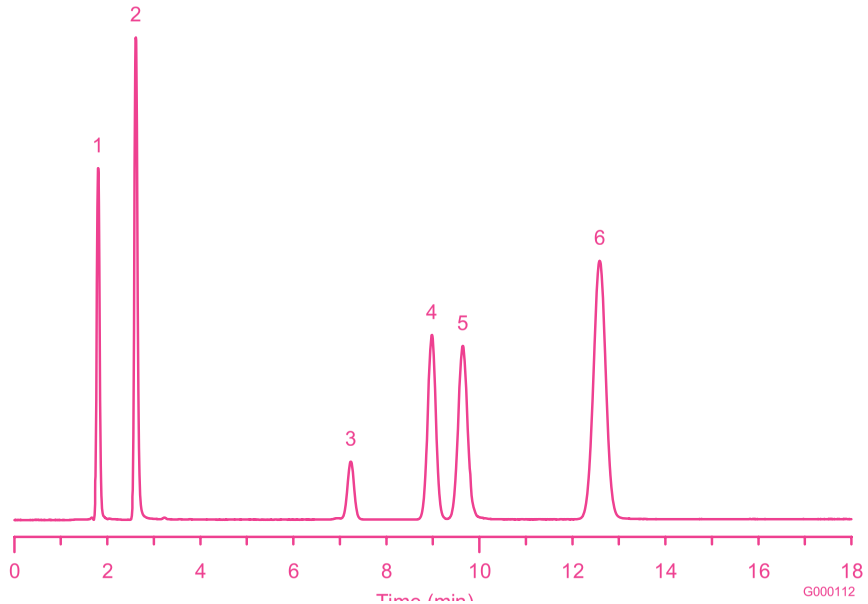


Verapamil



G001255, G001256, G000245, G001257, G000244, G001258

RP-AMIDE



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

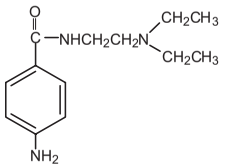
Discovery RP-AmideC16
15cm x 4.6mm column,
5µm particles,
MeOH:25mM KH_2PO_4,
pH 7.0 (40:60)
1mL/min
35°C
UV, 214nm
5µL, 0.5µg/mL of each analyte

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

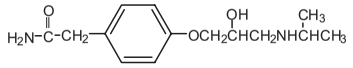
Antihypertensive/Antiarrhythmic Compounds from Serum

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.

Procainamide



Atenolol



G000095, G000613

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

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

Antihypertensive/ Antiarrhythmic Compounds from Serum

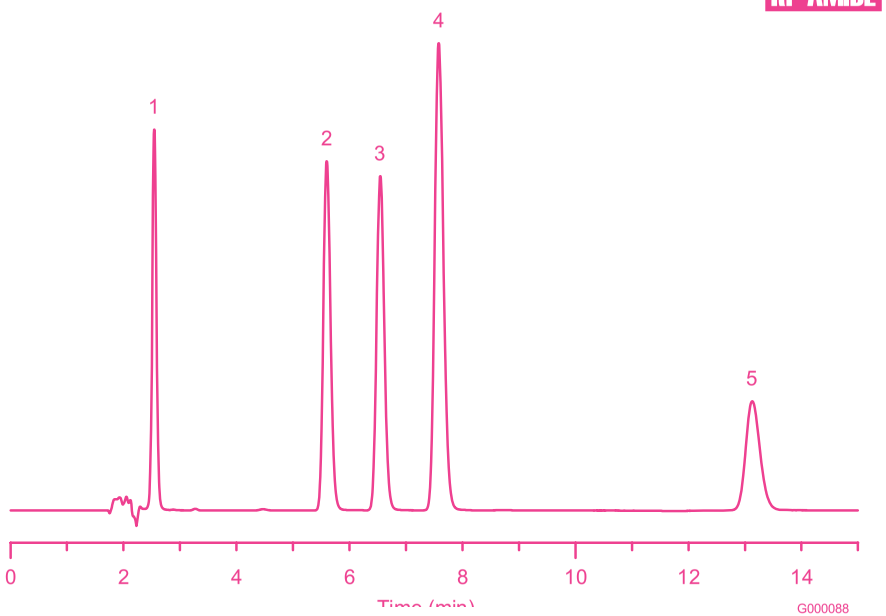
HPLC Conditions:
Discovery C18,
15cm x 4.6mm column, 5µm particles, preceded by a 2cm C18 guard column and 0.5µm frit filter.
MeOH:25mM K_2HPO_4
pH 7.0 (10:90)
1mL/min
30°C
UV, 220nm
50µL reconstituted porcine serum extract

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

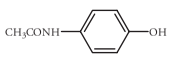
1. Acetaminophen
2. Aspirin
3. Sorbic acid
4. Benzoic acid
5. Salicylic acid



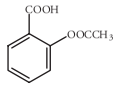
Antipyretics/Analgesics/Antifungals

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.

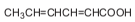
Acetaminophen



Aspirin



Sorbic acid



Benzoic acid

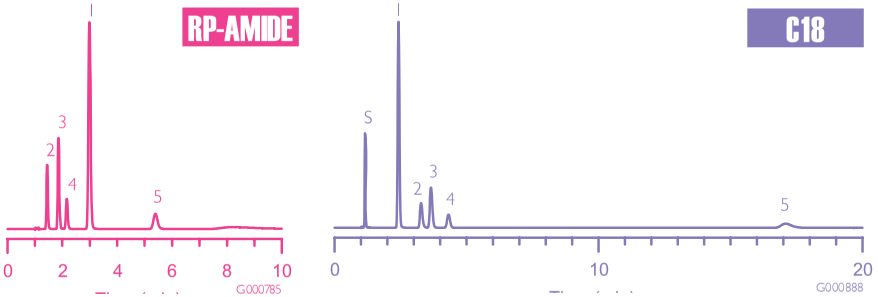
Salicylic acid

G001259, G001260, G000093
G001261, G000098

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

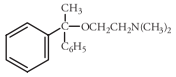


Antitussives/Antihistamines/Antipyretics

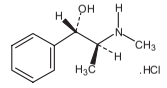
Many cold remedies contain various combinations of acetaminophen, pseudoephedrine, and an antihistamine as ingredients. The separation of a five component mix on Discovery columns is shown in this figure. It is striking that the RP-AmideC16 column elutes doxylamine, pseudoephedrine and codeine before 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

