Waters

ACQUITY UPLC™ Columns

C_{18} • Shield RP_{18} • C_8 • Phenyl BEH Column Chemistries

Acquity
Ultra Performance LC™

Ultra Performance LC™

Waters changed the landscape and future of chromatography with the ACQUITY Ultra Performance LC™ (UPLC™) system. Chromatographers need no longer choose between the speed of short columns and the resolution of long columns. Separations scientists can now enjoy the best of both worlds: speed and resolution with the bonus of increased sensitivity. UPLC™ delivers more information faster—without compromising data integrity.

Waters ACQUITY UPLC™ systems are holistically designed to dramatically improve resolution, sample throughout and sensitivity. Key innovations include:

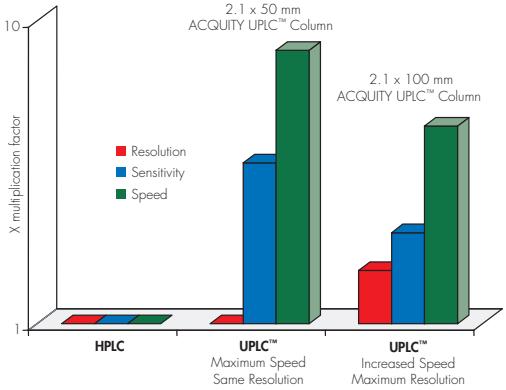
- Small, pressure tolerant particles
- High pressure fluidic modules
- Minimized system volume
- Negligible carryover
- Reduced cycle times
- Fast response detectors
- Integrated system software and diagnostics

UPLC™—Speed, Sensitivity and Resolution—Without Compromise

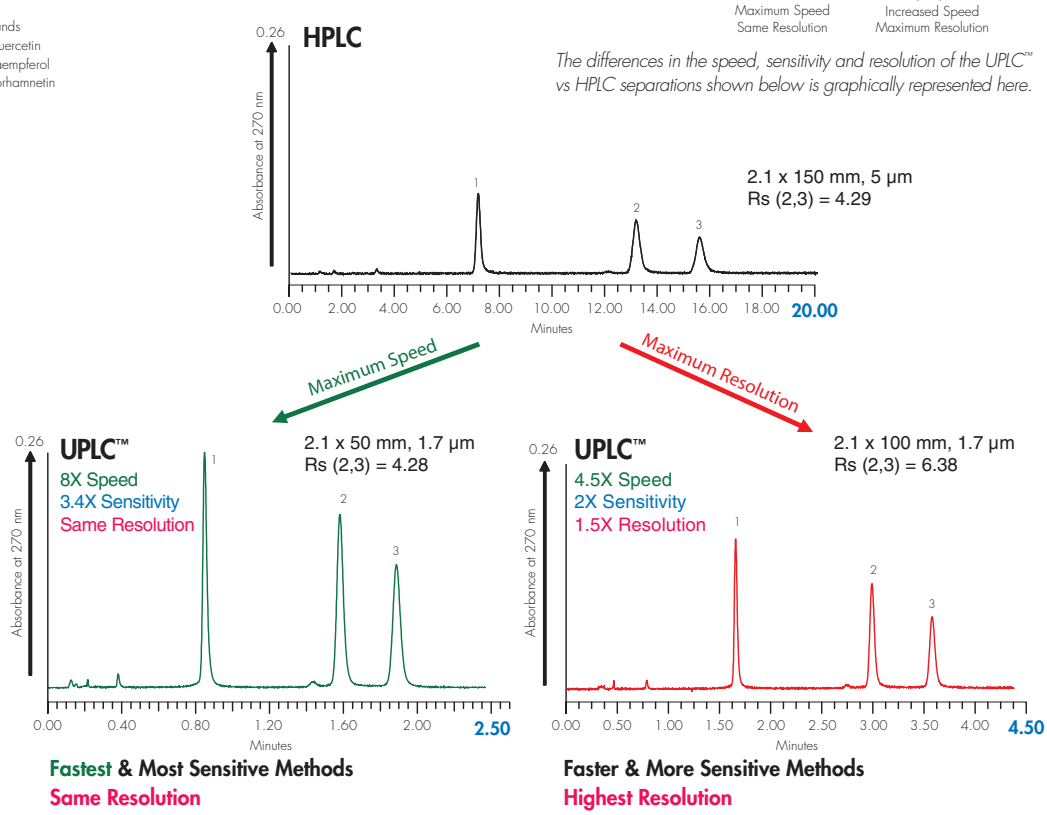
Conditions

HPLC Column:	XTerra® MS C_{18} 2.1 x 150 mm, 5 µm
UPLC™ Columns:	ACQUITY UPLC™ BEH C_{18} 2.1 x 50 mm, 1.7 µm ACQUITY UPLC™ BEH C_{18} 2.1 x 100 mm, 1.7 µm
Mobile Phase A:	0.1% HCOOH
Mobile Phase B:	ACN
Isocratic Mobile Phase Composition:	75% A; 25% B
Flow Rates:	HPLC: 0.20 mL/min UPLC™: 0.50 mL/min
Injection Volume:	5 µL
Sample Diluent:	0.1% HCOOH
Sample Concentration:	17 µg/mL
Temperature:	50 °C
Detection:	UV @ 270 nm
Instrument:	ACQUITY UPLC™ with ACQUITY UPLC™ 2996 PDA

Compounds
1. Quercetin
2. Kaempferol
3. Isorhamnetin



The differences in the speed, sensitivity and resolution of the UPLC™ vs HPLC separations shown below is graphically represented here.



