Choosing Preparative Columns

STEP 1

Once the analytical separation has been optimized, a loading study on the analytical column is performed to determine the capacity of the particular packing material. Because the large-scale separation should be identical to the small-scale separation, the maximum sample load will be dependent upon the complexity of the analytical separation.

STEP 2

Determine how much mass you need to purify or isolate.

STEP 3

Once the desired purified mass is established, some simple equations may be used to determine the required column size for purification. In addition, preparative HPLC system maximum flow rate and back pressure need to be considered and can limit column size.

Scale-up Factor

$$\text{Scale-up factor} = \frac{(\text{Diameter preparative})^2 \times \text{Length preparative}}{(\text{Diameter analytical})^2 \times \text{Length analytical}}$$

Consider scaling up from a **4.6 x 150 mm** column to a **19 x 150 mm** column:

$$\text{Scale-up factor} = \frac{(19)^2 \times 150}{(4.6)^2 \times 150} = 17.1$$

Applying the scale-up factor, we can predict that approximately 17–135 mg of sample could be applied to the larger column (packed with the same material as the analytical column). This range is based on an analytical (4.6 mm ID) mass load of 1–8 mg.

Flow Rate

$$\text{Flow rate (prep)} = \text{Flow rate (analytical)} \times \frac{\text{Diameter (prep)}^2}{\text{Diameter (analytical)}^2} \times \frac{\text{Particle Size (analytical)}}{\text{Particle Size (prep)}}$$

The calculated flow rate may be used for the larger column to ensure the same linear velocity of mobile phases as used in the analytical run. However, reasonable flow rates are based on column diameters. Systems will be limited by increasing backpressure with increasing column length and decreasing particle size.

Gradient Duration (GD)

$$\text{GD (prep)} = \frac{\text{GD (analytical)} \times \text{Length (prep)}}{\text{Length (analytical)}} \times \frac{\text{Diameter (prep)}^2}{\text{Diameter (analytical)}^2} \times \frac{\text{Flow rate (analytical)}}{\text{Flow rate (prep)}}$$

Mass Loading

Approximate Mass Loading Capacity (mg) for Preparative OBD Columns (Gradient Mode)

	Diameter (mm)				
Length (mm)	**4.6**	**10**	**19**	**30**	**50**
50	3	15	45	110	310
75	–	–	–	165	–
100	5	25	90	225	620
150	8	40	135	335	930
250	13	60	225	560	1550
Reasonable Flow Rate (mL/min)	1.4	6.6	24	60	164
Reasonable Injection Volume (µL)	20	100	350	880	2450

The calculated preparative gradient duration is entered into the pump's gradient separation over the same number of column volumes as was used in the analytical run.

Many factors affect the mass capacity of preparative columns. The listed capacities represent an 'average' estimate.

Capacity is:

- Higher for strongly retained materials
- Higher for simple mixtures
- Lower where higher resolution is required
- Very strongly dependent on loading conditions
  - limited by loading volume
  - limited by diluent solvent strength

Reasonable flow rates are based on column diameter. Systems will be limited by increasing backpressure with increasing column length and decreasing particle size.

Reasonable injection volumes are based on column diameter at a length of 50 mm with relatively strong solvents. Increased length is compatible with larger injection, but not proportionately so. Weaker solvents significantly increase injection volume.

Mass loading capacities for peptide purifications depend strongly on the sequence and may be estimated at 5–20% of listed values.

Did you know...

Waters offers a Preparative Chromatography Mix Standard to help you benchmark the performance of your preparative/purification system.

For more information, see page 310.