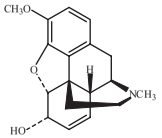
Doxylamine



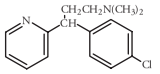
Pseudoephedrine



Codeine



Chlorpheniramine

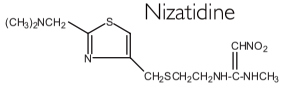


G000243, G001212, G000873, G000222, G001213

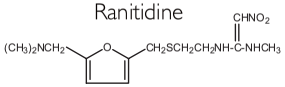
Antiulcer Compounds

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.

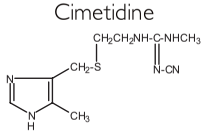
Nizatidine



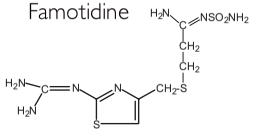
Ranitidine



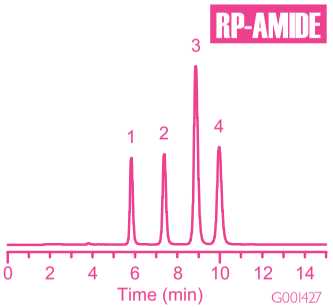
Cimetidine

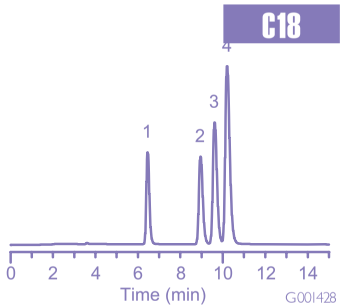


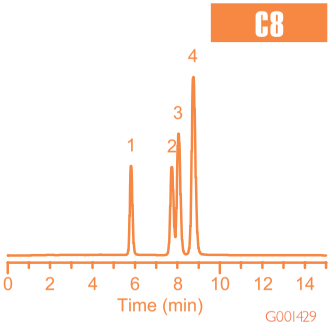
Famotidine

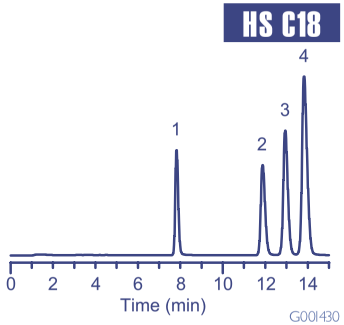


G000247, G000248, G000249, G000250









Antiulcer Compounds

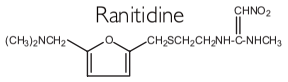
15cm x 4.6mm columns,
MeOH:25mM H_3PO_4/KOH,
pH 3.0 (15:85)
0.5mL/min
ambient temp.
215nm
3µL, 1.6µg/mL of each analyte

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

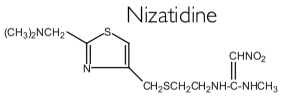
Antiulcer Compounds from Serum

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.

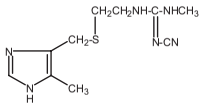
Ranitidine



Nizatidine



Cimetidine



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

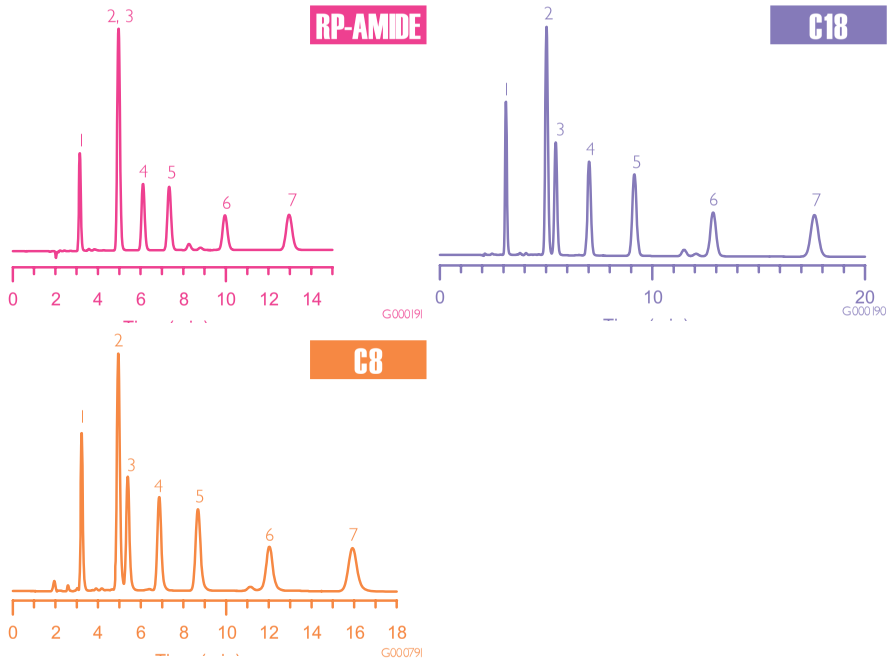
HPLC Conditions:
Discovery C18,
15cm x 4.6mm, 5µm particles,
preceded by 2cm C18 guard column and 0.5µm frit filter.
MeOH: 25mM K_2HPO_4,
pH 7.0 (20:80)
1.0mL/min, ambient temp.
UV, 235nm
60µL reconstituted porcine serum extract

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

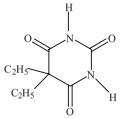
1. Barbital
2. Aprobarbital
3. Phenobarbital
4. Butabarbital
5. Mephobarbital
6. Pentobarbital
7. Secobarbital



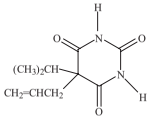
Barbiturates

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.

