



THE Essential

CHROMATOGRAPHY & SPECTROSCOPY

CATALOG

20¹¹₁₂
EDITION

LC AND LC/MS APPLICATIONS
PAGES 1058-1127

The Measure of Confidence



Agilent Technologies

Hi-Plex Retention Times

Compound	Retention (mins)					
	Ca (0.6 mL/min, 85 °C)	Ca (Duo) (0.4 mL/min, 85 °C)	K (0.6 mL/min, 85 °C)	Pb (0.6 mL/min, 70 °C)	H (0.6 mL/min, 70 °C)	Na (Octo) (0.6 mL/min, 70 °C)
Adonitol (Ribitol)	15.20	14.01	10.30	20.88	1.5	11
Arabinose	13.69	13.74	12.65	16.72	11.4	12.42
Erythritol	15.51	14.72	10.962	20.66	12.7	11.71
Fructose	13.49	13.29	11.55	19.33	10.6	11.59
Fucose	13.76	13.72	12.51	16.72	12.2	12.26
Galactose	12.32	12.41	11.5	15.29	10.7	11.28
Glucose	11.11	11.44	10.85	13.28	9.95	10.57
Glycerol	16.11	15.54	11.77	19.6	14.1	12.66
Lactose	9.68	9.77	8.6	12.07	8.5	8.58
Maltose	9.34	9.59	8.54	11.66	8.4	8.5
Maltotriose	8.46	8.76	7.55	11.09	7.7	7.61
Mannitol	17.43	15.05	9.96	30.66	11	10.64
Mannose	12.58	12.71	11.86	20.02	10.5	11.39
Raffinose	8.46	8.66	7.31	10.38	*8.2	7.38
Rhamnose	12.73	12.72	11.23	17.97	11.6	10.96
Sorbitol	21.47	17.19	10.31	—	11.1	11.16
Stachyose	7.82	8.16	6.77	9.84	7.4	6.83
Sucrose	9.25	9.51	8.24	10.95	*9.8	8.37
Xylose	12.12	12.47	11.76	14.44	10.6	11.53
Melezitose	8.29	8.56	7.22	9.86	*8.33	7.27
Xylitol	20.29	17.52	10.94	42.71	11.87	11.91
Dulcitol	19.68	16.56	10.14	45.92	11.03	10.98
Arabitol	17.5	15.62	10.52	30.54	11.7	11.34
Maltitol	12.74	11.41	8.19	27.05	8.78	8.66

* denotes partial hydrolysis.

By using Hi-Plex H columns at higher operating temperatures, closely eluting compounds can be resolved. Acid catalyzed hydrolysis of some oligosaccharides may occur.

Organic Acid H	Retention (mins)
Acetic acid	15.8
Citric acid	9.1
Formic acid	14.9
Fumaric acid	16.1
Lactic acid	13.8
Malic acid	10.6
Oxalic acid	7.2
Pyruvic acid	10.3
Succinic acid	13.0
Tartaric acid	9.6

Retention times (in minutes) were recorded under the following conditions:

Column: Hi-Plex H
PL1170-6830
7.7 x 300 mm, 8 µm

Mobile Phase: 0.005 M H₂SO₄

Flow Rate: 0.6 mL/min

Temperature: 55°C

Detector: UV, 210 nm

BioPharmaceutical Applications

NEW!**Consistent Ion-Exchange MAb Separation**

Column: Bio MAb, PEEK
5190-2411
2.1 x 250 mm, 5 μ m

Buffer: A: Sodium phosphate buffer, 20 mM
B: Buffer A + 400 mM NaCl

Gradient: 15-35% Buffer B from 0-30 min

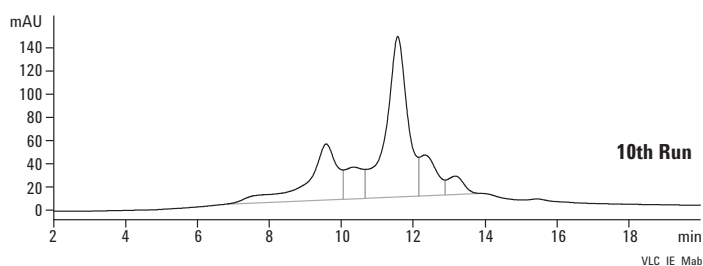
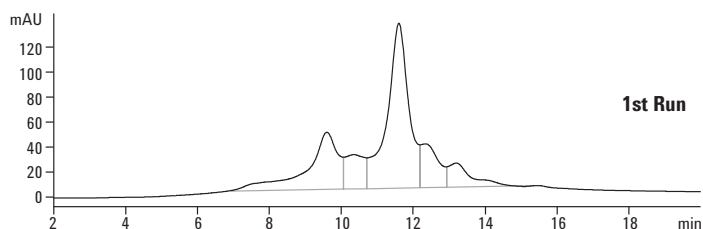
Flow Rate: 0.65 mL/min

Sample: CHO-humanized MAb, 1 mg/mL

Injection: 2.5 μ L

Detector: UV 220 nm

Temperature: Ambient

**NEW!****Intact MAb Monomer and Dimer Separation**

Column: Bio SEC-3, 300 \AA
5190-2511
7.8 x 300 mm, 3 μ m

Buffer: Sodium phosphate buffer, pH 7.0, 150 mM

Isocratic: 0-100% Buffer A from 0-30 min

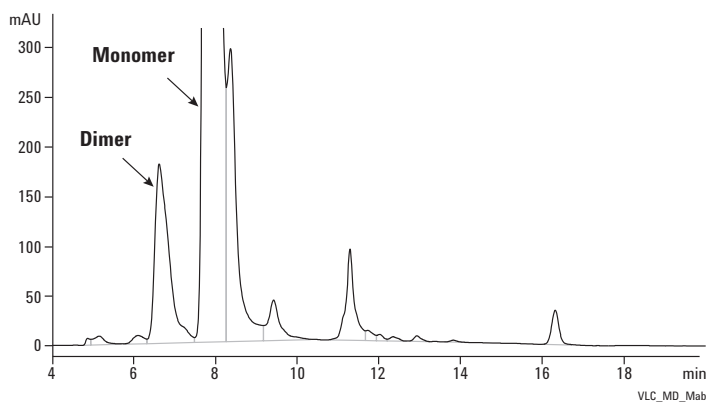
Flow Rate: 1.0 mL/min

Sample: CHO-humanized MAb, 5 mg/mL – intact

Injection: 5 μ L

Detector: UV 220 nm

Temperature: Ambient

**NEW!****MAb Separation of Heated, Stressed MAb**

Column: Bio SEC-3, 300 \AA
5190-2511
7.8 x 300 mm, 3 μ m

Buffer: Sodium phosphate buffer, pH 7.0,
150 mM +150 mM sodium sulfate

Isocratic: 0-100% Buffer A from 0-30 min

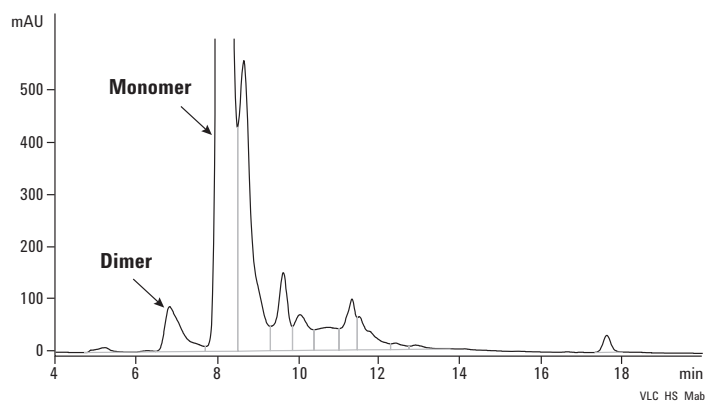
Flow Rate: 1.0 mL/min

Sample: CHO-humanized MAb, 5 mg/mL –
stressed at 60 $^{\circ}$ C

Injection: 5 μ L

Detector: UV 220 nm

Temperature: Ambient



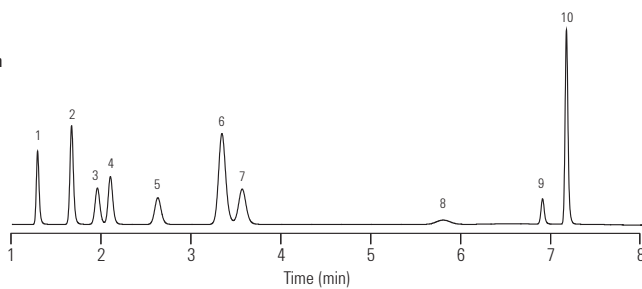
Nucleosides, Purines and Pyrimidines

Column: Eclipse Plus Phenyl Hexyl
959993-912
4.6 x 150 mm, 5 µm

Mobile Phase: 1% MeOH: 99% 20 mM Ammonium Acetate, pH 4.5

Flow Rate: 1 mL/min

Detector: UV 254 nm



1. Cytosine
2. Uracil
3. Cytidine
4. Guanine
5. Uridine
6. Adenine
7. Thymine
8. Guanosine
9. Thymidine
10. Adenosine

Amino Acid Standard Separation Eclipse Plus

Column: Eclipse Plus C18
959763-902
2.1 x 150 mm, 3.5 µm

Mobile Phase: A: 10 mM Na₂HPO₄, 10 mM Na₂B₄O₇, 0.5 mM NaN₃, pH 8.2
B: acetonitrile: methanol: water (45:45:10) (v/v/v)

Flow Rate: 0.42 mL/min

Temperature: 40°C

Detector: UV 338 nm, then switch to 280 nm at 15.7 min

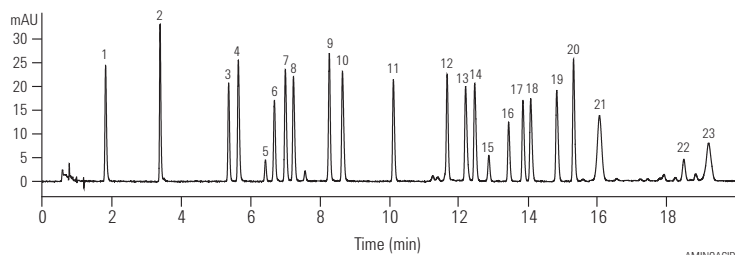
Sample: 900 µmol Amino Acids with extended Amino Acids and Internal Standards (500 µmol)

Derivatization: automated, online, OPA / FMOC

- | | | |
|--------|---------|---------|
| 1. ASP | 9. ARG | 17. PHE |
| 2. GLU | 10. ALA | 18. ILE |
| 3. ASN | 11. TYR | 19. LEU |
| 4. SER | 12. CY2 | 20. LYS |
| 5. GLN | 13. VAL | 21. HYP |
| 6. HIS | 14. MET | 22. SAR |
| 7. GLY | 15. NVA | 23. PRO |
| 8. THR | 16. TRP | |

Gradient

Time (min)	% B
0	2
0.5	2
20	57
20.1	100
23.5	100
23.6	2
25	stop



Antibodies: Fast Separation of IgM and IgG Antibodies

Column: ZORBAX GF-250
884973-701
4.6 x 250 mm, 4 µm

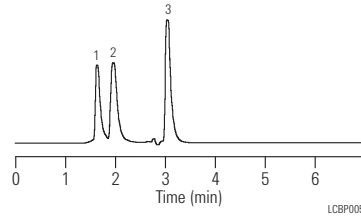
Mobile Phase: 200 mM Sodium Phosphate (pH 7), 0.01% Azide

Flow Rate: 0.94 mL/min

Temperature: Ambient

Detector: UV 230 nm

Sample: 2.5 µL (1mg/mL)



1. IgM, MOPC-104E
2. IgG2a, I HOPC-1
3. Buffer Solution

Glycosylated proteins: Large Molecules on Poroshell 300SB-C18 and 300SB-C8

Column A: Poroshell 300SB-C18
661750-902
1.0 x 75 mm, 5 µm

Column B: Poroshell 300SB-C8
661750-906
1.0 x 75 mm, 5 µm

Column C: ZORBAX 300SB-C18
865630-902
1.0 x 50 mm, 3.5 µm

Mobile Phase: A: 0.1% TFA in H₂O
B: 0.07% TFA in ACN

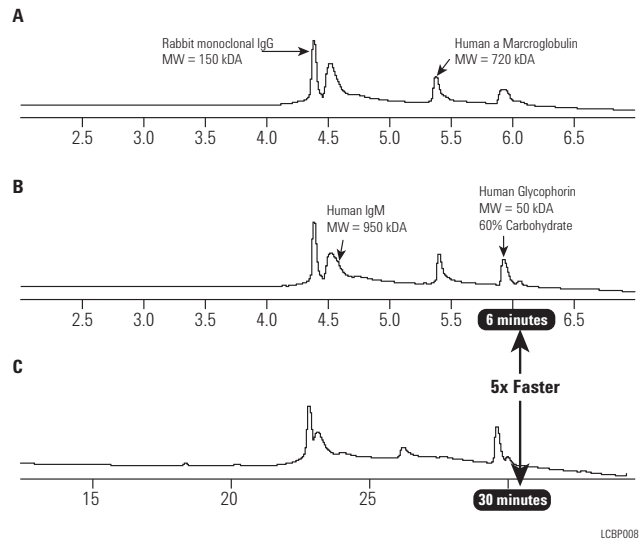
Flow Rate: A, B: 0.454 mL/min
C: 0.071 mL/min

Gradient: A, B: 0 min 5% B
10 min 100% B
C: 0 min 5% B
50 min 100% B

Temperature: 70°C

Detector: DAD 212 nm, 1.7 µL flow cell, <0.01 min peak width

Sample: Large Glycosylated Proteins



Courtesy of: Novartis Parma, Biotechnology, Basel, Dr. Kurt Forrer, Patrik Roethlisberger

HSA Tryptic Digest on ZORBAX Rapid Resolution HT 1.8 μm

Column A: ZORBAX SB-C18
883700-922
2.1 x 150 mm, 5 μm

Column B: ZORBAX SB-C18
822700-902
2.1 x 50 mm, 1.8 μm

Mobile Phase: A: Water w/0.1% TFA
B: ACN w/0.1% TFA

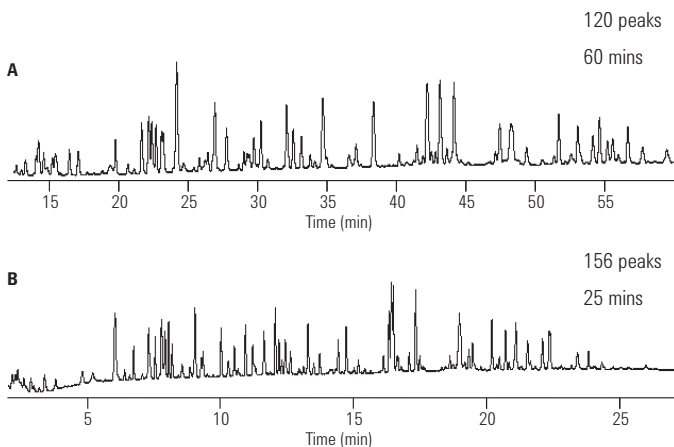
Flow Rate: A: 0.2 mL/min
B: 0.5mL/min

Gradient: A: 2 to 50% B in 70min
B: 2 to 50% B in 30min

Temperature: 50°C

Detector: UV 214 nm

Sample: HSA Tryptic Digest, 8 μL of 15 pmol / μL
(120 pmol on column)



LCBP013

Human Serum: Low Abundance Protein Isolation and Identification by LC/MS

Column: ZORBAX 300SB-C18
Trap: 0.3 x 5 mm, 5 μm , 5065-9913
Analytical: 0.3 x 150 mm, 5 μm , 5064-8263

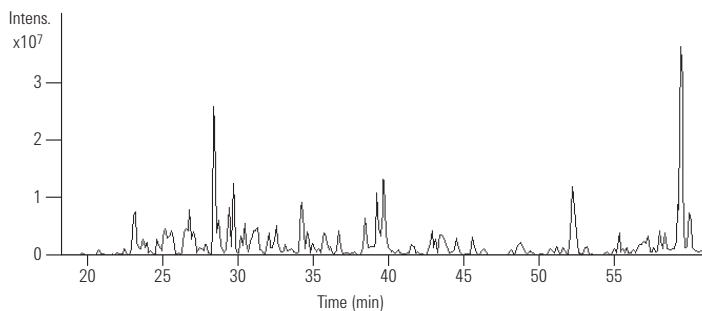
Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

Flow Rate: 6 $\mu\text{L}/\text{min}$

Gradient: 0 min 3% B
5 min 3% B (loading)
50 min 45% B
52 min 80% B
57 min 80% B
60 min 3% B

Sample: Band from 1-D in gel digest

Base Peak Chromatogram



LCBP014

Proteins Identified

1. Alpha-1-Antichymotrypsin
2. Antithrombin-III Precursor
3. Complement Factor B Precursor

Sample Preparation of Human Serum:
Major serum proteins removed using
Multiple Affinity Removal Column:
4.6 x 100 mm, P/N 5185-5985
Followed by 1-D gel digest

**Monoclonal IgG1 Chains:
Separation on Poroshell 300SB-C8**

Column: Poroshell 300SB-C8
660750-906
2.1 x 75 mm, 5 µm

Mobile Phase: A: 90% water: 10% ACN +
3 mL/L of MW 300 PEG
B: 10% water: 90% ACN +
3 mL/L of MW 300 PEG

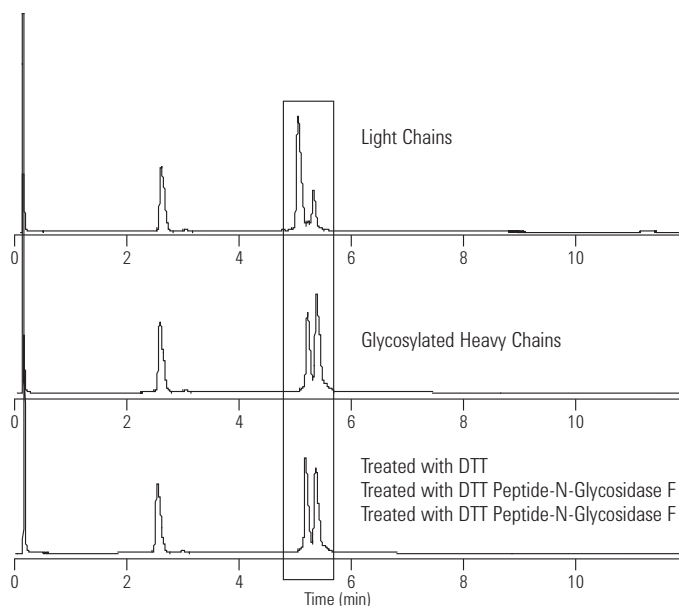
Flow Rate: 1.0 mL/min

Gradient: 0 min 25% B
10 min 40% B
10.1 min 25% B
12 min 25% B

Temperature: 70°C

Sample: Monoclonal IgG1

*Courtesy of: Novartis Pharma, Biotechnology, Basel,
Dr. Kurt Forrer, Patrik Roethlisberger*



LCBP015

**Use ZORBAX Extend-C18
for Alternate Selectivity at High pH**

Column: ZORBAX Extend-C18
773700-902
2.1 x 150 mm, 5 µm

Mobile Phase: A: 0.1% TFA in Water
B: 0.085% TFA in 80% ACN

A: 20 mM NH₄OH in Water
B: 20 mM NH₄OH in 80% ACN

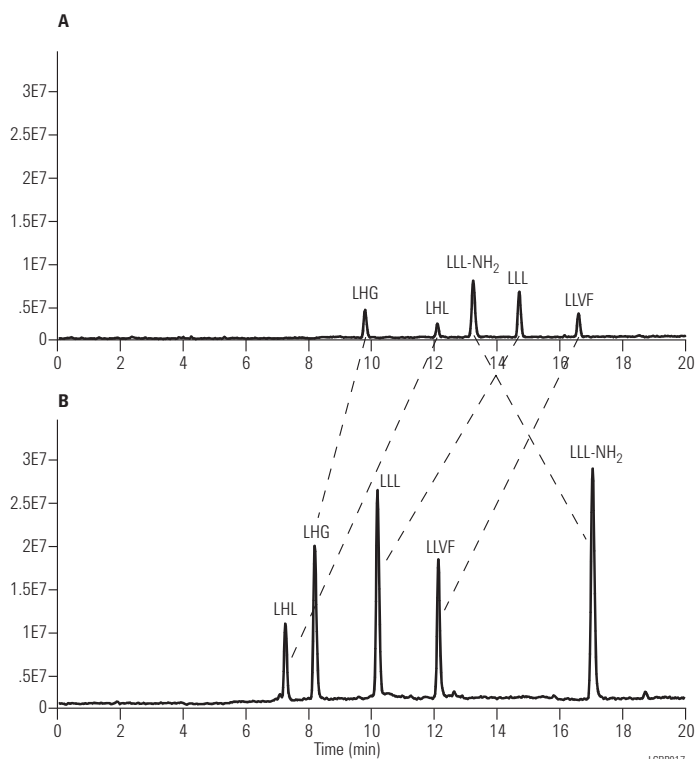
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25°C

MS Conditions: Pos. Ion ESI-Vf 70V, Vcap 4.5 kV
N₂ - 35 psi, 12 L/min, 300°C
4 µL (50 ng each peptide)

The Extend column can be used for high pH separations of peptides. At high and low pH, very different selectivity can result. Just by changing pH, a complimentary method can be developed and it is possible to determine if all peaks are resolved. The Extend column can be used at high and low pH, so the complimentary separation can be investigated with one column. Better MS sensitivity for this sample is also achieved at high pH.



LCBP017

Nucleosides: Separation of Deoxy and Ribonucleosides

Column: ZORBAX SB-C3
883975-909
4.6 x 150 mm, 5 µm

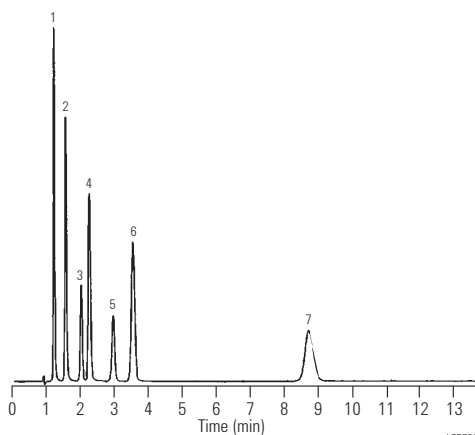
Mobile Phase: 4 mM Ammonium Phosphate
(pH 4.0 with Phosphoric Acid)

Flow Rate: 2.0 mL/min

Temperature: 35°C

Detector: UV 254 nm

Sample: 2 µL (1.6 µg each)



1. Cytidine
2. 2' Deoxycytidine
3. Inosine
4. Guanosine
5. 2' Deoxyinosine
6. 2' Deoxyguanosine
7. Adenosine

Nucleotides: Separation of Mononucleotides

Column: ZORBAX SAX
880952-703
4.6 x 250 mm, 5 µm

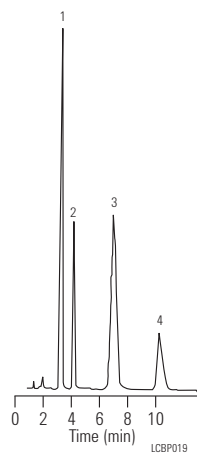
Mobile Phase: 0.1 M NH₄H₂PO₄

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV 254 nm

Sample: Orotic Acid, UMP, GMP, XMP



1. Orotic Acid
2. UMP
3. GMP
4. XMP



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

**Separation of Basic Peptides
on Bonus-RP versus Traditional Alkyl Phase**

Column A: ZORBAX Bonus-RP
883668-901
4.6 x 150 mm, 5 µm

Column B: Alkyl C8

Mobile Phase: A: 0.010 M ammonium phosphate,
pH 7 / 0.050 M sodium perchlorate
B: 0.010 M ammonium phosphate / 0.050 M
sodium perchlorate in 50% ACN

Flow Rate: 1.0 mL/min

Gradient: 0-100% B in 50 min.

