Future Directions

SFC instrumentation is maturing. The unique requirements for pumping these fluids is slowly becoming widely understood. There are unlikely to be further dramatic improvements in the core technologies. However, a wide range of accessories will appear. As more instruments are sold, the cost of production will tend to drop, making SFC more competitive with HPLC. Development of SFC detector interfaces will continue. Recent results with both mass spectrometry and evaporative light scattering detectors, finally indicate equal or better results than in HPLC-MS, or HPLC-ELSD, but with higher throughput.

User training has been another block to the spread of SFC in industry. One of the chief advantages of packed column SFC in industrial environments, particularly in the pharmaceutical industry, is its similarity with HPLC. Technicians familiar with HPLC have little trouble developing expertise in SFC.

Until recently, SFC has been viewed as a research tool, due largely to the relatively high cost of equipment and the need for an expert operator. As equipment is becoming less expensive, and easier to use, it is beginning to be used in routine analysis. Since the number of analysts involved in research is small compared to the number performing routine analysis, the incorporation of a technique into routine analysis represents a major expansion. This trend should continue, since the surprisingly low cost of operation and environmental friendliness are becoming more widely understood and appreciated.

See also: II/Chromatography: Supercritical Fluid: Historical Development; Theory of Supercritical Fluid Chromatography.

Further Reading


Large-Scale Supercritical Fluid Chromatography

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Introduction

Preparative supercritical fluid chromatography (Prep-SFC) came about at the same time as analytical supercritical fluid chromatography (SFC). During the 1960s and 1970s at the time of the early development of SFC, several authors included fraction collectors at the outlet of their analytical supercritical fluid (SF) chromatographs. However, the technological difficulties encountered kept these attempts somewhat marginal. The real interest in Prep-SFC appeared in 1982 when Perrut patented large-scale Prep-SFC with eluent recycling. Since then Prep-SFC has been studied and developed by several teams and on various scales. During the past decade commercial equipment has appeared and applications are reaching industry.

Principle

Prep-SFC is the result of the collaboration of three techniques:

- the principle of the separation is the same as in analytical SFC;
- the scale, the application and the industrial interest is the same as preparative high-performance liquid chromatography (Prep-HPLC);
the economical and practical interest of simple solvent recycling is the same as in industrial supercritical fluid extraction (SFE).

The extensive study of the properties and characteristics of supercritical fluids is not the subject of this article. The main properties relevant to their use in Prep-SFC are good solvent power (comparable to liquids), low viscosity and high diffusion coefficients (intermediate between gases and liquids) and straightforward modulation of solvent properties by pressure adjustment (it is possible to transform a supercritical fluid to a gas by isothermal depressurization).

The principle and characteristics of Prep-SFC can be described with a phase diagram. Figure 1 shows the eluent cycle in a Prep-SFC process.

At point 1, the eluent is supercritical and the chromatographic separation takes place in the column. At the column outlet, the eluent is depressurized (and heated) to the gas phase at point 2. While in a gas phase the eluent is cleaned and pure fractions collected. The cleaned gaseous eluent is then recycled, through condensation (point 3), recompression (point 4) and heating to the operating temperature (point 1).

This is a description of the simplest Prep-SFC process that uses a pure compound as a supercritical eluent. However, it is often necessary to use a mixture as the supercritical eluent. The secondary component of the eluent is most often a liquid solvent ("the modifier"). A Prep-SFC process that includes a modifier differs from the simple scheme without a modifier and both processes will be described here.

Prep-SFC processes can be classified according to scale. Micro-Prep-SFC is basically the adaptation of an analytical chromatograph to collect small fractions (microgram or milligram size) and is not described here. Laboratory-Prep-SFC is concerned with the purification of larger amounts (hundreds of milligrams or grams). It requires a specific technology, but cannot be considered to be large-scale SFC. It is described briefly later. Pilot-Prep-SFC is intended for the purifications of kilograms per day on columns having an internal diameter of between 5 and 15 cm. It can be used for scale-up studies or even for some small industrial productions. Production-Prep-SFC is for the purification of hundreds of kilograms to tens of metric tons per year. This scale of production takes full advantage of the economics of Prep-SFC.

**Important Characteristics**

The technique of Prep-SFC is best described by comparing it with Prep-HPLC and the emerging simulated moving bed (SMB) technique, an implementation of countercurrent continuous chromatography.

