Waters™

응용 자료

Demonstrating Chiral Scale-up and Purification Using the ACQUITY UPC^2 and Prep 80q SFC Systems

Jacquelyn Runco

Waters Corporation

Abstract

Supercritical fluid chromatography (SFC) has become a mainstream technology for chiral separations based on its higher efficiency, throughput, and wide applicability. For purification purposes, chiral SFC has seen increased interest and applicability, becoming the technique of choice in many cases. Purification of chiral compounds by SFC typically utilizes isocratic method conditions and stacked injections for optimal collection efficiency. In this application note, bucetin will be used as an example compound to demonstrate the typical work flow for chiral purification by SFC. The process will include gradient screening and isocratic method development on the ACQUITY UPC^2 System, scale-up and purification using stacked injections on the Prep 80q SFC System, and post purification analysis on the ACQUITY UPC^2 System.

Benefits

- Chiral method development and optimization for scale-up was fast and easy using the ACQUITY UPC^2 System saving time, sample and solvent.
- Successful scale-up of the isocratic chiral separation allows the user to take advantage of stacked injection

capabilities in preparative supercritical fluid chromatography (SFC), resulting in improved collection efficiency.

- Isolation of the enantiomers on the Prep 80q SFC System resulted in high purity fractions requiring less downstream workup due to the lack of organic solvent.

Introduction

Generally, when purification is the goal, it is more practical for method development to be performed at the analytical scale to save solvent, time and sample. Once the method has been optimized at the analytical scale, calculations based on column size and system volume are utilized to scale-up the separation for purification. For successful scale-up, identical sample concentration, diluents, mobile phase and average system pressure (specifically for SFC) are required. The analytical and preparative column chemistries must be identical and it is beneficial to maintain particle size and column length.[1]

Supercritical fluid chromatography (SFC) has become a mainstream technology for chiral separations based on its higher efficiency, throughput, and wide applicability. For purification purposes, chiral SFC has seen increased interest and applicability, becoming the technique of choice in many cases.[2] Purification of chiral compounds by SFC typically utilizes isocratic method conditions and stacked injections for optimal collection efficiency. In this application note, bucetin will be used as an example compound to demonstrate the typical work flow for chiral purification by SFC. The process will include gradient screening and isocratic method development on the ACQUITY UPC^2 System, scale-up and purification using stacked injections on the Prep 80q SFC System, and post purification analysis on the ACQUITY UPC^2 System.

Experimental

Sample description

The racemic bucetin sample was made up at a concentration of 10 mg/mL in HPLC grade methanol.

Instrumentation

Analytical chromatography, method development and supporting fraction analysis were performed on the ACQUITY UPC^2 System with a PDA Detector and controlled by MassLynx Software. Scale-up and purification was done on the Prep 80q SFC System with a 2489 UV/Vis Detector and ChromScope Software control.

Method conditions

General running conditions for the ACQUITY UPC^2 and the Prep 80q SFC System are listed below. All other conditions will be noted in the figures.

ACQUITY UPC^2 analytical method conditions

Control:	MassLynx Software
Mobile phase A:	CO_2
Mobile phase B:	Methanol (MS Grade)
Flow rate:	3 mL/min
Pressure:	120 bar
Temperature:	35 °C
PDA detector: Scan:	220 – 400 nm Extracted wavelength: 247 nm
Analytical column:	Chiralpak IA Column (4.6 x 150 mm, 5 µm)

Prep 80q SFC preparative method conditions

Control:	ChromScope Software

Prep 80q SFC preparative method conditions

Mobile phase A:	CO_2
Mobile phase B:	Methanol (HPLC grade)
Flow rate:	62 mL/min
Pressure:	120 bar
Temperature:	40 °C
UV/Vis detector:	247 nm
Preparative column:	Chiralpak IA Column (21 x 150 mm, 5 µm)

Results and Discussion

Method development in chiral SFC involves a column screening step using a gradient, followed by developing an isocratic method for purification on the selected column chemistry. Optimization is done to determine isocratic conditions that allow high loading and short cycle times. To determine a starting point for isocratic method development, the retention time and slope of the gradient are used to calculate the %B when the peak eluted. Typically, for better resolution and higher loading the calculated %B is adjusted 10% lower.

