Semipreparative Separation and Fractionation of Sulfonamides via Supercritical Fluid Chromatography

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Abstract

Three protocols with two different apparatus setups are evaluated to obtain a pure fraction from a supercritical fluid chromatographic separation of three sulfonamides. Results show that the addition of a rinse pump to flush the lines after the detector can decrease cross-contamination of the fractions. However, the location of the rinse pump in the system is a major factor. It is demonstrated that it is better to place the rinse pump immediately after the detector in order to obtain pure fractions. Evaluation of three collection protocols shows that collection at a constant interval can result in the isolation of a pure fraction. Minifractions collected every 0.2 min through the analyte peak show purities of 97, 92, and 73% for sulfamethazine (SMZ), sulfadimethoxine (SDM), and sulfaquinoxaline (SQX), respectively. However, collection of fractions at 0.5-min intervals throughout the entire chromatogram without regard to the onset of the peak shows purities of 92, 82, and 86% for SMZ, SDM, and SQX, respectively.

Introduction

The need for preparative- and semipreparative-scale chromatographic techniques for a variety of compound classes, especially pharmaceuticals, is increasing rapidly. It is often desirable, for example, to obtain a pure substance from a complex mixture for molecular identification or bioactivity testing. Several semipreparative scale techniques such as high-performance liquid chromatography (HPLC) (1), gel permeation chromatography (GPC) (2), and thin-layer chromatography (TLC) (3) have regularly been used to obtain a pure fraction. Preparative HPLC has matured and is widely used as a routine purification procedure in industry- and laboratory-scale applications.

In the past few years, several articles have described the advantages of using preparative supercritical fluid chromatography (SFC) (4–10) in the separation of fuels, crude oil, pharmaceuticals, and natural products. Fractionation is one of the unique advantages of SFC, especially with packed columns, because CO_2 is gaseous under normal ambient conditions, thus permitting the easy separation of analytes from the mobile phase by pressure reduction and subsequent recycling of CO_2. Also, the use of CO_2 can drastically decrease the volume of toxic and flammable organic solvent customarily used in preparative HPLC separations. Finally, preparative SFC is faster than preparative HPLC, and the former has a wider application range than preparative gas chromatography. If smaller amounts of material (less than 100 mg) are required for a special application such as structure analysis, commercial instrumentation is available with appropriate fraction collection and eluent removal techniques (11). Micropreparative SFC has been proposed as a rapid isolation method in screening processes to yield small amounts (0.5–50 mg) of unknown compounds for spectroscopic identification (12). Hanson (13) has used, for example, a commercially available Hewlett-Packard 1205A SFC system for the isolation of α-estradiol and β-estradiol from an estradiol mother liquor. A 4.6-mm-i.d. nitrophenyl column was loaded with 2.5 mg of liquor. An average of 0.4 mg of α-estradiol and 0.8 mg of β-estradiol were isolated in each run. Analytical results showed that the isolated steroids were more than 95% pure. The collection protocol was not discussed in the paper. Coleman and Verillon (14) have described the laboratory-scale preparative SFC of the two enantiomers of the β-blocker propranolol. Up to 50 mg of propranolol racemate was loaded onto a 4.6-mm-i.d. chiral column. Good recoveries were obtained with the addition of an outlet solvent (e.g., isopropanol) to keep the solutes dissolved after decompression of the mobile phase. A low flow rate of 0.25 mL/min of isopropanol, introduced downstream from the detector, was sufficient to obtain 80% recovery for each enantiomer with a purity of 99% for the first peak and 95% for the second one.

The object of our research was to evaluate a commercially available SFC system (i.e., Gilson SF-3) for micropreparative separation and fractionation of three sulfonamide drugs using methanol-modified CO_2. Different instrumental setups and peak collection protocols were employed in an effort to optimize collection efficiency of individual fractions.