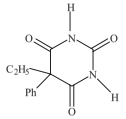
Barbital



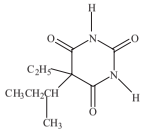
Aprobarbital



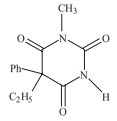
Phenobarbital



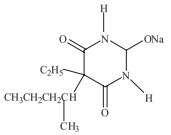
Butabarbital



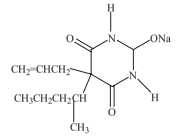
Mephobarbital



Pentobarbital



Secobarbital



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.

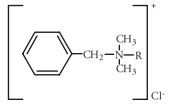
Efficiency of Recovery

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

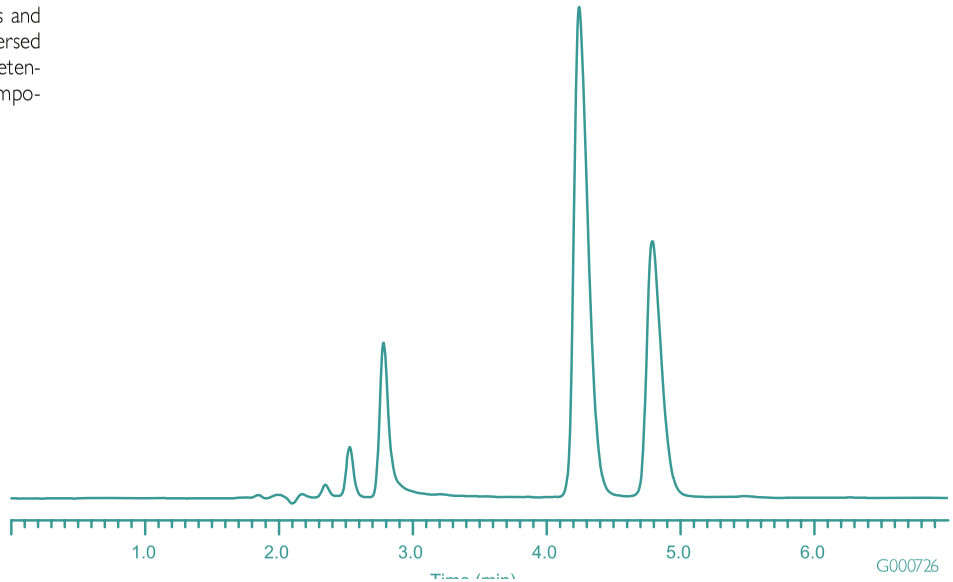
Benzalkonium Chlorides

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



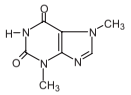
Benzalkonium Chlorides

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

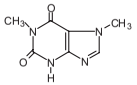
Bronchodilator (Caffeine Metabolites) from Serum

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.

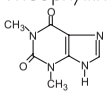
Theobromine



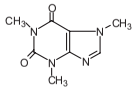
Paraxanthine



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

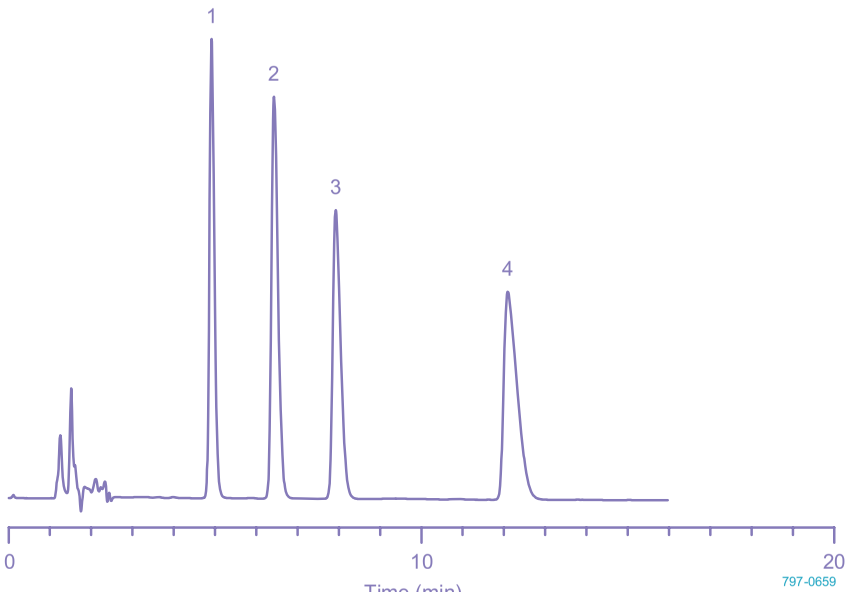
HPLC Conditions:
Discovery RP-AmideC16 column,
15cm x 4.6mm, 5µm particles,
preceded by 2cm RP-AmideC16 guard column and 0.5µm frit filter.
MeOH:1% acetic acid (17:83)
1.0mL/min
30°C
UV, 272nm
20µL reconstituted porcine serum extract

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

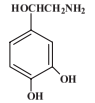


C18

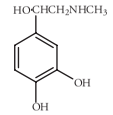
Catecholamines

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.

