

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

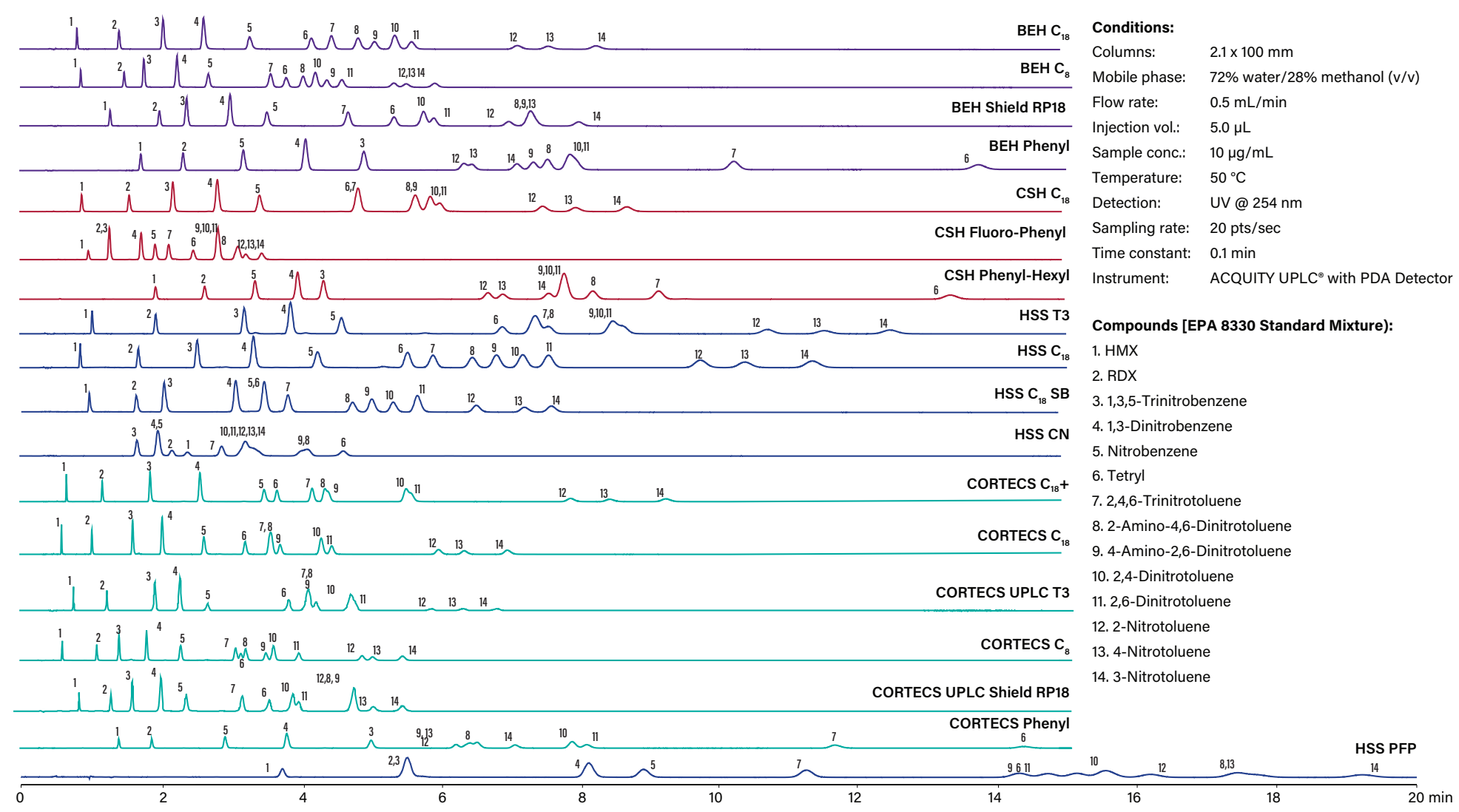
Solid-Core Technology

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

Wide pH Range	Wide Selectivity Range	High Efficiency
<p>BEH C₁₈ UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5, 10 µm</p> <p>BEH C₈ UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5, 10 µm</p> <p>BEH Shield RP18 UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5, 10 µm</p> <p>BEH Phenyl UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p> <p>BEH HILIC UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p> <p>BEH Amide UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p>	<p>CSH C₁₈ UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5, 10 µm</p> <p>CSH Phenyl-Hexyl UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p> <p>CSH Fluoro-Phenyl UPLC: 1.7 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p> <p>HSS C₁₈ UPLC: 1.8 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p> <p>HSS C₈ SB UPLC: 1.8 