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

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6.6					Mobile-Phase Chemical	pK _a	Buffer	Formula	Volume or Mass Required for	pH Adjustment	MS Compatible2	
	Bridge				C⊙RTECS	Acetic Acid (glacial)	4.8	Range	сн соон	0.571 ml	Acid/Base	Compatible?
	COLUMNS			Columns	COLUMNS	Ammonium Acetate nK 1	4.0	38-58		0.371 mL		
						Ammonium Acetate pK _a	4.0	8 2 10 2		0.770 g		• •
	BEH lechnology		HSS Technology	CSH lechnology	Solid-Core lechnology	Ammonium Ricarbonate	9.2 10.3	(8.2-11.3)		0.790 g		
						Ammonium Formate pK 1	3.8	2.8-4.8		0.640 g		
			 Good separations for basic compounds 	 Maximum efficiency 	Ammonium Formate pK _a 1	0.2	2.0-4.0		0.640 g		• •	
	 Excellent peak shape at elevated pH 	compounds ar	nd metabolites	under low pH conditions	 Increased sensitivity 	Ammonium Hydrovido	9.2	0.2-10.2		0.640 g		• •
	 Good universal column choice for a wide variety of compounds 	 Balanced retern hydrophobic a 	ntion of polar and nalytes	 Excellent MS performance with formic acid as a mobile-phase modifier 	■ Seamless scalability from UPLC [™] to UHPLC to HPLC	Ammonium Phoephote Dibasia	72.02	(6.2.10.2)		1.22 g		V
	 Stable across a wide pH range 	 High strength stability 	silica for mechanical	 Fast pH switching and column equilibration 		Earmia Aaid	2.9	(0.2-10.2)		0.420 ml		~
	 For separations at high temperatures (80 °C) 			- 4		N Mathulaurralidina	10.2	_		1.04 ml	_	
						R-methylpyrolidine Rhosphoric Acid	2.1	_		0.590 ml	_	v v
	Wide pH Range					Priosphoric Acid	2.1	(11.2.1)		0.580 IIIL		~
PREMIER	BEH C ₁₈ UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5, 10 μm	-0-5i -0-5i	Performance Benefits: General p to extreme pH stability and applica	burpose column ideally suited for method development due ability to the broadest range of compound classes.	Bonding : Trifunctional C ₁₈ , fully end-capped, bonded o Ethylene Bridged Hybrid (BEH) substrate.	Potassium Phosphate, Monobasic	2.1	(1.1-3.1)	KH ₂ PO ₄	1.30 g		X
~	BEH C ₈	-0-si	Performance Benefits : General p due to extreme pH stability and ap	purpose column ideally suited for method development E pplicability to the broadest range of compound classes. t	Bonding : Trifunctional C ₈ , fully end-capped, bonded o Ethylene Bridged Hybrid (BEH) substrate.	Potassium Phosphate, Dibasic	1.2	(0.2-8.2)		1.74 g		X
\frown	UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5, 10 μm BEH Shield RP18		Performance Benefits: Alternate	selectivity compared to straight chain C ₁₈ , E	Bonding: Monofunctional embedded polar C ₁₈ , fully end-		12.7	(11.7-13.7)		2.12 g	H_3PO_4 or KOH	×
	UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5, 10 μm	-0-51 <u>roter troop</u> + + + + + + + + + + + + + + + + + + +	particularly with phenolic analyte	s. Compatible with 100% aqueous-phase composition.	apped, bonded to Ethylene Bridged Hybrid (BEH) substrate.	Pyrrolidine	11.3	-		0.833 mL	-	V
	BEH Phenyl UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm	••	Performance Benefits: Excellent particularly in regard to polyarom bonded phase.	method development column for alternate selectivity, E atic compounds. Unique level of pH stability for a Phenyl t	Bonding : Trifunctional C ₆ Phenyl, fully end-capped, bonded o Ethylene Bridged Hybrid (BEH) substrate.		9.1, 12.7, 13.8	(8.2-14)		2.01 g	H ₃ BO ₄ or NaOH	X
	BEH HILIC		Performance Benefits: Excellent	for retention of very polar, basic, water soluble analytes.	Bonding: Unbonded Ethylene Bridged Hybrid (BEH)		3.1, 4.8, 6.4	(2.1-7.4)		2.58 g	Citric Acid or NaOH	X
	UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm		high concentrations of organic so	Ilvent.	Panding: Trifunctional amida bandad to Ethylana Pridaad	Iriethylamine (TEA)	11.01	_	(CH ₃ CH ₂) ₃ N	1.39 mL		✓