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Experimental

The SFC separation of sulfonamides was conducted using a commercial SFC system (model SF-3, Gilson, Inc., Middleton, WI) that employs two back-pressure regulators. Two different operating designs were studied, as shown in Figure 1. The two designs differed in the location of a T-valve where a pump was added to the system for rinsing the lines. In the first setup, the T-valve was placed between the first and second back-pressure regulators. In the second setup, the T-valve was placed after the ultraviolet–visible (UV–vis) detector and before the first back-pressure regulator. In both setups, the T-valve (Valco, Inc., Houston, TX) connected a model 306 rinse pump to the SFC system for flushing of the lines with methanol. A model 308 pump equipped with a cooling jacket and a thermostatic kit delivered the supercritical CO_2. Methanol (modifier) was added inline through the model 306 SFC pump. An Alltech (Deerfield, IL) amino Hypersil column (250 × 4.6 mm, 5-µm particle size) was placed inside the temperature regulator for isothermal operation at 70°C. Mobile phase pressure was optimized with a pressure regulator for isobaric operation at 300 atm. A model 119 UV–vis detector operating at 254 nm was used to detect eluting peaks. A model 233XL Sampler unit was employed for sample fractionation and collection. A 20-mL sample loop was used for all injections. A mixture of all three analytes was prepared in methanol at a concentration of 3 mg/mL. The total mobile phase (CO_2 and methanol) flow rate was 2 mL/min (liquid). The mobile phase composition was held constant at 85:15 (CO_2:methanol) during the first 5 min. Then the modifier percentage was increased to 20% over a 10-min period at a rate of 0.5% methanol/min.

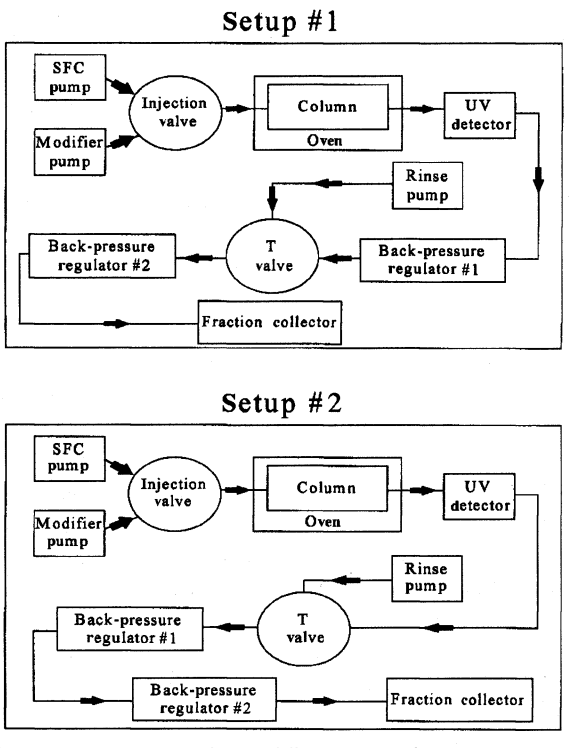

Figure 1. SFC system with two different setups for semipreparative minifractionation.

Three different protocols were used to collect the separated components. In the first method, fractions were collected starting from 0.3 min into the peak and continuing to 0.3 min after the detected peak. Within the peak, minifractions were collected at 0.3-min time intervals. This protocol was repeated at differential times of 0.4, 0.5, and 0.6 min. In the second method, fractions were collected from 0.4 min into to 0.4 min after the detected peak but with individual fractions collected every 0.2 min inside a peak. This protocol was also repeated for an individual minifraction collection time of 0.3 min inside a peak. In the third protocol, fractions were collected at 0.5-min intervals starting at a chromatographic run time of 5.0 min and ending at 15.0 min, regardless of peak elution times.

All sulfonamides were obtained in pure form from the U.S. Department of Agriculture's Agricultural Research Service in Philadelphia, PA. HPLC-grade methanol was purchased from EM Science (Gibbstown, NJ). SFE–SFC-grade CO_2 padded with helium (Air Products and Chemicals, Inc., Allentown, PA) was used for all studies.

Results and Discussion

The objective of this research was to carry out a semipreparative separation on a solution of three polar sulfonamides (i.e., sulfamethazine [SMZ], sulfaquinoxaline [SQX], and sulfadimethoxine [SDM]) and to isolate pure analyte fractions of each component. Three different peak collection methods were used as described in the Experimental section. After the fractions were collected and analyzed via SFC for purity, the total and individual peak areas from the three components were analyzed in order to determine the percentage of each sulfonamide analyte in each fraction. Each experiment was repeated at least three times, and excellent reproducibility was observed.

In the first part of this study, setup 1 (Figure 1) was used to collect separated components. Fractions were collected starting at 0.3 min after the onset of the peak and continuing to 0.3 min after the peak had passed. The chosen delay time was based on the system dead volume that existed from the

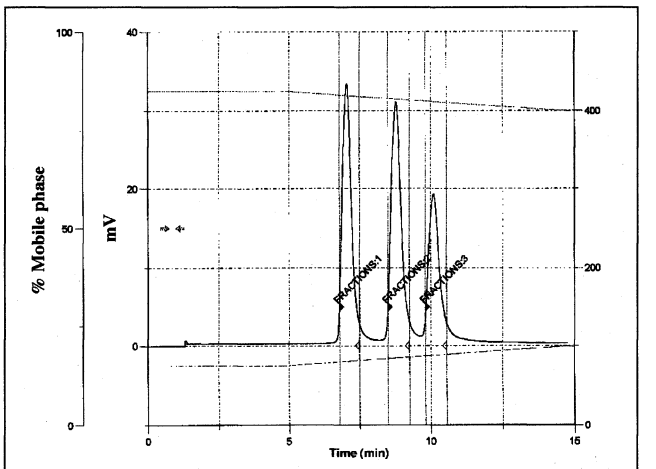

Figure 2. SFC separation of three sulfonamides with "tic marks" using setup 1 and protocol 1.

detector to the fraction collection system. The rinse pump flow rate during collection was set to 0.2 mL/min. Figure 2 shows the SFC separation of the three components with "tic marks" noting where the fraction collection started and where fraction collection stopped. Only one 20-mL injection was performed. Figure 3A shows the SFC–UV analysis of the first collected fraction, which contains only SMZ. Analysis of the second and third fractions (Figures 3B and 3C) showed the presence of the first and second components, respectively. Fraction 3 even showed evidence of the presence of the first component. Percentages of the first and second components in the second and third fractions relative to the total peak areas were 5 and 8%, respectively. This fraction contamination was believed to be due to the large dead volume between the detector and fraction collection. Similar results were obtained for fractions collected with longer delay times (e.g., 0.4, 0.5, and 0.6 min).

Next, in order to flush the dead volume after the detector, the rinse pump was moved behind the first regulation valve for setup 2 (Figure 1). Collection experiments similar to the first

