Watch

The Role of the Column in Preparative HPLC

This discussion of preparative chromatography focuses on the columns used in preparative HPLC, including how to select the appropriate mode, mobile-phase system, and operating conditions.

Ronald E. Majors
Column Watch Editor

In the course of writing this year's Pittcon coverage of high performance liquid chromatography (HPLC) columns (1,2), I observed that large numbers of columns and packing materials designed for preparative LC were introduced at the conference. I thought this might give me the opportunity to review some of the basics of preparative chromatography, emphasizing the role of the column in its successful implementation.

It often has been stated (or perhaps overstated) that the column is the heart of the liquid chromatograph. Choice of the wrong column and mobile-phase conditions for the sample at hand can trivialize all of the advantages of expensive, sophisticated instrumentation and data systems. In preparative chromatography, this statement also is true. Because one often is working with a sample that might have limited solubility in the injection solvent or mobile phase, and injections of large volumes of sample (for increased throughput) are used frequently, an unsuitable column–mobile-phase combination can be even more disastrous, with possible sample precipitation wreaking havoc on expensive wide-bore preparative columns and instrumentation. In this article, I will explore the columns used in preparative HPLC — how to select the appropriate mode, mobile-phase system, and operating conditions. I will assume that the reader already has a familiarity with analytical HPLC method development and separation optimization.

The definition of preparative chromatography always has been fuzzy and is dependent upon the eyes of the beholder because the mass of injected or collected sample often is dependent upon the amount available, sample complexity, or intended use. For some, a few micrograms of material are sufficient for further characterization or use, while for others, tens of grams represent preparative amounts. The purity requirement and sample throughput (that is, the amount of material or yield per unit time collected) also must be considered.

Analytical separations often assume Langmuir-like isotherms and that the resolution equation is obeyed strictly. In preparative HPLC, where columns are frequently (and sometimes heavily) overloaded, the actual isotherms and the commonly accepted relationships no longer apply quantitatively. For example, in analytical HPLC, a commonly accepted definition of column capacity is the mass of sample injected that causes a 10% decline in column efficiency. In preparative chromatography, column capacity is often not so precisely defined because the amount of sample injected could exceed this value by an order of magnitude. Instead, overload in preparative LC is defined as loading that no longer permits the isolation of product at the desired purity or recovery levels (3). Column capacity also must take into account other molecules in the sample, including the matrix, because these compete with the analytes of interest for active sites. Remember, when we discuss capacity, we mean the sample capacity and not the capacity factor, which is a measure of analyte retention. The goal of a preparative purification is the maximum production of purified product per injection.

Basics of Scaling Up from Analytical LC to Preparative LC

Scale-up from analytical LC to preparative-scale chromatography often is time-consuming and wasteful of materials unless an optimized scale-up strategy is employed. As depicted in Figure 1, a recommended method-development strategy is to develop and optimize the initial separation on an analytical size column, overload the column while maintaining adequate separation of components of interest, then scale-up accordingly to a preparative column of appropriate dimensions based upon the amount of purified compound needed. Thus, the choice of analytical column often is dictated by the availability of preparative

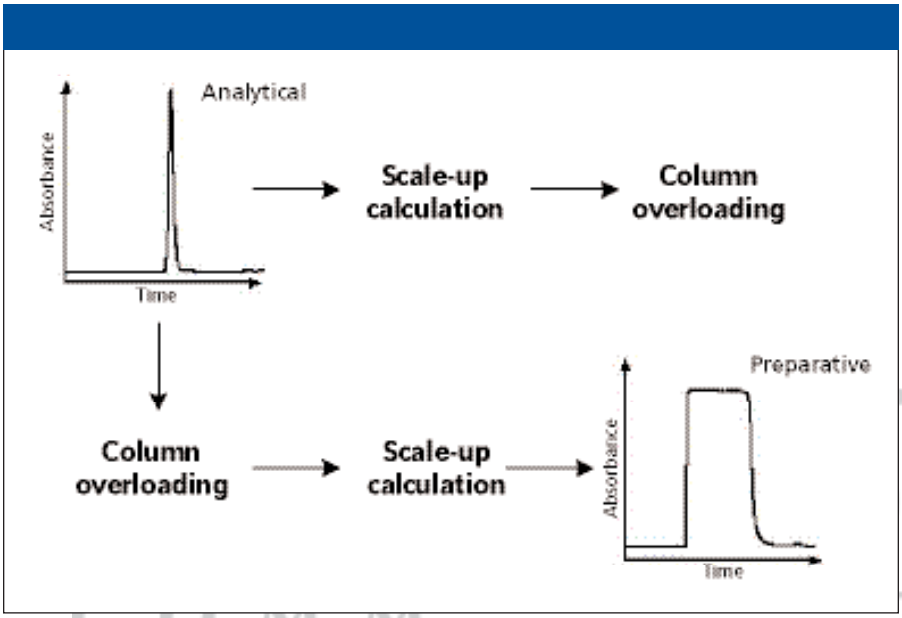


Figure 1: Method development strategy for scale-up.

columns containing the same column-packing material, either in prepacked columns or in bulk media that users can pack. Then, using the equations shown in Figure 2, the scale-up should be linear with perhaps only minor adjustments required to finalize the preparative method. It is highly recommended that one ensures that both analytical and preparative columns from the same line of packing material be readily available before beginning the preparative method development and optimization process.

