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5 Rules of Scaling LC Purification

Rule #3: Always be consistent with column volumes

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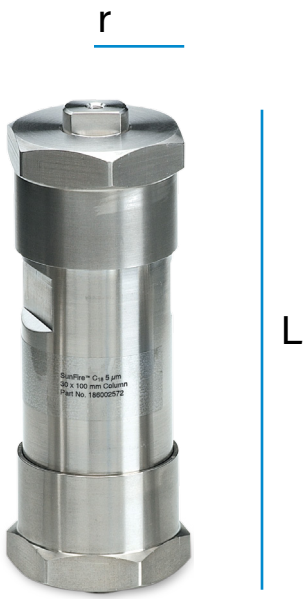
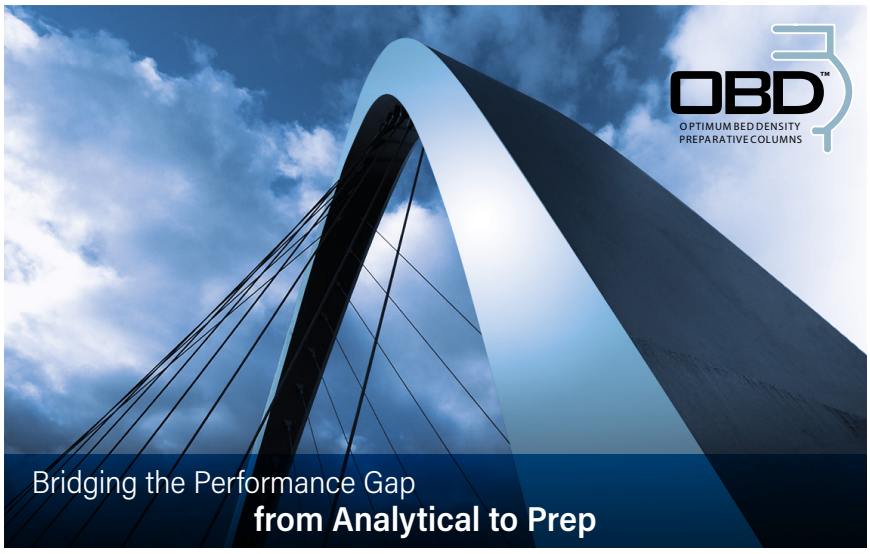


Although scaling up gradient separations to purify molecular targets is not difficult, successful scale up cannot be realized by simply changing the flow rate and copying the analytical gradient method for use in PREP. Such an overly-simplified approach to creating larger scale gradient methods usually results in undesirable and confusing chromatography, making target isolation difficult. However, creating a preparative gradient method for scale up, which delivers chromatography resembling the analytical scouting run, is straightforward when each gradient step is expressed in terms of column volumes (often referred to as CVs).

Because an LC column is essentially a cylinder, the empty LC column volume is calculated according to the equation $V = \pi r^2 L$, where r is the column radius and L is the length. Column specifications provided by the manufacturer are usually internal diameter (r = ½ internal column diameter) and length.

If the volume is calculated considering the porous packing contained within the column, then the revised formula $CV = \pi r^2 L(0.66)$ applies (a modification of the standard CV equation), and represents the volume inside the packed column that is not occupied by the media itself.

Although either volume equation is acceptable, the same equation needs to be applied in column volume calculations applied for all segments throughout the entire experiment to ensure consistency in results. While many factors, such as resolution, peak shape, run time, or final purity, define a favorable chromatographic profile, sometimes success is expressed in terms of the number of column volumes needed to achieve the separation. Generally, ten CVs or more might be required to attain the desired component separation for a complex mixture.



When scaling up to PREP, use the same media in the larger column as was employed for analysis of the crude mixture. The media's volume per gram and, ultimately, the resolution at the PREP scale, will remain consistent with the analytical scale, enabling linear scaling. Similar, if not identical, chromatograms usually result, ensuring confidence in compound isolation in PREP.

In addition to using the same column packing at both the analytical and preparative scales, maintaining the same number of CVs per gradient method segment ensures consistent chromatography. Start by determining the number of CVs for each gradient segment in the analytical method and establish the same number of CVs for the PREP method. Calculate the duration of a gradient segment in column volumes using the equation shown below:

$$\text{Segment duration in CV} = \text{segment duration in minutes} \times \text{flow rate/column volume}$$[1]

A gradient delay hold time added at the beginning of the PREP method properly adjusts for system dwell volume differences between the analytical and PREP LC systems, thus allowing component elution times to match the analytical run.

References

1. Diehl, D. et.al, Method Migration from UPLC Technology to Preparative HPLC, 720002375, October 2007, Waters Corporation.

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