Practical Strategies for Successful Scaling from UPC² to Preparative SFC

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Techniques for scaling separation methods from analytical to preparative in traditional liquid chromatography are well understood and easily accomplished. Many of the same factors that influence successful scaling in HPLC are also important in SFC, such as maintaining identical column length, particle size and stationary phase, along with determining system and extra column volumes in order to account for and anticipate differences in peak shape and retention time [1]. Also, depending on the choice of diluent and injection volume, the sample injection strategy needs to be considered.

In SFC, there are additional factors that will influence successful scaling including pump control and pressure drop differences between analytical and preparative scale systems [2]. In most analytical systems, such as the ACQUITY UPC² system, the CO_2 pump uses volume-based flow control (mL/min), while in many preparative SFC systems, the CO_2 pump delivers a specific mass flow (g/min) and the co-solvent pump delivers volumetrically. Since the density of CO_2 is dependent upon pressure and temperature, the actual amount of CO_2 being delivered differs between the analytical and preparative pumps and must be taken into account [3].

The two injection modes common in SFC, mixed-stream and modifier-stream, influence peak shape and retention time differently and should be maintained [4] when scaling up, if possible. In addition, chromatographic performance is affected by the compressibility of CO_2 and the resulting pressure drop across the column (measured by the difference between the system front pressure and the back pressure). As the mobile phase flows through analytical and preparative instruments, the respective system volumes and tubing IDs result in different pressure and CO_2 density profiles across the systems, leading to changes in selectivity, peak shape, and retention times [2].

All of the steps used to account for these factors will be demonstrated to produce scaled chromatography from the ACQUITY UPC² system to the Prep SFC 150 Mgm System, which is capable of running 50-150 g/min. The initial scale-up study was performed using a 19 mm ID column, followed by geometric scaling to a 30 mm ID column.

Experimental

Instrumentation:

Analytical separations were performed on a Waters ACQUITY UPC² System with a 3.0 x 150 mm, 5 µm, Torus 2-PIC column using the conditions noted in Table 1. The UPC² system was configured with an ACQUITY PDA for UV detection, and was modified to perform modifier-stream injections.

Preparative separations were achieved using a Waters Prep SFC 150 Mgm System with both a 19 x 150 mm, 5 µm, Torus 2-PIC column or a 30 x 150mm, 5µm, Torus 2-PIC column using the conditions noted in Table 1. The Waters Prep SFC 150 Mgm System has a Modifier Stream Injector, gas-liquid separator (GLS) for low-pressure collection, Prep Fraction Selector, and a Prep Collection Cabinet that holds six 2 L bottles. The system is designed for processing and collecting grams of material using stacked injections or multiple repeated injections, and has a recommended flow range of 50 to 150 mL/min, making it ideal for 19 to 30 mm ID columns.

Sample:

A mixture of caffeine (1 mg/mL), theophylline (1 mg/mL), and theobromine (0.15 mg/mL) was prepared in methanol and used as the separation test mix. Methanol was used as the co-solvent across both platforms.

Results & Discussion

In preparative SFC, the samples are dissolved in solvents stronger than the CO_2-majority mobile phase. When large, preparative volume injections are made, the stronger solvent (diluent) can cause peak distortions and retention time shifts in the chromatography [4]. As a result, preparative SFC systems utilise modifier-stream injections to mitigate these chromatographic effects. This technique is similar to at-column dilution used in preparative LC for large volume loading. Analytical systems are more often configured to perform injections using the mixed-stream technique. In normal analytical operation, the low volume injections have very little impact on the peak shapes. However, loading studies to determine the maximum injection amount on column are

Table 1: Analytical and Preparative Instrument method conditions

	Analytical 3 mm ID	Preparative 19 mm ID	Preparative 30 mm ID
Flow Rate	1.5 mL/min	56.9 g/min	141 g/min
Co-solvent %	15%	15.9 %	15.9%
Detector Channel	254 nm	254 nm	254 nm
Pressure	124 Bar (1800 psi)	124 Bar (1800 psi)	124 Bar (1800 psi)
Injection Volume	5 µL	200 µL	500 µL
Column Temp.	40°C	40°C	40°C

usually performed on the analytical scale and this requires larger volume injections. These larger volume injections result in the same diluent effects as would be observed in mixed-stream preparative SFC injections. To perform loading studies, and to match injection modes between the analytical and preparative systems, the UPC² was plumbed to perform modifier-stream injections (Figure 1). The effect of the change in injection mode on the UPC² can be seen in Figure 2, with the modifier-stream injections showing significantly better peak shape, and increased retention with an injection volume of only 5 µL.