In this case, the gradient conditions were 5–40% methanol in 5 minutes. At those conditions the average retention time of the two bucetin peaks was 3.03 min. After adjusting for a 0.6 min offset due to system volume, the %B at the elution time was calculated to be 22% methanol. Figure 1 shows chromatograms of (A) the bucetin separation under the gradient conditions, and (B) the separation under 22% methanol isocratic conditions. The two methods show very similar resolution for relatively low loading (2 µL), but not enough resolution for purification purposes. To improve loading, a 12% isocratic method was investigated (10% lower than the calculated percentage), the resulting chromatograms are in Figure 2 (A) and (B). Higher loading was achieved

and the resolution was acceptable, however to improve the 10 µL separation even further a final 11% isocratic method was used (Figure 2 (C)).

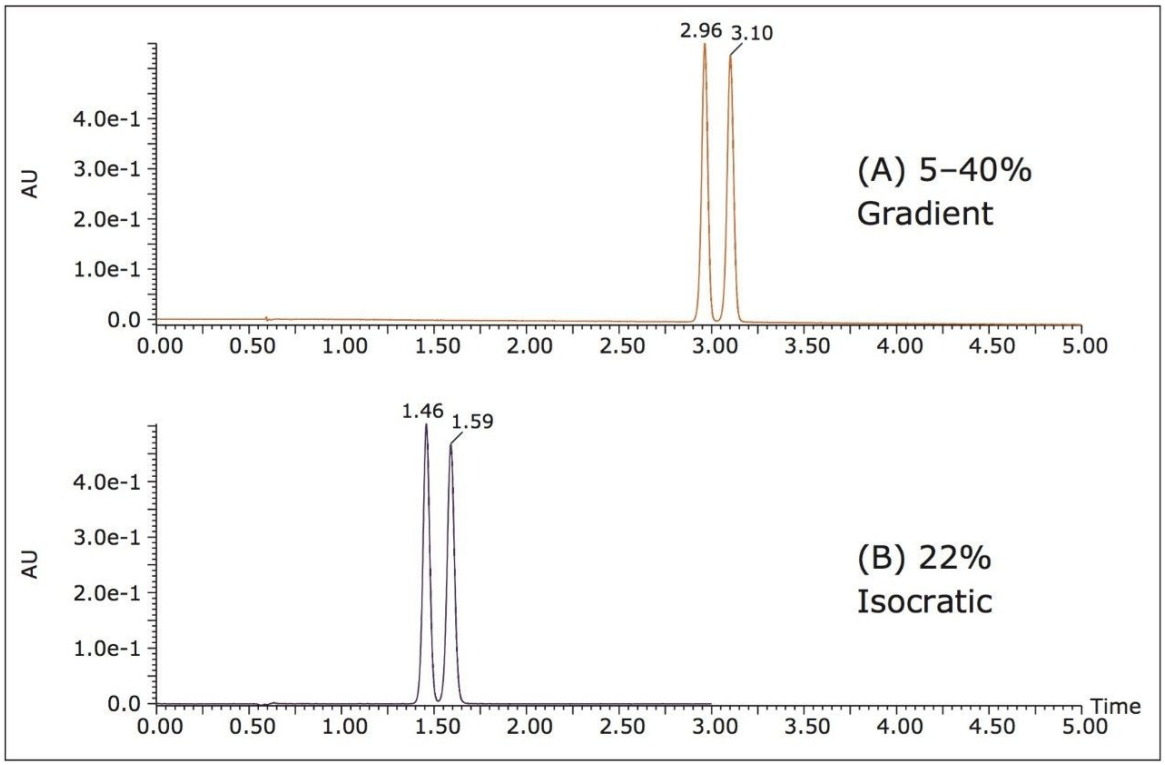

Figure 1. ACQUITY UPC^2 chromatograms of the bucetin chiral separation under gradient (A) and calculated isocratic (B) conditions. Conditions: (A) 3 mL/min, 5–40% gradient over 5 min, 120 bar, 35° C, 2 µL injection, (B) 3 mL/min, 22% isocratic, 120 bar, 35° C, 2 µL injection. PDA: extracted wavelength at 247 nm.