PREMIER	BEH Amide		 Performance Benefits: Rugged HiLLC 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. 		lybrid (BEH) substrate.	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 ₃ COOH	✓
\sim						Iriethylammonium Acetate (IEAA) 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	✓
Wide Selectivity Range				Iriethylammonium Formate (IEAF) pK _a 1	3.75	2.8-4.8	$(CH_3CH_2)_3N:NH_4COOH (1:2)$	0.695 mL 1EA/0.420 mL Formic Acid	TEA or HCOOH	✓		
						Triathy damage and una Farmanta (TFAF) m/ 0	11 01	10 0 10 0				
	CSH C ₁₈	-9 -0*	Performance Benefits : General p pH stability and rapid mobile-pha	purpose reversed-phase column that offers excellent se re-equilibration for method development. Charged t	Bonding: Trifunctional C ₁₈ ligand, fully end-capped, bonded o a Charged Surface Hybrid (CSH) particle substrate.	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	v
	CSH C₁₈ UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5, 10 μm	-0,-5i -0,-5i -0	Performance Benefits: General p pH stability and rapid mobile-pha Surface Hybrid (CSH™) Technolog capacity for basic compounds.	purpose reversed-phase column that offers excellent se re-equilibration for method development. Charged ty enables superior peak shape and increased loading	Bonding : Trifunctional C ₁₈ ligand, fully end-capped, bonded o a Charged Surface Hybrid (CSH) particle substrate.	Triethylammonium Formate (TEAF) pK _a 2 Trifluoroacetic Acid (TFA)	11.01 0.3	10.0-12.0 —	(CH ₃ CH ₂) ₃ N:NH₄COOH (2:1) CF ₃ COOH	1.39 mL TEA/0.210 mL Formic Acid 0.743 mL	TEA or HCOOH —	 ✓
	CSH C₁₈ 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	••••••••••••••••••••••••••••••••••••••	Performance Benefits: General p pH stability and rapid mobile-pha Surface Hybrid (CSH [™]) Technolog capacity for basic compounds. Performance Benefits: General pu interactions with polyaromatic co pH extremes. Charged Surface Hy increased loading capacity for basi	purpose reversed-phase column that offers excellent ise re-equilibration for method development. Charged gy enables superior peak shape and increased loading urpose alternative selectivity ligand that provides pi-pi impounds, while maintaining excellent reproducibility at ybrid (CSH) Technology enables superior peak shape and sic compounds.	Bonding: Trifunctional C₁8 ligand, fully end-capped, bonded o a Charged Surface Hybrid (CSH) particle substrate. Bonding: Trifunctional C6 Phenyl ligand, fully end-capped, bonded to a CSH particle substrate.	Triethylammonium Formate (TEAF) pK _a 2 Trifluoroacetic Acid (TFA) Importance of Mobile-Phase pH	0.3	10.0-12.0	(CH ₃ CH ₂) ₃ N:NH ₄ COOH (2:1) CF ₃ COOH Dependence of	1.39 mL TEA/0.210 mL Formic Acid 0.743 mL Retention on pH: Reversed-Phase Retention Map	TEA or HCOOH	 ✓
	CSH C ₁₈ 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	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array} $	Performance Benefits: General p pH stability and rapid mobile-pha Surface Hybrid (CSH [™]) Technolog capacity for basic compounds. Performance Benefits: General pu interactions with polyaromatic co pH extremes. Charged Surface Hy increased loading capacity for base Performance Benefits: General p of analyte selectivity, especially w Hybrid (CSH) Technology enables basic compounds	purpose reversed-phase column that offers excellent ise re-equilibration for method development. Charged gy enables superior peak shape and increased loading urpose alternative selectivity ligand that provides pi-pi impounds, while maintaining excellent reproducibility at ybrid (CSH) Technology enables superior peak shape and sic compounds. purpose column that provides a very high degree yhen using low-pH mobile phases. Charged Surface s superior peak shape and increased loading capacity for	Bonding: Trifunctional C ₁₈ ligand, fully end-capped, bonded o a Charged Surface Hybrid (CSH) particle substrate. Bonding: Trifunctional C ₆ Phenyl ligand, fully end-capped, bonded to a CSH particle substrate. Bonding: Trifunctional propyl fluorophenyl ligand, non-end-capped, bonded to a CSH particle substrate.	 