Without accounting for CO_2 density and mass flow control of the preparative CO_2 pump, the separation shown in Figure 2 (B) was directly scaled to the 19 mm column using a typical volume-to-volume scaling (Equation 1), resulting in a 60 mL/min flow rate at 15% co-solvent. To maintain peak shape and loading capacity, the injection volume was scaled for both the 19 mm and 30 mm ID columns (Equation 2) resulting in 200 µL and 500 µL injection volumes respectively.

$$F_{Prep}=F_{Analytical}*D^2_{Prep}/D^2_{Analytical}$$

Equation 1: Geometric flow rate scale-up equation from analytical to prep. F is flow rate (mL/min) and D is the inner diameter of the column (mm).

$$Vol_{Prep}=Vol_{Analytical}*D^2_{Prep}/D^2_{Analytical}*L_{Prep}/L_{Analytical}$$

Equation 2. Injection volume scale-up equation from analytical to prep. Vol is the injection volume (µL), D is the inner diameter of the column (mm), and L is the column length (mm).

It is important to determine the system volumes to account for timing differences in the chromatography. The extra-column system volumes (injector to detector) were determined to be 0.180 mL on the UPC² and 1.2 mL on the preparative system, resulting in a 0.10 min delay between the preparative system and the UPC². The chromatography was aligned to account for the delay. The resulting chromatography, using the typical volume-to-volume scaling, is shown in Figure 3.

Although the chromatography from the preparative separation very closely resembled the chromatography from the analytical separation, better agreement can be achieved when the difference in CO_2 pump control is taken into account [3]. On the UPC², the CO_2 pump uses a volumetric (mL/min) flow rate, while on the Prep SFC 150 Mgm System, the CO_2 pump uses a mass (g/min) flow rate. Converting the CO_2 flow on the UPC² system from volumetric to mass (using the temperature and pressure at the pump to determine density) before scaling ensures improved comparability between analytical and prep separations. Since both co-solvent pumps are controlled volumetrically (mL/min), the scale-up calculation has to be done separately for the CO_2 and the co-solvent. The resulting scaled flow rates (in g/min for the CO_2 and mL/min for the co-solvent) are then added for the total flow.

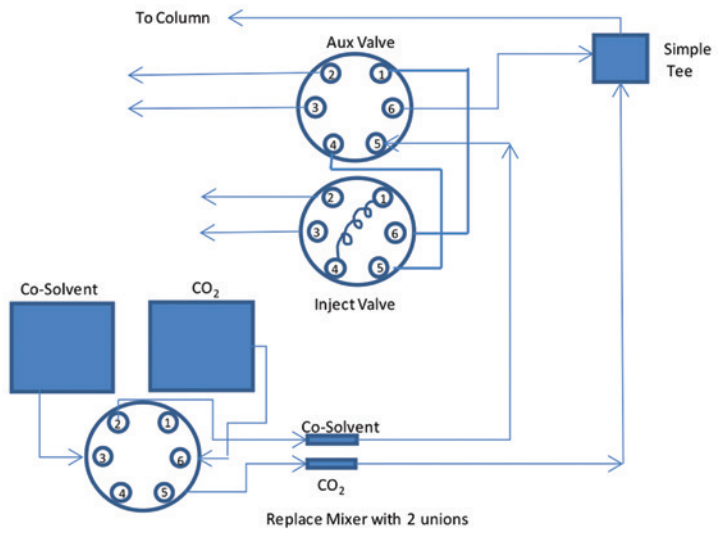

Figure 1: Plumbing diagram for UPC² using modifier stream injection.

