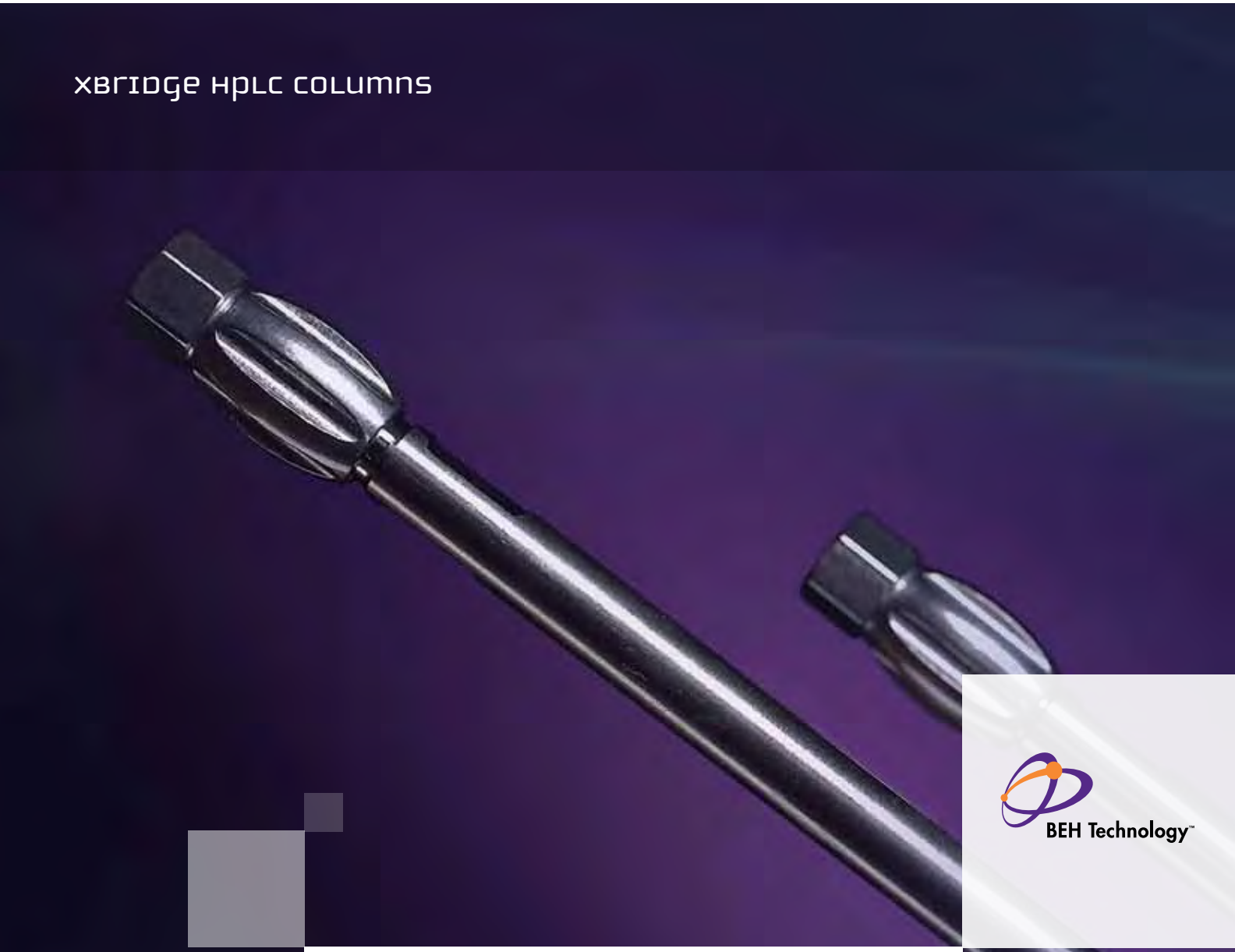
eXtreme flexibility

XBridge™ COLUMNS

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™



BEH Technology™

Reliable Performance

Separation scientists know that reproducible column performance is one of the most critical factors for method reliability. Each step of our column manufacturing process is monitored and tightly controlled to maintain unmatched product reproducibility. Every test from raw material characterization to final batch analysis ensures that the XBridge™ Column you use today will perform the same from column-to-column, batch-to-batch, and year-to-year.

Unrivaled Versatility

Designed for one purpose – to maximize your productivity.

Whether the goal is to create a quality control method or to develop a leading edge LC/MS assay, XBridge Columns help by:

- Improving pH Stability
  – increased column lifetime
- Enhancing Column Reliability
  – assay ruggedness
- Maximizing Particle Efficiency
  – unmatched peak shape and peak capacity

BEH C_{18}

BEH C_8

BEH Shield RP18

BEH Phenyl

BEH HILIC

BEH Amide

XBridge™ COLUMNS

OBD™ OPTIMUM BED DENSITY PREPARATIVE COLUMNS

BEH130 C_{18}

BEH300 C_{18}

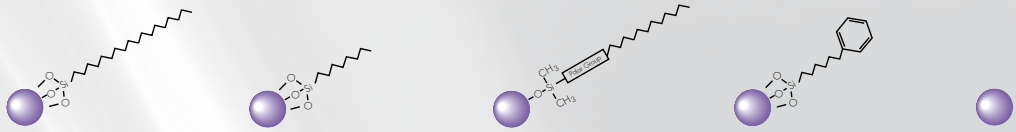
BEH300 C_4

BEH C_{18}

Unsurpassed Quality by Design

XBridge Columns are the benchmark for LC method ruggedness and longevity. They were designed to have superior pH stability over the widest pH range (1-12), high efficiencies and symmetrical peak shape. XBridge Column chemistries (C_{18}, C_8, Phenyl, Shield RP18, HILIC and Amide) are used by scientists to provide consistently accurate results for the most rigorous assays.

With a selection of 10-general-purpose and application-specific sorbents in the widest range of particle sizes, no other HPLC column family provides the necessary tools for the most demanding chromatographic challenges. Whether you require robust HPLC methods, seamless UPLC® transferability, or preparative scaling for product isolation, you can count on the versatility of XBridge Columns.



XBridge	C_{18}	C_8	Shield RP18	Phenyl	HILIC
Ligand Type	Trifunctional C_{18}	Trifunctional C_8	Monofunctional Embedded Polar	Trifunctional Phenyl-Hexyl	Unbonded BEH Particle
Ligand Density*	3.1 µmol/m²	3.2 µmol/m²	3.3 µmol/m²	3.0 µmol/m²	n/a
Carbon Load*	18%	13%	17%	15%	unbonded
Endcap Style	proprietary	proprietary	TMS	proprietary	n/a
USP Classification	L1	L7	L1	L11	L3
pH Range	1-12	1-12	2-11	1-12	1-9
Low pH Temp. Limit	80 °C	60 °C	50 °C	80 °C	45 °C
High pH Temp. Limit	60 °C	60 °C	45 °C	60 °C	45 °C
Pore Diameter*	130 Å	130 Å	130 Å	130 Å	130 Å
Surface Area*	185 m²/g	185 m²/g	185 m²/g	185 m²/g	185 m²/g
Particle Size	2.5, 3.5, 5, 10 µm	2.5, 3.5, 5, 10 µm	2.5, 3.5, 5, 10 µm	2.5, 3.5, 5 µm	2.5, 3.5, 5 µm

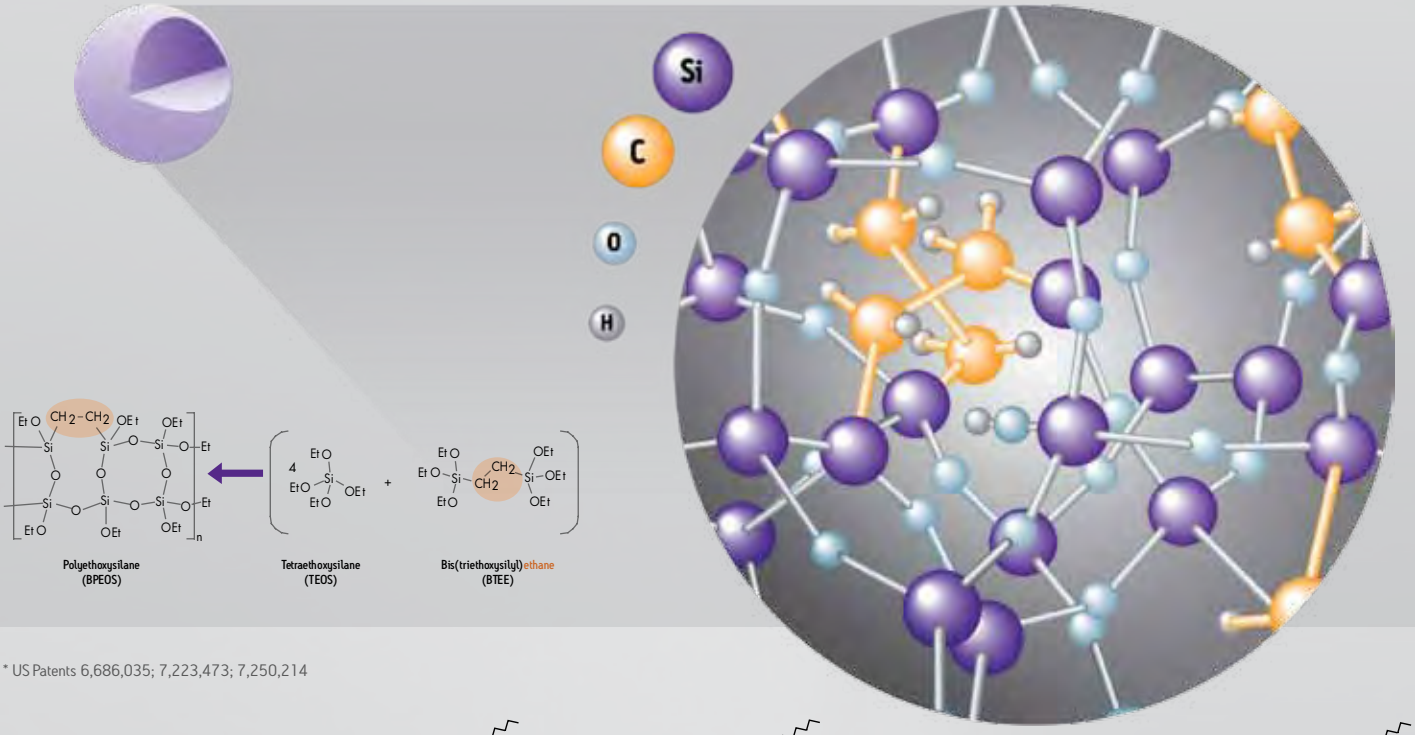
* Expected or approximate values.

