

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

[waters.com]

 BEH Technology	 HSS Technology	 Solid-Core Technology	 Atlantis Particles
<ul style="list-style-type: none">High retentivity for basic compoundsExcellent peak shape at elevated pHGood universal column choice for a wide variety of compoundsStable across a wide pH rangeFor separations at high temperatures (80 °C)	<ul style="list-style-type: none">High retentivity for polar organic compounds and metabolitesBalanced retention of polar and hydrophobic analytesHigh strength silica for mechanical stability	<ul style="list-style-type: none">Good separations for basic compounds under low pH conditionsExcellent MS performance with formic acid as a mobile-phase modifierFast pH switching and column equilibration	<ul style="list-style-type: none">Maximum efficiencyIncreased sensitivitySeamless scalability from UPLC™ to HPLC
Wide pH Range			
BEH C₈ UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5, 10 μm	BEH C₁₈ UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5, 10 μm	BEH Shield RP18 UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5, 10 μm	BEH Phenyl UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm
BEH HILIC UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm	BEH Amide UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm		

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

Polar Retention			
BEH C₈ AX UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 5 μm	BEH Z-HILIC UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 5 μm	Silica T3 HPLC: 3, 5, 10 μm	Silica HILIC HPLC: 3, 5 μm
Silica C₈ HPLC: 3, 5, 10 μm			

For more information on selectivity, see our **Simplified Column Selection and Method Development Wall Chart (72007934EN)**
Not sure where to start? Get help with the **Column Coach (https://find.waters.com/ColumnCoach/existingcolumn/column)**

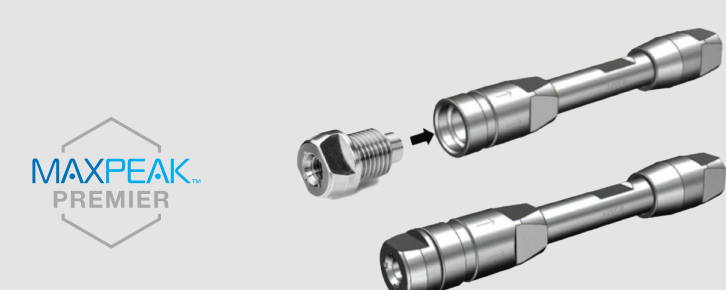
Extend Column Performance and Lifetime

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)



The VanGuard FIT Column design for the MaxPeak Premier Columns minimizes extra-column dispersion, enabling users to take advantage of the benefits of guard columns with an easy to use integrated design. Engineered to thread directly into the inlet of the MaxPeak Premier FIT Column, separation integrity is maintained.



