The Art of Column Packing

Introduction

When developing a new therapeutic the pharmaceutical industry performs chromatographic separations at all stages of drug development, to ensure the safety of drug products and to monitor clinical trials. In order to maintain the quality of separation and the integrity of the instrument, it is necessary to periodically change the column used for the separation. These changes are followed by extensive verification and validation procedures, such as system suitability tests, before an instrument can be returned to mainstream operation; even between columns manufactured in the same batch. This is particularly important when samples are required to meet regulatory standards and satisfy the guideline levels of sensitivity, selectivity and peak resolution between critical pairs. As such, it is a distinct advantage to reduce the instrument downtime when effecting a column change, by ensuring column consistency and allowing rapid validation of the system.

This article will outline the technological enhancements in automated column packing and of advanced, solid core stationary phase particles, and will discuss how these developments can enhance the sensitivity, specificity and resolution of pharmaceutical separations.

Regulated, Precise and Controlled

High performance liquid chromatography (HPLC) columns almost always employ small (typically 5 µm or smaller) stationary phase particle sizes, which do not favor dry packing methods. The manufactured stationary phase material is made into a paste, or slurry, using a suitable solvent which is selected for its compatibility with the stationary phase and packing protocol. Hexane is often used when packing C18 phases for example. The following criteria are important considerations in selecting the appropriate column packing process:

- Particles must not settle too quickly during the filling process
- Agglomeration of particles must be minimized
- The particle flow must be at high velocity as it hits the accumulating bed
- Particles should be allowed to settle into the bed before being buried
- Slurry solvents should be selected to be easily removed from the column and to not react with the stationary phase

Column packing methods are subdivided according to the method used to stabilize the stationary phase slurry material. The three commonly followed methods are physical, chemical and mechanical, and high and low viscosity [1].

- Physical stabilization is achieved using balanced or non-balanced density, and viscosity slurry methods
  - Balanced density methods utilize solvents such as tetrabromomethane, tetrachloroethylene, carbon tetrachloride and diiodoethane, to achieve a solvent density equivalent to that of the stationary phase particles
  - The non-balanced method uses lower density solvents, such as acetone, methanol and tetrahydrofuran
  - The viscosity slurry method uses viscous solvents, such as glycerol and glycerine to prevent particle settling
- Chemical stabilization uses a polar solvent and ionizing surfactant to electrostatically maintain the particulates
• Mechanical stabilization uses a specifically designed slurry vessel to continuously stir the material as it is pumped into the column
• Low viscosity methods use a low density suspension of particles to boost the homogeneity of the packing process, and use slurry solvents such as pentane, hexane and diethylether

Once prepared, the particulate slurry is packed into a reservoir, from which it is drawn by a pneumatic pump, and passed directly into the stainless steel body of the column at constant velocity and pressure. The flow rate of the material and pressure of the flow determine the rate of filling of the vessel. Manual packing methods involve the operation of a control valve to allow flow of the pressurized slurry and to start and stop the filling of individual columns. The length of time allowed for filling each column is therefore controlled manually and is calculated based on flow rate and pressure.

As a result, manual packing processes, while being optimized to produce high quality columns, introduce a potential source of variation between columns. In a pharmaceutical setting, it is the consistency of the separations which is critical, and so inter-column variations can represent significant analytical challenges.

One solution to this problem is to automate the packing process, using microprocessor control to accurately time and deliver the stationary phase slurry into the column body. When produced in this way columns are more consistently packed, reducing variation in results observed between maintenance column changes.

**Evolution of HPLC Particles**

HPLC columns are typically filled with an inert material, upon which the stationary phase is coated. There are a number of suitable materials that can be used as this scaffold, including silica, alumina and zirconia [1]. Silica based stationary phase supports are by far the most common, with its inertness, specific surface area and pore diameter properties making it an ideal substrate.

Silica may be directly used for certain applications, effectively separating a number of polar, aromatic, olefin and saturate hydrocarbon compounds. However, it is frequently necessary to deactivate the silica by derivitizing the surface of the particles, as the adsorption of many compounds onto the surface silanol groups is too strong. Such derivitization reactions are achieved by bonding chemicals to the silanol groups at the surface; typically alkyl groups of varying chain length and containing a range of functional groups.

There has recently been a focus on how the stationary phase packing materials can adversely influence a separation, with variations in particle size, porosity and stationary phase coating being mirrored in the performance of the column. Superior manufacturing techniques and column testing protocols have led to the development of Core Enhanced Technology™.
Previous advancements in stationary phase materials have moved from using large, irregular pellicular silica particles, through a range of spherical fully porous particles of decreasing size, down to sub 2 µm porous particles, which are frequently used in stationary phases today (Figure 1). Core Enhanced Technology produces particles with a diameter that is tightly regulated at 2.6 µm, constructed around a solid core of silica and a porous outer layer. These particles can be more consistently packed into a column with less void space between the particles. When combined with highly controlled, automated column packing techniques, Core Enhanced Technology enables the analyst to run faster, higher quality separations using existing instruments.

