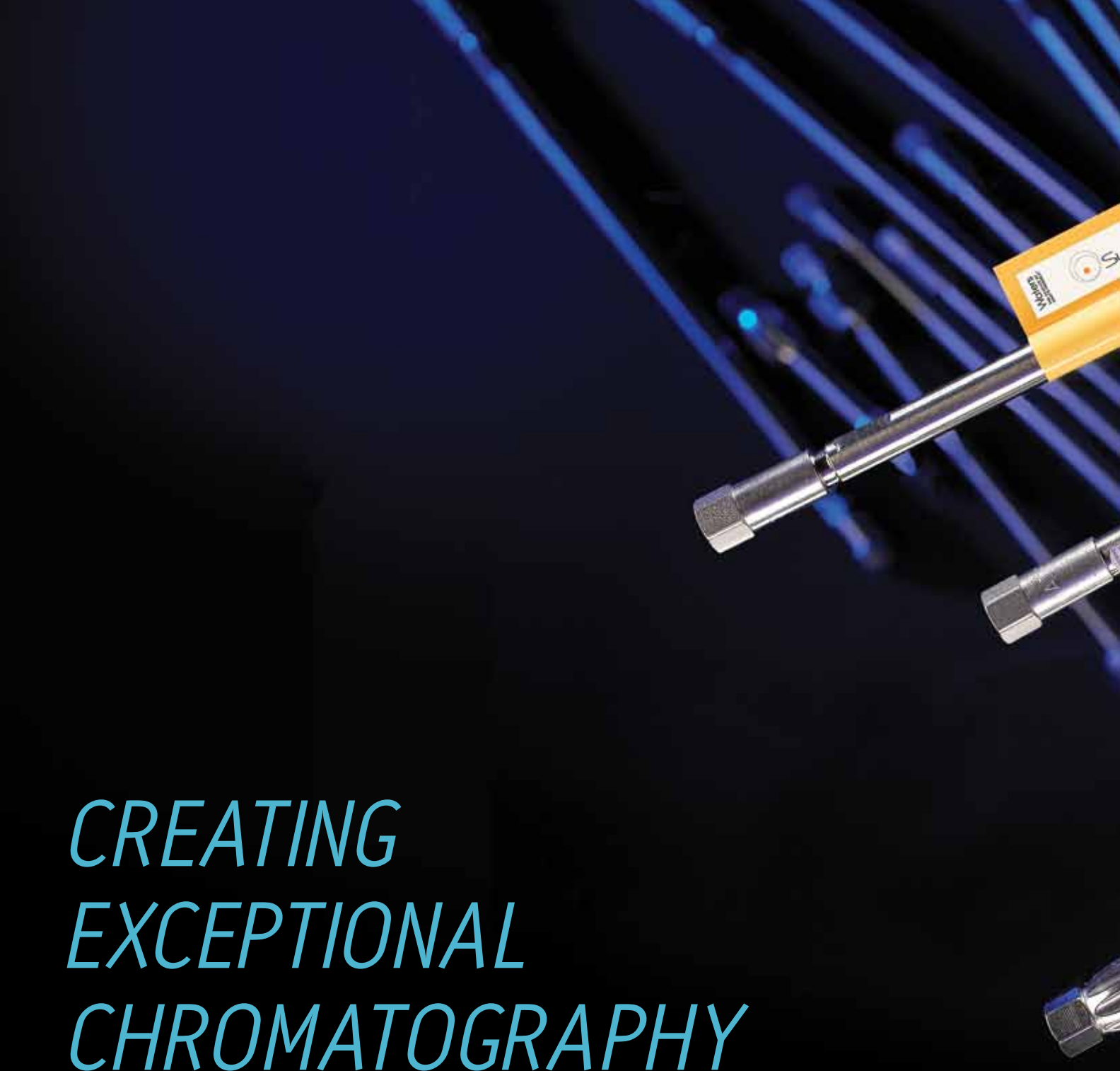
NEW INNOVATIONS ARE ELEVATING HPLC CAPABILITIES.

IS YOUR LABORATORY READY?

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™



CREATING EXCEPTIONAL CHROMATOGRAPHY

Waters reputation is based on chromatography, but we do not create chromatography — You do. Innovative thinking within your laboratory creates the chromatographic methods and the assays that sustain your business. The metric of your success is monitored by the methods and the results that you produce and the HPLC column that you choose today needs to support your success for the future. Waters full line of state-of-the-art reversed-phase and HILIC HPLC columns are chosen by scientists who understand that performance and innovation are linked and their success depends on them.