Based on BEH Technology

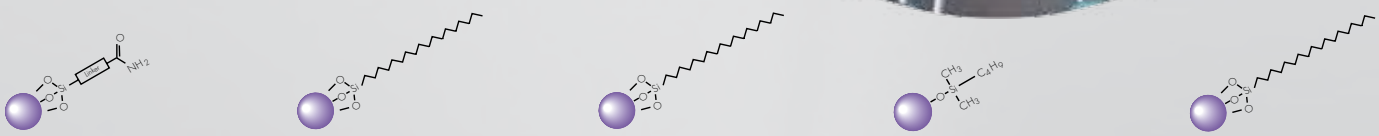
Ethylene Bridged Hybrid [BEH] Technology synthesis creates particles that ensure extreme column performance and long column lifetime under harsh operating conditions. The particle is prepared from two high purity monomers— tetraethoxysilane [TEOS] and bis(triethoxysilyl)ethane [BTEE]— resulting in a highly-stable, pH-resistant and mechanically-strong particle.

BEH Technology™

BEH Technology Particle Synthesis



* US Patents 6,686,035; 7,223,473; 7,250,214



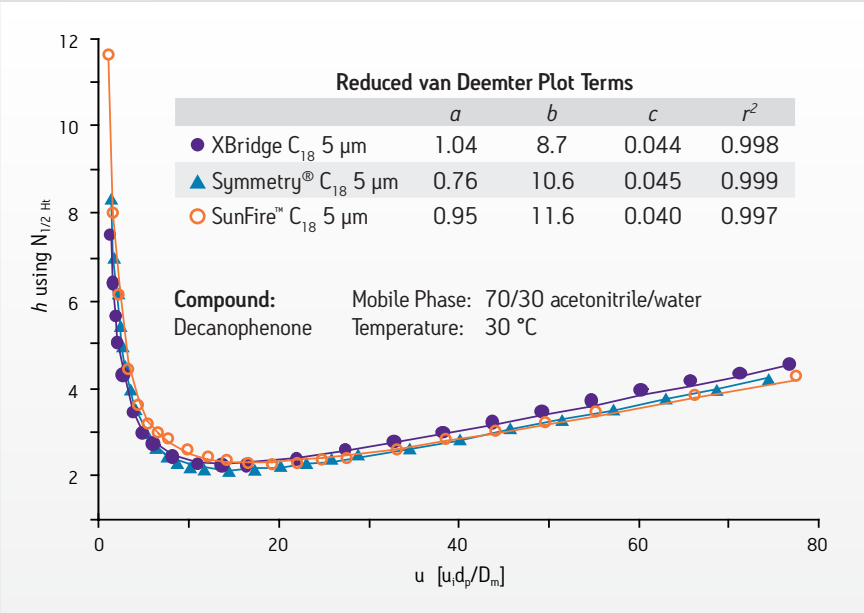
Amide	BEH130 C_{18}	BEH300 C_{18}	BEH300 C_4	BEH C_{18}
	Peptide Separation Technology	Peptide Separation Technology	Protein Separation Technology	Oligonucleotide Separation Technology
Amide	Trifunctional C_{18}	Trifunctional C_{18}	Monofunctional C_4	Trifunctional C_{18}
7.5 µmol/m²	3.1 µmol/m²	3.1 µmol/m²	2.4 µmol/m²	3.1 µmol/m²
12%	18%	12%	8%	18%
none	proprietary	proprietary	none	proprietary
-	L1	L1	L26	L1
2-11	1-12	1-12	1-10	1-12
90 °C	80 °C	80 °C	80 °C	80 °C
90 °C	60 °C	60 °C	50 °C	60 °C
130 Å	130 Å	300 Å	300 Å	130 Å
185 m²/g	185 m²/g	90 m²/g	90 m²/g	185 m²/g
2.5, 3.5 µm	3.5, 5, 10 µm	3.5, 5, 10 µm	3.5 µm	2.5 µm

Maximizing Column Efficiency and Column Lifetime

One of the most important parameters in designing the BEH particle was to significantly improve the chromatographic performance of the base particle. The origins of band spreading, which decreases separation efficiency, are described by the van Deemter equation.

The c-term in the van Deemter equation describes the mass transfer characteristics of an analyte as it interacts with the internal surface of the stationary phase. State-of-the-art silica-based phases have excellent mass transfer; however, they are limited to a narrow range of chromatographic conditions. A comparison of silica to the BEH particle reveals that hybrid materials maintain the efficiency while extending the range of usable chromatographic conditions.

van Deemter Curve Comparison



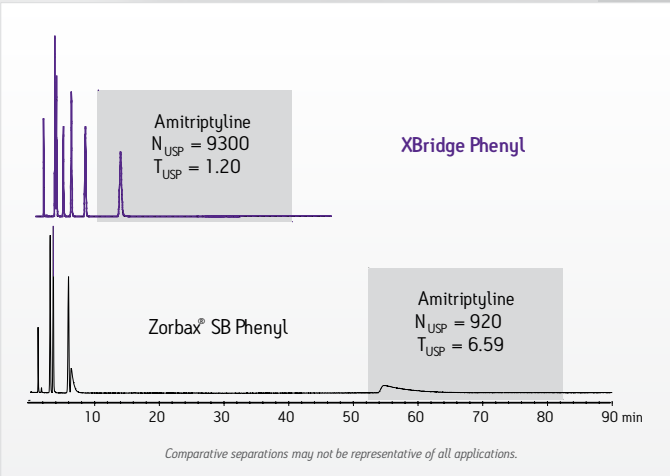
The reduced plate height, h, is a function of the reduced linear velocity, v, (both normalized for particle size) and a, b, and c summarize the contributions of eddy diffusion, longitudinal diffusion, and the sum of stationary- and mobile-phase mass transfer terms, respectively.

$$h = a + b/u + cu$$

CONTROLLED BONDING TO IMPROVE PEAK SHAPE

The ethylene bridge used during the BEH particle synthesis plays a critical role in providing improved chromatographic peak shape. The ethylene bridge links adjoining silanols. This not only increases particle strength, it reduces free silianol sites to minimize the adverse interactions with the injected sample. Traditional methods such as excessive end capping are limited to the steric hindrance of the end capping agent and bonded ligand to the active site. As a result, free silanol sites may be exposed creating broad and tailing chromatographic peaks. The ethylene bridge reduces the number of free silanols to provide a sterically favorable ratio for bonding and end capping the ligand. Controlling this process is one of the ways that XBridge Columns can provide unsurpassed peak shape performance.

Excellent Peak Shape



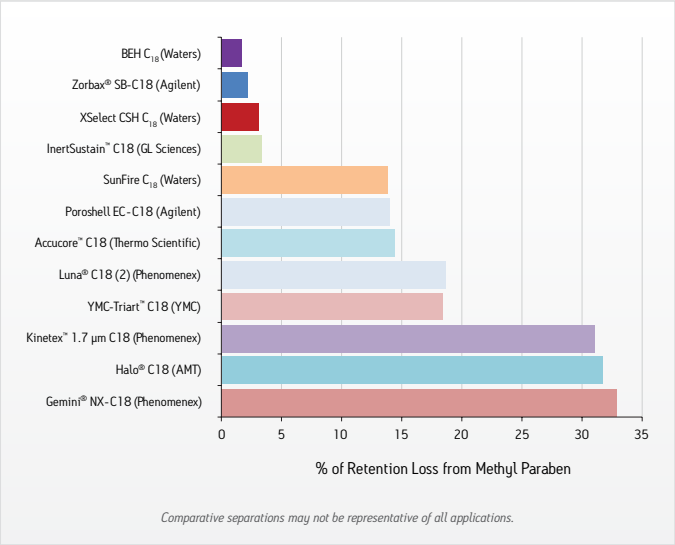
XBridge Phenyl Columns combine trifunctional bonding of the phenyl-hexyl ligand with proprietary end capping to produce industry-leading stability and exceptional peak shape.

Stability Under Extreme pH Conditions

IMPROVED PERFORMANCE AT LOW pH

The major cause of poor column lifetime in acidic (low-pH) mobile phases is due to the acid hydrolysis of the bonded phase. XBridge packing incorporates state-of-the-art, proprietary procedures for bonding and end capping resulting in ligand stability and chromatographic reproducibility at low pH. Compared to conventional materials, using an accelerated acidic mobile-phase stability test, XBridge C_{18} Columns show very little retention loss or peak shape degradation and exhibit exceptional column lifetime without resorting to sterically hindering the stationary phase.

Accelerated Acid Stability Test of Competitive Columns



XBridge packings incorporate well-characterized, state-of-the-art bonding and endcapping procedures to provide columns that are more stable using acidic-pH mobile phases compared to conventionally prepared columns.

TAKING ADVANTAGE OF PHOSPHATE BUFFERS

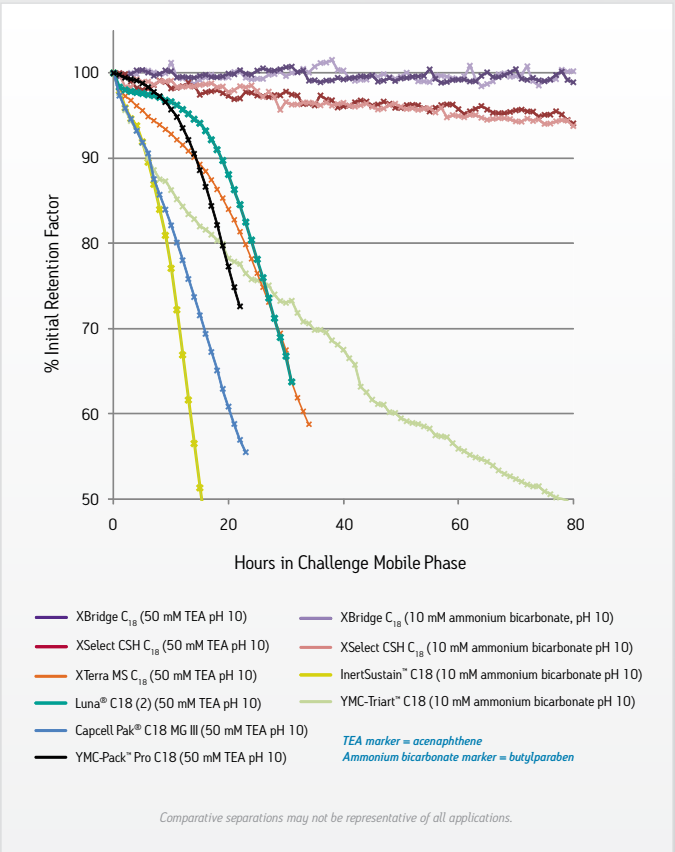
Phosphate buffers possess unique selectivity, excellent UV transparency and good buffering capacity at multiple pKa values. However, due to the aggressive nature of phosphate, traditional silica columns often exhibit significant reduced column lifetimes. XBridge Columns demonstrate excellent performance using phosphate buffers across the entire pH range by utilizing advanced bonding and end capping (acid stability) and the chemical resistance (alkaline stability) of BEH particle technology.

