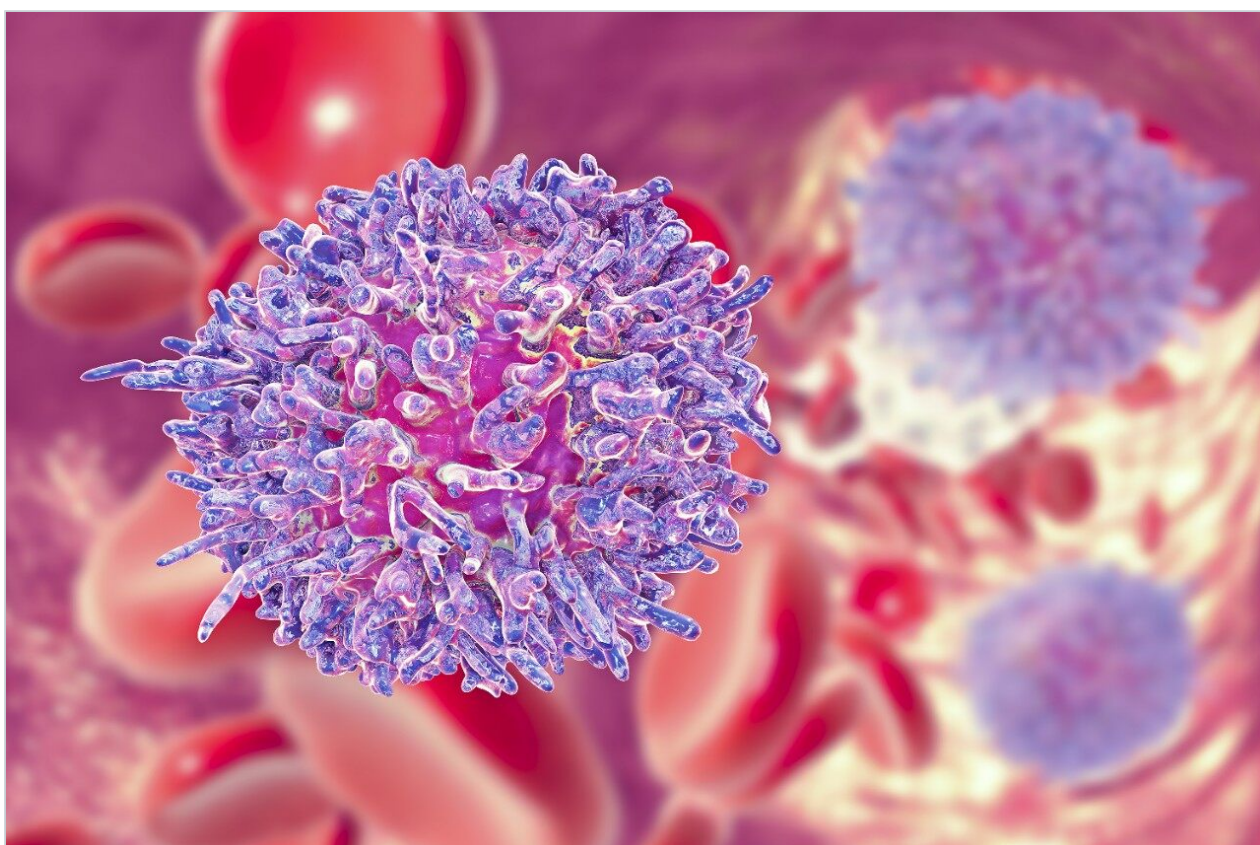




UPC² Strategy for Scaling SFC Methods: Applications for Preparative Chromatography

Christopher J. Hudalla, Abhijit Tarafder, Jo-Ann M. Jablonski, Roman Roshchin, Kenneth J. Fountain, Manisha A. Patel, Mark A. Hardink, Tony Yan, Frank W. Riley

Waters Corporation, Pfizer Inc.



Abstract

The first study focuses on the achiral analysis and purification of a reaction intermediate and product for the synthesis of the active pharmaceutical ingredient (API), Imatinib. The second study is for the purification of a proprietary pharmaceutical chiral compound. The ability to scale methods predictably and efficiently enables the rapid screening of methods on the faster analytical scale, with the direct transfer of the final method to preparative chromatography while maintaining chromatographic integrity between separations. The net result is a significant savings in time and mobile phase costs (raw materials and disposal of waste).

Benefits

- Scaling strategy based on density modulation can be used to efficiently transfer methods across different system/column configurations.
- Enables the fast method development of SFC applications on the analytical scale with subsequent transfer to preparative SFC while maintaining chromatographic integrity.
- Use of focused gradients facilitates purification of targeted analytes.

Introduction

In a previous application note, the use of density modulation was presented as an effective strategy for scaling SFC separations for preparative SFC purification.¹ This strategy is rooted in the principle that, for separations utilizing CO₂ as the principal mobile phase component, analyte retention factors are influenced largely by the mobile phase density and temperature. Modulation of the mobile phase density for the scaled separation, to approximate the average density of the original separation, yields a predictable separation that maintains the chromatographic integrity of the original separation. Modulation of the density can be accomplished by adjusting, in an appropriate way, any of the parameters which would impact the overall system pressure of the separation, and therefore the density of the mobile phase; e.g., the automated back-pressure regulator (ABPR) setting, the flow rate, or the column configuration. Of these options, adjusting the ABPR is more convenient and will be used as an example in this application note. In some cases, scaling by maintaining the same ratio of column length to particles size (L/d_p), which is a common practice for LC

scaling methodologies, is a useful first step for modulating density for scaling of SFC methods. In each case, density simulations can be used to understand the density profiles for the separations so that density modulation can be accomplished in a systematic manner. In this application note, we execute this strategy in real-life applications, highlighting two case studies, using the ACQUITY UltraPerformance Convergence Chromatography (UPC²) System for the fast development of methods on the analytical scale with the subsequent scale-up to preparative SFC purification. The first study focuses on the achiral analysis and purification of a reaction intermediate and product for the synthesis of the active pharmaceutical ingredient (API), Imatinib. The second study is for the purification of a proprietary pharmaceutical chiral compound. The ability to scale methods predictably and efficiently enables the rapid screening of methods on the faster analytical scale, with the direct transfer of the final method to preparative chromatography while maintaining chromatographic integrity between separations. The net result is a significant savings in time and mobile phase costs (raw materials and disposal of waste).

