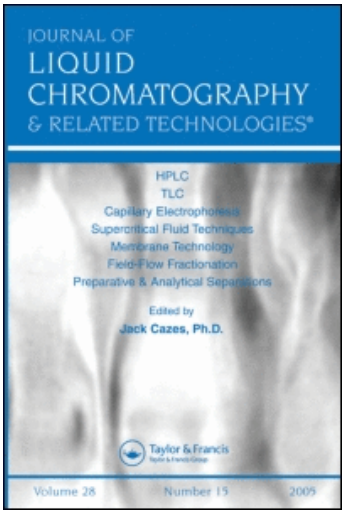


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# Principle and Applications of Supercritical Fluid Chromatography

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**Abstract:** One of the most challenging problems encountered during the development of a new pharmaceutical compound is linked to purification. The synthesis schemes are more and more complex and the request for rapid and efficient methods to isolate the target molecule from mixtures is crucial to ensure the success of the future drug.

Preparative chromatography is widely used at the developmental stages. The technology offers a perfect answer to the main challenge of the developers: "go fast and be efficient." The development of a chromatographic separation is easy at this stage; it is often possible to modify an analytical HPLC method to quickly isolate the purified product on a larger column.

At the production scale, preparative chromatography can also be applied. An optimization of the chromatographic conditions at an analytical scale (selection of stationary phase and solvent), together with the selection of the appropriate chromatographic process (discontinuous or continuous), lead to a cost effective, efficient, and robust industrial process. Preparative chromatography is applied at a very large scale for the purification of small organic molecules, peptides and proteins, carbohydrates, and chiral molecules under conditions required for pharmaceutical applications.

Even though preparative gas chromatography is successfully implemented for some applications at the industrial scale concerning low molecular weight and/or very volatile compounds, liquid eluents are by far the most common and competitive choice for the preparative chromatography process.

**Keywords:** SFC, Principles, Applications

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## INTRODUCTION

In the 60's, Klesper proposed the use of supercritical carbon dioxide for eluting a chromatographic column and developed the first supercritical fluid chromatography (SFC) equipment.[1] This development opened a new window for potential applications of preparative chromatography.

SFC primarily uses supercritical $CO_2$ as eluent. This compound has an acceptable critical pressure (73.8 bar) and its critical temperature is close to ambient conditions (31.1°C).[2]

The "solvent power" of a supercritical fluid is strongly linked to its density (controlled by pressure and temperature) and can also be adjusted by addition of an organic solvent (referred to as co-solvent) such methanol, acetonitrile, etc. This makes it possible to "tune" solvent properties for optimizing chromatographic separations. Due to the lower viscosity and higher diffusivity of supercritical fluids, compared to common solvents, a higher mobile phase velocity can be used in the column, leading to a higher process throughput, compared to liquid chromatography.

$CO_2$ can be easily removed from the purified product and recycled when decreasing the pressure of the collected fractions (the products are not soluble in the then gaseous $CO_2$). It reduces or, in some cases, eliminates the problem of organic solvent removal (and recovery) encountered with liquid eluents. It must also be stressed, that the use of $CO_2$ does not increase the greenhouse effect. It comes either from the chemical industry as a by product or from natural processes like beverage fermentation. $CO_2$, being a natural "ingredient" of the eco-system, is a "green", physiologically compatible solvent.

The commercial development of preparative SFC started in the 1980's.[3] This new technology already achieved some success. SFC will certainly not replace liquid chromatography, but supercritical $CO_2$ should certainly be considered as an alternative to classical liquid solvents when optimizing a chromatographic purification process.

## PRINCIPLE OF SFC

As shown in Figure 1, SFC can be considered as a cycle of the eluent around its critical point. First, the eluent (liquefied gas, for example $CO_2$) is compressed to the desired pressure, and adjusted to the required solvent power and separation selectivity (as a rule $P_c < P < 4P_c$, $P_c$ being the critical pressure).[4] Then, the compressed and heated eluent enters a chromatographic column which is maintained at the same temperature as the eluent. This temperature should be near the critical temperature $T_c$, for which a supercritical fluid exhibits its highest "tuneable" properties (significant changes in density and solvent power vs. pressure). The choice of the right elution

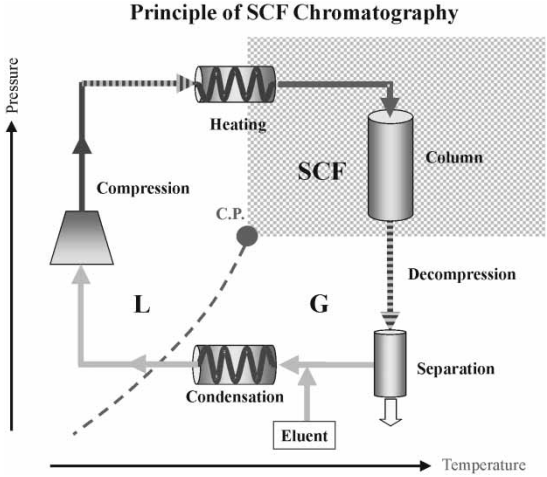


***Figure 1.*** Principle of chromatography with supercritical eluent.



pressure and temperature must be made carefully, because the variation of the solvent power modifies the retention of the products, and also the selectivity.