Temperature: 40°C

Detector: 215 nm

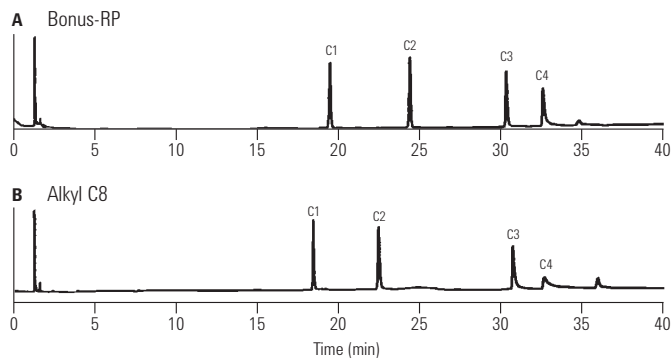
Sample: Basic 11-residue peptides
with net +1, +2, +3, +4
positive charges at neutral pH

C1: Ac-Gly-Gly-Gly-Leu-Gly-Gly-Ala-Gly-Gly-Leu-Lys-amide

C2: Ac-Lys-Tyr-Gly-Leu-Gly-Gly-Ala-Gly-Gly-Leu-Lys-amide

C3: Ac-Gly-Gly-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-amide

C4: Ac-Lys-Tyr-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-amide



LCBP020

Peptides: Effect of TFA Concentration

Column: ZORBAX 300SB-C8
883995-906
4.6 x 150 mm, 5 µm

Mobile Phase: A: Water and TFA
B: ACN and TFA

Flow Rate: 1.0 mL/min

Gradient: 0 min 0% B
30 min 30% B

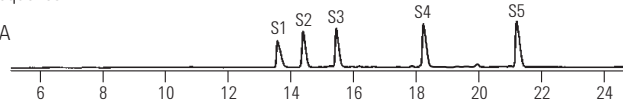
Temperature: 40°C

Detector: UV 254 nm

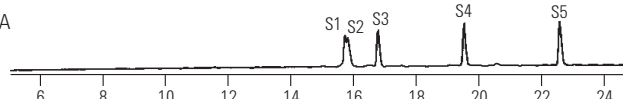
Sample: Peptide Standards S1-S5, decapeptides
differing slightly in hydrophobicity, 6 µL

Peptide Sequence

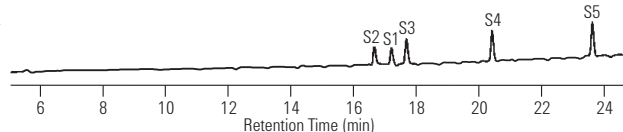
0.05% TFA



0.25% TFA



1.0% TFA



LCBP021

Exploiting chemical stability – TFA concentration

Column: PLRP-S 100Å
PL1512-5500
4.6 x 250 mm, 5 µm

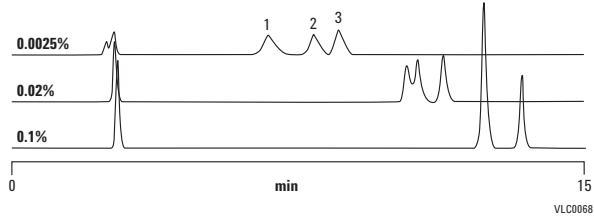
Mobile Phase: A: TFA (various %) in water
B: TFA (various %) in ACN

Gradient: Linear 12-40% B in 15 min

Flow Rate: 1.0 mL/min

Detector: ELS (neb=75°C, evap=85°C, gas=1.0 SLM)

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



Peptides: Separation of Antiotensins I, II, III with TFA and NH₄OH

Column: ZORBAX Extend-C18
773700-902
2.1 x 150 mm, 5 µm

Mobile Phase: A: Acidic Conditions
A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN
B: Basic Conditions
A: 10 mM NH₄OH in water
B: 10 mM NH₄OH in 80% ACN

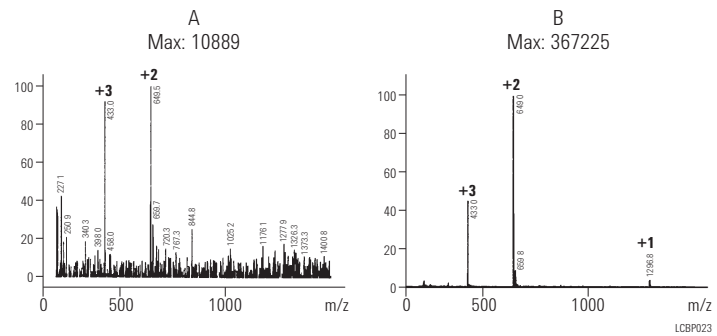
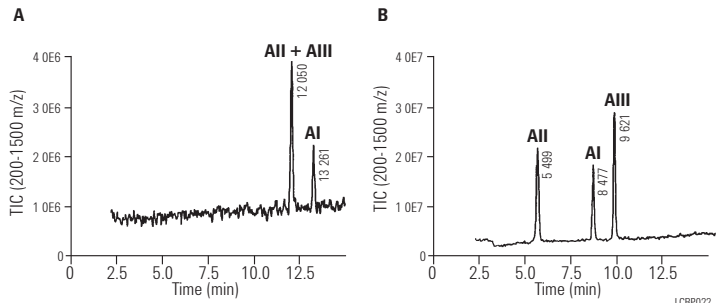
Flow Rate: 0.2 mL/min

Gradient: 15-50% B in 15 min

Temperature: 35°C

MS Conditions: Pos. Ion ESI - Vf 70V, Vcap 4.5 kV
N₂-35 psi, 12 L/min, 325°C

Sample: 2.5 µL sample (50 pmol each)



**Peptides/Proteins:
Equivalent Gradient Separations**

Column: ZORBAX 300SB-C8
883995-906
4.6 x 150 mm, 5 µm

Column: ZORBAX 300SB-C8
883750-906
2.1 x 150 mm, 5 µm

Mobile Phase: A: 95% Water: 5% ACN with 0.1% TFA
B: 5% Water: 95% ACN with 0.085% TFA

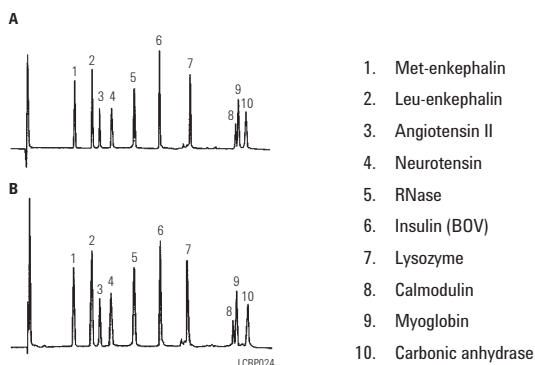
Flow Rate: A: Analytical
1 mL/min
B: Narrow Bore
0.2 mL/min

Gradient: 10-60% B in 30 min

Temperature: 35°C

Detector: UV 215 nm

Sample: 10 µL injection, Concentration 2-6 µg



**Peptides/Proteins: Effect of
Elevated Temperature**

Column: ZORBAX 300SB-C3
883995-909
4.6 x 150 mm, 5 µm

Mobile Phase: A: 5:95 ACN:Water
with 0.10% TFA (v/v%)
B: 95:5 ACN:Water
with 0.085% TFA (v/v%)

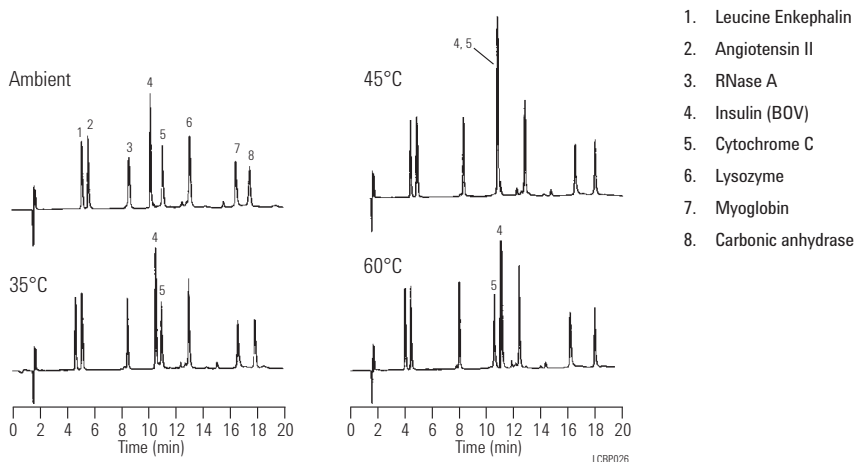
Flow Rate: 1.0 mL/min

Gradient: 15-53% in 20 min,
posttime 12 min

Temperature: Ambient – 60°C

Detector: UV 215 nm

Sample: Polypeptides



Separation of Polypeptides in Under 1 Minute

Column: Poroshell 300SB-C18
660750-902
2.1 x 75 mm, 5 µm

Mobile Phase: A: 0.1% TFA, H₂O
B: 0.07% TFA, ACN

Flow Rate: 3 mL/min.

Gradient: 0-100% B in 1.33 min

Temperature: 70°C

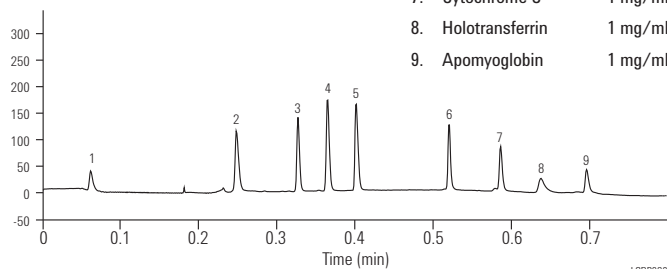
Detector: DAD 215/16 nm, ref = 310/10 nm

Sample: peptides/proteins, 0.5 µL

Mixer bypassed with P/N G1312-67301; Loop-bypass program

Sample (peptides/proteins)

- | | |
|--------------------|-------------|
| 1. gly-tyr | 0.125 mg/mL |
| 2. Val-tyr-val | 0.5 mg/mL |
| 3. Met-enkephalin | 0.5 mg/mL |
| 4. Leu-enkephalin | 0.5 mg/mL |
| 5. Angiotensin II | 0.5 mg/mL |
| 6. RNase A | 1 mg/mL |
| 7. Cytochrome C | 1 mg/mL |
| 8. Holotransferrin | 1 mg/mL |
| 9. Apomyoglobin | 1 mg/mL |



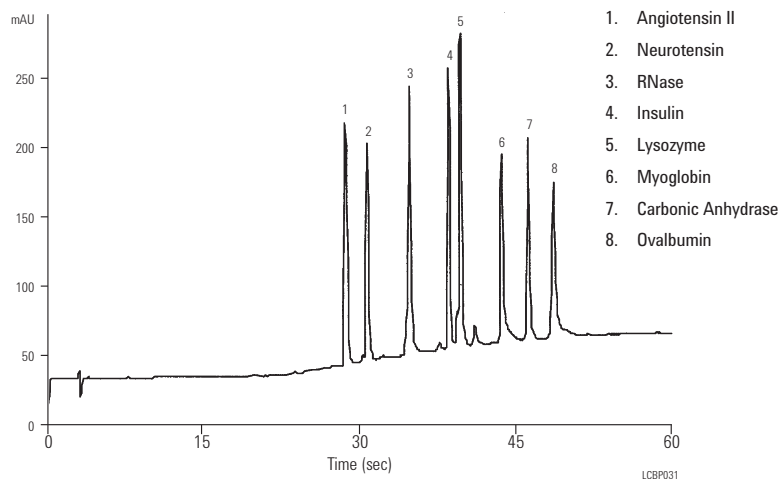
For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

Fast, High-Resolution Separation of Peptides and Proteins with Poroshell 300SB-C18

Column: Poroshell 300SB-C18
660750-902
2.1 x 75 mm, 5 µm

Mobile Phase: A: 0.1% TFA
B: 0.07% TFA in ACN
Flow Rate: 3.0 mL/min (360 bar pressure)
Gradient: 5-100% B in 1.0 min
Temperature: 70°C
Detector: UV 215 nm

Spaces between solutes indicate good peak capacity for rapidly separating complex samples.

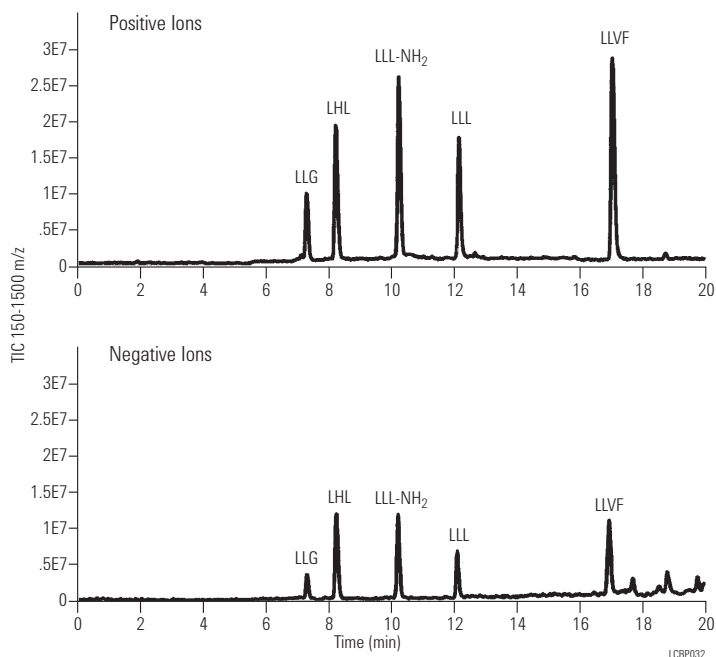


Peptide RP-HPLC/ESI-MS Using NH₄OH Mobile Phase Yields Both Positive and Negative Ion Spectra

Column: ZORBAX Extend-C18
773700-902
2.1 x 150 mm, 5 µm

Flow Rate: 0.25 mL/min
Gradient: 5-60% B in 20 min
Temperature: 25°C
MS Conditions: Pos. Ion ESI – Vf 70 V, Vcap 4.5 kV,
N₂– 35 psi, 12 L/min, 300°C
TIC 150-1500 m/z

Sample: 4 µL (50 ng each peptide)



Comparison of A β Peptide RP-HPLC Separations at Low and High pH

Column: ZORBAX Extend-C18
773700-902
2.1 x 150 mm, 5 μ m

Mobile Phase: A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN

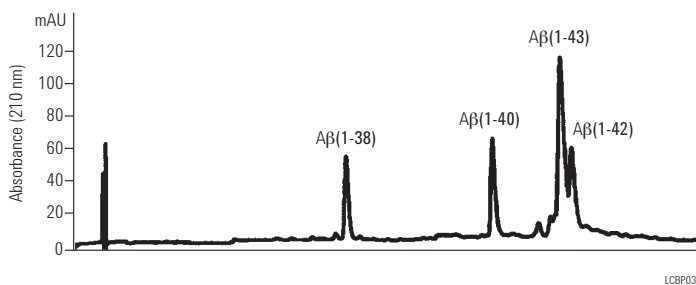
Flow Rate: 0.25 mL/min.

Gradient: 29-41% B in 30 min.

Temperature: 80°C

Detector: UV 210 nm

Sample: 5 μ L sample (100 pmol each)



Mobile Phase: A: 20 mM NH₄OH in water
B: 20 mM NH₄OH in 80% ACN

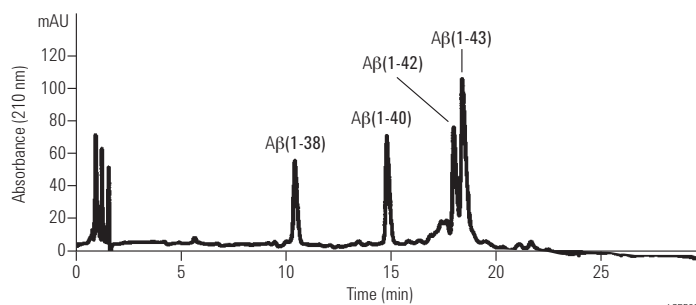
Flow Rate: 0.25 mL/min

Gradient: 26-38% B in 30 min

Temperature: 25°C

Detector: UV 210 nm

Sample: 5 μ L sample (100 pmol each)



Selectivity Comparison of TFA and NH₄OH for Peptide RP-HPLC\ESI-MS Analysis

Column: ZORBAX Extend-C18
773700-902
2.1 x 150 mm, 5 μ m

Mobile Phase: TFA Conditions:
A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN
NH₄OH Conditions:
A: 20 mM NH₄OH in water
B: 20 mM NH₄OH in 80% ACN

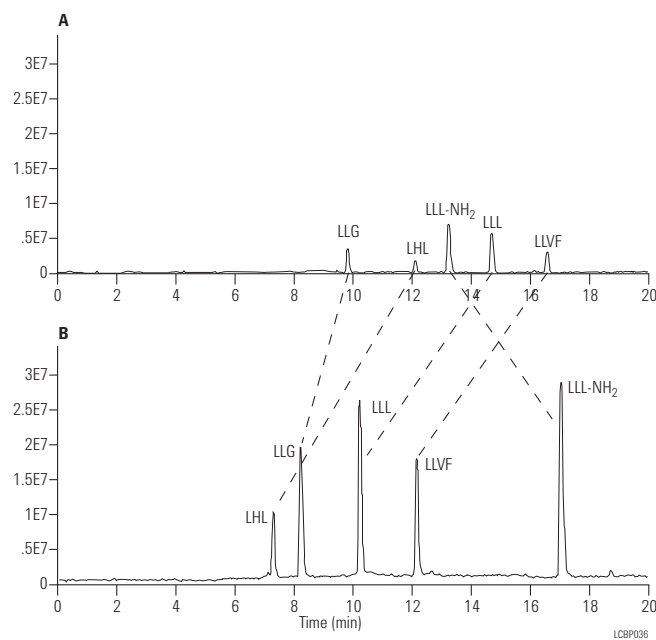
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25°C

MS Conditions: Pos. Ion ESI – Vf 70V, Vcap 4.5 kV,
N₂ – 35 psi, 12 L/min., 300°C
TIC 150-1500 m/z

Sample: 4 μ L (50 ng each peptide)



Peptide Phosphorylation Sites LC and LC/MS using Capillary LC Columns

Column: ZORBAX 300SB-C18
5064-8268
0.5 x 150 mm, 3.5 μm

Mobile Phase: A: 0.1% formic acid in water
B: 0.1% formic acid in ACN

Flow Rate: 5.5 μL/min

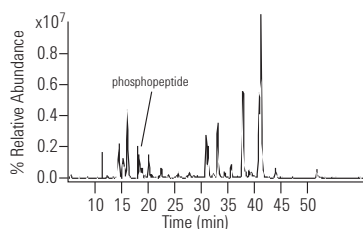
Gradient: 5-55% B in 50 min, to 85% B from 55-57 min

Detector: UV 206 nm

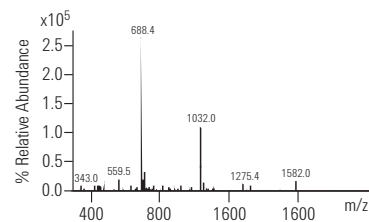
MS Conditions: LC/MS: Pos. Ion ESI with LC/MSD trap
Vcap: 4000 V
Drying gas flow: 7L/min
Drying gas temperature: 250°C
Nebulizer: 15 psi
Capillary Exit Volt: 50 V Max
Accum Time: 300 ms
Total Averages: 3
Isolation Width: 3 m/z
Frag Amplitude: 1.0 V

Sample: Beta case in digest, 100 nL (4 pmol)

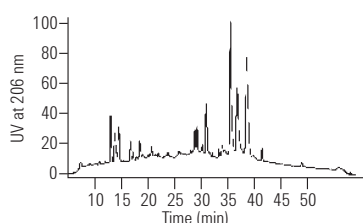
MS



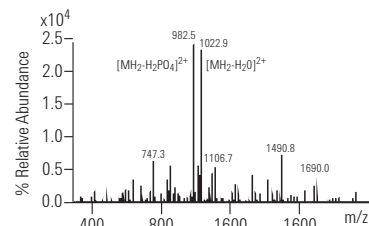
Full Scan MS



UV



MS/MS of [M+2H]²⁺ at m/z 1032



LCBP037

Proteins: Effect of Bonded Phase, RP

Column A: ZORBAX 300SB-C8
883995-906
4.6 x 150 mm, 5 μm

Column B: ZORBAX 300SB-CN
883995-905
4.6 x 150 mm, 5 μm

Mobile Phase: A: 0.1% TFA in Water,
B: 0.1% TFA in 50/50 ACN/Water

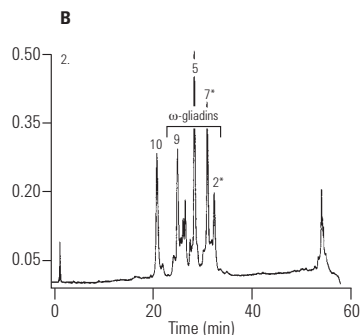
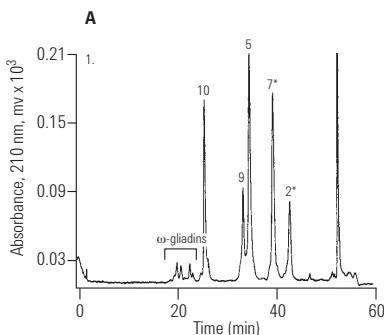
Flow Rate: 1.0 mL/min

Gradient: 1. 46-96% B in 60 min. 23-48% ACN
2. 50-86% B in 60 min. 25-43% ACN

Temperature: 50°C

Detector: UV 210 nm

Sample: Wheat proteins, including w-gliadins



LCBP038

Proteins: Effect of Bonded Phase

Column A: ZORBAX 300SB-C18
883995-902
4.6 x 150 mm, 5 µm

Column B: ZORBAX 300SB-C8
883995-906
4.6 x 150 mm, 5 µm

Column C: ZORBAX 300SB-C3
883995-909
4.6 x 150 mm, 5 µm

Column D: ZORBAX 300SB-CN
883995-905
4.6 x 150 mm, 5 µm

Mobile Phase: A: 0.1% TFA in H₂O
B: 0.09% TFA in 80% ACN/20% Water

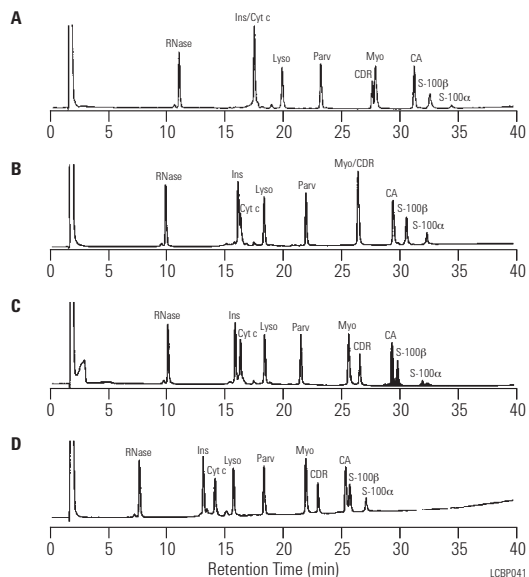
Flow Rate: 1.0 mL/min

Gradient: 25-70% B in 40 min

Temperature: 60°C

Detector: UV 210 nm

Sample: Polypeptides, 3 µg each



Standard proteins by reverse phase

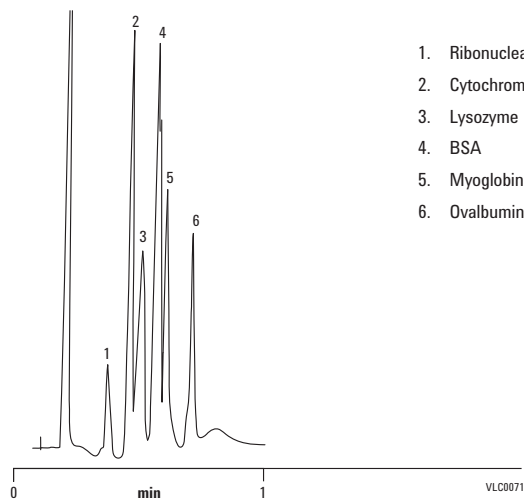
Column: PLRP-S 4000Å
PL1512-1803
4.6 x 50 mm, 8 µm

Mobile Phase: A: 0.1% TFA in 95% water : 5% ACN
B: 0.1% TFA in 5% water : 95% ACN

Gradient: Linear 18-60% B in 1 min

Flow Rate: 4.0 mL/min

Detector: UV, 280 nm



Standard ion-exchange protein separation

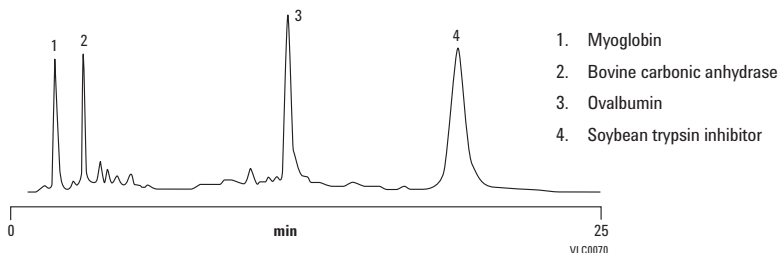
Column: PL-SAX 1000Å
 PL1551-1502
 4.6 x 50 mm, 5 µm

Mobile Phase: A: 10 mM Tris HCl pH 8
 B: A+0.35 M NaCl pH 8

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm


**Deoxynucleosides:
 Using Rapid Resolution 3.5 µm Columns**

Column A: ZORBAX SB-CN
 883975-905
 4.6 x 150 mm, 5 µm

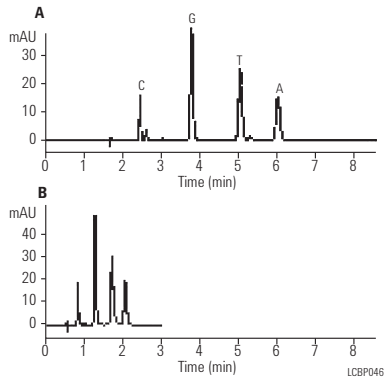
Column B: ZORBAX SB-CN
 835975-905
 4.6 x 50 mm, 3.5 µm

Mobile Phase: A: 0.1% TFA
 B: 90/10 v/v Methanol/Water (0.1% TFA)
 Isocratic, 97.5% A, 2.5% B

Flow Rate: 1.0 mL/min.

Temperature: 30°C

Detector: UV 254 nm



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

BSA Tryptic Digest on RRHT

Column: ZORBAX SB-C18
820700-902
2.1 x 150 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA, 5% ACN
B: 0.08% TFA, 95% ACN

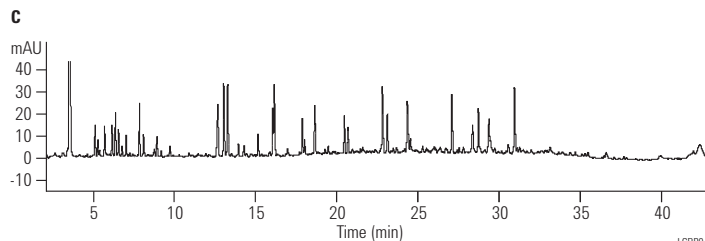
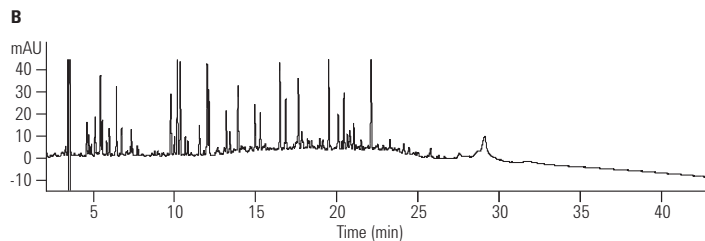
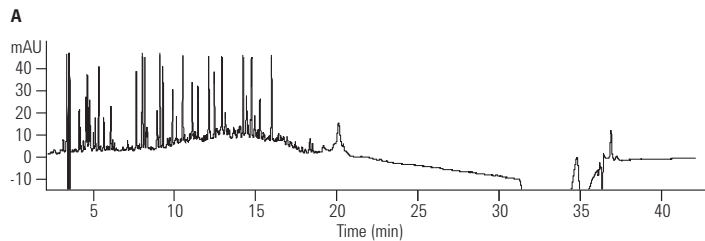
Flow Rate: 0.5 mL/min

Gradient: A: Time 0 %B 5, Time 30 %B 60
B: Time 0 %B 5, Time 45 %B 60
C: Time 0 %B 5, Time 67.5 %B 60

Temperature: 80°C

Detector: UV 214 nm

Sample: BSA Tryptic Digest



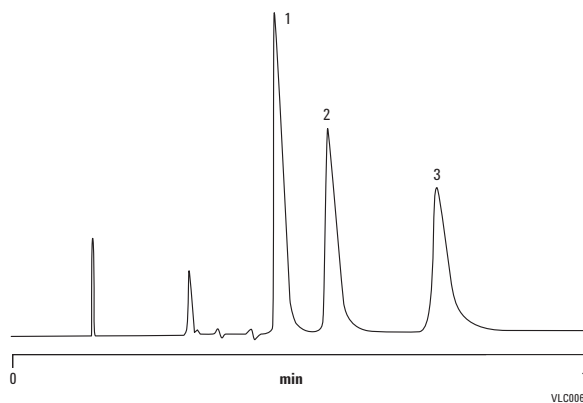
Catecholamines

Column: PLRP-S 100Å
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: 95% 25 mM citric acid,
25 mM Na₂HPO₄, 1 mM heptane
sulfonic acid : 5% ACN, pH 2.85

Flow Rate: 1.0 mL/min

Detector: UV, 280 nm



1. Noradrenaline
2. Adrenaline
3. Dopamine

Whey proteins in dairy samples – milk

Column: PLRP-S 300Å
PL1512-3801
4.6 x 150 mm, 8 µm

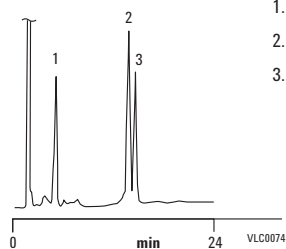
Mobile Phase: A: 0.1% TFA in 99% water : 1% ACN
B: 0.1% TFA in 1% water : 99% ACN

Gradient: 36-48% B, 0-24 min, 48-100% B, 24-30 min
100% B, 30-35 min, 100-36% B, 35-40 min

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Detector: UV, 220 nm



1. α-Lactalbumin
2. β-Lactoglobulin (B chain)
3. β-Lactoglobulin (A chain)

Temperature as a tool to enhance mass transfer and improve resolution of oligonucleotides in ion pair reverse phase HPLC

Column: PLRP-S 100Å
PL1512-1300
4.6 x 50 mm, 3 µm

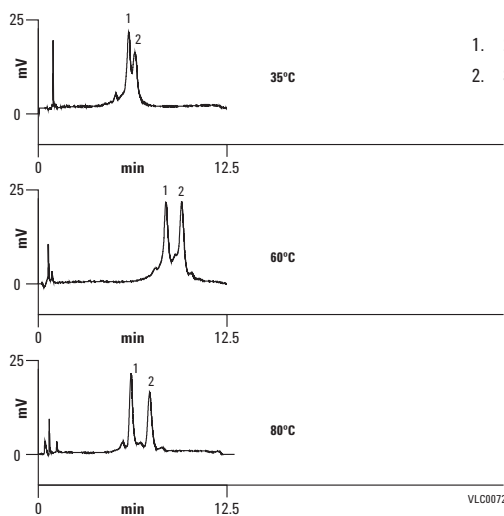
Mobile Phase: A: 100 mM TEAA
B: 100 mM TEAA in 25% ACN

Gradient: 5% change in buffer B over 5 min

Flow Rate: 1.0 mL/min

Temperature: 35°C, 60°C,
or 80°C

Detector: UV, 254 nm



1. 29 mer
2. 30 mer

Hydrophilic Purine/Pyrimidine Separation

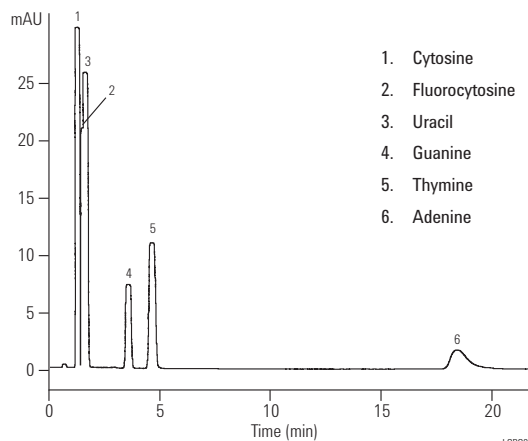
Column: ZORBAX SB-Aq
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 50 mM NaOAc, pH 4.6

Flow Rate: 2.0 mL/min

Temperature: 35°C

Detector: UV 254 nm



1. Cytosine
2. Fluorocytosine
3. Uracil
4. Guanine
5. Thymine
6. Adenine

Chemical/Industrial Applications

Analysis of Biocides in Hand Sanitizer

Column: Eclipse Plus C18
959757-902
2.1 x 50 mm, 1.8 μm

Mobile Phase: A: H₂O (0.5% TFA)
B: ACN (0.04% TFA)

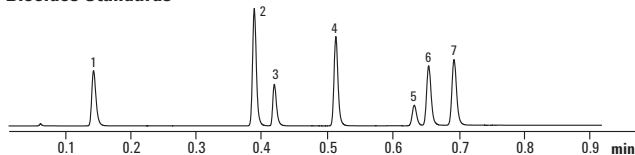
Flow Rate: 1.7 mL/min

Gradient: Time 0.0 95/5 A/B DAD: 275 nm (0 min)
Time 1.0 55/45 A/B 225 nm (0.46 min)
Time 1.1 0/100 A/B 255 nm (0.67 min)

Sample: 1 μL injection of 50 ppm std.

