



# 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 Positive/negative – Difference in refractive index of injection solvent Negative direction (gradient) – Absorbance of "A" mobile phase solvent Positive direction (gradient) – Absorbance of "B" mobile phase solvent Random – Temperature changes Random – Temperature changes Wavy or undulating – Temperature changes in room	Flush column, clean up sample, use pure solvents Use mobile phase for sample solvent Use non-absorbing or HPLC-grade or better solvent Use non-absorbing or HPLC-grade or better solvent Insulate column and tubing Thermostat column and tubing Monitor room temperature and control
Ghost peaks	Peaks from previous injection Contamination Unknown interferences in samples Ion-pair – Upset equilibrium Peptide mapping – Oxidation of TFA Reversed-phase – Contaminated water Spikes – Bubbles in solvent	Flush column to remove contaminants Sample cleanup or pre-fractionation Sample cleanup or pre-fractionation Prepare sample in actual mobile phase to minimize disturbance Prepare fresh daily; use anti-oxidant Check suitability of water by running different amount through reversed-phase column and measure peak height with elution; use HPLC grade solvents De-gas solvents
High column backpressure	Column blockage, adsorbed sample Mobile phase viscosity too high Particle size too small Plugged inlet frit Plugged inlet frit	Better sample cleanup; use guard column Use lower viscosity solvents or higher temperature Use larger $d_p$ packing Replace column 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**

<b>Symptom Type</b>	<b>Possible Cause</b>	<b>Solution</b>
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
	Beginning of peak doubling	See "peak doubling"
Peak tailing	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**

<b>Symptom Type</b>	<b>Possible Cause</b>	<b>Solution</b>
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**

<b>Symptom Type</b>	<b>Possible Cause</b>	<b>Solution</b>
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 thoroughly degassed Make sure LC backpressure is steady; this indicates a steady solvent flow

(Continued)

**LC/MS Troubleshooting**

<b>Symptom Type</b>	<b>Solution</b>
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 thoroughly 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 ( $> 18 \text{ M}\Omega \text{ cm}$ )
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  $\mu$ m

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

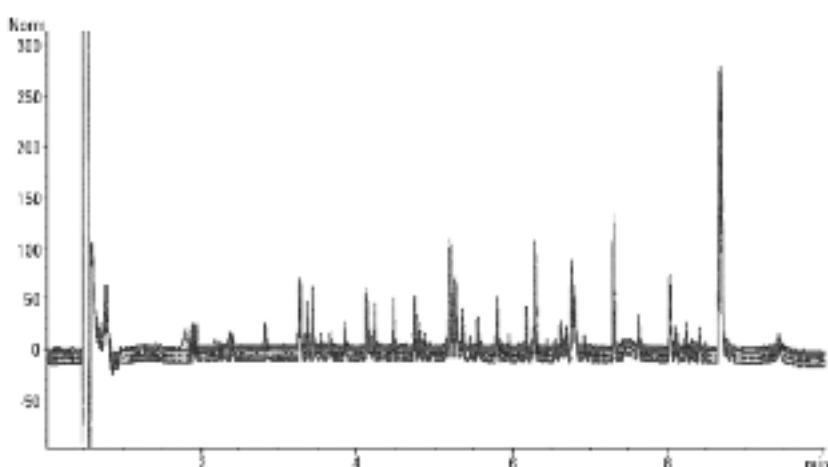
Temperature: 50 °C

Detector: Agilent 1290 Infinity LC

Injection: 5  $\mu$ L

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  $\mu$ m

Mobile Phase: A: 0.1% TFA in water  
B: 80% ACN + 0.01% TFA in water

Flow Rate: 1.0 mL/min

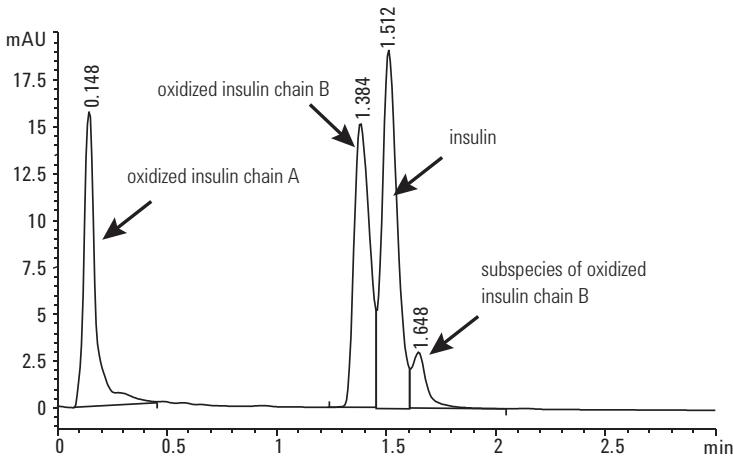
Pressure: 650-700 bar

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  $\mu$ L

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  $\mu$ m

Mobile Phase: A: 0.1% TFA in water  
B: 0.01% TFA in ACN

Flow Rate: 1.0 mL/min

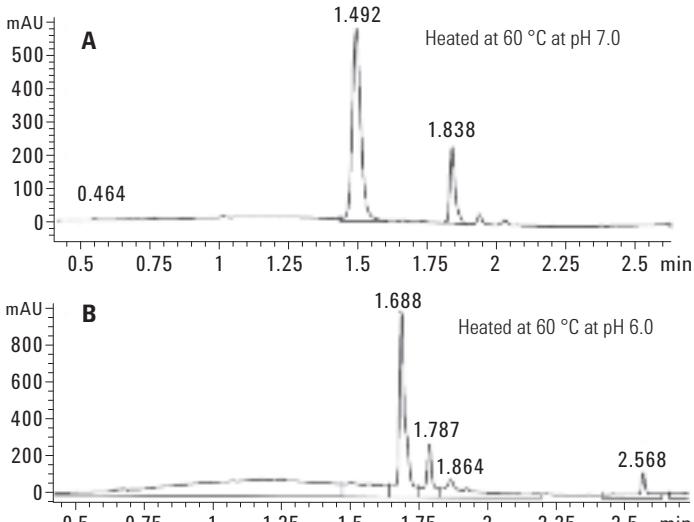
Pressure: 650 bar

Gradient: 5 to 100% B solvent from 0 to 2.5 min

Detector: UV, 280 nm  
Agilent 1290 Infinity LC

Sample: Recombinant human EPO protein (rEPO)

Sample Conc: 1.0 mg/mL

Injection: 3  $\mu$ L

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  $\mu$ m**

**Mobile Phase:** (Various)  
A: H<sub>2</sub>O + 0.1% TFA (v/v)  
B: n-propanol:ACN:H<sub>2</sub>O (80:10:10) + 0.1% TFA (v/v)

**Injection:** 1-3  $\mu$ L

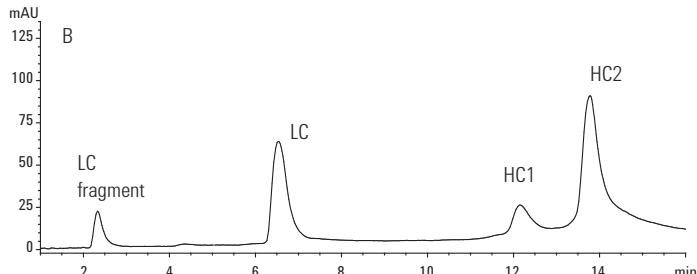
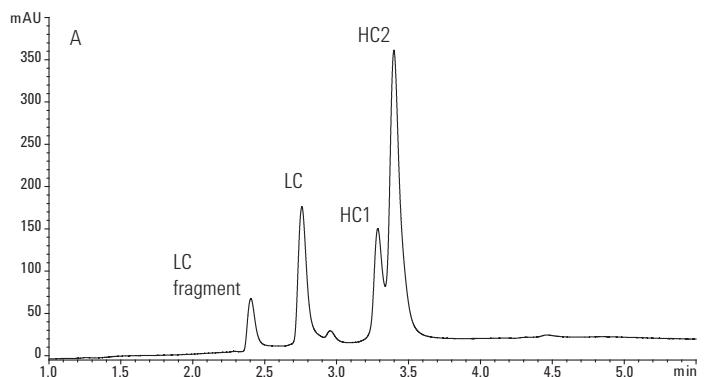
**Flow Rate:** 0.5 mL/min

**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.

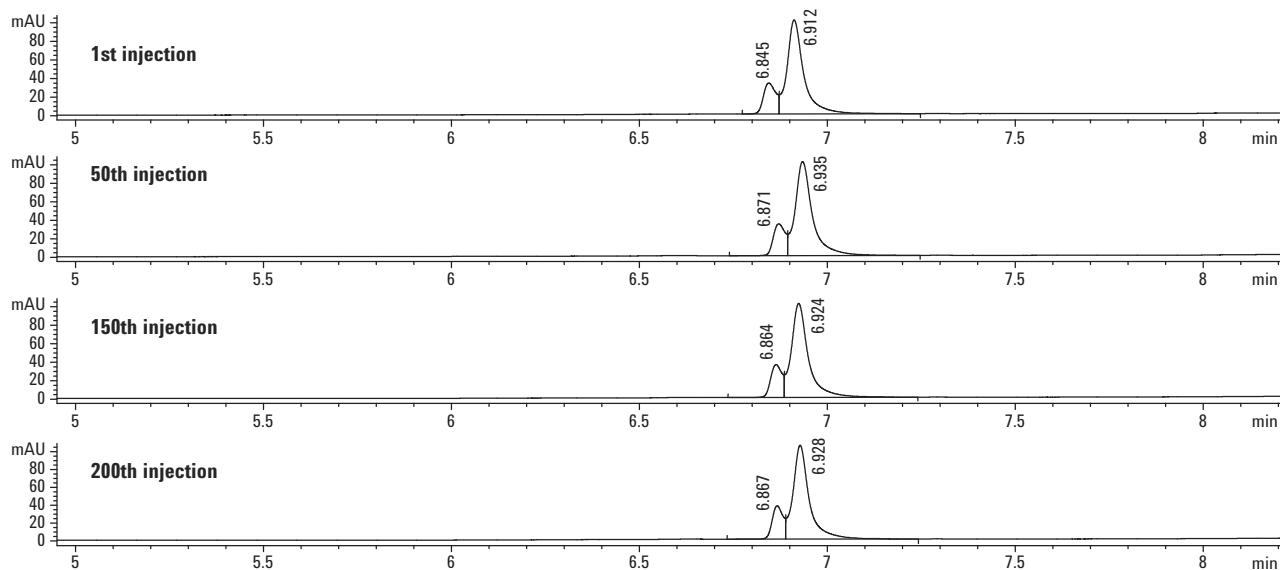


Comparison of two optimized gradients for the ultra fast separation of reduced and alkylated monoclonal antibodies on an Agilent ZORBAX RRHD 300SB-C8 column. The top panel details a rapid separation of the light and heavy chain variants in a shortened run time of less than 4 minutes. The bottom panel 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:	<b>Agilent ZORBAX RRHD 300SB-C3 858750-909 2.1 x 100 mm, 1.8 <math>\mu</math>m</b>	Temperature:	75 °C
Mobile Phase:	A: 0.1% TFA in water B: 80% n-propyl alcohol, 10% ACN, 9.9% water and 0.1% TFA	Detector:	UV, 280 Agilent 1290 Infinity LC
Flow Rate:	0.4 mL/min	Sample:	Reduced monoclonal antibody (IgG1) (1.0 mg/mL) - Agilent BL05 IgG1
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	Injection:	2 $\mu$ L



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](http://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  $\mu$ m**

**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

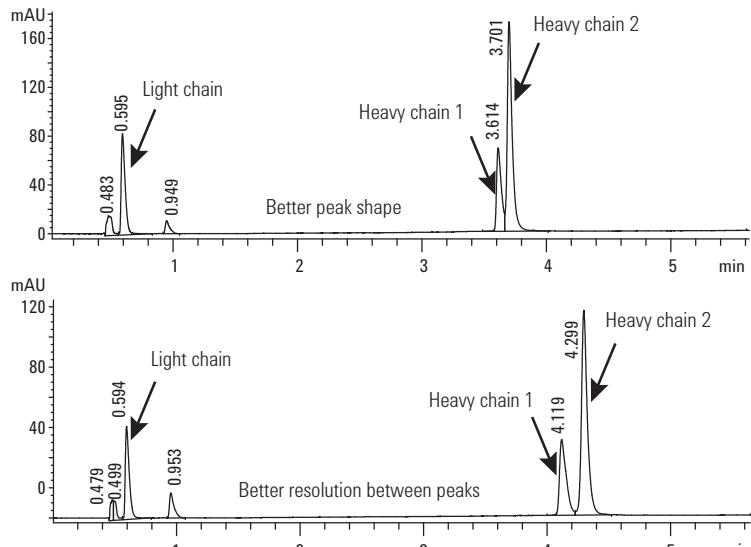
**Gradient:** 1st condition: 0 min-1% B,  
2 min-20% B,  
5 min-70% B  
2nd condition: 0 min-1% B,  
2 min-20% B,  
5 min-50% B

**Temperature:** 74 °C

**Detector:** UV, 280 nm

**Sample:** Reduced monoclonal antibody (IgG1)  
(1.0 mg/mL) - BioCreative IgG1

**Injection:** 2  $\mu$ L



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](http://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  $\mu$ m

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

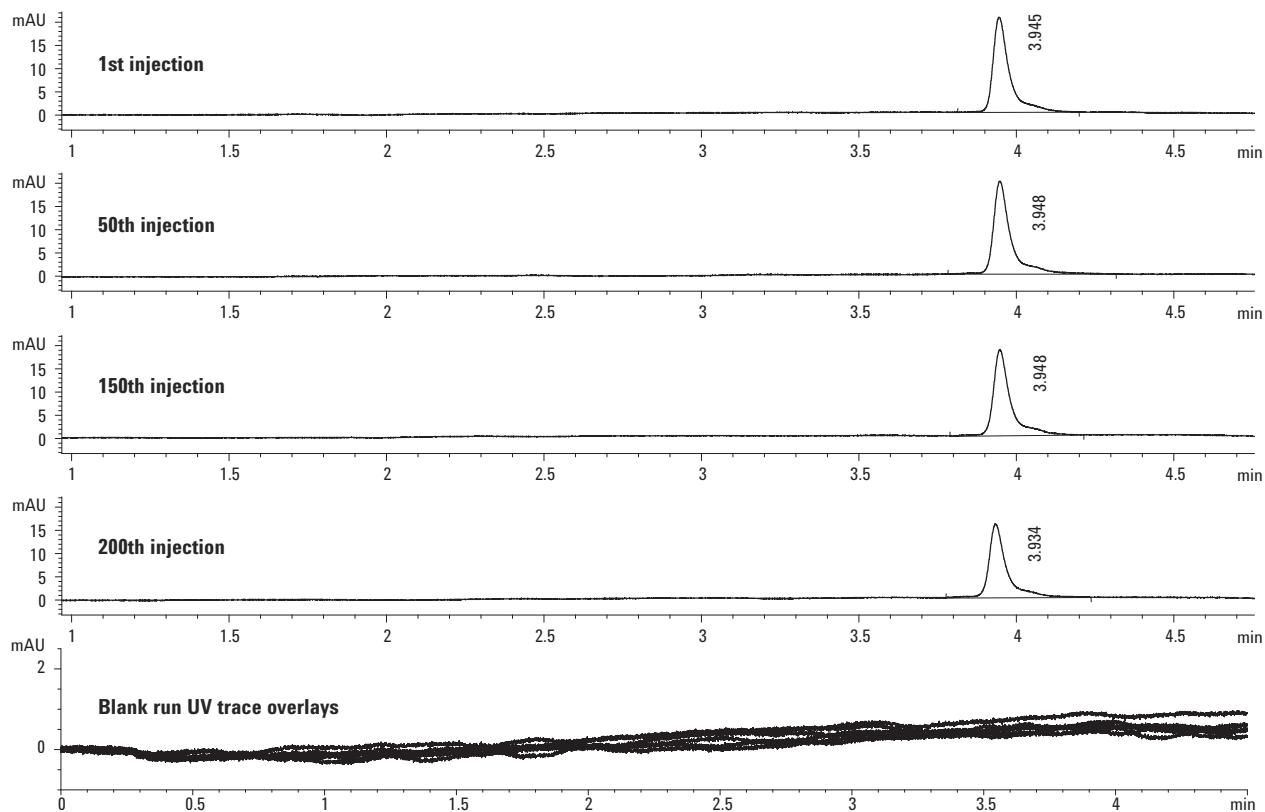
**Temperature:** 74 °C

**Detector:** UV, 280 nm

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

**Flow Rate:** 1.0 mL/min

**Injection:** 1  $\mu$ L



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

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

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

Temperature: Ambient

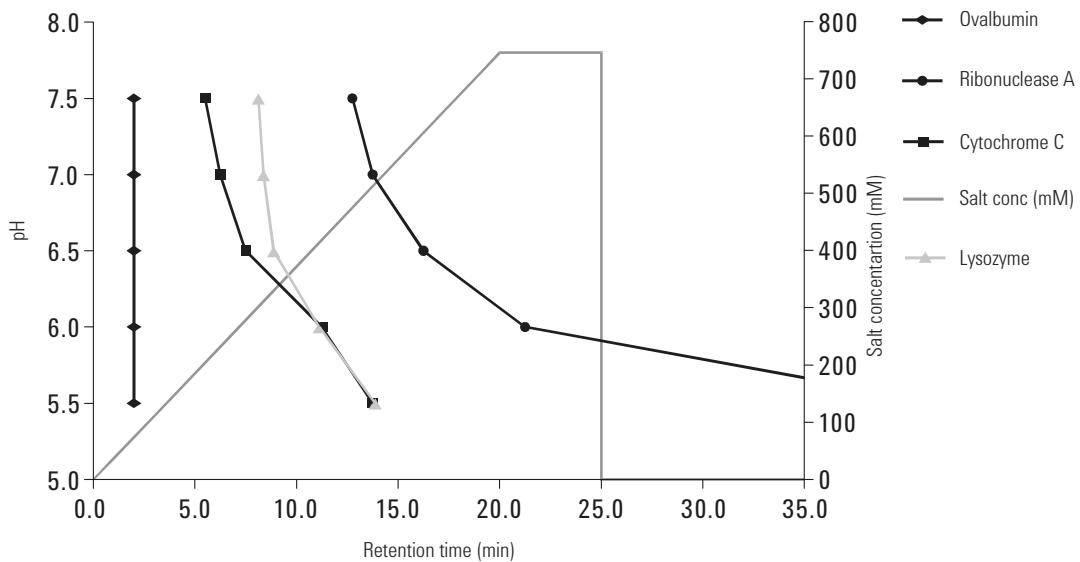
Mobile Phase: A: water  
B: 1.6 M NaCl

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

C: 40.0 mM Na<sub>2</sub>HPO<sub>4</sub>  
D: 40.0 mM Na<sub>2</sub>HPO<sub>4</sub>

Sample: Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme  
Sample Conc: 2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)

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)



Effect of pH on retention time of protein standards using an 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  $\text{NaH}_2\text{PO}_4$   
D: 40.0 mM  $\text{Na}_2\text{HPO}_4$   
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

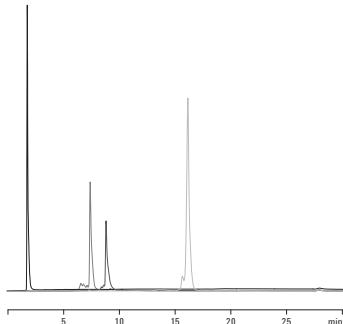
**Temperature:** Ambient

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

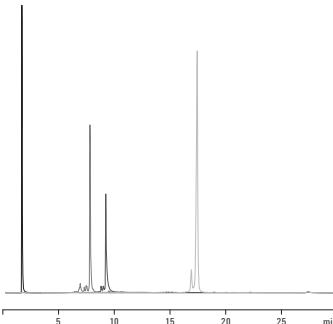
**Sample:** Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme

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

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



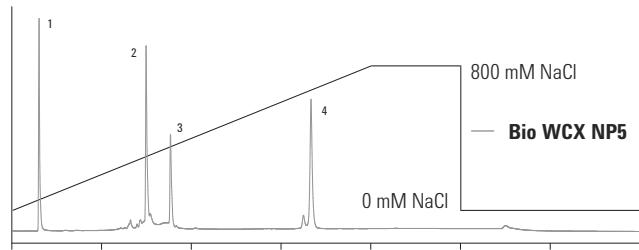
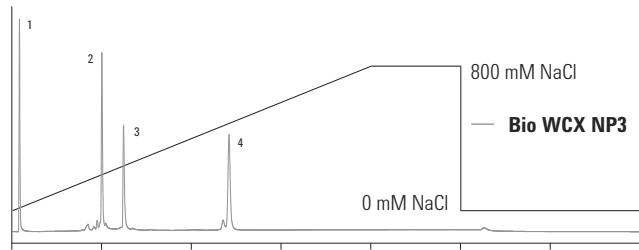
Separation of protein standards at pH 6.5  
using an Agilent Bio WCX, NP10 column.



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](http://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  $\mu$ m

**Column:** Agilent Bio WCX, stainless steel  
5190-2443  
4.6 x 50 mm, 3  $\mu$ m

**Column:** Agilent Bio WCX, stainless steel  
5190-2441  
4.6 x 50 mm, 1.7  $\mu$ m

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

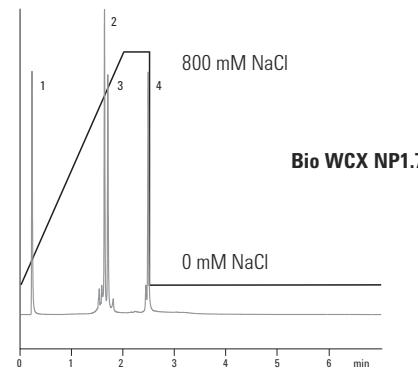
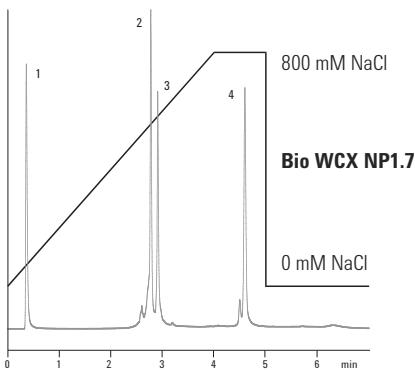
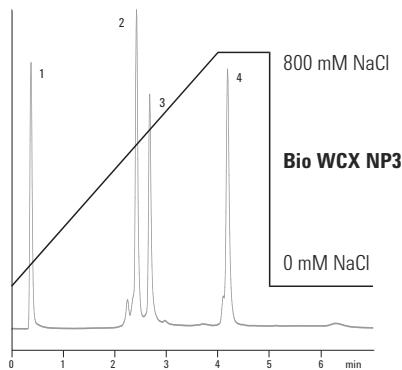
Temperature: Ambient

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<sub>2</sub>PO<sub>4</sub>  
D: 100 mM Na<sub>2</sub>HPO<sub>4</sub>  
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

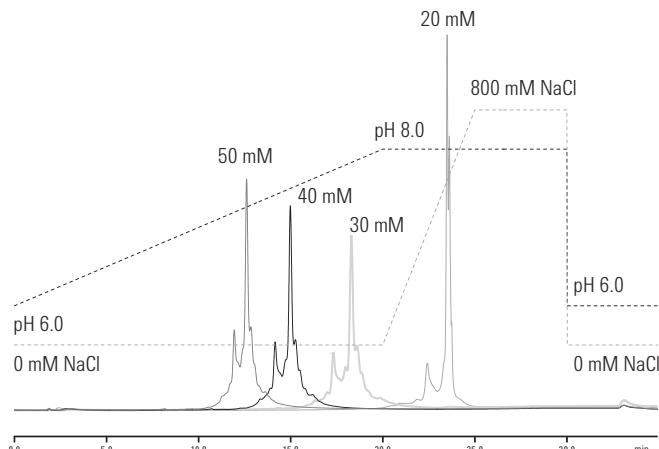
**Gradient:** pH 6.0 to 8.0, 0 to 20 minutes  
0 to 800 mM NaCl, 20 to 25 minutes  
800 mM NaCl, 25 to 30 minutes

**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](http://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

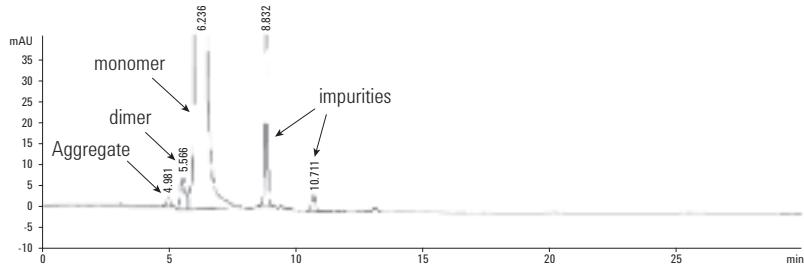
Mobile Phase: 150 mM sodium phosphate buffer, pH 7.0

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

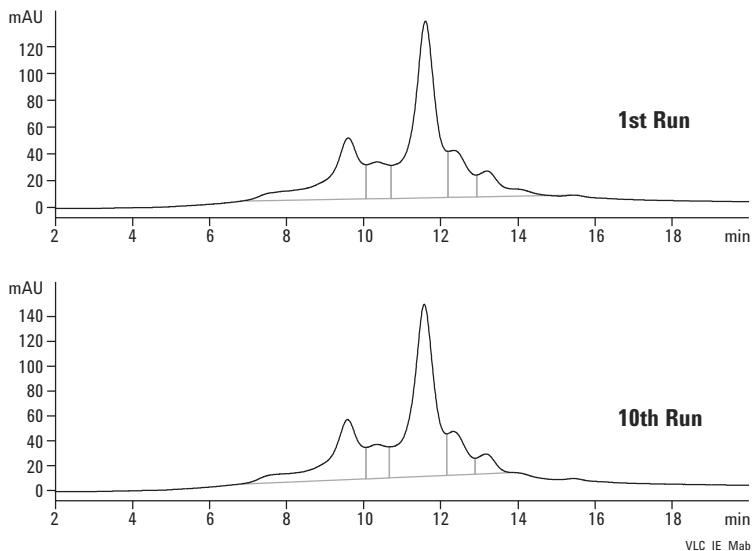
Flow Rate: 0.65 mL/min

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

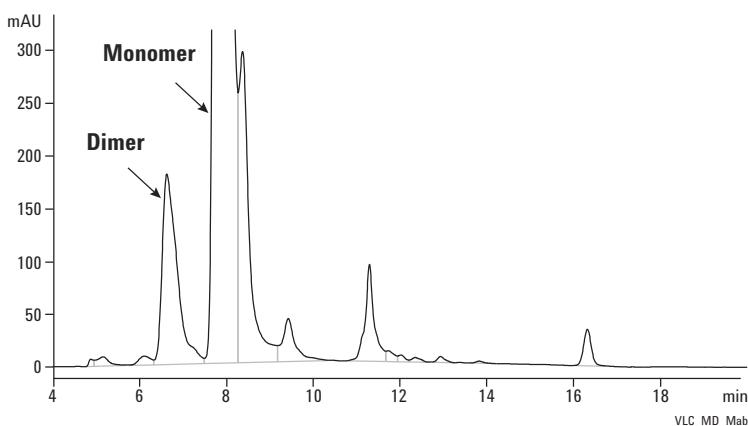
**Flow Rate:** 1.0 mL/min

**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

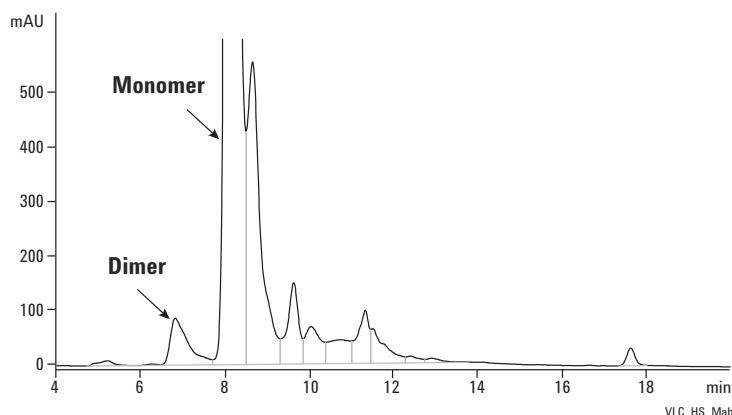
**Flow Rate:** 1.0 mL/min

**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](http://www.agilent.com/chem/library)

**Nucleosides, purines and pyrimidines**

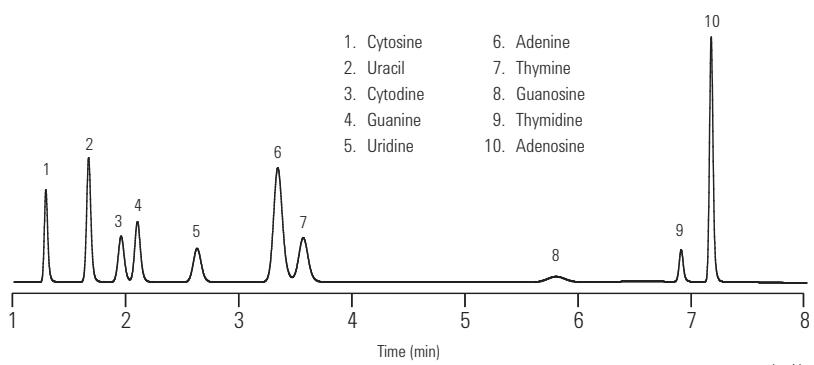
**Column:** Eclipse Plus Phenyl Hexyl  
959993-912  
**4.6 x 150 mm, 5 µm**

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

**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<sub>2</sub>HPO<sub>4</sub>, 10 mM Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, 0.5 mM NaN<sub>3</sub>, 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

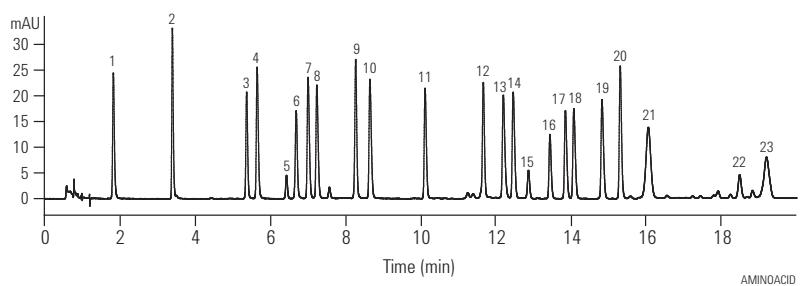
Sample: 900 pmol Amino Acids with extended Amino Acids and Internal Standards (500 pmol)