Atlantis
XBridge
XSelect

XBRIDGE HPLC COLUMNS

XBridge™ HPLC columns are designed for one purpose – to maximize your productivity. Whether your goal is to create a quality control method or to develop a leading edge LC/MS assay, XBridge columns are designed to help you by:

Improving pH Stability

- increased column lifetime

Improving Column Reliability

- assay ruggedness

Maximizing Particle Efficiency

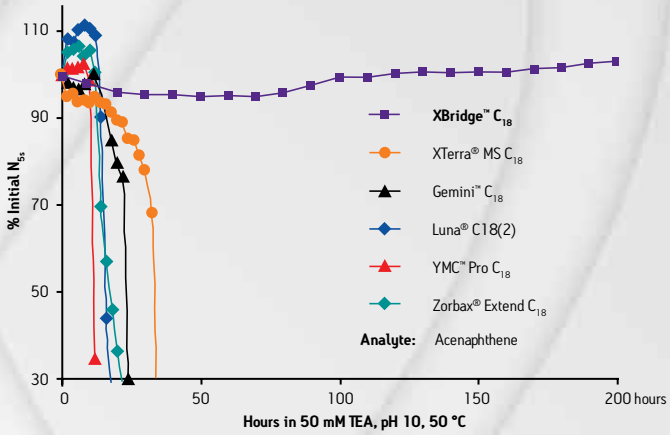
- unmatched peak shape and peak capacity

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

pH Stability

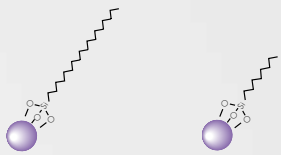
XBridge columns have been specifically designed to contain the most chemically stable chromatographic sorbent available allowing you to explore the full benefits of a wide pH (1-12) mobile-phase range. Chemical stability, especially for the extremes of pH, is built into the particle during the synthesis process and it cannot be duplicated using a conventional silica-based bonding process. No other column can match the chemical stability of an XBridge column.

Accelerated High pH Stability Test of Competitive Columns



Chromatograms, run at regular intervals during the high-pH lifetime study, verify that 86% of the original XBridge column efficiency remains after 300 hours at pH 10 and elevated temperature, with little change in peak shape or retention time.

Comparative separations may not be representative of all applications.



Ligand Type	Trifunctional C_{18}	Trifunctional C_8
Particle Size	2.5, 3.5, 5, 10 µm	2.5, 3.5, 5, 10 µm
Ligand Density*	3.1 µmol/m²	3.2 µmol/m²
Carbon Load*	18%	13%
Endcap Style	Proprietary	Proprietary
pH Range	1-12	1-12
Low pH Temp. Limit	80 °C	60 °C
High pH Temp. Limit	60 °C	60 °C
Pore Diameter*	130Å	130Å
Surface Area*	185 m²/g	185 m²/g

** Expected or approximate value.*

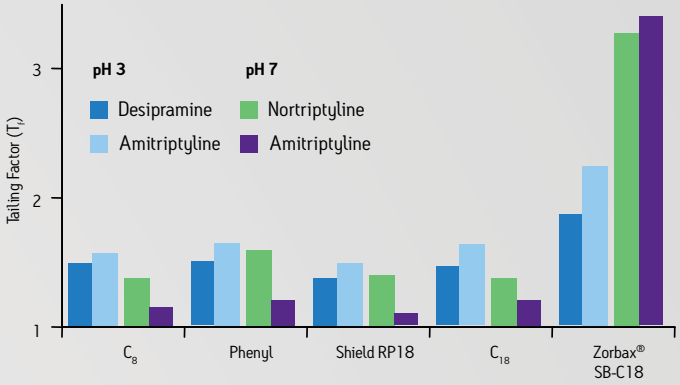
Column Reliability

Much of the cost when developing a chromatographic method is associated with the rigorous testing and validation of the final method. We understand that revalidation of your method is not an option, so we thoroughly test each batch of sorbent and final column product to ensure that you get the most reproducible columns available. With an XBridge column, you have the confidence that the method you develop today will be repeatable for the lifetime of your assay.

Particle Efficiency

The BEH (Ethylene-Bridged Hybrid) particle offers many advantages over conventional silica-based particles, including the ability to control the silanol activity with great precision. By controlling the silanol activity, you control and reduce unwanted silanol interactions that increase peak tailing.