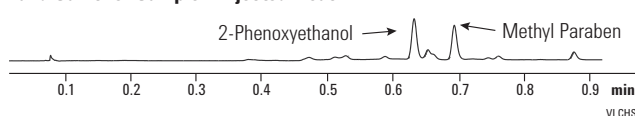
Temperature: 30°C

Biocides Standards



1. Kathon 1A
2. Kathon 1B
3. Carbendazim
4. 1,2-Benzisothiazol-3(2H)-one
5. 2-Phenoxyethanol
6. Benzoic Acid
7. Methyl Paraben

Hand Sanitizer Sample - Injected Neat



Triton X-114: Decreasing Run-time by Changing Bonded Phase

Column A: ZORBAX SB-C3
883975-909
4.6 x 150 mm, 5 μm

Column B: ZORBAX SB-C18
883975-902
4.6 x 150 mm, 5 μm

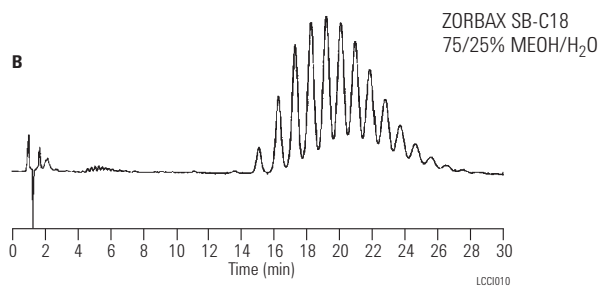
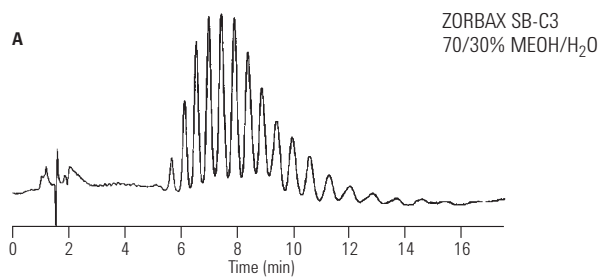
Mobile Phase: MeOH and H₂O (as indicated)

Flow Rate: 1.0 mL/min

Temperature: 50°C

Detector: UV 225 nm

Sample: Triton X-114



Organic Acids Separated on ZORBAX SB-Aq

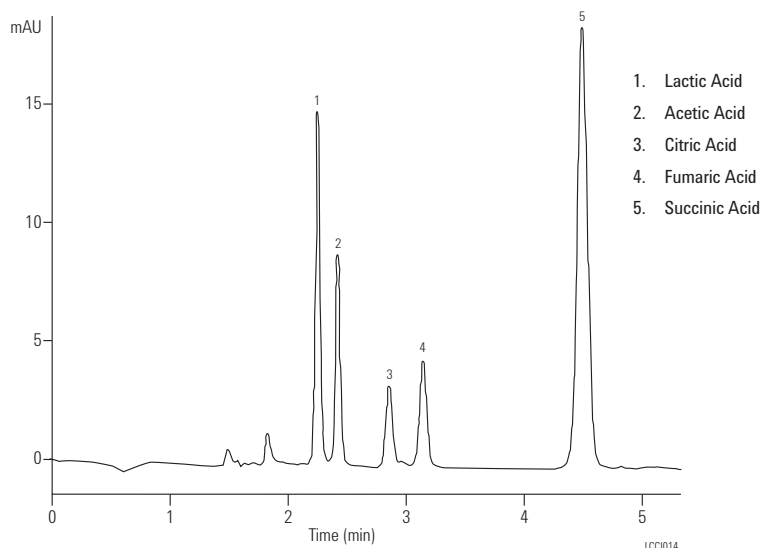
Column: ZORBAX SB-Aq
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 99% 20 mM NaH₂PO₄, pH2, 1% ACN

Flow Rate: 1.0 mL/min

Temperature: 35°C

Detector: UV 210 nm



Brij 35

Column: PLRP-S 100Å
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: A: Water
B: ACN

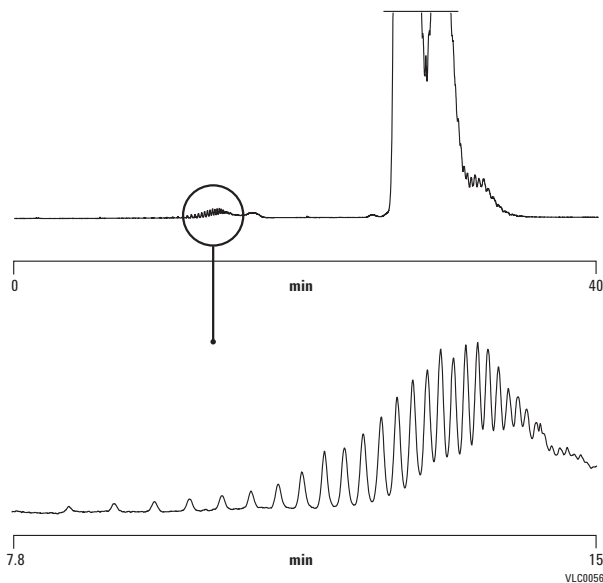
Gradient: 0-100% B in 40 min

Flow Rate: 0.8 mL/min

Injection Volume: 10 µL

Sample Conc: 1 mg/mL

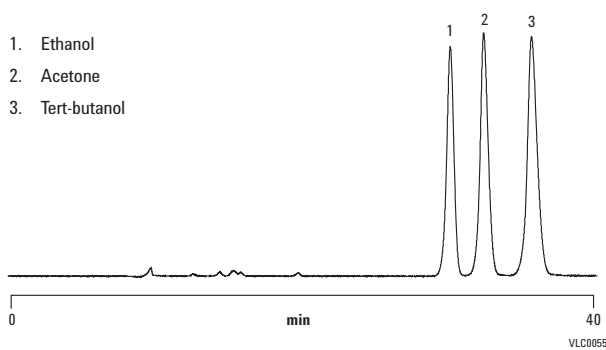
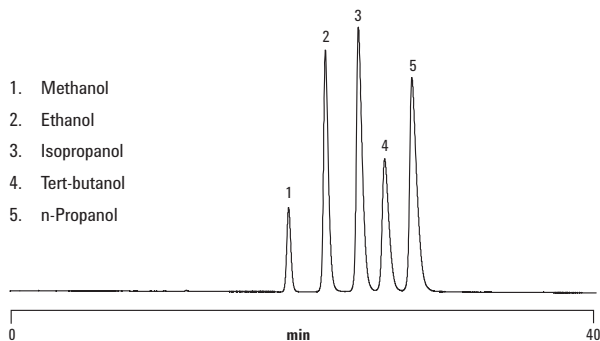
Detector: ELS (neb=50°C, evap=70°C, gas=1.5 SLM)



Alcohols and Aliphatic Compounds

Column: Hi-Plex H
 PL1170-6830
 7.7 x 300 mm, 8 µm

Mobile Phase: Water
Flow Rate: 0.6 mL/min
Temperature: 40°C
Detector: 356-LC RI



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

Environmental Applications

NEW!

Comparison of Phenols Separation with Poroshell 120

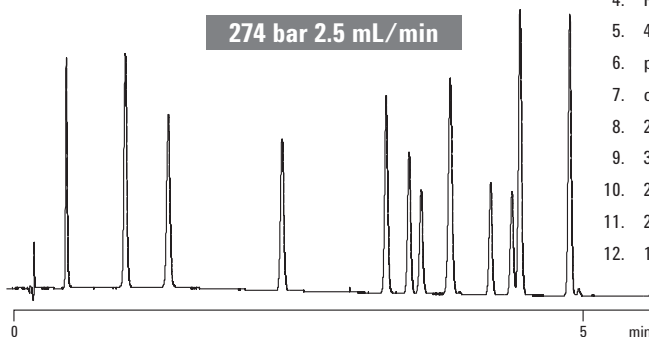
Column: Poroshell 120 EC-C18
699975-902
4.6 x 50 mm, 2.7 µm

Mobile Phase: A: Water with 0.1% Formic Acid
B: Acetonitrile

Gradient:

Time	%B
0.8	5%
6.8	60%

1200 SL controlled temperature at 25°C 2 mm flow cell



1. Hydroquinone
2. Resourcinol
3. Catechol
4. Phenol
5. 4-Nitrophenol
6. p-cresol
7. o-cresol
8. 2-Nitrophenol
9. 3,4 di methyl phenol
10. 2,3 di methyl phenol
11. 2,5 di methyl phenol
12. 1-napthol

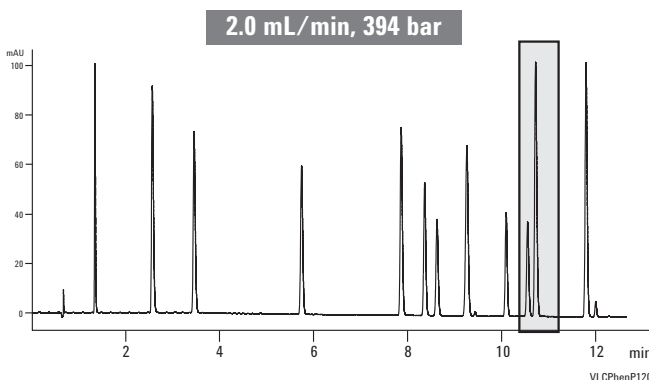
Column: Poroshell 120 EC-C18
695975-902
4.6 x 100 mm, 2.7 µm

Mobile Phase: A: Water with 0.1% Formic Acid
B: Acetonitrile

Gradient:

Time	%B
2.0	5%
17	60%

1200 RRLC SL controlled temperature at 25°C 2 mm flow cell



VLCPhenP120

NEW!

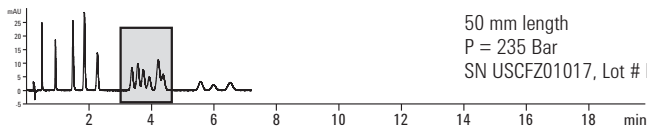
Comparison of EPA 8330 Separation on Poroshell 120 Columns

Column: Poroshell 120 EC-C18, 2.7 µm

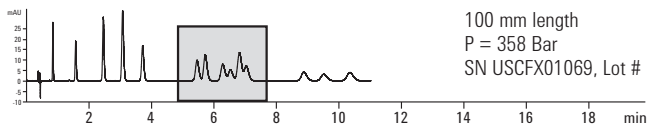
Mobile Phase: 25% Methanol: 75% Water

Flow Rate: 1 mL/min

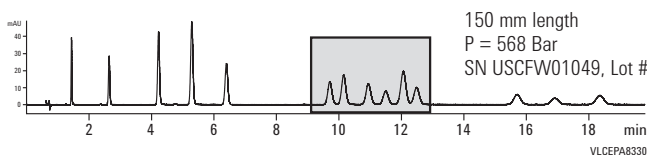
Temperature: 44°C



50 mm length
P = 235 Bar
SN USCFZ01017, Lot # B10016



100 mm length
P = 358 Bar
SN USCFX01069, Lot # B10034



150 mm length
P = 568 Bar
SN USCWF01049, Lot # B10022

VLCPEA8330

DNPH: Derivatized Aldehydes Obtained from Air

Column: ZORBAX ODS
884950-543
4.6 x 250 mm, 5 µm

Mobile Phase: A: 100% Water
B: 100% ACN

Flow Rate: 1.0 mL/min

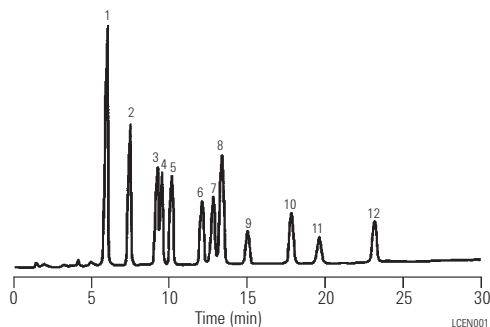
Gradient: 60-75% B in 30 min;
Wash: From 75-100% B in 5 min,
after 5 min return to 60% B

Temperature: 35°C

Detector: UV 230 nm

Sample: DNPH Derivatized Aldehydes

- | | |
|---------------------------|---|
| 1. Formaldehyde - DNPH | 7. 2-Butanone (MEK) - DNPH |
| 2. Acetaldehyde - DNPH | 8. Methacrolein - DNPH n-Butyraldehyde - DNPH |
| 3. Acetone - DNPH | 9. Benzaldehyde - DNPH |
| 4. Acrolein - DNPH | 10. Valeraldehyde - DNPH |
| 5. Propionaldehyde - DNPH | 11. m-Tolualdehyde - DNPH |
| 6. Crotonaldehyde - DNPH | 12. Hexaldehyde - DNPH |



Amitrol in Water by LC/MS, 0.05 ppb

Column: ZORBAX SB-C18
863954-302
3.0 x 150 mm, 3.5 µm

Mobile Phase: A: 10 mM ammonium acetate
B: MeOH

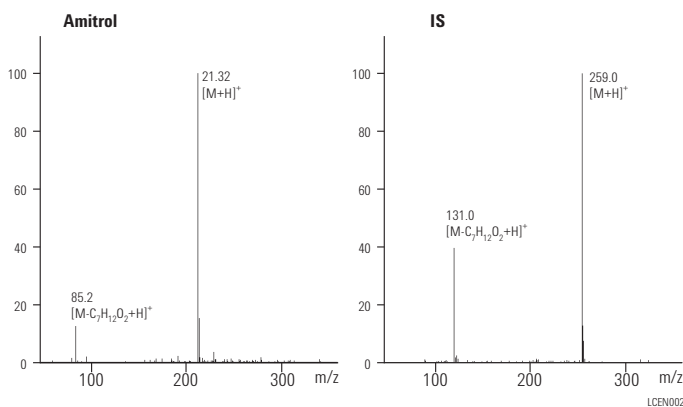
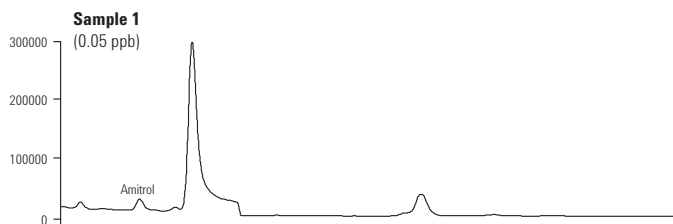
Flow Rate: 0.4 mL/min

Gradient: 0 min, 65% B; 10 min, 65% B;
15 min, 100% B; 20 min, 65% B

Temperature: 30°C

MS Conditions: Ionization Mode: APCI, positive polarity
SIM parameters: Ion: 213 Amitrol
Ion: 259 IS
Fragmentor: 100 V
SIM Resolution: Low
Vaporizer: 325°C
Drying Gas (N₂): 5.0 L/min
Gas Temperature: 350°C
Nebulizer pressure: 60 psig
Vcap: 4000 V
Corona: 4.0 uA

Sample: Amitrol in water, 100 µL



Anilines, Substituted: Rapid Separation

Column: ZORBAX Rx/SB-C8
866953-906
4.6 x 75 mm, 3.5 µm

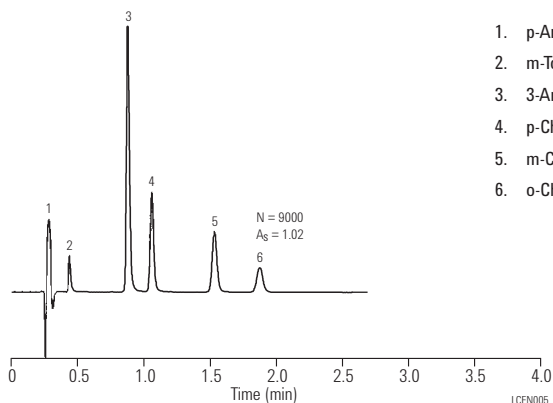
Mobile Phase: 20% ACN/80% 25 mM phosphate buffer, pH 2.5

Flow Rate: 3.0 mL/min

Temperature: 60°C

Detector: UV 254 nm

Sample: Anilines



1. p-Anisidine
2. m-Toluidine
3. 3-Amino-benzonitrile
4. p-Chloroaniline
5. m-Chloroaniline
6. o-Chloroaniline

Explosives and Related Compounds: Qualitative and Quantitative Analysis

Column A: ZORBAX SB-C18
883700-922
2.1 x 150 mm, 5 µm

Column B: ZORBAX SB-CN
883700-905
2.1 x 150 mm, 5 µm

Mobile Phase: A = ACN + 5% H₂O + 5 mM CF₃COONH₄
B = H₂O + 5% ACN + 5 mM CF₃COONH₄, pH 2.7 (CF₃COOH)

Flow Rate: 0.23 mL/min

Gradient: A:
0 min 80% B
2 min 80% B
10 min 70% B
20 min 65% B
25 min 60% B
35 min 30% B
40 min 30% B
42 min 80% B

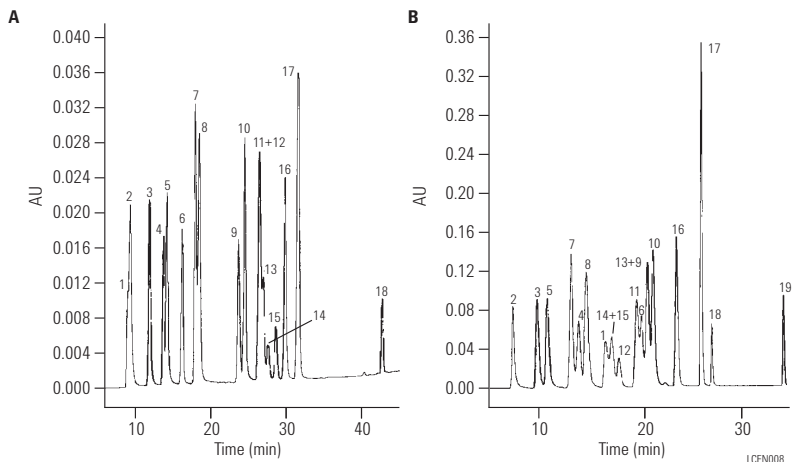
B:
0 min 80% B
1 min 80% B
15 min 70% B
30 min 20% B
35 min 20% B
37 min 80% B

Temperature: 18°C

Detector: UV 210, 240, 360 nm, wavelength switching for each compound

Sample: 10 µL of 19 explosive compounds in ACN/H₂O (20/80)

1. Picric acid
2. 4-Amino-2-nitrotoluene
3. 2-Amino-6-nitrotoluene
4. RDX
5. 2-Amino-4-nitrotoluene
6. HMX
7. 1,3-Dinitrobenzene
8. 1,3,5-Trinitrobenzene
9. 2-Amino-4,6-dinitrotoluene
10. 2,4-Dinitrotoluene
11. 4-Amino-4,6-dinitrotoluene
12. 2-Nitrotoluene
13. 2,6-Dinitrotoluene
14. 4-Nitrotoluene
15. 3-Nitrotoluene
16. 2,4,6-Trinitrotoluene
17. Tetryl
18. Diphenylamine
19. Hexyl



Explosives from Soil Extract

Column: ZORBAX SB-C18
880975-302
3.0 x 250 mm, 5 µm

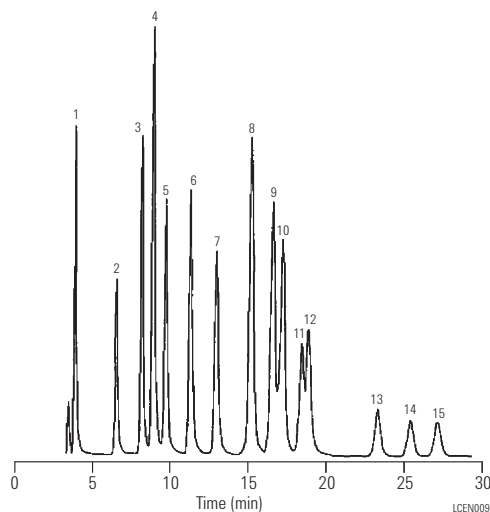
Mobile Phase: Methanol/Water (50/50) (v/v)

Flow Rate: 0.3 mL/min

Temperature: Ambient

Detector: UV 230 nm

Sample: 10 µL Explosives Mix



1. Octogen (HMX)
2. Hexogen (RDX)
3. 2-Amino-6-nitrotoluene
4. 1,3,5-Trinitrobenzene
5. 2-Amino-4-nitrotoluene
6. 1,3-Dinitrobenzene
7. Tetryl
8. 2,4,6-Trinitrotoluene
9. 4-Amino-2,6-dinitrotoluene
10. 2-Amino-4,6-dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2-Nitrotoluene
14. 4-Nitrotoluene
15. 3-Nitrotoluene

Herbicides on Different Bonded Phases

Column A: ZORBAX SB-CN
883975-905
4.6 x 150 mm, 5 µm

Column B: ZORBAX SB-Phenyl
883975-912
4.6 x 150 mm, 5 µm

Column C: ZORBAX SB-C8
883975-906
4.6 x 150 mm, 5 µm

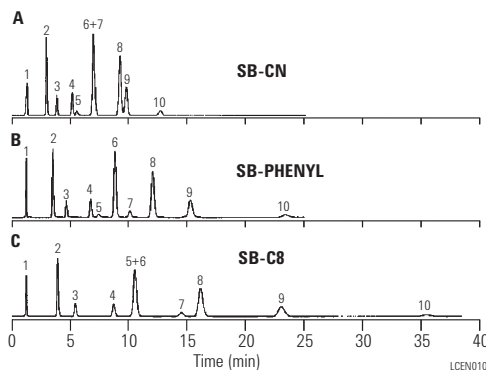
Mobile Phase: 35% ACN, 65% Water

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV 254 nm

Sample: Herbicides



1. Bentazon
2. Tebuthiuron
3. Simazine
4. Atrazine
5. Prometon
6. Diuron
7. Propazine
8. Propanil
9. Prometryne
10. Metolachlor

Herbicide/Pesticide Standards: Effect of Bonded Phase

Column: Eclipse XDB-C8
993967-906
4.6 x 150 mm, 5 µm

Mobile Phase: Water/Acetonitrile

Flow Rate: 1.0 mL/min

Gradient: 20-60% in 15 min.

Temperature: 50°C
40°C
30°C
20°C

Detector: DAD 240/30

Sample: Herbicide & Pesticide Standards

Column: Eclipse XDB-C18
993967-902
4.6 x 150 mm, 5 µm

Mobile Phase: Water/Acetonitrile

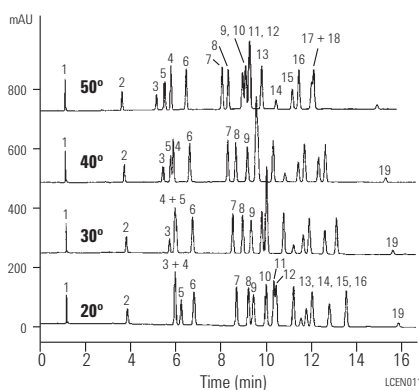
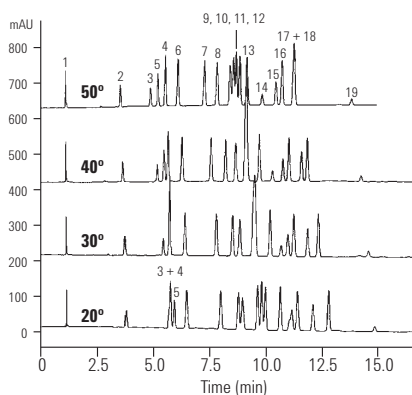
Flow Rate: 1.0 mL/min

Gradient: 20-60% in 15 min.