Choosing the Appropriate Mode and Stationary Phase in Preparative Chromatography

In preparative scale-up, the same separation modes that were used in analytical-scale chromatography can be employed. However, due to cost and availability of high performance preparative packing materials, the cost of mobile phase and mobile-phase additives, the high throughput requirements, and the need to recover isolated fractions in a high purity state, users often limit themselves to the more popular modes of adsorption and reversed-phase chromatography. Size-exclusion chromatography (SEC) and ion-exchange chromatography (IEC) sometimes are used for preparative scale-up and purification. A typical preparative use for SEC might be for the elimination of high molecular weight compounds or for protein purifications. The IEC technique is used for the preparation of inorganic or organic ionic compounds such as proteins. Both techniques are used sometimes in conjunction with other preparative modes. Of course, chiral preparative separations can be achieved on preparative versions and such columns can be purchased, but the cost of such columns relegates them to the specialty column category. Supercritical fluid chromatography has found use in large-scale purification of enantiomeric materials.

For decades, adsorption chromatography on silica gel or other liquid–solid media (such as alumina and kieselguhr) was the main technique for purification of a variety of synthetic organic mixtures as well as other sample types. However, the overwhelming popularity of reversed-phase chromatography in the analytical world has shifted the emphasis to this mode of operation for preparative LC. Also, many analytical HPLC users are not familiar with the principles of adsorption chromatography; they are more comfortable with reversed-phase chromatography and for this reason, tend to gravitate to it when facing a preparative need.

Liquid–solid (and normal phase) HPLC uses organic solvents that are more volatile compared with the aqueous-based solvent systems used in reversed-phase chromatography. In addition, normal-phase solvents have a higher solubility for many compounds, and reversed-phase chromatography often displays a lower sample capacity for compounds due to lower solubility. For compound recovery, the isolated fractions from a normal-phase separation can be recovered quickly by evaporation of the volatile organic solvent. On the other hand, at the conclusion of reversed-phase chromatography purification, the collected solutes are in a predominantly aqueous medium. The removal of the water to concentrate and recover the purified fraction might take much longer or necessitate additional isolation steps such as desalting or solid-phase extraction. The use of higher temperatures to evaporate aqueous media can stress the isolated drug or biomolecule or even destroy it during the concentration process. Thus, it is important to test the temperature stability during the analytical-scale method development before scaling up to preparative scale.

One consideration in preparative chromatography that usually is of lower importance in analytical chromatography is the sample capacity of the packing material. Because throughput is a primary criterion in preparative LC, columns with higher capacity can handle more material per injection. For adsorption chromatography, the surface area of an adsorbent dictates the capacity. A higher surface area sorbent will allow injection of larger sample masses than a lower surface area sorbent. In reversed-phase chromatography, in addition to solubility of the analyte, the bonded-phase coverage determines the sample capacity. Although one might think that the capacity of the reversed-phase chromatography media is dictated by the alkyl chain length of the bonded phase (for example, C_{18} versus C_4), it is more influenced by the bonded-phase coverage.

Surface coverage often is expressed as micromoles per meter squared. For a typical silica-gel packing, there are roughly 8 $\mu mol/m^2$ of surface silanols available for bonding. Because in adsorption chromatography the silanol group is responsible for analyte retention, the larger the surface area is, the more silanols that are present and the greater the retention. In reversed-phase chromatography, the mechanism is hydrophobic interaction between the alkyl and aryl groups on the analyte with the bonded phase. For a typical monomeric C18 bonded phase, for steric reasons, the bonded-phase coverage usually is in the range of 2.5–3 $\mu mol/m^2$. Due to its smaller footprint on the surface, a monomeric C8

Table I Guideline for capacity of preparative LC columns

Column i.d.	$\alpha < 1.2$	$\alpha > 1.5$
4.6-mm	2–3 mg	20–30 mg
9.4	10–20	100–200
21.2	50–200	500–2000

