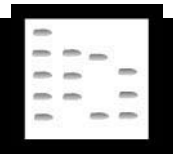
PREPARATIVE SUPERCRITICAL FLUID CHROMATOGRAPHY



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Introduction

Supercritical fluid chromatography (SFC) on a preparative scale is of interest because of its advantages over high performance liquid chromatography (HPLC). The high diffusivity and low viscosity of supercritical fluids allow rapid separation. The solvent strength can easily be controlled by changing pressure and/or temperature, so that mobile phases with a fixed composition can be used to separate many types of solute. Furthermore, many supercritical fluids are volatile or gaseous under normal ambient conditions, so that solutes can easily be recovered from the collected fractions by depressurization.

Preparative-scale SFC is now being used to separate high-value materials where preparative HPLC can be difficult to use. Peaks are narrower in SFC and for these difficult separations, very little overloading can be done to take advantage of this. Consequently, the maximum amount of material obtained in a run is of the order of 100 mg in preparative SFC, compared with the much larger amounts which are sometimes obtainable in preparative HPLC. Large-scale SFC systems can either be built in the laboratory or purchased commercially.

The unique physicochemical properties of supercritical fluids have convinced many workers that preparative SFC might be useful and relatively simple compared with preparative gas chromatography (GC), which is unsuitable for involatile and thermolabile compounds, and preparative liquid chromatography (LC), in which fraction-eluent separation can be problematic.

Preparative SFC is based on the following steps: periodic injection of the feed into a continuous flow of eluent, chromatographic separation due to selective interactions of the components of the sample with both the eluent and the stationary phase, detection at the column outlet, fraction collection, separation of the fractionated compounds from the eluent, further purification of the compounds and, optionally, recycling the eluent (**Figure 1**).

Preparative-scale SFC can be divided into two types: small-scale and large-scale. Small-scale can be defined as the isolation of milligrams to a gram of pure product for, say, structure analysis. Bench-scale equipment derived from common analytical apparatus can be used with the adoption of nondestructive detection, fraction collection and eluent removal. However, for industrial-scale production (1 g h^{-1} to 1 kg h^{-1}), large-scale equipment has to be used, posing different problems, even if the same concept is applied. In preparative SFC, most studies have been devoted to the former aspect.

History and Development

Preparative-scale SFC was first suggested in 1962 by Klesper *et al.* and an appropriate collection apparatus was constructed 10 years later by Jentoft and Gouw (**Table 1**). However, it was not until 1982, 20 years after it was first suggested, that a patent appeared on the technique from Perrut. Two years later, Chapelet-Letourneux and Perrut were the authors of a meeting abstract on preparative SFC. In 1986, the first papers were published by Ecknig and Polster, followed months later by an article from Jusforgues *et al.*

In the 1980s, predictably, reports of preparative SFC were small in number. However, during the 1990s, the total amount of material published on

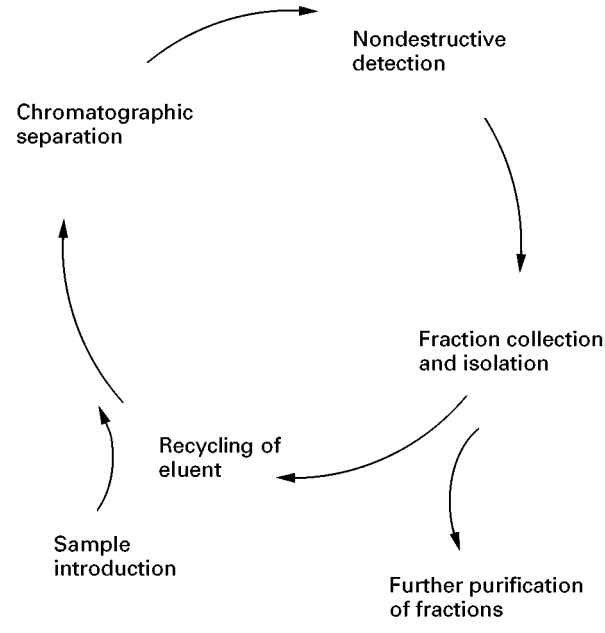

Figure 1 Flow diagram of the basic steps of preparative SFC.

Table 1 Historical perspective of preparative-scale supercritical fluid chromatography (SFC)

Year	*Development*
1962	SFC was suggested as a preparative tool
1972	Preparative SFC collection apparatus constructed
1982	First patent on preparative SFC published
1984	First meeting abstract on preparative SFC appeared
1986	First papers on industrial-scale preparative SFC published
1988	Jasco produce first commercial semipreparative SFE-SFC system
1990	First commercial process-scale SFC system by Prochrom

SFE, supercritical fluid extraction.

preparative-scale SFC has slowly increased; recently, publications have remained steady at three or four per year (**Figure 2**).

