Injection by Extraction: A Novel Sample Introduction Technique for Preparative SFC

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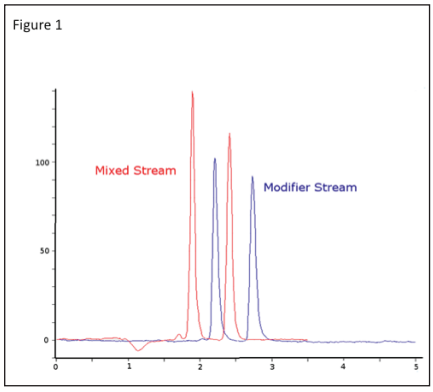
Comparison of injections using mixed-stream (in which the sample is introduced to the mixed, supercritical mobile phase) and modifier stream (where the sample is introduced to the modifier stream prior to the point of mixing with the CO_2) techniques in preparative SFC shows that both have their advantages and under most conditions there is little to choose between them. Neither technique, however, is perfect and neither can eliminate the possibility of sample precipitation on injection. An injection technique which uses an extractor to dissolve the dry sample in the supercritical fluid prior to injection has been developed and use of which is shown to result in reproducible separations with excellent peak shapes. Further, because the sample is dissolved in the mobile phase, the possibility of sample precipitation is eliminated. The quantity of sample injected can be varied by changing both the time during which the contents of the extractor are swept into the column and – with care – by changing the temperature of the extraction. The procedure is compatible with stacked injections and can be implemented on at least one commercial SFC system.

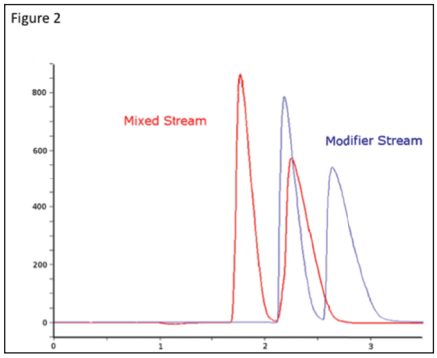
Sample introduction in preparative chromatography is always a compromise between the need to load the maximum amount of sample into the column and the effects of mass and volume overload on the separation. This is exacerbated when the sample solvent is not the same as the eluent in the system as solvent strength effects can also affect the situation. This has been known for many years in HPLC separations, where a solvent chromatographically stronger than the eluent can lead to 'fronting' of the peaks due to the slow mixing of the sample band with the eluent at the head of the column. Under these circumstances, the solute at the centre of the band experiences a higher solvent strength for a longer time than that at the edges of the band where diffusional mixing takes place. Thus, the centre of the band travels through the column at a faster speed than the edges of the band, distorting the peaks. Where the solvent strength of the sample solute is lower than that of the mobile phase, a similar effect is seen in which the centre of the band now experiences a lower solvent strength and therefore moves more slowly, leading to band tailing.

In SFC, because of the complexities of dissolving samples in the supercritical fluid, the usual sample solvent is the polar component of the mobile phase, in some cases with admixture of another solvent to enhance sample solubility. Conventionally, sample introduction is made from such a solution in two ways. One is to inject the sample solution directly into the mixed mobile phase (mixed stream injection). Under certain conditions, usually when injection volumes are large, this can lead to the expected fronting of the peaks with deleterious effects on the sample load. The other is to inject the sample into the modifier stream (modifier stream injection) [1]. This technique eliminates the mixing of a sample band with the mixed eluent and – providing the mixing of the modifier with the supercritical CO_2 is efficient – results in an undistorted injection band. The disadvantage of this technique lies in the fact that the modifier flow is generally a small proportion of the total eluent flow; for example, at 10% modifier composition, the injected band is diluted 10-fold by the CO_2 stream and thus the effective injected volume at the column head is 10 times larger than the original sample volume. At low modifier flow rates, therefore, there can be significant band broadening due to this effect.

A further problem which arises from the dissolution of the sample in the mobile phase modifier lies in the fact that some samples are much less soluble in the

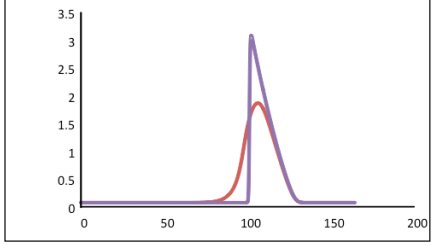


1. Analytical separation of trans-stilbene oxide (TSO) enantiomers by mixed stream and modifier stream injections. Sample TSO, 1 g/l, injection volume 100 µl. Column: CHIRALCEL OD-H 150 x 21.2 mm. Mobile phase 15% methanol in CO_2 at 60 ml/min flow rate, 40°C, back pressure 100 bar.