This is the reason why pure supercritical solvent ($CO_2$) is often mixed with a "classical" liquid co-solvent, which is also called "co-solvent" or "modifier" or "entrainer." A correctly chosen co-solvent can increase both the solvent power and the selectivity of chromatographic separations and strongly influences the solubilities of the products in the selected eluent.

The eluent leaving the column is then decompressed below its critical pressure and the supercritical solvent is transformed into a gas phase. For a separation with pure $CO_2$ eluent, the solvent power of the fluid drastically decreases, leading to solute precipitation and eluent-solute separation. When a co-solvent is used, the gaseous $CO_2$ is removed and the product is recovered in the liquid co-solvent. Gaseous eluent to be recycled is liquefied and is ready to be compressed again.

## SFC EQUIPMENT

### Solvent Delivery

Carbon dioxide, a typical fluid used for SFC, is delivered in high pressure cylinders for analytical and lab-scale instruments, or in high capacity tanks for larger units. Liquefied $CO_2$ inside the container is under the pressure corresponding to its gas-liquid equilibrium (about 57 bar at 20°C, see Figure 2).

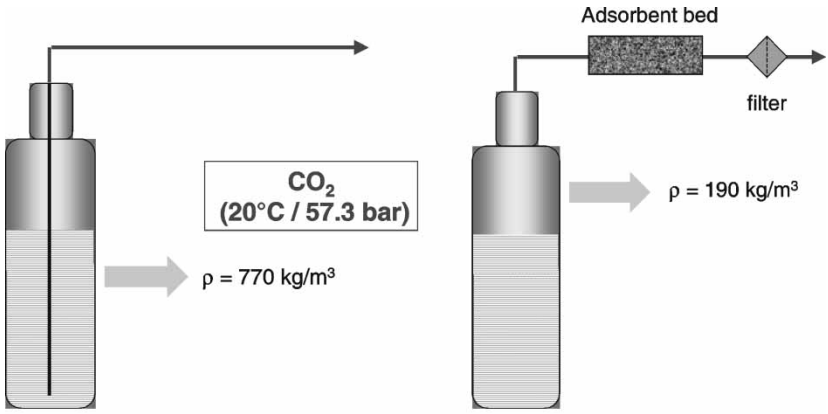


***Figure 2.*** $CO_2$ delivery systems: direct liquefied gas withdrawal and gas purification.



If the bottle is equipped with a dip tube, liquid $CO_2$ can be withdrawn without any significant pressure drop due to liquid phase evaporation. If not, at high $CO_2$ flow rates, there is a risk of freezing the bottle. Liquid modifiers used as polar additives in supercritical fluid chromatography are typically HPLC or analytical grade solvents.

## Pumping System

SFC systems require high pressure pumps. As a rule, piston or membrane pumps are used (except some analytical instruments operated with a syringe type of pump). Membrane pumps offer the advantages of zero leakage and contamination.

A typical solvent pumping system for SFC is presented in Figure 3. It consists of the main $CO_2$ pump and a supplementary pump for liquid

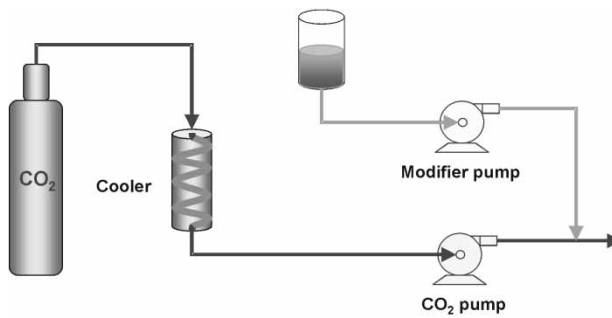


***Figure 3.*** Typical flow-sheet of eluent pumping system.

co-solvent. To avoid cavitation leading to a pumping capacity loss, pressure shocks, or even diaphragm damage, a sub-cooler is placed between the $CO_2$ source (even if it is a bottle with dip tube delivering liquefied gas) and the pump's suction valve.

## Feed Injection

Three different modes of injection can be applied (Figure 4):

a. Loop injection is a direct transposition of what is applied in analytical SFC. A low pressure feed pump is used to fill the loop. This is mostly recommended for preliminary tests of column performance and elution parameters.
b. An "in-line" injection mode is more versatile: the system offers a better flexibility for changing the injected volume. A high-pressure pump is required to inject the feed solution, but the injected stream is dissolved in the eluent flow.
c. The "in-column" injection mode is an alternative which permits injection of the feed solution directly onto the column, without any dilution.