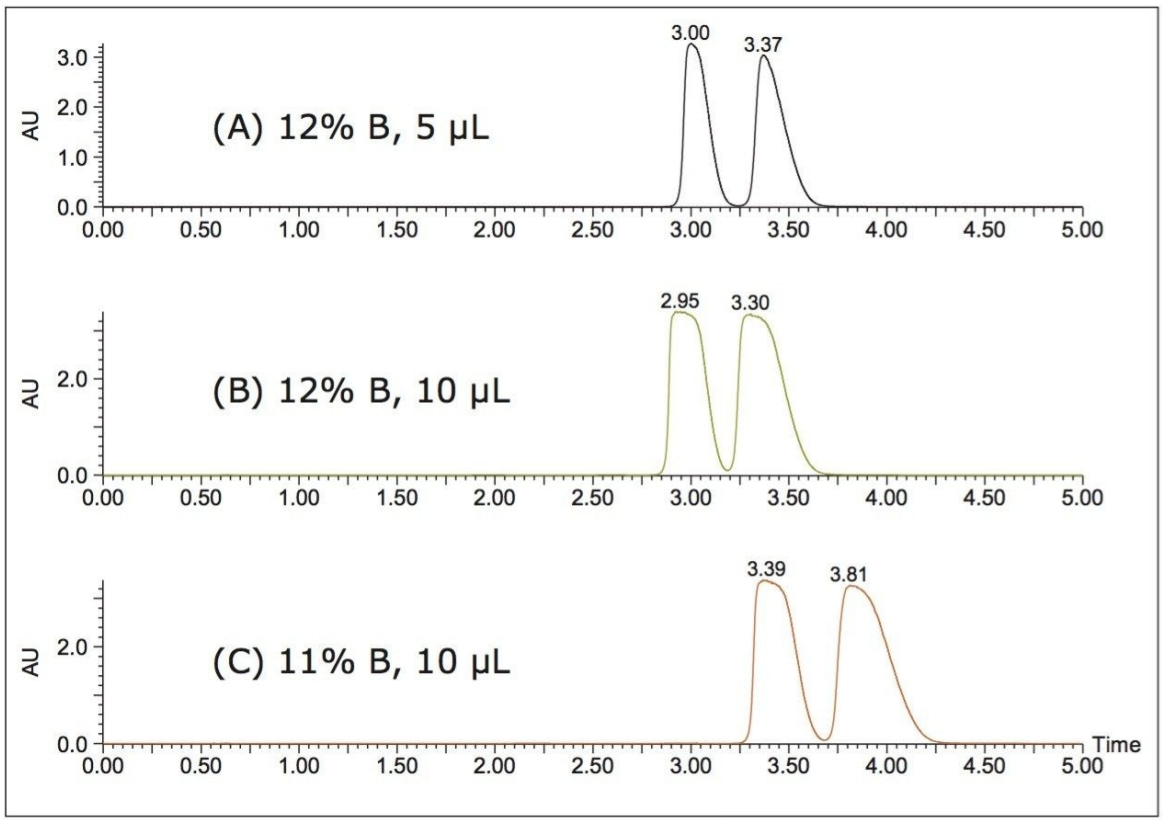

Figure 2. Chromatograms of the bucetin loading on the ACQUITY UPC² System. Conditions: (A) 3 mL/min, 12% isocratic, 120 bar, 35° C, 5 µL injection, (B) 3 mL/min, 12% isocratic, 120 bar, 35° C, 10 µL injection (C) 3 mL/min, 11% isocratic, 120 bar, 35° C, 10 µL injection. PDA: extracted wavelength at 247 nm.

Proper scaling ensures that the chromatographic profile of a sample at the preparative scale will be identical to the chromatography obtained at the analytical scale.[3] To maintain separation quality, the columns were identical in chemistry, particle size and length, and the system pressure was matched between the two systems. Geometric scaling of the flow rate and injection volume was performed based on the inner diameters (I.D.s) of the analytical and preparative columns using the Waters Prep Calculator. The flow rate of 3 mL/min and injection volume of 10 µL on the 4.6 mm analytical column equated to a flow rate of 62 mL/min and injection volume of 200 µL on the 21 mm prep column.