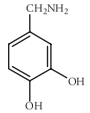
Norepinephrine



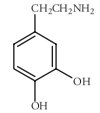
Epinephrine



3,4-Dihydroxybenzylamine



Dopamine



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

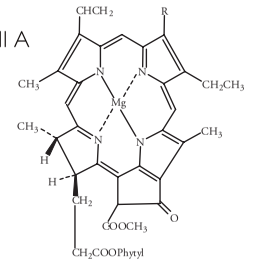
Fraction	Absorbance at 660nm (AU)	Appearance
1	0.081	Clear
2	0.178	Clear
3	0.255	Clear
4	0.286	Clear
5	0.330	Clear
6	0.402	Slightly green
7	0.503	Slightly green
2% chlorophyll standard	0.399	Slightly green

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

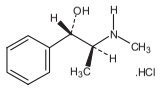


G001273

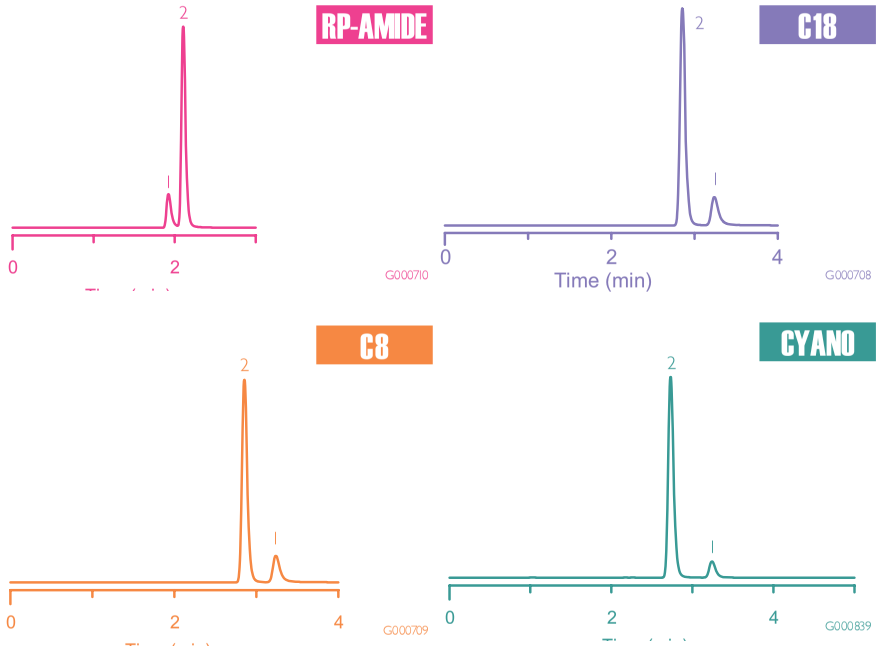
Cold Remedy Ingredients

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

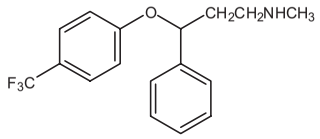
15cm x 4.6mm columns
5µm particles,
MeCN:25mM KH_2PO_4
pH 7.0 (15:85)
1mL/min
20°C
UV, 214nm
1µL

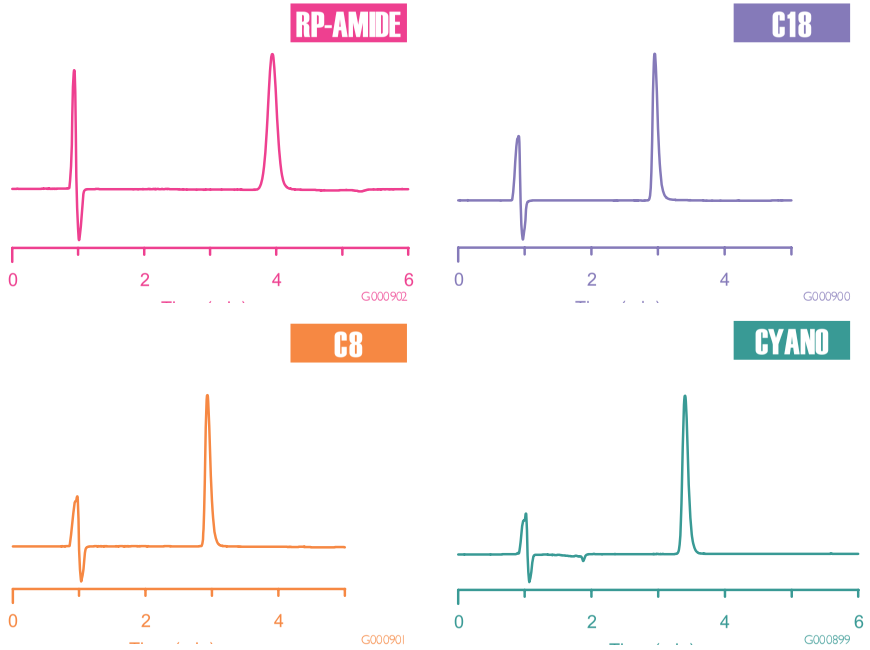
1. Pseudoephedrine
2. Acetaminophen

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®)





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

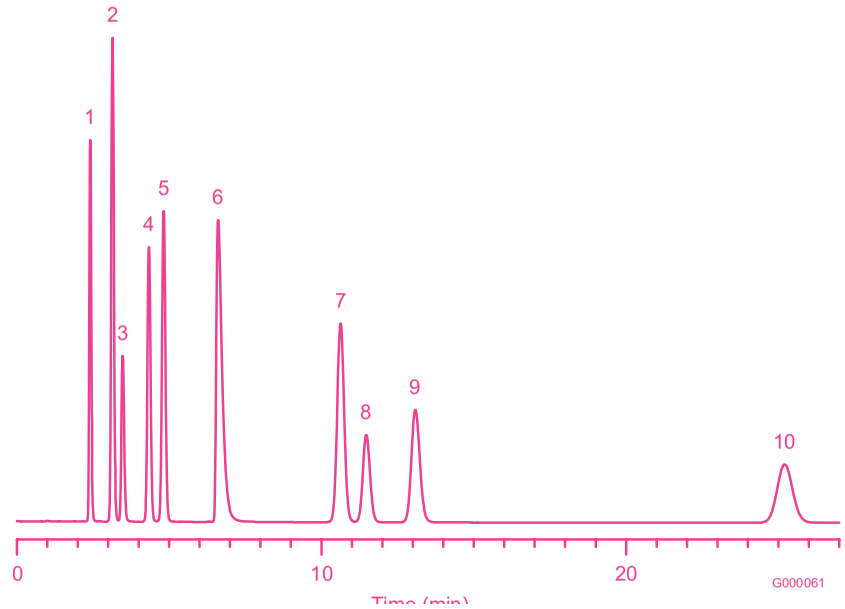
Humic acids are found in soils, coals, and peat. They are brown-colored, mixtures of not-well-defined macromolecules with polymeric phenolic and heterocyclic structures containing carboxyl groups and nitrogen functionalities. They are soluble in water and bases, but insoluble in mineral acids and alcohols. 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

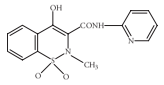
RP-AMIDE



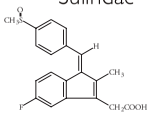
Nonsteroidal Antiinflamatory Drugs

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.

