Column Watch

Practical Aspects of Preparative HPLC in Pharmaceutical Development and Production

Guest Authors
**A. Brandt and
S. Kueppers**

Preparative high performance liquid chromatography (HPLC) can be used in pharmaceutical development for troubleshooting purposes or as part of a systematic scale-up process. In pharmaceutical production, the importance of preparative HPLC as a purification tool has been increasing. This month's "Column Watch" discusses the practical aspects of the scale-up process and the problems and costs of preparative separations within a midsize pharmaceutical company.

Two contrasting dynamics in the pharmaceutical industry are furthering interest and method development in preparative high performance liquid chromatography (HPLC). On one hand, regulatory agencies continue to press for more stringent requirements for the purity of pharmaceutical products and, especially, drug substances. On the other hand, pharmaceutical companies are developing compounds of increased complexity — drugs with numerous functional groups in a single molecule, polymeric compounds, biopharmaceuticals such as peptides, proteins, and many others — that must be purified, especially in production quantities. An acute need exists for other tools in addition to crystallization, the classic tool for purification, to address a growing number of purification problems.

Analysts can use chromatography; ultra-, micro-, and nanofiltration; solid-phase extraction; distillation; and other techniques as tools. Currently, preparative HPLC is the most powerful and versatile method for purification tasks in the pharmaceutical industry (1).

For some of the more difficult tasks, analysts can combine different purification tools. For example, a chromatographic separation could remove impurities of a differing polarity or reduce the content of one enantiomer in a racemic mixture. In both of these cases, crystallization might be used to prepare the pure product (2).

In a typical pharmaceutical company, the research department prepares only small amounts (milligrams to grams) of a desired purified new drug substance. Therefore, chromatographic purifications usually are performed in glass columns using gravity, and analytes are collected manually. These small-scale purifications require no pumps, valves, or automation. The development department, which conducts the next stage of scale-up, might produce a few kilograms to a few hundred kilograms of drug substance. Finally, larger amounts, from 100 kg to tons of drug substance per year, generally are produced in the production department and some form of automation is necessary for this production-scale work. Developing well-automated preparative chromatographic methods is a necessary and demanding task. Automating the scale-up processes starts in early development and culminates with the successful transfer of a method to the production plant.

In this installment of "Column Watch," we will present the current status of scale-up procedures for chromatographic separations at Schering AG (Berlin, Germany). Schering, a midsize pharmaceutical company, produces specialty drugs with an annual drug substance production ranging from as little as 10 kg to approximately 10 tons. The two primary applications for preparative LC in the development of a new drug are as a troubleshooting tool and in process development.

Preparative HPLC as a Troubleshooting Tool

Chromatographers must perform the troubleshooting in finding drug impurities in early development stages, when non–good manufacturing practices (GMP) material is produced. This material, which is not intended for use in humans, sometimes must be purified if initial chemical reactions provide insufficient yields of reasonably pure materials. In this case, users must have an excellent knowledge of chromatography and a wide range of available stationary and

mobile phases for method development. Because the amount of sample material to be purified is relatively small, little or no optimization of the separation can be performed before the start of the project, because time delays are problematic during the development process. These separations usually are performed in the laboratory scale on 5-, 6-, or even 8-cm i.d. columns. Sometimes, we perform the separations in the pilot plant on 15- or 30-cm columns.

Preparative LC in Process Development

In many cases, purification requirements dictate that chromatography be used as a purification step in the production process. In this situation, systematic method development must be performed before processing raw material at the pilot-plant scale. As a possible basis for preparative separations, analytical HPLC methods are tested first. Initial analytical HPLC runs and the final preparative requirements have two key differences: the necessity of buffer removal from the mobile phase and the requirement of using larger particle sizes to decrease cost and lower the pressure drop in the preparative separation. We found that thin-layer chromatography (TLC) often provides important purity information during the scale-up process.

