



Atlantis™

Columns

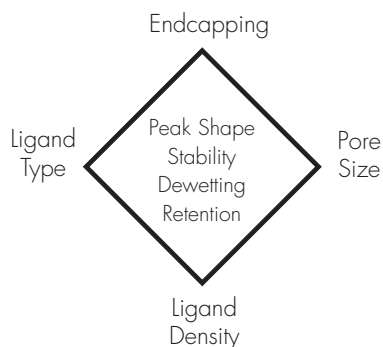
APPLICATIONS NOTEBOOK

Most chromatographers have experienced problems retaining and separating polar compounds using conventional reversed-phase (RP) chromatography. These difficult-to-analyze compounds either pass through the column unretained or, if retained at all, co-elute at the beginning of the chromatogram. Although today's sensitive and selective mass spectrometers (MS) may help identify these early co-eluting compounds, MS ion suppression often occurs if these analytes are not sufficiently separated from the solvent front. Waters Atlantis™ columns are designed for these types of challenging separations. Atlantis™ columns are available in two fully LC/MS compatible chemistries: dC₁₈ and HILIC Silica.

Atlantis™ dC₁₈ Columns – The Ideal Reversed-Phase Column

Waters spent two years designing a new and unique stationary phase material that would offer the perfect balance of RP retention for polar and non-polar compounds. Atlantis™ dC₁₈ columns exhibit superior retention of polar compounds as compared to conventional RP columns while not exhibiting excessive retention of hydrophobic compounds. Stationary phase physical attributes such as endcapping, silica pore size, bonded phase ligand density and ligand type were all studied and optimized in order to create a column that exhibits superior peak shape, low pH stability, resistance to dewetting (hydrophobic collapse) and enhanced polar compound retention.

Atlantis™ dC₁₈ Columns—An Intelligent Design



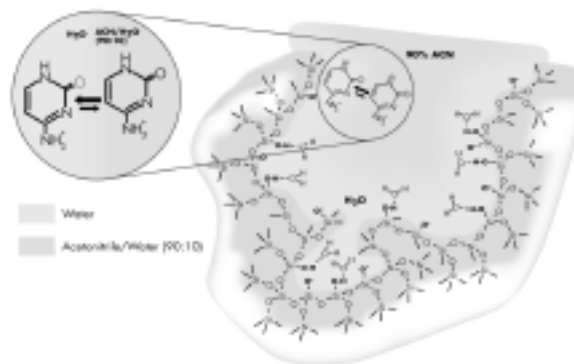
Optimizing key stationary phase attributes results in the optimal combination of peak shape, low pH stability, resistance to dewetting and polar compound retention.

One result of this stationary phase creation project was a thorough understanding of the stationary phase's role in polar compound retention. Only by studying and creating such a stationary phase can all the desirable characteristics of an ideal RP column be combined. Atlantis™ dC₁₈ columns are compatible with aqueous mobile phases, provide enhanced low pH stability, and are suitable for separating polar compounds as well as standard RP applications. Atlantis™ dC₁₈ columns are available in a wide range of column configurations ranging from nanoscale to preparative including Intelligent Speed (IS™) columns for fast, high-throughput analysis and Optimum Bed Density (OBD™) isolation and purification columns which provide analytical column performance in preparative column dimensions.

Atlantis™ HILIC Silica Columns – Hydrophilic Interaction Chromatography for Very Polar Basic Compounds

Another result of the Atlantis™ dC₁₈ columns stationary phase creation project was the realization that no single RP material can retain all polar, water-soluble compounds. Another mode of retention and separation needed to be considered for very polar compounds such as actives, metabolites, and peptides. Hydrophilic Interaction Chromatography (HILIC) is a variation of normal-phase chromatography that uses RP solvents. HILIC is also referred to as "aqueous normal phase" or "reverse reversed-phase" since elution is in the order of increasing hydrophilicity and the organic portion of the mobile phase (typically acetonitrile) is the weak solvent and the aqueous portion is the strong eluting solvent.

Retention Mechanisms in Hydrophilic Interaction Chromatography (HILIC)



The combination of partitioning and weak cation-exchange results in retention of polar bases with Atlantis™ HILIC Silica columns.

The reason for developing Atlantis™ HILIC Silica columns was to offer a simple, rugged stationary phase which provided improved LC/ESI-MS response, direct SPE solvent compatibility and complementary selectivity to RP. This complementary selectivity is important to AR&D and drug metabolism scientists since the impurity or metabolite is often more polar and present at much lower concentrations than the parent compound. With Atlantis™ HILIC Silica columns, these very polar compounds elute later than the higher concentration hydrophobic parent compound, thus minimizing the MS ion suppression that can occur at the beginning of the chromatogram.

For More Information

For more information about the Atlantis™ family of columns please visit the Waters website at www.waters.com.



*Atlantis™ Columns Brochure
Literature Code 720000793EN*

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List of Applications

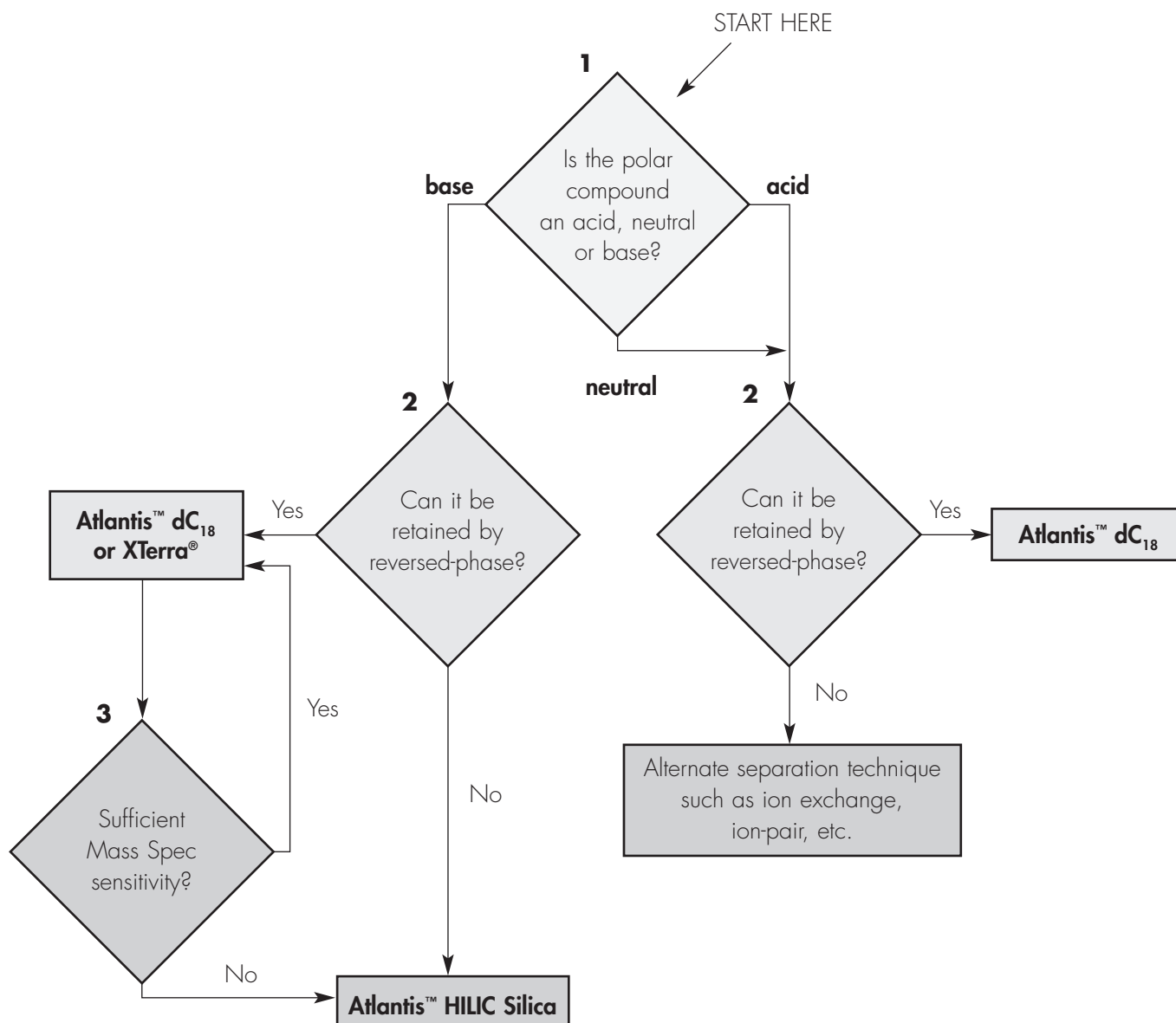
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Problem Solving and Troubleshooting Using Atlantis™ dC₁₈ Columns

Problem	Impact	Solution and Benefit
Little or no retention of polar compounds	<ul style="list-style-type: none"> • Re-run samples using separate methods for polar compounds • Increased method development time and labor 	<ul style="list-style-type: none"> • Polar compounds are retained longer with Atlantis™ dC₁₈ columns • One Atlantis™ dC₁₈ column and method can be used for polar and non-polar compounds • Decreased labor costs
Method requires 100% aqueous mobile phase for desired separation	<ul style="list-style-type: none"> • Loss of retention is observed 	<ul style="list-style-type: none"> • Atlantis™ dC₁₈ packing material is tested with highly polar analytes in 100% aqueous conditions, thereby ensuring its utility in aqueous conditions
Sudden loss of analyte retention observed when using highly aqueous mobile phase	<ul style="list-style-type: none"> • Run organic modifier through column to rewet and regenerate column • Increased labor and solvent costs • Decreased throughput • Reproducibility issues 	<ul style="list-style-type: none"> • Atlantis™ dC₁₈ columns do not lose retention in 100% aqueous mobile phases • Less time spent rewetting columns resulting in lower labor costs • Increased throughput
Short column lifetime in acidic mobile phases	<ul style="list-style-type: none"> • High cost due to frequent column replacement • Increased instrument downtime • Retention time reproducibility issues 	<ul style="list-style-type: none"> • The proprietary difunctional bonding chemistry of Atlantis™ dC₁₈ columns results in low pH stability and longer column lifetime • Decreased costs associated with column replacement and instrument maintenance
Retaining polar compounds on a conventional C ₁₈ column results in increased or infinite retention of non-polar compounds	<ul style="list-style-type: none"> • Multiple columns are required to separate analytes with a wide range of polarities • Increased method development time, labor and column costs • Decreased throughput 	<ul style="list-style-type: none"> • One Atlantis™ dC₁₈ column and method can be used for polar and non-polar compounds • Easier and faster method development • Increased throughput
Severe peak tailing for polar bases is observed	<ul style="list-style-type: none"> • Method fails system suitability guidelines for peak tailing • Increased method development time 	<ul style="list-style-type: none"> • Atlantis™ dC₁₈ columns are optimally endcapped and provide excellent peak shapes using MS compatible mobile phases • Easier and faster method development
Column bleed is observed on MS	<ul style="list-style-type: none"> • Frequent cleaning of MS source • Incorrect or inconsistent results 	<ul style="list-style-type: none"> • Atlantis™ dC₁₈ columns do not exhibit MS detectable column bleed • Decreased instrument downtime and maintenance costs
Column to column reproducibility is inconsistent (e.g., selectivity, retention, etc.)	<ul style="list-style-type: none"> • Increased labor costs due to individual column QC testing • Revalidate/redevelop method with each new batch of columns 	<ul style="list-style-type: none"> • The stringent Atlantis™ dC₁₈ packing material QC batch test separates highly polar analytes in 100% aqueous mobile phase conditions • Decreased method revalidation and development time

Problem	Impact	Solution and Benefit
Polar metabolites or contaminants not retained by reversed-phase HPLC	<ul style="list-style-type: none"> • Re-run samples using alternate, non-MS compatible chromatographic techniques • Metabolites or contaminants are not detected • Increased method development time 	<ul style="list-style-type: none"> • Atlantis™ HILIC Silica columns retain polar metabolites that cannot be retained by reversed-phase HPLC • Faster and easier method development
Severe peak tailing for polar bases is observed on reversed-phase column	<ul style="list-style-type: none"> • Method fails system suitability guidelines for peak tailing • Increased method development time 	<ul style="list-style-type: none"> • Atlantis™ HILIC Silica columns provide superior peak shapes for polar bases • Faster and easier method development
Evaporation and reconstitution step in sample preparation is too time consuming	<ul style="list-style-type: none"> • Increased labor costs and higher cost per analysis • Decreased sample throughput 	<ul style="list-style-type: none"> • Evaporation and reconstitution step is not necessary with Atlantis™ HILIC Silica columns since the mobile phases used are compatible with sample preparation organic solvents • Lower analysis costs • Increased throughput
Evaporation and reconstitution step in sample preparation results in poor analyte recoveries	<ul style="list-style-type: none"> • Unstable or mobile analytes are lost and/or not detected • Evaporated sample does not completely reconstitute • Method fails recovery and limits of detection requirements 	<ul style="list-style-type: none"> • Evaporation and reconstitution step is not necessary with Atlantis™ HILIC Silica columns since the mobile phases used are compatible with sample preparation organic solvents • Greater analyte recoveries • Lower limits of detection
Insufficient MS sensitivity due to highly aqueous reversed-phase mobile phases	<ul style="list-style-type: none"> • Samples need to be concentrated and re-analyzed • Low concentration metabolites or contaminants not detected 	<ul style="list-style-type: none"> • Highly volatile mobile phases used with Atlantis™ HILIC Silica columns provide increased ESI-MS sensitivity • Lower limits of detection
Poor polar stationary phase column lifetime (e.g., amino, diol, etc.)	<ul style="list-style-type: none"> • Frequent column replacement • Retention time reproducibility issues 	<ul style="list-style-type: none"> • Atlantis™ HILIC Silica columns do not have an unstable polar bonded phase • Decreased costs associated with frequent column replacement
Polar bonded phase bleed is observed on MS, UV and/or ELSD	<ul style="list-style-type: none"> • Noisy baselines resulting in poor sensitivity • Incorrect false positive peaks • Frequent system cleaning 	<ul style="list-style-type: none"> • Atlantis™ HILIC Silica columns do not exhibit detectable column bleed • Increased sensitivity • Decreased instrument downtime and maintenance costs
Column to column reproducibility is inconsistent (e.g., selectivity, retention, etc.)	<ul style="list-style-type: none"> • Increased labor costs due to individual column QC testing • Revalidate/redevelop method with each new batch of columns 	<ul style="list-style-type: none"> • Atlantis™ HILIC Silica columns are tested under actual HILIC conditions • Decreased method revalidation and development time
Difficult separation requires complementary chromatographic selectivity	<ul style="list-style-type: none"> • Multiple dedicated LC systems required • Separation techniques must be developed independently 	<ul style="list-style-type: none"> • Atlantis™ HILIC Silica columns use reversed-phase solvents • Single LC system running reversed-phase and HILIC separations can be easily automated • Greater flexibility, lower instrumentation costs, faster method development

Retaining Polar Compounds



To learn more about polar compound retention, contact your local Waters representative or visit us online at www.waters.com/atlantis

NOTE:

Atlantis™ dC₁₈ = High aqueous, Low pH

Atlantis™ HILIC Silica = High organic, Low pH

XTerra® = High aqueous, High pH

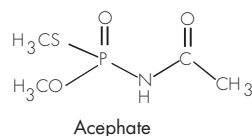
LC Conditions:

Column: Atlantis™ dC₁₈, 2.1 x 100 mm, 3 μm
 Part Number: 186001295
 Mobile Phase: 15% MeOH in H₂O
 Flow Rate: 0.2 mL/min
 Injection Volume: 20 μL
 Temperature: 25 °C
 Instrument: Alliance® HT 2795

MS Conditions:

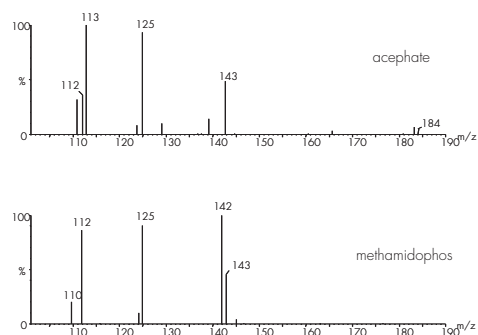
Instrument: Quattro micro™
 Ion Source: Electrospray positive
 Source Temp: 150 °C
 Desolvation Temp: 450 °C
 Cone Gas: 50 L/hr
 Desolvation Gas: 500 L/hr
 Collision Gas: Argon

Compound	MRM	Cone(V)	Coll.Energy(eV)
methamidophos	142>112	27	15
acephate	184>143	20	10



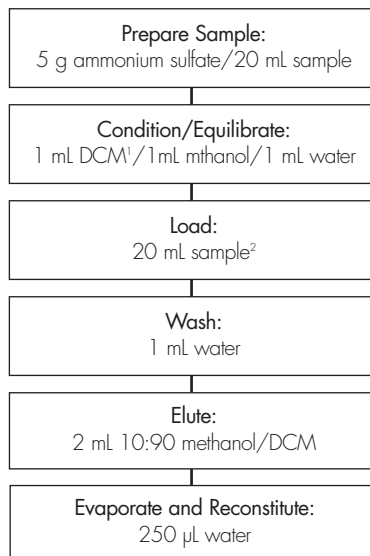
Acephate/Methamidophos Daughter Ion Spectra

200ng/L spiked river water - same injection as MRM data



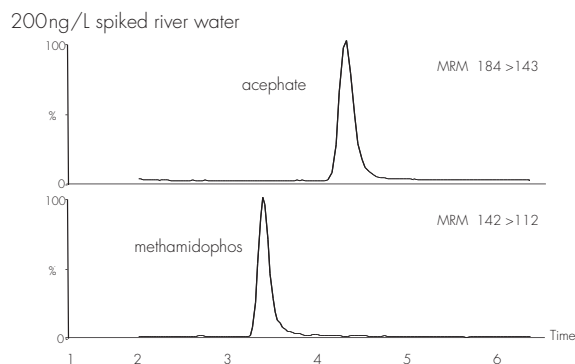
Oasis® HLB Extraction Method

Oasis® HLB Extraction Cartridge, 6 cc/200 mg



¹ methylene chloride

² 25 mL maximum sample size



Acephate/Methamidophos Recovery Data

External standard calculation (n=5)

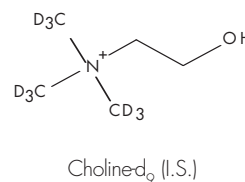
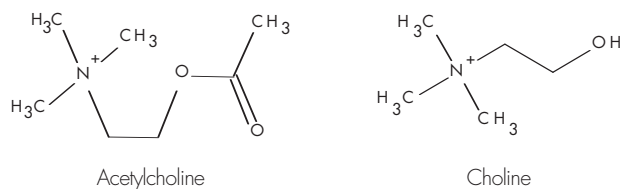
Methamidophos			Acephate		
Spike Level (ng/L)	Recovery ¹	RSD	Spike Level (ng/L)	Recovery ¹	RSD
50	50%	25%	50	92%	12%
200	62%	9.8%	200	91%	5.4%
400	66%	12%	400	90%	15%
800	60%	6.8%	800	105%	12%

¹ Calculated against standard prepared in matrix. Matrix suppression was approximately 40%. Without Oasis® HLB cleanup, matrix suppression was approximately 80%.

Acetylcholine and Choline

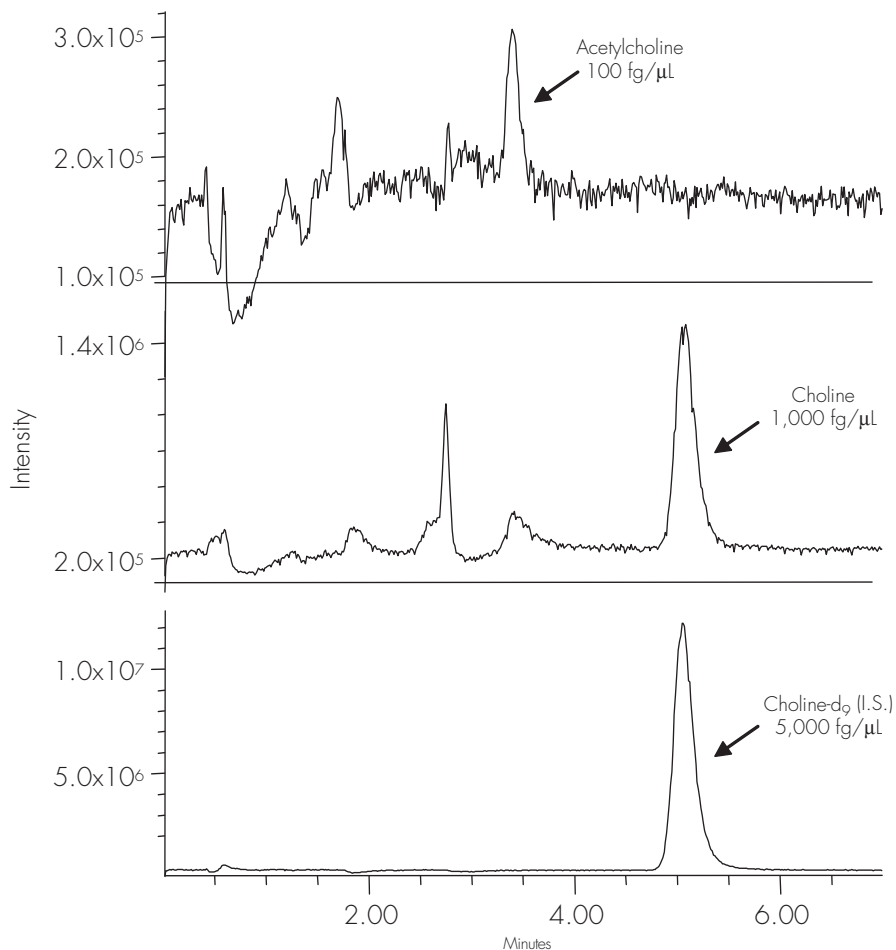
LC Conditions:

Column: Atlantis™ HILIC Silica 2.1 x 50 mm, 3 μm
 Part Number: 186002011
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 200 mM NH₄COOH, pH 3.0
 Flow Rate: 0.3 mL/min
 Isocratic Mobile Phase Composition: 9% A; 86% B; 5% C
 Injection Volume: 20 μL
 Sample Diluent: 60:40 IPA:ACN with 0.2% HCOOH
 Temperature: Ambient
 Instrument: Alliance® HT 2795 with Waters ZQ™



MS Conditions:

Ionization Mode: ES+
 Capillary (kV): 1.0
 Cone (V): 15 (ACh); 30 (Ch, Ch-d³)
 Extractor: 3 V
 RF Lens: 0.3 V
 Source Temp (°C): 150
 Desolvation Temp (°C): 350
 Cone Gas Flow (L/Hr): 50
 Desolvation Gas
 Flow (L/Hr): 700
 SIR m/z: 146.2 (ACh)
 103.9 (Ch)
 113.1 (Ch-d³)



LC Conditions:

Column: Atlantis™ dC₁₈ 2.1 x 150 mm, 3 μm
 Part Number: 186001299
 Mobile Phase A: 0.1% CH₃COOH
 Mobile Phase B: MeOH
 Isocratic Mobile Phase Composition: 99.5% A; 0.5% B
 Flow Rate: 0.2 mL/min
 Injection Volume: 20 μL
 Temperature: 26° C
 Instrument: Alliance® 2695, Quattro Premier™

MS Conditions:

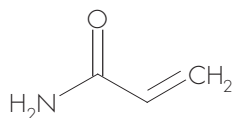
The mass detector was calibrated between m/z 19 to m/z 2000 with unit resolution.

MS/MS Analysis Function (MRM of 4 mass pairs)

Transition	Dwell Time	Collision Energy	Delay
71.7 > 43.9	0.4	11.0	0.01
71.7 > 54.8	0.4	10.0	0.01
74.7 > 44.9	0.4	11.0	0.01
74.7 > 57.8	0.4	10.0	0.01

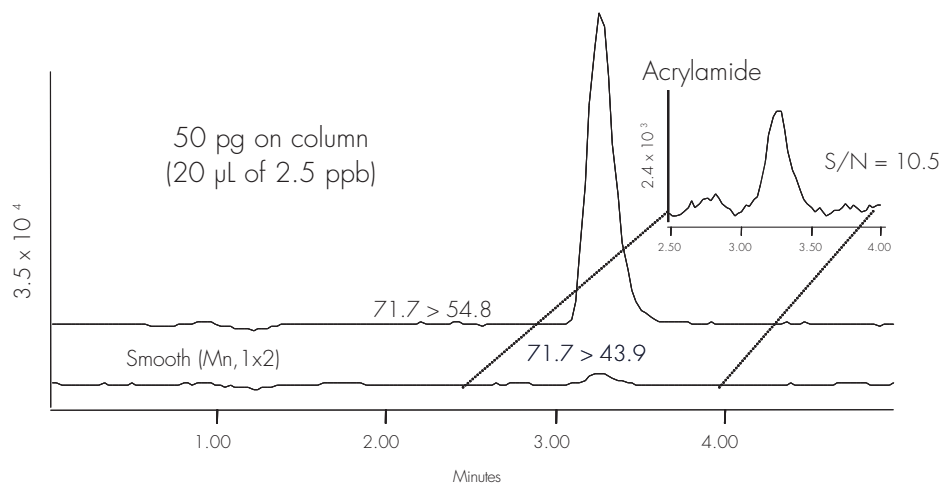
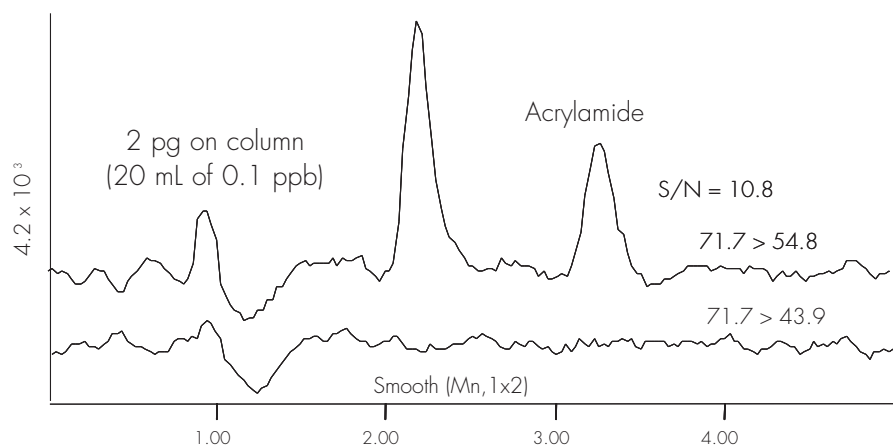
MS Conditions:

Ionization Mode: ES+
 Capillary (kV): 3.5
 Cone (V): 20
 Extractor: 5 V
 RF Lens: 0.0 V
 Source Temperature (°C): 120
 Desolvation Temp (°C): 450
 Desolvation Gas Flow (L/hr): 800
 Cone Gas Flow (L/hr): 50
 Gas Cell Pressure: 1.1 x 10⁻² mbar



Acrylamide

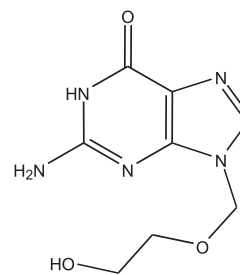
The 74.7>57.8 and 71.7>44.9 for the ¹³C₃-Acrylamide Internal Standard at 50 ppb.



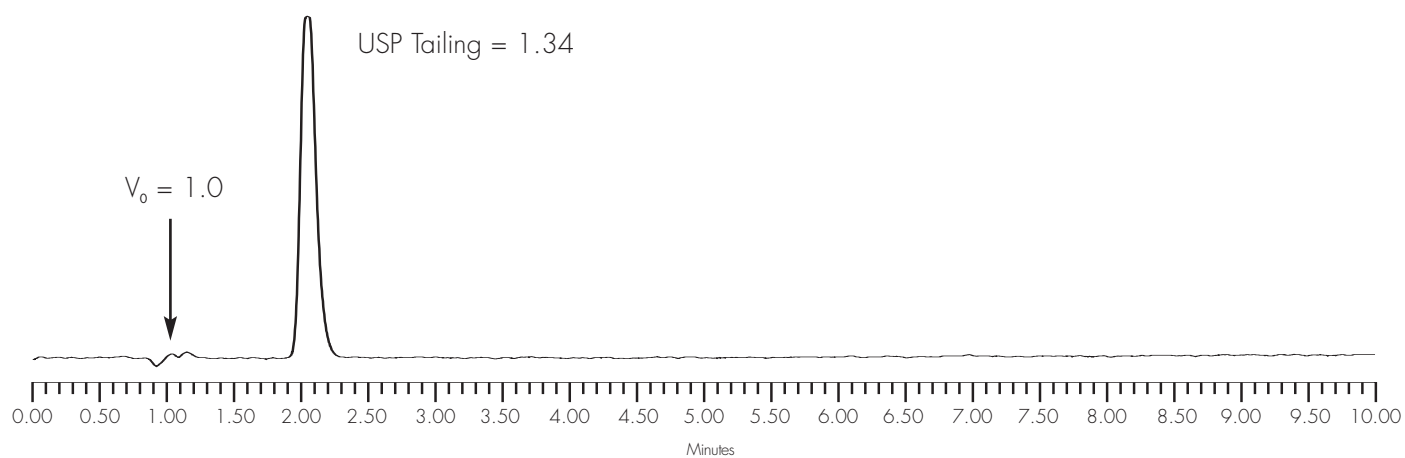
Acyclovir - Isocratic

LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm
 Part Number: 186001329
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 1.0 mL/min
 Isocratic Mobile
 Phase Composition: 87% A; 3% B; 10% C
 Injection Volume: 5 μL
 Sample Concentration: 10 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® HT 2795 with 2996 PDA



Acyclovir

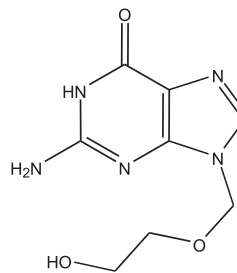


LC Conditions:

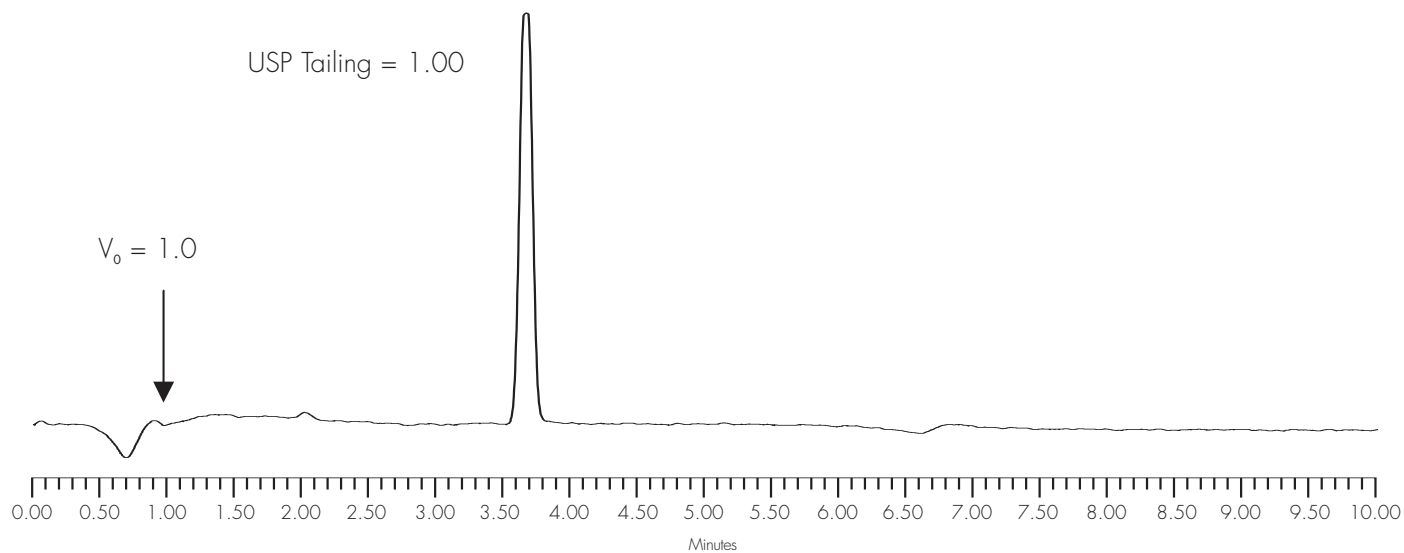
Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm
 Part Number: 186001329
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	90	0	10
5.0	85	5	10

Injection Volume: 5 μL
 Sample Concentration: 10 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® HT 2795 with 2996 PDA



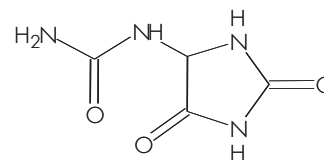
Acyclovir



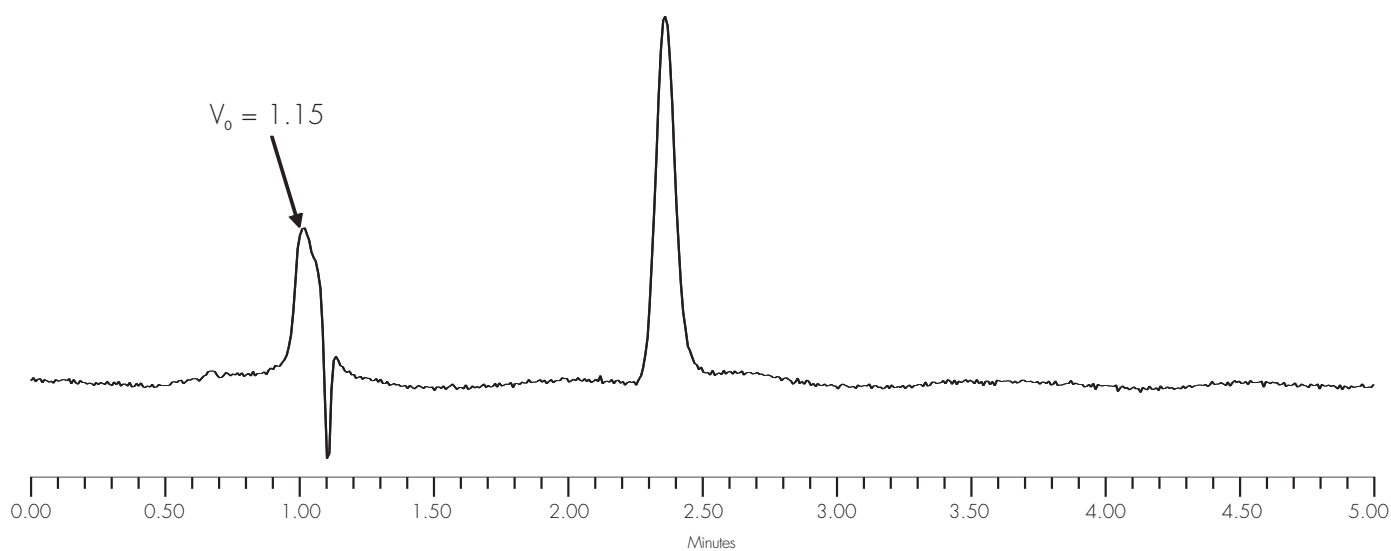
Allantoin

LC Conditions:

Column: Atlantis™ HILIC Silica 4.6 x 50 mm, 3 μm
 Part Number: 186002027
 Mobile Phase A: 100 mM NH₄COOH, pH 3.0
 Mobile Phase B: ACN
 Flow Rate: 1.0 mL/min
 Isocratic Mobile
 Phase Composition: 5% A; 95% B
 Injection Volume: 5 μL
 Injection Solvent: 75:25 ACN:H₂O
 Sample Concentration: 250 μg/mL
 Temperature: 30° C
 Detection: UV @ 205 nm
 Instrument: Alliance® 2695 with 2996 PDA



Allantoin

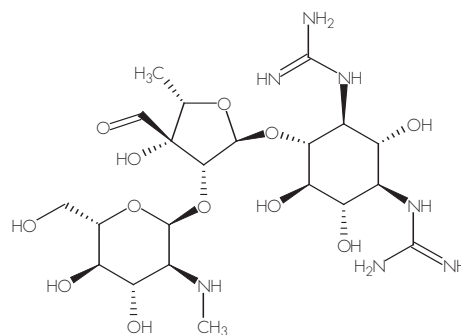


LC Conditions:

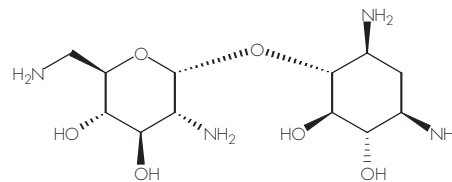
Column: Atlantis™ dC₁₈ 4.6 x 20 mm IS™, 3 μm
 Part Number: 186002062
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 0.50% Hexafluorobutyric acid in H₂O
 Flow Rate: 3.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	75	5	20
3.0	20	60	20

Injection Volume: 10 μL
 Sample Concentration: 0.1 mg/mL
 Temperature: 35° C
 Detection: Evaporative Light Scattering
 Instrument: Alliance® 2695 with 2420 ELSD

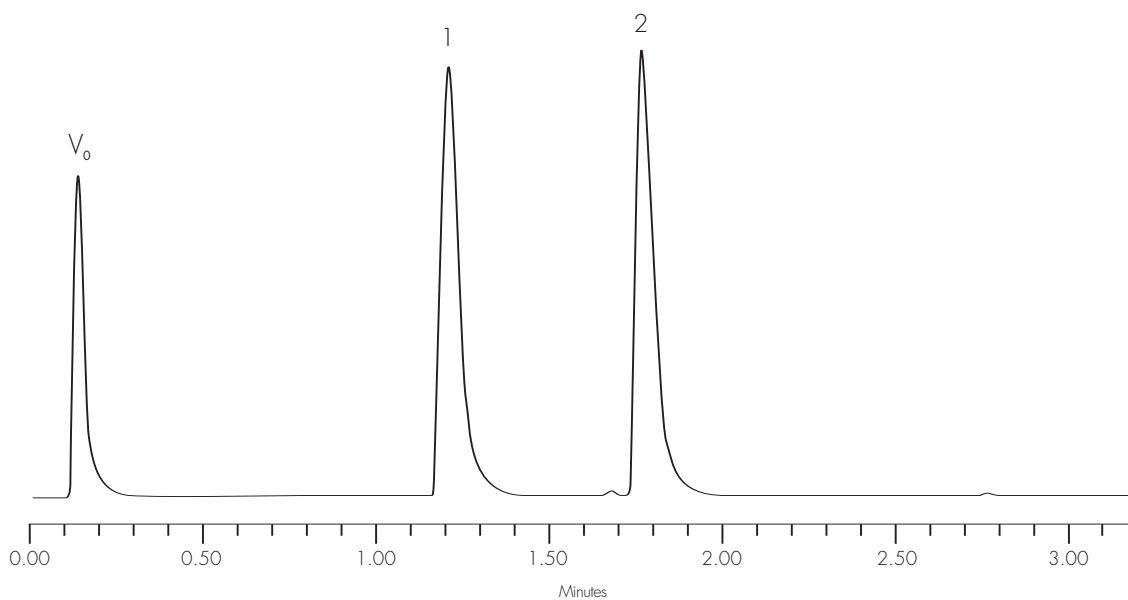


Streptomycin



Neomycin

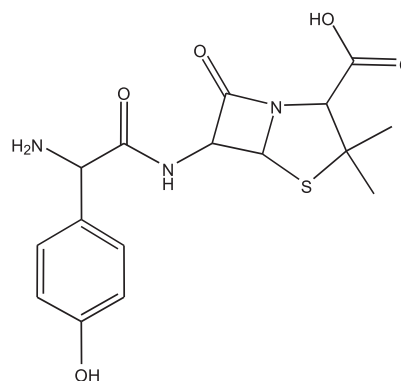
- Compounds:
1. Streptomycin
 2. Neomycin



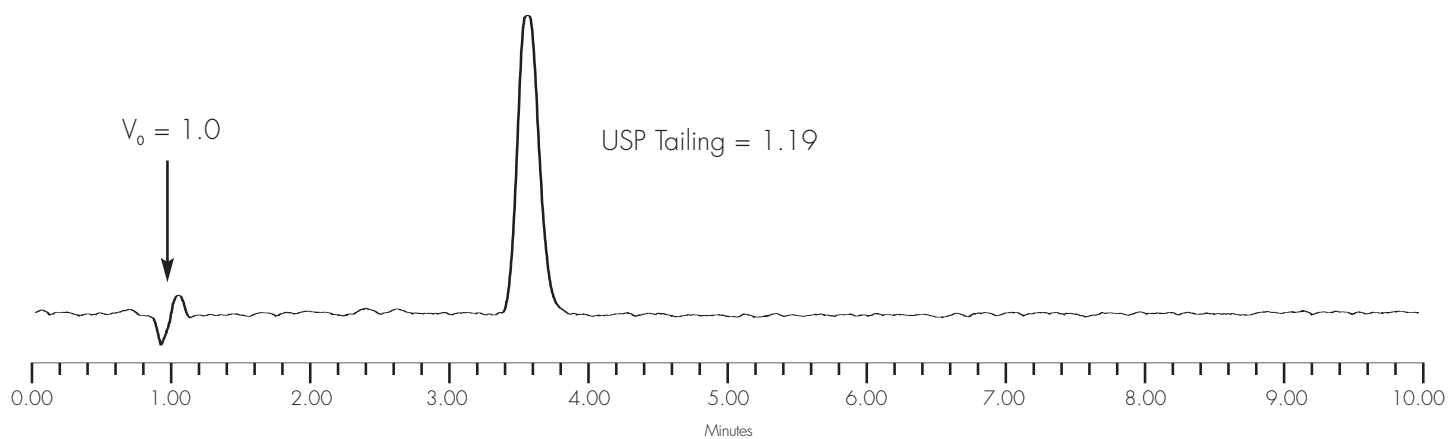
Amoxicillin—Isocratic

LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm
 Part Number: 186001329
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow rate: 1.0 mL/min
 Isocratic Mobile
 Phase Composition: 87% A; 3% B; 10% C
 Injection Volume: 10 μL
 Sample Concentration: 40 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® HT 2795 with 2996 PDA



Amoxicillin

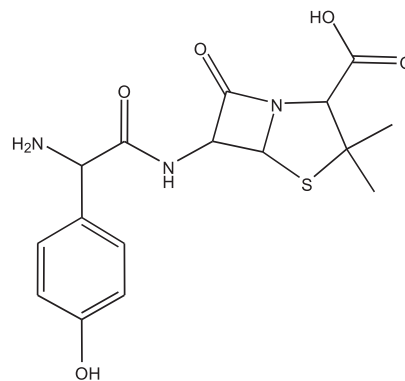


LC Conditions:

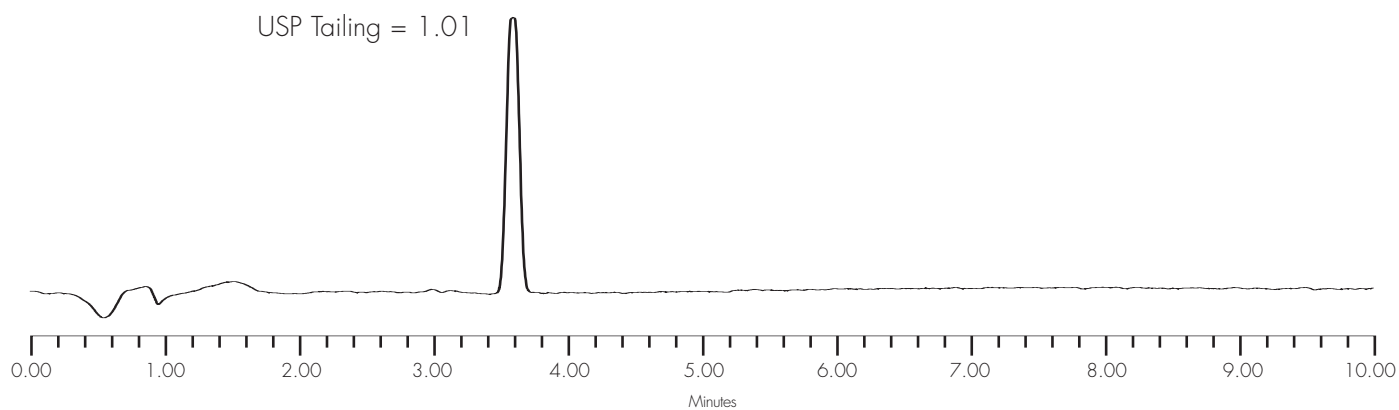
Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm
 Part Number: 186001329
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	90	0	10
5.0	85	5	10

Injection Volume: 10 μL
 Sample Concentration: 40 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® HT 2795 with 2996 PDA



Amoxicillin



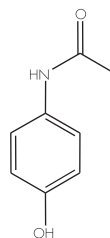
Analgesics

LC Conditions:

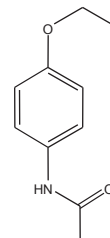
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 1% HCOOH, pH 2.3
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	75	15	10
10.0	30	60	10

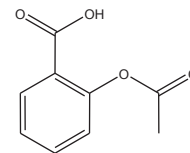
Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 260 nm
 Instrument: Alliance® 2695 with 2996 PDA



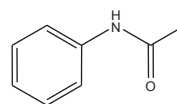
Acetaminophen



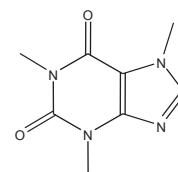
Phenacetin



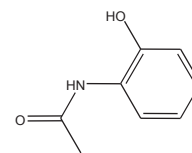
Acetylsalicylic acid



Acetanilide

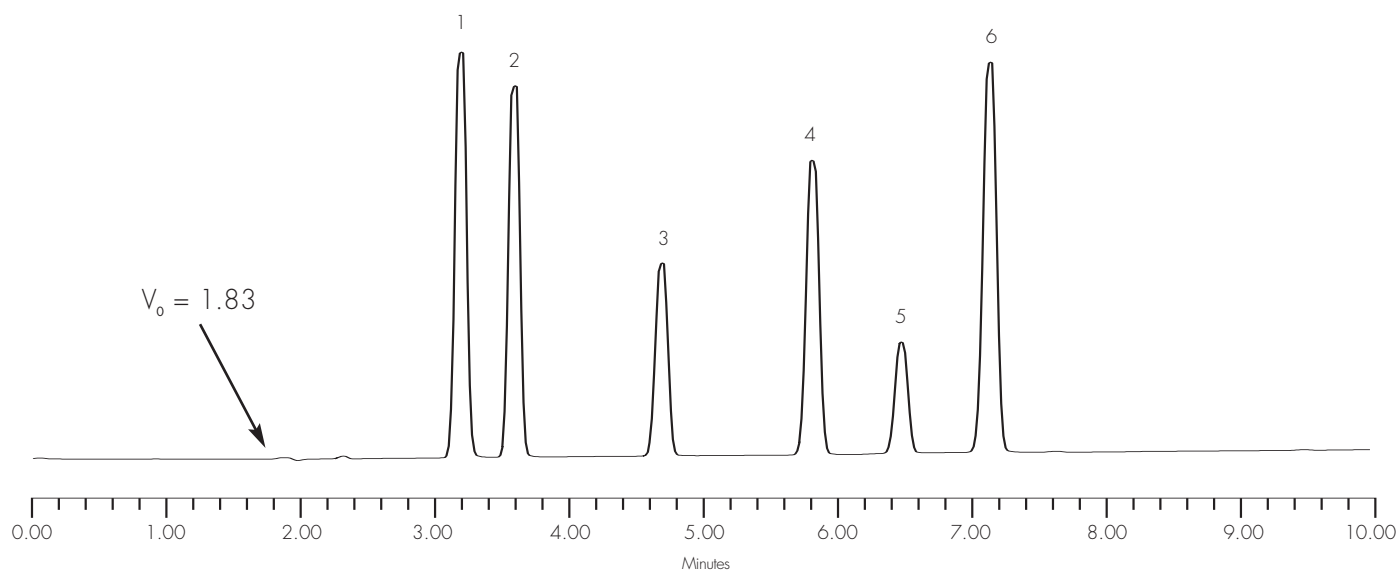


Caffeine



2-Acetamidophenol

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Acetaminophen	1.00	16
2. Caffeine	0.99	18
3. 2-Acetamidophenol	0.98	55
4. Acetanilide	1.00	22
5. Acetylsalicylic acid	0.96	55
6. Phenacetin	0.96	18