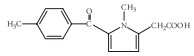
Piroxicam



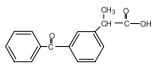
Sulindac



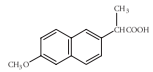
Tolmetin



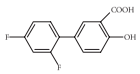
Ketoprofen



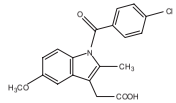
Naproxen



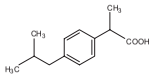
Diflunisal



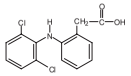
Indomethacin



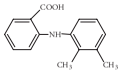
Ibuprofen



Diclofenac



Mefanamic acid

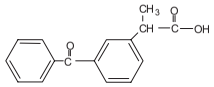


G001265, G001266, G001267, G000240, G001268, G001269, G001137, G000603, G000604, G001270

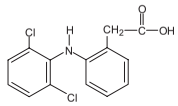
Nonsteroidal Antiinflammatory Drugs from Serum

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.

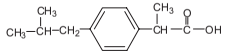
Ketoprofen



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

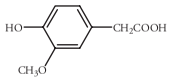
HPLC Conditions:
Discovery RP-AmideC16 column,
15cm x 4.6mm, 5µm particles,
preceded by 2cm
RP-AmideC16 guard column and
0.5µm frit filter.
MeCN: 25mM KH_2PO_4
pH 3.0 (50:50)
1.0mL/min
30°C
UV, 230nm
20µL reconstituted porcine serum extract

SPE Tube:
DSC-18
500mg/3mL

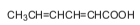
Organic Acids

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

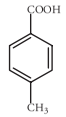


Sorbic acid



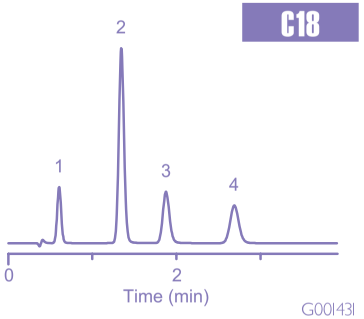
Salicylic acid

p-Toluic acid



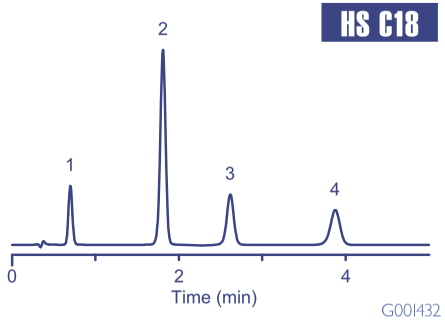
G001271, G000093, G000098, G001272

C18



G001431

HS C18



G001432

Organic Acids

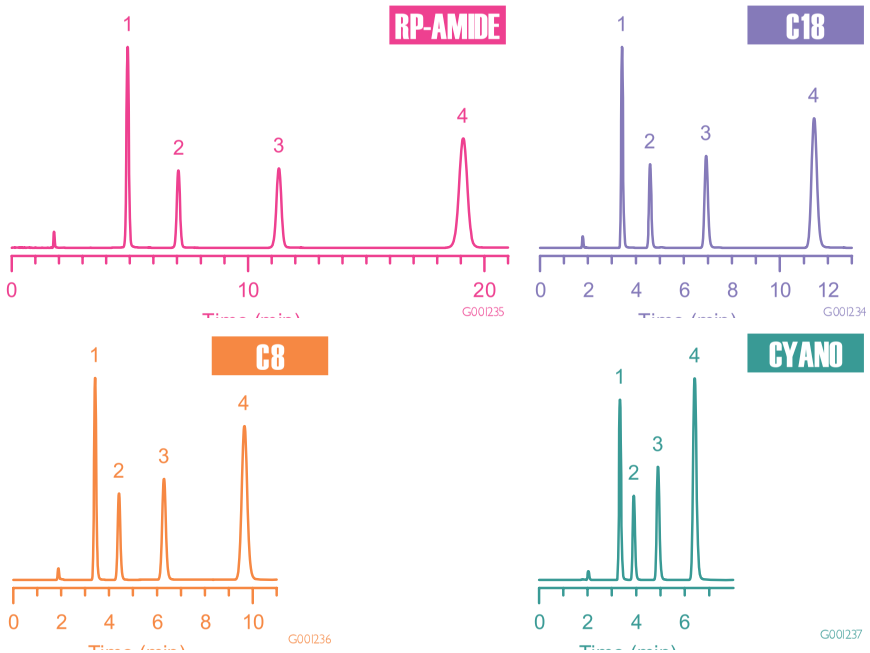
5cm x 4.6mm column,
MeOH:H_2O, 0.1% TFA (40:60)
2.0mL/min
20°C
UV, 254nm
10µL

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

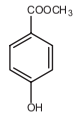
1. Methyl paraben
2. Ethyl paraben
3. Propyl paraben
4. Butyl paraben



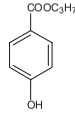
Parabens

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.