Whether your objective is maximum speed (with the same resolution) or maximum resolution (in less time), UPLC™ allows you to achieve both – in addition to increased sensitivity – without compromising chromatographic fidelity.

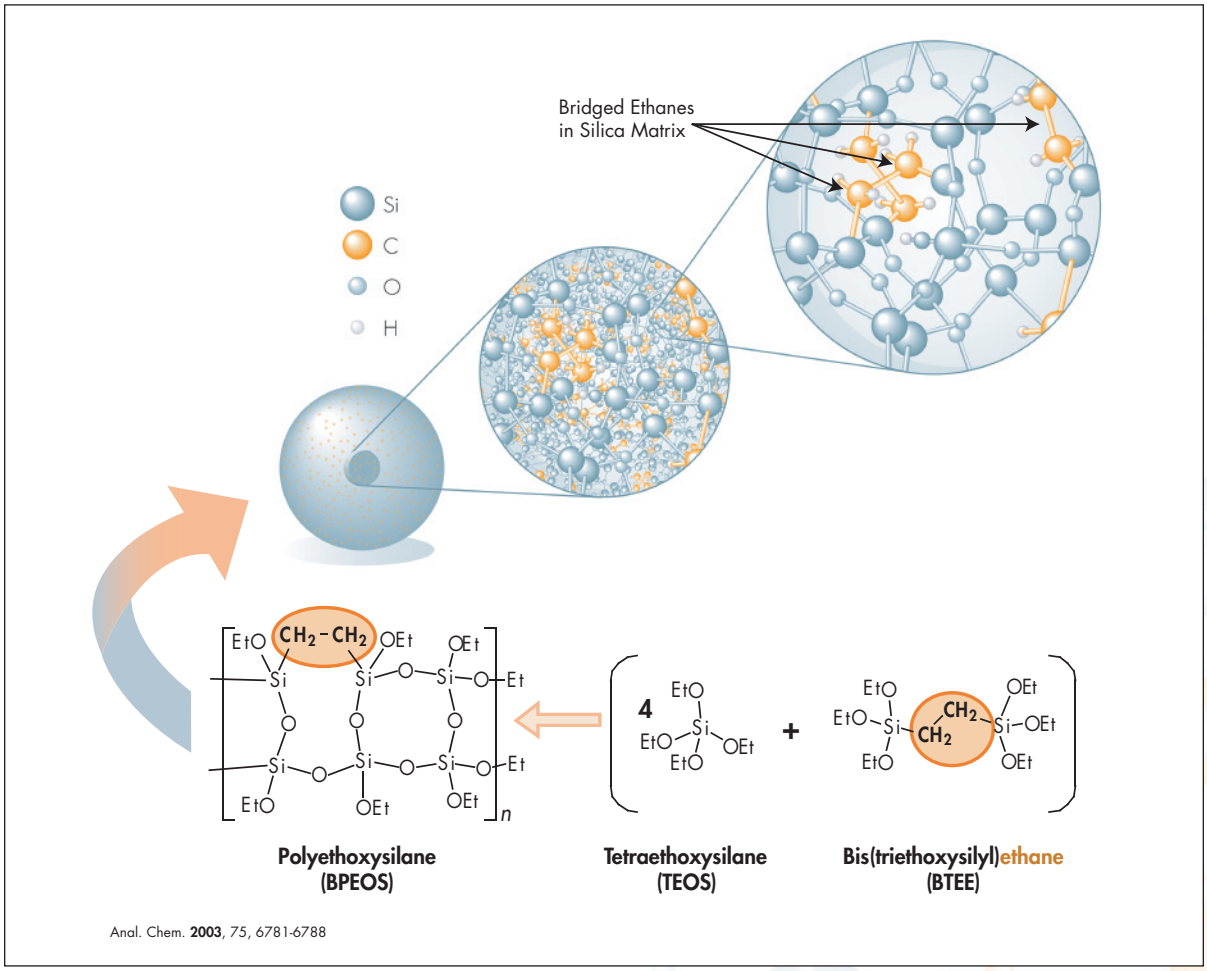
BEH Technology™

In 1999, Waters launched the XTerra® family of HPLC columns featuring first generation hybrid particle technology (HPT). Waters patented* HPT enabled XTerra® columns to become the most successful column product in the history of Waters. In HPT, the best properties of inorganic (silica) and organic (polymeric) packings are combined to produce a material that has superior mechanical strength, efficiency, high pH stability and peak shape for bases.