The selected injection mode depends on the application, but the right selection is crucial to avoid injection profile distortion that may occur due



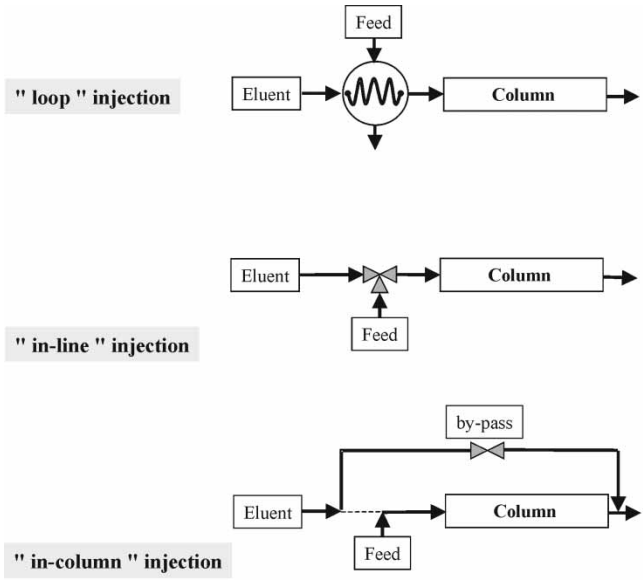


***Figure 4.*** Feed injection modes for NOVA*SEP* Super*Sep* systems.

to partial solute precipitation or to the injected solvent used to dissolve the feed.

## Chromatographic Column

Generally speaking, all HPLC stationary phases can be used for SFC. In addition, there can be more of SFC advantages compared to liquid eluent: significantly lower pressure drop, higher flow-rates, longer columns and/or small particle size, leading to improved separations.[5] There has been great progress in packed columns for supercritical fluid chromatography.[6,7]

## Detection

SFC is compatible with both HPLC and GC detectors:

a. On line pressure withstanding detectors
   - UV variable wavelength detectors
   - UV diode array detectors
   - Special chiral detectors

b. Off line low pressure detectors
   - Mass spectrometric detector
   - Light scattering detector
   - Flame ionization detector

A review of detectors applied to supercritical fluid chromatography has been reported by Chester and Pinskton.[8]

## Eluent/Solute Separation

It must be stressed that an instantaneous solute-eluent separation is a specific feature of SFC. In HPLC, as a rule, solute recovery and solvent regeneration are done in a separate downstream process.

As the solvent power of a supercritical fluid strongly depends on pressure, this effect is commonly used for solute recovery from the eluent stream. The transition from "excellent" to "poor" solvent state (compressed gas) results in solute precipitation. Under ideal conditions, an adiabatic decompression of supercritical $CO_2$ leads to the formation of a biphasic system, either liquid-gas (above triple point pressure of about 5 bar) or solid-gas below this pressure.

Typical decompression pressure is above 5 bar to avoid any dry ice (solid $CO_2$) formation. Because phase stratification is due to the difference in their densities, the efficiency of a separation can be strongly influenced by the operating temperature and pressure. Figure 5 shows the densities of gas and

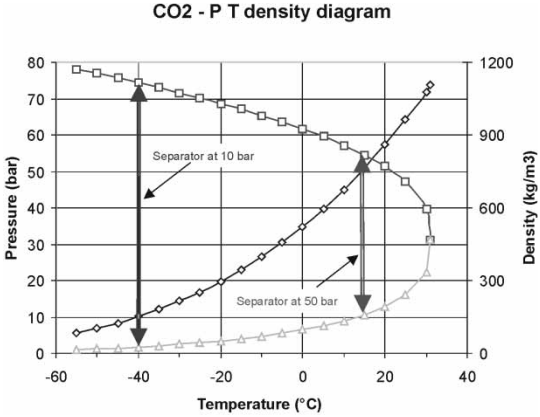


***Figure 5.*** Change in density of gas and liquid $CO_2$ vs. pressure and temperature.



coexisting liquid $CO_2$ for decompression (at 10 and 50 bar), performed under adiabatic conditions.

One can see, from Figure 5 that, due to a much higher difference in densities, the liquid and gas separation should be easier at low pressure.

Several systems are available to remove liquids and solids from a gas stream. A simple gravity settler requires a large vessel size to lower gas velocity; this leads to an excessive cost of the pressure system. It is not recommended if high separation efficiency is needed. In cyclonic separators (Figure 6), the centrifugal force acts on liquid droplets (aerosols) or solid particles as an "increased gravity." The solute containing gas stream enters via a tangential inlet, creating a vortex which causes the particles to migrate to the separator wall. The cleaned gas forms a vortex in the center of the cyclone, and leaves through a vortex finder at the top of the device. Generally, the cyclonic separators remove particles greater than 100 μm or

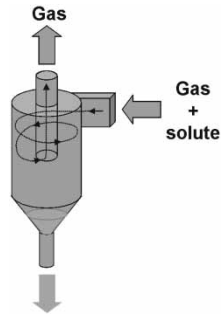


***Figure 6.*** Cyclonic separators.

even 10 μm.[9] Their performance strongly depends on cyclone sizing for the desired gas velocities (one can say that smaller cyclones capture smaller particles), but small cyclonic separators also have low capacity for solute storage and must be frequently drained to avoid reentrainment of the collected product by the gas vortex. A cyclonic separator that is designed specially for supercritical fluid applications has been patented.[10]

## Eluent Recycling

Except for analytical and small preparative instruments, eluent recycling after solute separation should be common practice. If not, $CO_2$ consumption can easily exceed 30 kg of liquefied gas per hour with a 50 mm i.D. column. In the following, we discuss gaseous fluid recycling and related problems.