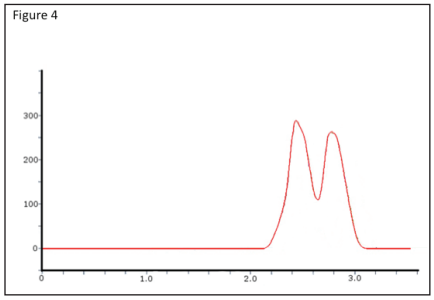
2. Preparative separation of guaiphenesin enantiomers by mixed stream and modifier stream injections. Sample 100 g/l, injection volume 390 µl. Conditions as Figure 1.

Figure 3



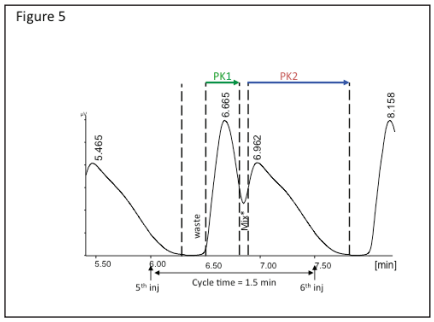
3. Computer simulation of an injection of 500 µl of guaiphenesin with and without solvent effects. See text for a discussion of the parameters used.

Figure 4



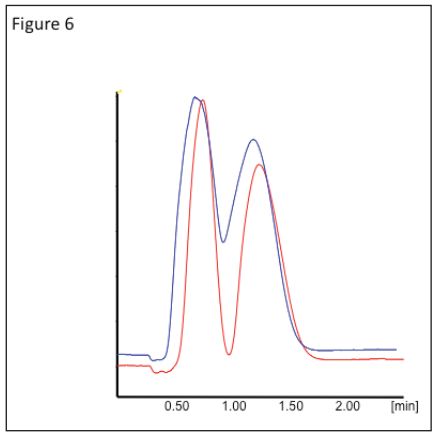
4. Analytical separation of trans-stilbene oxide (TSO) enantiomers by mixed stream and modifier stream injections. Sample TSO, 5 g/l, injection volume 2 ml. Column: CHIRALPAK IA 250 x 21.2 mm. Mobile phase 15% methanol in CO_2 at 100 ml/min flow rate, 40°C, back pressure 100 bar.

Figure 5



5. Preparative separation of guaiphenesin enantiomers by mixed stream injection. Sample 150 g/l, injection volume 2.7 ml. Column: CHIRALCEL OD 20µm, 250 x 21.2 mm. Mobile phase 20% ethanol in CO_2 at 180 g/min flow rate, 0°C, back pressure 150 bar.

Figure 6



6. Overlays of chromatograms of 112 mg guaiphenesin using mixed stream injection (5 ml) and samples dissolved in 20% ethanol in hexane (red line) and 100% ethanol (blue line). Conditions as Figure 5.

supercritical mobile phase than they are in the pure modifier and precipitation on mixing the sample and mobile phase streams is a not unusual occurrence. This can manifest itself as an increase in operating pressure on injection, sometimes resulting in incremental pressure increases with each successive injection, or can be sufficiently serious as to block the column inlet completely.

Dissolution of the sample in the supercritical fluid or in a solvent with similar solvent strength prior to injection should eliminate the disadvantages of the current sample injection procedures.