Triethylammonium Formate (TEAF) pK_a 2 Trifluoroacetic Acid (TFA) Importance of Mobile-Phase pH Using a wide mobile-phase pH range is an compound selectivity. Increase selectivity for: 	11.01 0.3 n effective approach	10.0-12.0 — to change	(CH ₃ CH ₂) ₃ N:NH ₄ COOH (2:1) CF ₃ COOH Dependence of The pH of the mobi choose a mobile-p	1.39 mL TEA/0.210 mL Formic Acid 0.743 mL Retention on pH: Reversed-Phase Retention Map le phase has the greatest impact on analyte retention. For hase pH that corresponds to the plateau regions of the rete	TEA or HCOOH — the most robust separations ention map.	 ✓ S,
PREMIER	CSH C ₁₈ 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 C ₁₈ UPLC: 1.8 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \end{array}\\ \begin{array}{c} \end{array}\\ \end{array}\\ \end{array}$ \left(\begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \left(\begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \left(\begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \left(\begin{array}{c} \end{array}\\ \end{array}\\ \end{array}\\ \left(\begin{array}{c} \end{array}\\ \left(\begin{array}{c} \end{array}\\ \left(\begin{array}{c} \end{array}\\ \left(\begin{array}{c} \end{array}\\ \end{array}\\ \left(\begin{array}{c} \end{array}\\ \left(\end{array}) \left(\end{array}) \left(\end{array}) \left(\end{array}) \left(\end{array}) \left(\end{array}) \left(\end{array}) \left(\end{array})	 Performance Benefits: General p pH stability and rapid mobile-pha Surface Hybrid (CSH™) Technolog capacity for basic compounds. Performance Benefits: General pu interactions with polyaromatic co pH extremes. Charged Surface Hy increased loading capacity for base Performance Benefits: General p of analyte selectivity, especially w Hybrid (CSH) Technology enabless basic compounds. Performance Benefits: Ultra perf peak shape, resists acid hydrolysi 	purpose reversed-phase column that offers excellent use re-equilibration for method development. Charged gy enables superior peak shape and increased loading urpose alternative selectivity ligand that provides pi-pi mpounds, while maintaining excellent reproducibility at ybrid (CSH) Technology enables superior peak shape and sic compounds. purpose column that provides a very high degree then using low-pH mobile phases. Charged Surface s superior peak shape and increased loading capacity for formance C ₁₈ chemistry, increased retention, superior is at low pH.	Bonding: Trifunctional C ₁₈ ligand, fully end-capped, bonded o a Charged Surface Hybrid (CSH) particle substrate. Bonding: Trifunctional C ₆ Phenyl ligand, fully end-capped, bonded to a CSH particle substrate. Bonding: Trifunctional propyl fluorophenyl ligand, on-end-capped, bonded to a CSH particle substrate. Bonding: Trifunctional propyl fluorophenyl ligand, on-end-capped, bonded to a CSH particle substrate. Bonding: High coverage trifunctional C ₁₉₇ fully end-capped, bonded to a High Strength Silica (HSS) particle substrate.	 Triethylammonium Formate (TEAF) pK_a 2 Trifluoroacetic Acid (TFA) Importance of Mobile-Phase pH Using a wide mobile-phase pH range is an compound selectivity. Increase selectivity for: Acids (Peaks 3 and 6) Bases (Peaks 1 and 4) Neutrals (Peaks 2 and 5) are largely unaffed 	11.01 0.3 n effective approach	10.0-12.0 — to change Ise pH.	(CH ₃ CH ₂) ₃ N:NH ₄ COOH (2:1) CF ₃ COOH Dependence of The pH of the mobi choose a mobile-p Non-ionized f Retention of r	1.39 mL TEA/0.210 mL Formic Acid 0.743 mL Retention on pH: Reversed-Phase Retention Map the phase has the greatest impact on analyte retention. For mase pH that corresponds to the plateau regions of the reter orm of acids and bases give most retention. eutral analytes not affected by pH.	TEA or HCOOH — the most robust separations ention map.	↓ •