Stationary-Phase Selection in the Scale-Up Process

One important criterion for initial method development is that the chromatographic stationary phase used in scale-up must be commercially available both in analytical columns (250 mm × 4 mm) and as bulk preparative material in large quantities (kilogram quantities and greater). Typically, packing materials with 10- to 20-µm parti-

cle diameters are used for preparative columns. The accompanying sidebar, "Typical Silica Packing Materials for Preparative Separations," lists materials commonly used for scale-up separations in our laboratory.

For additional method development information, we sometimes perform systematic column evaluation tests of different stationary phases using a column-switching valve. Simulated moving bed method development is started in parallel to the batch separation method for separation problems involving only two components or two groups of components.

Although approximately 84% of analytical HPLC separations are performed on reversed-phase silica materials (3), at Schering almost 85% of the preparative chromatography separations are performed on normal-phase silica gel columns.

Normal-phase methods are always our first choice because

- a direct transfer from normal-phase TLC or HPLC to preparative LC is possible;
- the costs of reversed-phase packing materials are still high and some of the packing materials are unavailable as bulk materials;
- cleaning normal-phase silica is much easier because the material is much more robust; and
- removing organic solvents typically used in normal-phase chromatography from the final product solution is easier than removing water from a reversed-phase chromatographic fraction and can be achieved at lower temperatures and provide higher product quality and lower energy costs.

Reversed-phase chromatographic methods work well, too, if analysts can use nanofiltration as part of the work-up procedure. Some specialized separation problems dictate the need for chiral stationary phases or even custom-made stationary phases.

Practical Considerations in Preparative HPLC Scale-Up

If the tests on analytical columns with analytical loadings show good separations, a scale-up to a larger column diameter can be performed using the configurations given in Table I. A 25-cm bed length usually is used in both preparative and analytical procedures.

Instead of jumping directly to the largest column diameter anticipated to be required eventually, the first step in our scale-up process is the transfer of the new separation

procedure to a 5-cm i.d. laboratory preparative LC column. These columns usually are packed in our own facilities with 300 g of bulk media using a slurry packing procedure. The separation is optimized in this size column. The sample injected onto the column starts usually at 1 g and increases to as much as 20 g, depending upon the quality (resolution) of the separation achieved, the quality of the raw material, and the specifications for the pure product. Starting with the 1-g injection, we collect fractions and reanalyze them for purity using the analytical test procedure. With an increase in the sample loading, the resolution decreases. The collection of impurities on the column can decrease the resolution of the subsequent separation, and late-eluted impurities can spoil the collected fractions of the subsequent separation. Therefore, washing steps often are implemented between chromatographic runs. Solvent gradients and recycling steps sometimes are necessary to increase the resolution for difficult separation problems. In the case of preparative chiral separations, we have found that temperature optimization can be of special importance.

At this stage of method scale-up, the productivity of the separation is fixed. The variables that have been optimized by this time include the sample loading, separation time, solvent composition, generation of side fractions, washing steps, and injection solvent. The injection solvent should be investigated because sharp peaks and high loadability are important goals, and choosing the proper solvent can aid in improving

Table I: Column inner diameter configurations used for preparative HPLC

Capacity	Column i.d. (cm)
2× Semipreparative LC	2–3.3
5× Preparative laboratory–LC	5–6
1× Preparative LC	8
1× Preparative LC	15
2× Preparative LC	30
1× Preparative LC	45*
1× Simulated moving bed	10 × 2.5 or 8 × 5

*Production only.

these parameters. The main goal of method development is a simple, well-automated, and robust separation process able to run 24 h per day. A separation process that is too sophisticated might turn our sleeping time into night shifts.

An important experimental consideration in scaling up from a 4-mm i.d. column to a 5-cm i.d. column is the flow cell used in UV detection, which is our most commonly used form of detection. The analytical cell is optimized for sensitivity, whereas the UV cell in the 5-cm i.d. semi-preparative instrument is optimized for the high loadings of the preparative separation. Therefore, detection in preparative chromatography also is possible using slightly absorbing solvents at low UV wavelengths, for example, ethyl acetate at 210 nm.

The mass balance of the processed material and the overall productivity must be checked during development. At the end of this optimization, we perform a reproducibility test with at least three injections. If these injections show the same quality for the fractions, we can transfer the method to the pilot plant.