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**

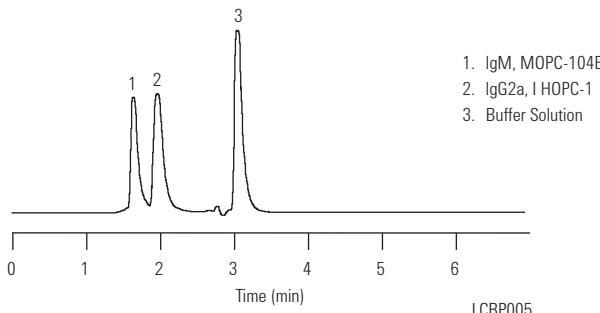
Time (min)	% B
0	2
0.5	2
20	57
20.1	100
23.5	100
23.6	2
25	stop



**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<sub>2</sub>O  
B: 0.07% TFA in ACN

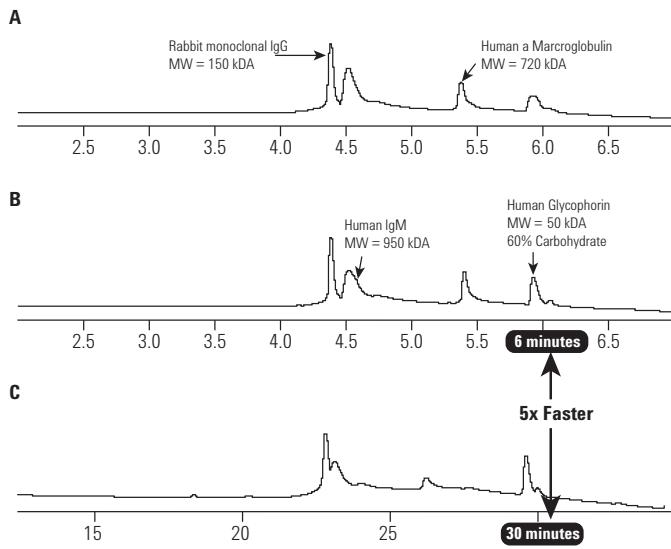
**Flow Rate:** A, B: 0.454 mL/min  
C: 0.071 mL/min

**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](http://www.agilent.com/chem/library)

**HSA tryptic digest  
on ZORBAX Rapid Resolution HT 1.8  $\mu$ m**

**Column A:** ZORBAX SB-C18  
883700-922  
2.1 x 150 mm, 5  $\mu$ m

**Column B:** ZORBAX SB-C18  
822700-902  
2.1 x 50 mm, 1.8  $\mu$ m

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

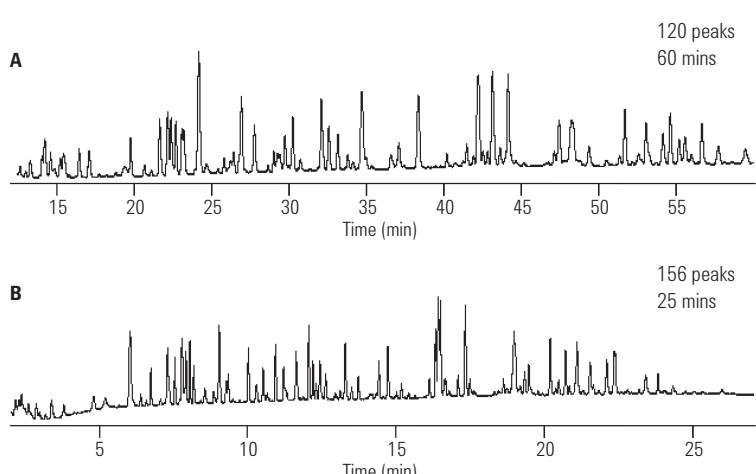
Flow Rate: A: 0.2 mL/min  
B: 0.5mL/min

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  $\mu$ L of 15 pmol/ $\mu$ L  
(120 pmol on column)



LCBP013

**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  $\mu$ m, 5065-9913  
**Analytical:** 0.3 x 150 mm,  
5  $\mu$ m, 5064-8263

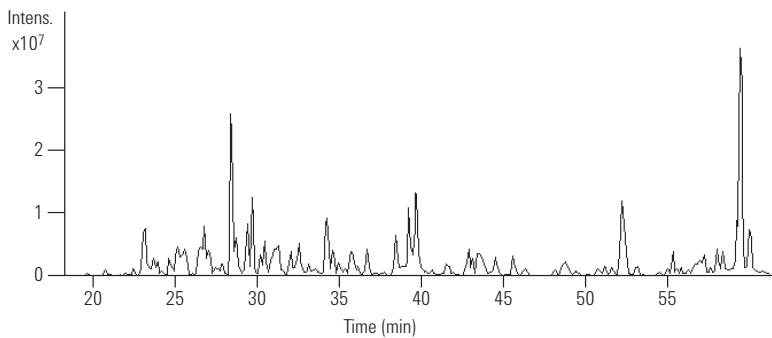
Mobile Phase: A: Water + 0.1% Formic acid  
B: Acetonitrile + 0.1% Formic acid

Flow Rate: 6  $\mu$ L/min

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

Sample: Band from 1-D in gel digest

**Base Peak Chromatogram**



LCBP014

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

**Proteins Identified**

1.  $\alpha$ -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  $\mu$ m

**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

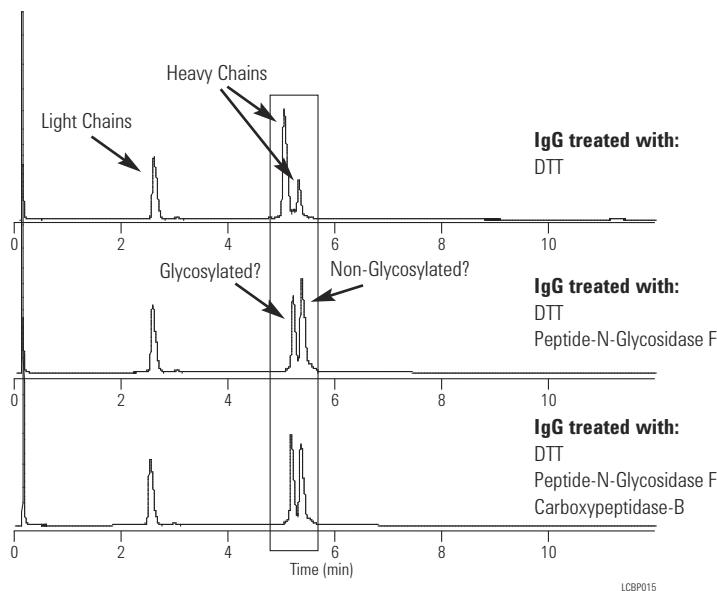
**Flow Rate:** 1.0 mL/min

**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*



LCBP015

## Use ZORBAX Extend-C18 for alternate selectivity at high pH

**Column:** ZORBAX Extend-C18  
773700-902  
2.1 x 150 mm, 5  $\mu$ m

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

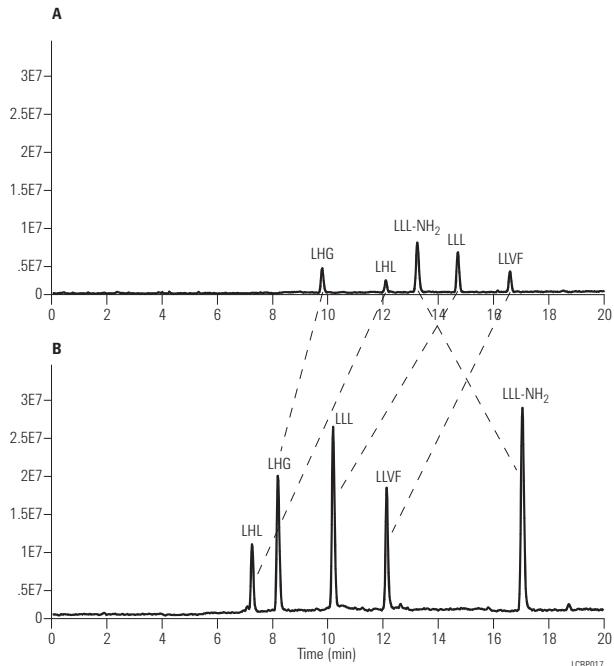
A: 20 mM NH<sub>4</sub>OH in Water  
B: 20 mM NH<sub>4</sub>OH in 80% ACN

**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<sub>2</sub> – 35 psi, 12 L/min, 300 °C  
4  $\mu$ L (50 ng each peptide)



LCBP017

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**

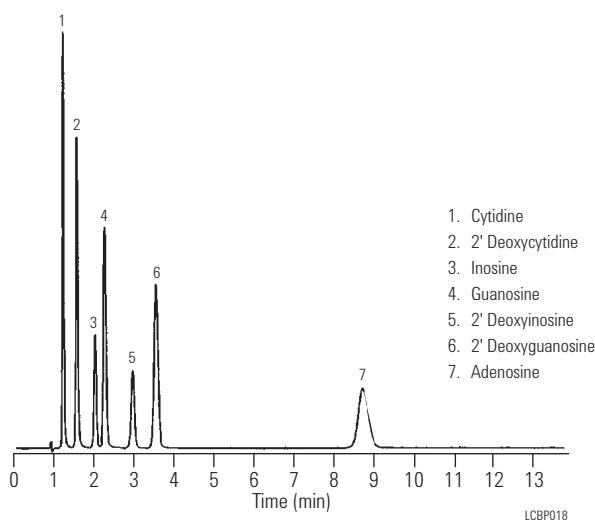
Mobile Phase: 4 mM Ammonium Phosphate (pH 4.0 with Phosphoric Acid)

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: 2 µL (1.6 µg each)

**Nucleotides: Separation of mononucleotides**

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

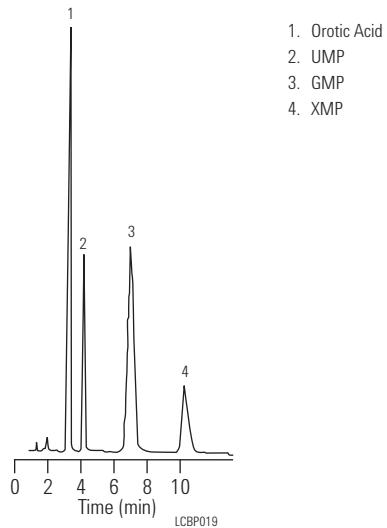
Mobile Phase: 0.1 M NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Orotic Acid, UMP, GMP, 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

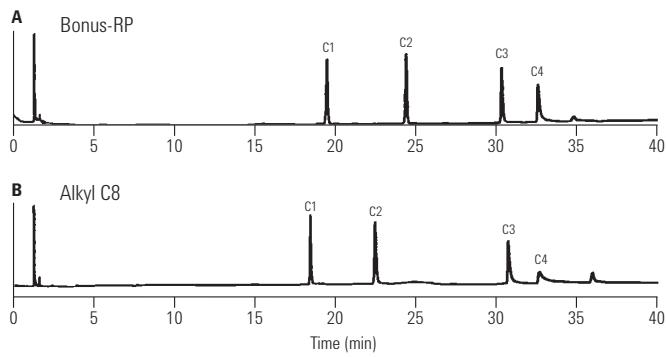
Gradient: 0-100% B in 50 min

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-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

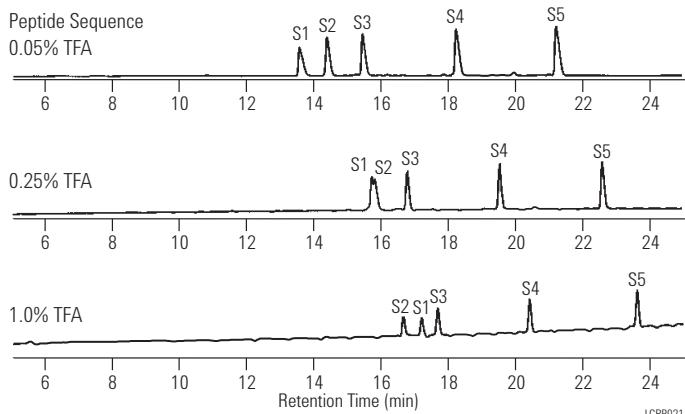
Flow Rate: 1.0 mL/min

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

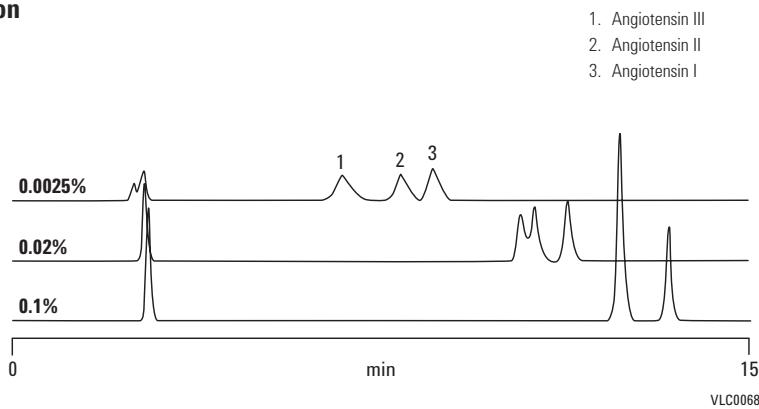


LCBP021

**Exploiting chemical stability – TFA concentration**

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

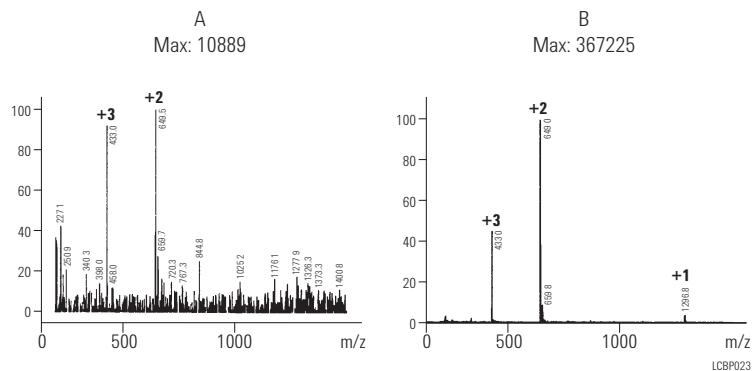
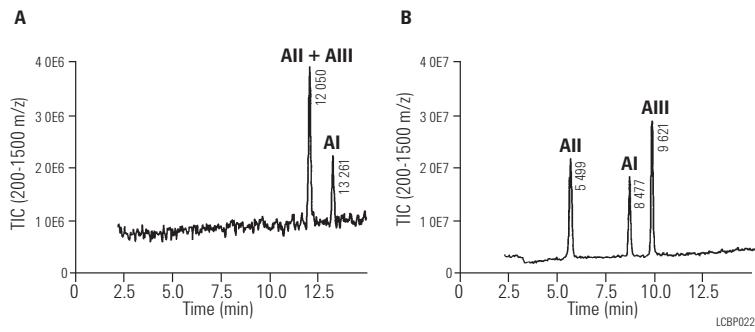
**Mobile Phase:** A: TFA (various %) in water  
B: TFA (various %) in ACN  
**Gradient:** Linear 12-40% B in 15 min  
**Flow Rate:** 1.0 mL/min  
**Detector:** ELS (neb=75 °C, evap=85 °C, gas=1.0 SLM)

**Peptides:****Separation of Antiotensins I, II, III with TFA and NH<sub>4</sub>OH**

**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<sub>4</sub>OH in water  
B: 10 mM NH<sub>4</sub>OH 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<sub>2</sub>-35 psi, 12 L/min, 325 °C  
**Sample:** 2.5 µL sample (50 pmol each)



## Peptides/proteins: Equivalent gradient separations

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

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

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

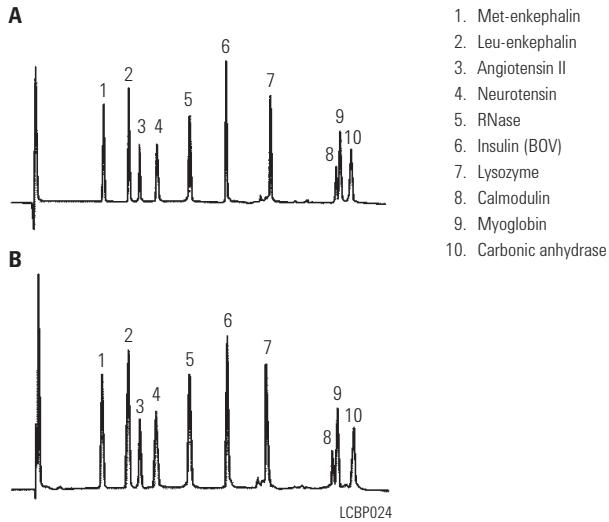
Flow Rate: A: Analytical  
1 mL/min  
B: Narrow Bore  
0.2 mL/min

Gradient: 10-60% B in 30 min

Temperature: 35 °C

Detector: UV, 215 nm

Sample: 10 µL injection, concentration 2-6 µg



## Peptides/proteins: Effect of elevated temperature

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

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

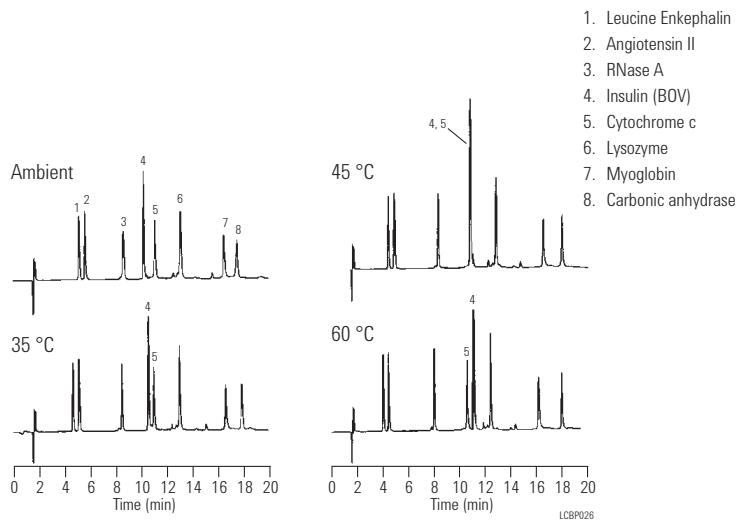
Flow Rate: 1.0 mL/min

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

Temperature: Ambient – 60 °C

Detector: UV, 215 nm

Sample: Polypeptides



**Separation of polypeptides in under 1 minute**

**Column:** Poroshell 300SB-C18  
660750-902  
2.1 x 75 mm, 5  $\mu$ m

Mobile Phase: A: 0.1% TFA, H<sub>2</sub>O  
B: 0.07% TFA, ACN

Flow Rate: 3 mL/min

Gradient: 0-100% B in 1.33 min

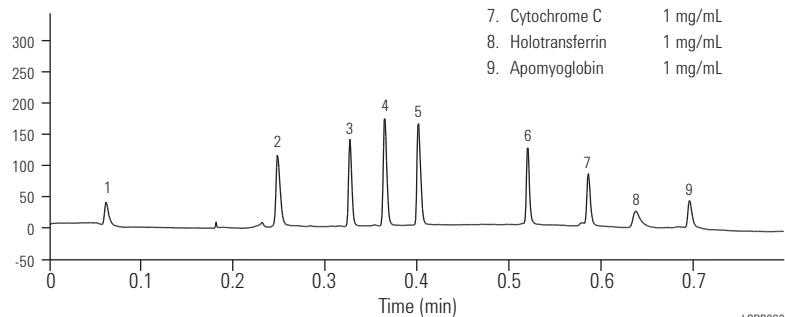
Temperature: 70 °C

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

Sample: Peptides/proteins, 0.5  $\mu$ L

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  $\mu$ m

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

Flow Rate: 3.0 mL/min (360 bar pressure)

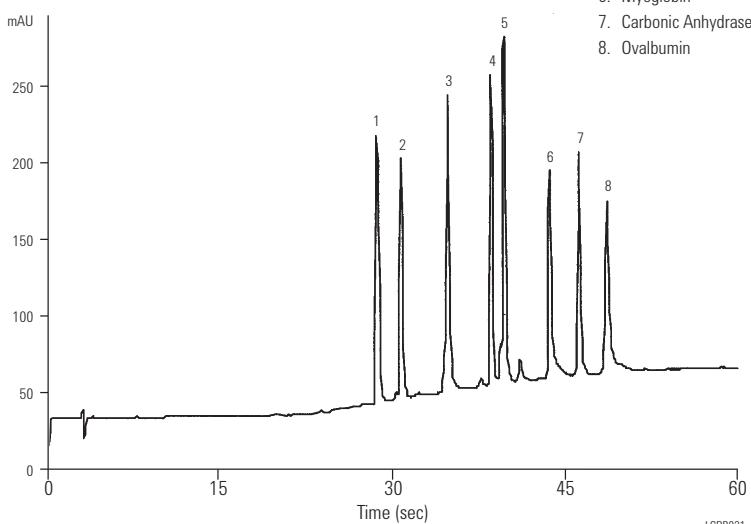
Gradient: 5-100% B in 1.0 min

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

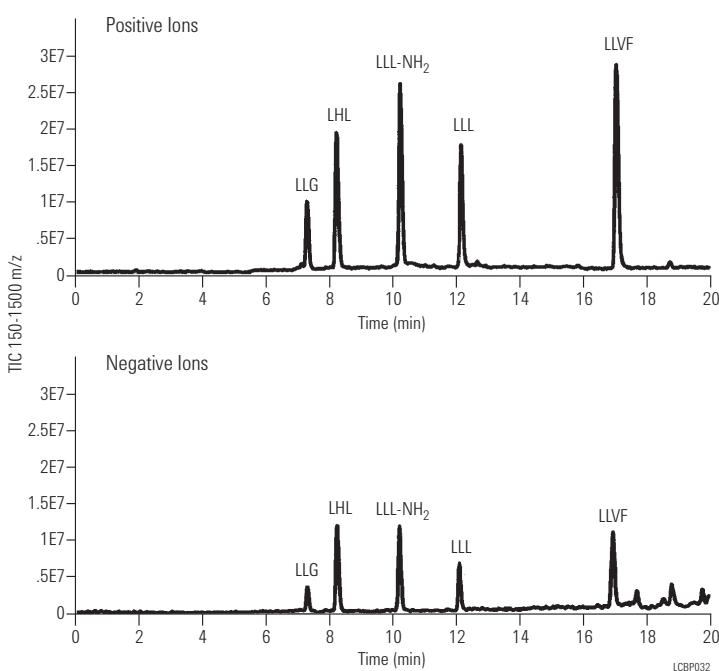


LCBP031

**Peptide RP-HPLC/ESI-MS  
using NH<sub>4</sub>OH mobile phase  
yields both positive and negative ion spectra**

**Column:** ZORBAX Extend-C18  
773700-902  
2.1 x 150 mm, 5  $\mu$ m

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<sub>2</sub> – 35 psi, 12 L/min, 300 °C  
TIC 150-1500 m/z  
Sample: 4  $\mu$ L (50 ng each peptide)

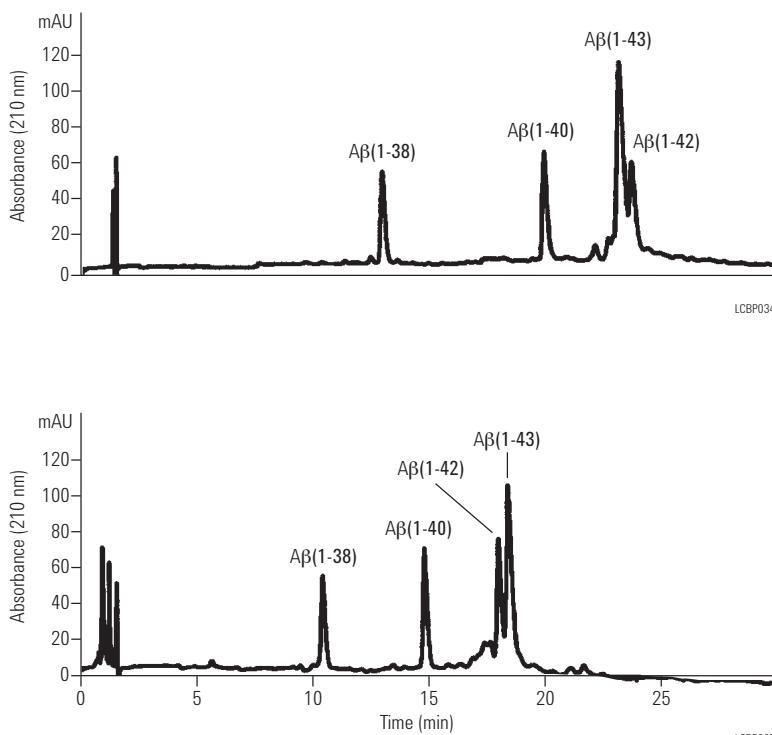


**Comparison of A $\beta$  peptide RP-HPLC  
separations at low and high pH**

**Column:** ZORBAX Extend-C18  
773700-902  
2.1 x 150 mm, 5  $\mu$ m

Mobile Phase: A: 0.1% TFA in water  
B: 0.085% TFA in 80% ACN  
Flow Rate: 0.25 mL/min  
Gradient: 29-41% B in 30 min  
Temperature: 80 °C  
Detector: UV, 210 nm  
Sample: 5  $\mu$ L sample (100 pmol each)

Mobile Phase: A: 20 mM NH<sub>4</sub>OH in water  
B: 20 mM NH<sub>4</sub>OH 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  $\mu$ L sample (100 pmol each)



### Selectivity comparison of TFA and NH<sub>4</sub>OH for peptide RP-HPLC\ESI-MS analysis

**Column:** ZORBAX Extend-C18  
773700-902  
2.1 x 150 mm, 5  $\mu$ m

**Mobile Phase:** TFA Conditions:  
A: 0.1% TFA in water  
B: 0.085% TFA in 80% ACN  
NH<sub>4</sub>OH Conditions:  
A: 20 mM NH<sub>4</sub>OH in water  
B: 20 mM NH<sub>4</sub>OH in 80% ACN

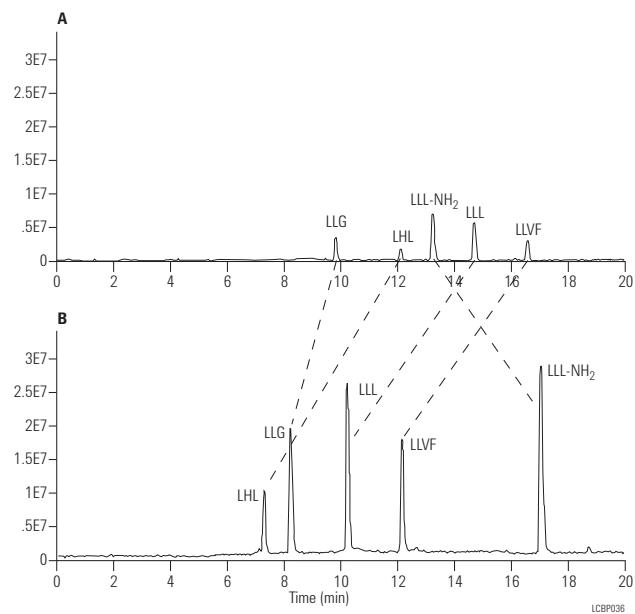
**Flow Rate:** 0.25 mL/min

**Gradient:** 5-60% B in 20 min

**Temperature:** 25 °C

**MS Conditions:** Pos. Ion ESI – Vf 70V, Vcap 4.5 kV,  
N<sub>2</sub> – 35 psi, 12 L/min., 300 °C  
TIC 150-1500 m/z

**Sample:** 4  $\mu$ L (50 ng each peptide)



### Peptide phosphorylation sites LC and LC/MS using Capillary LC columns

**Column:** ZORBAX 300SB-C18  
5064-8268  
0.5 x 150 mm, 3.5  $\mu$ m

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

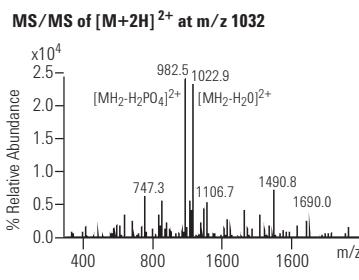
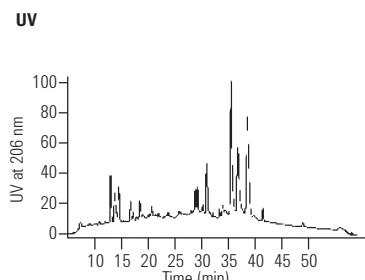
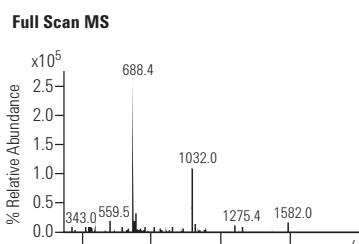
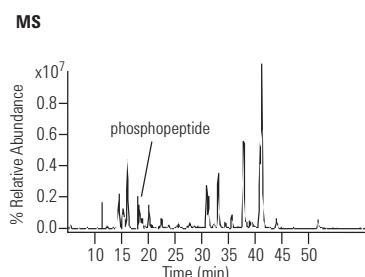
**Flow Rate:** 5.5  $\mu$ L/min

**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 Amplitude: 1.0 V

**Sample:** Beta casein 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

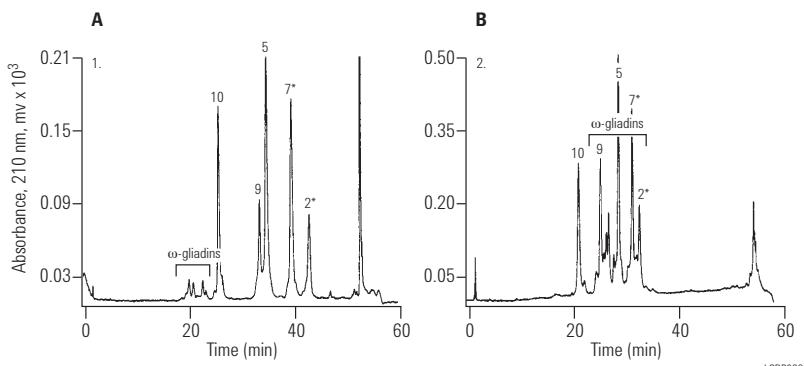
Flow Rate: 1.0 mL/min

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<sub>2</sub>O  
B: 0.09% TFA in 80% ACN/20% Water

