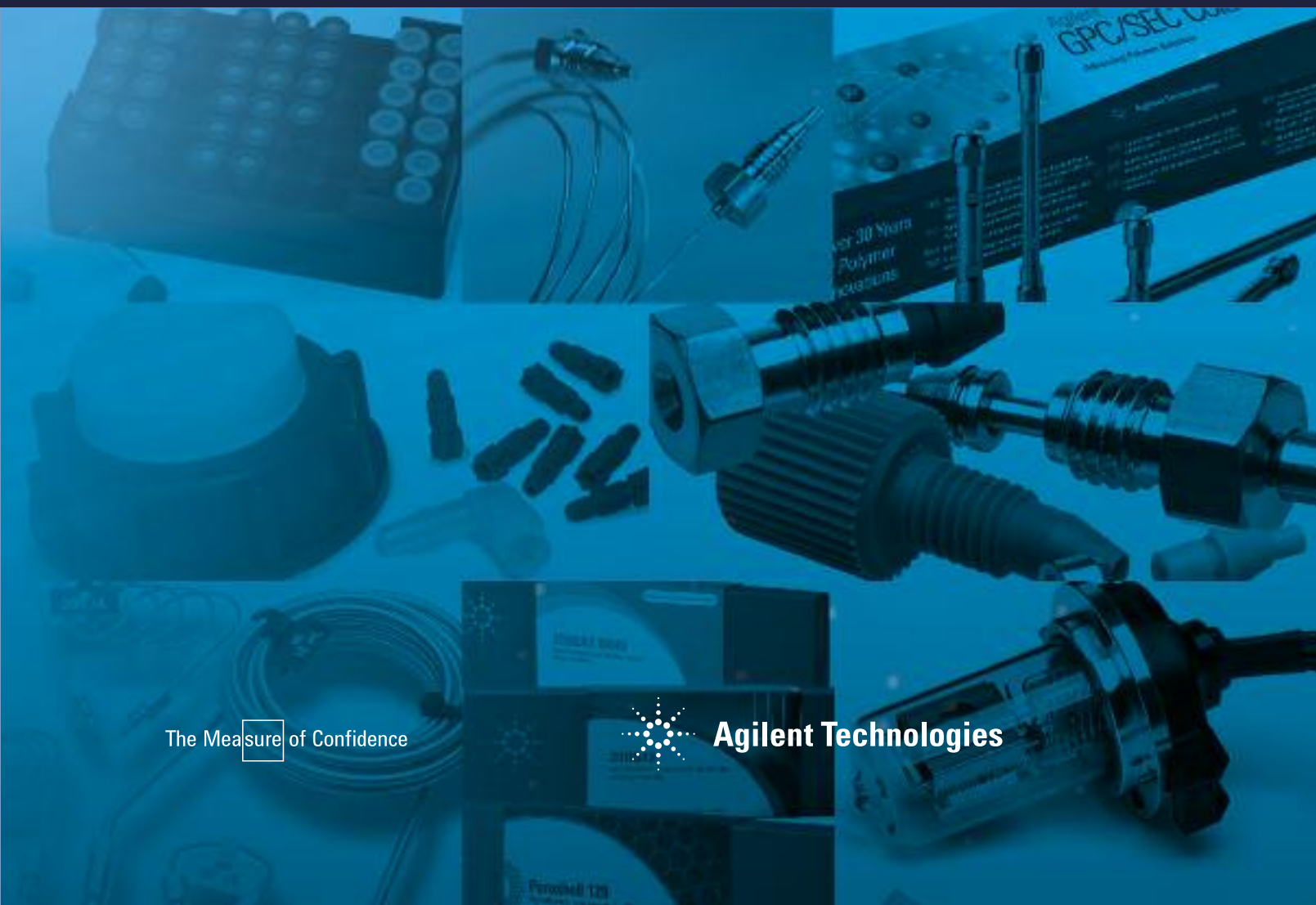
LC AND LC/MS

Your Essential Resource for Columns & Supplies



The Measure of Confidence

Agilent Technologies

LC and LC/MS Troubleshooting

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Baseline disturbance at void time	Positive/negative – Difference in refractive index of injection solvent	Use mobile phase for sample solvent
Detector leaks	Plugged inlet frit	Replace seals/gaskets
Drifting baseline	Positive direction – Contaminant buildup/elution	Flush column, clean up sample, use pure solvents
	Positive/negative – Difference in refractive index of injection solvent	Use mobile phase for sample solvent
	Negative direction (gradient) – Absorbance of "A" mobile phase solvent	Use non-absorbing or HPLC-grade or better solvent
	Positive direction (gradient) – Absorbance of "B" mobile phase solvent	Use non-absorbing or HPLC-grade or better solvent
	Random – Temperature changes	Insulate column and tubing
	Random – Temperature changes	Thermostat column and tubing
	Wavy or undulating – Temperature changes in room	Monitor room temperature and control
Ghost peaks	Peaks from previous injection	Flush column to remove contaminants
	Contamination	Sample cleanup or pre-fractionation
	Unknown interferences in samples	Sample cleanup or pre-fractionation
	Ion-pair – Upset equilibrium	Prepare sample in actual mobile phase to minimize disturbance
	Peptide mapping – Oxidation of TFA	Prepare fresh daily; use anti-oxidant
	Reversed-phase – Contaminated water	Check suitability of water by running different amount through reversed-phase column and measure peak height with elution; use HPLC grade solvents
	Spikes – Bubbles in solvent	De-gas solvents
High column backpressure	Column blockage, adsorbed sample	Better sample cleanup; use guard column
	Mobile phase viscosity too high	Use lower viscosity solvents or higher temperature
	Particle size too small	Use larger d_p packing
	Plugged inlet frit	Replace column
	Plugged inlet frit	Reverse solvent flow
Leaks	Subtle – White powder at fitting/loose fitting	Tighten fittings, cut tubing, or replace ferrules
Leaks, injection valve	Catastrophic – Worn valve rotor	Replace rotor in valve
Leaks, column or other fittings	Catastrophic – Loose fittings	Tighten or replace fittings
Leak, pump	Catastrophic – Pump seal failure	Replace pump seal

(Continued)

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Negative peaks	RI detector – solute refractive index less than solvent	No problem; reverse polarity to make positive
	UV detector – solute absorbance less than mobile phase	Use mobile phase with lower UV absorbance; do not recycle solvent too long
Noisy baseline	Random – Contaminant buildup	Flush column; clean up sample; use HPLC-grade solvent
	Continuous – Detector lamp problem	Replace detector lamp
	Occasional – External electrical interference	Use voltage stabilizer for LC system
Peak doubling	Sample volume too large	Reduce the volume e.g. by half and re-inject
	Injection solvent too strong	Use weaker injection solvent or mobile phase
	Blocked frit	Replace and use 0.5 µm porosity in-line filter
	Column void or channeling	Replace column; for some columns, fill in void with packing
	Unswept injector flowpath	Replace injector rotor
	Void at head of column	Replace column, top off column with packing
	Column overloaded with sample	Use higher capacity stationary phase Increase column diameter Decrease sample size
	Single peak – interfering components	Sample cleanup; pre-fractionation
Peak tailing	Beginning of peak doubling	See "peak doubling"
	Unswept dead volumes	Minimize number of connections Ensure injector seal is tight Ensure fittings are properly seated
	Basic compounds – Silanol interactions	Choose endcapped bonded phase Switch to polymeric phase
	Basic substances – Silanol interactions	Use stronger mobile phase or add competing base (e.g. TMA)
	Silica-based – Column degradation	Use specialty column; polymeric column or sterically protected

(Continued)

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Peaks are broad	Injection volume too large	Decrease solvent strength of injection solvent to focus solute
	Peak dispersion in injector valve	Introduce air bubble in front/back of sample to decrease dispersion
	Sampling rate of data system too slow	Increase frequency of sampling
	Slow detector time constant	Adjust time constant to match peak width
	Mobile phase viscosity too high	Increase column temperature
	Detector cell volume too large	Use smallest possible cell volume with no heat exchanger in system
	Injector volume too large	Decrease injection volume
	Long retention times	Use gradient elution or stronger mobile phase
Pressure fluctuation	Leaky check valve	Replace check valve
	Pump seal leaks	Replace pump seals
	Buildup of particulates	Filter sample; in-line filter; filter mobile phase
Pressure increasing	Buildup of particulates	Filter sample; in-line filter; filter mobile phase
	Water/organic systems – buffer precipitation	Test buffer-organic mixtures; ensure compatibility
Retention beyond total permeation volume	Size exclusion – Specific interactions	Add mobile phase modifiers or change solvent
Retention times changing	Column temperature varying	Thermostat column; insulate column; ensure lab temperature constant
	Equilibration time insufficient with gradient run or changes in isocratic mobile phase	Make sure at least 10 column volumes pass through column after solvent change or gradient conclusion
	Selective evaporation of mobile phase component	Less vigorous helium sparging; keep solvent reservoirs covered; prepare fresh mobile phase
	Buffer capacity insufficient	Use >20 mM concentration of buffer
	Inconsistent on-line mobile phase mixing	Ensure gradient system delivering constant composition; check vs. manual prep of mobile phase
	Contamination buildup	Occasionally flush column with strong solvent to remove contaminants
	First few injections – Adsorption on active sites	Condition column by initial injection of concentrated sample

(Continued)

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Retention times decreasing	Flow rate increasing	Check pump to make sure correct; if not, reset
	Column overloaded with sample	Decrease sample size
	Loss of bonded stationary phase	Keep mobile phase pH between 2 and 8.5
Retention times increasing	Flow rate is slowing	Fix leaks in liquid lines, replace pump seals, check for pump cavitation or air bubbles
	Active sites on silica packing	Use mobile phase modifier
	Loss of bonded stationary phase	Keep mobile phase pH between 2 and 8.5
	Mobile phase composition changing	Make sure mobile phase container is covered
	Active sites on silica packing	Add competing base to mobile phase
	Active sites on silica packing	Use higher coverage packing for stationary phase
Sensitivity problem	Peaks are outside of linear range of detector	Dilute/concentrate to bring into linear region
	First few sample injections – Absorption of sample in loop or column	Condition loop/column with concentrated sample
	Autosampler flow lines blocked	Check flow and make sure there are no blockages
	Injector sample loop underfilled	Make sure that loop is overfilled with sample
	Sample-related losses during preparation	Use internal standard during sample prep; optimize sample prep method
Slow column equilibration times (ion-pairing)	Equilibration time slow for long-chain ion-pairing reagents	Use shorter alkyl chain ion-pair reagent

LC/MS Troubleshooting

Symptom Type	Solution
No peaks	Spray from the nebulizer
	Make sure capillary voltage is set correctly
	Make sure LC/MSD is tuned correctly
	Make sure LC/MSD pressures are within normal ranges
	Check drying gas flow and temperature
	Make sure fragmentor is set correctly
Poor mass accuracy	Recalibrate the mass axis
	Make sure ions used for tuning span mass range of sample ions and show strong stable signals
Low signal	Check the solution chemistry; make sure solvent is appropriate for sample
	Make sure sample is fresh and has been stored correctly
	Make sure LC/MSD is tuned correctly
	Check the nebulizer condition
	Clean the capillary entrance
	Check the capillary for damage and contamination
Unstable signal	Make sure drying gas flow and temperature are correct for the solvent flow
	Make sure solvent is throroughly degassed
	Make sure LC backpressure is steady; this indicates a steady solvent flow

(Continued)

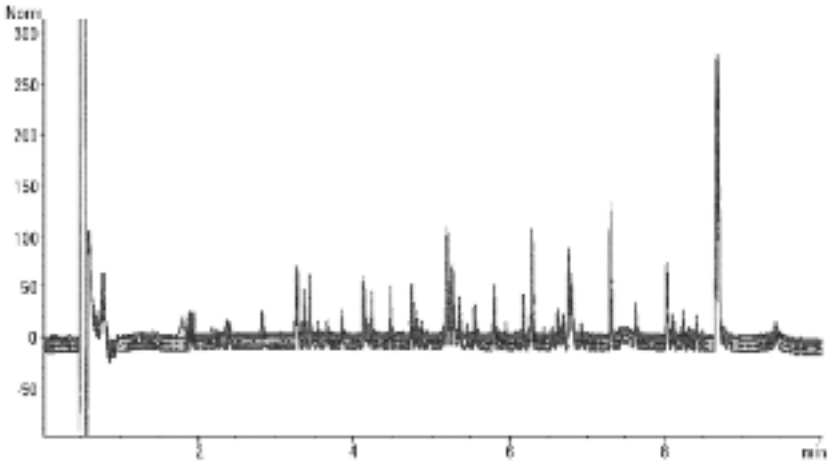
LC/MS Troubleshooting	
Symptom Type	**Solution**
High spectral noise	Use appropriate mass filter values
	Check spray shape; nebulizer may be damaged or set incorrectly
	Make sure drying gas flow and temperature are correct for the solvent flow
	Make sure solvent is throroughly degassed
	Make sure LC backpressure is steady; this indicates a steady solvent flow
	If you are using water as part of the mobile phase, make sure it is de-ionized (> 18 MΩ cm)
Droplets, not spray, exiting the nebulizer	Make sure nebulizing gas pressure is set high enough for the LC flow
	Check position of needle in nebulizer
	Stop solvent flow and remove nebulizer assembly
	Examine end of nebulizer for damage
No flow	Make sure LC is on and there is sufficient solvent in correct bottle
	Check for LC error messages
	Check for blockages
	Repair or replace any blocked components
	Check for leaks
	Make sure MS stream selector valve is set to LC to MSD
Undesired fragmentation	(APCI vs. Electrospray)
	APCI temperature is too high
	Fragmentor voltage is set too high

BioPharmaceutical Applications

NEW!

Protein digest analysis

Column:	**ZORBAX 300SB-C18 858750-902 2.1 x 100 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 0.085% TFA in ACN
Flow Rate:	0.5 mL/min
Pressure:	640 bar
Gradient:	2% B 1 min, 2-45% B 8.8 min, 45-95% B 0.2 min, 95% B 2 min, 98-2% B 0.2 min, 2% B 1.8 min
Temperature:	50 °C
Detector:	Agilent 1290 Infinity LC
Injection:	5 µL
Sample:	Protein digest
Sample Conc:	1 mg/mL

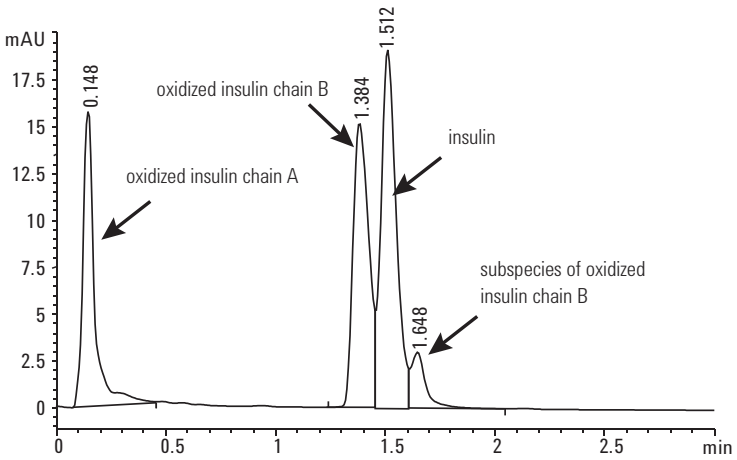


Overlaid chromatograms of 30 runs of a protein digest on an Agilent ZORBAX RRHD 300SB-C18 column.

NEW!

Analysis of oxidized insulin chains

Column:	**ZORBAX RRHD 300SB-C18 857750-902 2.1 x 50 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 80% ACN + 0.01% TFA in water
Flow Rate:	1.0 mL/min
Pressure:	650-700 bar
Gradient:	33-50% B, 0-4 min; 33% B, 4-5 min
Detector:	UV, 280 nm Agilent 1290 Infinity LC
Sample:	Insulin, oxidized insulin chain A and chain B from bovine pancreas (Sigma Aldrich, St. Louis, MO)
Sample Conc:	1 mg/mL
Injection:	2 µL

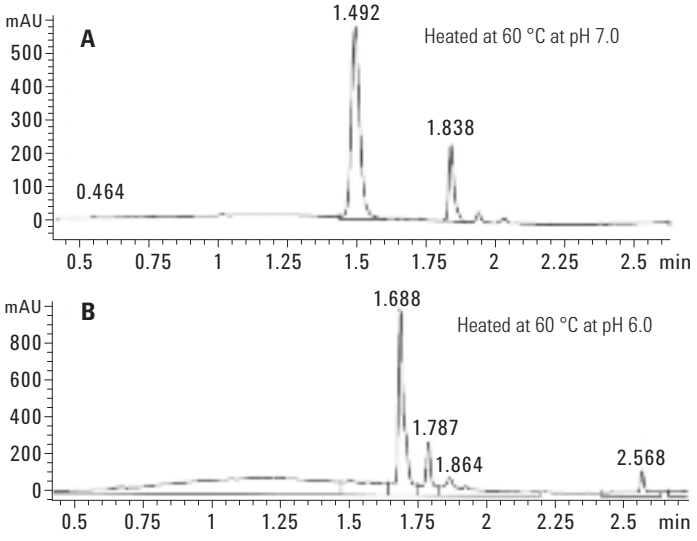


Insulin and oxidized insulin A and B chains are resolved quickly but insulin and oxidized chain B often co-elute.

NEW!

Fast separation of recombinant human erythropoietin

Column:	**ZORBAX RRHD 300SB-C18 857750-902 2.1 x 50 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 0.01% TFA in ACN
Flow Rate:	1.0 mL/min
Pressure:	650 bar
Gradient:	5 to 100% B solvent from 0 to 2.5 min
Detector:	UV, 280 nm Agilent 1290 Infinty LC
Sample:	Recombinant human EPO protein (rEPO)
Sample Conc:	1.0 mg/mL
Injection:	3 µL



Heat-treated rEPO protein are well resolved by the Agilent ZORBAX RRHD 300SB-C18 column. The column separated these heat-treated rEPO proteins.

NEW!

Separation optimization for ultra fast analysis of reduced and alkylated monoclonal antibody

Column: **ZORBAX RRHD 300SB-C8**
858750-906
2.1 x 100 mm, 1.8 µm

Mobile Phase: (Various)
A: H_2O + 0.1% TFA (v/v)
B: n-propanol:ACN:H_2O (80:10:10) + 0.1% TFA (v/v)

Injection: 1-3 µL

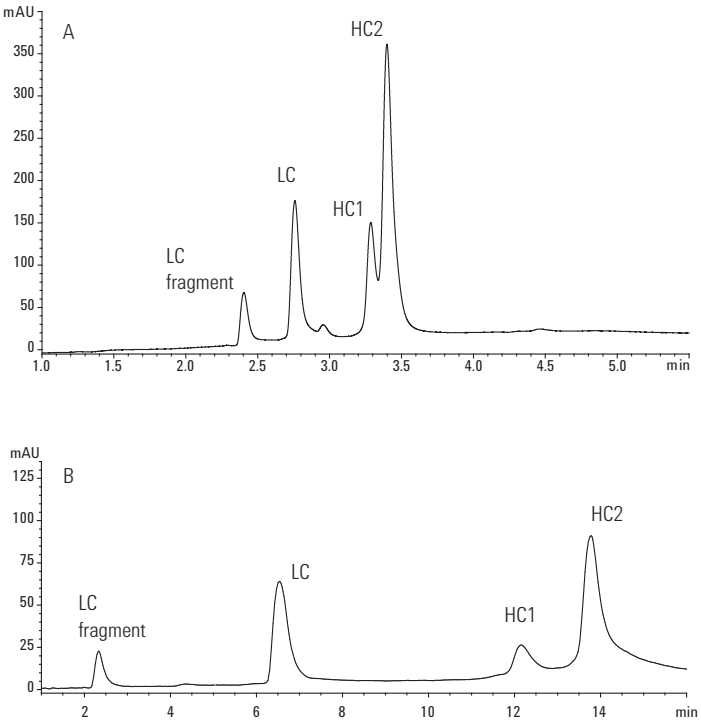
Flow Rate: 0.5 mL/min

Gradient: Multi-segmented
A (optimized for speed): 0 min-20% B, 3 min-35% B, 4 min-40% B, 5 min-40% B, 5.1 min-90% B, 5.5 min-90% B, 6 min-25% B
B (optimized for resolution): 0 min-25% B, 15 min-32% B, 16 min-32% B, 17 min-90% B, 17.5 min-90% B, 18 min-25% B

Temperature: 75 °C

Detector: UV, 225 nm
Agilent 1290 Infinity LC

For consecutive chromatographic runs, a 2-minute post run was added to re-equilibrate the column.



Comparison of two optmimized gradients for the ultra fast separation of reduced and alkylated monoclonal antibodies on an Agilent ZORBAX RRHD 300SB-C8 column. The top panel details a rapid separation of the light and heavy chain variants in a shortened run time of less than 4 minutes. The bottom panel displays complete baseline resolution of the two heavy chain variants during a longer runtime using a shallower gradient profile. Both separations were performed at 75 °C and completed with a fast 90% 1-propanol wash step (UV not shown).

NEW!

Column reproducibility – 200 injections of reduced monoclonal antibody using an Agilent ZORBAX RRHD 300SB-C3 column

Column: **Agilent ZORBAX RRHD 300SB-C3**
858750-909
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA in water
B: 80% n-propyl alcohol, 10% ACN, 9.9% water and 0.1% TFA

Flow Rate: 0.4 mL/min

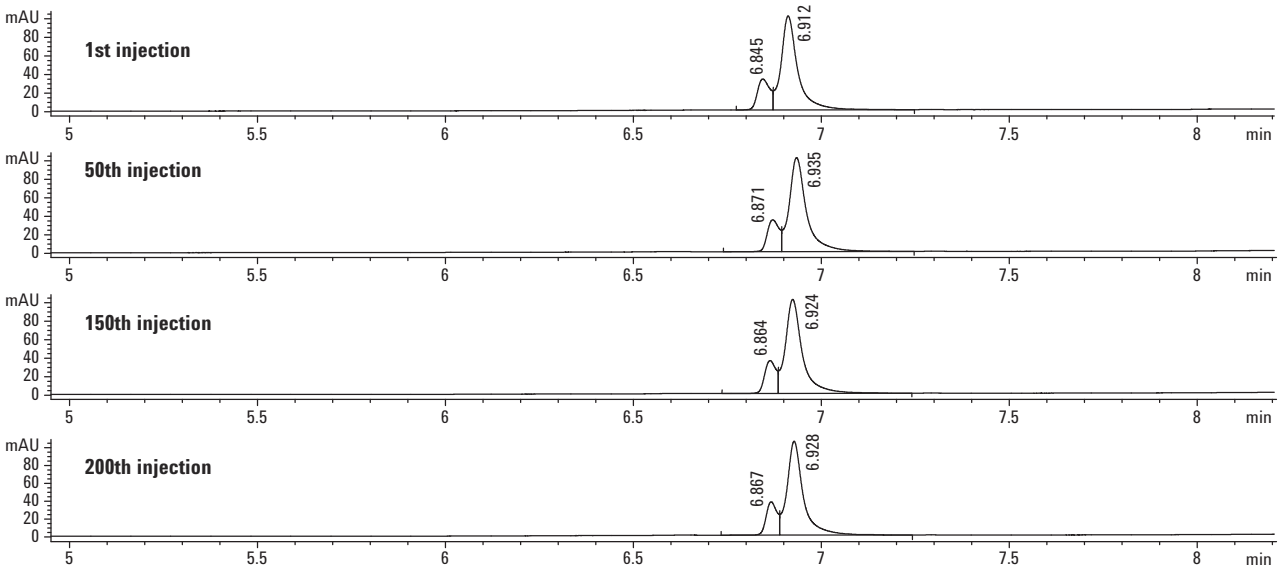
Gradient: 0 min-1% B, 2 min-20% B, 5 min-50% B, 7 min-50% B, 8.0 min-90% B, 8.3 min-1% hold for 2 min

Temperature: 75 °C

Detector: UV, 280
Agilent 1290 Infinity LC

Sample: Reduced monoclonal antibody (IgG1) (1.0 mg/mL) - Agilent BL05 IgG1

Injection: 2 µL



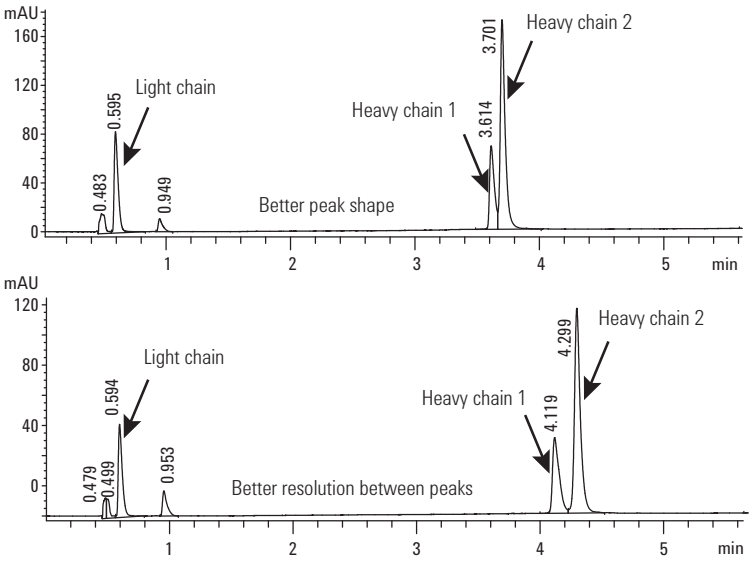
Reduced and alkylated mAb profiling during 200 repeated injections.

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

NEW!

Gradient optimizations for ultra fast analysis of reduced monoclonal antibody

Column:	**Agilent ZORBAX RRHD 300SB-Diphenyl 858750-944 2.1 x 100 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 80% propyl alcohol, 10% ACN, 9.9% water and 0.1% TFA
Flow Rate:	0.5 mL/min
Gradient:	1st condition: 0 min-1% B, 2 min-20% B, 5 min-70% B 2nd condition: 0 min-1% B, 2 min-20% B, 5 min-50% B
Temperature:	74 °C
Detector:	UV, 280 nm
Sample:	Reduced monoclonal antibody (IgG1) (1.0 mg/mL) - BioCreative IgG1
Injection:	2 µL



Comparison of two ultra-fast separations of reduced monoclonal antibodies was achieved on a Agilent ZORBAX RRHD 300SB-Diphenyl under different optimized conditions. The top panel separation delivered narrow peak widths with shorter retention times. The bottom panel separation displays higher resolution between the two heavy chain peaks, but with less efficiency.

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

NEW!

Ultra high speed and high resolution of intact monoclonal antibodies

Column: **Agilent ZORBAX RRHD 300-Diphenyl**
858750-944
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA in water
B: 80% n-propyl alcohol, 10% ACN, 9.9% water and 0.1% TFA

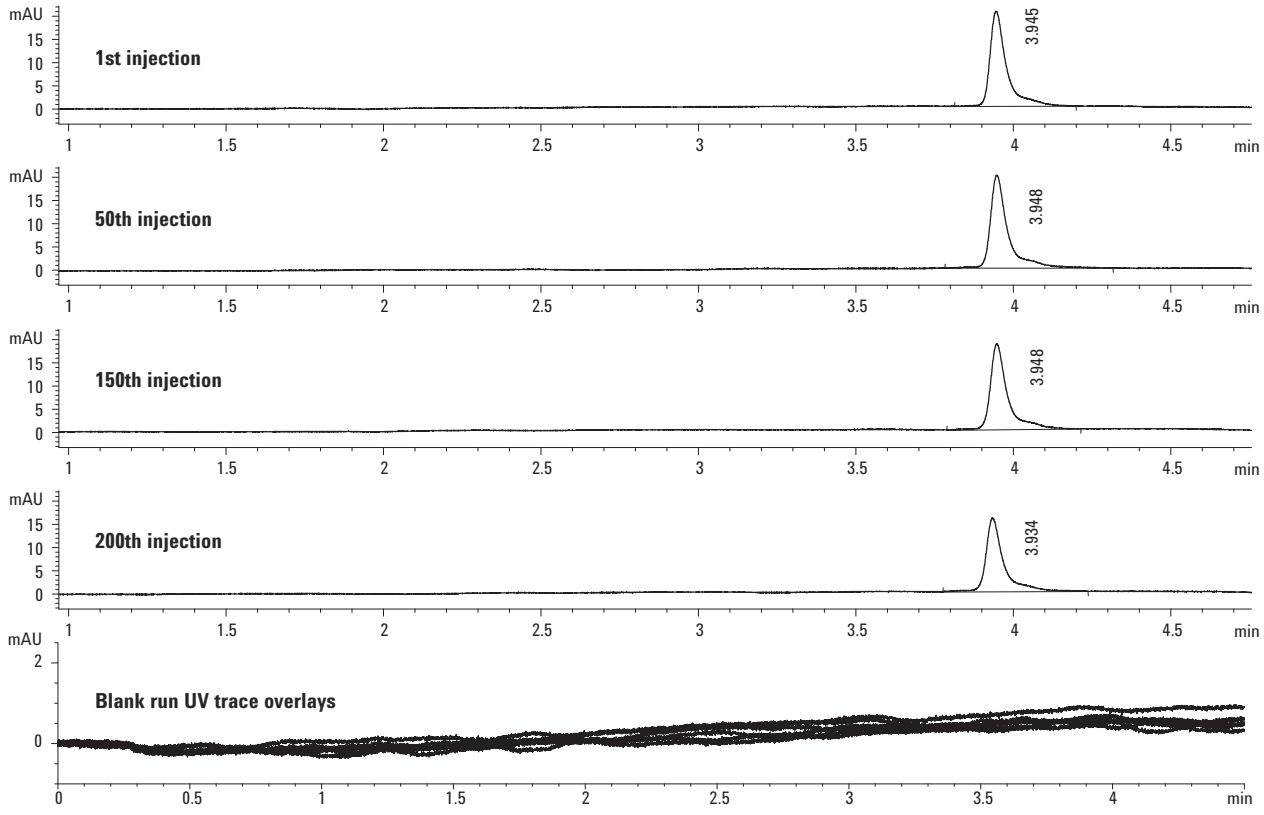
Flow Rate: 1.0 mL/min

Temperature: 74 °C

Detector: UV, 280 nm

Sample: Monoclonal antibody (IgG1) (1.0 mg/mL) - BioCreative IgG1 and Agilent Standard IgG1

Injection: 1 µL



Details of intact mAb profiling during 200 repeated injections. Intact mAb separations shown were collected at 1, 50, 150, and 200th run intervals. The bottom panel displays 5 UV blank run trace overlays collected every 20th run during the column evaluation (**note:** overlay traces are scaled to 2 mAu).

NEW!