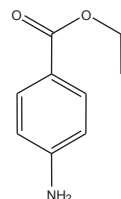


LC Conditions:

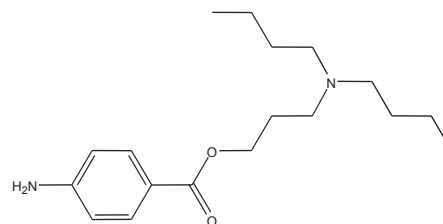
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 100 mM HCOONH₄, pH 3.75
 Flow Rate: 1.4 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	65	25	10
5.0	40	50	10
10.0	40	50	10

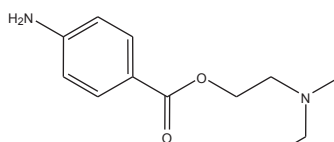
Injection Volume: 20 μL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA



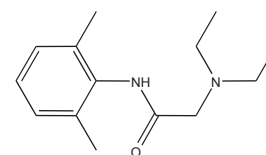
Benzocaine



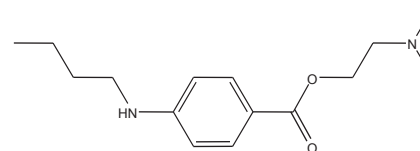
Butacaine



Procaine

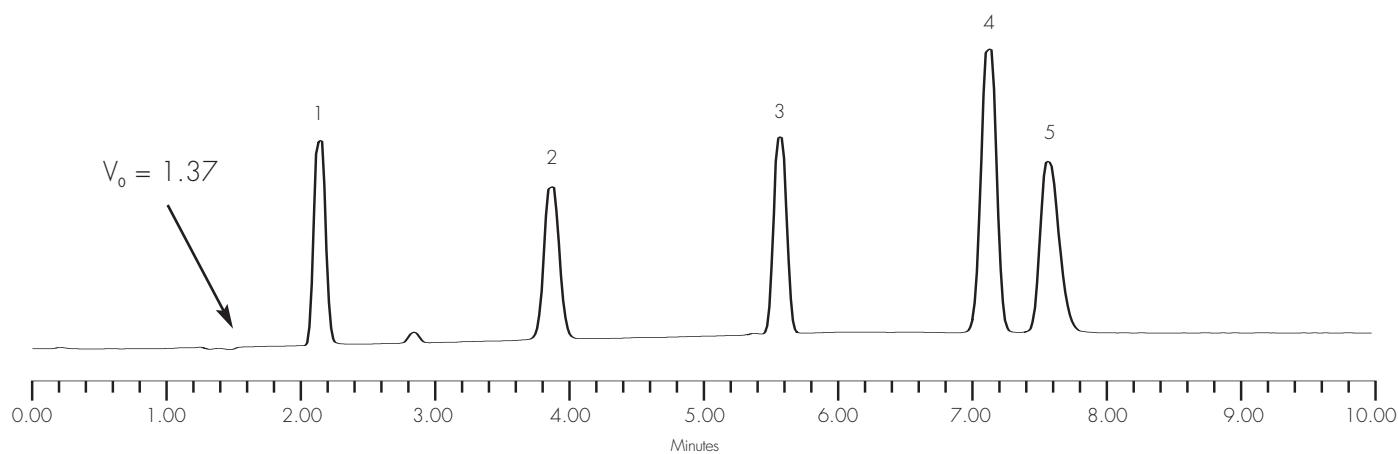


Lidocaine



Tetracaine

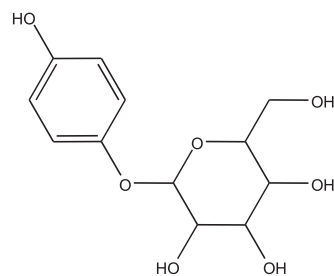
Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Procaine	1.07	7
2. Lidocaine	1.04	9
3. Butacaine	1.04	59
4. Benzocaine	0.98	59
5. Tetracaine	1.20	59



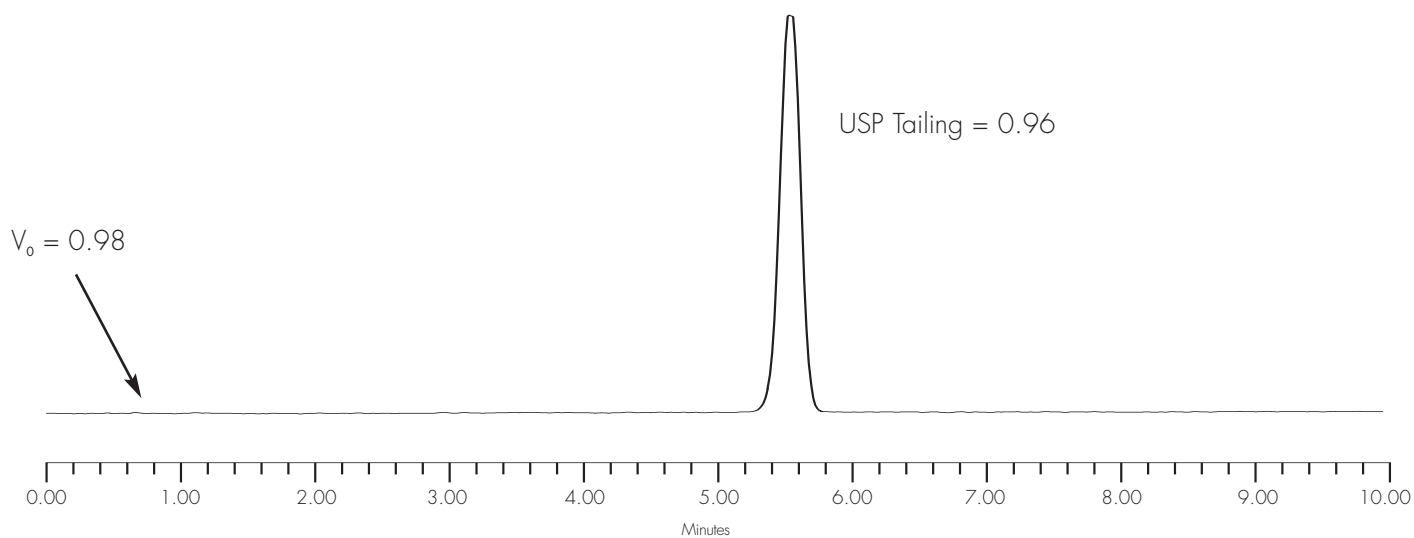
Arbutin

LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Flow Rate: 2.0 mL/min
 Isocratic Mobile
 Phase Composition: 10 mM HCOONH₄, pH 3.75
 Injection Volume: 5 μL
 Sample Concentration: 175 μg/mL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA



Arbutin

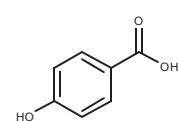


LC Conditions:

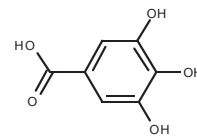
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 2.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	90	0	10
10.0	65	25	10

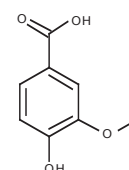
Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 285 nm
 Instrument: Alliance® 2695 with 2996 PDA



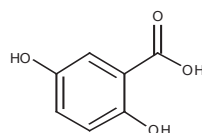
4-Hydroxybenzoic Acid



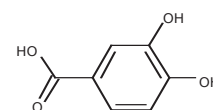
Gallic Acid



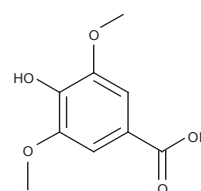
Vanillic Acid



Gentisic Acid

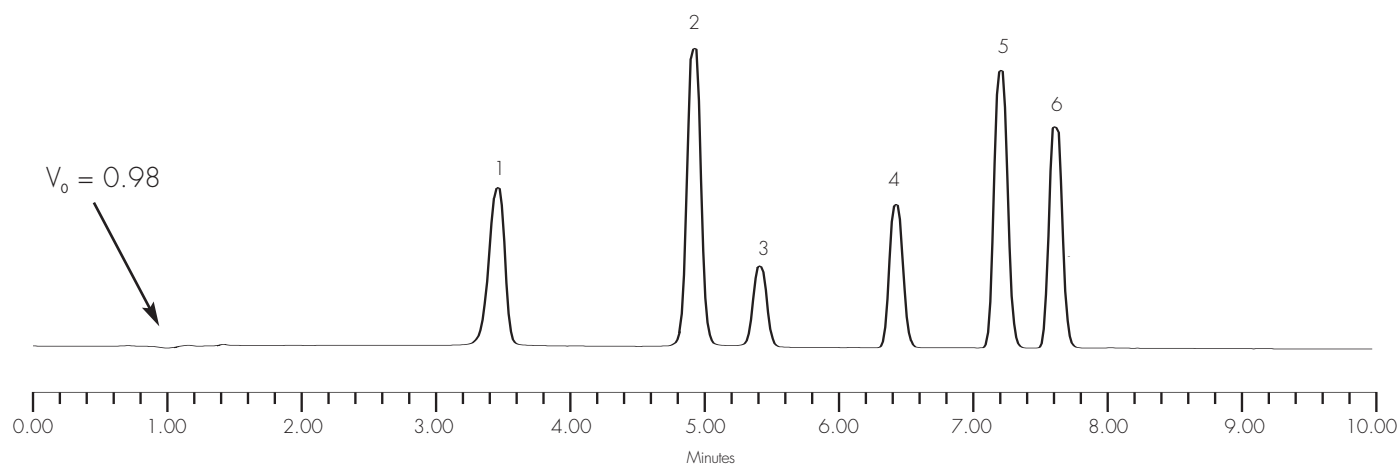


Protocatechuic Acid



Syringic acid

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Gallic acid	0.87	17
2. Protocatechuic acid	1.03	40
3. Gentisic acid	1.02	80
4. 4-Hydroxybenzoic acid	1.10	67
5. Vanillic acid	1.08	33
6. Syringic acid	1.09	17



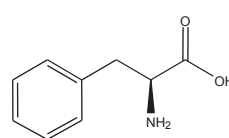
Aromatic Amino Acids

LC Conditions:

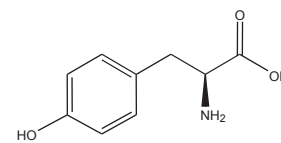
Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm
 Part Number: 186001329
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	90	0	10
10.0	10	80	10

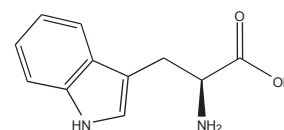
Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA



Phenylalanine

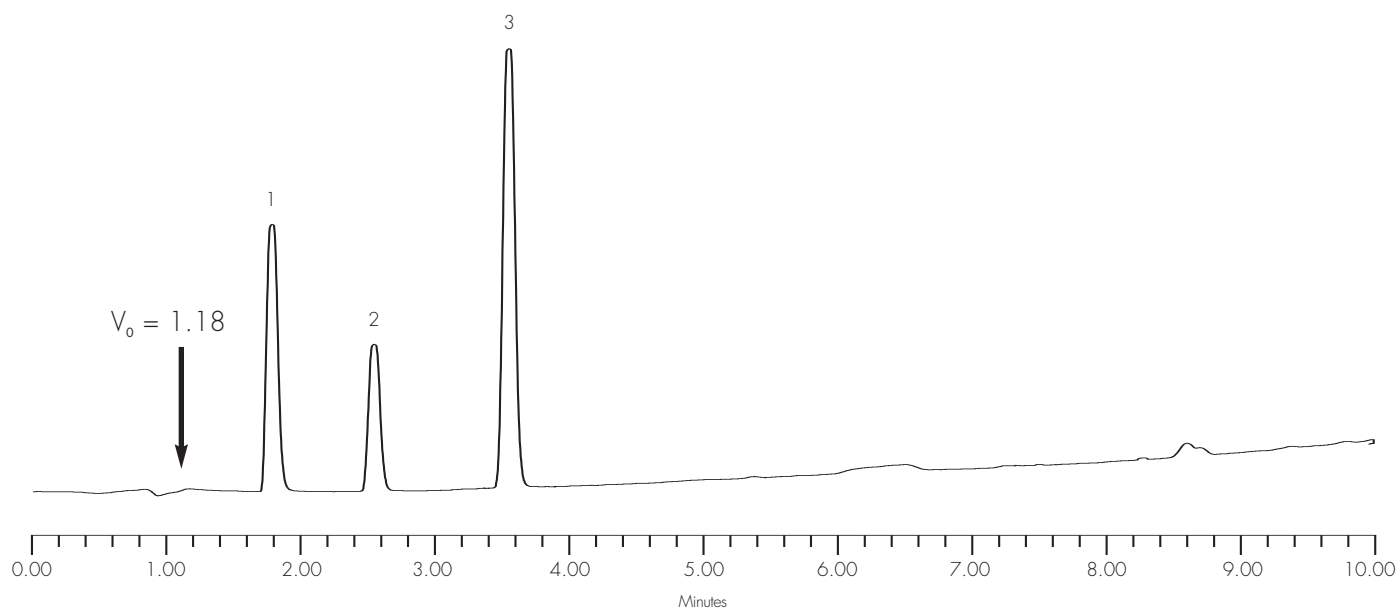


Tyrosine



Tryptophan

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Tyrosine	1.18	150
2. Phenylalanine	1.13	200
3. Tryptophan	1.11	30



LC Conditions:

Column: Atlantis™ d C₁₈, 2.1 x 100 mm, 3 μm
 Part number: 186001295
 Mobile Phase A: 15 mM HCOONH₄, pH 4.5
 Mobile Phase B: ACN
 Gradient:

Time (min)	%A	%B
0.0	90	10
19.0	10	90

 Flow rate: 0.2 mL/min
 Injection volume: 20 μL
 Instrument: Alliance® 2695 Separations Module

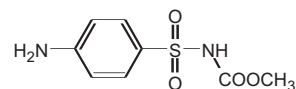
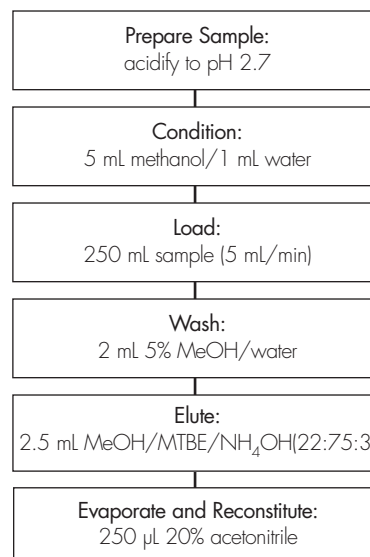
MS Conditions:

Instrument: Quattro Premier™
 Ion Source: Electrospray positive and negative
 Mode: Multiple Reaction Monitoring
 Source Temp: 150 °C
 Desolvation Temp: 450 °C
 Cone Gas: 50 L/Hr
 Desolvation Gas: 500 L/hr
 Collision Gas: Argon

MRM (ESI+)	Cone(V)	Coll. Energy (eV)
231>156	30	10

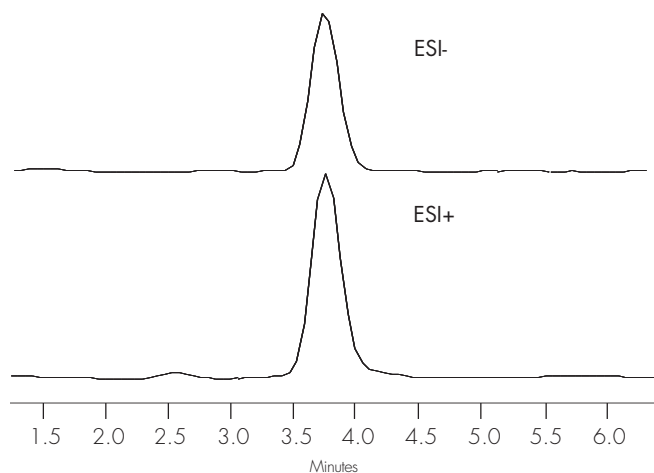
MRM (ESI+)	Cone(V)	Coll. Energy (eV)
229>197	25	15

Oasis® MCX Extraction Method
 Oasis® MCX Extraction Cartridge, 6 cc/150 mg (LP)
 Part Number: 186000255



Asulam

250 ng/L Spiked River Water



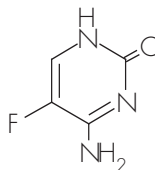
Asulam Recovery 1 (n=4)

50 ng/L	81% (14%RSD)
250 ng/L	78% (7.6%RSD)
1000 ng/L	71% (12%RSD)

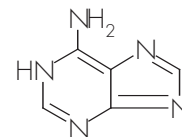
Atlantis™ dC₁₈ 100% Aqueous QC Batch Test

LC Conditions:

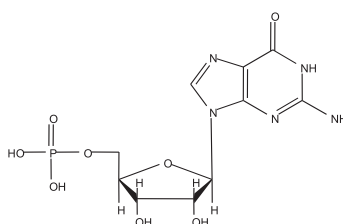
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Flow Rate: 1.2 mL/min
 Isocratic Mobile
 Phase Composition: 10 mM HCOONH₄, pH 3.0
 Injection Volume: 7 μL
 Temperature: Ambient
 Detection: UV @ 254 nm
 Instrument: Alliance® 2690 with 2996 PDA



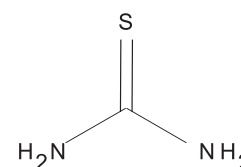
5-Fluorocytosine



Adenine

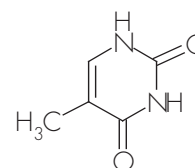


Guanosine-5'-monophosphate

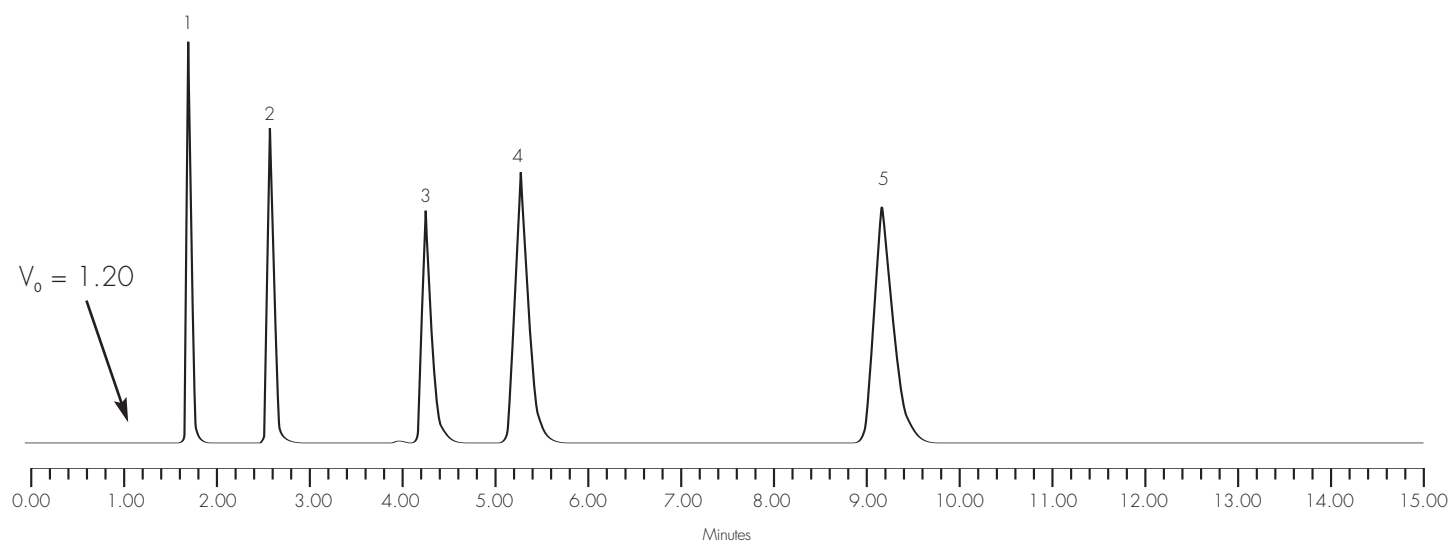


Thiourea

Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. Thiourea	1.11	35
2. 5-Fluorocytosine	1.09	35
3. Adenine	1.13	12
4. Guanosine-5'-monophosphate	1.11	71
5. Thymine	1.03	43



Thymine



LC Conditions:

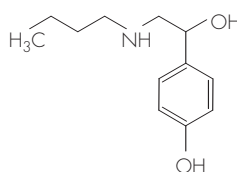
Column: Atlantis™ HILIC Silica 2.1 x 50 mm, 3 µm
 Part Number: 186002011
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 200 mM NH₄COOH, pH 3.0
 Flow Rate: 0.2 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	0	95	5
5.0	45	50	5

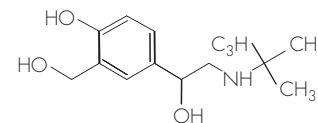
Injection Volume: 10 µL
 Sample Diluent: 75:25 ACN:MeOH with 0.2% HCOOH
 Sample Concentration: 100 ng/mL Albuterol, 50 ng/mL Bamethan
 Temperature: Ambient
 Instrument: Alliance® HT 2795, Waters ZQ™

MS Conditions:

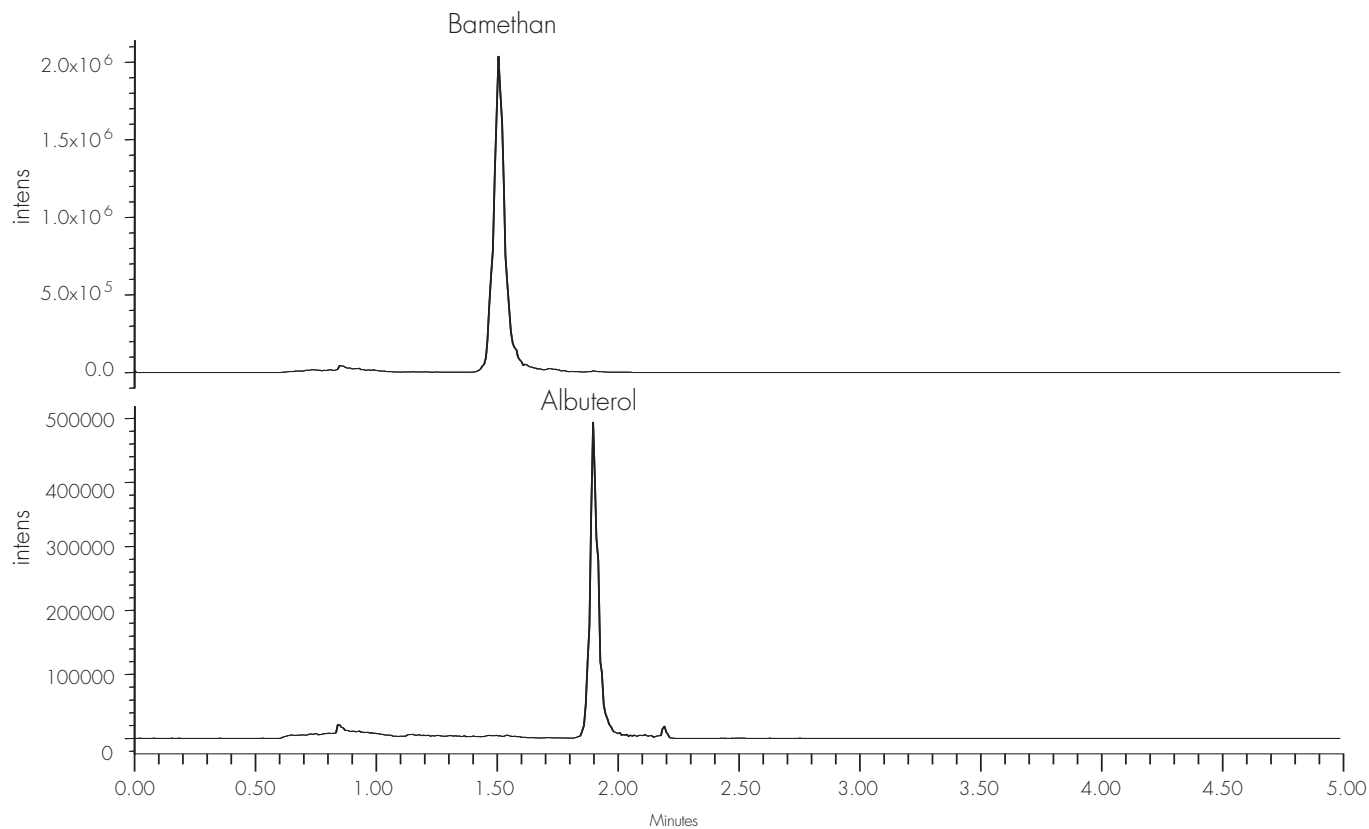
Ionization Mode: ES+
 Capillary (kV): 3.5
 Cone (V): 20
 Extractor: 3 V
 RF Lens: 0.3 V
 Source Temperature (°C): 100
 Desolvation Temperature (°C): 250
 Cone Gas Flow (L/Hr): 50
 Desolvation Gas Flow (L/Hr): 450
 SIR m/z: 209.9 (Bamethan)
 239.9 (Albuterol)



Bamethan



Albuterol



Bamethan and Albuterol—LC/UV HILIC vs. Reversed-Phase

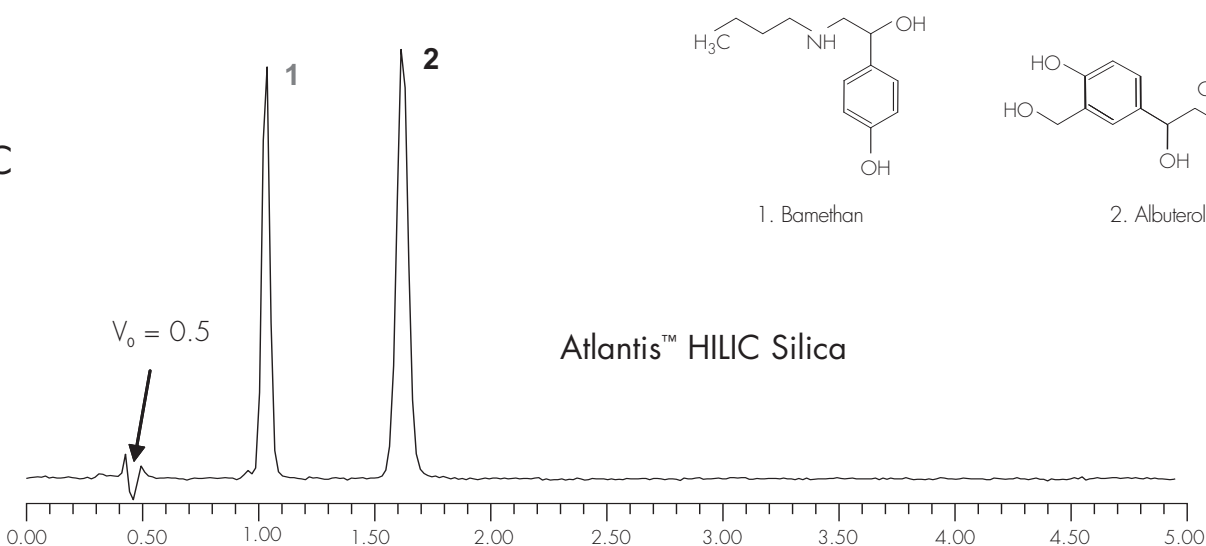
HILIC Conditions:

Column: Atlantis™ HILIC Silica 4.6 x 50 mm, 3 μm
Part Number: 186002027
Mobile Phase A: 100 mM NH₄COOH, pH 3.0
Mobile Phase B: ACN
Flow Rate: 2.0 mL/min
Isocratic Mobile
Phase Conditions: 10% A; 90% B
Injection Volume: 2 μL
Injection Solvent: 75:25 ACN:MeOH
Sample Concentration: 125 μg/mL
Temperature: 30° C
Detection: UV @ 280 nm
Instrument: Alliance® 2695 with 2996 PDA

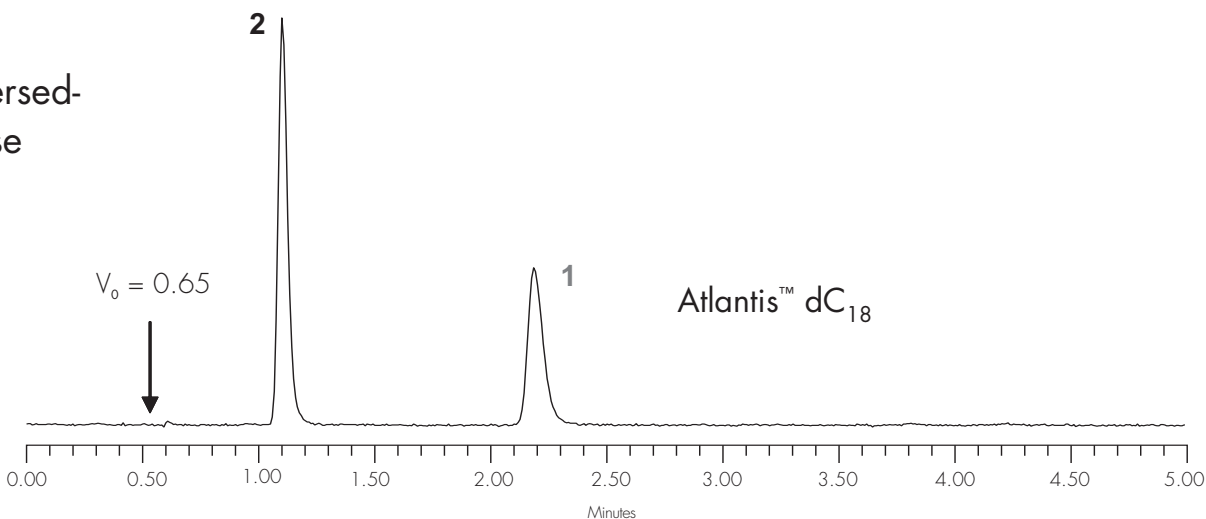
Reversed Phase Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm
Part Number: 186001329
Mobile Phase A: H₂O
Mobile Phase B: ACN
Mobile Phase C: 100 mM NH₄COOH, pH 3.0
Flow Rate: 1.4 mL/min
Isocratic Mobile
Phase Conditions: 82% A; 8% B; 10% C
Injection Volume: 2 μL
Injection Solvent: 75:25 H₂O:ACN
Sample Concentration: 125 μg/mL
Temperature: 30° C
Detection: UV @ 280 nm
Instrument: Alliance® 2695 with 2996 PDA

HILIC



Reversed-
Phase



LC Conditions:

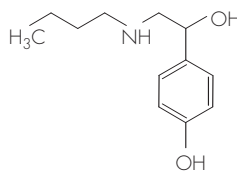
Column: Atlantis™ HILIC Silica 2.1 x 50 mm, 3 μm
 Part Number: 186002011
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 200 mM NH₄COOH, pH 3.0
 Flow Rate: 0.2 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	0	95	5
5.0	45	50	5

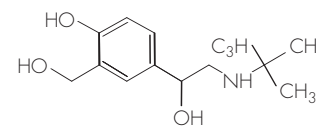
Injection Volume: 10 μL
 Sample Concentration: 50 pg/μL Albuterol
 10 pg/μL Bamethan
 Temperature: Ambient
 Instrument: Alliance® HT 2795 with Waters ZQ™

MS Conditions:

Ionization Mode: ES+
 Capillary (kV): 3.5
 Cone (V): 20
 Extractor: 3 V
 RF Lens: 0.3 V
 Source Temperature (°C): 100
 Desolvation Temperature (°C): 250
 Cone Gas Flow (L/Hr): 50
 Desolvation Gas Flow (L/Hr): 450
 SIR m/z: 209.9, 239.9

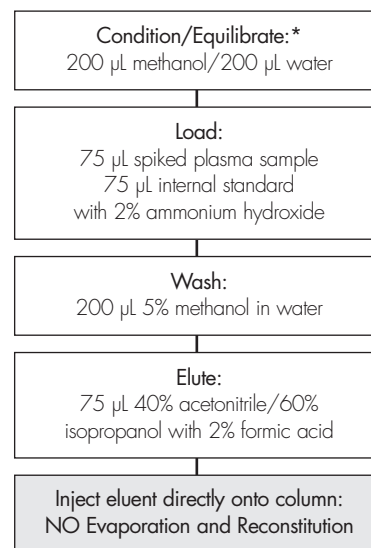


1. Bamethan

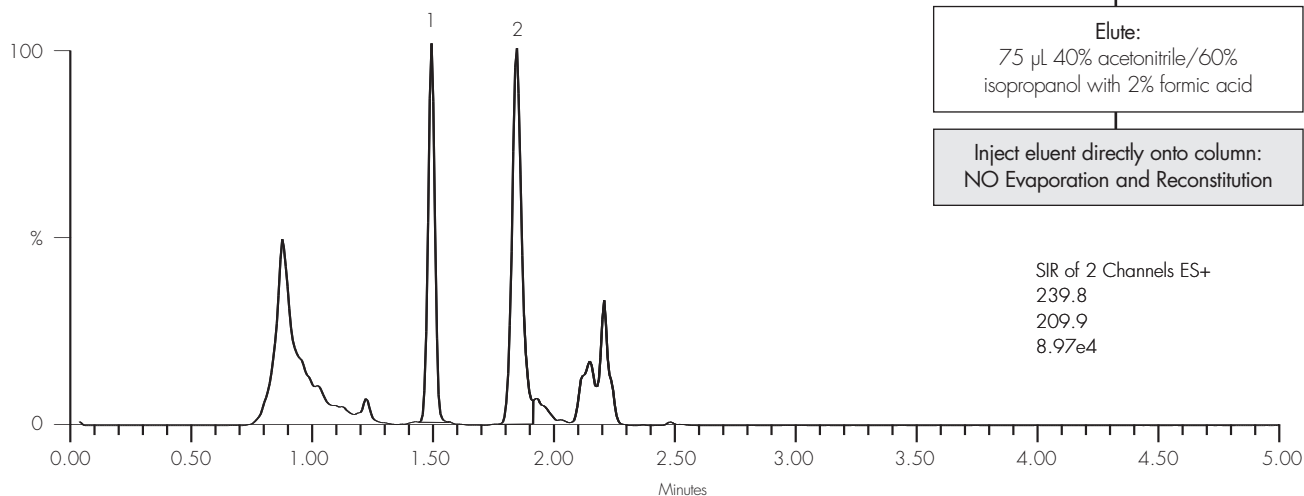


2. Albuterol

Oasis® HLB Extraction Method
 Oasis® HLB μElution Plate
 186001828BA



SIR of 2 Channels ES+
 239.8
 209.9
 8.97e4



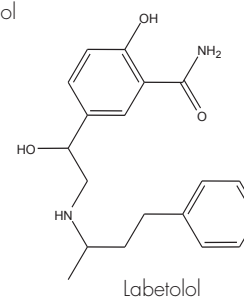
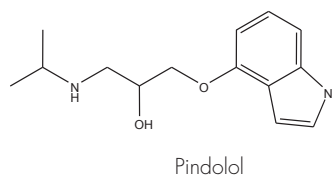
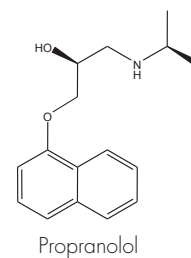
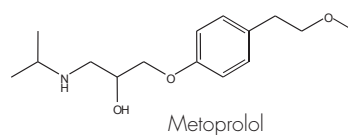
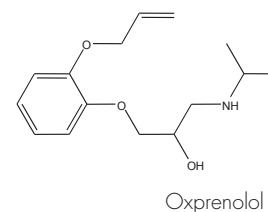
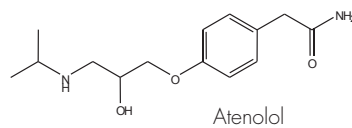
β-Blockers

LC Conditions:

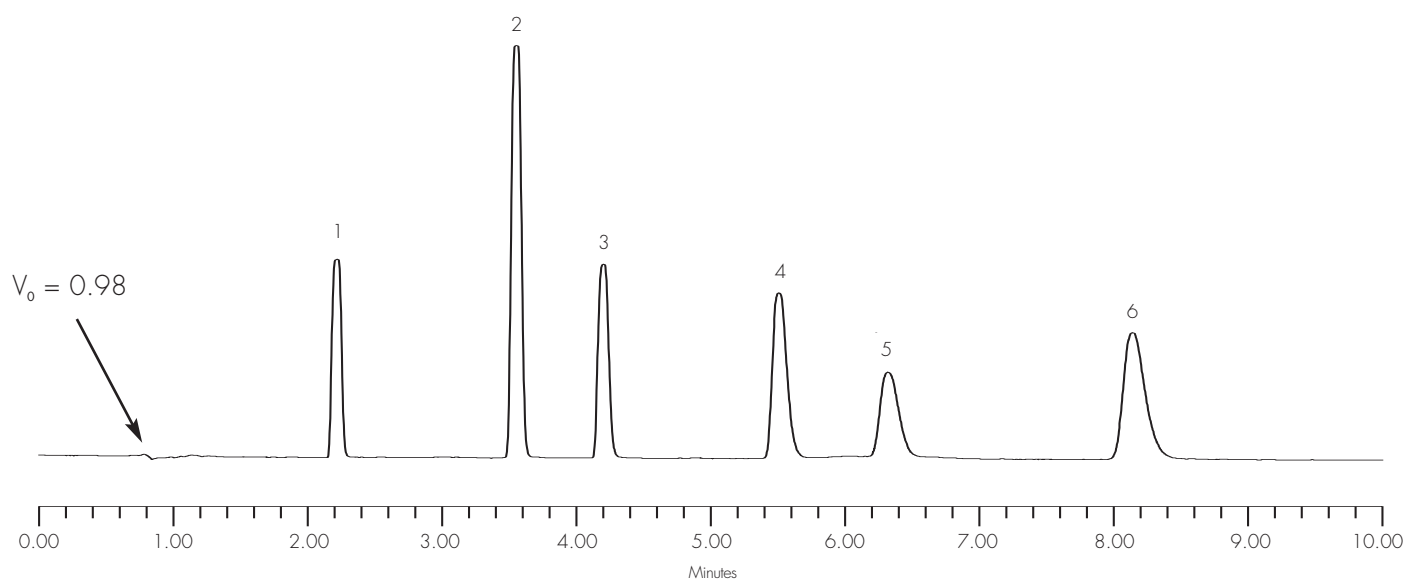
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 2.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	85	5	10
3.0	65	25	10
10.0	65	25	10

Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA

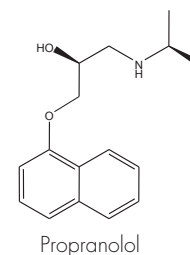
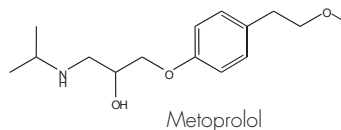
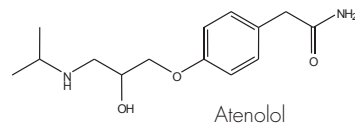


Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Atenolol	1.02	42
2. Pindolol	1.06	21
3. Metoprolol	1.13	63
4. Oxprenolol	1.32	42
5. Labetolol	1.31	63
6. Propranolol	1.41	21



LC Conditions:

Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: ACN/1% HCOOH (90/10)
 Sample Concentration: 5, 25, and 25 mg/mL in DMSO/MeOH (50/50)
 Temperature: Ambient
 Instrument: AutoPurification™ System
 Detection: UV @ 280 nm



Compounds:

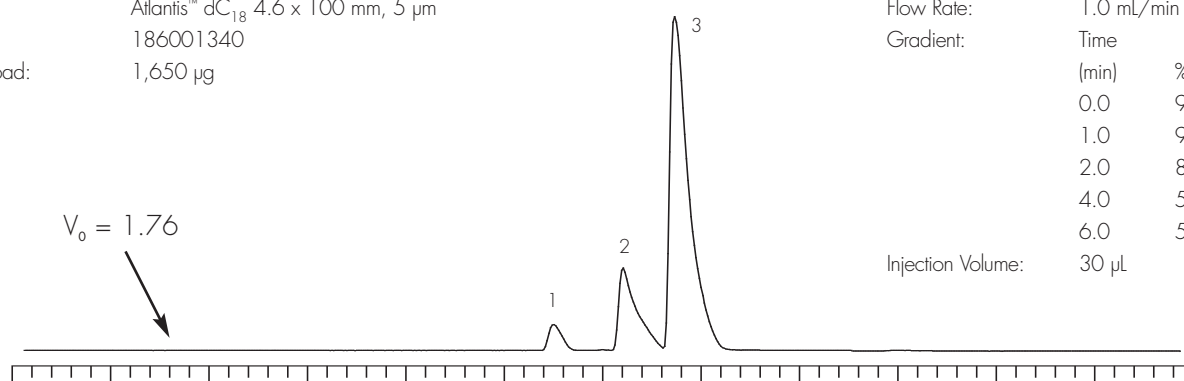
- Atenolol (5 mg/mL)
- Metoprolol (25 mg/mL)
- Propranolol (25 mg/mL)

Column: Atlantis™ dC₁₈ 4.6 x 100 mm, 5 μm
 Part Number: 186001340
 Total Mass Load: 1,650 μg

Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	95	5
1.0	95	5
2.0	80	20
4.0	50	50
6.0	50	50

Injection Volume: 30 μL

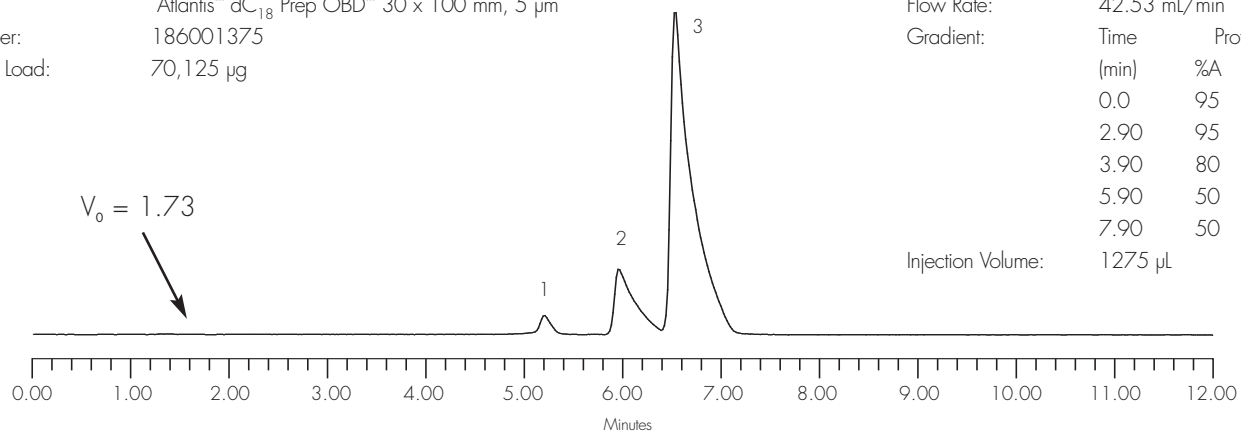


Column: Atlantis™ dC₁₈ Prep OBD™ 30 x 100 mm, 5 μm
 Part Number: 186001375
 Total Mass Load: 70,125 μg

Flow Rate: 42.53 mL/min
 Gradient:

Time (min)	%A	%B
0.0	95	5
2.90	95	5
3.90	80	20
5.90	50	50
7.90	50	50

Injection Volume: 1275 μL



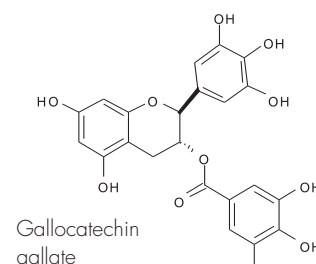
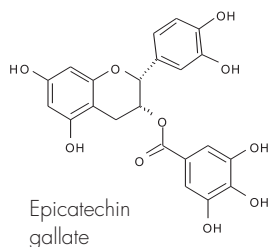
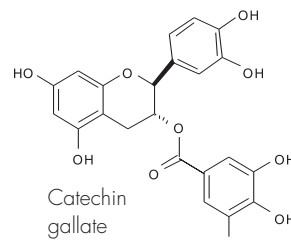
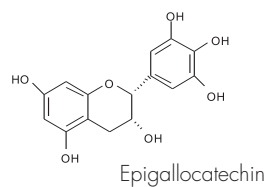
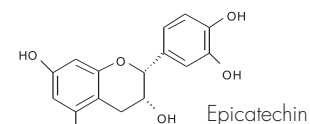
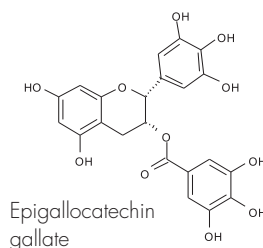
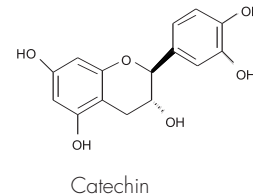
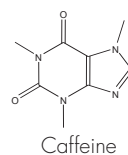
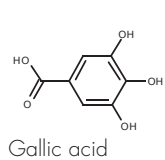
Catechins

LC Conditions:

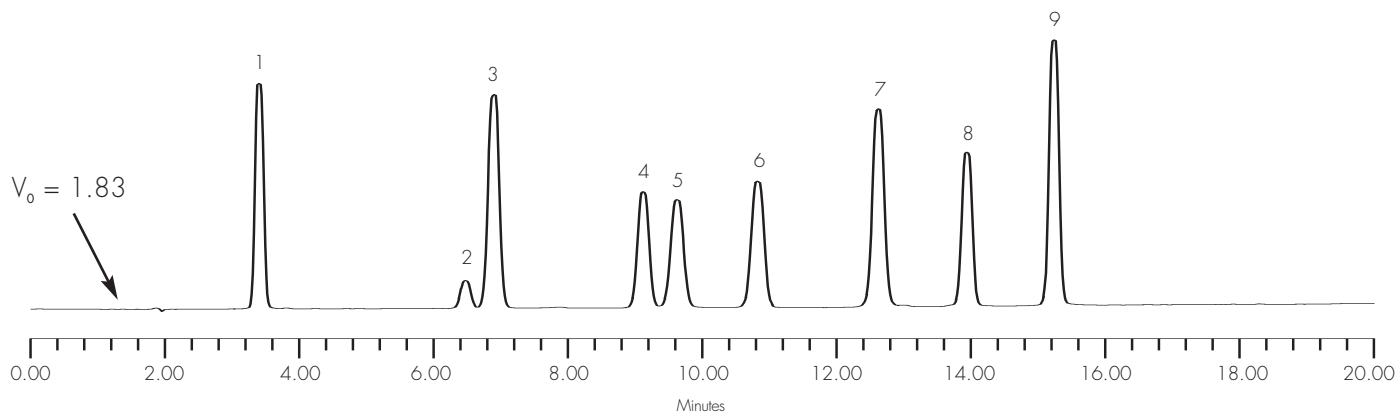
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 1% HCOOH, pH 2.3
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	70	20	10
10.0	60	30	10
20.0	30	60	10

Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA



Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Gallic Acid	1.05	11
2. Epigallocatechin	0.94	43
3. Catechin	1.00	22
4. Caffeine	0.97	11
5. Epigallocatechin gallate	1.08	22
6. Epicatechin	0.98	43
7. Gallocatechin gallate	0.98	33
8. Epicatechin gallate	0.98	22
9. Catechin gallate	0.99	33

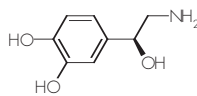


LC Conditions:

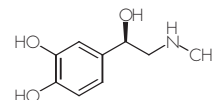
Column: Atlantis™ HILIC Silica 4.6 x 50 mm, 5 µm
 Part Number: 186002028
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 200 mM NH₄COOH, pH 3.0
 Flow Rate: 2.0 mL/min
 Gradient:

Time (min)	Profile		
	%A	%B	%C
0.0	0	95	5
5.0	25	70	5

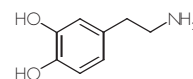
Injection Volume: 5 µL
 Injection Solvent: 75:25 ACN:MeOH with 0.2% HCOOH
 Analyte Concentrations: 42 µg/mL Norepinephrine
 17 µg/mL Epinephrine
 83 µg/mL Dopamine
 Detection: UV @ 280 nm
 Temperature: Ambient
 Instrument: Alliance® 2695 with 2996 PDA



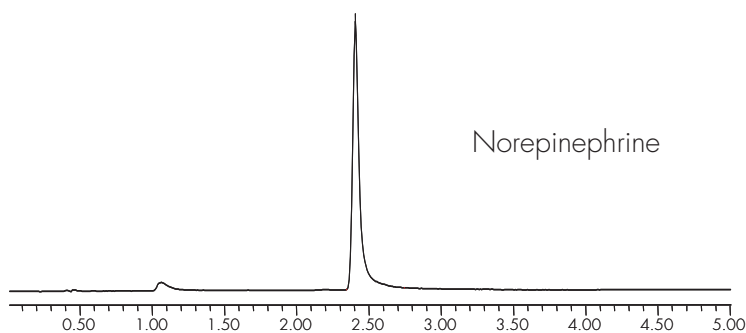
Norepinephrine



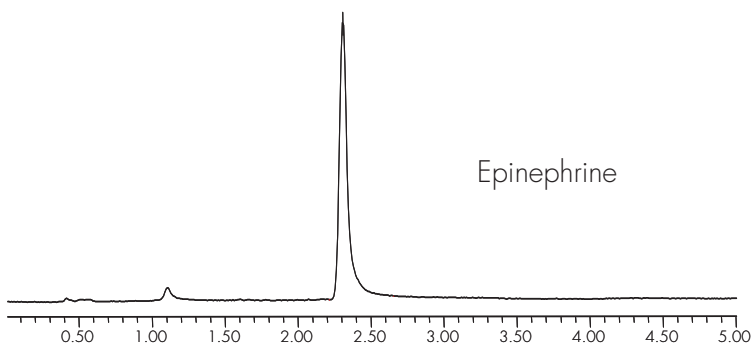
Epinephrine



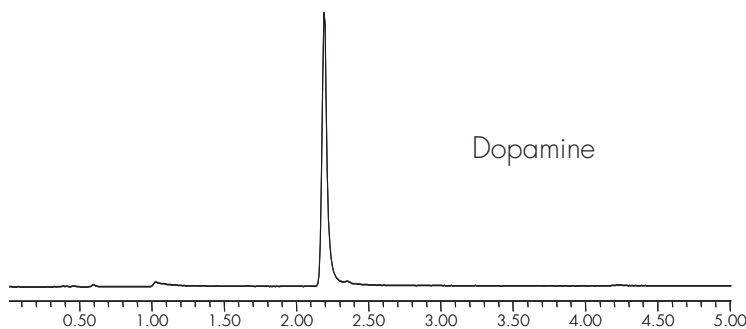
Dopamine



Norepinephrine



Epinephrine



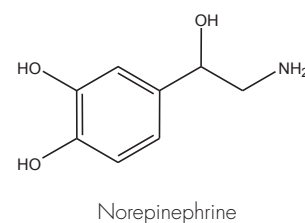
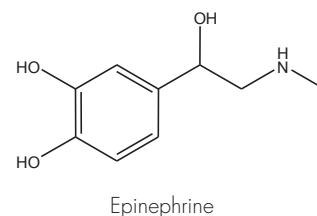
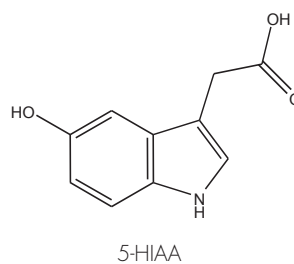
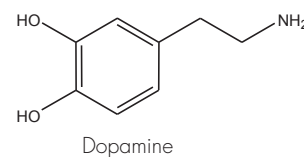
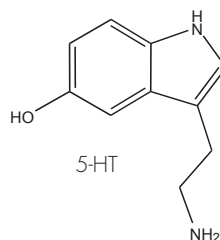
Dopamine

Minutes

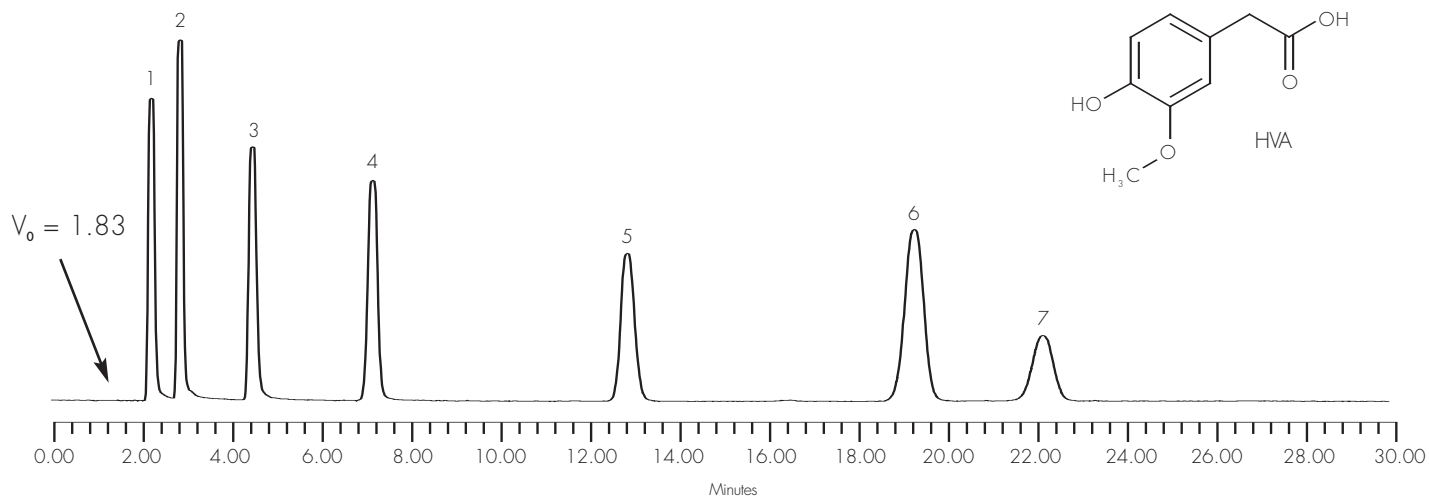
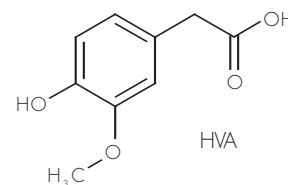
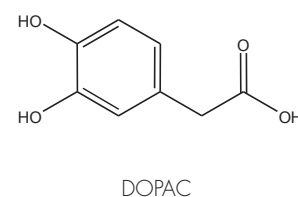
Catecholamines—Isocratic LC/UV

LC Conditions:

Columns: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM CH₃COONH₄, pH 5.0
 Flow Rate: 1.0 mL/min
 Isocratic Mobile
 Phase Composition: 88% A; 2% B; 10% C
 Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA



Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. Norepinephrine (NE)	1.21	23
2. Epinephrine (E)	1.20	9
3. Dopamine (DA)	1.21	23
4. 3,4-Dihydroxyphenylacetic acid (DOPAC)	1.00	23
5. Serotonin (5-HT)	1.10	27
6. 5-Hydroxy-3-indoleacetic acid (5-HIAA)	0.97	45
7. 4-Hydroxy-3-methoxyphenylacetic acid (HVA)	0.97	23

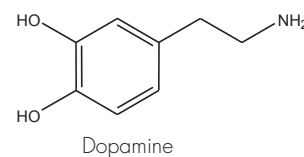
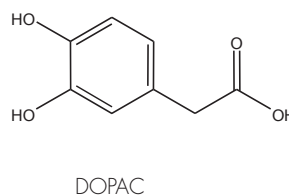
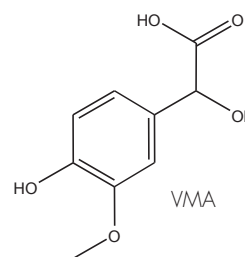
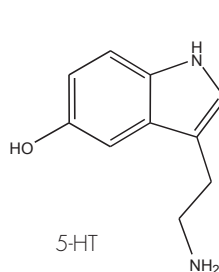


LC Conditions:

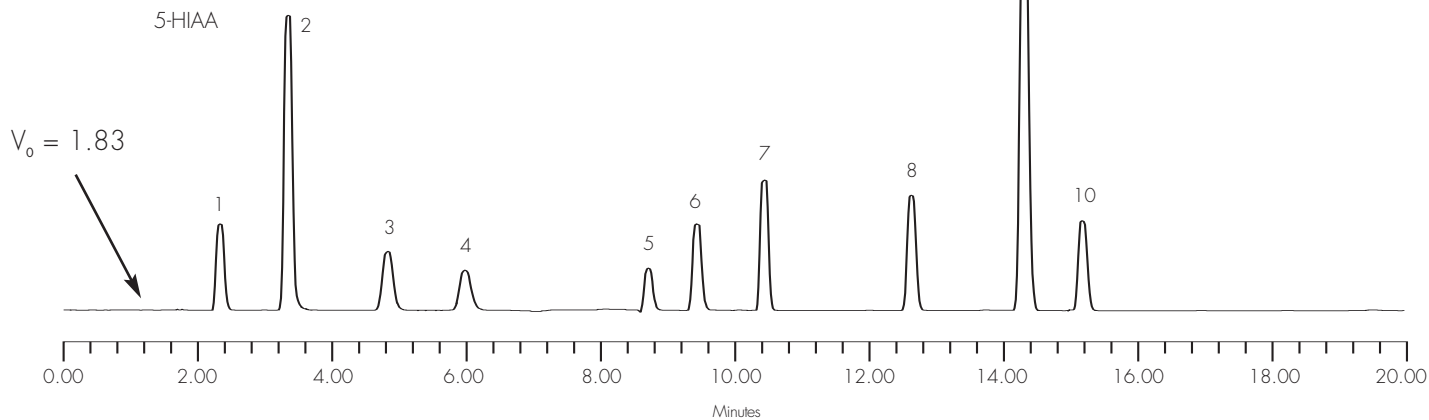
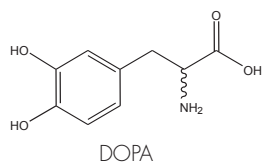
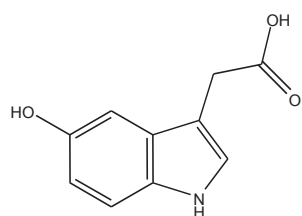
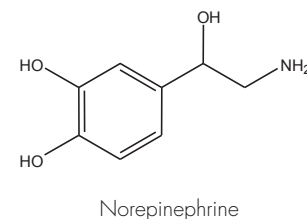
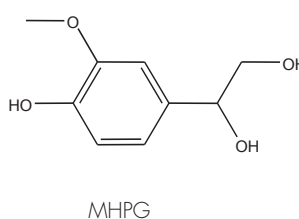
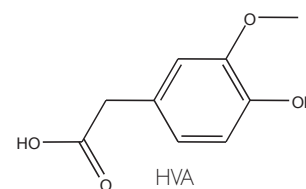
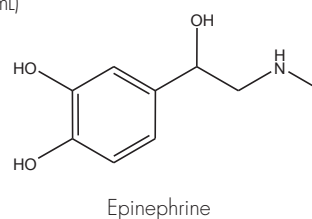
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	90	0	10
5.0	90	0	10
15.0	65	25	10
20.0	65	25	10

Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA



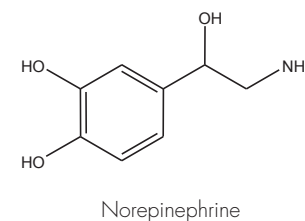
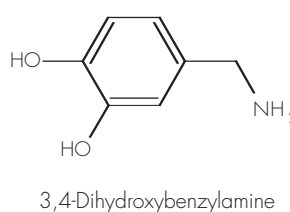
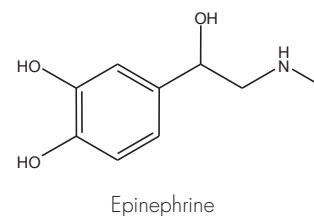
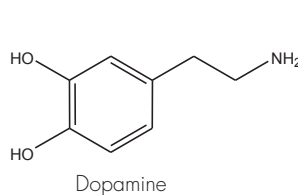
Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Norepinephrine (NE)	1.16	25
2. Epinephrine (E)	1.17	20
3. 3,4-Dihydroxyphenylalanine (DOPA)	1.15	20
4. Dopamine (DA)	1.04	25
5. 3-Methoxy-4-hydroxyphenylglycol (MHPG)	1.16	25
6. 4-Hydroxy-3-methoxymandelic acid (VMA)	1.17	25
7. Serotonin (5-HT)	1.10	30
8. 3,4-Dihydroxyphenylacetic acid (DOPAC)	1.09	25
9. 5-Hydroxy-3-indoleacetic acid (5-HIAA)	1.11	25
10. 4-Hydroxy-3-methoxyphenylacetic acid (HVA)	1.12	25



Catecholamines—LC/ECD

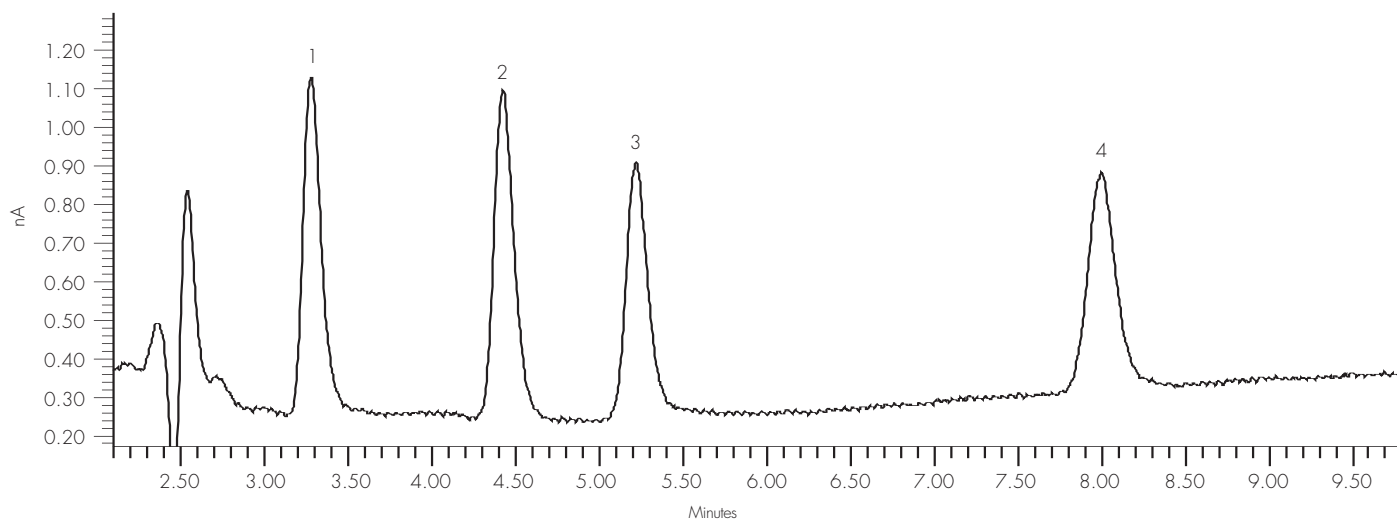
LC Conditions:

Column: Atlantis™ dC₁₈ 2.1 x 150 mm, 3 μm
 Part Number: 186001299
 Mobile Phase: 3% MeOH
 97% Buffer:
 10.5 g/L Citric Acid
 20 mg/L EDTA
 20 mg/L Octanesulfonic acid
 135 mg/L NaCl
 pH 2.9 with NaOH
 Flow Rate: 0.25 mL/min
 Injection Volume: 10 μL
 Sample Concentration: 2.0 ng/mL
 Temperature: 40° C
 Detection: Electrochemical, +600 mV
 (Glassy Carbon vs. ISSAC)
 Instrument: Alliance® 2695 with 2465 ECD



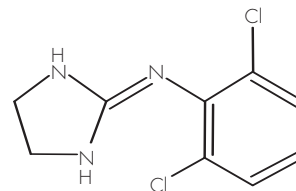
Compounds:

1. Norepinephrine (NE)
2. Epinephrine (E)
3. 3,4-Dihydroxybenzylamine (DHBA, Internal Standard)
4. Dopamine (DA)



LC Conditions:

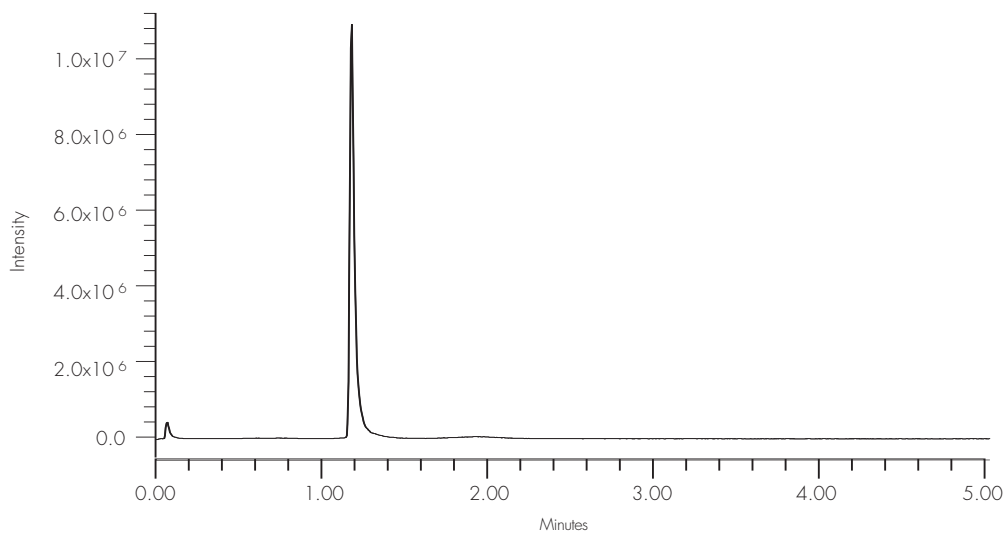
Column: Atlantis™ HILIC Silica 2.1 x 50 mm, 3 µm
Part Number: 186002011
Mobile Phase A: 200 mM NH₄COOH, pH 3.0
Mobile Phase B: ACN
Flow Rate: 0.3 mL/min
Isocratic Mobile
Phase Conditions: 5% A; 95% B
Injection Volume: 5 µL
Sample Diluent: 75:25 ACN:MeOH
Sample Concentration: 10 ng/mL
Temperature: Ambient
Instrument: Alliance® HT 2795 with Waters ZQ™



Clonidine

MS Conditions:

Ionization Mode: ES+
Capillary (kV): 2.0
Cone (V): 40
Extractor: 3 V
RF Lens: 0.3 V
Source Temperature (oC): 150
Desolvation Temperature (oC): 350
Cone Gas Flow (L/Hr): 50
Desolvation Gas Flow (L/Hr): 700
SIR m/z: 230.2



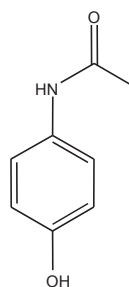
Cold Medicine Products

LC Conditions:

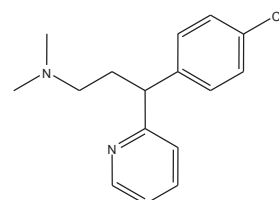
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: 0.1% TFA
 Mobile Phase B: ACN
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	77	23
1.0	77	23
3.0	52	48
10.0	52	48

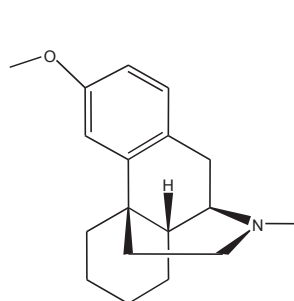
Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 265 nm
 Instrument: Alliance® 2695 with 2996 PDA



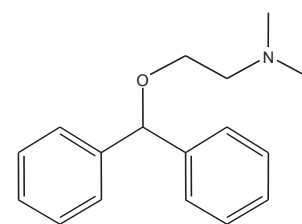
Acetaminophen



Chlorpheniramine

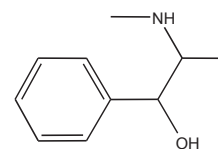


Dextromethorphan

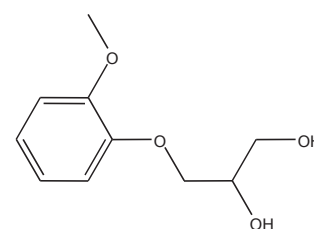


Diphenhydramine

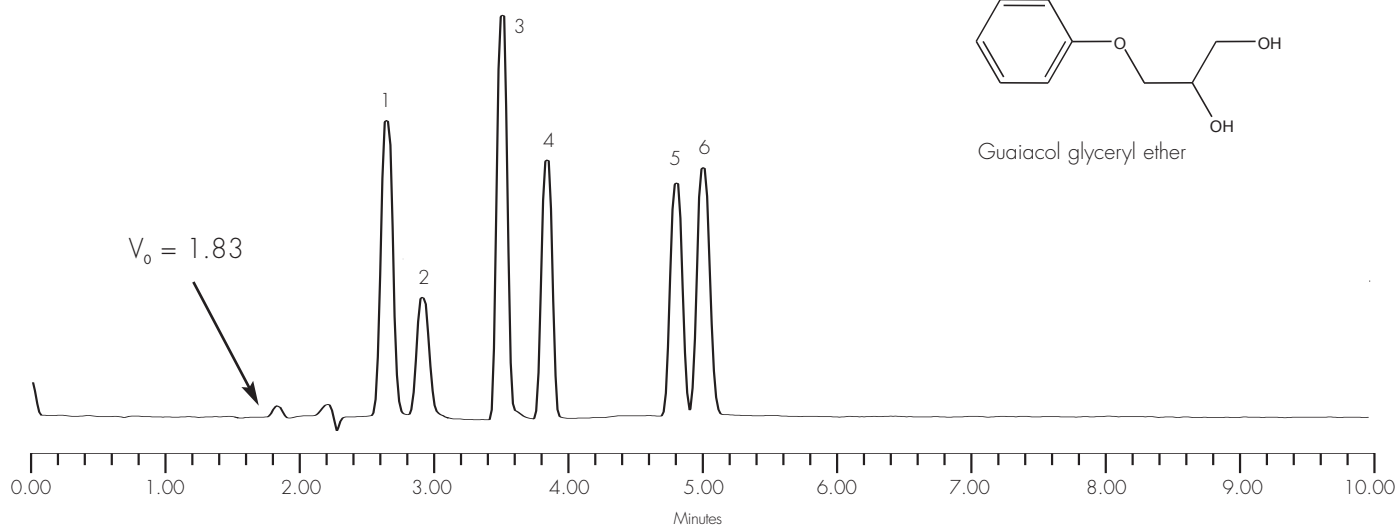
Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Acetaminophen	1.04	112
2. Pseudoephedrine	1.11	4
3. Chlorpheniramine	1.04	34
4. Guaiacol glyceryl ether	1.01	10
5. Dextromethorphan	1.00	34
6. Diphenhydramine	1.08	10



Pseudoephedrine



Guaiacol glyceryl ether



HILIC Conditions:

Column: Atlantis™ HILIC Silica 4.6 x 50 mm, 5 µm
 Part Number: 186002028
 Mobile Phase A: 90:10 ACN:NH₄CH₃COOH, pH 4.5
 Mobile Phase B: 6.5 mM NH₄CH₃COOH, pH 4.5
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	90	10
45.0	60	40

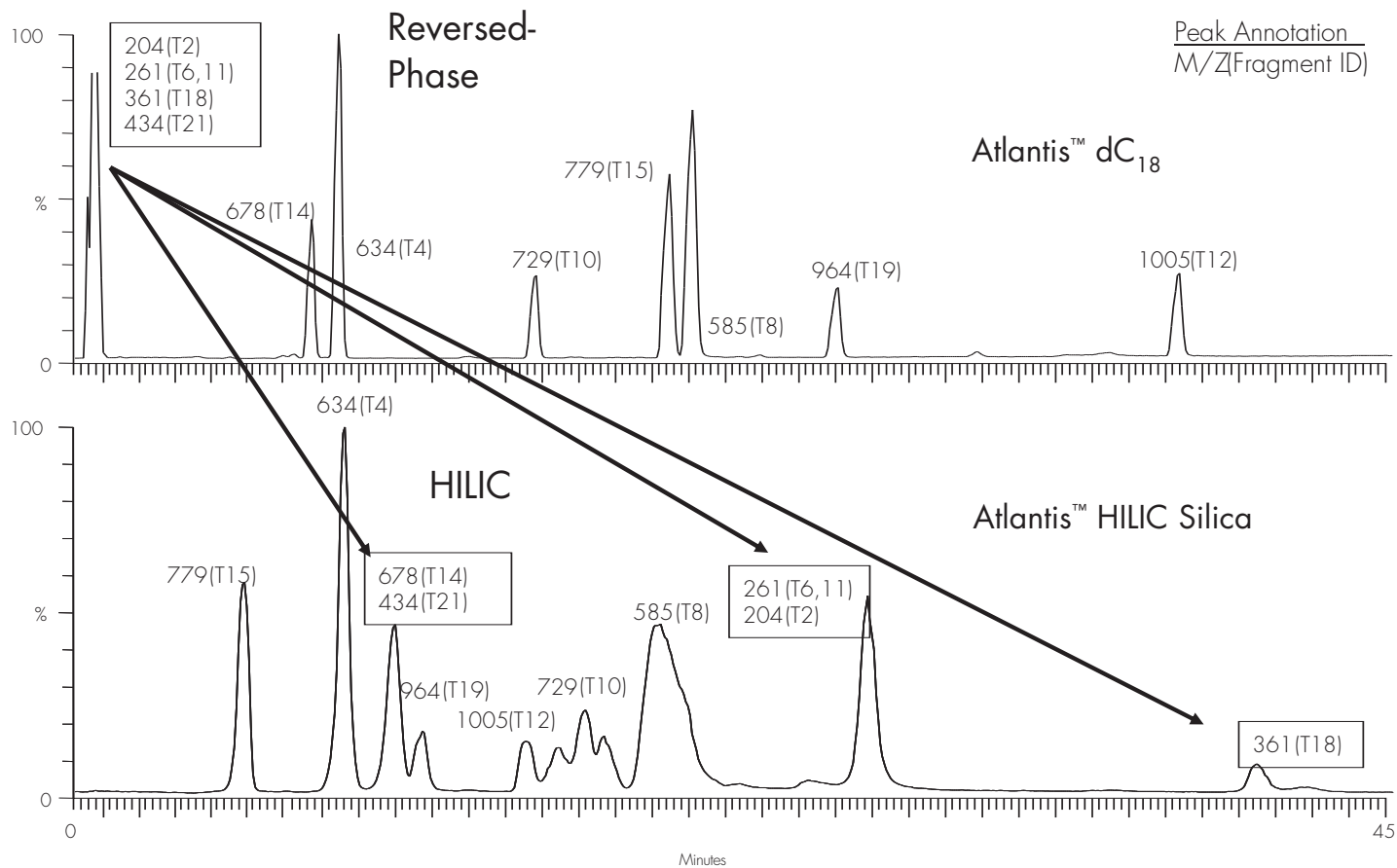
Injection Volume: 10 µL
 Sample Concentration: 1 µg/µL
 Temperature: 40° C
 Instrument: Alliance® 2690 with Waters ZQ™

Reversed-Phase Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 5 µm
 Part Number: 186001331
 Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: 0.1% HCOOH in ACN
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	100	0
45.0	60	40

Injection Volume: 10 µL
 Sample Concentration: 1 µg/µL
 Temperature: 40° C
 Instrument: Alliance® 2690 with Waters ZQ™



Dextromethorphan and N-Dealkylated Metabolite

LC Conditions:

Column: Atlantis™ dC₁₈ 2.1 x 150 mm, 5 μm

Part Number: 186001301

Mobile Phase A: 0.1% HCOOH in H₂O

Mobile Phase B: 0.1% HCOOH in ACN

Flow Rate: 0.3 mL/min

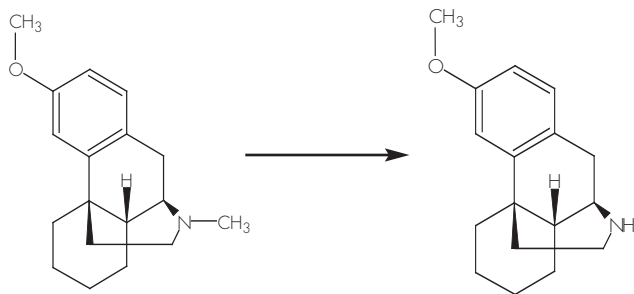
Gradient Time (min)	Profile	
	%A	%B
0.0	90	10
1.5	90	10
7.0	15	85
8.0	15	85
8.1	90	10
13.0	90	10

Injection Volume: 10 μL

Temperature: 30° C

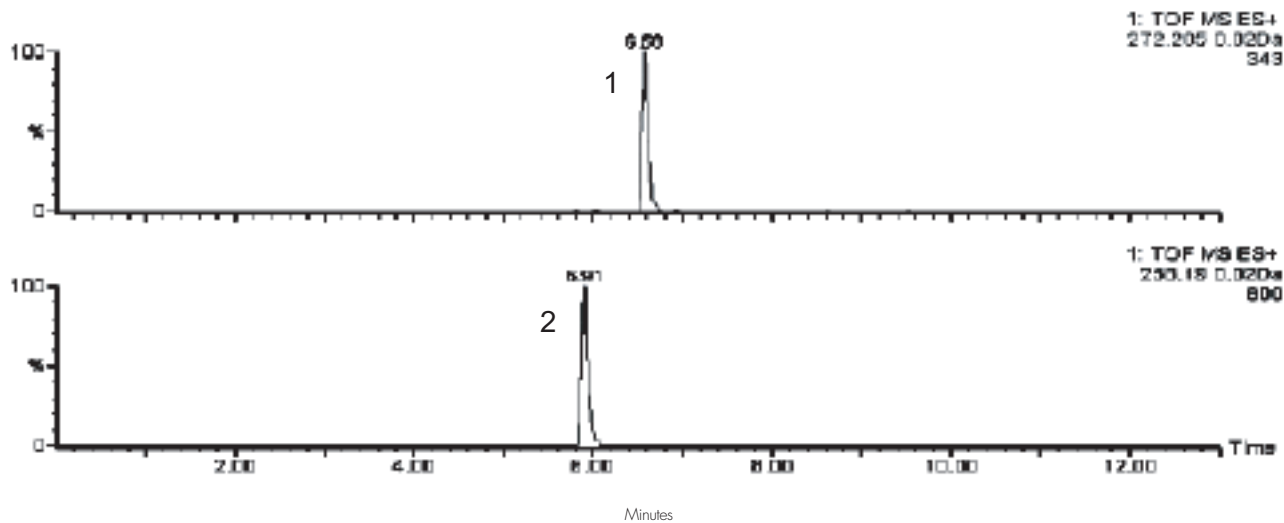
Instrument: Alliance® HT 2795, Q-ToF micro™ ES+

Sample: Dextromethorphan rat microsomal incubation at 0.5 mM



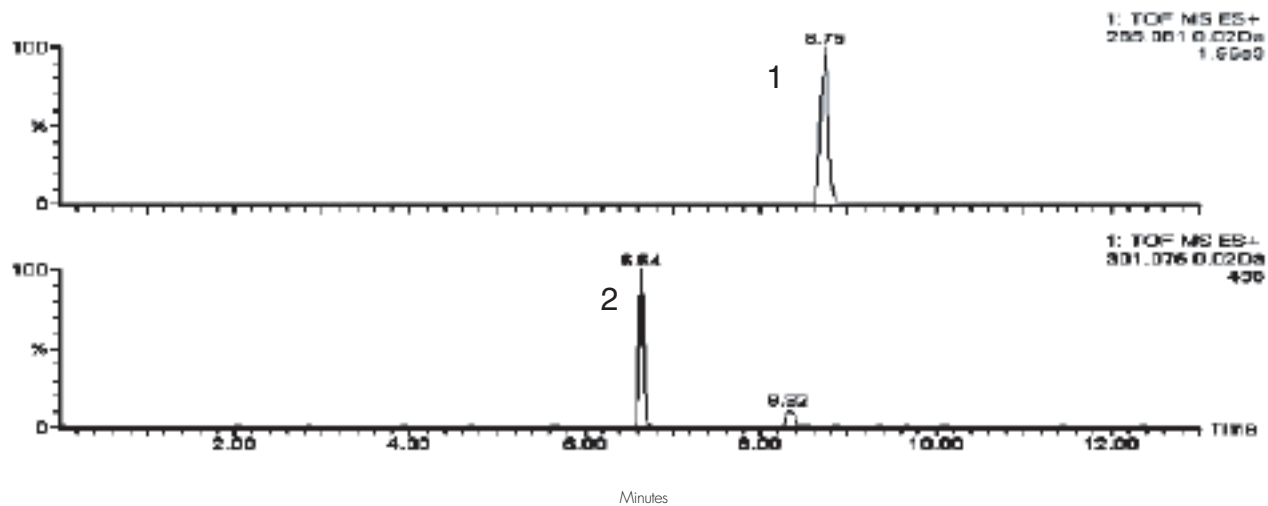
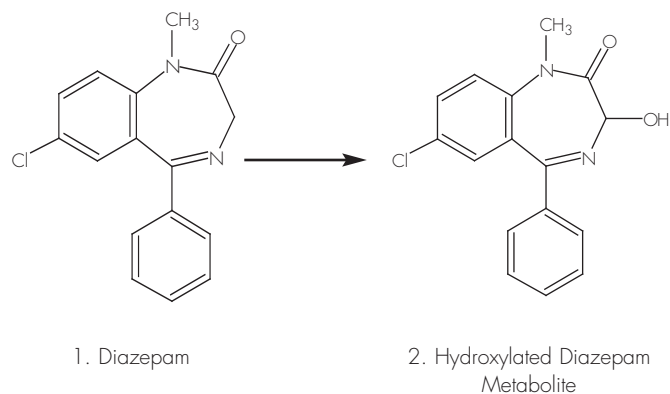
1. Dextromethorphan

2. N-Dealkylated Dextromethorphan Metabolite



LC Conditions:

Column:	Atlantis™ dC ₁₈ 2.1 x 150 mm, 5 µm																					
Part Number:	186001301																					
Mobile Phase A:	0.1 % HCOOH in H ₂ O																					
Mobile Phase B:	0.1% HCOOH in ACN																					
Flow Rate:	0.3 mL/min																					
Gradient:	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%A</th> <th>%B</th> </tr> </thead> <tbody> <tr> <td>0.0</td> <td>90</td> <td>10</td> </tr> <tr> <td>1.5</td> <td>90</td> <td>10</td> </tr> <tr> <td>7.0</td> <td>15</td> <td>85</td> </tr> <tr> <td>8.0</td> <td>15</td> <td>85</td> </tr> <tr> <td>8.1</td> <td>90</td> <td>10</td> </tr> <tr> <td>13.0</td> <td>90</td> <td>10</td> </tr> </tbody> </table>	Time (min)	%A	%B	0.0	90	10	1.5	90	10	7.0	15	85	8.0	15	85	8.1	90	10	13.0	90	10
Time (min)	%A	%B																				
0.0	90	10																				
1.5	90	10																				
7.0	15	85																				
8.0	15	85																				
8.1	90	10																				
13.0	90	10																				
Injection Volume:	10 µL																					
Temperature:	30° C																					
Instrument:	Alliance® HT 2795, Q-ToF micro™ ES+																					
Sample:	Diazepam rat microsomal incubation at 1 mM																					



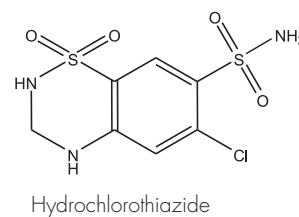
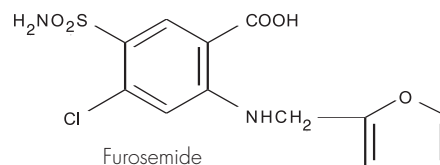
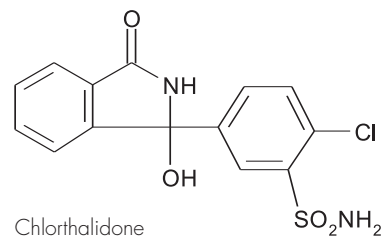
Diuretics

LC Conditions:

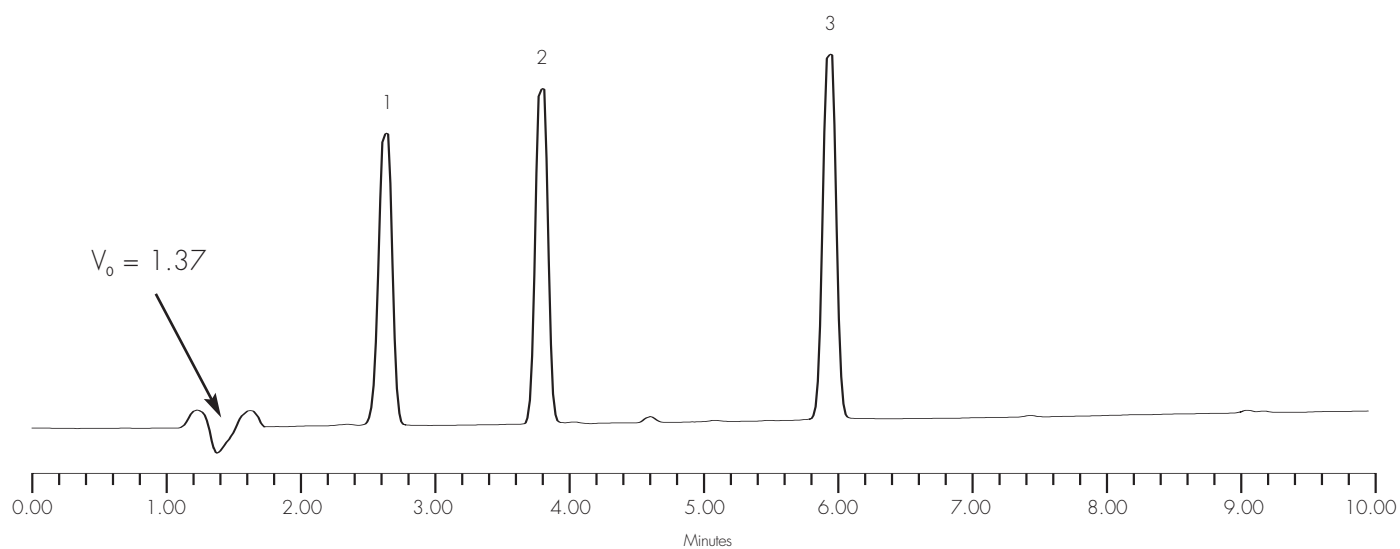
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.75
 Flow Rate: 1.4 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	67	23	10
10.0	35	55	10

Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 248 nm
 Instrument: Alliance® 2695 with 2996 PDA



Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Hydrochlorothiazide	0.94	70
2. Chlorthalidone	0.99	70
3. Furosemide	0.99	60

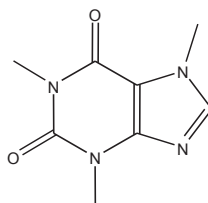


LC Conditions:

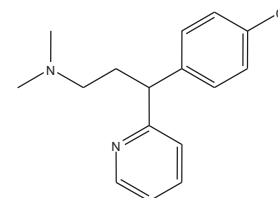
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 1% HCOOH, pH 2.3
 Flow Rate: 1.4 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	90	0	10
10.0	50	40	10

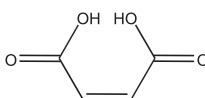
Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA



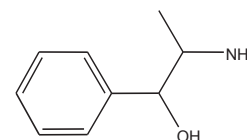
Caffeine



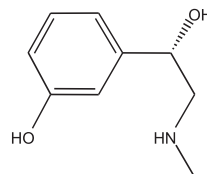
Chlorpheniramine



Maleic acid

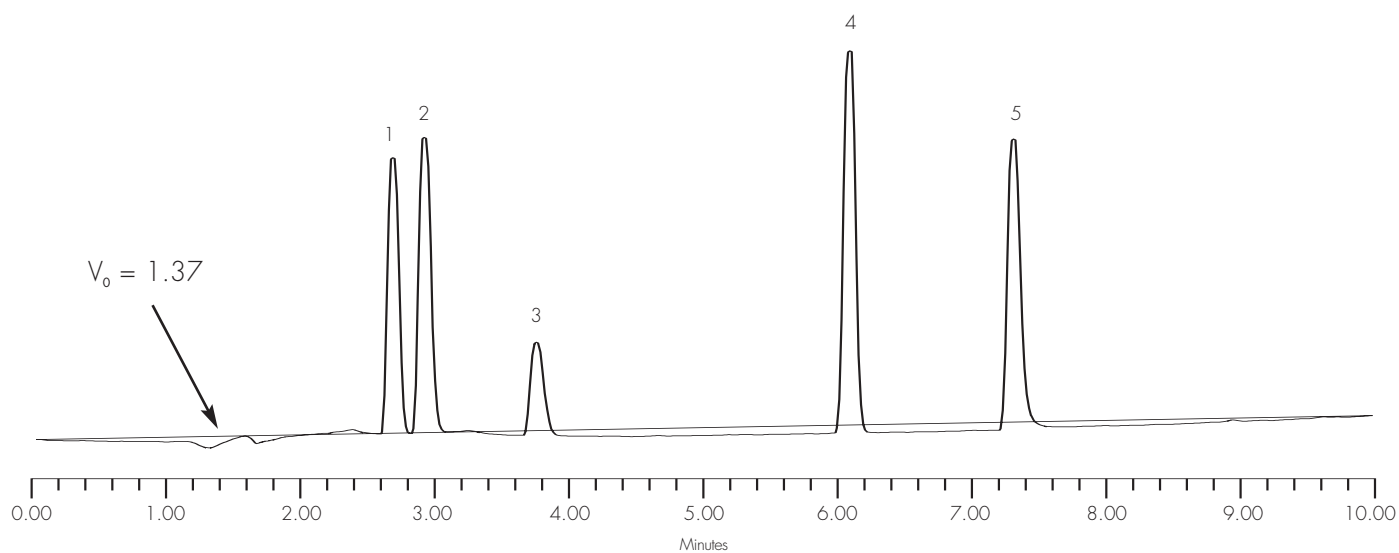


Phenylpropanolamine



Phenylephrine

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Phenylephrine	1.10	80
2. Maleic acid	1.17	33
3. Phenylpropanolamine	1.26	88
4. Caffeine	1.05	11
5. Chlorpheniramine	1.26	22



Fluoroquinolone Antibiotics in Beef Kidney (LC/MS/MS)

LC Conditions:

Column: Atlantis™ dC₁₈, 4.6 x 150 mm, 5 µm
 Part number: 1860001344
 Mobile Phase A: 0.2% NFPA* in H₂O
 Mobile Phase B: MeOH
 Flow rate: 0.8 mL/min
 Gradient:

Time (min)	%A	%B
0.0	60	40
10.0	20	80

 Injection volume: 50 µL
 Temperature: 30 °C
 Instrument: Alliance® 2695

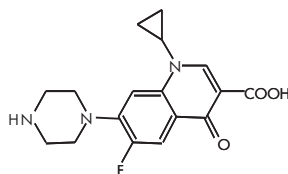
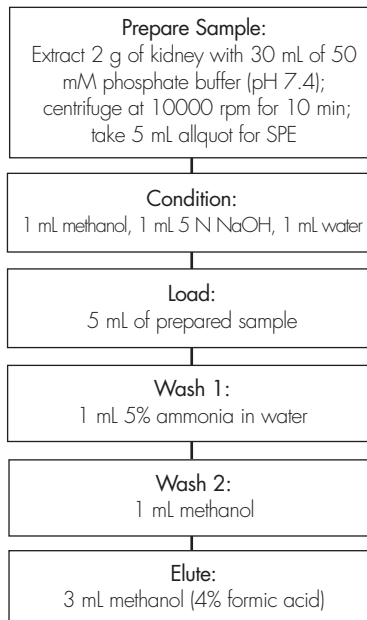
*NFPA: nonafluoropentanoic acid (NFPA) - C₄F₉COOH

MS Conditions:

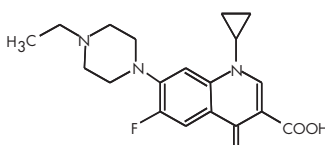
Instrument: Quattro micro™
 Ion Source: APCI+
 Mode: Multiple Reaction Monitoring
 Corona: 0.8 V
 Source Temp: 150 °C
 Desolvation Temp: 625 °C
 Cone Gas (N₂): 175 L/Hr
 Desolvation Gas (N₂): 250 L/Hr
 Collision Gas: Argon

Oasis® MAX Extraction Method

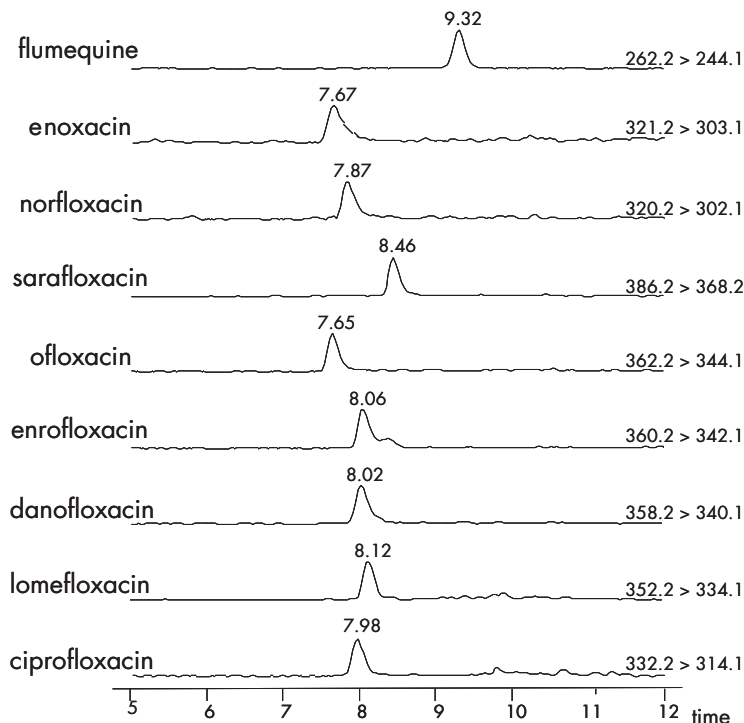
Oasis® MAX Extraction Cartridge, 6 cc/150 mg, 30 µm
 Part Number: 186000369