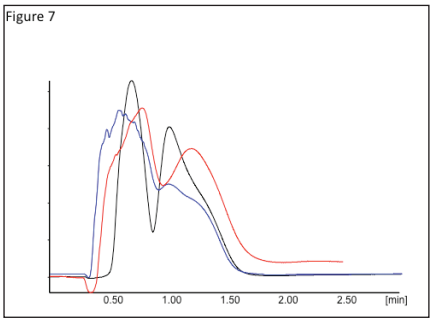
Results and Discussion

The first concern was to investigate briefly the differences between mixed-stream and modifier-stream injections. While the theory of the two processes indicates some limitations for each [2], there are little data existing which compare the two in practice under the usual conditions employed for smaller scale separations. We compared the standard mixed-stream injector on a SFC-PICLab Hybrid 10-100 system with a modified system which introduced the sample into the modifier stream. In this case, as the unit uses the CO_2 pump as an efficient mixer for the mobile phase with the modifier being introduced to the liquid CO_2 prior to the pump, the injected sample had to pass through the CO_2 pump and heat exchanger volume. However, given that when the streams are mixed at high pressure a mixer is needed in the solvent line it was not felt that this delay would be significant. Analytical injections of 100µl of a solution 1g/l in trans-stilbene oxide (TSO) were made though both injectors. These are shown in Figure 1. The modifier stream injection is delayed in the system due to the extra volume as noted above. To minimise the effects of this extra-column volume on the measured efficiency, the plate number of the second eluting peak was measured. When corrected for the hold-up time, the number of plates for the modifier stream injection was close to that of the mixed-stream injection (5840 vs 5363), as expected. In order to compare the preparative performance, injections of guaiphenesin at a sample load expected to result in touching band separation were made through the two systems. The overlays are shown in Figure 2; apart from the longer retention of the modifier stream injection, there is little difference between the separations.

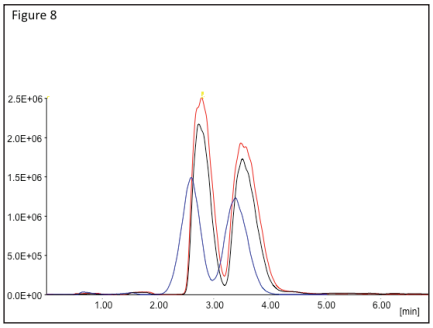
In order to arrive at some estimation of the potential peak distortion under the conditions of mixed-stream injections, some computer simulations of injections with and without modifier effects were carried out. The estimation of the isotherm parameters for methanol under the supercritical fluid conditions is not simple; for the current purposes, however, only a qualitative picture was needed. The Henry constant from the vacancy peak observed on injection of methanol alone was taken and the saturation capacity was estimated from values observed for larger solutes with allowance made for the relative molecular dimensions. The comparison for injections corresponding to 500 µl in the experimental set-up with and without modifier effects is shown in Figure 3. The fronting of the peak is clear, although it is more pronounced than is usually seen in practice; an effect of similar magnitude is seen in the chromatogram in Figure 4, which resulted from a 2 ml injection. The conclusion from these experiments is that for smaller injections at high sample concentration there is little to choose between the techniques. For samples with low solubility, however, the necessary injection of large volumes, for example in excess of 1 to 2 ml in a column 150 x 21.2 mm, can lead to some peak distortion in the mixed stream injection.

SFC is a normal phase technique in which a polar modifier, usually an alcohol, is used to adjust the eluting power of a relatively non-polar mobile phase. In some ways this is analogous to the situation in normal phase HPLC, in which hexane or heptane is used as the non-polar component of the mobile phase while an alcohol is used as modifier. Thus, our first attempts to ameliorate the effects of sample solvent on mixed-stream injection were directed towards trying to approximate the solvent strength of the supercritical mobile phase by dissolving the sample in mixtures of alcohol and hexane.

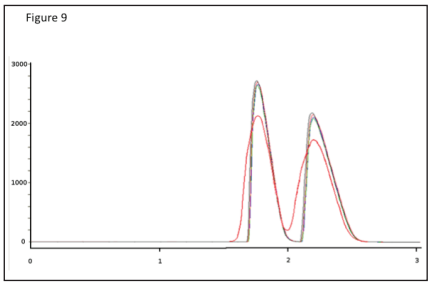
The application chosen for this investigation was a highly productive separation of the enantiomers of guaiphenesin using a CHIRALCEL OD column with 20% ethanol mobile phase. The sample in this case was dissolved at a concentration of 150 g/l in methanol; although the elution strength of methanol was similar to that of ethanol, the selectivity in the ethanol-based eluent was better than that in a methanol – CO_2 mixture but the solubility in methanol was sufficiently superior to predicate the use of this solvent for the sample. This separation, along with the separation conditions, is shown in Figure 5. The throughput for the separation was 8 kg racemate/kg CSP / day with product



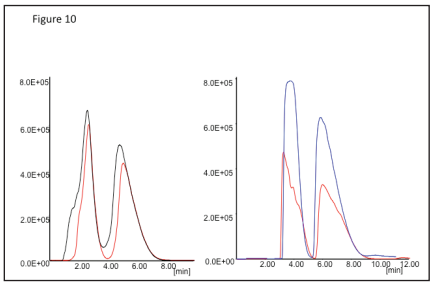
7. Overlays of chromatograms of 300 mg guaiphenesin in methanol (150 g/l, 2 ml, black line), in 20% hexane-alcohol (22.4 g/l, 13.4 ml, red line) and in pure ethanol (22.4g/l, 13.4 ml, black line). Conditions as Figure 5.