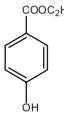
Methyl paraben



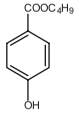
Propyl paraben



Ethyl paraben



Butyl paraben

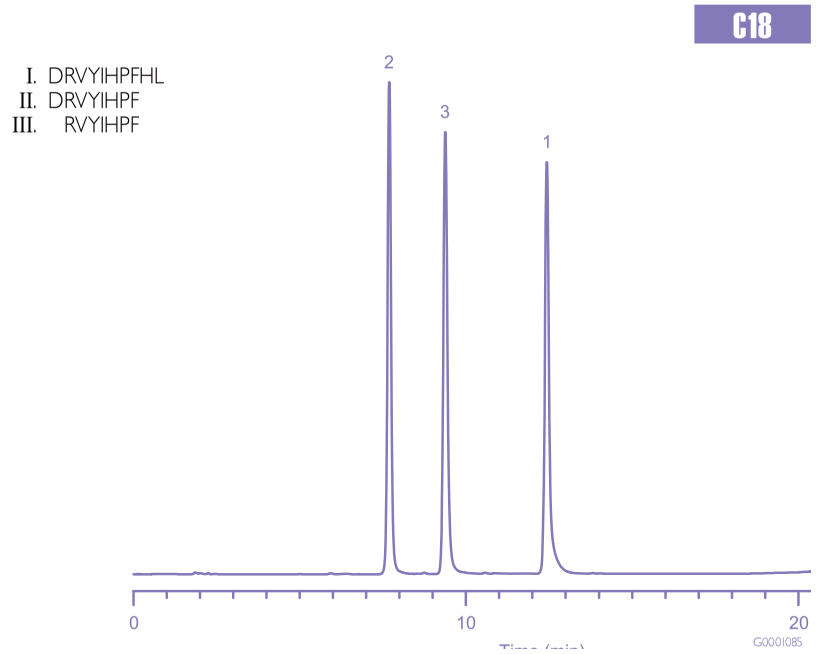


G000194, G000195, G000196, G000197

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

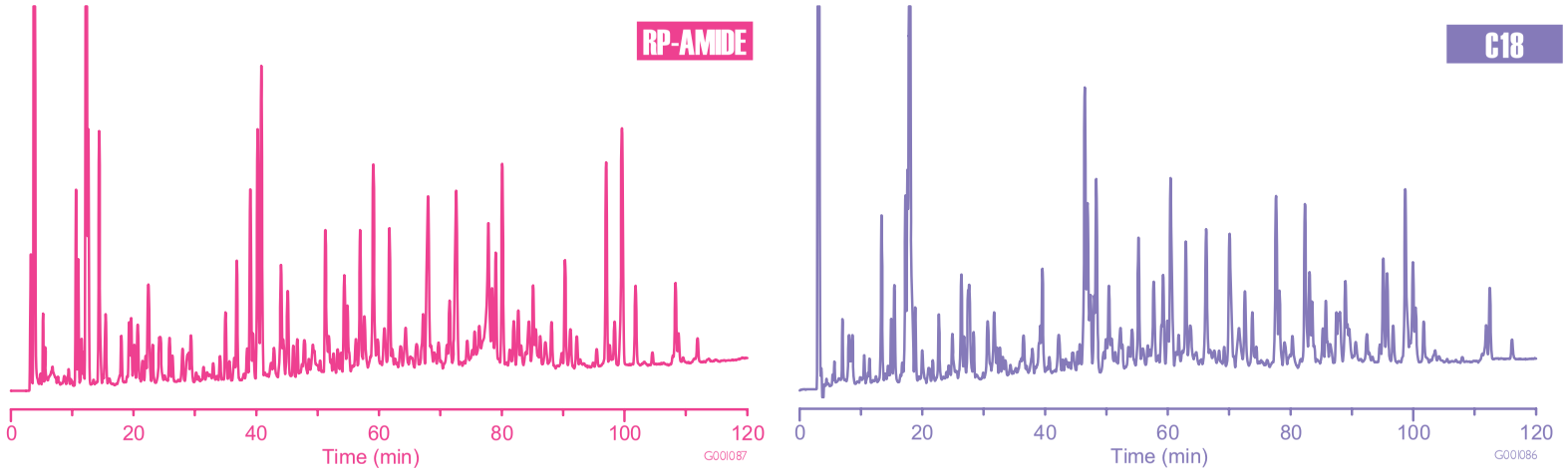


Peptides (Angiotensins)

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.

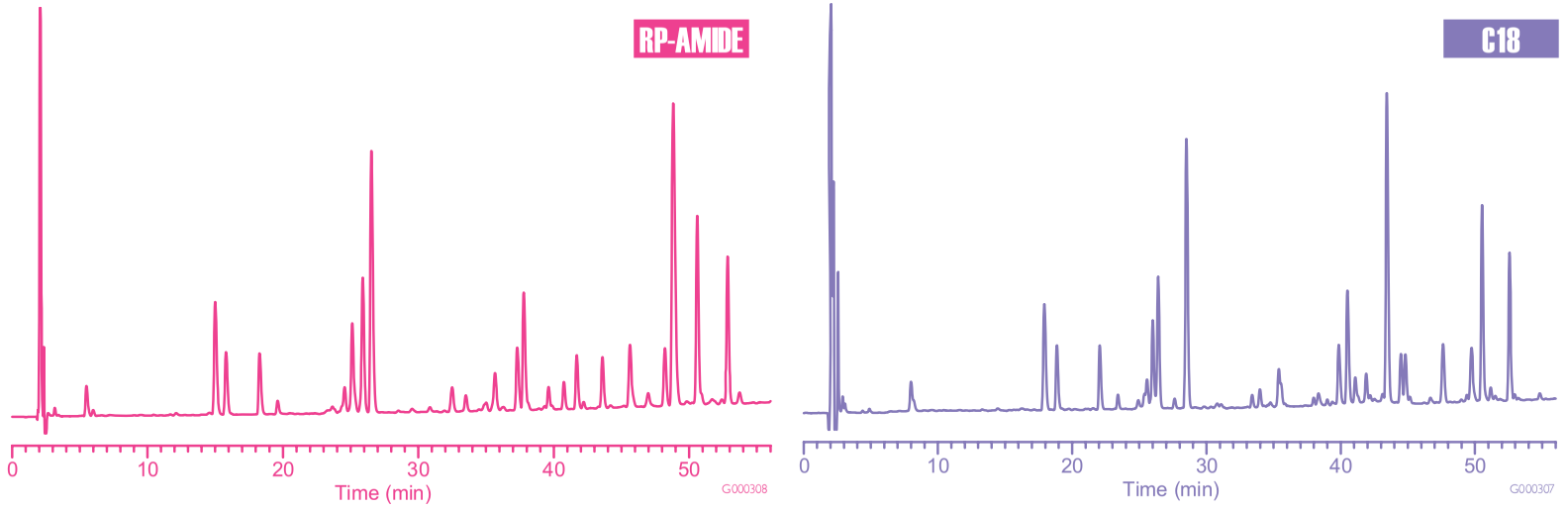


Peptides (Carboxyamido-methylated BSA Tryptic Digest)

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.



