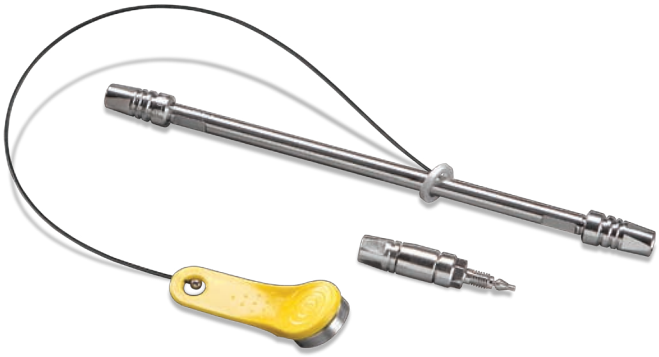
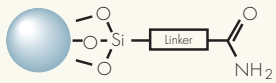
ACQUITY UPLC BEH AMIDE COLUMNS



Acquity
UltraPerformance LC®



Particle Type:	Ethylene Bridged Hybrid [BEH]
Ligand Type:	Trifunctional Amide
Particle Size:	1.7 µm
Ligand Density:	7.5 µmol/m²
Carbon Load:	12%
Endcap Style:	None
pH Range:	2-11
Pressure Tolerance:	1000 bar

[HYDROPHILIC INTERACTION CHROMATOGRAPHY]

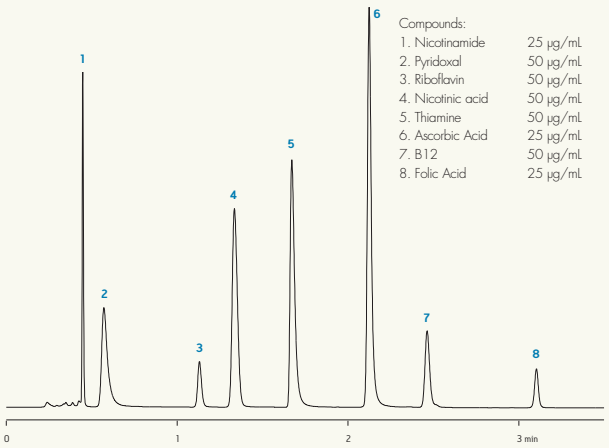
Since 2003, Waters has developed innovative stationary phases for Hydrophilic Interaction Chromatography [HILIC] to overcome the challenge of retaining and separating extremely polar compounds. Based on Waters novel ethylene bridged hybrid [BEH] particle technology, New ACQUITY UPLC® BEH Amide columns utilize a chemically stable, trifunctionally-bonded amide phase, enabling a new dimension in stability and versatility for HILIC separations.

Designed to retain polar analytes and metabolites that are too polar to retain by reversed-phase [RP] chromatography, ACQUITY UPLC BEH Amide columns facilitate the use of a wide range of mobile phase pH [2 –11] to facilitate the exceptional retention of polar analytes spanning a wide range in polarity, structural moiety and pK_a.

In addition to enhanced retention of polar compounds, ACQUITY UPLC BEH Amide columns provide increased mass spectrometry response, direct compatibility with sample preparation eluates [PPT, LLE and SPE] as well as orthogonal selectivity compared to reversed-phase materials, making HILIC an attractive alternative separation technique.

Separation of Water Soluble Vitamins

Column:	**ACQUITY UPLC BEH Amide**, 1.7 µm 2.1 x 50 mm
Part Number:	186004800
Mobile Phase A:	50/50 ACN/H_2O with 10 mM CH_3COONH_4 and 0.04% NH_4OH, pH 9.0
Mobile Phase B:	90/10 ACN/H_2O with 10 mM CH_3COONH_4 and 0.04% NH_4OH, pH 9.0
Flow Rate:	0.5 mL/min
Gradient:	Time (min) / Profile %A / %B
	0.00 / 0.1 / 99.9
	3.50 / 70.0 / 30.0
	3.51 / 0.1 / 99.9
	7.50 / 0.1 / 99.9
Injection Volume:	5.0 µL (PLNO)
Sample Diluent:	75/25 ACN/MeOH with 0.2% HCOOH
Column Temp.:	30 °C
Weak Ndl. Wash:	ACN/H_2O 95/5
Detection:	UV @ 265 nm
Sampling Rate:	20 points/sec
Filter Time Constant:	Normal
Instrument:	Waters ACQUITY UPLC with ACQUITY UPLC PDA Detector



Compounds:

1. Nicotinamide	25 µg/mL
2. Pyridoxal	50 µg/mL
3. Riboflavin	50 µg/mL
4. Nicotinic acid	50 µg/mL
5. Thiamine	50 µg/mL
6. Ascorbic Acid	25 µg/mL
7. B12	50 µg/mL
8. Folic Acid	25 µg/mL

[CARBOHYDRATE ANALYSIS]

One of the most abundant and diverse classes of compounds analyzed by HILIC are carbohydrates [e.g., monosaccharides, disaccharides, oligosaccharides and polysaccharides].