HIGH pH ENDURANCE

XBridge Columns are engineered to be the most pH stable and highest performing chromatographic phases commercially available. Approaches that claim high pH resistance, due to special surface modifications, cannot match the lifetime of XBridge HPLC Columns. This industry-leading stability is the product of combining the BEH particle synthesis process with advanced bonding and end-capping technologies.

Under accelerated pH 10 stability test conditions, a direct comparison to some of the most popular chromatographic phases, claiming to have extended high-pH stability, clearly shows that the XBridge C_{18} Column lifetime exceeds that of the next best column by over 1000% with very little degradation in chromatographic performance.

Accelerated High-pH Stability Test of Competitive Columns



XBridge Columns resist base particle dissolution and ligand hydrolysis when used with high-pH mobile phases. No other column family has the extended lifetime of an XBridge HPLC Column at elevated pH.

To learn more about BEH Technology,
visit **www.waters.com** and reference 720001159EN.

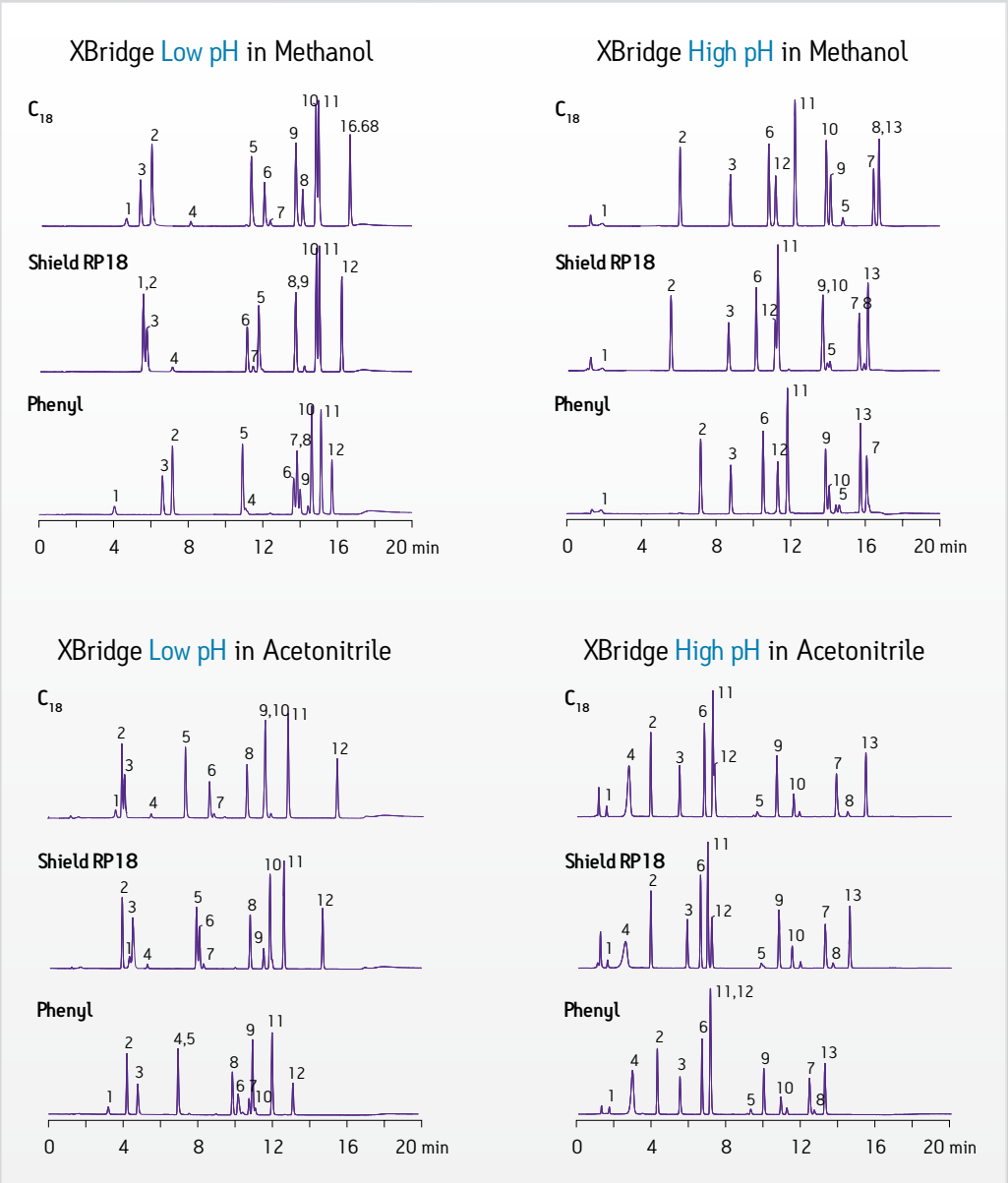
Method Development Flexibility

THE UNIVERSAL FAMILY OF HPLC COLUMNS

Selecting the most suitable column and separation conditions can be extremely difficult. XBridge HPLC Columns are designed to eliminate the compromises of sorbent selection and deliver the flexibility to work under any mobile phase, temperature and pH conditions necessary to achieve the desired separation.

A simple and effective reversed-phase method development approach consists of XBridge Columns, two mobile-phase pHs and two organic solvents. The availability of robust columns with wide useable pH ranges can quickly define the starting conditions for rugged method development. Optimized approaches save you time, effort and expense.

Recommended Method Screening using Column Chemistry, pH and Organic Modifier



By systematically screening a standard set of conditions, the separation scientist can quickly determine the best starting point for further method optimization. Using the results from this matrix, you can eliminate guesswork and maximize your method development productivity.

BENEFITS OF WIDER pH RANGE FOR INCREASED SELECTIVITY

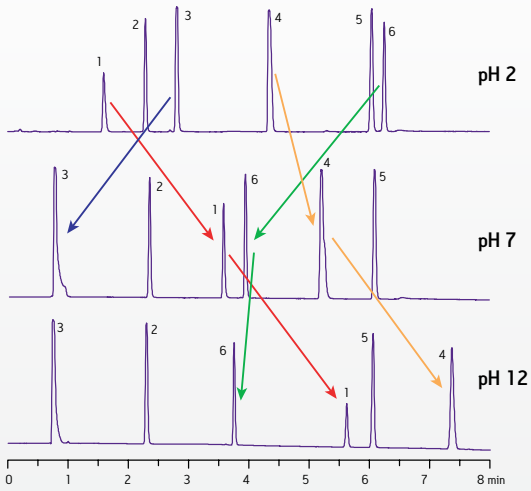
Systematic changes over a wide range of mobile-phase pH gives you the most control over analyte retention and selectivity. For maximum retention, the ionizable analyte should be in its most neutral form. For example, an acidic compound will have the most retention using acidic mobile phases. As the mobile-phase pH increases, acidic compound retention will decrease. The opposite is true for basic compound retention. High-pH mobile phases will provide the greatest retention, while acidic mobile phases will provide the least retention. For a neutral compound, mobile-phase pH has little effect.

Column: XBridge C_{18}, 4.6 x 100 mm, 3.5 µm
Mobile Phase A: 30 mM potassium phosphate buffer (pH 2, 7, 12)
Mobile Phase B: Acetonitrile
Flow Rate: 1.4 mL/min
Gradient:

Time (min)	Profile %A	%B
0.0	90	10
7.0	20	80
8.0	20	80

Instrument: Alliance® 2695 with 2996 PDA

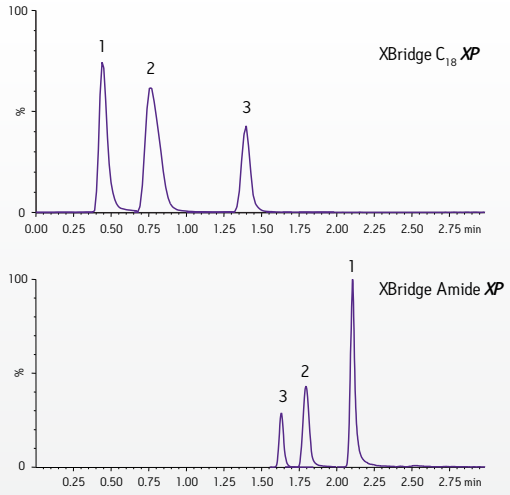
Compounds:
1. Doxylamine
2. Benzamide
3. Hydroxyisophthalic acid
4. Doxepine
5. Flavone
6. Fenoprofen



XBridge Columns are unique in their ability to withstand these aggressive conditions (even at pH 12) allowing complete flexibility for the method development chemist, while maintaining the highest efficiency values associated with silica columns.

IDEAL COLUMNS FOR LC/MS

The most sensitive bioanalytical separations rely on mass spectrometry (MS) instrumentation to achieve the low detection limits required for these assays. At low analyte concentrations, detection is much more susceptible to adverse column interactions like ligand bleed and particle shedding, both of which interfere with MS response. The BEH Technology™ incorporated into XBridge Columns virtually eliminates LC/MS bleed by providing increased hydrolytic stability and particle strength. The following example shows the chromatographic results from an analysis of neurotransmitters in a plasma sample. Note in both HILIC and reversed-phase conditions, signal integrity is maintained without MS signal suppression.



Method Conditions (RP Analysis)
Column: XBridge C_{18} ***XP***, 2.1 x 75 mm, 2.5 µm
Part Number: 186003085
Mobile Phase A: 0.1% formic acid in water
Mobile Phase B: 0.1% formic acid in acetonitrile
Flow Rate: 0.5 ml/min
Gradient:

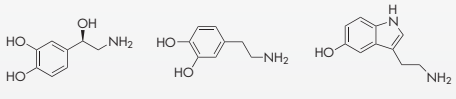
Time (min)	Profile %A	%B
Initial	100	0
0.5	100	70
2.0	70	30
2.1	100	0
3.0	100	0

Inj. Volume: 5 µL
Sample Diluent: 50/50 water/acetonitrile with 0.1% formic acid
Column Temp: 30 °C

Method Conditions (HILIC Analysis)
Column: XBridge Amide ***XP***, 2.1 x 75 mm, 2.5 µm
Part Number: 186006090
Mobile Phase A: 10 mM ammonium formate, pH 3.0
Mobile Phase B: 95/5 acetonitrile/water with 10 mM ammonium formate, pH 3.0
Flow Rate: 0.5 ml/min
Gradient:

Time (min)	Profile %A	%B
Initial	0	100
2.5	30	70
2.6	0	100
4.0	0	100

Inj. Volume: 5 µL
Column Temp.: 30 °C

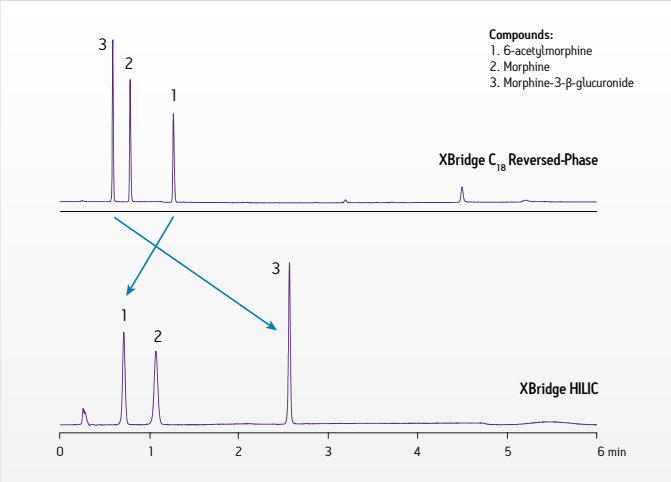


1) Norepinephrine 2) Dopamine 3) Serotonin

XBridge HILIC

COMPLEMENTARY SELECTIVITY FOR METHOD DEVELOPMENT

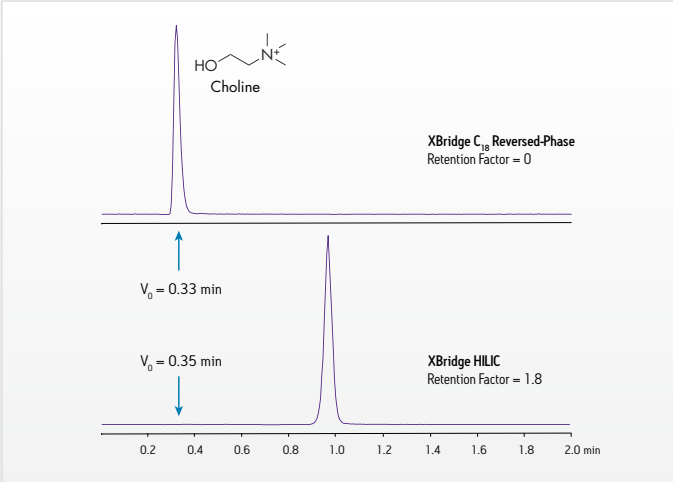
An effective method development approach uses both an XBridge HILIC Column and an XBridge Reversed-Phase Column to screen analyte selectivity and retentivity. In some cases, the results of this approach will reveal a reversal in elution order or improved retentivity, which can be advantageous when developing separations for polar analytes.



XBridge HILIC Columns provide orthogonal selectivity when compared to reversed-phase HPLC columns. As seen here, the polar metabolites of morphine are retained longer under HILIC conditions (vs. a reversed-phase separation).

ENHANCED RETENTION FOR POLAR BASES

HILIC retention mechanisms are a complex combination of partitioning, ion-exchange and hydrogen bonding, resulting in enhanced retention for polar analytes. Retention occurs when using a polar stationary phase in combination with mobile phases composed of acetonitrile concentrations greater than 80%.

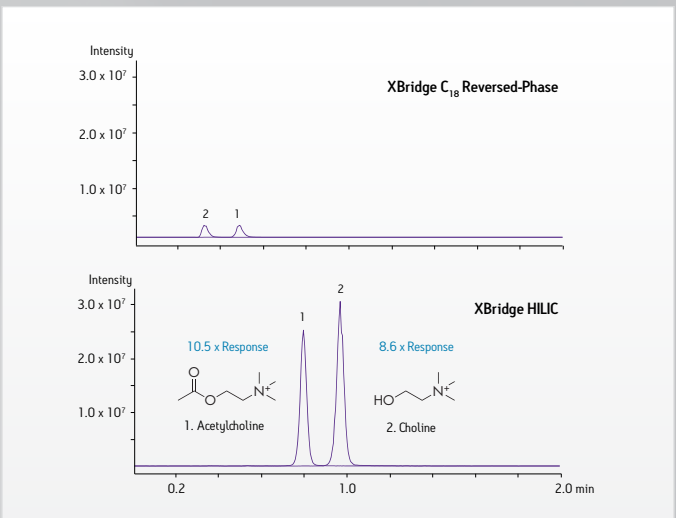


XBridge HILIC Columns provide retention for polar compounds that are too weakly retained using a traditional reversed-phase method.

ENHANCED MASS SPECTROMETRY SENSITIVITY

Utilization of HILIC has grown in popularity, primarily due to the extensive adoption of mass spectrometry as a detector and the necessity of improving sensitivity for the quantification of polar analytes. Unlike reversed-phase methods which utilize high aqueous mobile phases to induce retention of polar compounds, HILIC methods employ an acetonitrile-rich mobile phase. This high organic mobile phase is easily desolvated, resulting in improved ionization efficiency and mass spectrometry response.

Learn more about Hydrophilic-Interaction Chromatography, visit **www.waters.com** and reference 715002531.



XBridge HILIC provides enhanced mass spectrometry response resulting in lower limits of detection.

XBridge Amide

XBridge Amide Columns utilize a chemically stable, trifunctionally-bonded amide phase, enabling a new dimension in stability and versatility for HILIC separations. In addition to enhanced retention of highly polar compounds, XBridge Amide Columns are equally suited for sugar (saccharide) analysis. Some of the benefits are:

- 3.5 µm particle size for high resolution, high speed analysis of carbohydrates in complex sample matrices while maintaining or improving chromatographic resolution.
- Increased chemical stability for high-pH and high-temperature conditions to collapse anomers without the loss of reducing sugars.
- Exceptional column lifetimes and method robustness from a trifunctionally-bonded amide phase combined with BEH Technology.
- Improved quantification accuracy since XBridge Amide Columns (unlike amine-based columns) are immune to Schiff-base formation.

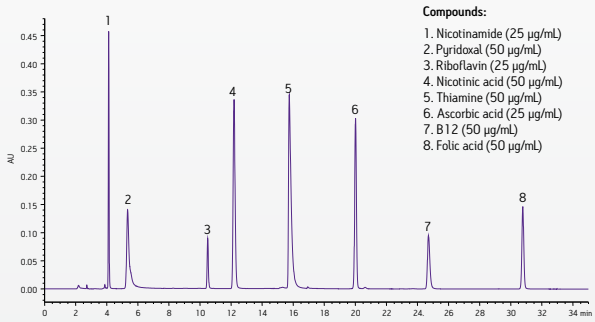
When Waters began developing HILIC chemistries in 2003, the sorbents shared one common characteristic: unbonded particles. The main separation mechanism for unbonded HILIC phases is dominated by weak-ion exchange with some partitioning as a secondary interaction. This promotes and favors retention of basic analytes. However, for a very polar compound that is acidic, neutral, or one that does not have a significant positive charge, an unbonded HILIC column may not provide the desired retention. The lack of bonded phases for HILIC separations prompted Waters to develop the amide phase for enhanced retention of acidic and neutral compounds.

Separation of Water Soluble Vitamins

Column:	XBridge Amide, 4.6 x 250 mm, 3.5 µm	Inj. Volume:	60 µL
Part Number:	186004870	Sample Diluent:	75/25 acetonitrile/methanol with 0.2% formic acid
Mobile Phase A:	50/50 acetonitrile/water with 10 mM ammonium acetate, pH 9.0	Column Temp.:	30 °C
		Needle Wash:	95/5 acetonitrile/water
		Seal Wash:	50/50 acetonitrile/water
Mobile Phase B:	90/10 acetonitrile/water with 10 mM ammonium acetate, pH 9.0	Detection:	UV @ 265 nm
		Sampling Rate:	20 points/sec
		Filter Time Constant:	0.2
Flow Rate:	1.2 mL/min	Instrument:	Alliance with 2998 PDA

Gradient:

Time (min)	Profile %A	%B
0.0	0.1	99.9
35.0	70	30
35.1	0.1	99.9
45.0	0.1	99.9



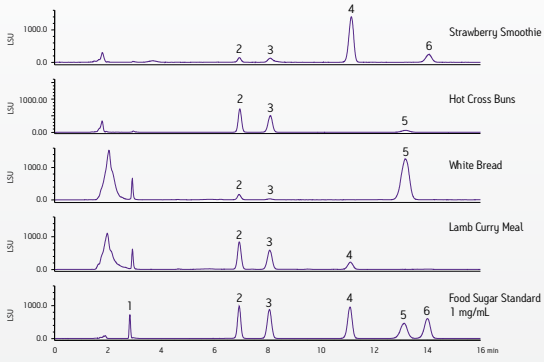
Very polar compounds, such as water soluble vitamins, are easily retained and separated using an XBridge Amide HPLC Column. The high-pH mobile phase ionizes the acidic compounds to provide more interaction between the amide ligand and analyte.

SUGAR ANALYSIS

The use of high-pH mobile phases with the XBridge Amide chemistry enables analysis of carbohydrates using electrospray negative (ES-) ionization, improving sensitivity 2-fold compared to ELS or RI detection. No metal addition, derivitization or post-column addition is necessary. These benefits cannot be achieved under acidic-pH conditions.

Column:	XBridge Amide, 4.6 x 250 mm, 3.5 µm
Part Number:	186004870
Mobile Phase A:	80/20 acetonitrile/water with 0.2 % triethylamine (TEA)
Mobile Phase B:	30/70 acetonitrile/water with 0.2 % triethylamine (TEA)
Flow Rate:	1.0 mL/min
Flow Profile:	90% A/10% B (75% acetontrile with 0.2% TEA)
Inj. Volume:	15 µL
Sample Concentration:	1 mg/mL each
Column Temp.:	35 °C
Instrument:	Alliance with 2424 ELSD

Compounds:
1. p-Toluamide
2. Fructose
3. Glucose
4. Sucrose
5. Maltose
6. Lactose

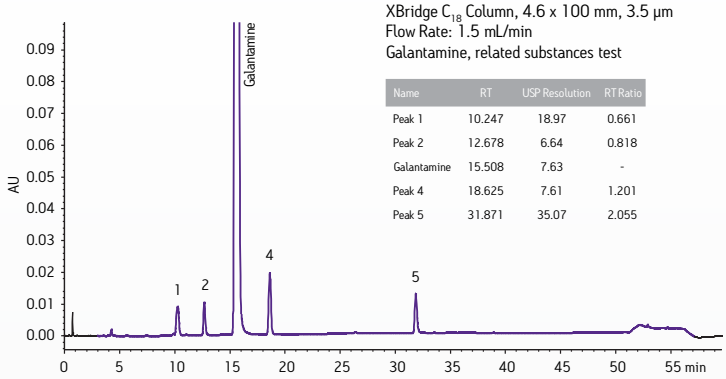


SCALABILITY

UPLC TECHNOLOGY

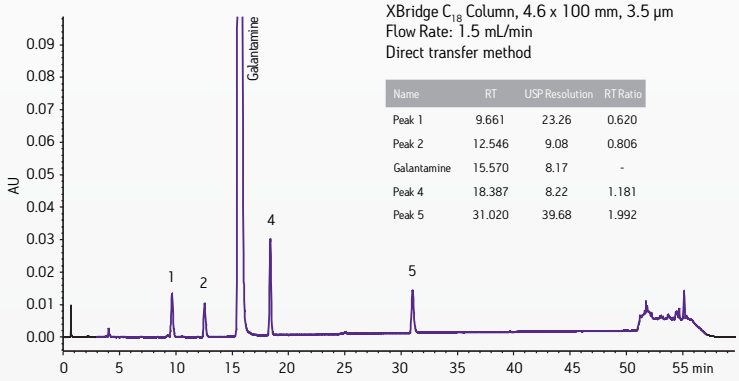
BEH Technology is one of the key enablers for UPLC separations— ACQUITY UPLC® BEH Columns and XBridge HPLC Columns are designed using a common, scalable base particle. The mechanical strength and particle integrity of BEH stationary phases provide column platforms for seamless transferability between UPLC, analytical HPLC and preparative HPLC separations. Chromatographers can take advantage of a wide range of particle sizes (i.e., 1.7, 2.5, 3.5, 5 and 10 µm) to preserve method efficacy.

