Video Article

Analytical HPLC to Preparative HPLC: Scale Up Techniques using a Natural Product Extract - ADVERTISEMENT

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Abstract

Using the Waters AutoPurification System, separation methods can be developed on an analytical scale and transferred to preparatory scale on the same system, reducing a laboratory's overall capital investment. Here we illustrate a systematic approach to scale-up using the separation of kudzu (Pueraria lobata) root extract to move from a 4.6 mm I.D. analytical column separation through 10, 19, and 30 mm I.D. preparatory column separations.

Video Link

The video component of this article can be found at http://www.jove.com/video/2885/

Protocol

1. Sample Preparation

Kudzu root pieces (20 g) were added to 100 mL of water/methanol 9:1 and shaken for 1 hour, allowed to stand overnight, and shaken for an additional hour. This extract was centrifuged at 3000 RPM for 20 minutes and used without further treatment.

2. Experimental Conditions

Chromatographic separations, at all scales, were carried out using the Waters AutoPurifcation System, which consisted of the following Waters components:

Pump:	2545 Binary Gradient Module
Detectors:	2998 Photodiode Array 3100 Mass Detector
Injector/Collector:	2767 Sample Manager
Column Management:	System Fluidics Organizer

An initial analytical scale separation was developed on a Waters SunFire C_{18}, 4.6 X 50 mm, 5 µm Column, using the conditions described below.

Column Temp:	Ambient
Flow Rate:	1.5 mL/min
Mobile Phase A:	Water + 0.1% Formic Acid
Mobile Phase B:	Methanol
Gradient:	5% to 70% B over 7 minutes
Injection Volume:	20 µL
Detection:	UV (200 to 400 nm) and MS Full Scan 150 to 700 m/z

The resulting chromatogram (Figure 1) showed a number of resolved compounds and was considered an acceptable candidate for scale-up.

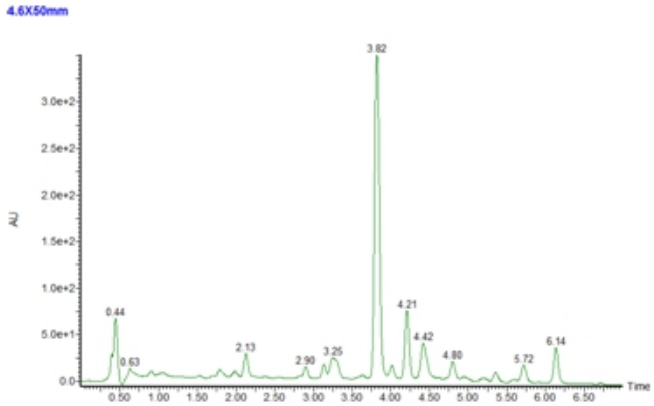

Figure 1. Analytical Separation (4.6 mm I.D.) of kudzu root extract.

Results and Discussion

Scale-Up Method

By using methods tailored to their compounds, chemists are able to obtain higher quality fractions from their mixtures in the shortest possible time speeding up the investigation process. There are a number of key factors to consider when approaching this scale-up process.

Column Chemistry

The heart of the separation is the column. Ideally you should choose column chemistries that are identical. If the analytical and preparative columns are of different chemistry, it becomes very difficult to predict the preparative separation based on the analytical results. Waters offers a wide range of column chemistry choices available in analytical and preparative scale dimensions. In addition to chemistry itself, particle size should also be considered. Columns of the same particle size will provide similar resolution of critical pairs at both separation scales. Column length also influences the separation efficiency; columns of identical length, when scaled, give similar separation power. It is possible to scale to shorter or longer column but keep in mind that the separation will change.

Injection Volume

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

$$Vol_{PREP} = Vol_{ANALYTICAL} \bullet \frac{D^2{}_{PREP}}{D^2{}_{ANALYTICAL}} \bullet \frac{L_{PREP}}{L_{ANALYTICAL}},$$

Where Vol is the injection volume (µL), D is the inner diameter of the column (mm), and L is the column length (mm). For example, a 20 µL injection on a 4.6 x 50 mm column corresponds to a 341 µL injection on a 19 x 50 mm preparative column.

Flow Rate

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:

$$F_{PREP} = F_{ANALYTICAL} \bullet \frac{D^2{}_{PREP}}{D^2{}_{ANALYTICAL}},$$

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.