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

Selection Guide

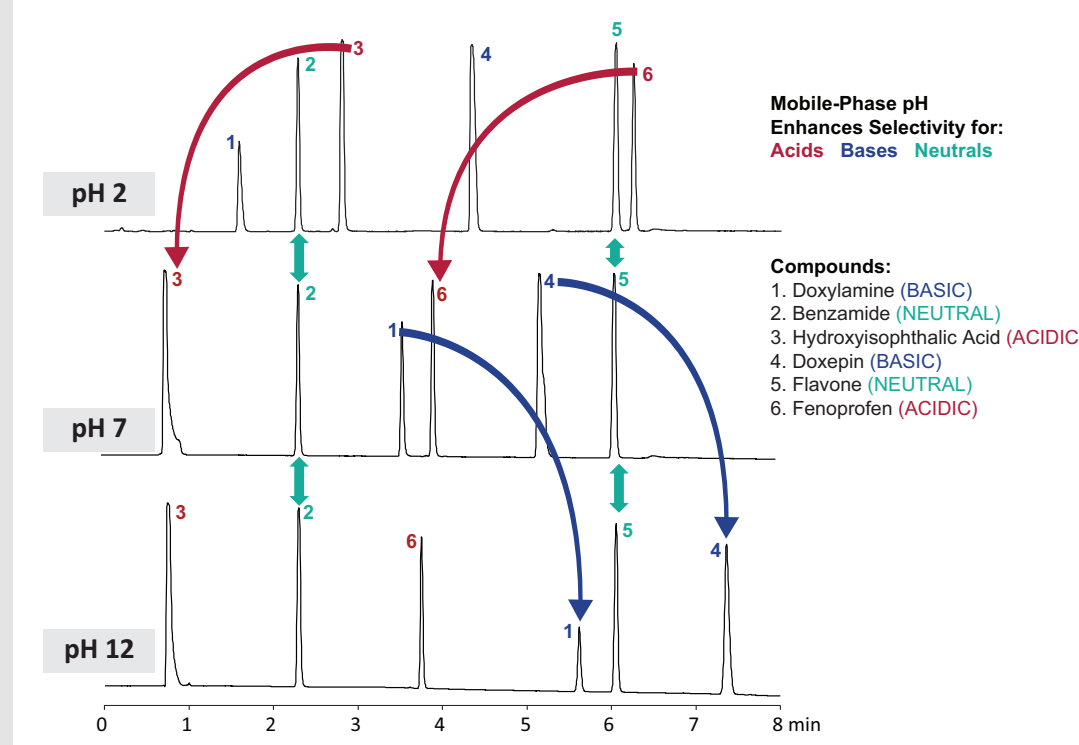
Column I.D.	Particle Size	VanGuard Format*	VanGuard Dimension
2.1 mm	<2 μm	VanGuard FIT Cartridge	2.1 x 5 mm
2.1 mm	>2 μm	VanGuard FIT Cartridge	2.1 x 5 mm
3.9 mm	>2 μm	VanGuard FIT Cartridge	3.9 x 5 mm

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 Carbonate	6.35, 9.25	5.9-6.9 and 8.8-9.8	(NH ₄) ₂ CO ₃	0.961 g	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 ₄ H ₉ 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) ₃ (CH ₃ COONa) ₃	2.58 g	Citric Acid or NaOH	✗
Trifluoroacetic Acid (TFA)	0.3	—	CF ₃ COOH	0.743 mL	—	✓