Optimizing protein separations with Agilent weak cation-exchange columns

Column: **Agilent Bio WCX, stainless steel**
5190-2453
4.6 x 250 mm, 10 µm

Column: **Agilent Bio WCX, stainless steel**
5190-2445
4.6 x 250 mm, 5 µm

Mobile Phase: A: water
B: 1.6 M NaCl
C: 40.0 mM NaH_2PO_4
D: 40.0 mM Na_2HPO_4
By combining predetermined proportions of C and D, 20 mM buffer solutions at the desired pH range were produced (proportions determined using Buffer Advisor software)

Flow Rate: 1.0 mL/min

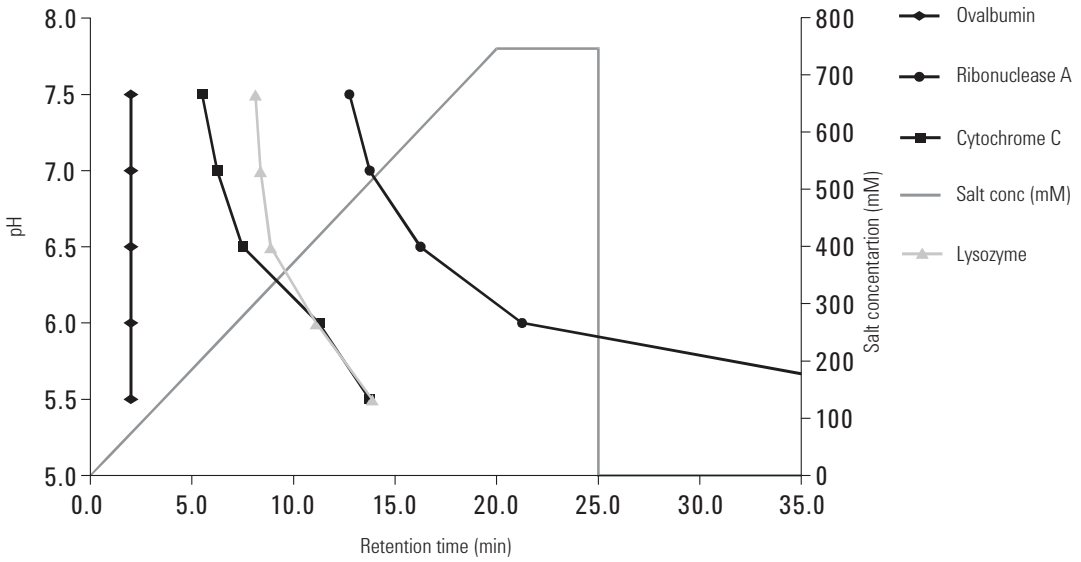
Gradient: 0 to 50% B, 0 to 20 min
50% B, 20 to 25 min
0% B, 25 to 35 min

Temperature: Ambient

Detector: UV, 220 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme

Sample Conc: 2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)



Effect of pH on retention time of protein standards using a Agilent Bio WCX column.

NEW!

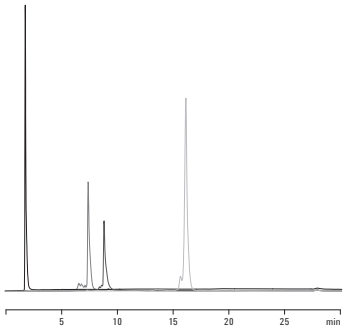
Improved resolution with smaller particle size with Agilent weak cation-exchange columns

Column: **Agilent Bio WCX, stainless steel**
5190-2453
4.6 x 250 mm, 10 µm

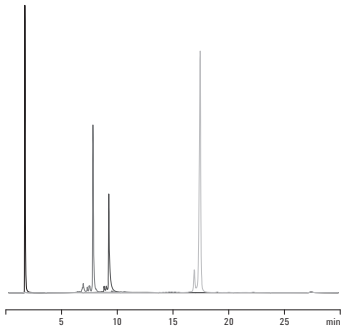
Column: **Agilent Bio WCX, stainless steel**
5190-2445
4.6 x 250 mm, 5 µm

Mobile Phase:	A: water B: 1.6 M NaCl C: 40.0 mM NaH_2PO_4 D: 40.0 mM Na_2HPO_4 By combining predetermined proportions of C and D, 20 mM buffer solutions at the desired pH range were produced (proportions determined using Buffer Advisor software)
Gradient:	0 to 50% B, 0 to 20 min 50% B, 20 to 25 min 0% B, 25 to 35 min
Temperature:	Ambient
Detector:	UV, 220 nm Agilent 1260 Infinity Bio-inert Quaternary LC
Sample:	Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme
Sample Conc:	2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)

1. Ovalbumin
2. Ribonuclease A
3. Cytochrome c
4. Lysozyme



Separation of protein standards at pH 6.5 using an Agilent Bio WCX, NP10 column.

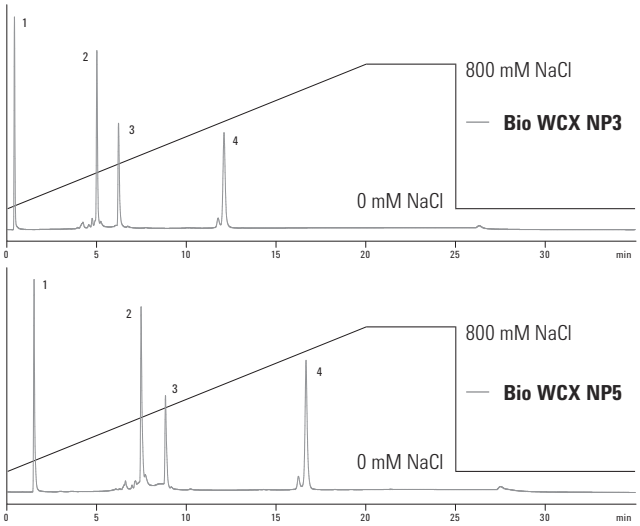


Separation of protein standards at pH 6.5 using an Agilent Bio WCX, NP5 column.

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

NEW!

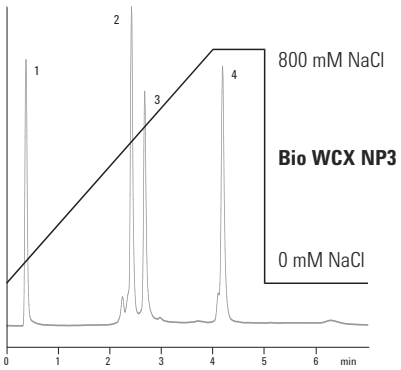
Faster separations using Agilent weak cation-exchange columns

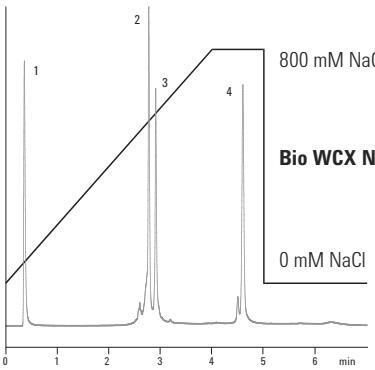


Protein separation on Agilent Bio WCX NP5 versus Agilent Bio WCX NP3.

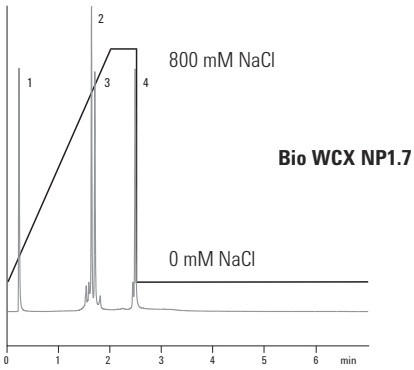
Column:	**Agilent Bio WCX, stainless steel 5190-2445 4.6 x 250 mm, 5 µm**
Column:	**Agilent Bio WCX, stainless steel 5190-2443 4.6 x 50 mm, 3 µm**
Column:	**Agilent Bio WCX, stainless steel 5190-2441 4.6 x 50 mm, 1.7 µm**
Mobile Phase:	A: 20 mM sodium phosphate, pH 6.5 B: A + 1.6 M NaCl
Flow Rate:	1.0 mL/min
Gradient:	0 to 50% B
Temperature:	Ambient
Detector:	UV, 220 nm Agilent 1260 Infinity Bio-inert Quaternary LC
Sample:	Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme
Sample Conc:	0.5 mg/mL

1. Ovalbumin
2. Ribonuclease A
3. Cytochrome c
4. Lysozyme





Comparison of Agilent Bio WCX NP3 versus Agilent Bio WCX NP1.7 (flow rate 1.0 mL/min).



Agilent Bio WCX NP1.7 for protein separations under 3 minutes (flow rate 1.7 mL/min).

NEW!

pH gradient elution for improved separation of monoclonal antibody charged variants

Column: **Bio MAb, stainless steel**
5190-2405
4.6 x 250 mm, 5 µm

Mobile Phase: A: water
B: 1.6 M NaCl
C: 100 mM NaH_2PO_4
D: 100 mM Na_2HPO_4
By combining predetermined proportions of C and D, buffer solutions at the desired pH range were produced at the selected buffer strengths.

Flow Rate: 1.0 mL/min

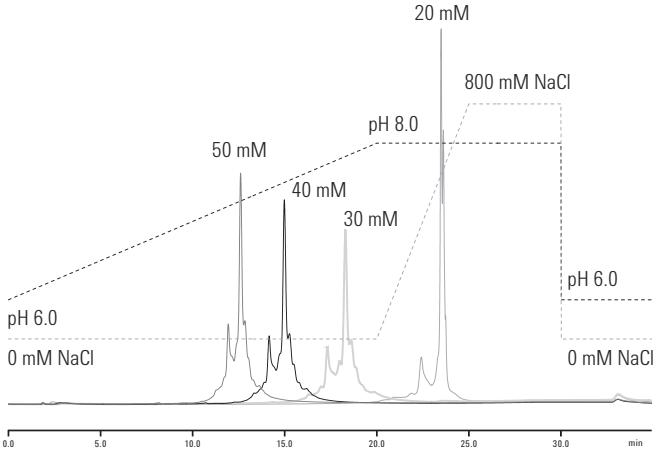
Gradient: pH 6.0 to 8.0, 0 to 20 minutes
0 to 800 mM NaCl, 20 to 25 minutes
800 mM NaCl, 25 to 30 minutes

Temperature: Ambient

Detector: UV, 220 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: IgG monoclonal antibody

Sample Conc: 2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)



Chromatograms of IgG monoclonal antibody at different ionic strengths.

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

NEW!

Separation of recombinant human erythropoietin (rEPO) using Agilent Bio SEC-3

Column: **Bio SEC-3, 100Å**
5190-2503
4.6 x 300 mm, 3 µm

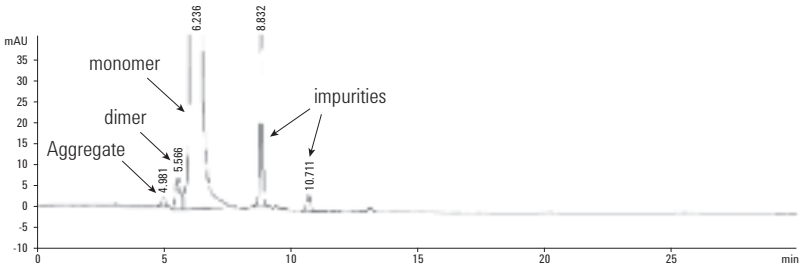
Mobile Phase: 150 mM sodium phosphate buffer, pH 7.0

Flow Rate: 0.35 mL/min

Detector: UV, 225 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: Recombinant human EPO protein (rEPO)

Sample Conc: 1.0 mg/mL



Consistent ion-exchange MAb separation

Column: **Bio MAb, PEEK**
5190-2411
2.1 x 250 mm, 5 µm

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

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

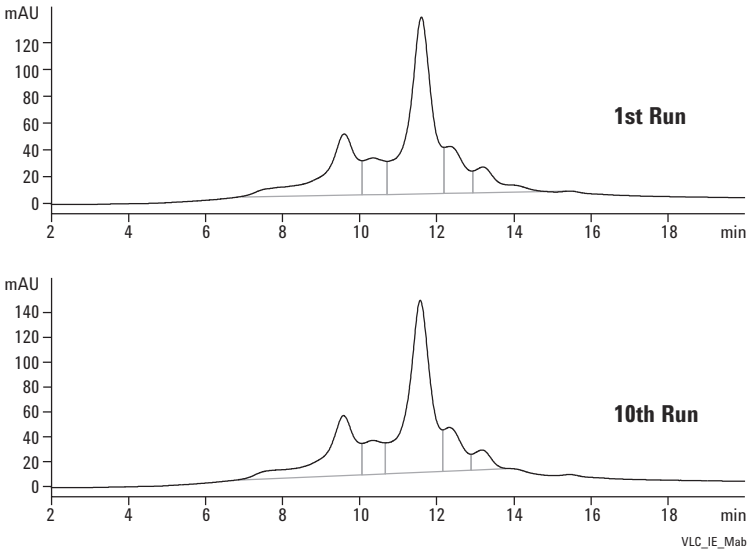
Flow Rate: 0.65 mL/min

Sample: CHO-humanized MAb, 1 mg/mL

Injection: 2.5 µL

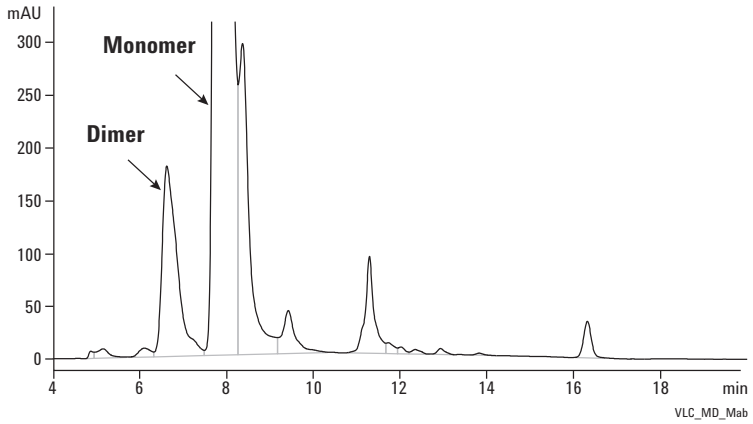
Detector: UV, 220 nm

Temperature: Ambient



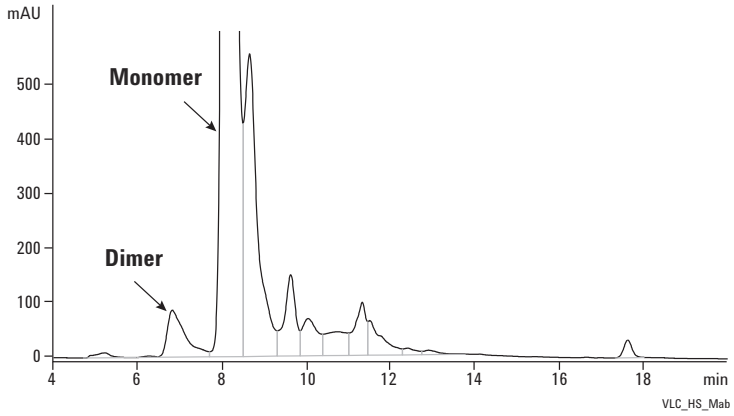
Intact MAb monomer and dimer separation

Column:	**Bio SEC-3, 300Å 5190-2511 7.8 x 300 mm, 3 µm**
Buffer:	Sodium phosphate buffer, pH 7.0, 150 mM
Gradient:	0-100% Buffer A from 0-30 min
Flow Rate:	1.0 mL/min
Sample:	CHO-humanized MAb, 5 mg/mL – intact
Injection:	5 µL
Detector:	UV, 220 nm
Temperature:	Ambient



Separation of heated, stressed MAb

Column:	**Bio SEC-3, 300Å 5190-2511 7.8 x 300 mm, 3 µm**
Buffer:	Sodium phosphate buffer, pH 7.0, 150 mM +150 mM sodium sulfate
Gradient:	0-100% Buffer A from 0-30 min
Flow Rate:	1.0 mL/min
Sample:	CHO-humanized MAb, 5 mg/mL – stressed at 60 °C
Injection:	5 µL
Detector:	UV, 220 nm
Temperature:	Ambient



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

Nucleosides, purines and pyrimidines

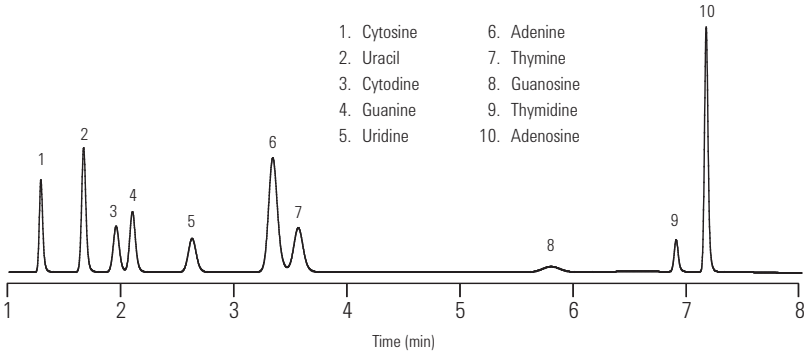
Column: **Eclipse Plus Phenyl Hexyl**
959993-912
4.6 x 150 mm, 5 µm

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. Cytodine
4. Guanine
5. Uridine
6. Adenine
7. Thymine
8. Guanosine
9. Thymidine
10. Adenosine



nucleosides

Amino acid standard separation on Eclipse Plus C18

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

Mobile Phase: A: 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, 0.5 mM NaN_3, 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

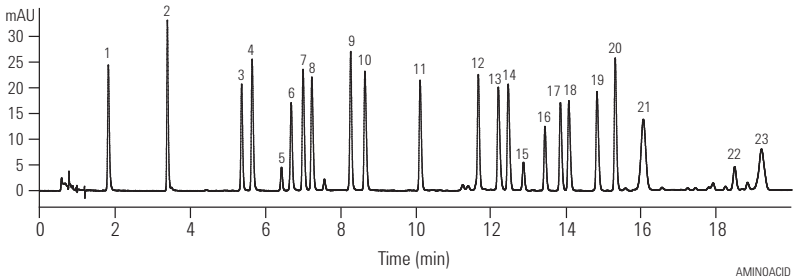
Sample: 900 pmol Amino Acids with extended Amino Acids and Internal Standards (500 pmol)

Derivatization: Automated, online, OPA / FMOC

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

Gradient

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



AMINOACID

Antibodies: Fast separation of IgM and IgG antibodies

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

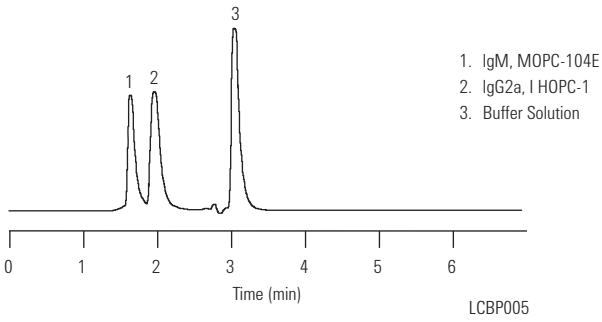
Mobile Phase: 200 mM Sodium Phosphate (pH 7), 0.01% Azide

Flow Rate: 0.94 mL/min

Temperature: Ambient

Detector: UV, 230 nm

Sample: 2.5 µL (1 mg/mL)



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 H_2O
B: 0.07% TFA in ACN

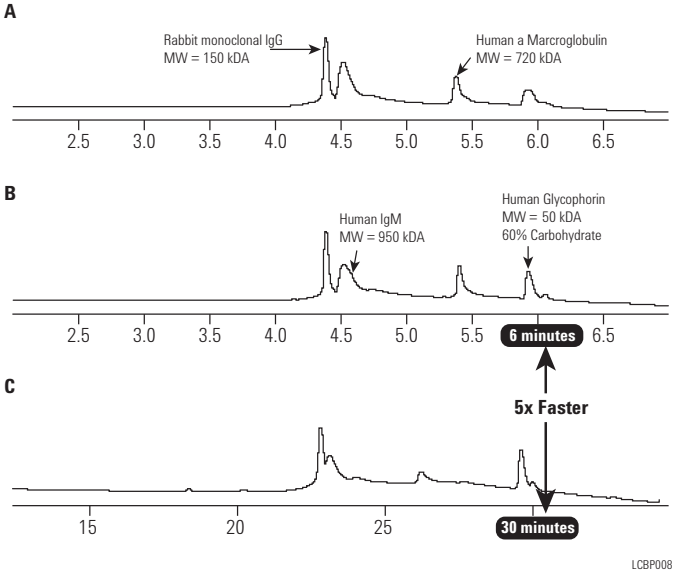
Flow Rate: A, B: 0.454 mL/min
C: 0.071 mL/min

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 AG, Basel.
Dr. Kurt Forrer
Patrik Roethlisberger

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

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

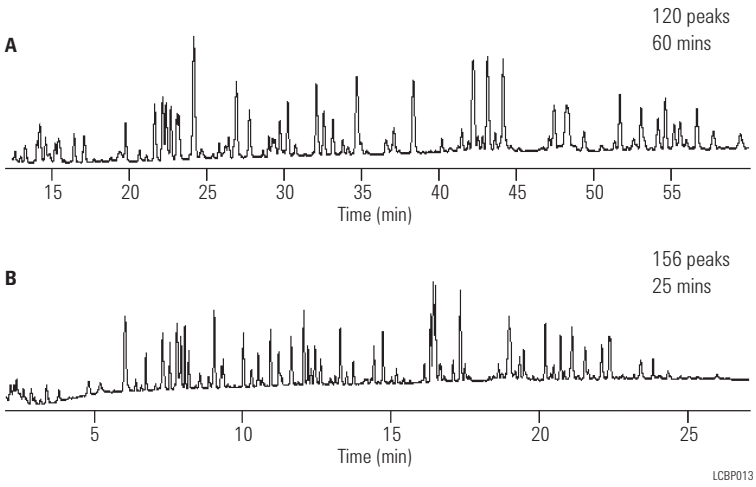
Flow Rate: A: 0.2 mL/min
B: 0.5mL/min

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)



Human serum: Low abundance protein isolation and identification from 1-D gel band 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

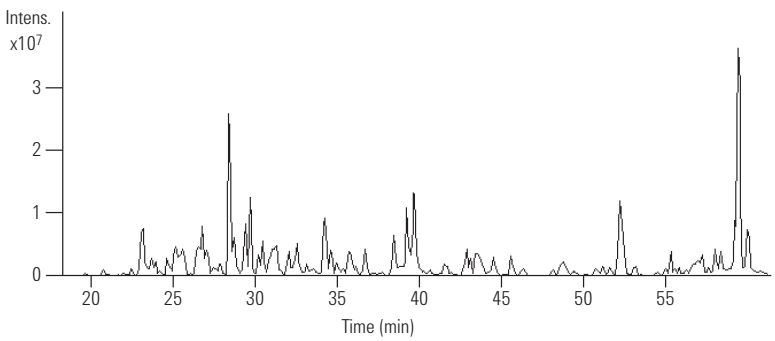
Flow Rate: 6 µL/min

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

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

Base Peak Chromatogram



Proteins Identified

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

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

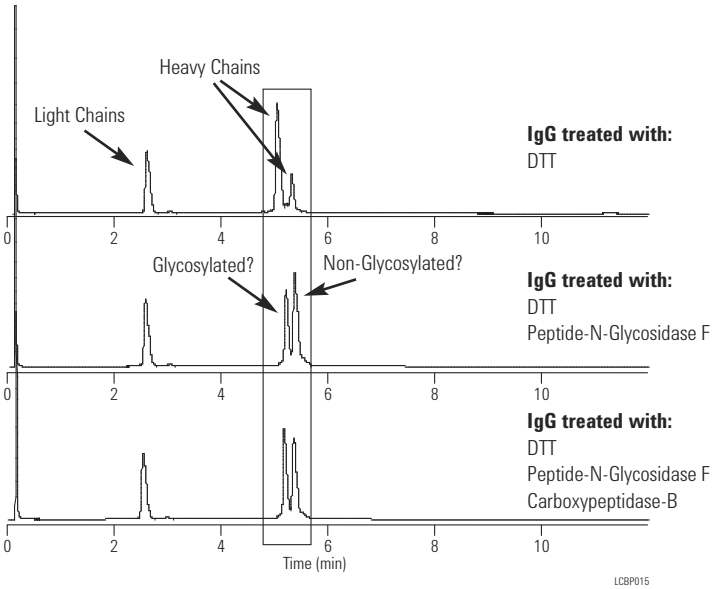
Flow Rate: 1.0 mL/min

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 AG, Basel.
Dr. Kurt Forrer
Patrik Roethlisberger



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

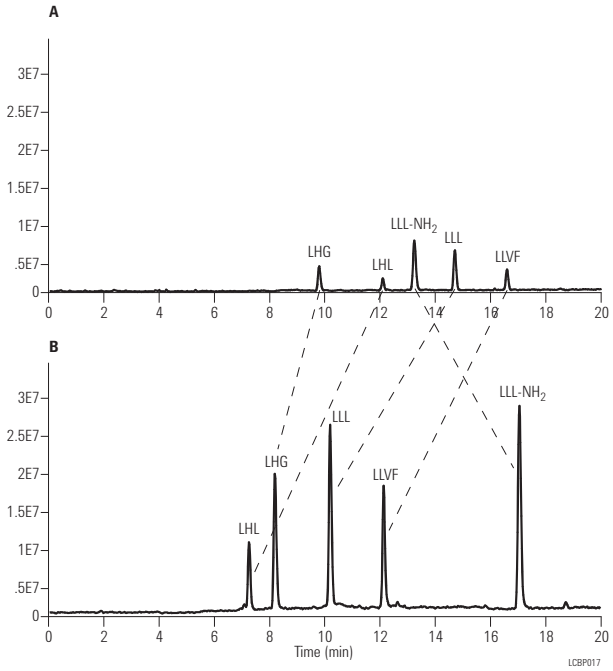
A: 20 mM NH_4OH in Water
B: 20 mM NH_4OH in 80% ACN

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
N_2 – 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 complementary 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 complementary separation can be investigated with one column. Better MS sensitivity for this sample is also achieved at high pH.

Nucleosides: Separation of deoxy and ribonucleosides

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

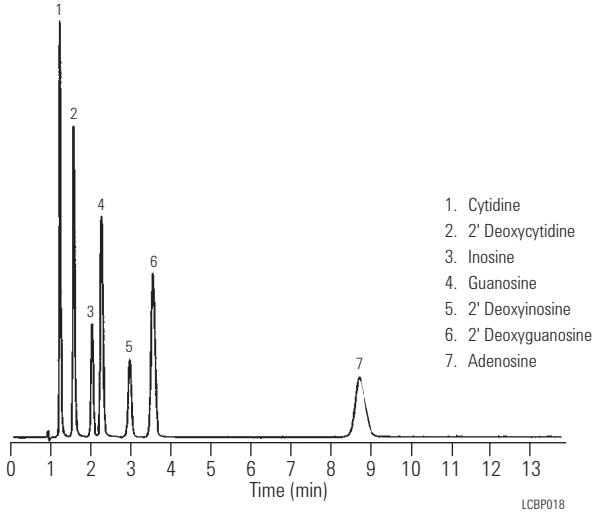
Mobile Phase: 4 mM Ammonium Phosphate (pH 4.0 with Phosphoric Acid)

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

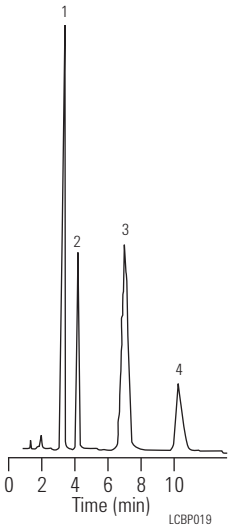
Mobile Phase: 0.1 M $NH_4H_2PO_4$

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

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

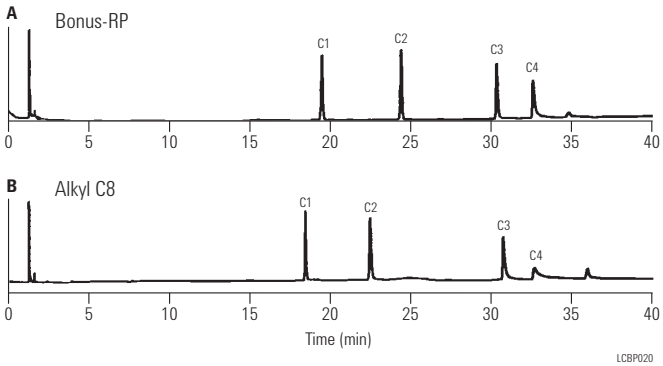
Gradient: 0-100% B in 50 min

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

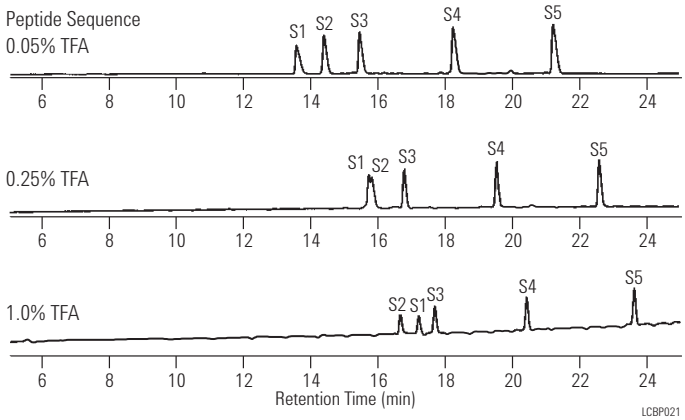
Flow Rate: 1.0 mL/min

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



LCBP021

Exploiting chemical stability – TFA concentration

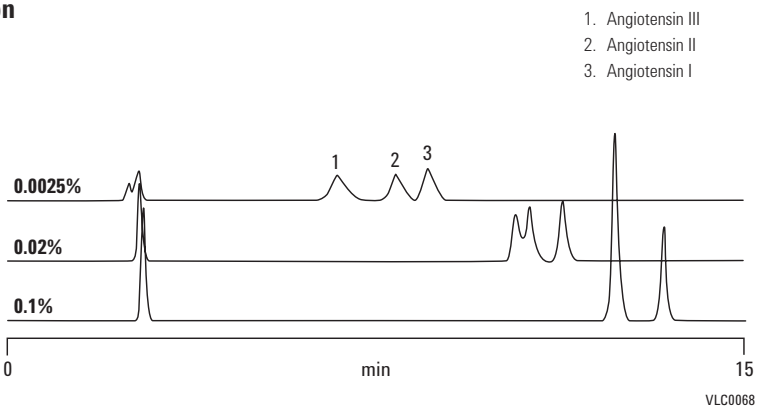
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

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)



Peptides: Separation of Antiotensins I, II, III with TFA and NH_4OH

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 NH_4OH in water
B: 10 mM NH_4OH in 80% ACN

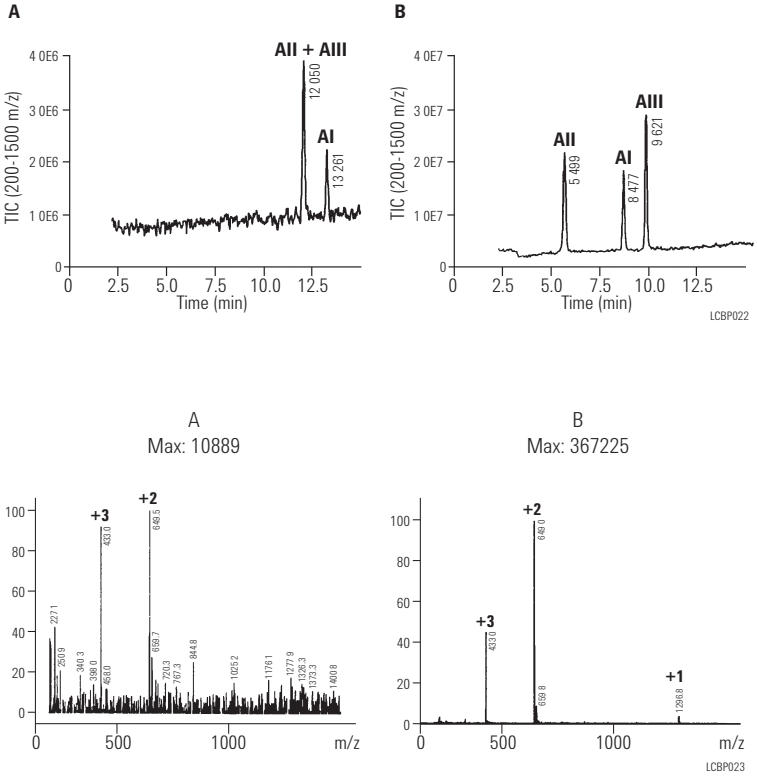
Flow Rate: 0.2 mL/min

Gradient: 15-50% B in 15 min

Temperature: 35 °C

MS Conditions: Pos. Ion ESI - Vf 70V, Vcap 4.5 kV
N_2-35 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

