

1. Introduction

Supercritical fluid chromatography (SFC) and supercritical fluid extraction (SFE) techniques have been developed following the appearance of supercritical fluids. These unique materials have led novel analytical applications in chromatography and extraction fields. Supercritical fluid chromatography is one of the most important column chromatography methods after gas chromatography (GC) and high-performance liquid chromatography (HPLC). SFC combines the advantages of GC and HPLC thanks to the superior properties of supercritical fluids. Also, supercritical fluid extraction is an advanced analytical technique. SFE does not have the problems experienced with liquids.

2. Definition and Formation of Supercritical Fluids

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 motion of the molecules are quite similar to gas molecules. On the other hand, a supercritical fluid behaves like a liquid because its density property is near liquid and, thus, supercritical fluid shows a similarity to a liquid in terms of dissolving effect.

The characteristic properties of a supercritical fluid are density, diffusivity and viscosity. Supercritical values for these features take place between liquids and gases.

Table 1. Supercritical fluid properties compared to liquids and gases

	Gas	Supercritical Fluid	Liquid
Density (gr/cm ³)	$0.6x 10^{-3} - 2x 10^{-3}$	0.2-0.5	0.6-2.0
Diffusivity (cm ² /s)	$1x 10^{-1} - 4x 10^{-1}$	$10^{-3} - 10^{-4}$	$0.2x 10^{-5} - 2x 10^{-5}$
Viscosity (gr/cm.s)	$1x 10^{-4} - 3x 10^{-4}$	$1x 10^{-4} - 3x 10^{-4}$	$0.2x10^{-2} - 3x10^{-2}$

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.

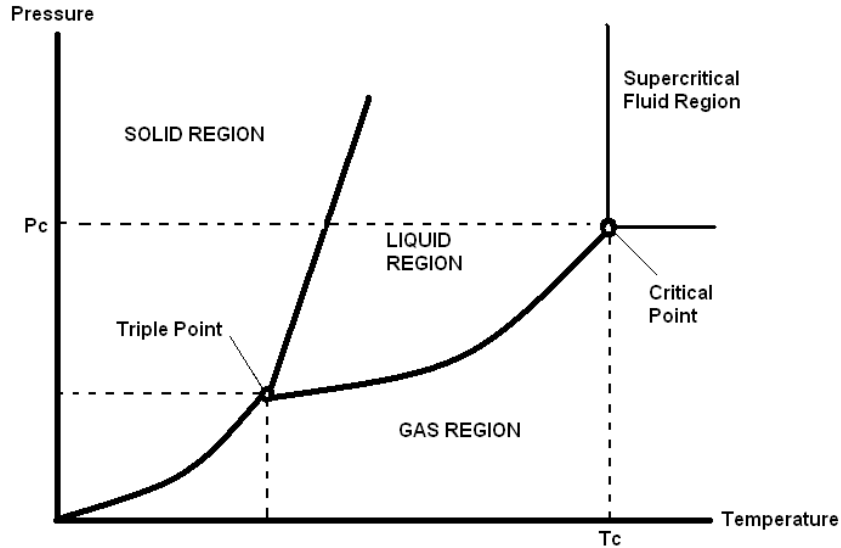


Figure 1. Idealized Phase Diagram.

For definition of SF, phase diagram is used. For a pure material, 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).

There is another characteristic point in the phase diagram, critical point (CP). This point is obtained at critical temperature (T_c) and critical pressure (P_c). After CP, no matter how much pressure or temperature is increased, the material cannot transform from gas to liquid or from liquid to gas phase. This form is supercritical fluid form. Increasing temperature cannot result in turning to gas, and increasing pressure cannot result in turning to liquid at this point. In the phase diagram, the field above T_c and P_c values is defined as supercritical region.

In theory, supercritical region can be reached in two ways. One is increasing the pressure above P_c value of the material by keeping temperature stable and then increasing the temperature above T_c value at a stable pressure value. Other way is increasing the temperature first above T_c value and then increasing the pressure above P_c value.

Critical point is characteristic for each material. It results from characteristic T_C and P_C values for each substance.

3. