Addressing the Need for Faster Screening and Fraction Collection for Chiral and Achiral SFC

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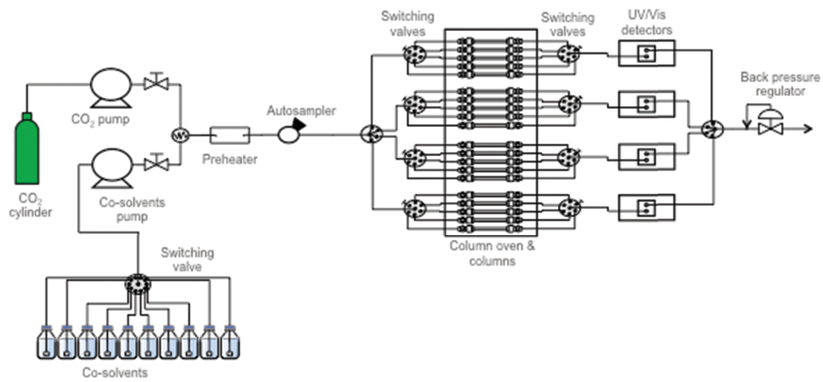
Introduction

Supercritical Fluid Chromatography (SFC) has become a well established technique in modern laboratories and is the preferred technique [1, 2] for both analytical and preparative enantiomeric separations for many reasons, including its speed and resolution advantages over normal phase HPLC. In addition to chiral applications, there has been steady growth over the past decade in the use of SFC for achiral analysis in numerous industries [2]. The continued expansion of applications employing SFC has led to expansive achiral column development and the optimisation of hardware platforms to enable more rigorous screening of solvent and column combinations for analytical SFC.

In reversed-phase HPLC the C18 column is so widely used it is often identified as the universal column [3]. In SFC there is a lack of universal or 'go to' column and at least 70 columns to choose [4]. Therefore, chiral and achiral SFC analysis requires column screening to narrow down the many available columns to determine the best column to achieve the desired resolution of the peaks of interest. The growing number of possible solvent [2]:column [4] combinations available today, combined with the increase in the number of samples requiring analysis, means higher throughput is required to keep pace with the demand. When column screening is performed to determine the selection of the most efficienct chromatographic condition to scale up, time is of the essence and the answer is desired as rapidly as possible.

The rapid analytical SFC screening provides the solvent and column combination for direct scale up to purification of the target analytes. Preparative SFC enables rapid purifications, using minimal solvents, along with easy fraction recovery due to the physical properties of the CO2 enabling that portion of the mobile phase to convert to the gaseous state upon exiting the column and encountering pressure less than its critical pressure. In enantiomeric purifications, only two fractions are needed to collect each of the enantiomers. The use of SFC for achiral purification requires an increase in the number of fraction vessels

Figure 1. Parallel SFC System Layout



available for use which can be accomplished through utilisation of an open-bed fraction collector. The use of such an open-bed fraction collector allows for greater capacity which ensures selection of fractions to satisfy the user's desired purity or recover criteria.

Method and Materials

Flavanone, ketoprofen, sulphamethazine, methanol, ethanol, acetonitrile and tetrahydrofuran were purchased from Sigma-Aldrich (St. Louis, MO, USA). The test compounds flavanone, ketoprofen and sulphamethazine were identified by a pharmaceutical customer as the ones they use for internal chiral and achiral SFC system testing. The CO2 cylinder was purchased from Airgas (PA, USA). Columns were acquired from multiple suppliers: Princeton Chromatography (Princeton, NJ, USA), Phenomenex (Torrance, CA, USA) and Chiral Technologies (PA, USA). The Jasco Analytical SFC (CO2 pump, co-solvent pump, automsampler, column oven, PDA detector and back pressure regulator) and Parallel SFC system (CO2 pump, co-solvent pump, solvent selector valve, autosampler, column oven, column selection valves, 4 UV detectors and back pressure regulator) were used for the analytical screening and development. The parallel SFC injects the sample and splits the sample and flow to 4 columns simultaneously as shown in Figure 1 with the screening capacity of 20 columns and 10 solvents. The Jasco Open-Bed Preparative SFC (CO2 pump, co-solvent pump, automsampler, column oven, UV detector, back pressure regulator and open-bed fraction collector) was used for the preparative purifications.

Figure 2. Column-solvent screening results

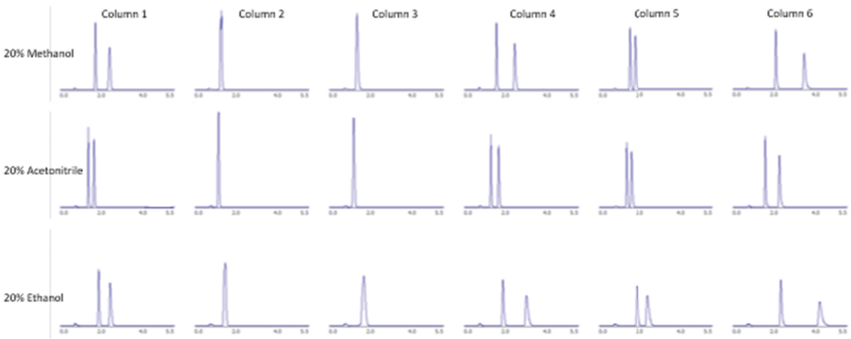


Figure 3. Parallel 16 column screening results

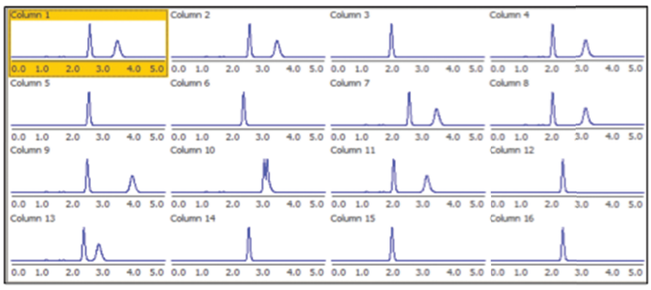
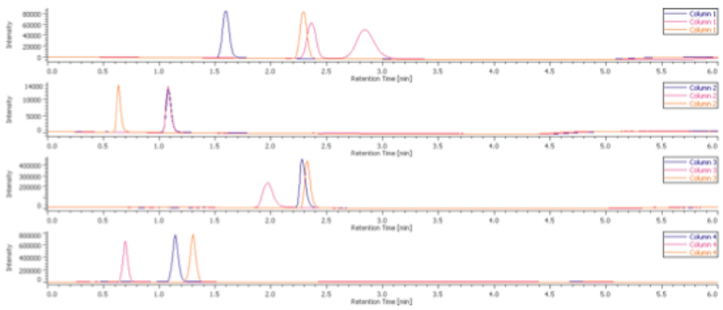


Figure 4. Customer sample 1



Analytical Conditions

Sample:	Flavanone 1 mg/mL
Injection:	10 µL
Flow Rate:	2 mL/min on Analytical SFC, 8 mL/min on Parallel SFC
Isocratic:	80% CO2, 20% Co-solvent (v/v)
Wavelength:	254 nm
Back Pressure:	120 bar
Columns:	6 chiral columns on Analytical SFC (Phenomenex and Chiral Technologies)
	16 chiral columns on Parallel SFC (Phenomenex and Chiral Technologies)

AD-H 4.6 x 250mm 5um, OD-H 4.6 x 250mm 5um, AS-3 4.6 x 150mm 3um, OJ-3 4.6 x 150mm 3um, IA-3 4.6 x 150mm 3um, IB-3 4.6 x 150mm 3um, IC-3 4.6 x 150mm 3um, ID-3 4.6 x 150mm 3um, IE-3 4.6 x 150mm 3um, IF-3 4.6 x 150mm 3um, Lux Cellulose-1 4.6 x 250mm 5um, Lux Cellulose-2 4.6 x 250mm 5um, Lux Cellulose-3 4.6 x 250mm 5um, Lux Cellulose-4 4.6 x 250mm 5um, Lux Amylose-1 4.6 x 250mm 5um, Lux Amylose-2 4.6 x 250mm 5um.