Prep-HPLC is a very useful tool in the field of high-performance separations. The technique’s high flexibility and efficiency means that most separation problems can be solved technically (but not necessarily economically). The main drawback of Prep-HPLC is the huge quantity of expensive solvent that needs to be used. Several strategies have been proposed to eliminate the solvent problem. One of them is SMB which cuts solvent use by up to 10-fold, but needs a higher level of economic investment and is limited to binary separations. Another is Prep-SFC, which reduces the solvent required by a factor of five to 20 (if a modifier is used), or uses no solvent at all (if no modifier is used) but also involves a high level of economic investment and has a limited range of applications.

**Selectivity**

The range of selectivities accessible by SFC is the same as for HPLC. This is not surprising since selectivity depends on the physical and chemical nature of the stationary and mobile phases. The stationary phases used in Prep-SFC are exactly the same as the ones used in Prep-HPLC. Theoretically, some stationary phases specific to SFC could be used (e.g. cross-linked polymers deposited on a silica support), but phases developed for HPLC are the only ones available in large quantities and, thus, they are the only ones used. Supercritical eluents are fewer in number than the mobile phases used in HPLC and, indeed, carbon dioxide is almost the only supercritical eluent used for Prep-SFC. But the possible use of modifiers, which can be almost any liquid solvent or any mixture, is a powerful parameter for variation of selectivity. Moreover, by changing the operating pressure it
is possible to adjust the solvent strength of the supercritical eluent, since it is compressible, without changing its composition.

The foregoing does not mean that there is no selectivity difference between the two techniques: for a given application, selectivity obtained by SFC can be much higher than by HPLC and vice versa.

**Efficiency and Speed**

Prep-SFC can attain efficiencies as good as those for Prep-HPLC. A reduction in plate heights of a factor of between two and four are easily obtained leading to efficiencies of between 10,000 and 70,000 plates per metre depending on particle size. Moreover, Prep-SFC has, just like analytical SFC, the advantage of being able to combine high efficiencies with high speed. Indeed, as can be seen in Figure 2, in any type of chromatography, a plate height increase (corresponding to a loss of efficiency) is observed when the eluent speed is increased beyond the minimum point. The slope of the curve is correlated to the diffusion coefficient of the sample in the eluent (the higher the diffusivity the smaller the slope). Since diffusion coefficients of supercritical fluids are much smaller than in liquids, the curve is flatter and it is possible to increase the eluent speed (and the productivity) without losing much efficiency. Moreover, in HPLC eluent speed is limited by the pressure drop in the column while in SFC the viscosity of the eluent is much less and such a limitation does not apply. To obtain high efficiencies in large diameter columns, the same difficulties are encountered in Prep-SFC as in Prep-HPLC. For diameters over 5 cm prepacked columns are not stable enough and lose more than half of their efficiency after a few hours use. This is due to rearrangement of the particles in the column induced by the friction of the flowing solvent. As for Prep-HPLC the solution to this problem is dynamic axial compression columns in which a piston continuously compresses the chromatographic bed, thus preventing the occurrence of voids and the loss of efficiency.

**Thermal Degradation**

There is no theoretical limit to the operating temperature range of supercritical fluids and some applications use temperatures as high as 200°C. However, the practical temperature range applied to Prep-SFC is from 0 to 100°C and most of the applications are made between 25 and 50°C. These moderate temperatures allow the processing of thermolabile molecules.

**Flexibility**

The flexibility of use of Prep-SFC is comparable to that of Prep-HPLC. Adjustment of operating parameters to a new feedstock and the start up of a system are simply and quickly performed in a few hours. Prep-SFC is thus well adapted to the type of batch purification often encountered in the pharmaceutical industry. Moreover, separations are not limited to binary separations and it is possible to remove a minor impurity or to isolate one or several compounds from a complex mixture.

**Absence or Reduced Use of Solvent**

When no modifier is used Prep-SFC can be considered as a ‘no-solvent’ process because carbon dioxide is used in most cases. The gas is recycled on-line thus there is no solvent consumption, no large storage necessity, no evaporation devices required for downstream processing and no problems with undesirable solvent traces left in the final product. Absence of solvent is also an advantage when it comes to the economics of large-scale separations (see below).