Ciprofloxacin



Enrofloxacin



Compound	10µg/kg % Recovery (± % RSD)	50µg/kg % Recovery (± % RSD)
flumequin	74 (± 9.1)	70 (± 17)
enoxacin	63 (± 5.7)	65 (± 13)
norfloxacin	64 (± 8.1)	65 (± 9.9)
sarafloxacin	68 (± 9.5)	71 (± 9.8)
ofloxacin	72 (± 7.0)	80 (± 8.6)
enrofloxacin	73 (± 5.3)	76 (± 8.9)
danofloxacin	64 (± 8.8)	68 (± 8.2)
lomefloxacin	76 (± 6.9)	76 (± 7.9)
ciprofloxacin	70 (± 8.7)	62 (± 6.1)

External standard calculation
 Results calculated against standards in matrix (n=5)

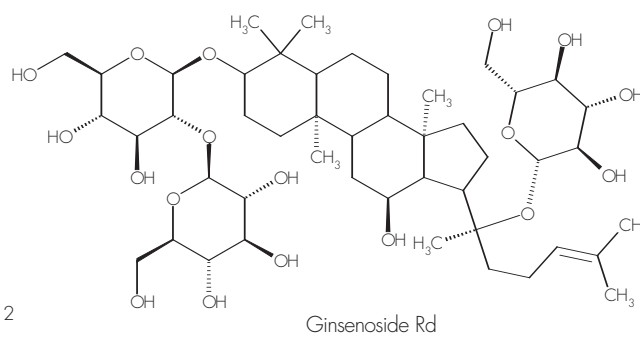
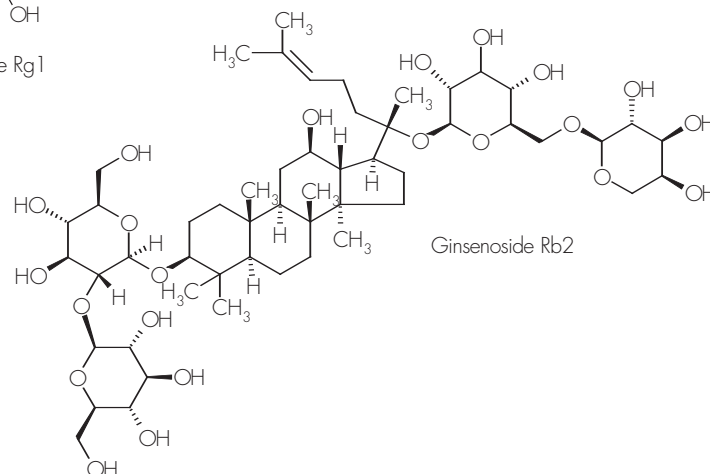
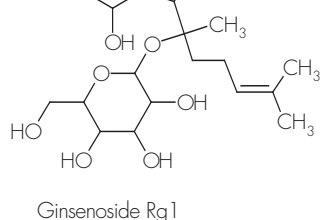
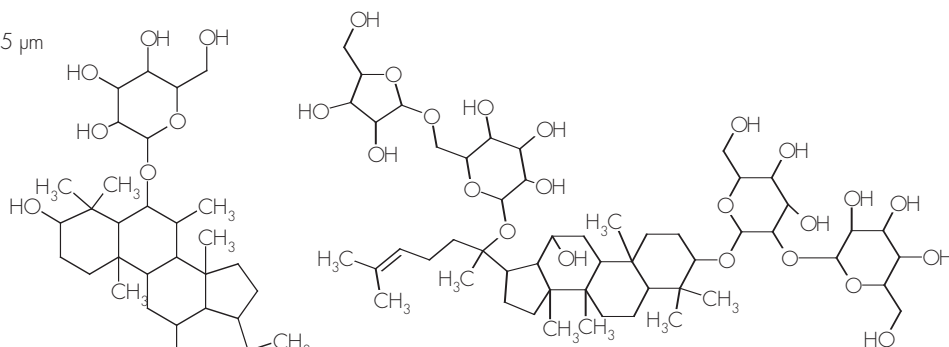
Compound	MW	(MRM)	Cone(V)	Coll.
flumequine	261	262 > 244	50	20
enoxacin	320	321 > 303	50	20
norfloxacin	319	320 > 302	50	23
sarafloxacin	385	386 > 368	50	25
ofloxacin	361	362 > 344	50	20
enrofloxacin	359	360 > 342	50	20
danofloxacin	357	358 > 340	50	25
lomefloxacin	351	352 > 334	50	20
ciprofloxacin	331	332 > 214	50	20

LC Conditions:

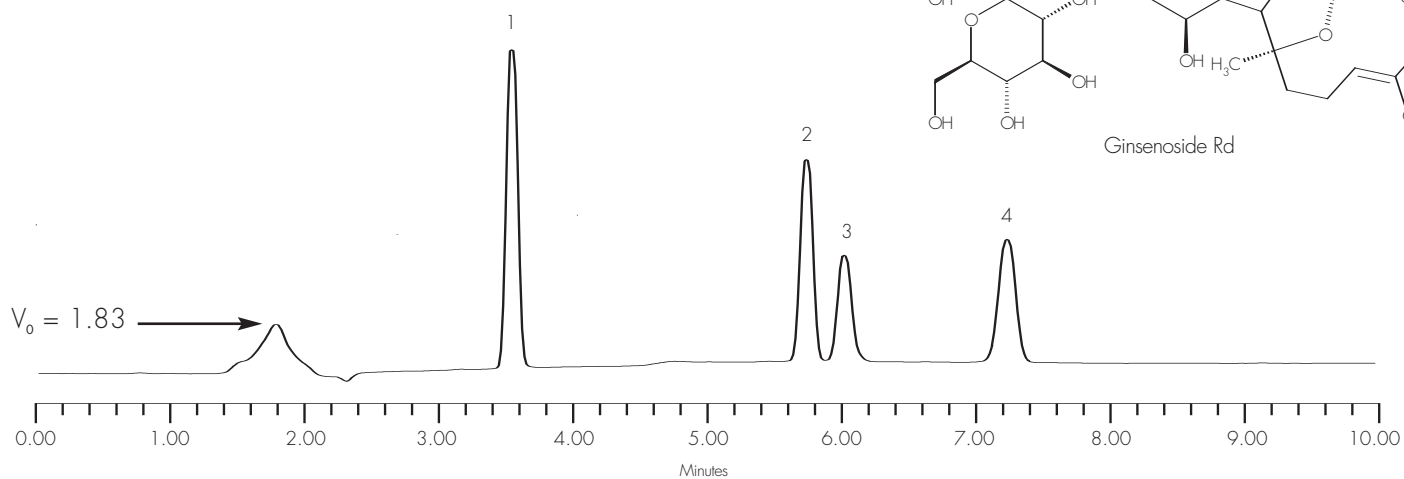
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	Profile %A	%B
0.0	75	25
4.0	60	40
10.0	60	40

Injection Volume: 10 μL
 Sample Concentrations: 63 μg/mL each
 Temperature: 30° C
 Detection: UV @ 203 nm
 Instrument: Alliance® 2695 with 2996 PDA



Compounds:	USP Tailing
1. Ginsenoside Rg1	1.02
2. Ginsenoside Rc	1.01
3. Ginsenoside Rb2	1.13
4. Ginsenoside Rd	0.99



Guanine and Acyclovir— HILIC vs. Reversed-Phase

HILIC Conditions:

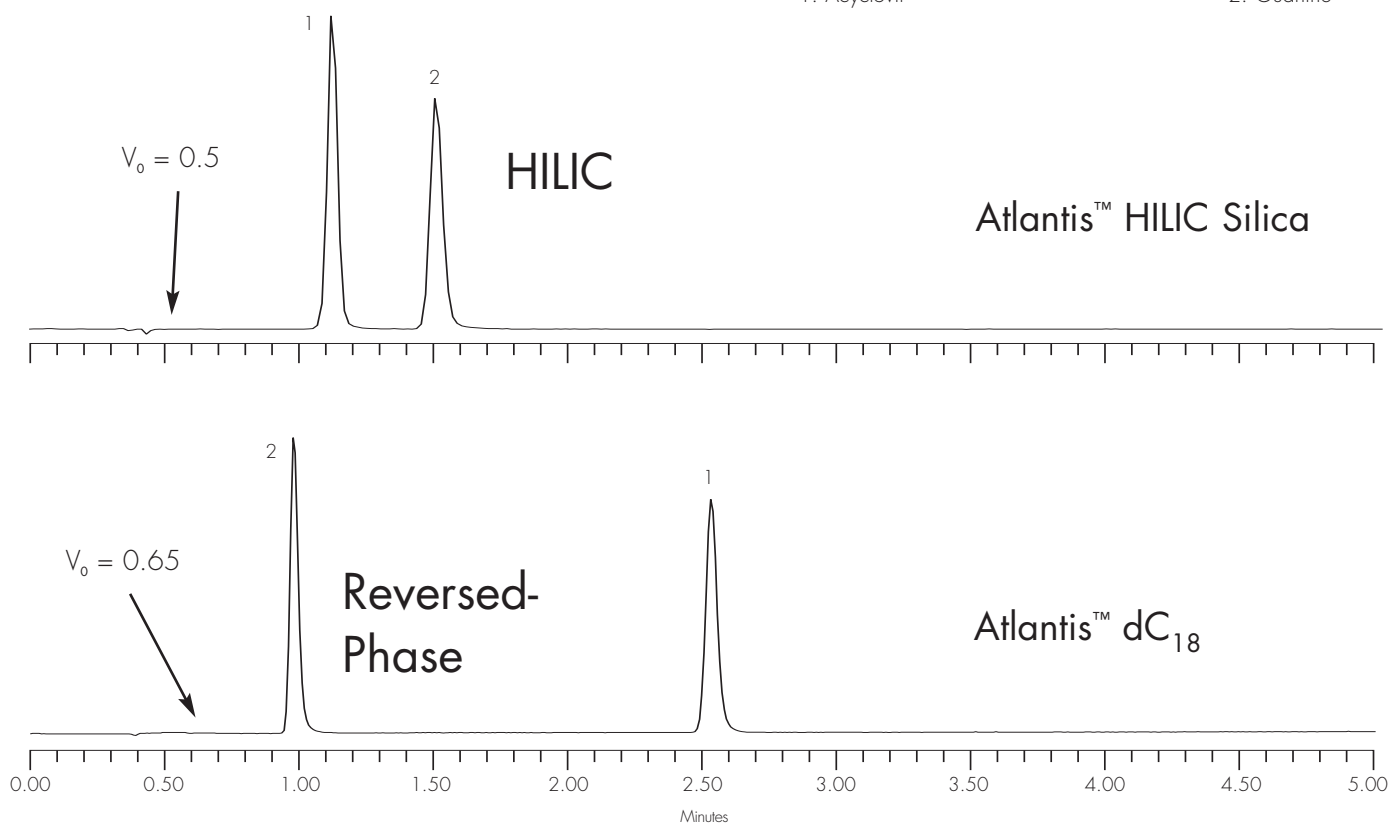
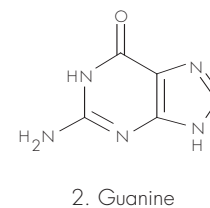
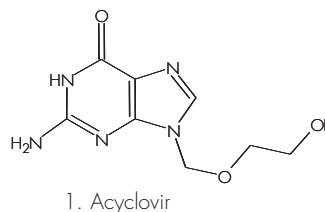
Column: Atlantis™ HILIC Silica 4.6 x 50 mm, 3 μm
 Part Number: 186002027
 Mobile Phase A: 0.2% HCOOH in H₂O
 Mobile Phase B: 0.2% HCOOH in ACN
 Flow Rate: 2.0 mL/min
 Isocratic Mobile
 Phase Composition: 10% A; 90% B
 Injection Volume: 1 μL
 Injection Solvent: 60:40 ACN:0.02N NaOH
 Sample Concentration: 83 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA

Reversed-Phase Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm
 Part Number: 186001329
 Mobile Phase A: 0.2% HCOOH in H₂O
 Mobile Phase B: 0.2% HCOOH in ACN
 Flow Rate: 1.4 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	100	0	0
5.0	95	5	0

 Injection Volume: 1 μL
 Sample Diluent: 0.02N NaOH
 Sample Concentration: 83 μg/mL
 Temperature: 30° C
 Detection: UV @ 205 nm
 Instrument: Alliance® 2695 with 2996 PDA



LC Conditions:

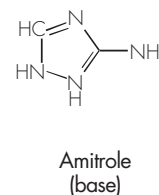
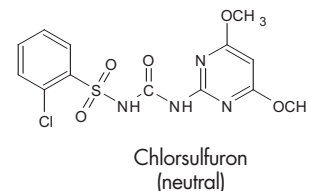
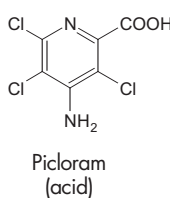
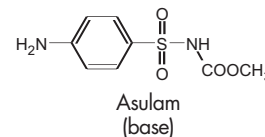
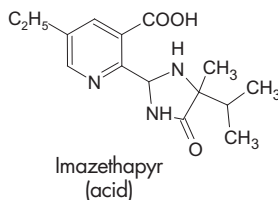
Column: Atlantis™ dC₁₈, 2.1 x 100 mm, 3 μm
 Part Number: 186001295
 Mobile Phase A: 15mM HCOONH₄, pH 4.5
 Mobile Phase B: ACN
 Flow rate: 200 μL/min
 Gradient:

Time (min)	%A	%B
0.0	90	10
19.0	10	90

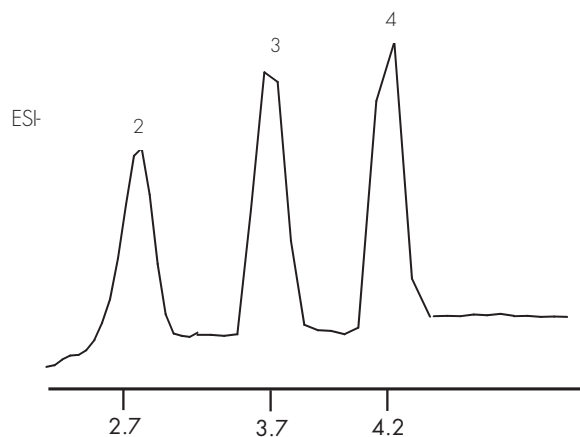
Injection Volume: 20 μL
 Instrument: Alliance® 2690

MS Conditions:

Mass spectrometer: Quattro Ultima™
 Instrument: Electrospray (ESI- and ESI+)
 Multiple Reaction Monitoring

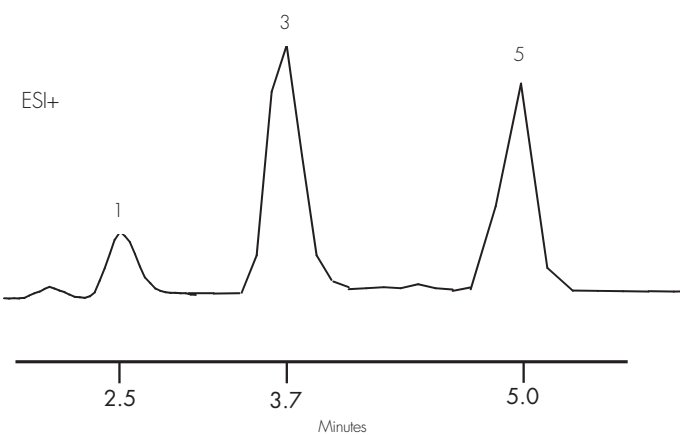


200 ng/L Spiked River Water Sample



Compounds

1. Amitrole (80%)
2. Picloram (75%)
3. Asulam (60%)
4. Imazethapyr (75%)
5. Chlorsulfuron (90%)



Compound	MW	Mode	(mrm)	cone (V)	Coll. Energy(ev)
Picloram	241.5	ESI-	239-195	20	10
Imazethapyr	289	ESI-	288-244	25	15
Asulam	230	ESI-	229-197	30	10
Asulam	230	ESI+	231-156	30	10
Chlorsulfuron	357	ESI+	358-141	20	17
Amitrole	84	ESI+	85-57	20	15

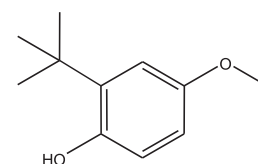
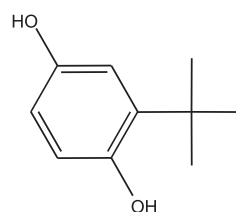
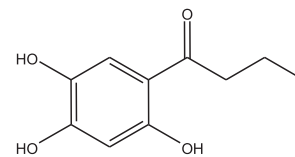
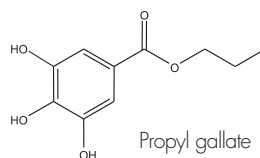
Lipid Soluble Antioxidants

LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 1% HCOOH, pH 2.3
 Mobile Phase D: MeOH
 Flow Rate: 2.0 mL/min
 Gradient:

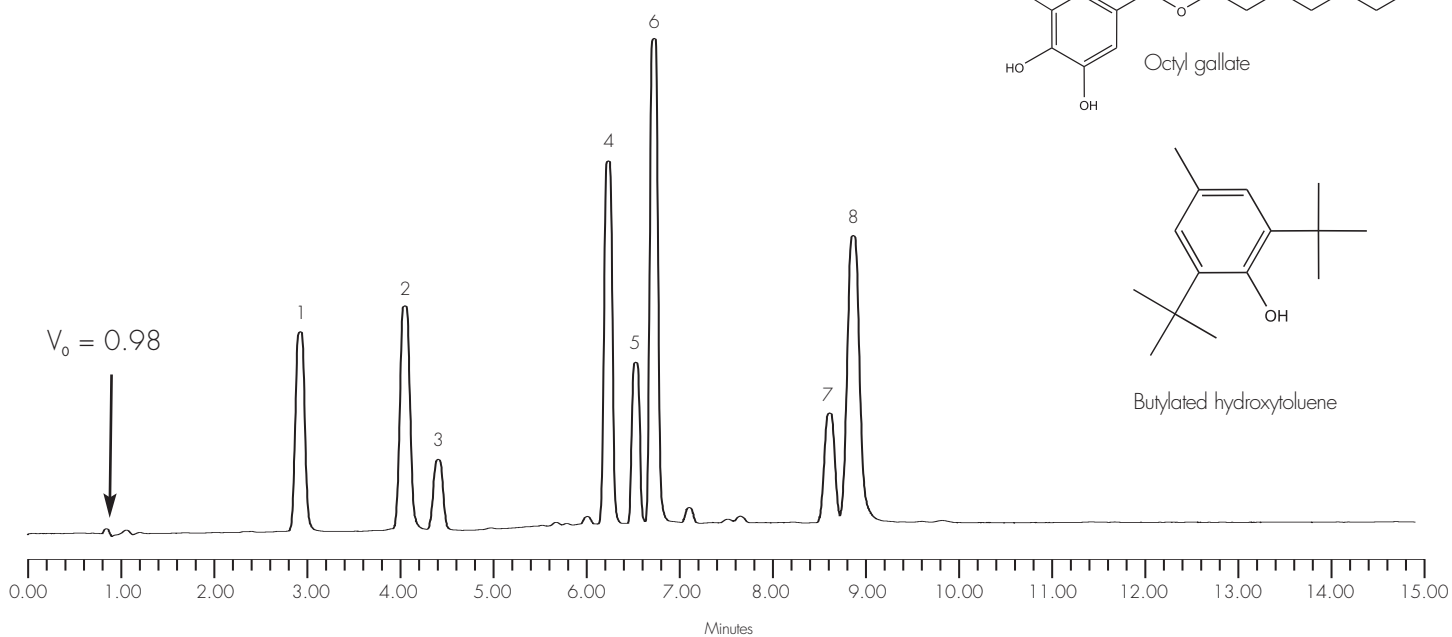
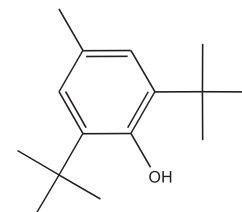
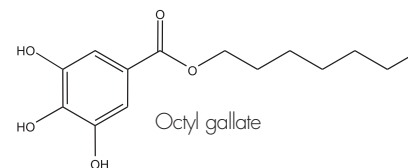
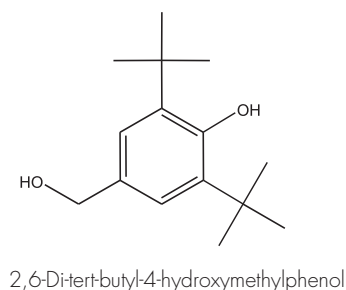
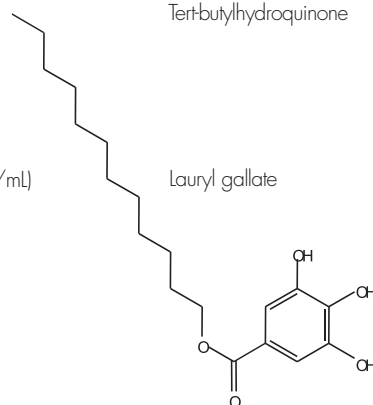
Time (min)	Profile			
	%A	%B	%C	%D
0.0	55	17.5	10	17.5
4.0	35	27.5	10	27.5
6.0	4	45	10	4
15.0	4	45	10	4

Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA



Compounds:

Compounds	USP Tailing	Sample Concentrations (μg/ml)
1. Propyl gallate (PG)	1.08	10
2. 2,4,5-Trihydroxybutyrophenone (THBP)	1.09	10
3. Tert-butylhydroquinone (TBHQ)	1.01	19
4. Butylated hydroxyanisole (BHA)	1.02	38
5. 2,6-Di-tert-butyl-4-hydroxymethylphenol	1.06	38
6. Octyl gallate (OG)	1.11	19
7. Butylated hydroxytoluene (BHT)	0.95	38
8. Lauryl gallate (LG)	1.16	23



HILIC Conditions:

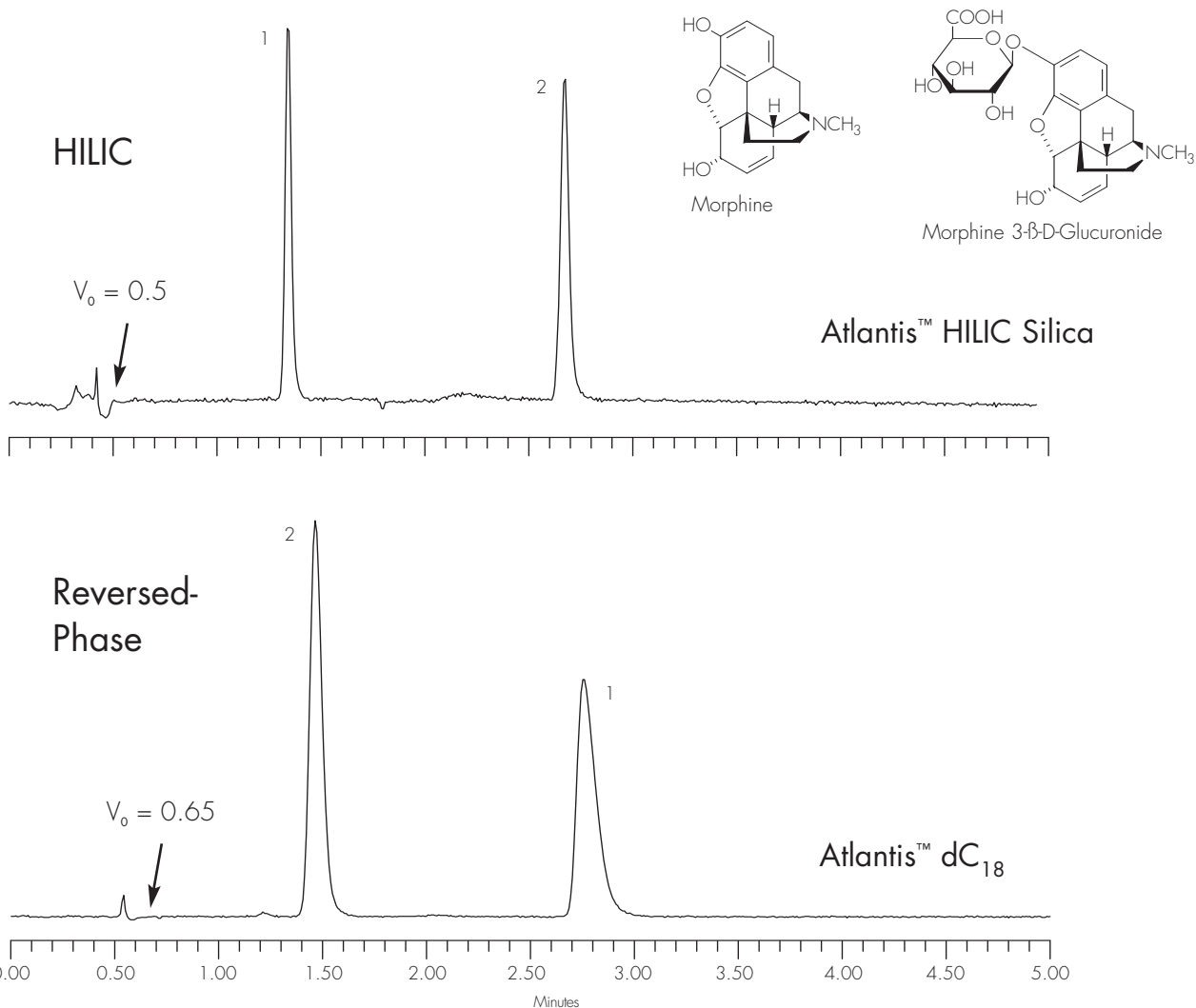
Column: Atlantis™ HILIC Silica 4.6 x 50 mm, 3 μm
 Part Number: 186002027
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM NH₄COOH, pH 3.0
 Flow Rate: 2.0 mL/min
 Gradient:

Time	Profile		
(min)	%A	%B	%C
0.0	0	90	10
5.0	40	50	10

Injection Volume: 5 μL
 Injection Solvent: 75:25 ACN:MeOH
 Sample Concentration: 125 μg/mL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA

Reversed-Phase Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm
 Part Number: 186001329
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM NH₄COOH, pH 3.0
 Flow Rate: 1.4 mL/min
 Isocratic Mobile
 Phase Conditions: 88% A: 2% B: 10% C
 Injection Volume: 5 μL
 Injection Solvent: 75:25 H₂O:MeOH
 Sample Concentration: 125 μg/mL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA



Nalidixic Acid Antibiotics by LC/MS

1.0 Minute Gradient

LC Conditions:

Column: Atlantis™ dC₁₈, 2.1 x 20 mm I_S™, 3 μm
 Part Number: 186002058
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 1% HCOOH in H₂O
 Flow Rate: 0.4 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	50	40	10
1.0	30	60	10

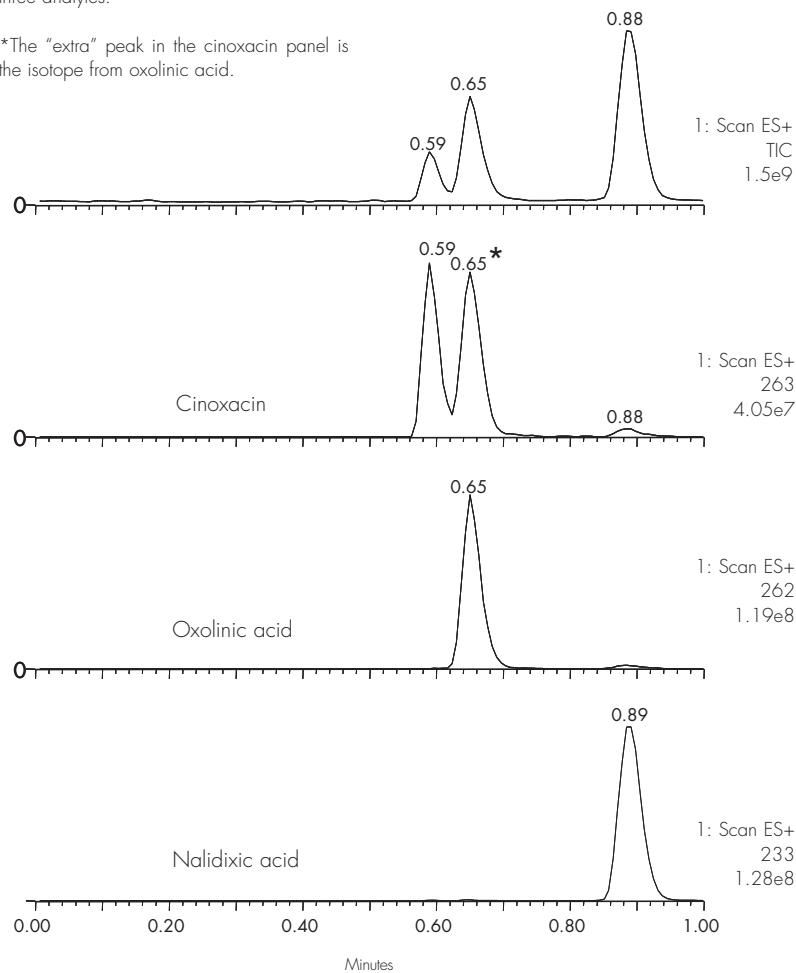
Injection Volume: 2 μL
 Sample Concentration: 10 μg/mL
 Temperature: 30° C
 Instrument: Alliance® HT 2795 with Waters ZQ™

MS Conditions:

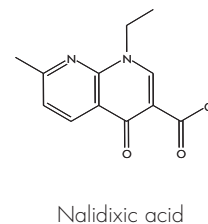
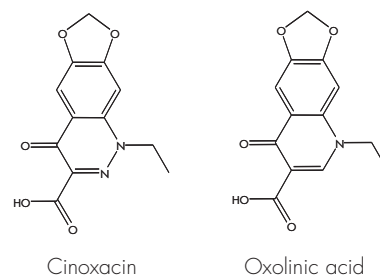
Waters ZQ™
 ES+
 Capillary (kV) 3.5
 Cone (V) 5.0
 Extractor 3.0
 RF Lens 0.1
 Source Temp (°C) 150
 Desolvation Temp (°C) 400
 Cone Gas Flow (L/Hr) 50
 Desolvation Gas Flow(L/Hr) 500
 LM Resolution 15
 HM Resolution 15
 Ion Energy 0.5
 Multiplier (V) 650

The top figure is the total ion current, followed by the extracted ion signals for each of the three analytes.

*The "extra" peak in the cinoxacin panel is the isotope from oxolinic acid.



Compounds	MW
1. Cinoxacin	262.2
2. Oxolinic Acid	261.2
3. Nalidixic Acid	232.2



LC Conditions:

Column: Atlantis™ dC₁₈, 2.1 x 20 mm I_S™, 3 μm
 Part Number: 186002058
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 1% HCOOH in H₂O
 Flow Rate: 0.4 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	60	30	10
2.0	40	50	10

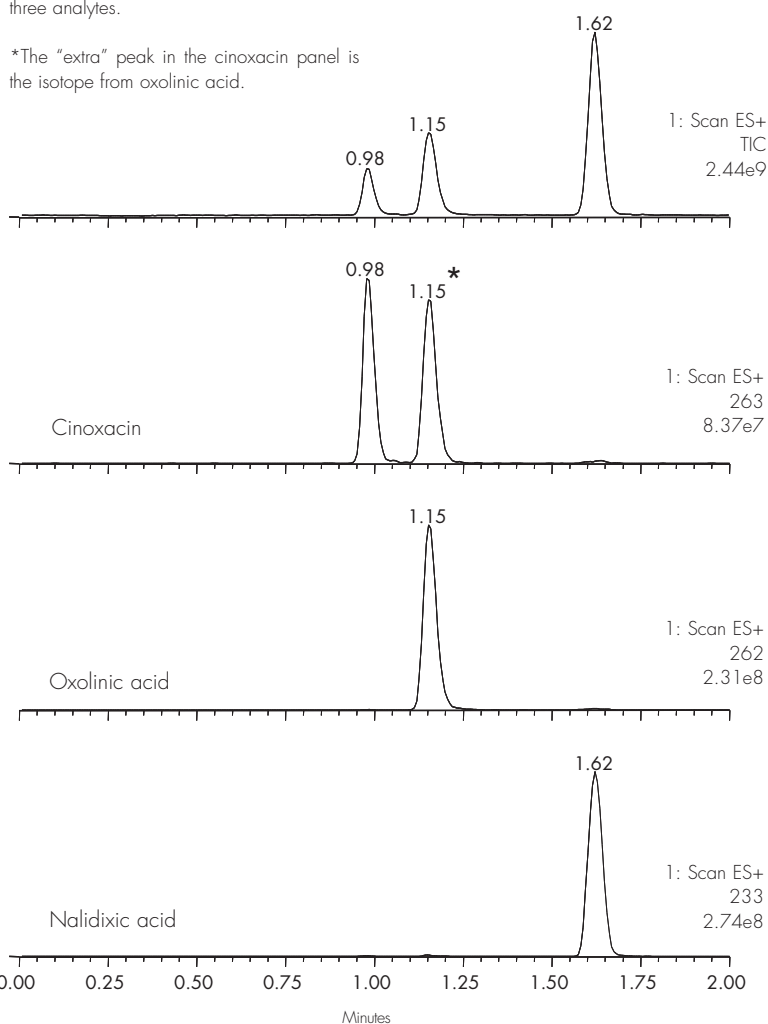
Injection Volume: 2 μL
 Sample Concentration: 10 μg/mL
 Temperature: 30° C
 Instrument: Alliance® HT 2795 with Waters ZQ™

MS Conditions:

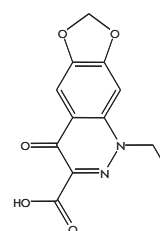
Waters ZQ™
 ES+
 Capillary (kV) 3.5
 Cone (V) 5.0
 Extractor 3.0
 RF Lens 0.1
 Source Temp (°C) 150
 Desolvation Temp (°C) 400
 Cone Gas Flow (L/Hr) 50
 Desolvation Gas Flow(L/Hr) 500
 LM Resolution 15
 HM Resolution 15
 Ion Energy 0.5
 Multiplier (V) 650

The top figure is the total ion current, followed by the extracted ion signals for each of the three analytes.

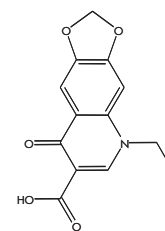
*The "extra" peak in the cinoxacin panel is the isotope from oxolinic acid.



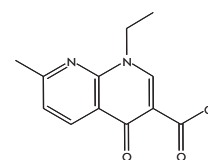
Compounds	MW
1. Cinoxacin	262.2
2. Oxolinic Acid	261.2
3. Nalidixic Acid	232.2



Cinoxacin



Oxolinic acid



Nalidixic acid

Nalidixic Acid Antibiotics by LC/MS

3.0 Minute Gradient

LC Conditions:

Column: Atlantis™ dC₁₈, 2.1 x 20 mm I^S™, 3 μm
 Part Number: 186002058
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 1% HCOOH in H₂O
 Flow Rate: 0.4 mL/min
 Gradient:

Time (min)	%A	Profile %B	%C
0.0	60	30	10
3.0	40	50	10

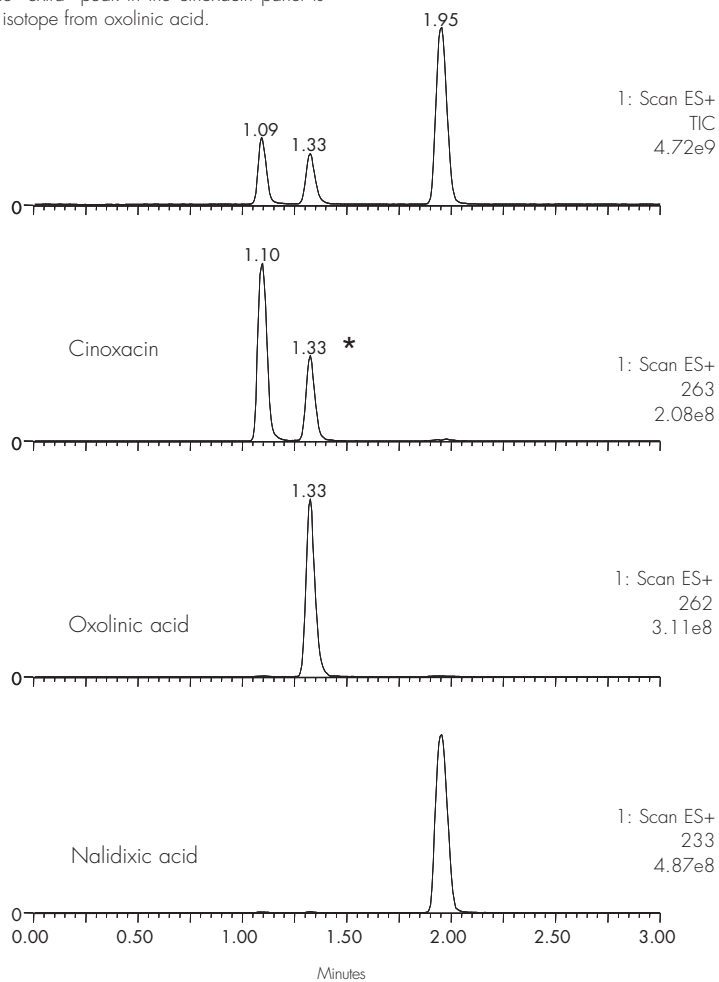
Injection Volume: 2 μL
 Sample Concentration: 10 μg/mL
 Temperature: 30°C
 Instrument: Alliance® HT 2795 with Waters ZQ™

MS Conditions:

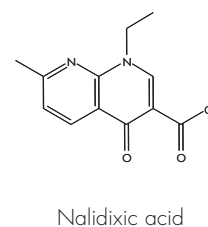
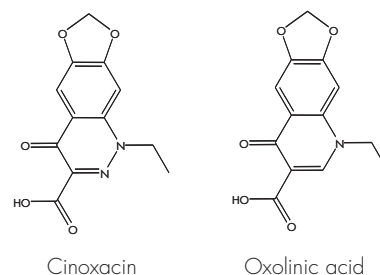
Waters ZQ™
 ES+
 Capillary (kV) 3.5
 Cone (V) 5.0
 Extractor 3.0
 RF Lens 0.1
 Source Temp (°C) 150
 Desolvation Temp (°C) 400
 Cone Gas Flow (L/Hr) 50
 Desolvation Gas Flow (L/Hr) 500
 LM Resolution 15
 HM Resolution 15
 Ion Energy 0.5
 Multiplier (V) 650

The top figure is the total ion current, followed by the extracted ion signals for each of the three analytes.

*The "extra" peak in the cinoxacin panel is the isotope from oxalinoic acid.



Compounds	MW
1. Cinoxacin	262.2
2. Oxolinic Acid	261.2
3. Nalidixic Acid	232.2



LC Conditions:

Column: Atlantis™ dC₁₈, 2.1 x 20 mm IS™, 3 μm
 Part Number: 186002058
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 1% HCOOH in H₂O
 Flow Rate: 0.2 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	60	30	10
5.0	40	50	10

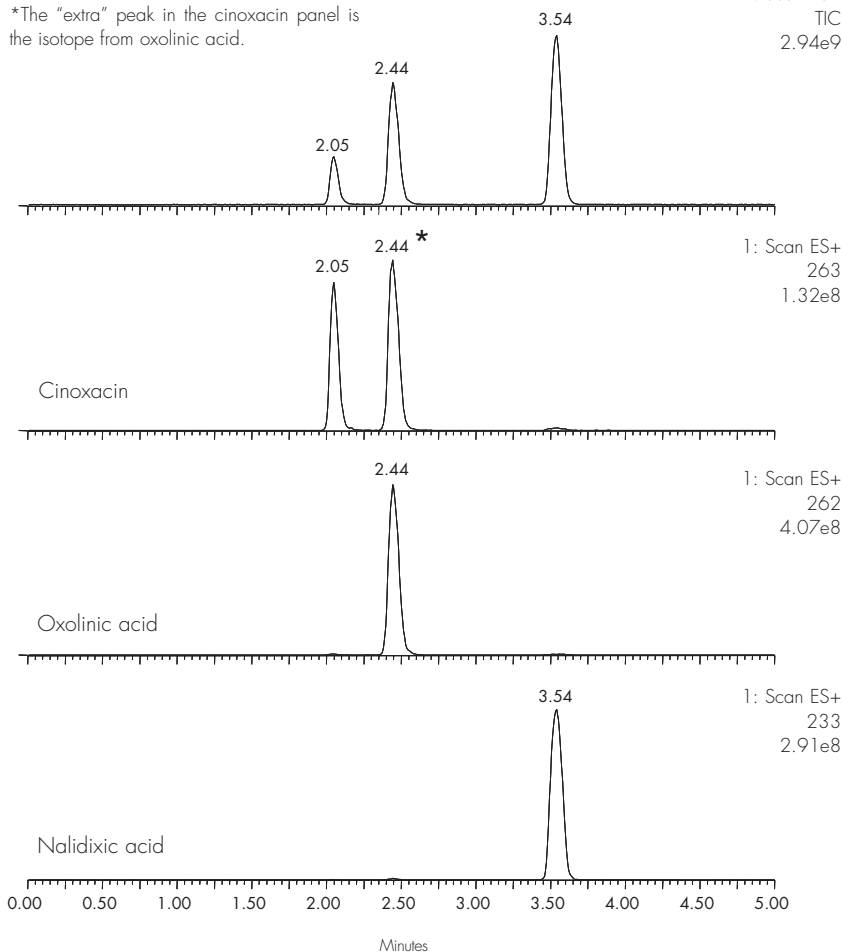
Injection Volume: 5 μL
 Sample Concentration: 10 μg/mL
 Temperature: 30°C
 Instrument: Alliance® HT 2795 with Waters ZQ™

MS Conditions:

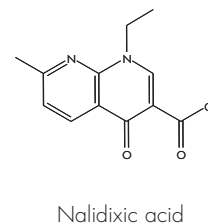
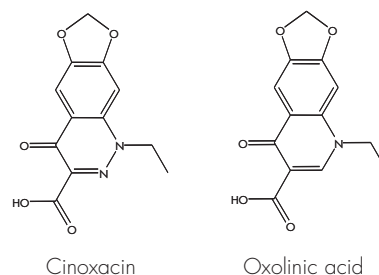
Waters ZQ™
 ES+
 Capillary (kV): 3.5
 Cone (V): 5.0
 Extractor: 3.0
 RF Lens: 0.1
 Source Temp (°C): 150
 Desolvation Temp (°C): 400
 Cone Gas Flow (L/Hr): 50
 Desolvation Gas Flow (L/Hr): 500
 LM Resolution: 15
 HM Resolution: 15
 Ion Energy: 0.5
 Multiplier (V): 650

The top figure is the total ion current, followed by the extracted ion signals for each of the three analytes.

*The "extra" peak in the cinoxacin panel is the isotope from oxolinic acid.