Experimental

Case Study 1 (Achiral purification)

Sample preparation

The final intermediate and product were collected from the reactions, shown in Figure 1, as part of the chemical synthesis of Imatinib, a tyrosine-kinase inhibitor used in the treatment of multiple cancers.^{2,3} Samples for preparative purification were prepared at a concentrations of 10 mg/mL with a diluent of 2-propanol/hexane/DMSO/acetonitrile (40:13:27:20). An additional dilution to 0.2 mg/mL with the same diluent was used for analytical injections.

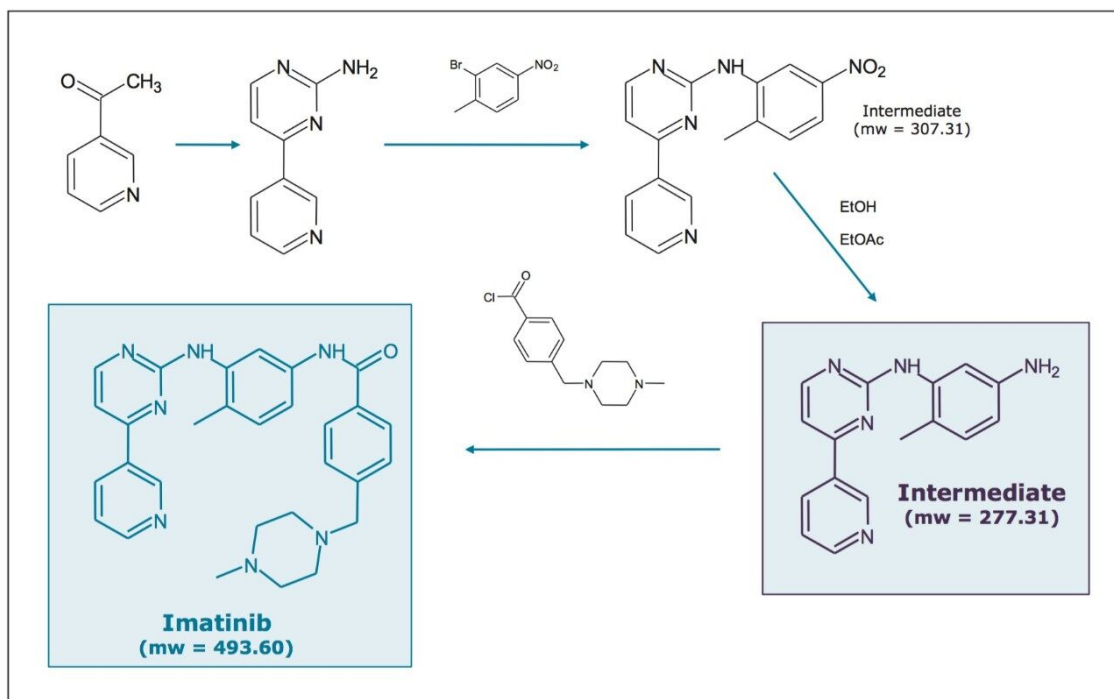


Figure 1. The synthetic pathway for the production of the tyrosine-kinase inhibitor, Imatinib. For this study, samples of the final intermediate and product were collected for method development and purification.

Method conditions

UPC² conditions

System:

ACQUITY UPC² with ACQUITY UPC² PDA
Detector

Columns:

ACQUITY UPC² BEH 2-Ethylpyridine, 1.7 μ m, 2.1 x 50 mm, p/n: 186006576
ACQUITY UPC² BEH, 1.7 μ m, 2.1 x 50 mm, p/n: 186006558
ACQUITY UPC² CSH Fluoro-Phenyl, 1.7 μ m, 2.1 x 50 mm, p/n: 186006567

Mobile phase A:

CO₂ (tank, medical grade)

UPC² conditions

Mobile phase B:	Methanol or methanol with 0.3% ammonium hydroxide (NH ₄ OH)
Flow rate:	1.5 mL/min
Gradient:	4–40% Modifier in 2 minutes (~26 column volumes)
Column temp.:	40 °C
ABPR:	1800 psi
UV detection:	235 nm (Compensated 380–480 nm) [40 pts/sec]
Injection volume:	2.0 µL
Strong needle wash:	2-Propanol (IPA)
Weak needle wash:	2-Propanol (IPA)
Seal wash:	2-Propanol (IPA)
Vials:	LCMS Certified Max Recovery Vials

Preparative SFC Conditions

System:	Prep 100q SFC System with PDA and MS detection
Columns:	Viridis BEH 2-Ethylpyridine OBD Prep, 5 µm, 19 x 150 mm, p/n: 186005764

Mobile phase A:	CO ₂ (house CO ₂ delivery system)
Mobile phase B:	Methanol with 0.3% ammonium hydroxide (NH ₄ OH) as additive
Flow rate:	80 mL/min
Gradient:	4–40% Modifier in 9.2 minutes (~26 column volumes)
Column temp.:	40 °C
ABPR:	1800 psi
UV detection:	235 nm
Injection volume:	120 µL
Wash solvent:	Methanol

Case Study 2 (Chiral purification)

Sample preparation

The sample is a proprietary chiral compound that degrades readily on exposure to water or alcohols. For that reason, the sample was prepared using acetonitrile as the diluent. The ability to address water-labile compounds is one of the additional benefits of SFC.