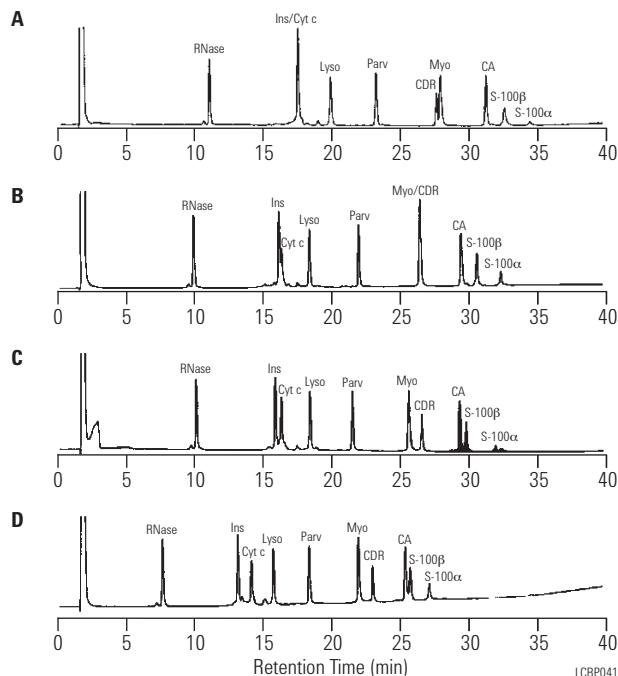
Flow Rate: 1.0 mL/min

Gradient: 25-70% B in 40 min

Temperature: 60 °C

Detector: UV, 210 nm

Sample: Polypeptides, 3 µg each



**Standard proteins by reversed-phase**

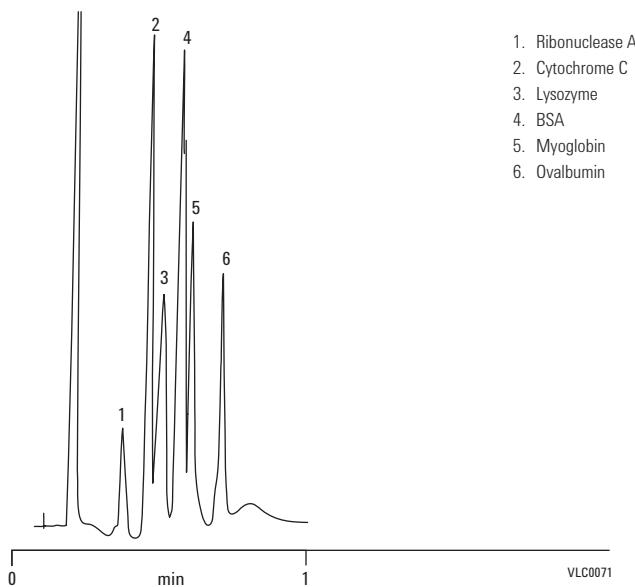
**Column:** PLRP-S 4000Å  
PL1512-1803  
4.6 x 50 mm, 8 µm

**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

**Standard ion-exchange protein separation**

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

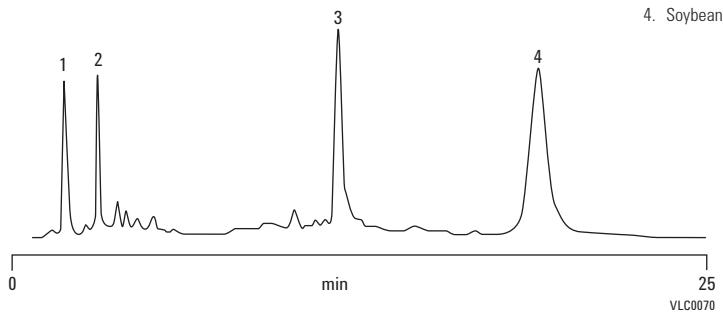
**Mobile Phase:** A: 10 mM Tris HCl pH 8  
B: A+0.35 M NaCl pH 8

**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**

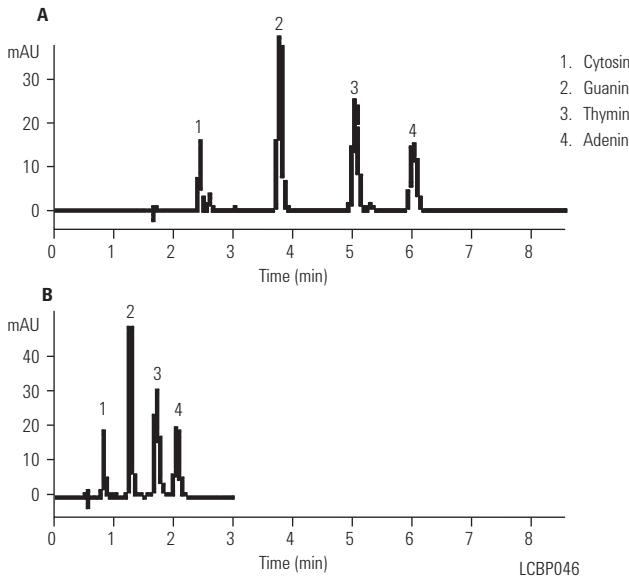
**Column B:** ZORBAX SB-CN  
**835975-905**  
**4.6 x 50 mm, 3.5 µm**

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

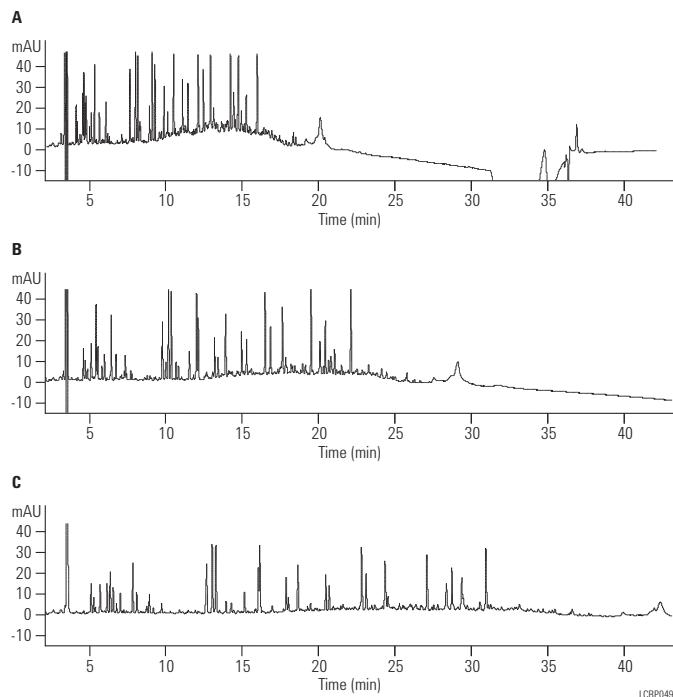
Flow Rate: 0.5 mL/min

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



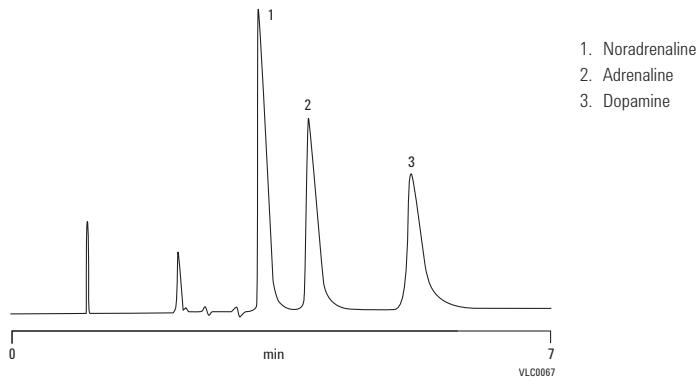
**Catecholamines**

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

**Mobile Phase:** 95% 25 mM citric acid,  
 25 mM Na<sub>2</sub>HPO<sub>4</sub>, 1 mM heptane  
 sulfonic acid:5% ACN, pH 2.85

**Flow Rate:** 1.0 mL/min

**Detector:** UV, 280 nm

**Whey proteins in dairy samples – milk**

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

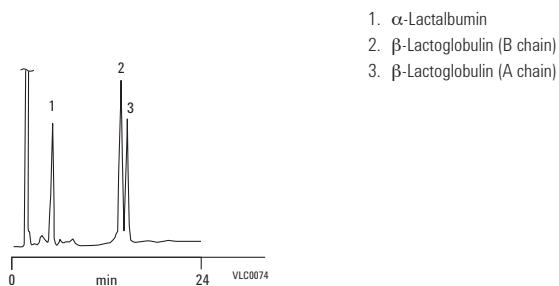
**Mobile Phase:** A: 0.1% TFA in 99% water:1% ACN  
 B: 0.1% TFA in 1% water:99% ACN

**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



**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

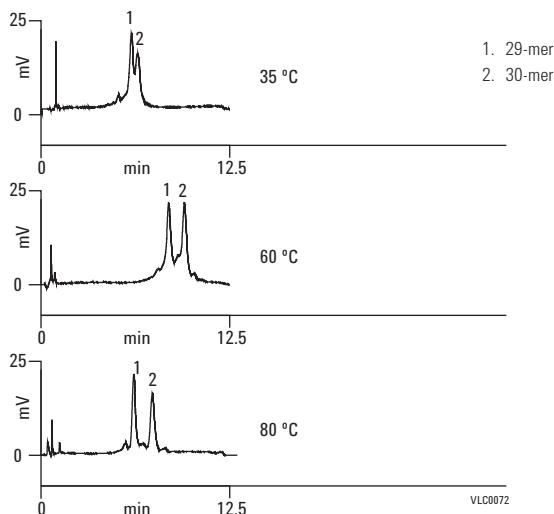
Mobile Phase: A: 100 mM TEAA  
B: 100 mM TEAA in 25% ACN

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



**Hydrophilic purine/pyrimidine separation**

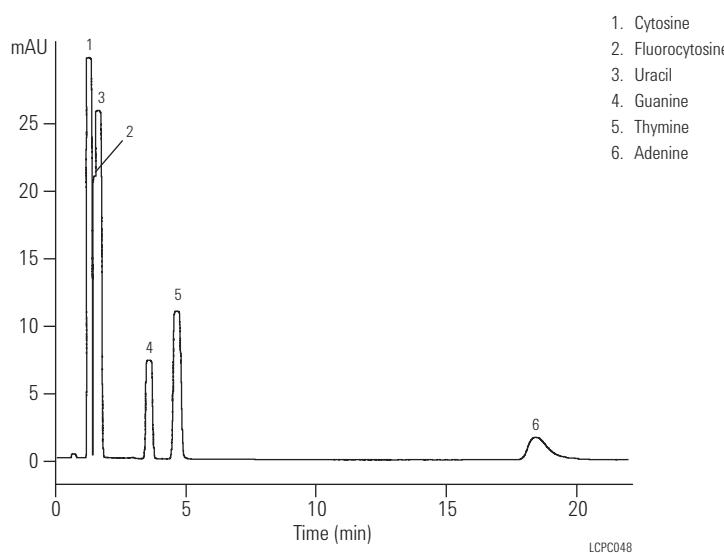
**Column:** ZORBAX SB-Aq  
883975-914  
4.6 x 150 mm, 5 µm

Mobile Phase: 50 mM NaOAc, pH 4.6

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm



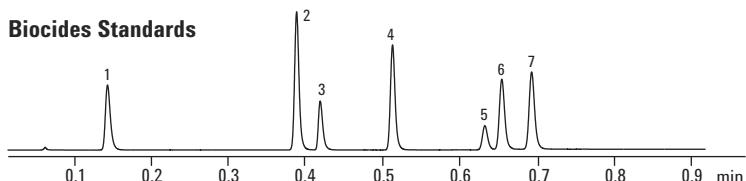
For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at [www.agilent.com/chem/library](http://www.agilent.com/chem/library)

# Chemical/Industrial Applications

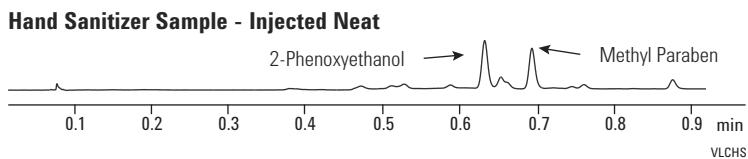
## Analysis of biocides in hand sanitizer

**Column:** ZORBAX RRHD Eclipse Plus C18  
959757-902  
2.1 x 50 mm, 1.8  $\mu$ m

Mobile Phase: A: H<sub>2</sub>O (0.5% TFA)      Gradient: Time 0.0 95/5 A/B      DAD: 275 nm (0 min)  
B: ACN (0.04% TFA)      Time 1.0 55/45 A/B      225 nm (0.46 min)  
Flow Rate: 1.7 mL/min      Time 1.1 0/100 A/B      255 nm (0.67 min)  
Sample: 1  $\mu$ L injection of 50 ppm std.  
Temperature: 30 °C

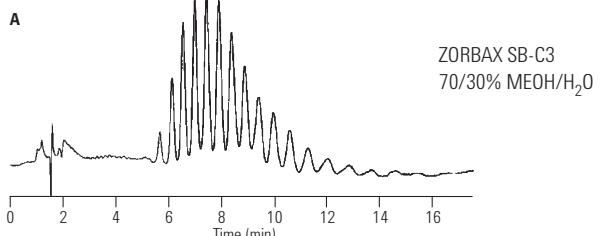


1. Kathon 1A
2. Kathon 1B
3. Carbendazim
4. 1,2-Benzisothiazol-3(2H)-one
5. 2-Phenoxyethanol
6. Benzoic Acid
7. Methyl Paraben

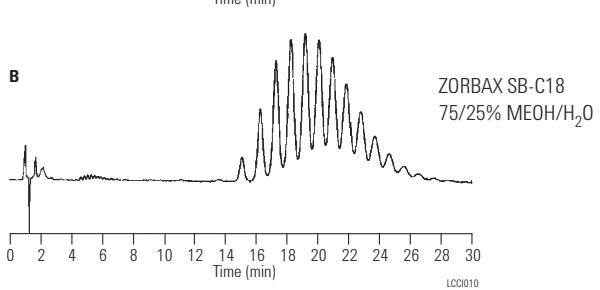


## Triton X-114: Decreasing run-time by changing bonded phase

**Column A:** ZORBAX SB-C3  
883975-909  
4.6 x 150 mm, 5  $\mu$ m



**Column B:** ZORBAX SB-C18  
883975-902  
4.6 x 150 mm, 5  $\mu$ m



**Organic acids separated on ZORBAX SB-Aq****Column:** ZORBAX SB-Aq

883975-914

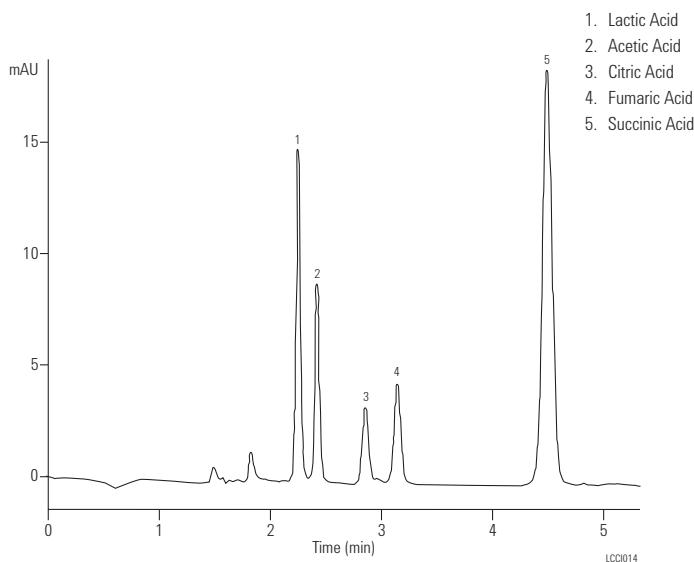
4.6 x 150 mm, 5 µm

Mobile Phase: 99% 20 mM NaH<sub>2</sub>PO<sub>4</sub>, 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

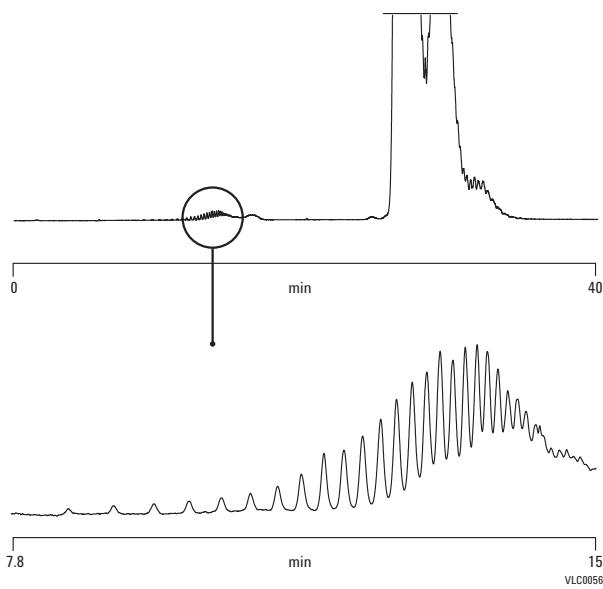
Gradient: 0-100% B in 40 min

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**

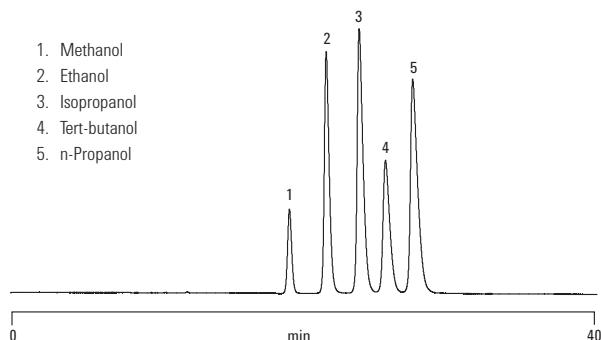
Mobile Phase: Water

Flow Rate: 0.6 mL/min

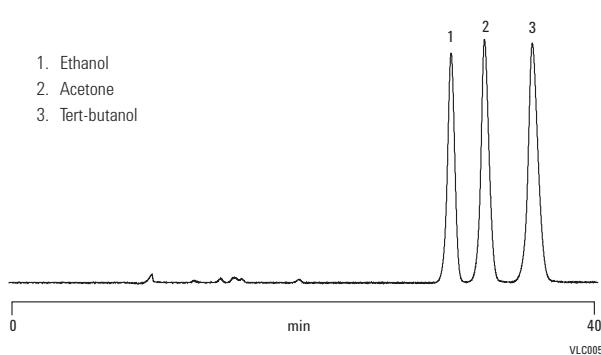
Temperature: 40 °C

Detector: 356-LC RI

1. Methanol
2. Ethanol
3. Isopropanol
4. Tert-butanol
5. n-Propanol



1. Ethanol
2. Acetone
3. Tert-butanol



VLC0055



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

# 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

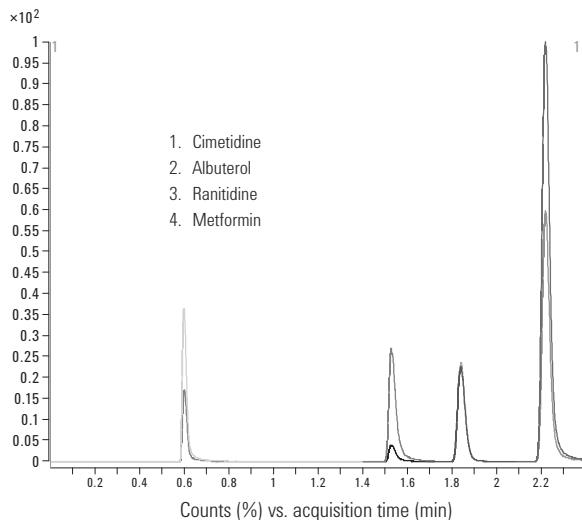
**Mobile Phase:** A: 10 mM ammonium acetate in water, pH 6.7  
B: acetonitrile

**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

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

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

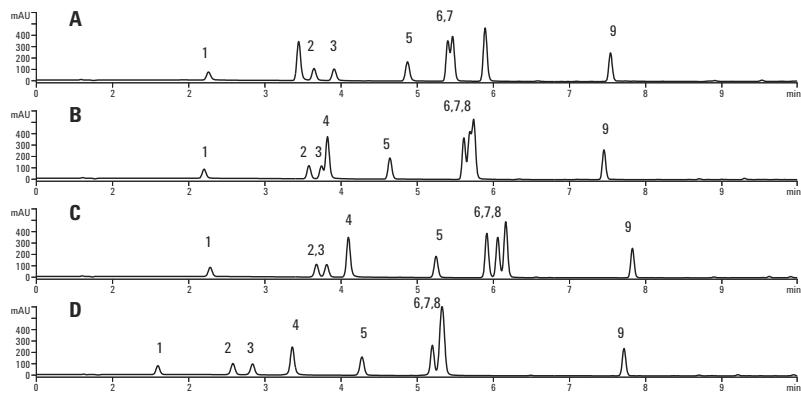
**Column C:** Poroshell 120 Phenyl-Hexyl  
695775-912  
2.1 x 100 mm, 2.7 µm

**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



### 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%

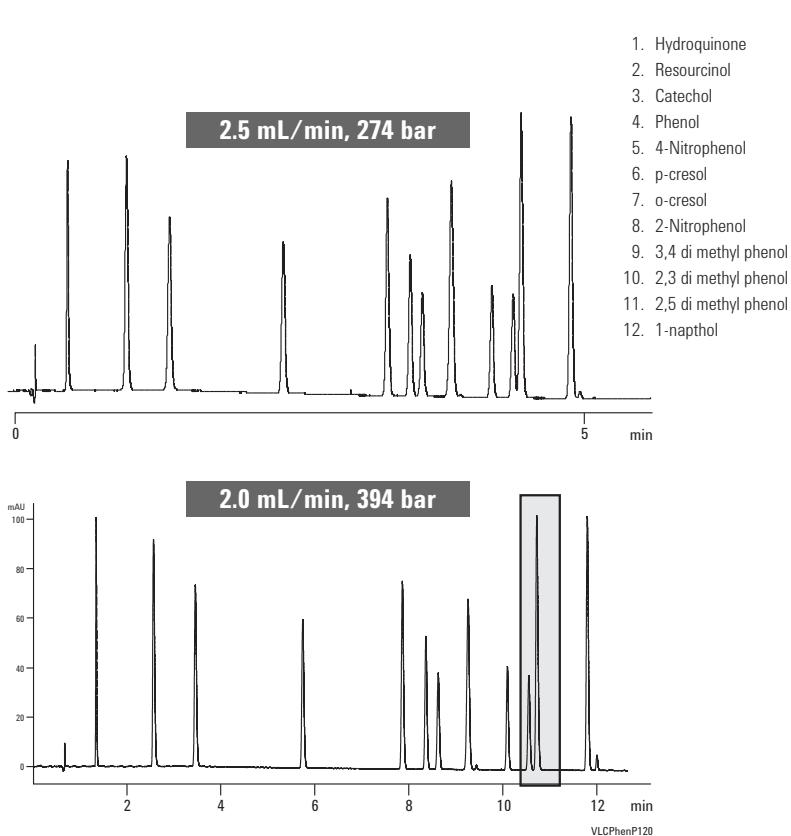
1200 SL controlled temperature at 25 °C 2 mm flow cell

**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



### 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

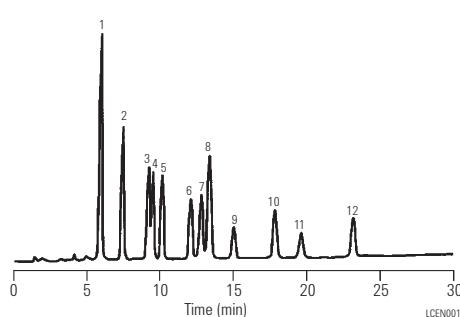
Gradient: 60-75% B in 30 min; Wash: From 75-100% B in 5 min, after 5 min return to 60% B

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. Hexanaldehyde – DNPH



**Amitrol in water by LC/MS, 0.05 ppb**

**Column:** ZORBAX SB-C18  
863954-302  
3.0 x 150 mm, 3.5  $\mu$ m

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

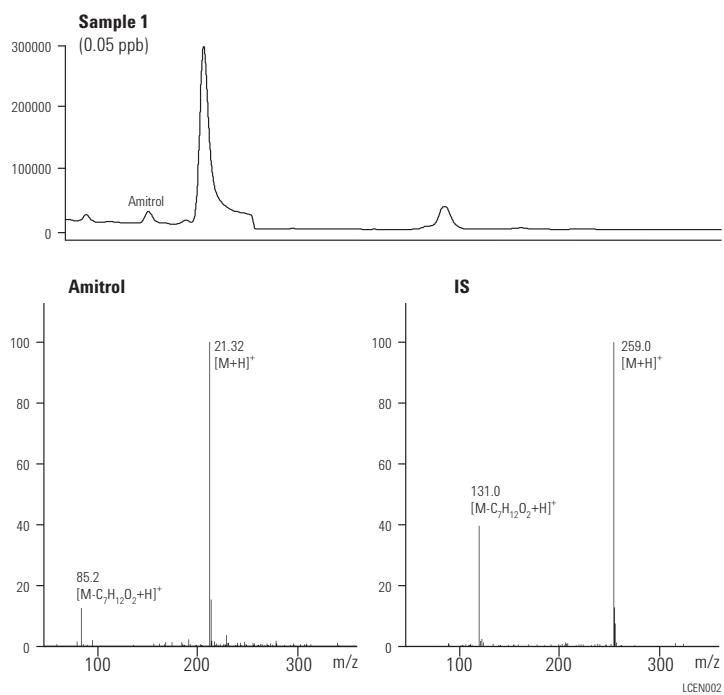
**Flow Rate:** 0.4 mL/min

**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  $\mu$ L

**Anilines, substituted: Rapid separation**

**Column:** ZORBAX Rx/SB-C8  
866953-906  
4.6 x 75 mm, 3.5  $\mu$ m

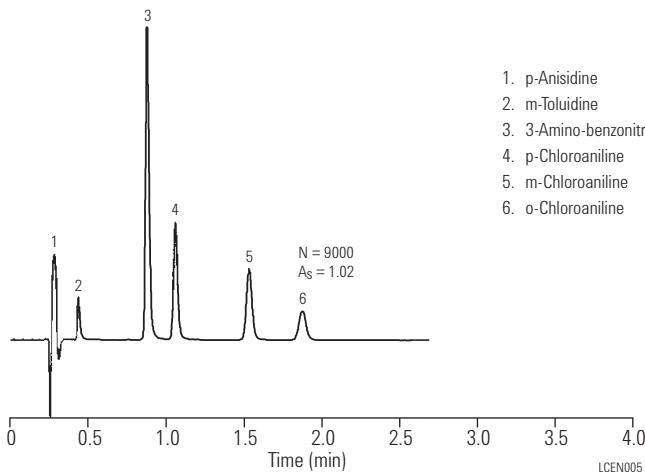
**Mobile Phase:** 20% ACN/80% 25 mM phosphate buffer, pH 2.5

**Flow Rate:** 3.0 mL/min

**Temperature:** 60 °C

**Detector:** UV, 254 nm

**Sample:** Anilines



**Explosives and related compounds:**  
**Qualitative and quantitative analysis**

**Column A:** ZORBAX SB-C18  
883700-922  
2.1 x 150 mm, 5  $\mu$ m

**Column B:** ZORBAX SB-CN  
883700-905  
2.1 x 150 mm, 5  $\mu$ m

Mobile Phase: A = ACN + 5% H<sub>2</sub>O + 5 mM CF<sub>3</sub>COONH<sub>4</sub>  
B = H<sub>2</sub>O + 5% ACN + 5 mM CF<sub>3</sub>COONH<sub>4</sub>,  
pH 2.7 (CF<sub>3</sub>COOH)

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

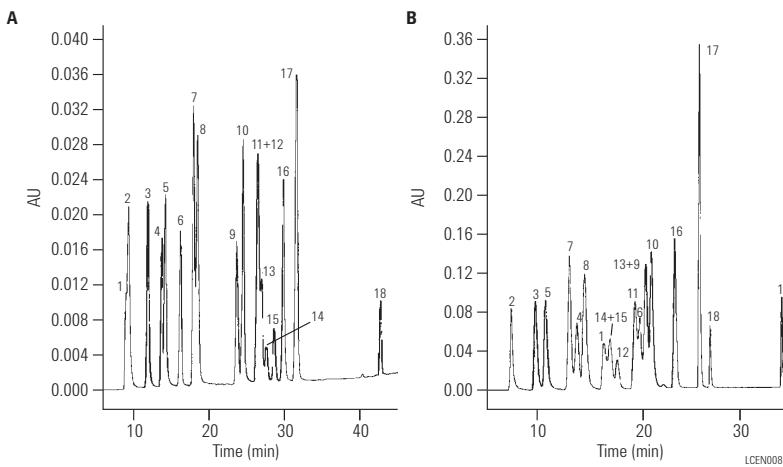
B:  
0 min 80% B  
1 min 80% B  
15 min 70% B  
30 min 20% B  
35 min 20% B  
37 min 80% B

Temperature: 18 °C

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

Sample: 10  $\mu$ L of 19 explosive compounds  
in ACN/H<sub>2</sub>O (20/80)

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



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

**Explosives from soil extract**

**Column:** ZORBAX SB-C18  
880975-302  
3.0 x 250 mm, 5  $\mu$ m

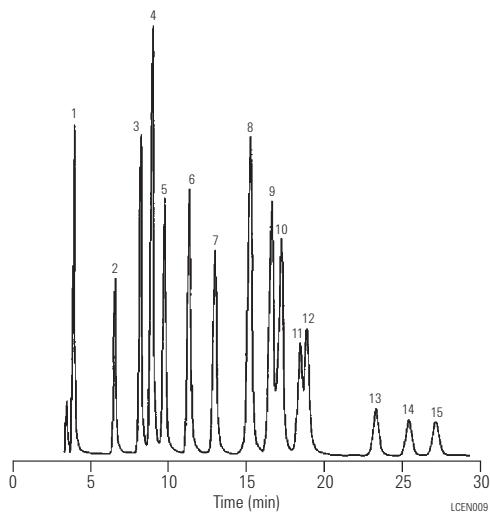
Mobile Phase: Methanol/Water (50/50) (v/v)