HPLC Analysis on an Alliance HPLC System
XBridge C_{18} Column, 4.6 x 100 mm, 3.5 µm
Flow Rate: 1.5 mL/min
Galantamine, related substances test



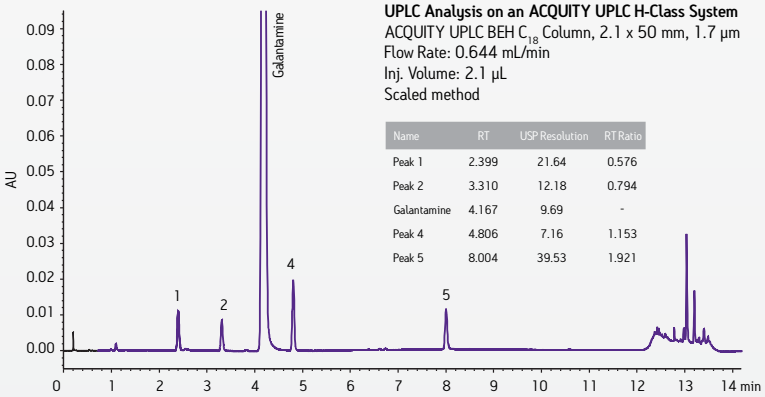
Name	RT	USP Resolution	RT Ratio
Peak 1	10.247	18.97	0.661
Peak 2	12.678	6.64	0.818
Galantamine	15.508	7.63	-
Peak 4	18.625	7.61	1.201
Peak 5	31.871	35.07	2.055

HPLC Analysis on an ACQUITY UPLC H-Class System
XBridge C_{18} Column, 4.6 x 100 mm, 3.5 µm
Flow Rate: 1.5 mL/min
Direct transfer method



Name	RT	USP Resolution	RT Ratio
Peak 1	9.661	23.26	0.620
Peak 2	12.546	9.08	0.806
Galantamine	15.570	8.17	-
Peak 4	18.387	8.22	1.181
Peak 5	31.020	39.68	1.992

UPLC Analysis on an ACQUITY UPLC H-Class System
ACQUITY UPLC BEH C_{18} Column, 2.1 x 50 mm, 1.7 µm
Flow Rate: 0.644 mL/min
Inj. Volume: 2.1 µL
Scaled method



Name	RT	USP Resolution	RT Ratio
Peak 1	2.399	21.64	0.576
Peak 2	3.310	12.18	0.794
Galantamine	4.167	9.69	-
Peak 4	4.806	7.16	1.153
Peak 5	8.004	39.53	1.921

Seemlessly transfer methods from HPLC to UPLC using XBridge HPLC and ACQUITY UPLC BEH Columns.

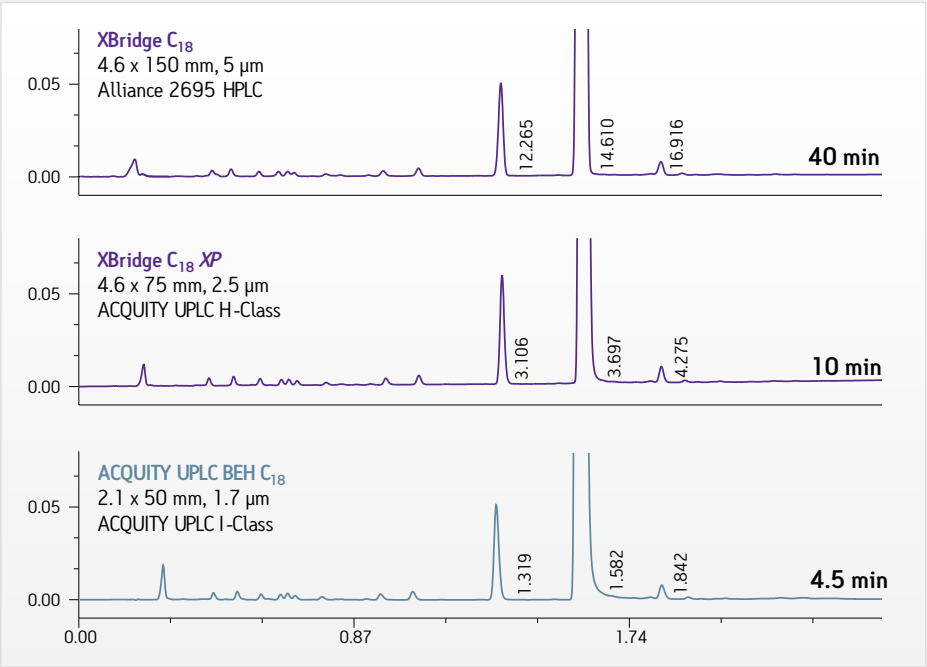
XBRIDGE *XP* 2.5 µm Columns

The availability of multiple particle sizes and dimensions allows the optimization of the total cycle time without sacrificing resolution. Methods can easily be transferred from HPLC to UPLC and from UPLC to HPLC. XBridge e*X*tended *P*erformance [*XP*] 2.5 µm Columns offer exceptional separation performance, robustness and throughput for HPLC assays while being fully compatible with all HPLC, UHPLC and UPLC Technology platforms.

Improve your existing HPLC productivity 2-4X with unmatched selectivity and flexibility:

- Directly scalable to 1.7 µm ACQUITY UPLC BEH Columns and larger 3.5/5 µm XBridge HPLC Columns
- Designed to withstand higher pressures of 9000 psi (4.6 mm ID) and 15,000 psi (2.1 and 3.0 mm ID)
- Selection of column lengths (30, 50, 75 and 100 mm) for the correct balance between resolution and throughput

High Throughput. Lower Backpressure. Improved Productivity.



*Make your HPLC system more productive with **XP** 2.5 µm Columns.. Unlike core-shell columns, methods developed on e**X**tended **P**erformance 2.5 µm Columns can be scaled and transferred to larger 3.5 and 5 µm HPLC columns, or to smaller sub-2-µm UPLC Columns, providing flexibility and method consistency between laboratories.*

Learn more about ***XP*** Columns, visit www.waters.com and reference 720004195EN.

SEAMLESSLY TRANSFER HPLC METHODS TO UPLC USING THE ACQUITY UPLC COLUMNS CALCULATOR

Available with all ACQUITY UPLC Systems, the ACQUITY UPLC Columns Calculator eliminates the guesswork of transferring methods based on gradient, column dimension and particle size.

www.waters.com/myuplc

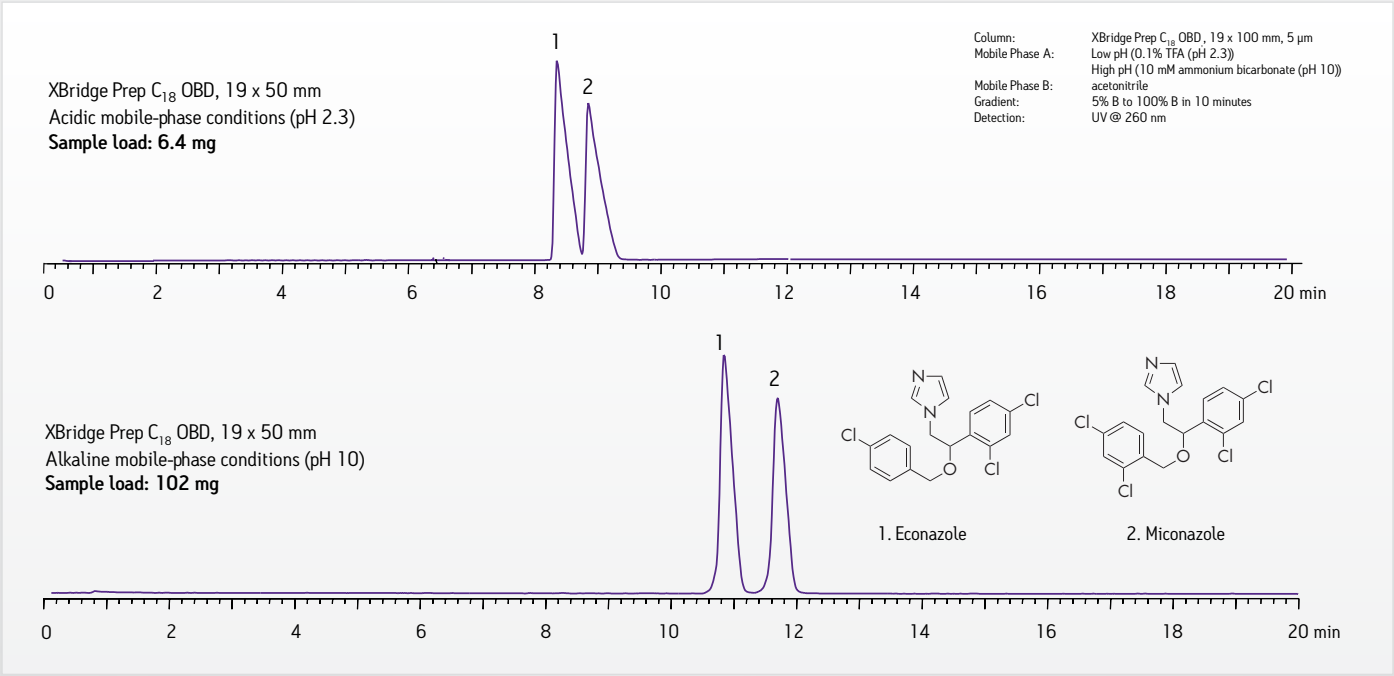
A Particle Designed for Purification

XBridge HPLC Preparative Columns contain the same packing materials that are available in UPLC and analytical HPLC dimensions, giving the purification chemist the ideal column family for quickly screening and developing high-load preparative methods. Purification chemists rely on XBridge Preparative Columns as the first choice for a highly-efficient, pH-stable column that provides low backpressures and exceptionally long column lifetimes.

XBridge Preparative Columns are designed with:

- 5- and 10-µm BEH particles providing the highest loadability with the lowest backpressure
- OBD™ Column stability to provide unsurpassed column lifetime by eliminating bed collapse
- Enhanced mass transfer that maintains particle efficiency from 1.7 µm analytical to 10 µm preparative packing materials.