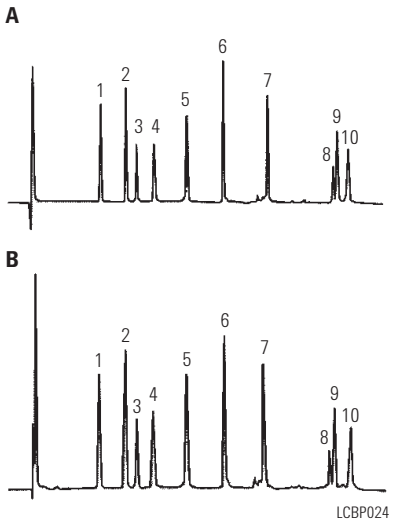
Flow Rate: A: Analytical
1 mL/min
B: Narrow Bore
0.2 mL/min

Gradient: 10-60% B in 30 min

Temperature: 35 °C

Detector: UV, 215 nm

Sample: 10 µL injection, concentration 2-6 µg



LCBP024

1. Met-enkephalin
2. Leu-enkephalin
3. Angiotensin II
4. Neurotensin
5. RNase
6. Insulin (BOV)
7. Lysozyme
8. Calmodulin
9. Myoglobin
10. Carbonic anhydrase

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%)

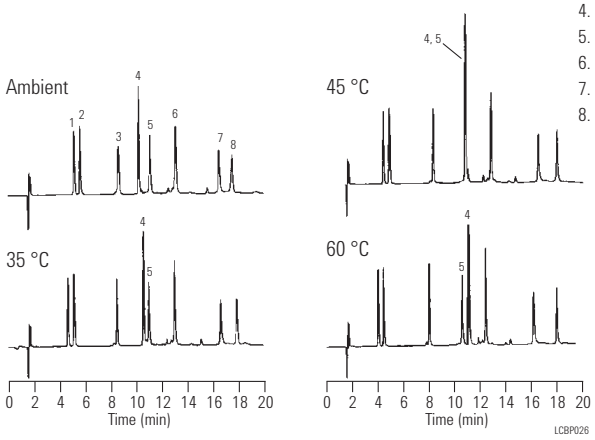
Flow Rate: 1.0 mL/min

Gradient: 15-53% in 20 min, post time 12 min

Temperature: Ambient – 60 °C

Detector: UV, 215 nm

Sample: Polypeptides



LCBP026

1. Leucine Enkephalin
2. Angiotensin II
3. RNase A
4. Insulin (BOV)
5. Cytochrome c
6. Lysozyme
7. Myoglobin
8. Carbonic anhydrase

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, H_2O
B: 0.07% TFA, ACN

Flow Rate: 3 mL/min

Gradient: 0-100% B in 1.33 min

Temperature: 70 °C

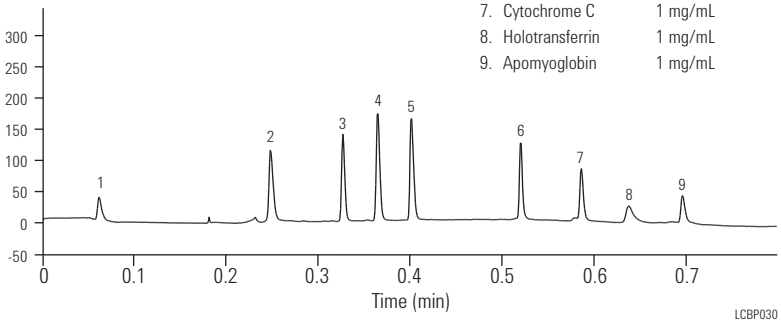
Detector: DAD 215/16 nm, ref = 310/10 nm

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



LCBP030

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)

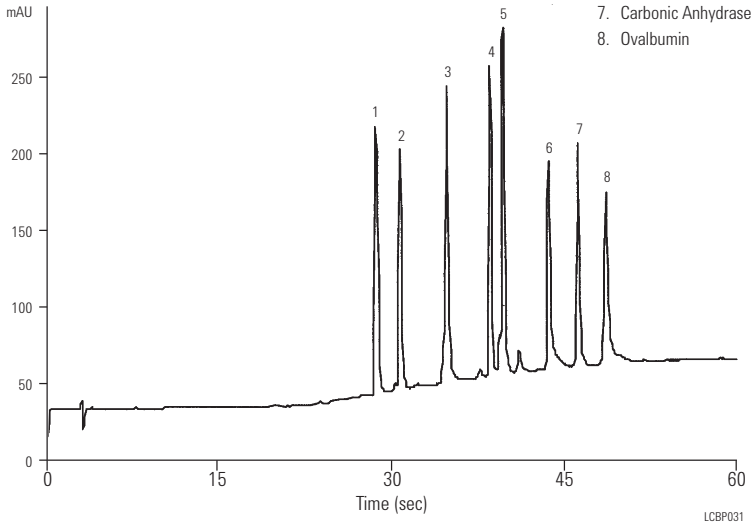
Gradient: 5-100% B in 1.0 min

Temperature: 70 °C

Detector: UV, 215 nm

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

1. Angiotensin II
2. Neurotensin
3. RNase
4. Insulin
5. Lysozyme
6. Myoglobin
7. Carbonic Anhydrase
8. Ovalbumin



LCBP031

Peptide RP-HPLC/ESI-MS using NH_4OH mobile phase yields both positive and negative ion spectra

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

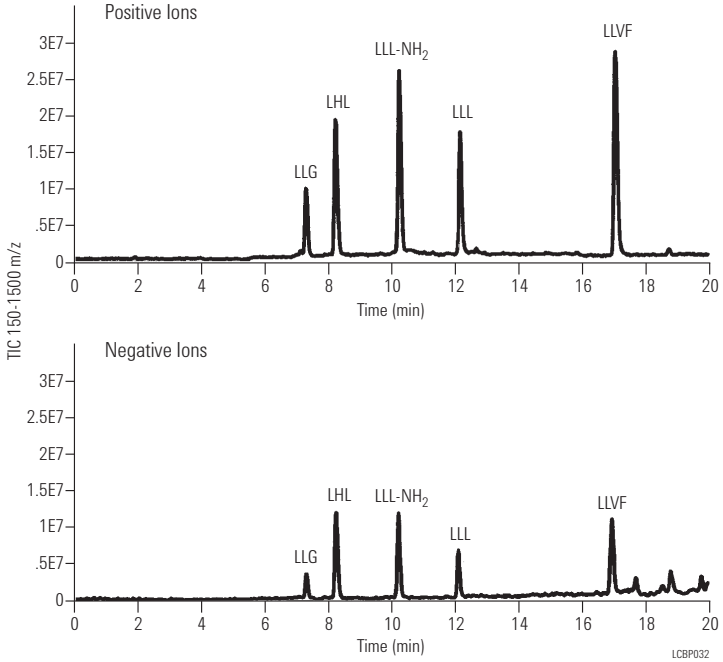
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS Conditions: Pos. Ion ESI – Vf 70 V, Vcap 4.5 kV,
N_2 – 35 psi, 12 L/min, 300 °C
TIC 150-1500 m/z

Sample: 4 µL (50 ng each peptide)



Comparison of Aβ peptide RP-HPLC separations at low and 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

Flow Rate: 0.25 mL/min

Gradient: 29-41% B in 30 min

Temperature: 80 °C

Detector: UV, 210 nm

Sample: 5 µL sample (100 pmol each)

Mobile Phase: A: 20 mM NH_4OH in water
B: 20 mM NH_4OH in 80% ACN

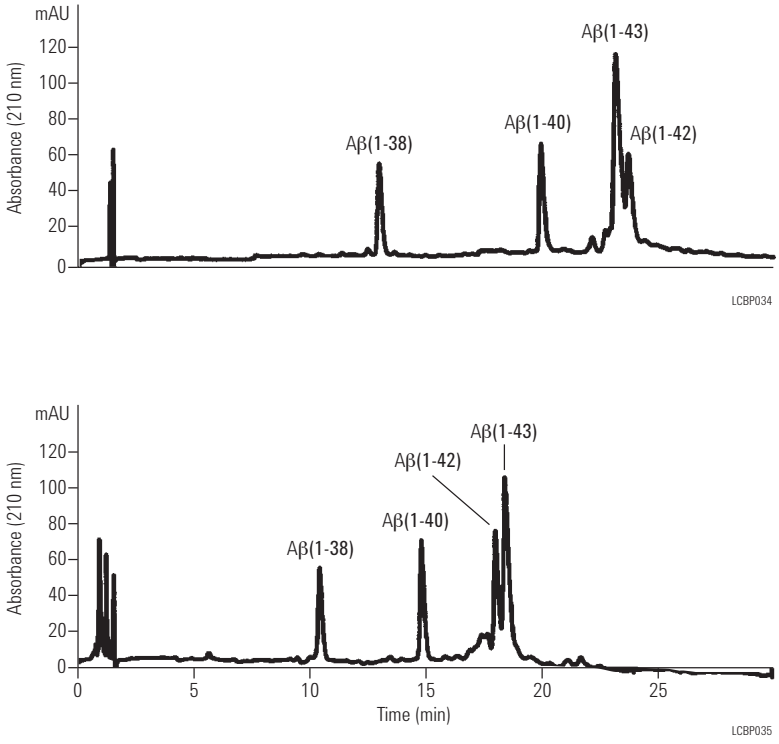
Flow Rate: 0.25 mL/min

Gradient: 26-38% B in 30 min

Temperature: 25 °C

Detector: UV, 210 nm

Sample: 5 µL sample (100 pmol each)



Selectivity comparison of TFA and NH_4OH for peptide RP-HPLC\ESI-MS analysis

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

Mobile Phase: TFA Conditions:
A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN
NH_4OH Conditions:
A: 20 mM NH_4OH in water
B: 20 mM NH_4OH in 80% ACN

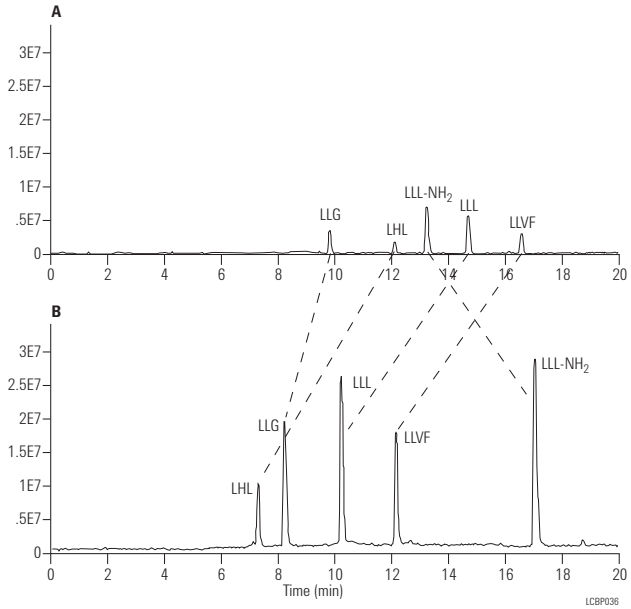
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS Conditions: Pos. Ion ESI – Vf 70V, Vcap 4.5 kV,
N_2 – 35 psi, 12 L/min., 300 °C
TIC 150-1500 m/z

Sample: 4 µL (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: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

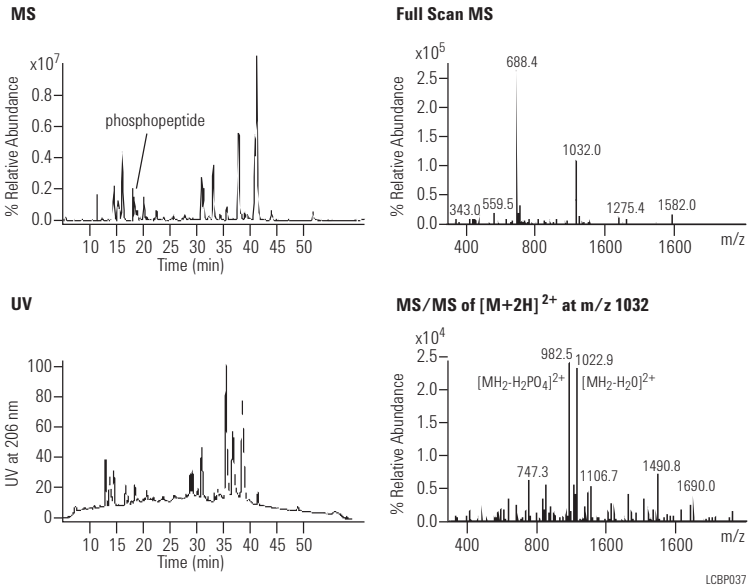
Flow Rate: 5.5 µL/min

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: 7 L/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 Ampitude: 1.0 V

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



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

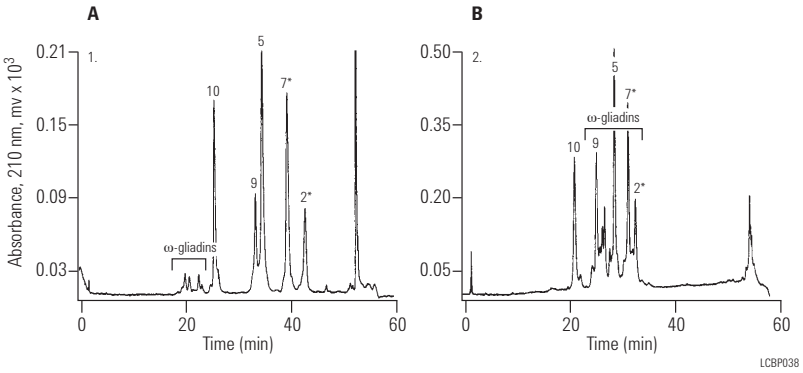
Flow Rate: 1.0 mL/min

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



Proteins: Effect of bonded phase

Column A: **ZORBAX RRHD 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 H_2O
B: 0.09% TFA in 80% ACN/20% Water

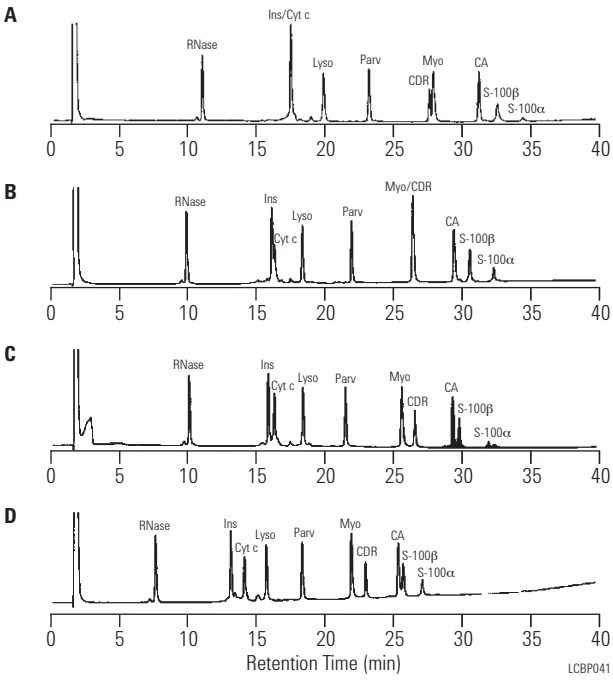
Flow Rate: 1.0 mL/min

Gradient: 25-70% B in 40 min

Temperature: 60 °C

Detector: UV, 210 nm

Sample: Polypeptides, 3 µg each



Standard proteins by reversed-phase

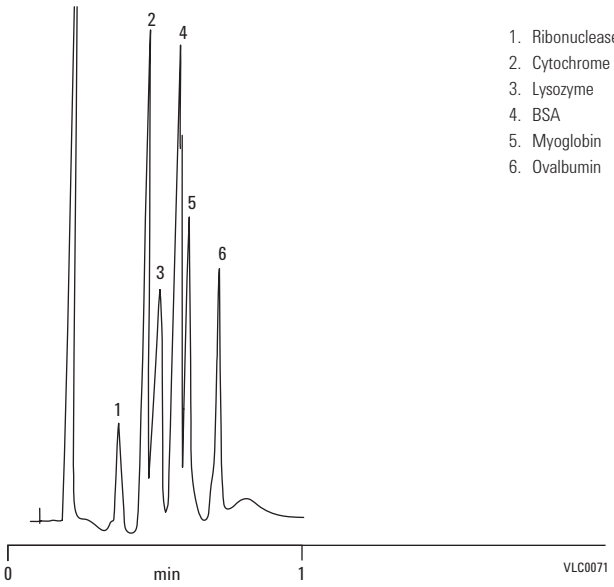
Column: **PLRP-S 4000Å**
PL1512-1803
4.6 x 50 mm, 8 µm

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



1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
4. BSA
5. Myoglobin
6. Ovalbumin

Standard ion-exchange protein separation

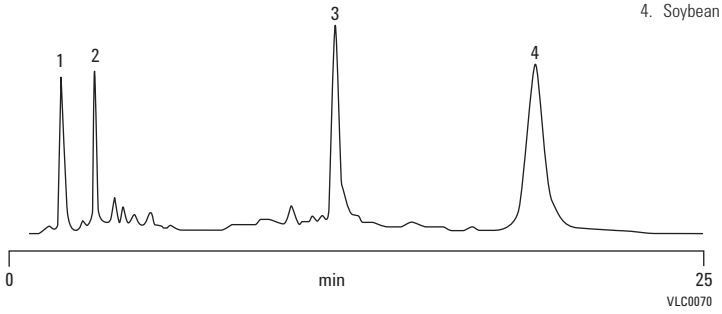
Column: **PL-SAX 1000Å**
PL1551-1502
4.6 x 50 mm, 5 µm

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



1. Myoglobin
2. Bovine carbonic anhydrase
3. Ovalbumin
4. Soybean trypsin inhibitor

Deoxynucleosides: Using rapid resolution 3.5 µm columns

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

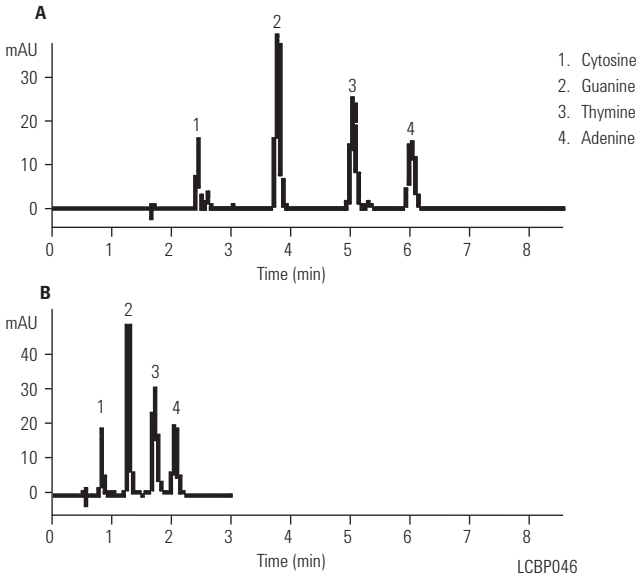
Column B: **ZORBAX SB-CN 835975-905 4.6 x 50 mm, 3.5 µm**

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



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

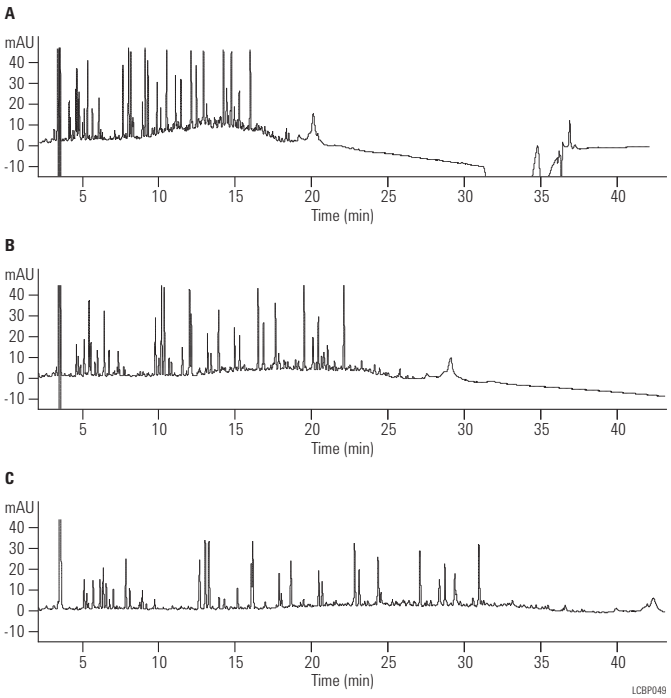
Flow Rate: 0.5 mL/min

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

Temperature: 80 °C

Detector: UV, 214 nm

Sample: BSA tryptic digest



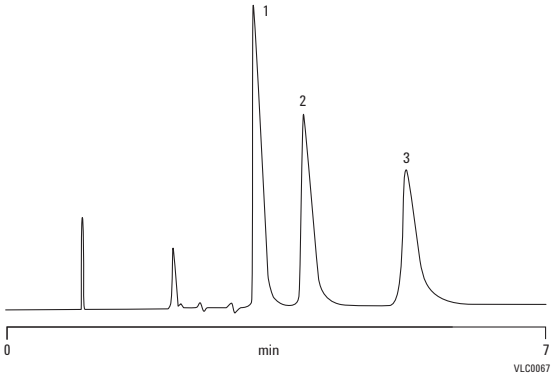
Catecholamines

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

Mobile Phase: 95% 25 mM citric acid, 25 mM Na_2HPO_4, 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

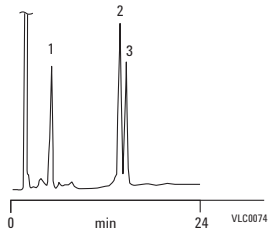
Mobile Phase: A: 0.1% TFA in 99% water:1% ACN
B: 0.1% TFA in 1% water:99% ACN

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 reversed-phase HPLC

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

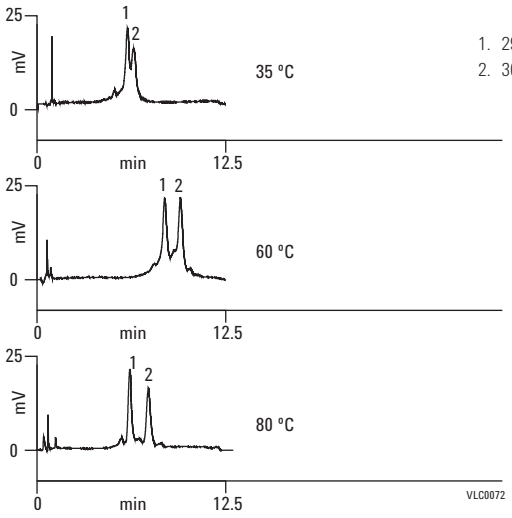
Mobile Phase: A: 100 mM TEAA
B: 100 mM TEAA in 25% ACN

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

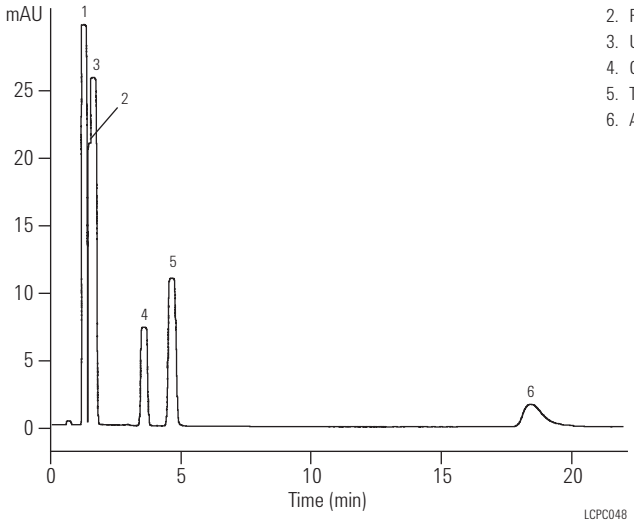
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

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

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Chemical/Industrial Applications

Analysis of biocides in hand sanitizer

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

Mobile Phase: A: H_2O (0.5% TFA)
B: ACN (0.04% TFA)

Flow Rate: 1.7 mL/min

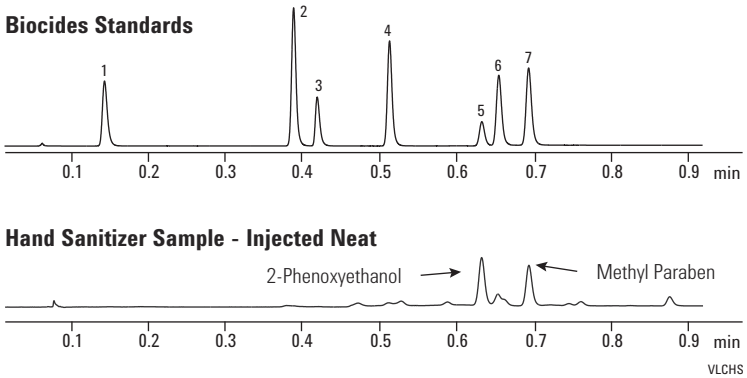
Gradient:

Time 0.0	95/5 A/B	
Time 1.0	55/45 A/B	
Time 1.1	0/100 A/B	

DAD: 275 nm (0 min)
225 nm (0.46 min)
255 nm (0.67 min)

Sample: 1 µL injection of 50 ppm std.

Temperature: 30 °C



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

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

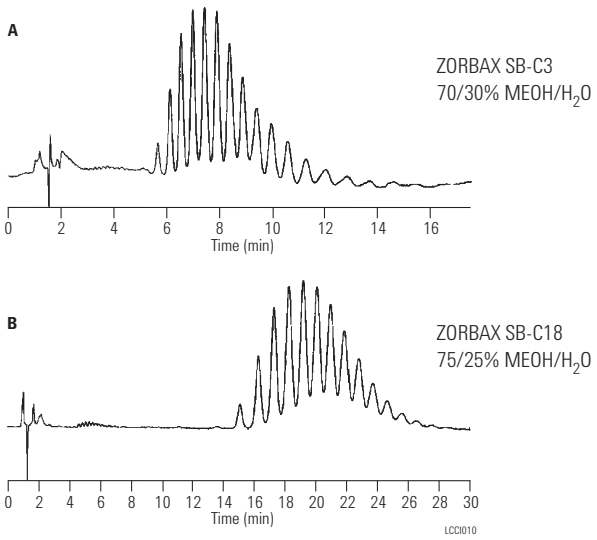
Mobile Phase: MeOH and H_2O (as indicated)

Flow Rate: 1.0 mL/min

Temperature: 50 °C

Detector: UV, 225 nm

Sample: Triton X-114



Organic acids separated on ZORBAX SB-Aq

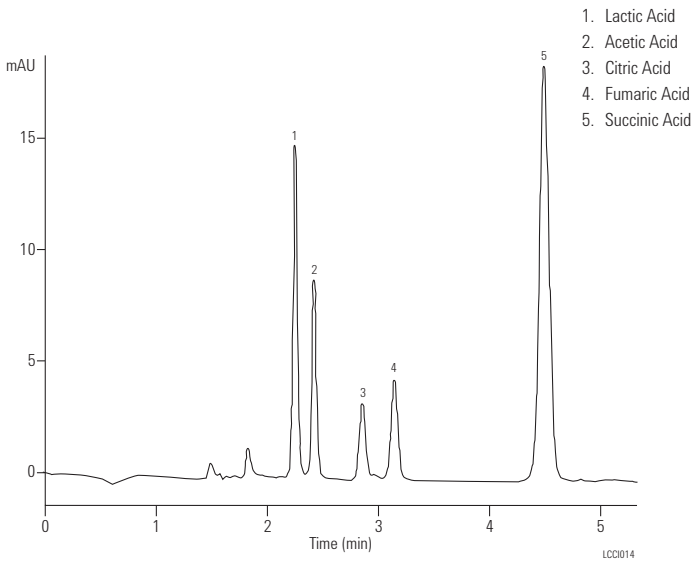
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 99% 20 mM NaH_2PO_4, pH 2, 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

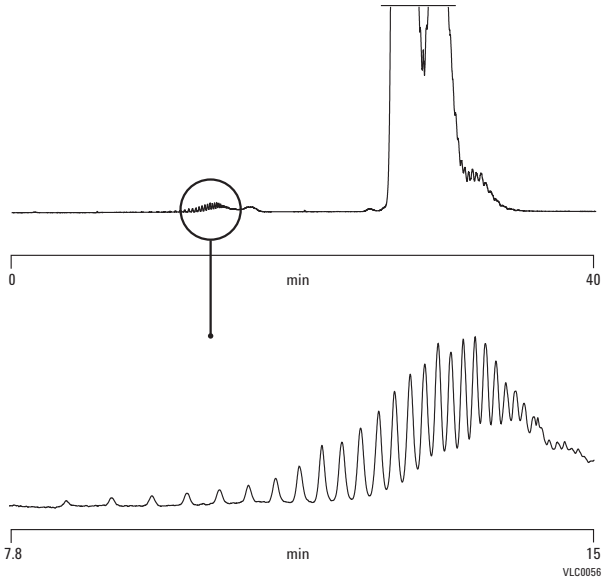
Gradient: 0-100% B in 40 min

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

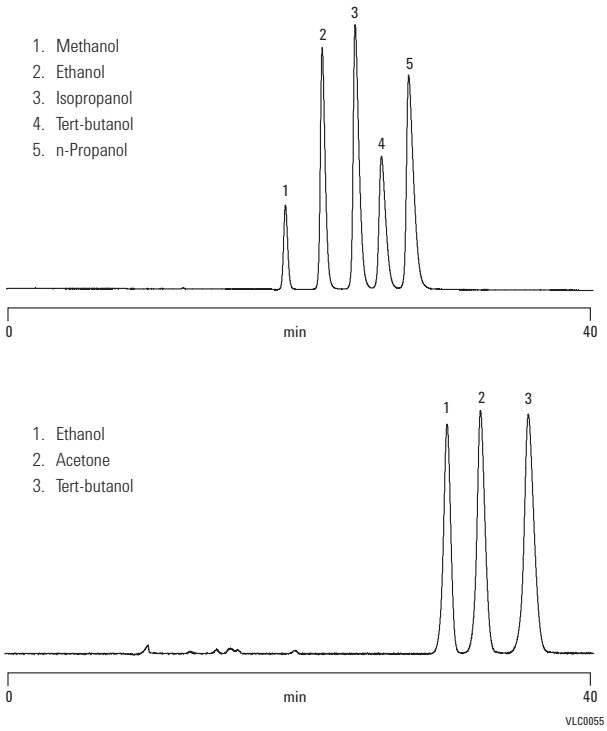
Column: **Hi-Plex H**
PL1170-6830
7.7 x 300 mm, 8 µm

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 40 °C

Detector: 356-LC RI



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Environmental Applications

NEW!

