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Preparative Chromatography Really Is Easy!

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Preparative chromatography is a technique that can greatly extend the capability of your HPLC system. The conventional picture of preparative chromatography is large solvent reservoirs and pumps that are pumping mobile phase at hundreds of milliliters per minute. One of the major reasons for doing preparative chromatography is to isolate sufficient quantities of material to further characterize the compound of interest. Analytical techniques have generally become more sophisticated and more sensitive over the last decade, so the mass required to accurately characterize a compound has reduced significantly. Hence, recent developments in column technology now make small scale preparative HPLC feasible using a conventional "analytical" HPLC.

First, we need to define preparative chromatography. It is not large volumes, quantities, or flow rates. It is simply a function of what happens to the outlet stream from the detector. If the eluent goes to a bottle marked waste, it is probably analytical chromatography. If it goes to a fraction collector, whether it be automated or manual, it is preparative chromatography. Some very elegant preparative work is done on 2 mm ID columns!

Here are some answers to typical questions asked about preparative chromatography.

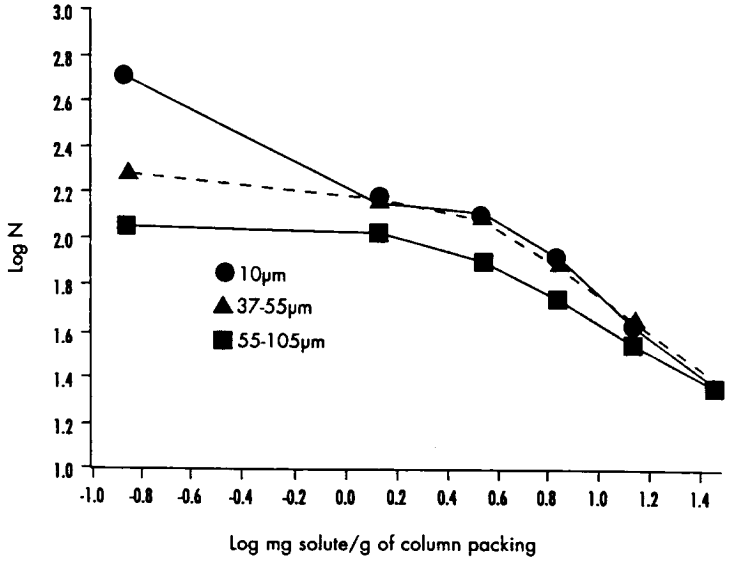
Q: How much can I load on the column?

A: The answer to this question is "it depends"! In the last edition of the *Waters Column*, I discussed some of the interplay between particle size, load and resolution. The larger particle size packings are less sensitive to mass overload than the small particle diameter packings (i.e. chromatography performed on columns of equivalent dimension degrades more rapidly with increasing load when the column is packed with small diameter packings), as shown in Figure 1. However, the resolving power of the large diameter packings is significantly inferior to that of small diameter packings.

In general terms, the amount that can be loaded onto a column is a function of the difficulty of the separation. If the alpha separating the component of interest and the nearest impurity is 1.04, then the maximum load without overlapping of peaks is going to be much less than if the alpha was 5. Time spent developing and optimizing the small scale separation will pay dividends when increasing the load on the column.

A good way of determining the load on a preparative column is to do your methods development and loading study on an analytical sized column filled with the same packing as will be used in the preparative run. This conserves solvent and makes scaling easier. First, develop the separation and then increase the sample mass to the point where the resolution is barely acceptable. Then, with the use of a few simple equations, the sample

Figure 1: Impact of increasing load on efficiency for three different particle diameters