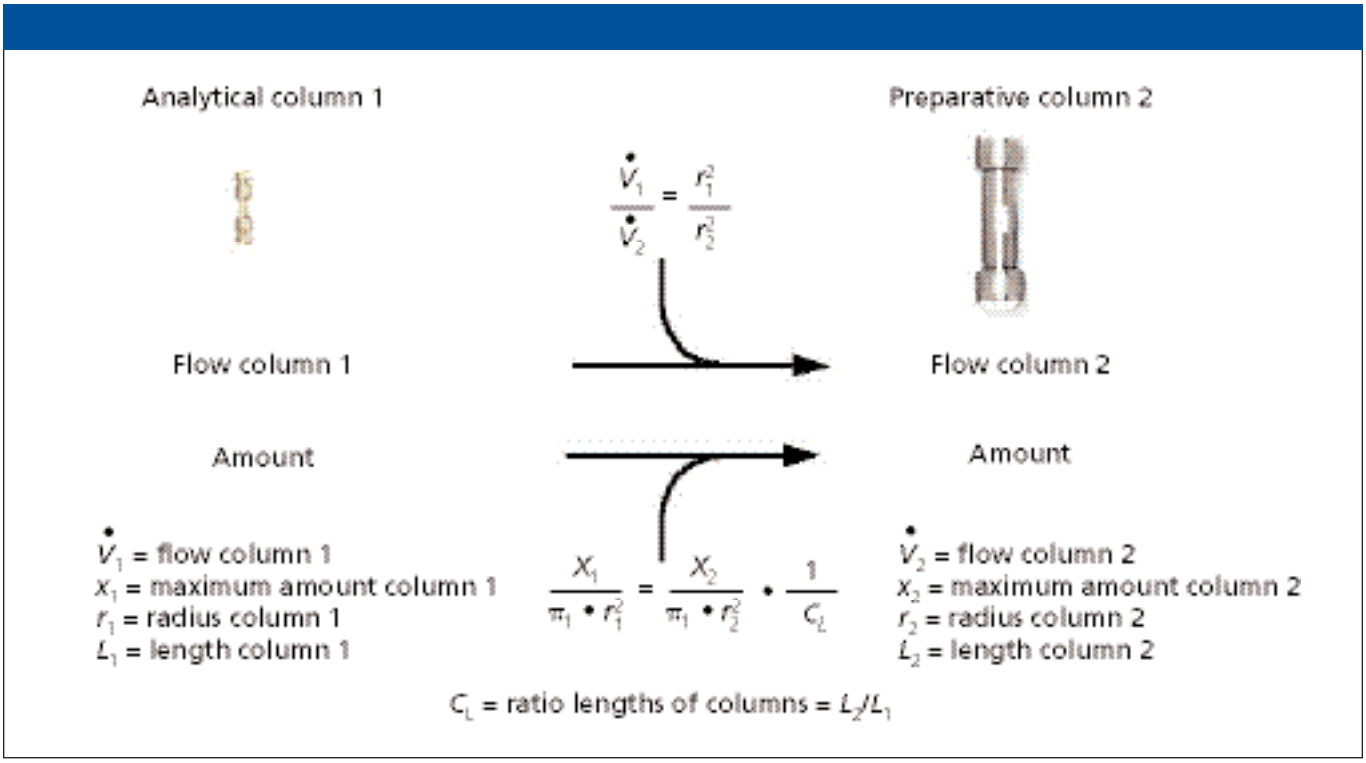


Figure 2: Calculations for scale-up method development.

phase might have a slightly larger coverage and a C4 an even larger coverage still. So on some reversed-phase packings, the coverage of a shorter chain alkyl phase actually can exceed that of a C18 bonded phase. Thus, the amount of available carbon for the hydrophobic interaction with the analyte actually might provide a better measure of surface coverage than the alkyl chain length. Most manufacturers provide the level of carbon coverage for their particular reversed-phase packings. In addition to bonded-phase density, the density of the particles in the packed column also can affect the sample capacity.

Resolution is seen as the most important factor in analytical chromatography and is equally important in preparative chromatography. However, because columns frequently are overloaded in preparative LC and peaks are broadened, the selectivity of the separation often is a very important factor in successfully using preparative LC. If the selectivity between two sample components that are to be isolated is high, one can overload the column to a much greater extent than if the selectivity is low. Thus, choice of stationary phase can be critical to providing the best selectivity for the components of interest. Table I provides some rough guidelines on sample capacity for a typical reversed-phase column as a function of α (selectivity factor). The actual sample capacity for your individual sample components might have to be determined by trial-and-error measurement.

Particle Size and Column Dimensions

Particle size is an important parameter for analytical HPLC. Generally, smaller particle sizes allow greater efficiency and permit the use of shorter columns to increase separation speed. In preparative chromatography, the particle size is important, but the column often is used in an overloaded state, the smaller and more expensive particles of 1.8-, 3.0-, and 3.5-µm average diameters that are used in analytical columns generally are not used in larger-scale preparative columns. If a sample is very complex with poor resolution (and selectivity) among compounds of interest and overloading sometimes is difficult, then 5-µm particles frequently are used. For well-resolved samples, larger particles of 7 and 10 µm can be used. Sometimes, even larger particles are used, but a tradeoff of yield, purity, and throughput must be achieved. Because pressure drop is inversely proportional to the particle diameter squared, larger particles give lower pressure drop, allowing higher flow rates, which in turn increases the throughput of preparative columns. Generally, the cost of packing material is inversely proportional to particle size, so the larger particles usually

Table II Typical operating conditions for columns of various dimensions

Parameter	Column Diameter*				
Length (cm)	4.6 mm	9.4 mm	21.2 mm	30 mm	50 mm
5	1 mg	3–5 mg	30–50 mg	80–100 mg	250–300 mg
10	4–6	15–20	80–90	200–250	500–700
15	8–10	30–40	140–160	300–350	800–900
25	12–15	50–60	200–250	475–525	1200–1400
Injection volume (µL)	10–20	50–75	350–400	700–800	1500–2500
Flow rate (mL/min)†	1.0	4.2	21.2	42.5	118

* Blue shaded area represents approximate sample mass that can be injected onto a column of these dimensions.
† Flow rate required to keep linear velocity and separation time the same.