BEH Technology™

The first generation methyl hybrid particles of XTerra® columns did not possess the mechanical strength or efficiency necessary to realize fully the potential speed, sensitivity and resolution capabilities of UPLC™ technology. Therefore, a new pressure-tolerant particle needed to be created. A new, second generation hybrid material was developed which utilizes a bridged ethylsiloxane/silica hybrid (BEH) structure. Compared to the first generation methyl hybrid particle of XTerra® columns, the BEH particle of ACQUITY UPLC™ BEH columns exhibits improved efficiency, strength and pH range. BEH Technology™ is a key enabler of the speed, sensitivity and resolution of UPLC™ separations.

ACQUITY UPLC™ Column Performance—Enabled by BEH Technology™



Anal. Chem. **2003**, 75, 6781-6788

*Patent No. 6,686,035 B2

Why Additional ACQUITY UPLC™ BEH Chemistries?

The fundamental resolution equation for isocratic separations states that resolution (Rs) is proportional to the square root of column efficiency (N). According to chromatographic theory, column efficiency (N) is inversely proportional to particle size (dp). Thus, smaller particles provide higher resolution. The highly efficient 1.7 µm BEH particles allow chromatographers to maximize the efficiency (N) of their separation when used in the ACQUITY UPLC™ system.

$$Rs = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right)$$

System Efficiency — Selectivity — Retentivity

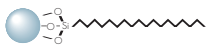
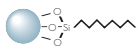
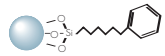
$$N \propto \frac{1}{d_p}$$

The improved efficiency (N) of a UPLC™ separation makes small changes in selectivity (α) a very powerful method development tool. These changes are now further enabled by a new expanded range of ACQUITY UPLC™ BEH chemistries.

However, the fundamental resolution equation also states that by changing the selectivity (α) or retentivity (*k*), resolution can also be improved. Lower efficiency HPLC separations required chromatographers to try at random one of the many different types of column chemistries in order to obtain an adequate separation. Since the efficiencies of UPLC™ separations are 2-3 times higher than those of HPLC separations, only a few ligands need to be explored to provide the desired separation.

The chemistries of the ACQUITY UPLC™ BEH column family were carefully chosen to produce the ideal combination of ultra-performance efficiencies, wide pH range and complementary selectivities. When combined with the increased efficiencies of UPLC™ technology, the ACQUITY UPLC™ BEH C_{18}, C_8, Shield RP_{18} and Phenyl column chemistries enable the rapid development of faster and more robust separations.

ACQUITY UPLC™ BEH Chemistry and Particle Information

	ACQUITY UPLC™ BEH Chemistry	C_{18}	C_8	Shield RP_{18}	Phenyl
					
Bonded Phase	**Ligand Type**	Trifunctional C_{18}	Trifunctional C_8	Monofunctional Embedded Polar Group	Trifunctional C_6 Phenyl
	Ligand Density*	3.1 µmol/m²	3.2 µmol/m²	3.3 µmol/m²	3.0 µmol/m²
	Carbon Load*	18%	13%	17%	15%
	Endcap Style	Proprietary	Proprietary	TMS	Proprietary
	pH Range	1-12	1-12	2-11	1-12
BEH Particle	**Pore Diameter***	130Å	130Å	130Å	130Å
	Pore Volume*	0.7 mL/g	0.7 mL/g	0.7 mL/g	0.7 mL/g
	Surface Area*	185 m²/g	185 m²/g	185 m²/g	185 m²/g
	90/10 Ratio*	1.5	1.5	1.5	1.5