Temperature: 50°C
40°C
30°C
20°C

Detector: DAD 240/30

Sample: Herbicide & Pesticide Standards



1. Desethylisopropylatrazine
2. Desethylatrazine
3. Benzthiazuron
4. Hexazinon
5. Metoxuron
6. Simazine
7. Methabenzthiazuron
8. Simazine
9. Atrazine
10. Isoproturon
11. Diuron
12. Monlinuron
13. Metobromuron
14. Metazachlor
15. Propazine
16. Sebutylazine
17. Terbutylazine
18. Linuron
19. Metolachlor



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

Separation of EPA 610 PAH Mix on 3.0 x 250 mm, 5 µm Eclipse PAH Column

Column: Eclipse PAH
959990-318
3.0 x 250 mm, 5 µm

Mobile Phase: A: Water
B: Acetonitrile
Initial %B = 40

Flow Rate: 0.85 mL/min

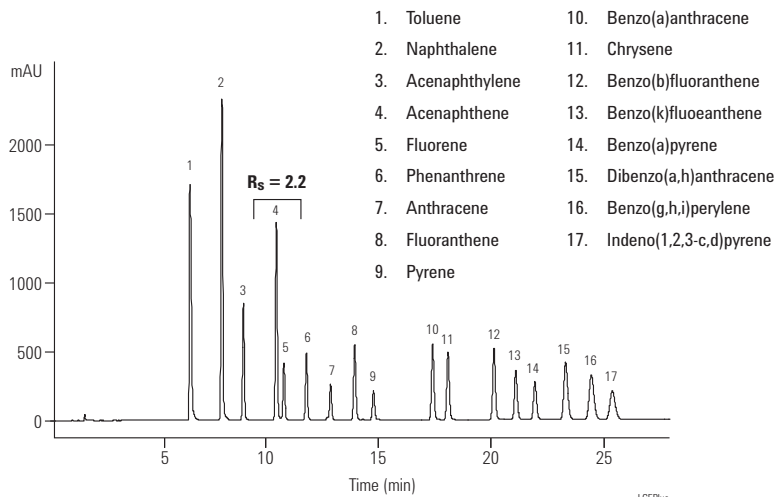
Gradient:

Time (Min)	%B
0.00	45
17.5	100
24.0	100
25.5	40
27.5	40

Stop Time = 25.0

Temperature: 25° C

Detector: 220.4 nm No Ref.; Stop time = 26.0 min



Polycyclic aromatic hydrocarbons according to EPA Method 610

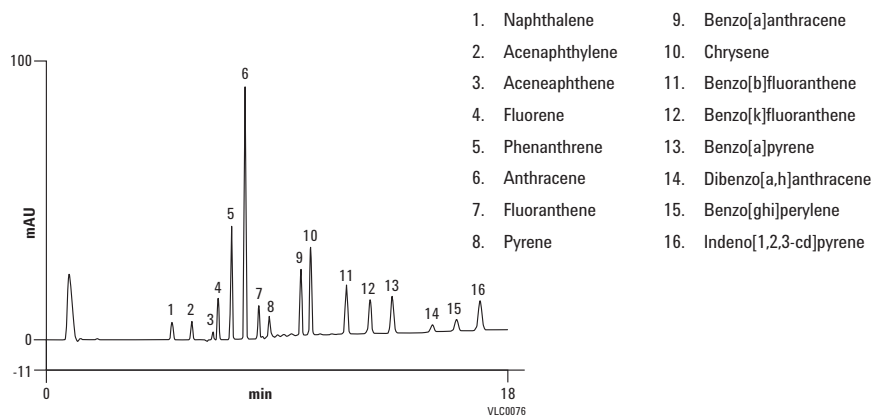
Column: Pursuit PAH
A7001100X046
4.6 x 100 mm, 3 µm

Sample: NIST 16473 Standard

Mobile Phase: A: ACN:water, 25:75
B: ACN

Flow Rate: 2.0 mL/min

Detector: UV, 254 nm



Separation of 20 PAHs on Eclipse PAH

Column: Eclipse PAH
959964-918
4.6 x 100 mm, 1.8 μm

Mobile Phase: A: Water
B: Acetonitrile

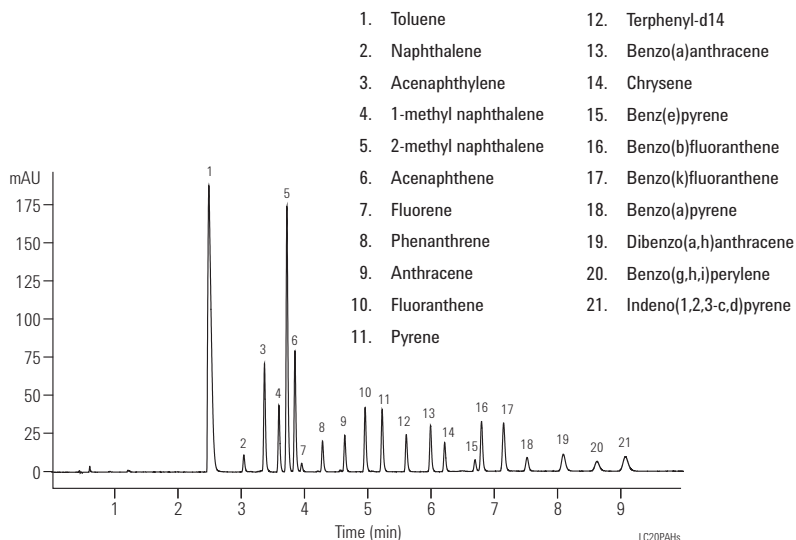
Flow Rate: 1.8 mL/min

Gradient:

Time (Min)	% B
0	40
6	100
9.5	100
10	40
Stop Time = 12	

Temperature: 25°C

Detector: 230.8 nm No Ref.; Data rate 0.2 s, micro flow cell



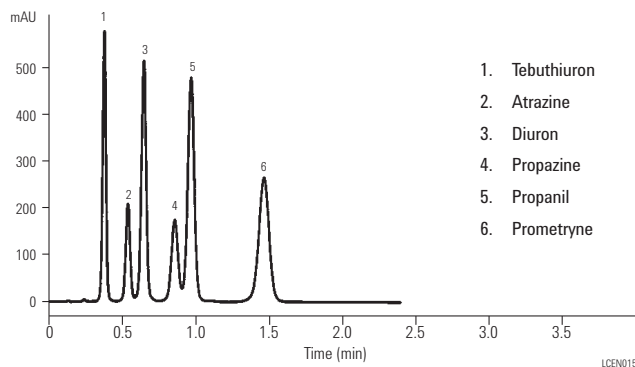
Herbicides: Rapid Separation

Column: Eclipse XDB-C18
933975-902
4.6 x 30 mm, 3.5 μm

Mobile Phase: MeOH:H₂O (60:40)

Flow Rate: 2 mL/min

Temperature: Ambient



Phenoxyacid herbicides

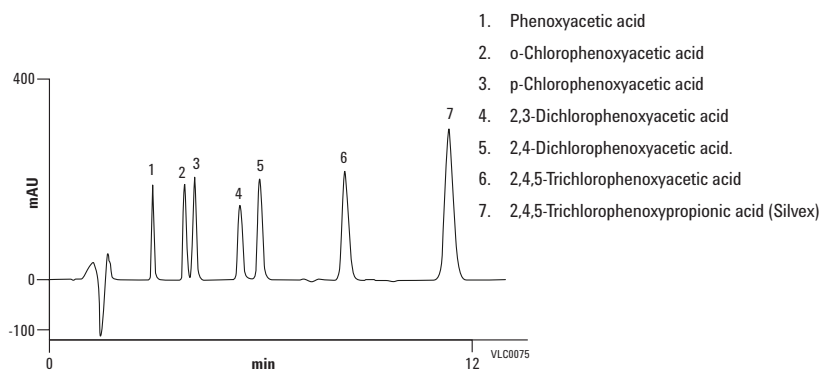
Column: Pursuit XR8 C8
A6010150X046
4.6 x 150 mm, 5 μm

Mobile Phase: MeCN:water+0.1%
HCOOH, 50:50

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm



Triazine Pesticides on Bonus-RP and Alkyl C8 Phase

Column: ZORBAX Bonus-RP
883668-901
4.6 x 150 mm, 5 µm

Mobile Phase: MeOH: 0.1% TFA (70:30)*

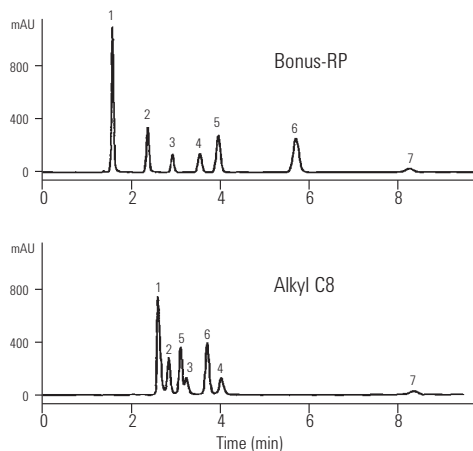
Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: 254 nm

Sample: Triazine pesticides, 2 µL

1. Prometryne
2. Tebuthion
3. Atrazine
4. Propazine
5. Diuron
6. Propanil
7. Dacthal



* For low pH work with Bonus-RP, a TFA mobile phase is often preferred over phosphate, and is compatible with LC/MS.

LCEN017

Phenols, Substituted

Column: ZORBAX SB-C18
883975-902
4.6 x 150 mm, 5 µm

Mobile Phase: 20% ACN/80% 0.01 M H₃PO₄
to 45% ACN in 7.5 min.

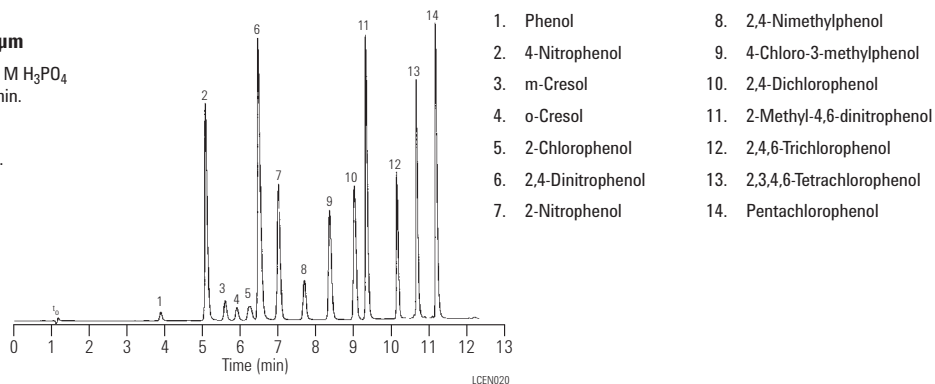
Flow Rate: 1.5 mL/min

Gradient: 80% ACN in 2.0 min.

Temperature: 35°C

Detector: UV 254 nm

Sample: Phenols



LCEN020

**Plant Hormones:
Rapid Gradient Elution Separation**

Column: ZORBAX Rx/SB-C8
866953-906
4.6 x 75 mm, 3.5 µm

Mobile Phase: A: Water with 0.1% TFA
B: Acetonitrile with 0.1% TFA

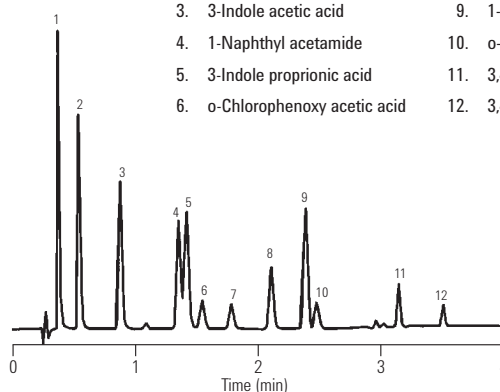
Flow Rate: 3.0 mL/min

Temperature: 60°C

Detector: UV 245 nm

Sample: Plant hormones

- | | |
|--------------------------------|---|
| 1. Kinetin | 7. p-Chlorophenoxy acetic acid |
| 2. n-6-Benzyl adenine | 8. 3-Indole butyric acid |
| 3. 3-Indole acetic acid | 9. 1-Naphthyl acetic acid |
| 4. 1-Naphthyl acetamide | 10. o-Chlorophenoxy propionic acid |
| 5. 3-Indole propionic acid | 11. 3,4,5-Trichlorophenoxy acetic acid |
| 6. o-Chlorophenoxy acetic acid | 12. 3,4,5-Trichlorophenoxy propionic acid |



LCEN022

VX Nerve Agent Metabolites by LC/MS-IS Standard (C13 labeled)

Column: ZORBAX NH2
860700-708
2.1 x 50 mm, 5 µm

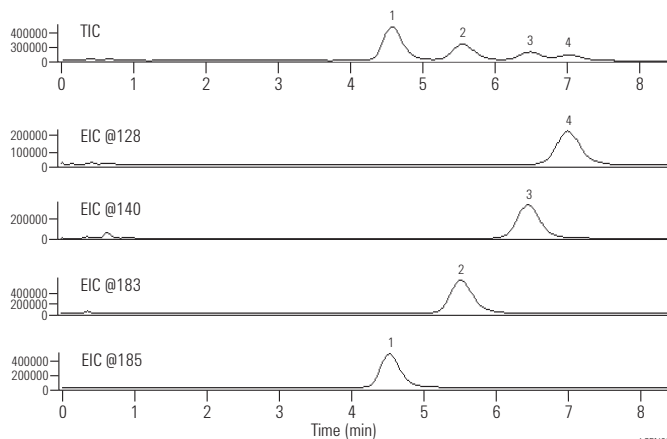
Mobile Phase: 1:1 (20 mM Ammonium Acetate pH 4.5/Acetonitrile)

Flow Rate: 0.5 mL/min, 1 µL injection (prepared std in ACN)

Temperature: 35°C

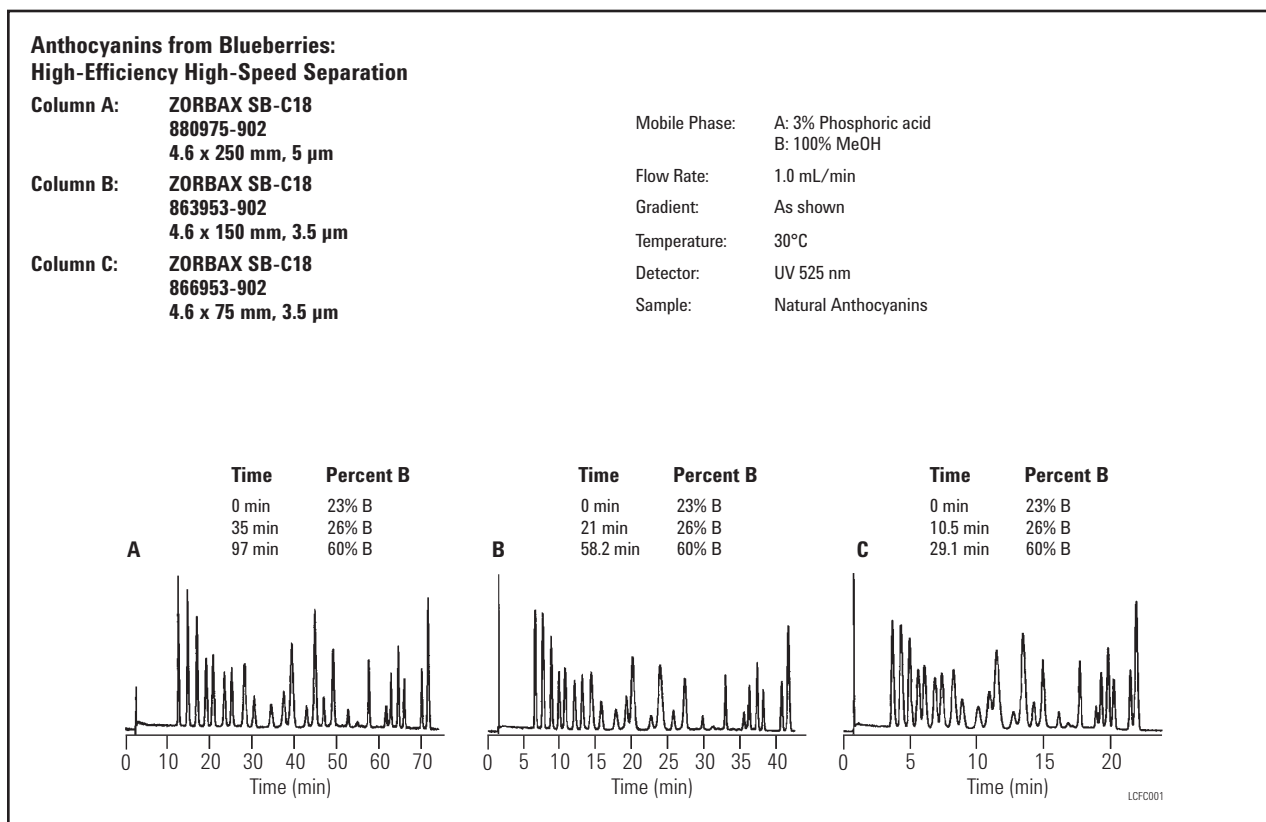
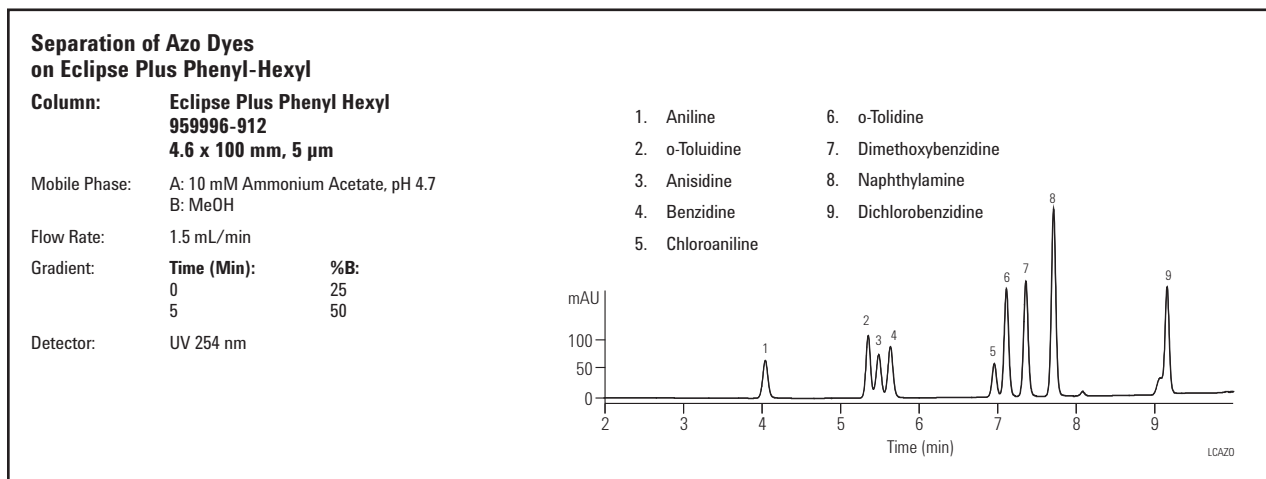
Detector: ESI-Negative Ion, Gas Flow 12 L/min, Nebulizer 60 psi

Sample	MW
1. Cyclohexyl methylphosphonic acid	178
2. Pinacolyl methylphosphonic acid	180
3. Isopropyl methylphosphonic acid	138
4. Ethyl methylphosphonic acid	124



LCEN025

Food and Consumer Product Applications



Aromatics II

Column: Eclipse XDB-Phenyl
963967-912
4.6 x 150 mm, 3.5 µm

Mobile Phase: H₂O: MeOH, 40:60

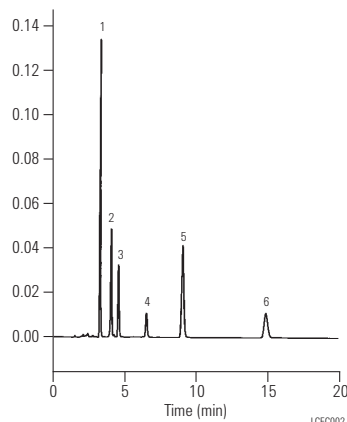
Flow Rate: 1.0 mL/min

Temperature: 35°C

Detector: 254 nm

Publication: FD13

Sample: Aromatic Sample



1. Acetophenone
2. Cinnamaldehyde
3. Eugenol
4. Cinnamaldehyde Impurity
5. Ethyl cinnamate
6. p-Cymene

Aspartame: Metabolites and Applications

Column: ZORBAX SB-C18
866953-902
4.6 x 75 mm, 3.5 µm

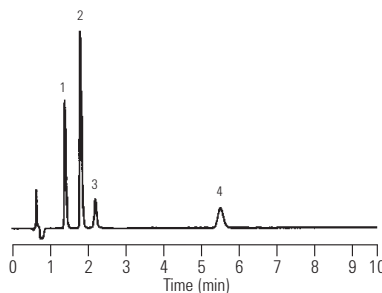
Mobile Phase: 85/15, 0.1% TFA/ACN

Flow Rate: 1.0 mL/min

Temperature: 35°C

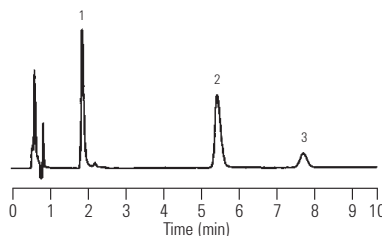
Detector: UV 210 nm

Sample: Aspartame



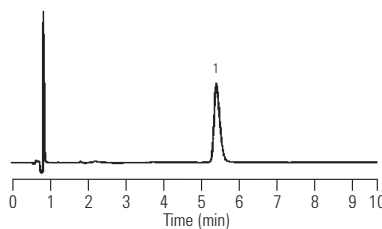
Aspartame and Its Metabolites

1. Phenylalanine
2. 5-benzyl-3,6-dioxo-2-piperazineacetic acid
3. Aspartic acid-phenylalanine dipeptide
4. Aspartame



Diet Coke

1. Caffeine
2. Aspartame
3. Unknown



Equal Sweetener

1. Aspartame

Carbohydrates: Carbohydrate Standards

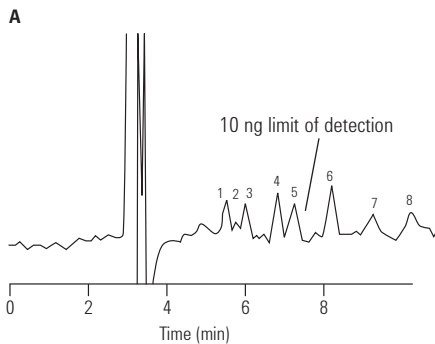
Column: ZORBAX Carbohydrate Analysis
843300-908
4.6 x 150 mm, 5 µm

Mobile Phase: 63% CH₃CN/H₂O
Flow Rate: 0.5 mL/min.

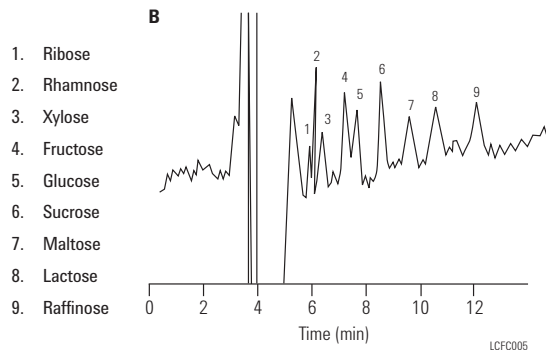
Detector: Agilent RID

Sample: Carbohydrate standard:
A: 25 ng/L, 1 µL injected
B: 500 µg/L, 50 µL injected

Carbohydrates: Separation Showing High Sensitivity



Sensitivity of High Injection Volume (50 µL)



Carbohydrates: Effect of Mobile Phase Strength

Column: ZORBAX NH2
880952-708
4.6 x 250 mm, 5 µm

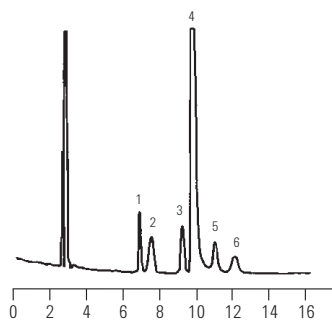
Mobile Phase: ACN/Water, as indicated
Flow Rate: 1.0 mL/min

Temperature: Ambient

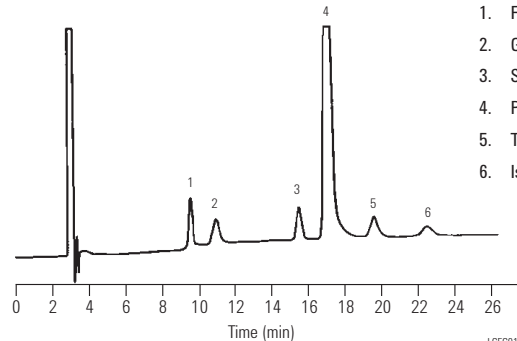
Detector: RI

Sample: Mono- and Disaccharides

ACN/H₂O: 70/30



ACN/H₂O: 75/25



1. Fructose
2. Glucose
3. Saccharose
4. Palatinose
5. Trehalulose
6. Isomaltose

Carbohydrates in Colas

Column: ZORBAX Carbohydrate Analysis
843300-908
4.6 x 150 mm, 5 µm

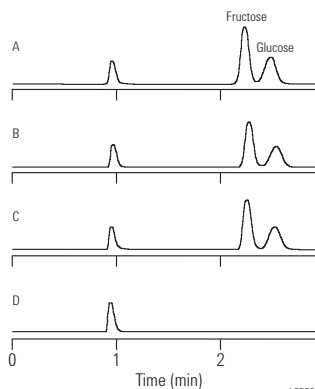
Mobile Phase: 75% ACN:25% H₂O

Flow Rate: 2.0 mL/min

Temperature: 30°C

Detector: RID

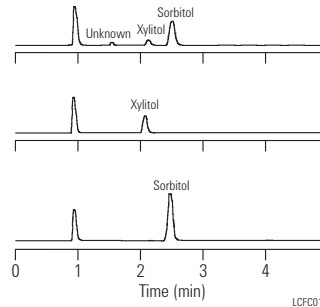
Sample: No dilution
A: COLA, Fountain
B: COLA, Can, Brand A
C: COLA, Brand B
D: COLA, Brand B, diet



Carbohydrates: Sugar Alcohols

Column: ZORBAX Carbohydrate Analysis
843300-908
4.6 x 150 mm, 5 µm

Mobile Phase: 75% ACN:25% H₂O
Flow Rate: 2.0 mL/min
Temperature: 30°C
Detector: RID
Sample: Chewing gum, sugar-free

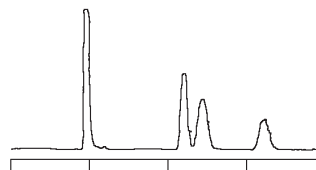


LCFC014

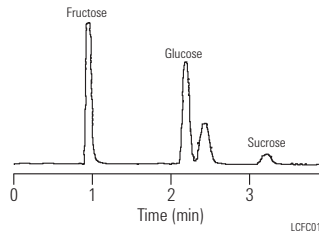
Carbohydrates in Juices

Column: ZORBAX Carbohydrate Analysis
843300-908
4.6 x 150 mm, 5 µm

Mobile Phase: 75% ACN/25% H₂O
Flow Rate: 2.0 mL/min
Temperature: 30°C
Detector: RID
Sample: diluted to 0.1X in 50:50 ACN:H₂O



Apple Drink
36.8% Fructose
24.9% Sucrose
38.3% Glucose



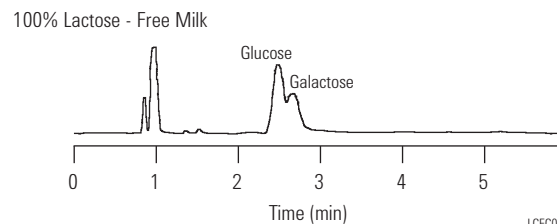
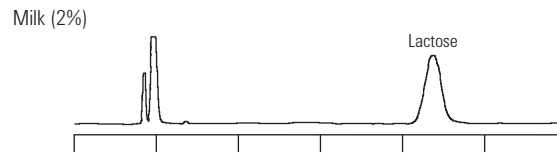
Apple Juice
58.7% Fructose
9.9% Sucrose
33.4% Glucose

LCFC016

Carbohydrates in Milk

Column: ZORBAX Carbohydrate Analysis
843300-908
4.6 x 150 mm, 5 µm

Mobile Phase: 75% ACN/25% H₂O
Flow Rate: 2.0 mL/min
Temperature: 30°C
Detector: RID
Sample: Partitioned between MeCl₂: H₂O



LCFC015

Flavoring Agents

Column: ZORBAX SB-Phenyl
860975-912
2.1 x 50 mm, 5 µm

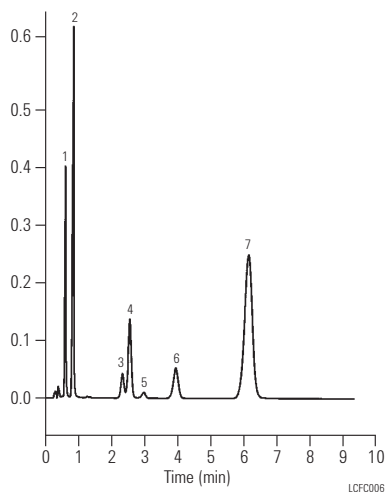
Mobile Phase: 0.3% TFA: ACN, 65:35

Flow Rate: 0.3 mL/min.