Method conditions

UPC² conditions

System:	ACQUITY UPC ² with ACQUITY UPC ² PDA Detector
Column:	Cellulose tris 3,5- dimethylphenylcarbamate

UPC² conditions

	stationary phase, 3 µm, 4.6 x 150 mm
Mobile phase A:	CO ₂
Mobile phase B:	Acetonitrile
Flow rate:	2 mL/min
Gradient:	20–50% Modifier in 3 minutes (~3.6 column volumes)
Column temp.:	40 °C
ABPR:	2175 psi
UV detection:	212 nm
Injection volume:	1.0 µL
Strong needle wash:	2-Propanol (IPA)
Weak needle wash:	2-Propanol (IPA)
Seal wash:	2-Propanol (IPA)

Preparative SFC conditions

System:	Prep 100q SFC system with PDA and MS detection
Column:	Cellulose tris 3,5- dimethylphenylcarbamate stationary phase, 5 µm, 21 x 250 mm

Mobile phase A:	CO ₂ (house CO ₂ delivery system)
Mobile phase B:	Acetonitrile
Flow rate:	70 mL/min
Gradient:	20–50% Modifier in 2.8 minutes (~3.6 column volumes)
Column temp.:	40 °C
ABPR:	1827 psi
UV detection:	212 nm
Injection volume:	300 µL

Results and Discussion

Density simulations

The simulation of the density profiles presented here was conducted based on the assumption that the variation of the pressure profile along the column is linear. The densities of the CO₂/methanol mixtures were calculated using the REFPROP software from NIST.⁴ REFPROP calculates the neat CO₂ density following the Span and Wagner equation of state (EOS) and calculates the CO₂/MeOH mixture density using the Kunz and Wagner model.^{5,6} For gradient methods, the density not only varies along the column, but also changes with time. The increasing modifier concentration during the gradient results in increases in mobile phase viscosity and therefore pressure, which in turn impacts the mobile phase density profile. For gradient separations, the density modulation strategy should consider the changing density profile as the modifier transitions from lower to higher concentrations.

Figure 2 shows an example of this approach for a mixture of standards separated at the analytical scale and at the preparative scale with modulated density. The density profiles were calculated for the inlet and outlet

of the columns, for both the analytical and preparative configurations. For the preparative configuration, the density was modulated to yield approximately the same average density profile as that calculated for the analytical configuration. The resulting chromatography (Figure 2, left) demonstrates the ability to achieve similar separations with this approach, yielding similar resolution and selectivity for the individual compounds. Differences observed between the two examples can be attributed to differences in system dwell volume or injection mode (modifier stream vs. mixed stream injection), neither of which were considered for these experiments.

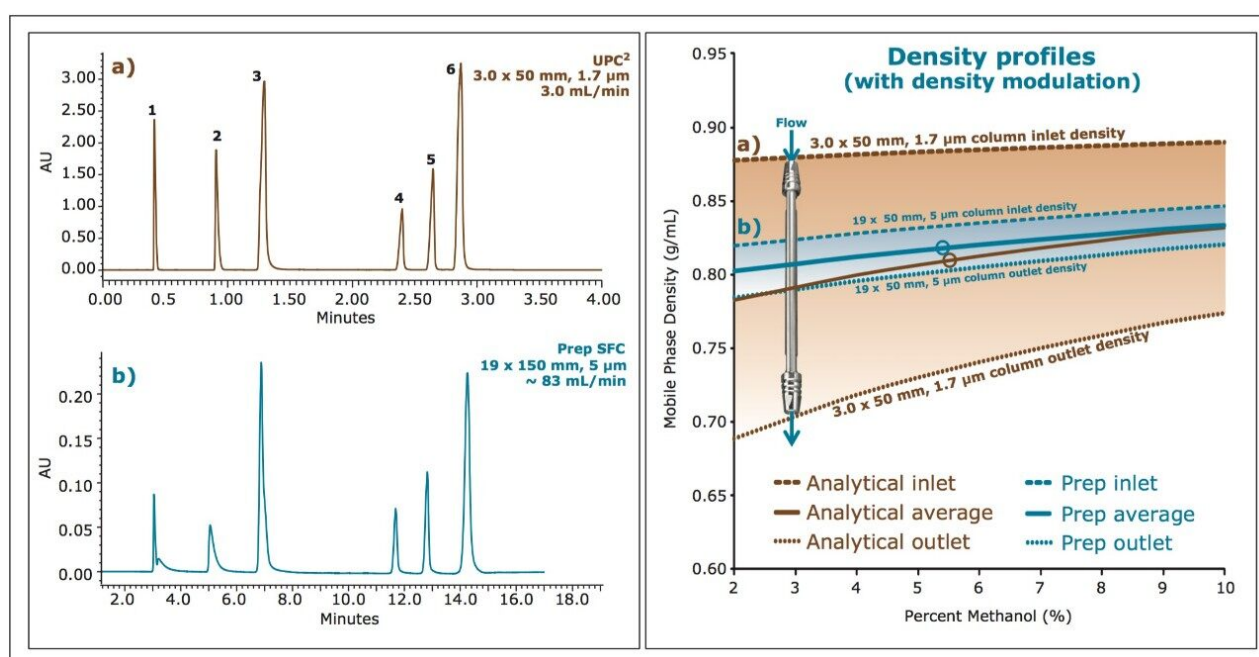


Figure 2. Gradient separations of a standard mix on the ACQUITY UPC² BEH 2-EP, 1.7 µm Column (a), and on Viridis BEH 2-EP, 5 µm OBD Prep Column (b) configurations. Density modulation was used for the direct scale up from analytical to preparative conditions, using a gradient from 2–10% methanol at flow rates of 3.0 mL/min and 83 mL/min. The gradient times (3 minutes and 13.5 minutes) were scaled to yield the same number of column volumes for each gradient separation. The ABPR settings were 1500 and 2103 psi, respectively. Analytes are: caffeine (1), carbamazepine (2), uracil (3), hydrocortisone (4), prednisolone (5), and sulfanilamide (6), each 0.2 mg/mL in methanol.