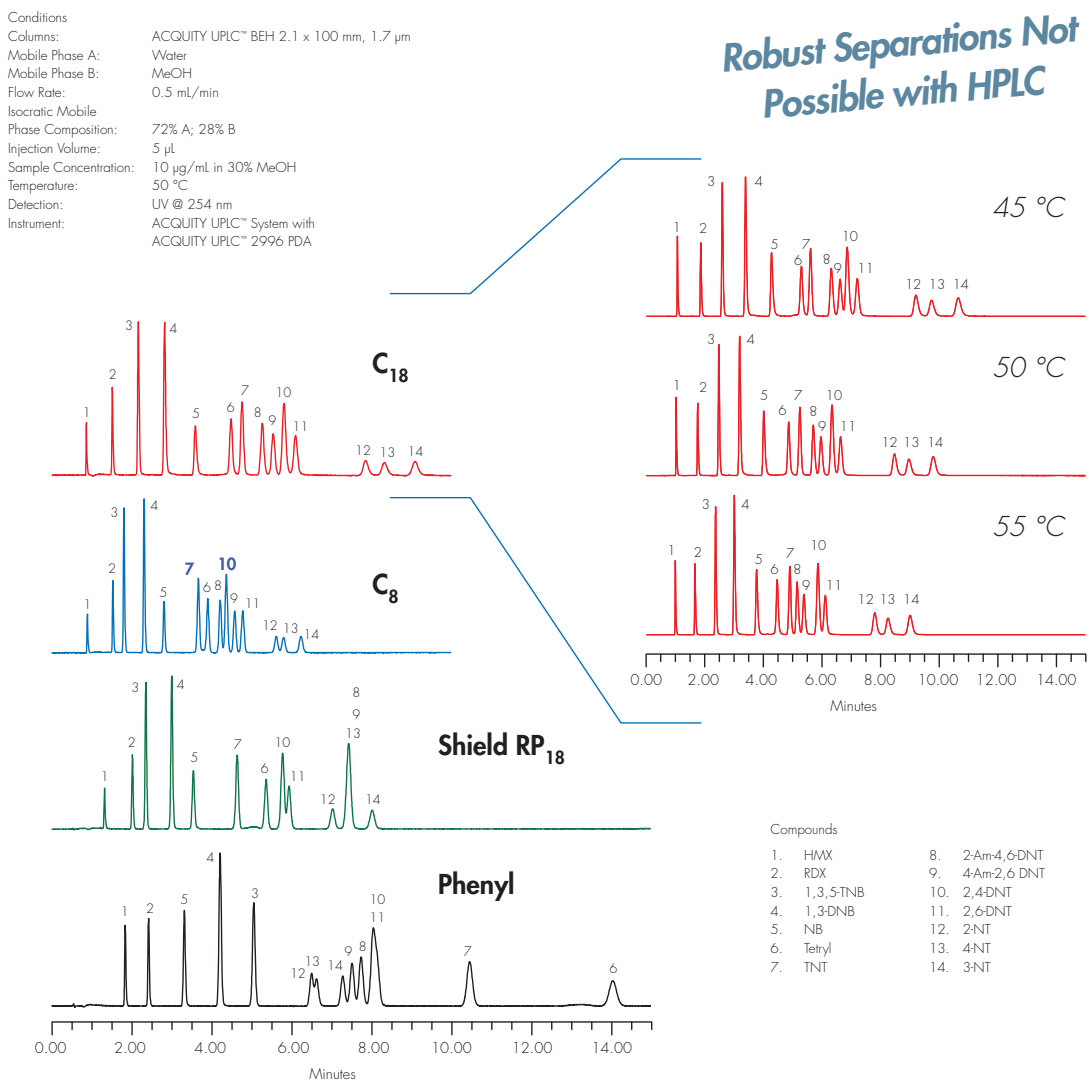
** Expected or Approximate Values*

ACQUITY UPLC™ BEH C_{18} and C_8 Columns

The vast majority of reversed-phase LC separations take place on columns that contain C_{18} or C_8 bonded stationary phases due to their stability, retentivity and reproducibility. In addition, these hydrophobic ligands most often provide the desired separation. ACQUITY UPLC™ BEH C_{18} and C_8 columns were designed to be the universal columns of choice for most UPLC™ separations by providing the widest pH range.

ACQUITY UPLC™ BEH C_{18} and C_8 columns incorporate trifunctional ligand bonding chemistries which produce superior low pH stability and ultra-low column bleed. This low pH stability is combined with the high pH stability of the 1.7 µm BEH particle to deliver the widest usable pH operating range. In addition, these new chemistries also utilize new, proprietary endcapping processes which produce outstanding peak shape for bases. These bonding chemistries and particle synthesis innovations produce the sharpest peaks, highest efficiencies and maximum MS sensitivities.

Explosives Separations on ACQUITY UPLC™ BEH Chemistries



EPA Method 8330 HPLC separations usually require temperature control of ± 1 °C since large shifts in selectivity can occur with small changes in temperature. The increased resolution of the ACQUITY UPLC™ BEH C_{18} column allows for a robust and less temperature-sensitive (up to ± 5 °C) separation.

