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5 Rules of Scaling LC Purification

Rule #1: Scale flow rate and sample load proportionately

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Chemists have been diligent in their pursuit to uncover best practices for achieving more reliable scale up from analysis to PREP LC. PREP LC, or preparative liquid chromatography, is a technique used to isolate target compound(s) from complex reaction mixtures.

To prevent unintended sample loss, the purification scientist must establish practical guidelines, which maximize compound yield and purity while working efficiently.

Identifying and collecting the correct target peaks is effortless when the preparative chromatography mirrors the analytical scouting run. Geometric scaling from analytical to prep, with a properly adjusted flow rate and sample load, ensures that the resolution and chromatographic profile of the separation is maintained.

Employing these simple scaling equations is an important step for direct scale up to PREP

Variables are defined as follows:
1 = Analytical column (used for scouting the crude mixture and method development)
2 = Preparative column (the one to be employed for the PREP LC separation)
F = flow rate; D= column internal diameter; d= particle size of packing material;
V = injection volume; M = crude mass; L = column length.

Why not just skip the equations?

First and foremost, maintaining linear velocity (by scaling the flow rate appropriately) when scaling to PREP prevents peak broadening, changes in target peak elution time, and resolution. Second, keeping the sample mass load on the PREP column proportional to that loaded on the analytical column ensures consistent peak widths and resolution between scales.

Flow Rate

$$F_2 = F_1 \times (D_2^2 / D_1^2)\,(d_{p1}/d_{p2})$$

Sample Load

Injection Volume

$$V_2 = V_1 \times (D_2^2 / D_1^2) \times (L_2 / L_1)$$

Crude Mass

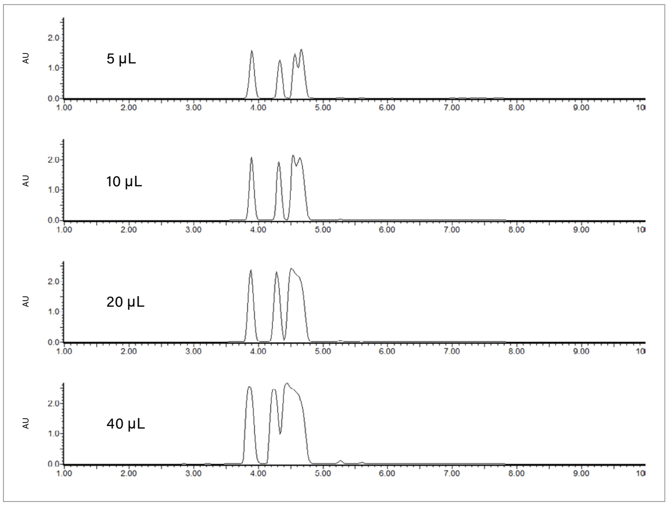
$$M_2 = M_1 \times (L_2 / L_1) \times (D_2^2 / D_1^2)$$

Don't know how much crude to load on the preparative column?

Perform a simple loading study on the analytical column to determine the amount you can load and scale accordingly. The analytical column sample loading study results, shown right, illustrate how increased injection volumes of the same sample mixture impact the chromatography.

Although the 40µL injection volume shows the loss of resolution between the second (target peak) and the later eluting peaks, the 20 µL injection volume maintains that resolution. Thus, the 20 µL volume injected on the analytical column can then be scaled to determine the maximum injection volume for the preparative run. Use the injection volume equation (shown above) or the online Preparative Calculator (available at www.waters.com/prep) to complete the scale-up calculation.

If a loading study cannot be performed, select a PREP column that is likely to accommodate the approximate crude mass available. The mass loading chart for small molecules shown below represents conservative, estimated loads by column dimension. Request a printed Preparative OBD™ Columns Wall Chart for obtaining the mass loading chart, the scale-up equations, and other essential information for scaling up.



Results of loading study on scouting column at various injection volumes of crude mixture.

Approximate Mass Loading Capacity (mg) for Prep OBD Columns

	Diameter (mm)				
Length (mm)	**4.6**	**10**	**19**	**30**	**50**
50	3	15	45	110	310
75	–	–	–	165	–
100	3	25	90	225	620
150	8	40	135	560	1550
Reasonable Flow Rate (mL/min)	1.4	6.6	24	60	164
Reasonable Injection Volume (µL)	20	100	350	880	2450

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