In Figure 3, the ACQUITY UPC² (A) and scaled-up Prep 80q SFC System (B) chromatograms are shown along with the final purification utilizing stacked injections and timed collections (C). The use of stacked injections

along with 200 µL loading allowed for 10 mg of sample to be processed in about 14 minutes. The first three sets of peaks show injection spikes from the injections made during the run, when no injections were made (sets 4 and 5) no injection spike is observed. Given that the initial sample was a racemic mixture, each enantiomer started out at a purity of 50%. The fractions obtained on the Prep 80q SFC System, were analyzed on the ACQUITY UPC^2 System (Figure 4), and the results revealed purities of 100% for peak 1 and 95% for peak 2.

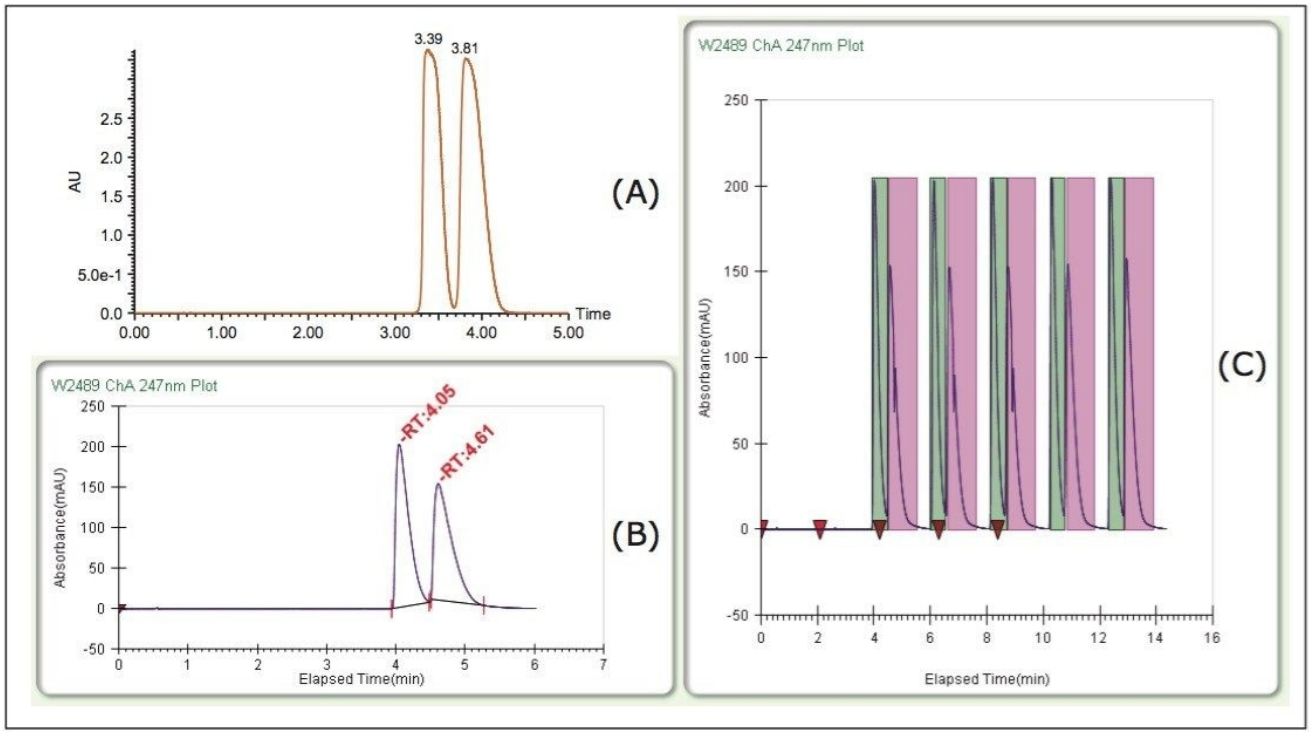

Figure 3. Chromatograms showing scale-up from the ACQUITY UPC^2 (A) to the Prep 80q SFC (B) systems, and stacked injections with collection marks on the Prep 80q SFC System (C). Conditions: (A) 3 mL/min, 11% isocratic, 120 bar, 35° C, 10 µL injection, (B) and (C) 62 mL/min, 11% isocratic, 120 bar, 35° C, 200 µL injection. PDA: extracted wavelength at 247 nm.