8. Chromatograms of benzoin enantiomers. Blue line: 36 mg (3 ml @ 12g/l) in methanol; black line: 48 mg extraction injection, red line: 62 mg extraction injection. Extractor operated at 40°C. Column: CHIRALCEL OD 20 µm, 250 x 21.2 mm. Mobile phase 3% ethanol in CO_2 at 100 g/min flow rate, 25°C, back pressure 110 bar.



9. Overlays of chromatograms of 52 mg TSO in methanol (52 g/l, 1 ml, red line) and 4 consecutive injections of 1 second (= 1 ml) from 2 g of TSO in an extractor 100 x 10 mm. Conditions as Figure 1.0°C, back pressure 150 bar.



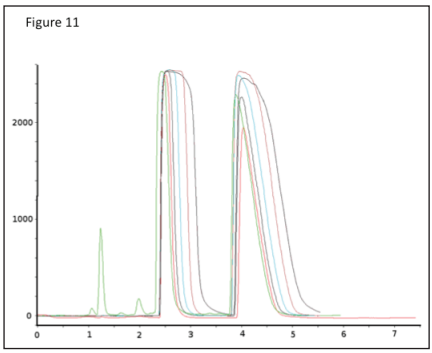
10. Separations of warfarin enantiomers. (a) overlays of 90 (6 ml) and 135 mg (9 ml) of warfarin in ethanol @ 15 g/l and (b) 90 and 150 mg warfarin from extraction injection. Column: CHIRALCEL OD 20 µm, 250 x 21.2 mm. Mobile phase 20% ethanol in CO_2 at 100 g/min flow rate, 25°C, back pressure 105 bar.

purities and recoveries of 99.3%ee and 91% recovery for the first eluting enantiomer and 98.4%ee and 96% recovery for the second. The solubility of guaiphenesin in a mixture of 20% ethanol in hexane was only 22.4 g/l. The resulting chromatogram from injection of 112 mg of guaiphenesin from a 5 ml injection using this mixture is shown in Figure 6 in comparison with the injection of the same quantity of sample from a solution at the same concentration in ethanol. Use of the hexane-ethanol solvent clearly gave an improved resolution over the use of ethanol as sample solvent under these otherwise identical conditions although the throughput was reduced to 2.2 kg racemate / kg CSP / day. In order to inject the same quantity of sample as used in the original separation, it was necessary to use a 13.4 ml injection volume of the hexane-ethanol mixture which resulted in significant volume overload in the 21.2 x 250 mm column used in the experiment. Figure 7 shows an overlay of chromatograms arising from the injection of 300 mg of guaiphenesin from methanol (2ml @ 150 g/l), hexane-ethanol (13.4ml @ 22.4 g/l) and ethanol (13.4 ml @ 22.4 g/l). Again, the injection with the mixed sample solvent gave a better resolution than that in pure ethanol which showed significant distortion over that of simple volume overload, much as would be expected.

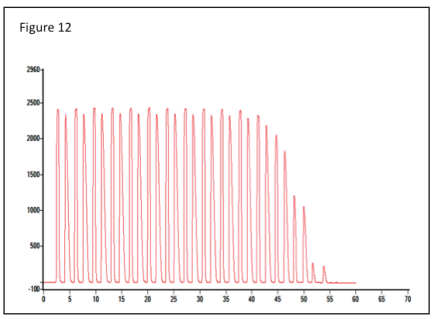
In practice, it is unfortunately rare to find samples with solubility as high as 150 g/l in any solvent. This means that injection volumes will normally be large and a way in which to introduce samples with as little peak distortion as possible, whether from introduction of a large volume of strong solvent or from the dilution effects of modifier stream injection into eluents with low modifier concentration, would be advantageous. Dissolution of the sample in the supercritical fluid would avoid both the band distortion due to injection in the polar modifier and also would avoid the problem of sample precipitation. Thus, an injection methodology involving dissolution of the sample in the mobile phase was developed. The dry sample is placed into an extraction cartridge which replaces the loop in the injection system. Prior to injection, the cartridge is pressurised with the supercritical fluid to dissolve the sample. Equally, pressure on the cartridge is maintained during operation. This dissolves more of the sample after each injection to maintain a saturated solution in the cartridge during the experiment. Injection of aliquots of the sample solution in the cartridge is made by diverting the mobile phase flow through the cartridge into the column for a suitable time. The instrument used for the experiments already had the provision to pressurise the injection loop prior to each injection so the modification continuously to pressurise the cartridge involved a simple software adjustment. Thus, the injected quantity can be varied by changing the time during which the cartridge is opened to the column; all other functions, such as stacked injection, collection etc are the same as for a conventional sample injection system.