XBridge Family USP Tailing Factors

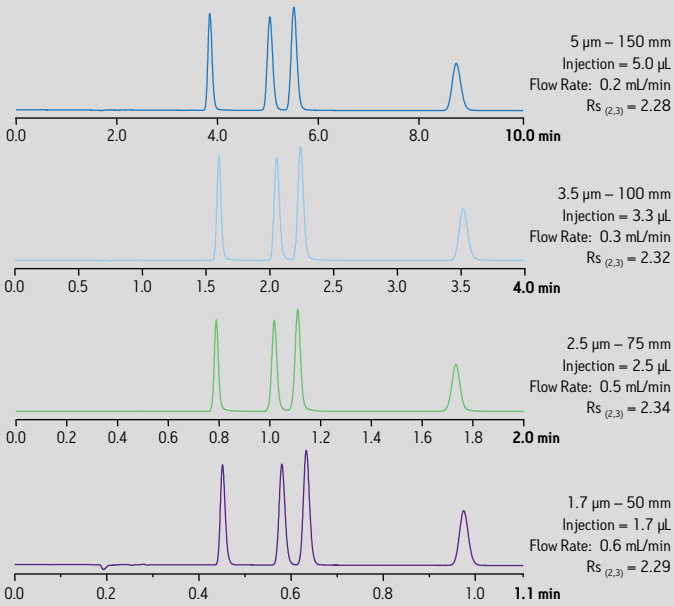


The combination of excellent particle and ligand stability as well as high chromatographic efficiencies makes XBridge columns an ideal choice for low and intermediate pH methods.

Comparative separations may not be representative of all applications.

Methods Transfer Using *XP* 2.5 µm Columns

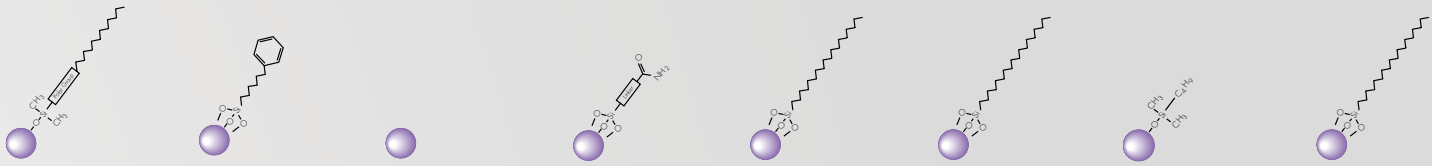
All XBridge and XSelect HPLC columns are offered in e*X*tended *P*erformance (*XP*) 2.5 µm column formats to help you transfer methods from HPLC to UPLC instrumentation. The *XP* 2.5 µm columns improve the performance of your current HPLC instrumentation and provide you with a pathway to gain maximum separation efficiency using sub-2-µm ACQUITY UPLC® technology.



Columns of different lengths and particle sizes were used to successfully reduce run times and maintain resolution.

LC Conditions

Columns:	XBridge C_{18} 2.1 x 150 mm, 5.0 µm XBridge C_{18} 2.1 x 100 mm, 3.5 µm XBridge C_{18} 2.1 x 75 mm, 2.5 µm ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm	Sample Diluent:	3% acetonitrile in water with 0.1% formic acid
Mobile Phase A:	0.1% formic acid in water	Sample Conc.:	25 µg/mL
Mobile Phase B:	0.1% formic acid in acetonitrile	Column Temp.:	38 °C
Flow Rate:	see chromatogram above	Detection:	UV @ 280 nm
Injection Volume:	see chromatogram above	Sampling Rate:	40 pts/sec
Isocratic:	95% A:5% B	Time Constant:	0.05
		Instrument:	ACQUITY UPLC with TUV detector



Monofunctional Embedded Polar Group (Shield RP18)	Trifunctional C_6 Phenyl	HILIC	Amide	BEH130 C_{18} (Peptide Separation Technology)	BEH300 C_{18} (Peptide Separation Technology)	BEH300 C_4 (Protein Separation Technology)	BEH C_{18} (Oligonucleotide Separation Technology)
2.5, 3.5, 5, 10 µm	2.5, 3.5, 5 µm	2.5, 3.5, 5 µm	2.5, 3.5 µm	3.5, 5, 10 µm	3.5, 5, 10 µm	3.5 µm	2.5 µm
3.3 µmol/m²	3.0 µmol/m²	N/A	7.5 µmol/m²	3.1 µmol/m²	3.1 µmol/m²	2.4 µmol/m²	3.1 µmol/m²
17%	15%	unbonded	12%	18%	12%	8%	18%
TMS	Proprietary	N/A	None	Proprietary	Proprietary	None	Proprietary
2-11	1-12	1-9	2-11	1-12	1-12	1-10	1-12
50 °C	80 °C	45 °C	90 °C	80 °C	80 °C	80 °C	80 °C
45 °C	60 °C	45 °C	90 °C	60 °C	60 °C	50 °C	60 °C
130Å	130Å	130Å	130Å	130Å	300Å	300Å	130Å
185 m²/g	185 m²/g	185 m²/g	185 m²/g	185 m²/g	90 m²/g	90 m²/g	185 m²/g

XSELECT HPLC COLUMNS

XSelect™ HPLC columns are designed for the method development scientist. Scientists who develop chromatographic methods demand the most diverse selection of sorbents to easily separate the most difficult analyte co-elutions. XSelect columns are tools that are:

Designed for Selectivity

- improve your ability to separate closely eluting peaks

Intended for Isolation & Purification

- highest analyte mass loading available

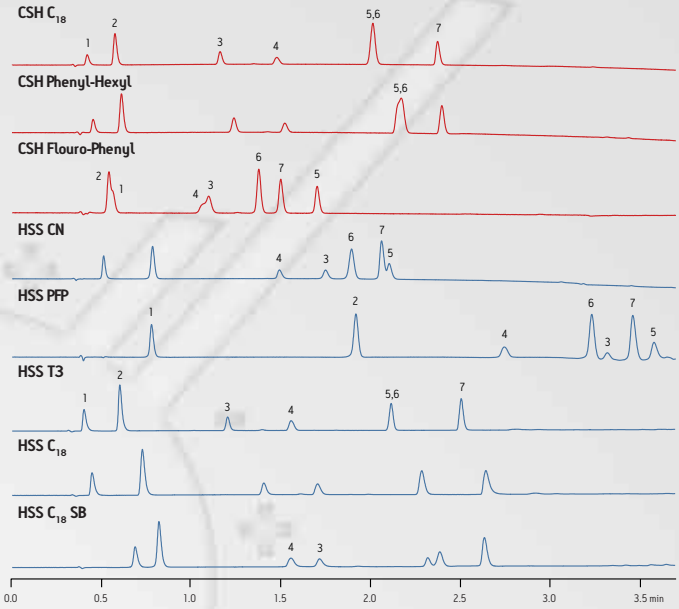
Ideal for Rapid Method Development

- reduce the time and cost spent developing methods

The base particle or substrate critically influences analyte selectivity; the bonded ligand has a secondary influence. Each, when used alone does not create dramatic selectivity changes; however, in combination, the substrate and ligand create the ultimate tools for enhancing analyte selectivity. It is for this reason that the XSelect family of columns contains both High Strength Silica (HSS) and Charged Surface Hybrid (CSH™) technologies with a unique optimization of bonded ligands. The result is reproducibility and high selectivity.

Enhanced Selectivity

Selectivity and retentivity are the most powerful tools a method developer has to influence chromatographic behavior. The XSelect family offers a diverse range of reversed-phase C_{18} columns (e.g., CSH C_{18}, HSS C_{18}, HSS C_{18} SB) for general purpose separations; as well as, columns that offer improved polar retention (T3) and greater selectivity options (Phenyl-Hexyl, Fluoro-Phenyl and Cyano) for method development.



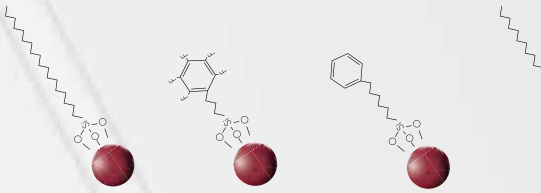
Observed selectivity differences for a mixture of basic analytes. Compounds: [1] aminopyrazine, [2] pindolol, [3] quinine, [4] labetalol, [5] verapamil, [6] diltiazem, [7] amitriptyline.

LC Conditions

Columns:	2.1 x 50 mm
Mobile Phase A:	10 mM ammonium formate, pH 3.0
Mobile Phase B:	methanol
Flow Rate:	0.4 mL/min
Injection Volume:	1 µL
Sample Diluent:	water
Column Temp.:	30 °C

Gradient:	Time (min)	%A	%B
	0.00	70	30
	3.00	15	85
	3.50	15	85
	3.51	70	30
	4.50	70	30

Detection:	UV @ 260 nm
Sampling Rate:	20 pts/sec
Filter Response:	normal
System:	ACQUITY UPLC with ACQUITY UPLC PDA detector

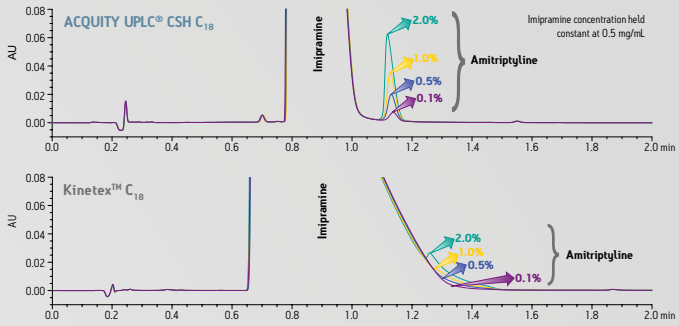


Ligand and Particle Type	CSH C_{18}	CSH Fluoro-Phenyl	CSH Phenyl-Hexyl
Particle Size	2.5, 3.5, 5 µm	2.5, 3.5, 5 µm	2.5, 3.5, 5 µm
Ligand Density*	2.3 µmol/m²	2.3 µmol/m²	2.3 µmol/m²
Carbon Load*	15%	10%	14%
Endcap Style	Proprietary	None	Proprietary
pH Range	1-11	1-8	1-11
Low pH Temp. Limit	80 °C	60 °C	80 °C
High pH Temp. Limit	45 °C	45 °C	45 °C
Pore Diameter*	130Å	130Å	130Å
Surface Area*	185 m²/g	185 m²/g	185 m²/g