Gas leaving the separators should be brought back into the same physical state and be at the same pressure as a fresh fluid delivered from the supply unit. Since most of the time liquid pumps are used in SFC equipment, gaseous eluent must be liquefied prior to recycling.

It is clear that $CO_2$ recycling is much more difficult when the solute-eluent separation is performed at low pressure; for example, at 10 bar, $CO_2$ condenses at $-40^{\circ}C$ instead of $+14^{\circ}C$ at 50 bar. To avoid cavitation phenomena, condensed $CO_2$ subcooling is strongly recommended. Our own experiences show that the temperature of the liquefied gas at the inlet of the eluent pump should be at least 5–10 degrees below its dew-point (about $-50^{\circ}C$ at 10 bar). Evidently, the cost of the cooling utility unit, operated under such cryogenic conditions is not negligible. It is not recommended to use compressors to increase the pressure from 10 bar to a higher value.

The preferred solution, easily adapted for SFC, is $CO_2$ recycling at a pressure of about 40–50 bar, slightly below the typical pressure inside the $CO_2$ cylinders stored at ambient conditions. Eluent recycling and condensation under the above pressure requires cooling utilities for a temperature range of $0-5^{\circ}C$ at a reasonable price. It is obvious that, under these conditions, the solute-eluent separation parameters must be carefully controlled to reach the highest efficiency of solute removal.

However, even the best designed separators and optimized working conditions cannot reach a 100% solute recovery. It should be stressed that the gaseous eluent leaving the separators *always contains traces of solute and non negligible amounts of co-solvent.* This means that the eluent has to be cleaned before recycling. The design of the cleaning system depends on the eluent composition and is different for pure $CO_2$ and $CO_2$ modified with a co-solvent.

## Cleaning $CO_2$ without Co-Solvent

Considering that the solute is not volatile, $CO_2$ leaving the separators may only contain some mechanically entrained droplets (aerosols) of product. Such droplets must be removed using an appropriate technique.

## Cleaning $CO_2$ with Co-Solvent (Liquid Modifier)

Cleaning of eluent containing co-solvent is not more complex than for pure $CO_2$, however, it is different. There are two kinds of co-solvent losses: mechanical and thermodynamic. Mechanical losses are due to the fact that some droplets of co-solvent are not trapped in the cyclones. Thermodynamic losses are related to the fact that the eluent leaving the separators contains co-solvent vapors. The theoretical value of the co-solvent vapor pressure can be easily calculated, according to the Gibbs law (see data for ethanol, Figure 7).

As can be seen, the content of volatile co-solvent in $CO_2$ depends considerably upon temperature and, to a lesser extent, upon pressure. In the case of ethanol as a co-solvent, under correctly chosen separation conditions, the thermodynamic loss into compressed $CO_2$ (from 10 to 50 bar) does not exceed 0.5%. As the retention times in chromatography are usually very sensitive to the co-solvent content in the eluent, this parameter has to be carefully controlled. A cleaning device for $CO_2$-liquid solvent systems has been patented[12] and proven experimentally.[13]



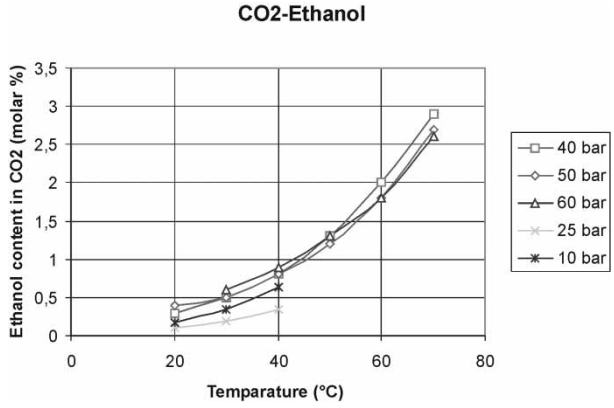


***Figure 7.*** Thermodynamic losses of ethanol vs. separator operating conditions.[11]

## COMPARISON WITH HPLC

HPLC is still the method of choice for chromatographic purifications.

In many practical situations in preparative chromatography, the productivity of the unit is limited by feed solubility in the mobile phase. For many SFC applications, the solvent power of supercritical $CO_2$ is limited and only low polarity compounds are soluble at acceptable levels. The problem can be partially overcome by the addition of a polar co-solvent such a methanol. However, in certain cases, 20% of the co-solvent, or more, is necessary to reach acceptable levels of solubility. Obviously, this fact decreases the advantage of the use of supercritical fluid $CO_2$, often claimed to be performed without use of organic solvents. Additionally, increasing co-solvent content complicates the separation and product recovery processes.