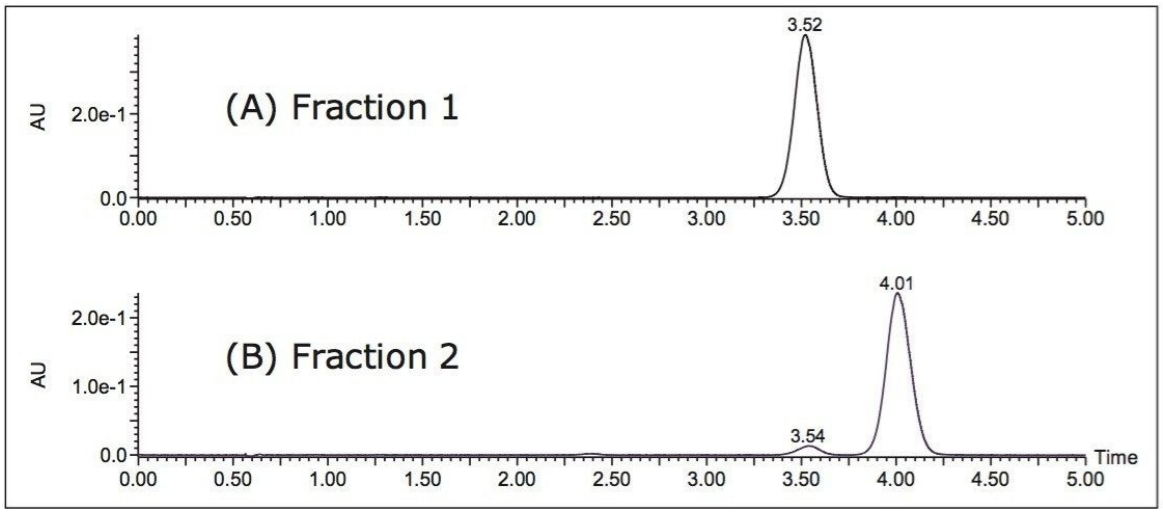

Figure 4. ACQUITY UPC^2 chromatograms showing analysis of the Prep 80q SFC System fractions. Conditions: (A) 3 mL/min, 11% isocratic, 120 bar, 35° C, 10 µL injection. PDA: extracted wavelength at 247 nm.

Conclusion

- Using the ACQUITY UPC^2 System, the gradient separation of the bucetin was successfully converted to an isocratic separation useful for stacked injection purification.
- Successful scale-up of a chiral SFC separation from the ACQUITY UPC^2 System to the Prep 80q SFC System was demonstrated using simple geometric scaling, calculated using the Waters Prep Calculator.
- The use of identical column chemistry, length and particle size along with maintaining the same pressures between systems resulted in similar separation profiles.
- The isocratic separation maximized loading while minimizing cycle time for stacked injections on the Prep 80q SFC System, resulting in a high efficiency purification workflow.
- Purification of the bucetin enantiomers was successful using timed collections on the Prep 80q SFC System; resulting in 100% and 95% pure fractions.

References

1. Aubin A, Jablonski J. Prep 150 LC System: Considerations for analytical to preparative scaling. Waters Application Note 720005458EN. July 2015.
2. Zulli S, Rolle D, Wang Z, Martin T, Chen R, and Sidhu H. Chiral purification with stacked injections and collections using the Prep 100 SFC MS directed system. Waters Application Note 720003773EN. November 2010.
3. Jablonski J, Aubin A. Peptide isolation using the Prep 150 LC system. Waters Application Note 720005455EN. July 2015.

Featured Products

SFC 80q/200q and 350 Preparative Systems <https://www.waters.com/10145745>

2489 UV/Visible (UV/Vis) Detector for Purification and Modular LC Systems <https://www.waters.com/134721513>

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

ACQUITY UPC2 System <https://www.waters.com/134658367>

MassLynx MS Software <https://www.waters.com/513662>

720005475, August 2015