Flow Rate: 0.3 mL/min

Temperature: Ambient

Detector: UV, 230 nm

Sample: 10  $\mu$ L 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  $\mu$ m

**Column B:** ZORBAX SB-Phenyl  
883975-912  
4.6 x 150 mm, 5  $\mu$ m

**Column C:** ZORBAX SB-C8  
883975-906  
4.6 x 150 mm, 5  $\mu$ m

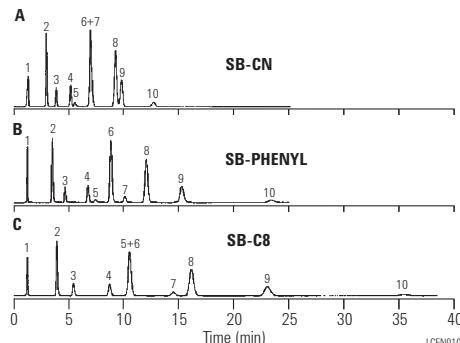
Mobile Phase: 35% ACN, 65% Water

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Herbicides



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

**Herbicide/pesticide standards:****Effect of bonded phase**

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

Mobile Phase: Water/Acetonitrile

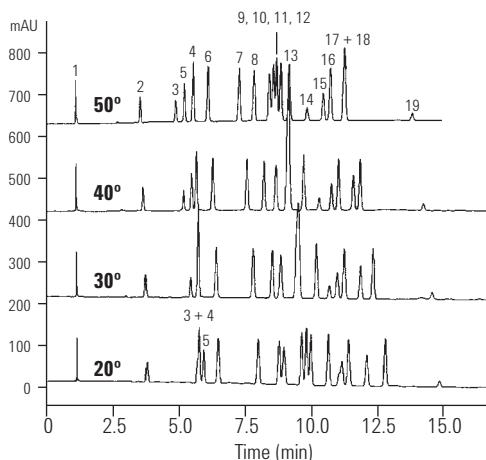
Flow Rate: 1.0 mL/min

Gradient: 20-60% in 15 min

Temperature: 50 °C  
40 °C  
30 °C  
20 °C

Detector: DAD 240

Sample: Herbicide & pesticide standards



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

Mobile Phase: Water/Acetonitrile

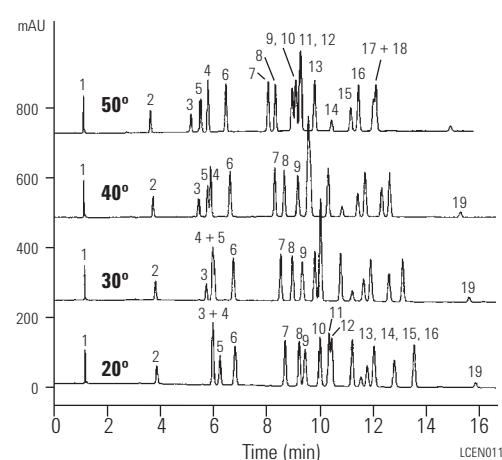
Flow Rate: 1.0 mL/min

Gradient: 20-60% in 15 min

Temperature: 50 °C  
40 °C  
30 °C  
20 °C

Detector: DAD 240

Sample: 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. Monoluronuron
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](http://www.agilent.com/chem/library)

**Separation of EPA 610 PAH Mix**

**Column:** Eclipse PAH  
959990-318  
3.0 x 250 mm, 5  $\mu$ m

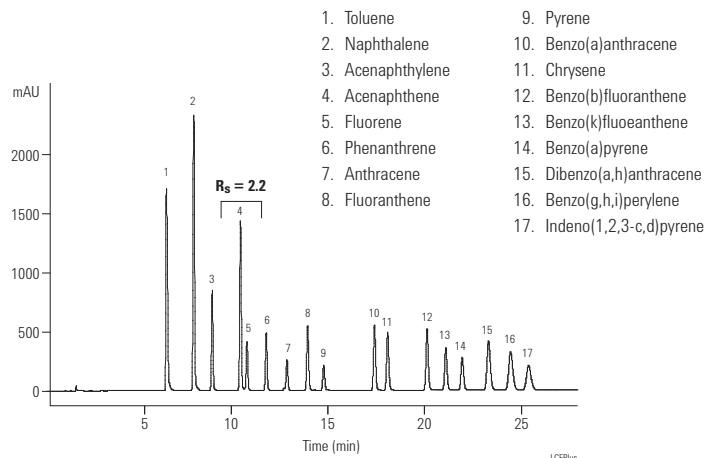
Mobile Phase: A: Water  
B: Acetonitrile  
Initial %B = 40

Flow Rate: 0.85 mL/min

Gradient: Time (Min) %B  
0.00 45  
17.5 100  
24.0 100  
25.5 40  
27.5 40  
Stop Time = 25.0

Temperature: 25 °C

Detector: 220, 4 nm No Ref.; Stop time = 26.0 min

**Polycyclic aromatic hydrocarbons according to EPA Method 610**

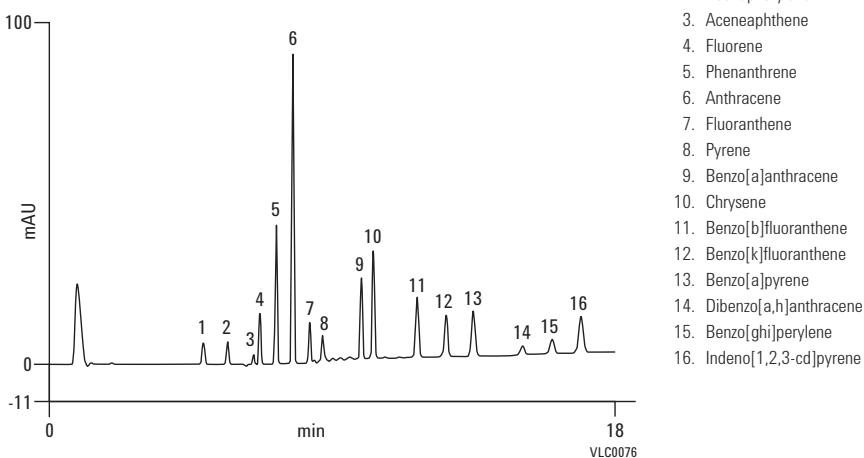
**Column:** Pursuit PAH  
A7001100X046  
4.6 x 100 mm, 3  $\mu$ m

Sample: NIST 16473 Standard

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

Flow Rate: 2.0 mL/min

Detector: UV, 254 nm



**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  $\mu$ m

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

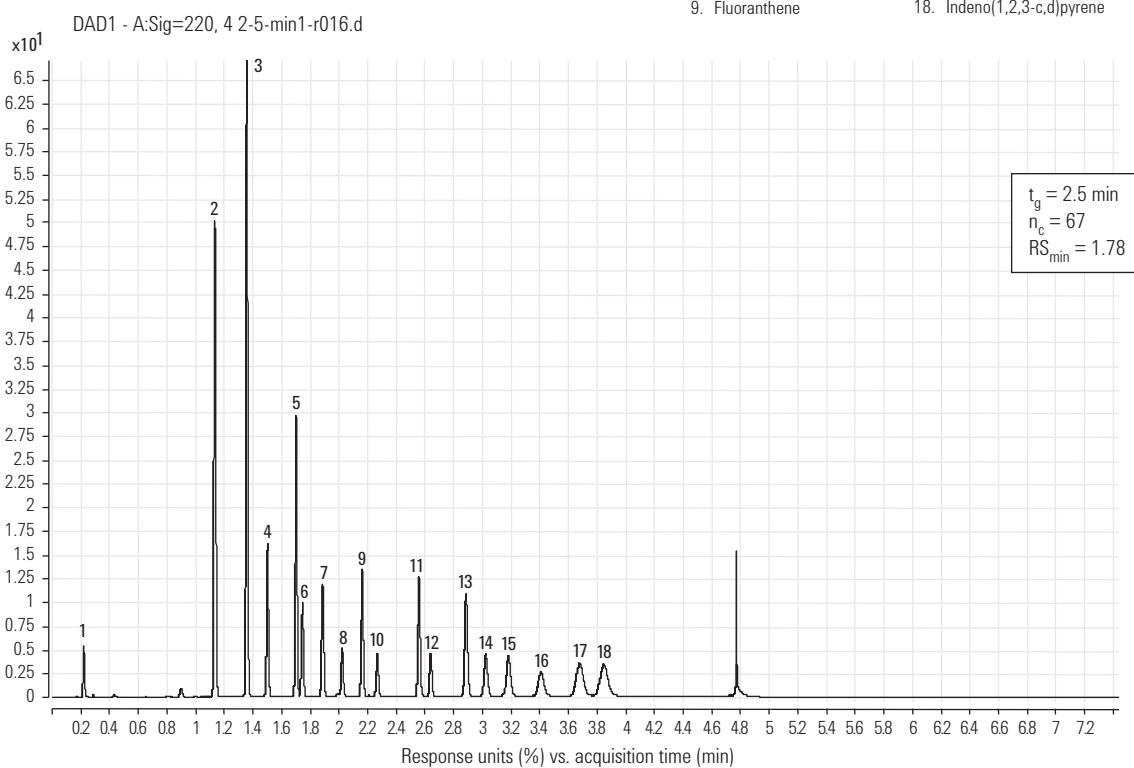
Temperature: 25 °C

Detector: Agilent 1290 Infinity LC

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

Sample: 0.5  $\mu$ L 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. Dibenz(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  $\mu$ m**

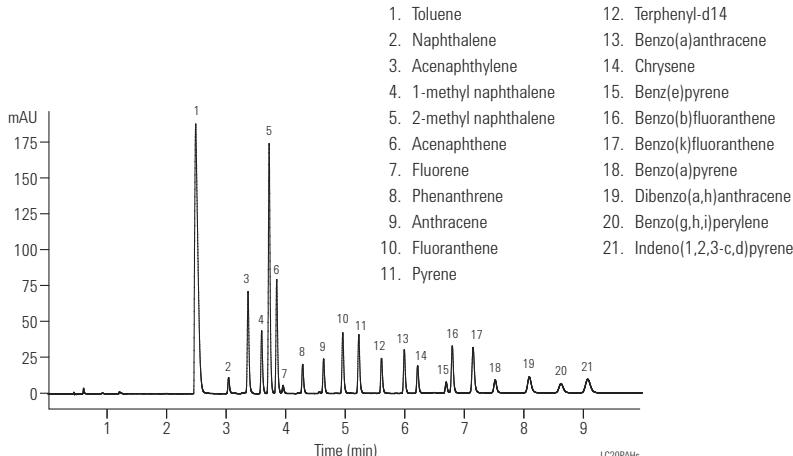
Mobile Phase: A: Water  
B: Acetonitrile

Flow Rate: 1.8 mL/min

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

Temperature: 25 °C

Detector: 230, 8 nm No Ref.; Data rate 0.2 s,  
micro flow cell

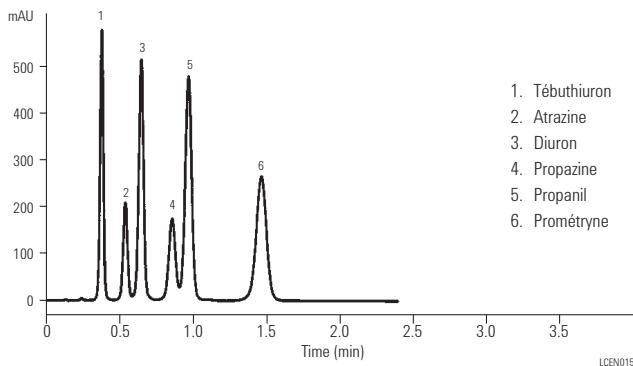
**Herbicides: Rapid separation**

**Column:** Eclipse XDB-C18  
933975-902  
**4.6 x 30 mm, 3.5  $\mu$ m**

Mobile Phase: MeOH:H<sub>2</sub>O (60:40)

Flow Rate: 2 mL/min

Temperature: Ambient

**Phenoxyacid herbicides**

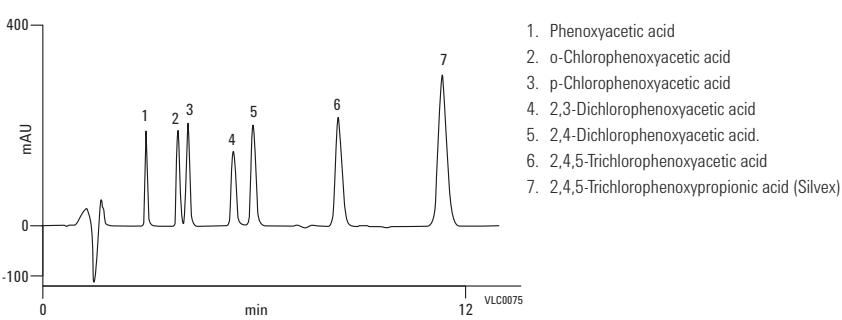
**Column:** Pursuit XR<sub>s</sub> C8  
A6010150X046  
**4.6 x 150 mm, 5  $\mu$ m**

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

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm



### Triazine pesticides on Bonus-RP and Alkyl C8 phase

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

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

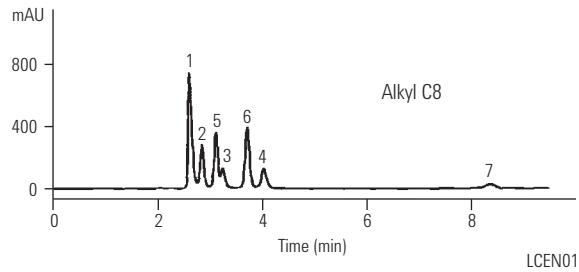
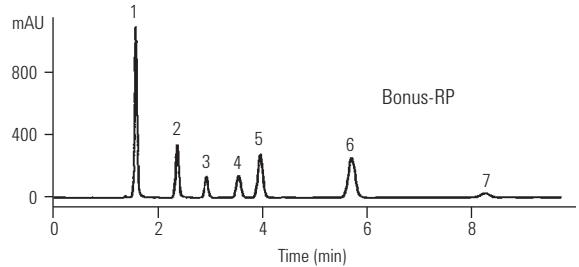
Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: 254 nm

Sample: Triazine pesticides, 2 µL

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



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

### Phenols, substituted

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

Mobile Phase: 20% ACN/80% 0.01 M H<sub>3</sub>PO<sub>4</sub> to 45% ACN in 7.5 min

Flow Rate: 1.5 mL/min

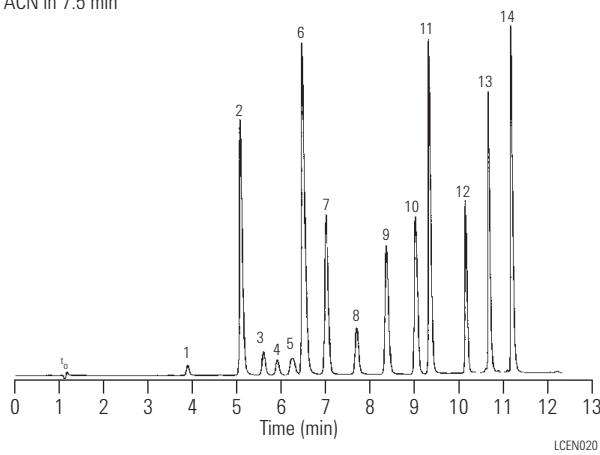
Gradient: 80% ACN in 2.0 min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Phenols

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**

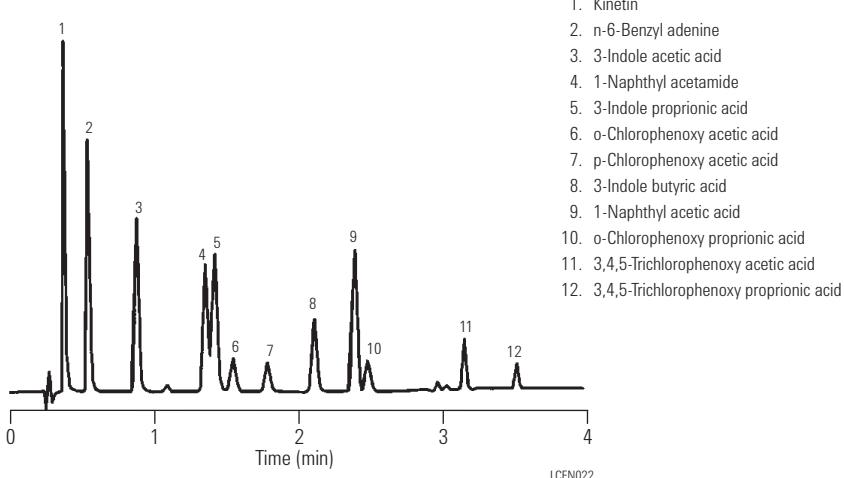
**Mobile Phase:** A: Water with 0.1% TFA  
B: Acetonitrile with 0.1% TFA

**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 propionic acid
6. o-Chlorophenoxy acetic acid
7. p-Chlorophenoxy acetic acid
8. 3-Indole butyric acid
9. 1-Naphthyl acetic acid
10. o-Chlorophenoxy propionic acid
11. 3,4,5-Trichlorophenoxy acetic acid
12. 3,4,5-Trichlorophenoxy propionic acid

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

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

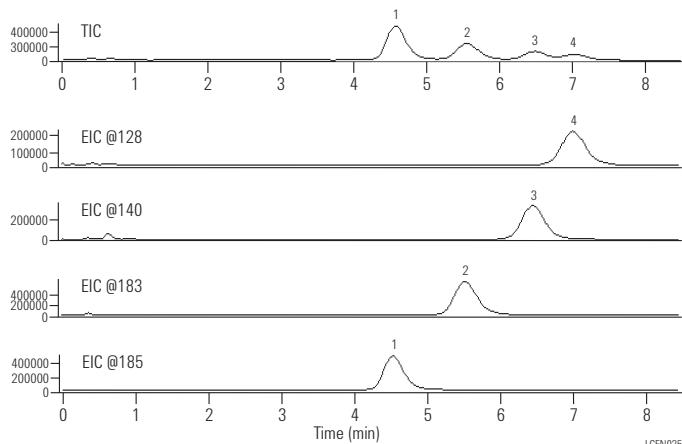
**Mobile Phase:** 1:1 (20 mM Ammonium Acetate pH 4.5/Acetonitrile)

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

**Temperature:** 35 °C

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

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



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

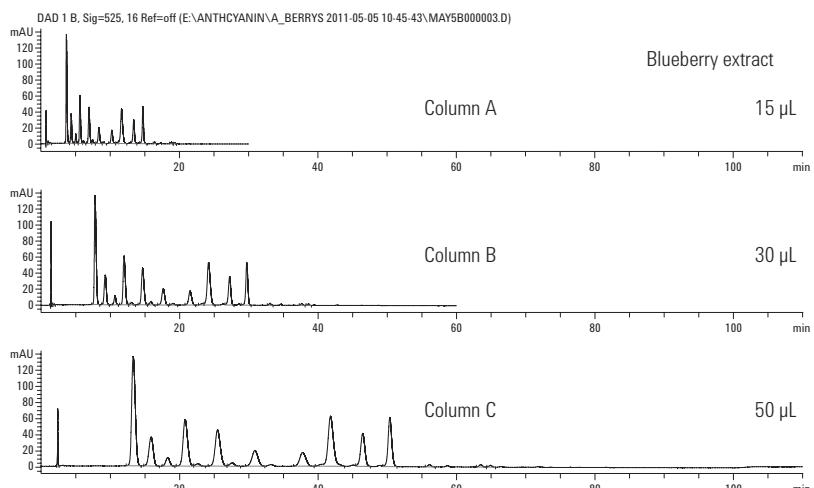
**Column B:** ZORBAX SB-C18  
863953-902  
4.6 x 150 mm, 3.5 µm

**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

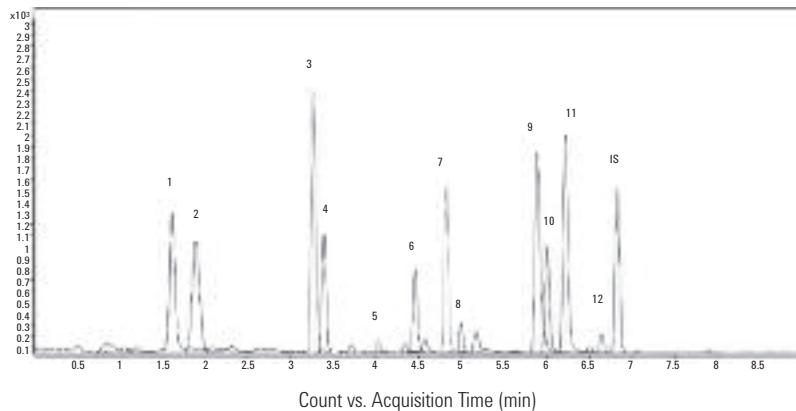
Flow Rate: 0.4 mL/min

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

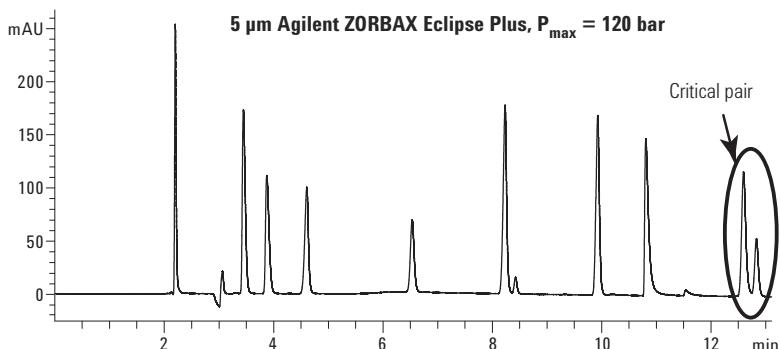
MRM chromatograms of 50 ng/g fortified sample processed by EN method.

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



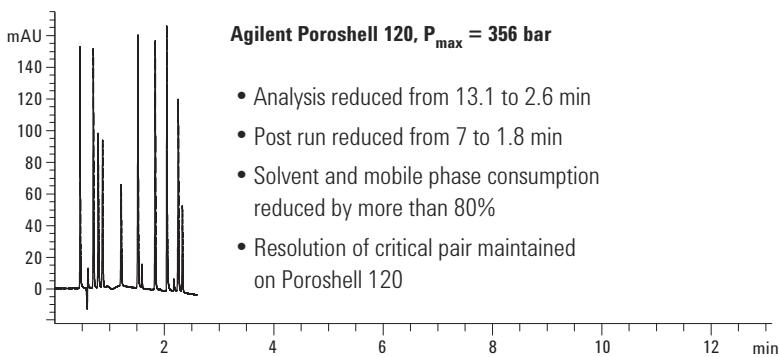
**NEW!**

An overlay of the original ZORBAX Eclipse Plus 5  $\mu\text{m}$  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  $\mu\text{m}$  ZORBAX Eclipse Plus method



**Column:** Eclipse Plus C18  
959990-902  
4.6 x 250 mm, 5  $\mu\text{m}$

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  $\mu\text{m}$

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](http://www.agilent.com/chem/library)

**NEW!****Fast analysis of sulfa drugs**

**Column:** Eclipse Plus C18  
959990-902  
4.6 x 250 mm, 5  $\mu$ m

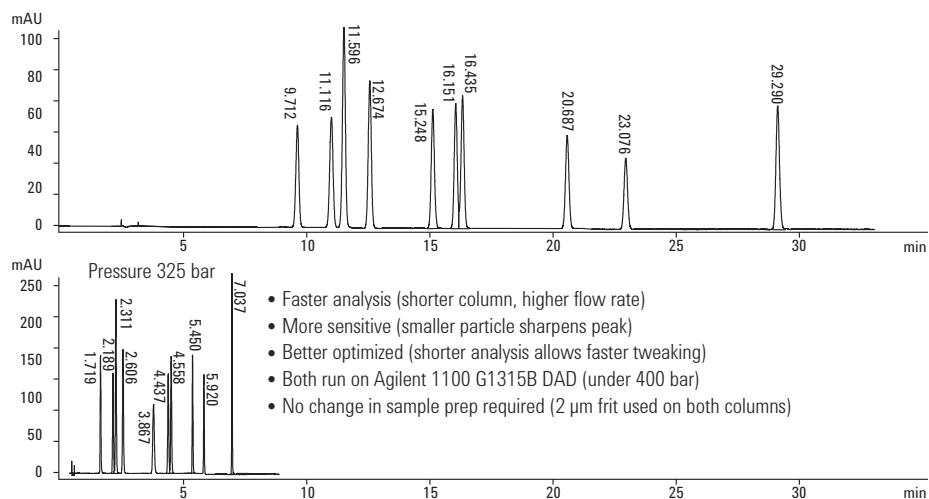
**Column:** Poroshell 120 EC-C18  
695975-902  
4.6 x 100 mm, 2.7  $\mu$ m

**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  $\mu$ m 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](http://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  $\mu$ m

**Column:** ZORBAX RRHD Eclipse Plus Phenyl-Hexyl  
959758-912  
2.1 x 100 mm, 1.8  $\mu$ m

**Column:** ZORBAX RRHD SB-Aq  
858700-914  
2.1 x 100 mm, 1.8  $\mu$ m

**Column:** ZORBAX RRHD SB-Phenyl  
858700-912  
2.1 x 100 mm, 1.8  $\mu$ m

**Mobile Phase:** A: 5% HCOOH in H<sub>2</sub>O  
B: CH<sub>3</sub>CN

**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

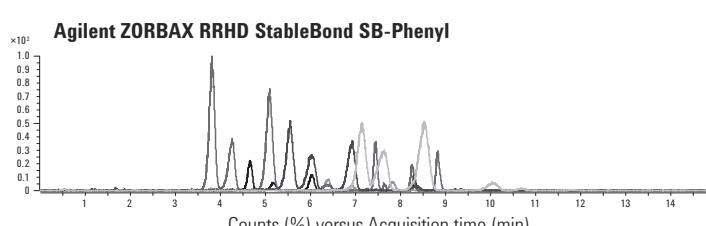
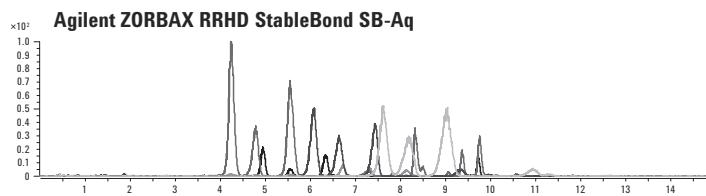
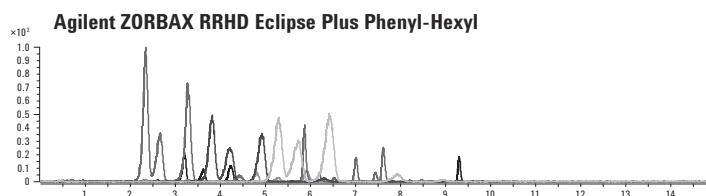
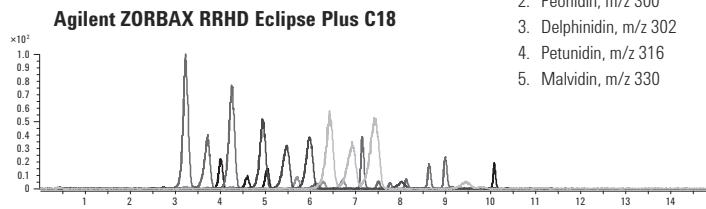
Drying gas: 10 L/min, 350 °C

Nebulizer Pressure: 50 psig

Capillary Voltage: 3500

**Sample:** 5  $\mu$ L injection of blueberry extract

1. Cyanidin, m/z 286
2. Peonidin, m/z 300
3. Delphinidin, m/z 302
4. Petunidin, m/z 316
5. Malvidin, m/z 330



Counts (%) versus Acquisition time (min)

**Separation of Azo Dyes**

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

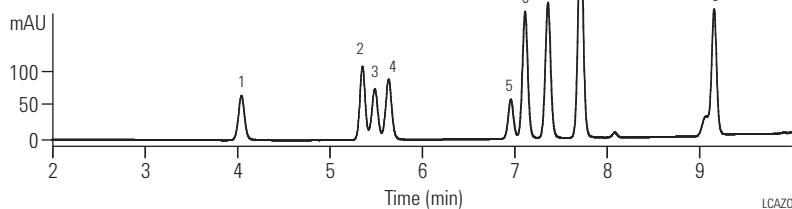
**Mobile Phase:** A: 10 mM Ammonium Acetate, pH 4.7  
B: MeOH

**Flow Rate:** 1.5 mL/min

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

**Detector:** UV, 254 nm

1. Aniline
2. o-Tolidine
3. Anisidine
4. Benzidine
5. Chloroaniline
6. o-Tolidine
7. Dimethoxybenzidine
8. Naphthylamine
9. Dichlorobenzidine



LCAZO

**Anthocyanins from blueberries:  
High-efficiency high-speed separation**

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

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

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

**Flow Rate:** 1.0 mL/min

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

**Gradient:** As shown

**Temperature:** 30 °C

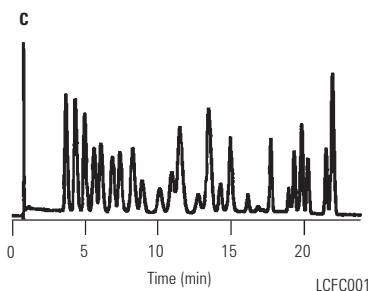
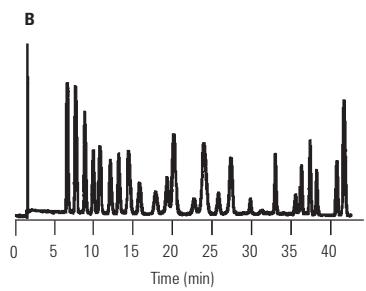
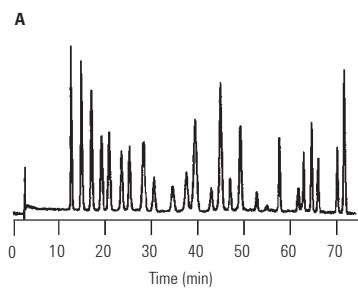
**Detector:** UV, 525 nm