Importance of Mobile-Phase pH

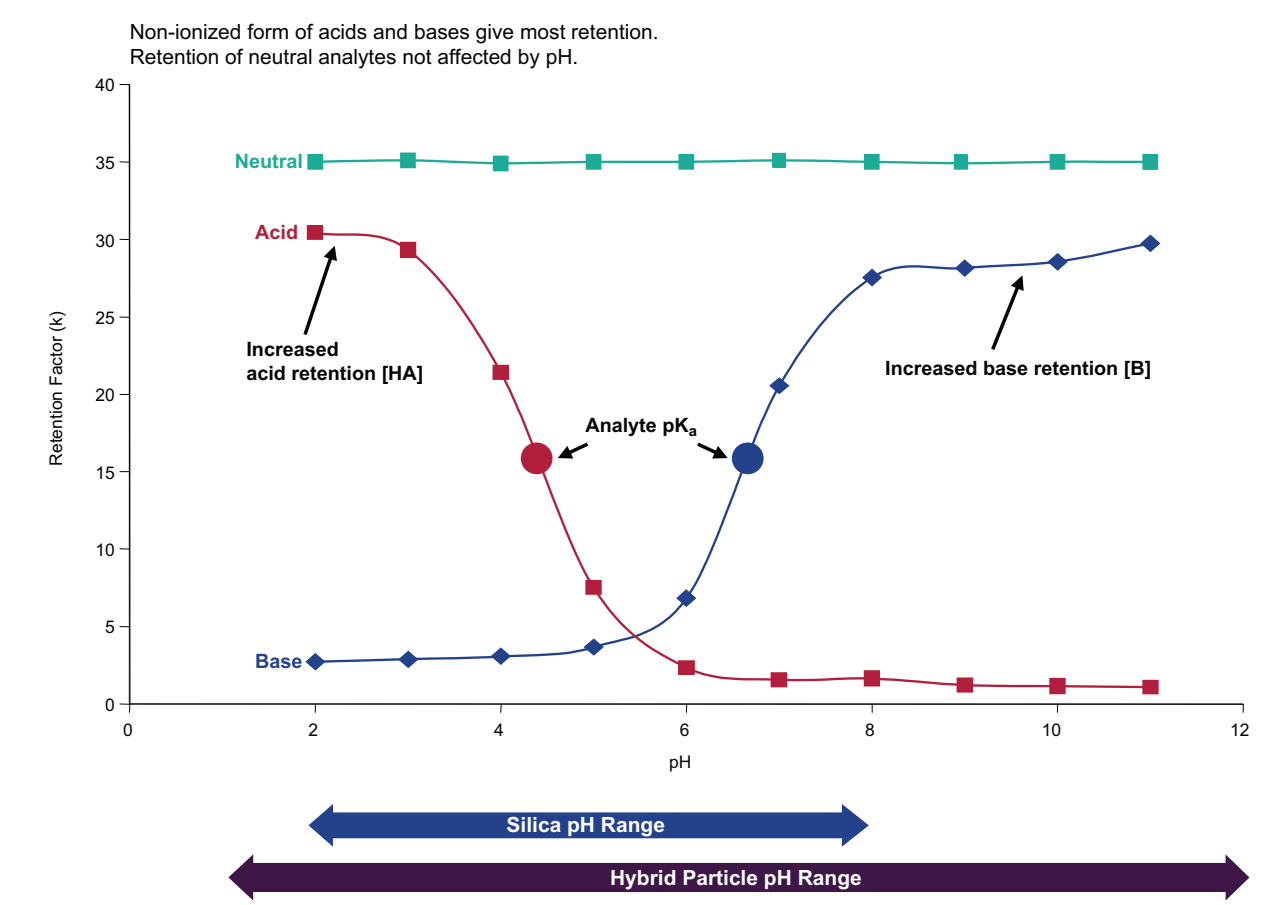
- Using a wide mobile-phase pH range is an effective approach to change compound selectivity.
- Increase selectivity for:
 - Acids (Peaks 3 and 6)
 - Bases (Peaks 1 and 4)
- Neutrals (Peaks 2 and 5) are largely unaffected by mobile-phase pH.

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.