Preparative Conditions

Sample:	Ketoprofen 10 mg/mL, Sulphamethazine 20 mg/mL
Injection:	Total concentration 30 mg/mL (200 µL injections)
Column:	Princeton Silica 30 mm x 150 mm, 100 Å, 5 µm
Flow rate:	100 mL/min
Isocratic:	65% CO2, 35% methanol, make-up flow 100% methanol 1 mL/min.
Gradient:	5-50% in 5 minutes (5-50% in 5 minutes, hold 50% for 1 minute, 50-5% in 30 seconds, hold 5% for 2 minutes), make-up flow rate 5 mL/min.

Back Pressure: 120 bar

Fraction collection triggering based on threshold into 200 mL fraction vials

Results

The traditional SFC single column, single co-solvent screening results are shown in Figure 2. Six columns and 3 solvents are screened, requiring 18 injections at 6 minutes each (108 minutes total). The enantiomers were successfully resolved on 4 of the 6 columns. Since the 3 solvents screened showed similar acceptable separation performance, methanol was used on the parallel SFC with 16 columns. Figure 3 shows the results of the 16-column parallel screen with methanol as the co-solvent. The 4-channel parallel screen required 4 injections at 6 minutes each (24 minutes total). The equivalent 16-column, 6 minute method screen would require 96 minutes illustrating the significant reduction in screening time.

To serve as an example, a proprietary sample submitted by a customer was analysed on the parallel SFC to illustrate the application in a small molecule, drug discovery environment. The samples required the addition of tetrahydrofuran (THF) to dissolve completely and thus, only 12 immobilised columns were screened to avoid damaging the more fragile, coated polysaccharide columns. The gradient employed ranged from 5% methanol to 50% methanol over 3 minutes with re-equilibration following. Figures 4, 5 and 6 show the overlaid results for the 12 columns. Each sample required just 18 minutes for 12 columns, equating to an average of 1.5 minutes per column compared to 6 minutes per column on the standard screening procedure.

Figure 5. Customer sample 2

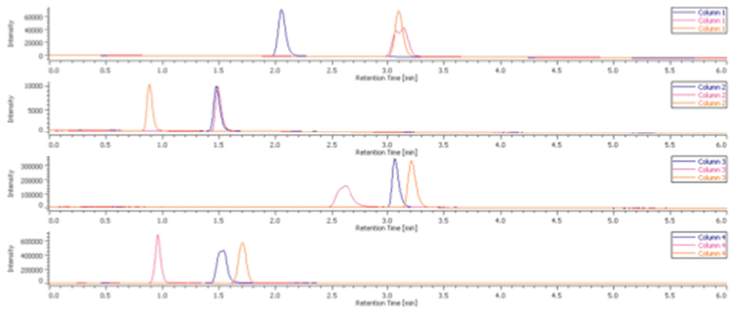


Figure 6. Customer sample 3.

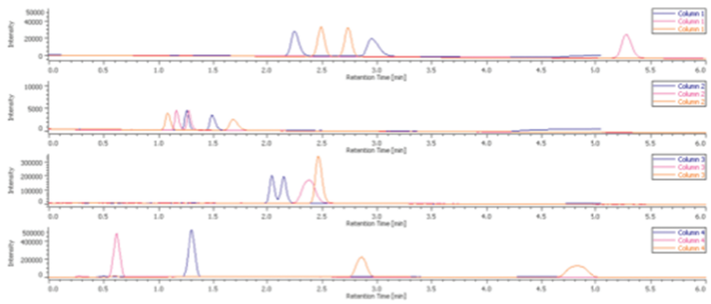


Figure 7.