Benefits of pH in Isolation and Purification



On column sample loading for basic compounds can often be increased by using high-pH mobile phases. As this example shows, conventional methods using acidic mobile phases and ion-pairing reagents provide inferior load, retention, and resolution compared to the same method using a high-pH mobile phase.

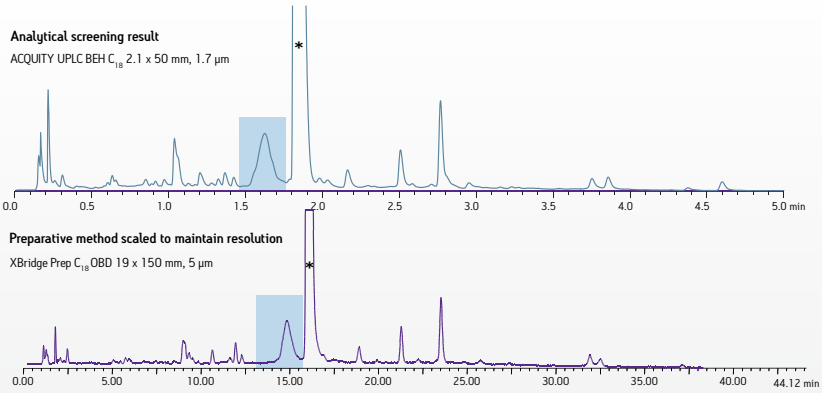
OBD TECHNOLOGY

REACHING A NEW LEVEL OF PREPARATIVE COLUMN SCALABILTY

After many years of research on preparative column packing, Waters developed the Optimum Bed Density (OBD) Preparative Column design* to effectively improve column lifetime and packed-bed stability. The OBD format revolutionized the industry by delivering the most stable, efficient, and reproducible preparative columns available. The combination of rugged XBridge packings and OBD Column design takes preparative column performance to a new level, ensuring direct scalability, maximum efficiency, and long column lifetimes.

OBD™
OPTIMUM BED DENSITY
PREPARATIVE COLUMNS

Direct Scalability



UPLC Method
Gradient:

Time	%A	%B
0.0	95	5
1.0	85	15
5.0	60	40
6.0	10	90
6.5	95	5
10.0	95	5

Flow Rate: 0.8 mL/min
Injection Volume: 5 µL

Preparative Direct Scale Method
Gradient:

Time	%A	%B
0.0	95	5
0.92	95	5
9.74	85	15
44.12	60	40
52.94	10	90
57.35	95	5
88.23	95	5

Flow Rate: 22.3 mL/min
Injection Volume: 1227 µL

Columns:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm (part number 186002350) XBridge Prep OBD C_{18}, 19 x 150 mm, 5 µm (part number 186002977)
Mobile Phase A:	10 mM ammonium bicarbonate, pH 10
Mobile Phase B:	acetonitrile
Gradients:	As indicated in the chromatograms
Sample Concentration:	2.0 mg/mL degradation sample
Detection:	UV @ 235 nm
Instrument:	ACQUITY UPLC with ACQUITY UPLC PDA, AutoPurification™ with 2996 PDA and ZQ™

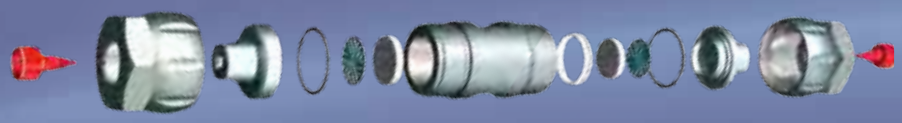
Scaling and optimization of preparative separations using XBridge Preparative OBD Columns. This example of a degradant isolation from ranitidine (API) (degradant highlighted in blue) shows that by maintaining column length to particle size ratio (L/dp), you can geometrically scale the separation to achieve a high sample load without compromising resolution.

Learn more about OBD Technology, visit **www.waters.com** and reference 720001939EN.

*UK Patent # GB2408469, US Patent # 7,399,410

OBD COLUMN DESIGN

The Prep OBD Column is designed to incorporate a pair of specially-designed distributors and chemically-inert seals made to prevent leaks at high operating pressures.



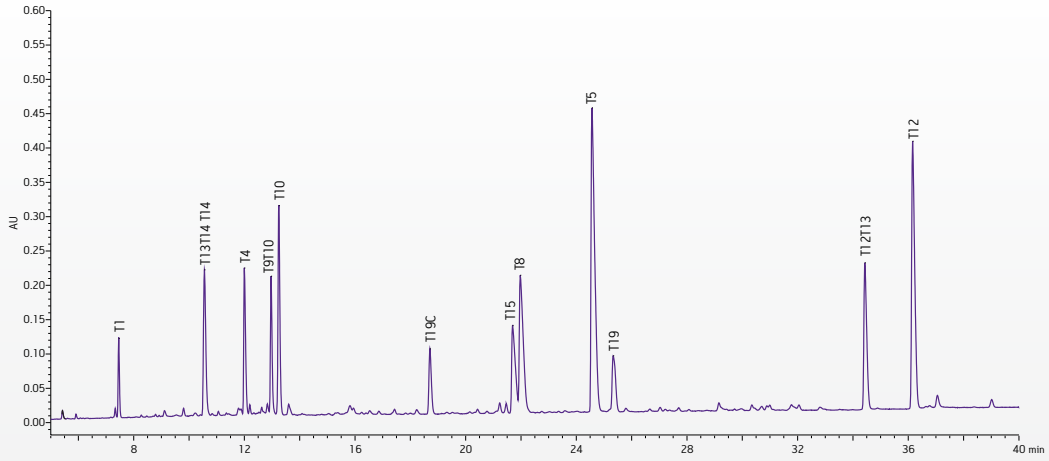
An exploded view of the elements of an empty OBD Column.

Peptide Separation Technology

Waters Peptide Separation Technology (PST) columns are quality tested with a peptide map in order to ensure the stability of peptide separation methods as well as predictable behavior with the variety of samples encountered in proteomics, protein characterization and peptide synthesis. Available in either 130 Å or 300 Å pore sizes and in particle sizes from 1.7 µm to 10 µm, XBridge PST Columns provide:

- Narrow, symmetrical peaks for maximum resolution
- Excellent separation of a wide variety of peptides
- Superior peak shape and retention in formic acid and trifluoracetic-acid mobile phases for optimal chromatography
- Seamless method migration from sub-2-µm UPLC Technology to 10 µm preparative HPLC separations.

Higher Resolution Peptide Mapping

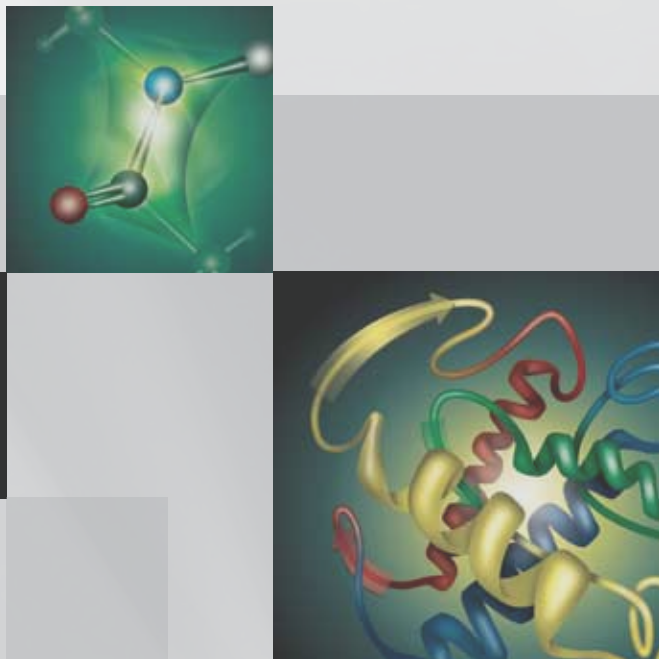


Column:	XBridge BEH130 C_{18}, 4.6 x 150 mm, 3.5 µm
Mobile Phase A:	0.045% TFA in water
Mobile Phase B:	0.045% TFA in acetonitrile
Gradient:	0% to 15% B in 9 minutes; 15 to 36% B in 30 minutes
Flow Rate:	0.96 mL/min
Column Temp.:	35 °C
Detection:	UV @ 214 nm

Compounds:

T1	N-AcGDVEK
T13-T14	KYIPGTK
T14	YIPGTK
T4	IFVQK
T9-T10	KTGQAPGFSYTDANK
T10	TGQAPGFSYTDANK
T19C	EDLIAY
T8	TGPNLHGLFGR
T15	MIFAGIK
T5	CAQCHTVEK (heme attached)
T19	EDLIAYLK
T12-T13	GITWGEETLMEYLENPKK
T12	GITWGEETLMEYLENPK

Retention time and method reproducibility are critical considerations for choosing a column suitable for peptide mapping. All XBridge PST C_{18} Columns are rigorously tested using a QC sample based on a tryptic digest of bovine cytochrome c. This insures that the column provides peptide separations spanning a broad range of hydrophobicity isoelectric points.



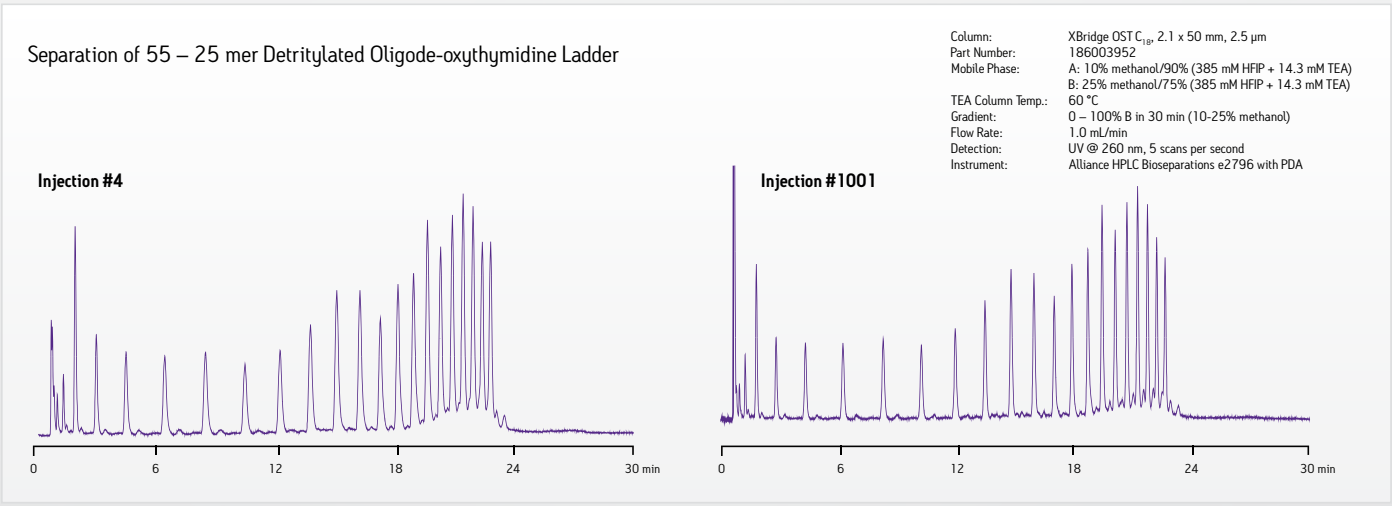
Learn more about Bioseparation Columns, visit **www.waters.com** and reference 720002148EN.