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Triethylammonium Formate (TEAF) pK _a 2 Trifluoroacetic Acid (TFA) Importance of Mobile-Phase pH Using a wide mobile-phase pH range is an compound selectivity. Increase selectivity for: Acids (Peaks 3 and 6) Bases (Peaks 1 and 4) Neutrals (Peaks 2 and 5) are largely unaffer The Importance of Mobile-Phase pH: Rapid	11.01 0.3 n effective approach ected by mobile-pha d Method Developm	10.0-12.0 — to change ise pH. ent bbile-Phase pH	(CH ₃ CH ₂) ₃ N:NH ₄ COOH (2:1) CF ₃ COOH Dependence of The pH of the mobil choose a mobile-p Non-ionized f Retention of the 30 - No	1.39 mL TEA/0.210 mL Formic Acid 0.743 mL Retention on pH: Reversed-Phase Retention Map le phase has the greatest impact on analyte retention. For hase pH that corresponds to the plateau regions of the reter orm of acids and bases give most retention. eutral analytes not affected by pH.	TEA or HCOOH	↓ 5,
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PREMIER	CSH C ₁₈ 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 UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm HSS C ₁₈ UPLC: 1.8 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm HSS C ₁₈ UPLC: 1.8 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm HSS C ₁₈ 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 C ₁₈ UPLC: 1.6 μm / UHPLC: 2.7 μm T3 UPLC: 1.6 μm / UHPLC: 2.7 μm	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ & - & 0 \\ & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} $ \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\	 Performance Benefits: General p pH stability and rapid mobile-pha Surface Hybrid (CSH[™]) Technolog capacity for basic compounds. 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Increase selectivity for: Acids (Peaks 3 and 6) Bases (Peaks 1 and 4) Neutrals (Peaks 2 and 5) are largely unaffe The Importance of Mobile-Phase pH: Rapid pH 2 pH 2 pH 12 pH	11.01 0.3 In effective approach ected by mobile-pha d Method Developm 5 6 6 7 8	10.0-12.0 	(CH ₃ CH ₂) ₃ N:NH ₄ COOH (2:1) CF ₃ COOH Dependence of The pH of the mobil choose a mobile-p Non-ionized f Retention of the 10- 10- 5- 0 0 0 15- 10- 5- 0 0 0 15- 10- 15- 10- 15- 10- 15- 15- 15- 15- 15- 15- 15- 15	1.39 mL TEA/0.210 mL Formic Acid 0.743 mL Retention on pH: Reversed-Phase Retention Map e phase has the greatest impact on analyte retention. For hase pH that corresponds to the plateau regions of the reter orm of acids and bases give most retention. eutral analytes not affected by pH.	TEA or HCOOH	5,
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Bonding: Trifunctional C ₁₀ bonding and endcapping, bonded o a silica solid-core partic	Triethylammonium Formate (TEAF) pK _a 2 Trifluoroacetic Acid (TFA) Using a wide mobile-phase pH range is an compound selectivity for: Acids (Peaks 3 and 6) Bases (Peaks 1 and 4) Neutrals (Peaks 2 and 5) are largely unaffer The Importance of Mobile-Phase pH: Rapion	11.01 0.3 In effective approach ected by mobile-phase d Method Developm 5 6 6 7 8	10.0-12.0 	(CH ₃ CH ₂) ₃ N:NH ₄ COOH (2:1) CF ₃ COOH Dependence of The pH of the mobile-p Non-ionized f Retention of r 40 35 0 (3) 15 10 5 0 0 (4) 15 15 15 15 15 15 15 15 15 15	1.39 mL TEA/0.210 mL Formic Acid 0.743 mL Retention on pH: Reversed-Phase Retention Map the phase has the greatest impact on analyte retention. 