The early work was performed on SFC systems built in the laboratory. At the end of the 1980s, commercial equipment became available from Jasco and Prochrom.

During the development of preparative-scale SFC, it was used for the separation of relatively simple, low-cost test substances, such as paraffins. Once the technique had been refined, it was applied to high-value complex samples, such as pharamaceuticals.

Instrumentation

A general preparative-scale SFC consists of a pumping system, a core chromatographic section (an injection loop, a column and a detector) followed by the back-pressure regulator (BPR) and fraction collection system. Depending on the type used, the collection system may be located either before or after the BPR (**Figure 3**). The discussion in this section will be limited to the example in Figure 3, with further examples being explored later.

Pumping System

The pumping system must be capable of delivering the mobile phase (e.g. carbon dioxide modified with methanol) at a total flow rate of above 30 mL min^{-1} and at a pressure of up to 400 bar. Such a system requires judicious choice of pump with an efficient cooling system to ensure that the carbon dioxide is pumped as a liquid. The carbon dioxide pump, the modifier pump and the mixer may be integrated into one pumping system or may be separate components (as in Figure 3). An accurate method of delivering modified carbon dioxide must be used for reproducible results. The use of a single and dual pumping system has been investigated. Many methods have been developed to allow the addition of a modifier to a premixed mobile phase. The former can deliver an accurate flow of modifier while the composition of a premixed mobile phase can vary with use. A flow-splitting method has been used where a fraction of the carbon dioxide displaces the modifier, after which the flows are recombined. The dual pumping systems allow one pump to control the carbon dioxide flow while the other controls modifier flow.

Syringe pumps have been used, but with the high flow rate required on larger preparative systems, their relatively small capacity limits their use. Thus, a reciprocating dual pumping system is becoming the

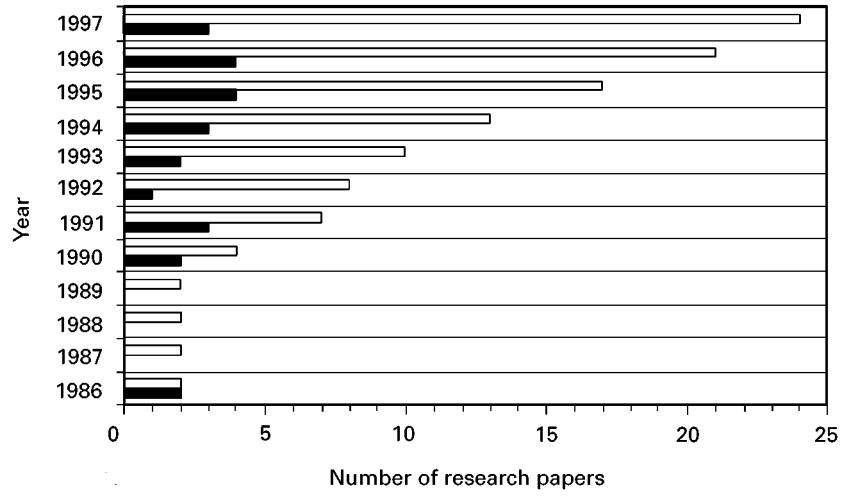

Figure 2 Growth in the number of research papers published on preparative SFC. The numbers quoted do not include review articles, poster presentations or meeting abstracts. Open boxes, cumulative, filled boxes, annual.

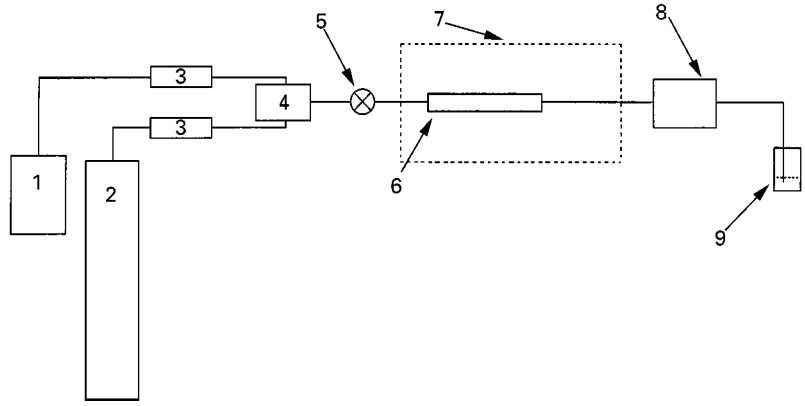

Figure 3 Schematic diagram of a preparative SFC system. 1, Modifier; 2, CO_2 cylinder; 3, pump; 4, mixer; 5, injector; 6, column; 7, oven; 8, detector; 9, collection vessel.

most common because it is able to deliver a continuous stable flow.