Temperature: Ambient

Detector: UV 254 nm

Sample: Cool Mint Listerine Sample



1. Unknown
2. Benzoic acid
3. Methyl salicylate
4. Carvone
5. Unknown
6. Thymol
7. Anethole

Food Colors, FD&C

Column: ZORBAX Eclipse XDB-C18
935967-902
4.6 x 50 mm, 3.5 µm

Mobile Phase: A: 0.1% TF A, pH to 4.4 with TEA, B: MeOH

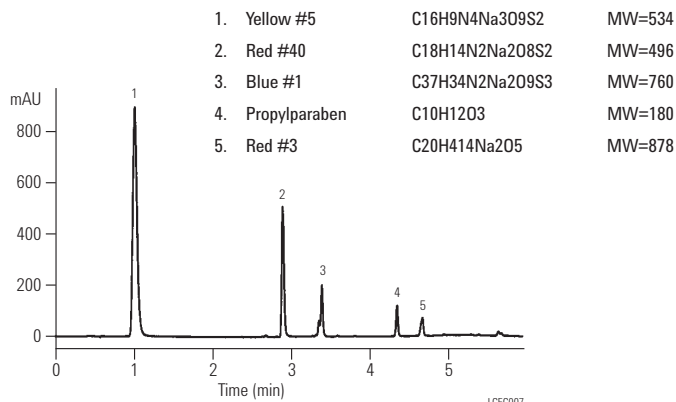
Flow Rate: 1.0 mL/min

Gradient: 17 to 100% B/4 min

Temperature: Ambient

Detector: UV 254 nm

Publication: LI FD16



- | | | |
|------------------|-----------------|--------|
| 1. Yellow #5 | C16H9N4Na3O9S2 | MW=534 |
| 2. Red #40 | C18H14N2Na2O8S2 | MW=496 |
| 3. Blue #1 | C37H34N2Na2O9S3 | MW=760 |
| 4. Propylparaben | C10H12O3 | MW=180 |
| 5. Red #3 | C20H414Na2O5 | MW=878 |

Neutraceuticals: Extract from Green Tea

Column: ZORBAX SB-C8
 863953-906
 4.6 x 150 mm, 3.5 µm

Mobile Phase: 75% 0.1% Trifluoroacetic acid: 25%
 Methanol

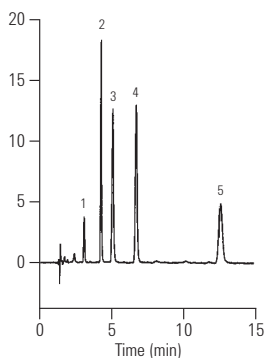
Injection: 1 mL/min

Temperature: 40°C

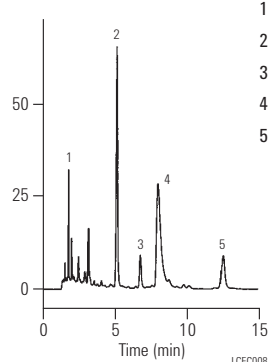
Detector: UV 280 nm

Sample: Green tea extract, 5 µL

Catechin Mixture



Green Tea Extract



1. Epigallocatechin
2. Epicatechin
3. Epigallocatechin gallate
4. Catechol
5. Epicatechin gallate

LCFC008

Tocopherols by LC/MS with APPI

Column: Eclipse XDB-C18
 993967-302
 3.0 x 150 mm, 5 µm

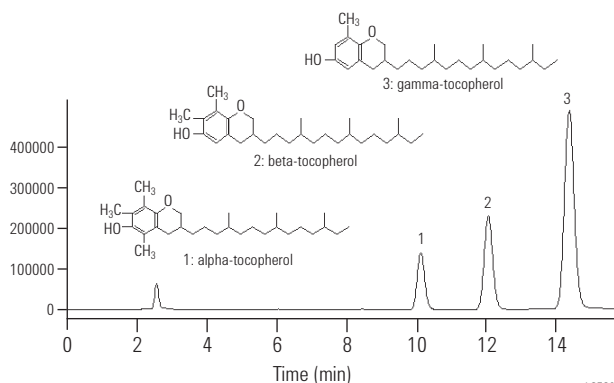
Mobile Phase: 97% MeOH: 3% 10 mM CH₃COONH₄

Flow Rate: 0.5 mL/min

Temperature: 40°C

MS Conditions: MS: Agilent 1100MSD SL
 Ionization: APPI (Positive)
 Scan range: m/z 100-500
 Vcap: 1500 V
 SIM ion: base peak
 Drying gas: 7 L/min at 350°C
 Nebulizer gas: 60 psi
 Vaporizer temp: 350°C
 Fragmentor: 140 V
 EM gain: 4

Sample Volume: 10 µL



LCFC011

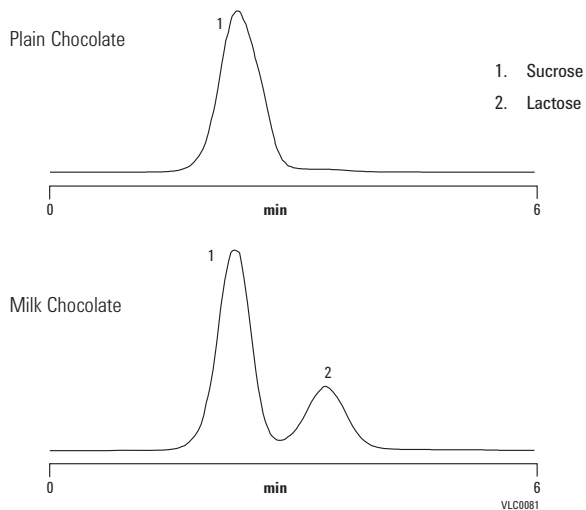


For a comprehensive listing of chromatograms searchable by compound name, visit our
 online Chromatogram Library at www.agilent.com/chem/library

Sugars in plain and milk chocolate

Column: Hi-Plex Pb
PL1170-6820
7.7 x 300 mm, 8 µm

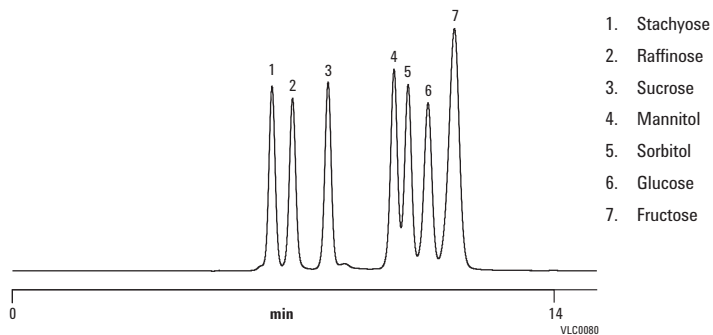
Mobile Phase: Water
Flow Rate: 0.6 mL/min
Temperature: 80°C
Detector: RI



Sugars

Column: Hi-Plex K
PL1170-6860
7.7 x 300 mm, 8 µm

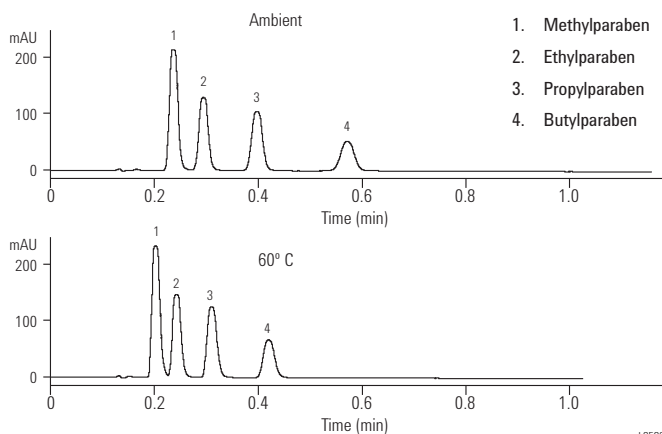
Sample: Sugars mixture (all 10 mg/mL), 20 µL injection
Mobile Phase: Water
Flow Rate: 0.6 mL/min
Temperature: 85°C
Detector: 356-LC RI



Parabens: High Speed Separation

Column: ZORBAX SB-C18 Rapid Resolution
Cartridge
833975-902
4.6 x 30 mm, 3.5 µm

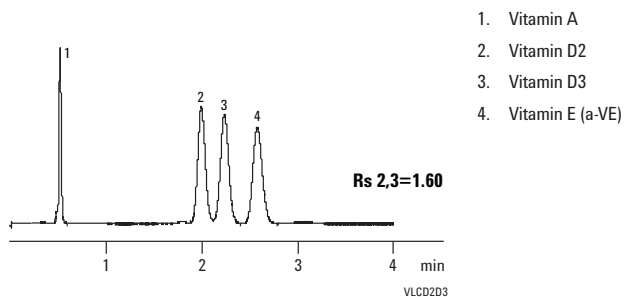
Mobile Phase: 0.1% H₃PO₄: ACN, (50:50)
Flow Rate: 2 mL/min
Temperature: top: ambient, bottom: 60°C
Detector: UV 254 nm with standard flow cell (13 µL)
Sample: Parabens, 1 µL



Separation of Vitamin D2/D3

Column: Eclipse PAH
959941-918
4.6 x 50 mm, 1.8 μm

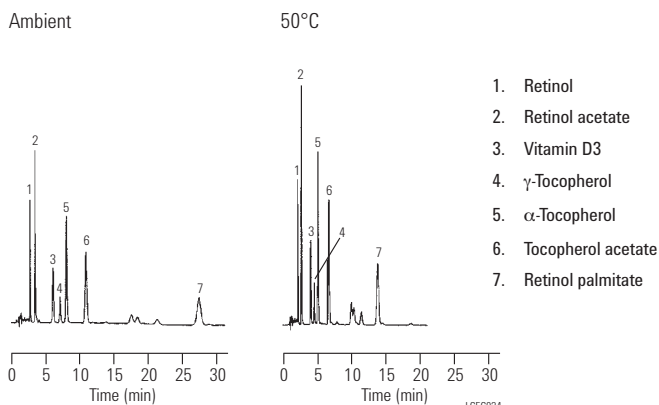
Mobile Phase: 92% MeOH, 8% water
Flow Rate: 2 mL/min
Temperature: 40°C
Detector: 325 nm for VA/280 nm for VD and VE



Fat-Soluble Vitamins on ZORBAX Eclipse XDB-C8

Column: Eclipse XDB-C8
993967-906
4.6 x 150 mm, 5 μm

Mobile Phase: 5/95 Water/MeOH
Flow Rate: 1.0 mL/min
Temperature: A: Ambient
B: 50°C
Detector: UV 280 nm
Sample: Fat Soluble Vitamins



Water-Soluble Vitamins

Column: ZORBAX SB-C8
883975-906
4.6 x 150 mm, 5 µm

Mobile Phase: A: 50 mM Sodium Phosphate, pH 2.5/MeOH (90/10)
B: 50 mM Sodium Phosphate, pH 2.5/MeOH (10/90)

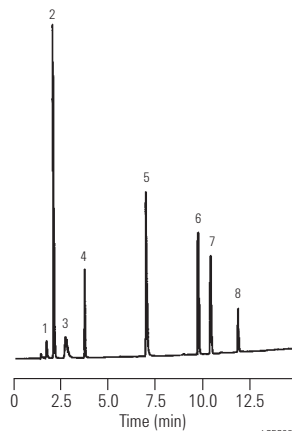
Flow Rate: 1.0 mL/min

Gradient: 0-70% B in 18 min

Temperature: Ambient

Detector: UV 245 nm

Sample: Water soluble vitamins



1. B₁-Thiamine
2. Vitamin C
3. B₃-Niacin
4. B₆-Pyridoxine
5. Pantothenic acid
6. Folic acid
7. B₁₂-Cyanocobalamin
8. B₂-Riboflavin

Water-Soluble Vitamins: High Speed Separation using Ion-Pairing

Column: ZORBAX Rx/SB-C8
866953-906
4.6 x 75 mm, 3.5 µm

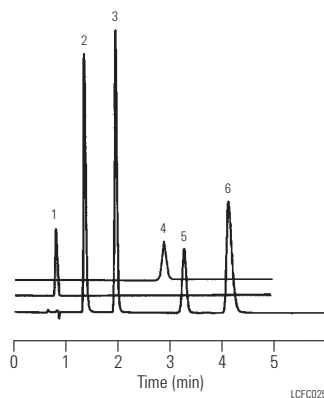
Mobile Phase: 10 mM Hexane Sulfonate with 0.1% Phosphoric Acid: MeOH (74:26)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV 245 nm

Sample: Water soluble vitamins



1. Vitamin C
2. B₃-Niacin
3. B₆-Pyridoxine
4. Folic acid
5. B₂-Riboflavin
6. B₁-Thiamine



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

**Water-Soluble Vitamins
using the USP 23 Method**

Column: ZORBAX SB-C18
880975-902
4.6 x 250 mm, 5 µm

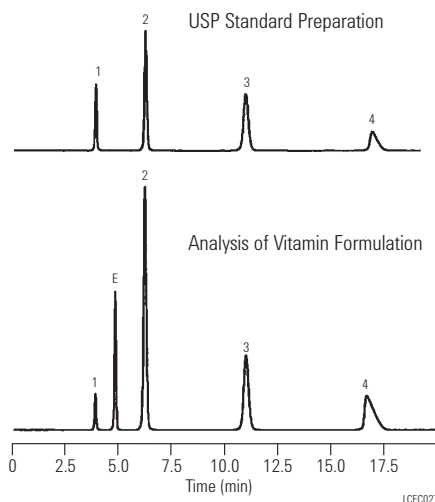
Mobile Phase: 7.2 mM Hexane Sulfonate/MeOH/Acetic Acid
(73/27/1) (ratio to 101)

Flow Rate: 1.0 mL/min

Temperature: 30° C

Detector: UV 280 nm

Sample: Water soluble vitamins



- 1. B₃-Niacin
- 2. B₆-Pyridoxine
- 3. B₂-Riboflavin
- 4. B₁-Thiamine
- E. Excipient

**Water Soluble B Vitamins
Separated on ZORBAX SB-Aq**

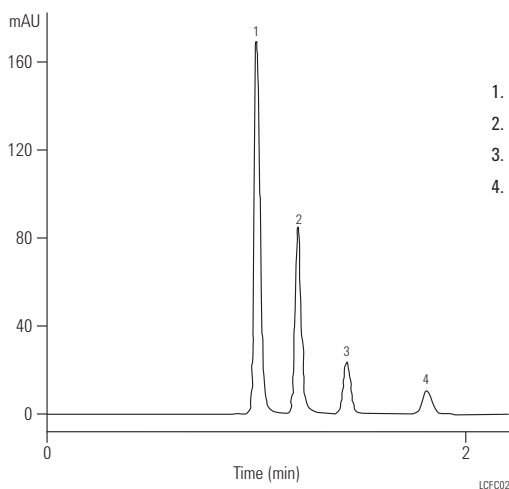
Column: ZORBAX SB-Aq
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 5% MeOH/95% TFA (0.1%)

Flow Rate: 2.0 mL/min

Temperature: 35° C

Detector: UV 254 nm



- 1. Thiamine
- 2. Nicotinic Acid
- 3. Pyridoxine
- 4. Niacinamide

Sunscreen Ingredients: Perform conventional, fast and ultra-fast separations on the same column family

Column A: Eclipse XDB-C18
993967-902
4.6 x 150 mm, 5 μ m
6 μ L inj

Column B: Eclipse XDB-C18
961967-902
4.6 x 100 mm, 3.5 μ m
4 μ L inj

Column C: Eclipse XDB-C18
927975-902
4.6 x 50 mm, 1.8 μ m
2 μ L inj

Mobile Phase: A: 15% water
B: 85% MeOH

Flow Rate: 1.0 mL/min

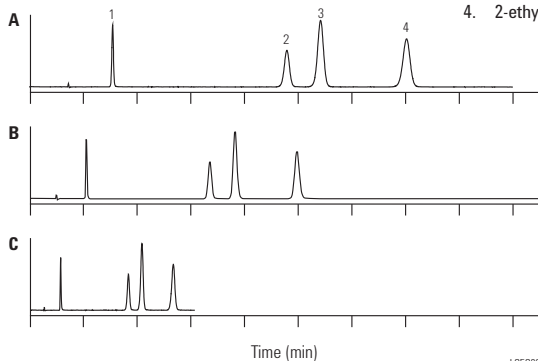
Temperature: Ambient

Detector: UV 254 nm

Publication: 5989-4721EN

Sample: Sunscreens

1. 2-hydroxy-4-methoxybenzophenone
2. Padimate O
3. 2-ethylhexyl trans-4-methoxycinnamate
4. 2-ethylhexyl salicylate



LCFC029

Fast Vitamin E Analysis on Rapid Resolution HT

Column A: Eclipse XDB-C18
927975-902
4.6 x 50 mm, 1.8 μ m

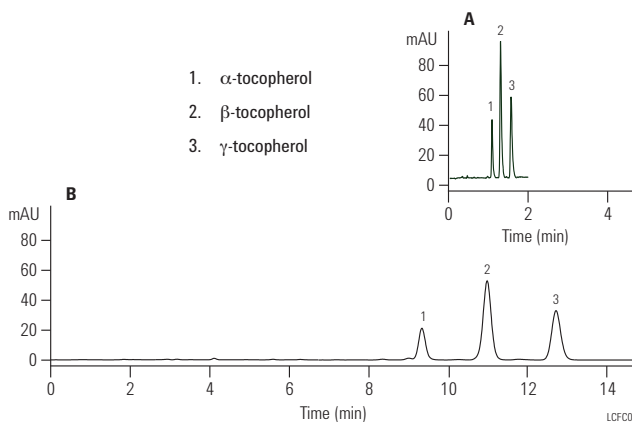
Column B: Eclipse XDB-C18
993967-902
4.6 x 150 mm, 5 μ m

Mobile Phase: A: 5% water
B: 95% MeOH

Flow Rate: 3 mL/min, 1 mL/min

Temperature: Ambient

1. α -tocopherol
2. β -tocopherol
3. γ -tocopherol



LCFC030

Theobromine in Beverages

Column: ZORBAX SB-C18
827975-901
4.6 x 50 mm, 1.8 µm

Mobile Phase: A: 92% 0.1% formic acid
B: 8% 0.1% formic acid in ACN

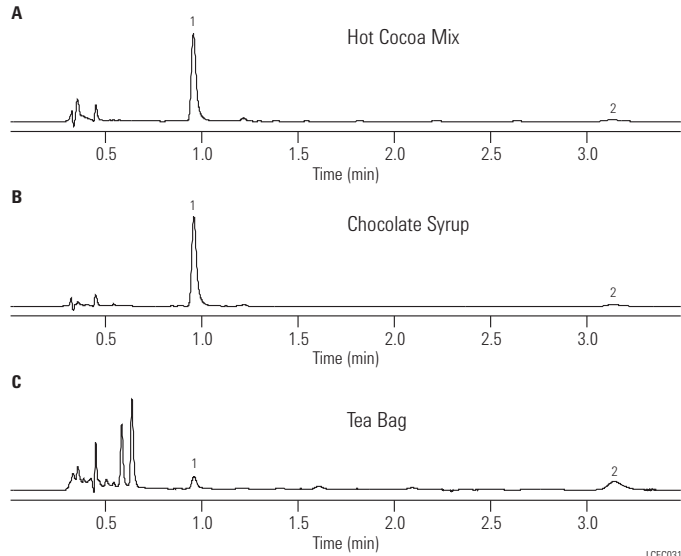
Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV 254 nm, flow cell 2 µL, 3 mm flow path

Sample: Theobromine

- 1. Theobromine
- 2. Caffeine



Kava kava analysis

Column: Pursuit UPS^{2.4} C18
A8100050X020H
2 x 50 mm, 2.4 µm

Mobile Phase: A: 0.5% formic acid in water
B: 0.5% formic acid in MeOH

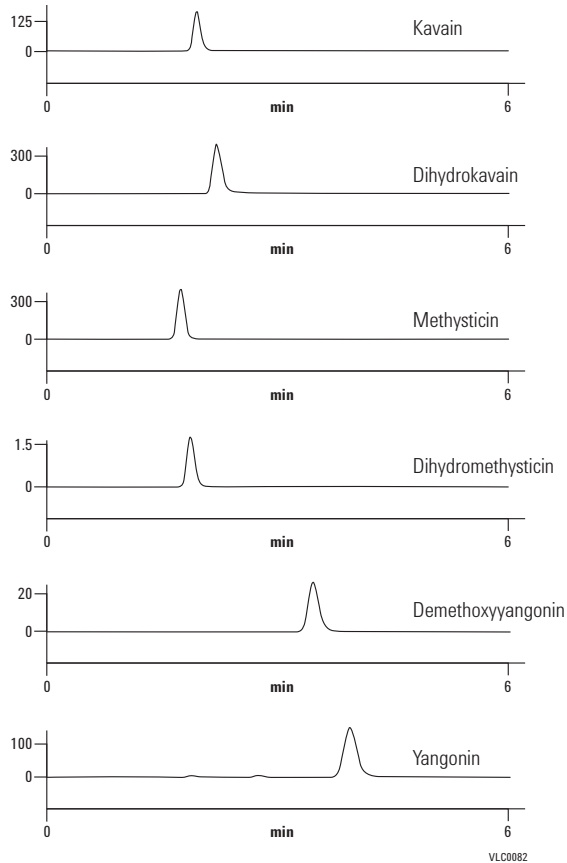
Gradient: 50% B isocratic

Flow Rate: 0.5 mL/min

Col Temp: 30°C

Sample Temp: Ambient

Detector: 320-MS

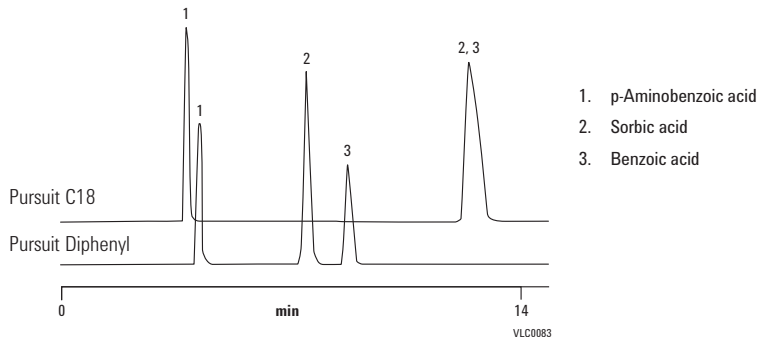


Benzoic acid/sorbic acid

Mobile Phase: 0.1% formic acid in water:
0.1% formic acid in MeCN, 80:20

Flow Rate: 0.7 mL/min

Detector: UV, 254 nm



Quantification and qualification of vitamin C and citric acid in fresh grapefruit juice

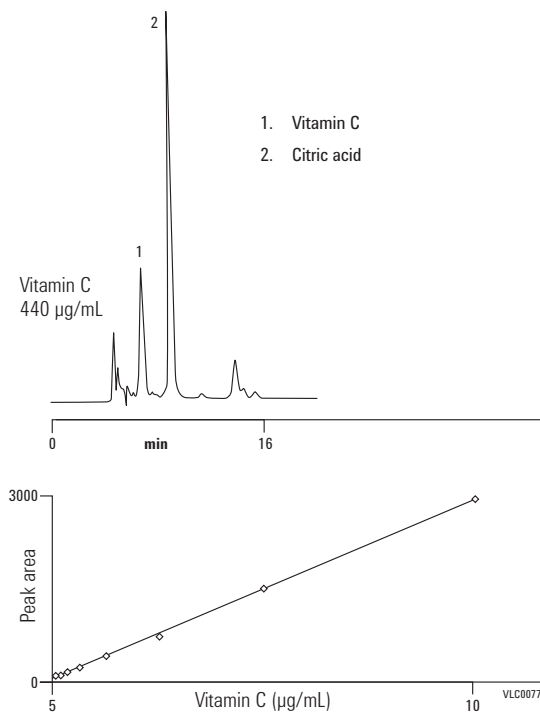
Column: PLRP-S 100Å
PL1512-5500
4.6 x 250 mm, 5 µm

Sample: Diluted 1:50 in eluent

Mobile Phase: 0.2M NaH₂PO₄, pH 2.14

Flow Rate: 0.5 mL/min

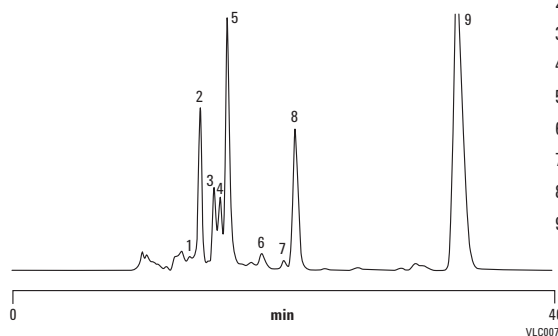
Detector: UV, 220 nm



Rose wine

Column: Hi-Plex H
 PL1170-6830
 7.7 x 300 mm, 8 µm

Mobile Phase: 0.004M H₂SO₄
 Flow Rate: 0.4 mL/min
 Pressure: 13 bar
 Temperature: 75°C
 Detector: 356-LC refractive index detector

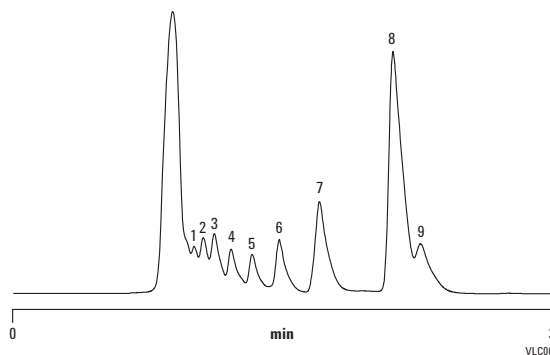


1. Citric acid
2. Tartaric acid
3. Glucose
4. Malic acid
5. Fructose
6. Succinic acid
7. Lactic acid
8. Glycerol
9. Ethanol

Sports drink

Column: Hi-Plex Na
 PL1171-6140
 7.7 x 300 mm, 10 µm

Sample: High energy orange flavor non-carbonated sports drink
 Mobile Phase: Water
 Flow Rate: 0.3 mL/min
 Temperature: 80°C
 Detector: RI

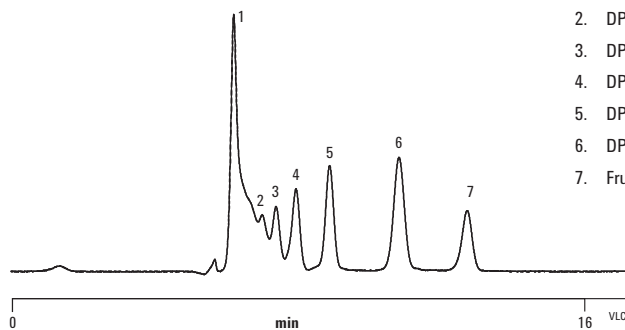


1. Dp8
2. Dp7
3. Dp6
4. Dp5
5. Dp4
6. Dp3
7. Dp2
8. Dp1 (Glucose)
9. Fructose

Oligosaccharides

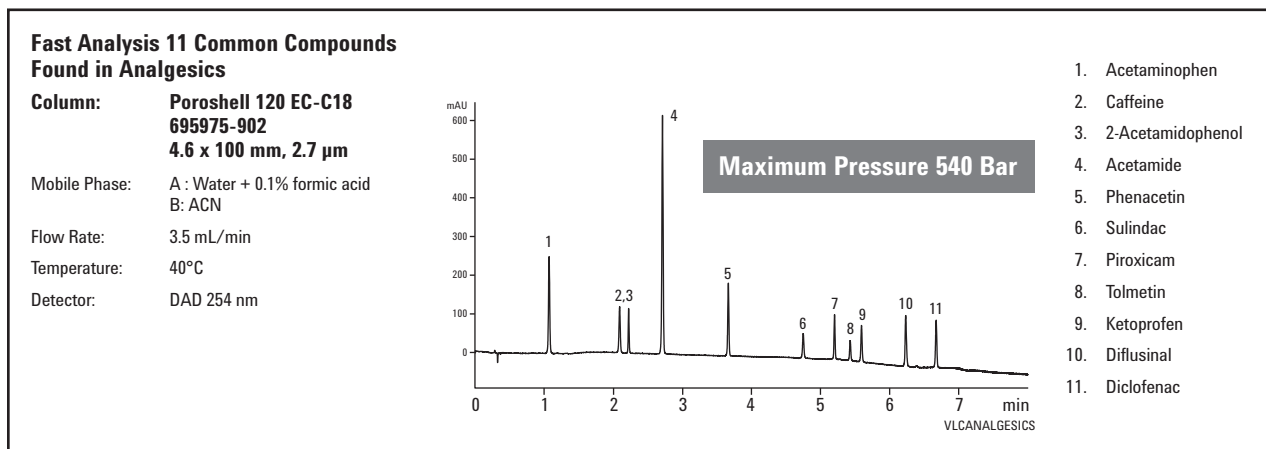
Column: Hi-Plex Ca (Duo)
 PL1F70-6850
 6.5 x 300 mm, 8 µm

Mobile Phase: DI water
 Flow Rate: 0.5 mL/min
 Temperature: 90°C
 Detector: 356-LC RI



1. Higher MW sugars
2. DP5
3. DP4
4. DP3
5. DP2
6. DP1
7. Fructose

Pharmaceutical Applications



NEW!