Compounds	MW
1. Cinoxacin	262.2
2. Oxolinic Acid	261.2
3. Nalidixic Acid	232.2



Nalidixic Acid Antibiotics

—LC/MS

LC Conditions:

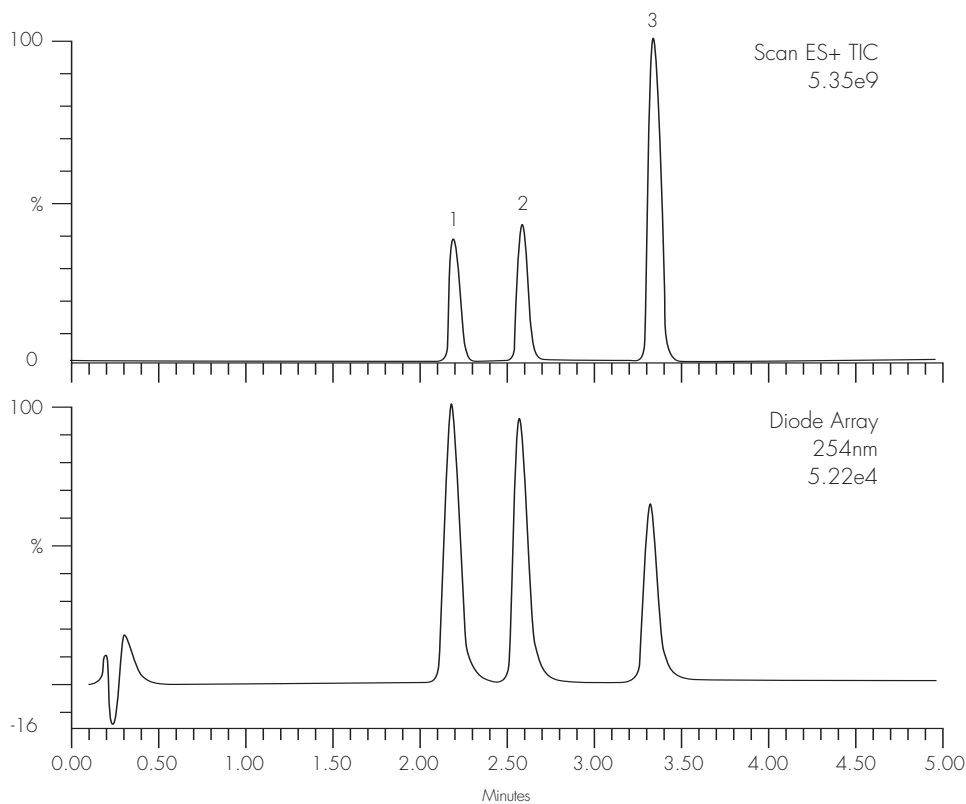
Column: Atlantis™ dC₁₈ 2.1 x 30 mm, 3 μm
 Part Number: 186001287
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 1% HCOOH in H₂O
 Flow Rate: 0.8 mL/min with 0.2 mL/min to MS
 Gradient:

Time (min)	%A	%B	%C
0.0	75	15	10
5.0	40	50	10

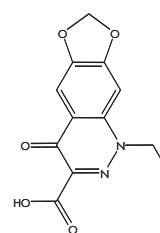
Injection Volume: 10 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm and MS ESI+
 Instrument: Alliance® 2795 with 2996 PDA and Waters ZQ™

Waters ZQ Settings:

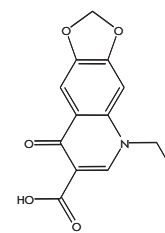
Source: ESI+
 Capillary (kV): 3.5
 Cone (V): 50
 Extractor: 3.0
 RF Lens: 0.1
 Source Temp: 150
 Desolvation Temp: 400
 Cone gas Flow (L/Hr): 50
 Desolvation Gas Flow (L/Hr): 500
 LM Resolution: 15
 HM Resolution: 15
 Ion Energy: 0.5
 Multiplier (V): 650



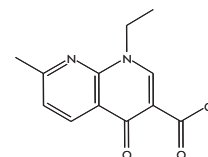
Compounds	MW
1. Cinoxacin	262.2
2. Oxolinic Acid	261.2
3. Nalidixic Acid	232.2



Cinoxacin



Oxolinic acid

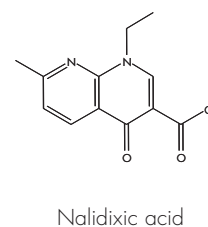
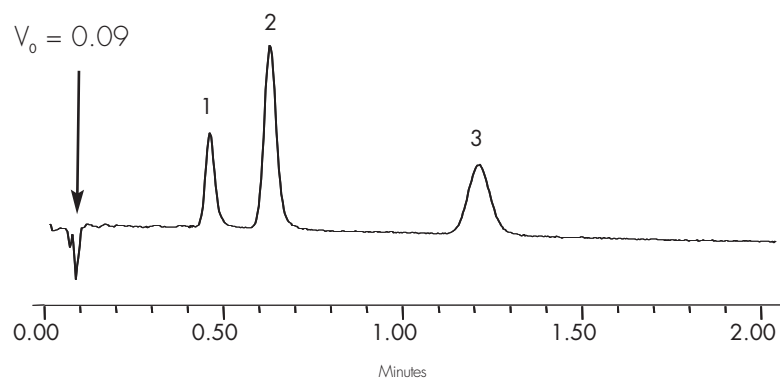
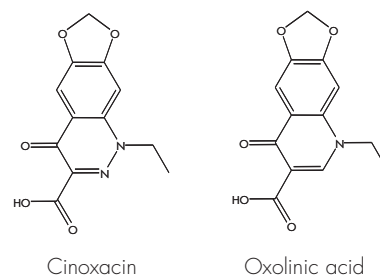


Nalidixic acid

LC Conditions:

Column: Atlantis™ dC₁₈, 4.6 x 20 mm I_S™, 3 μm,
 Part Number: 186002062
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: ACN
 Mobile Phase D: 1% HCOOH in H₂O
 Flow Rate: 4 mL/min
 Isocratic Mobile-
 Phase Composition: 55% A, 30% B, 5% C, 10% D
 Injection Volume: 5 μL
 Sample Concentration: 5 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2965 with 2996 PDA

Compounds	MW
1. Cinoxacin	262.2
2. Oxolinic Acid	261.2
3. Nalidixic Acid	232.2



Nucleic Acid Bases—Gradient

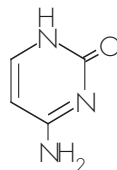
Effect of Polar Solvent on Retention in HILIC

LC Conditions:

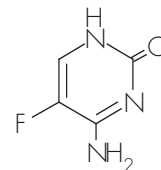
Column: Atlantis™ HILIC Silica, 4.6 x 50 mm, 3 μm
 Part Number: 186002027
 Mobile Phase A: H₂O, MeOH, or IPA
 Mobile Phase B: ACN
 Mobile Phase C: 200 mM NH₄COOH, pH 3.0
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	5	90	5
5.0	45	50	5

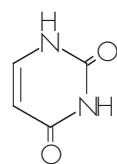
Injection Volume: 2 μL
 Injection Solvent: 75:25 ACN:MeOH
 Sample Concentration: 25 μg/mL
 Temperature: 30° C
 Detection: UV @ 260 nm
 Instrument: Alliance® 2695 with 2996 PDA



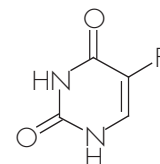
Cytosine



5-Fluorocytosine



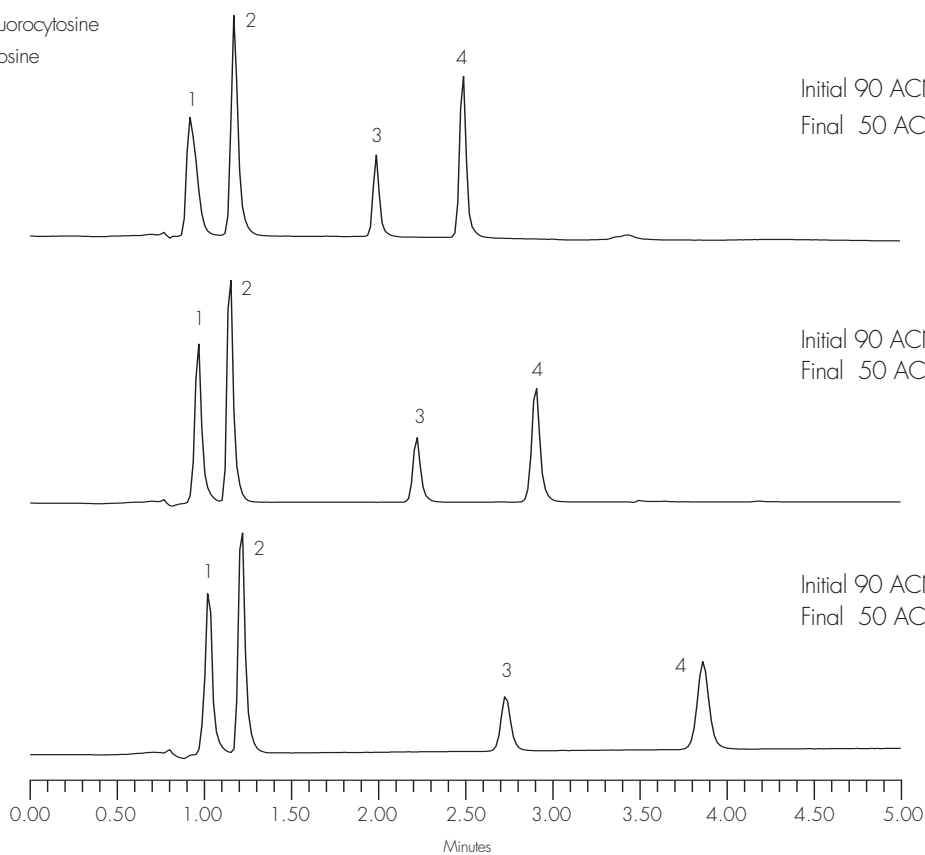
Uracil



5-Fluorouracil

Compounds

1. 5-Fluorouracil
2. Uracil
3. 5-Fluorocytosine
4. Cytosine



Initial 90 ACN: 5 H₂O: 5 buffer
 Final 50 ACN: 45 H₂O: 5 buffer

**Replace H₂O
with MeOH**

Initial 90 ACN: 5 MeOH: 5 buffer
 Final 50 ACN: 45 MeOH: 5 buffer

**Replace MeOH
with IPA**

Initial 90 ACN: 5 IPA: 5 buffer
 Final 50 ACN: 45 IPA: 5 buffer

HILIC Conditions:

Column: Atlantis™ HILIC Silica 4.6 x 50 mm, 3 µm
 Part Number: 186002027
 Mobile Phase A: MeOH
 Mobile Phase B: ACN
 Mobile Phase C: 200 mM CH₃COONH₄, pH 5.0
 Flow Rate: 2.0 mL/min
 Gradient:

Time	%A	%B	%C
0.0	3	95	2
5.0	40	50	10

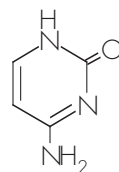
Injection Volume: 2 µL
 Injection Solvent: 75:25 ACN:MeOH
 Sample Concentration: 25 µg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA

Reversed-Phase Conditions:

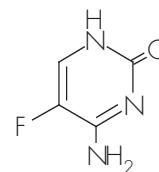
Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 µm
 Part Number: 1860001329
 Mobile Phase: 10 mM CH₃COONH₄, pH 5.0
 Flow Rate: 1.4 mL/min
 Isocratic Mobile
 Phase Conditions: 100% A
 Injection Volume: 2 µL
 Injection Solvent: H₂O
 Sample Concentration: 25 µg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA

Compounds

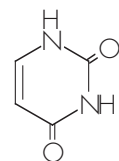
1. 5-Fluorouracil
2. Uracil
3. 5-Fluorocytosine
4. Cytosine



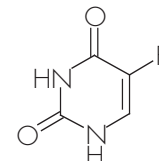
Cytosine



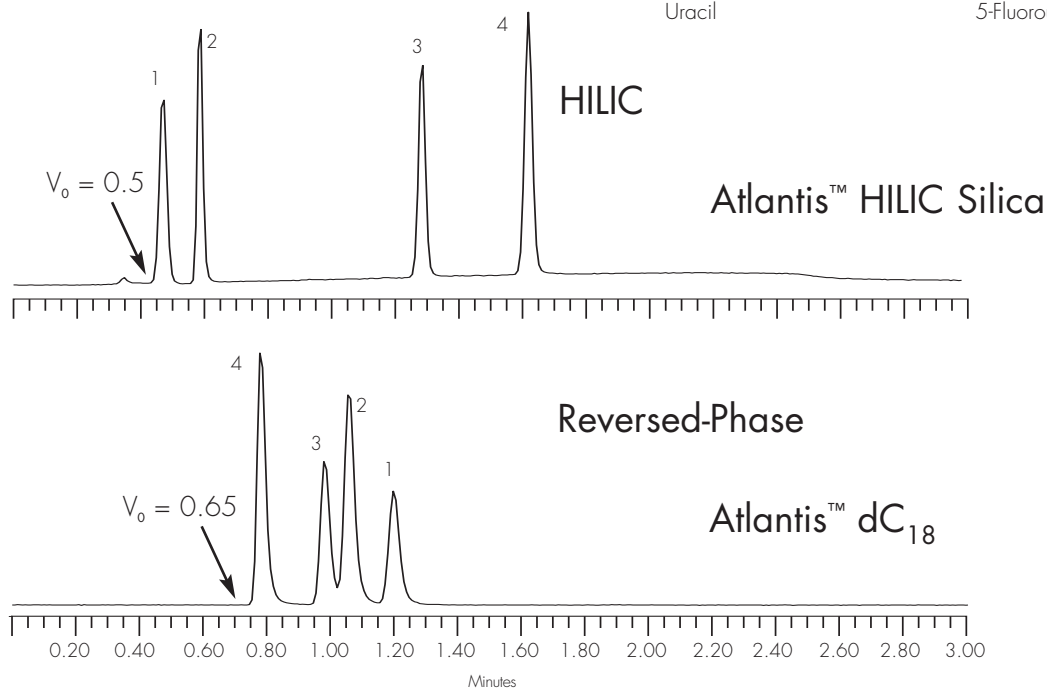
5-Fluorocytosine



Uracil



5-Fluorouracil



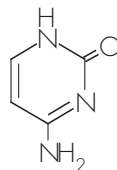
Nucleic Acid Bases —Gradient

LC Conditions:

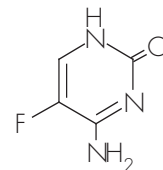
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM CH₃COONH₄, pH 5.0
 Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	90	0	10
10.0	84	6	10

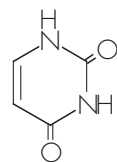
Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA



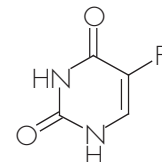
Cytosine



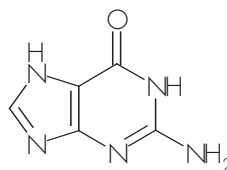
5-Fluorocytosine



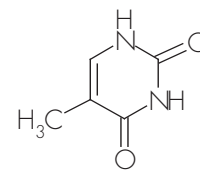
Uracil



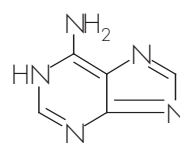
5-Fluorouracil



Guanine

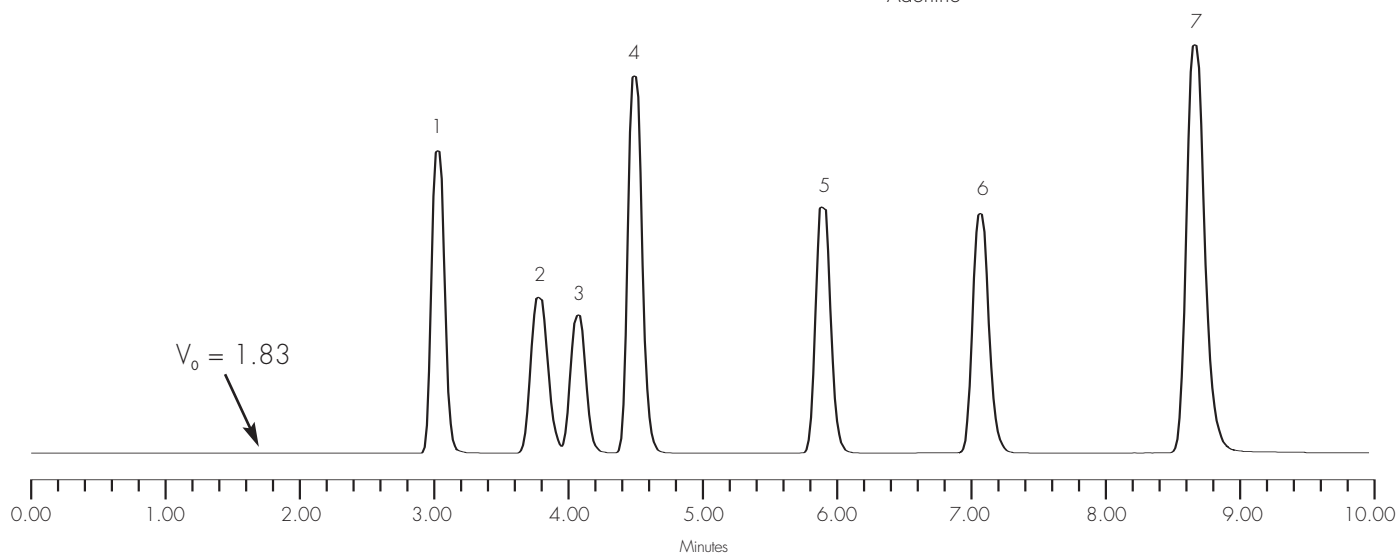


Thymine



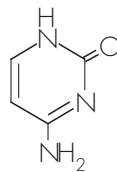
Adenine

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Cytosine	1.11	82
2. 5-Fluorocytosine	1.08	82
3. Uracil	1.05	4
4. 5-Fluorouracil	1.18	41
5. Guanine	1.16	10
6. Thymine	1.18	82
7. Adenine	1.25	164

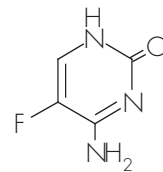


LC Conditions:

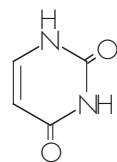
Column: Atlantis™ HILIC Silica, 4.6 x 50 mm, 3 μm
 Part Number: 186002027
 Mobile Phase A: 100 mM NH₄COOH
 Mobile Phase B: ACN
 Flow Rate: 1.0 mL/min
 Isocratic Mobile
 Phase Composition: 12% A; 88% B
 Injection Volume: 10 μL
 Injection Solvent: 75:25 ACN:MeOH
 Sample Concentration: 25 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA



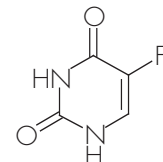
Cytosine



5-Fluorocytosine



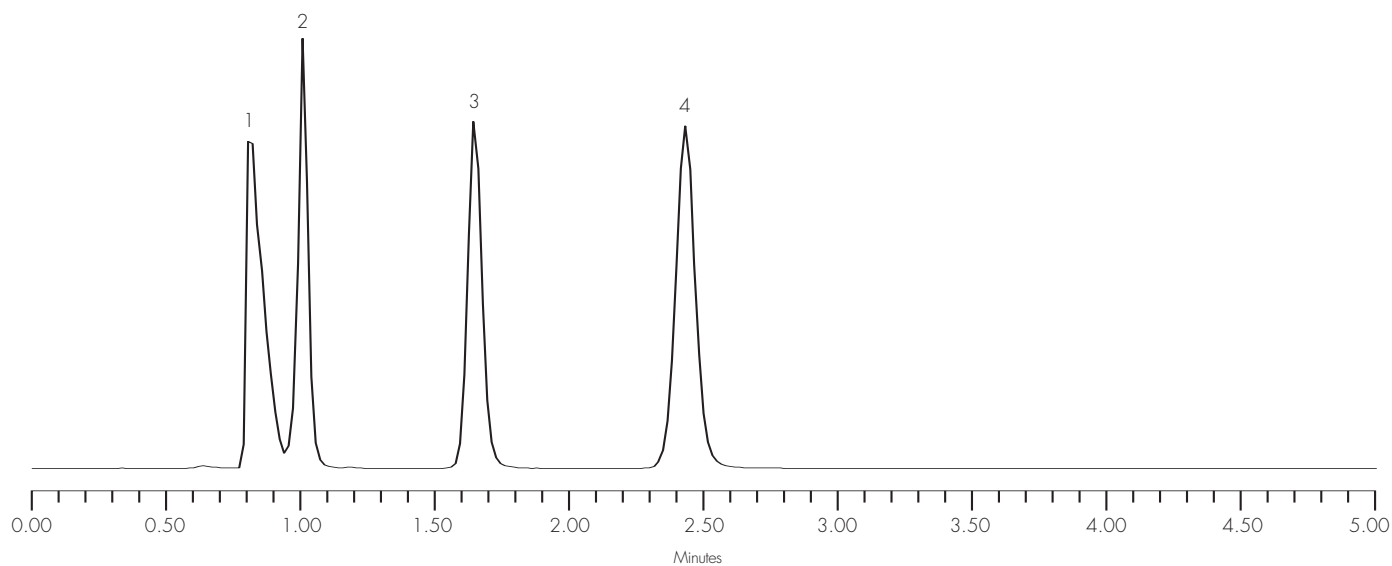
Uracil



5-Fluorouracil

Compounds

1. 5-Fluorouracil
2. Uracil
3. 5-Fluorocytosine
4. Cytosine



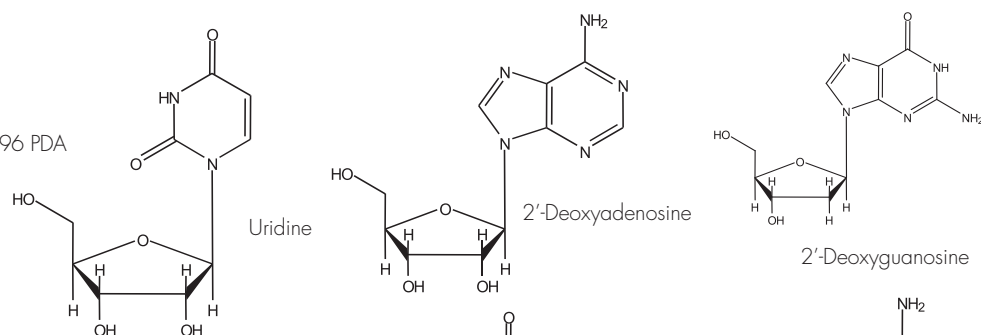
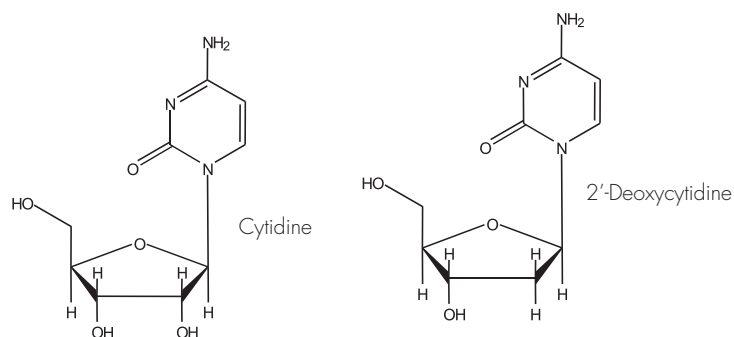
Nucleosides

LC Conditions:

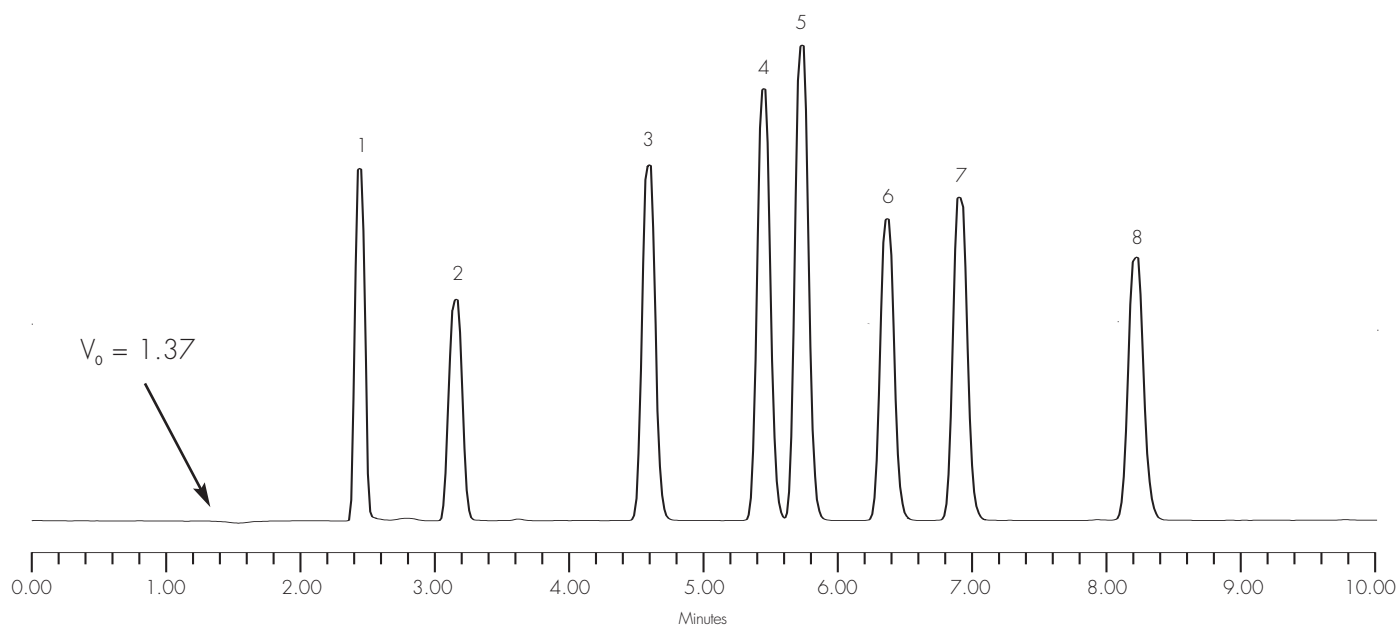
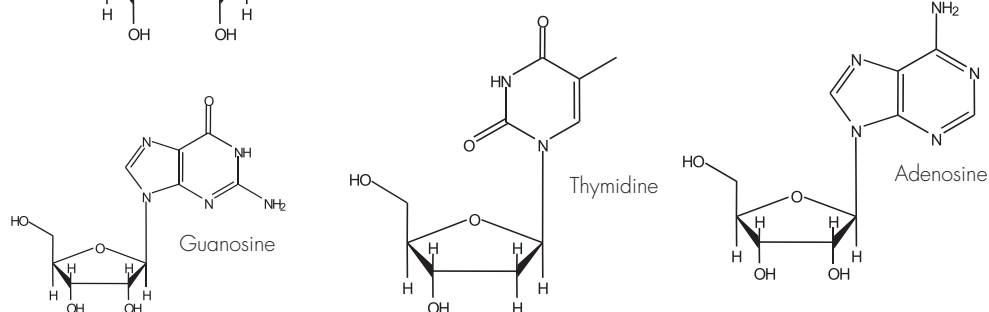
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 1% HCOOH, pH 2.3
 Flow Rate: 1.4 mL/min
 Gradient:

Time	Profile
(min)	%A %B %C
0.0	90 0 10
10.0	82 8 10

Injection Volume: 10 μL
 Sample Concentration: 19 μg/mL
 Temperature: 30° C
 Detection: UV @ 260 nm
 Instrument: Alliance® 2695 with 2996 PDA

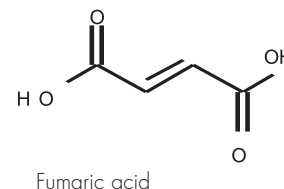
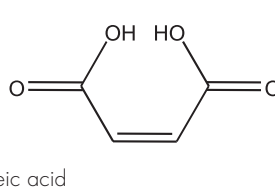
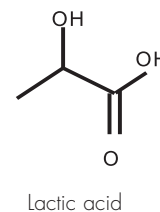
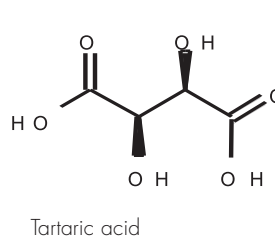
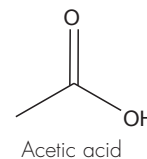
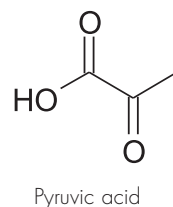
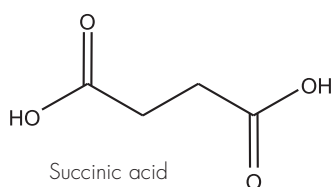
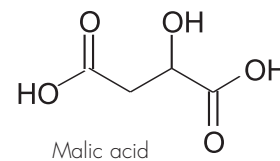
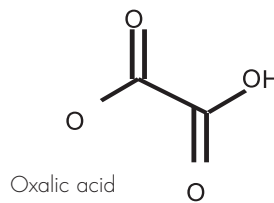


Compounds:	USP Tailing
1. Cytidine	1.05
2. 2'-Deoxycytidine	1.02
3. Uridine	1.08
4. Adenosine	1.07
5. 2'-Deoxyadenosine	1.08
6. Guanosine	1.09
7. 2'-Deoxyguanosine	1.10
8. Thymidine	1.10

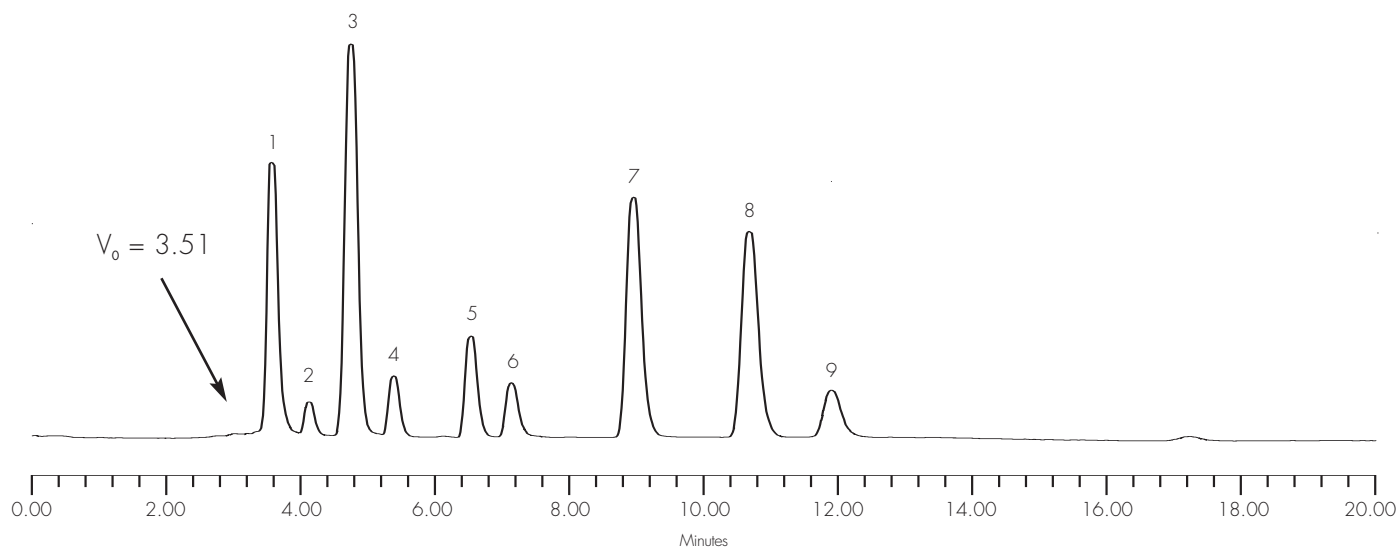


LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Flow Rate: 0.5 mL/min
 Isocratic Mobile
 Phase Composition: 20 mM NaH₂PO₄, pH 2.7
 Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 210 nm
 Instrument: Alliance® 2695 with 2996 PDA



Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Oxalic acid	1.35	25
2. Pyruvic acid	1.12	30
3. Tartaric acid	1.13	15
4. Malic acid	1.19	61
5. Lactic acid	1.24	243
6. Acetic acid	1.30	243
7. Maleic acid	1.24	3
8. Fumaric acid	1.23	3
9. Succinic acid	1.26	158

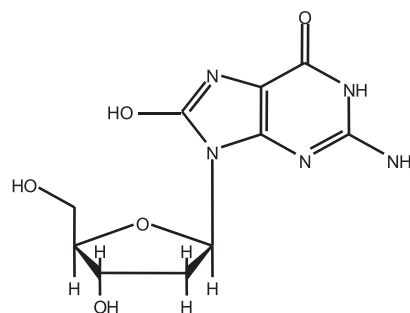
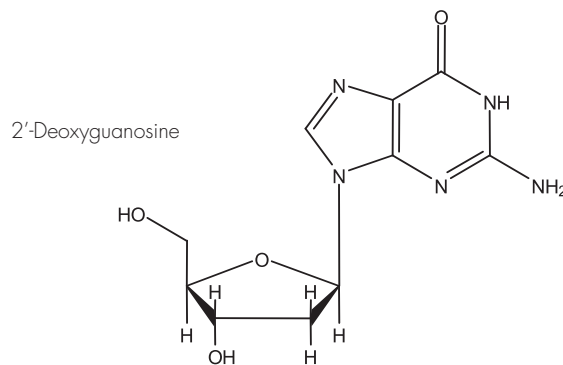


Oxidative Stress Markers

—5% ACN, pH 3.0

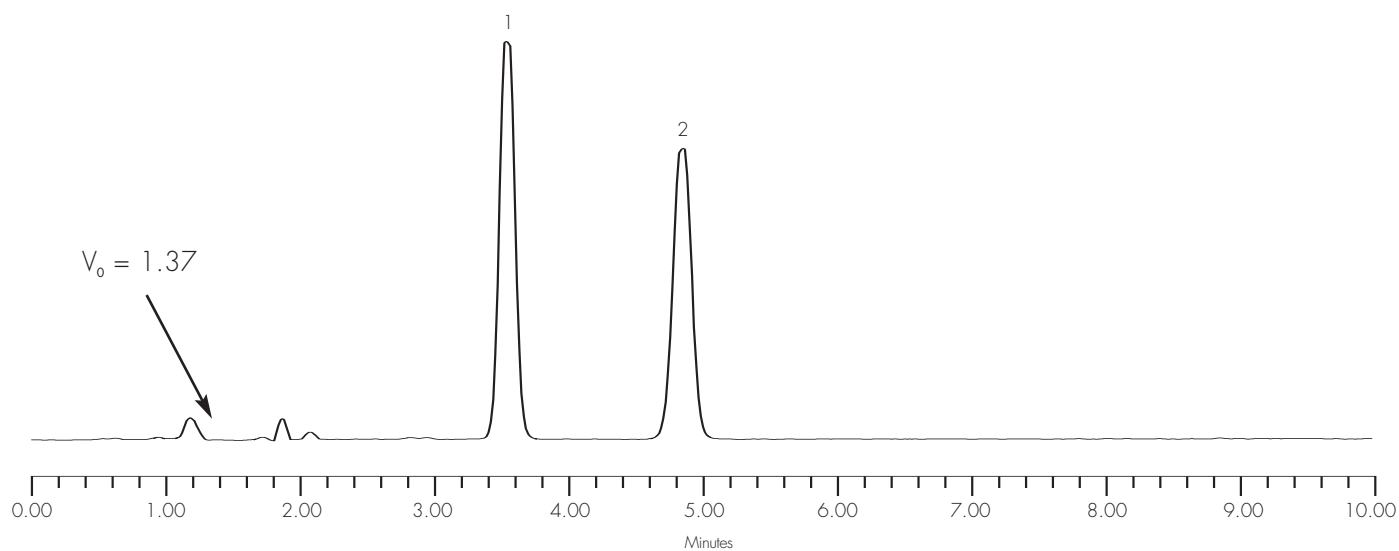
LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 1.4 mL/min
 Isocratic Mobile
 Phase Composition: 85% A; 5% B; 10% C
 Injection Volume: 10.0 μL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA



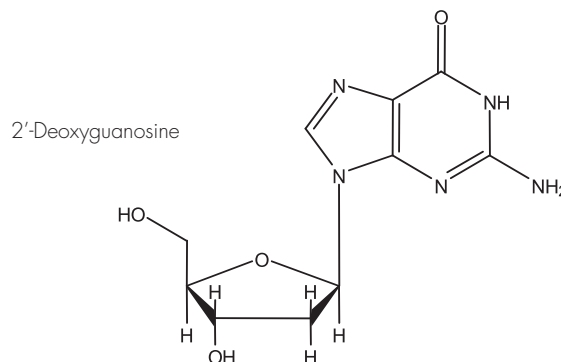
8-Hydroxy-2'-Deoxyguanosine

Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. 2'-Deoxyguanosine	1.04	75
2. 8-Hydroxy-2'-deoxyguanosine	0.99	75

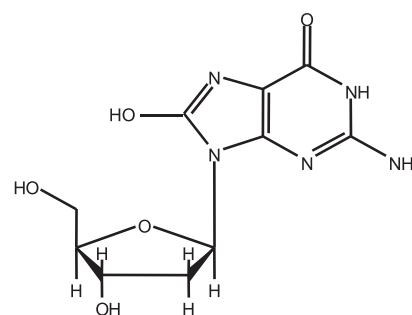


LC Conditions:

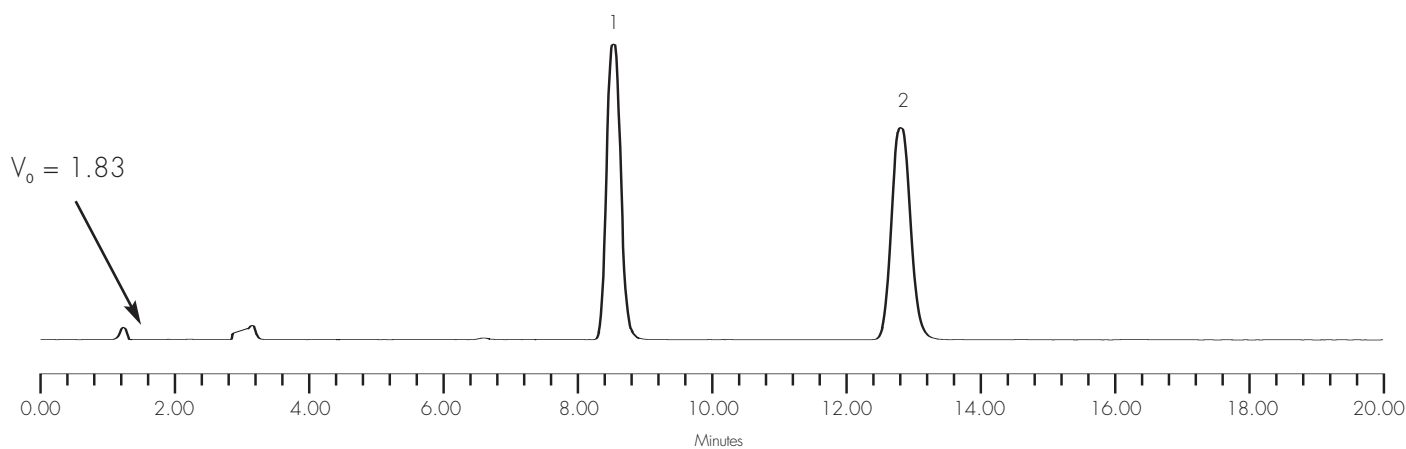
Column: Atlantis™ dC₁₈ 4.6 x 100 mm, 3 μm
 Part Number: 186001337
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 100 mM CH₃COONH₄, pH 5.0
 Flow Rate: 1.0 mL/min
 Isocratic Mobile
 Phase Composition: 85% A; 5% B; 10% C
 Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 285 nm
 Instrument: Alliance® 2695 with 2996 PDA



Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. 2'-Deoxyguanosine	1.12	75
2. 8-Hydroxy-2'-deoxyguanosine	1.11	75



8-Hydroxy-2'-Deoxyguanosine

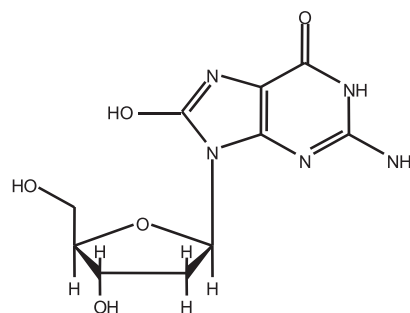
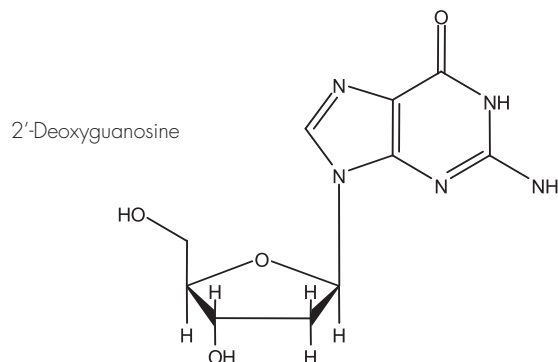


Oxidative Stress Markers

—10% MeOH, pH 5.0

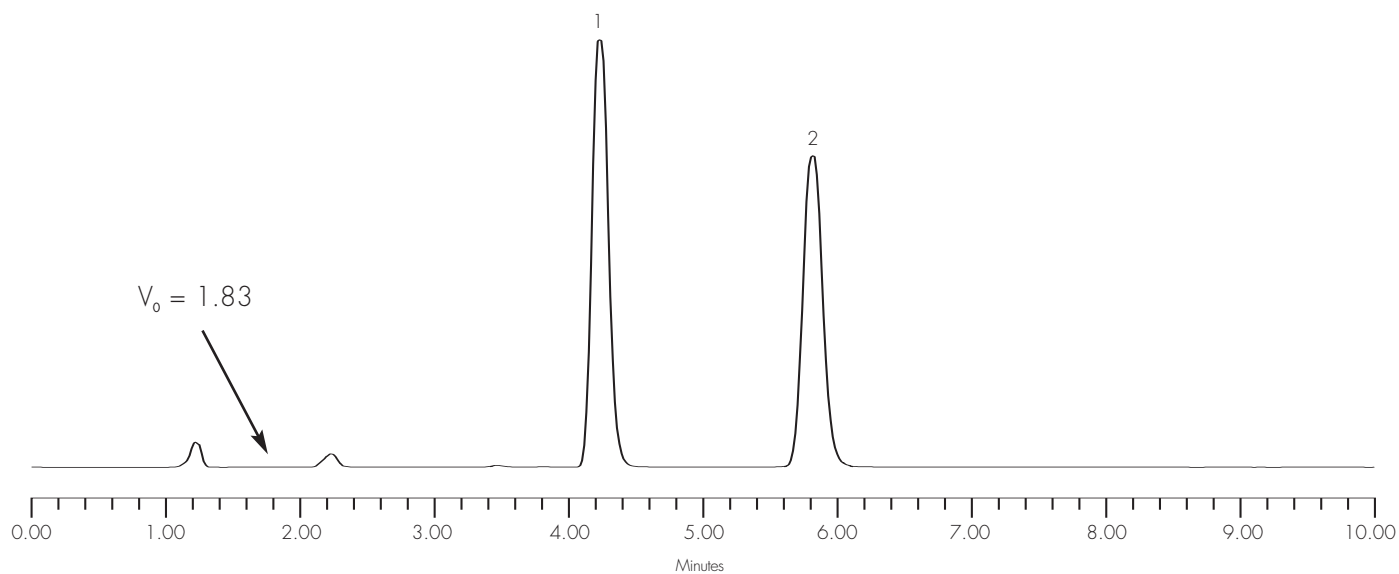
LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 100 mM CH₃COONH₄, pH 5.0
 Flow Rate: 1.0 mL/min
 Isocratic Mobile
 Phase Composition: 80% A; 10% B; 10% C
 Injection Volume: 20 μL
 Temperature: 30° C
 Detection: UV @ 285 nm
 Instrument: Alliance® 2695 with 2996 PDA



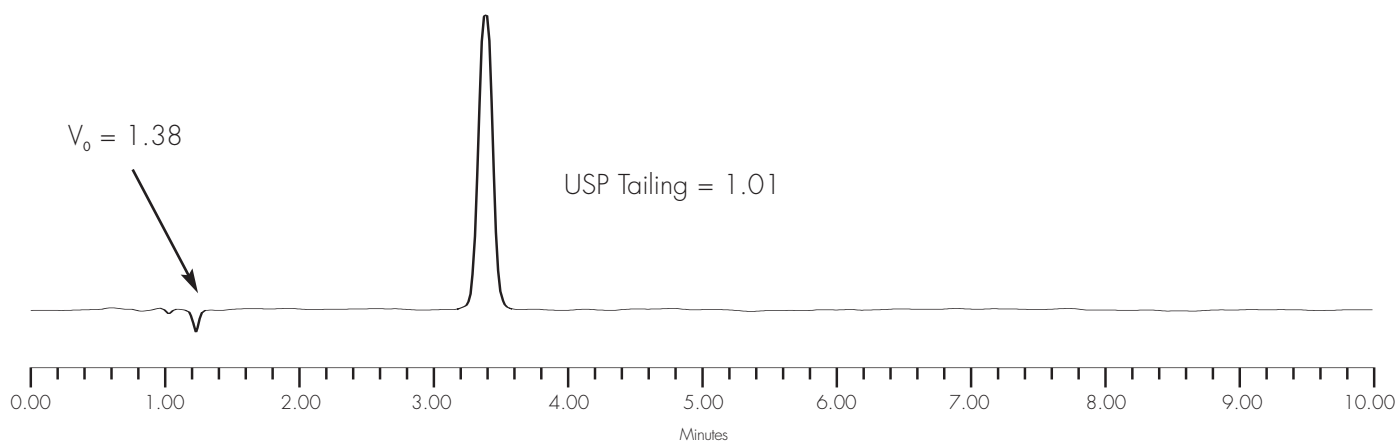
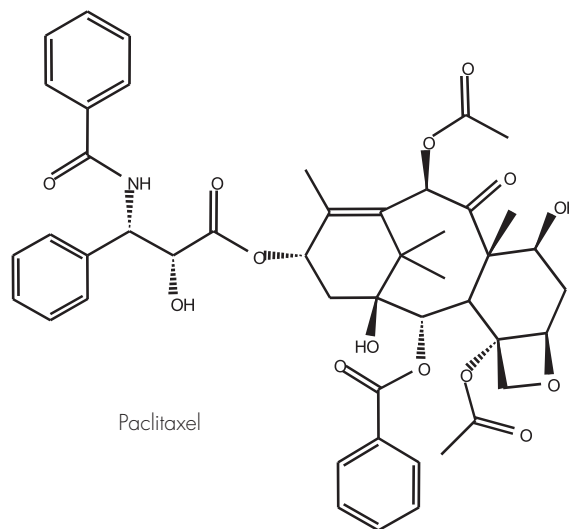
Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. 2'-Deoxyguanosine	1.15	75
2. 8-Hydroxy-2'-deoxyguanosine	1.11	75

8-Hydroxy-2'-Deoxyguanosine



LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 1.4 mL/min
 Isocratic / Mobile
 Phase Composition: 30% A; 60% B; 10% C
 Injection Volume: 2 μL
 Sample Concentration: 0.25 μg/mL
 Temperature: 30° C
 Detection: UV @ 238 nm
 Instrument: Alliance® 2695 with 2996 PDA



Peptides

LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 5 μm

Part Number: 186001331

Mobile Phase A: 0.02% TFA in H₂O

Mobile Phase B: 0.016% TFA in ACN

Flow Rate: 0.75 mL/min

Gradient:	Time		Profile	
	(min)	%A	%B	%B
	0.0	100	0	
	25.0	50	50	

Injection Volume: 20 μL

Temperature: 40° C

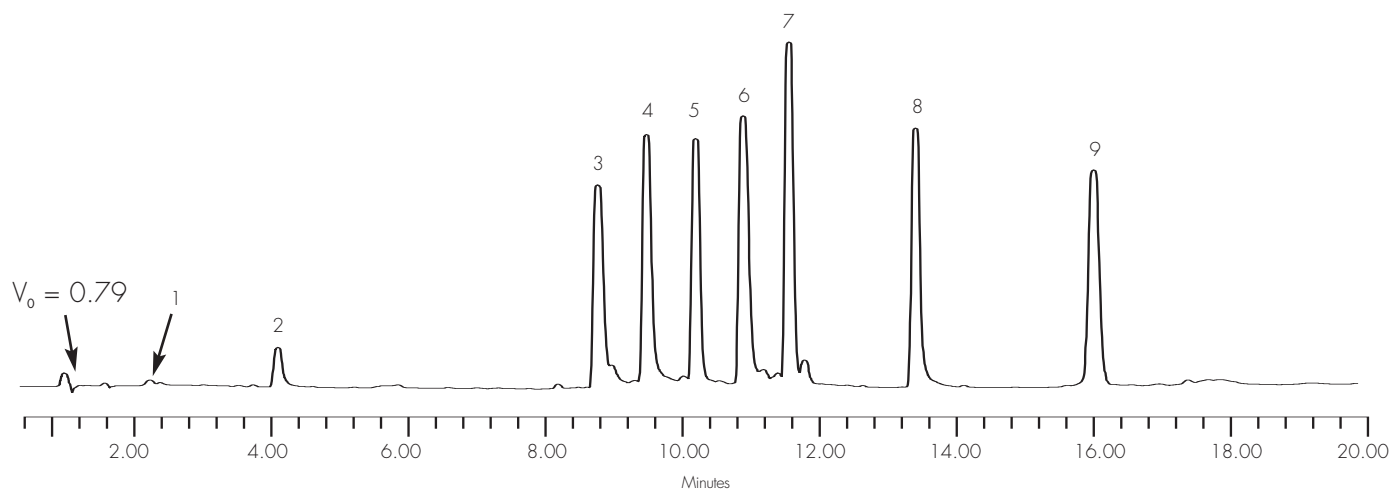
Total Mass Load: 18 μg

Detection: UV @ 220 nm

Instrument: Alliance® 2690 with 2996 PDA

Compounds:

1. I2275
2. A6677
3. Bradykinin
4. Angiotensin II
5. Angiotensin I
6. Substance P
7. Renin Substrate
8. Insulin B Chain
9. Melittin

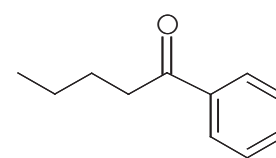
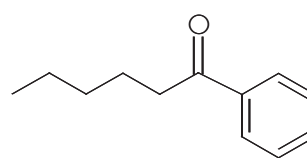
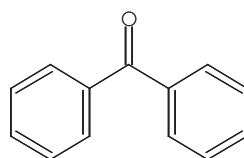
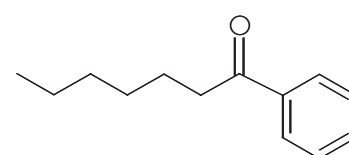
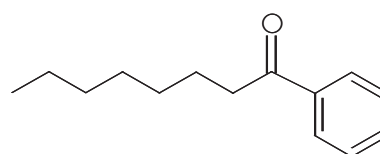
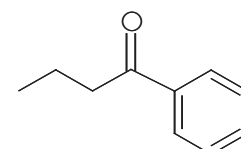
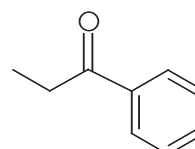
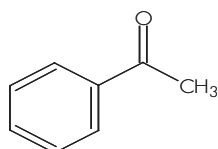
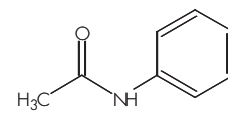
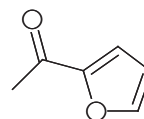
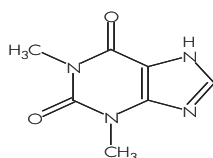