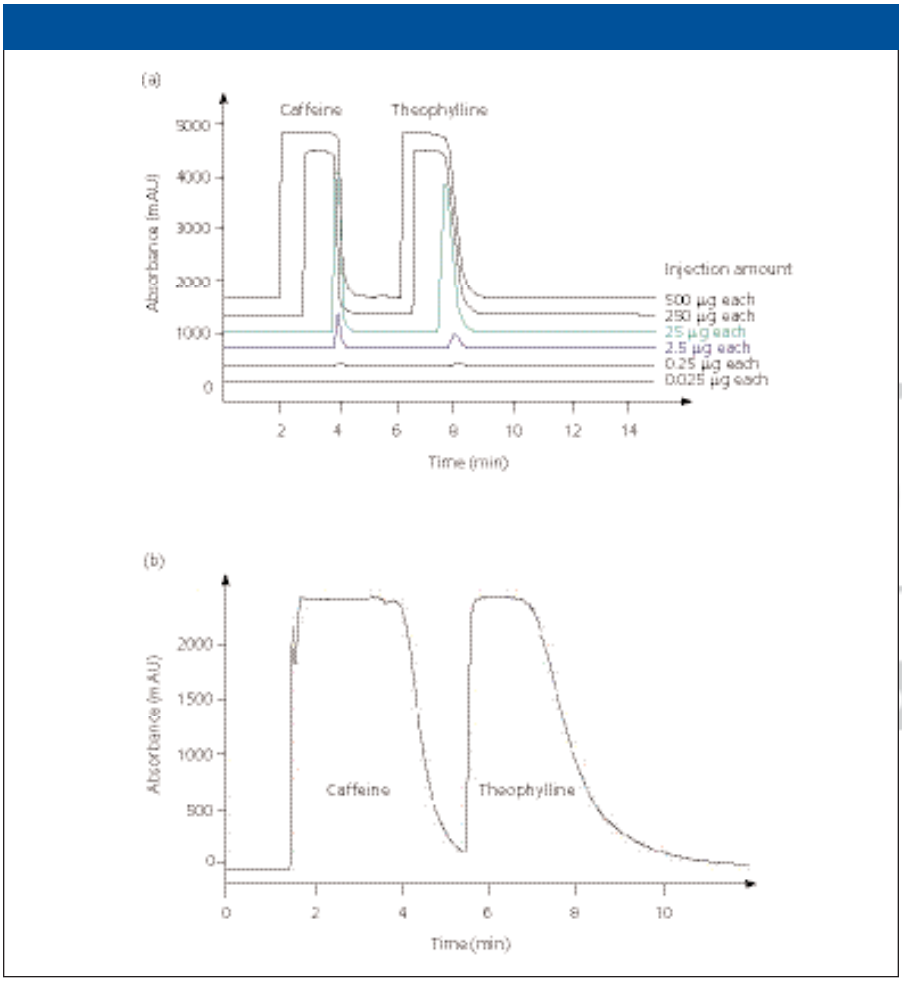


Figure 3: Scale-up of a reversed-phase separation of two xanthines. Shown are (a) analytical column separations and (b) the preparative separation resulting from scale-up calculations. (a) Column: 150 mm × 3 mm, 5-µm d_p Zorbax SB-C18 (Agilent Technologies, Wilmington, Delaware); mobile phase: 90:10 (v/v) water–acetonitrile; flow rate: 0.6 mL/min; detection: UV absorbance at 270 nm; pathlength: 10 mm; temperature: ambient. (b) Column: 150 mm × 21.2 mm, 5-µm d_p Zorbax SB-C18; mobile phase: 90:10 (v/v) water–acetonitrile; flow rate: 25 mL/min; detection: UV absorbance at 270 nm; pathlength: 3 mm; temperature: ambient.

are less expensive than the smaller particles. However, the need for high purity for complex samples with poor resolution between the important analytes can require small-particle packings.

Column dimensions are dictated by the amount of material per injection that one desires to inject. The amount of sample that can be injected increases with column internal diameter (and length), so using the equations in Figure 2, one can calculate the column diameter that fits the sample size required. Typically, 4.6-mm i.d. columns are used for small-scale preparative work, 7.8-mm i.d. columns are for semipreparative work, and 21.2-mm i.d. columns are for larger scale preparative applications. Columns with 30-mm, 50-mm, and even greater internal diameters are available for even higher levels of scale-up. Beyond these diameters, very large-scale preparative and process instruments capable of extremely high flow rates (hundreds of milliliters per minute) might be required, and thus, have a higher degree of solvent usage.

Amount of Sample Injected

The amount (mass) of sample that can be injected onto a column can vary widely based upon its solubility in the injection solvent. A compromise must be made between the relative strength of the injection solvent compared with the strength of the chromatographic mobile phase. If the injection solvent is too strong relative to the chromatography mobile phase, the column can be volume overloaded and the sample will spread out longitudinally on the column at the point of injection. In an isocratic mobile phase, this band spreading might not be recoverable — bands will only get wider. In gradient chromatography, depending upon the initial mobile-phase strength, analytes can be refocused and still provide good efficiency. The best condition is when the injection solvent is weaker than the chromatographic mobile phase, because the analyte is focused at the head of the column. In some cases, however, there can be stationary-phase overloading with the injected sample mass exceeding the capacity of the initial packing in the column. The concepts of volume overloading and concentration overloading are beyond the scope of this article. See references 3 and 4 for coverage.

Table II gives a rough idea of the sample masses and volumes that might be injected on reversed-phase columns of various dimensions. The masses were estimated for compounds with reasonable water solubility and only serve as guidelines. There are many experimental variables that affect the mass that can be injected. For compounds with relatively high k values displaying good resolution, for gradient elution separations, or for simple sample mixtures, one might be able to inject larger amounts. If one is attempting to separate complex samples showing poor resolution, compounds with low solubility in the injection solvent, or if one is using a strong injection solvent (relative to the mobile phase), then much smaller amounts could be required to achieve adequate separation with the absence of overloading.