Acquity
Ultra Performance LC™

Ordering Information

ACQUITY UPLC™ BEH Chemistry	Description	Packaging	Part Number
C_{18}	1.0 x 50 mm, 1.7 µm	Column	186002344
	1.0 x 100 mm, 1.7 µm	Column	186002346
	2.1 x 50 mm, 1.7 µm	Column	186002350
	2.1 x 100 mm, 1.7 µm	Column	186002352
	1.0 x 50 mm, 1.7 µm	3-Pack	176000861
	1.0 x 100 mm, 1.7 µm	3-Pack	176000862
	2.1 x 50 mm, 1.7 µm	3-Pack	176000863
	2.1 x 100 mm, 1.7 µm	3-Pack	176000864
C_8	1.0 x 50 mm, 1.7 µm	Column	186002875
	1.0 x 100 mm, 1.7 µm	Column	186002876
	2.1 x 50 mm, 1.7 µm	Column	186002877
	2.1 x 100 mm, 1.7 µm	Column	186002878
	1.0 x 50 mm, 1.7 µm	3-Pack	176000882
	1.0 x 100 mm, 1.7 µm	3-Pack	176000883
	2.1 x 50 mm, 1.7 µm	3-Pack	176000884
	2.1 x 100 mm, 1.7 µm	3-Pack	176000885
Shield RP_{18}	1.0 x 50 mm, 1.7 µm	Column	186002851
	1.0 x 100 mm, 1.7 µm	Column	186002852
	2.1 x 50 mm, 1.7 µm	Column	186002853
	2.1 x 100 mm, 1.7 µm	Column	186002854
	1.0 x 50 mm, 1.7 µm	3-Pack	176000874
	1.0 x 100 mm, 1.7 µm	3-Pack	176000875
	2.1 x 50 mm, 1.7 µm	3-Pack	176000876
	2.1 x 100 mm, 1.7 µm	3-Pack	176000877
Phenyl	1.0 x 50 mm, 1.7 µm	Column	186002882
	1.0 x 100 mm, 1.7 µm	Column	186002883
	2.1 x 50 mm, 1.7 µm	Column	186002884
	2.1 x 100 mm, 1.7 µm	Column	186002885
	1.0 x 50 mm, 1.7 µm	3-Pack	176000905
	1.0 x 100 mm, 1.7 µm	3-Pack	176000906
	2.1 x 50 mm, 1.7 µm	3-Pack	176000907
	2.1 x 100 mm, 1.7 µm	3-Pack	176000908

ACQUITY UPLC™ BEH Shield RP_{18} Columns

Embedded polar group (Shield) RP columns contain stationary phases that combine the hydrophobicity of an alkyl ligand with the hydrophilicity of an embedded polar group. Features of embedded polar group columns include alternate selectivity to that of alkyl reversed-phase columns, excellent peak shape for bases and aqueous mobile phase compatibility. The unique selectivity of Shield RP phases, especially for polyphenolic compounds, has been attributed to the embedded polar groups acting as hydrogen bond acceptors. ACQUITY UPLC™ BEH Shield RP_{18} columns are designed to provide selectivities that complement the ACQUITY UPLC™ BEH C_{18} and C_8 phases.

ACQUITY UPLC™ BEH Shield RP_{18} columns combine Waters patented Shield Technology* with BEH Technology™ by incorporating an embedded carbamate group into the bonded phase ligand. Waters shield ligands consistently demonstrate increased retention of phenolic compounds versus straight chain alkyl columns. The alternate selectivity and excellent peak shape from the embedded polar group ligand, when combined with the wide pH range and ultra-efficiency of the 1.7 µm BEH particle, provide a necessary and powerful tool for UPLC™ method development.

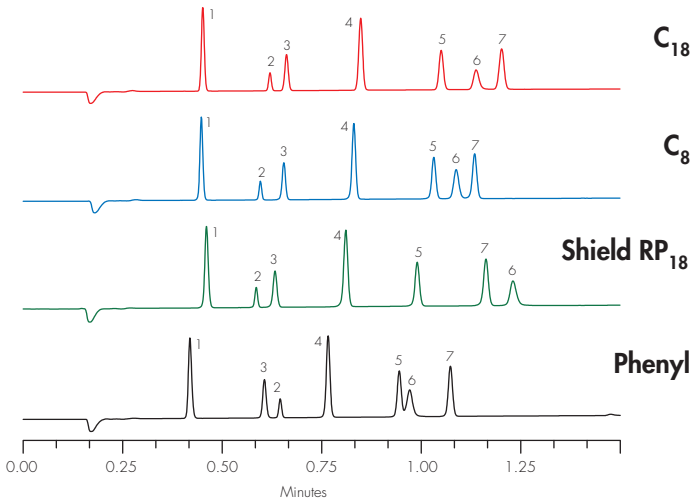
Fast Analgesics Separations

Conditions

Columns:	ACQUITY UPLC™ BEH 2.1 x 50 mm, 1.7 µm
Mobile Phase A:	0.1% HCOOH in Water
Mobile Phase B:	0.1% HCOOH in ACN
Flow Rate:	1.0 mL/min
Gradient:	Time (min) Profile %A %B Curve
	Initial 98 2 -
	1.50 75 25 5
	1.51 98 2 6
Injection Volume:	1 µL
Sample Concentration:	0.01 - 0.02 mg/mL
Temperature:	35 °C
Detection:	UV @ 240 nm
Instrument:	ACQUITY UPLC™ System with ACQUITY UPLC™ 2996 PDA

Compounds

1. Acetaminophen
2. 2-Acetamidophenol
3. Caffeine
4. Acetanilide
5. Acetylsalicylic Acid
6. Salicylic Acid
7. Phenacetin



Over-the-counter analgesics are designed purely for pain relief. Because of their low cost, effectiveness and safety, analgesics are recommended as a first line option by most physicians. Shown here are separations of seven popular analgesics in less than 1.3 minutes.