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PREMIER	CSH C ₁₈ 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 UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm HSS C ₁₈ UPLC: 1.7 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm HSS C ₁₈ UPLC: 1.8 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm HSS C ₁₈ 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 HSS CN UPLC: 1.8 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm T3 UPLC: 1.8 μm / UHPLC: 2.5 μm / HPLC: 3.5, 5 μm T3 UPLC: 1.6 μm / UHPLC: 2.7 μm T4 UPLC: 1.6 μm / UHPLC: 2.7 μm T4 UPLC: 1.6 μm / UHPLC: 2.7 μm T4 UPLC: 1.6 μm / UHPLC: 2.7 μm T4	$ \begin{array}{c} \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \begin{array}{c} & - & 0 \\ \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} $ \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \begin{array}{c} \end{array} \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array} \\ \end{array}	 Performance Benefits: General p pH stability and rapid mobile-pha Surface Hybrid (CSH™) Technolog capacity for basic compounds. Performance Benefits: General pu interactions with polyaromatic co pH extremes. Charged Surface Hy increased loading capacity for basic of analyte selectivity, especially w Hybrid (CSH) Technology enabless basic compounds. Performance Benefits: Ultra perf peak shape, resists acid hydrolysic Performance Benefits: Unique, n method development scientists. C under low-pH conditions. Performance Benefits: A general differences for Lewis bases throug additional selectivity based on shapes and normal-phase separational phase and normal-phase separational perficiency. A charged-surface-silic basic compounds at low pH, especially performance Benefits: General p efficiency. Balanced retention of at Performance Benefits: General p efficiency. Provides balanced retention perficiency. Alternative selectivity Performance Benefits: Caneral p efficiency. Alternative selectivity of performance Benefits: Excellent efficiency. Alternative selectivity of performance Benefits: Excellent efficiency. Alternative selectivity of compounds. 	purpose reversed-phase column that offers excellent tise re-equilibration for method development. Charged typ enables superior peak shape and increased loading It urpose alternative selectivity ligand that provides pi-pi mpounds, while maintaining excellent reproducibility at ybrid (CSH) Technology enables superior peak shape and sic compounds. It purpose column that provides a very high degree superior peak shape and increased loading capacity for formance C ₁₈ chemistry, increased retention, superior is at low pH. It toon-end-capped C ₁₈ chemistry designed specifically for Differs unique Selectivity for Bases (SB) when operating It purpose column designed to maximize selectivity gh pi-pi interactions. The rigid aromatic ring provides ape, dipole moment, and hydrogen bonding interactions. It purpose column that shows contrasting analyte phases. This column can be used for both reversed- ions. It purpose reversed-phase column designed to maximize cacsolid-core particle enables excellent peak shape for triality in low concentration modifier mobile phases. to traditional C ₁₈ columns. It purpose column designed to maximize acids, bases, and neutrals at low- and mid-range pH. It mobile-phase compatible column designed to maximize to traditional C ₁₈ columns. It purpose reversed-phase column designed to maximize there on the polar and non-polar compounds. It purpose column designed to maximize there on poth polar and non-polar compounds. It </td <td>Bonding: Trifunctional C₁₈ ligand, fully end-capped, bonded Bonding: Trifunctional C₆ Phenyl ligand, fully Ind-capped, bonded to a CSH particle substrate. Bonding: Trifunctional propyl fluorophenyl ligand, non-end-capped, bonded to a CSH particle substrate. Bonding: High coverage trifunctional C₁₉₉ fully end-capped, bonding: High coverage trifunctional C₁₉₉ fully end-capped, bonding: Intermediate coverage trifunctionally bonded C₁₉₉ bonding: T3 (C₁₈) bonding and endcapping, bonded bonding: T3 (C₁₈) bonding and endcapping, bonded bonding: Tifunctional pentafluorophenyl ligand, non-end-capped, bonded to High Strength Silica