Choice of Mobile Phase

Often, a linear transfer of the chromatographic conditions can be achieved during scale-up. The solvent system that will be used should be decided during the analytical method development. Factors influencing solvent choice are stationary-phase and mobile-phase conditions with optimum selectivity for compounds of interest; spectroscopic characteristics of mobile-phase solvents (that is, UV transparency, fluorescence properties, and mass spectroscopic compatibility); volatility for easy removal from isolated fractions; viscosity for low column back pressure; purity for low levels of nonvolatile contaminants; good solubility properties for maximum sample loads; and cost of solvents employed.

Not surprisingly, solvent systems in normal phase chromatography often fulfill these criteria, but nevertheless, preparative reversed-phase chromatography still commands the most attention. In analytical reversed-phase chromatography, nonvolatile buffer salts frequently are used to ensure proper pH and prevent tailing and

poor peak shape. In the development of the analytical separation destined for preparative scale-up, it is recommended that a volatile buffer or mobile-phase additive such as ammonium formate or ammonia be used for the initial method because removal will be much easier in the final stages of the method. Also, if mass spectrometry (MS) is used for confirmation, such a system will be more compatible. If one already has an analytical method employing a nonvolatile buffer, a volatile buffer usually can be substituted with only minor adjustments. Expect some differences in efficiency and selectivity from this substitution. A short list of recommended volatile buffer salts is included in Table III.

Because buffer volatility also is a primary concern with LC–MS interfacing, buffers used for preparative chromatography often are the same. For example, if using a phosphoric acid-based buffer at low pH, then a trifluoroacetic acid or formic acid system can be used with preparative LC and LC–MS, although mass spectroscopists prefer formic acid because trifluoroacetic acid might suppress ionization of certain analytes in the MS ion source.

Examples of Scale-up from Analytical to Preparative Chromatography

Xanthines: The basic principle of scaling up from an analytical chromatography column to a preparative column will be illustrated for the simple separation of two xanthines: caffeine and theophylline. These compounds easily can be separated using reversed-phase chromatography, as depicted in Figure 3a, which shows increasing amounts (from 0.025 µg each to 500 µg each) of the two xanthines on a 150 mm × 3.0 mm Zorbax StableBond C18 analytical column. Note that the smallest injection mass of 0.025 µg cannot be observed in Figure 3 because full-scale absorbance is quite insensitive The separation was performed isocratically at a flow rate of 0.6 mL/min using a water–acetonitrile mobile-phase system without any additives. Note that as the injected sample mass increases, there is a slight decrease in the retention time of the two compounds, indicating that some mass overloading is occurring. Because a standard 10-mm pathlength UV flow cell was used at a wavelength of 270 nm, the detector electronics saturated at the higher sample amounts, resulting in the expected flattop peaks. Sometimes, one can tune the detector to a nonoptimum spectroscopic absorption wavelength to prevent electronic saturation and obtain better peak-shape characteristics. The two chromatographic peaks also were well resolved, even at the highest loading, because these two compounds displayed very good separation selectivity. Such good selectivity predicted good overloading in the next stage of scale-up. Using the calculation formula from Figure 2, we next determined the maximum mass that could be loaded on our preparative column that contained the same packing material but with column dimensions of 150 mm × 21.2 mm, the same length as our analytical column. Figure 3b shows the preparative chromatogram for a 25-mg injection of each xanthine onto the larger column but this time using a shorter pathlength preparative flow cell with the UV detector. This shorter pathlength was needed to prevent early detector electronics saturation and allowed us to observe the eluted xanthines, even at the 25-mg injected level. Note that the flow rate was adjusted to 25 mL/min instead of the 30 mL/min that was calculated. The separa-