The HPLC technique also remains the method of choice for reversed phase separations, due to the non-polarity of $CO_2$

As yet, SFC remains in the shadow of HPLC, although the merits of SFC, especially in the pharmaceutical industry, are becoming increasingly recognized from day to day, due to the potential benefits of supercritical eluents compared to liquid ones.



It is often said that SFC is 3–5 times faster than HPLC. Low viscosity, together with increased diffusivity, lead to much shorter retention times and increased productivity for supercritical fluid chromatography, as opposed to classical HPLC, because the mobile phase velocity can be significantly larger. Also, under supercritical conditions, the chromatographic column is equilibrated in a few minutes instead of hours. Additionally, longer columns, packed with smaller particles, can be used in contrast to HPLC.

Retention in SFC separations is more easily adjustable than in HPLC. Pressure and co-solvent addition are the most frequently evaluated parameters for the screening of optimal operating conditions. The temperature effect in SFC is stronger, compared to HPLC, which means that temperature can often be used to fine-tune selectivity.

SFC also offers a much wider choice of mobile phases. For example, there is no problem to make a supercritical homogenous eluent containing a mixture of methanol + heptane, which are immiscible under HPLC conditions.

SFC is more flexible with respect to suitable detector choices. All HPLC detectors equipped with high pressure cells, but also many common GC detectors, can be used, either “on line” or “off line,” to analyze column effluent composition.

### Continuous Chromatography with Supercritical Eluent

It is commonly recognized, in the field of liquid chromatography, that the advantage of continuous chromatography, compared to elution

chromatography, is the higher process throughput and system robustness. This fact is also true when supercritical eluents are used.

A continuous SFC unit (Figure 8) has been built at NOVA*SEP* labs in Nancy, France, including eight columns of 20 cm bed length and 3.3 cm internal diameter.[11,14] This system can be operated with a uniform pressure or with a pressure gradient (for productivity improvement).

The recycling of eluent from zone 4 to zone 1 is performed by an external loop. The Extract, Feed, Raffinate and Recycling streams are controlled by a loop consisting of a flowmeter and a regulating valve. The eluent flow is adjusted automatically by adjusting the pressure at the inlet of zone 1. This simple method always fulfills the mass balance of the unit for any internal pressure and shortens the time required to adjust the pressure in the different sections of the system after the periodic switching of the inlet/outlet ports. The injection of modifier into the inlet ports (eluent and feed) is performed by one HPLC pump; another HPLC pump allows for injections of solute to be made into the feed stream.

The "tuneable" properties of a supercritical solvent are particularly attractive in continuous processes, where each of the four zones of the unit plays a different role:

a. in zone 1, the most retained component has to be desorbed;
b. in zone 2, the less retained component has to be desorbed;
c. in zone 3, the most retained component has to be adsorbed;
d. in zone 4, the less retained component has to be adsorbed.

These zones, therefore, require different elution strengths. In the isocratic pressure mode, as in liquid chromatography, the mobile phase flow rates are

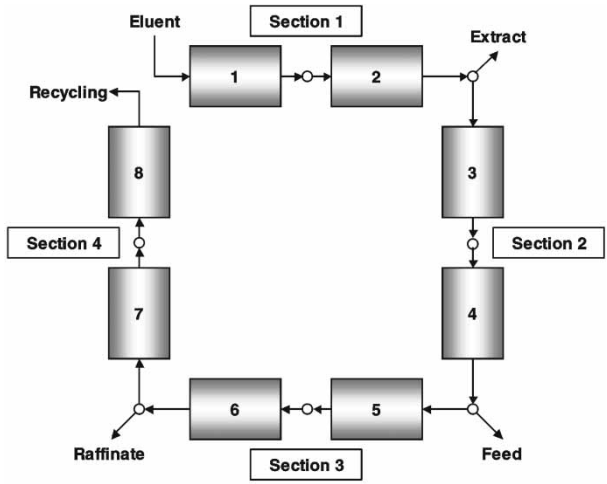


***Figure 8.*** Scheme of a four-zone, eight-column 2/2/2/2 SF-SMB Unit.

adjusted in each zone to fulfill these rules.[15] Under linear and non linear conditions, the adsorption of a solute tends to decrease as the supercritical eluent density increases. From these observations, it can be concluded that a decreasing pressure gradient along the four sections of the continuous system, operated under supercritical conditions, leads to a decreasing elution strength and can, therefore, improve the performance, compared to a system operated at constant pressure. This has been verified experimentally and theoretically for the chiral resolution of $\alpha$-tetralol.[16]

## APPLICATIONS OF SFC

After the pioneering work of Klesper in 1962,[1] the main applications of SFC focused on analytical chromatography. The main objective was to use the specific properties of the supercritical fluid to improve peak resolution (by improvement of mass transfer) and to speed up analysis.