Gradient Scaling

When columns are of identical length, no changes to the gradient profile are required. If scaling to longer or shorter columns, the gradient segment volume must be maintained to preserve the separation profile.

The Waters OBD Prep Calculator, a free download, (Figure 2) provides an easy to use tool that aids in these analytical-to-preparative scaling calculations (www.waters.com/prepcalculator). This calculator was used to convert the analytical separation method to the preparatory separation methods described in this application note.

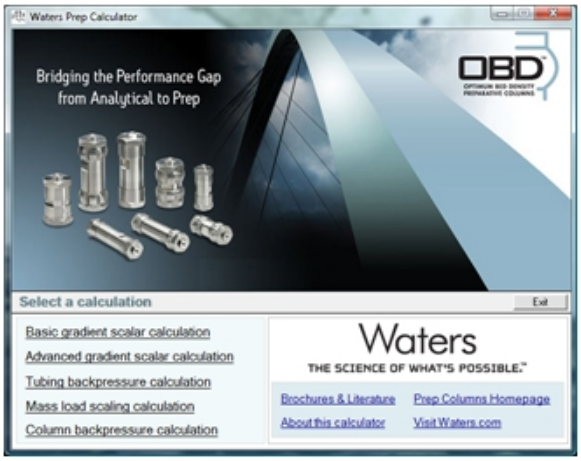

Figure 2. Waters OBD Prep Calculator.

Using the Waters OBD Prep Calculator

To calculate injection volume and flow rates, select the mass load scaling calculation (Figure 3) from the opening screen. Input your analytical and preparative column dimensions, analytical flow rate, and injection volume; the calculator returns the correct preparative values.

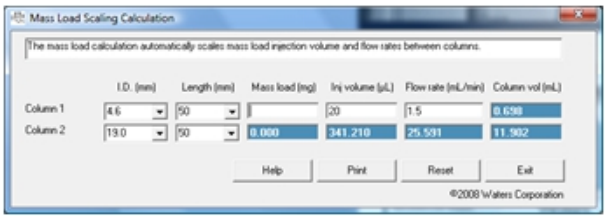

Figure 3. Waters OBD Prep Calculator mass load scaling calculation.

If your column lengths are identical you can simply input the preparative flow rates into your gradient table using the same gradient segment times as your analytical method. Alternatively, for gradient methods, choose the basic gradient scalar calculation (Figure 4) from the opening screen, select your analytical and preparative column dimensions, input your analytical gradient table, and click the calculate button. The preparative gradient table is automatically calculated and shown on the bottom half of the page. The Waters OBD Prep Calculator User Guide gives detailed instructions on use of all calculator functions.

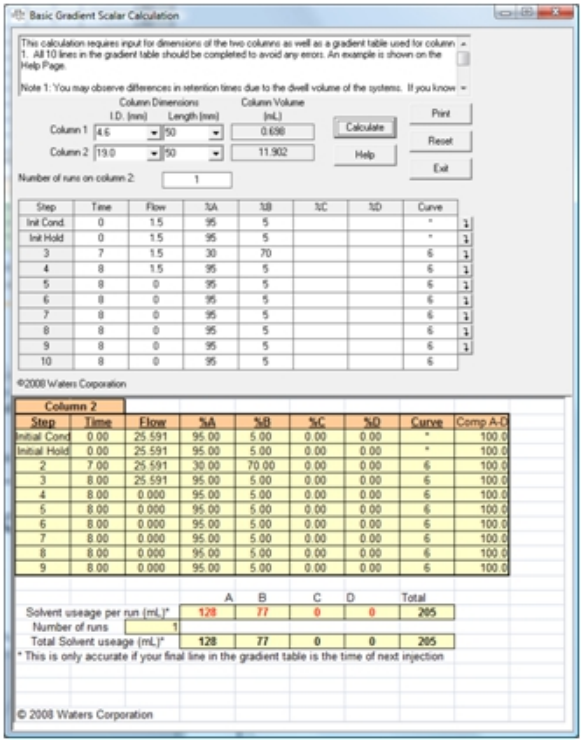

Figure 4. Waters OBD Prep Calculator basic gradient scalar calculation.