** Expected or approximate value.*

Isolation and Purification

High mass loading applications like compound purification and dissolution testing demand superior column performance. For these types of applications, column loading is limited by its inability to maintain symmetrical peak shape. This manifests itself as severe tailing of the main compound peak, which often overwhelms the trace impurities that you are trying to remove in the purification. XSelect CSH columns consistently provide narrow peaks under high loading conditions that allow the chromatographer the ability to separate trace-level impurities or degradants giving you more loading capacity with less time and solvent.



The improved mass loading of XSelect columns permits the separation, identification and quantification of closely eluting impurities or degradants.

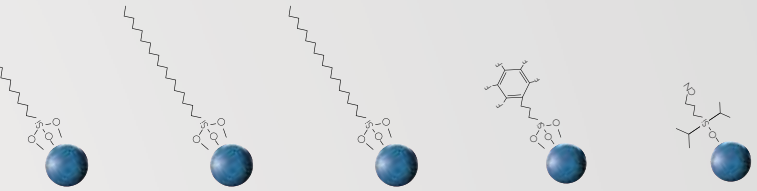
Comparative separations may not be representative of all applications.

LC Conditions

Columns: 2.1 x 50 mm
Mobile Phase A: water
Mobile Phase B: acetonitrile
Mobile Phase C: 2% formic acid in water
Gradient:

Time (min)	Flow (mL/min)	%A	%B	%C	Curve
Initial	0.6	70	25	5	Initial
2.0	0.6	60	35	5	6
3.0	0.6	0	95	5	6
3.1	0.6	70	25	5	6
5.0	0.6	70	25	5	6

Injection Volume: 5 µL
Sample Diluent: water
Sample Conc.: imipramine: 0.5 mg/mL; amitriptyline: as indicated (% of imipramine)
Column Temp.: 40 °C
Detection: UV @ 254 nm
Wash Solvent: 50/50 acetonitrile/water
Purge Solvent: 50/50 acetonitrile/water
System: ACQUITY UPLC H-Class with ACQUITY UPLC PDA detector



HSS C_{18}	HSS C_{18} SB	HSS T3	HSS PFP	HSS CN
2.5, 3.5, 5 µm	2.5, 3.5, 5 µm	2.5, 3.5, 5 µm	2.5, 3.5, 5 µm	2.5, 3.5, 5 µm
3.2 µmol/m²	1.6 µmol/m²	1.6 µmol/m²	3.2 µmol/m²	2.0 µmol/m²
15%	8%	11%	7%	5%
Proprietary	None	Proprietary	None	None
1-8	2-8	2-8	2-8	2-8
45 °C	45 °C	45 °C	45 °C	45 °C
45 °C	45 °C	45 °C	45 °C	45 °C
100Å	100Å	100Å	100Å	100Å
230 m²/g	230 m²/g	230 m²/g	230 m²/g	230 m²/g

Method Development and Transfer

When developing methods, skilled chromatographers realize that any method developed using uniquely selective columns must be easily transferable across laboratories, independent of the LC system platform used. XSelect columns are engineered for method development and are fully compatible with all modern detection modes.

Reproducibility and scalability for gradient separations on 2.1 x 50 mm columns containing nine different batches of CSH Fluoro-Phenyl representing three (1.7, 3.5, and 5 µm) particle sizes. Compounds: [1] thiourea, [2] resorcinol, [3] metoprolol, [4] 3-nitrophenol, [5] 2-chlorobenzoic acid, [6] amitriptyline, [7] diethylphthalate, [8] fenoprofen, [9] dipropylphthalate, [10] pyrenesulfonic acid.

LC Conditions

Columns: 2.1 x 50 mm
Flow Rate: 0.5 mL/min
Mobile Phase A: 15.4 mM ammonium formate, pH 3.0
Mobile Phase B: acetonitrile
Gradient: 5 to 90% B linear in 5 minutes
Injection Volume: 5 µL
Sample Diluent: water
Column Temp.: 30 °C
Detection: UV @ 254 nm
Sampling Rate: 20 pts/sec
Filter Response: normal
System: ACQUITY UPLC with ACQUITY UPLC PDA detector

ATLANTIS HPLC COLUMNS

Atlantis® HPLC columns provide exceptional performance, versatility and retention for polar compounds, while also affording balanced retention for broad analyte mixtures. Built for polar compound retention, Atlantis HPLC columns provide you with:

Polar Compound Retention without Ion-Pairing Reagents

Eliminating ion-pairing reagents improves detection limits, method reproducibility and robustness, while reducing instrument maintenance due to harsh mobile-phase environments.