Injection System

The injection system may be located inside the column oven to allow injection into a supercritical fluid or outside, as in Figure 3. The injection system most commonly used is as in HPLC, but much work is being applied to the development of novel injection methods for preparative SFC. It has been noted that large-volume injections, such as those required in preparative separations, may cause phase separations of the mobile phase and the injection solvent. Although not always so, this may have detrimental effects on the efficiency of separation. To overcome this, much work has been successfully focused on the development of a solventless injection system, where the injection solvent is removed prior to introduction to the column. The sample can then be focused on a pre-column, thus eliminating any band broadening.

Columns

The most common columns used in preparative SFC are standard HPLC columns. For small-scale preparative work, analytical columns can be used. However, for larger-scale separations, preparative columns are necessary. A wide range of packing is available; the most common type is octadecylsilane bonded phases.

Detection

Preparative SFC is compatible with the detection methods available in both GC and HPLC. The most common detector for preparative SFC is the ultraviolet-visible (UV/Vis) detector. Although more sensitive, the flame ionization detector (FID) is not often used because of restrictor plugging, which occurs when the highly concentrated effluent is introduced. In addition, most organic modifiers cannot be used with the FID. However, less sensitive refractive index detectors or an evaporative light-scattering sensor can be used as a universal detector if necessary. To enhance and allow many separations in SFC, a polar modifier must be used as carbon dioxide alone does not have sufficient polarity. The use of a modifier thus reduces the number of detection methods available, unless specific modifiers are chosen, for example, an FID can still be used with carbon dioxide modifier with formic acid or formamide.

Back-pressure Regulators

The BPR is used to maintain SFC system pressure above the critical pressure of the mobile phase. The BPR can be a simple restrictor like a capillary tube of either fused silica or stainless steel having appropriate dimensions for the required back-pressure and flow rate. However, in order to change the back-pressure using the same piece of restrictor, one needs to change the flow rate because the back-pressure is only produced by flow resistance. Although fused silica restrictors are the most common and cheapest BPR, they can be blocked by certain solutes or samples during use, and they can break when some organic solvents are used, or if scratched.

Another type of back-pressure device is a mechanical or electrical feedback regulator. This is a complex regulator which consists of a pressure-sensing device and a needle valve. The regulator can control the back-pressure irrespective of the mass flow rate of the fluid. Although more expensive than a restrictor, it is much less likely to become blocked. The supercritical fluid depressurizes in the BPR and emerges at atmos-

pheric pressure as a gas (carbon dioxide) and as a liquid (modifier), and it is then that the separated material is deposited into or on a collector.

Collection Methods

Many trapping methods have been tested and developed in preparative SFC. The mobile-phase modifier can be used as a trapping fluid. This was demonstrated by Heaton and co-workers who fractionated polycarboxylic acid mixtures. They found that purities were close to 100%. In-line trapping methods have proved to be just as efficient. Many different collection methods have been investigated using pressurized pre-BPR and depressurized post-BPR traps. Comparison of four depressurized collection methods showed that a collection solvent and/or cooling of the collection vessels was required to achieve good recoveries. Without these measures, recoveries fell dramatically. Collection into pressurized vessels was compared with deposition on to semipreparative thin-layer chromatography (TLC) plates and the latter gave poorer recoveries.

Principles of Separation

The principles of preparative SFC can be illustrated by taking a mixture of two compounds present in equal proportions. Providing their peaks are well resolved, a graph of purity against the fraction of material collected in total will look like curve 1 in **Figure 4**. The first half recovered will be a pure fraction of one of the compounds. The second half, collected separately, will contain the other compound. This is the ideal situation. For a poorer separation, curve 2 is encountered. Here, the second compound begins to elute before all the first has been collected. In the worst case, illustrated by curve 3, both compounds elute together.

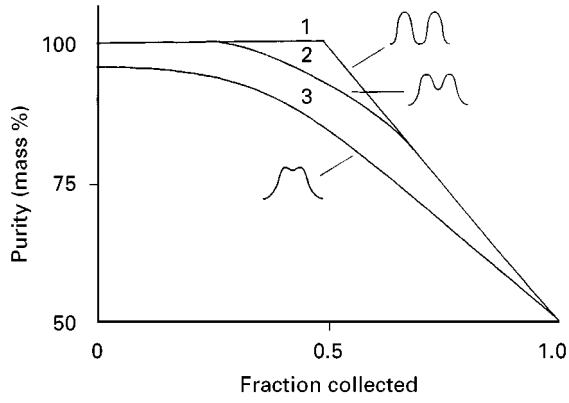

Figure 4 Plot of purity versus fraction collected for different degrees of separation.