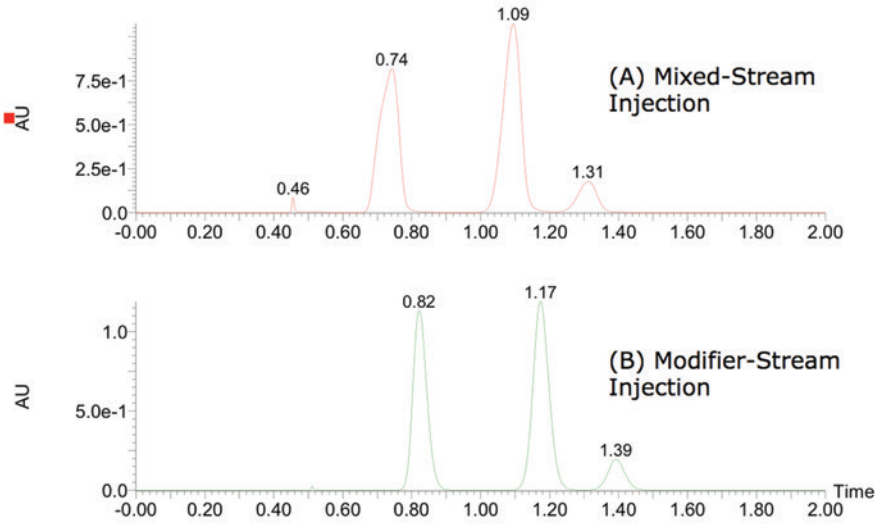

Figure 2: Comparison of UPC² chromatograms using mixed-stream and modifier-stream injection techniques.

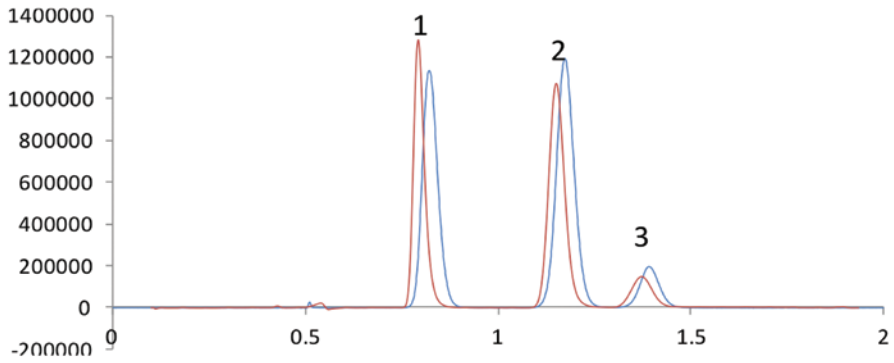

Figure 3: Overlaid chromatograms of the volume-to-volume scaled-up separation on the 19 mm Torus 2-PIC column (red) and on the 3 mm Torus 2-PIC column (blue). Preparative method conditions: 60 mL/min total flow, 15% methanol, 40°C, 124 bar, 200 µL injection volume. Analytical method conditions: 1.5 mL/min total flow, 15% methanol, 40°C, 124 bar, 5 µL injection volume. Peaks: (1) Caffeine, (2) Theophylline, (3) Theobromine

Table 2: Strategy used to scale-up the separation when converting the CO_2 flow from mL/min to g/min.

	CO_2 Flow	Co-solvent Flow
Method on UPC²	1.275 mL/min	0.225 mL/min
CO_2 mL to g conversion (density=0.936 g/mL)	1.1934 g/min	0.225 mL/min
Scaled to 19mm (19mm/3mm)² x UPC² Flow	47.87 g/min	9.025 mL/min
Final Prep Method	47.87 + 9.0205 = 56.9 g/min (Total Flow) 9.025/56.9*100=15.9% co-solvent	

In this case, the temperature and pressure of the CO_2 pump on the UPC² system was 13°C and 146 bar resulting in a CO_2 density of 0.936. With the converted volume to mass flow rate adjustment, the preparative CO_2 flow rate was 47.87 g/min, and the co-solvent flow was 9.025 mL/min for a total of 56.9 g/min and 15.9% methanol. The calculations and values are shown in Table 2.

In SFC, the differences in tubing diameter, flow rate, and column size between the analytical and preparative systems can produce changes in the pressure drops across the systems. Because CO_2 is compressible, these pressure changes create different density profiles across the columns, which in turn, affects the chromatography. When these differences in pressure are observed, the back pressure on the preparative system should be adjusted so the two systems operate at the same average pressure.

Average Pressure = (pump inlet pressure + back pressure)/2

Back pressure = pressure at the automated back pressure regulator (ABPR) (user settable).