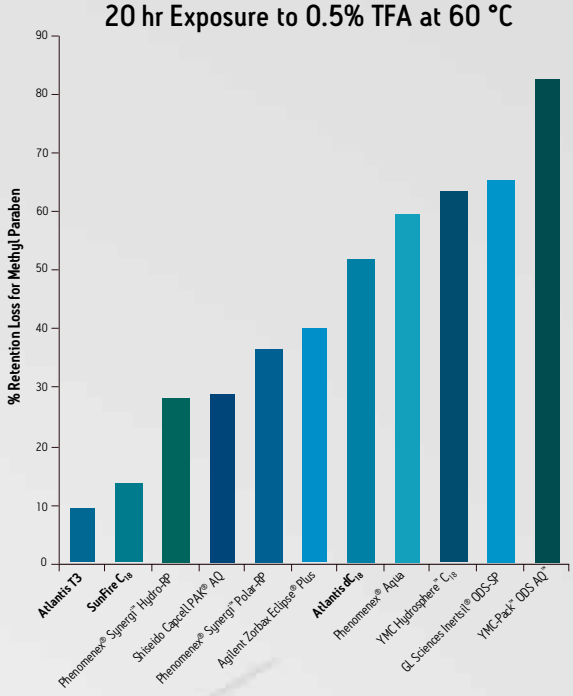
Compatibility with 100% Aqueous Mobile Phases

To maximize polar compound retention in reversed-phase methods, it is possible to use Atlantis reversed-phase HPLC columns with highly aqueous mobile phases and buffers without the risk of pore dewetting and hydrophobic collapse of the stationary phase.

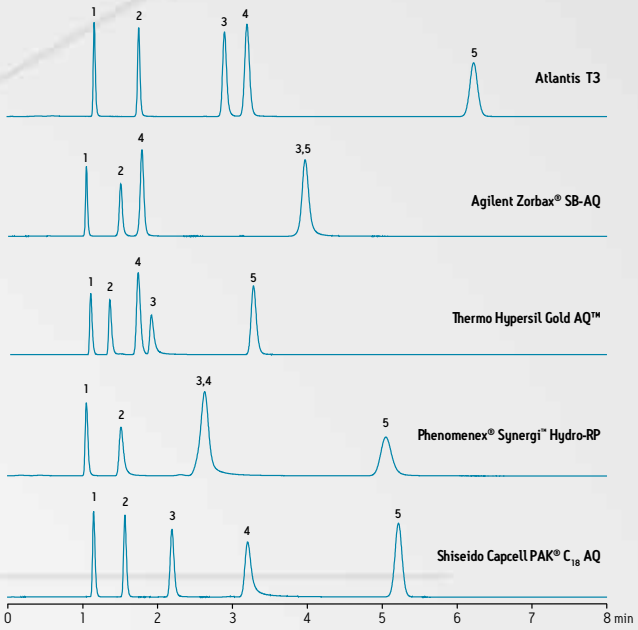
Long Column Lifetimes Using Low-pH Mobile Phases

Atlantis columns resist ligand hydrolysis when using strongly acidic mobile phases, thus maintaining method efficiency, compound retention, and critical analyte selectivity.

20 hr Exposure to 0.5% TFA at 60 °C



Polar Compound Retention



Separating highly polar analytes on the Atlantis T3 column compared to competitive brands. Scientists rely on the uncompromised peak shape and retention that only Atlantis columns can provide. Compounds: [1] thiourea, [2] 5-fluorocystine, [3] adenine, [4] guanosine-5'-monophosphate, [5] thymine.

Comparative separations may not be representative of all applications.

LC Conditions

Column:	4.6 x 150 mm	Detection:	254 nm
Mobile Phase:	10 mM ammonium formate, pH 3.0	Sampling Rate:	10 pts/sec
Flow Rate:	1.3 mL/min for 3 µm	System:	Alliance® 2695 with 2487 Dual-Wavelength Absorbance detector
Injection Volume:	2.0 µL		
Column Temp.:	30 °C		

Stationary Phase Characteristics

Intended Use	HPLC
Particle Type	Atlantis Silica
Available Chemistries	T3, dC_{18}, HILIC Silica
pH Range	T3: 2-8; dC_{18}: 3-7; HILIC Silica: 1-5
Maximum Rated Pressure	6000 psi [~400 bar]
Particle Size	3, 5, 10 µm
Pore Diameter / Volume	100Å/1.0 mL/g
Surface Area	330 m^2/g

www.waters.com/atlantis

SUNFIRE HPLC COLUMNS

SunFire™ columns set the standard for state-of-the-art bonded C_{18}- and C_8- silica HPLC columns. Benefiting from years of research and product development, SunFire columns represent the best in particle and bonding expertise and deliver industry-leading levels of chromatographic performance. Since their development in 2004, SunFire HPLC columns have gained the reputation for:

Excellent Low-pH Stability

Under low-pH mobile-phase conditions, SunFire columns exhibit superior column lifetimes that exceed many silica-based HPLC column brands.

