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应用纪要

Prep 150 LC System: Considerations for Analytical to Preparative Scaling

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Abstract

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.

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:

System:	Alliance 2695 with a 2998 PDA Detector
Flow rate:	1.5 mL/min
Mobile phase A:	Water + 0.05% TFA
Mobile phase B:	Acetonitrile + 0.05% TFA
Gradient:	10 to 60% B over 15 minutes

Analytical scale separation:

Injection volume:	25 µL
Detection:	UV at 220 nm
Data:	Empower 3
Column:	XBridge Protein BEH C_4 Column, 300Å, 5 µm, 4.6 mm x 150 mm

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

Configuration 1

Pump:	2545 Binary Gradient Module
Detector:	2489 UV Detector with Semi Prep Flow Cell
Injector:	Manual Prep Injector configured with a 2 mL loop
Collector:	Fraction Collector 3

Configuration 2

Pump:	2545 Quaternary Gradient Module
Detector:	2998 Photo Diode Array with Semi Prep Flow Cell

Injector:	2707 Autosampler configured with a 10 mL loop
Collector:	Fraction Collector 3
Both Prep 150 LC System configurations were controlled using ChromScope Software, v1.4.1.	
Column temp.:	Ambient
Flow rate:	25.6 mL/min
Mobile phase A:	Water + 0.05% TFA
Mobile phase B:	Acetonitrile + 0.05% TFA
Gradient on configuration 1:	10 to 60% B over 15 minutes following a 0.27 min isocratic hold
Gradient on configuration 2:	10 to 60% B over 14.75 minutes
Injection volume:	426 µL
Detection:	UV at 220 nm
Column:	XBridge Protein BEH C_4 OBD Prep Column, 300Å, 5 µm, 19 mm x 150 mm

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:

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

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.

Factor 4: Injection volume

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

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

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 25 µL injection on a 4.6 x 150 mm column corresponds to a 426 µL 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.[2] A version of the columns calculator is also embedded in Waters ChromScope Software.

Figure 1. Waters Preparative OBD Columns Calculator home screen.

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 been 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.

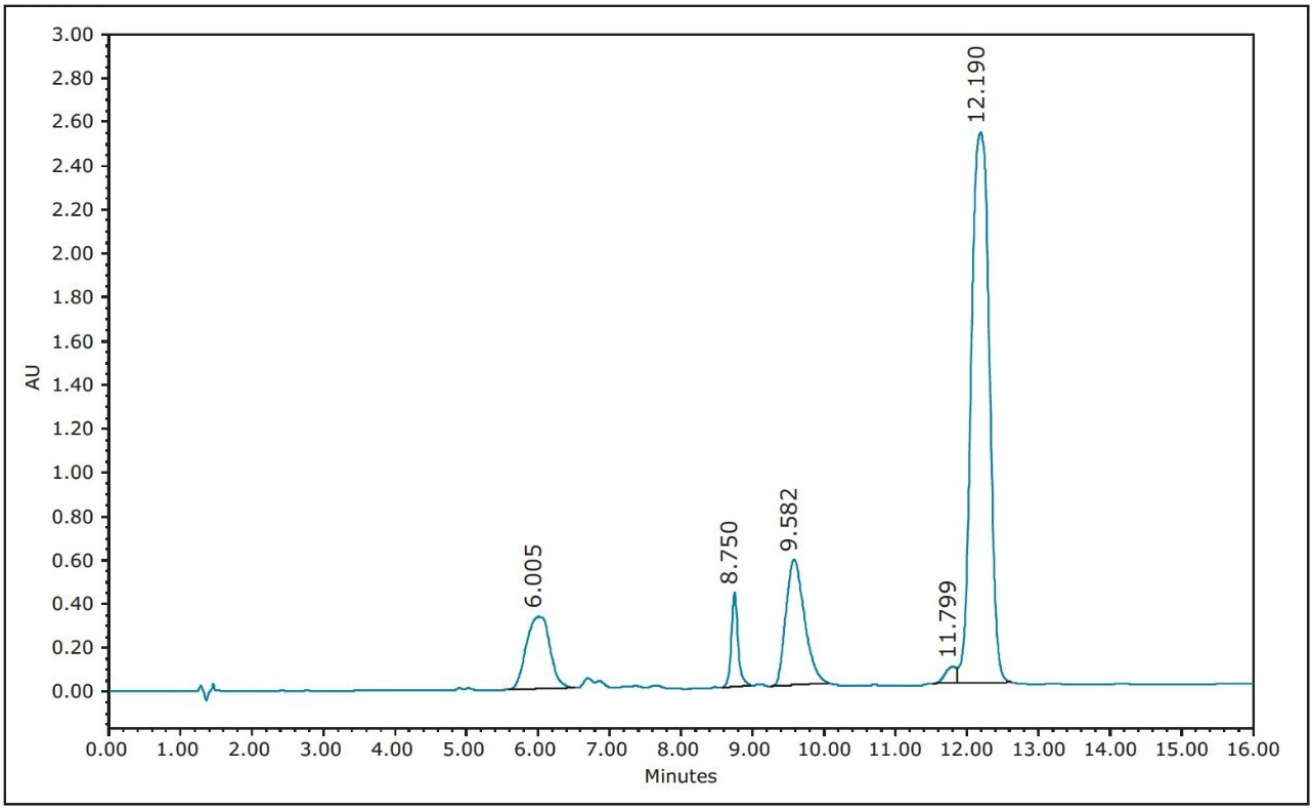

Figure 2. Separation of a chicken egg white sample using a XBridge Protein BEH C_4 Column, 300Å, 5 µm, 4.6 mm x 150 mm on an Alliance HPLC System.