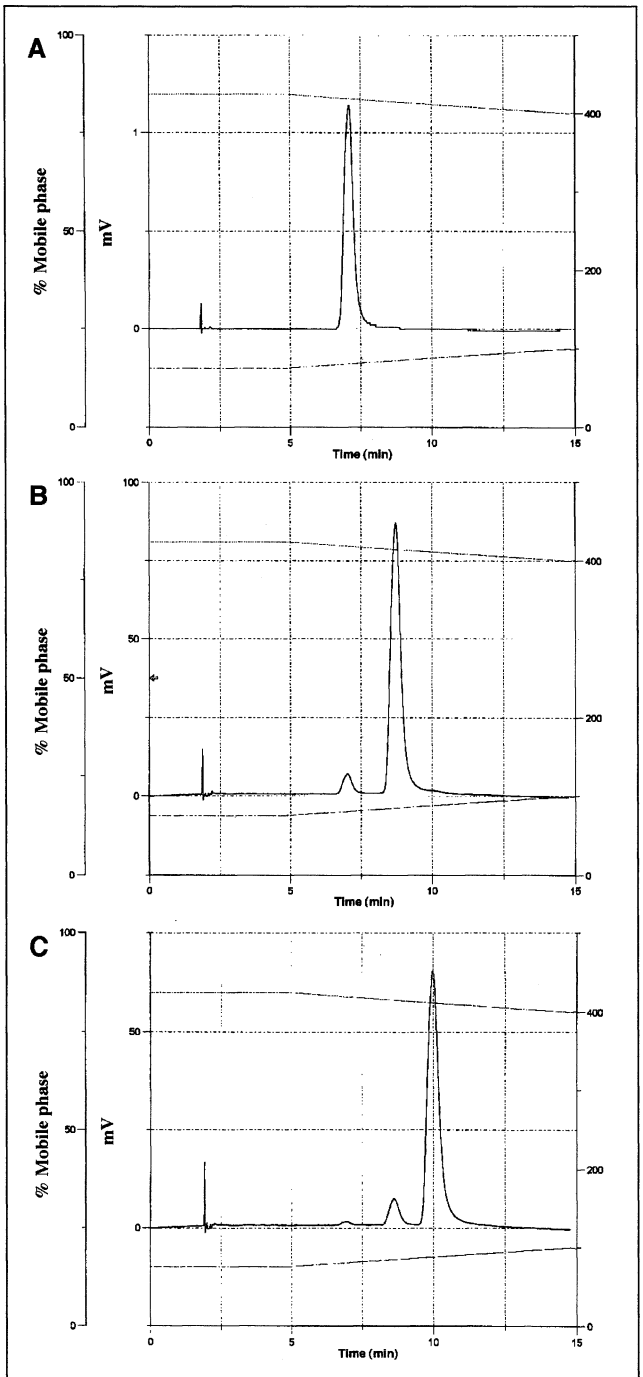

Figure 3. SFC analysis of three minifractions using setup 1 and protocol 1. (A) first minifraction, (B) second minifraction, (C) third minifraction.