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p> <p>HSS T3 UPLC: 1.8 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p> <p>HSS PFP UPLC: 1.8 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p> <p>HSS CN UPLC: 1.8 µm / UHPLC: 2.5 µm / HPLC: 3.5, 5 µm</p>	<p>C₁₈+ UPLC: 1.6 µm / UHPLC: 2.7 µm</p> <p>C₁₈ UPLC: 1.6 µm / UHPLC: 2.7 µm</p> <p>T3 UPLC: 1.6 µm / UHPLC: 2.7 µm</p> <p>C₈ UPLC: 1.6 µm / UHPLC: 2.7 µm</p> <p>Shield RP18 UPLC: 1.6 µm / UHPLC: 2.7 µm</p> <p>Phenyl UPLC: 1.6 µm / UHPLC: 2.7 µm</p> <p>HILIC UPLC: 1.6 µm / UHPLC: 2.7 µm</p>

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.



Extend Column Performance and Lifetime

VANGUARD Columns 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.

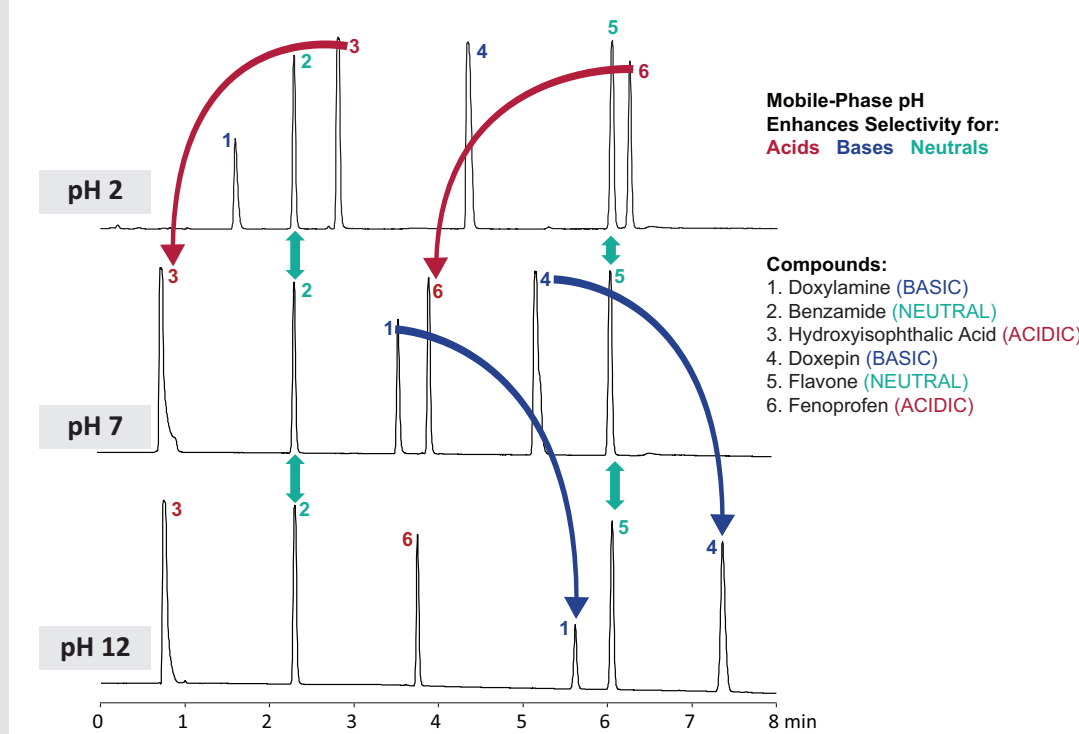
Column I.D.	Particle Size	VanGuard Format	VanGuard Dimension
2.1 mm	<2 µm	Pre-column	2.1 x 5 mm
2.1 mm	>2 µm	Cartridge Column	2.1 x 5 mm
3.0 mm	>2 µm	Cartridge Column	2.1 x 5 mm
3.9 mm	>2 µm	Cartridge Column	3.9 x 5 mm
4.6 mm	>2 µm	Cartridge Column	3.9 x 5 mm