**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



LCFC001

**Aromatics II**

**Column:** Eclipse XDB-Phenyl  
963967-912  
4.6 x 150 mm, 3.5  $\mu$ m

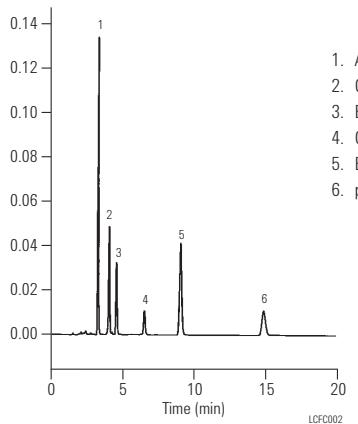
Mobile Phase: H<sub>2</sub>O: MeOH, 40:60

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Aromatic Sample

**Aspartame: Metabolites and applications**

**Column:** ZORBAX SB-C18  
866953-902  
4.6 x 75 mm, 3.5  $\mu$ m

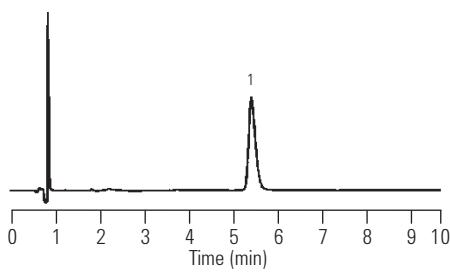
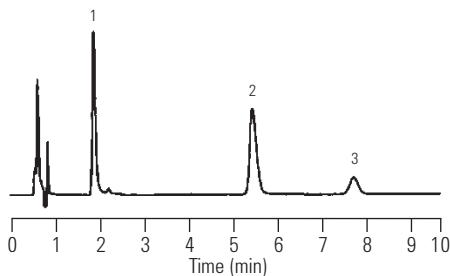
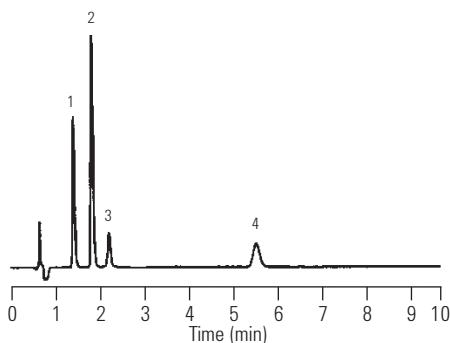
Mobile Phase: 85/15, 0.1% TFA/ACN

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 210 nm

Sample: Aspartame

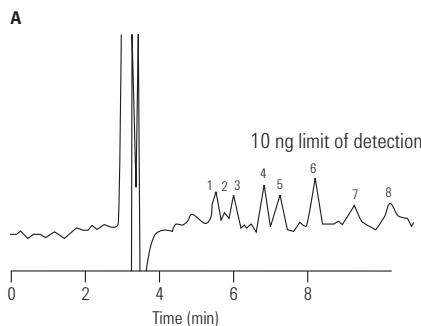


**Carbohydrates: Carbohydrate standards**

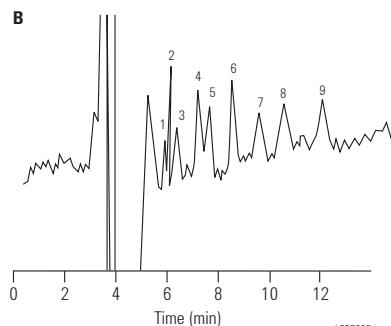
**Column:** ZORBAX Carbohydrate Analysis  
843300-908  
4.6 x 150 mm, 5  $\mu$ m

Mobile Phase: 63% CH<sub>3</sub>CN/H<sub>2</sub>O  
Flow Rate: 0.5 mL/min

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

**Carbohydrates: Separation showing high sensitivity**

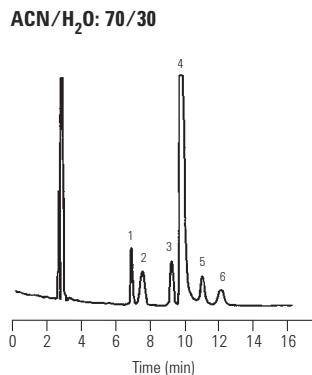
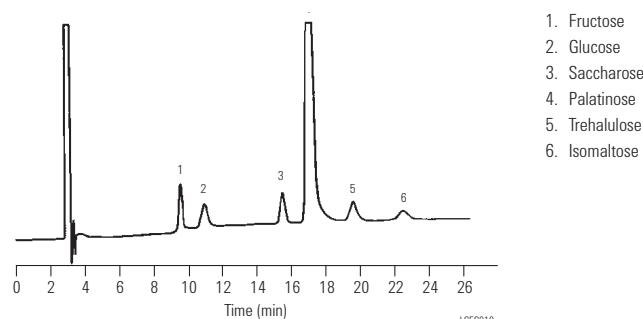
1. Ribose
2. Rhamnose
3. Xylose
4. Fructose
5. Glucose
6. Sucrose
7. Maltose
8. Lactose
9. Raffinose

**Sensitivity of high injection volume (50  $\mu$ L)****Carbohydrates: Effect of mobile phase strength**

**Column:** ZORBAX NH<sub>2</sub>  
880952-708  
4.6 x 250 mm, 5  $\mu$ m

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

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

**ACN/H<sub>2</sub>O: 75/25**

**Carbohydrates in colas**

**Column:** ZORBAX Carbohydrate Analysis  
843300-908  
4.6 x 150 mm, 5  $\mu$ m

Mobile Phase: 75% ACN:25% H<sub>2</sub>O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

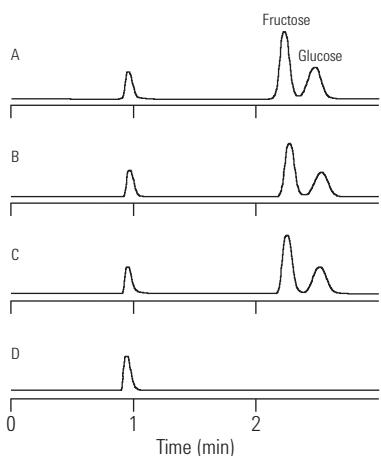
Sample: No dilution

A: COLA, Fountain

B: COLA, Can, Brand A

C: COLA, Brand B

D: COLA, Brand B, diet



LCFC013

**Carbohydrates: Sugar alcohols**

**Column:** ZORBAX Carbohydrate Analysis  
843300-908  
4.6 x 150 mm, 5  $\mu$ m

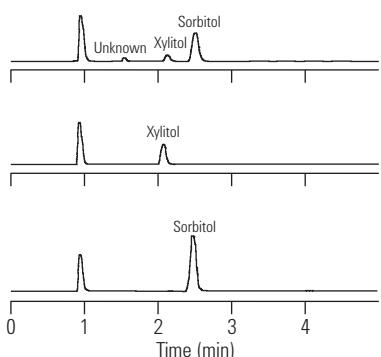
Mobile Phase: 75% ACN:25% H<sub>2</sub>O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Chewing gum, sugar-free



LCFC014

**Carbohydrates in juices**

**Column:** ZORBAX Carbohydrate Analysis  
843300-908  
4.6 x 150 mm, 5  $\mu$ m

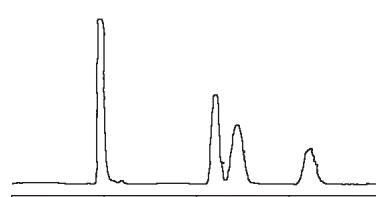
Mobile Phase: 75% ACN/25% H<sub>2</sub>O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Diluted to 0.1X in 50:50 ACN:H<sub>2</sub>O

**Apple Drink**

36.8% Fructose

24.9% Sucrose

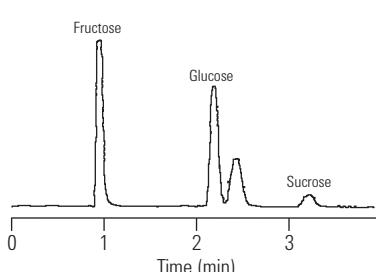
38.3% Glucose

**Apple Juice**

58.7% Fructose

9.9% Sucrose

33.4% Glucose



LCFC016

**Carbohydrates in milk**

**Column:** ZORBAX Carbohydrate Analysis  
843300-908  
4.6 x 150 mm, 5  $\mu$ m

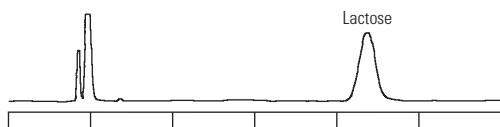
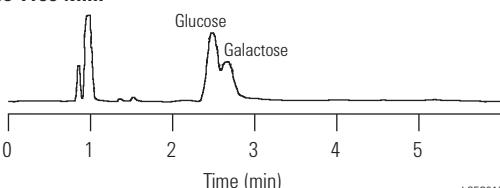
Mobile Phase: 75% ACN/25% H<sub>2</sub>O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Partitioned between CH<sub>3</sub>Cl<sub>2</sub>: H<sub>2</sub>O

**Milk (2%)****100% Lactose-Free Milk**

Time (min)

LCFC015

**Flavoring agents**

**Column:** ZORBAX SB-Phenyl  
860975-912  
2.1 x 50 mm, 5  $\mu$ m

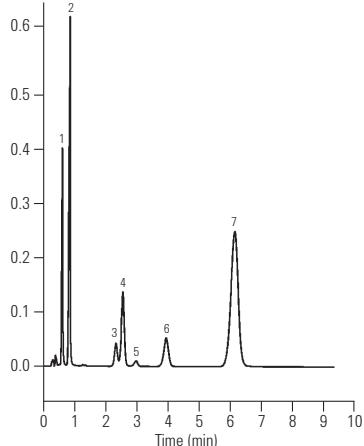
Mobile Phase: 0.3% TFA: ACN, 65:35

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

LCFC006

**Food colors, FD&C**

**Column:** ZORBAX Eclipse XDB-C18  
935967-902  
4.6 x 50 mm, 3.5  $\mu$ m

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

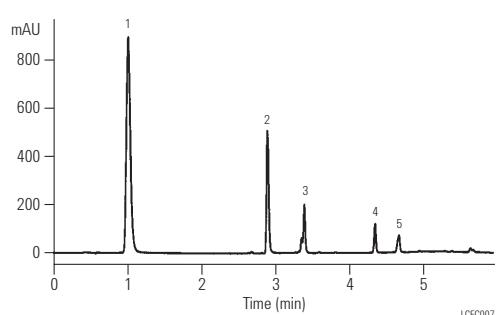
Flow Rate: 1.0 mL/min

Gradient: 17 to 100% B/4 min

Temperature: Ambient

Detector: UV, 254 nm

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

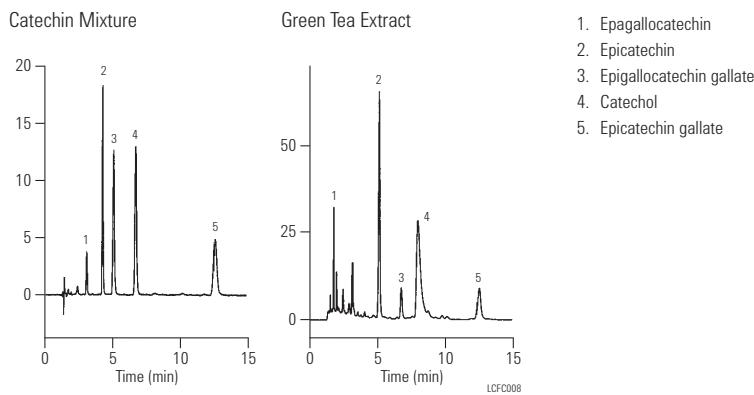


LCFC007

**Neutraceuticals: Extract from green tea**

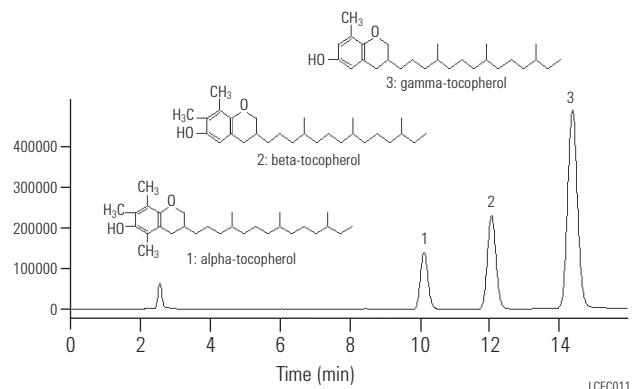
**Column:** ZORBAX SB-C8  
863953-906  
**4.6 x 150 mm, 3.5  $\mu$ m**

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  $\mu$ L

**Tocopherols by LC/MS with APPI**

**Column:** Eclipse XDB-C18  
993967-302  
**3.0 x 150 mm, 5  $\mu$ m**

Mobile Phase: 97% MeOH: 3% 10 mM  $\text{CH}_3\text{COONH}_4$   
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  $\mu$ L



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

**Sugars in plain and milk chocolate**

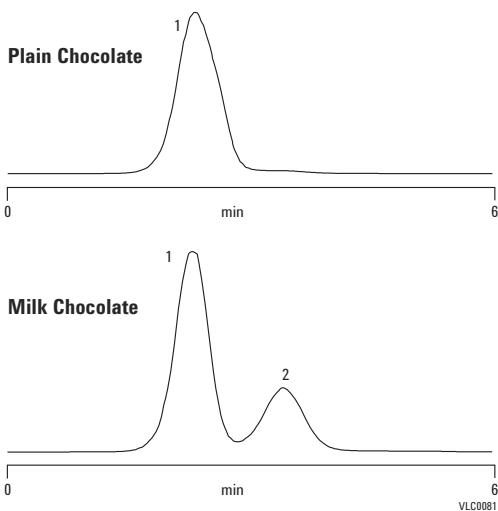
**Column:** Hi-Plex Pb  
PL1170-6820  
7.7 x 300 mm, 8  $\mu\text{m}$

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  $\mu\text{m}$

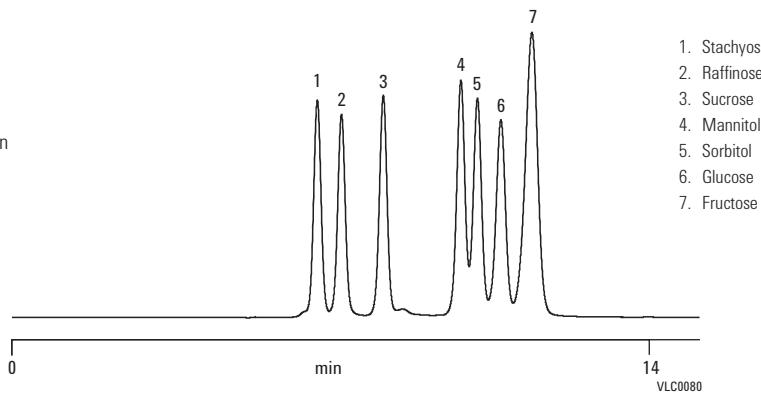
Sample: Sugars mixture (all 10 mg/mL), 20  $\mu\text{L}$  injection

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  $\mu\text{m}$

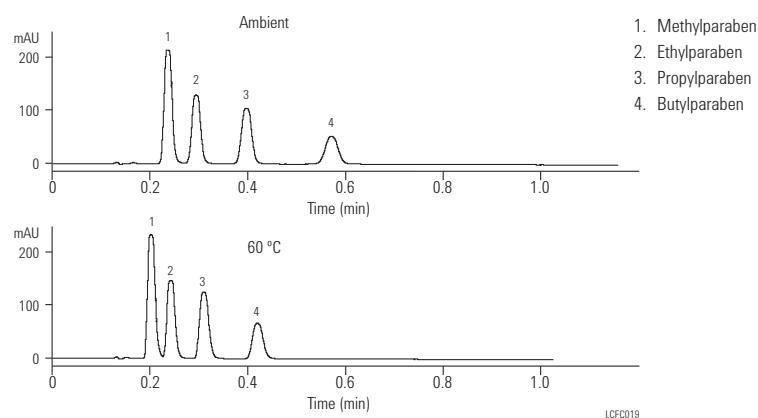
Mobile Phase: 0.1%  $\text{H}_3\text{PO}_4$ :ACN, (50:50)

Flow Rate: 2 mL/min

Temperature: Top: ambient, bottom: 60 °C

Detector: UV, 254 nm with standard flow cell (13  $\mu\text{L}$ )

Sample: Parabens, 1  $\mu\text{L}$

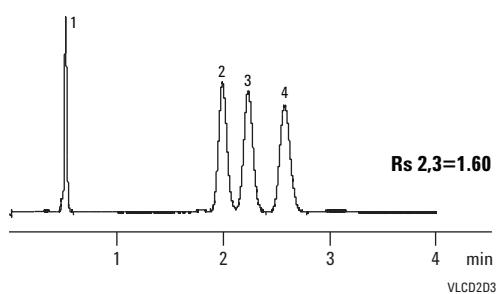


1. Methylparaben
2. Ethylparaben
3. Propylparaben
4. Butylparaben

**Separation of vitamin D<sub>2</sub>/D<sub>3</sub>**

**Column:** Eclipse PAH  
959941-918  
**4.6 x 50 mm, 1.8  $\mu$ m**

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

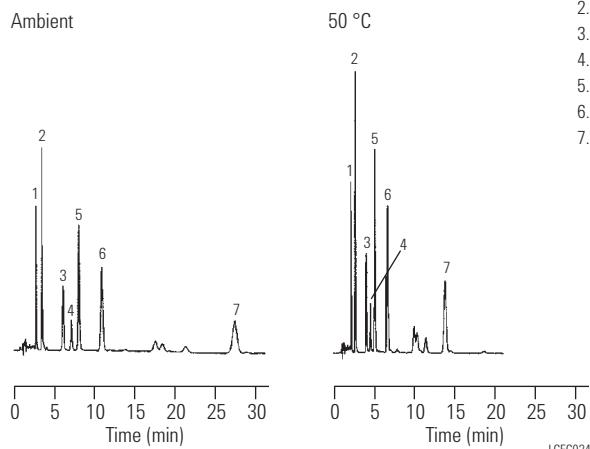


1. Vitamin A
2. Vitamin D2
3. Vitamin D3
4. Vitamin E (a-VE)

**Fat-soluble vitamins on ZORBAX Eclipse XDB-C8**

**Column:** Eclipse XDB-C8  
993967-906  
**4.6 x 150 mm, 5  $\mu$ m**

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

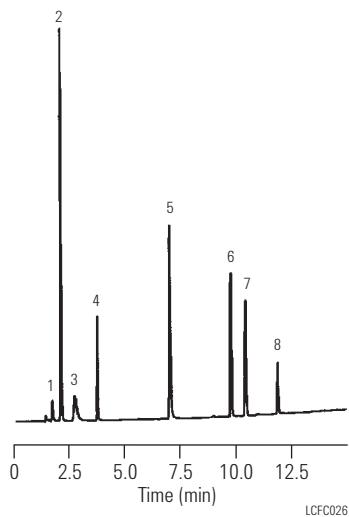


1. Retinol
2. Retinol acetate
3. Vitamin D3
4.  $\gamma$ -Tocopherol
5.  $\alpha$ -Tocopherol
6. Tocopherol acetate
7. Retinol palmitate

**Water-soluble vitamins**

**Column:** ZORBAX SB-C8  
883975-906  
**4.6 x 150 mm, 5  $\mu$ m**

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)

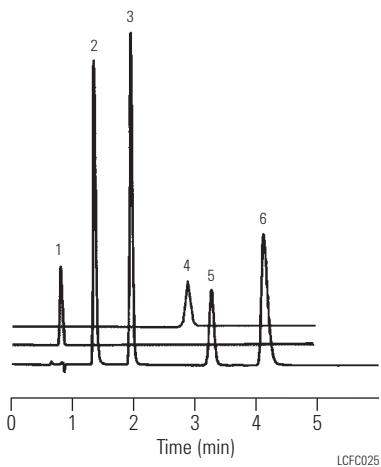
Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 245 nm

Sample: Water-soluble vitamins

1. Vitamin C
2. B<sub>3</sub>-Niacin
3. B<sub>6</sub>-Pyridoxine
4. Folic acid
5. B<sub>2</sub>-Riboflavin
6. B<sub>1</sub>-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)

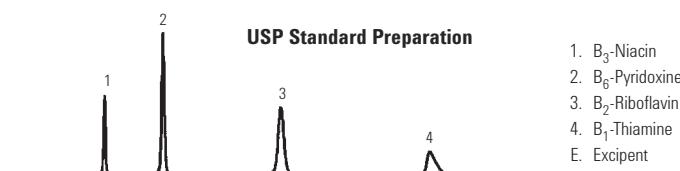
Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: UV, 280 nm

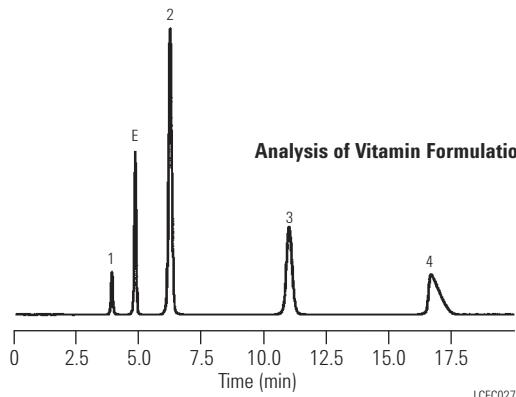
Sample: Water-soluble vitamins

**USP Standard Preparation**



1. B<sub>3</sub>-Niacin
2. B<sub>6</sub>-Pyridoxine
3. B<sub>2</sub>-Riboflavin
4. B<sub>1</sub>-Thiamine
- E. Excipient

**Analysis of Vitamin Formulation**



LCFC027

**Water-soluble B vitamins  
separated on ZORBAX SB-Aq**

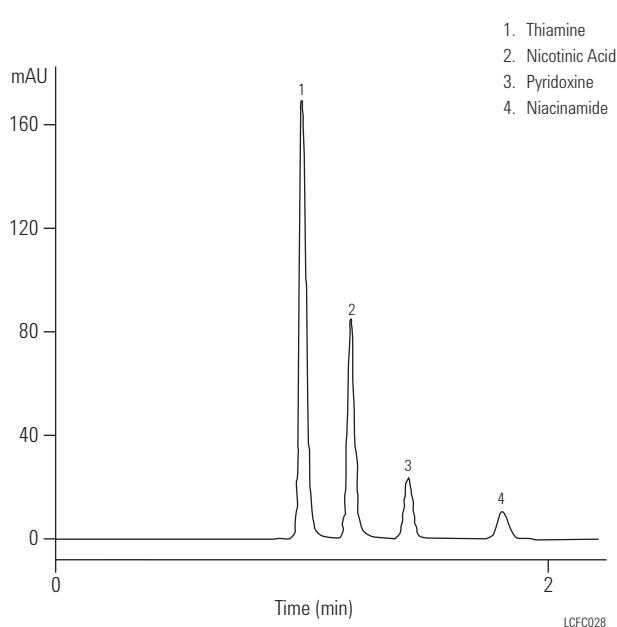
**Column:** ZORBAX SB-Aq  
883975-914  
**4.6 x 150 mm, 5 µm**

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

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm



**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

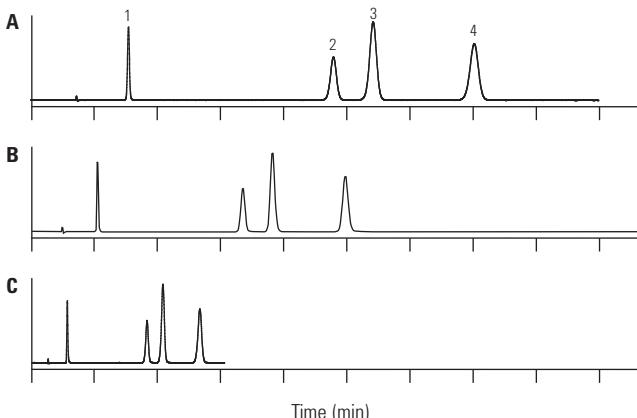
Flow Rate: 1.0 mL/min

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**

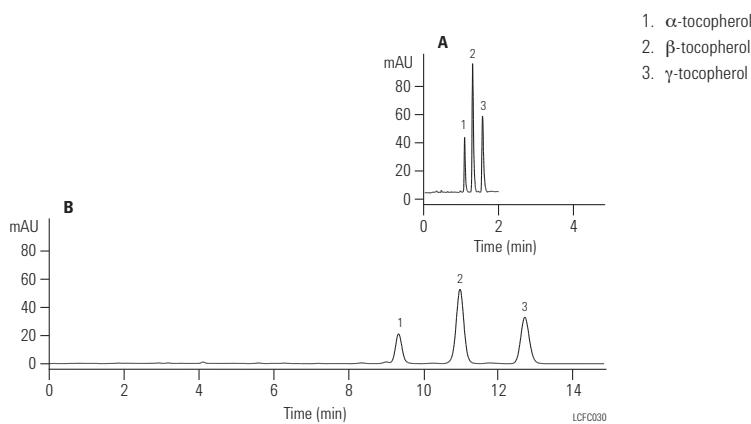
**Column A:** Eclipse XDB-C18  
927975-902  
4.6 x 50 mm, 1.8  $\mu$ m

**Column B:** Eclipse XDB-C18  
993967-902  
4.6 x 150 mm, 5  $\mu$ m

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

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

Temperature: Ambient

**Theobromine in beverages**

**Column:** ZORBAX SB-C18  
827975-902  
4.6 x 50 mm, 1.8  $\mu$ m

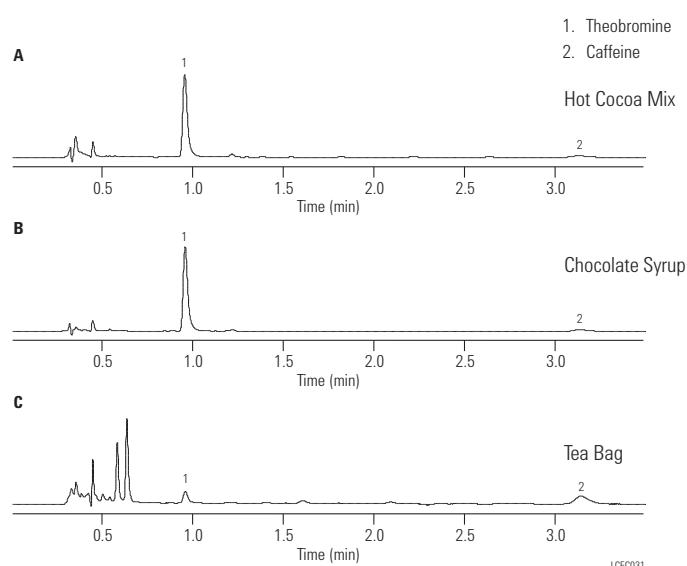
Mobile Phase: A: 92% 0.1% formic acid  
B: 8% 0.1% formic acid in ACN

Flow Rate: 1.5 mL/min

Temperature: Ambient

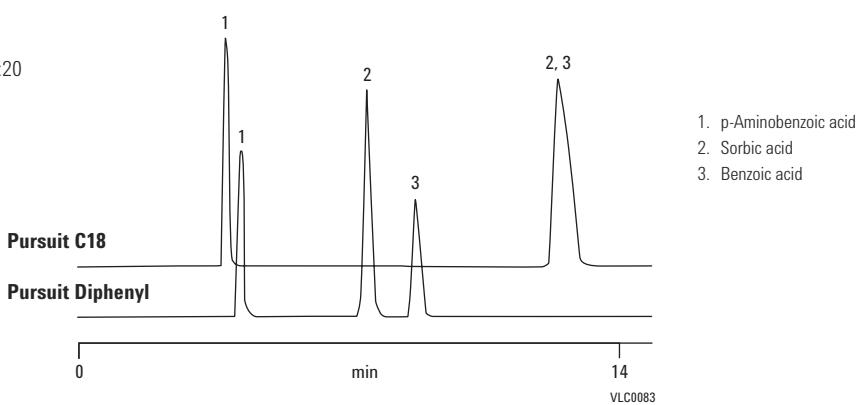
Detector: UV, 254 nm, flow cell 2  $\mu$ L,  
3 mm flow path

Sample: Theobromine



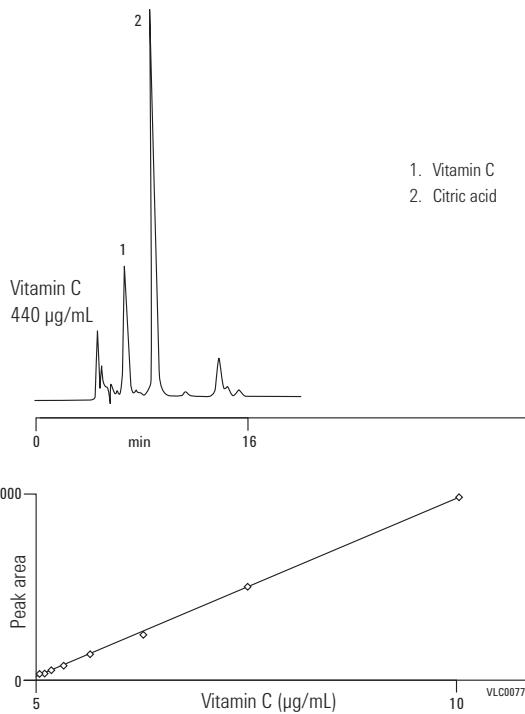
**Benzoic acid/sorbic acid**

Mobile Phase: 0.1% formic acid in water;  
0.1% formic acid in MeCN, 80:20  
Flow Rate: 0.7 mL/min  
Detector: UV, 254 nm

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

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

Sample: Diluted 1:50 in eluent  
Mobile Phase: 0.2M NaH<sub>2</sub>PO<sub>4</sub>, pH 2.14  
Flow Rate: 0.5 mL/min  
Detector: UV, 220 nm



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

**Rose wine**

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

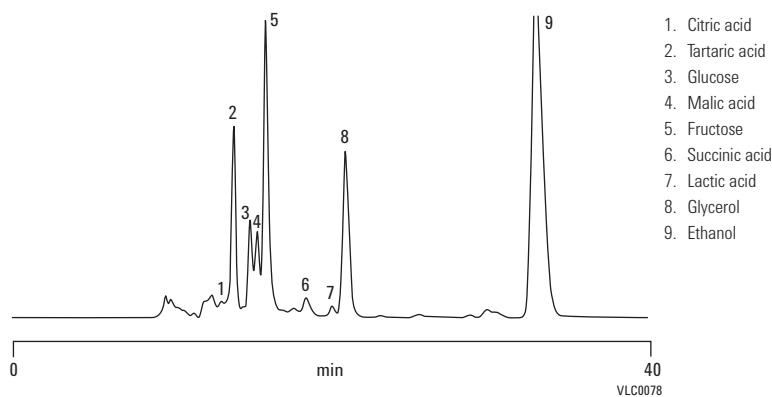
Mobile Phase: 0.004M H<sub>2</sub>SO<sub>4</sub>

