Waters UPLC, UHPLC, and HPLC Column Selection and Mobile-Phase Guide

[www.waters.com]

BEH Technology

- High retentivity for basic compounds
- Excellent peak shape at elevated pH
- Good universal column choice for a wide variety of compounds
- Stable across a wide pH range
- For separations at high temperatures (80 °C)

HSS Technology

- High retentivity for polar organic compounds and metabolites
- Balanced retention of polar and hydrophobic analytes
- High strength silica for mechanical stability

CSH Technology

- Good separations for basic compounds under low pH conditions
- Excellent MS performance with formic acid as a mobile-phase modifier
- Fast pH switching and column equilibration

Solid-Core Technology

- Maximum efficiency
- Increased sensitivity
- Seamless scalability from UPLC™ to UHPLC to HPLC

Wide pH Range

PREMIER **BEH C_{18}** **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5, 10 µm		**Performance Benefits**: General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compound classes.	**Bonding**: Trifunctional C_{18}, fully end-capped, bonded to Ethylene Bridged Hybrid (BEH) substrate.
BEH C_8 **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5, 10 µm		**Performance Benefits**: General purpose column ideally suited for method development due to extreme pH stability and applicability to the broadest range of compound classes.	**Bonding**: Trifunctional C_8, fully end-capped, bonded to Ethylene Bridged Hybrid (BEH) substrate.
PREMIER **BEH Shield RP18** **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5, 10 µm		**Performance Benefits**: Alternate selectivity compared to straight chain C_{18}, particularly with phenolic analytes. Compatible with 100% aqueous-phase composition.	**Bonding**: Monofunctional embedded polar C_{18}, fully end-capped, bonded to Ethylene Bridged Hybrid (BEH) substrate.
BEH Phenyl **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: Excellent method development column for alternate selectivity, particularly in regard to polyaromatic compounds. Unique level of pH stability for a Phenyl bonded phase.	**Bonding**: Trifunctional C_6 Phenyl, fully end-capped, bonded to Ethylene Bridged Hybrid (BEH) substrate.
BEH HILIC **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: Excellent for retention of very polar, basic, water soluble analytes. Specifically designed and tested for HILIC separations using mobile phases containing high concentrations of organic solvent.	**Bonding**: Unbonded Ethylene Bridged Hybrid (BEH) substrate.
PREMIER **BEH Amide** **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: Rugged HILIC stationary phase designed to separate a wide range of very polar compounds. Especially good at separating carbohydrates (saccharides) using high concentrations of organic modifier, elevated temperature, and high pH. Compatible with all modern detectors including MS, ELSD, UV, and fluorescence.	**Bonding**: Trifunctional amide bonded to Ethylene Bridged Hybrid (BEH) substrate.