LC Conditions:

Column: Atlantis™ dC₁₈ 2.1 x 20 mm IS™, 3 μm
 Part Number: 186002058
 Mobile Phase A: 0.1% HCOOH in Water
 Mobile Phase B: 0.1% HCOOH in ACN
 Flow Rate: 0.6 mL/min
 Gradient:

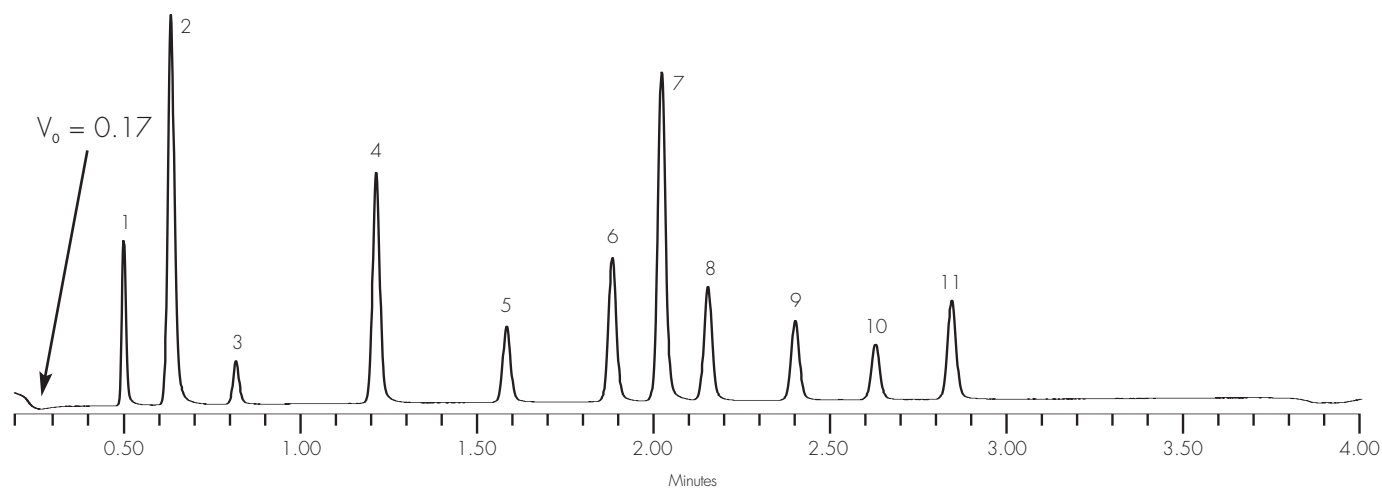
Time (min)	%A	%B
0.0	100	0
4.0	0	100

Injection Volume: 5 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2795 with 996 PDA



Compounds:

1. Theophylline
2. 2-Acetylfuran
3. Acetanilide
4. Acetophenone
5. Propiophenone
6. Butyrophenone
7. Benzophenone
8. Valerophenone
9. Hexanophenone
10. Heptanophenone
11. Octanophenone



Phenones— 4.6 x 20 mm IS™ Column

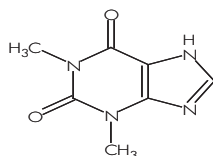
Atlantis™
Columns

Conditions:

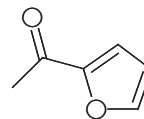
Column: Atlantis™ dC₁₈ 4.6 x 20 mm IS™, 3 μm
 Part Number: 186002062
 Mobile Phase A: 0.1% HCOOH in Water
 Mobile Phase B: 0.1% HCOOH in ACN
 Flow Rate: 3.0 mL/min
 Gradient:

Time (min)	Profile %A	%B
0.0	100	0
4.0	0	100

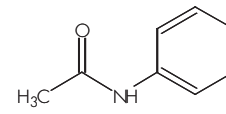
Injection Volume: 10 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2795 with 996 PDA



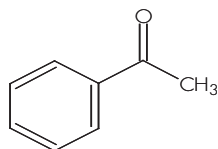
Theophylline



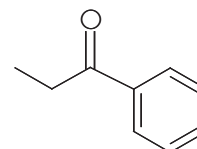
2-Acetylfuran



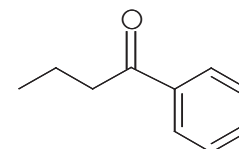
Acetanilide



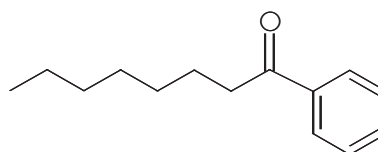
Acetophenone



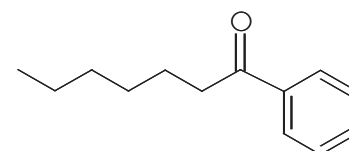
Propiophenone



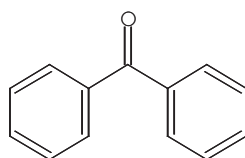
Butyrophenone



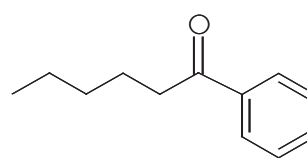
Octanophenone



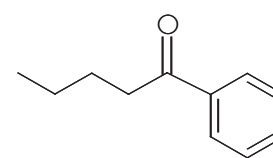
Heptanophenone



Benzophenone



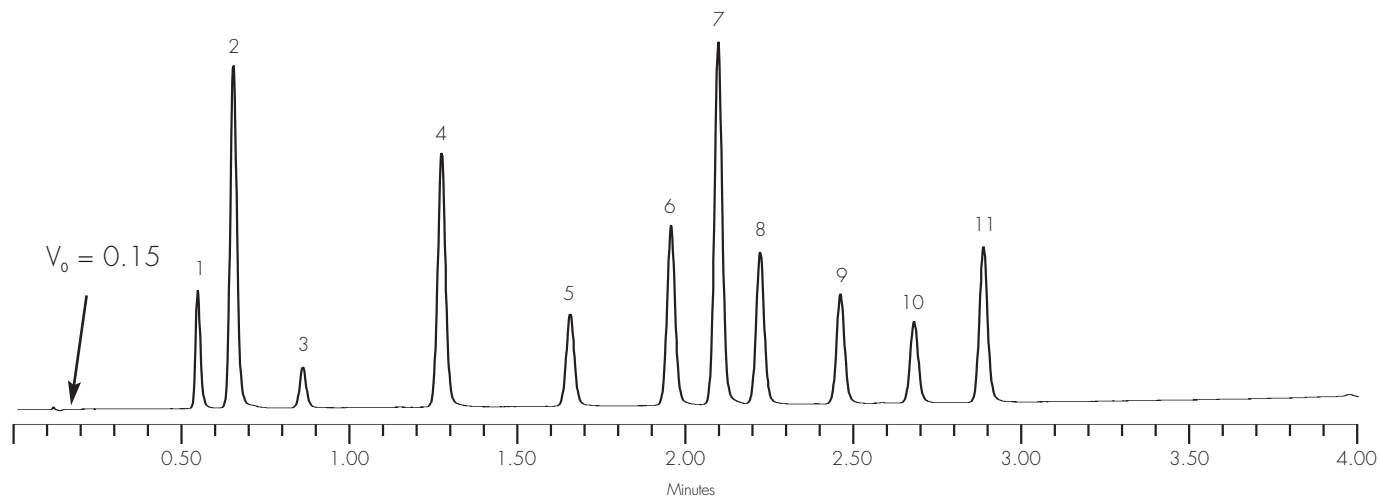
Hexanophenone



Valerophenone

Compounds:

1. Theophylline
2. 2-Acetylfuran
3. Acetanilide
4. Acetophenone
5. Propiophenone
6. Butyrophenone
7. Benzophenone
8. Valerophenone
9. Hexanophenone
10. Heptanophenone
11. Octanophenone

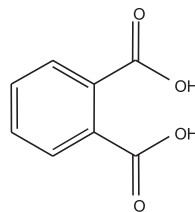


Conditions:

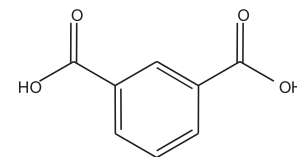
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 100 mM HCOONH₄, pH 3.75
 Flow Rate: 1.4 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	55	35	10
5.0	30	60	10

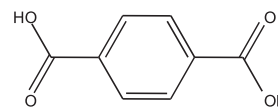
Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 280 nm
 Instrument: Alliance® 2695 with 2996 PDA



Phthalic acid

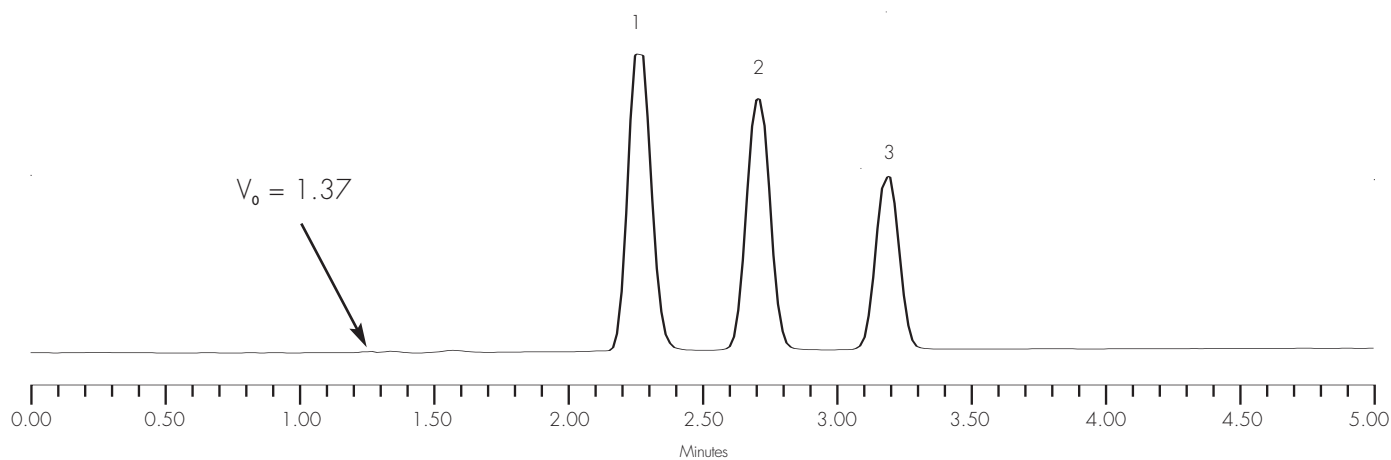


Isophthalic acid



Terephthalic acid

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Phthalic acid	1.10	43
2. Terephthalic acid	1.03	36
3. Isophthalic acid	1.00	43



Phthalic Esters

Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm

Part Number: 186001344

Mobile Phase A: H₂O

Mobile Phase B: ACN

Flow Rate: 1.0 mL/min

Gradient:	Time (min)	Profile	
		%A	%B
	0.0	10	90
	4.0	0	100
	10	0	100

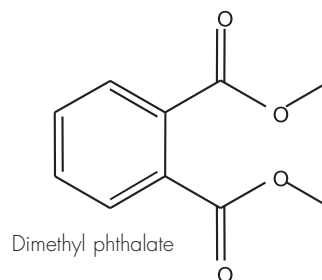
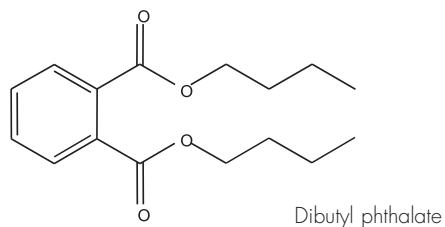
Injection Volume: 10.0 μL

Sample Concentrations: 200 μg/mL each

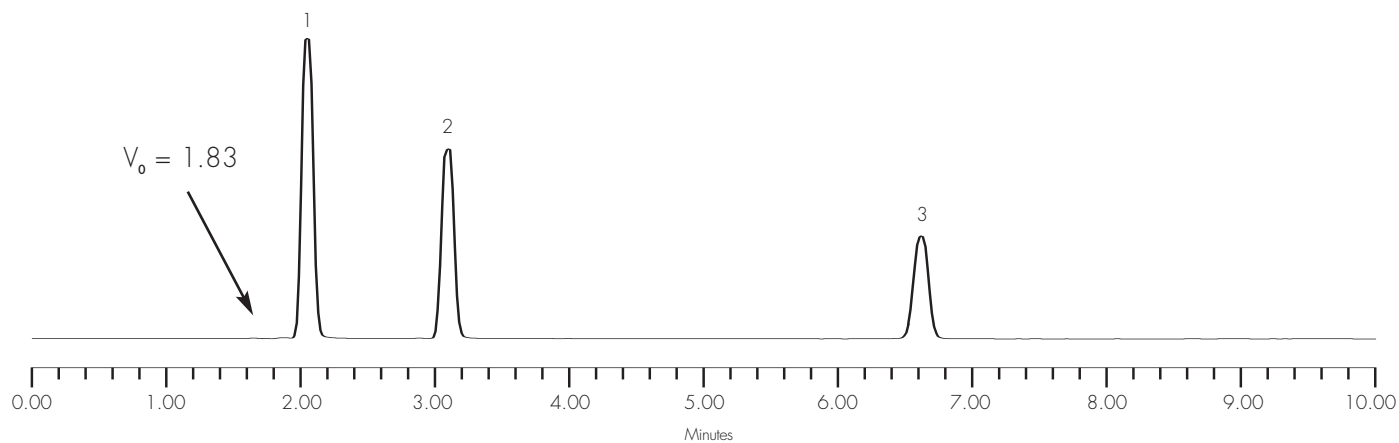
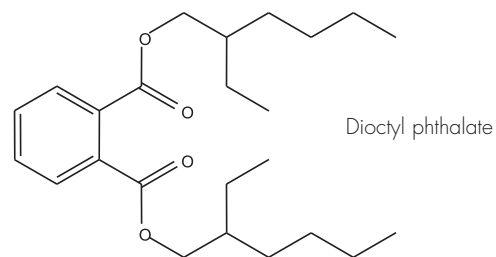
Temperature: 30° C

Detection: UV @ 285 nm

Instrument: Alliance® 2695 with 2996 PDA

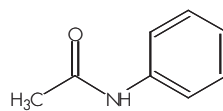


Compounds:	USP Tailing
1. Dimethyl phthalate	1.06
2. Dibutyl phthalate	1.04
3. Dioctyl phthalate	0.99

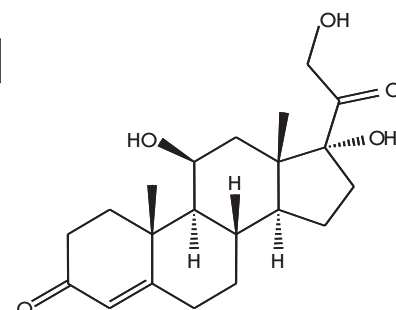


Conditions:

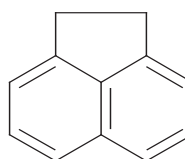
Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: ACN/1% HCOOH (90/10)
 Sample Concentration: 10 mg/mL each in DMSO
 Temperature: Ambient
 Instrument: AutoPurification™ System
 Detection: UV @ 260 nm



Acetanilide



Hydrocortisone



Acenaphthene

Compounds:

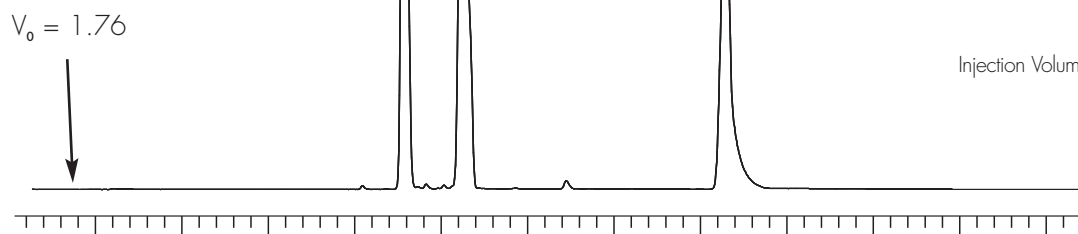
1. Acetanilide
2. Hydrocortisone
3. Acenaphthene

Column: Atlantis™ dC₁₈ 4.6 x 100 mm, 5 μm
 Part Number: 186001340
 Total Mass Load: 2,700 μg

Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	95	5
1.0	95	5
14.0	0	100
20.0	0	100

Injection Volume: 90 μL

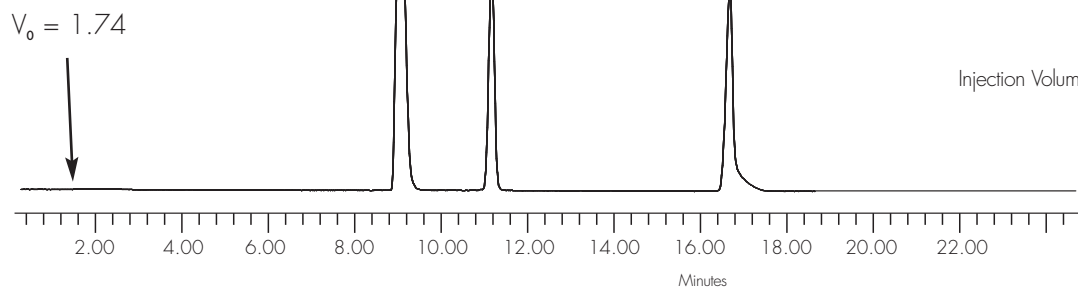


Column: Atlantis™ dC₁₈ Prep OBD™ 19 x 100 mm, 5 μm
 Part Number: 186002116
 Total Mass Load: 48,000 μg

Flow Rate: 17.06 mL/min
 Gradient:

Time (min)	%A	%B
0.0	95	5
3.02	95	5
16.02	0	100
22.02	0	100

Injection Volume: 1600 μL



Polar and Non-Polar Compound Test Mix— 2.1 x 20 mm IS™ Column

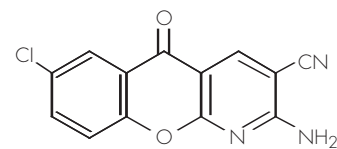
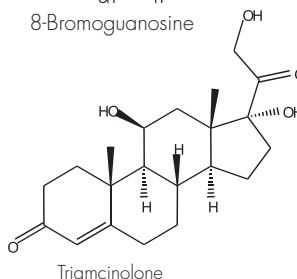
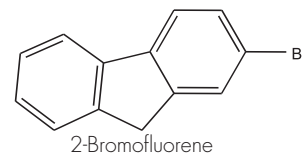
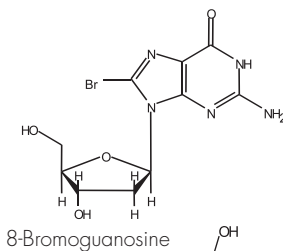
Atlantis™
Columns

Conditions:

Column: Atlantis™ dC₁₈ 2.1 x 20 mm IS™, 3 μm
 Part Number: 186002058
 Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: 0.1% HCOOH in ACN
 Flow Rate: 0.6 mL/min
 Gradient:

Time	Profile	
(min)	%A	%B
0.0	100	0
4.0	0	100

 Injection Volume: 5 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® HT 2795 with 996 PDA

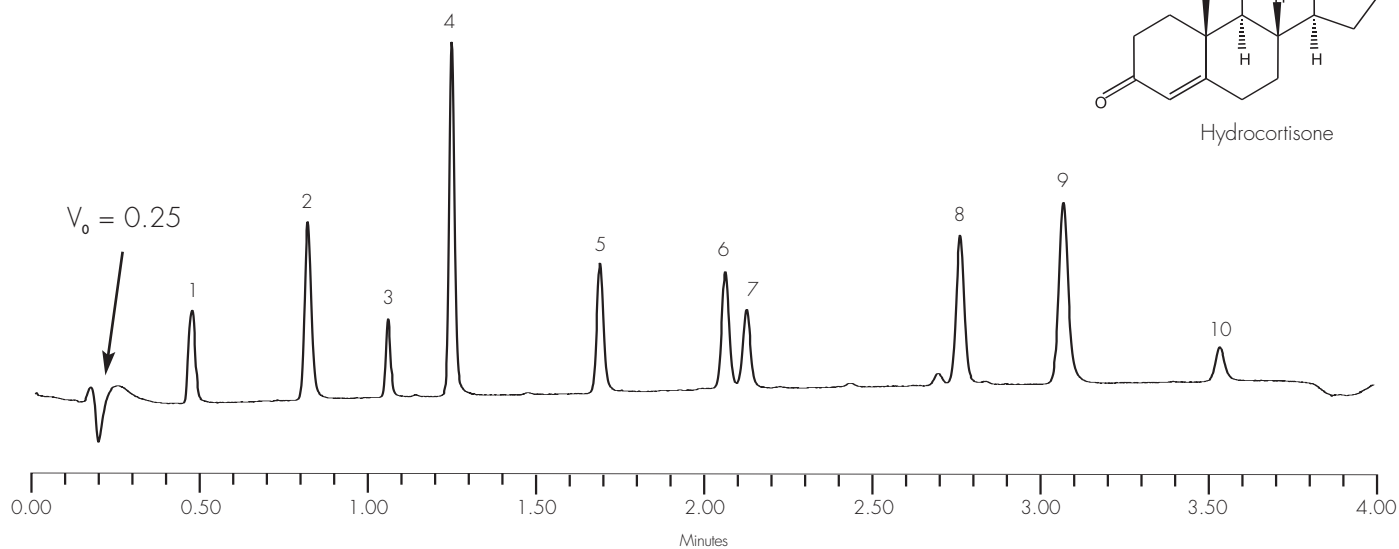
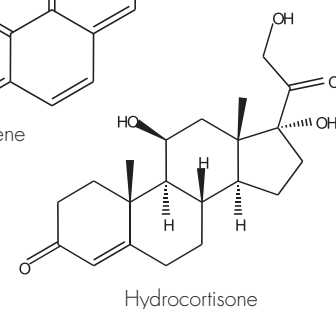
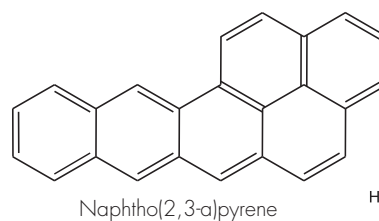
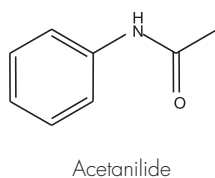
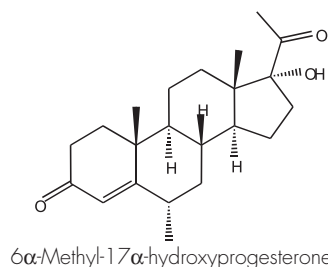
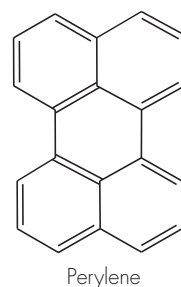
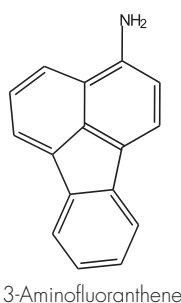


Compounds:

- 8-bromoguanosine
- Acetanilide
- Triamcinolone
- Hydrocortisone
- 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile
- 6α-Methyl-17α-hydroxyprogesterone
- 3-Aminofluoranthene
- 2-Bromofluorene
- Perylene
- Naphtho[2,3-a]pyrene

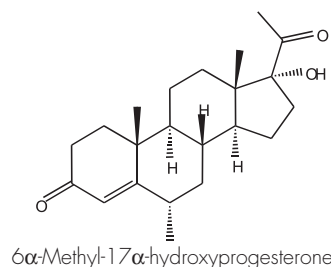
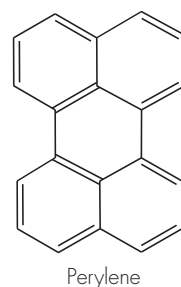
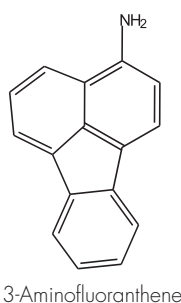
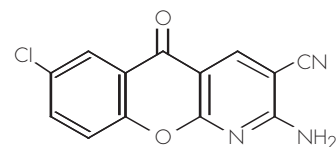
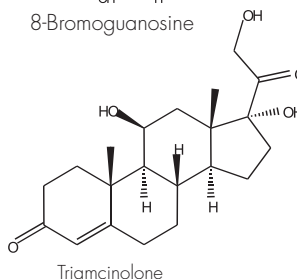
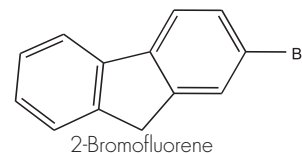
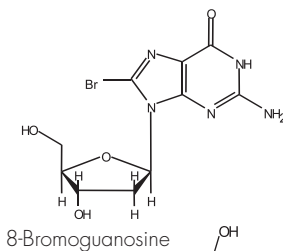
USP Tailing

- | | |
|---|------|
| 1. 8-bromoguanosine | 1.15 |
| 2. Acetanilide | 1.01 |
| 3. Triamcinolone | 0.99 |
| 4. Hydrocortisone | 1.01 |
| 5. 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile | 1.02 |
| 6. 6α-Methyl-17α-hydroxyprogesterone | 0.96 |
| 7. 3-Aminofluoranthene | 1.01 |
| 8. 2-Bromofluorene | 1.03 |
| 9. Perylene | 1.01 |
| 10. Naphtho[2,3-a]pyrene | 1.03 |



Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 20 mm IS™, 3 μm
 Part Number: 186002062
 Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: 0.1% HCOOH in ACN
 Flow Rate: 3.0 mL/min
 Gradient: Time Profile
 (min) %A %B
 0.0 100 0
 4.0 0 100
 Injection Volume: 10 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® HT 2795 with 996 PDA

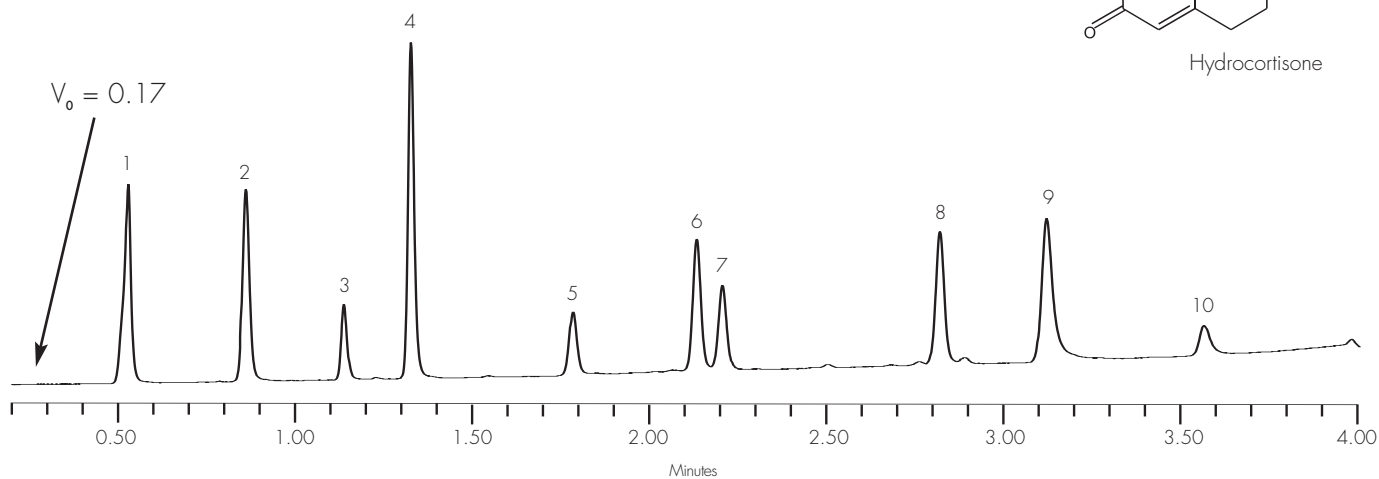
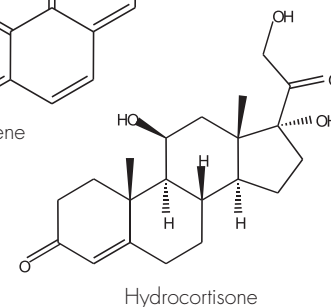
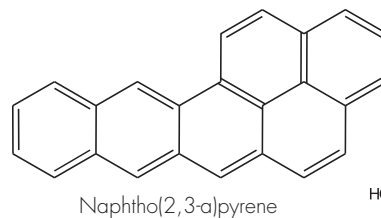
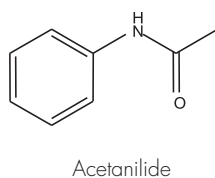


Compounds:

- 8-bromoguanosine
- Acetanilide
- Triamcinolone
- Hydrocortisone
- 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile
- 6α-Methyl-17α-hydroxyprogesterone
- 3-Aminofluoranthene
- 2-Bromofluorene
- Perylene
- Naphtho[2,3-a]pyrene

USP Tailing

- | | |
|---|------|
| 1. 8-bromoguanosine | 1.15 |
| 2. Acetanilide | 1.01 |
| 3. Triamcinolone | 0.99 |
| 4. Hydrocortisone | 1.01 |
| 5. 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile | 1.02 |
| 6. 6α-Methyl-17α-hydroxyprogesterone | 0.96 |
| 7. 3-Aminofluoranthene | 1.01 |
| 8. 2-Bromofluorene | 1.03 |
| 9. Perylene | 1.01 |
| 10. Naphtho[2,3-a]pyrene | 1.03 |



Polar and Non-Polar Compound Test Mix— 2.1 x 30 mm Column

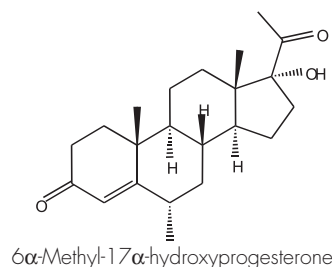
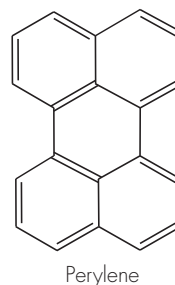
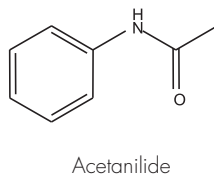
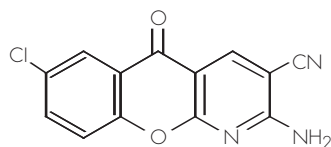
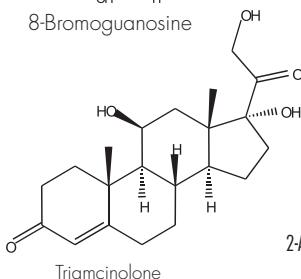
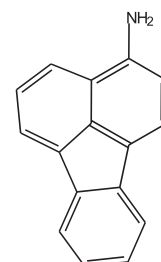
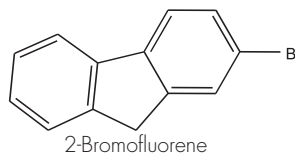
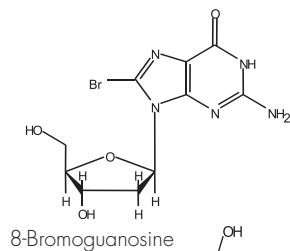
Atlantis™ Columns

Conditions:

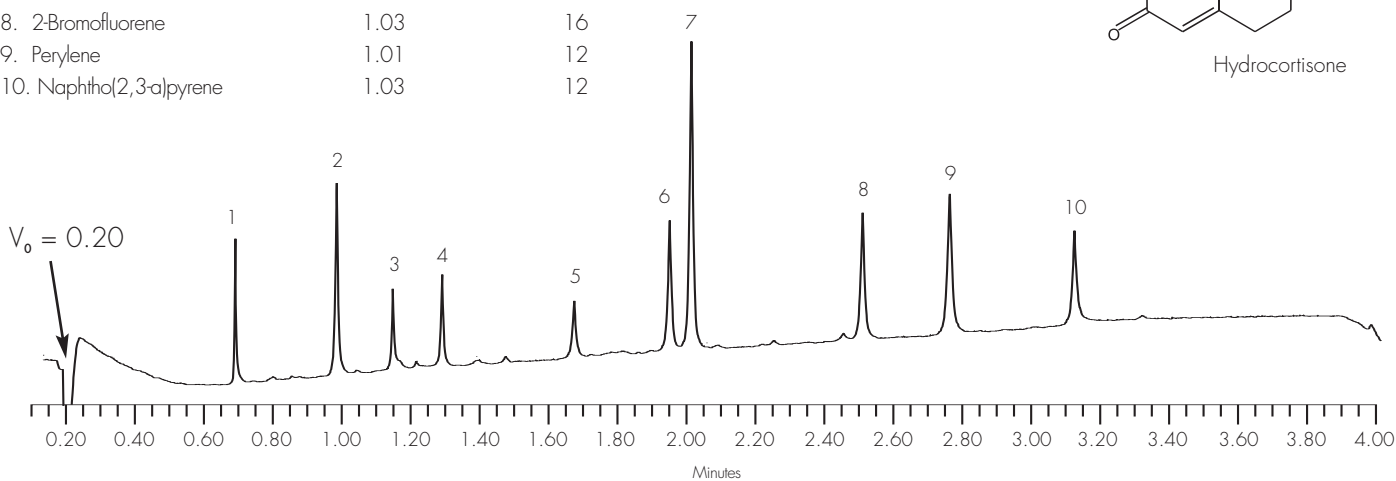
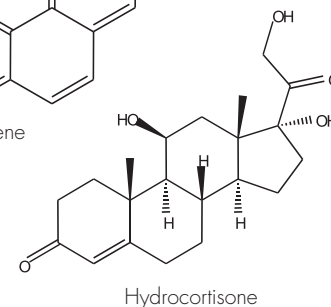
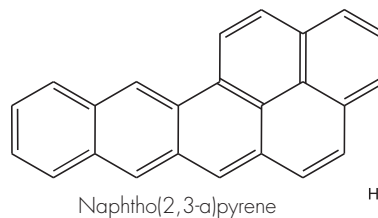
Column: Atlantis™ dC₁₈ 2.1 x 30 mm, 3 μm
 Part Number: 186001287
 Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: 0.1% HCOOH in ACN
 Flow Rate: 0.8 mL/min
 Gradient:

Time (min)	Profile %A	%B
0.0	100	0
0.2	100	0
3.2	0	100
4.0	0	100

Injection Volume: 2 μL
 Temperature: Ambient
 Detection: UV @ 254 nm
 Sampling Rate: 10 pts/sec
 Instrument: Alliance® 2795 with 2996 PDA



Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. 8-bromoguanosine	1.15	31
2. Acetanilide	1.01	19
3. Triamcinolone	0.99	31
4. Hydrocortisone	1.01	12
5. 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile	1.02	12
6. 6α-Methyl-17α-hydroxyprogesterone	0.96	25
7. 3-Aminofluoranthene	1.01	31
8. 2-Bromofluorene	1.03	16
9. Perylene	1.01	12
10. Naphtho[2,3-a]pyrene	1.03	12

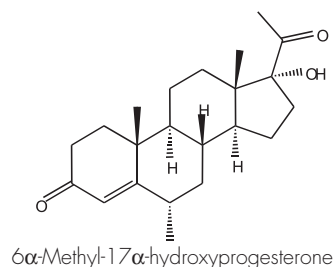
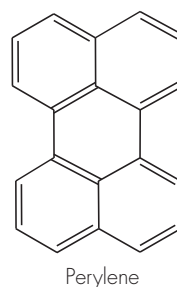
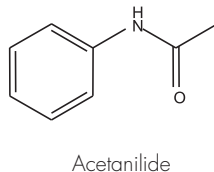
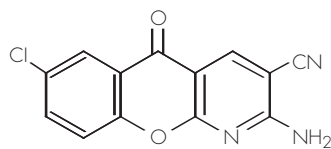
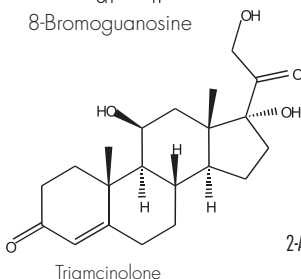
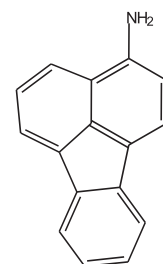
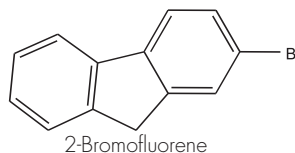
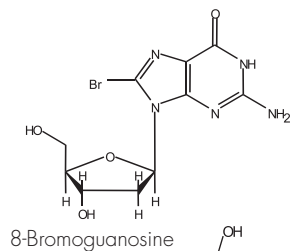


Conditions:

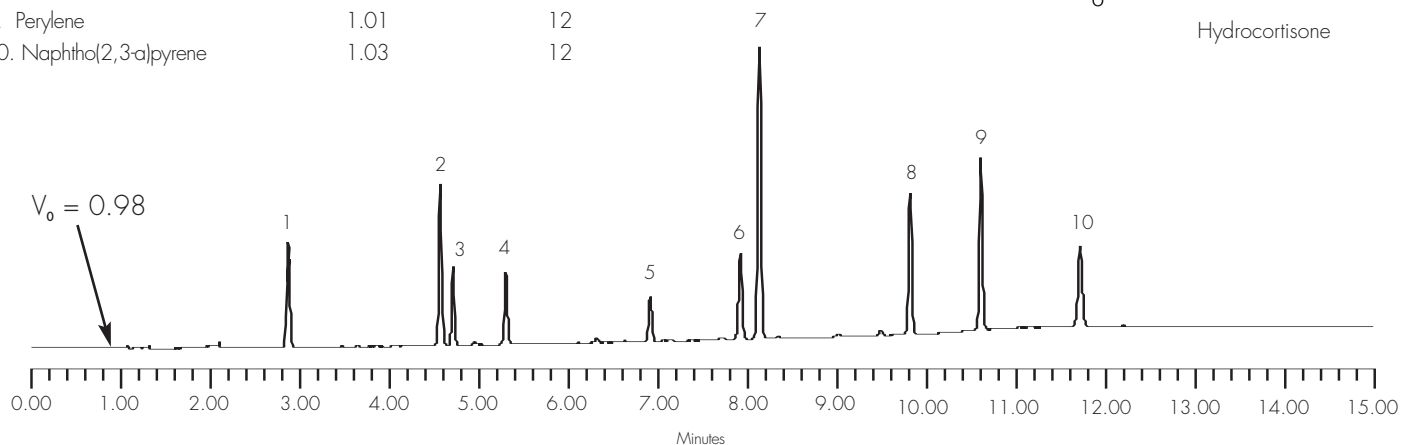
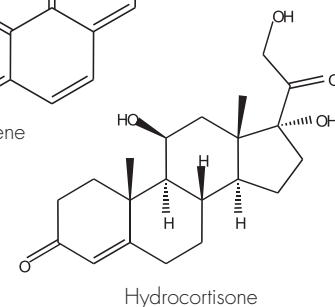
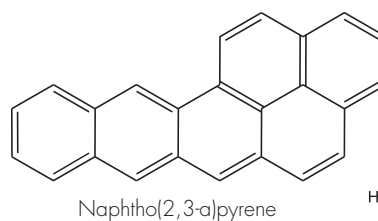
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: 0.1% HCOOH in ACN
 Flow Rate: 2.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	100	0
0.5	100	0
10.0	0	100
15.0	0	100

Injection Volume: 10 μL
 Temperature: Ambient
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA



Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. 8-bromoguanosine	1.15	31
2. Acetanilide	1.01	19
3. Triamcinolone	0.99	31
4. Hydrocortisone	1.01	12
5. 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile	1.02	12
6. 6α-Methyl-17α-hydroxyprogesterone	0.96	25
7. 3-Aminofluoranthene	1.01	31
8. 2-Bromofluorene	1.03	16
9. Perylene	1.01	12
10. Naphtho[2,3-a]pyrene	1.03	12



Polar and Non-Polar Compound Test Mix— (with Uracil), 4.6 x 150 mm Column

Atlantis™ Columns

Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm

Part Number: 186001344

Mobile Phase A: 0.1% HCOOH in H₂O

Mobile Phase B: 0.1% HCOOH in ACN

Flow Rate: 2.0 mL/min

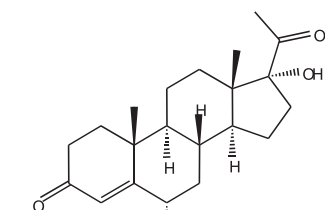
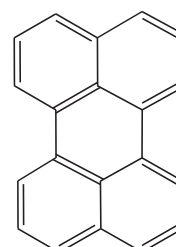
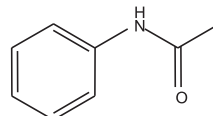
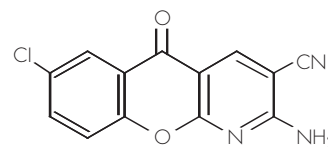
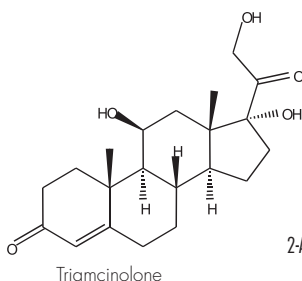
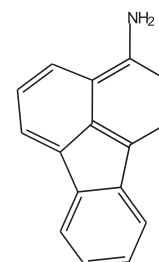
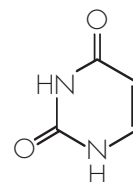
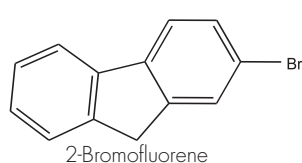
Gradient:	Time (min)	Profile	
		%A	%B
	0.0	100	0
	0.5	100	0
	10.0	0	100
	15.0	0	100

Injection Volume: 10 μL

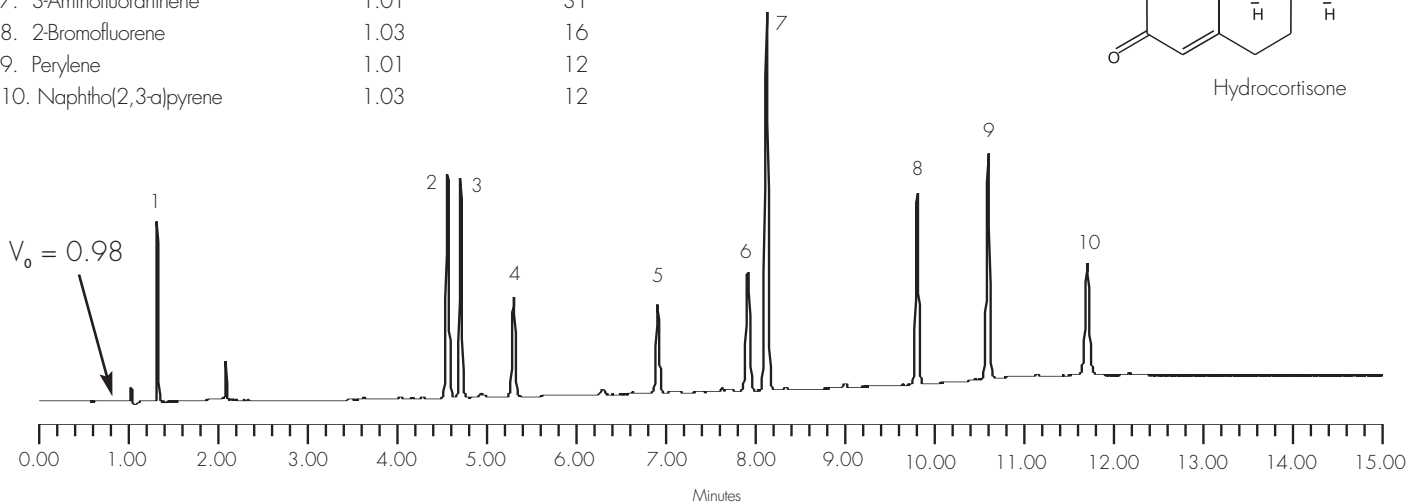
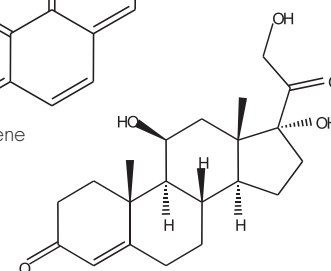
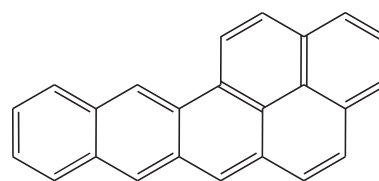
Temperature: Ambient

Detection: UV @ 254 nm

Instrument: Alliance® 2695 with 2996 PDA



Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. Uracil	1.15	31
2. Acetanilide	1.01	19
3. Triamcinolone	0.99	31
4. Hydrocortisone	1.01	12
5. 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile	1.02	12
6. 6α-Methyl-17α-hydroxyprogesterone	0.96	25
7. 3-Aminofluoranthene	1.01	31
8. 2-Bromofluorene	1.03	16
9. Perylene	1.01	12
10. Naphtho(2,3-a)pyrene	1.03	12

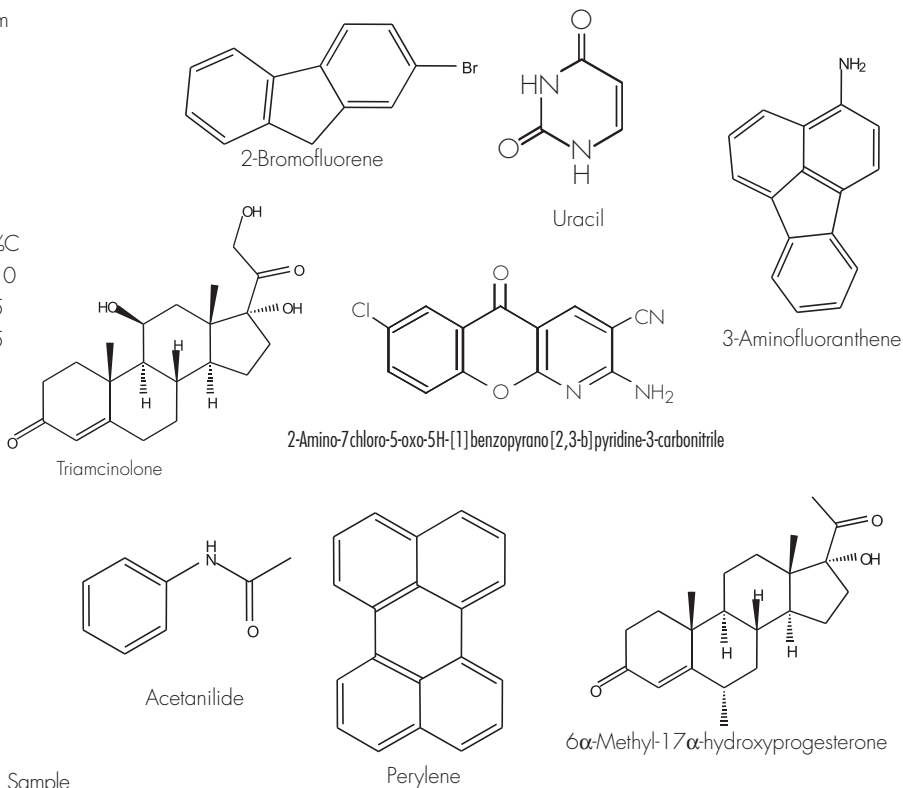


Conditions:

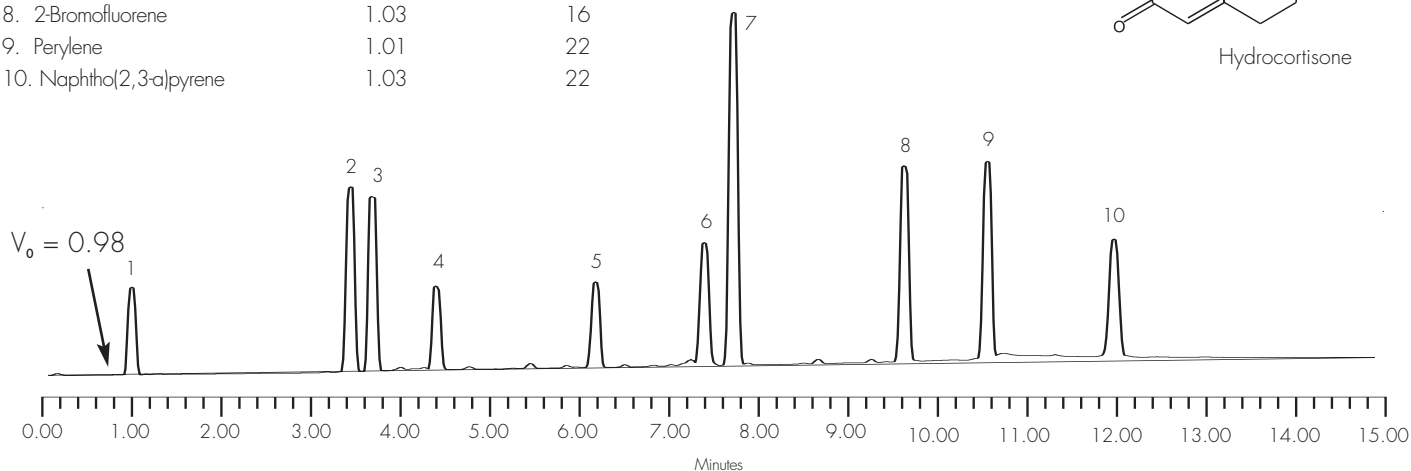
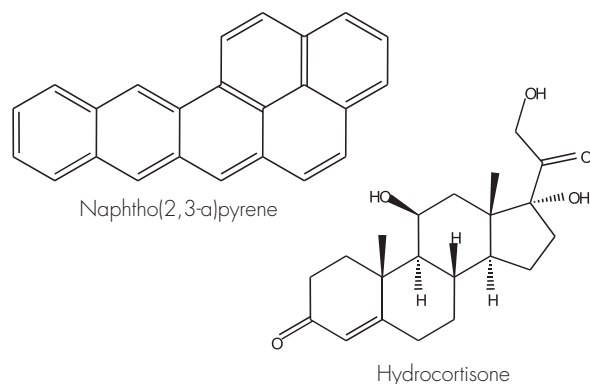
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.0
 Flow Rate: 2.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	80	10	10
10.0	0	95	5
15.0	0	95	5

Injection Volume: 5 μL
 Temperature: 30° C
 Detection: UV @ 254 nm
 Instrument: Alliance® 2695 with 2996 PDA



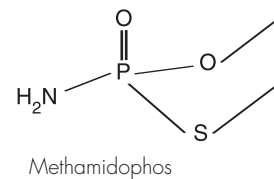
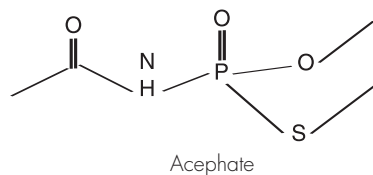
Compounds:	USP Tailing	Sample Concentrations (μg/ml)
1. Uracil	1.15	31
2. Acetanilide	1.01	19
3. Triamcinolone	0.99	31
4. Hydrocortisone	1.01	12
5. 2-Amino-7-chloro-5-oxo-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile	1.02	12
6. 6α-Methyl-17α-hydroxyprogesterone	0.96	25
7. 3-Aminofluoranthene	1.01	31
8. 2-Bromofluorene	1.03	16
9. Perylene	1.01	22
10. Naphtho(2,3-a)pyrene	1.03	22



Polar Pesticides

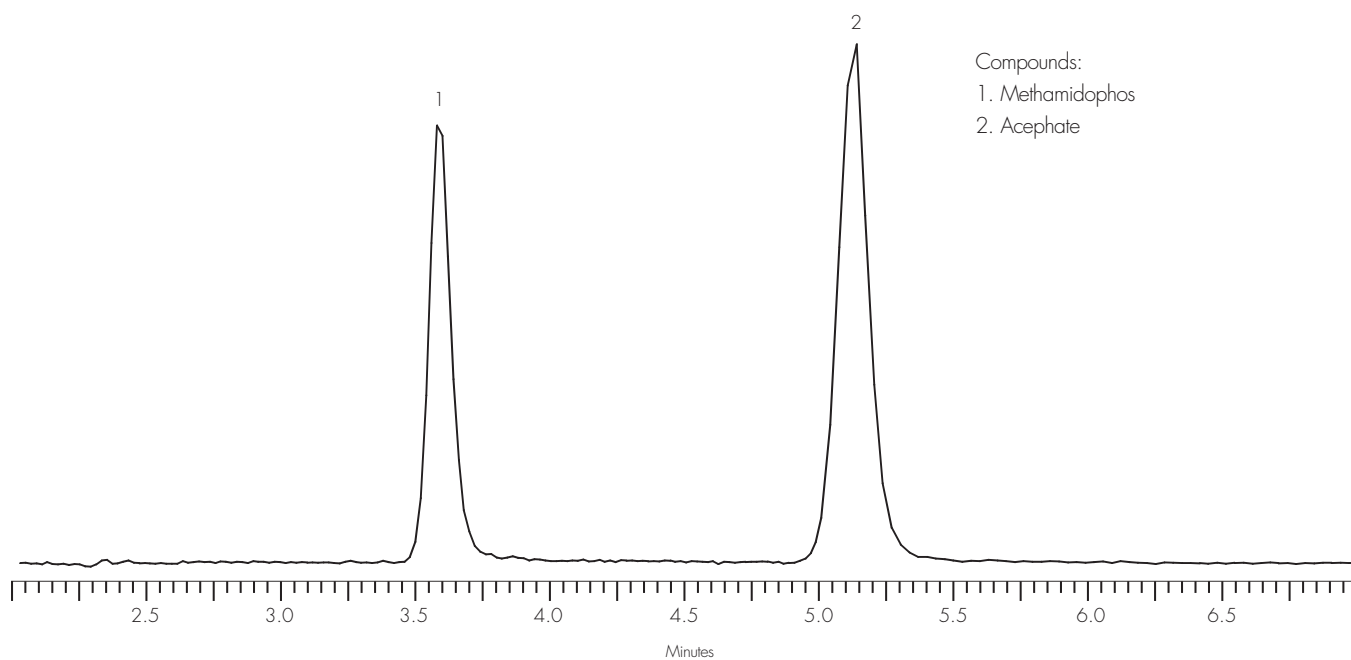
LC Conditions:

Column: Atlantis™ dC₁₈ 2.1 x 100 mm, 3 μm
 Part Number: 186001295
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Flow Rate: 0.17 mL/min
 Isocratic Mobile
 Phase Composition: 95% A; 5% B
 Injection Volume: 20 μL
 Sample Concentration: 10 ppb
 Temperature: 25° C
 Detection: MS(ESI+)
 Instrument: Alliance® 2795 with Waters ZQ™



MS Conditions:

ESI+		SIR of 5 Masses	
		mass (m/z)	cone (V)
Capillary (kV)	3.1	112 ^a	40
Extractor	3.0	113 ^b	40
RF Lens	0.1	142 ^a	20
Source Temp	150	143 ^b	25
Desolvation Temp	350	184 ^b	15
Desolvation Gas Flow (L/Hr)	500		
IM Resolution	12.5	^a ion for methamidophos	
HM Resolution	12.5	^b ion for acephate	
Ion Energy	1.0		
Multiplier (V)	650		

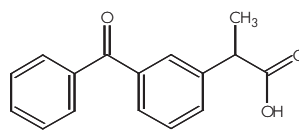


LC Conditions:

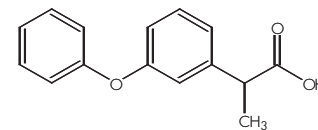
Column: Atlantis™ dC₁₈ 4.6 x 20 mm IS™, 3 μm
 Part Number: 186002062
 Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: 0.1% HCOOH in ACN
 Flow Rate: 3.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	65	35
2.0	60	40

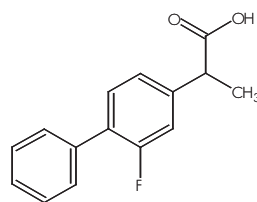
Injection Volume: 10 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 220 nm
 Instrument: Alliance® HT 2795 with 2996 PDA



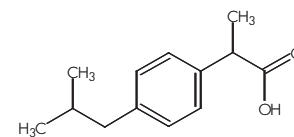
Ketoprofen



Fenoprofen



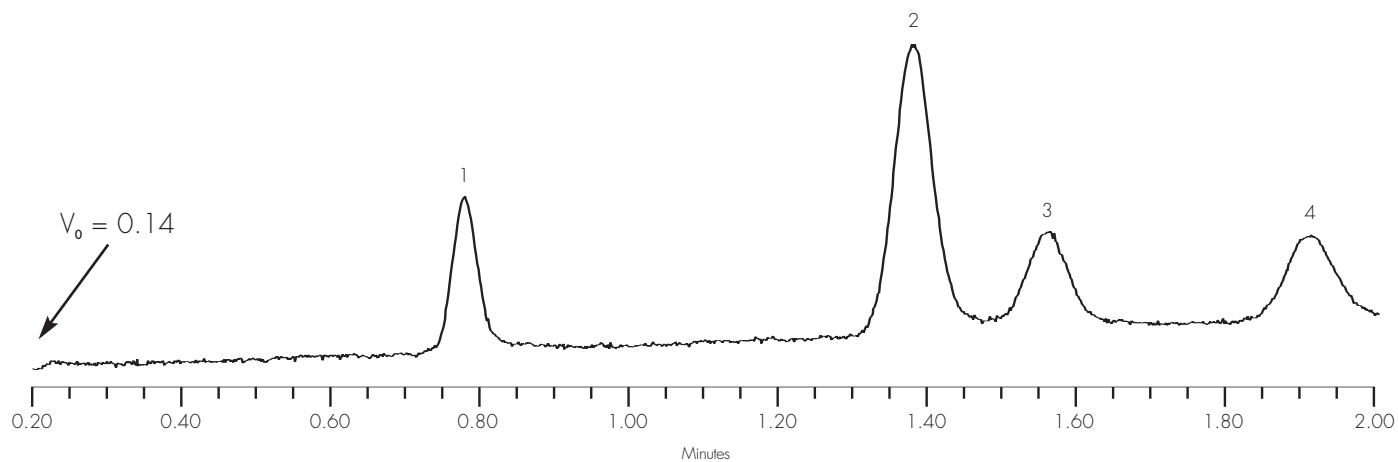
Flurbiprofen



Ibuprofen

Compounds:

1. Ketoprofen
2. Fenoprofen
3. Flurbiprofen
4. Ibuprofen

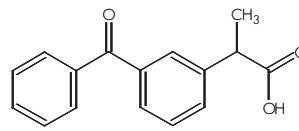


Profens—Isocratic 4.6 x 20 mm IS™ Column

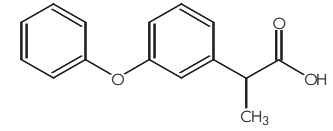
Atlantis™
Columns

LC Conditions:

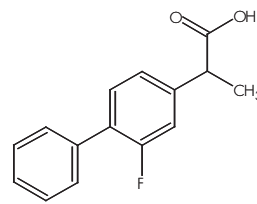
Column: Atlantis™ dC₁₈ 4.6 x 20 mm IS™, 3 μm
Part Number: 186002062
Mobile Phase A: 0.1% HCOOH in H₂O
Mobile Phase B: 0.1% HCOOH in ACN
Flow Rate: 3.0 mL/min
Isocratic Mobile Phase Conditions: 65% A, 35% B
Injection Volume: 10 μL
Sample Concentration: 20 μg/mL
Temperature: 30° C
Detection: UV @ 220 nm
Instrument: Alliance® 2695 with 2996 PDA



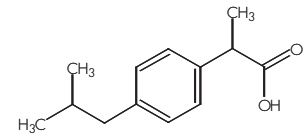
Ketoprofen



Fenoprofen



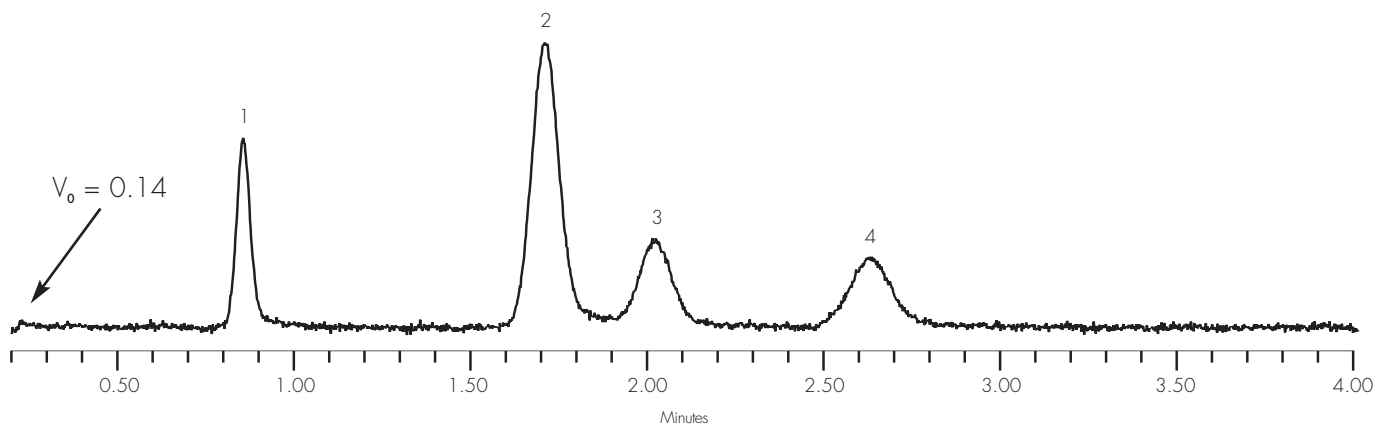
Flurbiprofen



Ibuprofen

Compounds:

1. Ketoprofen
2. Fenoprofen
3. Flurbiprofen
4. Ibuprofen

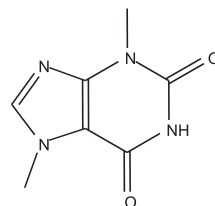


LC Conditions:

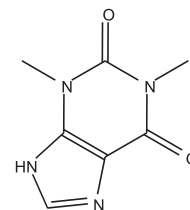
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 100 mM HCOONH₄, pH 3.75
 Flow Rate: 2.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	85	5	10
5.0	50	40	10

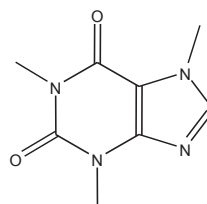
Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 270 nm
 Instrument: Alliance® 2695 with 2996 PDA



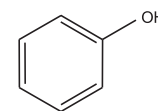
Theobromine



Theophylline

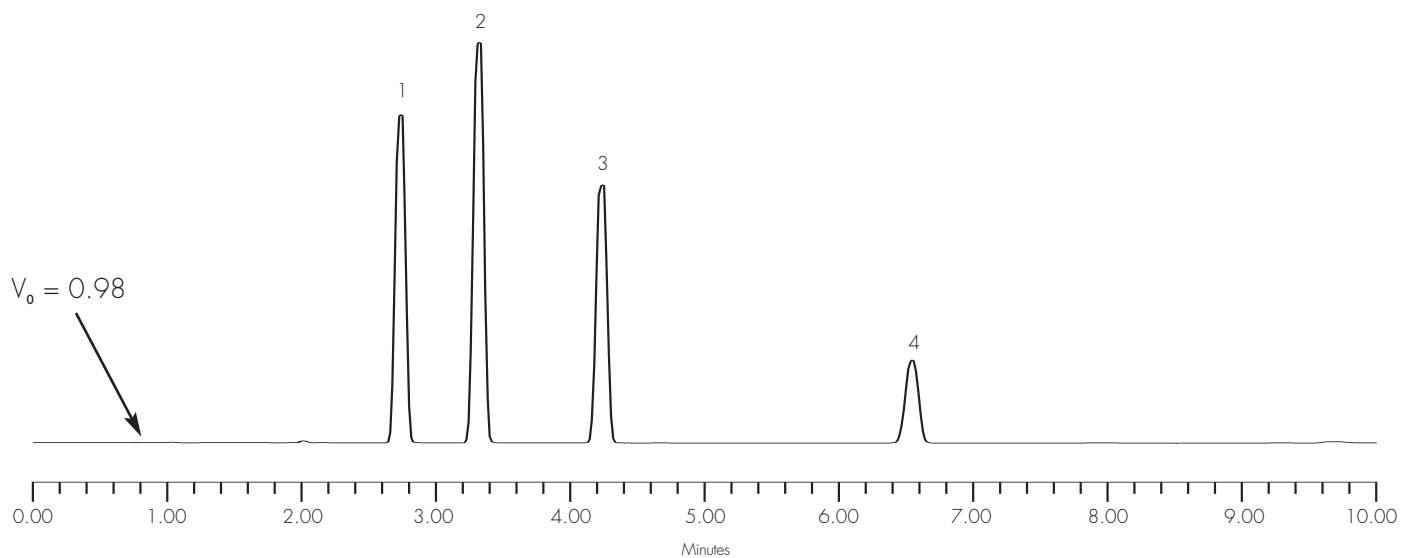


Caffeine



Phenol

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Theobromine	1.00	42
2. Theophylline	1.00	50
3. Caffeine	0.98	42
4. Phenol	0.97	42



Steroids— 2.1 x 20 mm IS™ Column

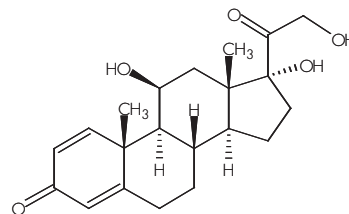
Atlantis™
Columns

LC Conditions:

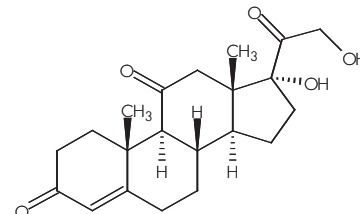
Column: Atlantis™ dC₁₈ 2.1 x 20 mm IS™, 3 μm
 Part Number: 186002058
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Flow Rate: 0.6 mL/min
 Gradient:

Time (min)	Profile	
	%A	%B
0.0	80	20
4.0	36	64

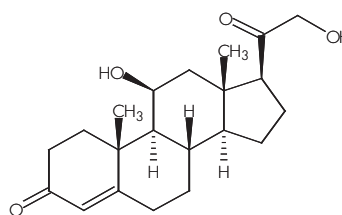
Injection Volume: 5 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 220 nm
 Instrument: Alliance® 2795 with 2996 PDA



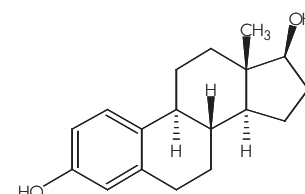
Prednisolone



Cortisone



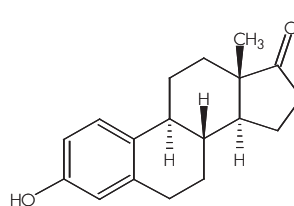
Corticosterone



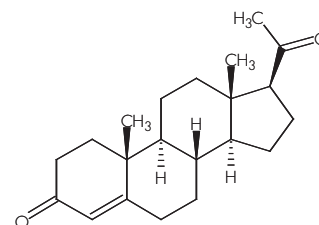
Estradiol

Compounds:

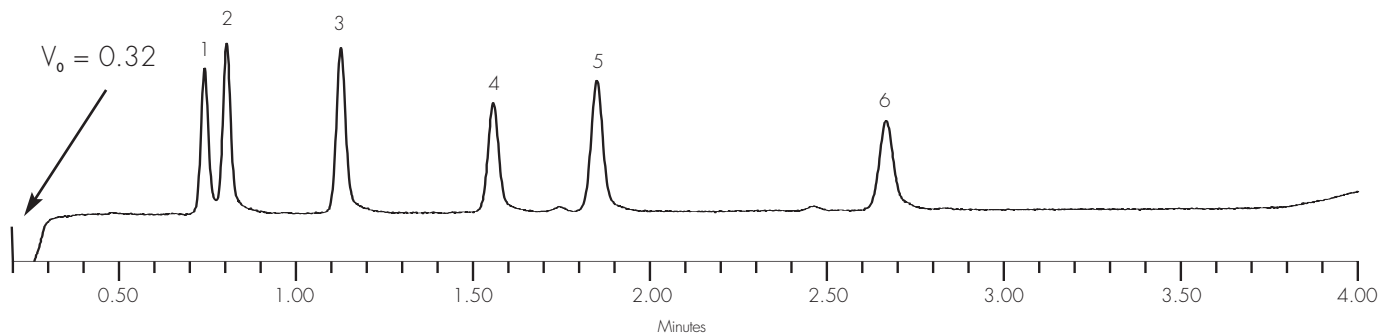
1. Prednisolone
2. Cortisone
3. Corticosterone
4. Estradiol
5. Estrone
6. Progesterone



Estrone



Progesterone

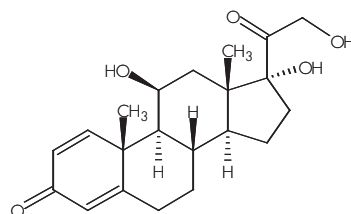


LC Conditions:

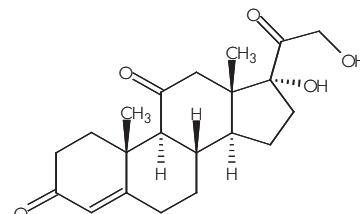
Column: Atlantis™ dC₁₈ 4.6 x 20 mm IS™, 3 μm
 Part Number: 186002062
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Flow Rate: 3.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	80	20
4.0	36	64

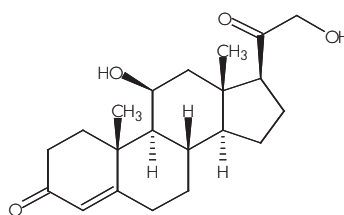
Injection Volume: 10 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 220 nm
 Instrument: Alliance® HT 2795 with 2996 PDA



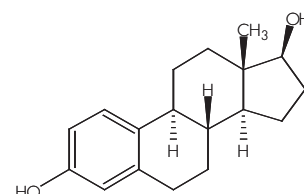
Prednisolone



Cortisone



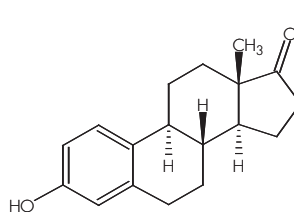
Corticosterone



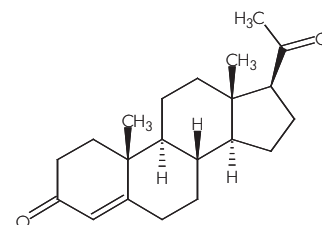
Estradiol

Compounds:

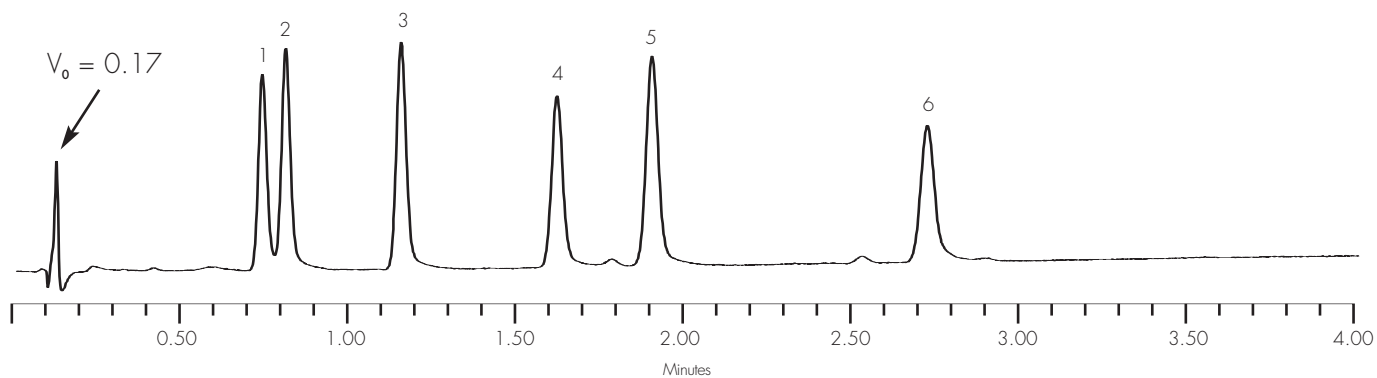
1. Prednisolone
2. Cortisone
3. Corticosterone
4. Estradiol
5. Estrone
6. Progesterone



Estrone



Progesterone



Sulfonamides— 2.1 x 20 mm IS™ Column

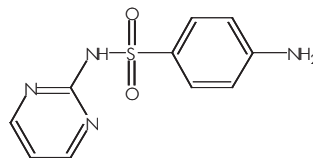
Atlantis™
Columns

LC Conditions:

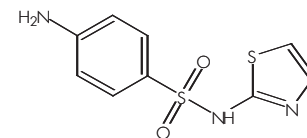
Column: Atlantis™ dC₁₈ 2.1 x 20 mm IS™, 3 μm
 Part Number: 186002058
 Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: 0.1% HCOOH in MeOH
 Flow Rate: 0.6 mL/min
 Gradient:

Time (min)	%A	Profile %B
0.0	100	0
4.0	50	50

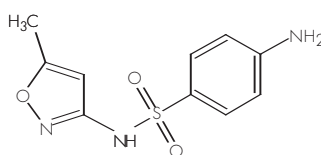
 Injection Volume: 10 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 270nm
 Instrument: Alliance® HT 2795 with 2996 PDA



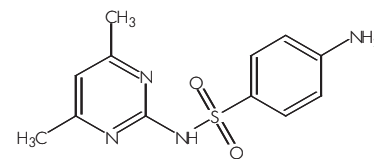
Sulfadiazine



Sulfathiazole



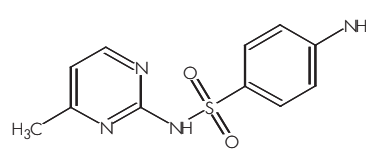
Sulfamethoxazole



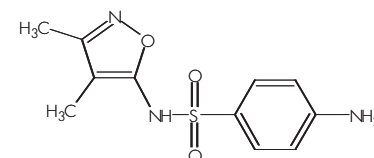
Sulfadimidine

Compounds:

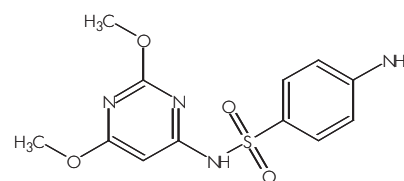
1. Sulfadiazine
2. Sulfathiazole
3. Sulfamerazine
4. Sulfadimidine
5. Sulfamethoxazole
6. Sulfisoxazole
7. Sulfadimethoxine



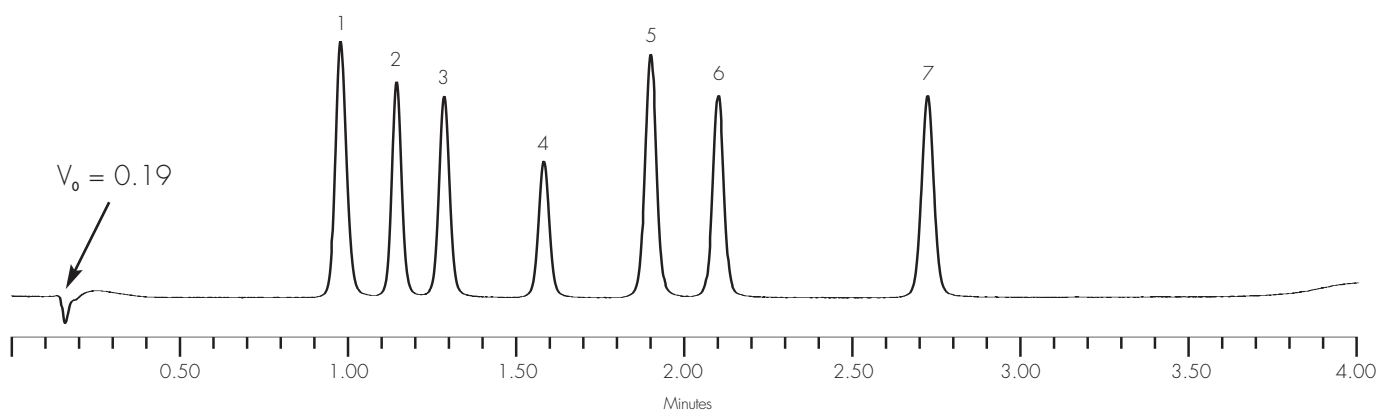
Sulfamerazine



Sulfisoxazole



Sulfadimethoxine

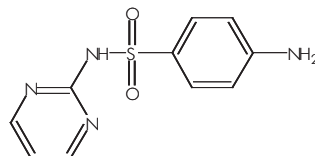


LC Conditions:

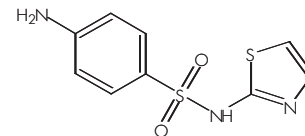
Column: Atlantis™ dC₁₈ 4.6 x 20 mm IS™, 3 μm
 Part Number: 186002062
 Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: 0.1% HCOOH in MeOH
 Flow Rate: 3.0 mL /min
 Gradient:

Time (min)	%A	%B
0.0	100	0
4.0	50	50

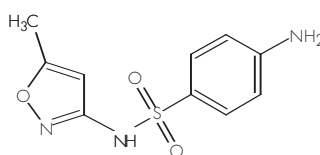
 Injection Volume: 10 μL
 Sample Concentration: 20 μg/mL
 Temperature: 30° C
 Detection: UV @ 270 nm
 Instrument: Alliance® HT 2795 with 2996 PDA



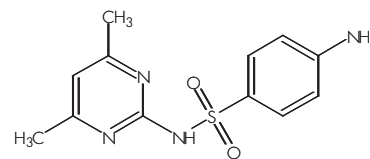
Sulfadiazine



Sulfathiazole



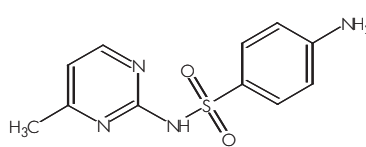
Sulfamethoxazole



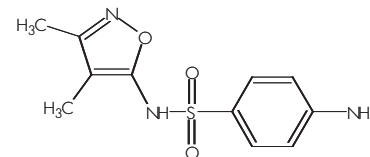
Sulfadimidine

Compounds:

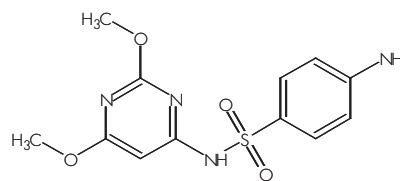
1. Sulfadiazine
2. Sulfathiazole
3. Sulfamerazine
4. Sulfadimidine
5. Sulfamethoxazole
6. Sulfisoxazole
7. Sulfadimethoxine



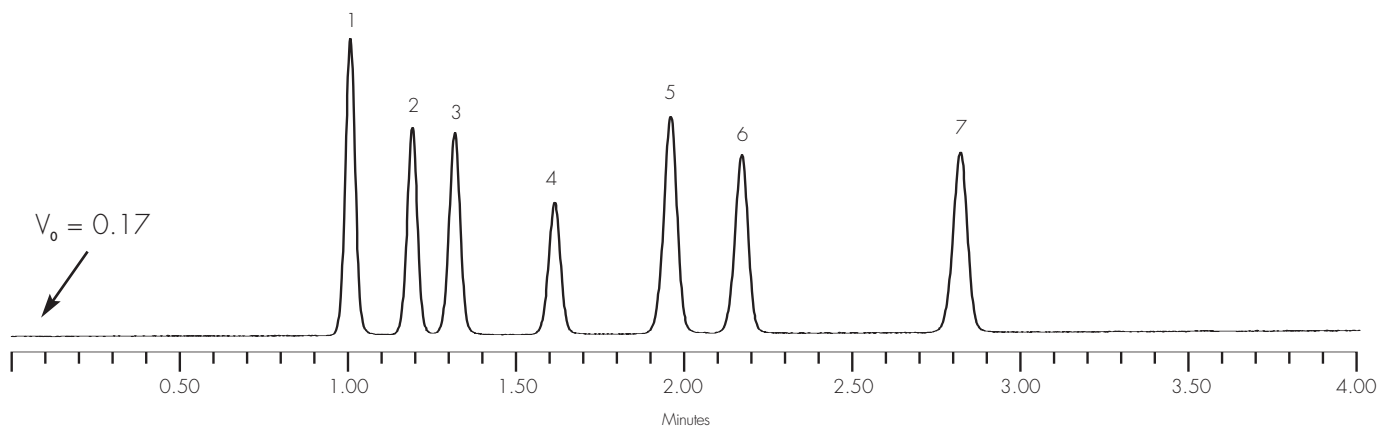
Sulfamerazine



Sulfisoxazole



Sulfadimethoxine



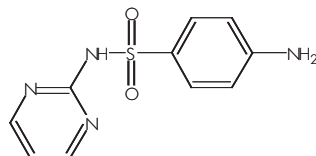
Sulfonamides

LC Conditions:

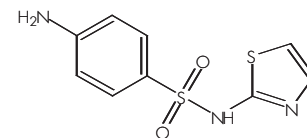
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: H₂O
 Mobile Phase B: MeOH
 Mobile Phase C: 1% HCOOH, pH 2.4
 Flow Rate: 2.0 mL/min
 Gradient:

Time (min)	%A	%B	%C
0.0	75	15	10
10.0	50	40	10

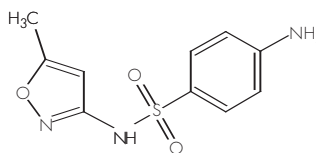
Injection Volume: 5 μL
 Temperature: 30° C
 Detection: UV @ 270 nm
 Instrument: Alliance® 2695 with 2996 PDA



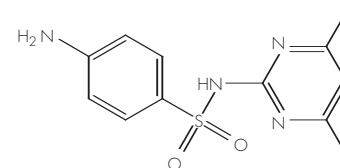
Sulfadiazine



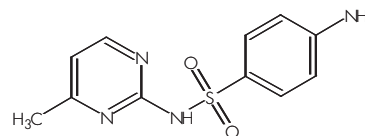
Sulfathiazole



Sulfamethoxazole

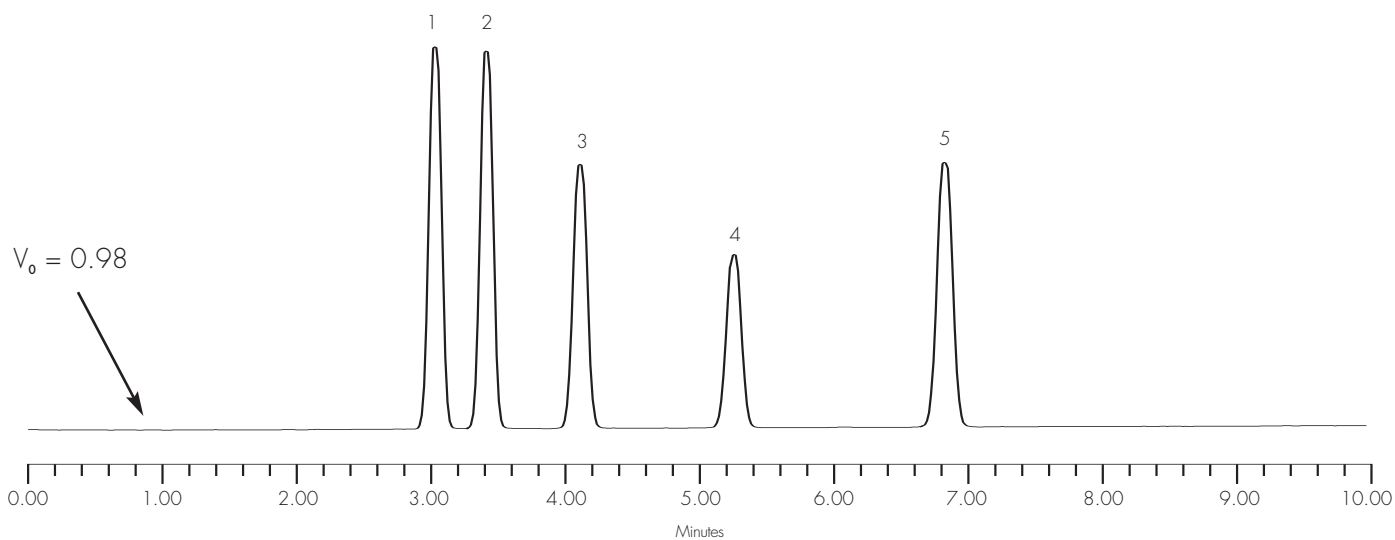


Sulfamethazine



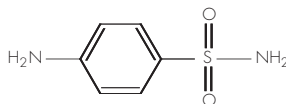
Sulfamerazine

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. Sulfadiazine	1.01	50
2. Sulfathiazole	1.02	67
3. Sulfamerazine	1.00	50
4. Sulfamethazine	0.99	33
5. Sulfamethoxazole	0.99	50

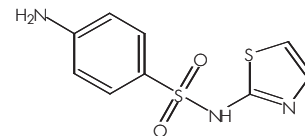


LC Conditions:

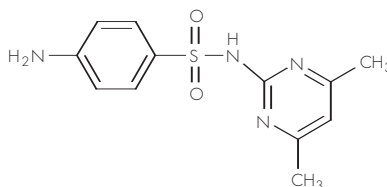
Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: ACN/1% HCOOH (90/10)
 Sample Concentration: 1.0 mg/mL each in DMSO
 Temperature: Ambient
 Instrument: AutoPurification™ System



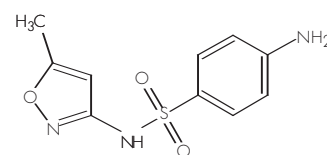
Sulfanilamide



Sulfathiazole



Sulfamethazine



Sulfamethoxazole

Compounds:

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

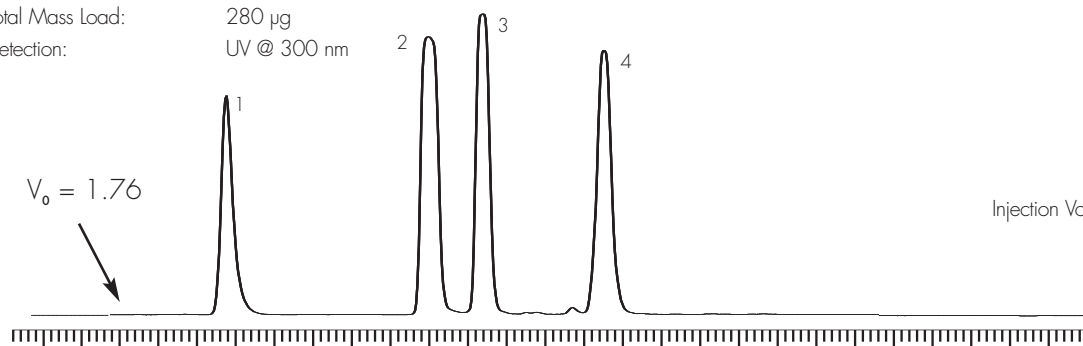
The flattened peak profiles on analytical chromatogram reflects the saturation of PDA detector, not column overload.

Column: Atlantis™ dC₁₈ 4.6 x 100 mm, 5 μm
 Part Number: 186001340
 Total Mass Load: 280 μg
 Detection: UV @ 300 nm

Flow Rate: 1.0 mL/min
 Gradient:

Time (min)	%A	%B
0.0	85	15
1.0	85	15
2.0	70	30
12.0	30	70
14.0	30	70

 Injection Volume: 70 μL

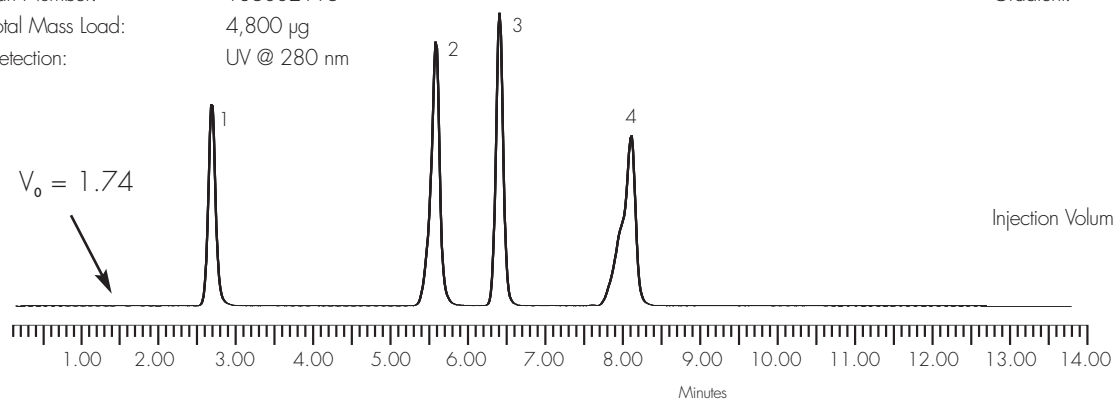


Column: Atlantis™ dC₁₈ Prep OBD™ 19 x 100 mm, 5 μm
 Part Number: 186002116
 Total Mass Load: 4,800 μg
 Detection: UV @ 280 nm

Flow Rate: 17.06 mL/min
 Gradient:

Time (min)	%A	%B
0.0	85	15
3.02	85	15
4.02	70	30
14.02	30	70
16.02	30	70

 Injection Volume: 1200 μL



Thiols

LC Conditions:

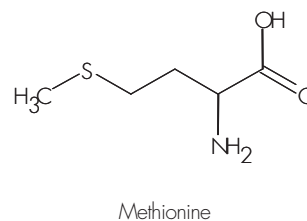
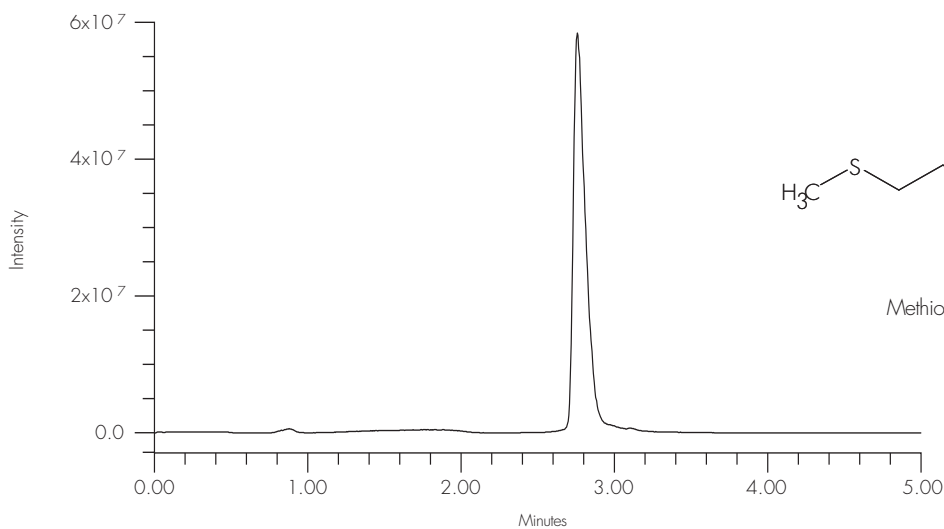
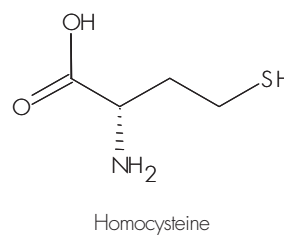
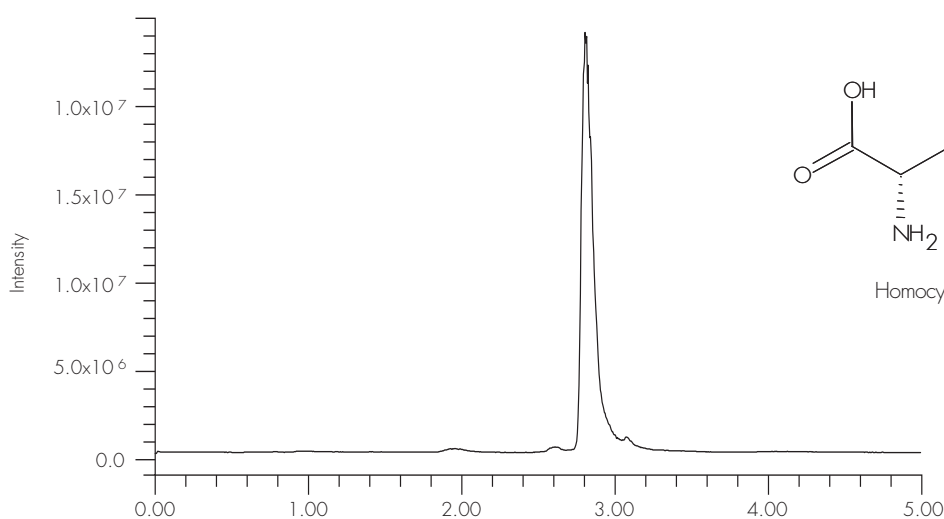
Column: Atlantis™ HILIC Silica 2.1 x 50 mm, 3 μm
 Part Number: 186002011
 Mobile Phase A: H₂O
 Mobile Phase B: ACN
 Mobile Phase C: 200 mM NH₄COOH, pH 3.0
 Flow Rate: 0.3 mL/min
 Gradient:

Time (min)	Profile		
	%A	%B	%C
0.0	0	95	5
5.0	45	50	5

Injection Volume: 5.0 μL
 Sample Diluent: 75:25 ACN:MeOH with 0.2% HCOOH
 Sample Concentration: 50 μg/mL
 Temperature: Ambient
 Instrument: Alliance® HT 2795 with Waters ZQ™

MS Conditions:

Ionization Mode: ES+
 Capillary (kV): 3.0
 Cone (V): 20
 Extractor: 3 V
 RF Lens: 0.3 V
 Source Temperature (°C): 150
 Desolvation Temperature (°C): 350
 Cone Gas Flow (L/Hr): 50
 Desolvation Gas Flow (L/Hr): 700
 SIR m/z: 136.1 (Homocysteine)
 150.2 (Methionine)



LC Conditions:

Column: Atlantis™ dC₁₈ 4.6 x 50 mm, 3 μm

Part Number: 186001329

Mobile Phase A: H₂O

Mobile Phase B: ACN

Mobile Phase C: 100 mM HCOONH₄, pH 3.0

Flow rate: 2.0 mL/min

Gradient:	Time (min)	Profile		
		%A	%B	%C
	0.0	90	0	10
	5.0	78	12	10

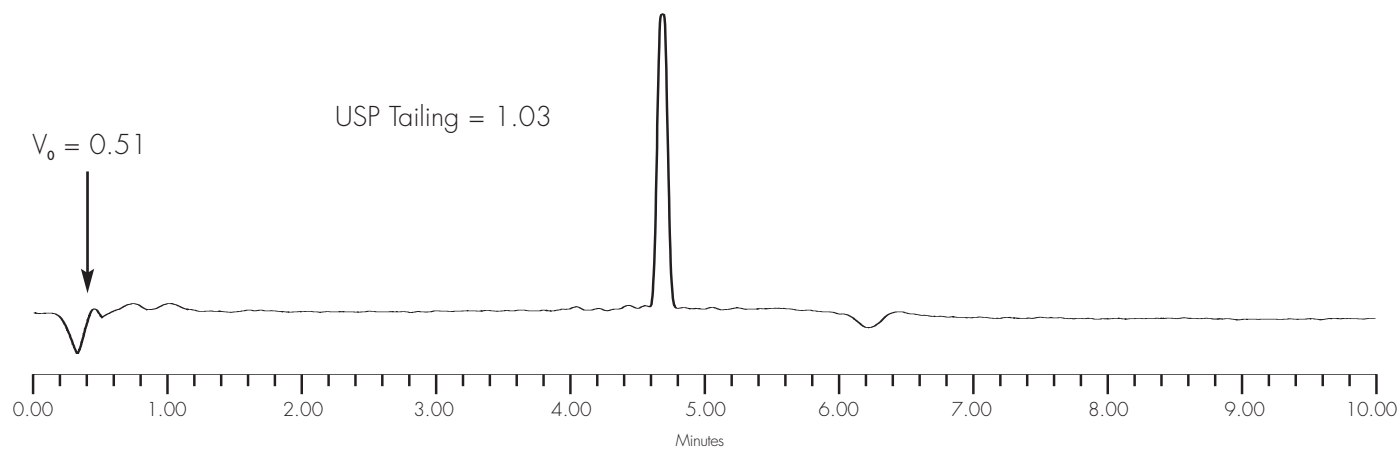
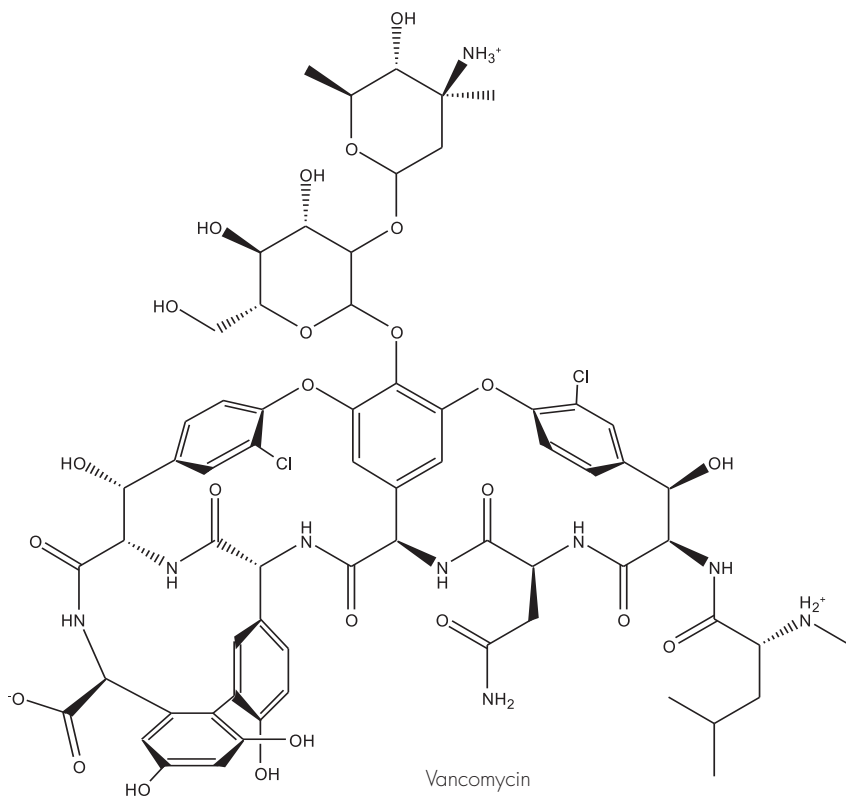
Injection Volume: 10 μL

Sample Concentration: 40 μg/mL

Temperature: 30° C

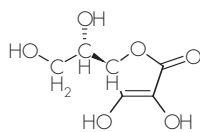
Detection: UV @ 254 nm

Instrument: Alliance® HT 2795 with 2996 PDA

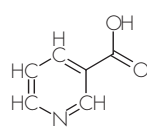


LC Conditions:

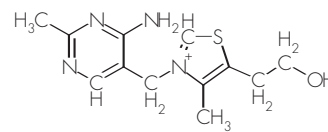
Column: Atlantis™ dC₁₈ 4.6 x 150 mm, 5 μm
 Part Number: 186001344
 Mobile Phase A: 0.1% TFA
 Mobile Phase B: ACN
 Flow Rate: 1.4 mL/min
 Gradient: Time Profile
 (min) %A %B
 0.0 100 0
 4.0 97 3
 6.0 85 15
 15.0 80 20
 Injection Volume: 10 μL
 Temperature: 30° C
 Detection: UV @ 260 nm
 Instrument: Alliance® 2695 with 2996 PDA



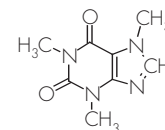
L-ascorbic acid
(Vitamin C)



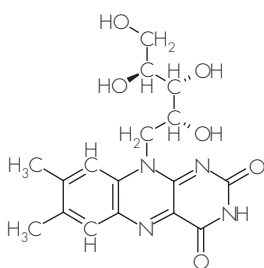
Nicotinic acid
(Niacin)



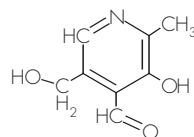
Thiamine
(Vitamin B1)



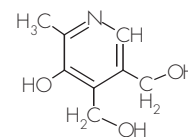
Caffeine



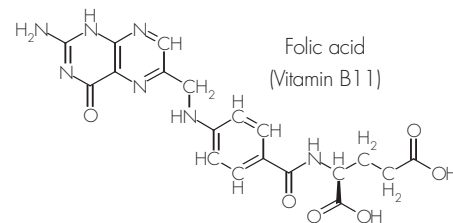
Riboflavin (Vitamin B2)



Pyridoxal



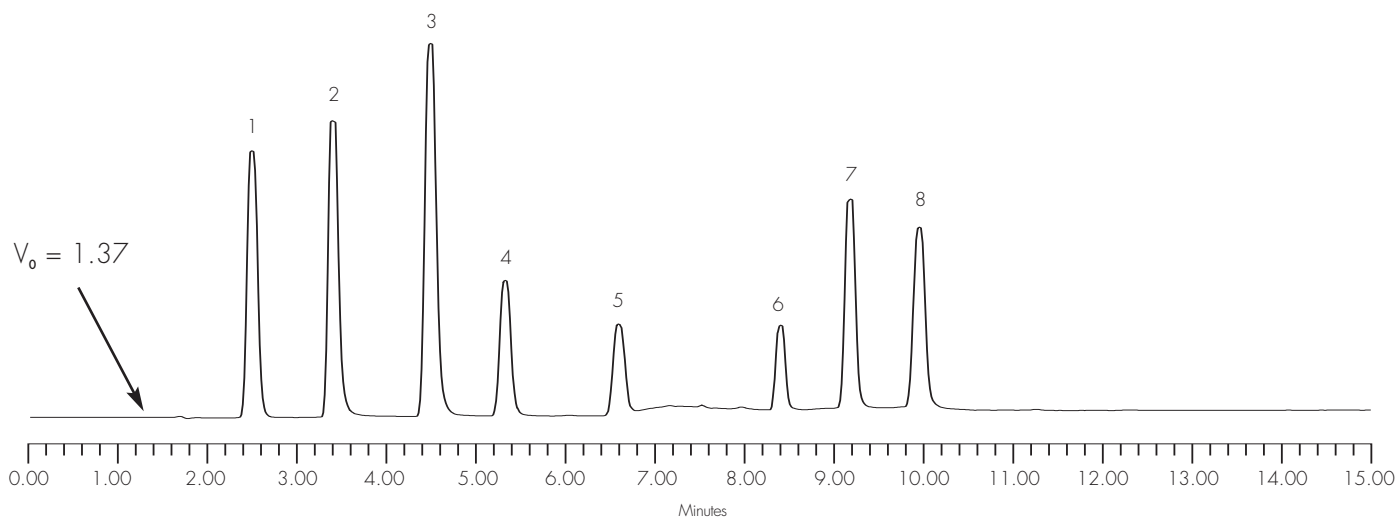
Pyridoxine



Folic acid
(Vitamin B11)

Compounds:

Compounds:	USP Tailing	Sample Concentrations (μg/mL)
1. L-ascorbic acid (vitamin C)	1.12	23.8
2. Nicotinic acid (niacin)	1.27	11.9
3. Thiamine (vitamin B1)	1.20	23.8
4. Pyridoxal	1.13	47.6
5. Pyridoxine	1.04	47.6
6. Folic acid (vitamin B11)	1.10	42.9
7. Caffeine	1.10	11.9
8. Riboflavin (vitamin B2)	1.14	6.0

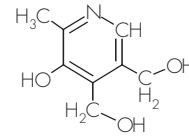


Water Soluble Vitamins— Analytical to Preparative Scale Up

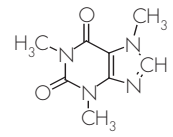
LC Conditions:

Mobile Phase A: 0.1% HCOOH in H₂O
 Mobile Phase B: ACN/1% HCOOH (90/10)
 Sample Concentration: 5, 25 mg/mL in water
 Temperature: Ambient
 Instrument: AutoPurification™ System
 Detection: UV @ 290 nm

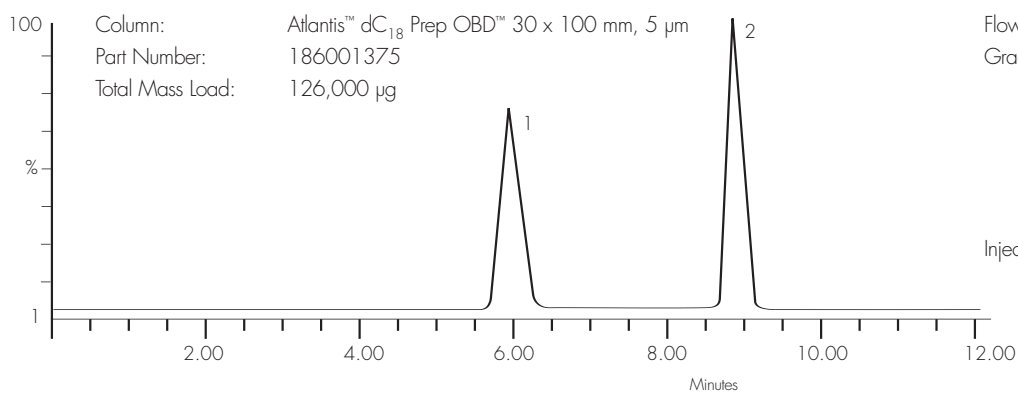
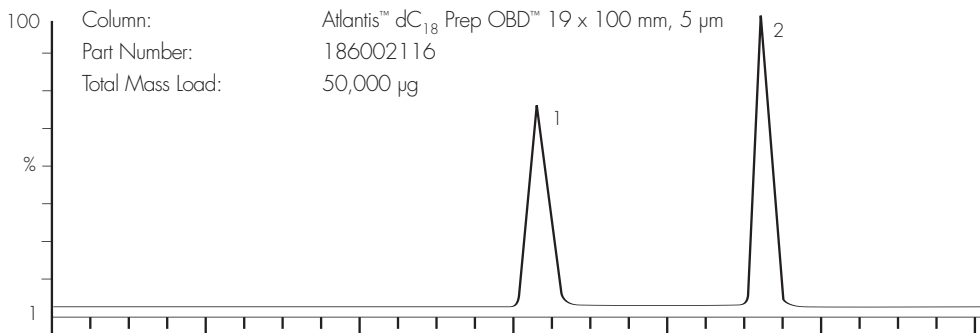
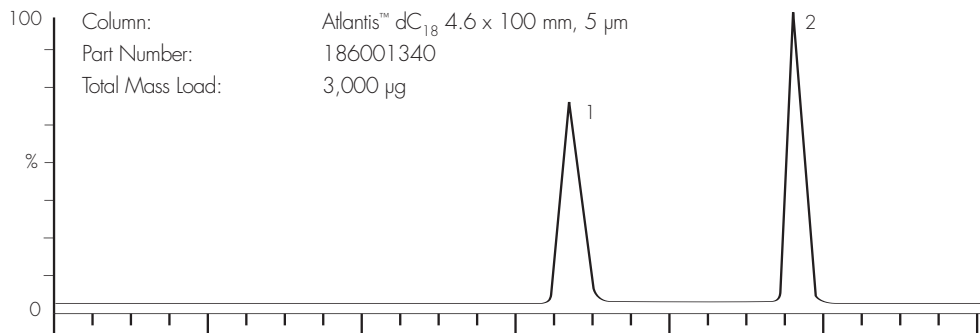
Compounds:
 1. Pyridoxine (5 mg/mL)
 2. Caffeine (25 mg/mL)



Pyridoxine



Caffeine



PLEASE READ CAREFULLY BEFORE USING ATLANTIS™ COLUMNS.

Thank you for choosing a Waters Atlantis™ column. The manufacture of Atlantis™ dC₁₈ and Atlantis™ HILIC Silica columns begins with ultrapure reagents to control the chemical composition and purity of the final product. Atlantis™ columns are manufactured in a cGMP, ISO 9002 certified plant with each step being conducted within narrow tolerances. Every column is individually tested and Certificates of Batch Analysis and a Performance Chromatogram are provided with each column.

Waters recommends the use of Waters Sentry™ guard columns to maximize column lifetime and protect the column from contaminants.

Connecting the Column or Cartridge to the HPLC

Column Connection

Handle the column with care. Do not drop or hit the column on a hard surface as it may disturb the bed and affect its performance.

1. Correct connection of 1/16 inch outer diameter stainless steel tubing leading to and from the column is essential for high-quality chromatographic results.
2. When using standard stainless steel compression screw fittings, it is important to ensure proper fit of the 1/16 inch outer diameter stainless steel tubing. When tightening or loosening the compression screw, place a 5/16 inch wrench on the compression screw and a 3/8 inch wrench on the hex head of the column endfitting.

Note: If one of the wrenches is placed on the column flat during this process, the endfitting will be loosened and leak.

3. If a leak occurs between the stainless steel compression screw fitting and the column endfitting, a new compression screw fitting, tubing and ferrule must be assembled.
4. An arrow on the column identification label indicates correct direction of solvent flow.

Cartridge Connection

Handle the cartridge with care. Do not drop or hit the cartridge on a hard surface as it may disturb the bed and affect its performance. Refer to Figure 1 for an exploded view of an Atlantis™ cartridge column with a Sentry™ guard column.

1. Unscrew end connectors from the old cartridge. Leave them connected to the inlet and outlet lines of the instrument.
2. Attach new cartridge column between connectors so that the direction of the flow arrow on the label is pointing in the direction of mobile phase flow (toward detector).
3. Fingertighten all fittings.

It is important to realize that extra column peak broadening can destroy a successful separation. The choice of appropriate column connectors and system tubing is discussed in detail below.

Column Connectors and System Tubing Considerations

Due to the absence of an industry standard, various column manufacturers have employed different types of chromatographic column connectors. The chromatographic performance of the separation can be negatively affected if the style of the column endfittings does not match the existing tubing ferrule setting. This section explains the differences between Waters style and Parker style ferrules and endfittings (Figure 2). Each endfitting style varies in the required length of the tubing protruding from the ferrule. The Atlantis™ column is equipped with Waters style endfittings, which require a 0.130 inch ferrule. If a non-Waters style column is presently being used, it is critical that ferrule depth be reset for optimal performance prior to installing an Atlantis™ column.

The presence of a void in the flow stream reduces column performance. This can occur if a Parker ferrule is connected to a Waters style endfitting (Figure 4).

Note: A void appears if tubing with a Parker ferrule is connected to a Waters style column.

Figure 1: Installation of Atlantis™ Cartridge Column with Sentry™ Guard Column

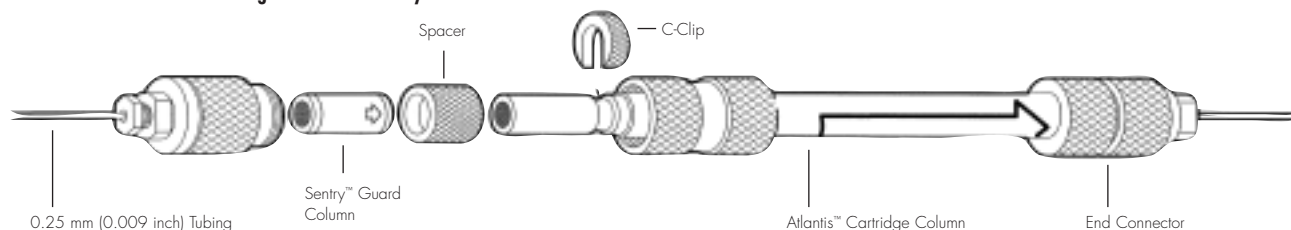


Figure 2: Waters and Parker Ferrule Types

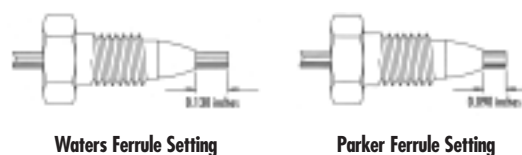


Figure 3: Proper Tubing/Column Connection

Tubing touches the bottom of the column endfitting, with no void between them.

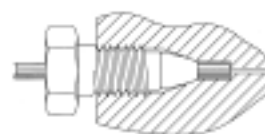
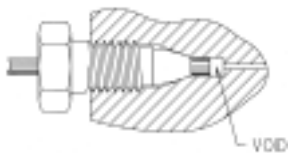


Figure 4: Parker Ferrule in a Waters Style Endfitting

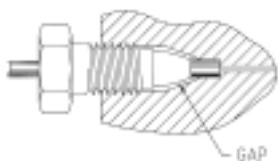


To fix this problem: Cut the end of the tubing with the ferrule, place a new ferrule on the tubing and make a new connection. Before tightening the screw, make sure that the tubing bottoms out in the endfitting of the column.

Conversely, if tubing with a Waters ferrule is connected to a column with Parker style endfitting, the end of the tubing will bottom out before the ferrule reaches its proper sealing position. This will leave a gap and create a leak (Figure 5).

Note: The connection leaks if a Waters ferrule is connected to a column with a Parker style endfitting.

Figure 5: Waters Ferrule in a Parker Style Endfitting



There are two ways to fix the problem:

1. Tighten the screw a bit more. The ferrule moves forward, and reaches the sealing surface. Do not overtighten since this may break the screw.
2. Cut the tubing, replace the ferrule and make a new connection.

Alternatively, replace the conventional compression screw fitting with an all-in-one PEEK fitting (Waters part number PSL613315) that allows resetting of the ferrule depth. Another approach is to use a Thermo Hypersil Keystone, Inc. SLIPFREE® connector to ensure the correct fit. The fingertight SLIPFREE® connectors automatically adjust to fit all compression screw type fittings without the use of tools (Figure 6).

Figure 6: Single and Double SLIPFREE® Connectors

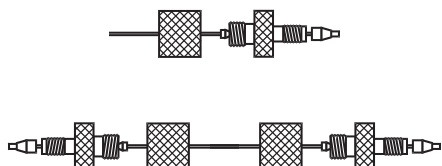


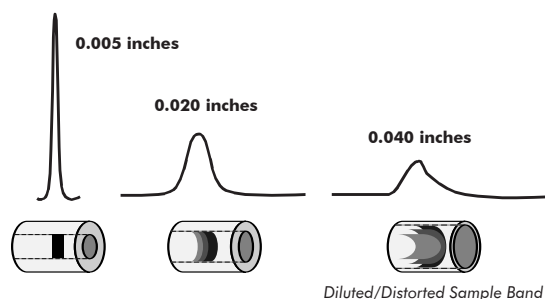
Table 1. Waters Part Numbers for SLIPFREE® Connectors

SLIPFREE® Type and Tubing Length	Tubing Internal Diameter		
	0.005"	0.010"	0.020"
Single 6 cm	PSL 618000	PSL 618006	PSL 618012
Single 10 cm	PSL 618002	PSL 618008	PSL 618014
Single 20 cm	PSL 618004	PSL 618010	PSL 618016
Double 6 cm	PSL 618001	PSL 618007	PSL 618013
Double 10 cm	PSL 618003	PSL 618009	PSL 618015
Double 20 cm	PSL 618005	PSL 618001	PSL 618017

Band Spreading Minimization

Internal tubing diameter influences system band spreading and peak shape. Larger tubing diameters cause excessive peak broadening and lower sensitivity (Figure 7).

Figure 7: Effect of Connecting Tubing on System



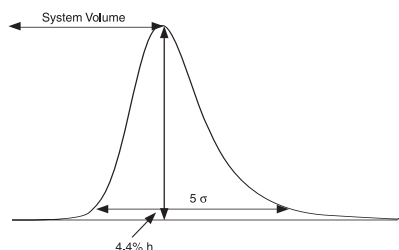
Measuring System Bandspread Volume

This test should be performed on an HPLC system with a single wavelength UV detector (not a Photodiode Array (PDA)).

1. Disconnect column from system and replace with a zero dead volume union.
2. Set flow rate to 1 mL/min.
3. Dilute a test mix in mobile phase to give a detector sensitivity 0.5 - 1.0 AUFS (system start up test mix can be used which contains uracil, ethyl and propyl parabens; Waters part number WAT034544).
4. Inject 2 to 5 µL of this solution.
5. Using 5 sigma method measure the peak width at 4.4% of peak height:

$$\begin{aligned} \text{Band Spread } (\mu\text{L}) &= \text{Peak Width (min)} \times \text{Flow Rate } (\mu\text{L}/\text{min}) \\ &= 0.1 \text{ min} \times 1000 \mu\text{L}/\text{min} \\ &= 100 \mu\text{L} \end{aligned}$$

Figure 8: Determination of System Bandspread Volume Using 5-Sigma Method



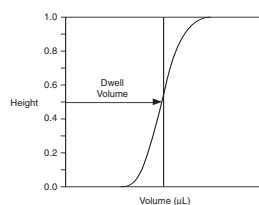
In a typical HPLC system, the Bandspread Volume should be $100 \mu\text{L} \pm 30 \mu\text{L}$.

In a microbore (≤ 2.1 mm i.d.) system, the Bandspread Volume should be no greater than 20 to $40 \mu\text{L}$.

Measuring Gradient Delay Volume

1. Replace the column with a zero dead volume union.
2. Prepare eluent A (pure solvent, such as methanol) and eluent B (solvent plus sample, such as 5.6 mg/mL propylparaben in methanol).
3. Equilibrate the system with eluent A until a stable baseline is achieved.
4. Switch to 100% eluent B.
5. Record the half height of the step and determine dwell volume (Figure 9).

Figure 9: Determination of Dwell Volume



The dwell volume should be less than 1 mL. If the dwell volume is greater than 1 mL, see **System Modification Recommendations** section on how to reduce system volume.

Use of Smaller i.d. Columns

A 3.0 mm i.d. narrow-bore column usually requires no system modifications. A 2.1 mm i.d. microbore column, however, requires modifications to the HPLC system to eliminate excessive system bandspread volume. Without proper system modifications, excessive system bandspread volume causes peak broadening and has a large impact on peak width as peak volume decreases.

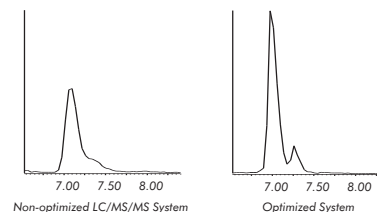
Impact of Bandspread Volume on 2.1 mm i.d. Column Performance

System with 70 μL bandspread:	10,000 plates
System with 130 μL bandspread:	8,000 plates (same column)

Note: Flow splitters after the column will introduce additional bandspreading.

System optimization, especially in a system that contains a flow splitter, can have dramatic effects on sensitivity and resolution. Optimization includes using correct-depth ferrules and minimizing tubing diameter and lengths. System optimization results in a doubling of sensitivity and resolution of the metabolite in an LC/MS/MS system (Figure 10).

Figure 10: Non-Optimized vs. Optimized LC/MS/MS System



System Modification Recommendations

1. Use a microbore detector flowcell with ≤ 2.1 mm i.d. columns.
Note: Detector sensitivity is reduced with the shorter flowcell pathlength in order to achieve lower bandspread volume.
2. Minimize injector sample loop volume.
3. Use 0.009 inch (0.25 mm) tubing between pump and injector.
4. Use 0.009 inch (0.25 mm) tubing for rest of connections in standard systems and 0.005 inch (0.12 mm) tubing for narrowbore (≤ 2.1 mm i.d.) systems.
5. Use perfect (pre-cut) connections (with a variable depth inlet if using columns from different suppliers).
6. Detector time constants should be shortened to less than 0.2 seconds.

Waters Small Particle Size (3 μm) Columns – Fast Chromatography

Waters columns with 3 μm particles provide faster and more efficient separations without sacrificing column lifetime. This section describes five parameters to consider when performing separations with columns containing 3 μm particles.

Note: Columns that contain 3 μm particles have smaller outlet frits to retain packing material. These columns should not be backflushed.

1. **Flow Rate** — Compared to columns with 5 μm particles, columns with 3 μm particles have higher optimum flow rates and are used when high efficiency and short analysis times are required. These higher flow rates, however, lead to increased backpressure.
Note: Use a flow rate that is practical for your system.
2. **Backpressure** — Backpressures for columns with 3 μm particles are higher than for 5 μm columns with the same dimensions. Waters suggests using a shorter column to compensate for increased backpressure and to obtain a shorter analysis time.
3. **Temperature** — Use a higher temperature to reduce backpressure caused by smaller particle sizes. The recommended temperature range for Atlantis™ columns is 20°C to 45°C. See **Column Usage** section for a discussion of elevated temperature use with Atlantis™ columns.
4. **Sampling Rate** — Use a sampling rate of about 10 points per second.
5. **Detector Time Constant** — Use a time constant of 0.1 seconds for fast analyses.

Column Performance Validation

Each Atlantis™ column comes with Certificates of Batch Analysis and a Performance Test Chromatogram. The Certificates of Analysis are specific to each batch of packing material and includes the gel lot batch number, analysis of unbonded particles, analysis of bonded particles (Atlantis™ dC₁₈), and chromatographic results and conditions. The Performance Test Chromatogram is specific to each individual column and contains information such as gel lot batch number, column serial number, USP plate count, USP tailing factor, capacity factor and chromatographic results and conditions.

These data should be stored for future reference.

Initial Column Efficiency Determination

1. Perform an efficiency test on the column before using it. Waters recommends using a suitable solute mixture, as found in the "Performance Test Chromatogram", to analyze the column upon receipt.
2. Determine the number of theoretical plates (N) and use for periodic comparison.
3. Repeat the test periodically to track column performance over time. Slight variations may be obtained on two different HPLC systems due to the quality of the connections, operating environment, system electronics, reagent quality, column condition and operator technique.

Column Equilibration

Atlantis™ columns are packed and shipped in 100% acetonitrile. It is important to ensure solvent compatibility before changing to a new solvent.

Atlantis™ dC₁₈ - Equilibrate with a minimum of 10 column volumes of the mobile phase to be used (refer to Table 2 for a listing of standard column volumes).

Atlantis™ HILIC Silica — Upon receipt, equilibrate with 50 column volumes of 50:50 acetonitrile:water with 10 mM final buffer concentration (refer to Table 2 for a listing of standard column volumes). Prior to the first injection, equilibrate with 20 column volumes of initial mobile phase conditions. See **HILIC Getting Started** section for additional information.

Table 2. Standard column volumes in mL (multiply by 10 for flush solvent volumes)

Column Length	Column Volume (mL)									
	Column internal diameter (mm)									
	1.0	2.1	3.0	3.9	4.6	7.8	10	19	30	50
15 mm	—	0.1	—	—	—	—	—	—	—	—
20 mm	—	0.1	0.1	—	0.3	—	—	—	—	—
30 mm	—	0.1	0.2	—	0.5	—	2.4	8	—	—
50 mm	0.1	0.2	0.3	—	0.8	2.4	4	14	35	98
100 mm	0.1	0.4	0.7	1.2	1.7	5	8	28	70	—
150 mm	0.1	0.5	1.0	1.8	2.5	7	12	42	106	294
250 mm	—	0.9	1.8	—	4	—	20	70	176	490
300 mm	—	—	—	—	—	14	24	85	212	589

Column Installation Procedure

*Note: The flow rates given in the procedure below are for a typical 4.6 mm i.d. column. Scale the flow rate up or down accordingly based upon the column i.d., length, particle size and backpressure of the Atlantis™ column being installed. See **Scaling Up/Down** section for calculating flow rates when changing column i.d. and/or length.*

1. Purge the pumping system and connect the inlet end of the column to the injector outlet.
2. Set the pump flow to 0.1 mL/min. and increase to 1 mL/min over 5 minutes.
3. When the mobile phase is flowing freely from the column outlet, attach the column to the detector. This prevents entry of air into the detector and provides more rapid baseline equilibration.
4. When the mobile phase is changed, gradually increase the flow rate of the new mobile phase from 0.0 mL/min to 1.0 mL/min in 0.1 mL/min increments.
5. Once a steady backpressure and baseline have been achieved, the column is ready to be used (or equilibrated).

*Note: If mobile phase additives are present in low concentrations (e.g., ion-pairing reagents), 100 to 200 column volumes may be required for complete equilibration. In addition, mobile phases that contain formate (e.g., ammonium formate, formic acid, etc.) may also require slightly longer initial column equilibration times. Please see additional equilibration information for Atlantis™ HILIC Silica columns in the **HILIC Getting Started** section.*

Column Usage

To ensure the continued high performance of Atlantis™ columns and cartridges, follow these guidelines:

Guard columns

Use a Waters Sentry™ guard cartridge of matching chemistry and particle size between the injector and main column. It is important to use a high-performance matching guard column to protect the main column while not compromising or changing analytical resolution.

Sample Preparation

1. Sample impurities often contribute to column contamination. Use Waters Oasis® or Sep-Pak® solid-phase extraction cartridges/columns of the appropriate chemistry to clean up the sample before analysis.
2. For reversed-phase separations (Atlantis™ dC₁₈) prepare the sample in mobile phase or a solvent that is weaker (less organic modifier) than the mobile phase. For Hydrophilic Interaction Chromatography (HILIC) separations (Atlantis™ HILIC Silica), the samples must be prepared in 100% organic solvents (e.g., acetonitrile). See **HILIC Getting Started** section for additional information.
3. If the sample is not dissolved in the mobile phase, ensure that the sample, solvent and mobile phases are miscible in order to avoid sample and/or buffer precipitation.
4. Filter sample through a 0.2 µm membrane to remove particulates. If the sample is dissolved in a solvent that contains an organic modifier (e.g., acetonitrile, methanol, etc.) ensure that the membrane material does not dissolve in the solvent. Contact the membrane manufacturer with solvent compatibility questions.

pH Range

Atlantis™ dC₁₈ - The pH range for Atlantis™ dC₁₈ columns is 2 to 7.

Atlantis™ HILIC Silica - The pH range for Atlantis™ HILIC Silica columns is 1 to 6.

Column lifetime will vary depending upon the temperature, type and concentration of buffer used. A listing of recommended and non-recommended buffers is given in Table 3. Please use this as a guideline when developing methods.

Note: Operating at the upper end of the pH range in combination with elevated temperatures will lead to shorter column lifetime.

Table 3: Buffer recommendations for using Atlantis™ columns from pH 1 to 7

Additive or Buffer	pKa	Buffer range (±1 pH unit)	Volatility	Used for Mass Spec?	Comments
TFA	0.3		Volatile	Yes	Ion pair additive, can suppress MS signal. Used in the 0.01-0.1% range.
Formic Acid	3.75		Volatile	Yes	Maximum buffering obtained when used with Ammonium Formate salt. Used in 0.1-1.0% range.
Acetic Acid	4.76		Volatile	Yes	Maximum buffering obtained when used with Ammonium Acetate salt. Used in 0.1-1.0% range.
Formate (NH ₄ COOH)	3.75	2.75 – 4.75	Volatile	Yes	Used in the 1-10mM range. Note: sodium or potassium salts are not volatile.
Acetate (NH ₄ CH ₃ COOH)	4.76	3.76 – 5.76	Volatile	Yes	Used in the 1-10mM range. Note: sodium or potassium salts are not volatile.
Phosphate 1	2.15	1.15 – 3.15	Non-volatile	No	Traditional low pH buffer, good UV transparency. Not recommended for HILIC
Phosphate 2	7.2	6.20 – 8.20	Non-volatile	No	Above pH 7, reduce temperature/concentration and use guard column to maximize lifetime. Not recommended for HILIC

* Phosphate salt buffers are not recommended for HILIC (phosphoric acid is OK).

Solvents

To maintain maximum column performance, use high quality chromatography grade solvents. Filter all aqueous buffers prior to use. Pall Gelman Laboratory Acrodisc® filters are recommended. Solvents containing suspended particulate materials will generally clog the outside surface of the inlet distribution frit of the column. This will result in higher operating pressure and poorer performance.

Degas all solvents thoroughly before use to prevent bubble formation in the pump and detector. The use of an on-line degassing unit is also recommended. This is especially important when running low pressure gradients since bubble formation can occur as a result of aqueous and organic solvent mixing during the gradient.

Pressure

Atlantis™ columns can tolerate pressures of up to 6,000 psi (400 bar or 40 Mpa) although pressures greater than 4,000 – 5,000 psi should be avoided in order to maximize column and system lifetimes.

Temperature

Temperatures between 20°C – 45°C are recommended for operating Waters Atlantis™ columns in order to enhance selectivity, lower solvent viscosity and increase mass transfer rates. However, any temperature rise above ambient will have a negative effect on lifetime which will vary depending on the pH and buffer conditions used.

Scaling Up/Down Isocratic Methods

The following formulas will allow scale up or scale down, while maintaining the same linear velocity (retention time), and provide new sample loading values:

$$\begin{aligned} \text{If column i.d. and length are altered:} \quad & F_2 = F_1 (r_2/r_1)^2 \\ & \text{or} \quad \text{Load}_2 = \text{Load}_1 (r_2/r_1)^2 (L_2/L_1) \\ & \text{or} \quad \ln j \text{ vol}_1 = \ln j \text{ vol}_2 (r_2/r_1)^2 (L_2/L_1) \end{aligned}$$

Where: r = Radius of the column, in mm

F = Flow rate, in mL/min

L = Length of column, in mm

1 = Original, or reference column

2 = New column

HILIC Getting Started

Equilibration of Atlantis™ HILIC Silica Columns

1. Upon receipt, equilibrate in 50% acetonitrile/50% aqueous buffer (10 mM final buffer concentration) for 50 column volumes.
 2. Prior to first injection, equilibrate with 20 column volumes of initial mobile phase conditions.
 3. When running gradients, equilibrate with 10 column volumes between injections.
- Failure to appropriately equilibrate the column could result in drifting retention times.*

HILIC Mobile Phase Considerations

1. Always maintain at least 5% polar solvent in the mobile phase or gradient (e.g., 5% water, 5% methanol or 3% methanol/2% aqueous buffer, etc.). This ensures that the Atlantis™ HILIC Silica particle is always hydrated.
2. Maintain at least 40% organic solvent (e.g., acetonitrile) in your mobile phase or gradient.
3. Avoid phosphate salt buffers to avoid precipitation in HILIC mobile phases (phosphoric acid is OK).
4. Buffers such as ammonium formate or ammonium acetate will produce more reproducible results than additives such as formic acid or acetic acid. If an additive (e.g., formic acid) must be used instead of a buffer, use 0.2% (v:v) instead of 0.1%.
5. For best peak shape, maintain a buffer concentration of 10 mM in your mobile phase/gradient at all times.

Injection Solvents for HILIC

1. If possible, injection solvents should be 100% organic solvent. Water must be eliminated or minimized. Choose weak HILIC solvents such as acetonitrile, isopropanol, methanol, etc.
2. A generic injection solvent is 75:25 acetonitrile:methanol. This is a good compromise between analyte solubility and peak shape.
3. Avoid water and dimethylsulfoxide (DMSO) as injection solvents. These solvents will produce very poor peak shapes.

4. Exchange water or DMSO with acetonitrile by using reversed-phase solid phase extraction. If this is not possible, dilute the water or DMSO with organic solvent.

Additional HILIC Recommendations

1. For initial scouting conditions, run a gradient from 95% acetonitrile to 50% acetonitrile. If no retention occurs, run isocratically with 95:3:2 acetonitrile:methanol:aqueous buffer.
2. Alternate polar solvents such as methanol, acetone or isopropanol can also be used in place of water in the mobile phase to increase retention.
3. Be sure that your needle wash solvent/purge solvent contains the same high organic solvent concentration as your mobile phase, else peak shapes will suffer.

Column Cleaning, Regenerating and Storage

Cleaning and Regeneration

A sudden increase in pressure or shift in retention or resolution may indicate contamination of the column.

Atlantis™ dC₁₈ – Flush with a neat organic solvent to remove the non-polar contaminant(s). If this flushing procedure does not solve the problem, purge the column with a sequence of progressively more non-polar solvents. For example, switch from water to tetrahydrofuran to methylene chloride. Return to the standard mobile phase conditions by reversing the sequence.

Atlantis™ HILIC Silica – Flush with 50:50 acetonitrile:water to remove the polar contaminant(s). If this flushing procedure does not solve the problem, purge the column with 5:95 acetonitrile:water.

Guard columns require replacement at regular intervals as determined by sample contamination. When system backpressure increases above a set pressure limit, it is usually an indication that the guard column should be replaced. A sudden appearance of split peaks is also indicative of a need to replace the guard column.

Storage

Atlantis™ dC₁₈ – For periods longer than four days, store the column in 100% acetonitrile. Do not store columns in buffered eluents. If the mobile phase contained a buffer salt, flush the column with 10 column volumes of HPLC grade water (see Table 2 for common column volumes) and replace with 100% acetonitrile for storage. Failure to perform this intermediate step could result in precipitation of the buffer salt in the column when 100% acetonitrile is introduced.

Atlantis™ HILIC Silica – For periods longer than four days, store the column in 95:5 acetonitrile: water. Do not store columns in buffered eluents. If the mobile phase contained a buffer salt, flush the column with 10 column volumes of 95:5 acetonitrile:water (see Table 2 for common column volumes) prior to storage.

Completely seal column to avoid evaporation and drying out of the bed.

Note: If a column has been run with a formate-containing mobile phase (e.g., ammonium formate, formic acid, etc.) and is flushed to remove the buffer, slightly longer equilibration times may be required after the column is re-installed and run again with a formate-containing mobile phase.

Troubleshooting

Changes in retention time, resolution, or backpressure are often due to column contamination. See the **Column Cleaning, Regeneration and Storage** section of this Care and Use Manual. Information on column troubleshooting problems may be found in *HPLC Columns Theory, Technology and Practice*, U.D. Neue, (Wiley-VCH, 1997) or the Waters HPLC Troubleshooting Guide (Literature Code 720000181EN).

Atlantis™ dC₁₈ Analytical Columns

Hardware Type	Dimensions	Partide Size	Part No.
NanoEase™	0.075 x 50 mm	3 µm	186002194
NanoEase™	0.075 x 100 mm	3 µm	186002195
NanoEase™	0.075 x 150 mm	3 µm	186002197
NanoEase™	0.100 x 50 mm	3 µm	186002207
NanoEase™	0.100 x 100 mm	3 µm	186002208
NanoEase™	0.100 x 150 mm	3 µm	186002209
NanoEase™	0.150 x 50 mm	3 µm	186002466
NanoEase™	0.150 x 100 mm	3 µm	186002467
NanoEase™	0.150 x 150 mm	3 µm	186002468
Column	0.320 x 50 mm	3 µm	186002304
Column	0.320 x 100 mm	3 µm	186002305
Column	0.320 x 150 mm	3 µm	186002306
Column	1.0 x 50 mm	3 µm	186001279
Column	1.0 x 50 mm	5 µm	186001281
Column	1.0 x 150 mm	3 µm	186001283
Column	1.0 x 150 mm	5 µm	186001285
Guard	2.1 x 10 mm	3 µm	186001377 ¹
Guard	2.1 x 10 mm	5 µm	186001379 ¹
Direct Connect	2.1 x 15 mm	3 µm	186002064
Direct Connect	2.1 x 15 mm	5 µm	186002065
Guard	2.1 x 20 mm	3 µm	186001381 ²
Guard	2.1 x 20 mm	5 µm	186001383 ³
1/5" Column	2.1 x 20 mm	3 µm	186002058
1/5" Column	2.1 x 20 mm	5 µm	186002059
Column	2.1 x 30 mm	3 µm	186001287
Column	2.1 x 30 mm	5 µm	186001289
Column	2.1 x 50 mm	3 µm	186001291
Column	2.1 x 50 mm	5 µm	186001293
Column	2.1 x 100 mm	3 µm	186001295
Column	2.1 x 100 mm	5 µm	186001297
Column	2.1 x 150 mm	3 µm	186001299
Column	2.1 x 150 mm	5 µm	186001301
1/5" Column	3.0 x 20 mm	3 µm	186002060
1/5" Column	3.0 x 20 mm	5 µm	186002061

Atlantis™ dC₁₈ Preparative Columns

Hardware Type	Dimensions	Partide Size	Part No.
Guard	10 x 10 mm	5 µm	186002300 ³
Guard	10 x 10 mm	10 µm	186002452 ²
Column	10 x 50 mm	5 µm	186002298
Column	10 x 100 mm	5 µm	186002299
Column	10 x 150 mm	10 µm	186002453
Column	10 x 250 mm	10 µm	186002454
Guard	19 x 10 mm	5 µm	186001361 ⁴
Guard	19 x 10 mm	10 µm	186001363 ⁴
Column	19 x 50 mm	5 µm	186001365
Column	19 x 100 mm	5 µm	186001367
Column	19 x 150 mm	10 µm	186001369
Column	19 x 250 mm	10 µm	186001371
Column	30 x 50 mm	5 µm	186001373
Column	30 x 100 mm	5 µm	186001375
Column	30 x 150 mm	10 µm	186002417
Column	30 x 250 mm	10 µm	186002418

Atlantis™ dC₁₈ Analytical Columns

Hardware Type	Dimensions	Partide Size	Part No.
Column	3.0 x 50 mm	3 µm	186001389
Column	3.0 x 50 mm	5 µm	186001391
Column	3.0 x 100 mm	3 µm	186001303
Column	3.0 x 100 mm	5 µm	186001305
Column	3.0 x 150 mm	3 µm	186001307
Column	3.0 x 150 mm	5 µm	186001309
Column	3.0 x 250 mm	5 µm	186001311
Guard	3.9 x 20 mm	3 µm	186001313 ³
Guard	3.9 x 20 mm	5 µm	186001315 ³
Cartridge Column	3.9 x 50 mm	3 µm	186001385 ⁴
Cartridge Column	3.9 x 50 mm	5 µm	186001387 ⁴
Column	3.9 x 100 mm	3 µm	186001393
Column	3.9 x 100 mm	5 µm	186001395
Column	3.9 x 150 mm	3 µm	186001317
Column	3.9 x 150 mm	5 µm	186001319
Guard	4.6 x 20 mm	3 µm	186001321 ³
Guard	4.6 x 20 mm	5 µm	186001323 ³
1/5" Column	4.6 x 20 mm	3 µm	186002062
1/5" Column	4.6 x 20 mm	5 µm	186002063
Column	4.6 x 30 mm	3 µm	186001325
Column	4.6 x 30 mm	5 µm	186001327
Column	4.6 x 50 mm	3 µm	186001329
Column	4.6 x 50 mm	5 µm	186001331
Column	4.6 x 75 mm	3 µm	186001333
Column	4.6 x 75 mm	5 µm	186001335
Column	4.6 x 100 mm	3 µm	186001337
Column	4.6 x 100 mm	5 µm	186001340
Column	4.6 x 150 mm	3 µm	186001342
Column	4.6 x 150 mm	5 µm	186001344
Column	4.6 x 250 mm	5 µm	186001346

¹ Requires Sentry Guard Holder WAT097958

² Requires Sentry Guard Holder 186000262

³ Requires Sentry Guard Holder WAT046910

⁴ Requires Cartridge End Fittings WAT037525

⁵ Requires Cartridge Guard Holder 289000779

⁶ Requires Cartridge Guard Holder 186000709

Atlantis™ dC₁₈ Method Validation Kits

Dimensions	Partide Size	Part No.
4.6 x 150 mm	3 µm	186002312
4.6 x 150 mm	5 µm	186002311
4.6 x 250 mm	5 µm	186002313

Ordering Information— Atlantis™ HILIC Silica Columns

Atlantis™ HILIC Silica Analytical Columns

Hardware Type	Dimensions	Partide Size	Part No.
Column	1.0 x 50 mm	3 µm	186002003
Column	1.0 x 50 mm	5 µm	186002004
Guard	2.1 x 10 mm	3 µm	186002005 ¹
Guard	2.1 x 10 mm	5 µm	186002006 ¹
Direct Connect	2.1 x 15 mm	3 µm	186002007
Direct Connect	2.1 x 15 mm	5 µm	186002008
Column	2.1 x 30 mm	3 µm	186002009
Column	2.1 x 30 mm	5 µm	186002010
Column	2.1 x 50 mm	3 µm	186002011
Column	2.1 x 50 mm	5 µm	186002012
Column	2.1 x 100 mm	3 µm	186002013
Column	2.1 x 100 mm	5 µm	186002014
Column	2.1 x 150 mm	3 µm	186002015
Column	2.1 x 150 mm	5 µm	186002016
Column	3.0 x 50 mm	3 µm	186002017
Column	3.0 x 50 mm	5 µm	186002018
Column	3.0 x 100 mm	3 µm	186002019
Column	3.0 x 100 mm	5 µm	186002020
Guard	3.9 x 20 mm	3 µm	186002021 ³
Guard	3.9 x 20 mm	5 µm	186002022 ³
Guard	4.6 x 20 mm	3 µm	186002023 ³
Guard	4.6 x 20 mm	5 µm	186002024 ³
Column	4.6 x 30 mm	3 µm	186002025
Column	4.6 x 30 mm	5 µm	186002026
Column	4.6 x 50 mm	3 µm	186002027
Column	4.6 x 50 mm	5 µm	186002028
Column	4.6 x 100 mm	3 µm	186002029
Column	4.6 x 100 mm	5 µm	186002030
Column	4.6 x 150 mm	3 µm	186002031
Column	4.6 x 150 mm	5 µm	186002032
Column	4.6 x 250 mm	5 µm	186002033

Atlantis™ HILIC Silica Method Validation Kits

Dimensions	Partide Size	Part No.
4.6 x 150 mm	3 µm	186002315
4.6 x 150 mm	5 µm	186002314
4.6 x 250 mm	5 µm	186002316

- ¹ Requires Sentry Guard Holder WAT097958
- ² Requires Sentry Guard Holder 186000262
- ³ Requires Sentry Guard Holder WAT046910
- ⁴ Requires Cartridge End Fittings WAT037525
- ⁵ Requires Cartridge Guard Holder 289000779
- ⁶ Requires Cartridge Guard Holder 186000709



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The quality management system of Waters' manufacturing facilities in Taunton, Massachusetts and Wexford, Ireland complies with the International Standard ISO 9002 Quality Management and Quality Assurance Standards. Waters' quality management system is periodically audited by the registering body to ensure compliance.

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