The ACQUITY UPLC BEH Amide column is an exceptional tool for the analysis of carbohydrates, providing the following benefits:

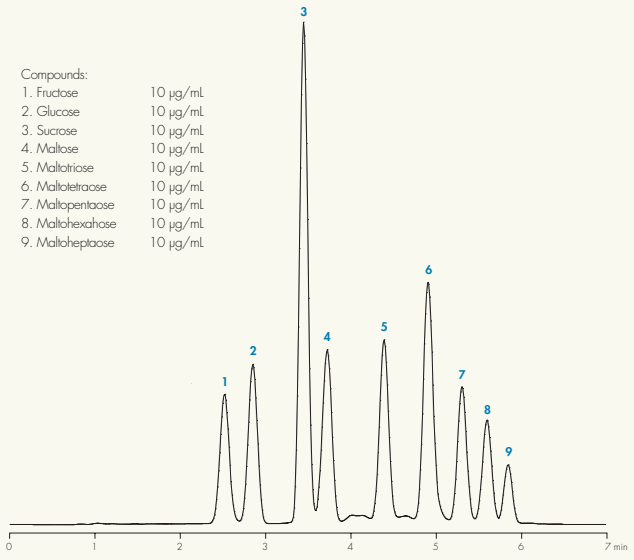
- The 1.7 µm particle size enables high resolution, high speed analysis of carbohydrates in complex sample matrices while maintaining or improving chromatographic resolution.
- Increased chemical stability enables the use of high pH and high temperature to collapse reducing sugar anomers, shorten analysis times and improve MS detection.
- BEH particle technology, in combination with a trifunctionally-bonded amide phase, provides exceptional column lifetime, thus improving assay robustness.
- Unlike amine-based columns used for carbohydrate analysis, the ACQUITY UPLC BEH Amide column is not susceptible to Schiff base formation, thus improving quantitation accuracy.
- High pH mobile phase compatibility facilitates MS detection without the need for derivatization, post column addition or complexing with a metal cation, thus dramatically simplifying sample pre-treatment before LC/MS analysis and improving assay sensitivity.

UPLC/MS Analysis of Saccharides

Column:	**ACQUITY UPLC BEH Amide**, 1.7 µm 2.1 x 50 mm
Part Number:	186004800
Mobile Phase A:	30/70 MeCN/H_2O with 0.10% NH_4OH
Mobile Phase B:	80/20 MeCN/H_2O with 0.10% NH_4OH
Flow Rate:	0.17 mL/min
Gradient:	Time (min) / %A / %B: Initial 0 / 100; 5.00 60 / 40; 5.01 0 / 100
Injection Volume:	0.7 µL (PLNO)
Sample Diluent:	50/50 MeCN/H_2O
Column Temp.:	35 °C
Needle Wash:	ACN/H_2O 75/25
Instrument:	Waters ACQUITY UPLC with ACQUITY UPLC TQD
Ionization Mode:	ES-
Capillary:	2.8 kV
Cone Voltage:	25 V
Source Temp.:	120 °C
Desolv. Temp.:	350 °C
Desolv. Gas Flow:	500 L/Hr
SIR m/z:	179.2 (fructose, glucose); 341.3 (sucrose, maltose); 503.4, 665.5, 827.6, 989.7, 115 (maltooligosaccharides [n=1 to 5])
Dwell Time:	0.08 s

Compounds:

1. Fructose	10 µg/mL
2. Glucose	10 µg/mL
3. Sucrose	10 µg/mL
4. Maltose	10 µg/mL
5. Maltotriose	10 µg/mL
6. Maltotetraose	10 µg/mL
7. Maltopentaose	10 µg/mL
8. Maltohexahose	10 µg/mL
9. Maltoheptaose	10 µg/mL



ACQUITY UPLC BEH Amide Columns

Chemistry	Particle Size	Dimension	Part Number 1 pack	Part Number 3 pack	Part Number Method Validation Kit
BEH Amide	1.7 µm	1.0 x 50 mm	186004848	176001914	—
BEH Amide	1.7 µm	1.0 x 100 mm	186004849	176001915	—
BEH Amide	1.7 µm	1.0 x 150 mm	186004850	176001916	—
BEH Amide	1.7 µm	2.1 x 30 mm	186004839	176001906	—
BEH Amide	1.7 µm	2.1 x 50 mm	186004800	176001907	186004807
BEH Amide	1.7 µm	2.1 x 100 mm	186004801	176001908	186004808
BEH Amide	1.7 µm	2.1 x 150 mm	186004802	176001909	—
BEH Amide	1.7 µm	3.0 x 30 mm	186004803	176001910	—
BEH Amide	1.7 µm	3.0 x 50 mm	186004804	176001911	186004809
BEH Amide	1.7 µm	3.0 x 100 mm	186004805	176001912	186004810
BEH Amide	1.7 µm	3.0 x 150 mm	186004806	176001913	—
BEH Amide	1.7 µm	2.1 x 5 mm*	—	186004799	—

* VanGuard Pre-column 3-pack

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