Case study 1: Achiral purification (imatinib)

For the initial method development, samples of the intermediate and product were screened on three different ACQUITY UPC² Column chemistries using methanol as a modifier, with a generic screening gradient of 4–40% modifier. Not surprisingly, due to the amine functionalities on both compounds, severe

tailing was observed for the intermediate, while no elution of the product was observed on two of the UPC² Column chemistries. Significant improvement in peak shape was observed when compounds were re-screened using an additional basic additive of 0.3% ammonium hydroxide in the methanol modifier. The screening results are shown in Figure 3.

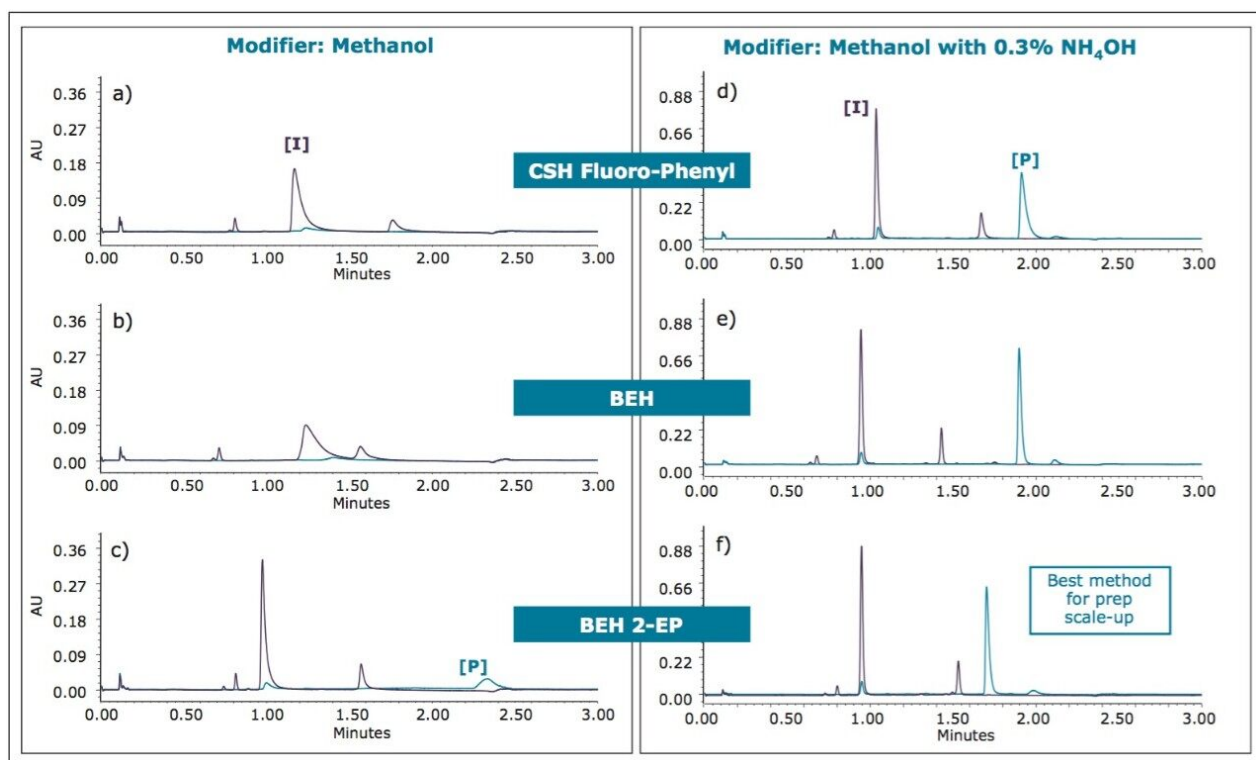


Figure 3. Initial column screening experiments using methanol (left) and methanol with 0.3% NH_4OH (right) as modifier. The intermediate [I] and final product [P] were screened using a 2.1 x 50 mm column configuration with a 1.7 μm particle size for three ACQUITY UPC² chemistries; CSH Fluoro-Phenyl (a and d), BEH (b and e), and BEH 2-Ethylpyridine (c and f). Screening gradients utilized a 2 minute gradient from 4–40% modifier at a flow rate of 1.5 mL/min. The temperature was 40°C with an automated backpressure regulator (ABPR) setting of 1800 psi

Based on these initial results, the BEH 2-Ethylpyridine chemistry was chosen for additional evaluations in scaling up for preparative purification, using a modifier of methanol with 0.3% ammonium hydroxide as additive.

Scaling to prep (geometric scaling)

As previously discussed, to scale SFC separations in a predictable manner, it is critical to understand the

density profiles for the individual separations. For the selected screening gradient shown in Figure 3(f), the density was simulated for both the column inlet and outlet at multiple points during the gradient, as the modifier transitioned from 4% to 40%. From these values, the average mobile phase density that would be experienced by an analyte during the separation was calculated to be 0.857 g/mL. For scaling to the preparative scale, the strategy of maintaining the same ratio of column length to particle size (L/d_p) was employed. Based on the 1.7 μm , 50 mm analytical column dimension, a preparative column was chosen in a 5 μm , 19 x 150 mm configuration. The method was scaled geometrically for separation on a Prep 100q SFC System equipped with both UV and MS detection. A preparative flow rate of 80 mL/min was chosen based on the optimum performance of the system at that flow rate. The gradient time (t_g) was scaled from the analytical conditions such that the total number of column volumes (CV) for each gradient were the same (~26 CV), resulting in a preparative gradient of 9.2 minutes. The gradient modifier concentrations were the same as for the analytical separation (4-40%). Chromatograms for both the intermediate [I] and product [P] were collected, at concentrations of 10 mg/mL, with the preparative method. As was performed for the analytical separation, the density profiles were calculated for the preparative column inlet and outlet at multiple points during the separation, taking into account the changing density of the mobile phase with changing concentration of the modifier. From those simulations, the average density for the preparative separation was calculated to be 0.847 mg/mL. The density simulations for the analytical and preparative scale, along with the resulting chromatography are shown below in Figure 4.