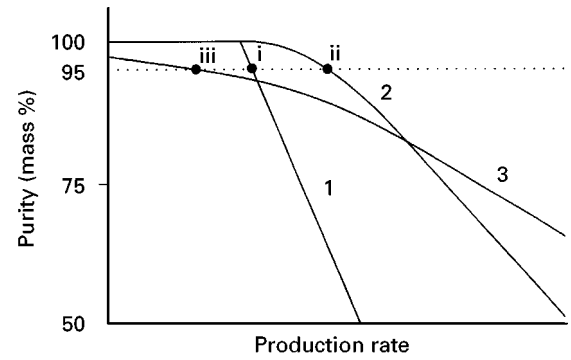

Figure 5 Plot of purity versus production rate for different degrees of separation.

Optimization

The variables to be optimized include the type of column, the concentration of modifier (if any), pressure, temperature, flow rate and loading.

Usually, optimization of column type, pressure, temperature and modifier concentration is conducted on an analytical scale. Initially, the chromatograms from experiments without trapping are scrutinized to help choose the best conditions.

However, for the optimization of flow rate and loading conditions, trapping experiments can be performed to obtain plots of purity against production rate for different conditions. The rate of production of the eluent from the end of the column can be improved by increasing flow rate or loading, for a given set of conditions. However, this can have a deleterious effect on separation. Consequently, if purity is presented versus rate of production, the curves 1, 2 and 3 (Figure 4) will become extended (**Figure 5**). The horizontal dotted line in Figure 5 signified 95% purity and it dissects the curves 1, 2 and 3 at i, ii and iii, respectively. These points can also be shown as a graph of production rate against loading or flow rate for a purity of 95% (**Figure 6**). Consequently, it can be seen how an optimum loading or flow rate can be chosen for a given purity.

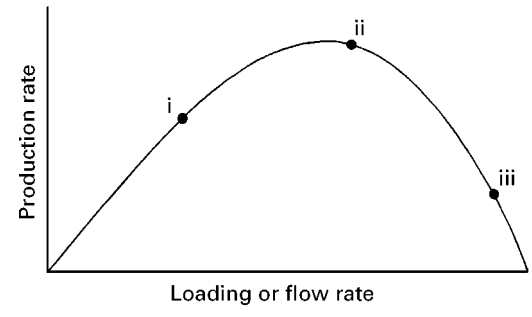

Figure 6 Plot of production rate versus loading or flow rate for a given required purity.

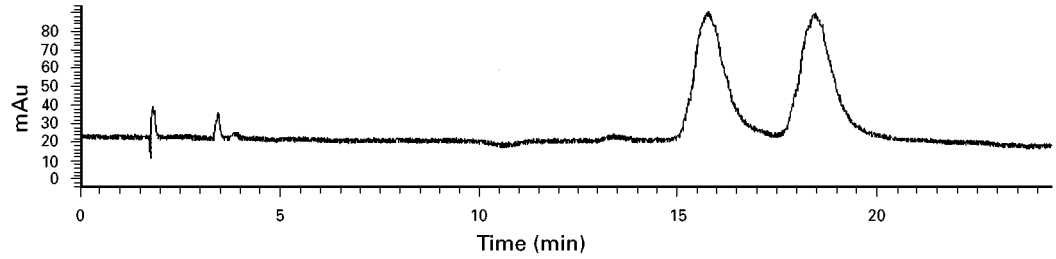

Figure 7 Chromatogram of the separation of a chiral benzimidazole.

For purities less than 100%, the purity versus production rate curves can be fitted to a quadratic. The resultant equations can be exploited to calculate values of production rate at a given purity for the various loadings. Plots can then be produced of production rate against loading. Consequently, an optimum loading can be obtained for a required purity.

Applications

The relatively low throughput of preparative SFC dictates that the technique is only economically viable for high-value substances, such as natural products, polymers and pharmaceuticals.

Probably the most promising area for preparative SFC is in chiral separations where several applications have been reported. The racemates of important biological compounds have been isolated into their optically pure forms. Shorter analysis times have also been observed when performing chiral separations of β-blockers, benzodiazepines, non-steroidal anti-inflammatory agents and β-agonists. An example of the separation of a chiral benzimidazole and 2,2,2-trifluoro-1-(9-anthryl)ethanol achieved using SFC is shown in **Figures 7** and 8.