Flow Rate: 0.4 mL/min

Pressure: 13 bar

Temperature: 75 °C

Detector: RI

**Sports drink**

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

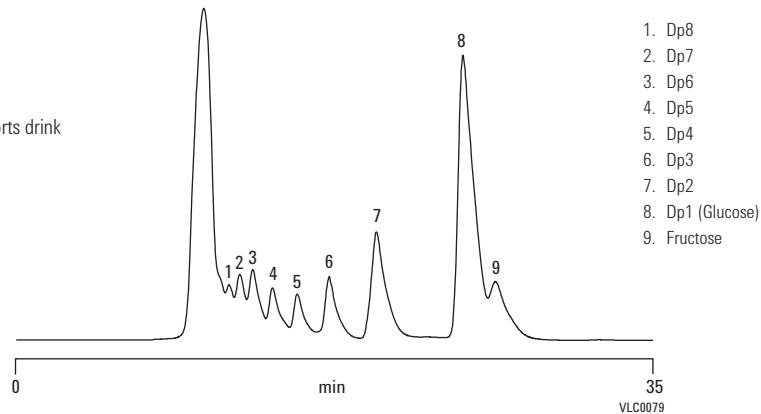
Sample: High energy orange flavor non-carbonated sports drink

Mobile Phase: Water

Flow Rate: 0.3 mL/min

Temperature: 80 °C

Detector: RI

**Oligosaccharides**

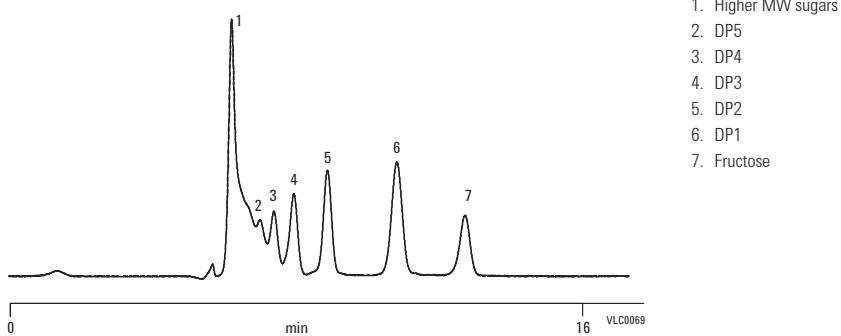
**Column:** Hi-Plex Ca (Duo)  
**PL1F70-6850**  
**6.5 x 300 mm, 8 µm**

Mobile Phase: DI water

Flow Rate: 0.5 mL/min

Temperature: 90 °C

Detector: RI



## Pharmaceutical Applications

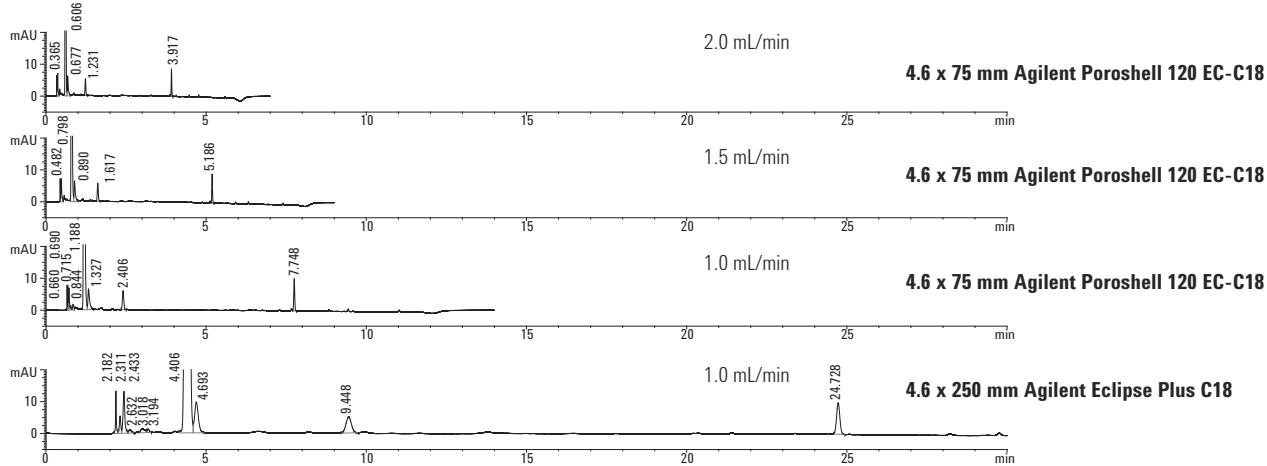
NEW!

## 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  $\mu$ m

Detector: Agilent 1200 Infinity Series



**NEW!**

## Naproxen analysis

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

Method requirement N > 4000 Bs better than 11.5

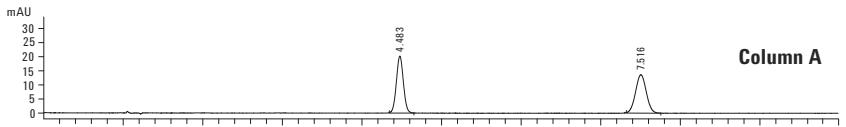
**Column B:** Poroshell 120 EC-C18  
699975-902  
4.6 x 50 mm, 2.7  $\mu$ m

Mobile Phase: 50:49:1 MeCN:H<sub>2</sub>O:Glacial acetic acid

Flow Rate: 1.2 mL/min

Injection: Column A: 20  $\mu$ L  
Column B: 6  $\mu$ L

Injection: Naproxen



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

**NEW!**

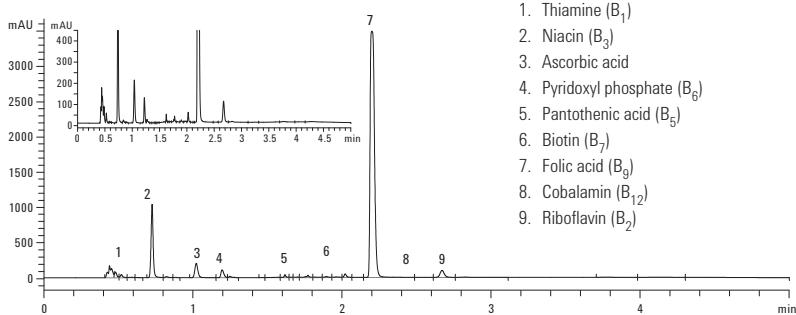
### Analysis of water soluble vitamins in multivitamin tablets

**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

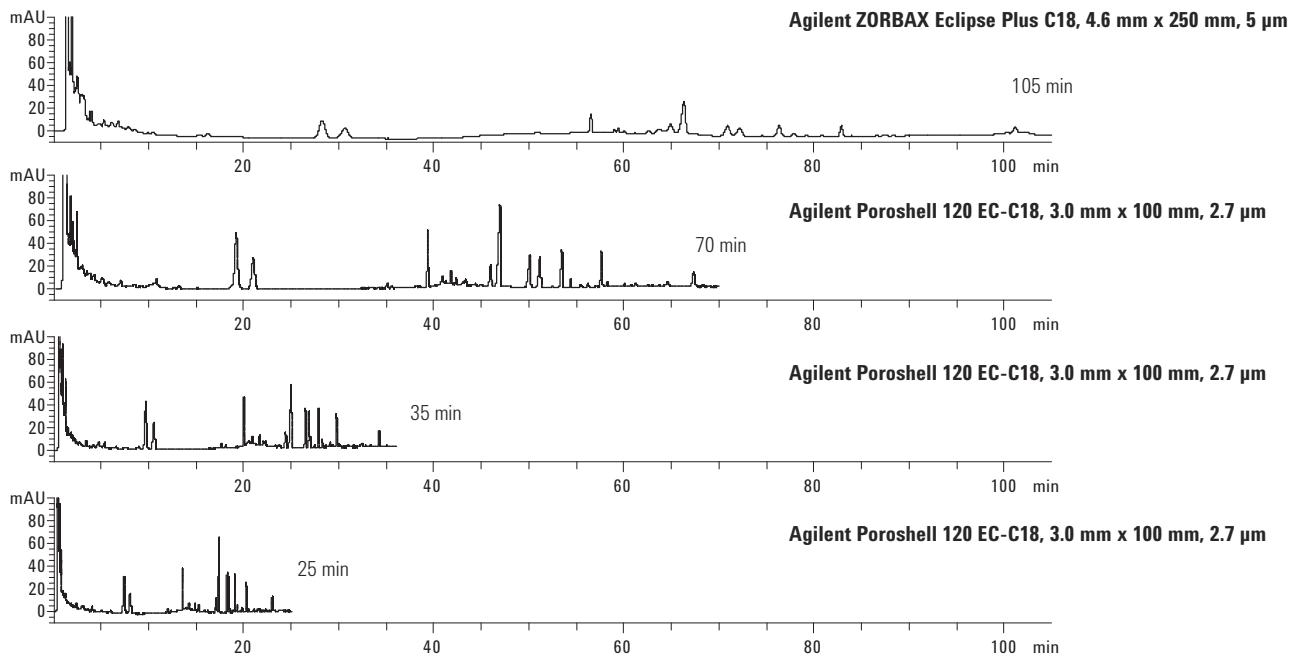
**NEW!**

### 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



**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  
695775-902  
2.1 x 100 mm, 2.7 µm

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

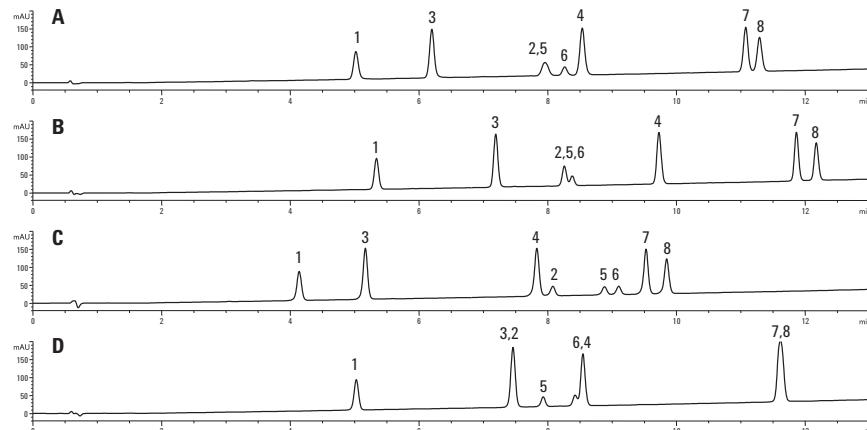
**Column D:** Poroshell 120 Bonus RP  
695775-901  
2.1 x 100 mm, 2.7 µm

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

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

Gradient: 40-80% MeOH in 14 min

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

**NEW!****Mixture of beta blockers**

**Column A:** Poroshell 120 Bonus RP  
695775-901  
2.1 x 100 mm, 2.7 µm

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

**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

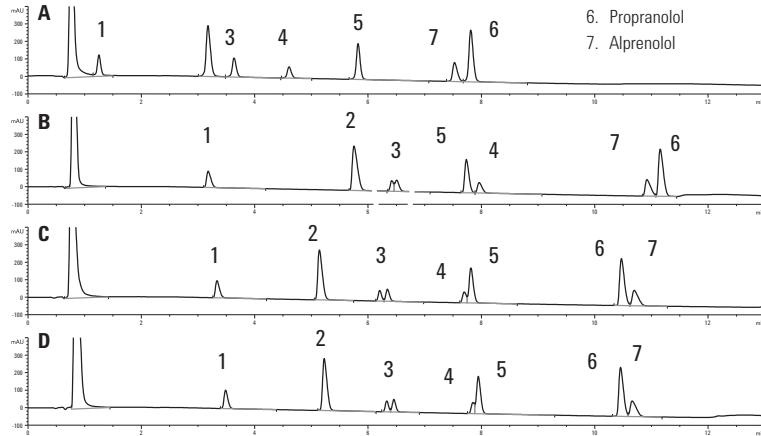
**Column D:** Poroshell 120 SB-C18  
695775-902  
2.1 x 100 mm, 2.7 µm

Mobile Phase: 10 mM pH 3.8 NH<sub>4</sub>HCO<sub>3</sub>, methanol

Flow Rate: 0.35 mL/min

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

\* Nadolol is isobaric and elutes as two peaks.



**NEW!**

**Several ZORBAX RRHD 1.8  $\mu$ m selectivities facilitate method development**

**Column:** ZORBAX RRHD Eclipse Plus C18  
959758-902

2.1 x 100 mm, 1.8  $\mu$ m

**Column:** ZORBAX RRHD Eclipse XDB-C18  
981758-902

2.1 x 100 mm, 1.8  $\mu$ m

**Column:** ZORBAX RRHD SB-C18  
858700-902

2.1 x 100 mm, 1.8  $\mu$ m

**Column:** ZORBAX RRHD Extend-C18  
758700-902

2.1 x 100 mm, 1.8  $\mu$ m

**Mobile Phase:** A: H<sub>2</sub>O  
B: CH<sub>3</sub>CN, each with 0.1% HCOOH

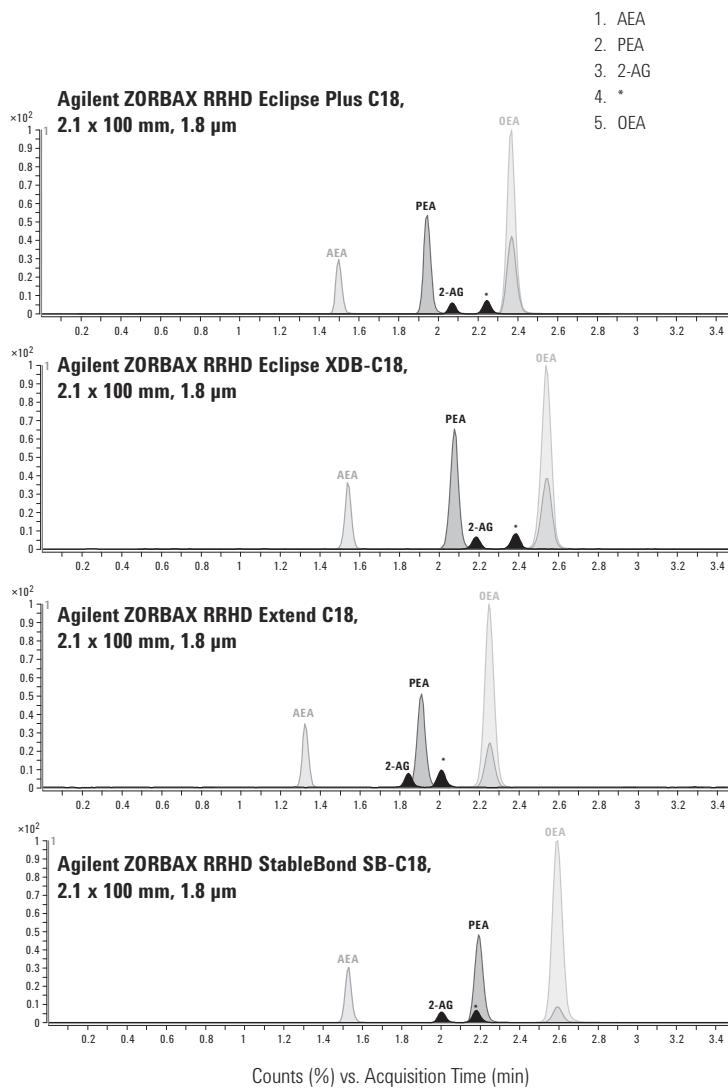
**Detector:** Agilent 1290 Infinity LC with an Agilent 6410 Triple Quadrupole Mass Spectrometer

**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.

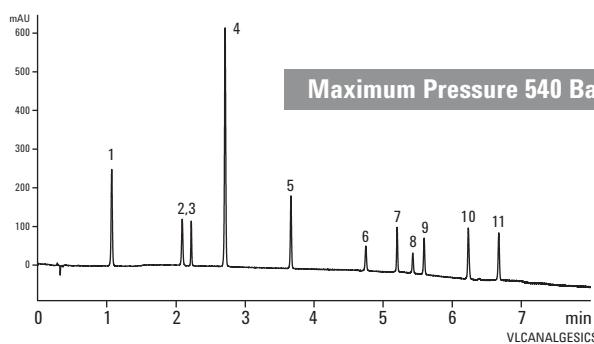


For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at [www.agilent.com/chem/library](http://www.agilent.com/chem/library)

### Fast analysis 11 common compounds found in analgesics

**Column:** Poroshell 120 EC-C18  
695975-902  
**4.6 x 100 mm, 2.7  $\mu$ m**

Mobile Phase: A : Water + 0.1% formic acid  
B: ACN  
Flow Rate: 3.5 mL/min  
Temperature: 40 °C  
Detector: DAD 254 nm



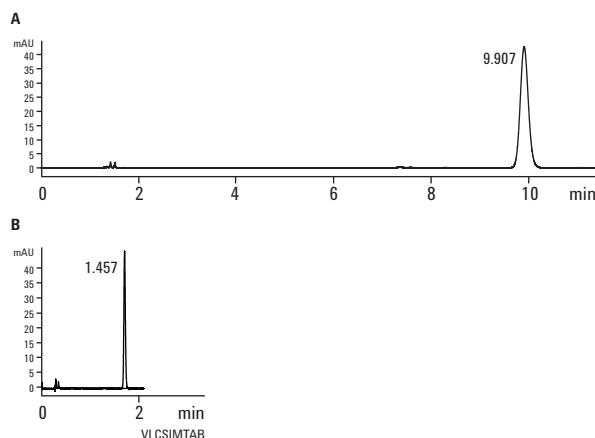
1. Acetaminophen
2. Caffeine
3. 2-Acetamidophenol
4. Acetamide
5. Phenacetin
6. Sulindac
7. Piroxicam
8. Tolmetin
9. Ketoprofen
10. Diflusinal
11. Diclofenac

### Faster analysis of USP Method for simvastatin tablet

**Column A:** Eclipse Plus C18  
959990-902  
**4.6 x 250 mm, 5  $\mu$ m**

**Column B:** Poroshell 120 EC-C18  
697975-902  
**4.6 x 75 mm, 2.7  $\mu$ m**

Mobile Phase: 65% CH<sub>3</sub>CN,  
35% 3.9 g/L NaH<sub>2</sub>PO<sub>4</sub> (pH 4.5)  
Flow Rate: 1.5 mL/min for 5  $\mu$ m column  
2.8 mL/min for 2.7  $\mu$ m Poroshell 120 column  
Temperature: 45 °C  
Detector: DAD Sig = 238, 8  
Ref = 360, 100 nm



	<b>USP Requirement</b>	<b>5 <math>\mu</math>m (1.5 mL/min)</b>	<b>2.7 <math>\mu</math>m (2.8 mL/min)</b>
T <sub>R</sub>	N/A	9.907	1.457
k'	> 3.0	5.962	5.122
N	> 4500	16939	14439
T <sub>f</sub>	< 2.0	1.09	1.10

**Faster separation of sulfa drugs**

**Column A:** Eclipse Plus C18  
959990-902  
4.6 x 250 mm, 5  $\mu$ m

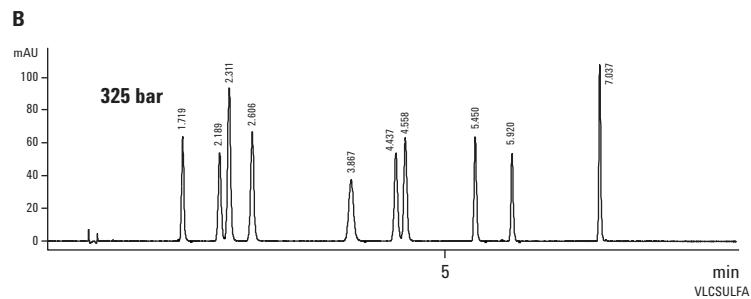
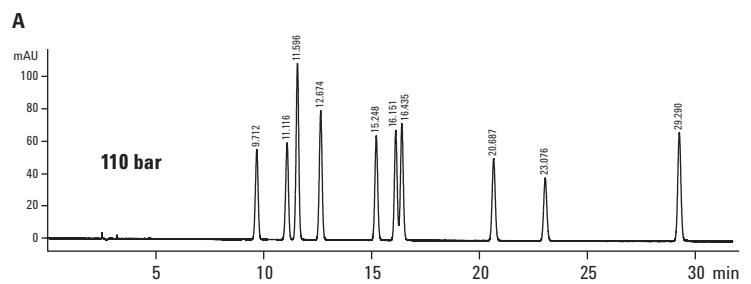
Time	%B
0	8
33	33
35	33

**Column B:** Poroshell 120 EC-C18  
695975-902  
4.6 x 100 mm, 2.7  $\mu$ m

Time	%B
0	8
12	33
13.2	33

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

Flow Rate: 1 mL/min

**Separation of pharmaceutical cardiac drugs**

**Column:** Eclipse Plus C18  
959996-902  
4.6 x 100 mm, 5  $\mu$ m

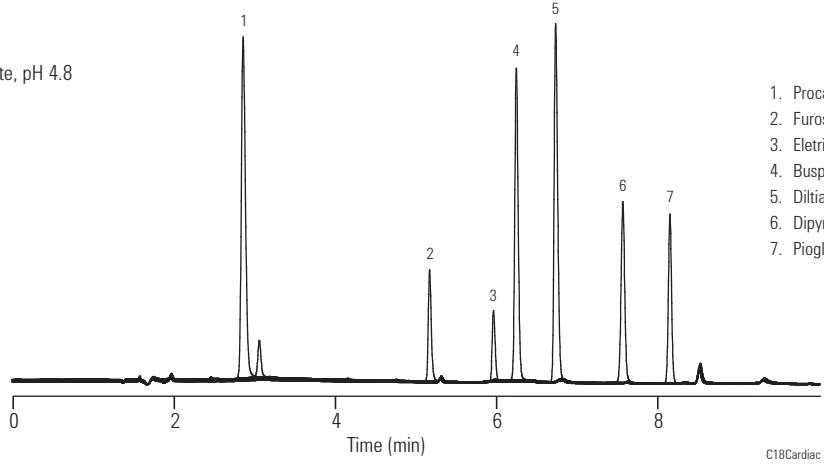
Mobile Phase: A: 20 mM Ammonium Acetate, pH 4.8  
B: ACN

Flow Rate: 1 mL/min

Gradient: 10-90% in 10 min

Detector: UV, 254 nm

1. Procainamide
2. Furosemide
3. Eletriptan
4. Buspirone
5. Diltiazem
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  $\mu$ m**

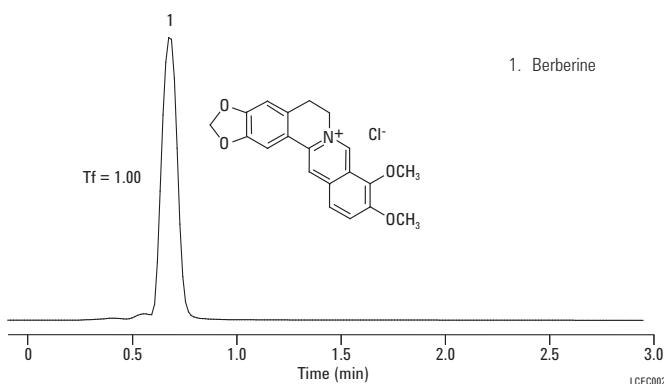
**Mobile Phase:** A: 50% 8 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7  
B: 50% ACN

**Flow Rate:** 1.0 mL/min

**Temperature:** Ambient

**Detector:** UV, 254 nm

**Sample:** Berberine, 0.4 mg/mL, 2  $\mu$ L

**Xanthines: Higher resolution, same selectivity with RRHT**

**Column A:** ZORBAX SB-C18  
846975-902  
**4.6 x 50 mm, 5  $\mu$ m**

**Column B:** ZORBAX SB-C18  
827975-902  
**4.6 x 50 mm, 1.8  $\mu$ m**

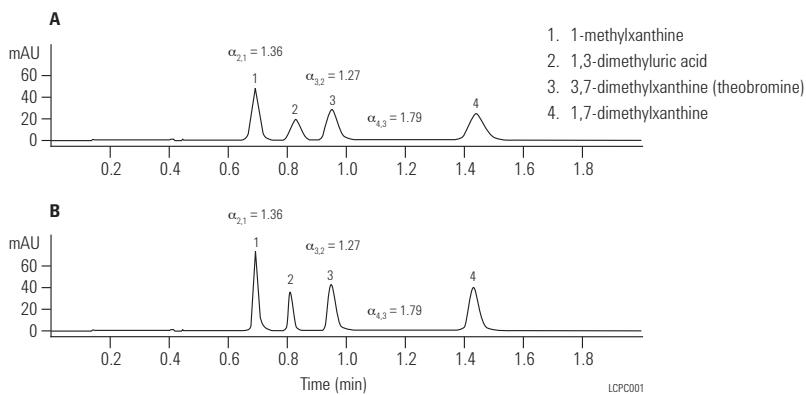
**Mobile Phase:** A: 92% 0.1% formic acid  
B: 8% 0.1% formic acid in ACN

**Flow Rate:** 1.5 mL/min

**Temperature:** Ambient

**Detector:** UV, 254 nm

**Sample:** Xanthines

**Antihistamines:  
Fast separations on RRHT Extend-C18**

**Column A:** ZORBAX Extend-C18  
773450-902  
**4.6 x 150 mm, 5  $\mu$ m**

**Column B:** ZORBAX Extend-C18  
727975-902  
**4.6 x 50 mm, 1.8  $\mu$ m**

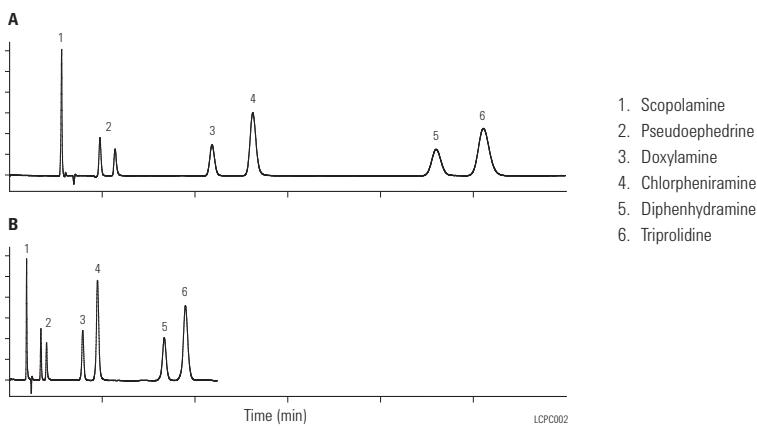
**Mobile Phase:** A: 30% 50 mM pyrrolidine buffer  
B: 70% MeOH

**Flow Rate:** 1.0 mL/min

**Temperature:** Ambient

**Detector:** UV, 220 nm

**Sample:** Antihistamines



**Ibuprofen:**  
Optimizing selectivity with RRHT Columns

**Column A:** SB-C8  
827975-906  
4.6 x 50 mm, 1.8  $\mu$ m

**Column B:** Eclipse XDB-C8  
927975-906  
4.6 x 50 mm, 1.8  $\mu$ m

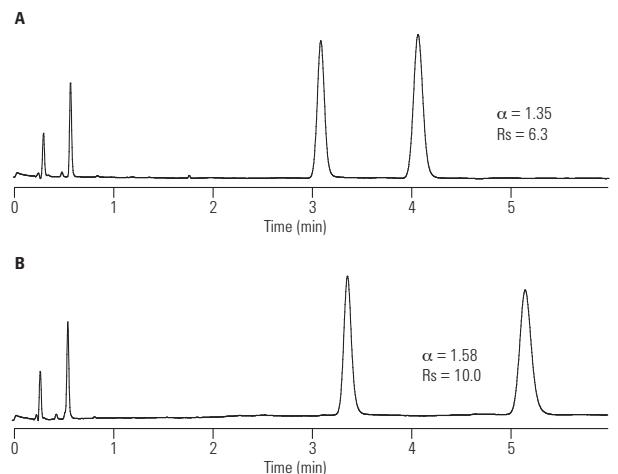
Mobile Phase: A: 63% water  
B: 37% acetonitrile + 1.8 mL H<sub>3</sub>PO<sub>4</sub>

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Ibuprofen oral suspension



1. Benzophenone  
2. Ibuprofen

LCPC003

### Analgesics

**Column:** Pursuit XR<sup>s</sup> Diphenyl  
A6020150X046  
4.6 x 150 mm, 5  $\mu$ m

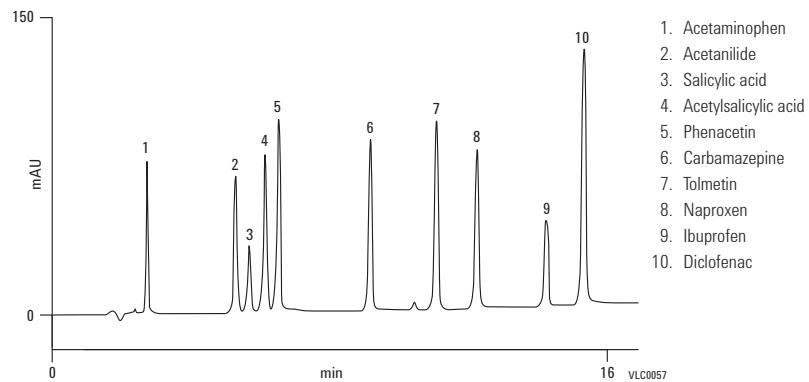
Mobile Phase: A: Water+0.1% HCOOH  
B: MeCN+0.1% HCCOH

