

ZORBAX Reversed-Phase HPLC Column Selection Flow Chart

For small and large molecules

Most chromatographers use reversed-phase HPLC as one of their key analysis techniques. Reversed-phase HPLC can be used to analyze ionic and nonionic analytes. Therefore, this ZORBAX Column Selection flow chart will focus on reversed-phase columns. To more easily select a reversed-phase column for method development of small and large molecules, follow the outline below.

This flow chart provides information on choosing an initial column for method development of small molecule and protein and peptide samples, and includes decisions on bonded phase and column configuration.

Small Molecules MW < 2000	Large Molecules MW > 2000
80-120Å	300Å
Packing Pore Size	
<p>First choice of a packing pore size is based on the size of molecules to be analyzed. Typical small molecules can diffuse easily in and out of standard 80-120Å pore packings, but peptides and proteins may not. For this reason, it is recommended to use 300Å pore-size packings (300SB) for isocratic or gradient separations of peptides and proteins.</p>	
Eclipse XDB-C18	StableBond 300SB-C18
Starting Column Bonded Phase	
<p>A C18 is recommended as the starting column bonded phase for most samples since it maximizes retention for moderately polar to non-polar compounds. Shorter chain phases should be considered if resolution cannot be optimized with a C18 phase or if you are analyzing larger proteins.</p>	
4.6mm	4.6 mm
& Column Diameter	
<p>Column diameter defines how much material (mass) you can inject on a column - the smaller the diameter, the less material that can be injected while maintaining good peak shape. Smaller diameter columns provide increased sensitivity and therefore are commonly used for LC/MS and sample limited applications.</p>	

Column Type	Inner Diameter (mm)	Sample Load (approx)	Typical Flow Rate	Increased Sensitivity	Applications
Analytical	4.5	0.1-1.5 mg	0.5-3 mL/min		Standard separations
Solvent Saver	3.0	150-500 ug	0.3-1.5 mL/min	+	Save solvent, uses standard HPLC instrument
NarrowBore	2.1	50-120 ug	0.1-0.5 mL/min	++	High sensitivity, limited sample, LC/MS, save solvent
Microbore	1.0	10-50 ug	10-100 µL/min	++++	High sensitivity, limited sample, LC/MS
Capillary	0.5, 0.3	1-10 ug	1-15 µL/min	+++++	Very high sensitivity, LC/MS, peptides and proteins
Nano	0.1, 0.075	100-200 ng	200-500 nL/min	+++++	Very high sensitivity, LC/MS, peptides and proteins
Semi-Prep	9.4	1-10 mg	5-10 mL/min		mg preparative separations
Preparative	21.2	20-250 mg	20-60 mL/min		Hundreds of milligrams up to 1 gram

Small Molecules MW < 2000	Large Molecules MW > 2000
5µm	5µm
Standard Porous Particle Size	
<p>For conventional analyses of small and large molecules, the standard particle size is 5 µm. However, smaller particle sizes are available and provide higher efficiency and higher resolution. They are also available in short column lengths.</p>	
Fast Analysis Particle Sizes	

Rapid Resolution 3.5µm Rapid Resolution HT 1.8µm Rapid Resolution, 3.5µm particles provide 60% greater efficiency than 5µm particles while Rapid Resolution HT columns, 1.8µm particles provide 200% greater efficiency. Shorter columns can be used to significantly reduce analysis time of small molecules.	Rapid Resolution 3.5µm Poroshell 5µm For peptides, Rapid Resolution, 3.5µm particles can be used with shorter columns for faster, high-resolution gradient analyses. But for proteins, Poroshell columns, with a solid core and porous shell, provide the fastest analysis.		
150mm	Column Length	150 mm	
A conventional column length is 150mm or 250mm for high resolution with 5µm particles. This is a good place to start a typical analysis because it will provide high resolution in a 20-30 minute analysis. For fast analysis, shorter columns (75mm and 50mm) with smaller particle sizes (3.5µm) should be used.	For proteins and peptide digests, 150mm is also a good starting column length. The typical gradient time on a column of this length will be 30-60 minutes for a high-resolution analysis. For fast analysis, shorter columns (50mm) with smaller particles (3.5µm) should be used.		
Starting Column Choices			
Small Molecules		Large Molecules	
Standard Analysis Eclipse XDB-C18 4.6 x 150 mm, 5µm PN 993967-902	Fast Analysis Rapid Resolution Eclipse XDB-C18 4.6 x 75 mm, 3.5µm PN 966967-902 Rapid Resolution HT Eclipse XDB-C18 4.6 x 50 mm, 1.8µm PN 922975-902	Standard Analysis 300SB-C18 4.6 x 150 mm, 5µm PN 883995-902	Fast Analysis Rapid Resolution 300SB-C18 4.6 x 50 mm, 3.5µm PN 865973-902 Poroshell 300SB-C18 2.1 x 75 mm, 5µm PN 660750-902