

A Review Article on Supercritical Fluid Chromatography

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ABSTRACT

Supercritical Fluid Chromatography (SFC) is a form of normal phase chromatography, first used in 1962. SFC typically utilizes carbon dioxide as the mobile phase; therefore the entire chromatographic flow path must be pressurized. Because the supercritical phase represents a state in which liquid and gas properties converge, supercritical fluid chromatography is sometimes called "convergence chromatography." Supercritical fluid chromatography is one of the most important column chromatography methods after gas chromatography (GC) and high-performance liquid chromatography (HPLC). Supercritical fluids combine useful properties of gas and liquid phases. The characteristic properties of a supercritical fluid are density, diffusivity and viscosity. SFC, the sample is carried through a separating column by a supercritical fluid where the mixture is divided into unique bands based on the amount of interaction between the individual analytes and the stationary phase in the column. As these bands leave the column, their identities and quantities are determined by a detector. SFC is a hybrid of gas and liquid chromatography because when the mobile phase is below its critical temperature and above its critical pressure, it acts as a liquid, so the technique is liquid chromatography (LC) and when the mobile phase is above its critical temperature and below its critical pressure, the instrumentation that is required for supercritical fluid chromatography is versatile because of its multi-detector compatibility. SFC has been applied to wide variety of materials including natural products, drugs, foods, pesticides, herbicides, surfactants, polymers and polymer additives, fossil fuels, petroleum, explosives and propellants.

Keywords: Critical pressure, critical temperature, supercritical fluid, diffusivity

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1. INTRODUCTION

Supercritical Fluid Chromatography (SFC) is a form of normal phase chromatography, first used in 1962, that is used for the analysis and purification of low to moderate molecular weight, thermally labile molecules. It can also be used for the separation of chiral compounds. Principles are similar to those of high performance liquid chromatography (HPLC), however SFC typically utilizes carbon dioxide as the mobile phase; therefore the entire chromatographic flow path must be pressurized. Because the supercritical phase represents a state in which liquid and gas properties converge, supercritical fluid chromatography is sometimes called "convergence chromatography."

Supercritical fluids (SF) have densities and dissolving capacities similar to those of

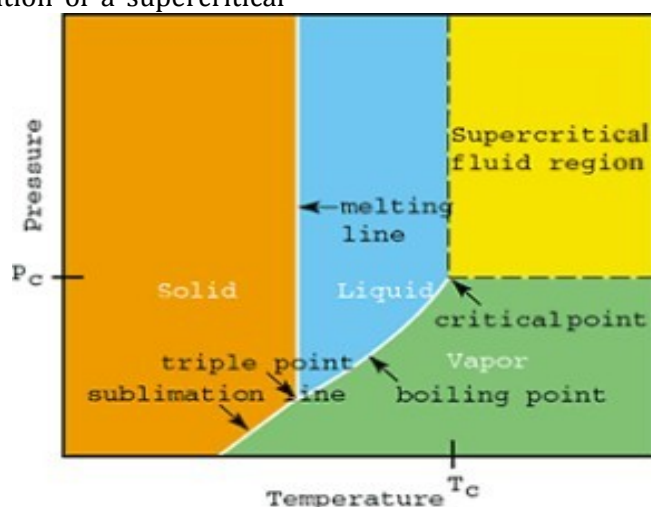
certain liquids, but lower viscosities and better diffusion properties. Accordingly, SF used as mobile phases in chromatography should act both as substance carriers like the mobile phases in gas chromatography (GC) and also dissolve these substances like the solvents in liquid chromatography (HPLC). This chromatographic variant is known as supercritical fluid chromatography (SFC).

They described in 1962 the separation of thermo-labile porphyrin derivatives using supercritical chlorofluoromethanes at pressures up to 140 bar and temperatures from 150 to 170°C. This method was further developed both theoretically and experimentally later by other workers in the 1960s. Unfortunately, the development of SFC during this period was not

comparable with the tempestuous growth of HPLC which occurred at about the same time. The initial major growth period for SFC, thus, occurred approximately 20-year later in the 1980s.