*Patent No. 5,374,755

ACQUITY UPLC™ BEH Phenyl Columns

Phenyl ligand-containing reversed-phase columns can provide complementary selectivities when compared to other straight-chain alkyl stationary phases, especially for analytes that contain aromatic rings. Traditional weaknesses of phenyl ligands include pH stability, reproducibility and peak shape. ACQUITY UPLC™ BEH Phenyl columns were intelligently designed to overcome these weaknesses and provide complementary selectivities, pH stability and excellent peak shape for all compounds.

ACQUITY UPLC™ BEH Phenyl columns utilize a trifunctional C_6 alkyl tether between the phenyl ring and the silyl functionality. This ligand, combined with the same proprietary endcapping processes as the ACQUITY UPLC™ BEH C_{18} and C_8 columns, provides ultra-low column bleed, long column lifetimes and excellent peak shape. This unique combination of ligand and endcap on the 1.7 µm BEH particle creates a new dimension in selectivity and efficiency for challenging UPLC™ separations.

Separations of Caffeic Acid Derivatives in Echinacea Purpurea

Conditions

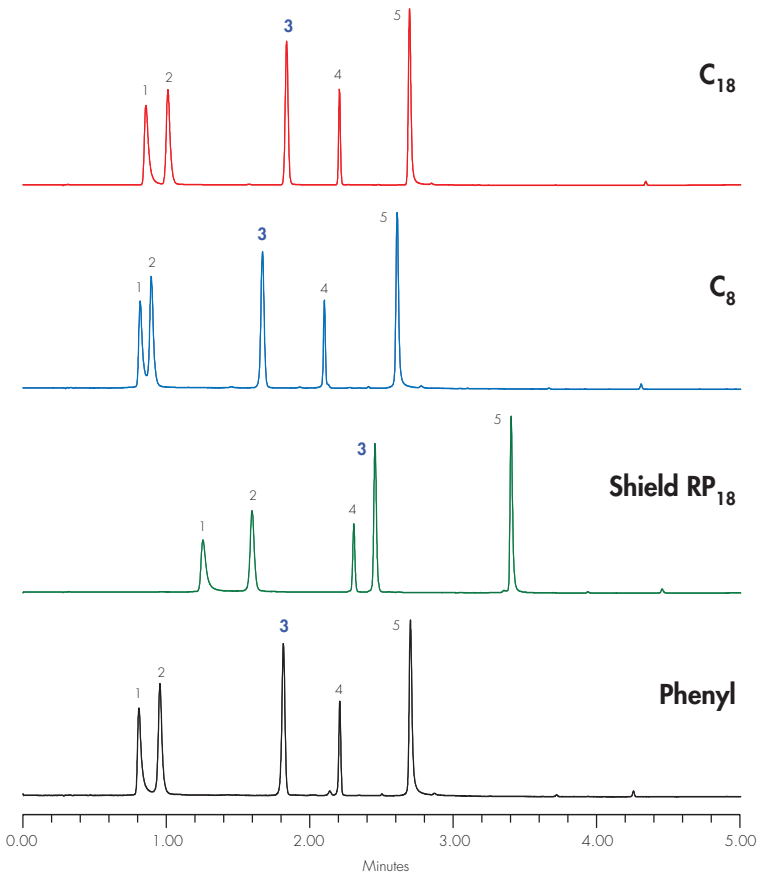
Columns:	ACQUITY UPLC™ BEH 2.1 x 50 mm, 1.7 µm
Mobile Phase A:	0.1% CF_3COOH in H_2O
Mobile Phase B:	0.08% CF_3COOH in ACN
Flow Rate:	0.5 mL/min
Gradient:	

Time (min)	Profile %A	%B	Curve
0.0	92	8	6
0.1	92	8	7
4.45	50	50	6
4.86	10	90	6
5.0	92	8	6
6.0	92	8	6

Injection Volume:	1 µL
Sample Diluent:	50:50 H_2O: MeOH with 0.05% CF_3COOH
Sample Concentration:	100 µg/mL
Temperature:	40 °C
Detection:	UV @ 330 nm
Instrument:	ACQUITY UPLC™ System with ACQUITY UPLC™ TUV

Compounds

1. Caftaric acid
2. Chlorogenic acid
3. Cynarin
4. Echinacoside
5. Cichoric acid



The active components in various Echinacea preparations can be divided into three major groups: caffeic acid derivatives, polysaccharides, and lipophilic components. Studies have shown that there is a variation in the quality of popular Echinacea-containing products. Thus, scientific monitoring of Echinacea's active ingredients is desired. Shown here are sub-four-minute separations of the primary caffeic acid derivatives—polyphenolic compounds that have been shown to possess antioxidant properties.