Based on the geometric scaling used for the method, with the column configurations chosen to maintain L/d_p , the resulting average densities calculated for the two separations were very similar, resulting in similar chromatography without the need for additional density modulation (see Figure 4a and 4b). While this is true for this particular example, maintaining L/d_p may not always yield similar average densities, due to differences in flow rates. Only through the density simulations can this be confirmed.

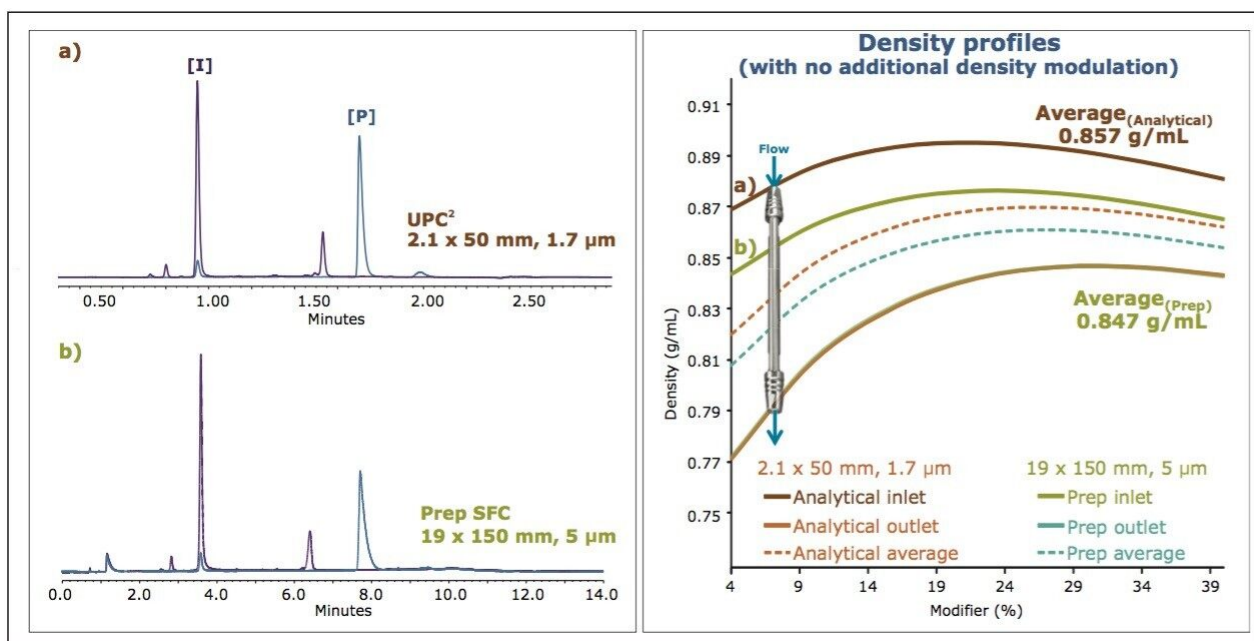


Figure 4. Evaluation of the intermediate [I] and product [P] from the synthesis of Imatanib, under gradient conditions on an ACQUITY UPC² BEH 2-EP, 1.7 μ m, 2.1 x 50 mm Column (a), and on an ACQUITY UPC² BEH 2-EP, 5 μ m, 19 x 150 mm Column (b). The ratio of column length to particle size (L/d_p) was maintained for the direct scale up from analytical to preparative conditions, using a gradient from 4–40% methanol (with 0.3% NH_4OH) at flow rates of 1.5 mL/min and 80 mL/min. The gradient times (2 minutes and 9.2 minutes) were scaled to yield the same number of column volumes for each gradient separation (~ 26 CV). At a temperature of 40°C and an ABPR setting of 1800 psi, the average mobile phase densities were 0.857 and 0.847 g/mL, respectively.

Focused gradients

The ideal separation scenario for purification is the fast screening of a method at the analytical scale to establish purification conditions, with a subsequent direct scale-up to a preparative purification using a focused gradient to target a specific analyte of interest. Focused or segmented gradients are commonly used in LC separations. However, for SFC separations, where mobile phase density plays a critical role, their use is not as well understood. To understand this better, the analytical screening separation (shown in Figure 3f) was used to develop an analytical scale focused gradient separation. This intermediate step of developing an analytical focused gradient method was done solely to test the concept of a focused gradient. In our ideal scenario, this intermediate step would not be required. While the resulting methods would be the same, it is important to note that the conditions to be used for the focused gradient at the preparative scale are derived from the analytical screening gradient (Figure 3f) and NOT from the analytical focused gradients of this

intermediate step. This would enable direct transfer from the analytical screening gradient to the preparative purification using a focused gradient.

Based on the gradient slope for the analytical separation and the retention time for each peak of interest, the modifier concentration at which each peak eluted was calculated; 14% modifier for the intermediate and 29% modifier for the product. Based on those calculations, 2-minute focused gradients were developed centered on those percentages, with starting and ending modifier concentrations that were 5% lower and higher, respectively, than the target percentages. For the synthesis intermediate, a gradient from 9-19% was used, while for the product, a gradient from 24-34% was used. All other analytical conditions were kept constant. The resulting chromatography for the intermediate [I] and product [P] using their respective focused gradients are shown in Figure 5, along with the results for the original screening gradient.