Among the numerous published applications of preparative SFC, the most important issues are listed below:

a. The most studied application of SFC is probably the separation of unsaturated fatty acids.[17–20] Pure $CO_2$ is perfectly suited for the separation of this class of compounds (low polarity). Production at a large scale has even been announced[21] for this application, where the separation cost of prep-SFC appears to be lower than the cost of prep-HLPC.
b. Cyclosporin: Prep-SFC appears to be more efficient than Prep-HPLC to purify Cyclosporin.[22] The separation has been patented.[23]
c. Phytol: the separation of cis-trans isomers has been developed, demonstrating that Prep-SFC can be cheaper then Prep-HPLC at a large scale.[24]
d. Enantiomers: Prep-SFC appears to be a promising technique for chiral resolution. The stationary phase used for liquid chromatography can be used. High productivities are obtained in certain cases.[25–27]

The flexibility and short development time required with SFC is often highlighted and lab-scale systems are often considered as an attractive tool, at the developmental stages, for the rapid purification of new substances.[28]

Application of continuous processes (SF-SMB) has also been published for the separation of fatty acids ethyl esters,[29] the purification of phytol,[30] the separation of bi-naphthol enantiomers,[31] or the chiral resolution of $\alpha$-tetralol.[16] The interest of a pressure gradient has been demonstrated experimentally.

### Example

The specific behaviour of supercritical fluid eluent is illustrated with the separation of trans-stilbene oxide (TSO) racemate.

The objective is to show the development of the separation using an analytical SFC system and the scale up results obtained on a pilot unit.

The separation is developed on the chiral stationary phase, Chiralcel OD 20 μm (Chiral Technologies Europe, France). An organic modifier (isopropanol) is used to increase the polarity of the eluent in order to achieve an acceptable retention of the two enantiomers.

The separation is developed on an analytical SFC system (Series SF3 Gilson System) with a 4.6 × 250 mm analytical column packed with the chiral stationary phase Chiralcel OD.

## Selection of the Chromatographic Conditions

The influence of the organic modifier, the operating temperature, and pressure are studied with the analytical equipment.

The change of retention time and selectivity with adjustment of the eluent composition (percentage of isopropanol) is presented in Figure 9. The back pressure of the column is set at 80 bar and the temperature is 20°C.

The organic modifier tends to increase the polarity of the eluent and is perfectly miscible with $CO_2$. The situation is identical to that which is observed with a liquid eluent under the present normal phase conditions: retention decreases with increasing percentage of IPA.

The influence of the eluent temperature is presented in Figure 10. Increasing temperature tends to increase retention. This behavior is not usually observed in liquid chromatography, considering the thermodynamics of the retention phenomenon described by the Gibbs equation. However,



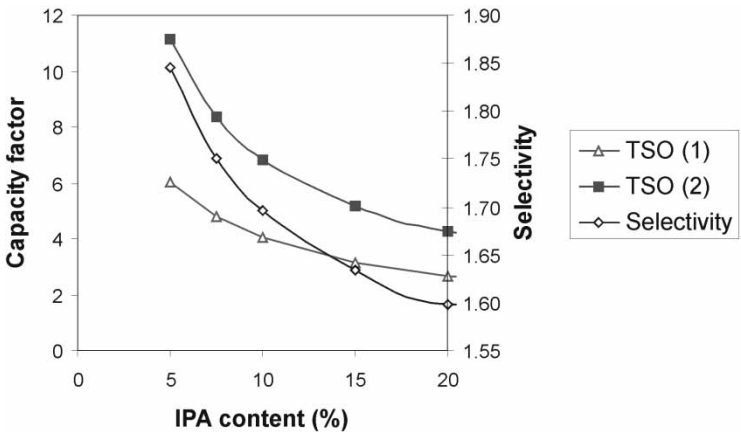


***Figure 9.*** Influence of %IPA on capacity factor $\overline{K} = ({}^{\varepsilon}ext/1 - {}^{\varepsilon}ext)((t_r - t_o)/t_o)$ and selectivity $\alpha = \overline{K}_2/\overline{K}_1$ of TSO isomers; back pressure = 80 bar, temperature = 20°C.

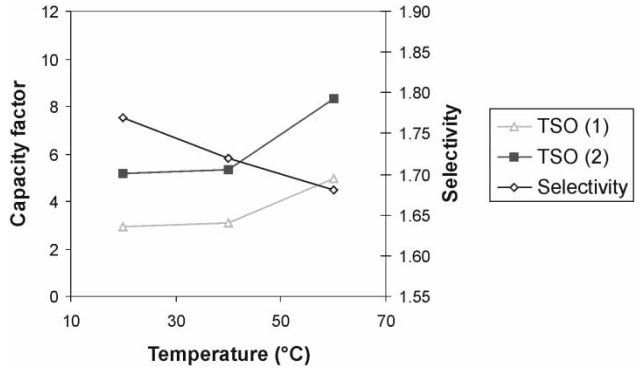


***Figure 10.*** Influence of temperature on retention and selectivity; back pressure = 80 bar, % IPA = 10%.



temperature has a antagonistic effect in SFC as the fluid density tends to decrease with temperature; this reduces the solvent power and tends to increase retention.