Oligonucleotide Separation Technology

Oligonucleotide Separation Technology (OST) columns are available in both 1.7 µm UPLC and 2.5 µm HPLC particle formats to provide the flexibility to meet a wide variety of isolation and analytical needs while still delivering exceptional resolution and column lifetime. XBridge OST Columns offer:

- Separation efficiencies equivalent or better than PAGE-CGE or ion-exchange HPLC methods
- The ability to resolve large oligonucleotide sequences due to the enhanced resolving power obtained using sub-3-µm particles
- Scalable column offerings for lab-scale isolation needs
- Exceptional column life for reduced cost per analysis.

Outstanding Column Lifetime of the XBridge OST Column

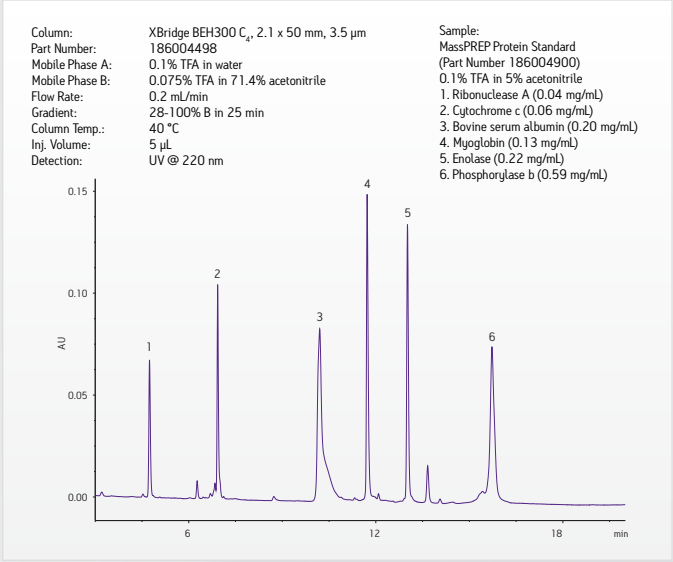


Protein Separation Technology

The analysis and characterization of protein samples requires the detection of small chemical differences between large molecules. Protein analyses use an array of chromatographic techniques including reversed-phase and size-exclusion chromatography (SEC), each being sensitive to a different property of the protein. XBridge BEH300 C_4 Columns provide:

- Protein separations based on size, hydrophobicities, and isoelectric points
- 3.5 µm packing for HPLC and 1.7 µm packing for UPLC methods
- Significant reduction of protein carryover
- Quality-control testing with a known protein mixture
- ESI-MS compatibility for protein identification.

300Å C_4 Columns Developed for Protein Chromatography



XBridge BEH300 C_4 Columns can be used with proteins that have a wide range of properties. This protein mix was chosen to represent a range of isoelectric points, molecular weights, and hydrophobicities.

Ordering Information

XBridge Analytical Columns

Dimension	Type	Particle Size	Qty.	C_{18}	C_8	Shield RP18	Phenyl	HILIC	Amide
1.0 x 50 mm	Column	2.5 µm	1/pk	186003118	186003164	186003136	186003306	—	—
2.1 x 10 mm	Guard	2.5 µm	1/pk	186003056[1]	186003074[1]	186003065[1]	186003359[1]	186004455[1]	—
2.1 x 20 mm *IS*™	Column	2.5 µm	1/pk	186003201	186003167	186003139	186003307	—	—
2.1 x 30 mm	Column	2.5 µm	1/pk	186003084	186003099	186003091	186003308	186004456	—
2.1 x 30 mm *XP*	Column	2.5 µm	1/pk	186006028	186006040	186006052	186006064	186006076	186006088
2.1 x 30 mm *XP*	Column	2.5 µm	3/pk	176002546	176002554	176002562	176002570	176002578	176002586
2.1 x 50 mm	Column	2.5 µm	1/pk	186003085	186003101	186003092	186003309	186004457	—
2.1 x 50 mm *XP*	Column	2.5 µm	1/pk	186006029	186006041	186006053	186006065	186006077	186006089
2.1 x 50 mm *XP*	Column	2.5 µm	3/pk	176002547	176002555	176002563	176002571	176002579	176002587
2.1 x 75 mm	Column	2.5 µm	1/pk	186005626	186005627	186005628	186005629	—	—
2.1 x 75 mm *XP*	Column	2.5 µm	1/pk	186006030	186006042	186006054	186006066	186006078	186006090
2.1 x 75 mm *XP*	Column	2.5 µm	3/pk	176002548	176002556	176002564	176002572	176002580	176002588
2.1 x 100 mm *XP*	Column	2.5 µm	1/pk	186006031	186006043	186006055	186006067	186006079	176002589
2.1 x 100 mm *XP*	Column	2.5 µm	3/pk	176002549	176002557	176002565	176002573	176002581	186006092
3.0 x 20 mm	Guard	2.5 µm	1/pk	186003057[2]	186003075[2]	186003066[2]	186003360[2]	—	—
3.0 x 20 mm *IS*	Column	2.5 µm	1/pk	186003087	186003168	186003140	186003310	—	—
3.0 x 30 mm	Column	2.5 µm	1/pk	186003121	186003169	186003141	186003311	—	—
3.0 x 30 mm *XP*	Column	2.5 µm	1/pk	186006032	186006044	186006056	186006068	186006080	186006092
3.0 x 30 mm *XP*	Column	2.5 µm	3/pk	176002550	176002558	176002566	176002574	176002582	176002590
3.0 x 50 mm	Column	2.5 µm	1/pk	186003122	186003170	186003142	186003312	186004458	—
3.0 x 50 mm *XP*	Column	2.5 µm	1/pk	186006033	186006045	186006057	186006069	186006081	186006093
3.0 x 50 mm *XP*	Column	2.5 µm	3/pk	176002551	176002559	176002567	176002575	176002583	176002591
3.0 x 75 mm	Column	2.5 µm	1/pk	186005630	186005631	186005632	186005633	—	—
3.0 x 75 mm *XP*	Column	2.5 µm	1/pk	186006034	186006046	186006058	186006070	186006082	186006094
3.0 x 75 mm *XP*	Column	2.5 µm	3/pk	176002552	176002560	176002568	176002576	176002584	176002592
3.0 x 100 mm *XP*	Column	2.5 µm	1/pk	186006035	186006047	186006059	186006071	186006083	186006095
3.0 x 100 mm *XP*	Column	2.5 µm	3/pk	176002553	176002561	176002569	176002577	176002585	176002593
4.6 x 20 mm	Guard	2.5 µm	1/pk	186003058[2]	186003076[2]	186003067[2]	186003361[2]	186004459[2]	—
4.6 x 20 mm *IS*	Column	2.5 µm	1/pk	186003088	186003172	186003144	186003313	—	—
4.6 x 30 mm	Column	2.5 µm	1/pk	186003089	186003173	186003145	186003314	—	—
4.6 x 30 mm *XP*	Column	2.5 µm	1/pk	186006036	186006048	186006060	186006072	186006084	186006096
4.6 x 50 mm	Column	2.5 µm	1/pk	186003090	186003174	186003096	186003315	186004460	—
4.6 x 50 mm *XP*	Column	2.5 µm	1/pk	186006037	186006049	186006061	186006073	186006085	186006097
4.6 x 75 mm	Column	2.5 µm	1/pk	186003124	186003175	186003146	186003316	186004461	—
4.6 x 75 mm *XP*	Column	2.5 µm	1/pk	186006038	186006050	186006062	186006074	186006086	186006098
4.6 x 100 mm *XP*	Column	2.5 µm	1/pk	186006039	186006051	186006063	186006075	186006087	186006099
1.0 x 50 mm	Column	3.5 µm	1/pk	186003126	186003177	186003148	186003317	186004429	186004871
1.0 x 100 mm	Column	3.5 µm	1/pk	186003127	186003178	186003149	186003318	—	—
1.0 x 150 mm	Column	3.5 µm	1/pk	186003128	186003179	186003150	186003319	—	—
2.1 x 10 mm	Guard	3.5 µm	1/pk	186003059[1]	186003077[1]	186003068[1]	186003362[1]	186004430[1]	186004857[1]
2.1 x 20 mm *IS*	Column	3.5 µm	1/pk	186003019	186003180	186003151	186003320	—	—
2.1 x 30 mm	Column	3.5 µm	1/pk	186003020	186003046	186003035	186003321	186004431	186004858
2.1 x 50 mm	Column	3.5 µm	1/pk	186003021	186003047	186003036	186003322	186004432	186004859
2.1 x 100 mm	Column	3.5 µm	1/pk	186003022	186003048	186003037	186003323	186004433	186004860
2.1 x 150 mm	Column	3.5 µm	1/pk	186003023	186003049	186003038	186003324	186004434	186004861
3.0 x 20 mm	Guard	3.5 µm	1/pk	186003060[2]	186003078[2]	186003069[2]	186003363[2]	—	—
3.0 x 20 mm *IS*	Column	3.5 µm	1/pk	186003024	186003181	186003152	186003325	—	—
3.0 x 30 mm	Column	3.5 µm	1/pk	186003025	186003182	186003153	186003326	—	186004862
3.0 x 50 mm	Column	3.5 µm	1/pk	186003026	186003050	186003039	186003327	186004435	186004863
3.0 x 100 mm	Column	3.5 µm	1/pk	186003027	186003051	186003040	186003328	186004436	186004864
3.0 x 150 mm	Column	3.5 µm	1/pk	186003028	186003052	186003041	186003329	—	—
4.6 x 20 mm	Guard	3.5 µm	1/pk	186003061[2]	186003079[2]	186003070[2]	186003364[2]	186004437[2]	186004865[2]
4.6 x 20 mm *IS*	Column	3.5 µm	1/pk	186003029	186003183	186003154	186003330	—	—
4.6 x 30 mm	Column	3.5 µm	1/pk	186003030	186003184	186003155	186003331	186004438	186004866
4.6 x 50 mm	Column	3.5 µm	1/pk	186003031	186003053	186003042	186003332	186004439	186004867
4.6 x 75 mm	Column	3.5 µm	1/pk	186003032	186003185	186003043	186003333	—	—
4.6 x 100 mm	Column	3.5 µm	1/pk	186003033	186003054	186003044	186003334	186004440	186004868
4.6 x 150 mm	Column	3.5 µm	1/pk	186003034	186003055	186003045	186003335	186004441	186004869
4.6 x 250 mm	Column	3.5 µm	1/pk	186003943	186003963	186003964	186003965	—	186004870
2.1 x 10 mm	Guard	5 µm	1/pk	186003062[1]	186003080[1]	186003071[1]	186003366[1]	186004442[1]	—
2.1 x 20 mm *IS*	Column	5 µm	1/pk	186003107	186003186	186003156	186003336	—	—

