Considerations for Column Selection in a Purification Workflow

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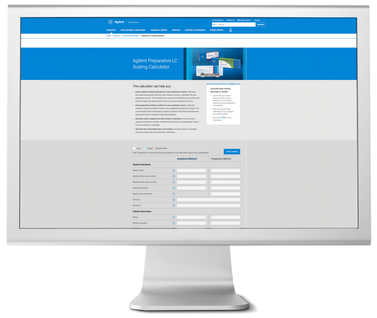
Stationary phase

Select a stationary phase that fits your objectives and applications

Objective	Analyte; Mobile phase conditions	Phase	Specifications				
			Pore size	Surface area	pH range	Carbon load	Endcapped
Throughput (Large number of samples; combinatorial libraries; open access or automated purification)	Low pH	Poroshell 120 SB-C18	120 Å	130 m²/g	1.0 – 8.0	9.0%	No
	High pH	Poroshell 120 HPH-C18	100 Å	95 m²/g	2.0 – 11.0	Proprietary	Double
Yield (Bulk purification; impurity isolation; large injection volumes)	Maximum loadability; high pH	Pursuit XRs C18	100 Å	440 m²/g	1.5 – 10.0	22.0%	Yes
	Lower retention of hydrophobic analytes vs C18	Pursuit XRs C8	100 Å	440 m²/g	1.5 – 10.0	15.0%	Yes
	Maximum loadability; alternate selectivity for aromatic compounds	Pursuit XRs Diphenyl	100 Å	440 m²/g	1.5 – 7.5	14.6%	Yes
Alternate selectivity for throughput or yield (General purpose)	General purpose; high pH	ZORBAX Eclipse Plus C18	95 Å	160 m²/g	2.0 – 9.0	9.0%	Double
	Lower retention of hydrophobic analytes vs C18	ZORBAX Eclipse Plus C8	95 Å	160 m²/g	2.0 – 9.0	7.0%	Double
	Low pH	ZORBAX StableBond C18	80 Å	180 m²/g	0.8 – 8.0	10.0%	No
	Lower retention of hydrophobic analytes vs C18	ZORBAX StableBond C8	80 Å	180 m²/g	1.0 – 8.0	5.5%	No
	Alternate selectivity for aromatic compounds	ZORBAX Eclipse Plus Phenyl-Hexyl	95 Å	160 m²/g	2.0 – 8.0	9.0%	Double



Preparative LC Scaling Calculator

- Scales analytical method parameters to the preparative column
- Scales preparative method conditions to the analytical column
- Calculates column capacity and total number of injections
- Calculates time and mobile phase consumption
- Accounts for differences in particle size and length

Purification workflow

1. Check sample formulation
- Check solubility by adding a few drops of sample solution into beaker of mobile phase at starting conditions.
- In the case of strong sample precipitation, please use sample sandwiching or at column dilution injection technique avoiding injector clogging. **Learn more**

2. Screen columns/mobile phases
- Screen several columns with complementary selectivities using a generic gradient (5-95% organic).
- Only use analytical columns available in matching preparative dimensions. **Learn more**
- Mobile phase: water:acetonitrile and water:methanol most common.
- pH: Avoid two peaks by operating at a pH 1-2 units away from compound's pK.
- Learn more about method development **here**

3. Optimize separation
- Optimizing the method on the analytical system saves sample and solvent.
- Focus on separation of the critical pair; impurities can coelute.
- The resolution of the critical pair determines sample loading capacity.

4. Determine maximum sample load
- Conducting mass and injection volume loading studies on analytical system to save sample and solvent.
- Learn more about loading studies **here**

5. Determine prep column size
- A preparative column that matches the analytical column's stationary phase, particle size and length will provide the most consistent results.
- Larger column ids can handle larger injection volumes and work well for purifying dilute samples in fewer injections.

6. Scale method to prep column
- Use linear scale up equations.
- Use a calculator to quickly scale preparative run conditions.

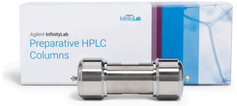
7. Purify
- Purify compounds and reinject for confirmation.

Instrument compatibility

Column internal diameter	Analytical	Semipreparative and preparative			
4.6 mm	1 - 2 mL/min				
9.4 or 10 mm		4 - 10 mL/min			
21.2 mm			18 - 42 mL/min		
30 mm				34 - 85 mL/min	
50 mm					94 - 236 mL/min
Purification range (mg)	0.5 - 15	5 - 50	20 - 400	40 - 800	100 - 2000
Injection volume (µl)	10	100	500	1000	2500
Instrumentation	1260/1290 Infinity II / III analytical-scale LC purification systems				
		1260 Infinity II preparative LC systems			
			1290 Infinity II preparative LC systems		

Helpful resources

- InfinityLab Preparative LC Columns Brochure
- InfinityLab Preparative LC Columns Quick Ordering Guide
- www.agilent.com/chem/prepcolumns
- www.agilent.com/lc/prep-systems
- Preparative LC Scaling Calculator – www.agilent.com/chem/prepcalc



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