Although pressure has no effect on the eluent strength when liquid solvents are used, under supercritical conditions, however, the solvent density increases strongly with increasing pressure. This offers an additional degree of freedom when selecting the chromatographic conditions for the process. The influence of the column back pressure is illustrated in Figure 11. The influence of pressure decreases as the amount of co-solvent increases, but a significant effect is still observed, even with 10% of IPA.

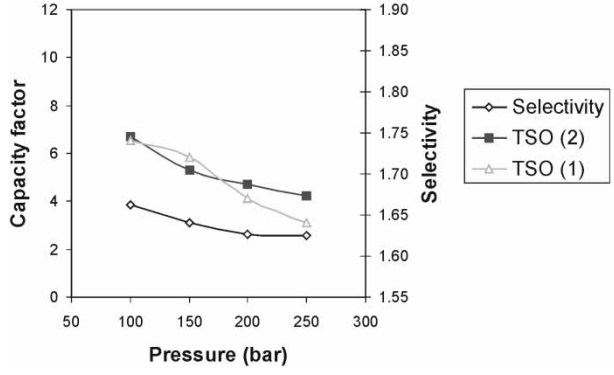


***Figure 11.*** Influence of pressure on retention and selectivity; Temperature = 20°C and % IPA = 10.

## Optimization of the Process Throughput

The optimization of a preparative process not only requires achieving good resolution between the two compounds, but also optimization of the injected quantity.

In the present case, the TSO racemate is injected in pure IPA (concentration = 31.7 g/L at 20°C).

The maximum injected amount is directly linked to the loading capacity of the stationary phase. Figure 12 shows chromatograms obtained when increasing the injected amount on the analytical column. A classical Langmuirian behavior is observed for the adsorption of the TSO enantiomers.

Considering that both enantiomers have to be purified with the maximum yield, the highest acceptable volume is 300 μL on the analytical column.



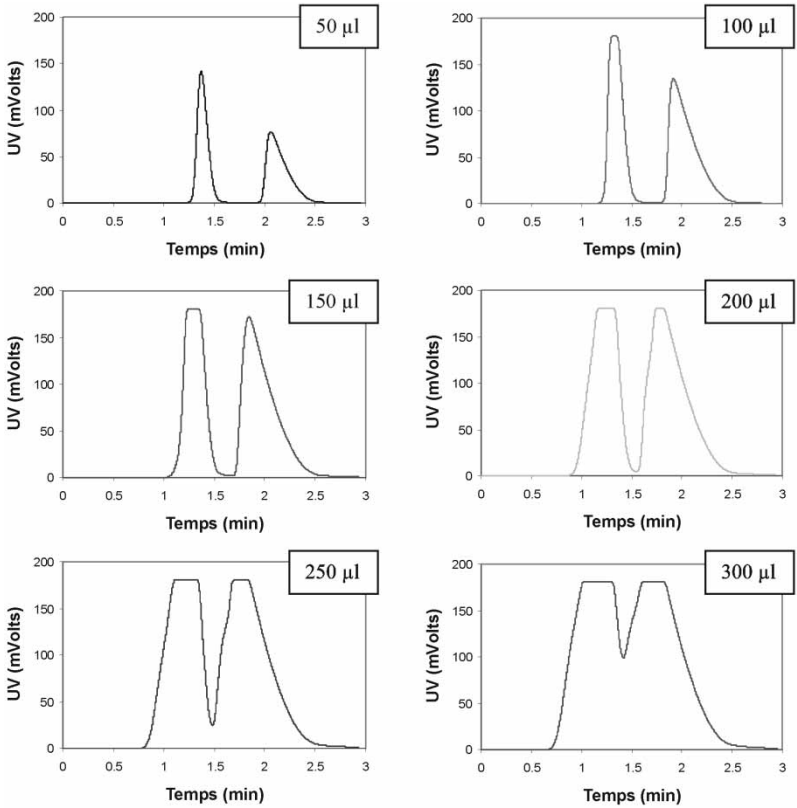


***Figure 12.*** Effect of the injected quantity (Chiracel OD, 20 μm: 20°C/110–80 bar eluent flow rate = 6.7 ml/min, 11% IPA (v/v), feed concentration = 31.7 g/L, equivalent to 5.9 g/min, 10 wt.% IPA.

The process daily throughput is linked to both the injected amount per run and the time between two successive injections. This time has to be minimized (e.g., by stacked-injections) in order to optimize the process productivity.

The minimum time between two successive injections corresponds to the duration for eluting the two enantiomer peaks. Under the selected conditions, this time is equal to 100 seconds.

## Scale-Up to a Pilot SFC Unit

Provided that the process technology is mastered to ensure an efficient scale-up, analytical SFC units are perfectly suited for optimizing the chromatographic conditions to maximize the process throughput.