Fast LC/MS/MS analysis of group 4 pharmaceuticals from EPA-1694

Column: **ZORBAX RRHD HILIC Plus**
959758-901
2.1 x 100 mm, 1.8 µm

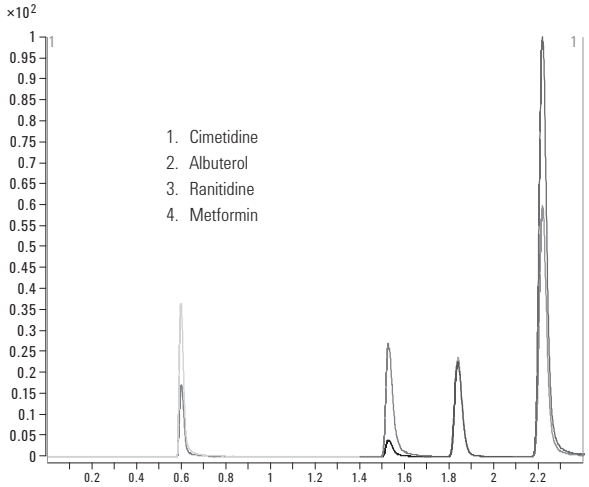
Mobile Phase: A: 10 mM ammonium acetate in water, pH 6.7
B: acetonitrile

Flow Rate: 1 mL/min

Detector: Agilent 1290 Infinity LC with an
Agilent 6410 Triple Quadrupole Mass Spectrometer

MS Conditions: TCC: 25 °C
dMRM, ESI positive mode, cycle time 35 ms
Drying Gas: 9 L/min, 300 °C
Nebulizer Pressure: 40 psig
Capillary Voltage: 4000

Sample: 0.1 µL injection of 0.1 mg/mL each in acetonitrile/water (3:1): cimetidine, albuterol, ranitidine and metformin



NEW!

Separation of azo dye degradation products

Column A: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

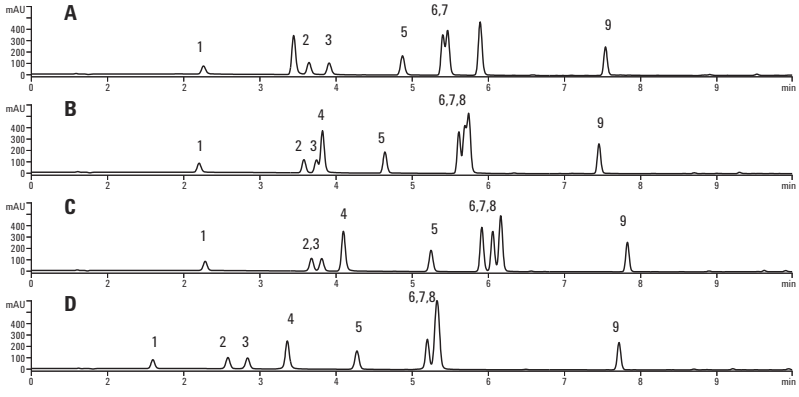
Column D: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Flow Rate: 0.4 mL/min

Gradient: 15 to 100% MeOH over 10 min

Solvent: 10 mM Ammonium acetate, pH 4.8

1. Aniline
2. o-Toluidine
3. Methoxyaniline
4. Chloroaniline
5. Benzidine
6. Dimethylbenzidine
7. 3,3'-Dimethoxybenzidine
8. Napthylamine
9. Dichlorobenzidine



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

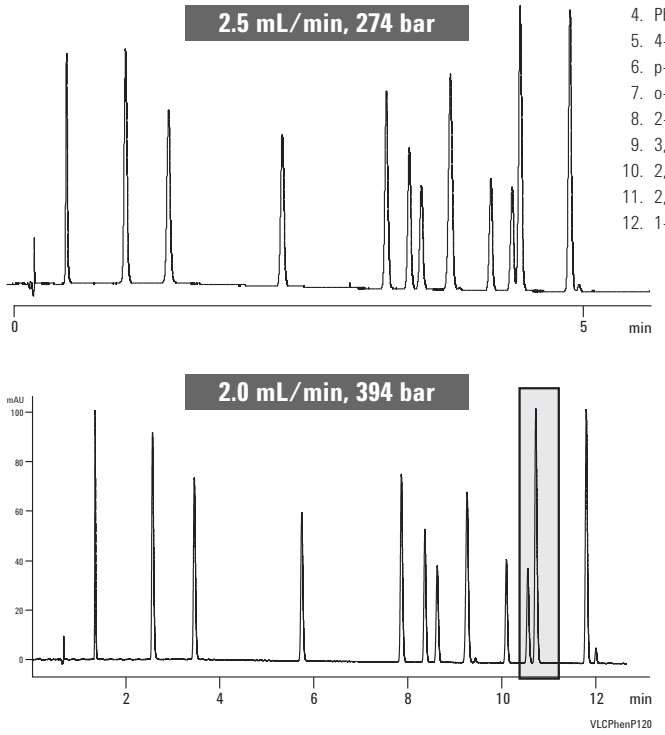
Column: **Poroshell 120 EC-C18**
695975-902
4.6 x 100 mm, 2.7 µm

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



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

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

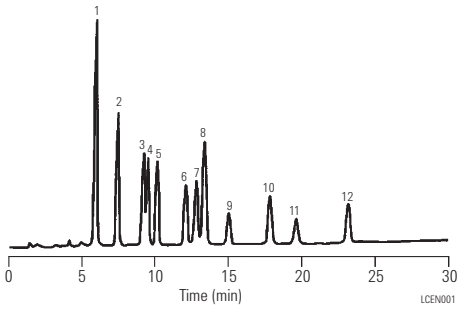
Flow Rate: 1.0 mL/min

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

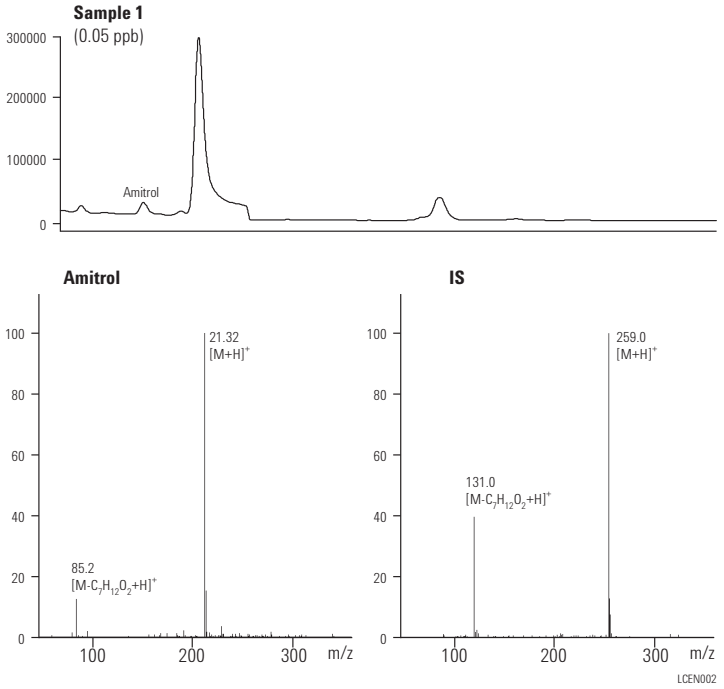
Gradient: 0 min, 65% B; 10 min, 65% B;
15 min, 100% B; 20 min, 65% B

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 (N_2):	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

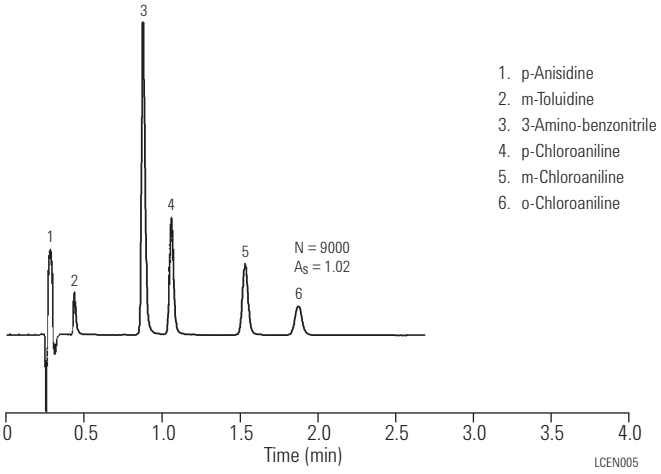
Mobile Phase: 20% ACN/80% 25 mM phosphate buffer, pH 2.5

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% H_2O + 5 mM CF_3COONH_4
B = H_2O + 5% ACN + 5 mM CF_3COONH_4,
pH 2.7 (CF_3COOH)

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

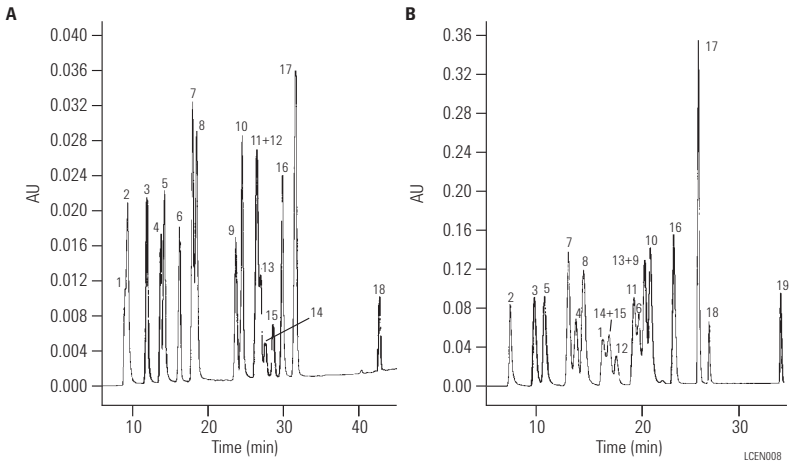
B:
0 min 80% B
1 min 80% B
15 min 70% B
30 min 20% B
35 min 20% B
37 min 80% B

Temperature: 18 °C

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

Sample: 10 µL of 19 explosive compounds in ACN/H_2O (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



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Explosives from soil extract

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

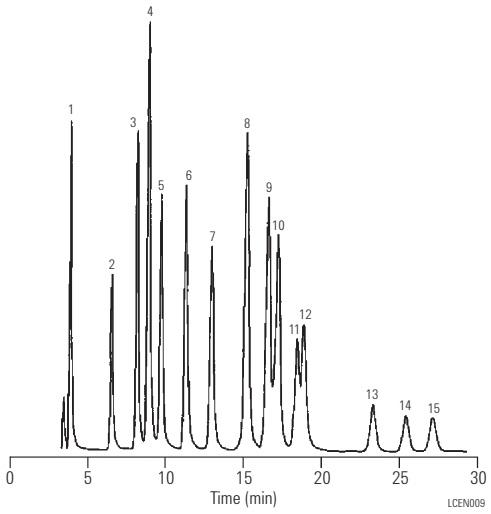
Mobile Phase: Methanol/Water (50/50) (v/v)

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

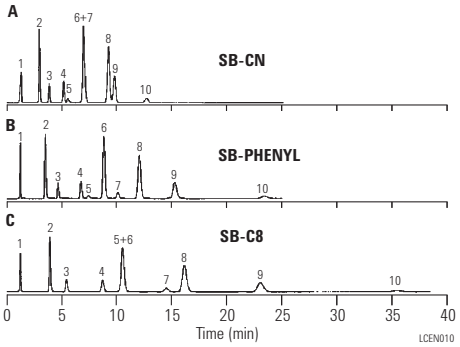
Mobile Phase: 35% ACN, 65% Water

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

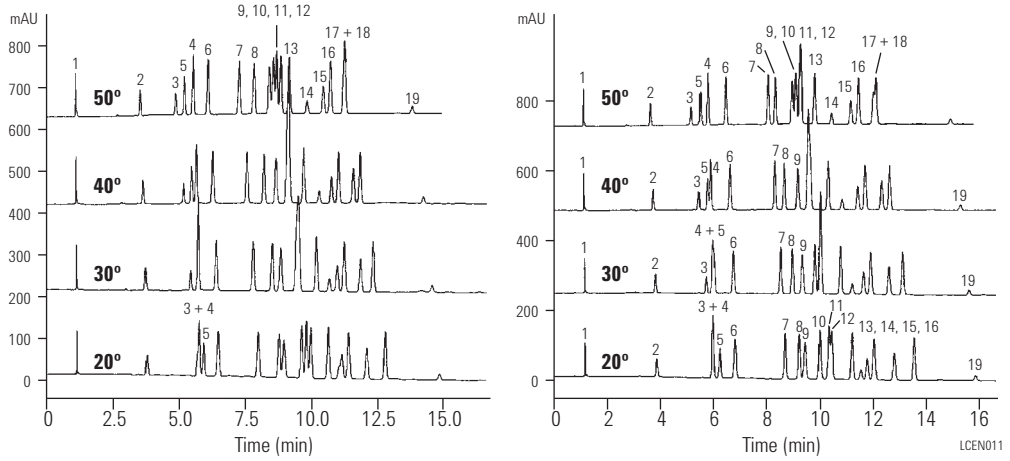
Sample: Herbicides



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	Column: Eclipse XDB-C18 993967-902 4.6 x 150 mm, 5 µm
Mobile Phase:	Water/Acetonitrile	Water/Acetonitrile
Flow Rate:	1.0 mL/min	1.0 mL/min
Gradient:	20-60% in 15 min	20-60% in 15 min
Temperature:	50 °C 40 °C 30 °C 20 °C	50 °C 40 °C 30 °C 20 °C
Detector:	DAD 240	DAD 240
Sample:	Herbicide & pesticide standards	Herbicide & pesticide standards



1. Desethyldesisopropylatrazine
2. Desethylatrazine
3. Benzthiazuron
4. Hexazinon
5. Metoxuron
6. Simazine
7. Methabenzthiazuron
8. Simazine
9. Atrazine
10. Isoproturon
11. Diuron
12. Monolonuron
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

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

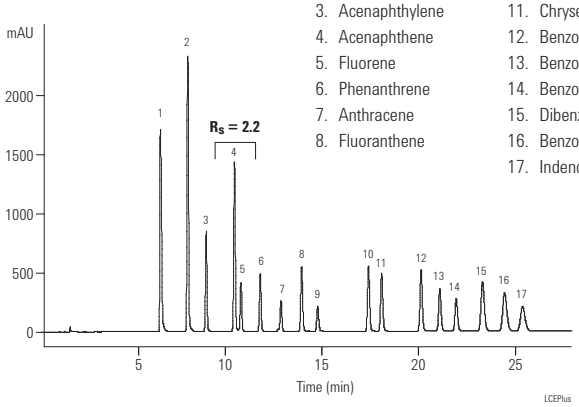
Gradient:

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



1. Toluene
2. Naphthalene
3. Acenaphthylene
4. Acenaphthene
5. Fluorene
6. Phenanthrene
7. Anthracene
8. Fluoranthene
9. Pyrene
10. Benzo(a)anthracene
11. Chrysene
12. Benzo(b)fluoranthene
13. Benzo(k)fluoeanthene
14. Benzo(a)pyrene
15. Dibenzo(a,h)anthracene
16. Benzo(g,h,i)perylene
17. Indeno(1,2,3-c,d)pyrene

Polycyclic aromatic hydrocarbons according to EPA Method 610

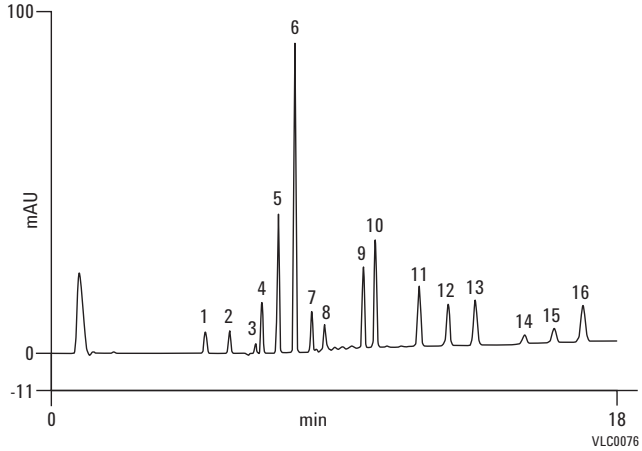
Column: **Pursuit PAH**
A7001100X046
4.6 x 100 mm, 3 µm

Sample: NIST 16473 Standard

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

Flow Rate: 2.0 mL/min

Detector: UV, 254 nm



1. Naphthalene
2. Acenaphthylene
3. Aceneaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Dibenzo[a,h]anthracene
15. Benzo[ghi]perylene
16. Indeno[1,2,3-cd]pyrene

NEW!

Rapid method development for 18 PAH compounds with an Agilent RRHD Eclipse PAH column

Column: **ZORBAX RRHD Eclipse PAH**
959758-918
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: Water
B: Acetonitrile

Flow Rate: 0.84 mL/min

Gradient: 40-100% B, gradient time (t_g) varies from 1 to 20 min; isocratic hold at 100% B for 2 min, re-equilibrate column at 40% B for 3 min

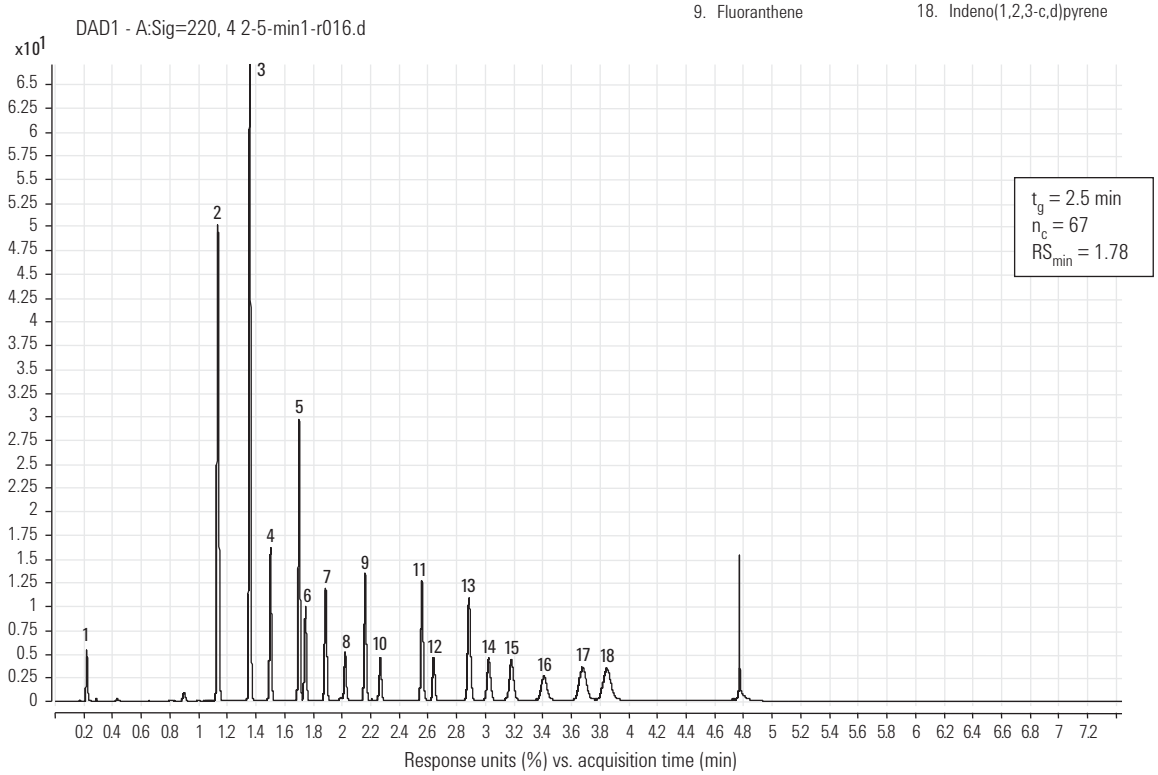
Temperature: 25 °C

Detector: Agilent 1290 Infinity LC

MS Conditions: Sig = 220, 4 nm; Ref = Off

Sample: 0.5 µL injection of diluted Agilent PAH Mixture (P/N 8500-6035) spiked with thiourea as a v_0 marker

1. Thiourea (V_0 marker)
2. Toluene
3. Naphthalene
4. Acenaphthylene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo(b)fluoranthene
14. Benzo(k)fluoranthene
15. Benzo(a)pyrene
16. Dibenzo(a,h)anthracene
17. Benzo(g,h,i)perylene
18. Indeno(1,2,3-c,d)pyrene



Gradient times are rapidly screened for the separation of 18 compounds.

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

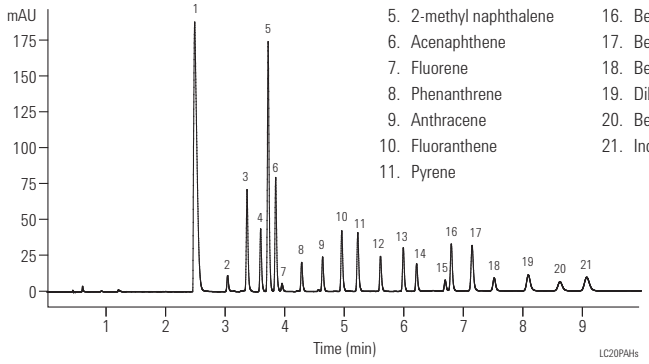
Gradient:

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



1. Toluene
2. Naphthalene
3. Acenaphthylene
4. 1-methyl naphthalene
5. 2-methyl naphthalene
6. Acenaphthene
7. Fluorene
8. Phenanthrene
9. Anthracene
10. Fluoranthene
11. Pyrene
12. Terphenyl-d14
13. Benzo(a)anthracene
14. Chrysene
15. Benz(e)pyrene
16. Benzo(b)fluoranthene
17. Benzo(k)fluoranthene
18. Benzo(a)pyrene
19. Dibenzo(a,h)anthracene
20. Benzo(g,h,i)perylene
21. Indeno(1,2,3-c,d)pyrene

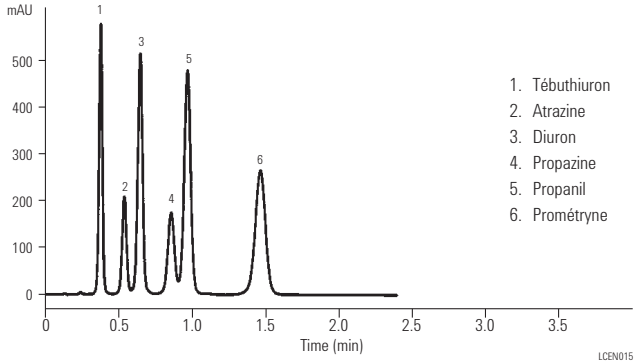
Herbicides: Rapid separation

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

Mobile Phase: MeOH:H_2O (60:40)

Flow Rate: 2 mL/min

Temperature: Ambient



1. Tébuthiuron
2. Atrazine
3. Diuron
4. Propazine
5. Propanil
6. Prométryne

Phenoxyacid herbicides

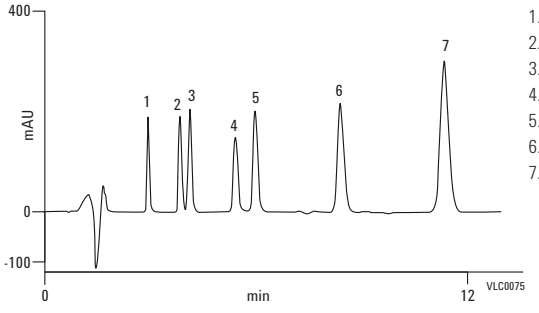
Column: **Pursuit XRs C8**
A6010150X046
4.6 x 150 mm, 5 µm

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

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm



1. Phenoxyacetic acid
2. o-Chlorophenoxyacetic acid
3. p-Chlorophenoxyacetic acid
4. 2,3-Dichlorophenoxyacetic acid
5. 2,4-Dichlorophenoxyacetic acid.
6. 2,4,5-Trichlorophenoxyacetic acid
7. 2,4,5-Trichlorophenoxypropionic acid (Silvex)

Triazine pesticides on Bonus-RP and Alkyl C8 phase

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

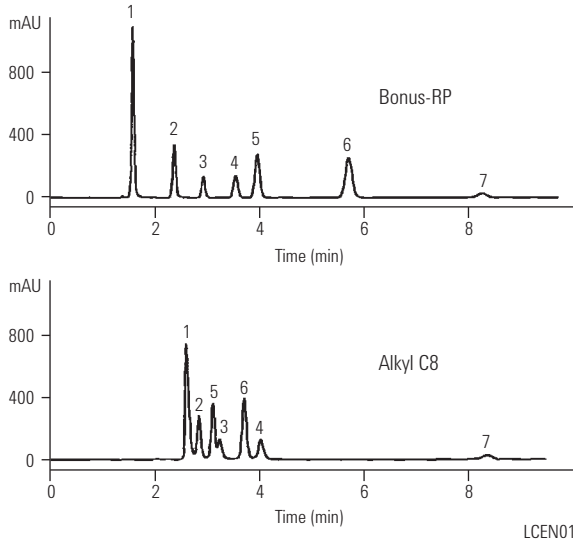
Mobile Phase: MeOH: 0.1% TFA (70:30)*

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: 254 nm

Sample: Triazine pesticides, 2 µL
1. Prometryne
2. Tebuthiuron
3. Atrazine
4. Propazine
5. Diuron
6. Propanil
7. Dacthal



LCEN01

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

Phenols, substituted

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

Mobile Phase: 20% ACN/80% 0.01 M H_3PO_4 to 45% ACN in 7.5 min

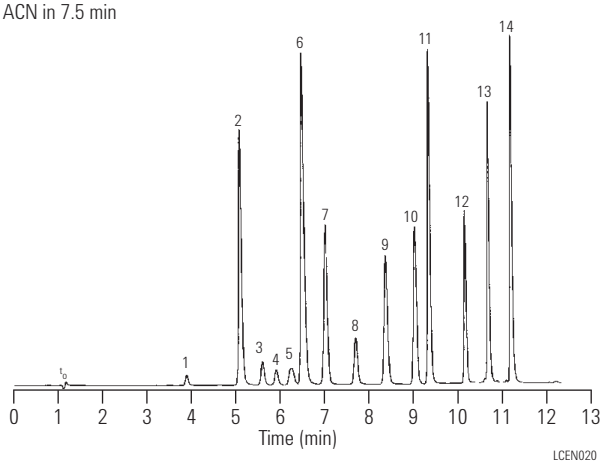
Flow Rate: 1.5 mL/min

Gradient: 80% ACN in 2.0 min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Phenols



LCEN020

1. Phenol
2. 4-Nitrophenol
3. m-Cresol
4. o-Cresol
5. 2-Chlorophenol
6. 2,4-Dinitrophenol
7. 2-Nitrophenol
8. 2,4-Dimethylphenol
9. 4-Chloro-3-methylphenol
10. 2,4-Dichlorophenol
11. 2-Methyl-4,6-dinitrophenol
12. 2,4,6-Trichlorophenol
13. 2,3,4,6-Tetrachlorophenol
14. Pentachlorophenol

Plant hormones: Rapid gradient elution separation

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

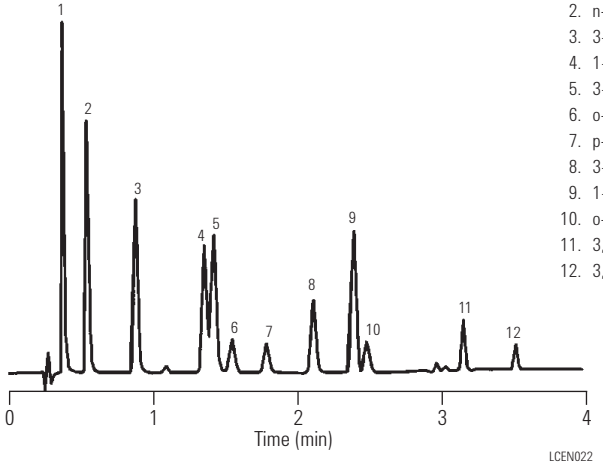
Mobile Phase: A: Water with 0.1% TFA
B: Acetonitrile with 0.1% TFA

Flow Rate: 3.0 mL/min

Temperature: 60 °C

Detector: UV, 245 nm

Sample: Plant hormones



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

VX nerve agent metabolites by LC/MS-IS standard (C13 labeled)

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

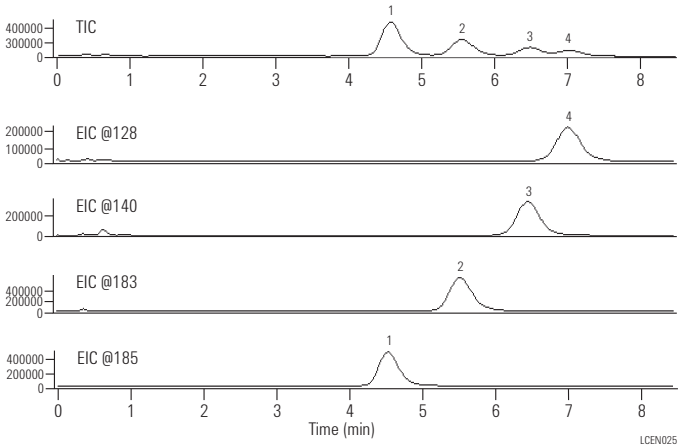
Mobile Phase: 1:1 (20 mM Ammonium Actetate pH 4.5/Acetonitrile)

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



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

Food and Consumer Product Applications

NEW!

Blueberry anthocyanin analysis

Column A: **Poroshell 120 SB-C18**
687975-902
4.6 x 75 mm, 2.7 µm

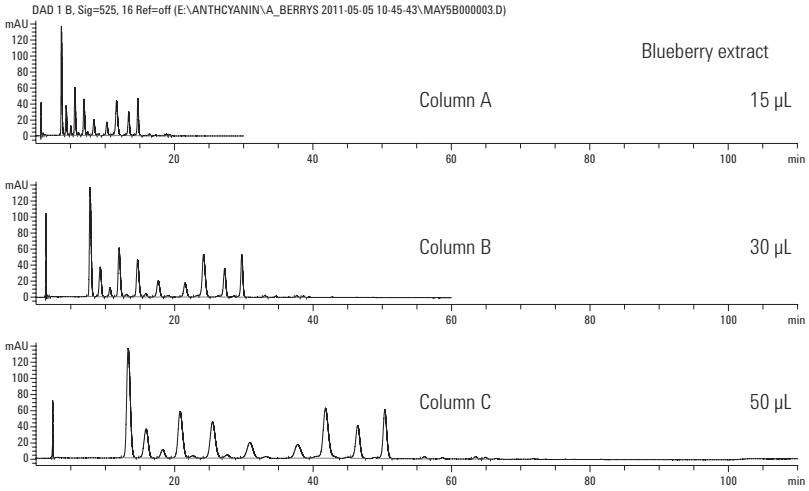
Column B: **ZORBAX SB-C18**
863953-902
4.6 x 150 mm, 3.5 µm

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

Flow Rate: 1 mL/min

Detector: Agilent 1260 Rapid Infinity LC

Blueberry anthocyanin analysis on totally porous and superficially porous StableBond C18 columns. Overlay of anthocyanin method with 250 mm 5 µm, 150 mm 3.5 µm, and 75 mm 2.7 µm at 1 mL/min.



NEW!