Preparative SFC has been applied successfully to polymers. Highly isotactic (*-it*) and highly syndiotactic (*-st*) fractions of poly(methyl methacrylates) (PMMA), where the degree of polymerization (DP) ranges from 25 to 50, have been isolated by Ute *et al.* (1993). This represents an improvement on earlier work where the same authors preparatively separated PMMAs with DP ranging from 19 to 29. An example of separation of a PMMA is shown in **Figure 9**.

Preparative-scale separations using supercritical fluids have been coupled to reaction systems to separate products of synthesis reaction. Jacobson *et al.* designed a supercritical fluid synthesis system with on-line preparative SFC using supercritical ammonia. ^{11}C-labelled anisole, L-methionine and 4-methoxyphenylguanidine were isolated and found to have a radiochemical purity of $>98\%$.

Several compounds of pharmaceutical and biological interest have been isolated by preparative SFC and it has been reported that the supercritical technique was faster and more efficient than preparative HPLC.

Large-scale preparative SFC is a promising method for producing valuable fractions free from solvent and, at first glance, it is surprising that there have been so few reports on semi-industrial preparative SFC separations. In practice, however, problems have been reported with mobile-phase recycling, mobile-phase product separation, sample injection, fraction collection and columns, all of which are more crucial than on a smaller scale.

Conclusions

Preparative SFC is still applied to only a minority of compounds and is underutilized, although the number of applications for which it has been used is increasing. As SFC is used on a wider variety of compounds, many of these applications will undoubtedly be transferred to the preparative scale.

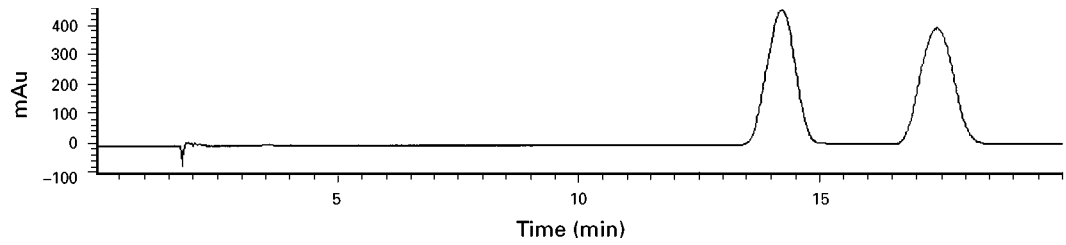

Figure 8 Chromatogram of the separation of 2,2,2-trifluoro-1-(9-anthryl)-ethanol.

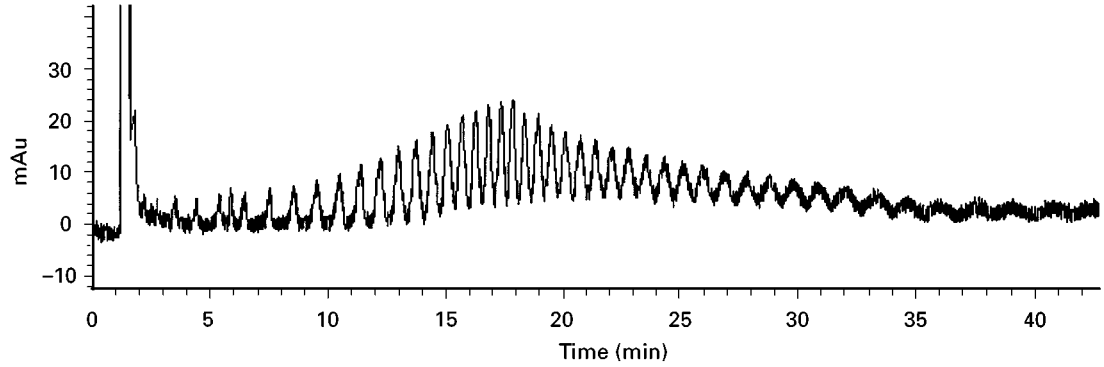

Figure 9 Chromatogram of the separation of a poly(methyl methacrylate).

The main disadvantages associated with using HPLC as a preparative separation technique are not experienced with SFC, namely the removal of the collected sample from the mobile phase and subsequent disposal of the mobile phase.

Further Reading

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PRESSURIZED FLUID EXTRACTION: NON-ENVIRONMENTAL APPLICATIONS

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Introduction

Pressurized fluid extraction (PFE), also referred to as accelerated solvent extraction (ASE), is a liquid