Wide Selectivity Range

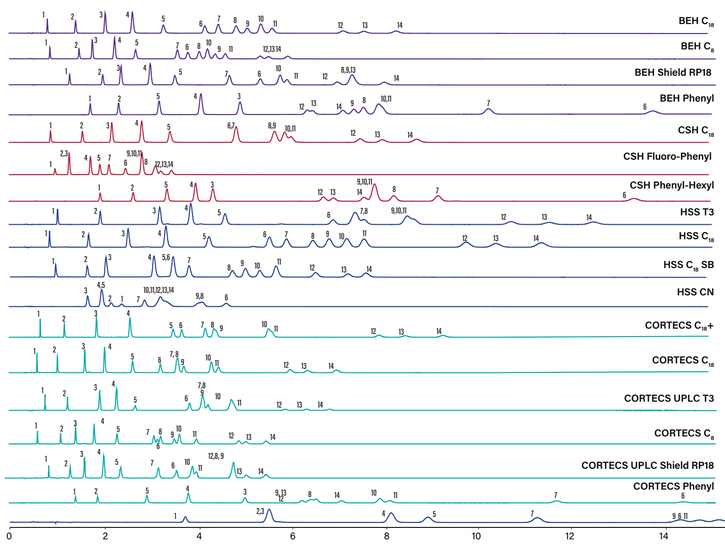
PREMIER **CSH C_{18}** **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5, 10 µm		**Performance Benefits**: General purpose reversed-phase column that offers excellent pH stability and rapid mobile-phase re-equilibration for method development. Charged Surface Hybrid (CSH™) Technology enables superior peak shape and increased loading capacity for basic compounds.	**Bonding**: Trifunctional C_{18} ligand, fully end-capped, bonded to a Charged Surface Hybrid (CSH) particle substrate.
PREMIER **CSH Phenyl-Hexyl** **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: General purpose alternative selectivity ligand that provides pi-pi interactions with polyaromatic compounds, while maintaining excellent reproducibility at pH extremes. Charged Surface Hybrid (CSH) Technology enables superior peak shape and increased loading capacity for basic compounds.	**Bonding**: Trifunctional C_6 Phenyl ligand, fully end-capped, bonded to a CSH particle substrate.
CSH Fluoro-Phenyl **UPLC:** 1.7 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: General purpose column that provides a very high degree of analyte selectivity, especially when using low-pH mobile phases. Charged Surface Hybrid (CSH) Technology enables superior peak shape and increased loading capacity for basic compounds.	**Bonding**: Trifunctional propyl fluorophenyl ligand, non-end-capped, bonded to a CSH particle substrate.
HSS C_{18} **UPLC:** 1.8 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: Ultra performance C_{18} chemistry, increased retention, superior peak shape, resists acid hydrolysis at low pH.	**Bonding**: High coverage trifunctional C_{18}, fully end-capped, bonded to a High Strength Silica (HSS) particle substrate.
HSS C_{18} SB **UPLC:** 1.8 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: Unique, non-end-capped C_{18} chemistry designed specifically for method development scientists. Offers unique Selectivity for Bases (SB) when operating under low-pH conditions.	**Bonding**: Intermediate coverage trifunctionally bonded C_{18}, no endcapping, bonded to High Strength Silica (HSS) particle substrate.
PREMIER **HSS T3** **UPLC:** 1.8 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: Aqueous mobile-phase compatible column designed for exceptional polar compound retention.	**Bonding**: T3 (C_{18}) bonding and endcapping, bonded to High Strength Silica (HSS) particle substrate.
HSS PFP **UPLC:** 1.8 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		**Performance Benefits**: A general purpose column designed to maximize selectivity differences for Lewis bases through pi-pi interactions. The rigid aromatic ring provides additional selectivity based on shape, dipole moment, and hydrogen bonding interactions.	**Bonding**: Trifunctional pentafluorophenyl ligand, non-end-capped, bonded to High Strength Silica (HSS) substrate.
HSS CN **UPLC:** 1.8 µm / **UHPLC:** 2.5 µm / **HPLC:** 3.5, 5 µm		Performance Benefits: A general purpose column that shows contrasting analyte selectivity when compared to C_{18} phases. This column can be used for both reversed-phase and normal-phase separations.	Bonding: Sterically hindered, mono-functional cyano-propyl ligand, non-end-capped, bonded to High Strength Silica (HSS) substrate.

High Efficiency

C_{18}+ **UPLC:** 1.6 µm / **UHPLC:**: 2.7 µm		**Performance Benefits**: General purpose reversed-phase column designed to maximize efficiency. A charged-surface-silica-solid-core particle enables excellent peak shape for basic compounds at low pH, especially in low concentration modifier mobile phases. Offers complementary selectivity to traditional C_{18} columns.	**Bonding**: Trifunctional C_{18} ligand, fully end-capped, bonded to a charged surface silica solid-core particle substrate.
C_{18} **UPLC:** 1.6 µm / **UHPLC:** 2.7 µm		**Performance Benefits**: General purpose reversed-phase column designed to maximize efficiency. Balanced retention of acids, bases, and neutrals at low- and mid-range pH.	**Bonding**: Intermediate coverage trifunctional-C_{18} ligand, fully end-capped, bonded to a silica solid-core particle substrate.
T3 **UPLC:** 1.6 µm / **UHPLC:** 2.7 µm		**Performance Benefits**: Aqueous mobile-phase compatible column designed to maximize efficiency. Provides balanced retention for both polar and non-polar compounds.	**Bonding**: T3 (C_{18}) bonding and endcapping, bonded to a silica solid-core particle substrate
C_8 **UPLC:** 1.6 µm / **UHPLC:** 2.7 µm		**Performance Benefits**: General purpose column designed to maximize efficiency. Similar selectivity, but shorter retentivity when compared to typical C_{18} phases.	**Bonding**: Trifunctional C_8, fully end-capped, bonded to a silica solid-core particle substrate.
Shield RP18 **UPLC:** 1.6 µm / **UHPLC:** 2.7 µm		**Performance Benefits**: Excellent method development column designed to give maximum efficiency. Alternative selectivity when compared to typical C_{18} phases, especially phenolic compounds.	**Bonding**: Monofunctional embedded polar C_{18}, fully end-capped, bonded to a silica solid-core particle substrate.
Phenyl **UPLC:** 1.6 µm / **UHPLC:** 2.7 µm		**Performance Benefits**: Excellent method development column designed to give maximum efficiency as well as alternate selectivity, particularly in regard to polyaromatic compounds.	**Bonding**: Trifunctional C_6 Phenyl, fully end-capped, bonded to a silica solid-core particle substrate.
HILIC **UPLC:** 1.6 µm / **UHPLC:** 2.7 µm		**Performance Benefits**: High efficiency column designed for retention of extremely polar, basic, water-soluble analytes.	**Bonding**: Unbonded high-purity silica solid-core particle substrate.