Gradient: 25-80% B in 20 min

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



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

**Anesthetics, local: Bonded phase selectivity**

**Column A:** ZORBAX SB-C18  
883975-902  
4.6 x 150 mm, 5  $\mu$ m

**Column B:** ZORBAX SB-C8  
883975-906  
4.6 x 150 mm, 5  $\mu$ m

**Column C:** ZORBAX SB-C3  
883975-909  
4.6 x 150 mm, 5  $\mu$ m

**Column D:** ZORBAX SB-Phenyl  
883975-912  
4.6 x 150 mm, 5  $\mu$ m

**Column E:** ZORBAX SB-CN  
883975-905  
4.6 x 150 mm, 5  $\mu$ m

Mobile Phase: A: 50 mM NaH<sub>2</sub>PO<sub>4</sub> pH 2.5 in 95% H<sub>2</sub>O/5% ACN  
B: 50 mM NaH<sub>2</sub>PO<sub>4</sub> pH 2.5 in 47% H<sub>2</sub>O/53% ACN

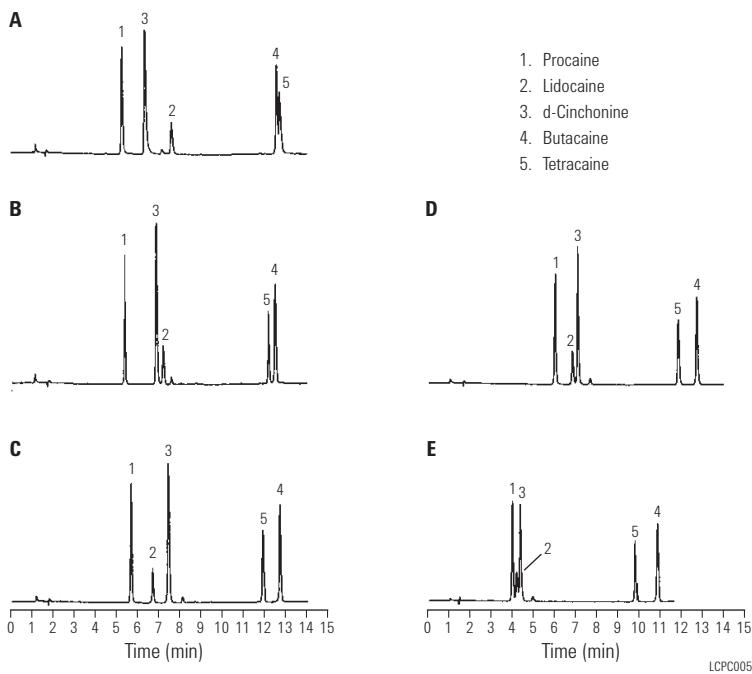
Flow Rate: 1.5 mL/min

Gradient: 0-100% B in 18.8 min

Temperature: 26 °C

Detector: UV, 254 nm

Sample: 10  $\mu$ L, 10  $\mu$ g/mL

**Local anesthetics**

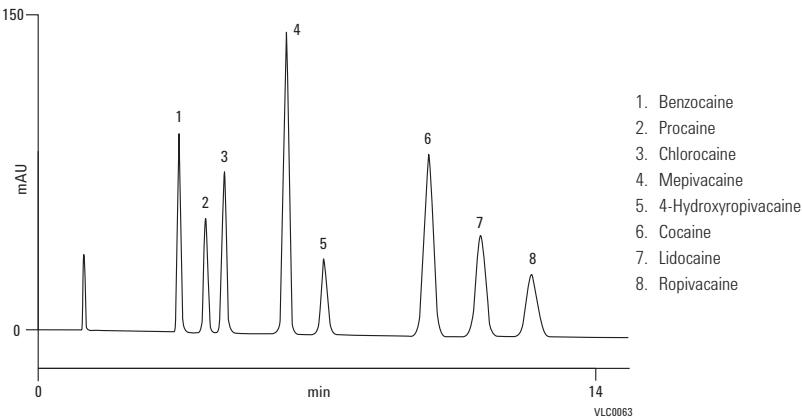
**Column:** Pursuit XR<sup>®</sup> C8  
A6010150X046  
4.6 x 150 mm, 5  $\mu$ m

Mobile Phase: 65:35 MeOH:5 mM NH<sub>4</sub>CO<sub>3</sub>, pH 10

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 210 nm



**Antibiotics: High speed separation**

**Column:** ZORBAX Rx/SB-C8  
866953-906  
4.6 x 75 mm, 3.5  $\mu$ m

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

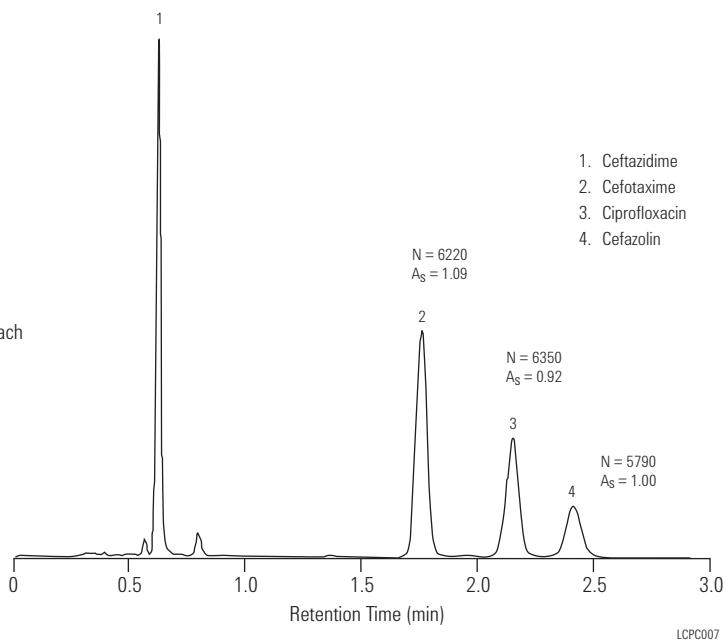
Flow Rate: 3.0 mL/min

Gradient: 45-70% B in 35 min

Temperature: 60 °C

Detector: UV, 260 nm

Sample: 1  $\mu$ L containing 0.40, 0.36, 0.10 and 0.37  $\mu$ g 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  $\mu$ m

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

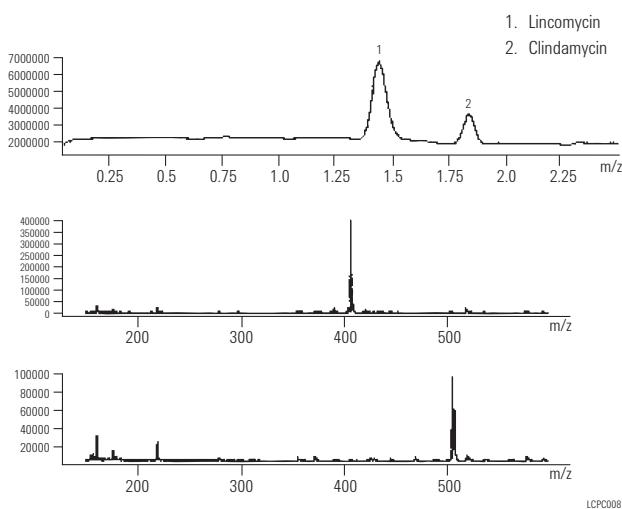
Gradient: Post time: 1.5 min

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  $\mu$ A

Sample: Antibiotics, 1  $\mu$ L



**Antifungal medications**

**Column:** ZORBAX Bonus-RP  
883668-901  
4.6 x 150 mm, 5  $\mu$ m

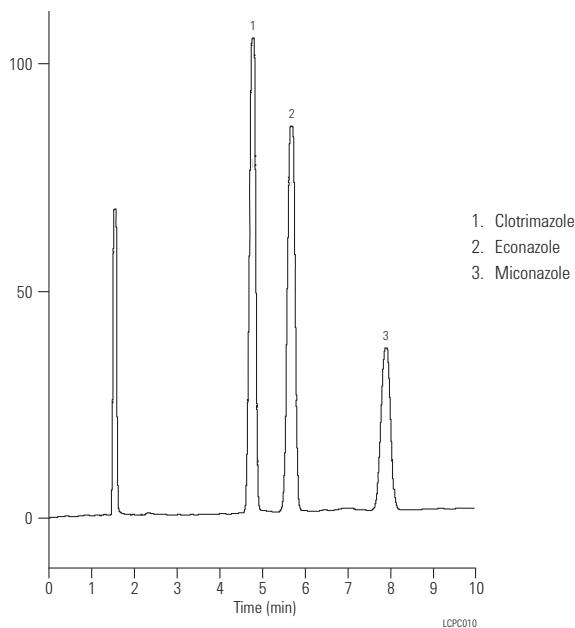
**Mobile Phase:** 35% 25 mM NaH<sub>2</sub>PO<sub>4</sub>, Dibasic (pH 6.5 with H<sub>3</sub>PO<sub>4</sub>):  
65% ACN

**Flow Rate:** 1 mL/min

**Temperature:** Ambient

**Detector:** UV, 220 nm

**Sample:** Antifungals, 2  $\mu$ L

**Antifungals**

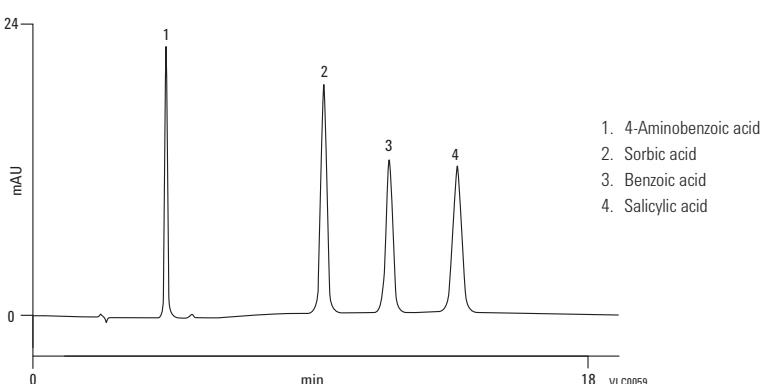
**Column:** Pursuit XR<sup>®</sup> Diphenyl  
A6020150X046  
4.6 x 150 mm, 5  $\mu$ m

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

**Flow Rate:** 1.0 mL/min

**Temperature:** Ambient

**Detector:** UV, 254 nm



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at [www.agilent.com/chem/library](http://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  $\mu$ m

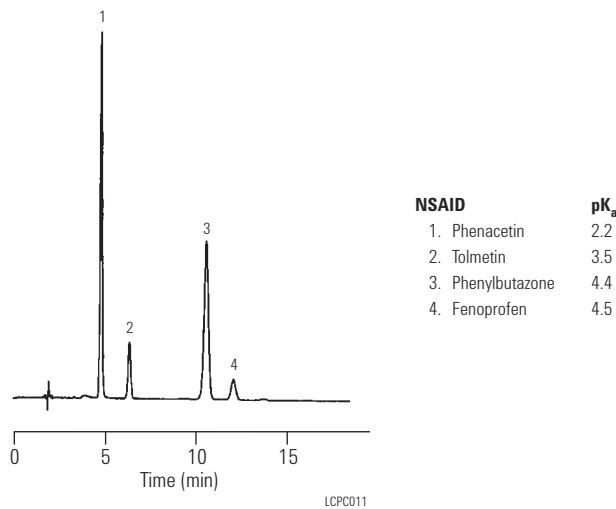
Mobile Phase: 50/50, 25 mM Sodium Phosphate  
(pH 7.0 with Phosphoric Acid), MeOH

Flow Rate: 0.2 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: 2  $\mu$ L, 10 ug/mL



**Separation of small molecule anorectics**

**Column A:** ZORBAX Bonus-RP  
883668-901  
4.6 x 150 mm, 5  $\mu$ m

**Column B:** Traditional Alkyl C8 Phase

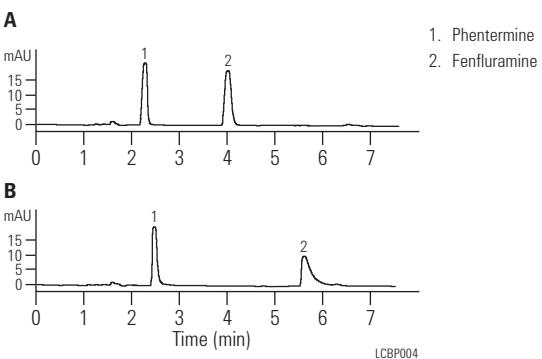
Mobile Phase: 25 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.2/MeOH: ACN (50:50), 45/55

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Anorectics "Fen-phen", 5  $\mu$ L



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

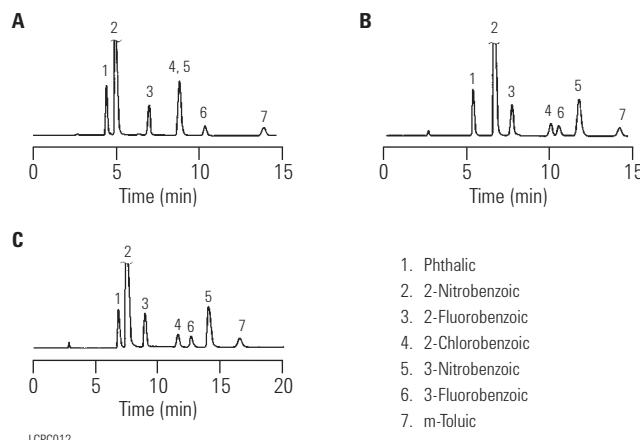
Mobile Phase: 30-45% methanol in 25 mM Na Phosphate, pH 2.5  
A: 45% Methanol  
B: 40% Methanol  
C: 30% Methanol

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Benzoic acids



## 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

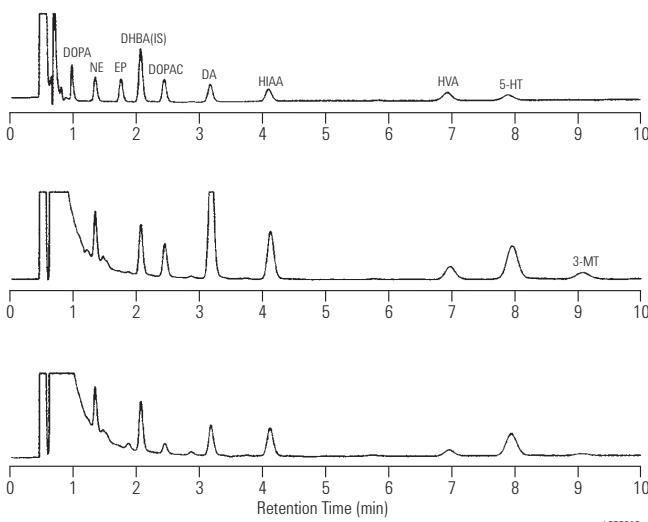
Flow Rate: 1.5 mL/min

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 Sratium  
C: Mouse Neocortex

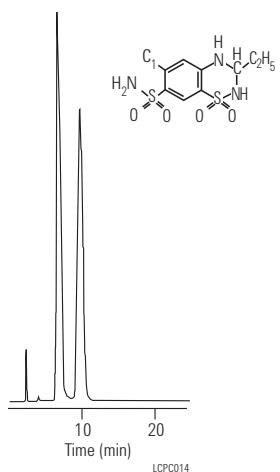
- |                                      |                                  |
|--------------------------------------|----------------------------------|
| 1. DOPA-Dihydroxyphenylalanine       | 6. HIAA-Hydroxyindoleacetic acid |
| 2. DHBA-Dihydroxybenzyl amine        | 7. EP-Epinephrine            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



**Chiral ethiazide (diuretic drug) separation**

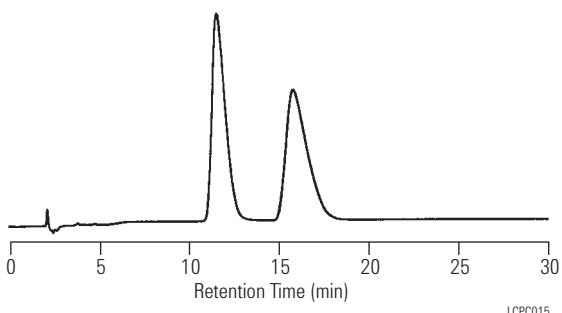
**Column:** Ultron ES-OVM Chiral  
**702111651**  
**4.6 x 150 mm, 5 µm**

**Mobile Phase:** 20 mM KH<sub>2</sub>PO<sub>4</sub> (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<sub>2</sub>PO<sub>4</sub>, 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](http://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

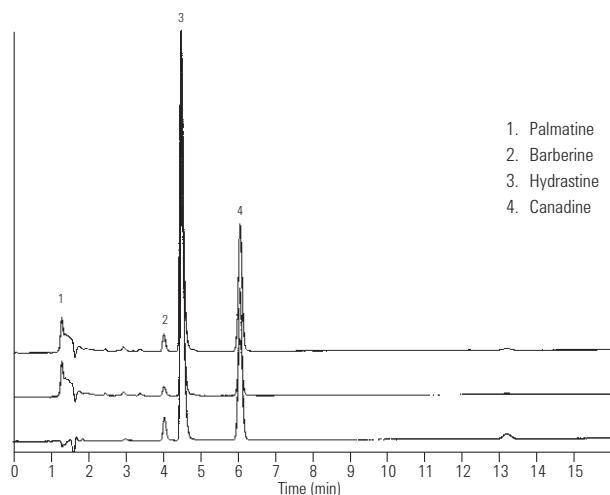
Flow Rate: 1.0 mL/min

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.



### 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

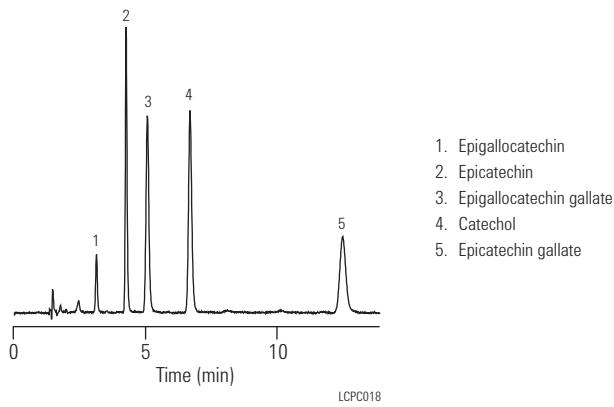
Flow Rate: 1.0 mL/min

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.



### Chiral separation of hexobarbital

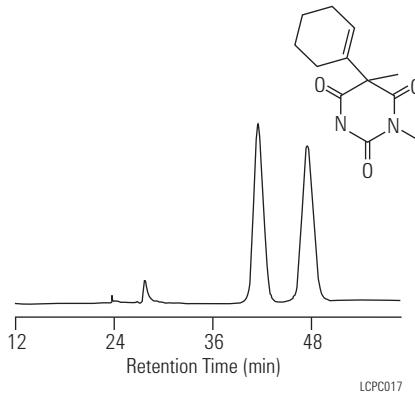
**Column:** Chiradex  
79925CB-584  
**4.0 x 250 mm, 5 µm**

Mobile Phase: Methanol/water, 20:80

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm

Sample: Hexobarbital



**Chiral separation of S- and R-Norfluoxetine**

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

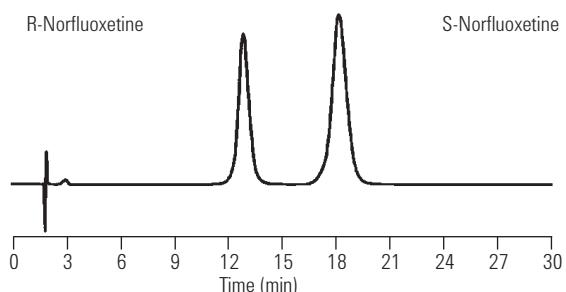
Mobile Phase: 6/94 (v/v) MeOH / 20 mM KH<sub>2</sub>PO<sub>4</sub>

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**

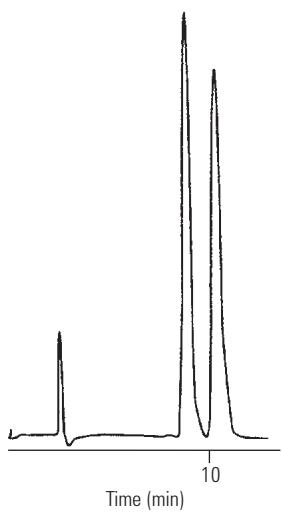
Mobile Phase: 20 mM phosphate buffer, pH 6.0

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 220 nm

Sample: 20 µL containing 0.35 µg salbutamol mixture

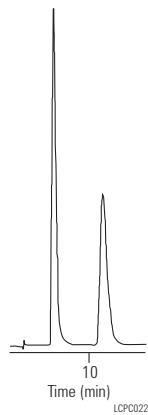


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**Chiral separation of tolperison enantiomers**

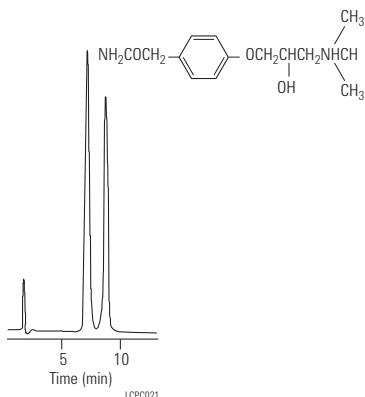
**Column:** Ultron ES-OVM Chiral  
**702111651**  
**4.6 x 150 mm, 5 µm**

Mobile Phase: 20 mM KH<sub>2</sub>PO<sub>4</sub> (pH 5.5), C<sub>2</sub>H<sub>5</sub>OH (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**

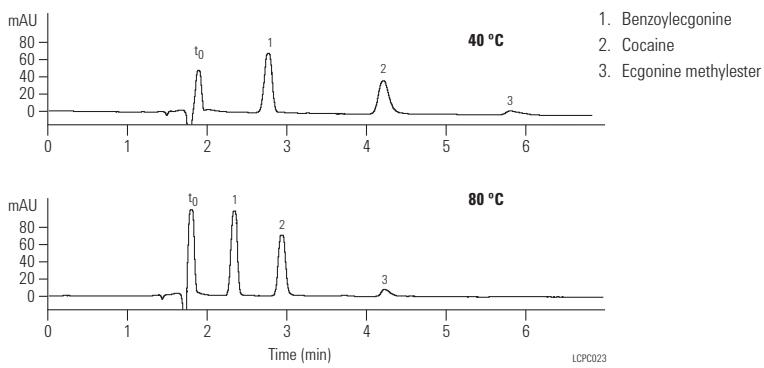
**Column:** Ultron ES-Pepsin  
**822111631A**  
**4.6 x 150 mm, 5 µm**

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<sub>4</sub> Acetate, 25 mM, pH 6 (70:30)  
Flow Rate: 1.0 mL/min  
Temperature: 40 and 80 °C  
Detector: UV, 210 nm



**Aspirin and cough remedy**

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

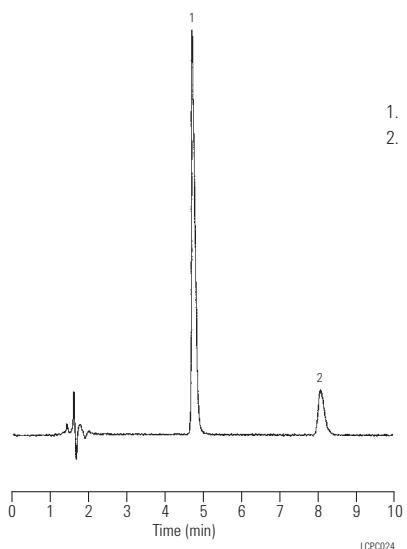
Mobile Phase: (75:25) 25 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 3.0): ACN

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: UV, 254 nm

Sample: 5 µL, 10 µg/mL



**Cough formula mixture:  
Fast and efficient separation**

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

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

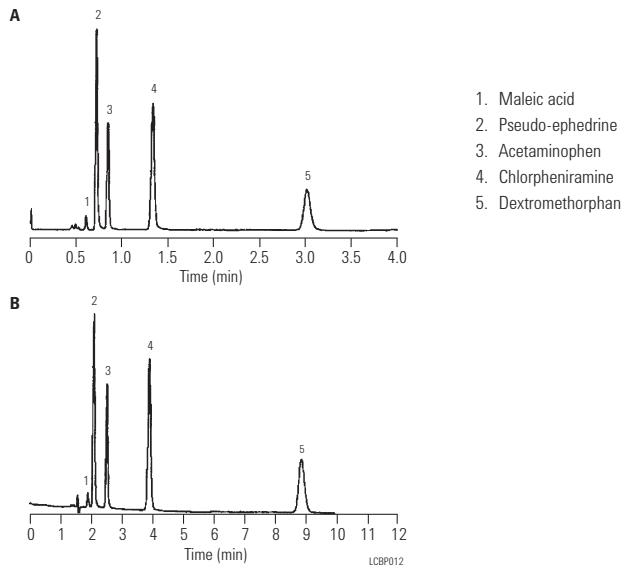
Mobile Phase: 20/80, Acetonitrile/150 mM Na Citrate, pH 2.6

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

Temperature: 35 °C

Detector: UV, 270 nm

Sample: 2 µL, cough formula



**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

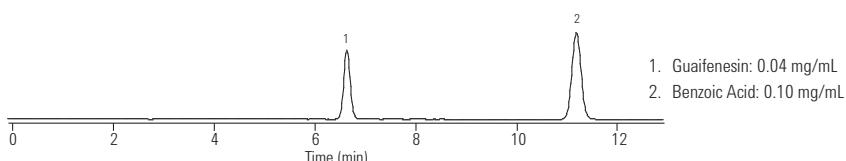
Sample: Guaifenesin

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



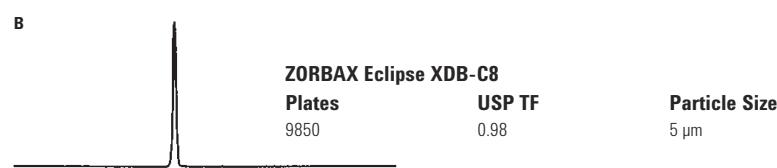
Minimum Resolution Required = 3.0

**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**

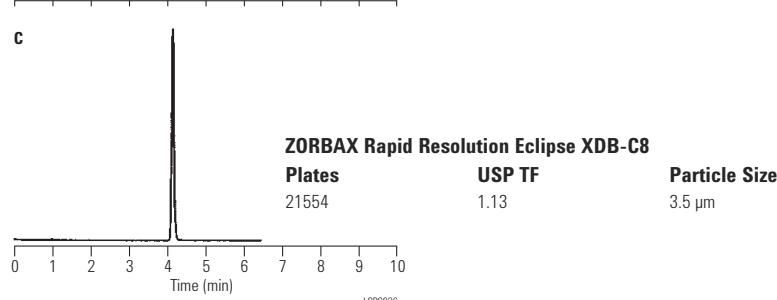
Mobile Phase: 80/20, Water/Methanol

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Metronidazole



**Morphine and metabolites:**  
**Extracted blood plasma sample separation**

**Column:** ZORBAX SB-C18  
 863953-902  
 4.6 x 150 mm, 3.5  $\mu$ m

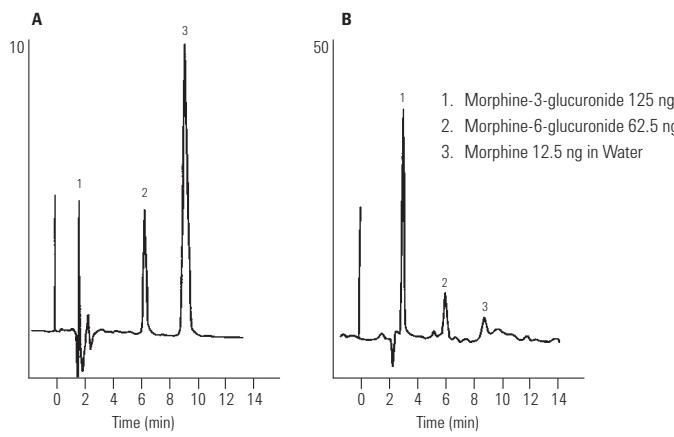
Mobile Phase: 97/3 70 mM KH<sub>2</sub>PO<sub>4</sub> + 1 mM EDTA/ACN, pH 4.5

Flow Rate: 1.5 mL/min

Temperature: Ambient

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

Sample: 50  $\mu$ L  
 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.