The separation of TSO, developed on an analytical system, is extrapolated to a pilot unit equipped with a 5 cm i.d. DAC column (SUPER*SEP* 50, from NOVA*SEP*, Pompey, France). The unit can be operated at a maximum $CO_2$ flow rate of 1 kg/min. Up to 30% of co-solvent can be added. Four cyclonic separators are available to recover the collected fractions. A recycling loop is integrated into the equipment to minimize the required amount of $CO_2$.

A picture of the SUPER*SEP* 50 unit is shown in Figure 13. A better resolution of the peaks has been observed on the preparative system,

***Figure 13.*** Pilot SFC unit: SUPER*SEP* 50.

compared to the analytical system. Thus, using a shorter column, the cycle time has been reduced and the injected volume has been increased. This small discrepancy can be explained by the fact that the analytical system is controlled by volume flow rates and the preparative system is controlled by mass flow rates; then, composition of co-solvent and column internal velocity may be slightly different. Table 1 presents the scaled up operating parameters.

Figure 14 presents the superimposed chromatograms for successive injections on a SUPER*SEP* 50 unit (NOVA*SEP*, Pompey France).

The purified enantiomers are recovered in the cyclonic separators. The purity obtained for both enantiomers exceeds 99%. The recovery yield (mass recovered in the cyclone/injected amount) is at least 94%. This result demonstrates the performance of the chromatographic column together with the efficiency of the cyclonic separators for recovering the purified products.

$CO_2$ is removed in the cyclones and the collected purified enantiomers are directly recovered in the organic co-solvent.

It is worth mentioning that the dilution factor is much less than what it would be with liquid chromatography: the two purified enantiomers are collected at a concentration of 12 and 11 g/L, respectively.

This example illustrates the potentials of SFC:

a. The chromatographic separation is developed just like a classical liquid chromatographic process, i.e., selection of the right eluent composition, adjustment of the separation temperature, optimization of the injected amount. SFC, however, possesses an additional parameter for process optimization, i.e., the average column pressure, which directly influences the solvent polarity and solubility properties.
b. The results obtained at the analytical scale can easily be extrapolated to preparative size. No specific adjustment is required, provided adequate technology is used at the preparative scale, particularly as far as the column is concerned.

***Table 1.*** Process scale up from analytical to 5 cm i.d. DAC column

| | Analytical column | SUPER*SEP* 50 |
|---|---|---|
| Column length (cm) | 25 | 20 |
| Column diameter (mm) | 4.6 | 50 |
| Injected volume (mL) | 0.3 | 32 |
| Cycle time (sec) | 100 | 80 |
| Eluent flowrate (g/min) | 5.9 | 700 |
| Column pressure drop (bar) | 30 | 25 |
| Column outlet pressure (bar) | 80 | 80 |

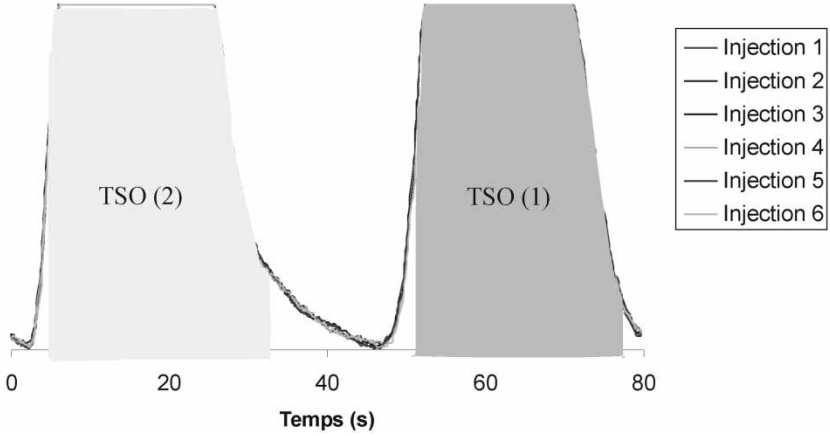


***Figure 14.*** Preparative chromatograms for 6 successive injections.



c. The purified products are recovered in the cyclonic separators. $CO_2$ is easily removed and recycled in the process and the products are recovered with a high collection yield and a low dilution in the organic modifier used as the co-solvent.

Compared to Prep-HPLC, Prep-SFC requires more sophisticated technology, but it brings significant advantages. $CO_2$ recycling drastically reduces $CO_2$ consumption.

## CONCLUSION

SFC was developed in the 1960s, and some commercially available instruments were introduced to the market in the beginning of the 1980s. However, after a period of great enthusiasm, when users found that instruments were difficult to operate, and poorer performance than HPLC were obtained, the interest in SFC considerably dropped.

There is, nowadays, increasing demand for new, environmentally friendly processes, trends to banish some classes of solvents (ozone depletion), to prevent or reduce the direct and indirect effects of emissions of volatile organic compounds (VOCs) in the environment and on human health. All these reasons favor the use of SFC.

Considering the numerous advantages of Prep-SFC, especially for rapid chiral separations of pharmaceutical compounds, its revival in the near future is very likely. The systems proposed by specialized companies will be more and more innovative and with high performance.

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