Selectivity Choices Nitroaromatic Compounds

The choice of stationary phase influences the selectivity of the separation. The bonded phases and particle substrates used in Waters™ HPLC Columns are developed to maximize the differences in analyte retention to help resolve the most demanding separation challenges.



Conditions:

Columns:	2.1 x 100 mm
Mobile phase:	72% water/28% methanol (v/v)
Flow rate:	0.5 mL/min
Injection vol.:	5.0 µL
Sample conc.:	10 µg/mL
Temperature:	50 °C
Detection:	UV @ 254 nm
Sampling rate:	20 pts/sec
Time constant:	0.1 min
Instrument:	ACQUITY™ UPLC with PDA Detector

Compounds [EPA 8330 Standard Mixture):

1. HMX
2. RDX
3. 1,3,5-Trinitrobenzene
4. 1,3-Dinitrobenzene
5. Nitrobenzene
6. Tetryl
7. 2,4,6-Trinitrotoluene
8. 2-Amino-4,6-Dinitrotoluene
9. 4-Amino-2,6-Dinitrotoluene
10. 2,4-Dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2-Nitrotoluene
13. 4-Nitrotoluene
14. 3-Nitrotoluene

Comparative separations may not be representative of all applications.

Extend Column Performance and Lifetime

VanGuard™ Column Protection

Using a guard column is an economical way to prolong analytical column lifetime without compromising chromatographic performance. VanGuard™ Column Protection Products are available in a wide selection of particle sizes and stationary phases making them ideally suited for the physical and chemical protection for all analytical columns.

- Minimal chromatographic effects and optimized performance
- Superior column protection for UPLC, UHPLC, and HPLC Columns and Sorbents with particle sizes ranging from 1.6 mm to 5 mm
- Compatible operating pressures up to 18,000 psi (1240 bar)

Selection Guide

VanGuard Column Protection Cartridge/Pre-column selection based on analytical column I.D.

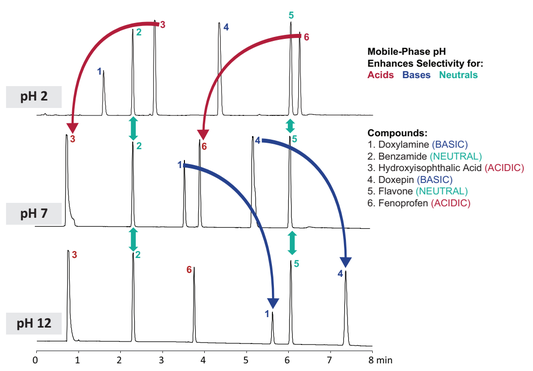
Column I.D.	Particle Size	VanGuard Format	VanGuard Dimension
2.1 mm	<2 µm	Pre-column	2.1 x 5 mm
2.1 mm	>2 µm	Cartridge Column	2.1 x 5 mm
3.0 mm	>2 µm	Cartridge Column	2.1 x 5 mm
3.9 mm	>2 µm	Cartridge Column	3.9 x 5 mm
4.6 mm	>2 µm	Cartridge Column	3.9 x 5 mm