A supercritical fluid is the phase of a material at critical temperature and critical pressure of the material. Supercritical fluids combine useful properties of gas and liquid phases. Their behaviors are near gas from some aspects and near liquid in terms of different features. A supercritical fluid provides a gas-like characteristic when it fills a container and it takes the shape of the container. The formation of a supercritical

fluid is the result of a dynamic equilibrium. When a material is heated until its specific critical temperature in a closed system, which means at constant pressure, a dynamic equilibrium is generated. This equilibrium includes the same number of molecules coming out of liquid phase to gas phase by gaining energy and going in to liquid phase from gas phase by losing energy. At this particular point, the phase curve between liquid and gas phases disappears and supercritical material appears. For definition of SF, phase diagram is used.



A phase diagram shows the fields where the material is in the form of solid, liquid and gas in terms of different temperature and pressure values. Some curves, where two phases (solid-gas, solid-liquid and liquid-gas) exist together, defines the boundaries of the phase regions. These curves, for

example, include sublimation for solid-gas boundary, melting for solid-liquid boundary, and vaporization for liquid-gas boundary. Other than these binary existence curves, there is a point where all three phases are present together in equilibrium; Triple Point (TP).

Table 1: Comparison of densities, viscosities and diffusivities for liquid, supercritical fluid and gas

State	Density (g/ml)	Viscosity (poise×10)	Diffusivity (cm ² /s×10 ³)
Liquid	0.8-1	3-24	0.005-0.02
Sup.fluid	0.2-0.9	0.2- 1	0.01-0.3
Gas	0.001	0.05-0.35	10-1000

2. Physical Properties of Supercritical Fluids

2.1. Density

Density characteristic of a supercritical fluid is between a gas and a liquid as near liquid. In the supercritical region, density of a supercritical fluid increases when pressure rises at a constant temperature. When

pressure is constant, density of the material decreases with increasing temperature. Dissolving effect of a supercritical fluid is dependent on its density value. Also, supercritical fluids are better carriers than gases thanks to their higher density. Therefore, density is an essential parameter for analytical techniques using supercritical fluids as solvents.

2.2. Diffusivity

Diffusivity of a supercritical fluid can be 100 times more than a liquid and 1,000-10,000 times less than a gas. Because supercritical fluids have more diffusivity than a liquid, a solute can show better diffusivity in a supercritical fluid than in a liquid. Diffusivity is parallel with temperature and contrary with pressure. Increasing pressure affects supercritical fluid molecules to become closer to each other and decreases diffusivity in the material. The greater diffusivity gives supercritical fluids the chance to be faster carriers for analytical applications. Hence, supercritical fluids play an important role for chromatography and extraction methods.

2.3. Viscosity

Viscosity for a supercritical fluid is almost the same to a gas and it is 10 times less than a liquid. Thus, supercritical fluids are less resistant than liquids towards the components flowing through themselves. The viscosity of supercritical fluids distinguish from liquids that temperature has a little effect on liquid viscosity while it can influence supercritical fluid viscosity in a considerable way. These three major properties are related to each other. The change in temperature and pressure can affect all of them in different combinations. For instance, increasing pressure causes a rise for viscosity and rising viscosity results in declining diffusivity.

3. Supercritical Fluid Chromatography (SFC)

SFC is the third column chromatography technique after HPLC and GC. SFC can be more advantageous than HPLC and GC when we analyze the compounds which are decomposed at high temperatures with GC and do not have functional groups to be detected by HPLC detection systems. SFC enables change of some properties during the chromatographic process. This tuning ability brings an advantage to optimize the analysis. Also, SFC have a broader range of detectors than HPLC. There are three major quality for column chromatographies:-

- Selectivity
- Efficiency
- Sensitivity

GC much better in terms of efficiency and sensitivity. HPLC is better at selectivity owing to changeable mobile phases and various stationary phases. Although SFC is not good enough in selectivity as much as HPLC, it has a good quality in terms of sensitivity and efficiency.

SF used as mobile phases in chromatography should act both as substance carriers like the mobile phases in gas chromatography (GC) and also dissolve these substances like the solvents in liquid chromatography(HPLC).

