APPLICATION BENEFITS

- The Prep 150 LC System is an affordable, highly reliable system for preparative chromatography and is suitable for large bio-molecule compound isolation.

- Using the Prep 150 LC System, analytical chromatography can be easily and successfully scaled to preparatory chromatography using a systematic approach.

- The Prep 150 LC System is controlled by ChromScope Software, an intuitive and easy-to-use software that enables users to quickly purify compounds, reducing the amount of time required for training.

INTRODUCTION

To minimize the consumption of sample and solvents, there is a benefit in developing separation methods on a small scale and transferring them to a larger scale. Taking into account the important parameters and applying appropriate scaling factors, users can successfully scale up from analytical chromatography to larger scale preparative separations. In this application note, the analytical scale separation of chicken egg white is used to demonstrate the calculations and techniques used to successfully transfer from a 4.6 mm I.D. analytical column separation to a 19 mm I.D. preparatory column separation.
EXPERIMENTAL

Sample description
Lyophilized chicken egg white was dissolved in mobile phase A at a concentration of 10 mg/mL. The sample was filtered through a 0.45-µm syringe tip filter prior to use.

Method conditions
Analytical scale separation:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>System</td>
<td>Alliance 2695 with a 2998 PDA Detector</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.5 mL/min</td>
</tr>
<tr>
<td>Mobile phase A</td>
<td>Water + 0.05% TFA</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>Acetonitrile + 0.05% TFA</td>
</tr>
<tr>
<td>Gradient</td>
<td>10 to 60% B over 15 minutes</td>
</tr>
<tr>
<td>Injection volume</td>
<td>25 µL</td>
</tr>
<tr>
<td>Detection</td>
<td>UV at 220 nm</td>
</tr>
<tr>
<td>Data</td>
<td>Empower 3</td>
</tr>
<tr>
<td>Column</td>
<td>XBridge Protein BEH C4 Column, 300Å, 5 µm, 4.6 mm x 150 mm</td>
</tr>
</tbody>
</table>

Preparative chromatographic separations were carried out using two different Waters Prep 150 LC System configurations to demonstrate scaling capability.

<table>
<thead>
<tr>
<th>Configuration 1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump</td>
<td>2545 Binary Gradient Module</td>
</tr>
<tr>
<td>Detector</td>
<td>2489 UV Detector with Semi Prep Flow Cell</td>
</tr>
<tr>
<td>Injector</td>
<td>Manual Prep Injector configured with a 2 mL loop</td>
</tr>
<tr>
<td>Collector</td>
<td>Fraction Collector 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Configuration 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump</td>
<td>2545 Quaternary Gradient Module</td>
</tr>
<tr>
<td>Detector</td>
<td>2998 Photo Diode Array with Semi Prep Flow Cell</td>
</tr>
<tr>
<td>Injector</td>
<td>2707 Autosampler configured with a 10 mL loop</td>
</tr>
<tr>
<td>Collector</td>
<td>Fraction Collector 3</td>
</tr>
</tbody>
</table>

Both Prep 150 LC System configurations were controlled using ChromScope Software, v1.4.1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column temp.</td>
<td>Ambient</td>
</tr>
<tr>
<td>Flow rate</td>
<td>25.6 mL/min</td>
</tr>
<tr>
<td>Mobile phase A</td>
<td>Water + 0.05% TFA</td>
</tr>
<tr>
<td>Mobile phase B</td>
<td>Acetonitrile + 0.05% TFA</td>
</tr>
<tr>
<td>Gradient on</td>
<td>configuration 1: 10 to 60% B over 15 minutes following a 0.27 min isocratic hold</td>
</tr>
<tr>
<td>Gradient on</td>
<td>configuration 2: 10 to 60% B over 14.75 minutes</td>
</tr>
<tr>
<td>Injection volume</td>
<td>426 µL</td>
</tr>
<tr>
<td>Detection</td>
<td>UV at 220 nm</td>
</tr>
<tr>
<td>Column</td>
<td>XBridge Protein BEH C4 OBD Prep Column, 300Å, 5 µm, 19 mm x 150 mm</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Successful method scaling from analytical HPLC to preparative HPLC requires a systematic approach and attention to several factors.

Factor 1: Analytical method

Users should try and develop the best analytical method possible. Better analytical methods mean better preparatory methods. Three of the most common issues users encounter when developing methods that will be scaled to prep are:

■ Column heating. Prep systems generally do not have column heating capability, therefore the analytical method should be developed at room temperature. Column heating is possible at a preparative scale, details of these techniques have been previously described.¹

■ No loading studies. If possible, it is helpful for users to do a loading study at the analytical scale to confirm that adequate resolution will be maintained at the preparative scale.

■ Inadequate re-equilibration time. Following a gradient separation (regardless of scale), the system and column need to be re-equilibrated prior to the next injection. The best rule of thumb for re-equilibration is to pump 3X system volume plus 5X column volume of the initial mobile phase composition prior to the injection.