Initial experiments were carried out using a dry sample of benzoin in a 100 x 10 mm extractor which was immersed in a temperature controlled ultrasonic bath to maximise the dissolution rate and to try to prevent channelling in the bed of sample. Figure 8 shows the result of the injection of 48 and 62 mg using the extraction-injection technique and of the injection of 36 mg as a 3 ml injection of a solution 12 g/l in methanol by the conventional approach [3]. Similar results have been obtained with guaiphenesin and trans-stilbene oxide (TSO); Figure 9 shows an overlay of 4 consecutive injections using 1 second extraction in comparison with a 1 ml injection of TSO at 52g/ml. The quantity injected in the extraction-injection experiment was determined by comparison of peak areas with standard injections of a solution of TSO in methanol. In the absence of solubility data in the supercritical fluid, this calibration is needed if one wishes to determine the exact quantity injected.

As noted, solubility is often a limiting factor in preparative chromatography. Warfarin, for example, is soluble at 15 g/l in ethanol which means that in order to inject a sufficiently large quantity for even a touching band separation, large volumes are required. An additional consideration in the chromatography of warfarin is that the sample slowly crystallises from the alcohol solution after dissolution. Figure 10(a) shows the separation of the enantiomers of 90 and 130 mg samples injected conventionally, whereas Figure 10(b) shows the separations of 90 and 150 mg samples injected by extraction. The higher sample load was attained under identical conditions to those of the 90 mg injection except that the extractor was heated to 40°C to enhance the sample solubility in the mobile phase. Further experiments with warfarin demonstrate that very large injection volumes can be made with little deleterious effect on peak shape. The overlaid chromatograms shown in Figure 11 result from injection times of 4 to 40



11. Extraction-injection of warfarin; overlays of chromatograms from 8, 12,20, 30 and 40 ml injected from an extractor (50 x 10 mm) packed with 400 mg warfarin coated on 2 g Daiso C-1 (50 µm). Column: CHIRALCEL OD-H 150 x 21.2 mm. Mobile phase 20% methanol in CO_2 at 60 ml/min flow rate, 40°C, back pressure 100 bar.



12. Stacked injection separation of warfarin enantiomers. 760 mg warfarin coated on 2 g Daiso C-1 (50 µm) packed into an extractor (100 x 10 mm) with 30 second injection time. Conditions as for Figure 11. Separation time 6.3 minutes, cycle time 3.5 minutes.

seconds, corresponding to volumes of 4 to 40 ml, into a 150 x 21.2 mm column. Volume overload effects can be clearly seen, although there is no evidence of other peak distortion. In this case the warfarin was coated on a C1 bonded phase of 50 micron particle size before being packed into the cartridge [4]. The purpose of this is to maximise the rate of sample dissolution as well as to reduce the void volume of the cartridge. This reduces the time spent at the end of the separation to flush the last traces of sample from the extractor. If one uses an empty tube as extraction cartridge it acts as an infinite dilution mixer (assuming the space inside is perfectly mixed) so that many injections are required completely to flush the remaining sample from the cartridge at the end of the separation. Use of the packed cartridge eliminates the dead volume and at the end of the separation the peak areas fall to zero within at most 5 injection cycles as the sample becomes exhausted. This can be seen from the chromatogram in Figure 12 where 750 mg of warfarin was coated on 4.3 g of packing material before being loaded into an extractor 100 x 10 mm. The run consisted of 14 stacked injections, each of a little over 40 mg with only 4 injections between the start of sample exhaustion and the end of the run. In this case threshold sample collection was used so the chromatographic system shut down at the conclusion of the separation as the mass loaded on the column diminished below a certain level. As is the case with other preparative separations the stacked injection run was preceded by three injections at 10, 20 and 30 second injection time to establish separation conditions and the collection parameters.

Conclusions

Injection by extraction offers some conveniences and advantages over conventional methods of sample introduction in preparative scale SFC. Because the sample is dissolved in the mobile phase, there is little chance of it precipitating on injection. Peak shapes are good, especially when samples of low solubility requiring large injection volumes are used, which can allow a larger injected quantity relative to other techniques. Injection volumes can be very large, while the problems experienced with very large injection loops are avoided.

When the sample solubility in the mobile phase is not known (although techniques exist to measure this [5]), there is uncertainty in the number of injection cycles needed for any one sample unless a calibration injection is made. Use of threshold collection resolves this issue as the system stops once the sample is exhausted.

References

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