**Advanced Column Testing**

Pharmaceutical applications demand consistent separations at all stages of the drug development process. Tanaka et al. have developed a series of model separations to characterize the stationary phase and packaging materials, outlining how the column performs against a number of standardized analytes [2]. Figure 2 provides a summary of these tests which, when performed on all columns, can identify any variation in results caused by column changes. Column variation can therefore be minimized by placing stringent specifications on these tests.
Figure 2: Diagrammatic representation of the silica characterization (purple), carbon load (grey) and Tanaka chromatographic tests (orange) for quality control of column production

**Resolving the Difference: Superior Separations**

The efficiency of a separation is described by the Van Deemter equation, which takes into consideration the longitudinal and eddy diffusion of analyte particles and mobile phase as they move through the column. The ultimate output of this equation is referred to as the height equivalent to a theoretical plate (H), which becomes smaller as the optimum conditions of flow rate (µ) and pressure are reached. Each column and stationary phase has different optimal conditions, as H is influenced by the mass transfer of analyte between stationary and mobile phases, which varies for each type of phase and analyte.

![Figure 3](image)

Figure 3: Upper Pane: a plot demonstrating how the height equivalent to a theoretical plate varies with mobile phase flow rate across a number of stationary phases. Lower pane: a plot to show how the pressure of a system can vary with flow rate of the mobile phase, across a number of column types.

Figure 3 shows how a column packed using an automated control process and with Core Enhanced Technology particles, performs as mobile phase velocity increases. In simple terms, the lower the value of H, the more efficient the separation. As such, these data indicate that the advancements in column technology allow for significantly greater efficiency at higher mobile phase velocities. Furthermore, the highly regulated particle engineering and controlled packing of the column bed enables rapid mass transfer of analytes between phases and reduces eddy diffusion within the column, further minimizing the value of H.
Enhanced Applications: Boosting Pharmaceutical Workflows

Pharmaceutical separations need to adhere to strict regulatory standards, as set out in the United States Pharmacopeia (USP) methods [3]. The advantages conferred to common pharmaceutical applications when used in conjunction with columns manufactured using Core Enhanced Technology and with highly optimized, automated microprocessor packing protocols, are outlined below.

Application Advantages

The non-steroidal anti-inflammatory drug ibuprofen and the monoamine transport inhibitor valerophenone are separated effectively by a C18 stationary phase. Figure 4a is a chromatogram showing peaks of these two compounds. The resolution of these two compounds is enhanced by the superior peak shape and minimal peak broadening.

The antihistamine cetirizine hydrochloride is most effectively isolated using a hydrophilic interaction liquid chromatography (HILIC) stationary phase, as seen in Figure 4b. As with the C18 phase this separation is fast, with the compound eluting in 0.7 minutes. This speed has not reduced the quality of the separation as the peaks which are produced are consistently narrow, giving excellent resolution.

Figure 4: a) Separation of 500 µg/ml of 1 valerophenone and 2 ibuprofen on a Thermo Scientific Accucore C18 100x4.6 mm, 2.6 µm column, at a flow rate of 2.0 mL/min and a pressure of 276 bar. b) Chromatogram for cetirizine hydrochloride retained on an Accucore HILIC 50x2.1 mm, 2.6 µm column at a flow rate of 0.4 mL/min and a pressure of 50 bar.

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<tr>
<th>Valerophenone</th>
<th>Ibuprofen</th>
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<tbody>
<tr>
<td>( t_1 (\text{min}) )</td>
<td>1.16</td>
</tr>
<tr>
<td>( t_2 (\text{min}) )</td>
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<th>RET Specifications</th>
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<tr>
<td>Retention factor</td>
<td>1.21</td>
</tr>
<tr>
<td>( %\text{RE} )</td>
<td>1.39</td>
</tr>
<tr>
<td>( %\text{RE} )</td>
<td>0.17</td>
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The elution of these three compounds demonstrates the advantages provided by the Core Enhanced Technology and automated column packing protocols. The uniform and narrow peak shapes, with little or no tailing and broadening, and the rapid elution from both stationary phase chemistries, completely satisfy the USP regulatory requirements for all compounds.

**Conclusions**

HPLC is a rugged and reliable analytical technique and is an essential component of the drug development process. Manufacturing, production and safety testing of compounds as they move through the pharmaceutical pipeline are all required to satisfy strict regulatory standards. HPLC column development has moved to satisfy the increasing demands to boost workflow efficiency and productivity and to reduce costs.

Highly controlled, automated packing protocols enable manufacturers to increase column efficiency and reduce inter-column variation. Core Enhanced Technology serves to improve analyte exchange between phases and gives narrow, consistent peaks with minimal tailing. Thorough and comprehensive testing of stationary phase materials for interactions between packing silica and analyte molecules, when performed on every batch of chromatographic media, can further reduce inconsistencies caused by interactions between packing materials and analyte molecules.

In this article we have demonstrated how columns produced using these technological advancements, such as the Thermo Scientific Accucore HPLC columns, provide separations of comparable or higher quality than existing technology and which more than satisfy regulatory requirements.

**References**