HSS) substrate. Bonding: Sterically hindered, mono-functional cyano-propyl gand, non-end-capped, bonded to High Strength Silica HSS) substrate. Bonding: Intermediate coverage trifunctional-C₁₈ ligand, fully sonding: Intermediate coverage trifunctional-C₁₈ ligand, fully sonding: Intermediate coverage trifunctional-C₁₈ ligand, fully sonding: T3 (C₁₈) bonding and endcapping, bonded o a charged surface silica solid-core particle substrate. Bonding: Trifunctional C₁₈ ligand, fully end-capped, bonded o a charged surface silica solid-core particle substrate. Bonding: T3 (C₁₈) b</td> <td>Triethylammonium Formate (TEAF) pK_a 2 Trifluoroacetic Acid (TFA) Importance of Mobile-Phase pH Using a wide mobile-phase pH range is an compound selectivity for: Acids (Peaks 3 and 6) Bases (Peaks 1 and 4) Neutrals (Peaks 2 and 5) are largely unaffer The Importance of Mobile-Phase pH: Rapid H 2 H 2 H 2 D 1 D 2 D 2 D 2 D 2 D 2 D 2 D 2 D 2</td> <td>11.01 0.3 In effective approach ected by mobile-pha d Method Developm 5 6 7 8</td> <td>10.0-12.0 </td> <td>(CH₃CH₂)₃N:NH₄COOH (2:1) CF₃COOH Dependence of The pH of the mobile-p Non-ionized f Retention of r 1 1 1 2 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1</td> <td>1.39 mL TEA/0.210 mL Formic Acid 0.743 mL Retention on pH: Reversed-Phase Retention Map e phase has the greatest impact on analyte retention. For hase pH that corresponds to the plateau regions of the reter orm of acids and bases give most retention. eutral analytes not affected by pH. utral Acid Analyte pK billica pH Range Cid retention [HA] Billica pH Range Hybrid Particle pH Range</td> <td>TEA or HCOOH</td> <td>\$</td>	Bonding: Trifunctional C ₁₈ ligand, fully end-capped, bonded Bonding: Trifunctional C ₆ Phenyl ligand, fully Ind-capped, bonded to a CSH particle substrate. Bonding: Trifunctional propyl fluorophenyl ligand, non-end-capped, bonded to a CSH particle substrate. Bonding: High coverage trifunctional C ₁₉₉ fully end-capped, bonding: High coverage trifunctional C ₁₉₉ fully end-capped, bonding: Intermediate coverage trifunctionally bonded C ₁₉₉ bonding: T3 (C ₁₈) bonding and endcapping, bonded bonding: T3 (C ₁₈) bonding and endcapping, bonded bonding: Tifunctional pentafluorophenyl ligand, non-end-capped, bonded to High Strength Silica HSS) substrate. Bonding: Sterically hindered, mono-functional cyano-propyl gand, non-end-capped, bonded to High Strength Silica HSS) substrate. Bonding: Intermediate coverage trifunctional-C ₁₈ ligand, fully sonding: Intermediate coverage trifunctional-C ₁₈ ligand, fully sonding: Intermediate coverage trifunctional-C ₁₈ ligand, fully sonding: T3 (C ₁₈) bonding and endcapping, bonded o a charged surface silica solid-core particle substrate. Bonding: Trifunctional C ₁₈ ligand, fully end-capped, bonded o a charged surface silica solid-core particle substrate. Bonding: T3 (C ₁₈) b	Triethylammonium Formate (TEAF) pK _a 2 Trifluoroacetic Acid (TFA) Importance of Mobile-Phase pH Using a wide mobile-phase pH range is an compound selectivity for: Acids (Peaks 3 and 6) Bases (Peaks 1 and 4) Neutrals (Peaks 2 and 5) are largely unaffer The Importance of Mobile-Phase pH: Rapid H 2 H 2 H 2 D 1 D 2 D 2 D 2 D 2 D 2 D 2 D 2 D 2	11.01 0.3 In effective approach ected by mobile-pha d Method Developm 5 6 7 8	10.0-12.0 	(CH ₃ CH ₂) ₃ N:NH ₄ COOH (2:1) CF ₃ COOH Dependence of The pH of the mobile-p Non-ionized f Retention of r 1 1 1 2 1 2 1 1 1 1 1 1 1 1 1 1 1 1 1	1.39 mL TEA/0.210 mL Formic Acid 0.743 mL Retention on pH: Reversed-Phase Retention Map e phase has the greatest impact on analyte retention. For hase pH that corresponds to the plateau regions of the reter orm of acids and bases give most retention. eutral analytes not affected by pH. utral Acid Analyte pK billica pH Range Cid retention [HA] Billica pH Range Hybrid Particle pH Range	TEA or HCOOH	\$