In this case, the pressure drops were the same (20 bar, 290 psi) on both the UPC² and the Prep SFC 150 Mgm System, so the pressure and density profiles across the columns were consistent and required no adjustment in the preparative system back pressure.

The resulting chromatography on the 19 mm prep column overlaid with the initial UPC² separation (Figure 4 (A)) shows excellent chromatographic agreement between the two scales. This demonstrates that if the differences between systems and scales are accounted for, successful method transfer between analytical and prep SFC can be accomplished.

As a further demonstration of scaling, the UPC² chromatography was also scaled up to a 30 mm i.d. column. In this case, flow rate and injection volumes were scaled using the previously described formulas. Figure 4 (B) shows the scaled-up chromatography generated on the 30 mm ID column using the same strategy and system as with the 19 mm column. The separation also shows good agreement with the UPC² separation (overlaid).

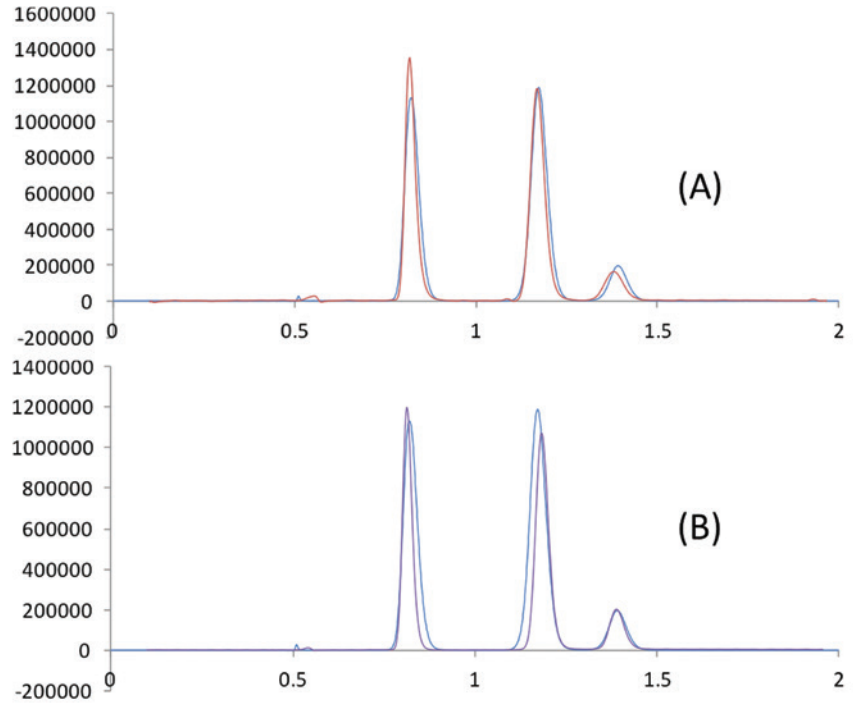

Figure 4: Preparative SFC chromatograms on both the 19 mm ((A), red) and 30 mm ((B), purple) 2-PIC prep columns using the CO_2 mass conversion before scale-up, overlaid with the UPC² chromatogram (blue). Preparative method conditions: (A) 56.9 g/min total flow, 15.9 % methanol, 124 bar, 40°C, 200 µL injection volume. (B) 141 g/min total flow, 15.9% methanol, 124 bar, 40°C, 500 µL injection volume. Analytical method conditions: 1.5 mL/min total flow, 15% methanol, 40°C, 124 bar, 5 µL injection volume.

The small differences in peak height can be explained by differences in times when aliquots of the sample mixture were taken from the source container. Also, the two preparative columns had different lifetime numbers of injections and overall run time.

Conclusions

Successful scaling from analytical to preparative SFC can be achieved by considering several factors:

- Start with columns of identical particle size, length and stationary phase for geometric scaling.
- Use geometric scaling of flow rate and injection volume, accounting for differences in CO_2 flow control by converting the volumetric flow to mass flow before scaling.
- Match injection modes on analytical and preparative systems (mixed-stream and modifier-stream).
- Observe the difference (if any) in pressure drop between systems and adjust the back pressure of the preparative system (matching the average pressure) so the pressure drops (CO_2 density profiles) are similar.

Following this practical strategy provides greater success in scaling chromatography from analytical SFC to preparative SFC.

References

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