When a modifier has to be mixed with the supercritical main eluent (carbon dioxide), some, but not all, of these advantages are lost. The modifier is added to the eluent concentrations ranging from 0.1 to 25% (more often 1–5%). Thus, only a fraction of the eluent is not recycled on-line which means there is reduced solvent storage and a smaller evaporation plant than for Prep-HPLC. Moreover, the modifier used will very often be a single solvent (not a mixture) which simplifies its evaporation and recycling. The nature of modifiers most often used (methanol, ethanol, isopropanol) means that the toxicity problem of solvent traces left in the final product is

![Figure 2](image-url) Influence of eluent speed on efficiency. Comparison between high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC).
reduced. Finally, the economic advantage over Prep-HPLC is not completely lost because of the reduced quantity of solvent and its ease of recycling.

**Scale-up**

Scaling up a method developed in the laboratory can be made easily by multiplying the size of the system (flow rates, injected quantity, section of the column, and so on) by the scale factor. This is possible only if the packing material used is the same and the efficiency of the column is the same (this is possible even on very large columns by dynamic axial compression). Figure 3 gives an example of the scaling up possibility.

**Limitations**

Large and/or polar compounds have a limited solubility in pure carbon dioxide. As has been already mentioned, in this case it is then necessary to add a modifier to the supercritical eluent to enhance the solubilities. The number of applications amenable to Prep-SFC is greatly increased by the use of modifiers. However, some classes of compounds are still insoluble in carbon dioxide and modifier. An example is that some peptides can be dissolved and processed by SFC, but proteins are outside of the range of Prep-SFC.

Another limitation of Prep-SFC is economic. Investment costs for Prep-SFC are much higher than for Prep-HPLC. Savings on solvent consumption will counterbalance the difference in investment cost only for large- or very large-scale applications. For small- or medium-size applications, where both techniques are applicable, HPLC will often be more economical than SFC.

**Implementation**

Figure 4 shows a schematic flow diagram of a Prep-SFC. Typical pressures and fluid physical states are also indicated.

**Eluent tank**

The eluent tank (1) contains carbon dioxide under gas-liquid equilibrium at 4.5 MPa and 10°C. It is a buffer volume in the carbon dioxide loop and its volume is about twice the volume of the column. A molecule of carbon dioxide will pass around the system about 15 times per hour. The external carbon dioxide storage tank needs to be only 50 times the volume of the column for one week of operation (compared with the required storage of solvent in HPLC which is 600–1000 times the volume of the column).
Pumps

The carbon dioxide pump (2) is a reciprocating plunger- or membrane-type metering pump. It is a high-pressure pump equipped with one or multiple heads. Pumping is only efficient if the carbon dioxide is kept liquid on the suction side and in the pump head. In order to avoid cavitation it is necessary to cool the eluent tank (1), the suction line and the pump head. Since carbon dioxide is somewhat compressible, reliable pumping is difficult to achieve (the real flow rate differs from the nominal flow rate of the pump) and the efficiency of the pump depends on both inlet and outlet pressures and inlet temperature. Thus, it is better not to rely on the pump to provide a constant flow rate. In the example given the pump is used as a pressure source only, the column inlet pressure is regulated by a pressure regulator and the flow rate is regulated through the depressurization valve (6) at the column outlet. The modifier pump is an ordinary high-pressure liquid metering pump.

Injection Device

For production purposes injections must be made periodically and automatically. The main component of the injection device (3) is a high-pressure metering pump that introduces the sample either directly into the flow of supercritical eluent or into an intermediate injection loop.

Chromatographic Column

The column (4) is similar to Prep-HPLC columns equipped with a dynamic axial compression (DAC) system. A greater thickness of stainless steel is required to withstand the high pressures and a water jacket maintains the operating temperature of the column. Note that, unlike in Prep-HPLC, column outlet pressure is not atmospheric but very close to column inlet pressure (e.g. inlet pressure = 20 MPa and outlet pressure = 18 MPa).

Detector

As in analytical SFC, almost any type of detector (5) can be used (except refractive index detectors): ultraviolet (UV) absorption and so on. UV absorption detectors equipped with a high-pressure cell are used most commonly. Due to the high flow rates, detectors at column outlet must be placed in a split line.