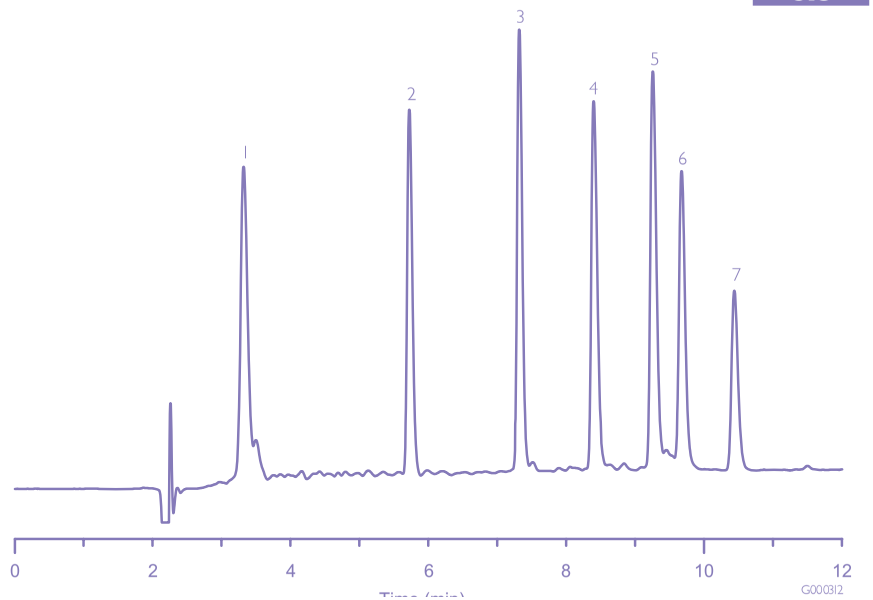
Peptides (Cytochrome c Tryptic Digest)

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

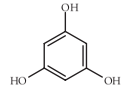
Fraction	Peak Height (mAU)
1	4.2
2	4.0
3	4.3
4	4.4
5	4.7
6	6.2
7	28.5
2% phloroglucinol standard	9.3

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.

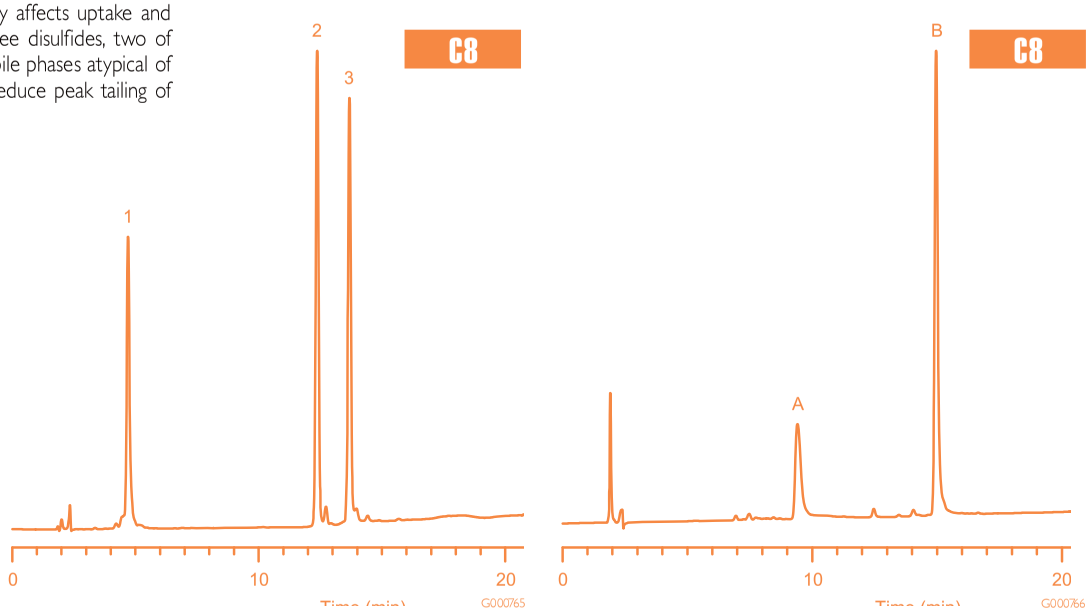
Phloroglucinol



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.

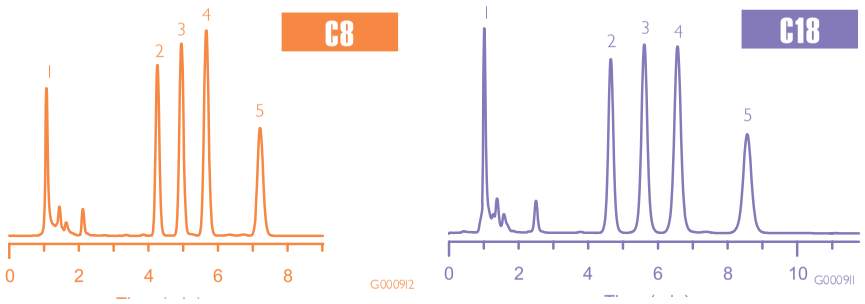
Fat Soluble Vitamins (A and E)

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

Discovery C18
MeOH:H_2O (95:5)

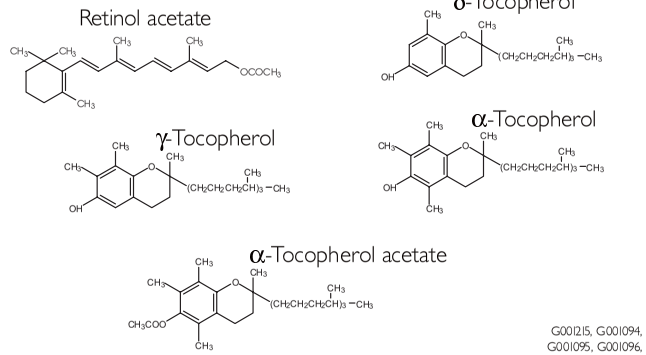
Discovery C8
MeCN:H_2O (90:10)

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.