Depending upon batch size and required sample loading, the separation method can be transferred to one of the columns in the GMP area. Typically, columns ranging from 5 to 30 cm (or even 45 cm) in diameter are used in our production environment. Table II lists the respective scale-up factors.

Our experience shows that most optimization work must be performed by transferring a separation method from an analytical-scale (4-mm i.d.) column to a 5-cm i.d. column. Scaling up further to 8-,15-, 30-, or even 45-cm i.d. columns seldom requires major method changes or causes problems. In large-scale preparative separations, the first two runs are controlled manually, and, if workers observe no technical problems, then subsequent runs can be performed automatically.

Problems Encountered in Preparative Scale-Up

A typical problem encountered in scale-up is that the material used during method development was produced in laboratory scale and differs in solubility and impurity profile from those of the material that must be processed in pilot-plant scale. The pilot-plant material can be either of a different quality or show larger amounts of the same impurities, and, in some cases, even new impurities can arise. In the case of an impurity profile that shows larger amounts of the same impurities or new impurities, analysts must retest the separation method at laboratory scale before starting the pilot-plant separation.

In the case of a pilot-plant material that shows higher purity, the solubility in the weak solvent chosen during optimization often is not good enough. In this case, the productivity can be lower than expected because the amount separated each run must be decreased. Because we achieve linear scale-up, the chromatographic run takes the same time in preparative scale as in laboratory scale, so the substance itself is not stressed longer in the separation equipment. The final work-up after the separation step includes the removal of the chromatographic solvent. The desired fraction collected is a solution that contains the substance of interest in the range of a few percent by weight and, therefore, large amounts of solvent must be removed. The evaporation of solvents, especially water, takes some time, so the purified drug substance can be stressed or even destroyed during the concentration process. Thus, it is useful to test the temperature stability of the substance during laboratory-scale method development.

Temperature also can influence the separation performance. For example, the mixing of organic solvents before they enter the column can result in a strong increase or decrease in the solvent temperature and can influence the operating temperatures of the

mixing unit and column. Temperature effects at the center of the column caused by heat dissipation also can influence the separation (3).

Another problem that often appears during the first separation in pilot-plant scale is that some impurities accumulate on the column during a series of sequential runs. The quality of the separation deteriorates during the sequence. Because the fraction collection commonly is controlled by peak height, a UV detector does not detect this problem and the purity of the fractions decreases. When impurities accumulate on the column, the peak shapes or the retention times of the components of interest might change, so operators can see quality problems. Unfortunately, sometimes peak shapes and retention times show no changes. An additional indicator that impurities have accumulated on the column is a pressure increase; therefore, it is helpful to monitor the column pressure. In our experience, however, most problems in preparative LC are technical problems related to instrument failure.

Costs of Preparative HPLC Separations

In preparative separations, the stationary phase usually is recovered and used again to purify the next batch of the same substance. Often the major operating cost in preparative LC is the solvent rather than the packing material. Therefore, the choice of solvent is important in method development and scale-up. Figure 1 illustrates the relative costs of typical organic solvents used in preparative separations.

In addition to the absolute solvent cost, the possibility of recovering the used solvent is important in the solvent choice. Solvents should be inexpensive, and solvent recovery, especially for larger separation projects, is recommended. If different solvents are used during the separation — for example, one solvent for the injection and a different solvent for elution — it might

Table II: Scale-up factors from 5-cm i.d. columns to larger-inner-diameter preparative HPLC columns

Column i.d. (cm)	Scale-Up Factor
From 5 to 8	2.56
From 5 to 15	9.00
From 5 to 30	36.0
From 5 to 45	81.0

Low costs ————————————————————— High costs

Methylene chloride
Acetone   Methyl-*tert*-butyl ether
Methanol   Ethyl acetate   Acetonitrile
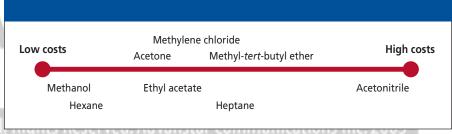Hexane   Heptane

Figure 1: Relative costs for organic solvents used in preparative HPLC.

make sense to collect these solvents separately to make recycling and processing the solvents easier. Some mixtures of certain solvents — hexane–methyl-*tert*-butyl ether, hexane–ether, and three-component mixtures — cannot be recovered as pure solvents.