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4. Instrumentation for SFC

SFC has a quite similar setup to HPLC instruments in terms of apparatus. Especially, they use similar stationary phases with similar column types. there are some differences. Temperature is critical for supercritical fluids, so there should be a heat control tool in the system like GC has. Also, there should be a pressure control mechanism, a restrictor, because pressure is another essential parameter for supercritical fluid materials to be kept at the required level.

4.1 Pumps

high-pressure pump used in SFC is determined by the column type. For packed columns, reciprocating pumps are generally used while for capillary SFC, syringe pumps are most commonly employed. Reciprocating pumps allow easier mixing of the mobile phase or introduction of modifier fluids. Syringe pumps provide consistent pressure for a neat mobile phase.

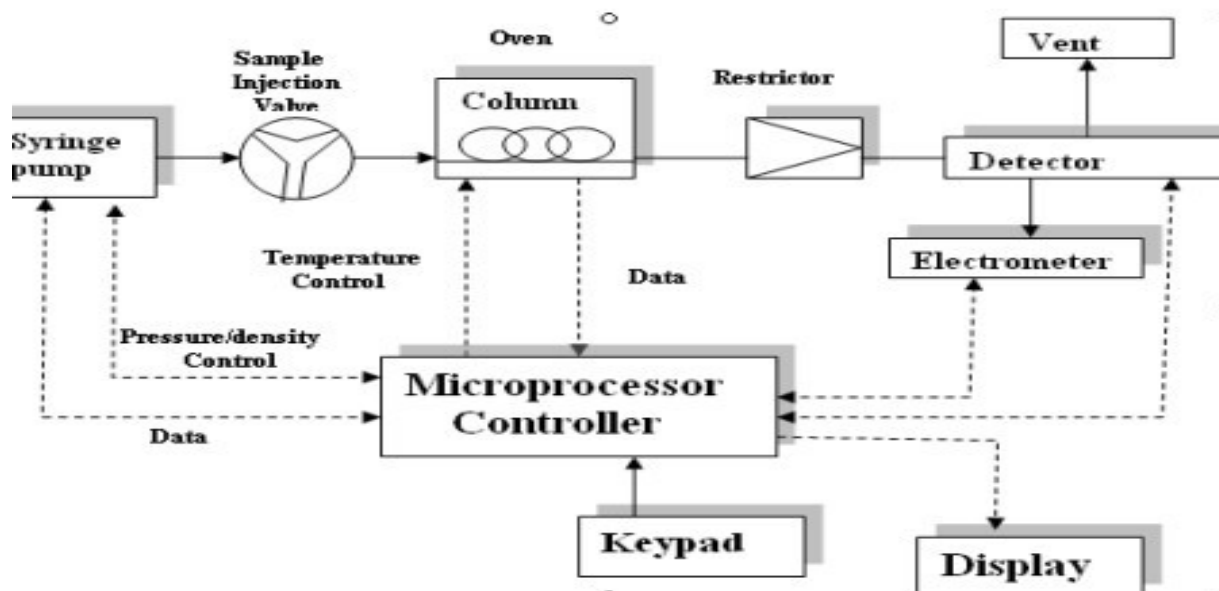
4.2 Injector

Injection in SFC is usually achieved by switching of the content of a sample loop into the carrier fluid at the column entrance by means of a suitable valve. For packed column SFC, a conventional HPLC injection system is adequate, but for the capillary column SFC, the sample volume depends on column diameters and small sample

volumes must be quickly injected into the column, therefore pneumatically driven valves are used.

4.3 Oven

A thermo stated column oven is required for precise temperature control of the mobile phase. Conventional GC or LC ovens are generally used.



4.4 Columns

The strong solvating abilities of mobile phase in SFC makes the careful selection of stationary phases imperative. Basically two types of analytical columns are used in SFC, packed and capillary. Earlier work employed absorbents such as alumina, silica or polystyrene or stationary phases insoluble in SC-CO₂. More recent packed column work has involved bonded non-extractable stationary phases such as octadecylsilyl (C₁₈) or aminopropyl bonded silica.

SFC columns are pretty similar to HPLC columns in terms of coating materials. there are two types of column used in sfc.