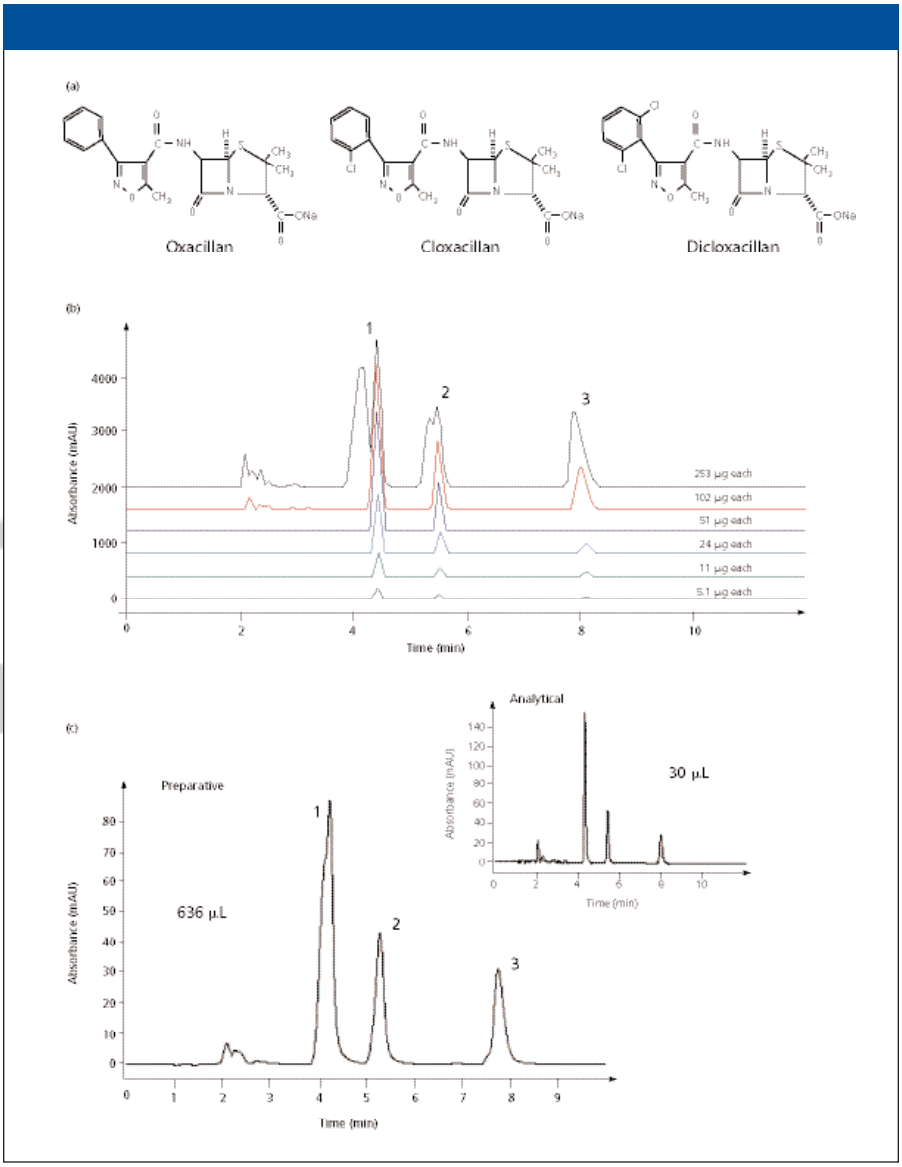


Figure 4: Scale-up of a separation of three antibiotics. Shown are (a) the structures of the antibiotics, (b) scale-up separations on an analytical column, and (c) the resulting scaled preparative separation. (b) Column: 150 mm × 4.6 mm, 5-µm d_p Zorbax Eclipse XDB-C18; mobile phase: 65:35 (v/v) water–acetonitrile, both with 0.1% trifluoroacetic acid; flow rate: 1 mL/min; detection: UV absorbance at 254 nm; injection volume: 30 µL; temperature: ambient. (b) Column: 150 mm × 21.2 mm, 5-µm d_p Zorbax Eclipse XDB-C18; mobile phase: 65:35 (v/v) water–acetonitrile, both with 0.1% trifluoroacetic acid; flow rate: 21.2 mL/min; detection: UV absorbance at 254 nm; injection volume: 636 µL containing 2.2 mg of each antibiotic; temperature: ambient. The conditions for the inset analytical chromatogram in (c) were specified in Figure 3b. Peaks: 1 = dicloxacillan, 2 = cloxacillan, 3 = oxacillan.