Benchmarking System Performance

useful troubleshooting tool.

1. Retention time range or reproducibility

2. Peak area range or reproducibility

Typical Criteria

3. Peak tailing range

4. Peak resolution

6. System pressure

5. Response

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	Column Selection Guide			
Shimadzu Prominence UFLC	HPLC	41 µL	XBridge 3.5, 5 µm; XSelect 3.5, 5 µm;				Orrama kaza
Alliance™ 2695 HPLC	HPLC	29 µL	CORTECS 2.7 µm				
Agilent 1260 UHPLC (600 bar)	HPLC	28 µL	3.0-4.6 mm I.D.		Alliance HPLC	ACQUITY Arc	ACQUITY UPLC H-Clas
Thermo Accela UHPLC	HPLC	21 µL	XBridge 2.5, 5 μm; XSelect 2.5, 5 μm;	System	HPLC	UHPLC	UPLC

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.

1 2 3 4 5 6 7 8 9 10 11 12 13 14	BEH C ₁₈	Conditions:
$1 \qquad 2 \qquad 1 \qquad 3 \qquad 4 \qquad 5 \qquad 7 \qquad 6 \qquad 8 \qquad 10 \qquad 9 \qquad 1 \qquad 12,1314 \qquad 2 \qquad $	BEH C ₈	Columns: 2.1 x 100 mm Mobile phase: 72% water/28% methanol (v/v)
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	BEH Shield RP18	Flow rate: 0.5 mL/min
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	BEH Phenyl	Injection vol.: 5.0 μL Sample conc.: 10 μg/mL
$1 \qquad 2 \qquad 3 \qquad 4 \qquad 5 \qquad 6.7 \qquad 8.9 \qquad 10.11 \qquad 12 \qquad 13 \qquad 14$	CSH C ₁₈	Temperature: 50 °C Detection: UV @ 254 nm
2,3) 4 5 7 9,10,1 1 4 5 7 6 8 12,13,14	CSH Fluoro-Phenyl	Sampling rate: 20 pts/sec
$1 \qquad 2 \qquad 5 \qquad 4 \qquad 3 \qquad 12 \qquad 13 \qquad 14 \qquad 8 \qquad 7 \qquad 7$	CSH Phenyl-Hexyl	Instrument: ACQUITY [™] UPLC with PDA Detector
	14 HSS T3	Compounds [EPA 8330 Standard Mixture):
$-\frac{1}{2} \frac{2}{3} \frac{4}{5} \frac{5}{5} \frac{5}{7} \frac{8}{7} \frac{9}{10} \frac{11}{12} \frac{12}{12} \frac{14}{14}$	HSS C ₁₈	1. HMX
1 2 1 4 5.6 7 8 9 10 11 12 13 14	HSS C ₁₈ SB	2. RDX 3. 1,3,5-Trinitrobenzene
$\begin{array}{c} 3 & 4,5 \\ 1 & 1,12,13,14 \\ 1 & 2 & 1 \\ 1 & 7 \\ 1 & 1,12,13,14 \\ 1 & 9,8 \\ 1 & 6 \\ 1 & 1 \\ 1 & 1,12,13,14 \\ 1 & 9,8 \\ 1 & 1 \\ 1 $	HSS CN	4. 1,3-Dinitrobenzene
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	CORTECS C ₁₈ +	6. Tetryl