Analysis of pesticide residues in green tea

Column: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Mobile Phase: A: 5 mM FA in water
B: 5 mM FA in ACN

Flow Rate: 0.4 mL/min

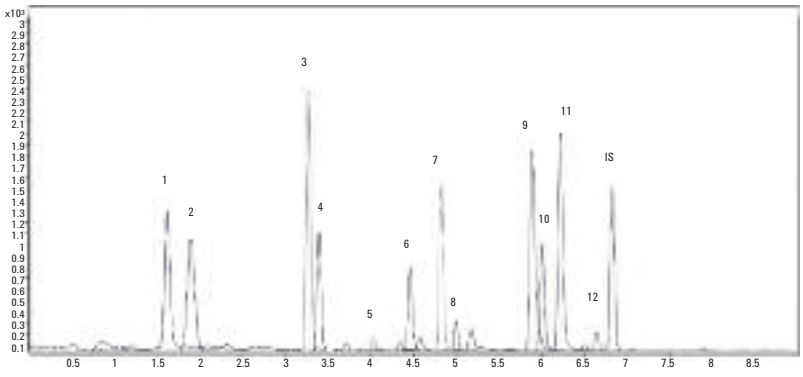
Gradient: 5% B in 1 min, 50% B in 3 min, 90% B in 7 min, 90% B in 8 min, 5% B in 8.2 min, 5% B in 9 min

Temperature: 30 °C

MRM chromatograms of 50 ng/g fortified sample processed by EN method.

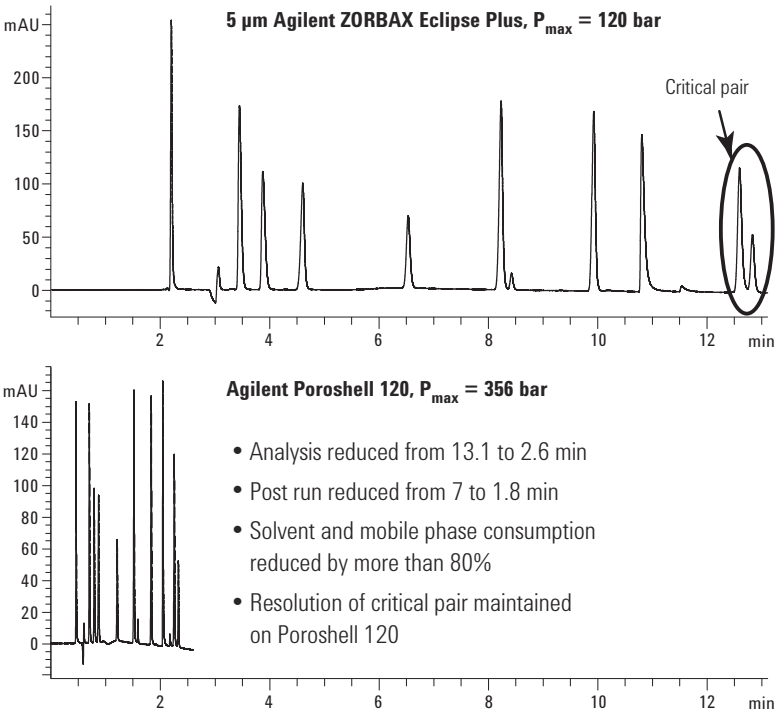
1. Acephate
2. Pymetrozine
3. Carbendazim
4. Thiabendazole
5. Imidacloprid
6. Imazalil
7. Propoxur
8. Carbaryl
9. Cyprodinil
10. Ethoprophos
11. Penconazole
12. Kresoxim-methyl

IS TPP



NEW!

An overlay of the original ZORBAX Eclipse Plus 5 µm method and Agilent Poroshell 120 method. All 11 peaks on Poroshell 120 are resolved by the time the first peak elutes on the original 5 µm ZORBAX Eclipse Plus method



- Analysis reduced from 13.1 to 2.6 min
- Post run reduced from 7 to 1.8 min
- Solvent and mobile phase consumption reduced by more than 80%
- Resolution of critical pair maintained on Poroshell 120

Column:	**Eclipse Plus C18 959990-902 4.6 x 250 mm, 5 µm**
Mobile Phase:	A: 20 mM ammonium acetate, pH 4.80 B: acetonitrile
Flow Rate:	1.000 mL/min
Gradient:	14% B at t_0, ramp to 52% B in 12.0 min
Temperature:	30 °C

Column:	**Poroshell 120 EC-C18 695975-302 3.0 x 100 mm, 2.7 µm**
Mobile Phase:	A: 20 mM ammonium acetate, pH 4.80 B: acetonitrile
Flow Rate:	0.851 mL/min
Gradient:	14% B at t_0, ramp to 52% B in 2.1 min
Temperature:	30 °C

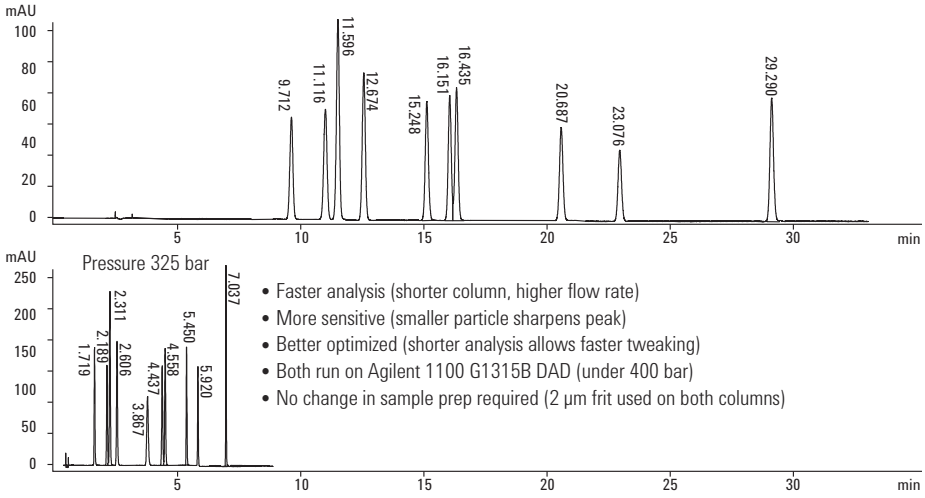
For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Fast analysis of sulfa drugs

Column:	**Eclipse Plus C18 959990-902 4.6 x 250 mm, 5 µm**
Column:	**Poroshell 120 EC-C18 695975-902 4.6 x 100 mm, 2.7 µm**
Gradient:	Formic acid/acetonitrile
Detector:	Agilent 1100 Series LC
Sample:	Ten sulfa drugs

A separation of ten sulfa drugs scaled from an Agilent ZORBAX Eclipse Plus C18 column to an Agilent Poroshell 120 EC-C18 column showing analysis time decreased from 30 min to 8 min using a formic acid/acetonitrile gradient.



- Faster analysis (shorter column, higher flow rate)
- More sensitive (smaller particle sharpens peak)
- Better optimized (shorter analysis allows faster tweaking)
- Both run on Agilent 1100 G1315B DAD (under 400 bar)
- No change in sample prep required (2 µm frit used on both columns)

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

NEW!

Determination of anthocyanins in blueberries

Column: **ZORBAX RRHD Eclipse Plus C18**
959758-902
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD Eclipse Plus Phenyl-Hexyl**
959758-912
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD SB-Aq**
858700-914
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD SB-Phenyl**
858700-912
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 5% HCOOH in H_2O
B: CH_3CN

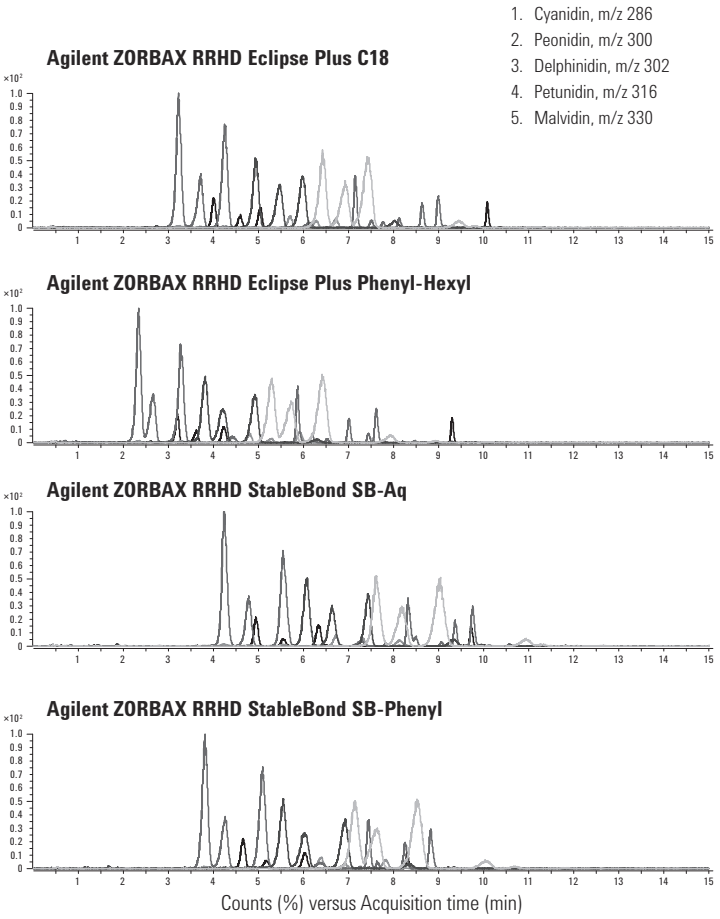
Flow Rate: 0.65 mL

Gradient: 10-50% B in 15 min

Detector: Agilent 1290 Infinity LC

MS Conditions: DAD: Sig = 525, 8 nm; Ref = Off
MS2 Scan: ESI + 200-1000
Scan time: 100 ms, 0.2 amu step
Fragmentor: 180 V
Drying gas: 10 L/min, 350 °C
Nebulizer Pressure: 50 psig
Capillary Voltage: 3500

Sample: 5 µL injection of blueberry extract



Separation of Azo Dyes

Column: **Eclipse Plus Phenyl Hexyl**
959996-912
4.6 x 100 mm, 5 µm

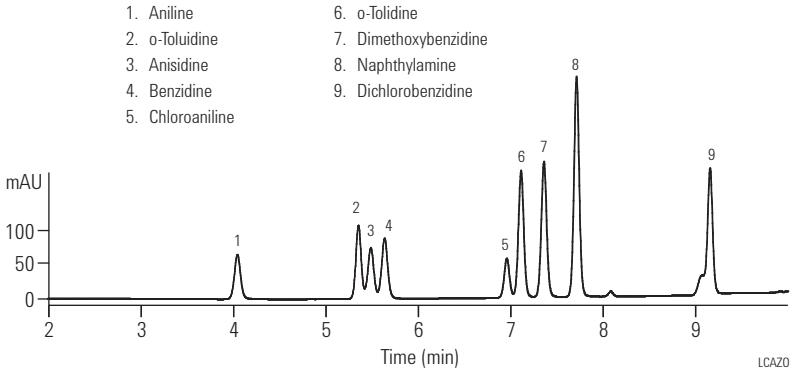
Mobile Phase: A: 10 mM Ammonium Acetate, pH 4.7
B: MeOH

Flow Rate: 1.5 mL/min

Gradient:

Time (Min):	%B:
0	25
5	50

Detector: UV, 254 nm



Anthocyanins from blueberries: High-efficiency high-speed separation

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

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

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

Mobile Phase: A: 3% Phosphoric acid
B: 100% MeOH

Flow Rate: 1.0 mL/min

Gradient: As shown

Temperature: 30 °C

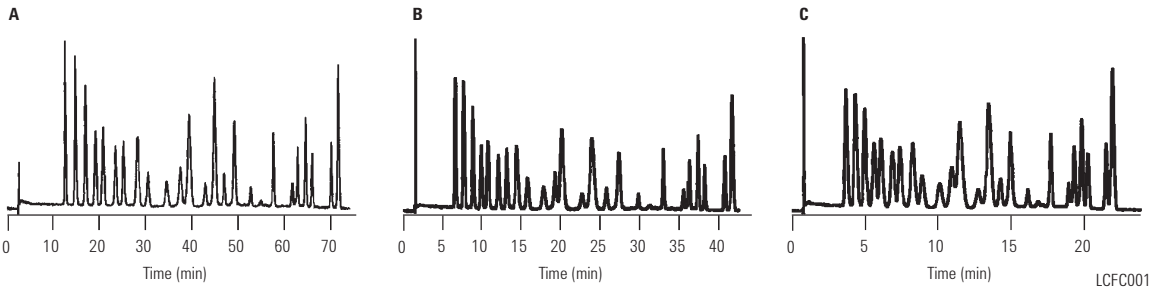
Detector: UV, 525 nm

Sample: Natural anthocyanins

Time	Percent B
0 min	23% B
35 min	26% B
97 min	60% B

Time	Percent B
0 min	23% B
21 min	26% B
58.2 min	60% B

Time	Percent B
0 min	23% B
10.5 min	26% B
29.1 min	60% B



Aromatics II

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

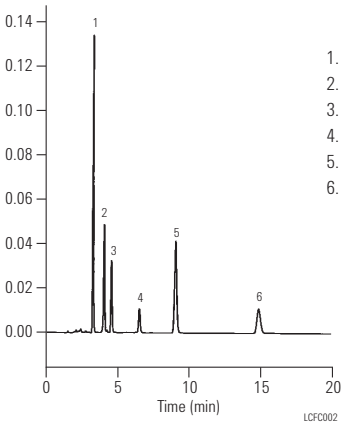
Mobile Phase: H_2O: MeOH, 40:60

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

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

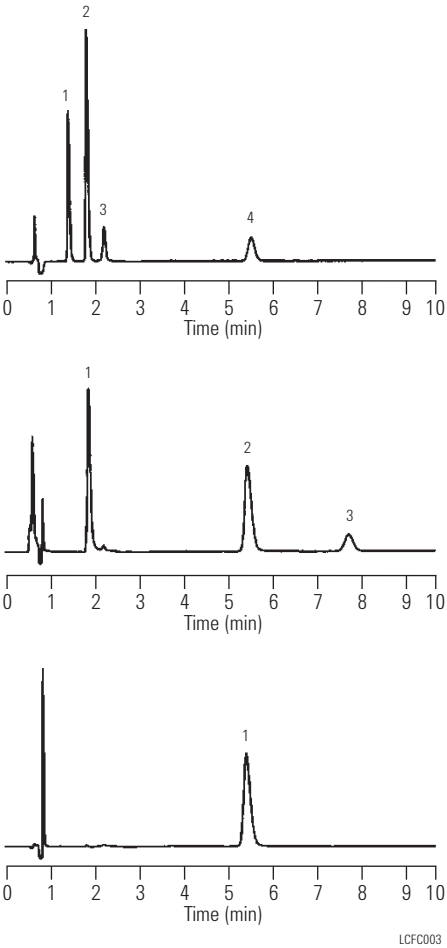
Mobile Phase: 85/15, 0.1% TFA/ACN

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

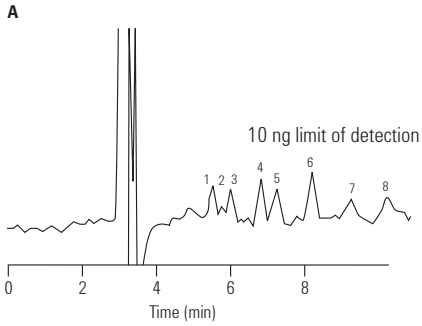
Carbohydrates: Carbohydrate standards

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

Mobile Phase: 63% CH_3CN/H_2O
Flow Rate: 0.5 mL/min

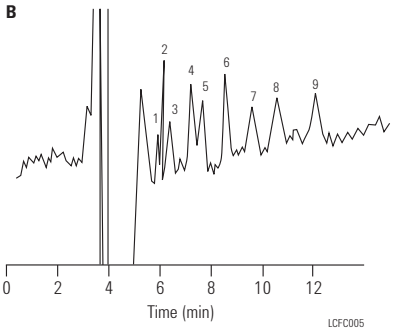
Detector: Agilent RID
Sample: Carbohydrate standard:
A: 25 ng/ L, 1 µL injected
B: 500 pg/ L, 50 µL injected

Carbohydrates: Separation showing high sensitivity



1. Ribose
2. Rhamnose
3. Xylose
4. Fructose
5. Glucose
6. Sucrose
7. Maltose
8. Lactose
9. Raffinose

Sensitivity of high injection volume (50 µL)



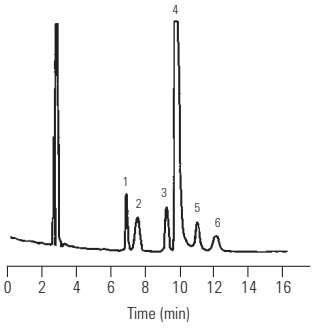
Carbohydrates: Effect of mobile phase strength

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

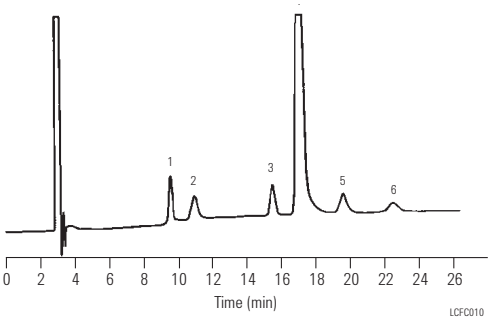
Mobile Phase: ACN/Water, as indicated
Flow Rate: 1.0 mL/min

Temperature: Ambient
Detector: RI
Sample: Mono- and Disaccarides

ACN/H_2O: 70/30



ACN/H_2O: 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**

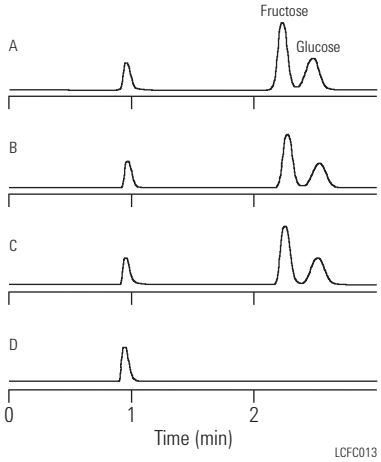
Mobile Phase: 75% ACN:25% H_2O

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**

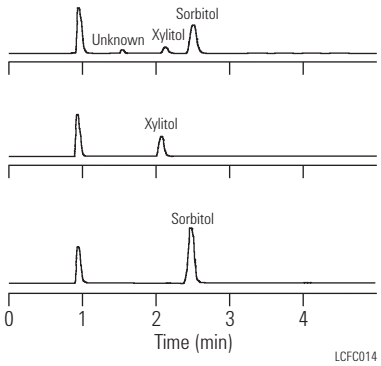
Mobile Phase: 75% ACN:25% H_2O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Chewing gum, sugar-free



Carbohydrates in juices

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

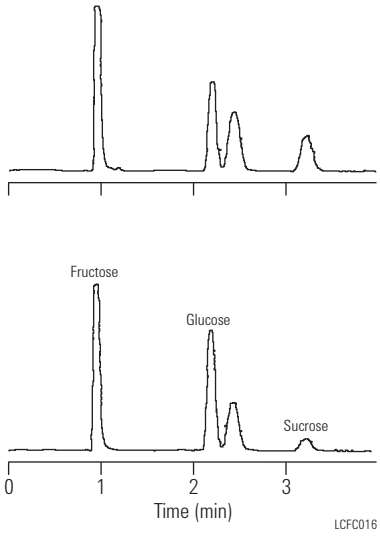
Mobile Phase: 75% ACN/25% H_2O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Diluted to 0.1X in 50:50 ACN:H_2O



Apple Drink
36.8% Fructose
24.9% Sucrose
38.3% Glucose

Apple Juice
58.7% Fructose
9.9% Sucrose
33.4% Glucose

Carbohydrates in milk

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

Mobile Phase: 75% ACN/25% H_2O

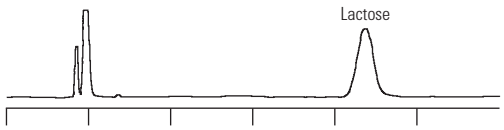
Flow Rate: 2.0 mL/min

Temperature: 30 °C

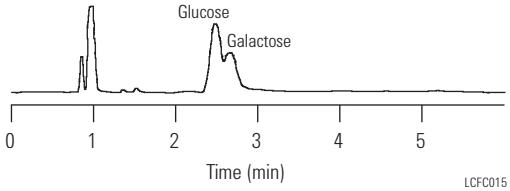
Detector: RID

Sample: Partitioned between CH_3Cl_2: H_2O

Milk (2%)



100% Lactose-Free Milk



Flavoring agents

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

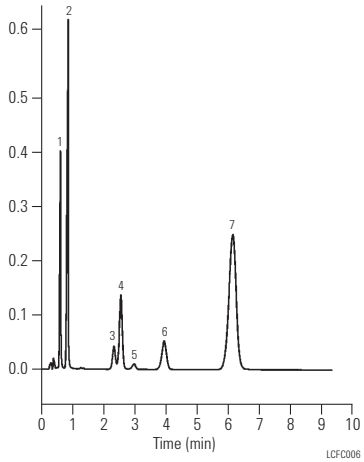
Mobile Phase: 0.3% TFA: ACN, 65:35

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% TFA, pH to 4.4 with TEA, B: MeOH

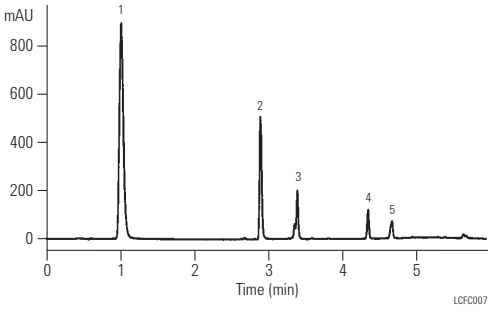
Flow Rate: 1.0 mL/min

Gradient: 17 to 100% B/4 min

Temperature: Ambient

Detector: UV, 254 nm

1. Yellow #5	$C_{16}H_9N_4Na_3O_9S_2$	MW=534
2. Red #40	$C_{18}H_{14}N_2Na_2O_8S_2$	MW=496
3. Blue #1	$C_{37}H_{34}N_2Na_2O_9S_3$	MW=760
4. Propylparaben	$C_{10}H_{12}O_3$	MW=180
5. Red #3	$C_{20}H_{14}Na_2O_5$	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

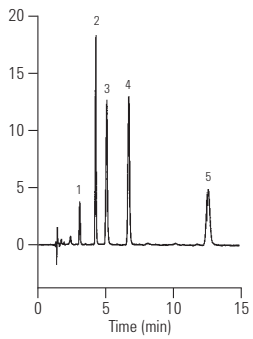
Injection: 1 mL/min

Temperature: 40 °C

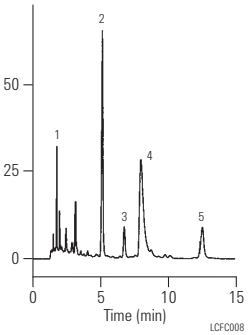
Detector: UV, 280 nm

Sample: Green tea extract, 5 µL

Catechin Mixture



Green Tea Extract



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

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 CH_3COONH_4

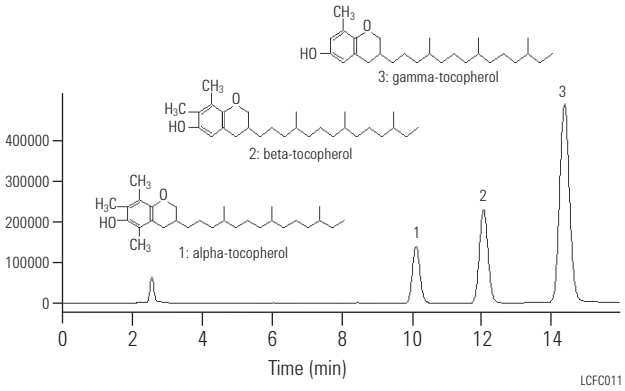
Flow Rate: 0.5 mL/min

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



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

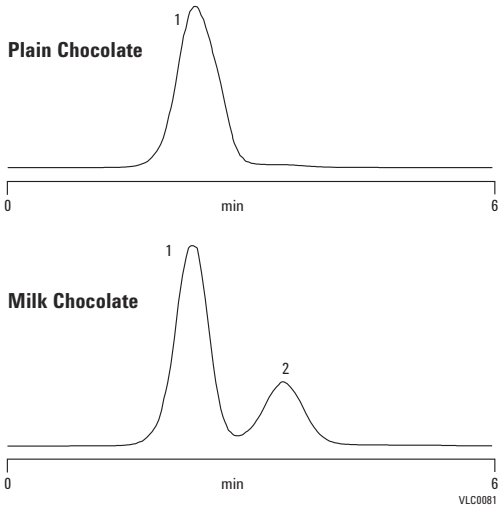
Column: **Hi-Plex Pb**
PL1170-6820
7.7 x 300 mm, 8 µm

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 80 °C

Detector: RI



1. Sucrose
2. Lactose

Sugars

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

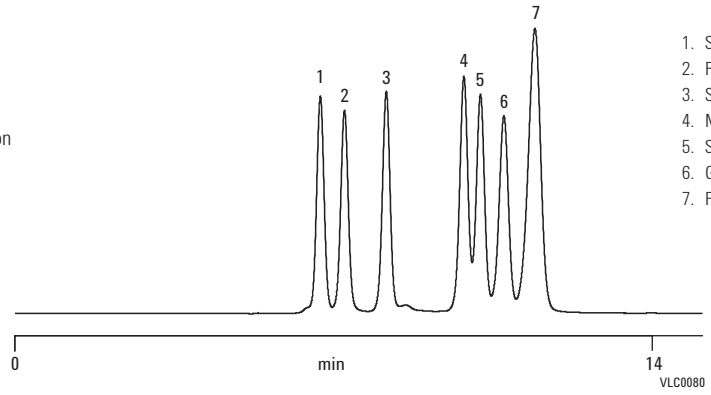
Sample: Sugars mixture (all 10 mg/mL), 20 µL injection

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 85 °C

Detector: 356-LC RI



1. Stachyose
2. Raffinose
3. Sucrose
4. Mannitol
5. Sorbitol
6. Glucose
7. Fructose

Parabens: High speed separation

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

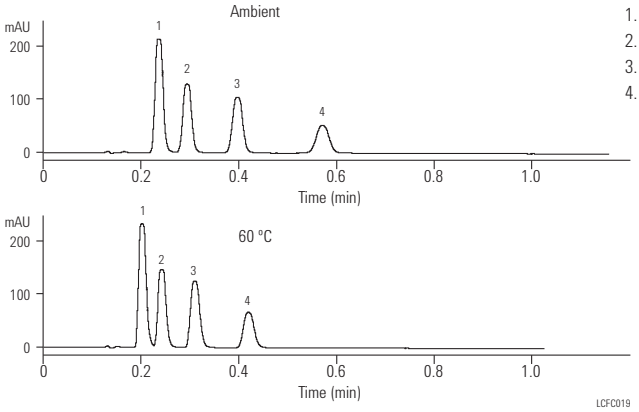
Mobile Phase: 0.1% H_3PO_4:ACN, (50:50)

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



1. Methylparaben
2. Ethylparaben
3. Propylparaben
4. Butylparaben

Separation of vitamin D2/D3

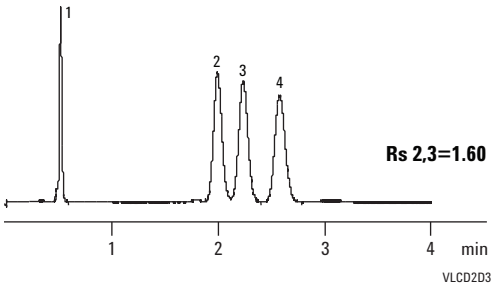
Column: **Eclipse PAH**
959941-918
4.6 x 50 mm, 1.8 µm

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



1. Vitamin A
2. Vitamin D2
3. Vitamin D3
4. Vitamin E (a-VE)

Fat-soluble vitamins on ZORBAX Eclipse XDB-C8

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

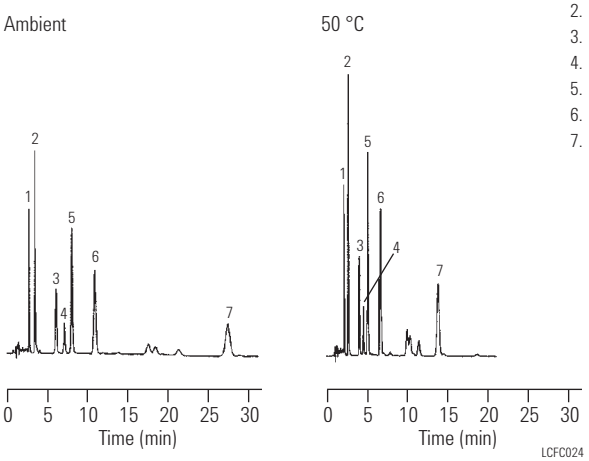
Mobile Phase: 5/95 Water/MeOH

Flow Rate: 1.0 mL/min

Temperature: A: Ambient
B: 50 °C

Detector: UV, 280 nm

Sample: Fat-soluble vitamins



1. Retinol
2. Retinol acetate
3. Vitamin D3
4. γ-Tocopherol
5. α-Tocopherol
6. Tocopherol acetate
7. Retinol palmitate

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)

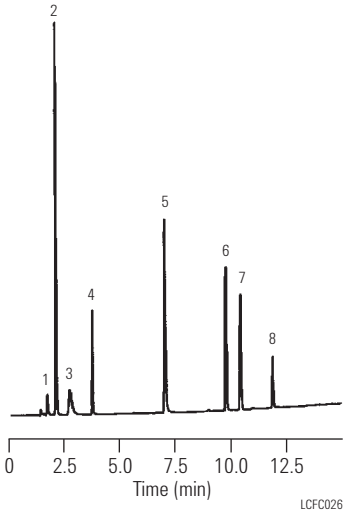
Flow Rate: 1.0 mL/min

Gradient: 0-70% B in 18 min

Temperature: Ambient

Detector: UV, 245 nm

Sample: Water-soluble vitamins



1. B_1-Thiamine
2. Vitamin C
3. B_3-Niacin
4. B_6-Pyridoxine
5. Pantothenic acid
6. Folic acid
7. B_{12}-Cyanocobalamin
8. B_2-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

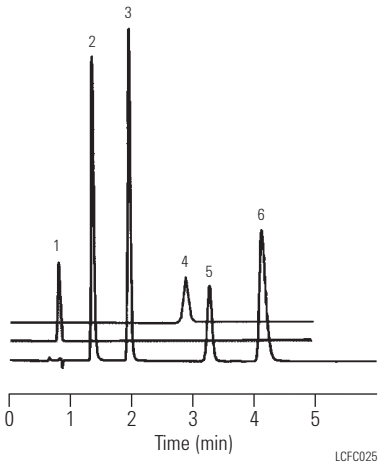
Mobile Phase: 10 mM Hexane Sulfonate with 0.1% Phosphoric Acid: MeOH (74:26)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 245 nm

Sample: Water-soluble vitamins



1. Vitamin C
2. B_3-Niacin
3. B_6-Pyridoxine
4. Folic acid
5. B_2-Riboflavin
6. B_1-Thiamine

Water-soluble vitamins using the USP 23 method

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

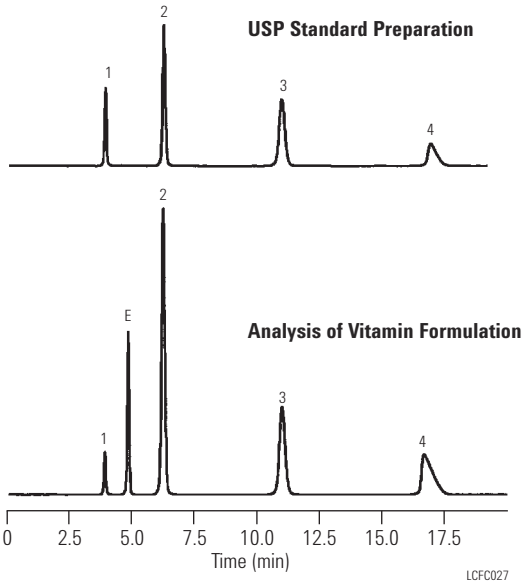
Mobile Phase: 7.2 mM Hexane Sulfonate/MeOH/Acetic Acid (73/27/1) (ratio to 101)