Long UPLC™ Column Lifetimes

The innovation of ACQUITY UPLC™ BEH columns does not stop with the development of rugged and efficient stationary phases. Another major focus was the production of stable UPLC™ columns that provide the longest possible lifetimes under UPLC™ conditions. New column hardware was designed to minimize band broadening and ensure leak-free connections. An innovative frit design was implemented in order to keep the 1.7 µm particles in the column and out of the detector or MS source. New proprietary column-packing stations and processes were developed to ensure a stable packed column bed and long, reproducible column lifetimes.

The result: Waters ACQUITY UPLC™ BEH columns provide column lifetimes under UPLC™ conditions which meet and/or exceed HPLC column lifetimes run under HPLC conditions.

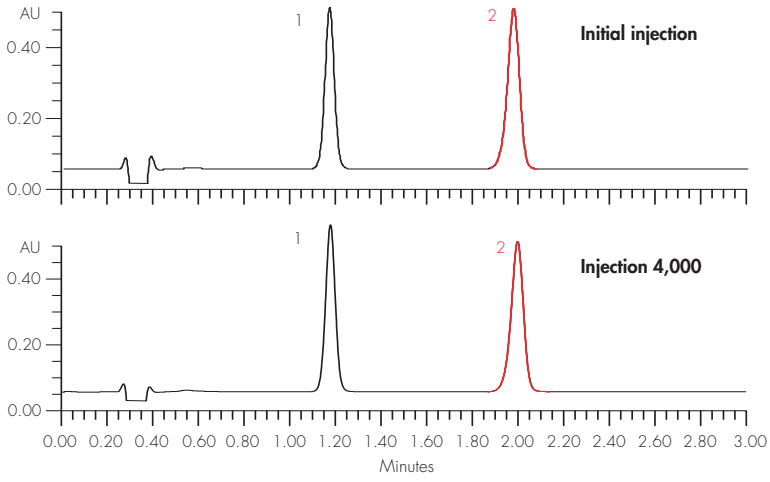
Long Column Lifetimes

Conditions

Column:	ACQUITY UPLC™ BEH Shield RP_{18} 2.1 x 50 mm, 1.7 µm
Mobile Phase A:	0.1% HCOOH in Water
Mobile Phase B:	0.1% HCOOH in ACN
Flow Rates:	Low Pressure: 0.5 mL/min (~6,000 psi)
	High Pressure: 1.25 mL/min (~13,000 psi)
Isocratic Mobile Phase Conditions:	Low Pressure: 67% A; 33% B
	High Pressure: 62% A; 38% B
Injection Volume:	5 µL
Sample Diluent:	MeOH
Temperature:	65 °C
Detection:	UV @ 225 nm
Instrument:	ACQUITY UPLC™ with ACQUITY UPLC™ TUV

Compounds
1. Ketorolac (208 µg/mL)
2. Naproxen (13 µg/mL)

Isocratic Column Aging Procedure:
1. Install and equilibrate column.
2. Set flow rate to 0.5 mL/min (6,000 psi), inject and record data.
3. Increase flow rate to 1.25 mL/min to achieve backpressure of 13,000 psi.
4. Inject 200 to 400 times.
5. Reduce flow rate to 0.5 mL/min (6,000 psi), inject and record data.
6. Cycle and repeat steps 2 – 5.



Over 4,000 Injections at 13,000 psi and 65 °C

No demonstrated losses in efficiency, peak shapes or retention times after 4,000 injections at 13,000 psi and 65 °C. The column was removed after 4,000 injections—still fully functional.

Data courtesy of Dr. Ken Wehmeyer, The Proctor & Gamble Company

Excellent Reproducibility

Waters continues to set the industry standard for column-to-column and batch-to-batch reproducibility. Beginning with the Symmetry® brand of HPLC columns in 1994 and continuing with the XTerra®, Atlantis® and SunFire™ brands, Waters columns provide consistent results, excellent peak shape and long column lifetimes. ACQUITY UPLC™ BEH columns are manufactured in the same cGMP, ISO 9002-certified facilities that produce these industry-leading HPLC column brands. Method development scientists can be assured that the UPLC™ separation produced this year can be reproduced year after year.