LCP027

**Opiates (drugs of abuse) by LC/MS**

**Column:** ZORBAX SB-AQ  
 830990-914  
 2.1 x 150 mm, 3.5  $\mu$ m

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

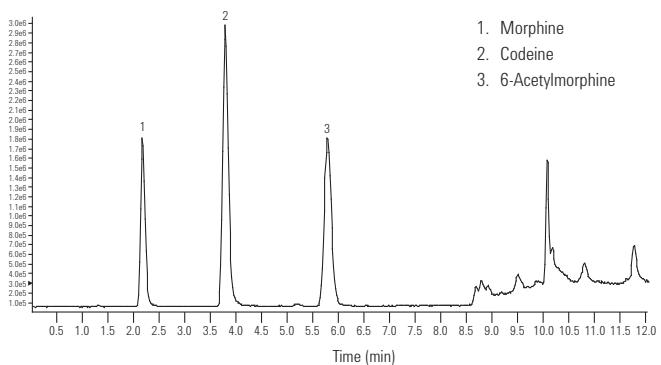
Flow Rate: 0.25 mL/min

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  $\mu$ M purine  
 and HP-921 calibrant to dual ESI for  
 continuous auto-calibration

Sample: Opiates

XIC of +TOF MS



LCP028



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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  $\mu$ m  
(Custom column)

**Column:** ZORBAX RRHD HILIC Plus  
959758-901  
2.1 x 100 mm, 1.8  $\mu$ m

Mobile Phase: A: 10 mM  $\text{NH}_4\text{HCO}_3$ , pH 3.2  
B:  $\text{CH}_3\text{CN}/100 \text{ mM } \text{NH}_4\text{HCO}_3$ , 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

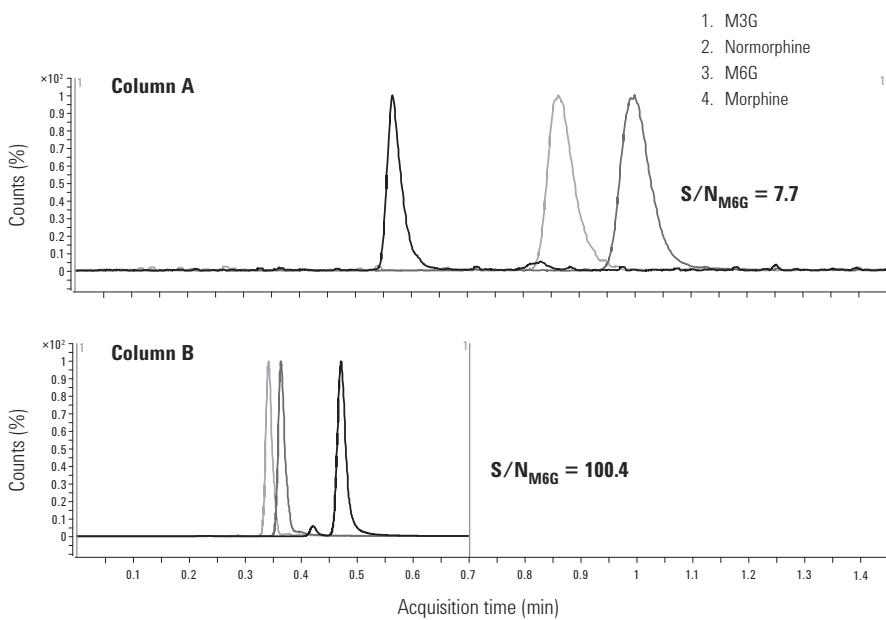
Detector: Agilent 1290 Infinity LC with an  
Agilent 6410A Triple Quadrupole Mass Spectrometer

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 varies with mobile phase flow rate  
Agilent MassHunter versions B.03.01, B.02.00 AND B.03.01 were used for data acquisition, qualitative, and quantitative analyses, respectively

Software: Sample: 2  $\mu$ L injection of 1  $\mu$ g/mL each of morphine, normorphine, morphine-3- $\beta$ -D-glucuronide: HILIC sample was prepared in  $\text{CH}_3\text{CN}$ ; RPLC sample was prepared in  $\text{H}_2\text{O}$

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**

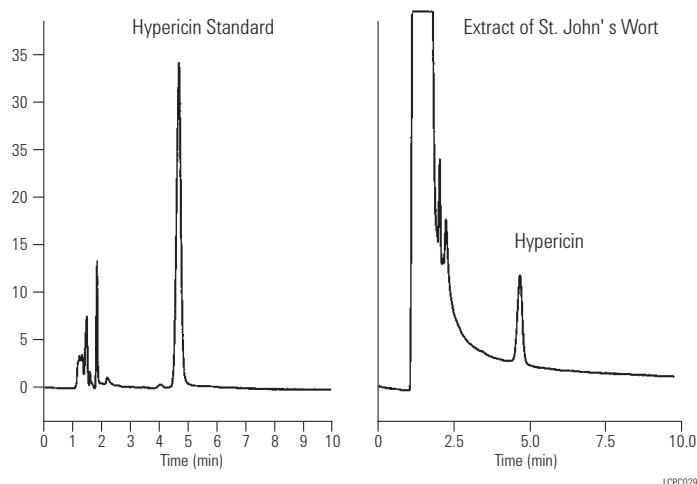
**Mobile Phase:** 23% 25 mM Na<sub>2</sub>HPO<sub>4</sub>, Dibasic (pH 7.0 with H<sub>3</sub>PO<sub>4</sub>); 77% MeOH

**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<sub>4</sub> Formate (pH = 3.6)  
B: ACN with 10 mM NH<sub>4</sub> Formate

**Flow Rate:** 0.6 mL/min

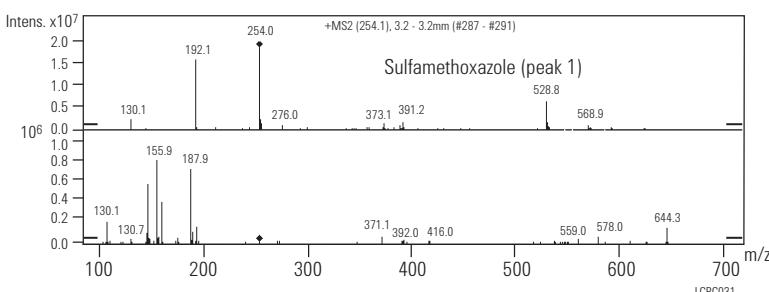
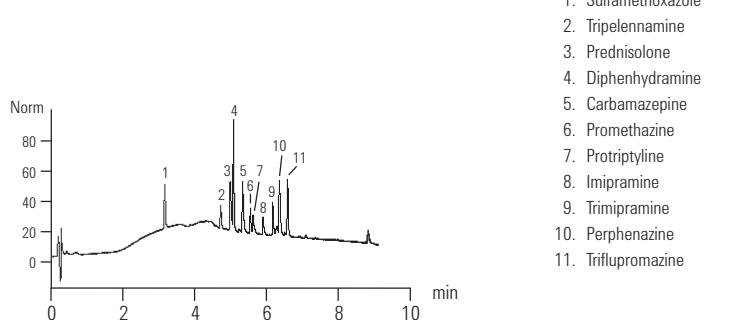
**Gradient:** 5% B to 70% B in 7.5 min, to 95% B in 8.5 min

**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



**Hormones/steroids**

**Column:** ZORBAX RRHT SB-C18  
823975-902  
**4.6 x 30 mm, 1.8  $\mu$ m**

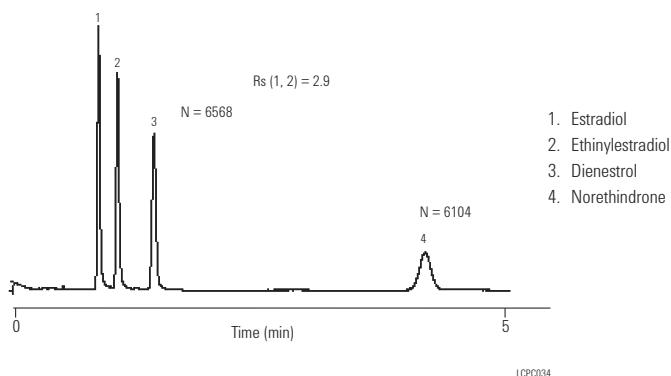
Mobile Phase: 50% 20 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 2.8: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: RT

Detector: UV, 230 nm

Sample: Hormones/steroids

**Steroids: Separation**

**Column:** Eclipse XDB-CN  
993967-905  
**4.6 x 150 mm, 5  $\mu$ m**

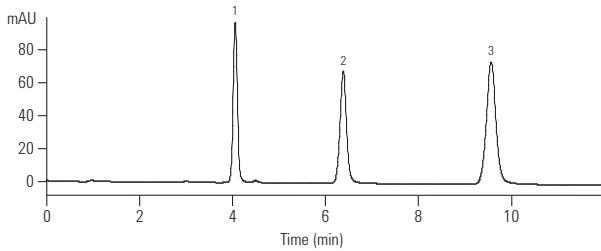
Mobile Phase: 40:60 ACN:Water

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



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

**Steroids**

**Column A:** Eclipse XDB-Phenyl  
993967-912  
4.6 x 150 mm, 3.5  $\mu$ m

**Column B:** Eclipse XDB-C18  
993967-902  
4.6 x 150 mm, 5  $\mu$ m

Mobile Phase: H<sub>2</sub>O:ACN, 60:40

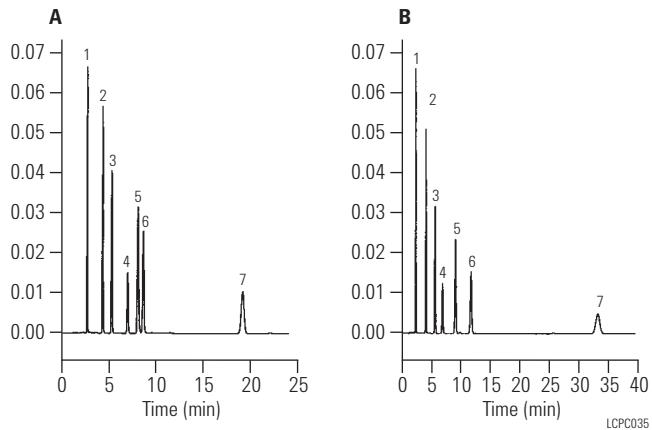
Flow Rate: 1.0 mL/min

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](http://www.agilent.com/chem/library)

**Triamcinolone – USP analysis of triamcinolone**

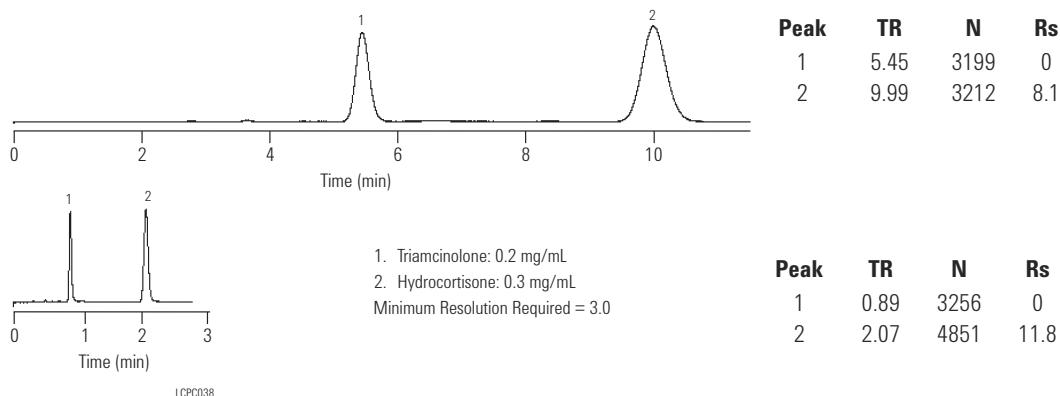
**Column:** Eclipse XDB-C18  
923975-902  
**4.6 x 30 mm, 1.8  $\mu$ m**

Mobile Phase: 47% Methanol:53% Water

Flow Rate: 1.5 mL/min

Temperature: 25 °C

Sample: Triamcinolone, 1  $\mu$ L

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

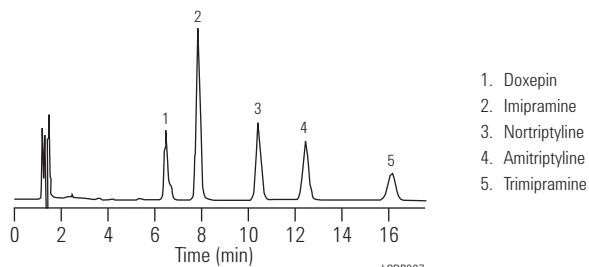
**Column:** ZORBAX Extend-C18  
773450-902  
**4.6 x 150 mm, 5  $\mu$ m**

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

Flow Rate: 0.5 mL/min

Temperature: 40 °C

Detector: UV, 215 nm



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.

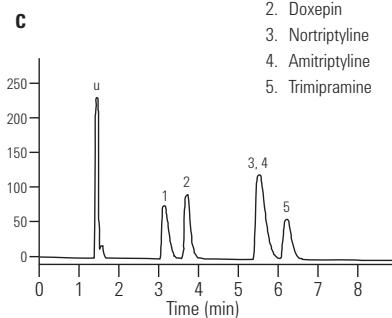
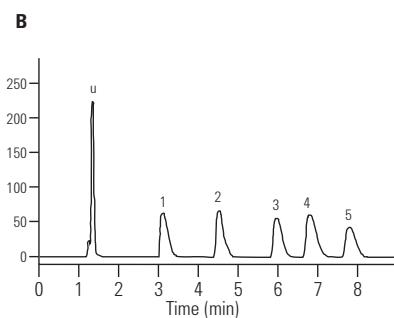
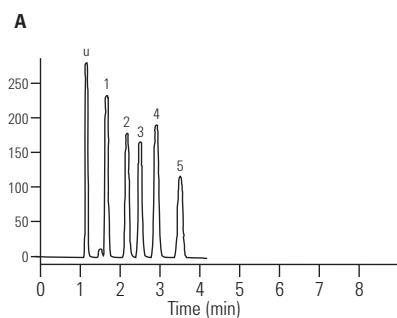
### Antidepressants, tricyclic: Comparative separation

**Column A:** ZORBAX Bonus-RP  
883668-901  
4.6 x 150 mm, 5  $\mu$ m

**Column B:** Brand A Polar-linked C8

**Column C:** Brand B Polar-linked C18

Mobile Phase: ACN: 20 mM Na Citrate, pH 6 (60:40)  
Flow Rate: 1.0 mL/min  
Temperature: Ambient  
Detector: UV, 254 nm  
Sample: Tricyclic antidepressants (u= uracil)



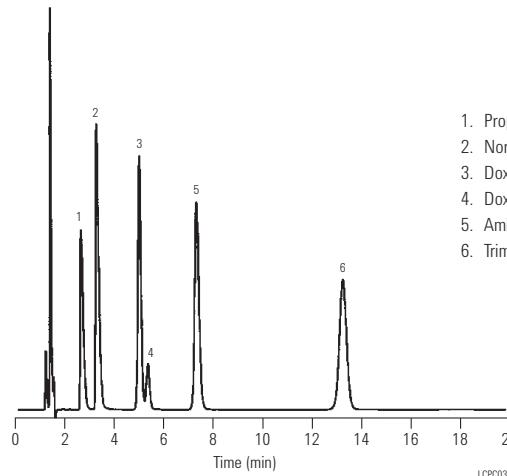
1. Propranolol
2. Doxepin
3. Nortriptyline
4. Amitriptyline
5. Trimipramine

LCBP011

### Tricyclic antidepressants

**Column:** Eclipse XDB-C8  
993967-906  
4.6 x 150 mm, 5  $\mu$ m

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  $\mu$ L, Antidepressant mix, 10  $\mu$ g/mL



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

LCP039

**Tricyclic antidepressants and metabolites:****Effect of pore size**

**Column A:** ZORBAX SB-C18  
863953-902  
4.6 x 150 mm, 3.5  $\mu$ m

**Column B:** ZORBAX RRHD 300SB-C18  
883995-902  
4.6 x 150 mm, 5  $\mu$ m

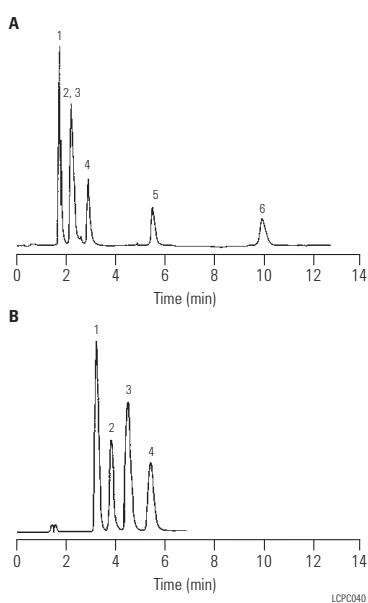
Mobile Phase: 40/60, 25 mM Phosphate Buffer,  
10 mM Triethylamine, pH 6.2/ACN

Flow Rate: 1.2 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: 10  $\mu$ L, Antidepressant mix, 10  $\mu$ g/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  $\mu$ m

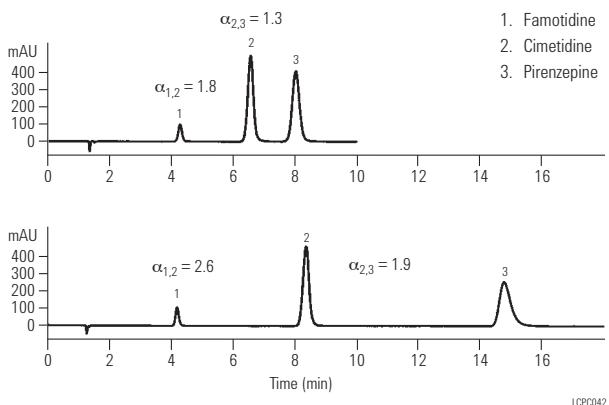
Mobile Phase: Na citrate, 20 mM, pH 6.1: MeOH, (80:20)

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  $\mu$ m

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

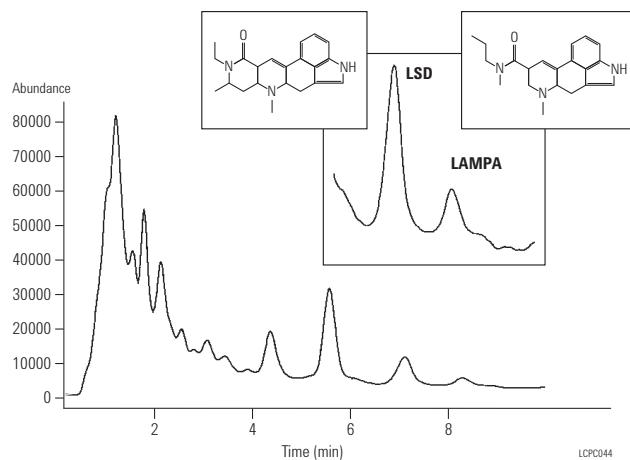
Flow Rate: 0.3 mL/min

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  $\mu$ m

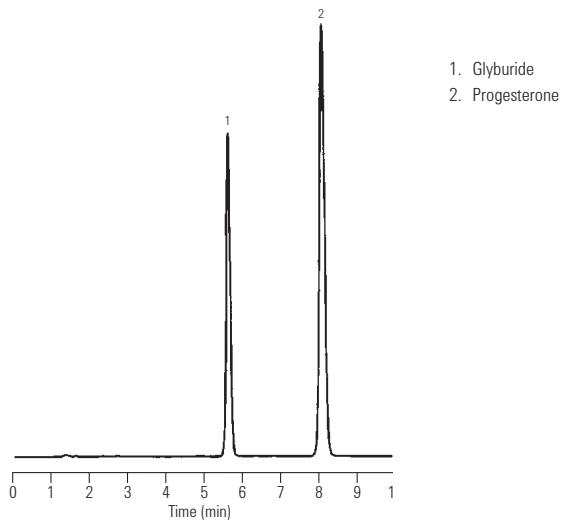
Mobile Phase: 45/55, 50 mM Ammonium Phosphate/ACN, Final pH 5.35

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: 5  $\mu$ L, 10 ug/mL each of standard



**Dexamethasone, USP method: Rapid analysis****Column A:** ZORBAX SB-C8

880975-906

4.6 x 250 mm, 5 µm

A

B

**Column B:** ZORBAX Rx/SB-C8

866953-906

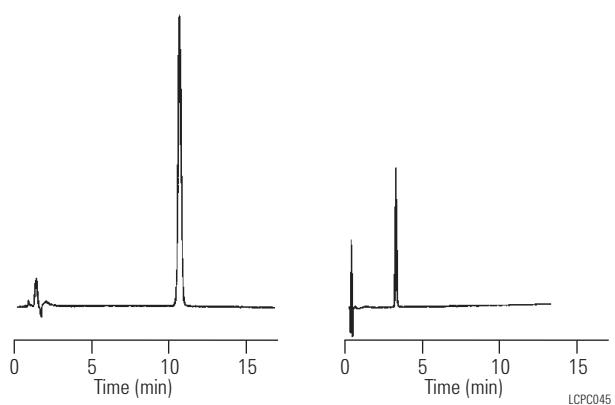
4.6 x 75 mm, 3.5 µm

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

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Dexamethasone  
10 µL and 5 µL, 10 µg/mL**USP analysis of tetracyclines****Column:** PLRP-S 100Å

PL1512-5500

4.6 x 250 mm, 5 µm

Sample: 20 mg tetracycline in 25 mL 0.01M HCl

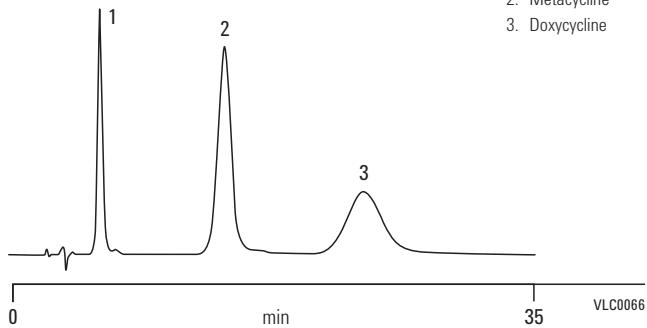
1. Oxytetracycline
2. Metacycline
3. Doxycycline

Mobile Phase: 60 g 2-Methyl-2-propanol + 200 mL UHP water + 400 mL 0.2 M K<sub>2</sub>HPO<sub>4</sub> 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

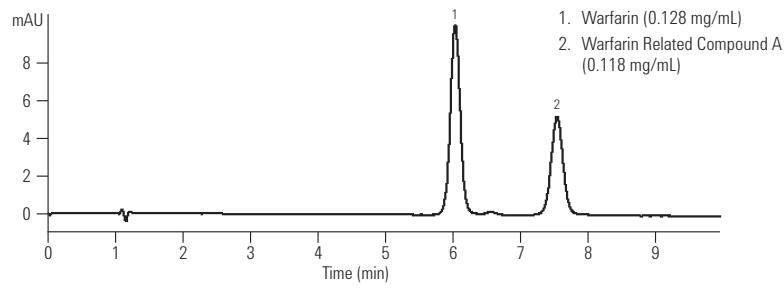


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### Warfarin: USP chromatographic purity method using Eclipse XDB-CN

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

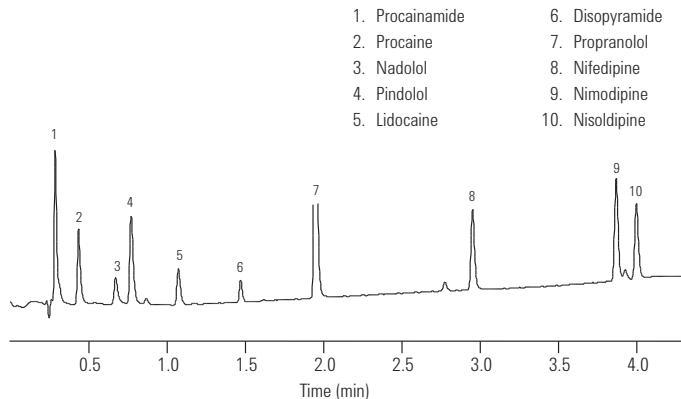
Mobile Phase: 32:68:1 Acetonitrile:Water:Glacial Acetic Acid  
 Flow Rate: 1.5 mL/min  
 Temperature: 25 °C  
 Detector: UV, 260 nm  
 Sample: Warfarin, 2 µL



### Ten cardiac drugs on Rapid Resolution HT SB-C18

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

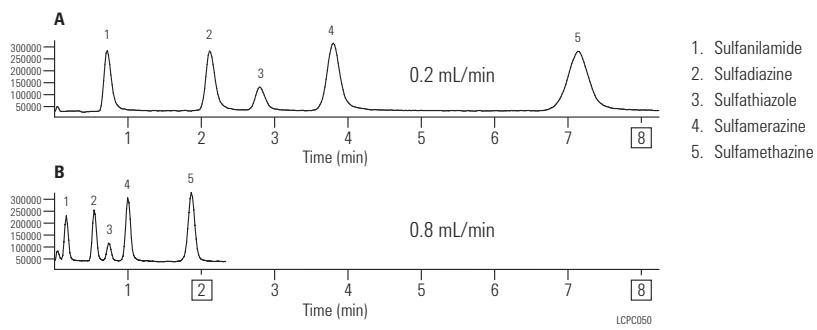
Mobile Phase: A: 0.1% TFA, 5% ACN  
 B: 0.08% TFA, 95% ACN  
 Flow Rate: 2 mL/min  
 Gradient: 0.0 min 12.5% B  
 10.5 min 60% B  
 12.0 min 60% B  
 Temperature: 70 °C  
 Detector: UV, 230 nm  
 Sample: Cardiac drugs



### 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 XR<sub>s</sub> Ultra C8  
A7511100X020  
**2.0 x 100 mm, 3.0  $\mu$ m**

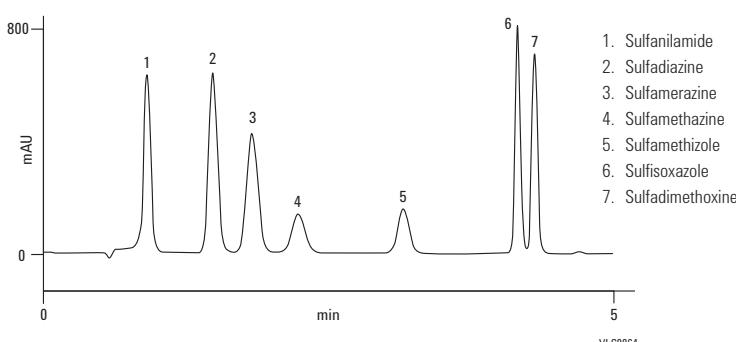
**Mobile Phase:** A: Water+0.1% TFA  
B: MeCN+0.1% TFA

**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

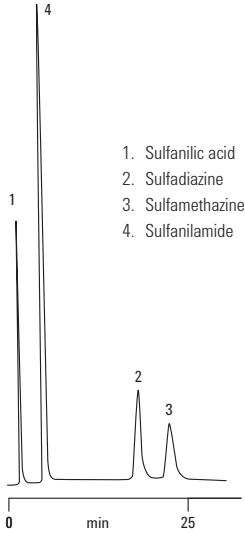
**Sulfa drugs**

**Column:** PLRP-S 100 $\text{\AA}$   
PL1111-3500  
**4.6 x 150 mm, 5  $\mu$ m**

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

**Flow Rate:** 1.0 mL/min

**Detector:** UV, 254 nm

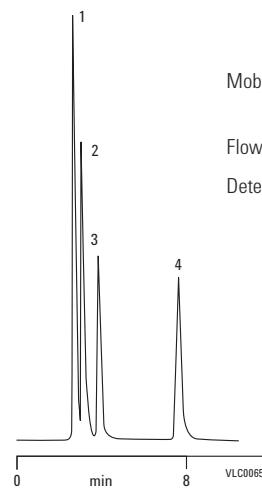


**Column:** PLRP-S 100 $\text{\AA}$   
PL1111-3500  
**4.6 x 150 mm, 5  $\mu$ m**

**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  $\mu$ m

**Column B:** ZORBAX SB-CN  
827975-905  
4.6 x 50 mm, 1.8  $\mu$ m

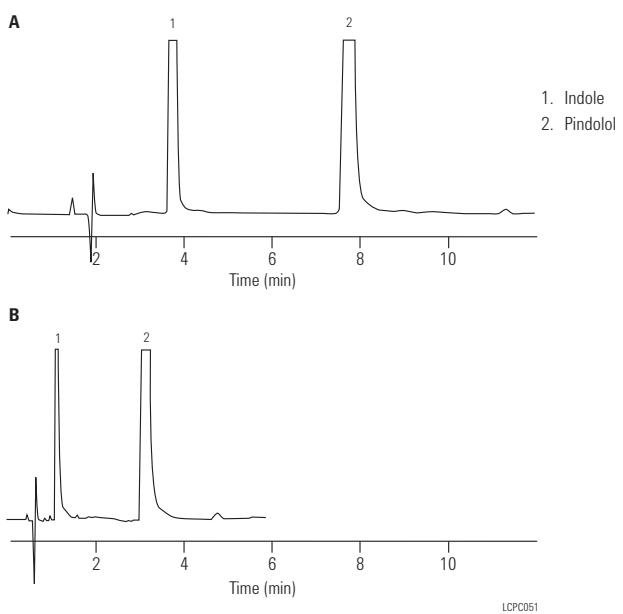
Mobile Phase: A: 70% 50 mM Na Acetate  
B: 30% ACN

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 219 nm

Sample: Pindolol, 2  $\mu$ L

**Lamotrigine**

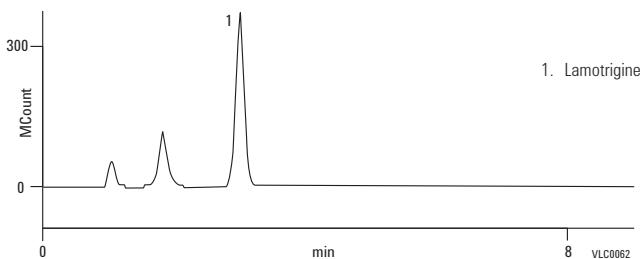
**Column:** Pursuit XR<sub>s</sub> Ultra C8  
A7511100X020  
2.0 x 100 mm, 3.0  $\mu$ m

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

Flow Rate: 0.2 mL/min

Injection Volume: 5  $\mu$ L, 50% MeOH

Detector: MS



**Barbiturates**

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

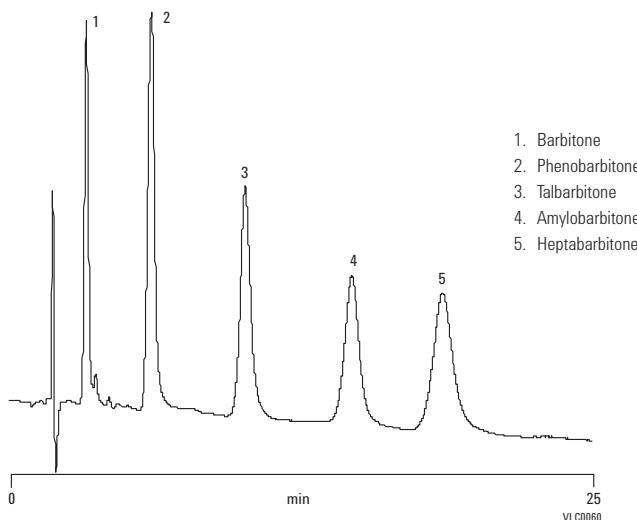
Mobile Phase: Water

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 elements for reversed-phase liquid chromatography. *LCGC Europe*, January 1999, 30-36. Used with permission.

**Analysis of ciprofloxacin and ciprofloxacin metabolites**

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

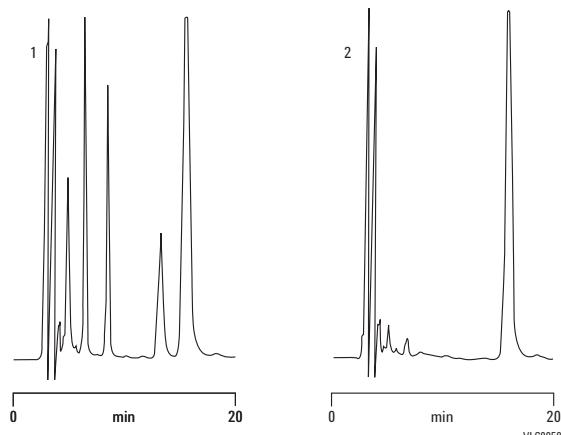
Mobile Phase: 74% 20 mM TCA:22% ACN:4% MeOH adjusted to pH 3

Flow Rate: 1.0 mL/min

Detector: UV, 277 nm

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

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](http://www.informaworld.com)).



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