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: UV, 280 nm

Sample: Water-soluble vitamins



1. B_3-Niacin
2. B_6-Pyridoxine
3. B_2-Riboflavin
4. B_1-Thiamine
E. Excipent

Water-soluble B vitamins separated on ZORBAX SB-Aq

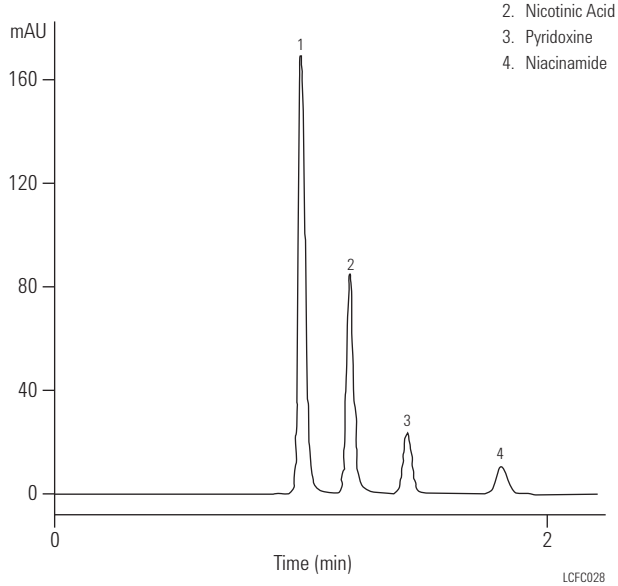
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

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

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 µm
6 µL inj

Column B: **Eclipse XDB-C18**
961967-902
4.6 x 100 mm, 3.5 µm
4 µL inj

Column C: **Eclipse XDB-C18**
927975-902
4.6 x 50 mm, 1.8 µm
2 µL inj

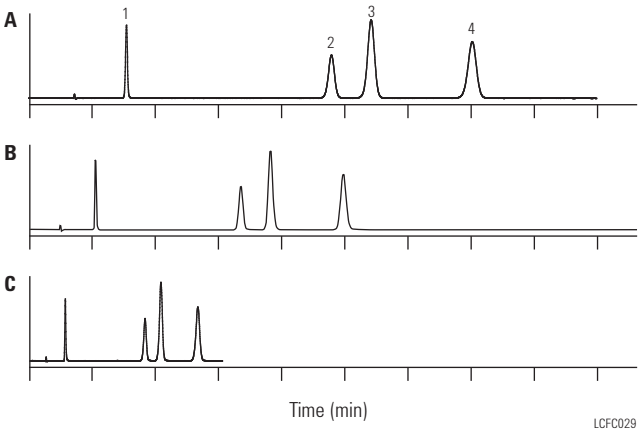
Mobile Phase: A: 15% water
B: 85% MeOH

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Sunscreens



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

Fast vitamin E analysis on Rapid Resolution HT

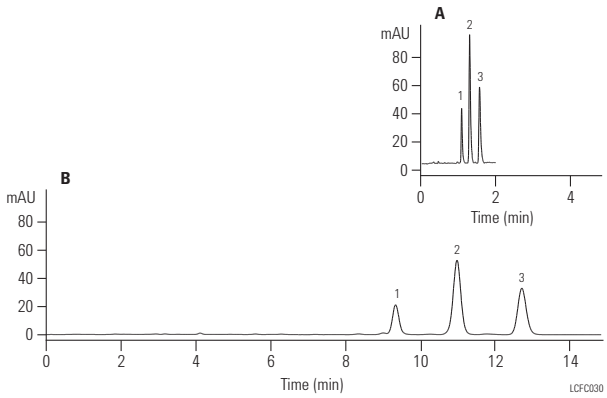
Column A: **Eclipse XDB-C18**
927975-902
4.6 x 50 mm, 1.8 µm

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

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

Theobromine in beverages

Column: **ZORBAX SB-C18**
827975-902
4.6 x 50 mm, 1.8 µm

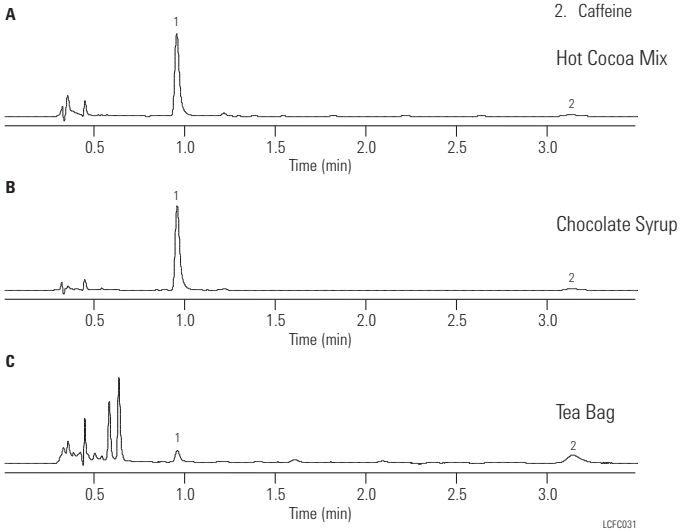
Mobile Phase: A: 92% 0.1% formic acid
B: 8% 0.1% formic acid in ACN

Flow Rate: 1.5 mL/min

Temperature: Ambient

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

Sample: Theobromine



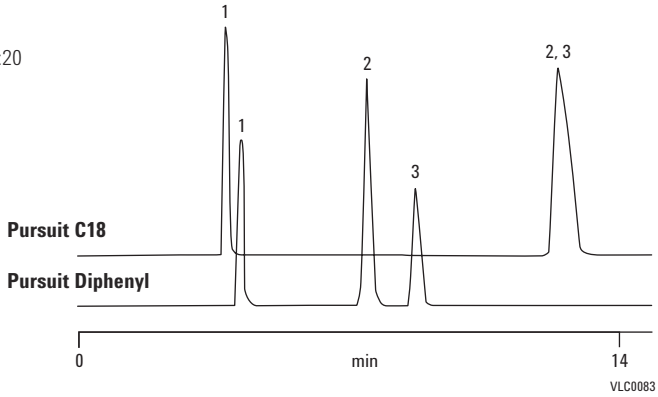
1. Theobromine
2. Caffeine

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



1. p-Aminobenzoic acid
2. Sorbic acid
3. Benzoic acid

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

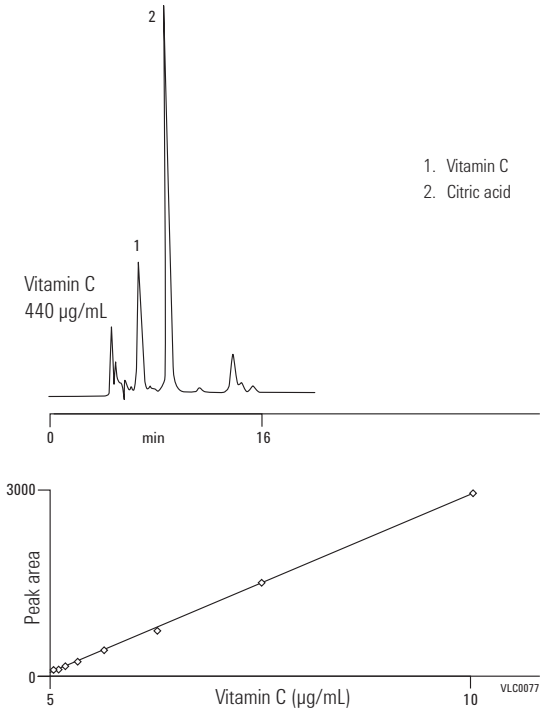
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

Sample: Diluted 1:50 in eluent

Mobile Phase: 0.2M NaH_2PO_4, pH 2.14

Flow Rate: 0.5 mL/min

Detector: UV, 220 nm



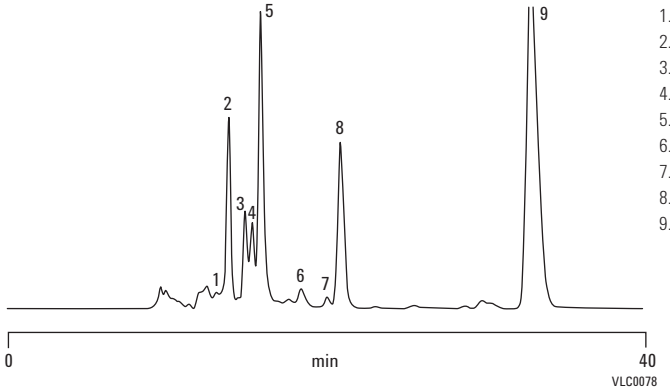
1. Vitamin C
2. Citric acid

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

Rose wine

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

Mobile Phase: 0.004M H_2SO_4
Flow Rate: 0.4 mL/min
Pressure: 13 bar
Temperature: 75 °C
Detector: RI

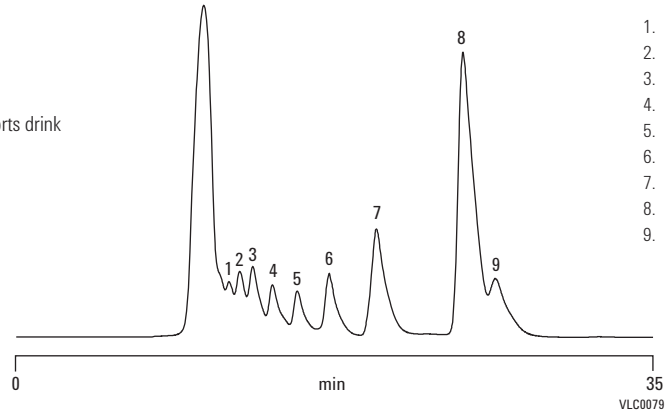


1. Citric acid
2. Tartaric acid
3. Glucose
4. Malic acid
5. Fructose
6. Succinic acid
7. Lactic acid
8. Glycerol
9. Ethanol

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

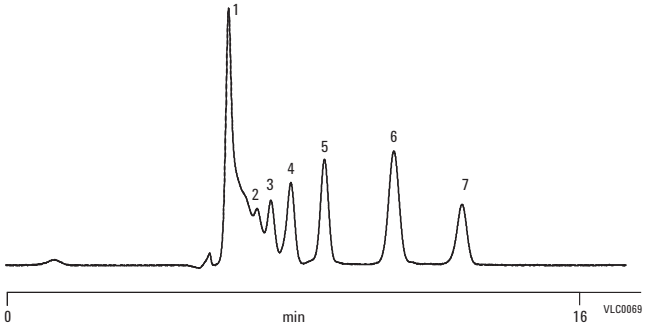


1. Dp8
2. Dp7
3. Dp6
4. Dp5
5. Dp4
6. Dp3
7. Dp2
8. Dp1 (Glucose)
9. Fructose

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: RI



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

Pharmaceutical Applications

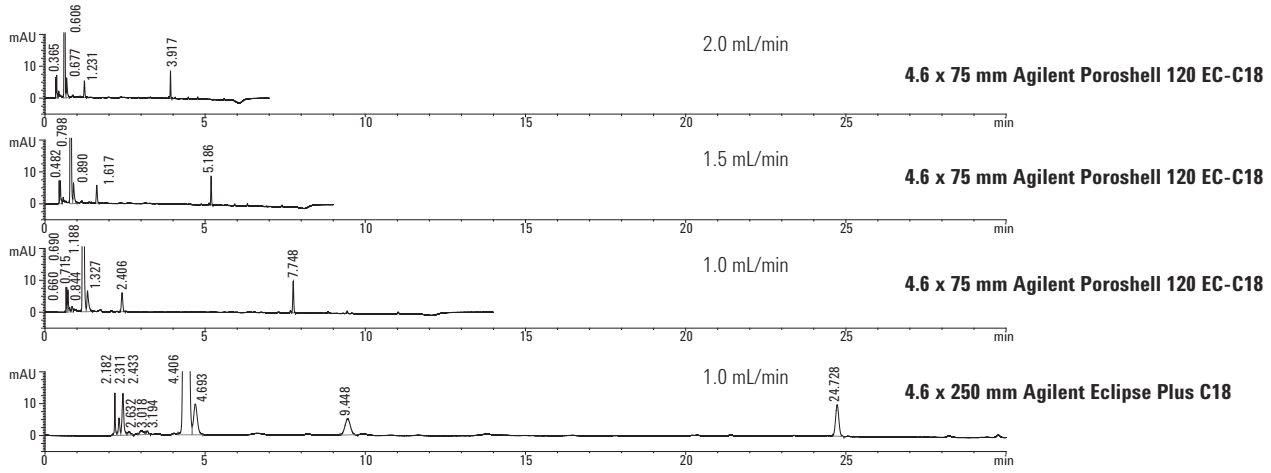
NEW!

Fast analysis of cefepime and related impurities

Column: **Poroshell 120 EC-C18**
697975-902
4.6 x 75 mm, 2.7 µm

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

Detector: Agilent 1200 Infinity Series



NEW!

Naproxen analysis

Column A: **Eclipse Plus C18**
959993-902
4.6 x 150 mm, 5 µm

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

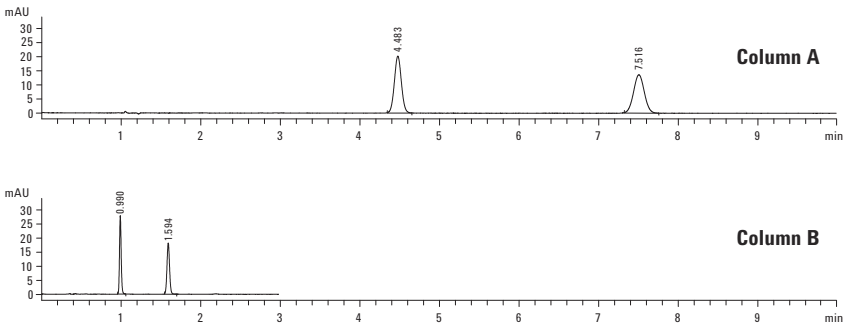
Mobile Phase: 50:49:1 MeCN:H_2O:Glacial acetic acid

Flow Rate: 1.2 mL/min

Injection: Column A: 20 µL
Column B: 6.7 µL

Injection: Naproxen

Method requirement N > 4000 Rs better than 11.5



4-fold reduction in analysis time for this method when transferring to Poroshell 120.

NEW!

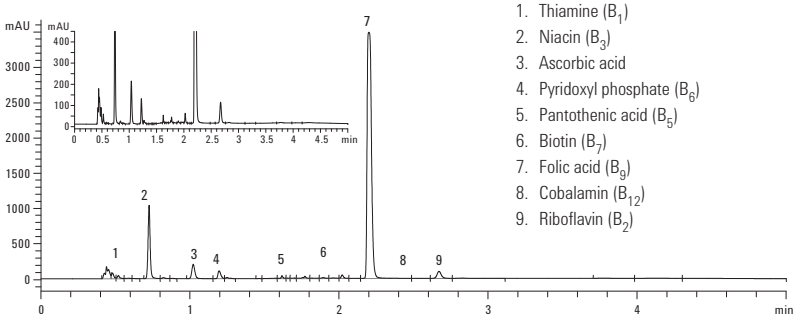
Analysis of water soluble vitamins in multivitamin tablets

Column: **Poroshell 120 EC-C18**
697975-902
4.6 x 75 mm, 2.7 µm

Flow Rate: 1.5 mL/min

Gradient: 0 min-1% B, 0.5 min-12% B, 0.52 min-30% B, 3.5 min-30% B, 4.5 min-1% B

Injection: 5 µL



1. Thiamine (B_1)
2. Niacin (B_3)
3. Ascorbic acid
4. Pyridoxyl phosphate (B_6)
5. Pantothenic acid (B_5)
6. Biotin (B_7)
7. Folic acid (B_9)
8. Cobalamin (B_{12})
9. Riboflavin (B_2)

NEW!

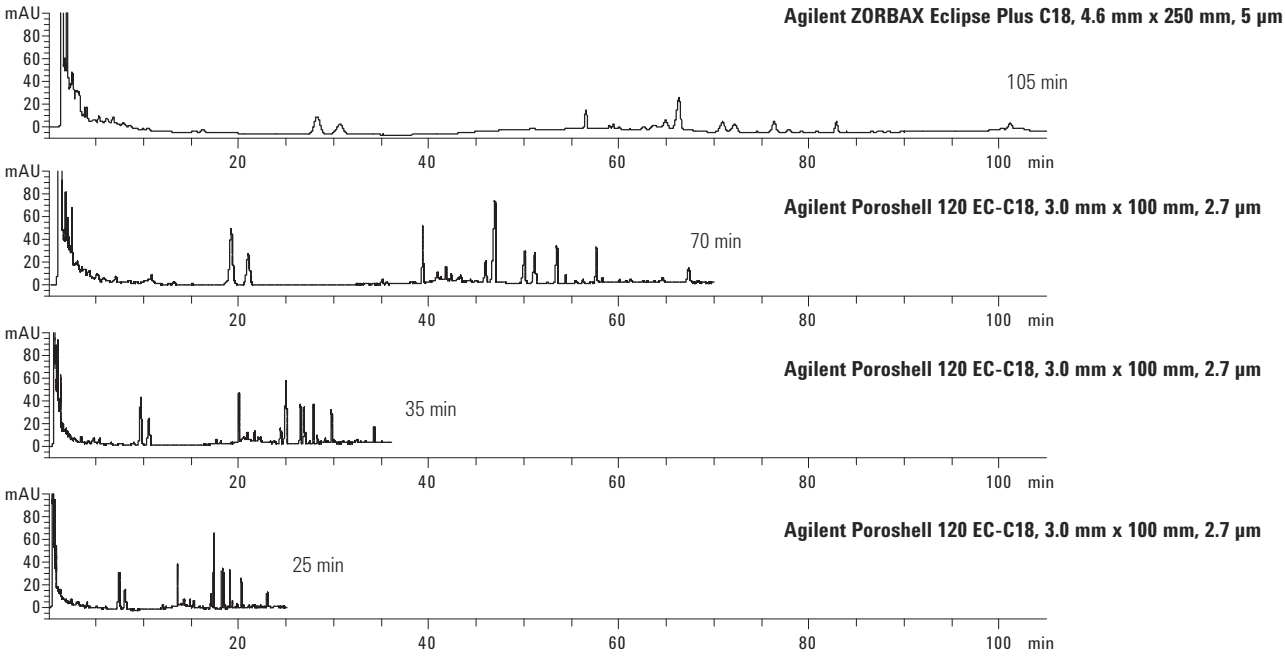
Fast method for ginseng analyses scaled from a traditional method

Column: **Eclipse Plus C18**
959993-902
4.6 x 150 mm, 5 µm

Column: **Poroshell 120 EC-C18**
695975-302
3.0 x 100 mm, 2.7 µm

Detector: 1200 Infinity Series

Sample: Ginsenoside



Agilent ZORBAX Eclipse Plus C18, 4.6 mm x 250 mm, 5 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

NEW!

Separation of 8 steroids

Column A: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

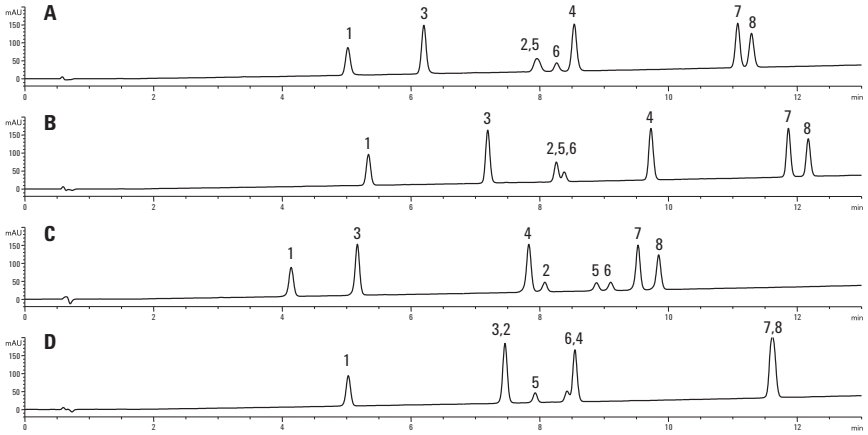
Column D: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Mobile Phase: 0.1% formic acid in both water and MeOH

Flow Rate: 0.4 mL/min, 25 ºC, 2.1 x 100 mm 40 ºC

Gradient: 40-80% MeOH in 14 min

1. Hydrocortisone
2. β-Estradiol
3. Androstadiene 3,17 dione
4. Testosterone
5. Ethynylestradiol
6. Estrone
7. Norethindrone acetate
8. Progesterone



NEW!

Mixture of beta blockers

Column A: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column D: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

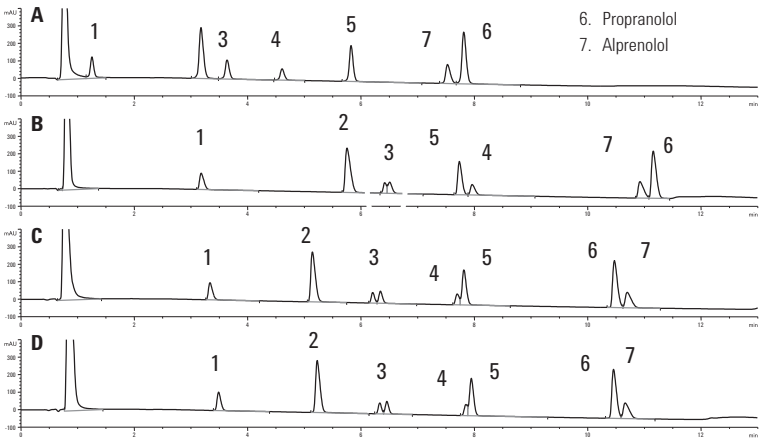
Mobile Phase: 10 mM pH 3.8 NH_4HCO_2, methanol

Flow Rate: 0.35 mL/min

Gradient: 90% B to 30% B over 12 min

* Nadolol is isobaric and elutes as two peaks.

1. Atenolol
2. Pindolol
3. Nadolol
4. Metoprolol
5. Acebutolol
6. Propranolol
7. Alprenolol



NEW!

Several ZORBAX RRHD 1.8 µm selectivities facilitate method development

Column: **ZORBAX RRHD Eclipse Plus C18**
959758-902
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD Eclipse XDB-C18**
981758-902
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD SB-C18**
858700-902
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD Extend-C18**
758700-902
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: H_2O
B: CH_3CN, each with 0.1% HCOOH

Detector: Agilent 1290 Infinity LC with an
Agilent 6410 Triple Quadrupole Mass Spectrometer

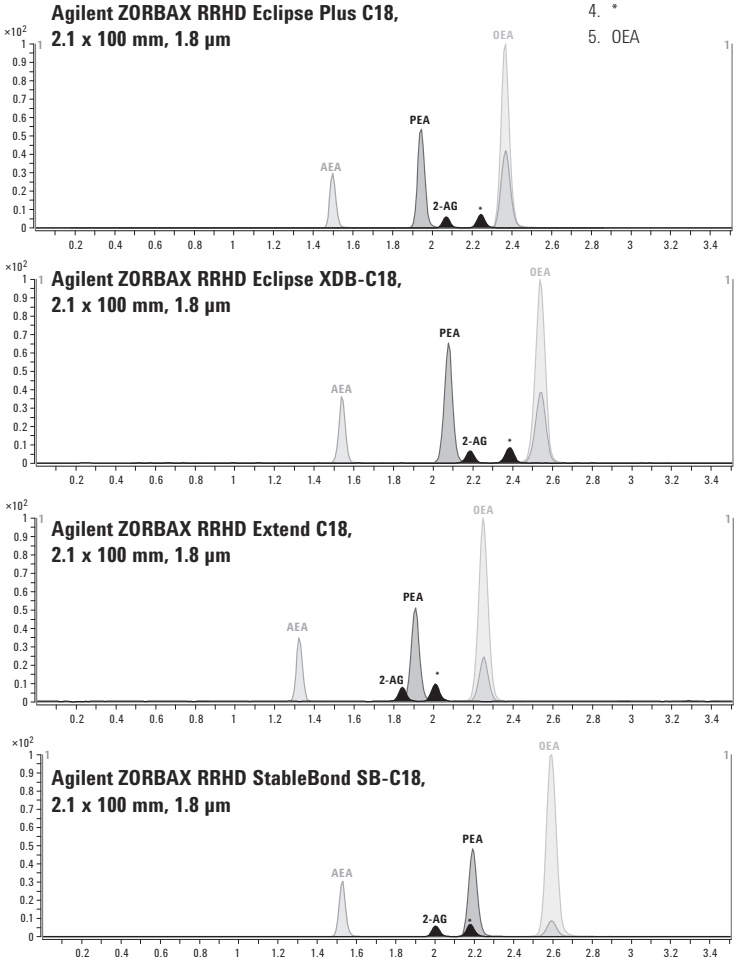
MS Conditions: TCC: 30 °C
MS Source: Electrospray AP-ESI
Drying-gas temperature and flow: 325 °C, 12 L/min
Nebulizer gas pressure: 35 psi
Capillary voltage: 3000 V

Sample: Four endocannabinoid fatty amides:
Arachidonoylglycerol (AEA)
2-Arachidonoylglycerol (2-AG)
Palmitoylethanolamide (PEA)
Oleoylethanolamide (OEA)

*** The second black peak is an impurity, believed to be 1,3-arachidonolyglycerol, a rearrangement of 2-AG**

The selectivity of four Agilent ZORBAX RRHD C18 columns is compared using a method for endocannabinoids.

1. AEA
2. PEA
3. 2-AG
4. *
5. OEA



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

Fast analysis 11 common compounds found in analgesics

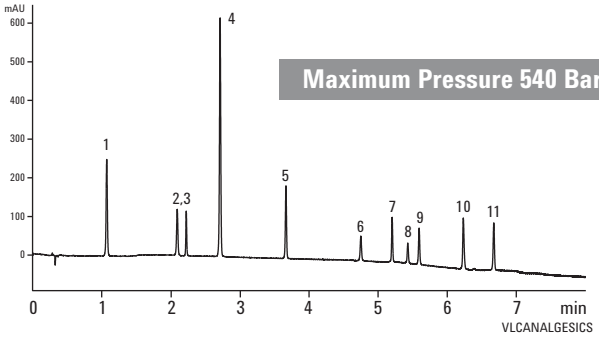
Column: **Poroshell 120 EC-C18**
695975-902
4.6 x 100 mm, 2.7 µm

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

Flow Rate: 3.5 mL/min

Temperature: 40 °C

Detector: DAD 254 nm



Maximum Pressure 540 Bar

1. Acetaminophen
2. Caffeine
3. 2-Acetamidophenol
4. Acetamide
5. Phenacetin
6. Sulindac
7. Piroxicam
8. Tolmetin
9. Ketoprofen
10. Diflusinal
11. Diclofenac

Faster analysis of USP Method for simvastatin tablet

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

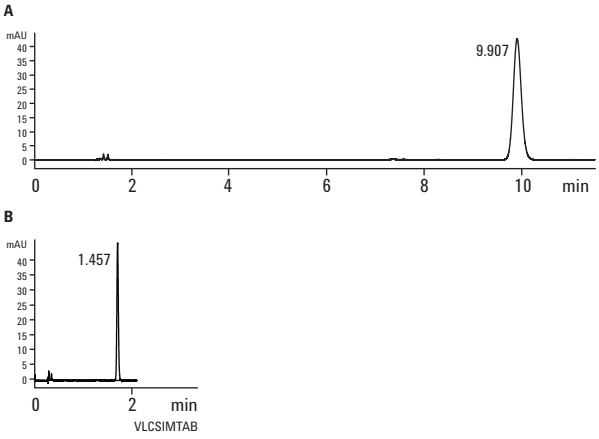
Column B: **Poroshell 120 EC-C18**
697975-902
4.6 x 75 mm, 2.7 µm

Mobile Phase: 65% CH_3CN,
35% 3.9 g/L NaH_2PO_4 (pH 4.5)

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

Temperature: 45 °C

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



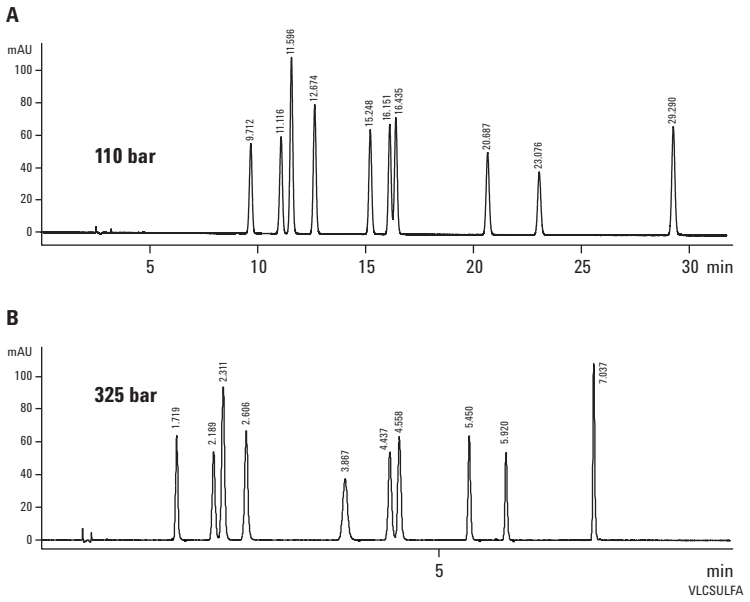
	USP Requirement	5 µm (1.5 mL/min)	2.7 µm (2.8 mL/min)
T_R	N/A	9.907	1.457
k'	> 3.0	5.962	5.122
N	> 4500	16939	14439
T_f	< 2.0	1.09	1.10

Faster separation of sulfa drugs

		Time	%B
Column A:	**Eclipse Plus C18**	0	8
	959990-902	33	33
	4.6 x 250 mm, 5 µm	35	33
Column B:	**Poroshell 120 EC-C18**	**Time**	**%B**
	695975-902	0	8
	4.6 x 100 mm, 2.7 µm	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

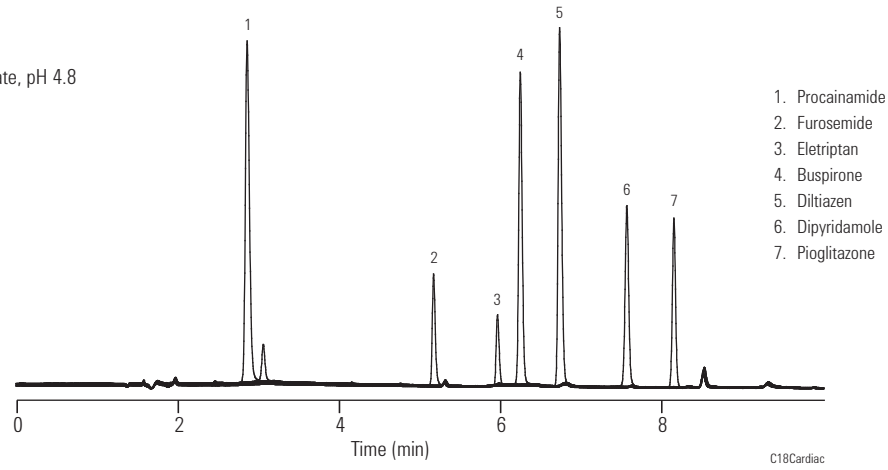
Column: **Eclipse Plus C18**
959996-902
4.6 x 100 mm, 5 µm