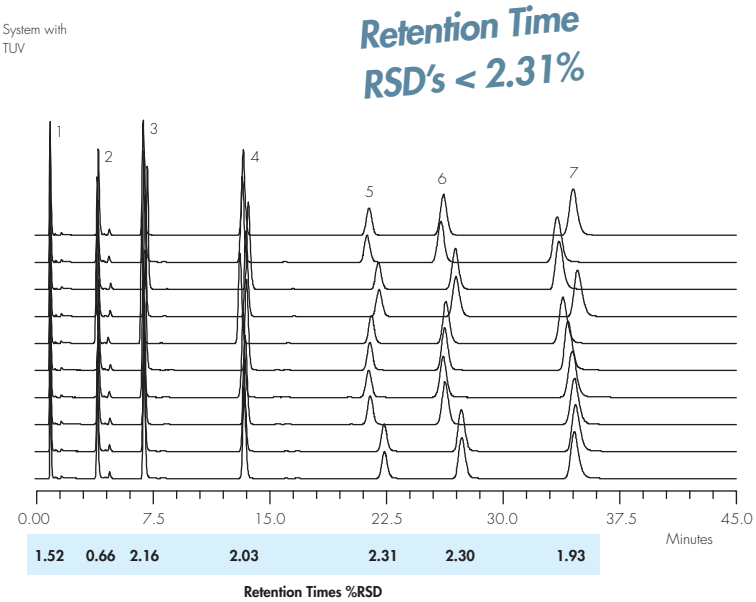
Excellent Batch-To Batch Reproducibility

Conditions

Columns:	ACQUITY UPLC™ BEH C_{18} 2.1 x 100 mm, 1.7 µm
Mobile Phase A:	20 mM KH_2PO_4/K_2HPO_4, pH 7.0
Mobile Phase B:	MeOH
Isocratic Mobile Phase Composition:	45% A; 55% B
Flow rate:	0.25 mL/min
Injection volume:	2 µL
Detection:	UV @ 254 nm
Temperature:	30.0 °C
Instrument:	ACQUITY UPLC™ System with ACQUITY UPLC™ TUV

Compounds

1. Uracil
2. Propranolol
3. Butylparaben
4. 2-Methylnaphthalene
5. Dipropylphthalate
6. Acenaphthene
7. Amitriptyline



Overlay of QC chromatograms from ten separate batches of ACQUITY UPLC™ BEH C_{18} packing material.

ACQUITY UPLC™ BEH Column Innovations

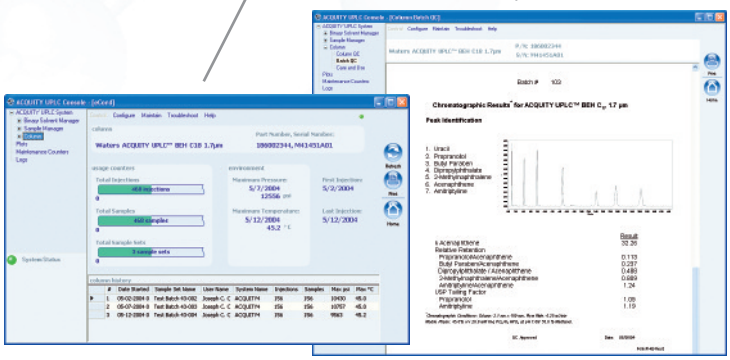
Engineering
- New column hardware
- Low band broadening
- Innovative frit design
- Leak-free connections

Bulk Synthesis
- Efficient 1.7 µm particles (BEH technology™)
- Most technologically advanced porous particles ever created
- Combination of highest efficiencies, widest pH range and superior mechanical strength
- Stable column beds at UPLC™ pressures

Software
- Paperless tracking of column history with new eCord™ technology

Column Packing
- Columns must withstand UPLC™ operating pressures
- New proprietary packing methods
- New test instruments

ACQUITY UPLC™ columns feature eCord™ technology



Columns optimized for UPLC™ separations require innovative hardware and manufacturing processes that are not apparent when simply looking at the outside of the column. Like the holistically designed ACQUITY UPLC™ system, attention to every detail was critical for success. ACQUITY UPLC™ columns were designed to be an integral part of the low bandspread UPLC™ system. The ACQUITY UPLC™ column and system could not be developed by simply "designing down" or re-engineering an existing HPLC column or system. Typical HPLC system extra-column volumes and pressure limits would severely compromise the performance of UPLC™ columns. Like the BEH particle, the ACQUITY UPLC™ column is the most technologically advanced LC column ever created.

A great deal of cutting edge manufacturing technology creates ACQUITY UPLC™ columns. Procedures for reproducibly producing and sizing commercial quantities of 1.7 µm BEH particles had to be developed. New packing stations and methods had to be invented and implemented since UPLC™ columns are packed and tested quite differently than HPLC columns. Additionally, UPLC™ instrumentation is necessary to test these columns, something that no other manufacturer possesses. Lastly, since UPLC™ technology was created with the future in mind, ACQUITY UPLC™ columns incorporate eCord™ technology — a step towards the paperless laboratory. Besides storing each column's unique Certificate of Analysis, the eCord™ tracks column usage such as date of installation, number of injections, number of sample sets, maximum temperature and pressure and the date that the column was last used. All of this information travels with the column and is easily printable. The eCord™ is permanently attached to the column and the data cannot be erased.

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For Complete Confidence

April 05, 720001140EN