- Open tubular columns
- packed columns

Open tubular

Ones are preferred more and they have similarities to HPLC fused-silica columns. This type of columns contain internal coating of cross-linked siloxane material as stationary phase. The thickness of that coatings can be 0,05-1 μm. The length of that columns can be in the range of 10-20 m.

Packed Columns

Similar to HPLC columns (10, 5, or 3 μm porous particles) Silica based chemically bonded phases Typically 10 cm long X 4.6 mm.

4.5 Restrictor or Back-Pressure Device

This is a device, which is used to maintain desired pressure in the column by a pressure-adjustable diaphragm or controlled nozzle so that the same column-outlet pressure is maintained irrespective of the mobile phase pump flow rate. It keeps the mobile phase supercritical throughout the separation and often must be heated to prevent clogging. The pressure restrictor is placed either after the detector or at the end of the column.

A typical restrictor for a 50 or 100 μm open tubular column consists of a 2-10 cm length of 5-19 capillary tubing attached to the column. Alternately the restriction may be integral part of the column formed by drawing down the end of the column in the flame.

4.6 Microprocessor

The commercial instruments for SFC are ordinarily equipped with one or more

microprocessors to control such variables as pumping pressures, oven temperature and detector performance.

4.7 Detector

SFC utilizes mobile phases, which can either be liquid like or gas like. Therefore it is compatible with both HPLC and GC detectors. Conventional gas-phase detectors such as flame ionization detectors and flame photometric detectors, liquid-phase detectors like refractive index detectors, ultraviolet-visible spectrophotometric detectors and light scattering detectors have been employed for SFC. Mass spectrometry and fourier transform infrared spectrometry can also be used effectively with SFC. The choice of detectors will depend upon the mobile phase composition, column type, flow rate and ability to withstand the high pressures of SFC. One of the biggest advantage of SFC over HPLC is regarding detectors. Flame ionization detector, which is normally present in GC setup, can be applied to SFC. This detector can contribute to the quality of analyses of SFC with its good features.

FID is a highly sensitive detector. It is not a trouble maker and this is critical for analytical processes. SFC can be coupled with mass spectrometer, ultraviolet spectrometer, infrared spectrometer more easily than HPLC. Some other detectors which are used with HPLC can be attached to SFC such as fluorescence emission spectrometer or thermionic.

4.8 Mobile phases

There is a wide variety of materials to be used as mobile phase in SFC. Mobile phase can be selected from the solvent groups of inorganic solvents, hydrocarbons, alcohols, ethers, halides; or can be acetone, acetonitrile, pyridine etc.

The most common supercritical fluid which is used in SFC is carbon dioxide because its critical temperature and pressure are easy to reach. Also, carbon dioxide is low-cost, easy to find, inert towards UV, non-poisonous and a good solvent for non-polar molecules. Other than carbon dioxide; ethane, n-butane, N₂O, dichlorodifluoromethane, diethyl ether, ammonia, tetrahydrofuran can be used.

Table 2: Properties of some solvents as mobile phase at the critical point

Critical Temperature (°C)	Critical Pressure (bar)
Carbon dioxide	31.1 72
N ₂ O	36.5 70.6
Ammonia	132.5 109.8
Ethane	32.3 47.6
n-Butane	152 70.6
Diethyl ether	193.6 63.8
Tetrahydrofuran	267 50.5

4.9 Effect of Pressure

Part of the theory of separation in SFC is based on the density of the supercritical fluid which corresponds to solvating power. As the pressure in the system is increased, the density of the supercritical fluid increases and correspondingly its solvating power increases. This in turn shortens the elution time for the eluent as pressure changes in SFC have a pronounced effect on the retention of analytes. This effect is general and similar to programmed temperature in GC or gradient elution in HPLC.