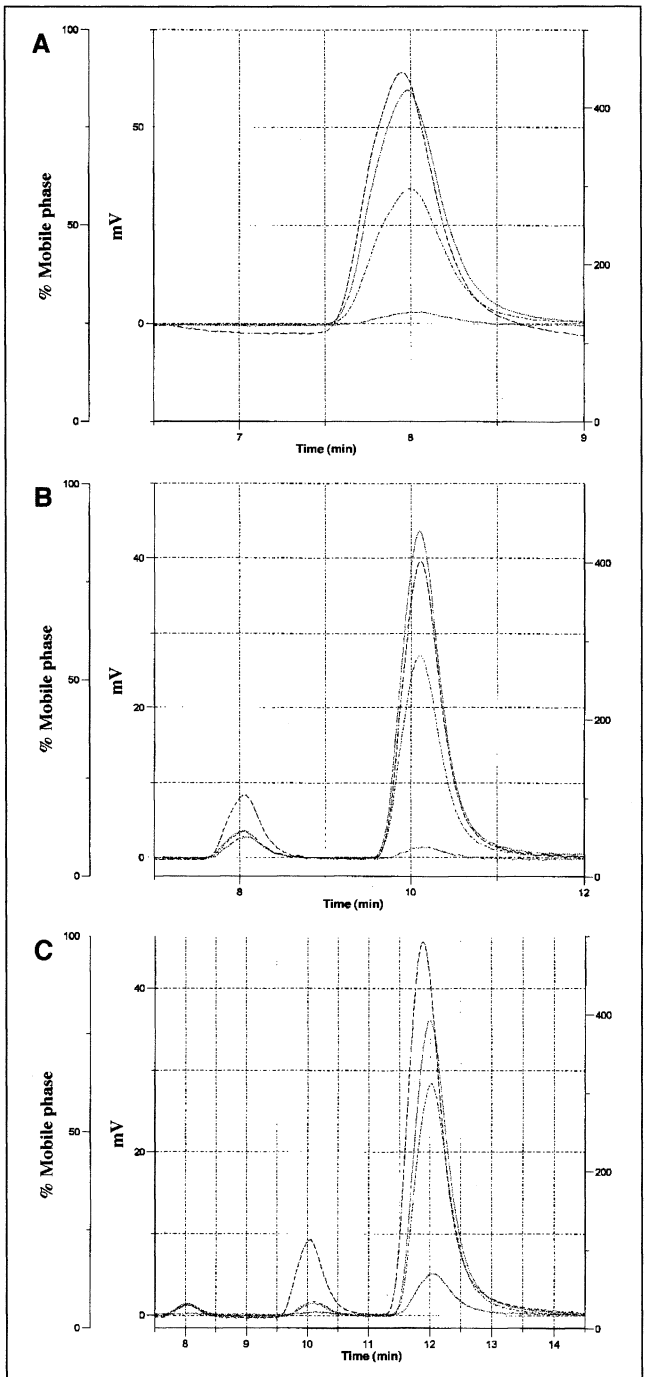

Figure 4. SFC analysis of 12 minifractions collected using setup 1 and protocol 2. (A) First four minifractions (SMZ), (B) second four minifractions (SDM), (C) third four minifractions (SQX).

part of the study were repeated. Separated components were collected using different delay times (0.3, 0.4, 0.5, and 0.6 min). Results were similar to those obtained in the first part of our study. However, percentages of the first and second peaks in the second and third fractions (less than 4%) were less than those percentages obtained in the first part of this study. Varying the rinse pump flow rate did not lower the percentages of contamination.

Next, both setups were tested by collecting a fraction starting at 0.4 min into the peak and extending the collection to 0.4 min after the peak, as stated before. However, within this chromatographic time period, individual fractions were collected at 0.2-min intervals within each eluted peak. For setup 1, a total of four minifractions were collected for each sulfonamide peak, whereas for setup 2, a total of five minifractions were collected for each peak. Figures 4A–4C show the SFC–UV analysis of the four minifractions obtained for each peak using setup 1. As expected, each minifraction of SMZ (Figure 4A) was not contaminated with SDM or SQX. On the other hand, the four fractions that were obtained for SDM (Figure 4B) were contaminated with SMZ, and the four fractions obtained for SQX (Figure 4C) were slightly contaminated with both SMZ and SDM. Figures 5A–5C show the analysis of the five minifractions obtained for each peak using setup 2. The first five fractions, which contained SMZ, were pure, as was expected. The first minifraction of SDM contained less than 3% of SMZ, whereas the other minifractions were free of SMZ and thus contained only SDM. Similar results were obtained for minifractions arising from elution of SQX. The first minifraction for SQX contained less than 4% of SDM, whereas the other four fractions were free of both SDM and SMZ. Table I shows the