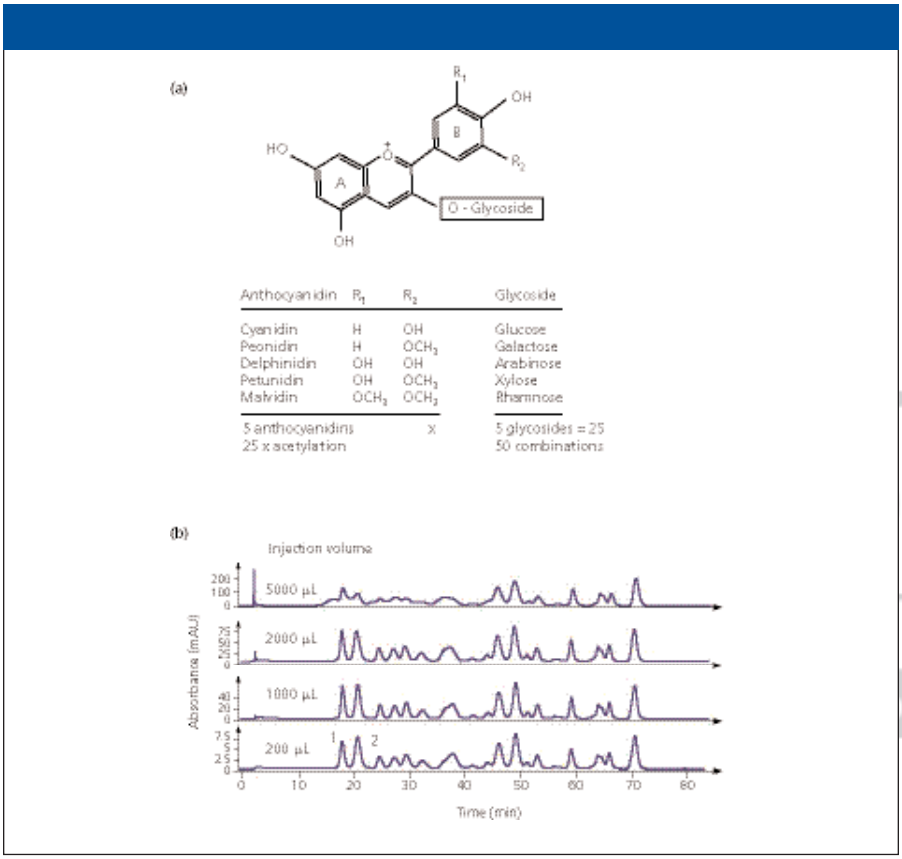


Figure 5: Preparative separation of anthocyanins in a blueberry extract (7). Shown are (a) anthocyanin structures and (b) chromatograms from the separation. Column: 250 mm × 21.2 mm, 7-µm d_p PrepHT Zorbax StableBond-C18 column. Mobile phase A: 0.1% trifluoroacetic acid in water; mobile phase B: 0.1% trifluoroacetic acid in methanol; gradient: 23–25% B over 35 min, 25–53.5% B over 50 min; flow rate 21.2 mL/min; detection: UV absorbance at 525 nm; sample: Sample: 46.1 mg/mL total dissolved solids (~5-mg/mL anthocyanins). Peaks: 1 = dephinindin-3-galactoside, 2 = delphinindin-3-glucoside.

tion of the two xanthines was almost to the baseline, meaning that both compounds could be collected at high purity.

Antibiotics: Let us consider a separation that is a bit more complex: the scale-up of three antibiotics — dicloxacillan, cloxacillan, and oxacillan — that also contain some low-level impurities. Their structures are shown in Figure 4a. Using analytical columns, we first investigated four bonded-phase options for optimum peak shape and maximum selectivity (5) and found the best separation in the shortest time on an endcapped C18 column.

Scaling up on an analytical column rather than a preparative column saves sample, solvent, and time. By injecting increasing amounts of sample onto the analytical column, one quickly can determine the point where the overload begins to affect peak shape and resolution. Figure 4b shows the injection of increasing amounts of our three antibiotics onto a 150 mm × 4.6 mm analytical column. Note that as the mass increases, there is a point where peak overload begins to occur. At the overload point, peaks begin to become nonsymmetrical and eventually can become very distorted, as evidenced by the uppermost chromatogram of Figure 4b. Therefore, when experimenting with a larger column, in some cases, one may want to scale back somewhat on the calculated injected sample mass to maintain good peak shape, resolution, and peak purity. On the other hand, to improve sample load and throughput, one might want to increase the sample size and actually overload the column until peaks start to overlap and purity is compromised.

In the case of the preparative scale-up, we used the preparative scale-up factors equivalent to the second chromatogram from the top in Figure 4b — the mass that was injected just before overloading occurred on the analytical column. The resulting scaled preparative separation is shown in Figure 4c. The flow rate used on this preparative column is 21.2 times faster that the flow rate used on the analytical column, and the amount injected was also increased by the same amount. On this larger bore column, 2.2 mg of each antibiotic could be isolated under nonoverloaded conditions. Thus, on the preparative column, the identical separation and analysis time of less than 9 min was achieved as with the analytical column.

Natural Products, Anthocyanins: Anthocyanins are complex mixtures of pigments found in fruits and other plant parts. They are associated with the colored components of red grapes, various berries, and so on. There is a great deal of interest in anthocyanins due to their antioxidant properties and potential health benefits. In nature, they occur most often as glycosides and might be acetylated, which gives rise to very heterogeneous mixtures (Figure 5a). They can be separated by reversed-phase chromatography using water (acidified with formic acid)–methanol gradients and detected in the visible region of the spectra due to their color.

Anthocyanins can be extracted from fruit matrices using 70% methanol in water (6). Unfortunately, this high percentage of methanol can wreak havoc on the chromatography if a large volume of extract is injected onto the column. To illustrate this effect, a commercial sample of blueberries was extracted using the standard method (6), and the extracts were injected into a 250 mm × 21.2 mm preparative column. The effect of injection volume can be seen clearly in Figure 5b, where volumes of extract (diluted 3:10) as large as 5 mL were injected onto a preparative column. With the largest injection volume, the front part of the chromatogram is affected by the methanol in the injection solvent with peaks spreading out and resolution lost, while the later peaks are unaf-

Table III Common volatile buffers used in preparative HPLC

Buffer Salt	pH Range	
Trifluoroacetate	xx–1.5	
Ammonium formate	3.0–5.0	
Pyridinium formate	3.0–5.0	
Ammonium acetate	3.8–5.8	
Ammonium carbonate	5.5–7.5	9.3–11.3
Ammonium hydroxide	8.3–10.3	

fected because their capacity factors are quite high in the initial mobile-phase composition. Nevertheless, fractions of the first two eluted anthocyanins (identified as peaks 1 and 2 in the chromatogram of Figure 5) were collected automatically from the preparative column and identified by their UV and mass spectra (7) as delphinindin-3-galactoside and delphinindin-3-glucoside, respectively. By reinjection onto an analytical column, their purities were determined to be greater than 99% and greater than 97%, respectively.

Successful Use of Preparative Chromatography

Many of the factors in successful analytical HPLC are prevalent in preparative HPLC also, but some are even more of a factor. Because samples in preparative applications often are crude mixtures, impurities can accumulate at the head of the column and, if not removed, can cause peak shape and retention time change. Sometimes accumulated impurities do not affect retention but can change the column pressure, so one must watch for increased column pressure. It is a good idea to flush the column occasionally with increasingly stronger solvents to remove bound impurities (8). Build-up of material in a packed column occurs most frequently when the injection solvent is weaker than the mobile phase and is especially noticeable when isocratic elution is used. The stronger solvent strength used in gradient elution tends to help with removal of strongly held impurities. Silica-gel adsorbent tends to hold onto more polar analytes, especially basic compounds, while reversed-phase packings tend to favor more hydrophobic impurities.