Depressurization Valve

The depressurization valve (6) is a critical piece of equipment. Its function is to reduce the eluent pressure from the operating pressure (e.g. 18 MPa) down to the recycling line pressure (e.g. 4.5 MPa) and to control the flow rate of the eluent. Depressurization through the valve is almost adiabatic so that it is accompanied by an intense cooling and the fluid at the outlet of the valve is not gaseous but is a gas–liquid mixture (e.g. for carbon dioxide, at 4.5 MPa and 10°C). If the outlet pressure were atmospheric, the physical state of the carbon dioxide would not be a gas–liquid equilibrium but a gas–solid equilibrium (triple point pressure is 0.5 MPa) and the solid would cause random plugging of the tubes. Since the valve is placed at the column outlet, special attention must be given to its design so that it does not include dead volumes that would cause the remixing of the purified fractions.

Traps Lines

A trap line (7) isolates a purified fraction, separates it from the eluent and collects it at atmospheric pressure. There are several ways to achieve this. One configuration is presented in the example which includes a stop valve to select which line is opened when a fraction elutes from the column, a heat exchanger to completely vaporize the carbon dioxide and a cyclone to separate the gaseous carbon dioxide from the liquid sample (or sample dissolved in the modifier). Indeed, the separation cannot be done by a simple decantation, since, after the depressurization, the liquid fraction is highly divided and behaves like a fog that is carried away with the gas. The cyclonic separator is a device that uses the fluid speed to centrifuge it. It has no mechanical moving parts so it is cheap and simple; when properly designed, it can trap 97–100% of the liquid fraction. Also included is a depressurization stage to reduce smoothly the sample pressure from 4.5 MPa to atmospheric pressure.

The number of trap lines required depends on the application. For most industrial processes three to four lines are sufficient. These are a waste line, one or two pure products lines and a mixture line for a fraction requiring recycling (its composition is similar to that of the crude feedstock).

Eluent Recycling

The gas phase is recycled (8). Optionally it can be cleaned with a bed of adsorbents to stop the traces of sample that have not been removed by the cyclones. It is then condensed before being sent back to the eluent tank.

Automation

Although it is possible to imagine a manual version of a Prep-SFC in practice it is almost essential to
automate. Two pressures, two temperatures and one flow-rate must be controlled. In addition, high-pressure safety and periodic chromatographic functions (injection and collects) must be managed. Finally, data must be acquired and logged.

**High-pressure Safety**

Supercritical fluids are high-pressure compressible fluids which contain a high level of stored energy that can cause damage if it is abruptly released to atmosphere (unlike high pressure liquids which store little energy because they are not compressible). To prevent any problems associated with pressure release all components are built to withstand pressures much higher than the operating pressure and are certified by regulatory authorities. Safety valves are placed at all critical points in the system and a pressure switch (electromechanical) stops the pressure source (the pump) in case of overpressure. The pump may also incorporate a device to prevent it reaching pressures higher than the maximum operating pressure. Finally, sensors placed all around the system are automatically monitored to prevent any safety problems.

**Prep-SFC: What Scale?**

Table 1 gives examples of the production scales obtained with different column diameters. These are only typical figures and, depending on the application, actual figures can be bigger or smaller. Pilot or production Prep-SFC can be used for purification of hundreds of grams in a week or for tens of tons in a year as is shown in Tables 2 and 3.

### Table 1

<table>
<thead>
<tr>
<th>Column diameter (mm)</th>
<th>Column length (cm)</th>
<th>Typical loading capacity (g h(^{-1}))</th>
<th>Carbon dioxide flow rate (kg h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10–30</td>
<td>60</td>
<td>100–200</td>
</tr>
<tr>
<td>200</td>
<td>10–30</td>
<td>240</td>
<td>400–800</td>
</tr>
<tr>
<td>300</td>
<td>10–30</td>
<td>540</td>
<td>900–1800</td>
</tr>
<tr>
<td>500</td>
<td>10–30</td>
<td>1500</td>
<td>2500–5000</td>
</tr>
</tbody>
</table>

**Table 2**

- 3 g (per injection on a 100 mm internal diameter column)
- ×6 (injections per hour)
- ×24 (hours per day)
- ×4 (days per week)

= 1.7 kg per week

**Table 3**

- 250 g (per injection on a 500 mm internal diameter column)
- ×6 (injections per hour)
- ×24 (hours per day)
- ×300 (days per year)

= 10.8 t year\(^{-1}\)

**Variants**

**Working Modes**

Various working modes can be implemented in Prep-SFC. They include pressure and/or composition gradients and simulated moving bed SFC (SMB-SFC). Although these techniques may be useful in theory, they are rarely justified by practice and economics. Pressure or composition gradients can be used for selectivity enhancement and/or elution time reduction when the feedstock components have a large range of retention factors. However, with gradients, one of the advantages of Prep-SFC, the speed, is lost since time is required between the two injections to restore the initial pressure or composition, whereas in isocratic Prep-SFC, one can inject the next sample before the previous one is completely eluted.