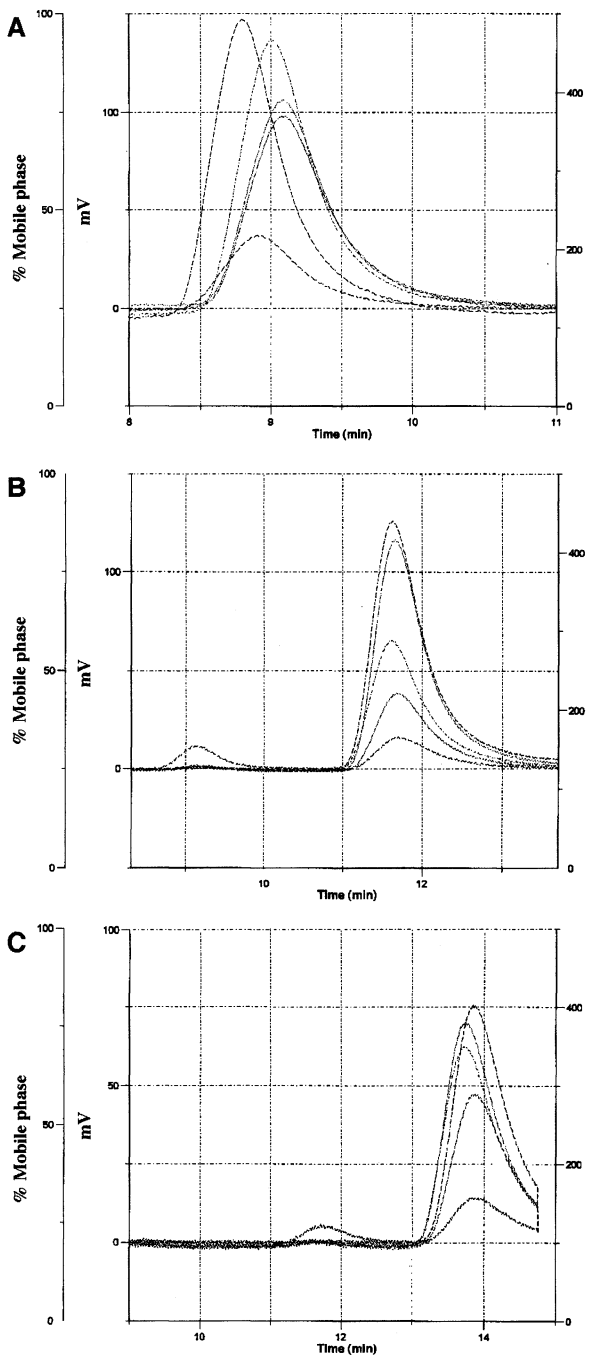

Figure 5. SFC analysis of 15 minifractions collected using setup 2 and protocol 2. (A) First five minifractions (SMZ), (B) second five minifractions (SDM), (C) third five minifractions (SQX).

Table I. Collection by 0.4-min Delay During and After an Eluted Peak with Fractions Collected at 0.2-min Intervals Inside the Peak Using Setup 2

Fraction collected	SMZ (%)	SDM (%)	SQX (%)
1	49.3		
2	34.9		
3	4.6		
4	5.7		
5	2.4		
6	3.1	6.3	
7		44.3	
8		25.0	
9		16.9	
10		5.6	
11		1.9	26.8
12			26.3
13			23.9
14			17.2
15			5.8

Table II. Fraction Collection at 0.5-min Intervals Regardless of Peak Elution Using Setup 1

Fraction collected	Time (min)	SMZ (%)	SDM (%)	SQX (%)
1	7.5–8.0	36.7		
2	8.0–8.5	40.5		
3	8.5–9.0	14.4		
4	9.0–9.5	8.4	8.2	
5	9.5–10.0		55.2	
6	10.0–10.5		26.7	
7	10.5–11.0		9.9	13.8
8	11.0–11.5			58.4
9	11.5–12.0			27.8

percentages of each sulfonamide in each fraction. As can be observed, the first minifraction of SDM contained 3% of SMZ and 6% of SDM; the first minifraction of SQX contained 2% of SDM and 26.8% of SQX. Overall, this collection protocol yielded a total peak purity percentage of 97% for peak 1, 92% for peak 2, and 73.2% for peak 3 when all respective minifractions were combined.

A third basic time configuration was attempted with setup 1. In this sequence, fractions were collected at 0.5-min intervals from elution times of 5.0–15.0 min without regard to the elution time of individual sulfonamide peaks. Pure SMZ fractions were obtained at collection times of 7.5, 8.0, and 8.5 min. The fraction collected at 9.0 min contained both SMZ and SDM. Pure fractions of SDM, however, were collected at 9.5 and 10 min. At 10.5 min, the fraction contained a mixture of SDM and SQX. Pure fractions for SQX were collected at 11.0 and 11.5 min. Table II shows the total percentage area of each analyte underneath each collected fraction in time interval fractions from 7.5 to 11.5 min. Altogether this collection process yielded fractions with a total peak purity percentage of 92% for SMZ, 82% for SDM, and 86% for SQX. However, the second collection technique, with a delay time of 0.4 min into the peak and individual fractions collection every 0.2 min, gave a higher value of SDM and a lower value for SQX.

Conclusion

Bench-scale separations of sulfa drugs can be accomplished with commercial analytical SFC systems employing packed columns. Rejection of only the first minifraction (e.g., 0.2 min) within the chromatographic peak gave essentially analytically pure sulfonamides. This approach affords sufficient sample for nuclear magnetic resonance, infrared, and mass spectrometric analysis off-line where optimized spectrometric parameters can be readily applied.

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