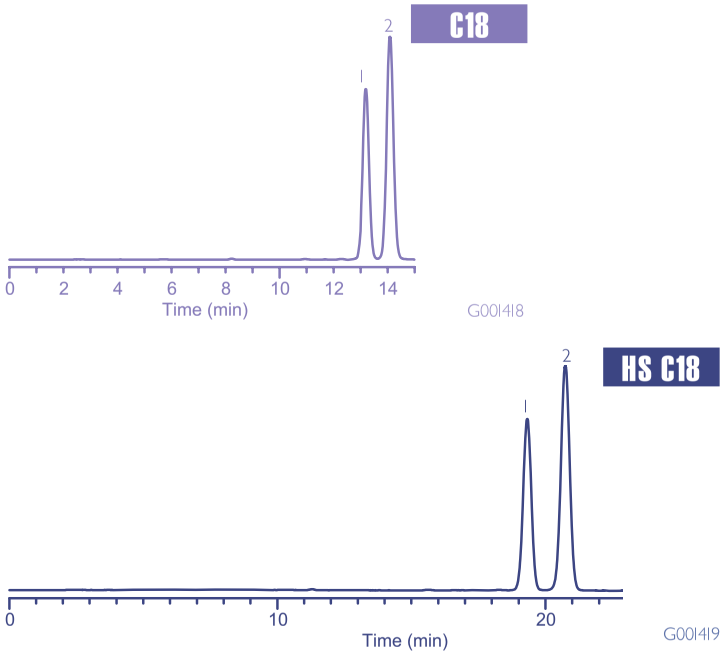


G001215, G001094, G001095, G001096, G001097

Fat Soluble Vitamins (D_2 and D_3)

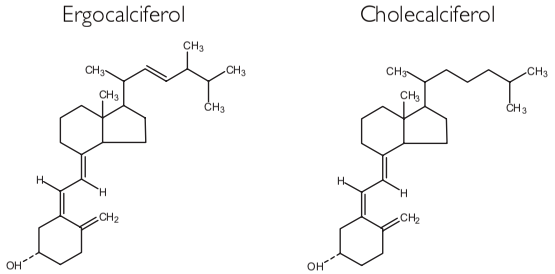
Discovery C18
15cm x 4.6mm column,
100% MeCN
0.8mL/min
30°C
UV, 290nm
10µL, 50µg/mL of each analyte

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.

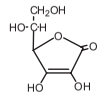


G001098, G001099

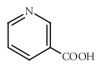
Water Soluble Vitamins

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.

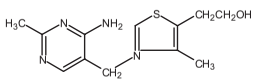
Ascorbic acid



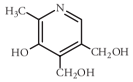
Nicotinic acid



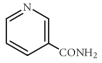
Thiamine



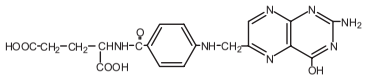
Pyridoxine



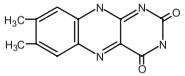
Nicotinamide

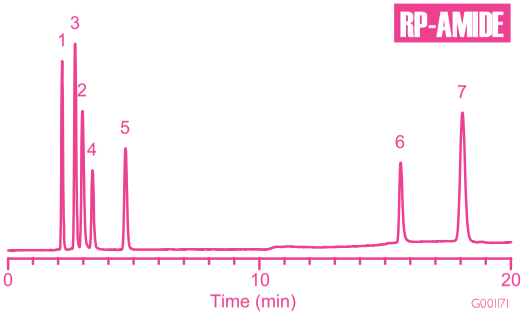


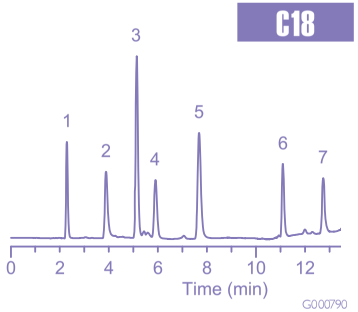
Folic acid

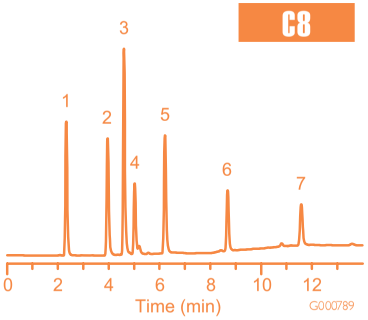


Riboflavin









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

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

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

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.

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.

SUPELCO, Supelco Park, Bellefonte, PA 16823-0048 • *phone:* 800-247-6628 / 814-359-3441 • *fax:* 800-447-3044 / 814-359-3044 • *internet:* www.sigma-aldrich.com/supelco • *email:* supelco@sial.com

ISO 9001 REGISTERED

The SIGMA-ALDRICH Family

SIGMA — Biochemicals and Reagents for Life Science Research

ALDRICH — Organics and Inorganics for Chemical Synthesis

Fluka — Specialty Chemicals and Analytical Reagents for Research

SUPELCO — Chromatography Products for Analysis and Purification

Riedel-de Haën — Laboratory Chemicals and Reagents for Research and Analysis

Sigma-Aldrich Research is the research sales and marketing division of Sigma-Aldrich, Inc. Sigma-Aldrich Fine Chemicals is the bulk division of Sigma-Aldrich, Inc.

T499127C
CJW