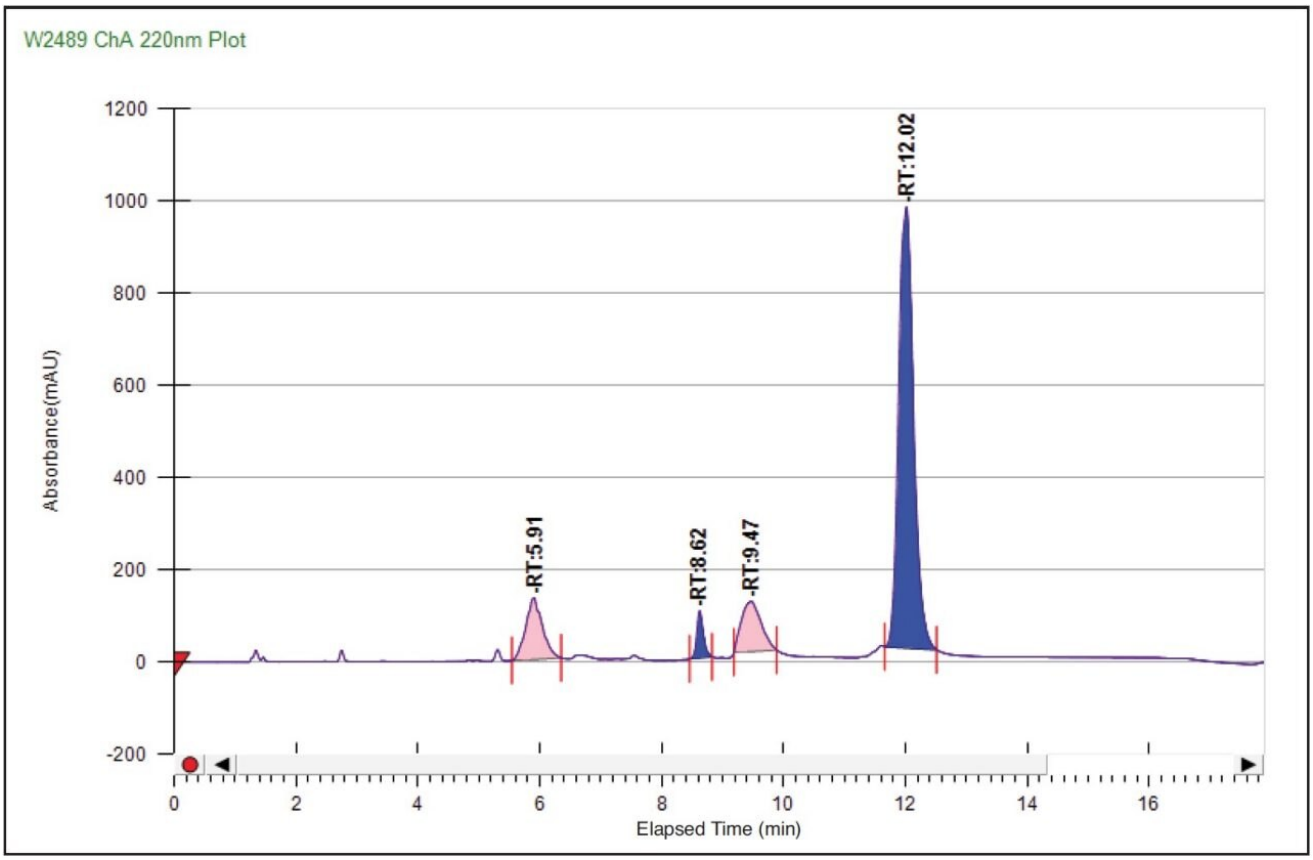

Figure 3. Separation of a chicken egg white sample using a XBridge Protein BEH C_4 OBD Prep Column, 300Å, 5 µm, 19 mm x 150 mm on Prep 150 LC System configuration 1.