5. Comparison of SFC with Other Types of Chromatography

SFC combines some of the characteristics of gas and liquid chromatography, as several physical properties of SCF are intermediate between gases and liquids. Like GC, SFC is inherently faster than LC because the lower viscosity makes use of higher flow rates. Diffusion rates in SCFs are intermediate between gases and liquids. As a consequence, band broadening is greater in SCFs but less, than in gases. Thus, the intermediate diffusivities and viscosities of SCFs result in faster separation than is achieved in LC, accompanied by lower zone

broadening than is encountered in GC. The mobile phases play different role in GC, LC and SCF. In GC, the mobile phase causes the zone movement. In LC, the mobile phase transports the solute molecule and also interacts with them thus influencing the selectivity. When a molecule dissolves in supercritical medium, the process resembles volatilization but at much lower temperature than that of GC. Thus, at a given temperature the vapor pressure for a large molecule in SCF may be 10 to 100 greater than in the absence of that fluid. As a consequence, high molecular weight compounds, thermally unstable species, polymers and large biological molecules can be eluted from a column at a reasonably low temperature. The biggest advantage that SFC holds over GC is the ability to separate thermally labile compounds. This is appreciated in the pharmaceutical fields since roughly 20% of all drugs candidates fall in this category. Unlike GC, by changing the mobile phase the selectivity can be varied in SFC.

6. SFC in chromatographic techniques

SFC is potentially useful for various applications. The ability to vary selectivity by programming the parameters P (pressure) and T (temperature) rather than by modifying the chemical composition of the eluent represents the technique's major difference. The low viscosity of the mobile phase permits an arrangement of several HPLC-type columns in series. The range of compounds analyzed by SFC includes lipids and oils, emulsifiers, oligomers and polymers compounds of molecular mass greater than 1000 which cannot be studied in GC. SFC offers superior speed and efficiency compared to HPLC. Finally, use of supercritical carbon dioxide as a mobile phase facilitates coupling with a mass spectrometer or an infrared spectrophotometer and even with an NMR spectrometer.

7. Advantages of Working with SFC

- The physical properties of supercritical fluids between liquids and gases enables the SFC technique to combine some of the strong aspects of HPLC and GC. Lower viscosity of supercritical fluids makes SFC a definitely faster method than HPLC.

Lower viscosity leads high flow speed for the mobile phase.

- The critical pressure of supercritical fluids, some fragile materials that are sensitive to high temperature can be analyzed through SFC. These materials can be the compounds which decompose at high temperatures, the materials which has low vapor pressure/volatility, polymers and large biological molecules.
- High pressure conditions provide a chance to work with lower temperature than normally needed; hence, the temperature-sensitive components can be analyzed via SFC.
- The diffusion of the components flowing through a supercritical fluid is higher than they have in HPLC due to the higher diffusivity of supercritical fluids than liquids. This brings better distribution into the mobile phase and a better separating at the end.

8. Drawbacks

There have been a few technical issues that have limited adoption of SFC technology,

- First of which is the high pressure operating conditions. High-pressure vessels are expensive and bulky, and special materials are often needed to avoid dissolving gaskets and O-rings in the supercritical fluid.
- A second drawback is difficulty in maintaining pressure (backpressure regulation). Whereas liquids are nearly incompressible, so their densities are constant regardless of pressure, supercritical fluids are highly compressible and their physical properties change with pressure - such as the pressure drop across a packed-bed column. Currently, automated backpressure regulators can maintain a constant pressure in the column even if flow rate varies, mitigating this problem.
- A third drawback is difficulty in gas/liquid separation during collection of product. Upon depressurization, the CO₂ rapidly turns into gas and aerosolizes any dissolved analyte in the process. Cyclone separators have lessened difficulties in gas/liquid separations.

9. Applications of SFC

There are applications for food, environmental and pharmaceutical products. Also, pesticides, herbicides, polymers, explosives and fossil fuels are other classes to be used. Through this method, analyses can be conducted for a wide variety of drug compounds such as antibiotics, prostaglandins, steroids, taxol, vitamins, barbiturates, non steroidal anti-inflammatory agents etc. Chiral separations can be done for many pharmaceutical compounds. SFC is dominantly used for non-polar compounds because of the weakness of carbon dioxide, which is the most common supercritical fluid mobile phase, in terms of dissolving polar solutes effectively. SFC can take place in petroleum industry with the applications on total aromatic content analysis or other hydrocarbon separations.

It is used for the analysis and purification of low to moderate molecular weight, thermally labile molecules. It can also be used for the separation of chiral compounds. SFC is used in industry primarily for separation of chiral molecules, and uses the same columns as standard HPLC systems. SFC is now commonly used for achiral separations and purifications in the pharmaceutical industry.