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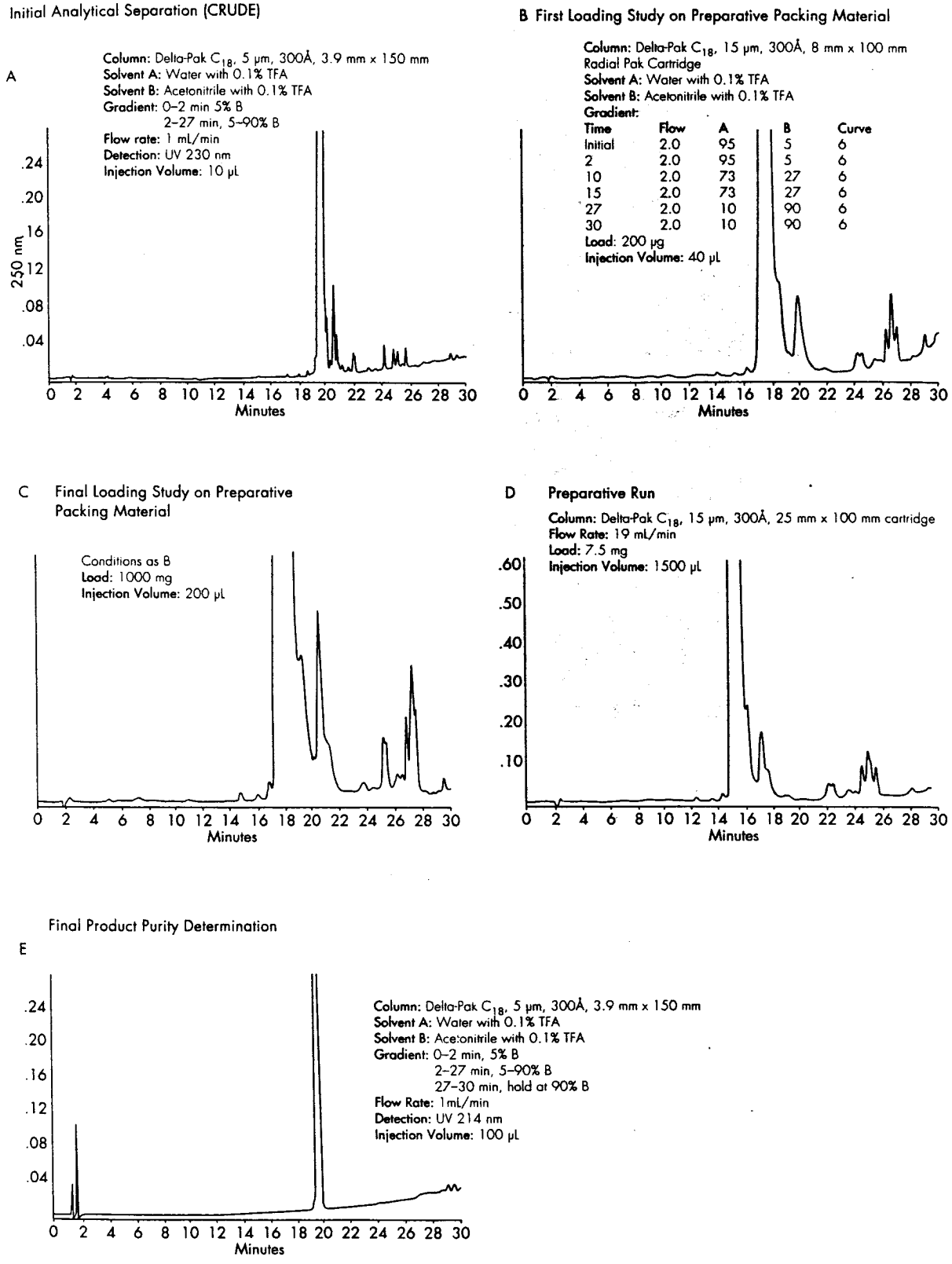
oad. Flow rate and gradient duration can be obtained for the proper size preparative column. The initial separation was performed on a 5 µm Delta-Pak™ column (Figure 2A). The methods development on the 15 µm column was followed by subsequent loading studies (Figures 2B – 2D). Through this study, it was determined that 1mg could be loaded without compromising the "analytical scale" resolution (Figure 2C). The subsequent preparative run (Figure 2D), was performed using the remainder of the synthetic peptide sample, and fraction

Figure 2



purity was confirmed by using the high resolution 5 µm column (Figure 2E), as compared to the initial analytical separation (crude) (Figure 2A).

Load was scaled up as a function of column volume, as per equation 1.

Equation 1

Sample Load

$$Load_p = Load_a \times \frac{(D_p)^2 L_p}{(D_a)^2 L_a}$$

D = Diameter
L = Length
a = analytical
p = preparative

Q: What flow rate do I need to use?

A: Optimally, the flow rate should be scaled up to maintain constant linear velocity. The calculation is shown in Equation 2. Many high performance analytical HPLC pumps such as the Waters™ 600 offer extended flow rates up to 45 mL/min when fitted with 225 µL heads. This is ideal for use with 25 mm ID preparative LC cartridges and columns without compromising analytical capabilities. However, if the HPLC pump is limited to 10 mL/min, this will not adversely affect the chromatography. Column efficiency does vary with linear

Equation 2

Flow rate (Constant Linear Velocity)