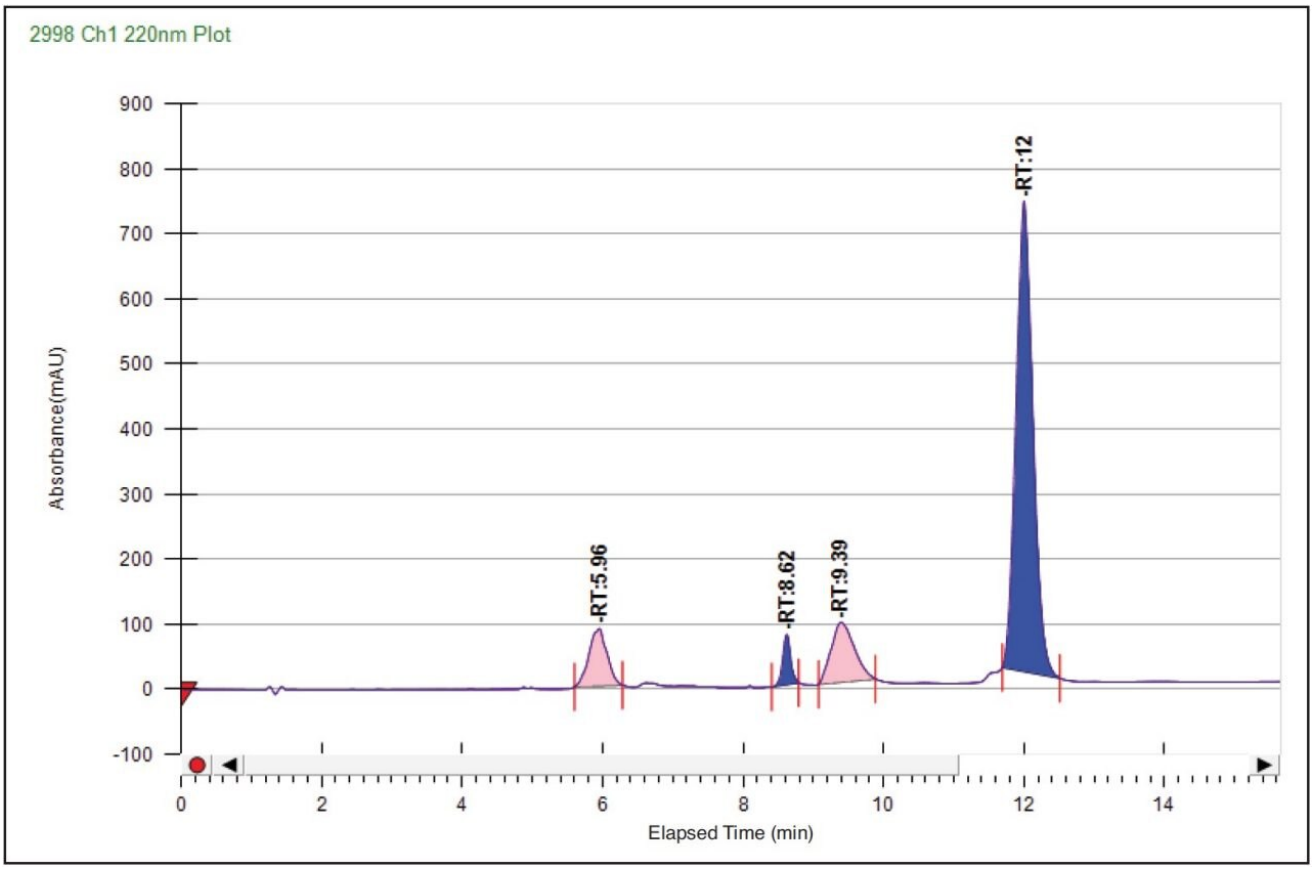

Figure 4. Separation of a chicken egg white sample using a XBridge Protein BEH C_4 OBD Prep Column, 300Å, 5 µm, 19 mm x 150 mm on Prep 150 LC System configuration 2.

Alliance HPLC	Alliance HPLC	Prep 150 LC Config. 1	Prep 150 LC Config. 1	Prep 150 LC Config. 2	Prep 150 LC Config. 2
RT (minutes)	Resolution	RT (minutes)	Resolution	RT (minutes)	Resolution
6.00	–	5.91	–	5.96	–
8.75	7.2	8.62	8.5	8.62	8.2
9.58	2.6	9.47	2.2	9.39	1.9
12.19	5.5	12.02	4.9	12.00	5.1

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

Conclusion

- 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

1. Effective Use of Temperature Control in Compound Isolation, Jo-Ann M. Jablonski, Thomas E. Wheat, Diane M. Diehl. Waters Application Note, 2009, Part Number: 720002954en
2. Developing Focused Gradients for Isolation and Purification, Jo-Ann M. Jablonski, Thomas E. Wheat, Diane M. Diehl, Waters Application Note, 2009, Part Number: 720002955en

Featured Products

Alliance HPLC System <https://www.waters.com/534293>

Prep 150 LC System <https://www.waters.com/134727002>

ChromScope Software <https://www.waters.com/134647658>

Empower 3 Chromatography Data Software <https://www.waters.com/513188>

2998 Photodiode Array (PDA) Detector <https://www.waters.com/1001362>

2489 UV/Visible (UV/Vis) Detector <https://www.waters.com/515198>

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