High Efficiency

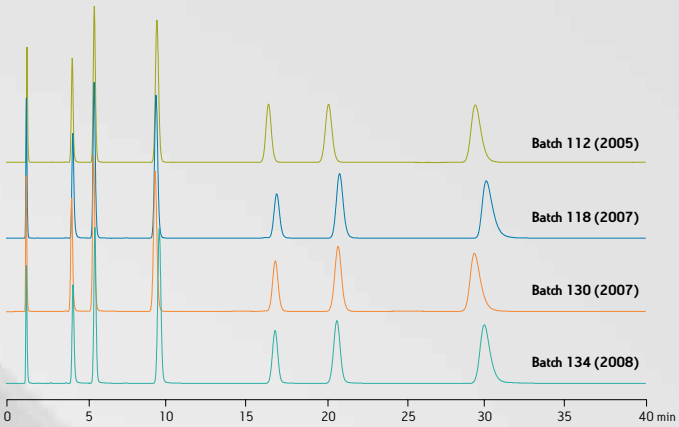
A synergistic combination of particle synthesis, packing technology, and hardware engineering is required for high efficiency. SunFire Intelligent Speed™ (*IS*™) and Optimum Bed Density (OBD™) columns were developed specifically from this knowledge.

Superior Peak Shape

For years, SunFire columns provide symmetrical peaks for improved resolution of acidic, neutral and basic compounds at low and moderate pH ranges (2-8).

Batch-to-Batch Reproducibility

Waters is dedicated to maintain the tightest specifications in the HPLC industry. Controlled manufacturing processes and column packing procedures ensure that you receive the best, most reproducible HPLC column available.



This excellent reproducibility is a result of our commitment to maintaining the tightest specifications in the HPLC column industry. SunFire columns start with high purity raw materials, and are produced using controlled manufacturing processes and column packing procedures that provide today's scientists with the best, most reproducible HPLC columns available.

Stationary Phase Characteristics

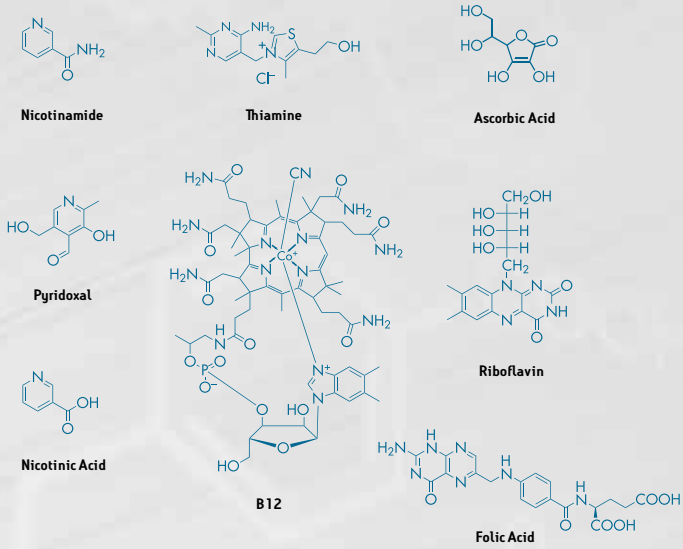
Intended Use	HPLC
Particle Type	SunFire Silica
Available Chemistries	C_8, C_{18}, Silica
pH Range	2-8
Maximum Rated Pressure	6000 psi [~400 bar]
Particle Size	2.5, 3.5, 5, 10 µm
Pore Diameter / Volume	100Å/1.0 mL/g
Surface Area	340 m^2/g