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 ₃ COOH	0.571 mL	—	✓
Ammonium Acetate pK _a 1	4.8	3.8-5.8	CH ₃ COONH ₄	0.770 g	CH ₃ COOH or NH ₄ OH	✓
Ammonium Acetate pK _a 2	9.2	8.2-10.2	CH ₃ COONH ₄	0.770 g	CH ₃ COOH or NH ₄ OH	✓
Ammonium Bicarbonate	9.2, 10.3	(8.2-11.3)	NH ₄ HCO ₃	0.790 g	HCOOH or NH ₄ OH	✓
Ammonium Formate pK _a 1	3.8	2.8-4.8	NH ₄ COOH	0.640 g	HCOOH or NH ₄ OH	✓
Ammonium Formate pK _a 2	9.2	8.2-10.2	NH ₄ COOH	0.640 g	HCOOH or NH ₄ OH	✓
Ammonium Hydroxide	9.2	—	NH ₄ OH	0.675 mL	—	✓
Ammonium Phosphate, Dibasic	7.2, 9.2	(6.2-10.2)	(NH ₄) ₂ HPO ₄	1.32 g	H ₃ PO ₄ or NH ₄ OH	✗
Formic Acid	3.8	—	HCOOH	0.420 mL	—	✓
N-Methylpyrrolidine	10.3	—	C ₅ H ₁₁ NO	1.04 mL	—	✓
Phosphoric Acid	2.1	—	H ₃ PO ₄	0.580 mL	—	✗
Potassium Phosphate, Monobasic	2.1	(1.1-3.1)	KH ₂ PO ₄	1.36 g	H ₃ PO ₄ or KOH	✗
Potassium Phosphate, Dibasic	7.2	(6.2-8.2)	K ₂ HPO ₄	1.74 g	H ₃ PO ₄ or KOH	✗
Potassium Phosphate, Tribasic	12.7	(11.7-13.7)	K ₃ PO ₄	2.12 g	H ₃ PO ₄ or KOH	✗
Pyrrolidine	11.3	—	C ₄ H ₉ N	0.833 mL	—	✓
Sodium Borate	9.1, 12.7, 13.8	(8.2-14)	Na ₂ B ₄ O ₇	2.01 g	H ₃ BO ₃ or NaOH	✗
Sodium Citrate, Tribasic	3.1, 4.8, 6.4	(2.1-7.4)	HOC(COONa)(CH ₂ COONa) ₂	2.58 g	Citric Acid or NaOH	✗
Triethylamine (TEA)	11.01	—	(CH ₃ CH ₂) ₃ N	1.39 mL	—	✓
Triethylammonium Acetate (TEAA) pK _a 1	4.76	3.8-5.8	(CH ₃ CH ₂) ₃ N:CH ₃ COONH ₄ (1:2)	0.695 mL TEA/0.571 mL Acetic Acid	TEA or CH ₃ COOH	✓
Triethylammonium Acetate (TEAA) pK _a 2	11.01	10.0-12.0	(CH ₃ CH ₂) ₃ N:CH ₃ COONH 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 ₃ CH ₂) ₃ N:NH ₄ COOH (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 ₃ CH ₂) ₃ N:NH ₄ COOH (2:1)	1.39 mL TEA/0.210 mL Formic Acid	TEA or HCOOH	✓
Trifluoroacetic Acid (TFA)	0.3	—	CF ₃ COOH	0.743 mL	—	✓