These solvents usually are recovered as azeotropic mixtures that can be readjusted to the desired composition before reuse. Some production plants are equipped with automated solvent recovery units that enable collection of used solvent, distillation of solvent mixtures, gas chromatography control of the quality and composition, readjustment of the composition by adding one solvent (if needed), and recycling the solvent into the HPLC process.

Methanol is a solvent often used in preparative separations. It is an inexpensive and strongly polar solvent commonly used in combination with water as a mobile phase in reversed-phase separations. Methanol also can be used for flushing normal-phase silica columns to remove adsorbed polar contaminants. It also can be recovered easily from many mixtures. In reversed-phase applications, acetonitrile yields better peaks but is far too expensive in most cases for larger-scale separations.

Of course, an initial goal of the scale-up process is to find an acceptable separation. If analysts find more than one set of separa-
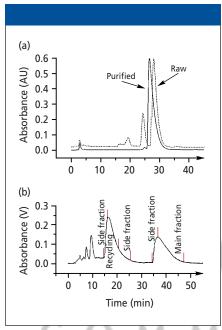
tion conditions, then the cost of solvents becomes a major criterion. The solvent selection usually is determined during the initial method development with the 4-mm i.d. analytical-scale columns or by TLC tests. Sometimes when the overall cost of goods is important to a final product, workers can perform a systematic solvent selection even in the later stages of development.

Figure 2 shows an example of scaling up to a preparative-scale separation. Figure 2a shows the analytical separation. The analytical separation was performed using a Hypersil BDS C8 column, an acetonitrile–water gradient, and UV detection at 250 nm. The separation of the mixture is quite difficult, because the main impurity in the raw material found on the leading edge of the main peak is an isomer. The product is a steroid with double bonds in the ring system. The isomers have the same overall structure; only the position of one double bond is different for these two isomers.

Therefore, we had to implement recycling and use a very long column (approximately 30 cm) to obtain a better preparative-scale separation (Figure 2b). The preparative separation was performed on a C18 column. The column was packed in-house with 1000 g of bonded silica on an 8-cm i.d. column. UV detection in the preparative scale was performed at 254 nm. The mobile phase was 45:55 (v/v) isopropanol–acetonitrile. The flow rate was 260 mL/min at a pressure drop of approximately 25 bar. The sample injected in each run was 1.7 g in 3.5 mL of tetrahydrofuran.

Conclusion

We discussed some practical aspects in the scale-up procedures of HPLC purification used in our laboratory, pilot plant, and production environments. We have found that a gradual scale-up starting with 4-mm i.d. analytical columns, followed by intermediate-size columns of 5-cm i.d. or so, with the ultimate goal of using 35–45 cm i.d. columns is the best approach for preparative optimization. Despite the fact that among the tools used in the large-scale purification of pharmaceuticals, preparative HPLC is one of the more expensive and

time-consuming approaches, it yields the highest-purity drug substance. After the scale-up from analytical to 5-cm columns has been optimized, most problems of additional scale-up are technical problems that also occur in other purification technologies.

The interest in preparative HPLC will continue to grow because of the increasing uncertainty in the market expectations for product purity. Its nearly linear scalability makes preparative HPLC one of the more viable approaches to compound purification.

References

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Stephan Kueppers is head of the central analytical chemistry department of the research center Forschungszentrum Jülich, ZCH, D-52425 Jülich, Germany, e-mail s.kueppers@fz-juelich.de. Andreas Brandt is head of a pilot plant in the process development of Schering AG (Berlin, Germany).

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 "Column Watch," LCGC, 859 Willamette Street, Eugene, OR 97401, USA, e-mail lcgcedit@lcgcmag.com.

Figure 2: Example of scaling up a separation from (a) analytical to (b) preparative scale.