HILIC HPLC COLUMNS

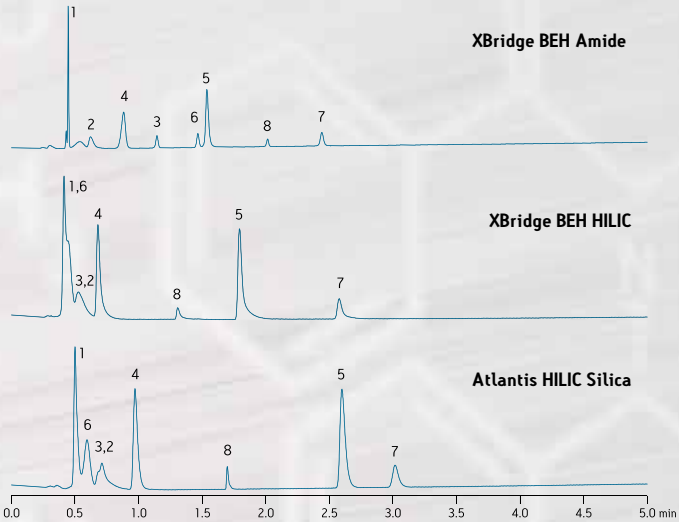
Hydrophilic Interaction Chromatography (HILIC) is a versatile and effective alternative to reversed-phase retention for highly polar compounds. HILIC outperforms reversed-phase techniques when you are primarily interested in retaining small organic acids and bases as well as neutral compounds, like water-soluble vitamins and sugars.

HILIC for Orthogonal Selectivity

HILIC techniques are gaining popularity as part of HPLC method development strategies. As a complementary technique to traditional reversed-phase chromatography, HILIC separations not only retain highly polar compounds but provide, in some cases, large selectivity differences where there can be a complete reversal in elution order compared to a reversed-phase chromatogram.



Chemical structures of water soluble vitamins.



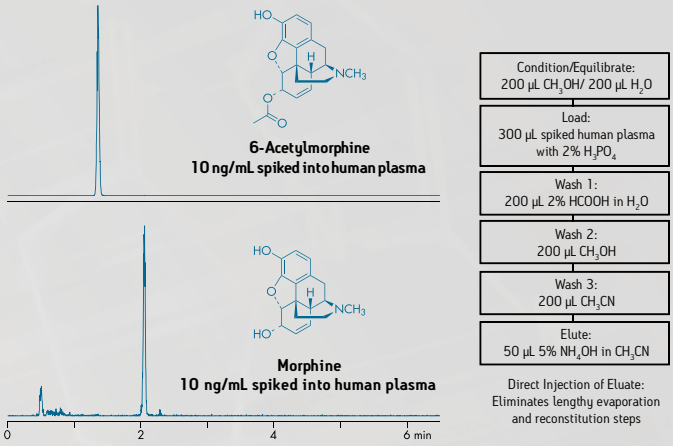
Column selectivity at low pH. Compounds: [1] nicotinamide, [2] pyridoxine, [3] riboflavin, [4] nicotinic acid, [5] thiamine, [6] ascorbic acid, [7] B12, [8] folic acid.

LC Conditions

Columns:	2.1 x 50 mm
Flow Rate:	0.5 mL/min
Mobile Phase A:	50/50 acetonitrile/water with 10 mM ammonium formate
Mobile Phase B:	95/5 acetonitrile/water with 10 mM ammonium formate
Gradient:	99.9% B to 0.1% B linear in 5 minutes
Injection Volume:	5 µL
Sample Diluent:	75/25 acetonitrile/methanol
Column Temp.:	30 °C
Sampling Rate:	20 pts/sec
System:	ACQUITY UPLC with ACQUITY UPLC PDA detector

HILIC for Sample Throughput

Many common sample preparation techniques such as protein precipitation and elution from solid-phase extraction devices include the use of organic solvents such as acetonitrile. For reversed-phase LC, injection of a strong solvent from this type of extract may produce a chromatogram with peak distortion and smearing. This problem is eliminated when using a HILIC column. This not only saves time, but also improves variability in sampling because tedious sample evaporation and reconstitution steps can be eliminated.



XBridge HILIC provides direct injection of SPE eluents, thus increasing sample throughput.

Ligand Type	Atlantis HILIC Silica	XBridge HILIC	XBridge Amide
Particle Size	3, 5 µm	2.5, 3.5, 5 µm	2.5, 3.5 µm
pH Range	1-5	1-9	2-11
Low pH Temp. Limit	45 °C	45 °C	90 °C
High pH Temp. Limit	45 °C	45 °C	90 °C
Pore Diameter*	100Å	130Å	130Å
Surface Area*	330 m^2/g	185 m^2/g	185 m^2/g

** Expected or approximate value.*

TECHNICAL RESOURCES

Literature References

HPLC Troubleshooting Guide
Part Number: WA20769

HPLC Columns Theory, Technology, and Practice
Part Number: WAT038216

Comprehensive Guide to HILIC
Part Number: 715002531

Beginners Guide to Liquid Chromatography
Part Number: 715001531

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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The quality management system of Waters' manufacturing facilities in Taunton, Massachusetts and Wexford, Ireland complies with the International Standard ISO 9001 Quality Management and Quality Assurance Standards. Waters' quality management system is periodically audited by the registering body to ensure compliance.

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