SMB-SFC offers some of the theoretical advantages of its related technique SMB-HPLC. In SMB-SFC, the feed introduction and the product removal are continuous. The stationary phase is used more efficiently due to the countercurrent process. In SMB-HPLC the high complexity of the system is counterbalanced by savings on the mobile phase consumption, but this is not the case in SMB-SFC because the eluent is already recycled. However, in SMB-HPLC, the elution is necessarily isocratic while in SMB-SFC it is possible to create a pressure gradient in the system and, in some cases, this advantage can bring a decisive productivity increase.

**Operating Options**

The system described earlier is not the only possible way to implement Prep-SFC. For instance, one can
choose to regulate the flow rate by controlling the pump speed and to control the column outlet pressure by the depressurization valve. The carbon dioxide pressure in the traps can be reduced to atmospheric pressure and the condenser can be replaced by a compressor. It is also possible to add the modifier on the suction side of the main eluent pump.

Applications

Although the principle of Prep-SFC is well established, its commercial availability is relatively new so that there are only a limited number of published applications. One of the first and most studied applications with an industrial opening is the purification of $\omega$-3 unsaturated fatty acids from fish-oil extracts. More precisely, the ethyl esters of eico-sapentaenoic acid ($C_{22:5}$) and docosahexaenoic acid ($C_{20:6}$) are required at a relatively high purity. Pure carbon dioxide is used as an eluent. There is no difficulty in solubilizing the ethyl esters. Large-scale production is envisaged (tens of tons per year). The costing of the process shows that separation by Prep-SFC is two to five times cheaper than Prep-HPLC and the quality of the final product is better.

More recently, other applications have been patented among which one important example is the purification of cyclosporine (a cyclic undecapeptide) with carbon dioxide and an alcohol as modifier. Among the emerging applications under study many chiral separations are candidates to be performed by Prep-SFC. One separation example described below in more detail has been developed in the authors’ facilities.

Cis-trans Isomer Separation of Phytol

The separation of the cis- and trans-isomers of phytol has been chosen as an application of Prep-SFC using carbon dioxide and a modifier (isopropanol) as eluent (see Figure 5).

Phytol is a fatty alcohol and its trans-isomer is used in perfumery. The separation has been done on laboratory-scale equipment and has been extrapolated to industrial production. The main steps of the optimization procedure are given below.

**Figure 5** Molecular structure of phytol.

Stationary phase Apolar bonded phases and polymeric phases have been rapidly eliminated because of a lack of selectivity. A classical silica stationary phase was found to give the best separation.

Mobile phase Pure carbon dioxide will not elute the sample so it is necessary to add a modifier. Three alcohols have been tested: methanol (MeOH), ethanol (EtOH) and isopropanol (IPA); IPA gives the best selectivity (see Figure 6).

Overloading The first overloading trials have shown that the thermodynamics of the separation is governed by a concave upward adsorption isotherm (low concentrations migrate faster through the column than high concentrations) even for rather small loadings (10 $\mu$L on a 10 mm internal diameter column). This phenomenon can be clearly seen in Figure 7.

Temperature A rapid screening of operating temperatures in the range of 10–80°C shows the influence of this parameter (see Figure 8). The intermediate temperature (50°C) was chosen to maintain good resolution and short cycle time.

Pressure and modifier content A rapid screening of these two parameters led us to choose 25 MPa and 5.5% (w/w) of IPA in carbon dioxide.

Preliminary trial After establishing the optimum conditions above a preliminary injection was carried out to give the chromatogram shown in Figure 9.