Importance of Mobile-Phase pH

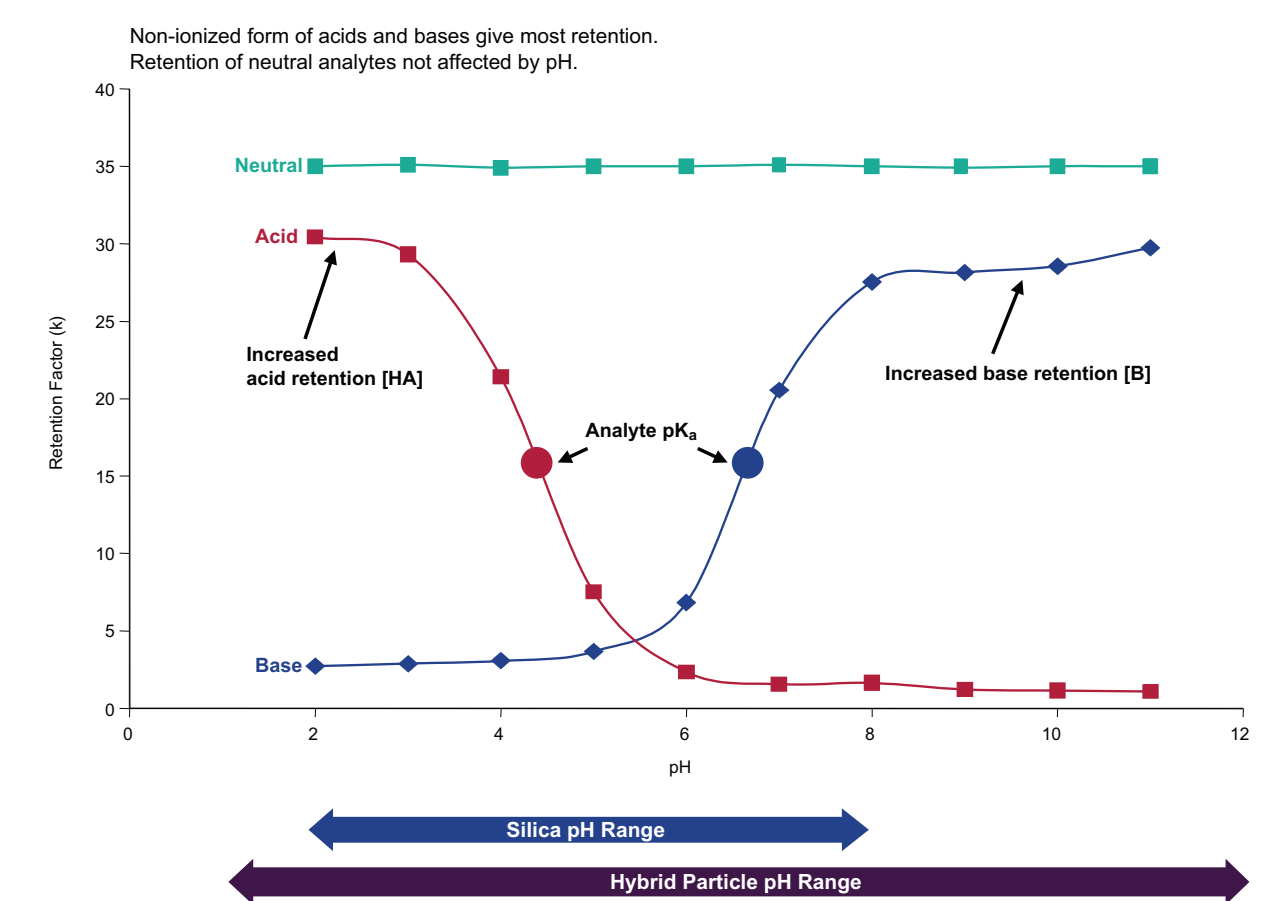
- Using a wide mobile-phase pH range is an effective approach to change compound selectivity.
- Increase selectivity for:
 - Acids (Peaks 3 and 6)
 - Bases (Peaks 1 and 4)
- Neutrals (Peaks 2 and 5) are largely unaffected by mobile-phase pH.