Mobile-Phase Chemical	pK_a	Buffer Range	Formula	Volume or Mass Required for 10 mM Mobile-Phase Concentration (per 1 L)	pH Adjustment Acid/Base	MS Compatible?
Acetic Acid (glacial)	4.8	—	CH_3COOH	0.571 mL	—	✓
Ammonium Acetate pK_a 1	4.8	3.8-5.8	CH_3COONH_4	0.770 g	CH_3COOH or NH_4OH	✓
Ammonium Acetate pK_a 2	9.2	8.2-10.2	CH_3COONH_4	0.770 g	CH_3COOH or NH_4OH	✓
Ammonium Bicarbonate	9.2, 10.3	(8.2-11.3)	NH_4HCO_3	0.790 g	HCOOH or NH_4OH	✓
Ammonium Formate pK_a 1	3.8	2.8-4.8	NH_4COOH	0.640 g	HCOOH or NH_4OH	✓
Ammonium Formate pK_a 2	9.2	8.2-10.2	NH_4COOH	0.640 g	HCOOH or NH_4OH	✓
Ammonium Hydroxide	9.2	—	NH_4OH	0.675 mL	—	✓
Ammonium Phosphate, Dibasic	7.2, 9.2	(6.2-10.2)	$(NH_4)_2HPO_4$	1.32 g	H_3PO_4 or NH_4OH	✕
Formic Acid	3.8	—	HCOOH	0.420 mL	—	✓
N-Methylpyrrolidine	10.3	—	$C_5H_{11}NO$	1.04 mL	—	✓
Phosphoric Acid	2.1	—	H_3PO_4	0.580 mL	—	✕
Potassium Phosphate, Monobasic	2.1	(1.1-3.1)	KH_2PO_4	1.36 g	H_3PO_4 or KOH	✕
Potassium Phosphate, Dibasic	7.2	(6.2-8.2)	K_2HPO_4	1.74 g	H_3PO_4 or KOH	✕
Potassium Phosphate, Tribasic	12.7	(11.7-13.7)	K_3PO_4	2.12 g	H_3PO_4 or KOH	✕
Pyrrolidine	11.3	—	C_4H_9N	0.833 mL	—	✓
Sodium Borate	9.1, 12.7, 13.8	(8.2-14)	$Na_2B_4O_7$	2.01 g	H_3BO_4 or NaOH	✕
Sodium Citrate, Tribasic	3.1, 4.8, 6.4	(2.1-7.4)	$HOC(COONa)(CH_2COONa)_2$	2.58 g	Citric Acid or NaOH	✕
Triethylamine (TEA)	11.01	—	$(CH_3CH_2)_3N$	1.39 mL	—	✓
Triethylammonium Acetate (TEAA) pK_a 1	4.76	3.8-5.8	$(CH_3CH_2)_3N$:CH_3COONH_4 (1:2)	0.695 mL TEA/0.571 mL Acetic Acid	TEA or CH_3COOH	✓
Triethylammonium Acetate (TEAA) pK_a 2	11.01	10.0-12.0	$(CH_3CH_2)_3N$:CH_3COONH_4 (2:1)	1.39 mL TEA/0.285 mL Acetic Acid	TEA or HCOOH	✓
Triethylammonium Formate (TEAF) pK_a 1	3.75	2.8-4.8	$(CH_3CH_2)_3N$:NH_4COOH (1:2)	0.695 mL TEA/0.420 mL Formic Acid	TEA or HCOOH	✓
Triethylammonium Formate (TEAF) pK_a 2	11.01	10.0-12.0	$(CH_3CH_2)_3N$:NH_4COOH (2:1)	1.39 mL TEA/0.210 mL Formic Acid	TEA or HCOOH	✓
Trifluoroacetic Acid (TFA)	0.3	—	CF_3COOH	0.743 mL	—	✓

Importance of Mobile-Phase pH

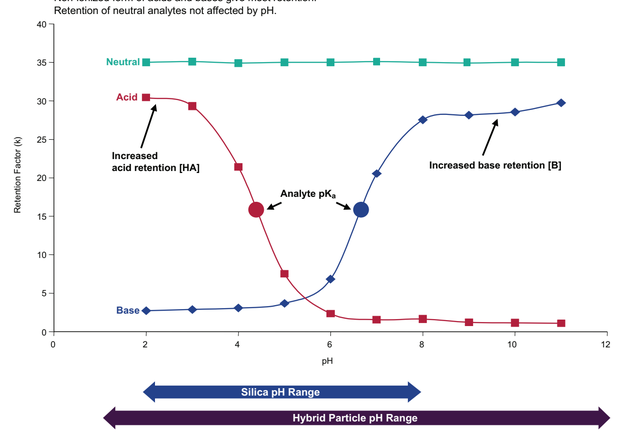
- Using a wide mobile-phase pH range is an effective approach to change compound selectivity.
- Increase selectivity for:
  - Acids (Peaks 3 and 6)
  - Bases (Peaks 1 and 4)
- Neutrals (Peaks 2 and 5) are largely unaffected by mobile-phase pH.