Knowing the history of the preparative column is of utmost importance. Because impurities from previous samples can show up unexpectedly when new preparative separation conditions are developed, it is advisable to start with a fresh column, or if this is not feasible, it is recommended to go through a solvent washing procedure at the very least. Of course, if the cost of the solvents required to regenerate the column is more than that of packing or column replacement, then it makes sense to replace the packing or replace the column. For self-packed columns, unpacking the column and externally cleaning the loose packing material might be easier than attempting to clean it in the packed column. Often, the first few centimeters of a column suffer the most contamination, and removing this material and replacing it with fresh packing can be performed relatively easily.

Since both adsorption and reversed-phase thin-layer chromatography (TLC) plates are now available, during method development and transfer to the preparative adsorption column, the use of TLC can be helpful for quickly optimizing the mobile-phase composition, for noting the presence of strongly retained compounds at the point of spotting (equivalent to the head of the packed bed in the preparative column), and for qualitatively monitoring the purity of collected fractions. With TLC, one can get an idea if the collected fraction is fairly pure or if additional method development is required. Of course, analytical HPLC can be used, but one is never certain of the presence of strongly retained compounds that can be slowly eluted from the column to contaminate subsequent isolated fractions.

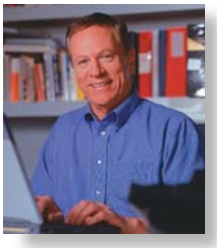
Some special techniques for ensuring purity in preparative chromatography that are not used in analytical liquid chromatography are heartcutting and recycle. In heartcutting, just the middle portion of a peak of interest is collected and those portions of the peak in which there is possible overlap with other sample components are discarded or sent to waste. Of course, in heartcutting, we sacrifice yield for purity. In recycle preparative chromatography, the effective length of a column is increased by reinjecting the column effluent that contains partially separated compounds of interest back into the head of the column, sometimes several times. Such a technique can be performed manually by collecting the fraction of interest and reinjecting it into the column. In modern HPLC-based systems, recycling can be done automatically using the alternate column-switching technique. In the latter technique, two preparative columns connected by multiport valves are used alternatively to effectively lengthen the time that a series of unresolved compounds spends on the column. Details of these special techniques are beyond the scope of this article.

Conclusions

Further information about preparative chromatography columns and proper usage can be found in reference books and reviews devoted to the principles and applications (3,4,9–12). Modern preparative instruments coupled to high-efficiency and high-throughput columns have made the job of purifying impure substances much easier. UV-based and mass-based fraction collectors can selectively cut eluted peaks to offer additional fractionation capability. The outlook for preparative chromatography looks bright, with rugged preparative columns that can withstand many injections now the norm, and further work in special preparative phases such as monoliths and high-capacity sorbents will continue.

References

(1) R.E. Majors, *LCGC* **22**(3), 230–242 (2004).
(2) R.E. Majors, *LCGC* **22**(4), 322–337 (2004).
(3) *Preparative Liquid Chromatography*, vol. 38, B.A. Bidlingmeyer, Ed., (Elsevier Scientific, New York, 1987).
(4) L.R. Snyder, J.J. Kirkland, and J.L. Glajch, *Practical HPLC Method Development*, 2nd ed. (Wiley & Sons, New York, 1997), pp. 50–56.
(5) W. Long and R. Majors, "Preparative HPLC Scale-Up of Antibiotics," Application Note, Agilent Technologies, Inc., Wilmington, Delaware, February 2004.
(6) W. Kalt, J.E. McDonald, R.D. Ricker, and X.Lu, Can. *J. Plant Sci.* **79**, 617–623 (1999).
(7) Clifford Woodward, "Scale-Up of Anthocyanin Separations and Re-Analysis of Collected Fractions on an Agilent Prep-C18 Column" Application Note, Agilent Technologies, Inc., Wilmington, Delaware, February 2004.
(8) R.E. Majors, *LCGC* **21**(1), 19–26 (2003).
(9) R.P.W. Scott, *Preparative Chromatography*, Chrom-Ed Book Series, [Online] available: http://www.library4science.com/.
(10) *Preparative-Scale Chromatography*, E. Grushka, Ed., (Marcel Dekker, New York, 1988).
(11) "Scale-Up and Optimization in Preparative Chromatography: Principles and Biopharmaceutical Applications," *Chromatographic Science*, vol. 88, A.S. Rathore, Ed., (Marcel Dekker, New York, 2003).
(12) *Preparative and Production Scale Chromatography*, G. Ganetsos and P.E. Barker, Eds., (Marcel Dekker, New York, 1992).



Ronald E. Majors

"Column Watch" Editor Ronald E. Majors is business development manager, Consumables and Accessories Business Unit, Agilent Technologies, Wilmington, Delaware, and is a member of LCGC*'s editorial advisory board. Direct correspondence about this column to "Sample Prep Perspectives,"* LCGC*, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail lcgcedit@lcgcmag.com.*