The 4-min cycle time refers to the time elapsed between two successive injections. The total chromatogram duration is 6 min but it is not necessary to wait for the previous injection to be completely eluted before the next injection. With this overlapping injection procedure, the late eluted impurities of the first injection will elute together with the early eluted impurities of the second injection and a 50% increase in productivity is obtained in this particular case. This short cycle time is an advantageous characteristic of Prep-SFC compared to Prep-HPLC where cycle times are closer to 30 min.

Small-scale production On the basis of these results production has been carried out. Figure 10 shows the chromatogram of the repeated injections and Table 4 gives the results of this production step.

Extrapolation of these results to large-scale production can be made directly with a 300 mm internal diameter column and gives 2.3 t year$^{-1}$ production of the trans-isomer from 4 t of feedstock with a product purity of 97.8% and a recovery of greater than 80%.
Figure 6  Phytol purification; modifier selection. Stationary phase: silica 15 μm, 100 Å, AMICON; column: 10 mm x 250 mm; temperature 50°C; pressure 25 MPa; carbon dioxide flow rate 13.5 g min⁻¹; modifier: MeOH, EtOH or IPA at 0.5 mL min⁻¹ (2.9% w/w); sample: phytol mixture 10 μL.

Figure 7  Phytol purification. Peaks distortion due to overloading. Modifier: IPA; sample: crude phytol 10, 20, 40, 60, and 100 μL; other conditions as in Figure 6.

Figure 8  Phytol separation. Influence of temperature. Temperature: 10, 50 and 80°C. Phytol crude: 10, 60, and 100 μL; other conditions as in Figure 7.
Chromatogram preliminary to production test. Stationary phase: silica 15 μm, 100 Å, AMICON; column: 10 mm × 250 mm; temperature 50 °C; pressure 25 MPa; carbon dioxide flow rate: 13.5 g min⁻¹; modifier: IPA at 1 mL min⁻¹ (5.5% w/w); sample: crude phytol 60 μL = 50.5 mg undiluted; detection: UV absorption at 220 nm.

**Economics of Prep SFC**

The economics of a separation process depend greatly on the specific application. Indeed, the same Prep-SFC equipment can purify 1 kg per day or 50 kg per day depending on the cycle time, the loadability of the column, the solubility of the sample, the selectivity between the compounds in the sample, and the required purity and recovery. However, it is possible to give the main features of the cost of a separation by Prep-SFC and a reasonable range of costs.

Figure 11 shows a cost breakdown of a typical industrial separation using pure carbon dioxide and no modifier. The total purification cost for this example is about US$100 per kilogram injected. The range for such a case is US$20–200 per kilogram. One can see that the capital outlay is half of the total cost while the eluent cost (carbon dioxide) is negligible (2%). The high capital cost is a consequence of working at high pressure and the low cost of the eluent is a consequence of the total on-line recycling of a cheap solvent.

Figure 12 shows the cost breakdown of a typical industrial separation using carbon dioxide and modifier. The total purification cost for this example is about US$280 per kilogram injected. The range for such a case is US$40–400 per kilogram. By comparison with the previous example, the capital cost is slightly higher (an additional pump and bigger traps) and the recycling of the modifier represents 15–30% of the total purification cost. The other costs are similar.

The structure of these cost breakdowns can be compared with Prep-HPLC where the cost of the solvent losses and solvent recycling can represent 60% of the total purification costs. Since an increase in production scale is accompanied by a large decrease of the relative costs (per kilogram of product) except for the consumables (solvent and stationary phase), the purification costs by Prep-SFC and Prep-HPLC will have the tendencies shown in Figure 13 and the choice between Prep-SFC and Prep-HPLC depends on the scale of the purification envisaged. Large scales of production are highly favourable to Prep-SFC.

**Lab-Prep-SFC**

Lab-Prep-SFC is the purification of hundreds of milligrams or gram quantities. The column internal diameter is typically 10 or 20 mm and the carbon dioxide flow rate is between 10 and 60 g min⁻¹. It is

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**Table 4** Phytol separation—preparative step results

<table>
<thead>
<tr>
<th>Impurities</th>
<th>cis-Isomer</th>
<th>trans-Isomer</th>
<th>Mass of fractions (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>7.6%</td>
<td>34.2%</td>
<td>58.2%</td>
</tr>
<tr>
<td>Fraction 2</td>
<td>0.2%</td>
<td>99.3%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Fraction 3</td>
<td>0%</td>
<td>77.2%</td>
<td>22.8%</td>
</tr>
<tr>
<td>Fraction 4</td>
<td>0.2%</td>
<td>2%</td>
<td>97.8%</td>
</tr>
<tr>
<td>Fraction 5</td>
<td>60.6%</td>
<td>5%</td>
<td>34.4%</td>
</tr>
</tbody>
</table>

One gram of crude has been injected in 1.5 hours on a 10 mm ID column. 95% of the injected material has been collected in four fractions. The cis- and trans-isomers have been collected with high purities. Only 68 g of IPA were used for the production.
used for two different purposes: application studies preliminary to scale up (see the earlier example) and sample purification for research purpose.