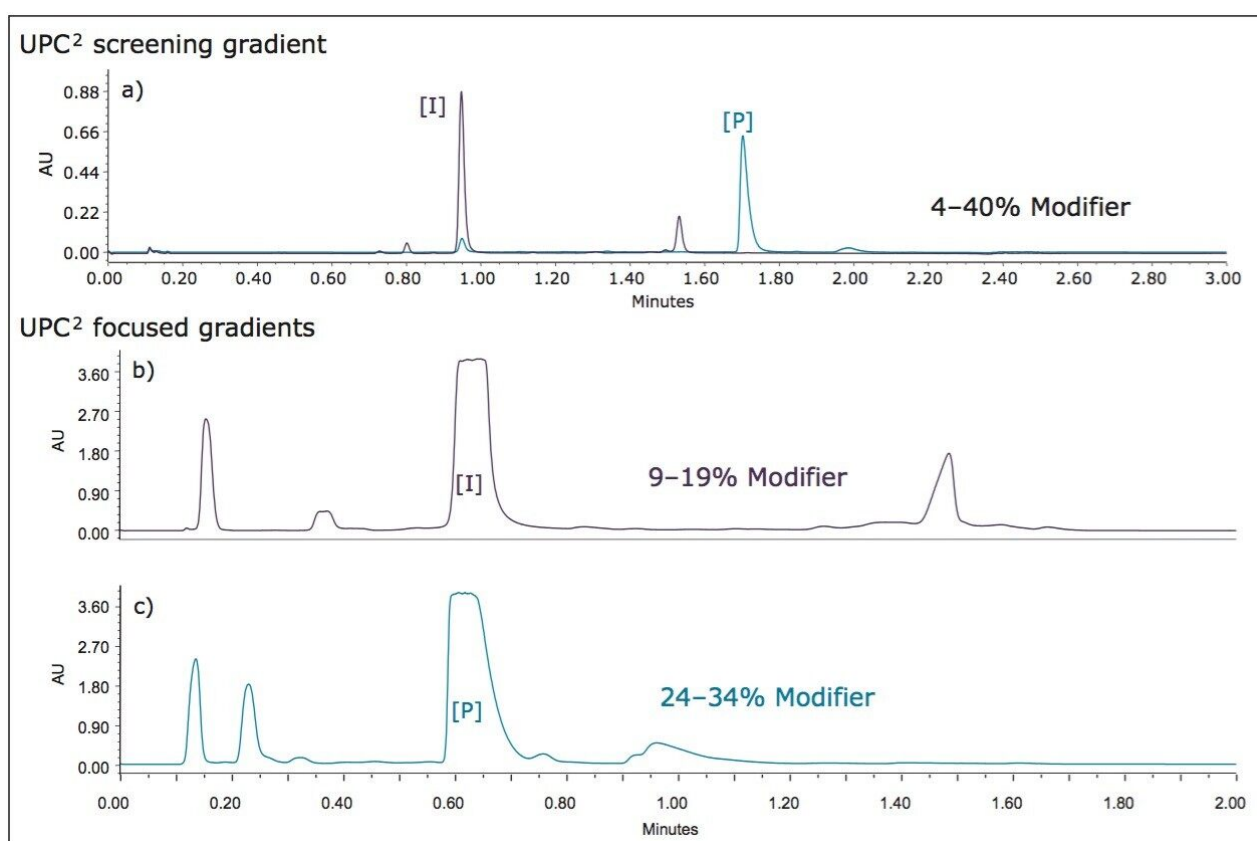


Figure 5. Evaluation of the intermediate [I] and product [P] by UPC² on an ACQUITY UPC² BEH 2-EP, 1.7 μ m, 2.1 x 50 mm Column. The separations used methanol with 0.3% NH₄OH as modifier with 2-minute gradients of 4–40%, used for the initial screening conditions (a), a 9–19% modifier gradient focused for the intermediate [I] peak (b), and a 24–34% modifier gradient focused for the product [P] peak (c).

These results seem to support the use of focused gradients for SFC, and are extended next to preparative separations. It is important to demonstrate the ability to move from the analytical screening directly to the focused preparative separation to maximize utility and time savings. Figure 6 shows the preparative separation of the intermediate (top) and product (bottom) with their respective focused gradients. Mass directed fraction collection was used to collect the fractions of interest for multiple injections of each sample mixture.

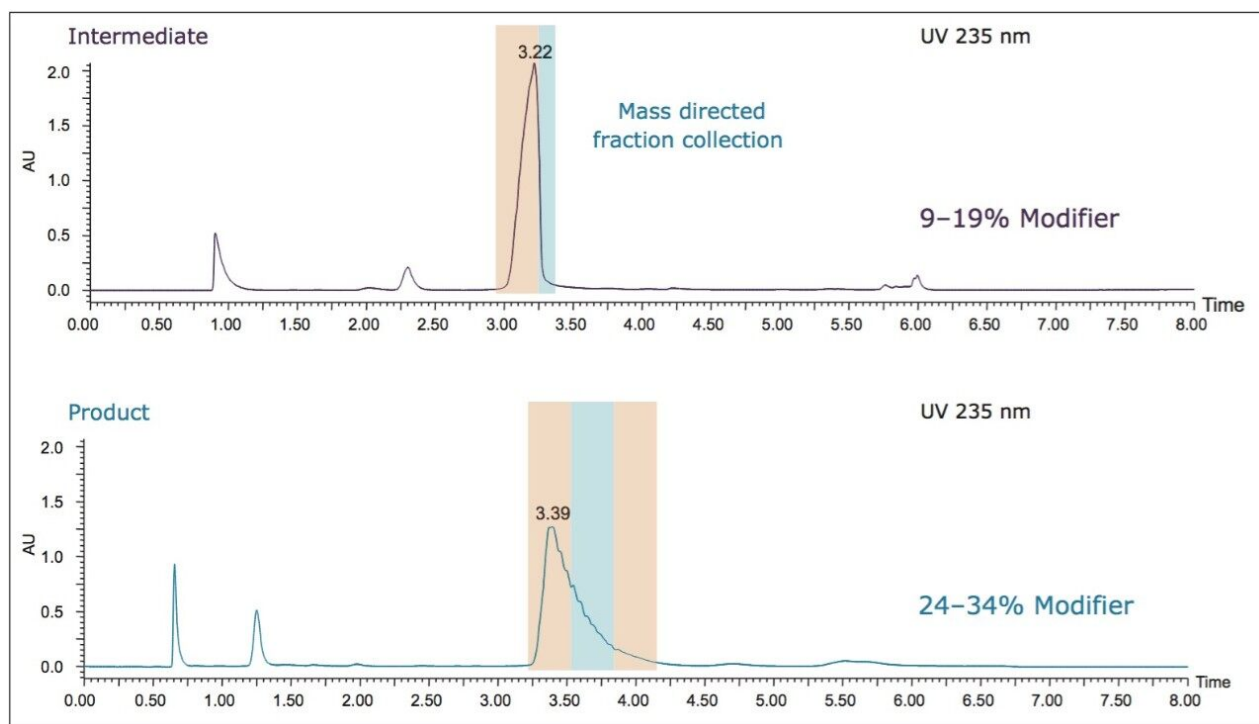


Figure 6. Preparative purification of the intermediate (top) and product (bottom) on a Viridis BEH 2-EP OBD Prep, 5 μ m, 19 x 150 mm Column. The separations used methanol with 0.3% NH_4OH as modifier with 5.1-minute gradients. A focused gradient of 9–19% modifier was used for the intermediate peak and a focused gradient of 24–34% modifier was used for the product peak. Mass directed fraction collection was used for the purification.