Faster Analysis of USP Method for Simvastatin Tablet with Poroshell 120

Column A: Eclipse Plus C18
959990-902
4.6 x 250 mm, 5 μ m

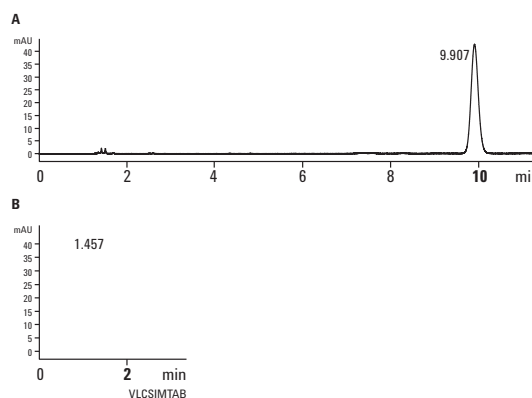
Column B: Poroshell 120 EC-C18
697975-902
4.6 x 75 mm, 2.7 μ m

Mobile Phase: 65% CH₃CN,
35% 3.9 g/L NaH₂PO₄ (pH 4.5)

Flow Rate: 1.5 mL/min for 5 μ m column
2.8 mL/min for 2.7 μ m Poroshell 120 column

Temperature: 45°C

Detector: DAD Sig = 238, 8
Ref = 360, 100 nm



	USP Requirement	5 μm (1.5 mL/min)	2.7 μm (2.8 mL/min)
T_R	N/A	9.907	1.457
k'	> 3.0	5.962	5.122
N	> 4500	16939	14439
T_f	< 2.0	1.09	1.10

NEW!**Faster Separation of Sulfa Drugs with Poroshell 120**

Column A: Eclipse Plus C18
959990-902
4.6 x 250 mm, 5 µm

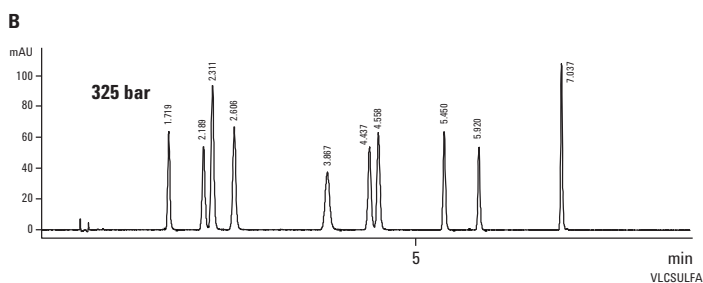
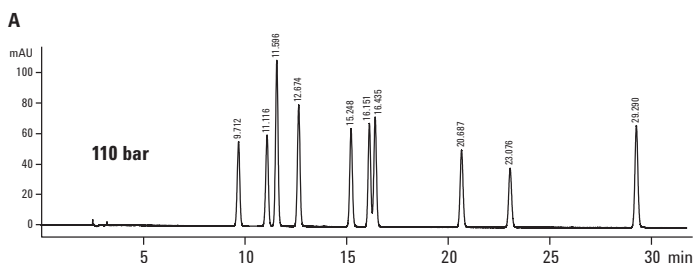
Time	%B
0	8
33	33
35	33

Column B: Poroshell 120 EC-C18
695975-902
4.6 x 100 mm, 2.7 µm

Time	%B
0	8
12	33
13.2	33

Mobile Phase: A: 0.1% formic acid in Water
B: 0.1% formic acid in ACN

Flow Rate: 1 mL/min

**Separation of Pharmaceutical Cardiac Drugs on Eclipse Plus C18**

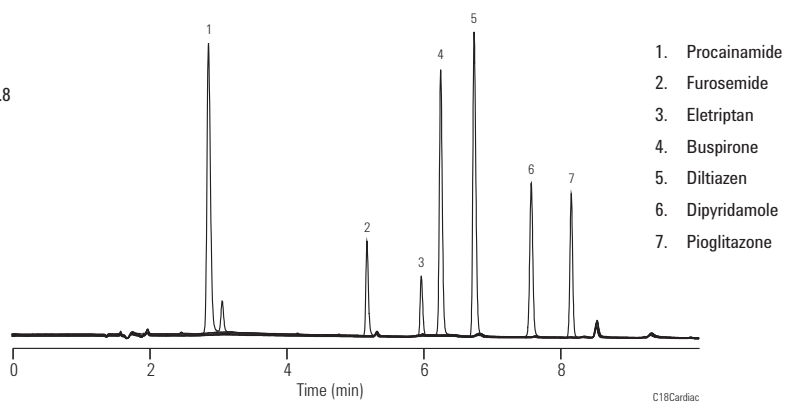
Column: Eclipse Plus C18
959996-902
4.6 x 100 mm, 5 µm

Mobile Phase: A: 20 mM Ammonium Acetate, pH 4.8
B: ACN

Flow Rate: 1 mL/min

Gradient: 10-90% in 10 min

Detector: UV 254 nm



Fast and Ultra-Fast Analysis of Basic Compounds on Eclipse Plus

Column: Eclipse Plus C18
959941-902
4.6 x 50 mm, 1.8 μ m

Mobile Phase: A: 50% 8 mM K_2HPO_4 , pH 7
B: 50% ACN

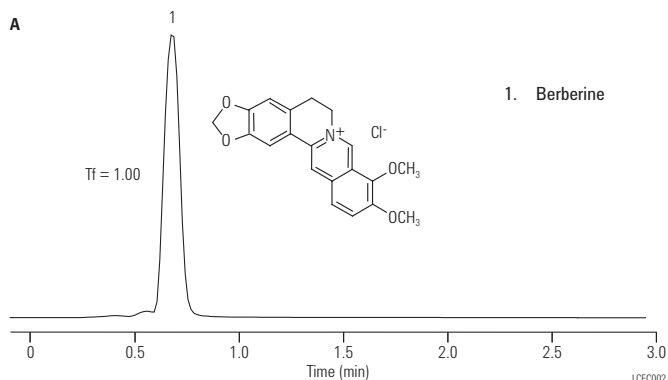
Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV 254 nm

Publication: 5989-4934EN

Sample: Berberine, 0.4 mg/mL, 2 μ L



Xanthines: Higher Resolution, Same Selectivity with RRHT

Column A: ZORBAX SB-C18
846975-902
4.6 x 50 mm, 5 μ m

Column B: ZORBAX SB-C18
827975-901
4.6 x 50 mm, 1.8 μ m

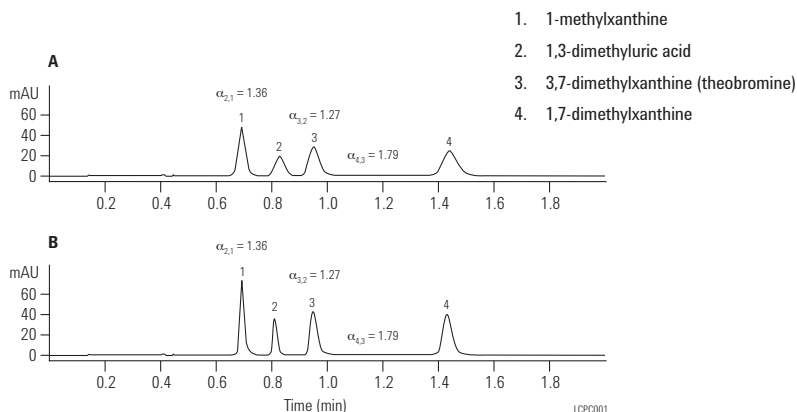
Mobile Phase: A: 92% 0.1% formic acid
B: 8% 0.1% formic acid in ACN

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV 254 nm

Sample: Xanthines



Antihistamines: Fast Separations on RRHT Extend-C18

Column A: ZORBAX Extend-C18
773450-902
4.6 x 150 mm, 5 μ m

Column B: ZORBAX Extend-C18
727975-902
4.6 x 50 mm, 1.8 μ m

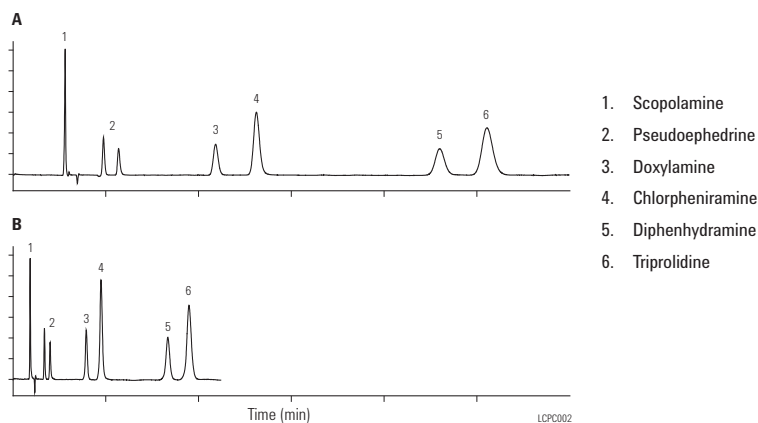
Mobile Phase: A: 30% 50 mM pyrrolidine buffer
B: 70% MeOH

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV 220 nm

Sample: Antihistamines



Ibuprofen: Optimizing Selectivity with RRHT Columns

Column A: SB-C8
827975-906
4.6 x 50 mm, 1.8 μ m

Column B: Eclipse XDB-C8
927975-906
4.6 x 50 mm, 1.8 μ m

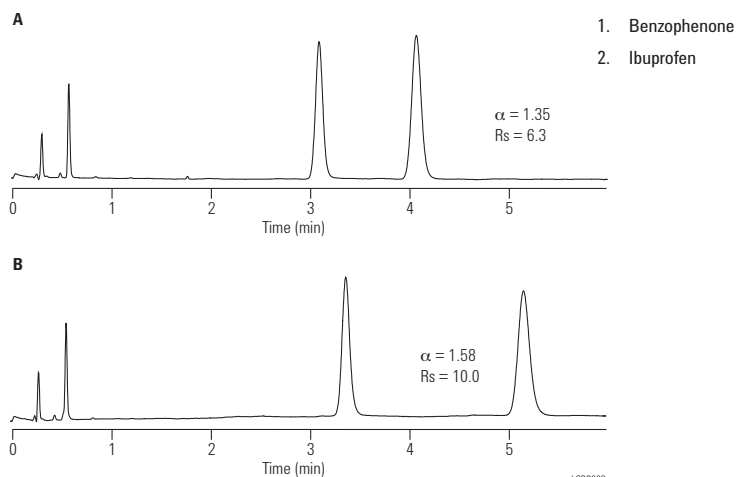
Mobile Phase: A: 63% water
B: 37% acetonitrile + 1.8 mL H₃PO₄

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV 254 nm

Sample: Ibuprofen Oral Suspension

**Analgesics**

Column: Pursuit XRs Diphenyl
A6020150X046
4.6 x 150 mm, 5 μ m

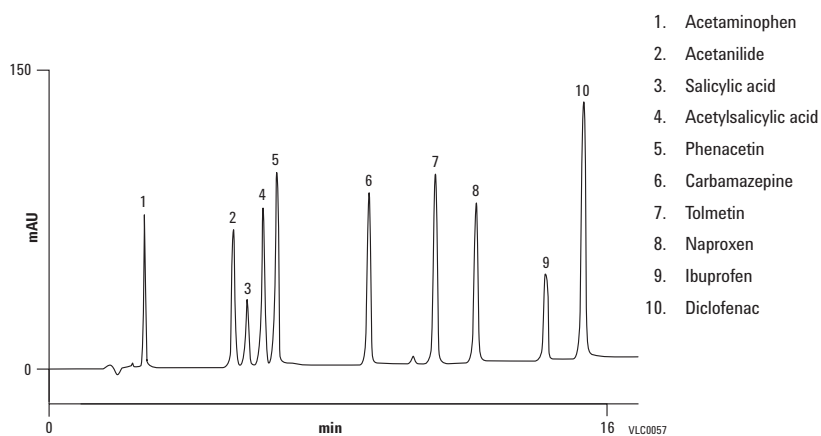
Mobile Phase: A: Water+0.1% HCOOH
B: MeCN+0.1% HCCOH

Gradient: 25-80% B in 20 min

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

Anesthetics, Local: Bonded Phase Selectivity

Column A: ZORBAX SB-C18
883975-902
4.6 x 150 mm, 5 µm

Column B: ZORBAX SB-C8
883975-906
4.6 x 150 mm, 5 µm

Column C: ZORBAX SB-C3
883975-909
4.6 x 150 mm, 5 µm

Column D: ZORBAX SB-Phenyl
883975-912
4.6 x 150 mm, 5 µm

Column E: ZORBAX SB-CN
883975-905
4.6 x 150 mm, 5 µm

Mobile Phase: A: 50 mM NaH₂PO₄ pH 2.5 in 95% H₂O/5% ACN
B: 50 mM NaH₂PO₄ pH 2.5 in 47% H₂O/53% ACN

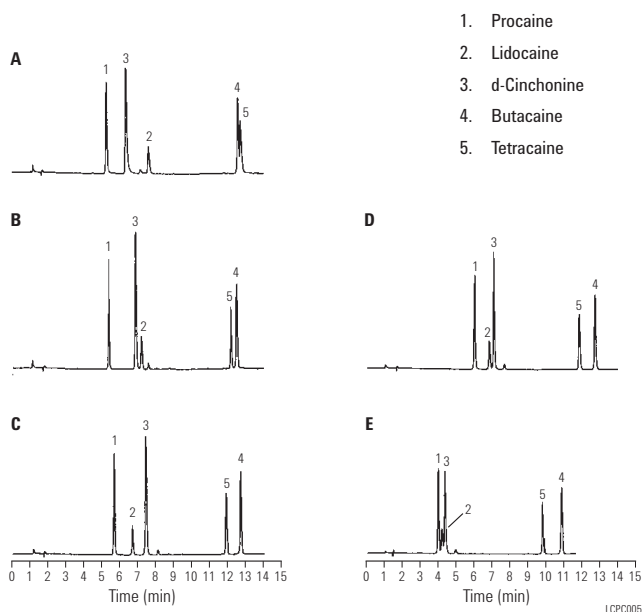
Flow Rate: 1.5 mL/min

Gradient: 0-100% B in 18.8 min

Temperature: 26°C

Detector: UV 254 nm

Sample: 10 µL, 10 µg/mL



Local anesthetics

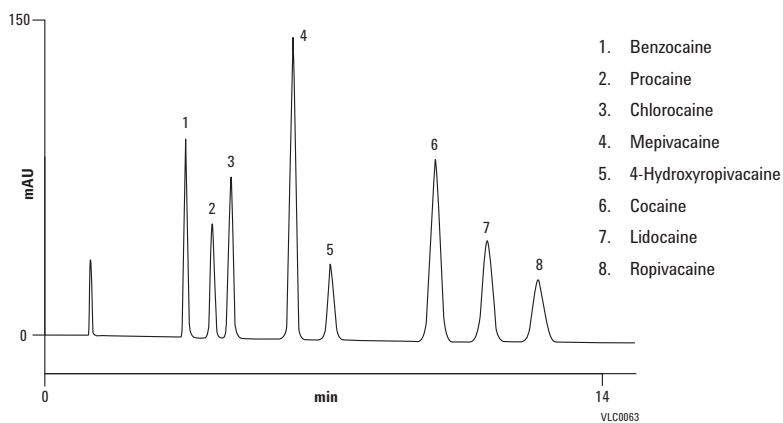
Column: Pursuit XR_s C8
A6010150X046
4.6 x 150 mm, 5 µm

Mobile Phase: MeOH:5 mM NH₄CO₃ 65:35, pH 10

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 210 nm



Antibiotics: High Speed Separation

Column: ZORBAX Rx/SB-C8
866953-906
4.6 x 75 mm, 3.5 µm

Mobile Phase: 8.0% acetonitrile/92% 0.1% aqueous TFA

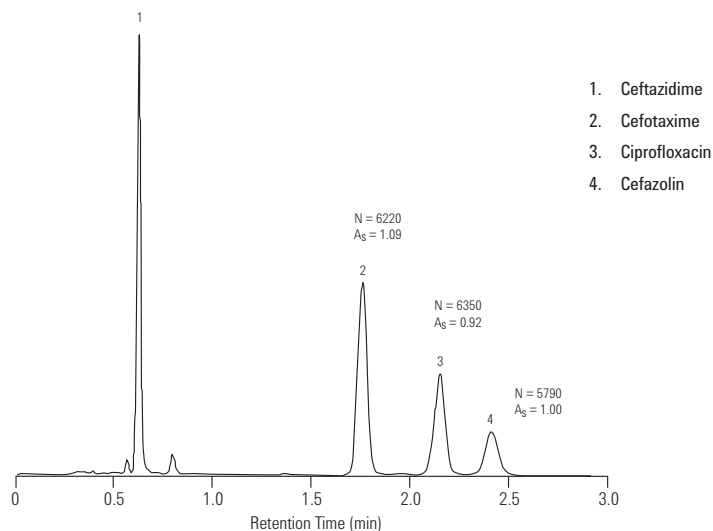
Flow Rate: 3.0 mL/min

Gradient: 45-70% B in 35 min

Temperature: 60°C

Detector: UV 260 nm

Sample: 1 µL containing 0.40, 0.36, 0.10 and 0.37 µg ea. of 1-4 resp.



- 1. Ceftazidime
- 2. Cefotaxime
- 3. Ciprofloxacin
- 4. Cefazolin

LCPC007

Antibiotics: Lincomycin and Clindamycin by LC-APCI-MS LC-TIC

Column: ZORBAX SB-C18 cartridge
823700-902
2.1 x 30 mm, 1.8 µm

Mobile Phase: Gradient: 15-50% B in 1 min, hold for 1.5 min.
A: 0.2% formic acid pH 2.8
B: ACN + 0.2% formic acid

Flow Rate: 0.5 mL/min

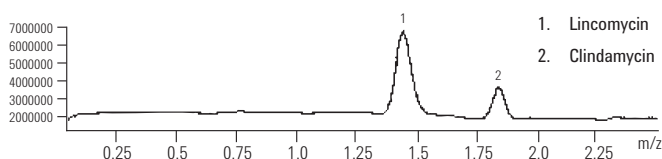
Gradient: Post time: 1.5 min

Temperature: Ambient

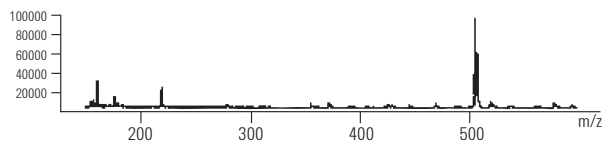
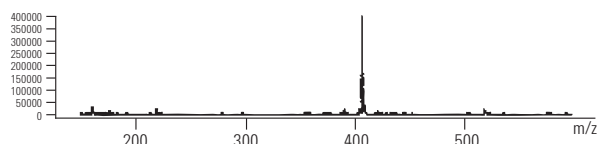
Detector: APCI, Positive ion

MS Conditions: Peak width: 0.10 min
Scan: 150-600 Da, step 0.1
Fragmentor: 70
Gas Temp: 350°C
Vaporizer: 350°C
Drying gas: 12 L/min
Nebulizer pres: 50 psi
Vcap: +3000 V
Corona: 4.0 µA

Sample: Antibiotics, 1 µL



- 1. Lincomycin
- 2. Clindamycin



LCPC008

Antifungal Medications

Column: ZORBAX Bonus-RP
883668-901
4.6 x 150 mm, 5 µm

Mobile Phase: 35% 25 mM NaH₂PO₄,
Dibasic (pH 6.5 with H₃PO₄); 65% ACN

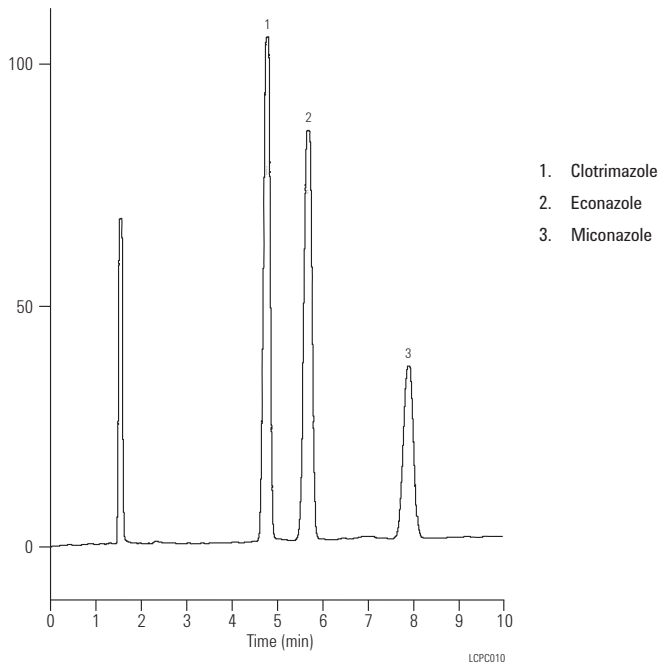
Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV 220 nm

Publication: LI PH46

Sample: Antifungals, 2 µL



Antifungals

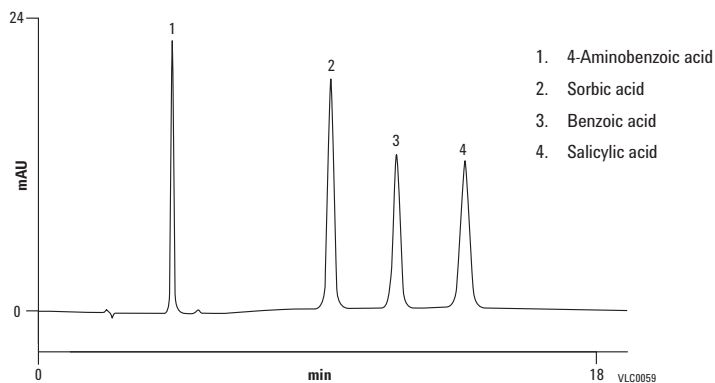
Column: Pursuit XRs Diphenyl
A6020150X046
4.6 x 150 mm, 5 µm

Mobile Phase: Water+0.1% HCOOH;
MeCN+0.1% HCOOH, 80:20

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



**Analgesics: Non-steroidal Anti-inflammatory
Drugs: Narrow Bore Separation**

Column: Eclipse XDB-C8
993700-906
2.1 x 150 mm, 5 μ m

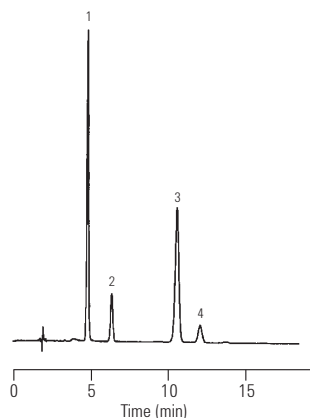
Mobile Phase: 50/50, 25 mM Sodium Phosphate
(pH 7.0 with Phosphoric Acid), MeOH

Flow Rate: 0.2 mL/min

Temperature: 35°C

Detector: UV 254 nm

Sample: 2 μ L, 10 ug/mL



NSAID	pK _a
1. Phenacetin	2.2
2. Tolmetin	3.5
3. Phenylbutazone	4.4
4. Fenoprofen	4.5

**Separation of Small Molecule Anorectics on
Bonus-RP and Traditional Alkyl Phase**

Column A: ZORBAX Bonus-RP
883668-901
4.6 x 150 mm, 5 μ m

Column B: Traditional Alkyl C8 Phase

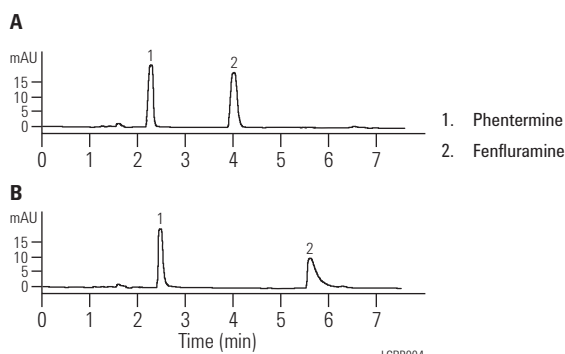
Mobile Phase: 25 mM K₂HPO₄, pH 7.2/MeOH: ACN
(50:50), 45/55

Flow Rate: 1 mL/min.