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



1. Procainamide
2. Furosemide
3. Eletriptan
4. Buspirone
5. Diltiazen
6. Dipyridamole
7. Pioglitazone

Fast and ultra-fast analysis of basic compounds

Column: **Eclipse Plus C18**
959941-902
4.6 x 50 mm, 1.8 µm

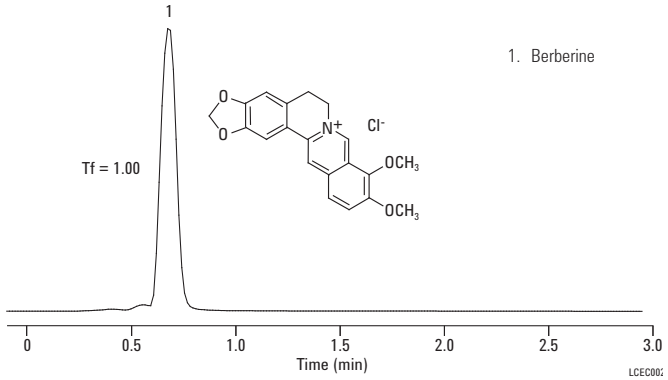
Mobile Phase: A: 50% 8 mM K_2HPO_4, pH 7
B: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Berberine, 0.4 mg/mL, 2 µL



1. Berberine

Xanthines: Higher resolution, same selectivity with RRHT

Column A: **ZORBAX SB-C18**
846975-902
4.6 x 50 mm, 5 µm

Column B: **ZORBAX SB-C18**
827975-902
4.6 x 50 mm, 1.8 µm

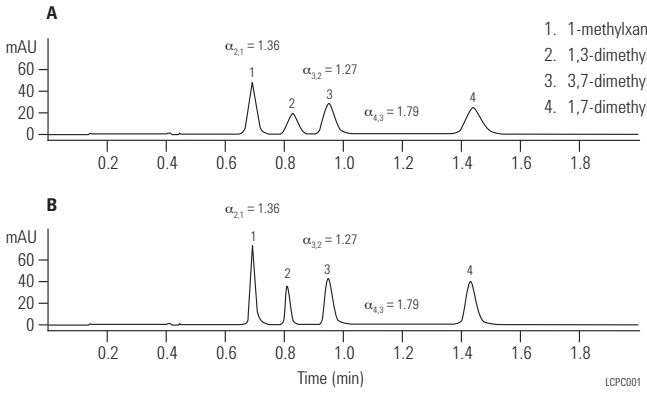
Mobile Phase: A: 92% 0.1% formic acid
B: 8% 0.1% formic acid in ACN

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Xanthines



1. 1-methylxanthine
2. 1,3-dimethyluric acid
3. 3,7-dimethylxanthine (theobromine)
4. 1,7-dimethylxanthine

Antihistamines: Fast separations on RRHT Extend-C18

Column A: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

Column B: **ZORBAX Extend-C18**
727975-902
4.6 x 50 mm, 1.8 µm

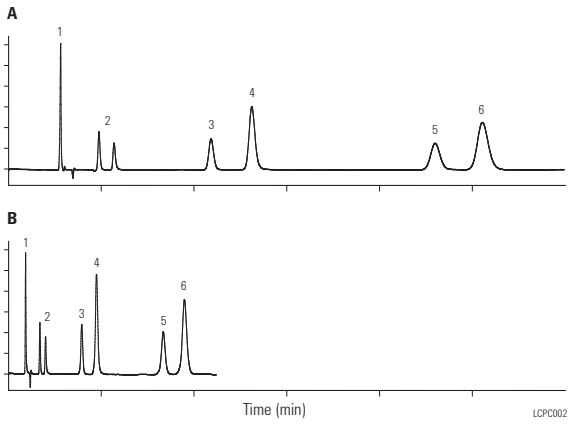
Mobile Phase: A: 30% 50 mM pyrrolidine buffer
B: 70% MeOH

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Antihistamines



1. Scopolamine
2. Pseudoephedrine
3. Doxylamine
4. Chlorpheniramine
5. Diphenhydramine
6. Triprolidine

Ibuprofen: Optimizing selectivity with RRHT Columns

Column A: **SB-C8**
827975-906
4.6 x 50 mm, 1.8 µm

Column B: **Eclipse XDB-C8**
927975-906
4.6 x 50 mm, 1.8 µm

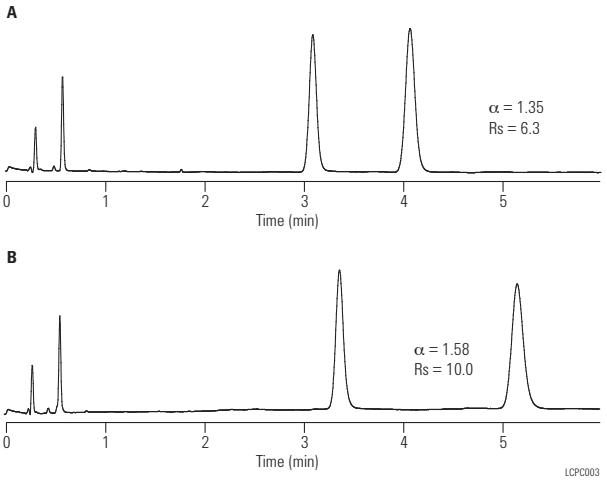
Mobile Phase: A: 63% water
B: 37% acetonitrile + 1.8 mL H_3PO_4

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Ibuprofen oral suspension



1. Benzophenone
2. Ibuprofen

Analgesics

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

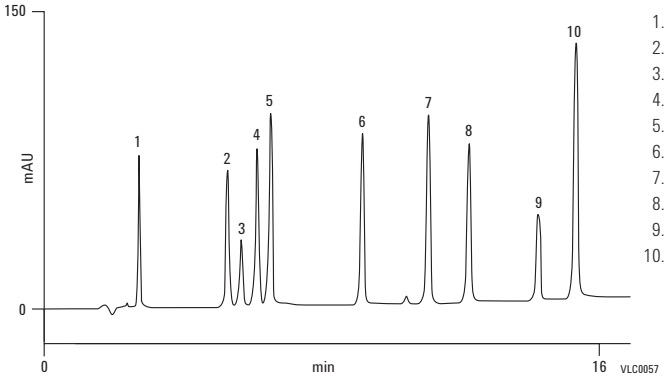
Mobile Phase: A: Water+0.1% HCOOH
B: MeCN+0.1% HCCOH

Gradient: 25-80% B in 20 min

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



1. Acetaminophen
2. Acetanilide
3. Salicylic acid
4. Acetylsalicylic acid
5. Phenacetin
6. Carbamazepine
7. Tolmetin
8. Naproxen
9. Ibuprofen
10. Diclofenac

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 NaH_2PO_4 pH 2.5 in 95% H_2O/5% ACN
B: 50 mM NaH_2PO_4 pH 2.5 in 47% H_2O/53% ACN

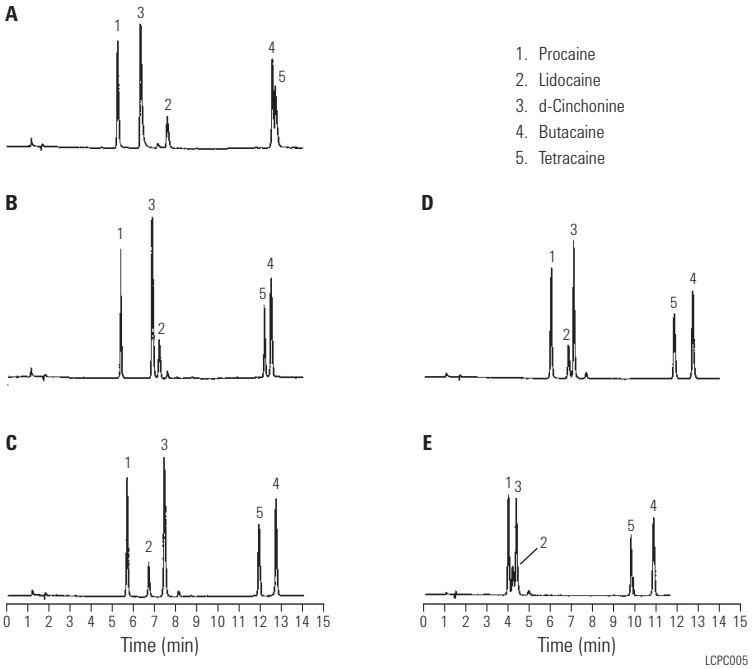
Flow Rate: 1.5 mL/min

Gradient: 0-100% B in 18.8 min

Temperature: 26 °C

Detector: UV, 254 nm

Sample: 10 µL, 10 ug/mL



1. Procaine
2. Lidocaine
3. d-Cinchonine
4. Butacaine
5. Tetracaine

Local anesthetics

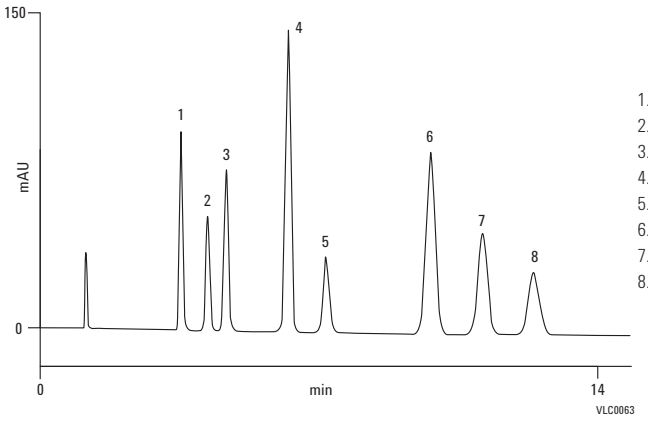
Column: **Pursuit XRs C8**
A6010150X046
4.6 x 150 mm, 5 µm

Mobile Phase: 65:35 MeOH:5 mM NH_4CO_3, pH 10

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 210 nm



1. Benzocaine
2. Procaine
3. Chlorocaine
4. Mepivacaine
5. 4-Hydroxyropivacaine
6. Cocaine
7. Lidocaine
8. Ropivacaine

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

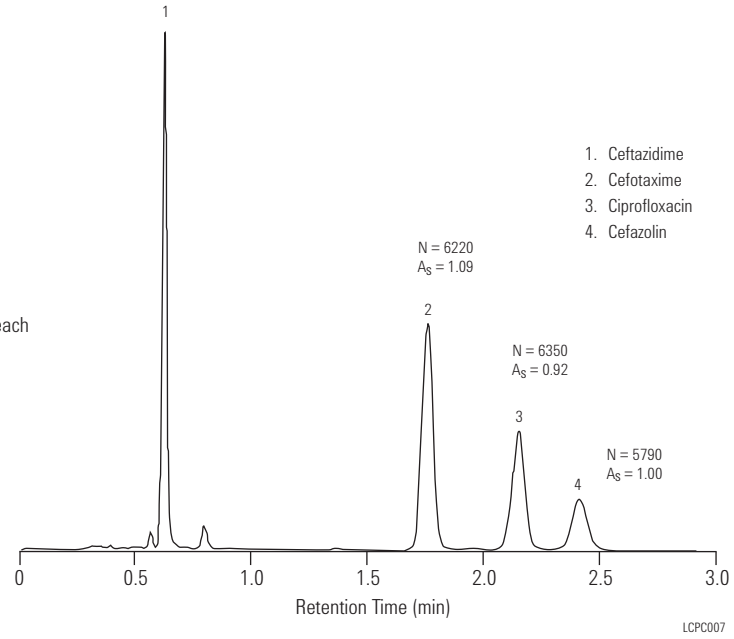
Flow Rate: 3.0 mL/min

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 each of 1-4 resp.



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

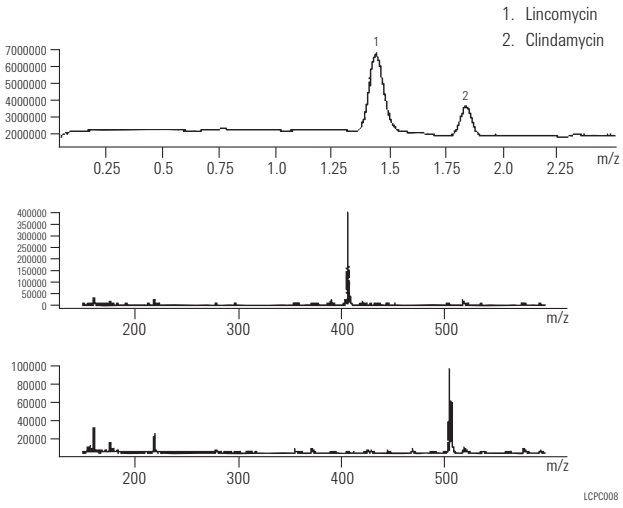
Temperature: Ambient

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



Antifungal medications

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

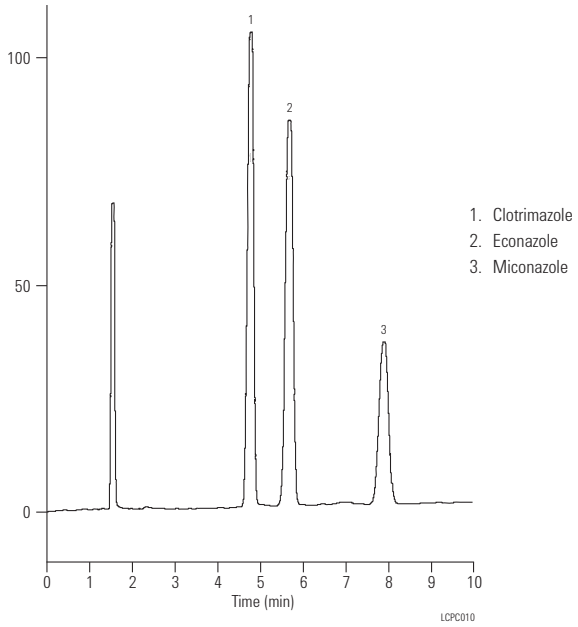
Mobile Phase: 35% 25 mM NaH_2PO_4, Dibasic (pH 6.5 with H_3PO_4): 65% ACN

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Antifungals, 2 µL



1. Clotrimazole
2. Econazole
3. Miconazole

Antifungals

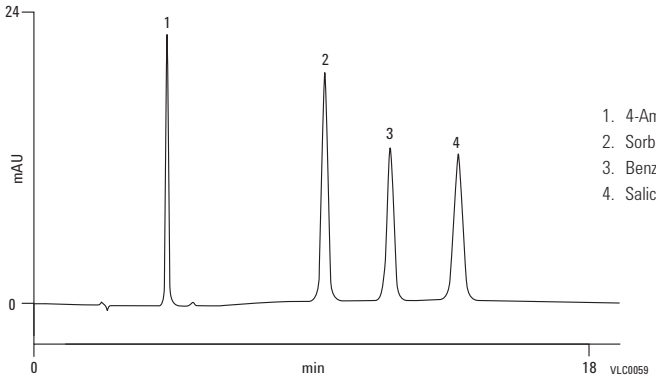
Column: **Pursuit XRs Diphenyl**
A6020150X046
4.6 x 150 mm, 5 µm

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

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



1. 4-Aminobenzoic acid
2. Sorbic acid
3. Benzoic acid
4. Salicylic acid

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

Analgesics: Non-steroidal anti-inflammatory drugs: Narrow bore separation

Column: **Eclipse XDB-C8**
993700-906
2.1 x 150 mm, 5 µm

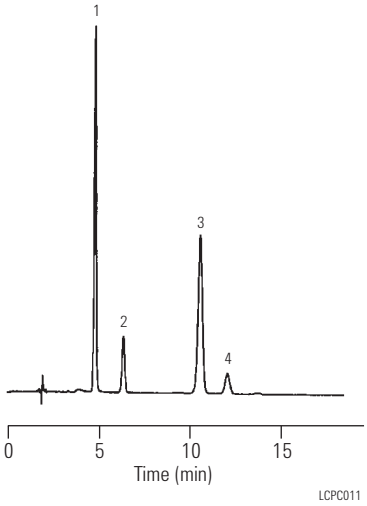
Mobile Phase: 50/50, 25 mM Sodium Phosphate (pH 7.0 with Phosphoric Acid), MeOH

Flow Rate: 0.2 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: 2 µL, 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

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

Column B: **Traditional Alkyl C8 Phase**

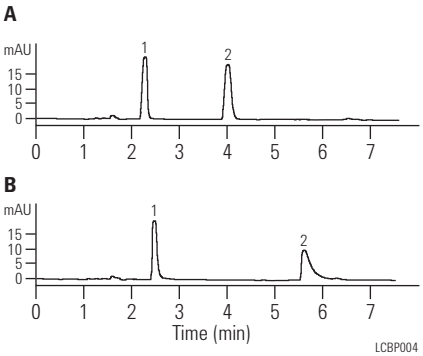
Mobile Phase: 25 mM K_2HPO_4, pH 7.2/MeOH: ACN (50:50), 45/55

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Anorectics "Fen-phen", 5 µL



1. Phentermine
2. Fenfluramine

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

Aromatic acids/benzoic acids: Selectivity differences

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

Column B: **ZORBAX SB-Phenyl**
880975-912
4.6 x 250 mm, 5 µm

Column C: **ZORBAX SB-CN**
880975-905
4.6 x 250 mm, 5 µm

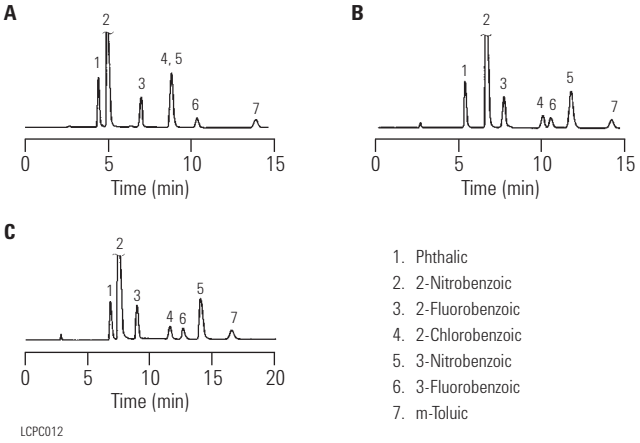
Mobile Phase: 30-45% methanol in 25 mM Na Phosphate, pH 2.5
A: 45% Methanol
B: 40% Methanol
C: 30% Methanol

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Benzoic acids



LCPC012

1. Phthalic
2. 2-Nitrobenzoic
3. 2-Fluorobenzoic
4. 2-Chlorobenzoic
5. 3-Nitrobenzoic
6. 3-Fluorobenzoic
7. m-Toluic

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 EDTA,
0.75 mM octyl sulfonate,
9% methanol pH 3.5

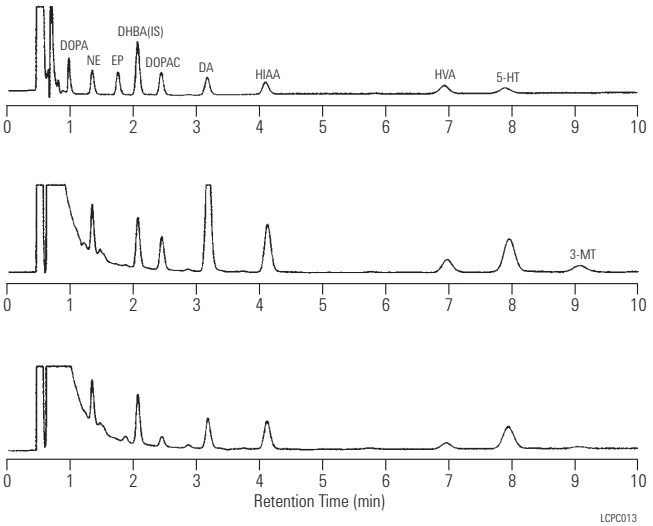
Flow Rate: 1.5 mL/min

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 Sriatum
C: Mouse Neocortex

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



LCPC013

Chiral ethiazide (diuretic drug) separation

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

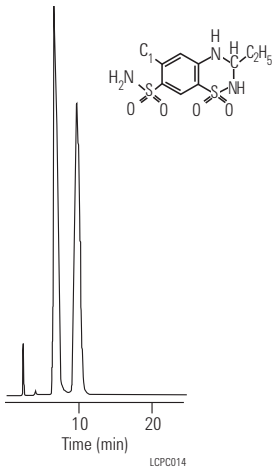
Mobile Phase: 20 mM KH_2PO_4 (pH 4.6)

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 enantioners (Prozac)

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

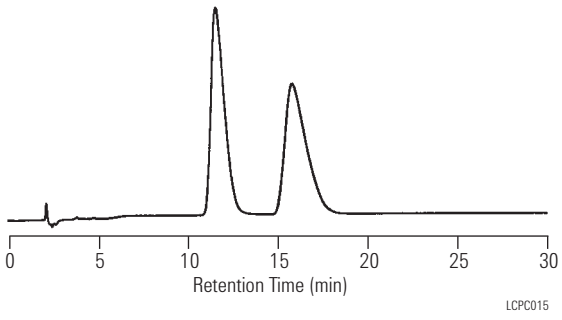
Mobile Phase: 25/75 (v/v) EtOH / 20 mM KH_2PO_4, pH 5.5 (adjusted with NaOH)

Flow Rate: 0.8 mL/min

Temperature: Ambient

Detector: UV, 225 nm

Sample: Mixture fluoxetine (Prozac) enantiomers



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

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

Goldenseal and related alkaloids on Rapid Resolution Eclipse XDB-C18

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

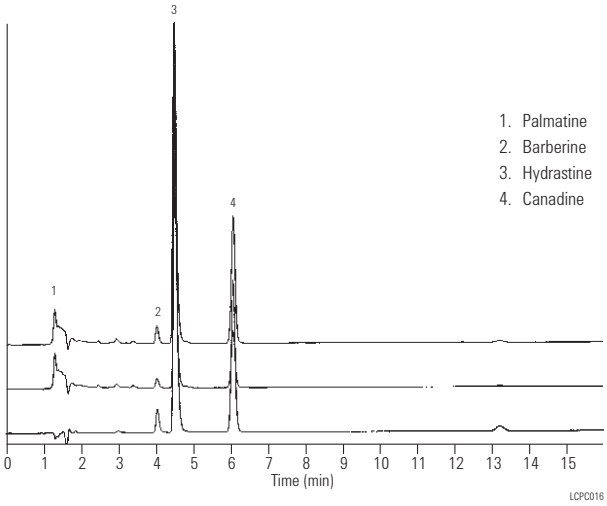
Flow Rate: 1.0 mL/min

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

Components of green tea separated on Rapid Resolution StableBond SB-C8

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

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

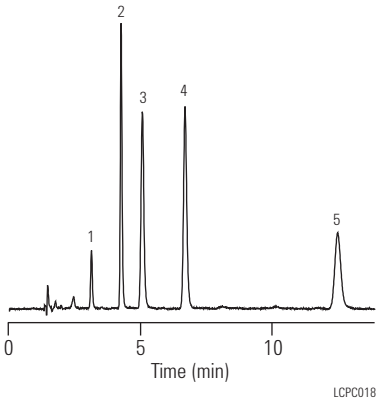
Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: 280 nm

Sample: Green tea

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



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

Chiral separation of hexobarbitol

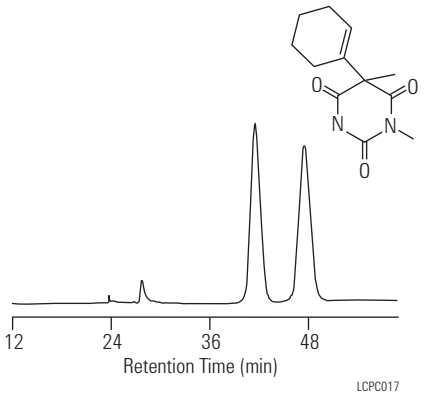
Column: **Chiradex**
79925CB-584
4.0 x 250 mm, 5 µm

Mobile Phase: Methanol/water, 20:80

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm

Sample: Hexobarbitol



Chiral separation of S- and R-Norfluoxetine

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

Mobile Phase: 6/94 (v/v) MeOH / 20 mM KH_2PO_4

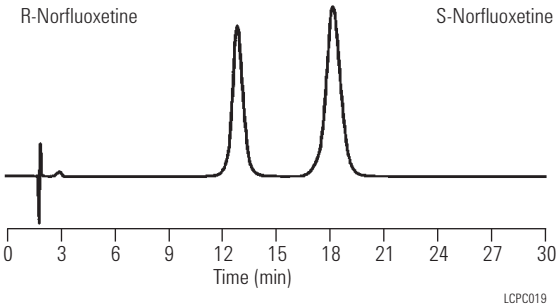
Flow Rate: 1.0 mL/min

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

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

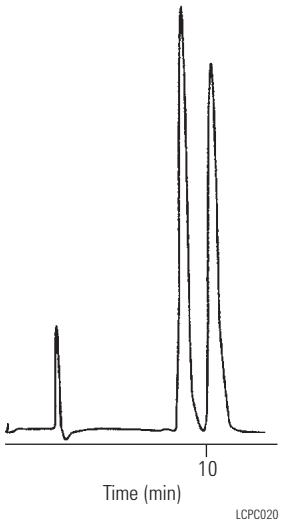
Mobile Phase: 20 mM phosphate buffer, pH 6.0

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 220 nm

Sample: 20 µL containing 0.35 µg salbutamol mixture



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Chiral separation of tolperison enantiomers

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

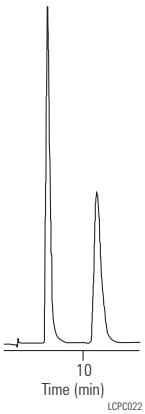
Mobile Phase: 20 mM KH_2PO_4 (pH 5.5), C_2H_5OH (100/4 v/v)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm, 0.04 AUFS

Sample: Tolperison, 5 µL



Chiral separation of atenolol

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

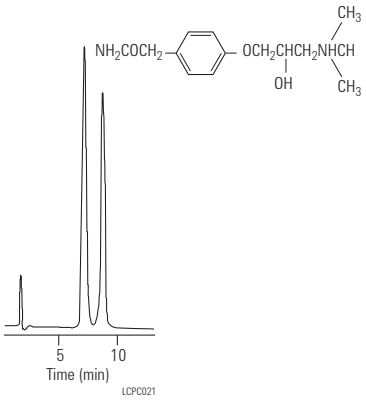
Mobile Phase: 20 mM phosphate buffer, pH 6.0/Ethanol (99/1)

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

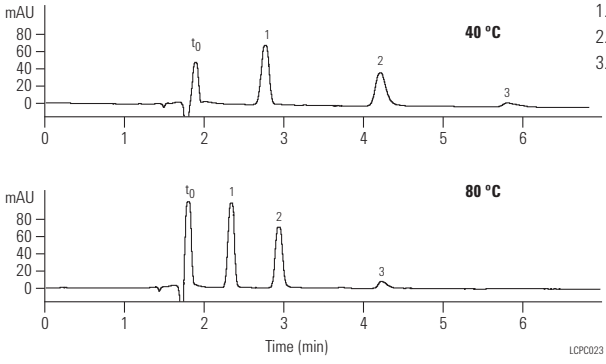
Column: **ZORBAX Rx-SIL**
883975-901
4.6 x 150 mm, 5 µm

Mobile Phase: MeOH: NH_4 Acetate, 25 mM, pH 6 (70:30)

Flow Rate: 1.0 mL/min

Temperature: 40 and 80 °C

Detector: UV, 210 nm



1. Benzoylecgonine
2. Cocaine
3. Ecgonine methylester

Aspirin and cough remedy

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

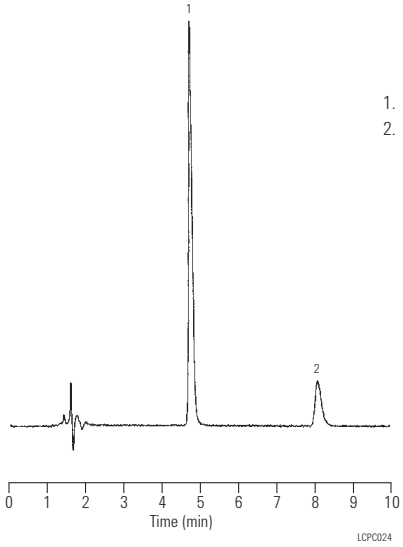
Mobile Phase: (75:25) 25 mM Na_2HPO_4 (pH 3.0): ACN

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: UV, 254 nm

Sample: 5 µL, 10 µg/mL



1. Acetylsalicylic acid
2. Dextromethorphan

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

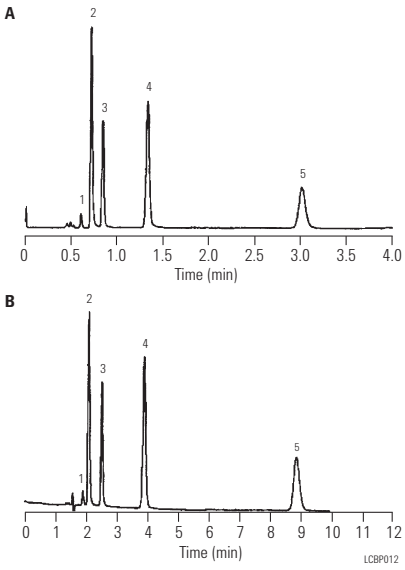
Mobile Phase: 20/80, Acetonitrile/150 mM Na Citrate, pH 2.6

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

Temperature: 35 °C

Detector: UV, 270 nm

Sample: 2 µL, cough formula



1. Maleic acid
2. Pseudo-ephedrine
3. Acetaminophen
4. Chlorpheniramine
5. Dextromethorphan

Guaifenesin: USP analysis of guaifenesin

Mobile Phase: 40% Methanol:60% Water:1.5% Glacial Acetic Acid

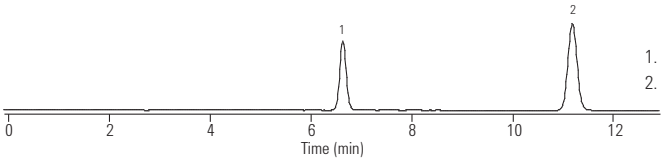
Flow Rate: 1.0 mL/min

Temperature: 25 °C

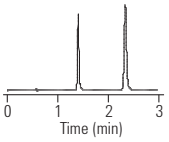
Sample: Guaifenesin
A: 8 µL
B: 2 mL

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

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

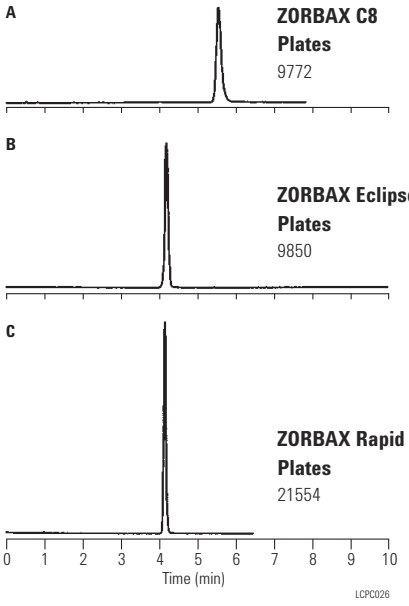
Mobile Phase: 80/20, Water/Methanol

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Metronidazole



A — **ZORBAX C8**

Plates	USP TF	Particle Size
9772	1.30	5 µm

B — **ZORBAX Eclipse XDB-C8**

Plates	USP TF	Particle Size
9850	0.98	5 µm

C — **ZORBAX Rapid Resolution Eclipse XDB-C8**

Plates	USP TF	Particle Size
21554	1.13	3.5 µm

Morphine and metabolites: Extracted blood plasma sample separation

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

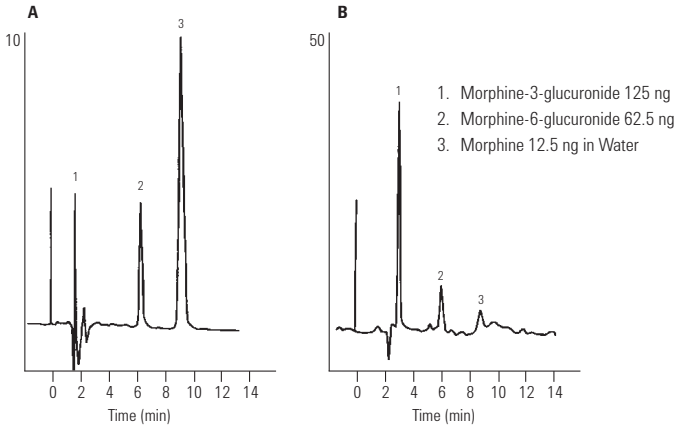
Mobile Phase: 97/3 70 mM KH_2PO_4 + 1 mM EDTA/ACN, pH 4.5

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.