The Importance of Mobile-Phase pH: Rapid Method Development



Dependence of Retention on pH: Reversed-Phase Retention Map

The pH of the mobile phase has the greatest impact on analyte retention. For the most robust separations, choose a mobile-phase pH that corresponds to the plateau regions of the retention map.



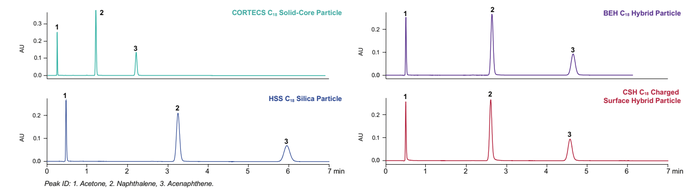
Benchmarking System Performance

Use Waters Quality Control (QC) Reference Materials to evaluate or verify key performance criteria with data generated when the system is known to be in good working order. The criteria chosen, along with the routine use of a control chart, provides an understanding of the capability of your system and can be a useful troubleshooting tool.

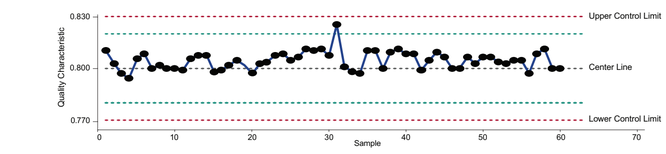
Typical Criteria

1. Retention time range or reproducibility
2. Peak area range or reproducibility
3. Peak tailing range
4. Peak resolution
5. Response
6. System pressure

Versatility of Neutrals QC Reference Material



Peak ID: 1. Acetone, 2. Naphthalene, 3. Acenaphthene.



Benchmarking System Performance

Instrument bandspread is one of the most practical LC instrument parameters to understand when transferring LC methods. Knowing the result of this simple measurement gives the separation scientist the ability to develop compatible methods that are independent of the LC instrument manufacturer. The following table gives recommendations on column configuration based on nominal instrument bandspread values.

System	LC Technique	Bandspread*	Recommended Column Particle Sizes and I.D.s
Shimadzu Prominence UFLC	HPLC	41 µL	XBridge 3.5, 5 µm; XSelect 3.5, 5 µm; CORTECS 2.7 µm 3.0–4.6 mm I.D.
Alliance™ 2695 HPLC	HPLC	29 µL	
Agilent 1260 UHPLC (600 bar)	HPLC	28 µL	
Thermo Accela UHPLC	HPLC	21 µL	XBridge 2.5, 5 µm; XSelect 2.5, 5 µm; CORTECS 2.7 µm 3.0 mm I.D.
Agilent 1290 UHPLC (1200 bar)	UHPLC	17 µL	
ACQUITY Arc™	UHPLC	23 µL	XBridge 2.5, 5 µm; XSelect 2.5, 5 µm; CORTECS 2.7 µm 3.0 mm I.D.
ACQUITY UPLC	UPLC	12 µL	ACQUITY UPLC BEH 1.7 µm; ACQUITY UPLC CSH 1.7 µm; CORTECS 1.6 µm 2.1 mm I.D.
ACQUITY UPLC H-Class w/Column Manager	UPLC	12 µL	
ACQUITY UPLC H-Class	UPLC	9 µL	

Note: The provided data is for reference only and is based on nominal values for unmodified systems. Any adjustment to the plumbing, connectivity, and configuration of the system will change the instrument bandspread and will influence the resulting chromatography.

Column Selection Guide

System	HPLC	UHPLC	UPLC
Dispersion	>40 µL	22–29 µL	<15 µL
Particle Size	3–5 µm	2–3 µm	<2 µm
Routine Pressure	<4000 psi	<10,000 psi	<18,000 psi
Column I.D.	4.6 mm (3.0 mm)	3.0 mm (2.1 mm)	2.1 mm (1.0 mm)
Column Length	75–250 mm	50–150 mm	≤150 mm

Optimized column dimension matched to Waters LC Systems.

PREMIER ***Select column configurations for chemistries that show the PREMIER symbol are available in the PREMIER column format. The PREMIER columns utilize MaxPeak™ High Performance Surface (HPS) Technology which increases reproducibility, improves peak shape, and enables more accurate recovery by minimizing unwanted analyte/surface interactions.***

Waters

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