Results

To demonstrate the previously described techniques, the analytical separation method described in the experimental section was scaled to three different preparative dimension columns (10.0, 19.0, and 30.0 mm I.D.). The scaled flow rates and injection volumes (all calculated using the Waters OBD Prep Calculator) are shown in Table 1.

Internal Diameter (mm)	Flow Rate (mL/min)	Injection Volume (µL)
4.6	1.5	20
10.0	7.1	95
19.0	25.6	341
30.0	63.8	851

Table 1. Waters OBD Prep Calculator scaled flow rates and injection volumes.

All of the preparative columns are SunFire Preparative C_{18}, 5 µm, 50 mm in length with OBD technology, and all of the separations were performed on the same system as the analytical scale chromatography. As can been seen from Figure 5, regardless of the scale, the chromatography (UV TIC) is very similar.

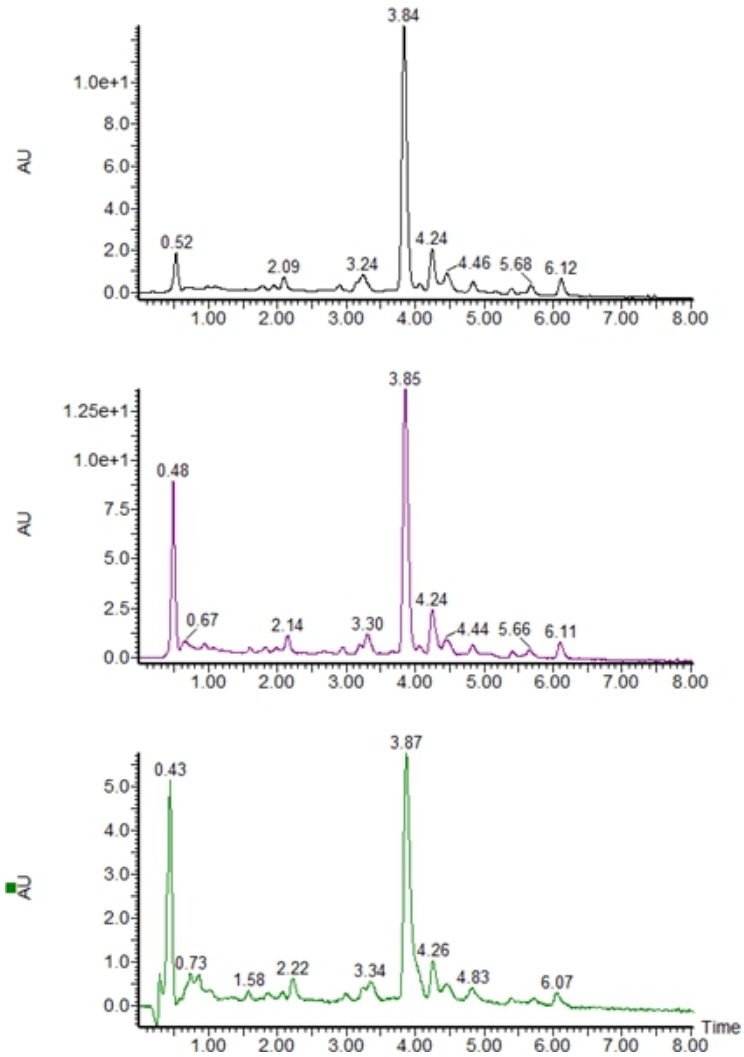

Figure 5. Scaled Preparatory Separations, 10 mm I.D. (top), 19 mm I.D. (middle), 30 I.D. mm (bottom)

When compared to the original 4.6 mm I.D. scale (Figure 1), it can be seen that in terms of resolution and retention time the chromatography is again very similar. 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. This exercise also demonstrates the unique capability of the Waters AutoPurification System, which allows users to perform both analytical and preparatory chromatography on the same system with no performance compromise.

Conclusions

- Analytical chromatography can be successfully and easily scaled to preparatory chromatography by using a systematic approach.
- The use of identical column chemistry and identical column lengths maintains separation quality.
- Waters proprietary Optimum Bed Density (OBD) column design offers excellent sample loading and column stability in an extensive array of chemistries and configurations.
- The Waters Prep OBD Calculator aids in scaling calculations.
- Using the Waters Autopurification System, separation methods can be developed on an analytical scale and transferred to preparatory scale on the same system, reducing the overall capital investment.
- 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 only at the preparatory scale.