Factor 2: Mobile phases, samples, and columns

Mobile phases need to be the same at both the analytical and preparative scales, identical A, identical B, and same additive concentration. Samples need to be made at the same concentration using the same diluent. For the greatest chance of success, try to use columns of the same length, chemistry, and particle size. Using matched columns will provide similar resolution of critical pairs at all separation scales. Waters offers a wide range of column chemistry choices available in analytical and preparative scale dimensions. Of course, it is possible to use columns of different lengths and particle sizes; the chromatography will be similar, but the resolution of some components will be different and loading capacity could also be affected.

Factor 3: Flow rates

To maintain separation quality, the flow rate must be scaled based on column dimensions. With columns of identical particle size, the following equation is used to geometrically scale flow rate:

\[ F_{\text{PREP}} = F_{\text{ANALYTICAL}} \cdot \frac{D^2_{\text{PREP}}}{D^2_{\text{ANALYTICAL}}} , \]

where \( F \) is flow rate (mL/min) and \( D \) is the inner diameter of the column (mm). For example, a 1.5 mL/min flow rate on a 4.6 mm I.D. column equates to a 25.6 mL/min flow rate on a 19 mm I.D. column.
Factor 4: Injection volume

To maintain peak shape and loading capacity, the injection volume needs to be suitably scaled using the following equation:

$$Vol_{PREP} = Vol_{ANALYTICAL} \cdot \frac{D_{PREP}^2}{D_{ANALYTICAL}^2} \cdot \frac{L_{PREP}}{L_{ANALYTICAL}},$$

where $Vol$ is the injection volume ($\mu$L), $D$ is the inner diameter of the column (mm), and $L$ is the column length (mm). For example, a 25 $\mu$L injection on a 4.6 x 150 mm column corresponds to a 426 $\mu$L injection on a 19 x 150 mm preparative column.

Factor 5: Gradient scaling

When columns are of identical length, changes to the gradient profile are required based on the system volume. To make these adjustments, the system volume must be measured for both the analytical and preparative system. Details on this procedure are included in the Preparative OBD Columns Calculator (Figure 1). The columns calculator provides an easy to use tool that aids in all of the analytical-to-preparative scaling calculations described in this application note. A version of the columns calculator is also embedded in Waters ChromScope Software.

To demonstrate the previously described techniques, the analytical separation method (developed on an HPLC system with a system volume of 0.65 mL) described in the experimental section was scaled to preparative 19 mm I.D. preparative column on two independent Prep 150 LC Systems, a manual injector configuration (system volume of 4.25 mL) and an automated injector based configuration (system volume of 17.5 mL). The scaled flow rates and injection volumes (all calculated using the Preparative OBD Columns Calculator) are shown in the experimental section.
As can be seen from Figure 2, the analytical method provides good separation of major peaks in the egg white sample. Regardless of the Prep 150 LC System configuration, the scaled preparative chromatography is very similar (Figures 3 and 4). When compared to the original 4.6 mm I.D. scale, it can be seen that in terms of resolution and retention time the chromatography is again very similar (Table 1). This simple experiment demonstrates that a systematic approach to scale up meets the goal of maintaining chromatographic resolution between key components and enables users to better predict chromatographic performance between analytical and preparative chromatography.

Figure 2. Separation of a chicken egg white sample using a XBridge Protein BEH C4 Column, 300Å, 5 µm, 4.6 mm x 150 mm on an Alliance HPLC System.

Figure 3. Separation of a chicken egg white sample using a XBridge Protein BEH C4 OBD Prep Column, 300Å, 5 µm, 19 mm x 150 mm on Prep 150 LC System configuration 1.
Figure 4. Separation of a chicken egg white sample using a XBridge Protein BEH C4 OBD Prep Column, 300Å, 5 µm, 19 mm x 150 mm on Prep 150 LC System configuration 2.

Table 1. Retention time and resolution comparison (minutes) of the 4 major peaks.

<table>
<thead>
<tr>
<th></th>
<th>Alliance HPLC</th>
<th>Alliance HPLC</th>
<th>Prep 150 LC Config. 1</th>
<th>Prep 150 LC Config. 1</th>
<th>Prep 150 LC Config. 2</th>
<th>Prep 150 LC Config. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT (minutes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td></td>
<td>5.91</td>
<td></td>
<td>5.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.75</td>
<td></td>
<td>8.62</td>
<td>8.5</td>
<td>8.62</td>
<td>8.2</td>
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<tr>
<td></td>
<td>9.58</td>
<td></td>
<td>9.47</td>
<td>2.2</td>
<td>9.39</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>12.19</td>
<td></td>
<td>12.02</td>
<td>4.9</td>
<td>12.00</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Table 1. Retention time and resolution comparison (minutes) of the 4 major peaks.
CONCLUSIONS

- Analytical chromatography can be successfully scaled to preparatory chromatography easily by using a systematic approach.
- The use of identical column chemistry and identical column lengths maintains separation quality.
- Knowing and using the system volume for both the analytical and prep systems aids in error-free scale-up.
- The Waters Prep OBD Calculator aids in the scaling calculations.
- Developing methods on the analytical scale and transferring them to preparatory scale reduces solvent and sample consumption while reducing waste disposal cost compared to developing separation methods at the preparatory scale only.

References