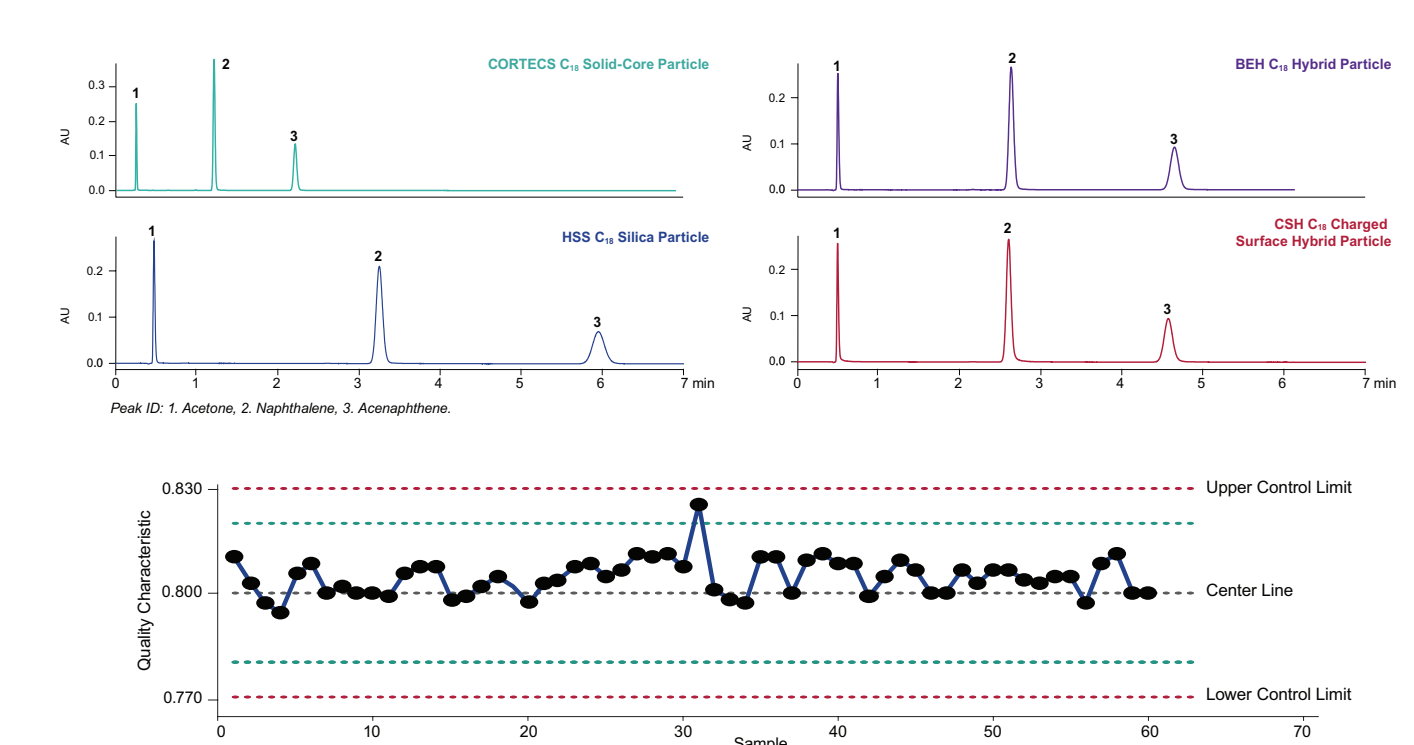
Typical Criteria

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



For QCRM recommendations, see our **Waters Columns, Analytical Standards & Reagents Selection Guide Wall Chart (72002241EN)**.

Versatility of Neutrals QC Reference Material



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.

Waters Systems	Band Spread (μL) 4 sigma	Recommended Column Particle Sizes and I.D.s
Alliance e2695 HPLC System	45-70	3.0-5.0 μm/4.6 mm ID
Arc HPLC System	50-70	3.0-5.0 μm/4.6 mm ID
Alliance IS HPLC System	19-26	3.0-5.0 μm/4.6 mm ID
ACQUITY Arc Systems	20-30	2.x μm/3.0 mm ID
Arc Premier Systems	≤30	2.x μm/3.0 mm ID
ACQUITY UPLC System (Classic)	10-14	<2 μm/2.1 mm ID
ACQUITY Premier Systems	9-14	<2 μm/2.1 mm ID

Other Systems	Band Spread (μL) 4 sigma	Recommended Column Particle Sizes and I.D.s
Agilent 1100 Series	30-45	See manufacturer recommendations
Agilent 1260 Infinity/Infinity II Series	17-25	See manufacturer recommendations
Thermo U 3000 Systems	17-28	See manufacturer recommendations
Shimadzu i-Series/Advanced i-Series	14-20	See manufacturer recommendations

Disclaimer: Configuration differences will impact dispersion, including column heaters, detectors, tubing, flow cells, etc. All values are guidelines. For accuracy measure the dispersion for your system.

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.



System category	HPLC	UHPLC	UPLC
Dispersion (5 sigma)	>40 μL	22-29 μL	<15 μL
Particle Size*	3-5 μm	2-3 μm	<2 μm
Routine Pressure	<9500 psi	<9500 psi	<18,000 psi
Column I.D.*	3.0-4.6 mm	2.1-3.0 mm	1.0-2.1 mm

Note: Measurements given are representative of the LC category, and do not necessarily represent the instruments pictured
*All column attributes are recommended to optimize performance