Temperature: Ambient

Detector: UV 254 nm

Sample: Anorectics "Fen-phen", 5 μ L


Aromatic Acids/Benzoic Acids—Selectivity Differences

Column A: ZORBAX SB-C8
880975-906
4.6 x 250 mm, 5 μ m

Column B: ZORBAX SB-Phenyl
880975-912
4.6 x 250 mm, 5 μ m

Column C: ZORBAX SB-CN
880975-905
4.6 x 250 mm, 5 μ m

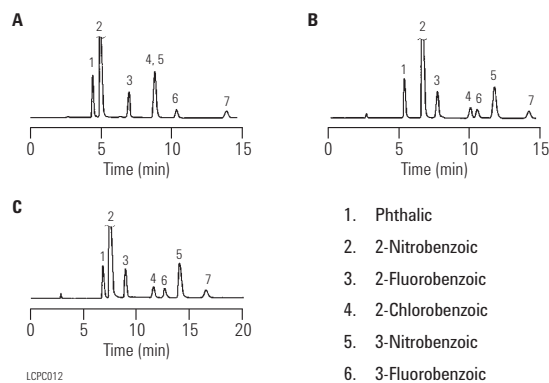
Mobile Phase: 30-45% methanol (above) in 25 mM Na Phosphate, pH 2.5
A: 45% Methanol
B: 40% Methanol
C: 30% Methanol

Flow Rate: 1.0 mL/min

Temperature: 35°C

Detector: UV 254 nm

Sample: Benzoic acids



Catecholamines/Biogenic Amines: Rapid Separation using Ion Pair Reagents

Column: ZORBAX Rx/SB-C8
866953-906
4.6 x 75 mm, 3.5 µm

Mobile Phase: 0.14 M sodium phosphate,
20 mM EDT A,
0.75 mM octyl sulfonate,
9% methanol pH 3.5

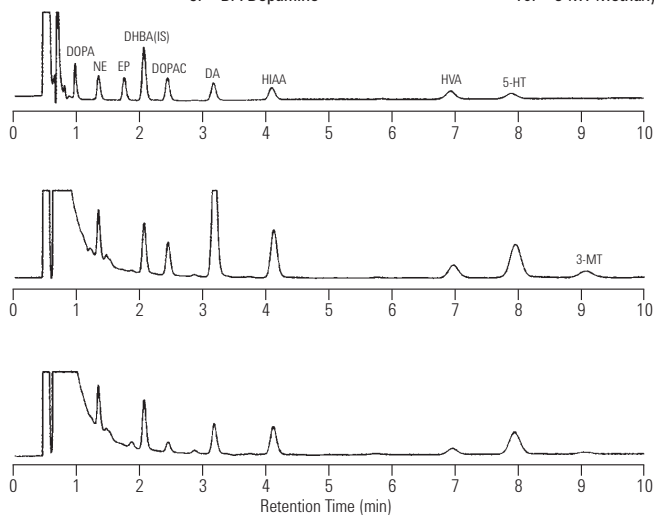
Flow Rate: 1.5 mL/min

Temperature: 26°C

Detector: 0.75 V vs Ag/AgCl with electro-
chemical detection

Sample: 10 µg/mL each standard; volume
20 µL (2 g tissue sample)
A. Standards (2pmol; DHBA 5pmol)
B. Mouse Satrium
C. Mouse Neocortex

- | | |
|--------------------------------------|----------------------------------|
| 1. DOPA-Dihydroxyphenylalanine | 6. HIAA-Hydroxyindoleacetic acid |
| 2. DHBA-Dihydroxybenzyl amine | 7. EP-Epinephrine |
| 3. DOPAC-Dihydroxyphenyl acetic acid | 8. HVA-Homovanillic acid |
| 4. NE-Norepinephrine | 9. 5-HT-Hydroxytryptamine |
| 5. DA-Dopamine | 10. 3-MT-Methoxytyrosine |



Chiral Ethiazide (Diuretic Drug) Separation on Ultron ES-OVM

Column: Ultron ES-OVM Chiral
702111651
4.6 x 150 mm, 5 µm

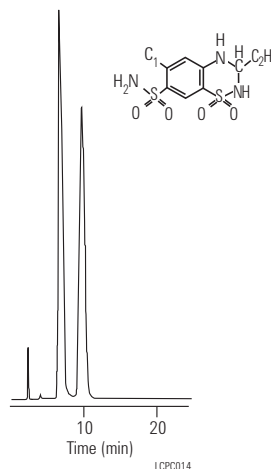
Mobile Phase: 20 mM KH₂PO₄ (pH 4.6)

Flow Rate: 1.0 mL/min

Temperature: 25° C

Detector: UV 220 nm

Sample: 20 µL containing 0.35 µg Ethiazide



Chiral Separation of Fluoxetine Enantiomers (Prozac) using Ultron ES-OVM

Column: Ultron ES-OVM Chiral
702111651
4.6 x 150 mm, 5 µm

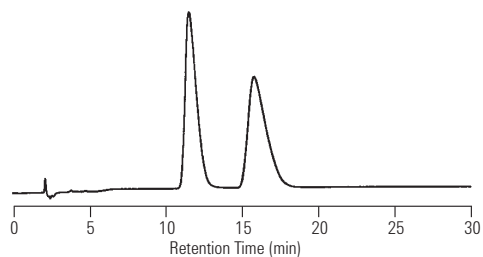
Mobile Phase: 25/75 (v/v) EtOH / 20 mM KH₂PO₄, pH 5.5
(adjusted with NaOH)

Flow Rate: 0.8 mL/min

Temperature: Ambient

Detector: UV 225 nm

Sample: Mixture Fluoxetine (Prozac) enantiomers



LCPC015

Courtesy of D.S. Ristry and V.S. Sharp, Eli Lilly and Co.

Goldenseal and Related Alkaloids on a Rapid Resolution Eclipse XDB-C18 Column

Column: Eclipse XDB-C18
963967-902
4.6 x 150 mm, 3.5 µm

Mobile Phase: 68% 30 mM ammonium acetate, 14 mM TEA, pH ~4.85
32% Acetonitrile

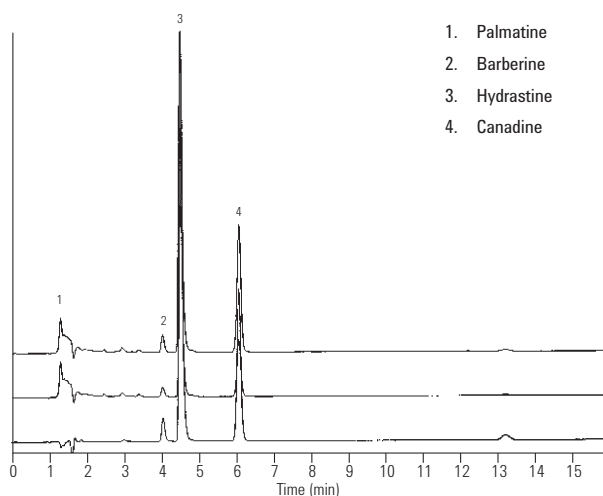
Flow Rate: 1.0 mL/min

Temperature: 30°C

Detector: 230 nm

Sample: Goldenseal and related Alkaloids

Alkaloids, such as the active components in Goldenseal and other related plants, are quickly and accurately separated using isocratic conditions on an Eclipse XDB-C18 Rapid Resolution column.



1. Palmatine
2. Barberine
3. Hydrastine
4. Canadine

LCPC016

Components of Green Tea Separated on a Rapid Resolution StableBond SB-C8 Column

Column: ZORBAX SB-C8
863953-906
4.6 x 150 mm, 3.5 µm

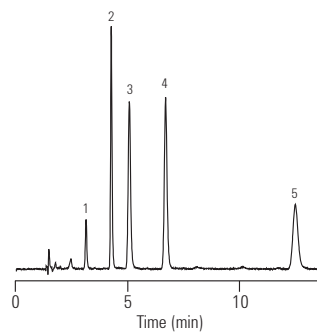
Mobile Phase: 75% 0.1% TFA : 25% MeOH

Flow Rate: 1.0 mL/min

Temperature: 40°C

Detector: 280 nm

Sample: Green tea



1. Epigallocatechin
2. Epicatechin
3. Epigallocatechin gallate
4. Catechol
5. Epicatechin gallate

LCPC018

Nutraceuticals, such as the components of green tea, are quickly separated on a StableBond SB-C8 Rapid Resolution column.

Chiral Separation of Hexobarbital

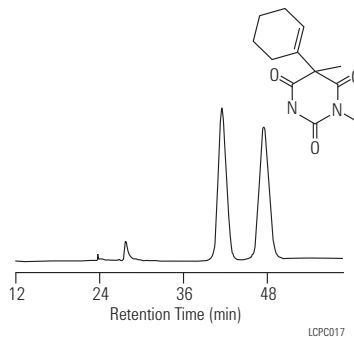
Column: Chiradex
79925CB-584
4.0 x 250 mm, 5 µm

Mobile Phase: Methanol/water, 20:80

Flow Rate: 1.0 mL/min

Detector: UV 220 nm

Sample: Hexobarbital



Chiral Separation of S- and R-Norfluoxetine using Ultron ES-Pepsin

Column: Ultron ES-OVM Chiral
724111653
4.6 x 250 mm, 10 µm

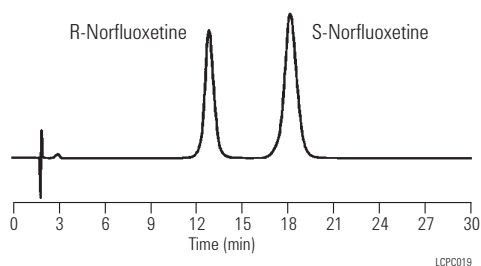
Mobile Phase: 6/94 (v/v) MeOH / 20 mM KH₂PO₄

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV 225 nm

Sample: 50 µg/mL of 2:3 mixture R : S-Norfluoxetine



Courtesy of D.S. Ristry and V.S. Sharp, Eli Lilly and Co.

Chiral Separation of Salbutamol on Ultron ES-Pepsin

Column: Ultron ES-Pepsin
822111631A
4.6 x 150 mm, 5 µm

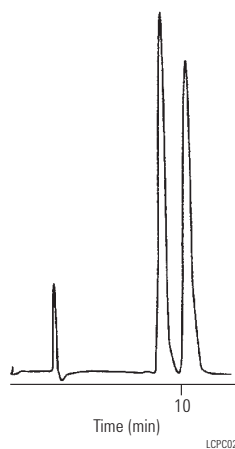
Mobile Phase: 20 mM phosphate buffer, pH 6.0

Flow Rate: 1.0 mL/min

Temperature: 25° C

Detector: UV 220 nm

Sample: 20 µL containing 0.35 µg Salbutamol Mixture



Chiral Separation of Tolperison Enantiomers on Ultron ES-OVM

Column: Ultron ES-OVM Chiral
702111651
4.6 x 150 mm, 5 µm

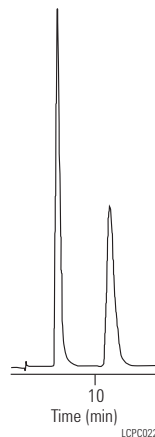
Mobile Phase: 20 mM KH₂PO₄ (pH 5.5), C₂H₅OH (100/4 v/v)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV 220 nm, 0.04 AUFS

Sample: Tolperison, 5 µL

**Chiral Separation of Atenolol on Ultron ES-Pepsin**

Column: Ultron ES-Pepsin
822111631A
4.6 x 150 mm, 5 µm

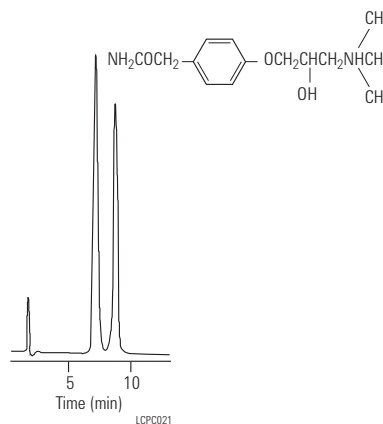
Mobile Phase: 20 mM phosphate buffer, pH 6.0/Ethanol (99/1)

Flow Rate: 1.0 mL/min

Temperature: 25° C

Detector: UV 220 nm, 0.04 AUFS

Sample: 1.5 µL, 0.25 mg/mL, Atenolol Racemic Mixture

**Cocaine and Metabolites**

Column: ZORBAX Rx-SIL
883975-901
4.6 x 150 mm, 5 µm

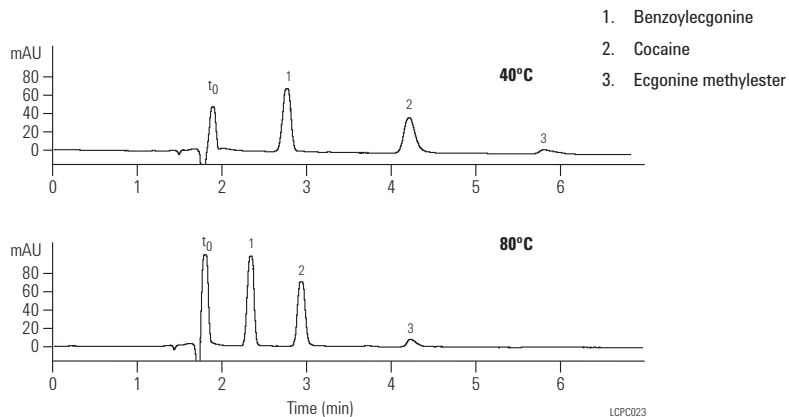
Mobile Phase: MeOH: NH₄ Acetate, 25 mM,
pH 6 (70:30)

Flow Rate: 1.0 mL/min

Temperature: 40 and 80°C

Detector: UV 210 nm

Publication: LI PH42



Aspirin and Cough Remedy on ZORBAX Eclipse XDB-C8

Column: Eclipse XDB-C8
993967-906
4.6 x 150 mm, 5 µm

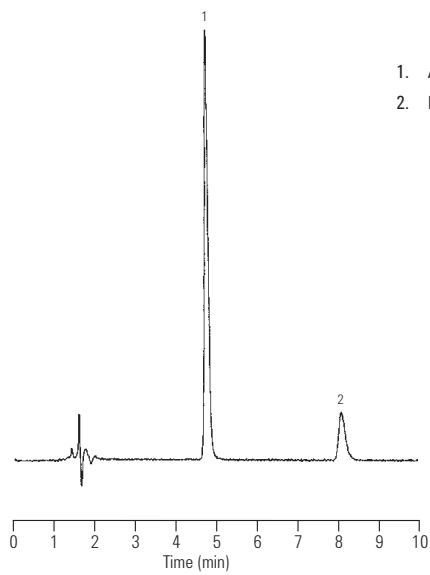
Mobile Phase: (75:25) 25 mM Na₂HPO₄ (pH 3.0): ACN

Flow Rate: 1.0 mL/min

Temperature: 40°C

Detector: UV 254 nm

Sample: 5 µL, 10 µg/mL



Cough Formula Mixture: Fast and Efficient Separation

Column A: ZORBAX SB-CN
866953-905
4.6 x 75 mm, 3.5 µm

Column B: ZORBAX SB-CN
883975-905
4.6 x 150 mm, 5 µm

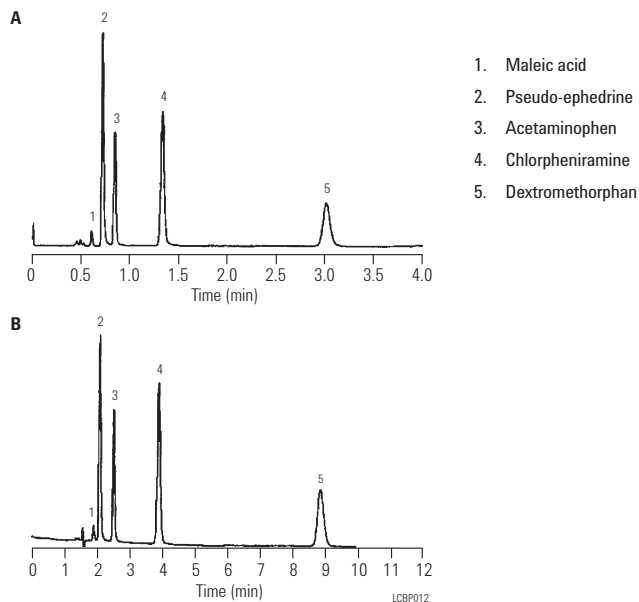
Mobile Phase: 20/80, Acetonitrile/150 mM NaCitrate,
pH 2.6

Flow Rate: 1.5 mL/min, 1.0 mL/min

Temperature: 35°C

Detector: UV 270 nm

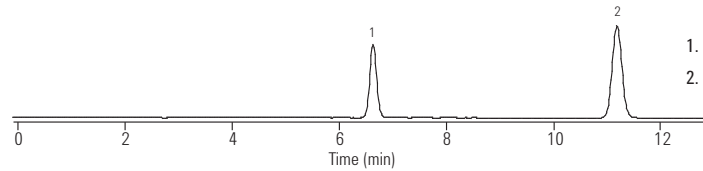
Sample: 2 µL, Cough Formula



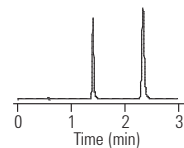
Guaifenesin: USP Analysis of Guaifenesin

Mobile Phase: 40% Methanol:60% Water:1.5% Glacial Acetic Acid
 Flow Rate: 1.0 mL/min
 Temperature: 25°C
 Sample: Guaifenesin
 A: 8 µL
 B: 2 mL

Column:	Eclipse XDB-C18 990967-902 4.6 x 250 mm, 5 µm	Peak	TR	N	Rs
		1	6.63	12,737	0
		2	11.19	18,552	15.8



1. Guaifenesin: 0.04 mg/mL
 2. Benzoic Acid: 0.10 mg/mL



Column:	Eclipse XDB-C18 922975-902 4.6 x 50 mm, 1.8 µm	Peak	TR	N	Rs
		1	1.4	11,421	0
		2	2.33	12,909	12.3

LCPC025

Minimum Resolution Required = 3.0

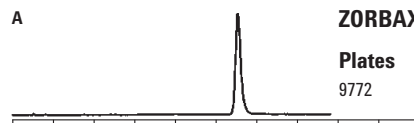
Metronidazole: Updating USP Methods

Column A: ZORBAX C8
883952-706
4.6 x 150 mm, 5 µm

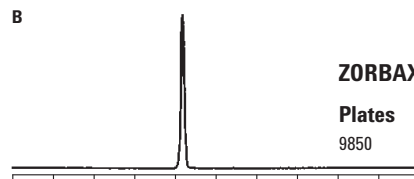
Column B: Eclipse XDB-C8
993967-906
4.6 x 150 mm, 5 µm

Column C: Eclipse XDB-C8
963967-906
4.6 x 150 mm, 3.5 µm

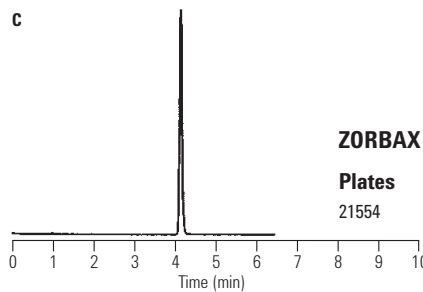
Mobile Phase: 80/20, Water/Methanol
 Flow Rate: 1.0 mL/min
 Temperature: Ambient
 Detector: UV 254 nm
 Sample: Metronidazole



Plates	USP TF	Particle Size
9772	1.30	5 µm



Plates	USP TF	Particle Size
9850	0.98	5 µm



Plates	USP TF	Particle Size
21554	1.13	3.5 µm

LCPC026

Morphine and Metabolites: Extracted Blood Plasma Sample Separation

Column: ZORBAX SB-C18
863953-902
4.6 x 150 mm, 3.5 µm

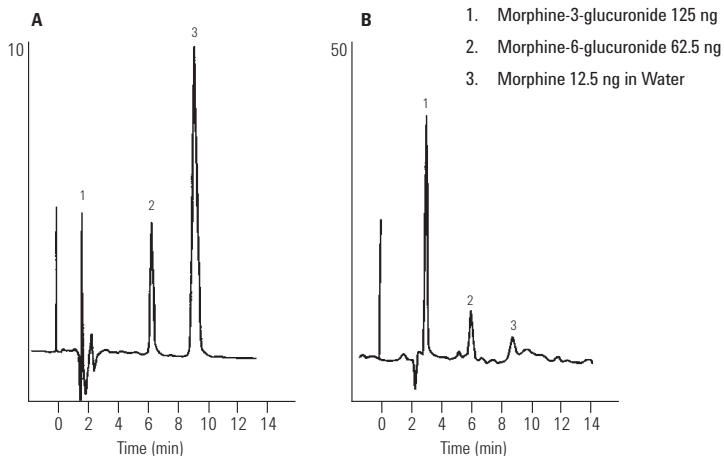
Mobile Phase: 97/3/70 mM KH₂PO₄ + 1 mM EDTA/ACN,
pH 4.5

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: A: Electrochemical, 720 mV
B: Fluorescence, Ex = 285 nm, Em = 352 nm

Sample: 50 µL
Morphine-3-glucuronide 125 ng
Morphine-6-glucuronide 62.5 ng
Morphine 12.5 ng in Water



Courtesy of J. Visser, Center for Pharmacy,
Univ. Groningen, The Netherlands.

LCPC027

Opiates (Drugs of Abuse) by LC/MS

Column: ZORBAX SB-AQ
830990-914
2.1 x 150 mm, 3.5 µm

Mobile Phase: A: Acetonitrile with 0.1% formic acid
B: Water with 0.1% formic acid

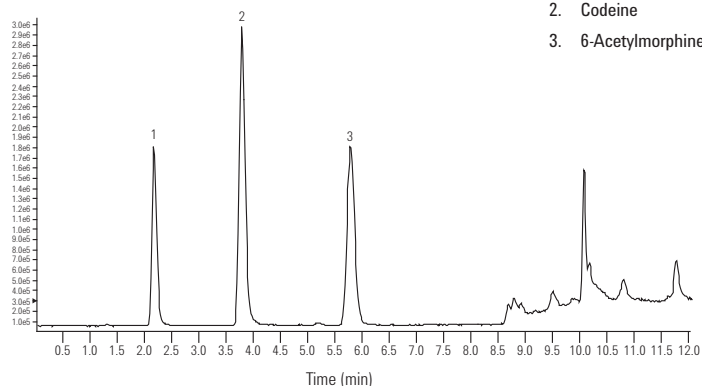
Flow Rate: 0.25 mL/min

Gradient: 0 min 10% B
5 min 35% B
5.1 min 100% B

MS Conditions: Time of Flight (TOF)
Standard with calibrant delivery system
providing constant low flow of ~2 µM purine
and HP-921 calibrant to dual ESI for
continuous auto-calibration

Sample: Opiates

XIC of +TOF MS



LCPC028



For a comprehensive listing of chromatograms searchable by compound name, visit our
online Chromatogram Library at www.agilent.com/chem/library

Neutraceuticals: Hypericin Separation in St. John's Wort

Column: Eclipse XDB-C8
993967-906
4.6 x 150 mm, 5 µm

Mobile Phase: 23% 25 mM Na₂HPO₄,
Dibasic (pH 7.0 with H₃PO₄): 77% MeOH

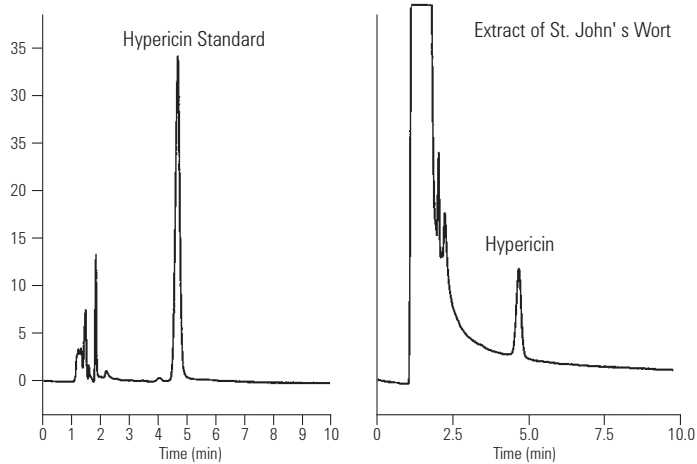
Flow Rate: 1.0 mL/min

Temperature: 35°C

Detector: 254 nm

Publication: LI PH47

Sample: Neutraceuticals



LPC0029

Pharmaceuticals: Rapid, High Sensitivity LC and LC/MS of 11 Drugs

Column: Eclipse XDB-C18
925700-902
2.1 x 50 mm, 1.8 µm

Mobile Phase: A: 10 mM NH₄ Formate (pH=3.6)
B: ACN with 10 mM NH₄ Formate

Flow Rate: 0.6 mL/min

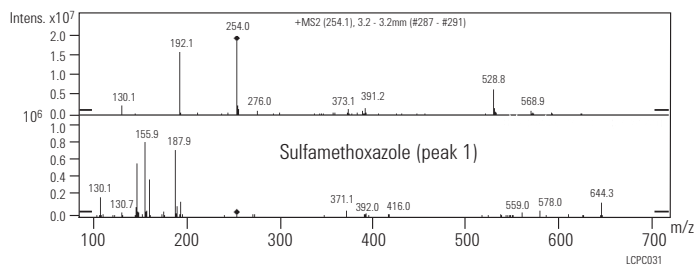
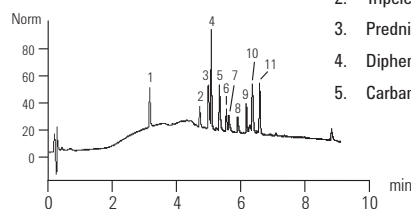
Gradient: 5% B to 70% B in 7.5 min,
to 95% B in 8.5 min

Temperature: 65°C

Detector: UV 230 nm and MSD Trap SL

MS Conditions: Pos. Dry Gas: 345°C
Neb.: 45 psi
HV Cap: 3500 V
Range: 100-700
Average: 5 Spectra
ICC: 30000
Charge Con: On
Smart Par. Settings: Tar Mas: 250 m/z
Comp. Stab.: 100%
Trap Drive: 100%
Frag. Options: Smart Frag: On
Frag. Width: 10 m/z

- | | |
|---------------------|---------------------|
| 1. Sulfamethoxazole | 6. Promethazine |
| 2. Tripeleminamine | 7. Protriptyline |
| 3. Prednisolone | 8. Imipramine |
| 4. Diphenhydramine | 9. Trimipramine |
| 5. Carbamazepine | 10. Perphenazine |
| | 11. Triflupromazine |



LPC0031

Hormones/Steroids on ZORBAX Rapid Resolution HT SB-C18

Column: ZORBAX SB-C18 RRHT cartridge
823975-902
4.6 x 30 mm, 1.8 μm

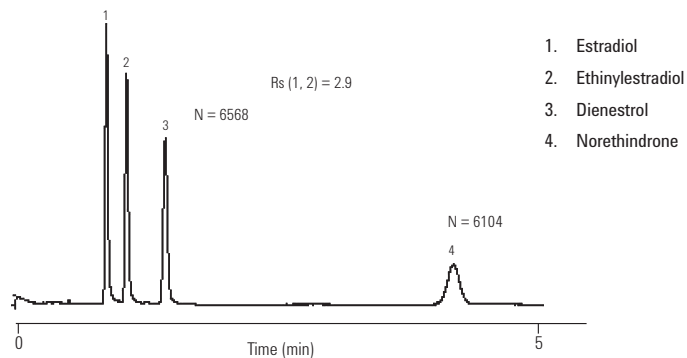
Mobile Phase: 50% 20 mM NaH₂PO₄, pH 2.8: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: RT

Detector: UV 230 nm

Sample: Hormones/Steroids



1. Estradiol
2. Ethinylestradiol
3. Dienestrol
4. Norethindrone

LCPC034

Steroids: Separation on Eclipse XDB-CN

Column: Eclipse XDB-CN
993967-905
4.6 x 150 mm, 5 μm

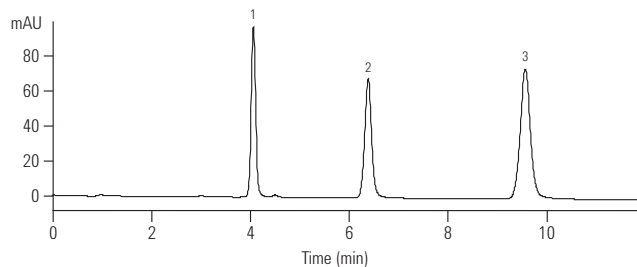
Mobile Phase: 40:60 ACN:Water

Flow Rate: 1.0 mL/min

Temperature: 25°C

Detector: UV 205 nm

Sample: 1. Norethindrone 0.514 mg/mL
2. Progesterone 0.407 mg/mL
3. Mestranol 0.057 mg/mL



LCPC036

Steroids

Column A: Eclipse XDB-Phenyl
963967-912
4.6 x 150 mm, 3.5 μm

Column B: Eclipse XDB-C18
993967-902
4.6 x 150 mm, 5 μm

Mobile Phase: H₂O : ACN, 60:40

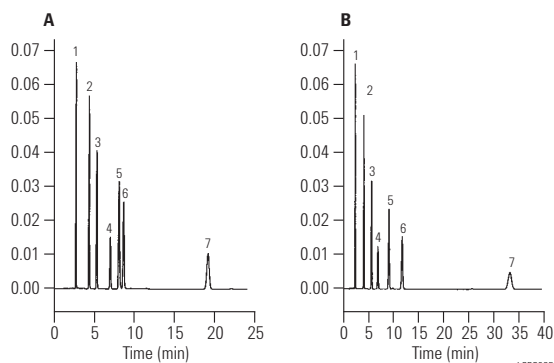
Flow Rate: 1.0 mL/min

Temperature: 35°C

Detector: 254 nm

Publication: LI PH36

Sample: Steroid Sample
1. Prednisolone
2. Corticosterone
3. 11 -hydroxyprogesterone
4. Cortisone acetate
5. Deoxycorticosterone
6. 17 hydroxyprogesterone
7. Progesterone



LCPC035

Triamcinolone - USP Analysis of Triamcinolone

Column: Eclipse XDB-C18
923975-902
4.6 x 30 mm, 1.8 μ m

Mobile Phase: 47% Methanol:53% Water

Flow Rate: 1.5 mL/min

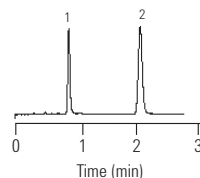
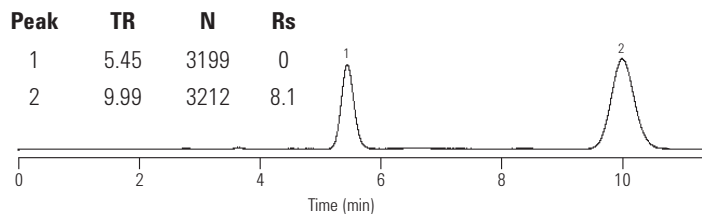
Temperature: 25°C

Sample: Triamcinolone, 1 μ L

1. Triamcinolone: 0.2 mg/mL

2. Hydrocortisone: 0.3 mg/mL

Minimum Resolution Required = 3.0



LCPC038

**Separation of Highly Basic Antidepressants
above their pKa in Free Base Form
(pKa 9.5-9.7)**

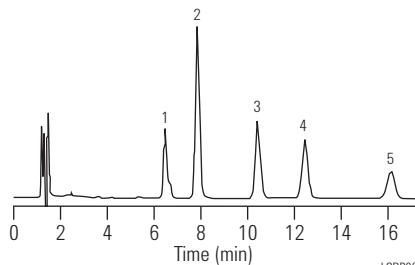
Column: ZORBAX Extend-C18
773450-902
4.6 x 150 mm, 5 μ m

Mobile Phase: 75% Methanol / 25% 50 mM Pyrrolidine
Buffer, pH 11.5

Flow Rate: 0.5 mL/min.