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.



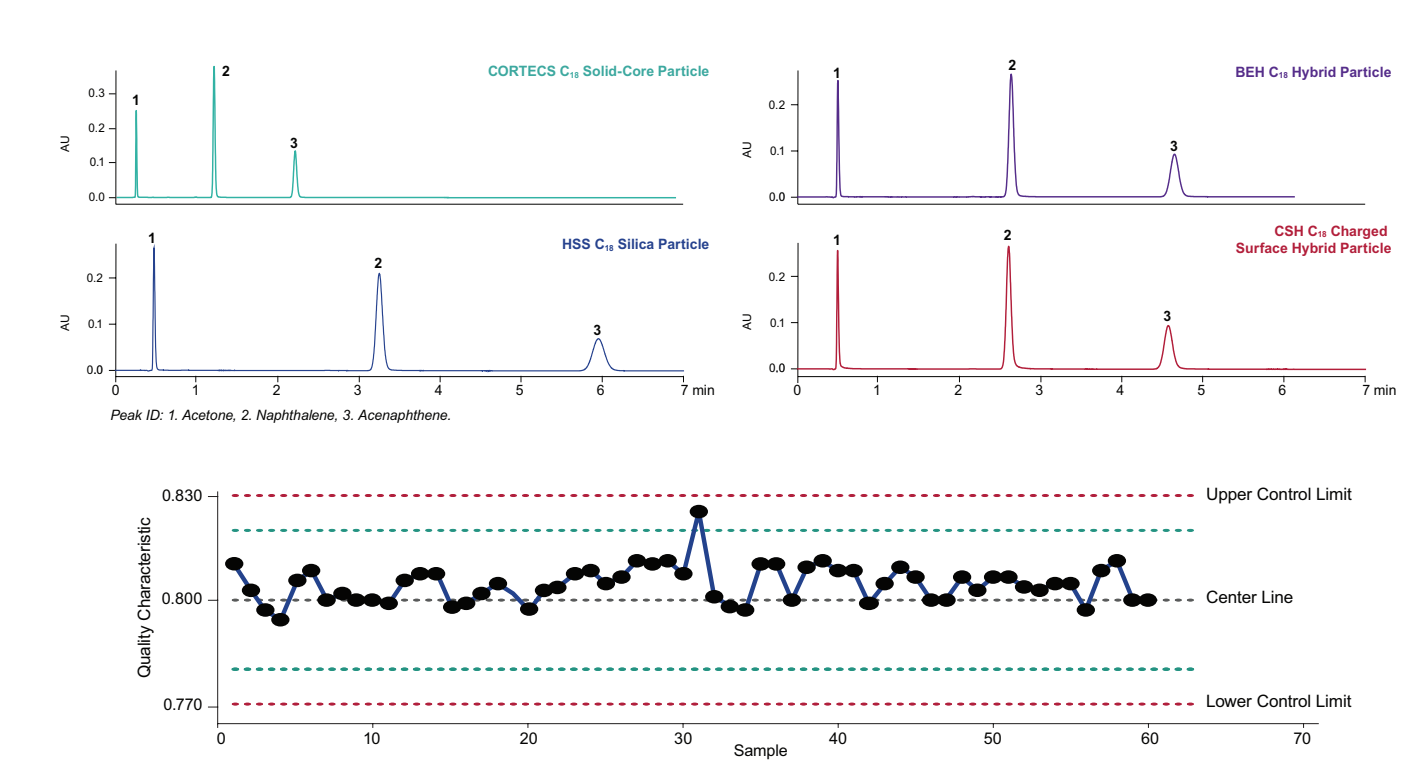
Benchmarking System Performance

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**
- Retention time range or reproducibility
 - Peak area range or reproducibility
 - Peak tailing range
 - Peak resolution
 - Response
 - System pressure



Versatility of Neutrals QC Reference Material



Benchmarking System Performance

Instrument bandwidth 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 bandwidth values.

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

Select column configurations for chemistries that show the MaxPeak™ Premier symbol are available in the MaxPeak Premier Column format. The MaxPeak 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.



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