$$Flow\ Rate_p = Flow\ Rate_a \times \frac{(D_p)^2}{(D_a)^2}$$

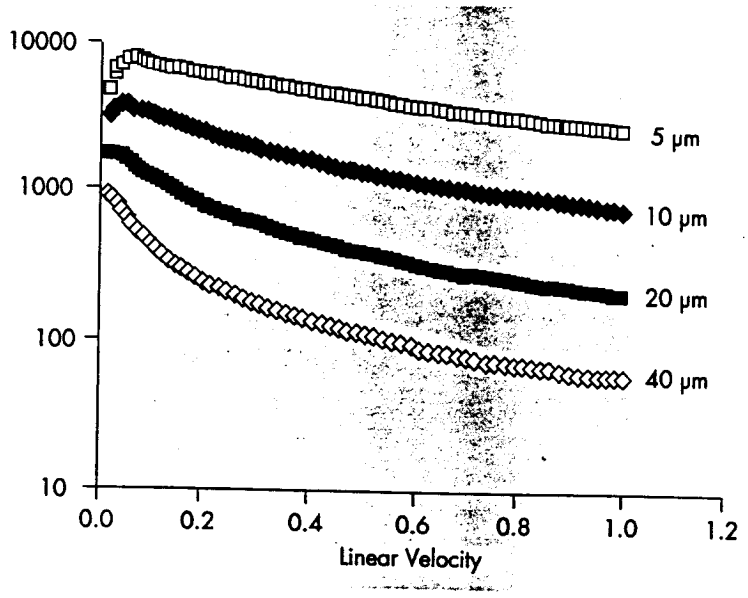
D = diameter
a = analytical p = preparative

velocity, but the effect is much more pronounced at very high or extremely low linear velocities. This is illustrated in Figure 3. Running a 25 mm ID prep column at 10 mL/min, while not the optimal flow rate, will certainly give acceptable results. It is important that the gradient duration be scaled up as shown in Equation 3.

Equation 3

$$Gradient\ Duration_{Prep} = \frac{(Void\ Vol_p)\ (Grad.\ Dur_a)\ (Flow\ Rate_a)}{(Void\ Vol_a)\ (Flow\ Rate_p)}$$

Figure 3: Dependence of the Plate-count on the linear velocity for 100 mm columns



These equations are approximate and don't take into account such phenomena as instrument delay volume, however, they usually give good results and illustrate that scaling up is simple.

In conclusion, preparative chromatography is very similar to the analytical technique with which many of us are familiar. And, the HPLC sitting in your lab can generate enough pure material for further characterization, which increases the utility of the system.

The next conversation in chemistry will address the role of radial compression in preparative chromatography and will show that preparative chromatography columns don't need to cost a fortune.

Technical Article Review

Pharmaceutical

"HPLC-Quantification of Diethylamine Salicylate and Methyl Nicotinate in Ointments"
M.A. Abounassif, E.M. Abdel-Moety and R.A. Gad-Kariem
Journal of Liquid Chromatography, 15(4), 1992.

"Simultaneous Ion Chromatographic Determination of Chloride and Calcium Contained in Ringer's Injection Using a Chelating Agent as Eluent"
Wei Zhou, Wenying Liu and Dengkui An
Journal of Chromatography, 589, 1992.

"Determination of 4-aminopyridine in Serum by Solid-phase Extraction and High Performance Liquid Chromatography"
A. van der Horst and P.N.F.C. deGode
Journal of Chromatography, 574, 1992.

"Simple and Rapid Screening Procedure for 27 Neuroleptics Using HPLC/DAD"
A. Tracqui, P. Kintz, P. Kreissig and P. Mangin
Journal of Liquid Chromatography, 15(8), 1992.

Food/Beverage/Agriculture

"Comparative Studies of the Degradation of Nonstarchy Polysaccharides by Sorghums and Barleys During Malting"
Okokon U. EtokAppan
Journal of Science, Food & Agriculture, 58, 1992.

"Sugar and Soluble Solids Changes in Refrigerated Sweet Corn (Zeqa mays L)"
S. Zhu, J.R. Mount and J.L. Collins
Journal of Food Science, 57(2), 1992.

"Liquid Chromatographic Determination of Apple Pulp Procyanidins"
F.J. Perez-Ilzarbe, V. Martinez, T. Hernandez and I. Estrella
Journal of Liquid Chromatography, 15(4), 1992.

Industrial

GPC:"A Review of the Application of HPLC and GPC to the Analysis of Synthetic Resins"
S. Podzimek
Chromatographia, 32(7/8), 1992.

IC:"The Analysis of Sulfur in Coal: An Ion Chromatography Experiment"
Edward Koubek and Alexander E. Stewart
Journal of Chemical Education.

Life Science

"Reversed-phase High Performance Liquid Chromatography of Underivatized Fatty Acids by Fatty Acid Analysis Column"
Jon I.E. Teng
Journal of Liquid Chromatography, 15(9), 1992.

"Reversed-phase Chromatography of Phenylthiocarbamyl Amino Acid Derivatives of Physiological Amino Acids: An Evaluation and a Comparison with Analysis by Ion-exchange Chromatography"
Andrew S. Feste
Journal of Chromatography, 574, 1992.

"Determination of Cysteine Plus Half-cystine in Protein and Peptide Hydrolysates: Use of Dithiodiglycolic Acid and Phenylisothiocyanate Derivatization"
John G. Hoogerheide and Carla M. Campbell
Analytical Biochemistry, 201, 1992.

"Use of Marfey's Reagent to Quantitate Racemization upon Anchoring of Amino Acids to Solid Supports for Peptide Synthesis"
J. Gordon Adamson, Thang Hoang, Anna Crivici and Gilles A. Lajoie
Analytical Biochemistry, 202, 1992.

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