The principle of Lab-Prep-SFC is the same as large-scale Prep-SFC but its technology is slightly different. It is possible, but not necessary, to recycle the carbon dioxide and the columns do not require dynamic axial compression and are slurry prepacked. A UV absorption detector can be installed on-line, and an automated injection device is required for repeated injections, but individual injection facilities by way of a manual injection loop are also necessary for evaluation study purposes.

The interest of Prep-SFC at this scale is not in terms of cost as we have seen that the effect of the savings on solvent is only sensible for large-scale applications. Users of Lab-Prep-SFC are mainly pharmaceutical laboratory researchers who find in SFC a way to reduce the quantity of solvents stored in their laboratory and who appreciate the speed of both method development and purification. Then, the interest of Prep-SFC is not measured in dollars per kilogram, but in the number of samples processed per week.

**Conclusions**

Prep-SFC is a powerful separation technique that gives high purities for difficult separations. The main advantages of this technique can be summed up by comparison with Prep-HPLC: it solves (or considerably reduces) the problems associated with solvents, it is economical at a large scale and fast at a small scale. Its limitations are the relatively high level of investment required and the restricted range of applications due to the low solubility of many biological macromolecules. Given this fact, one can conclude that the principal technical and economic domains of application of Prep-SFC are in the pharmaceutical industry and the high value compounds of the food and fine chemical industries. One can predict that Prep-SFC will not replace Prep-HPLC but should take 15–25% of its market share.

**Further Reading**


Theory of Supercritical Fluid Chromatography

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Introduction
The practising analyst requires of any separation technique that it should provide rapid and efficient resolution of mixtures. The relevant theory, therefore, is concerned with factors which influence chromatographic retention and resolution, and with variables influencing injection and detection.

The special properties of supercritical fluids make them unique chromatographic mobile phases, and the development of supercritical fluid chromatography (SFC) to make use of these properties has been discussed elsewhere. Here, the theory underlying SFC chromatographic parameters is described.

Retention in SFC
SFC is unique among chromatographic techniques in having four variables to change retention: temperature, density or pressure, and mobile-phase composition.

Effect of Column Temperature
The temperature, \( T \), at which an analysis is carried out has an important influence on retention. At constant pressure the retention factor, \( k \), may be either increased or decreased (Figure 1) by increasing the temperature, because of the combined influences of solubility in the stationary and mobile phases, and on vapour pressure, so that resolution may be tuned by adjusting \( T \). A rigorous expression which accounts for the variation of SFC retention with temperature at constant pressure is:

\[
\ln k - \ln \phi_m + \ln \left( \frac{\rho}{c^0} \right) = C + \frac{\Delta S^0}{R} - \frac{PV + \Delta H^0}{RT}
\]

[1] where \( \phi_m \) is the fugacity coefficient of the solute in the mobile phase, \( \rho \) is the density of the supercritical fluid, \( c^0 \) is the standard concentration and \( C \) is a constant. \( \Delta H^0 \) and \( \Delta S^0 \) are respectively the partial molar enthalpy and entropy of solution of the solute in the stationary phase.

The left-hand side of eqn [1] is obtained as follows: experimental values of \( k \); values of \( \rho/c^0 \) from the


Figure 1  Capacity ratios of pyrene as a function of column end pressure (\( P_e \)) and temperature (\( T \)). Three-dimensional network shown in two perspectives. Conditions: 25 cm \( \times \) 4.6 mm i.d. packed column, 10 \( \mu \)m silica particles; \( n \)-pentane. (Reprinted with permission from Lee ML and Markides KE (eds) (1990) Analytical Supercritical Fluid and Extraction. Provo, UT, USA: Chromatography Conferences, Inc.)