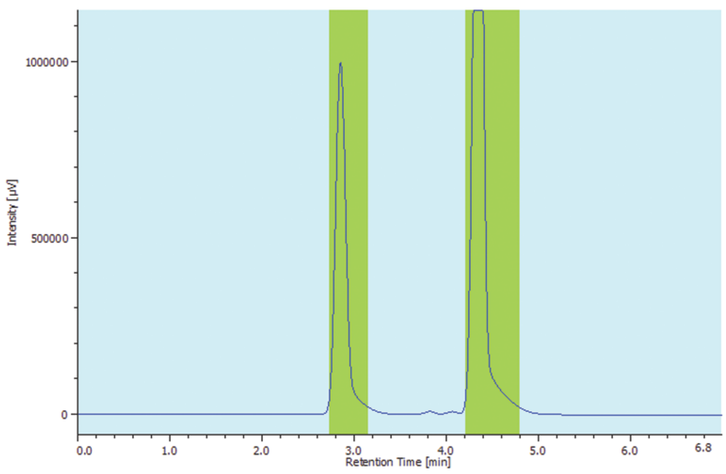


Table 1. Open-Bed preparative SFC recoveries

Open-Bed Recoveries			
Isocratic 35%	Amount injected on Column	Sample Recovered	Recovery
Ketoprofen	19.6 mg	18.12 mg	92.4 %
Sulfamethazine	40.48mg	39.24 mg	96.2 %
Gradient 5-50%	Amount injected on Column	Sample Recovered	Recovery
Ketoprofen	20.32 mg	19.62 mg	96.5 %
Sulfamethazine	42.09 mg	41.6 mg	99.0 %

The columns used varied in length and particle size, but as all parallel screens show, successful separations were achieved on multiple column. After this rapid screening process produces the optimised separation conditions, the next step is scaling the chromatographic parameters up to the larger preparative column for analyte purification.

An example chromatogram of the achiral purification of ketoprofen and sulphamethazine is shown in Figure 7. The green highlights show the collection windows which were triggered based on UV signal threshold. The recovery results of this purification are shown in Table 1 using both an isocratic and gradient method. The recovery is greater than 95% for both compounds under both separation conditions. In addition the purity was greater than 99.5% for both compounds.

This open-bed collection allows for immediate fraction gathering for dry down of the co-solvent and processing and also provides more than 3 times the number of fractions. The additional fractions are important for achiral purifications as there can be low level impurities of interest for compound development that need to be collected requiring more than 8 fractions. For preparative SFC to be applicable to library purifications, this increased fraction capacity (with autosampler option) is critical in providing the number of fractions required for the large number of samples.

Conclusions

Parallel screening of 4 columns offers a 4-fold reduction in screening time in SFC compared to traditional single column screening. This time savings can provide faster turn-around time to move to scale up on preparative or allows more columns and solvents to be screened when the initial do not provide the resolution. With more achiral samples being analysed on SFC, increased fraction capacity of an open-bed fraction collector provides collection of the principle compounds as well as potential impurities. The ability to automate the purification of multiple chiral and achiral samples with immediate sample dry down is critical to increasing sample throughput. As the number of potential drug compounds each year increases, faster sample processing is required to keep up with the modern pharmaceutical SFC lab.

1. K. L.Williams and L. C.Sander. Enantiomer separations on chiral stationary phases in supercritical fluid chromatography. Journal of Chromatography A. 1997. 785 (3) 149-158.
2. How Good is SFC for Polar Analytes?. Chromatography Today. 2013. June.
3. A Universal Reversed-Phase HPLC Method for Pharmaceutical Analysis. LCGC. 2016. 34 (6). 408-419.
4. Classification of Columns for SFC Use. LCGC. 2014. 10 (4).