[1] Requires 2.1 x 10 mm Universal Sentry Guard Holder, Part No. WAT097958

[2] Requires 3.0 x 20 mm/4.6 x 20 mm Universal Sentry Guard Holder, Part No. WAT046910

XBridge Analytical Columns

Dimension	Type	Particle Size	Qty.	C_{18}	C_8	Shield RP18	Phenyl	HILIC	Amide
2.1 x 30 mm	Column	5 µm	1/pk	186003129	186003187	186003157	186003337	186004443	—
2.1 x 50 mm	Column	5 µm	1/pk	186003108	186003011	186002999	186003338	186004444	—
2.1 x 100 mm	Column	5 µm	1/pk	186003109	186003012	186003002	186003339	186004445	—
2.1 x 150 mm	Column	5 µm	1/pk	186003110	186003013	186003003	186003340	186004446	—
3.0 x 20 mm	Guard	5 µm	1/pk	186003063[2]	186003081[2]	186003072[2]	186003367[2]	—	—
3.0 x 20 mm *IS*	Column	5 µm	1/pk	186003130	186003188	186003158	186003341	—	—
3.0 x 30 mm	Column	5 µm	1/pk	186003111	186003189	186003159	186003342	—	—
3.0 x 50 mm	Column	5 µm	1/pk	186003131	186003190	186003160	186003343	186004447	—
3.0 x 100 mm	Column	5 µm	1/pk	186003132	186003191	186003004	186003344	186004448	—
3.0 x 150 mm	Column	5 µm	1/pk	186003112	186003014	186003005	186003345	—	—
3.0 x 250 mm	Column	5 µm	1/pk	186003133	186003192	186003161	186003346	—	—
4.6 x 20 mm	Guard	5 µm	1/pk	186003064[2]	186003082[2]	186003073[2]	186003368[2]	186004449[2]	—
4.6 x 20 mm *IS*	Column	5 µm	1/pk	186003134	186003193	186003162	186003347	—	—
4.6 x 30 mm	Column	5 µm	1/pk	186003135	186003194	186003163	186003348	186004450	—
4.6 x 50 mm	Column	5 µm	1/pk	186003113	186003015	186003006	186003349	186004451	—
4.6 x 75 mm	Column	5 µm	1/pk	186003114	186003195	186003007	186003350	—	—
4.6 x 100 mm	Column	5 µm	1/pk	186003115	186003016	186003008	186003351	186004452	—
4.6 x 150 mm	Column	5 µm	1/pk	186003116	186003017	186003009	186003352	186004453	—
4.6 x 250 mm	Column	5 µm	1/pk	186003117	186003018	186003010	186003353	186004454	—

[1] Requires 2.1 x 10 mm Universal Sentry Guard Holder, Part No. WAT097958

[2] Requires 3.0 x 20 mm/4.6 x 20 mm Universal Sentry Guard Holder, Part No. WAT046910

XBridge Preparative Columns

Dimension	Type	Particle Size	C_{18}	C_8	Shield RP18	Phenyl	HILIC
10 x 10 mm	Guard	5 µm	186002972[3]	186002991[3]	186002983[3]	186003354[3]	186004720[3]
10 x 50 mm	Column	5 µm	186002973	186003264	186003257	186003271	186004721
10 x 100 mm	Column	5 µm	186003255	186003265	186003258	186003272	186004722
10 x 150 mm	Column	5 µm	186002974	186003266	186003259	186003273	—
10 x 250 mm	Column	5 µm	186003256	186003267	186003260	186003274	—
19 x 10 mm	Guard	5 µm	186002975[4]	186002992[4]	186002984[4]	186003355[4]	186004723[4]
OBD 19 x 50 mm	Column	5 µm	186002977	186002993	186002985	186003356	186004724
OBD 19 x 100 mm	Column	5 µm	186002978	186002994	186002986	186003357	186004725
OBD 19 x 150 mm	Column	5 µm	186002979	186002995	186002987	186003358	186004726
OBD 19 x 250 mm	Column	5 µm	186004021	186004023	186004022	186004024	186004730
OBD 30 x 50 mm	Column	5 µm	186002980	186002996	186002988	186003277	186004727
OBD 30 x 75 mm	Column	5 µm	186002981	186003269	186003262	186003278	—
OBD 30 x 100 mm	Column	5 µm	186002982	186002997	186002989	186003279	186004728
OBD 30 x 150 mm	Column	5 µm	186003284	186003083	186002990	186003276	186004729
OBD 30 x 250 mm	Column	5 µm	186004025	—	—	—	186004731
OBD 50 x 50 mm	Column	5 µm	186003933	186003934	186003935	186003936	186004732
OBD 50 x 100 mm	Column	5 µm	186003937	186003938	186003939	186003940	186004733
OBD 50 x 150 mm	Column	5 µm	186003929	—	—	—	186004734
OBD 50 x 250 mm	Column	5 µm	186004107	—	—	—	186004735
10 x 10 mm	Guard	10 µm	186003889[3]	186004003[3]	186003988[3]	—	—
10 x 150 mm	Column	10 µm	186003890	186004004	186003989	—	—
10 x 250 mm	Column	10 µm	186003891	186004005	186003990	—	—
19 x 10 mm	Guard	10 µm	186003892[4]	186004006[4]	186003991[4]	—	—
OBD 19 x 50 mm	Column	10 µm	186003893	186004007	186003992	—	—
OBD 19 x 100 mm	Column	10 µm	186003901	186004008	186003993	—	—
OBD 19 x 150 mm	Column	10 µm	186003894	186004009	186003994	—	—
OBD 19 x 250 mm	Column	10 µm	186003895	186004010	186003995	—	—
OBD 30 x 75 mm	Column	10 µm	186004711	—	—	—	—
OBD 30 x 100 mm	Column	10 µm	186003930	—	—	—	—
OBD 30 x 150 mm	Column	10 µm	186003896	186004011	186003996	—	—
OBD 30 x 250 mm	Column	10 µm	186003897	186004012	186003997	—	—
OBD 50 x 50 mm	Column	10 µm	186003898	186004013	186003998	—	—
OBD 50 x 100 mm	Column	10 µm	186003902	186004014	186003999	—	—
OBD 50 x 150 mm	Column	10 µm	186003899	186004015	186004001	—	—
OBD 50 x 250 mm	Column	10 µm	186003900	186004016	186004002	—	—

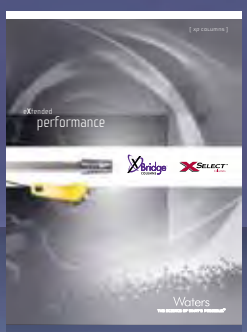
[3] Requires 10 x 10 mm Prep Guard Holder, Part No. 289000779

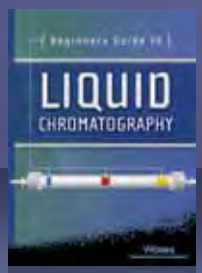
[4] Requires 19 x 10 mm Prep Guard Holder, Part No. 186000709

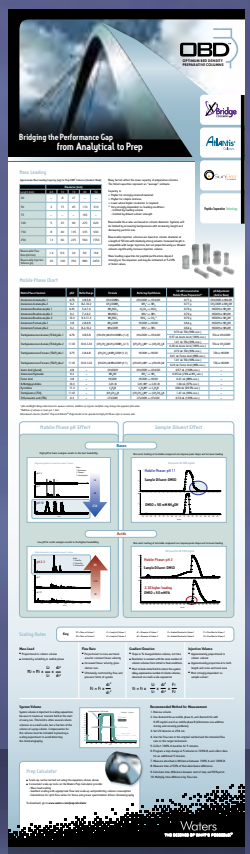
XBridge Columns Method Validation Kits— Each Method Validation Kit contains 3 columns, each from a different batch.					
Dimension	**Particle Size**	**C_{18}**	**C_8**	**Shield RP18**	**Phenyl**
2.1 x 100 mm	3.5 µm	186003766	186003777	186003788	186003799
3.0 x 100 mm	3.5 µm	186003767	186003778	186003789	186003800
3.0 x 150 mm	3.5 µm	186003768	186003779	186003790	186003801
4.6 x 100 mm	3.5 µm	186003769	186003780	186003791	186003802
4.6 x 150 mm	3.5 µm	186003770	186003781	186003792	186003803
2.1 x 150 mm	5 µm	186003771	186003782	186003793	186003804
3.0 x 100 mm	5 µm	186003772	186003783	186003794	186003805
3.0 x 150 mm	5 µm	186003773	186003784	186003795	186003806
4.6 x 100 mm	5 µm	186003774	186003785	186003796	186003807
4.6 x 150 mm	5 µm	186003775	186003786	186003797	186003808
4.6 x 250 mm	5 µm	186003776	186003787	186003798	186003809

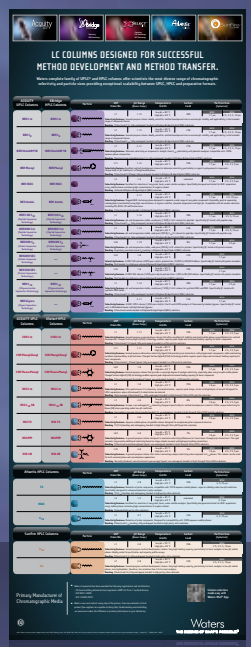
LITERATURE REFERENCES

Description	**Part Number**
Analytical Columns Wall Chart	720002241EN
A Review of Waters' Bonded Phase Shield Technology and its Use in High Performance Liquid Chromatography (HPLC) White Paper	720000207EN
A Review of Waters Hybrid Particle Technology. Part 2. Ethylene-Bridged [BEH Technology] Hybrids and Their Use in Liquid Chromatography White Paper	720001159EN
Beginner's Guide to Liquid Chromatography	715001531
Bioseparations and Analyses Brochure	720002148EN
Comprehensive Guide to HILIC	715002531
Optimum Bed Density (OBD) Columns: Enabling Technology for Laboratory-Scale Isolation and Purification White Paper	720001939EN
Preparative OBD Columns Wall Chart	720002117EN
Preparative Optimum Bed Density (OBD) Columns Brochure	720002336EN
XP Columns Brochure	720004195EN









Electronic Tools

Waters Reversed-Phase Column Selectivity Chart

www.waters.com/selectivitychart

Waters Column Advisor

www.waters.com/columnadvisor

Waters Part Selector & Selectivity Chart for iPad®

www.waters.com/apps

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January 2012 720001255EN SC-FP