The collected fractions for each analyte were pooled prior to analysis using the original screening gradient on the UPC² System to confirm purity. For each analyte, approximately 120 mg was collected with purities in excess of 99% (shown in Figure 7).

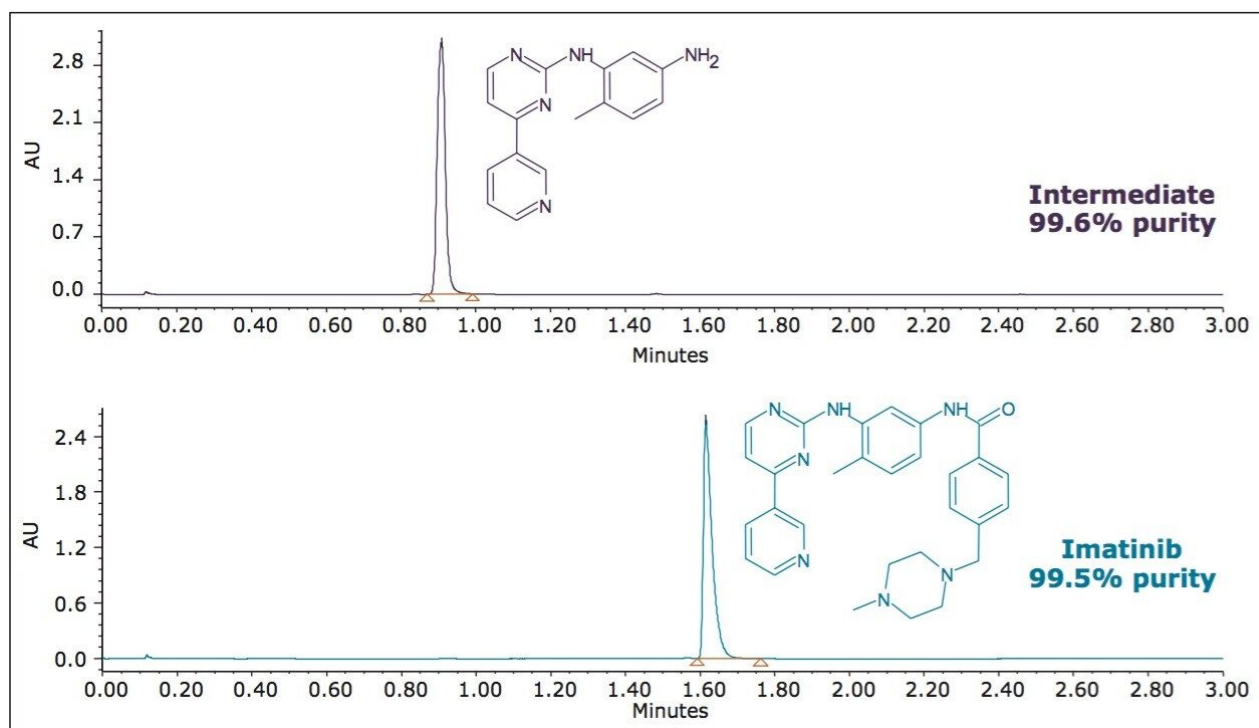


Figure 7. Evaluation of the pooled fractions from the preparative purification of the intermediate (top) and final product (bottom) from the synthesis of Imatinib, under analytical UPC² conditions using the initial screening parameters, with a modifier gradient of 4–40%. The purity of the resulting fractions were 99.6% and 99.5%, respectively.

This example highlights the total workflow potential for purification, with the ability to do fast method development at the analytical scale with subsequent scale-up directly to a preparative purification using a targeted focused gradient. The initial method from UPC² can then be used for final purity confirmation of the purified product. In this example, the combination of column dimensions (matched for L/d_p) and flow rates yielded similar density profiles for the analytical and preparative separations, enabling successful method transfer without the need for additional density modulation.

Case study 2: Chiral purification

Because of the unstable nature of the chiral compound analyzed in this case study, typical reversed-phase chromatographic methods could not be used. For the same reason, normal method development tools in UPC,² like modifier or additive variation, were not available for exploration. The method was limited to acetonitrile as the modifier, in which the compound showed the greatest stability. Method development was therefore limited to evaluation of the effects of column chemistry, temperature, pressure, and flow rate. The

column selection was chosen based on screening of several commercially available chiral columns. Once an acceptable method was achieved under analytical conditions using the UPC² system, parameters were scaled up for purification while maintaining L/d_p . Unlike the scale-up described in the case study for Imatinib, this particular combination of flow rates and column dimensions did not yield similar average density profiles for the analytical and preparative separations. The density simulations were employed to further guide the scale-up to preparative conditions, modulating the density of the preparative scale separation to approximate the same average density calculated for the analytical separation. The resulting chromatography and density calculations are shown below in Figure 8.