Temperature: 40°C

Detector: UV 215 nm



1. Doxepin
2. Imipramine
3. Nortriptyline
4. Amitriptyline
5. Trimipramine

LCBP007



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

Antidepressants, Tricyclic: Comparative Separation

Column A: ZORBAX Bonus-RP
883668-901
4.6 x 150 mm, 5 µm

Column B: Brand A Polar-linked C8

Column C: Brand B Polar-linked C18

Mobile Phase: ACN: 20 mM Na Citrate, pH 6 (60:40)

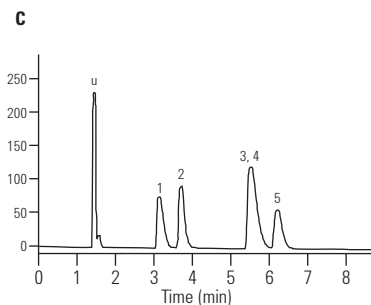
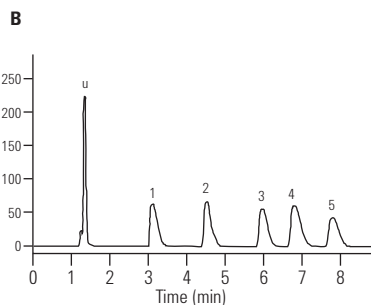
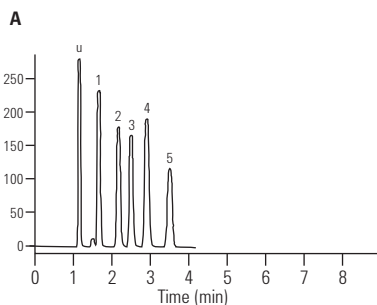
Flow Rate: 1.0 mL/min.

Temperature: Ambient

Detector: UV 254 nm

Sample: Tricyclic antidepressants (u= uracil)

1. Propranolol
2. Doxepin
3. Nortriptyline
4. Amitriptyline
5. Trimipramine



LCBP011

Tricyclic Antidepressants

Column: Eclipse XDB-C8
993967-906
4.6 x 150 mm, 5 µm

Mobile Phase: 38/62 THF/25 mM Potassium Phosphate, pH7

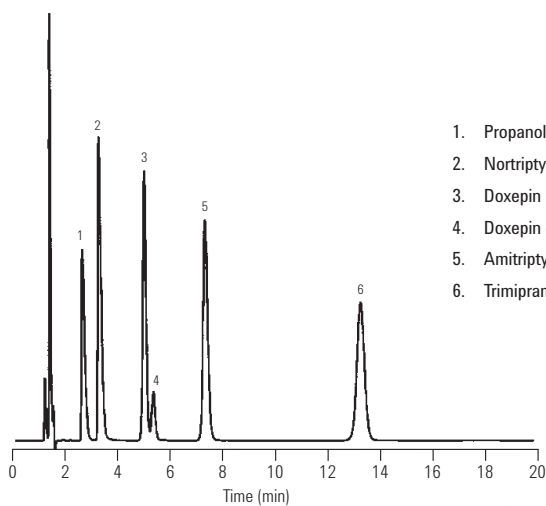
Flow Rate: 1.0 mL/min

Temperature: 23°C

Detector: UV 254 nm

Sample: 10 µL, Antidepressant Mix, 10 µg/mL

1. Propranolol
2. Nortriptyline
3. Doxepin
4. Doxepin dimer
5. Amitriptyline
6. Trimipramine



LCPC039

**Tricyclic Antidepressants and Metabolites:
Effect of Pore Size**

Column A: ZORBAX SB-C18
863953-902
4.6 x 150 mm, 3.5 μm

Column B: ZORBAX 300SB-C18
883995-902
4.6 x 150 mm, 5 μm

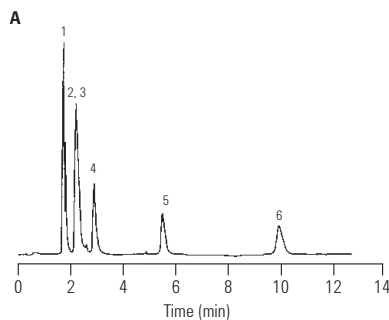
Mobile Phase: 40/60, 25 mM Phosphate Buffer,
10 mM Triethylamine, pH6.2/ACN

Flow Rate: 1.2 mL/min

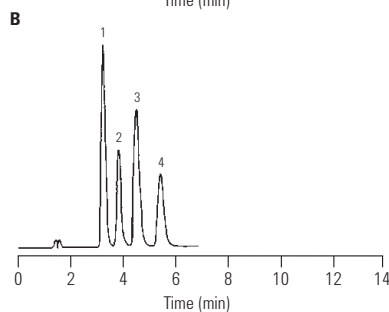
Temperature: Ambient

Detector: UV 254 nm

Sample: 10 μL, Antidepressant Mix, 10 μg/mL



1. trans- 10-OH - Nortriptyline
2. trans- 10-OH - Amitriptyline
3. cis- 10-OH - Nortriptyline
4. cis- 10-OH - Amitriptyline
5. Nortriptyline
6. Amitriptyline



LCPC040

Ulcer Treatment Drugs at Intermediate pH

Column: ZORBAX Bonus-RP
883668-901
4.6 x 150 mm, 5 μm

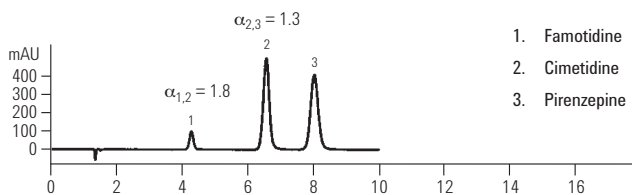
Mobile Phase: Na citrate, 20 mM, pH 6.1: MeOH, (80:20)

Flow Rate: 1.0 mL/min

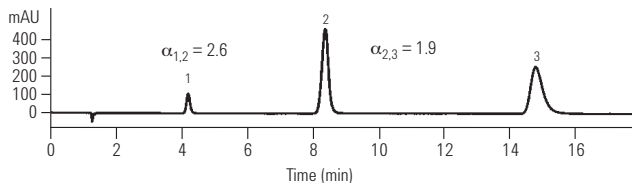
Temperature: Ambient

Detector: UV 220 nm

Sample: Ulcer treatment drugs



1. Famotidine
2. Cimetidine
3. Pirenzepine



LCPC042

Urine, LSD Analysis by LC/MS

Column: Eclipse XDB-C8
960967-906
2.1 x 50 mm, 5 µm

Mobile Phase: 15 : 85, ACN : 10 mM Ammonium Formate, pH 3.7

Flow Rate: 0.3 mL/min

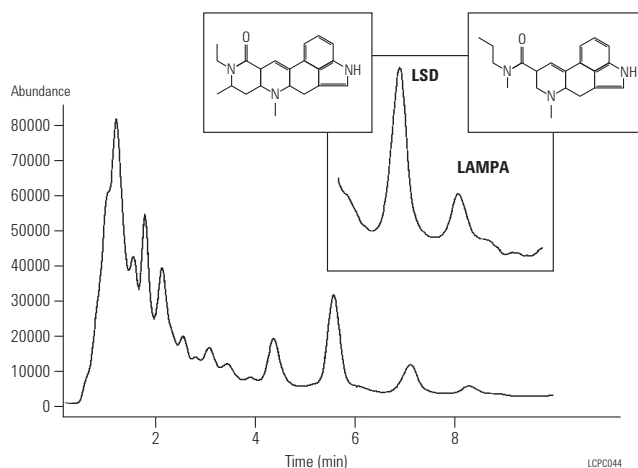
Temperature: 30° C

Detector: MS

MS Conditions: SIM mode, Ions : 324.2, 223.1, 208.1
Fragmentor (dynamically ramped) 100V
at 324.2, 148V at 223.1, 170V at 208.1

Publication: LI PH35

Sample: LSD



Hughes, J.M., C.A. Miller and S.M. Fischer, "Development of a Method for the Forensic Analysis of LSD in Urine", presented at the ASMS, Palm Springs, June 1997.

Benzodiazepines in urine

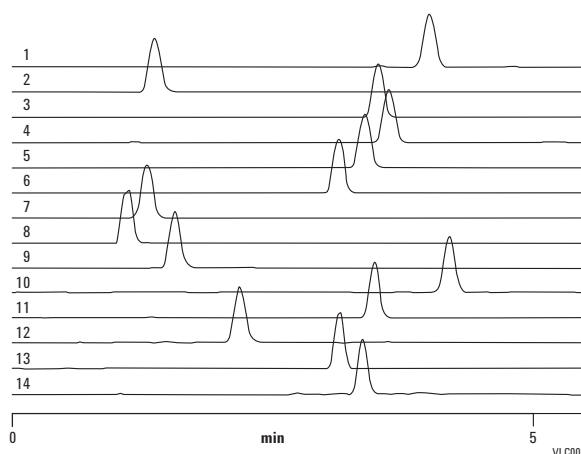
Column: Pursuit PAH
A7001100X046
4.6 x 100 mm, 3 µm

Mobile Phase: A: 0.1% formic acid
B: MeOH

Gradient: Hold 60% B for 1 min,
60-80% B in 1 min,
hold at 80% B for 2.5 min

Flow Rate: 0.2 mL/min

Sample Conc: Urine 100 ng/mL



1. Nordiazepam
2. 7-Aminoclonazepam
3. Desalkylflurazepam
4. Temazepam
5. Alprazolam
6. Clonazepam
7. Midazolam
8. Flurazepam
9. 7-Aminoflurazepam
10. Diazepam
11. Oxazepam
12. Chlordiazepam
13. Flunitrazepam
14. Lorazepam



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

USP Method: Glyburide and Internal Standard, Progesterone

Column: Eclipse XDB-C8
990967-906
4.6 x 250 mm, 5 µm

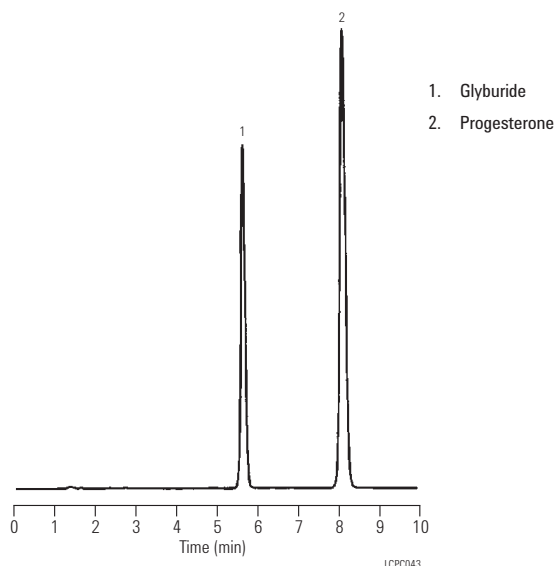
Mobile Phase: 45/55, 50 mM Ammonium Phosphate/ACN, Final pH 5.35

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV 254 nm

Sample: 5 µL, 10 µg/mL each of standard

**Dexamethasone, USP Method: Rapid Analysis**

Column A: ZORBAX SB-C8
880975-906
4.6 x 250 mm, 5 µm

Column B: ZORBAX Rx/SB-C8
866953-906
4.6 x 75 mm, 3.5 µm

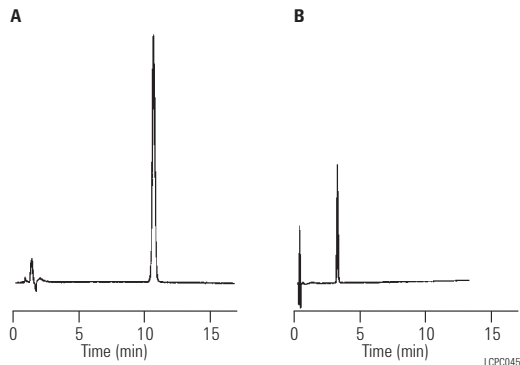
Mobile Phase: A = Water, B = ACN; Isocratic 30% B

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV 254 nm

Sample: Dexamethasone
10 µL and 5 µL, 10 µg/mL

**USP analysis of tetracyclines**

Column: PLRP-S 100Å
PL1512-5500
4.6 x 250 mm, 5 µm

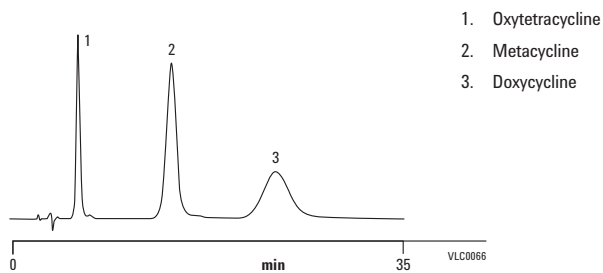
Sample: 20 mg tetracycline in 25 mL 0.01M HCl

Mobile Phase: 60 g 2-Methyl-2-propanol + 200 mL UHP water + 400 mL 0.2 M K₂HPO₄ at pH 8 + 50 mL 10 g/L tetrabutylammonium hydrogen sulphate at pH 8 + 10 mL 40 g/L sodium edetate at pH 8, made up to 1000 mL with water (adjust pH with dilute NaOH)

Flow Rate: 1.0 mL/min

Temperature: 60 °C

Detector: UV, 254 nm



Warfarin: USP Chromatographic Purity Method Using Eclipse XDB-CN

Column: Eclipse XDB-CN
993967-905
4.6 x 150 mm, 5 µm

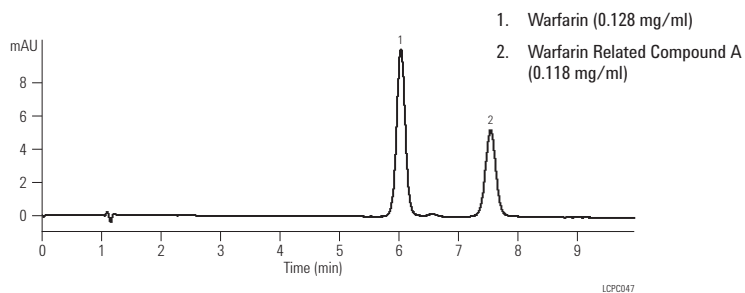
Mobile Phase: 32:68:1 Acetonitrile:Water:Glacial Acetic Acid

Flow Rate: 1.5 mL/min

Temperature: 25°C

Detector: UV 260 nm

Sample: Warfarin, 2 µL



Ten Cardiac Drugs on Rapid Resolution HT SB-C18

Column: SB-C18
829975-902
4.6 x 150 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA, 5% ACN
B: 0.08% TFA, 95% ACN

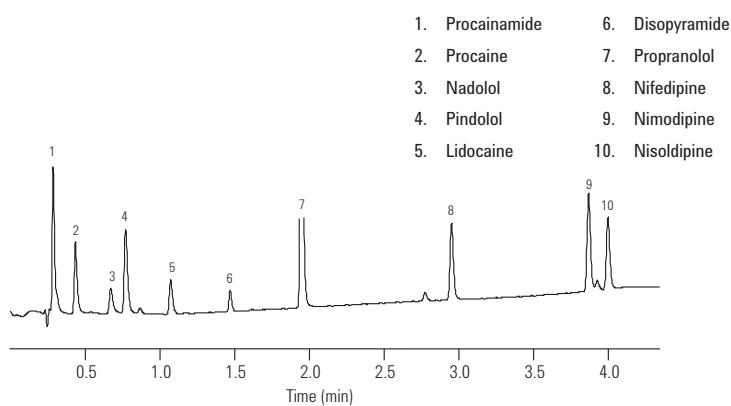
Flow Rate: 2 mL/min

Gradient: 0.0 min 12.5% B
10.5 min 60% B
12.0 min 60% B

Temperature: 70°C

Detector: UV 230 nm

Sample: Cardiac Drugs



Sulfonamides – Fast Analysis with RRHT Columns

Column: SB-C18
824700-902
2.1 x 30 mm, 1.8 µm

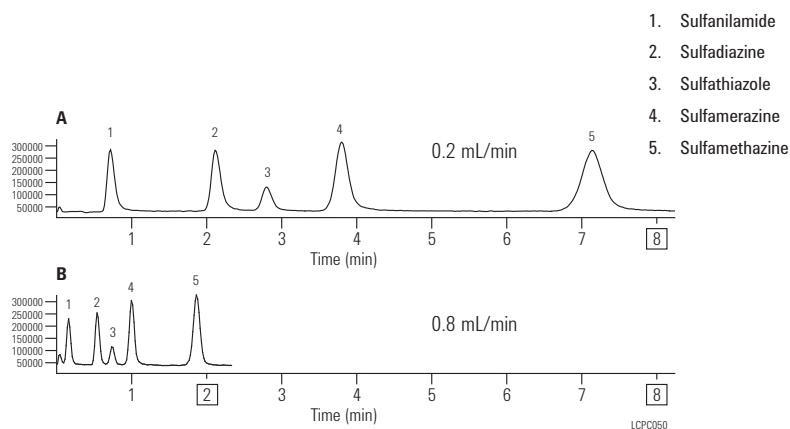
Mobile Phase: A: 90% 0.1% formic acid
B: 10% 0.1% formic acid in MeOH

Flow Rate: A: 0.2 mL/min
B: 0.8 mL/min

Temperature: 35°C

Detector: TIC, Single Quad

Sample: Sulfonamides



Sulfa drugs

Column: Pursuit XRs Ultra^{2.8} C8
A7511100X020
2 x 100 mm, 2.8 µm

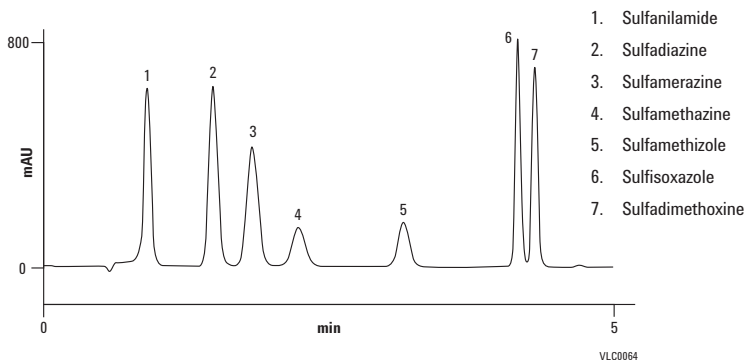
Mobile Phase: A: Water+0.1% TFA
B: MeCN+0.1% TFA

Gradient: 10% B for 10 min,
ramp to 45% B in 1 min and hold for 1 min,
return to 10% B in 1 min and hold for 1 min

Flow Rate: 0.65 mL/min

Temperature: Ambient

Detector: UV, 254 nm



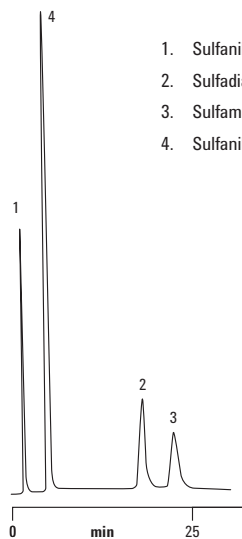
Sulfa drugs

Column: PLRP-S 100Å
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: Potassium sulfate:
ACN 7:1, pH 2.2

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm

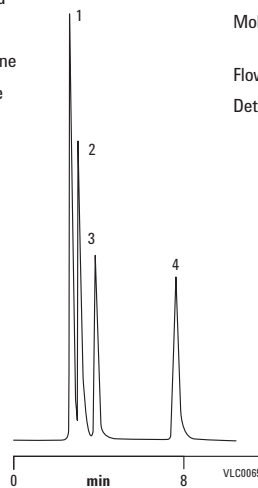


Column: PLRP-S 100Å
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: Disodium tetraborate: ACN 6:1,
pH 9.3

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm



Fast Analysis of Pindolol

Column A: ZORBAX SB-CN
863953-905
4.6 x 150 mm, 3.5 μ m

Column B: ZORBAX SB-CN
827975-905
4.6 x 50 mm, 1.8 μ m

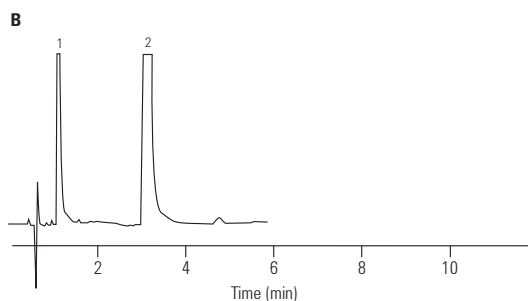
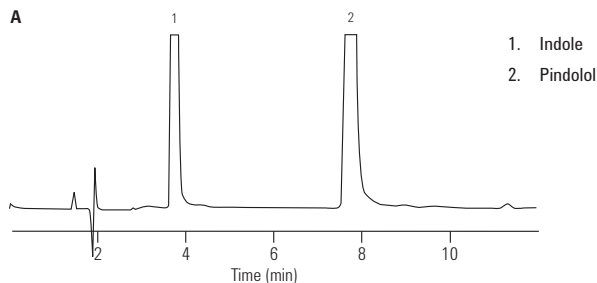
Mobile Phase: A: 70% 50 mM NaAcetate
B: 30% ACN

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV 219 nm

Sample: Pindolol, 2 μ L



LCPC051

Lamotrigine

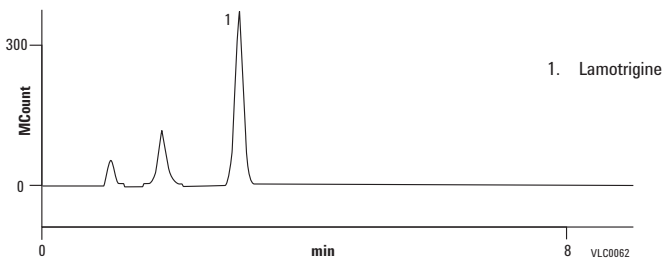
Column: Pursuit XRs Ultra^{2.8} C8
A7511100X020
2 x 100 mm, 2.8 μ m

Mobile Phase: ACN:water, 25:90 for 1 min

Flow Rate: 0.2 mL/min

Injection Volume: 5 μ L, 50% MeOH

Detector: MS



VLC0062



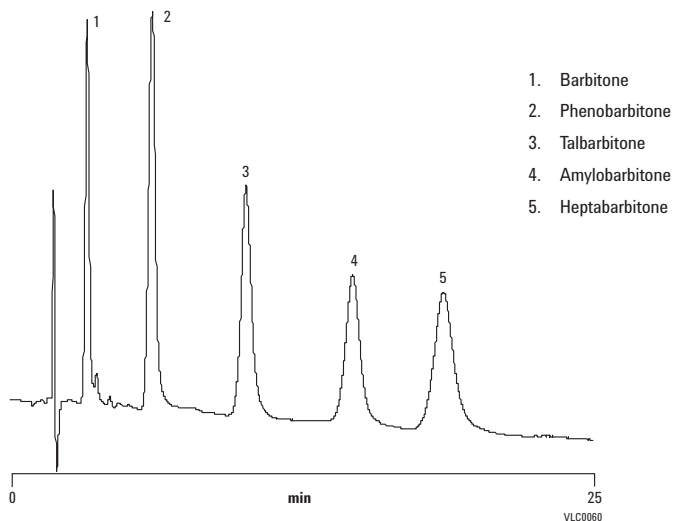
For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at www.agilent.com/chem/library

Barbiturates

Column: PLRP-S 100Å
PL1512-5500
4.6 x 250 mm, 5 µm

Mobile Phase: Water
Flow Rate: 1.0 mL/min
Temperature: 200°C
Detector: UV, 220 nm

Courtesy: Smith, RM, Burgess, RJ, Cheinthaovorn, O and Stuttard, JR (1999) Superheated water: a new look at chromatographic eluents for reversed-phase liquid chromatography. LCGC Europe, January 1999, 30-36. Used with permission

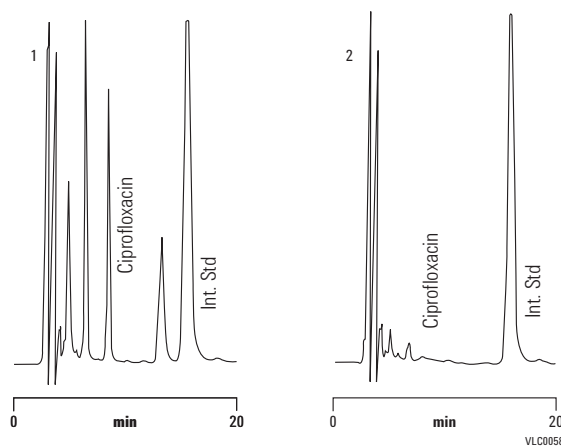
**Analysis of ciprofloxacin and ciprofloxacin metabolites**

Column: PLRP-S 100Å
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: 74% 20 mM TCA:22%
ACN:4% MeOH adjusted to pH 3
Flow Rate: 1.0 mL/min
Detector: UV, 277 nm

Krol GJ, Noe, AJ and Beerman, D (1986) Liquid chromatographic analysis of ciprofloxacin and ciprofloxacin metabolites in body fluids. Journal of Liquid Chromatography, 9(13), 2897-2919. Reprinted with permission of the publisher (Taylor & Francis Group, www.informaworld.com)

- Blank urine sample containing known concentrations of internal standard, ciprofloxacin and its metabolites
- Blank urine sample containing only internal standard





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