$\begin{bmatrix} 1 & 2 & 3 \\ 1 & 2 & 4 \\ 1 & 5 & 6 & 7,8 \\ 1 & 1 & 12 & 13 & 14 \\ 1 & 1 & 1 & 12 & 13 & 14 \\ 1 & 1 & 1 & 1 & 12 \\ 1 & 1 & 1 & 1 & 12 \\ 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1$	CORTECS C ₁₈	8. 2-Amino-4,6-Dinitrotoluene 9. 4-Amino-2 6-Dinitrotoluene
1 2 3 4 7,8 9 10 11 12 13 14	CORTECS UPLC T3	10. 2,4-Dinitrotoluene 11. 2,6-Dinitrotoluene
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	CORTECS C ₈	12. 2-Nitrotoluene 13. 4-Nitrotoluene
$\begin{bmatrix} 1 & 3 & 4 \\ 2 & 4 \end{bmatrix} = \begin{bmatrix} 3 & 10 & 12,8,9 \\ 1 & 2 & 4 \end{bmatrix} = \begin{bmatrix} 12,8,9 & 12,8,9 \\ 1 & 1 & 12,8,9 \end{bmatrix}$	CORTECS UPLC Shield RP18	14. 3-Nitrotoluene
$- 1 2 5 4 3 9_1 3 8 14 10 11 7$	CORTECS Phenyl ၆	HQQ DED
23 0		10 0.12



Selection Guide

based on analytical column I.D.

Comparative separations may not be representative of all applications.

Extend Column Performance and Lifetime



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)



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

Column I.D. Particle Size VanGuard Format VanGuard Dimension

VanGuard Column Protection Cartridge/Pre-column selection

Note: The provided data is for reference only and is based on nominal values for unmodified systems. Any adjustment to the plumbing,				Optimized column dimension matched to Waters LC Systems.				
ACQUITY UPLC H-Class	UPLC	9 µL	2.1 mm l.D.	Column Length	75–250 mm	50–150 mm	≤150 mm	
ACQUITY UPLC H-Class w/Column Manager	UPLC	12 µL	ACQUITY UPLC CSH 1.7 µm; CORTECS 1.6 µm	Column I.D.	4.6 mm (3.0 mm)	3.0 mm (2.1 mm)	2.1 mm (1.0 mm)	
ACQUITY UPLC	UPLC	12 µL	ACOUITY UPLC BEH 1.7 um:	Houtine Flessure	<4000 psi	<10,000 psi	<18,000 psi	
ACQUITY Arc™	UHPLC	23 µL	XBridge 2.5, 5 μm; XSelect 2.5, 5 μm; CORTECS 2.7 μm 3.0 mm I.D.	Boutine Pressure	<1000 psi	<10.000 psi	<18 000 psi	
				Particle Size	3–5 μm	2–3 µm	<2 µm	
				Dispersion	>40 µL	22–29 µL	<15 µL	
Agilent 1290 UHPLC (1200 bar)	UHPLC	17 uL	3.0 mm I D					

Select column configurations for chemistries that show the PREMIER symbol PREMIER 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.

connectivity, and configuration of the system will change the instrument bandspread and will influence the resulting chromatography.



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