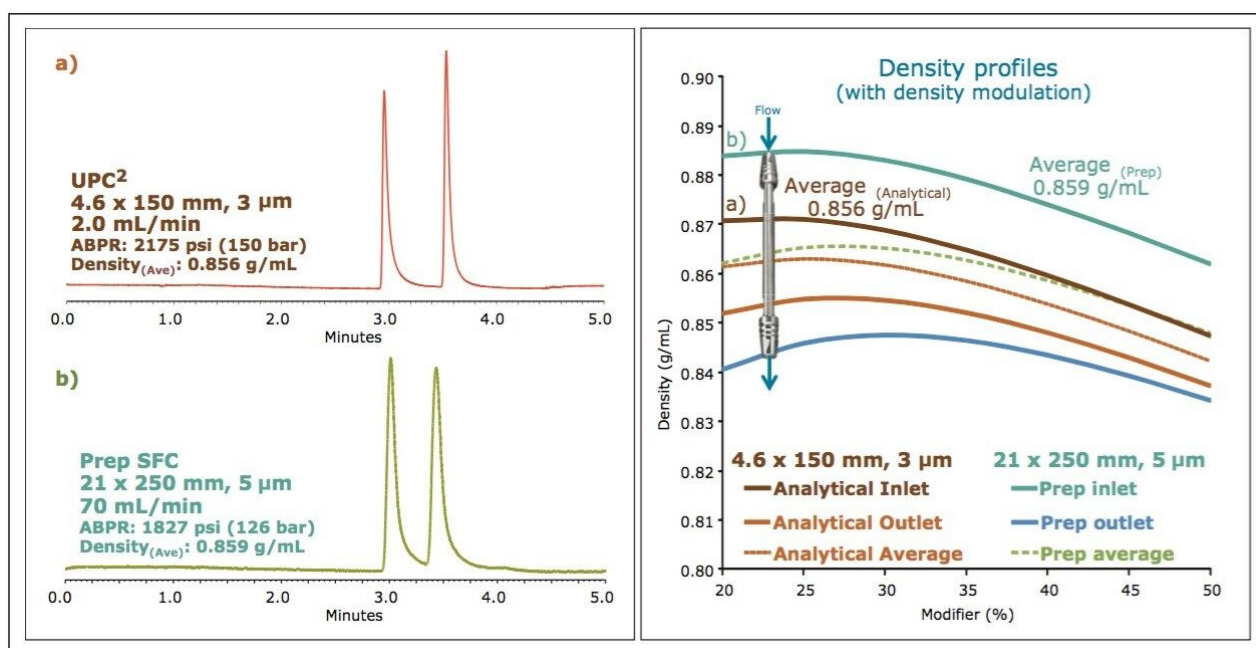


Figure 8. Gradient separations of a chiral compound on a 3 µm, 4.6 x 150 mm, chiral column (a), and on the equivalent 5 µm, 21 x 250 mm, chiral prep column (b). Density modulation was used for the direct scale up from analytical to preparative conditions, using a gradient from 20-50% acetonitrile at flow rates of 2.0 mL/min and 70 mL/min. The gradient times (3 minutes and 2.8 minutes) were scaled to yield the same number of column volumes for each gradient separation (~3.6 CV). The ABPR settings were 2175 and 1827 psi, and the average mobile phase densities were 0.856 and 0.859 g/mL, respectively. Injections volumes were 1 µL for the analytical and 300 µL for the preparative separations.

It is important to note that the density calculations for this exercise, using the NIST REFPROP database, are based on the physical properties of methanol, rather than acetonitrile. This is because properties of CO₂/acetonitrile binary mixtures are not available from NIST or from any other authentic sources. However, the

densities of the two solvents are comparable (methanol = 791.8 g/mL and acetonitrile = 786.0 g/mL at normal temperature and pressure). The intent of the density modulation approach is to minimize the DIFFERENCES between the average densities of the two systems, and does NOT depend on the ABSOLUTE density values of any of the systems. This means that, if we can assume a linear variation of the density values of the CO₂ /acetonitrile mixture from the CO₂/methanol mixture, calculation of the difference between the average density values, using CO₂/methanol properties, should not be very different from calculations based on CO₂ /acetonitrile properties. Even with this caveat, this example again highlights the ability to achieve predictable scaled separations, based on SFC methods developed at the analytical scale using UPC.²

Conclusion

The application of a systematic approach using density modulation for scaling facilitates the efficient, predictable transfer of SFC methods from the analytical to the preparative purification scale. In some cases, a cognizant choice of column dimensions and flow rates may be a logical first step before applying the density modulation strategy. In other cases, as in the Imatinib case study presented here, where column dimensions are based on column availability and maintaining L/d_p, and flow rates are based on optimum instrumental parameters, density profiles may be inherently matched, requiring no additional density modulation. In any case, density simulations are a valuable tool in understanding the nature of the separations, and can be used to guide additional density modulation by adjustments to the ABPR, to yield similar average density profiles between the analytical and preparative scale separations. In addition, as are frequently used for LC applications, focused gradients can be used as an effective tool for preparative SFC purification. The ability to transition directly from an analytical scale screening separation on UPC² to a preparative scale purification with a focused gradient and mobile phase density modulation represents a significant savings in time and costs associated with method development for preparative SFC purifications.

References

1. Hudalla C, Tarafder A, Jablonski J, and Fountain K. Waters Application Note 720004818en, 2013.
2. Novartis Pharma AG. Gleevec (imatinibmesylate) tablets prescribing information, Anon. drugs of choice for cancer, Treat Guidel Med Lett., East Hanover, NJ; 2006 Sep,1:41–52, 2003.

3. Goldman JM, Melo JV, *N. Engl. J. Med.*, 349 (15): 1451–64, 2003.
4. Lemmon EW, Huber M, McLinden MO, Friend DG, National Institute of Standards and Technology, Standard Reference Data Program, Gaithersburg 9.1.
5. Span R, Wagner W, *J. Phys. Chem. Ref. Data*, 25(6):1509–1596, 1996.
6. Kunz O, Wagner W, *J. Chem. Eng. Data*, 57(11):3032–3091, 2012.

Featured Products

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

Prep 100q SFC System <<https://www.waters.com/10145748>>

2998 Photodiode Array (PDA) Detector for Purification and Modular LC Systems <
<https://www.waters.com/134721663>>

720005064, May 2014