9.1 Applications in the material and polymer industry

Supercritical fluids are used extensively in the material and polymer industry. Rapid expansion from supercritical solutions across an orifice or nozzle is used commercially to precipitate solids. In this technique, a solute dissolved in supercritical fluid is depressurized rapidly. By controlling the operating variables carefully, the desired precipitated morphology can be attained. In a process, called gas anti-solvent, a supercritical fluid is rapidly added to a solution of a crystalline solid dissolved in an organic solvent. Since the solute has limited solubility in the fluid, the supercritical fluid acts as an anti-solvent to precipitate solid crystals. By varying the density of the fluid, the particle size distribution of final crystals can be finely controlled. Another process is the precipitation using a compressed fluid anti-solvent. In this process, the solution is

sprayed through a nozzle into a compressed fluid and the solvent diffuses rapidly into the supercritical fluid while the fluid swells the solution to precipitate the solute. This process has been used commercially to form nanometric, monodisperse microspheres of polymers. Another process that has been commercialized is the usage of supercritical fluid carbon dioxide to produce foamed parts. Since supercritical fluids depress the glass transition temperature of the polymer, polymer foams can be formed at room temperature by directly adding the supercritical fluid into the extruder.

9.2 Food applications

Carbon dioxide is the most common supercritical fluid in the food industry. Due to the non-toxicity and low critical temperature, it can be used to extract thermally labile food components and the product is not contaminated with residual solvent. Further, the extracts color, composition, odor, texture are controllable and extraction by supercritical fluid carbon dioxide retains the aroma of the product. Supercritical carbon dioxide extraction is used as a replacement for hexane in extracting soybean-oil and has been tested for extraction from corn, sunflower and peanuts. Supercritical fluid extraction provides a distinct advantage not only in the replacement but also extracts oils that are lower in iron and free fatty acid. To satisfy the consumer's need for 'lighter' foods, developmental work on supercritical extraction of oils from potato chips and other snack foods are being carried out. In addition, supercritical carbon dioxide has also been used to extract lilac, essential oils, black pepper, nutmeg, vanilla, basil, ginger, chamomile, and cholesterol.

A large amount of research has been concentrated on the decaffeination of coffee by supercritical carbon dioxide. Thus, it is not surprising to note that this was the first process to be commercialized (in 1978), whose primary step is supercritical extraction. Dry carbon dioxide cannot extract caffeine from dry coffee effectively and that the beans should be pre-wetted by water. Soaking of about 2 hours is necessary for efficient extraction of caffeine from coffee beans by supercritical carbon

dioxide. Commercial processes for decaffeination of coffee include the Kraft General Foods in USA and several processes in Germany.

9.3 Pharmaceutical applications

Since the residual solvent present in the extracted material is of critical importance in the pharmaceutical industry, supercritical fluid carbon dioxide has found several applications. The extraction of vitamin E from soybean oil and a purification method for vitamin E has been well studied. The latter process avoids the step of vacuum distillation, which usually results in the thermal degradation of the product. Solubilities and recrystallization of various drugs has been demonstrated in supercritical fluids.

9.4 Environmental applications

Due to strict environmental regulations, supercritical fluids are used as replacements for conventional hazardous chemicals such as hexane. Supercritical fluid extraction has been proposed as an alternative technique for soil remediation and activated carbon regeneration. Over 99% of a majority of organics can be removed from contaminated soil. Organics that have been successfully extracted include PAHs, PCBs, DDT and toxophene. Carbon dioxide has been used with entrainers for the extraction of highly polar compounds. A commercial process to separate oils from refinery sludge and contaminated soil has been developed by CF Systems Corporation, USA. Chelating moieties that dissolve into carbon dioxide have been developed for the extraction of heavy metals from soil.

10. CONCLUSION

Supercritical fluid chromatography are the techniques which take advantage of supercritical fluids and their unique physical properties to surpass other related methods in both chromatography and extraction fields. Sometimes, they take place as alternative instrumental analytical techniques while, in some other cases, they are used as complementary partners for binary systems. The ability of an analytical method to solve the real-world problems is the marker of its benefit that can be taken from.

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