Opiates (drugs of abuse) by LC/MS

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

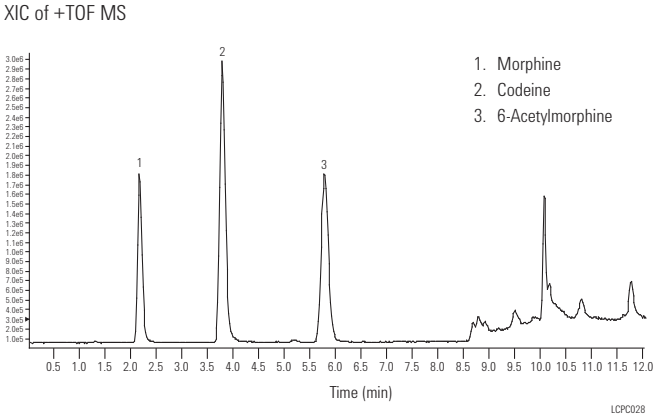
Mobile Phase: A: Acetonitrile with 0.1% formic acid
B: Water with 0.1% formic acid

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



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NEW!

Comparing HILIC and RPLC of morphine using Agilent ZORBAX RRHD columns with UHPLC/MS

Column: **Agilent ZORBAX Eclipse Plus C18**
2.1 x 100mm, 5 µm
(Custom column)

Column: **ZORBAX RRHD HILIC Plus**
959758-901
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 10 mM NH_4HCO_2, pH 3.2
B: CH_3CN/100 mM NH_4HCO_2, pH 3.2 (9:1)
Column A: 10% B isocratic
Column B: 70% B isocratic

Flow Rate: Column A: 0.4 mL/min
Column B: 1 mL/min

Pressure: Column A: 90 bar
Column B: 810 bar

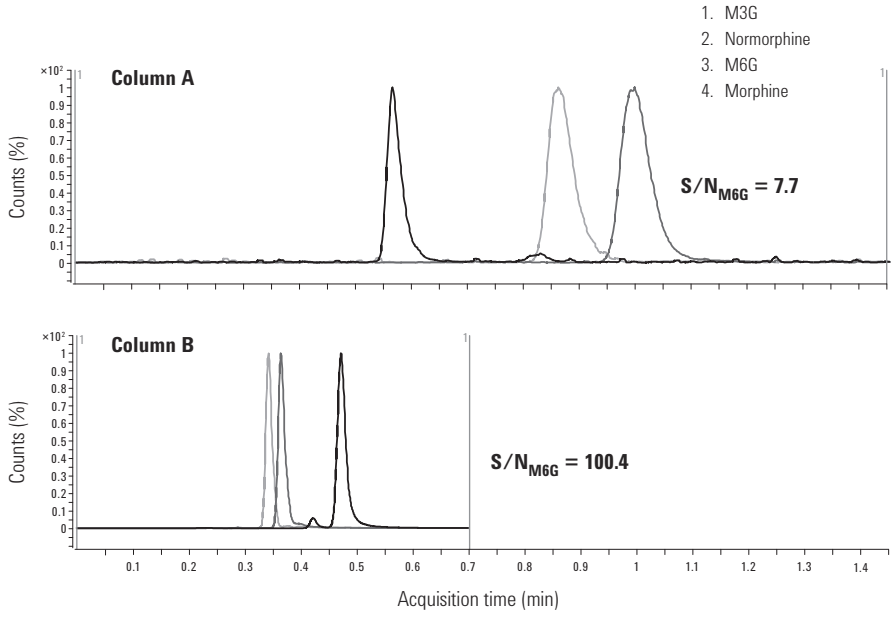
Temperature: 25 °C

Detector: Agilent 1290 Infinity LC with an Agilent 6410A Triple Quadrupole Mass Spectrometer

MS Conditions:
MS Source: Positive ESI, capillary 4000 V, drying gas temperature, flow rate and nebulizer pressure vary with mobile phase flow rate
MS Acquisition: Selected ion mode (SIM), delta EMV 200 V, MS dwell time caries with mobile phase flow rate
Software: Agilent MassHunter versions B.03.01, B.02.00 AND B.03.01 were used for data acquisition, qualitative, and quantitative analyses, respectively

Sample: 2 µL injection of 1 µg/mL each of morphine, normorphine, morphine-3-ß-D-glucuronide: HILIC sample was prepared in CH_3CN; RPLC sample was prepared in H_2O

HILIC mode with UHPLC columns cuts analysis time in half, while improving sensitivity by more than a factor of 10, compared to traditional LC columns in RPLC mode with MS detection.



Neutraceuticals: Hypericin separation in St. John's Wort

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

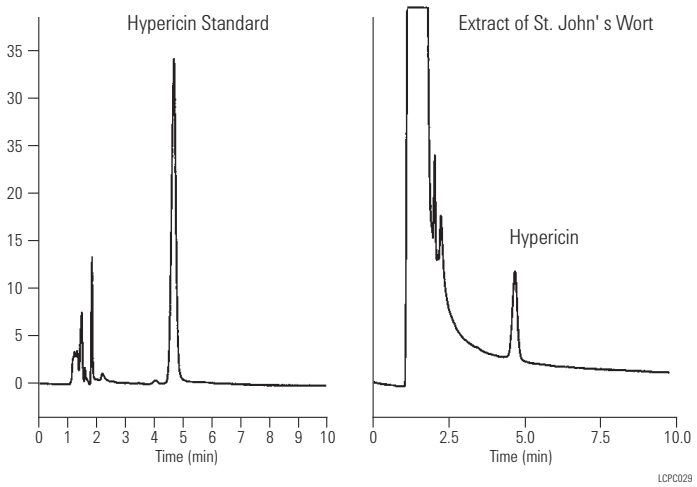
Mobile Phase: 23% 25 mM Na_2HPO_4 ,
Dibasic (pH 7.0 with H_3PO_4): 77% MeOH

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Neutraceuticals



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 NH_4 Formate (pH = 3.6)
B: ACN with 10 mM NH_4 Formate

Flow Rate: 0.6 mL/min

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

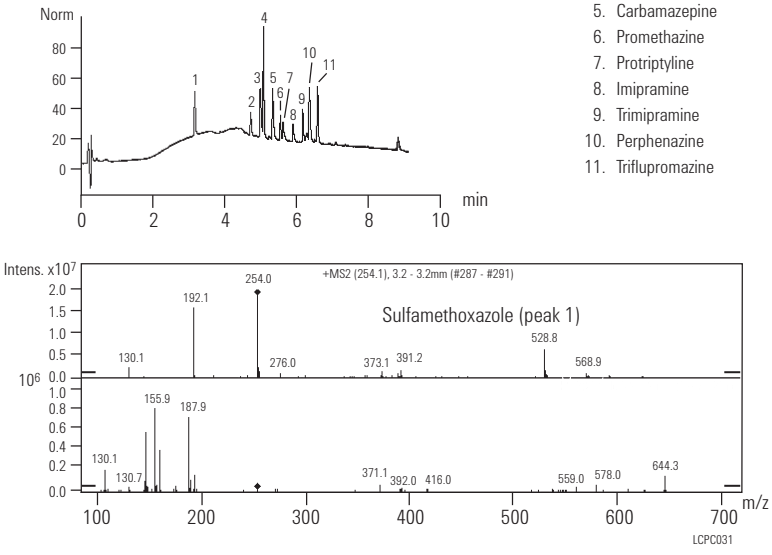
Temperature: 65 °C

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
2. Tripelennamine
3. Prednisolone
4. Diphenhydramine
5. Carbamazepine
6. Promethazine
7. Protriptyline
8. Imipramine
9. Trimipramine
10. Perphenazine
11. Triflupromazine



Hormones/steroids

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

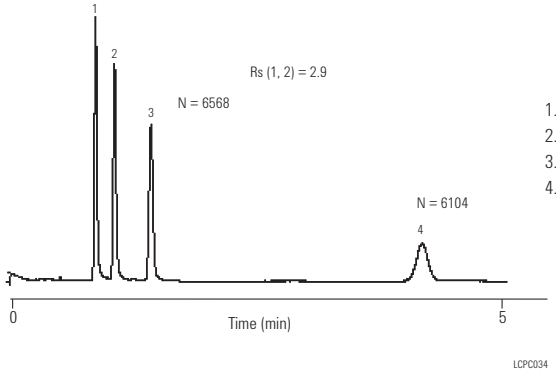
Mobile Phase: 50% 20 mM NaH_2PO_4, pH 2.8: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: RT

Detector: UV, 230 nm

Sample: Hormones/steroids



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

Steroids: Separation

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

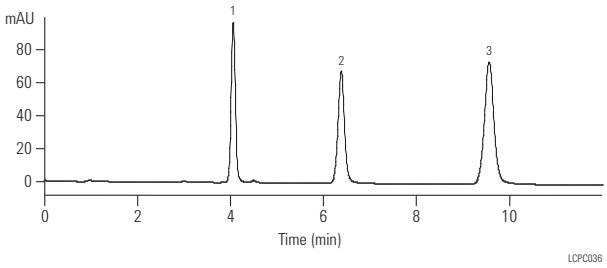
Mobile Phase: 40:60 ACN:Water

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



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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

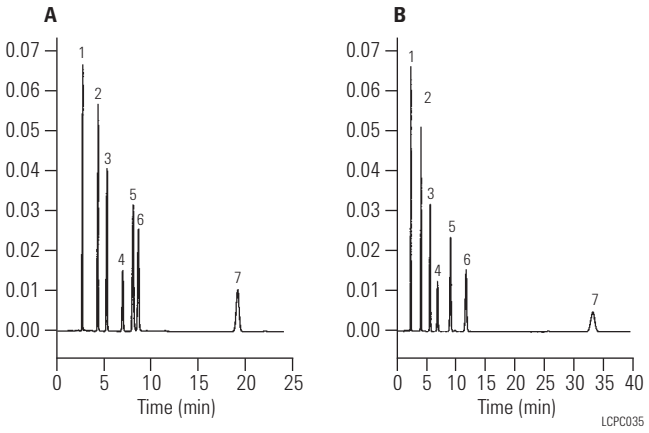
Mobile Phase: H_2O:ACN, 60:40

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

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



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

Triamcinolone – USP analysis of triamcinolone

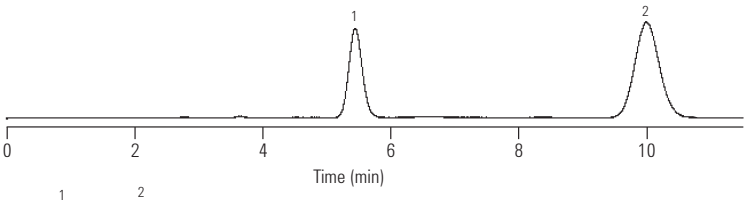
Column: **Eclipse XDB-C18**
923975-902
4.6 x 30 mm, 1.8 µm

Mobile Phase: 47% Methanol:53% Water

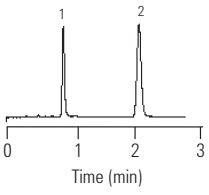
Flow Rate: 1.5 mL/min

Temperature: 25 °C

Sample: Triamcinolone, 1 µL



Peak	TR	N	Rs
1	5.45	3199	0
2	9.99	3212	8.1



1. Triamcinolone: 0.2 mg/mL
2. Hydrocortisone: 0.3 mg/mL

Minimum Resolution Required = 3.0

Peak	TR	N	Rs
1	0.89	3256	0
2	2.07	4851	11.8

LCPC038

Separation of highly basic antidepressants above their pKa in free base form (pKa 9.5-9.7)

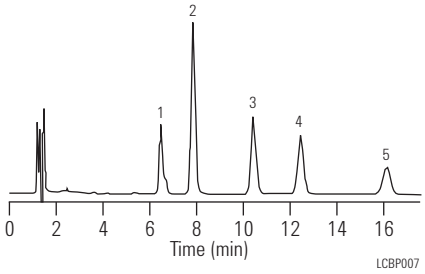
Column: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

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

Basic drugs can often be separated in their charged form at low pH with StableBond or at mid-range pH with Eclipse XDB or Bonus -RP columns. With Extend-C18, you can separate at high pH to improve solubility, improve retention, or obtain different selectivity.

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**

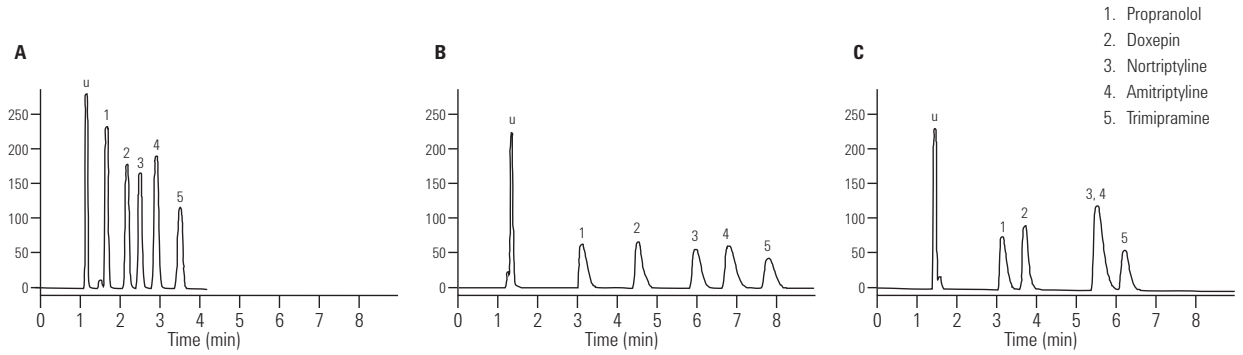
Mobile Phase: ACN: 20 mM Na Citrate, pH 6 (60:40)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Tricyclic antidepressants (u= uracil)



LCBP011

Tricyclic antidepressants

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

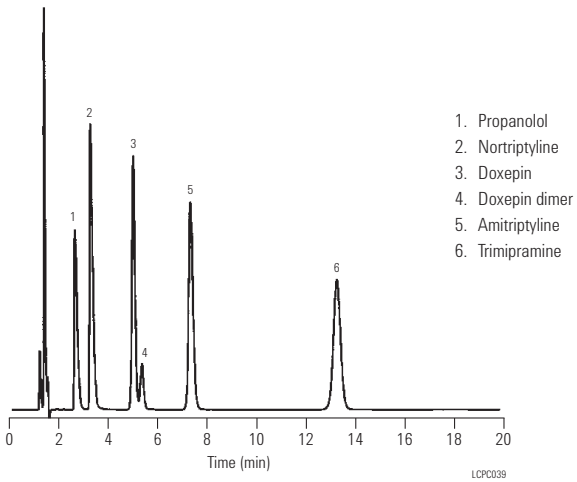
Mobile Phase: 38/62 THF/25 mM Potassium Phosphate, pH7

Flow Rate: 1.0 mL/min

Temperature: 23 °C

Detector: UV, 254 nm

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



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 RRHD 300SB-C18**
883995-902
4.6 x 150 mm, 5 µm

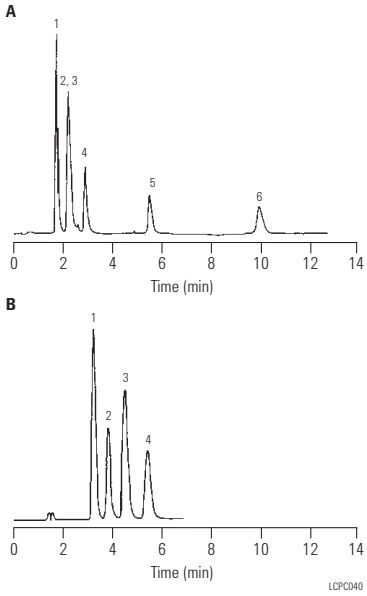
Mobile Phase: 40/60, 25 mM Phosphate Buffer, 10 mM Triethylamine, pH 6.2/ACN

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

Ulcer treatment drugs at intermediate pH

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

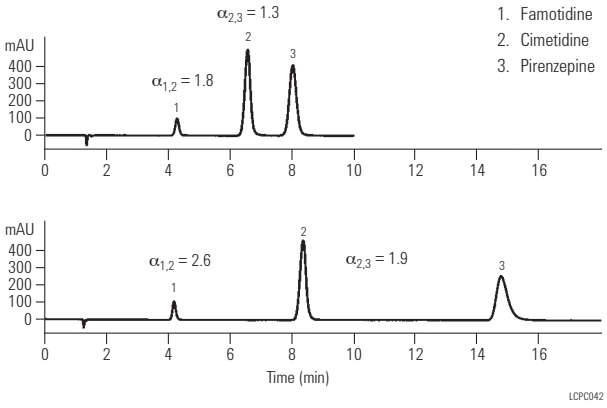
Mobile Phase: Na citrate, 20 mM, pH 6.1: MeOH, (80:20)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Ulcer treatment drugs



1. Famotidine
2. Cimetidine
3. Pirenzepine

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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

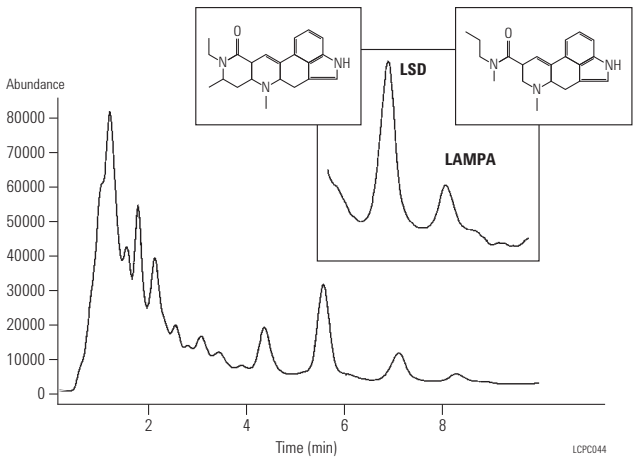
Temperature: 30 °C

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

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.



USP method:
Glyburide and internal standard, progesterone

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

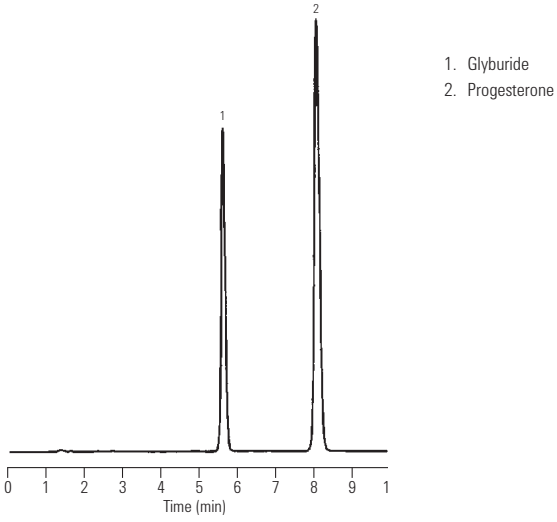
Mobile Phase: 45/55, 50 mM Ammonium Phosphate/ACN, Final pH 5.35

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm

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



1. Glyburide
2. Progesterone

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

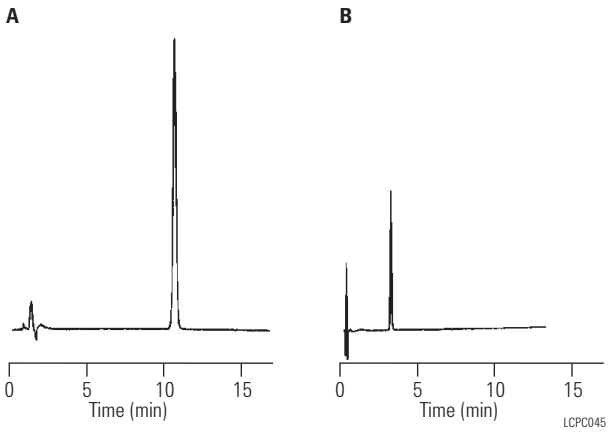
Mobile Phase: A = Water, B = ACN; Isocratic 30% B

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

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



USP analysis of tetracyclines

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

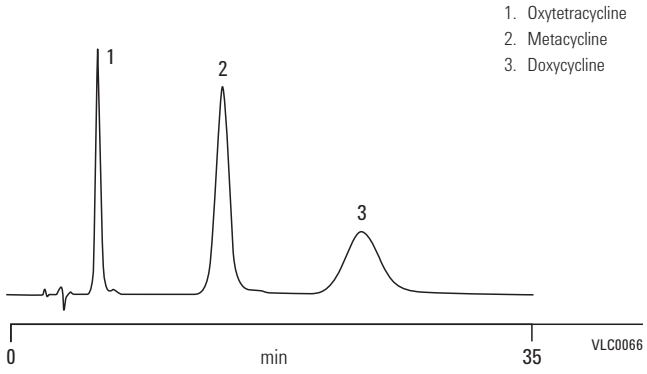
Sample: 20 mg tetracycline in 25 mL 0.01M HCl

Mobile Phase: 60 g 2-Methyl-2-propanol + 200 mL UHP water + 400 mL 0.2 M K_2HPO_4 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



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Warfarin: USP chromatographic purity method using Eclipse XDB-CN

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

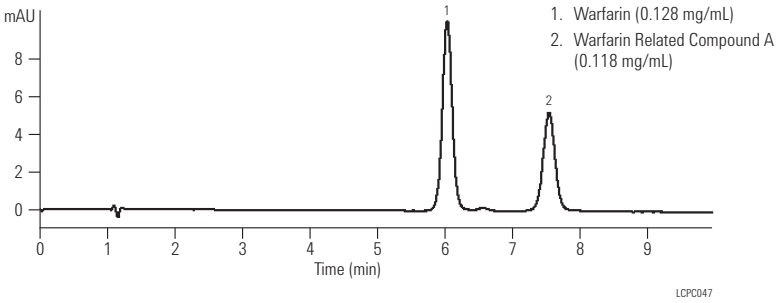
Mobile Phase: 32:68:1 Acetonitrile:Water:Glacial Acetic Acid

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

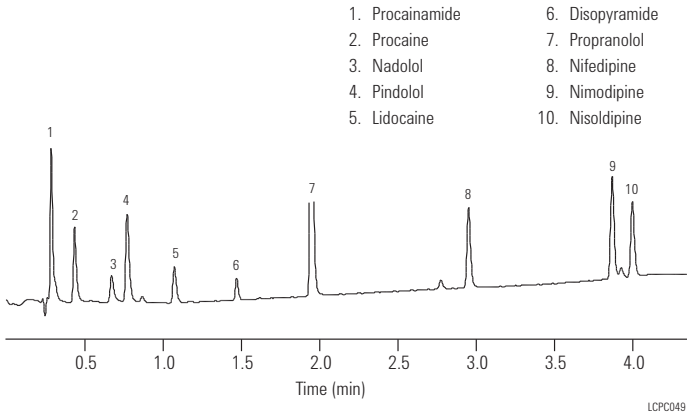
Flow Rate: 2 mL/min

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

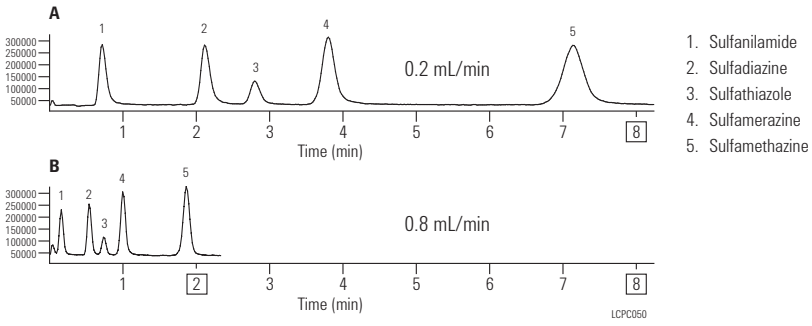
Mobile Phase: A: 90% 0.1% formic acid
B: 10% 0.1% formic acid in MeOH

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 C8**
A7511100X020
2.0 x 100 mm, 3.0 µm

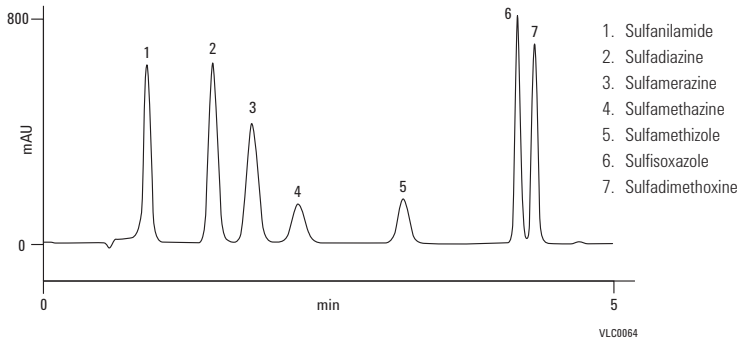
Mobile Phase: A: Water+0.1% TFA
B: MeCN+0.1% TFA

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



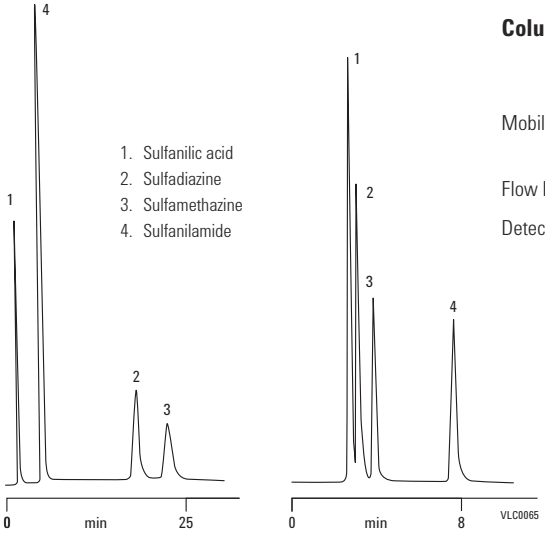
Sulfa drugs

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

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Fast analysis of Pindolol

Column A: **ZORBAX SB-CN**
863953-905
4.6 x 150 mm, 3.5 µm

Column B: **ZORBAX SB-CN**
827975-905
4.6 x 50 mm, 1.8 µm

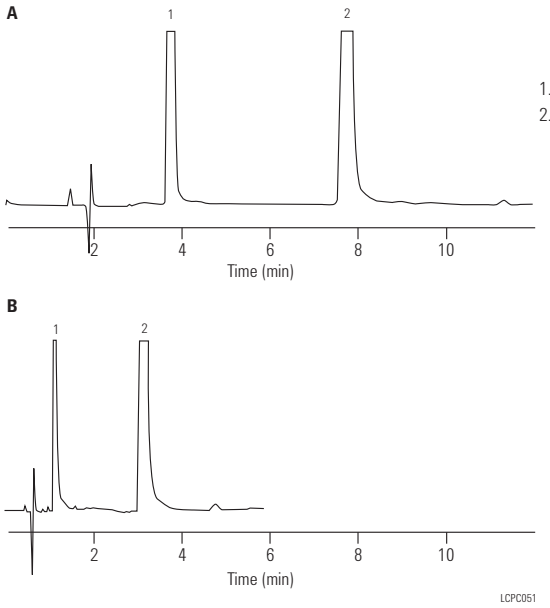
Mobile Phase: A: 70% 50 mM Na Acetate
B: 30% ACN

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 219 nm

Sample: Pindolol, 2 µL



1. Indole
2. Pindolol

Lamotrigine

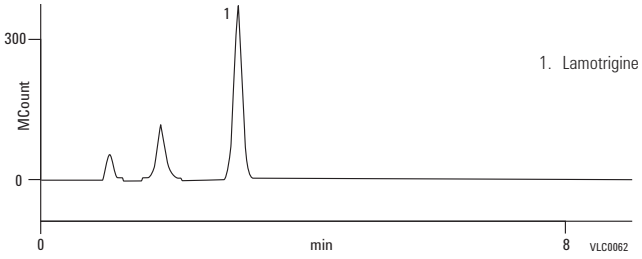
Column: **Pursuit XRs Ultra C8**
A7511100X020
2.0 x 100 mm, 3.0 µm

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

Flow Rate: 0.2 mL/min

Injection Volume: 5 µL, 50% MeOH

Detector: MS



1. Lamotrigine

Barbiturates

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

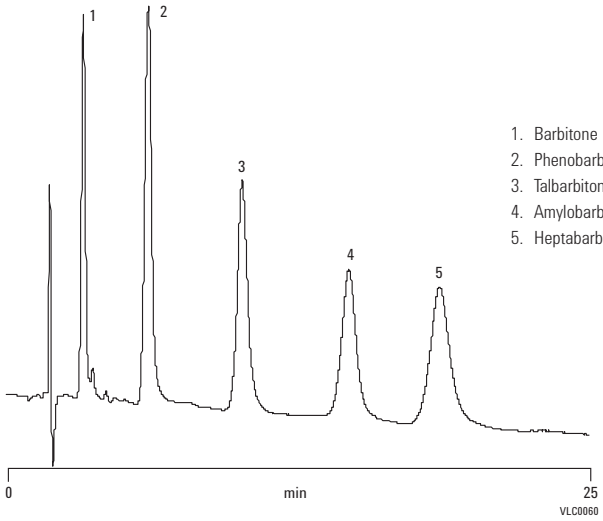
Mobile Phase: Water

Flow Rate: 1.0 mL/min

Temperature: 200 °C

Detector: UV, 220 nm

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



1. Barbitone
2. Phenobarbitone
3. Talbarbitone
4. Amylobarbitone
5. Heptabarbitone

Analysis of ciprofloxacin and ciprofloxacin metabolites

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

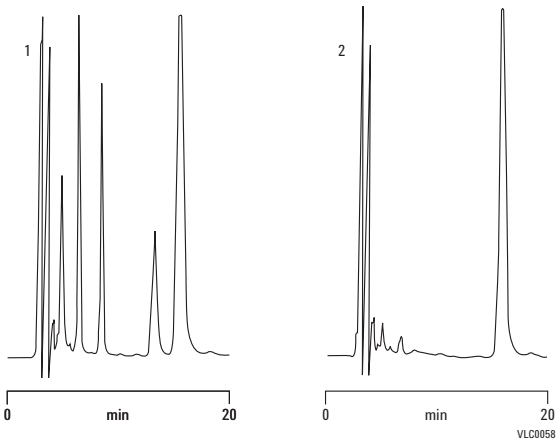
Mobile Phase: 74% 20 mM TCA:22% ACN:4% MeOH adjusted to pH 3

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).

1. Blank urine sample containing known concentrations of internal standard, ciprofloxacin and its metabolites
2. Blank urine sample containing only internal standard



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Part Number	Description	Quantity	Price	Total Cost

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City:		
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Deliver to:		

Part Number	Description	Quantity	Price	Total Cost

Special Instructions:

Subtotal:	
Tax:	
Total:	

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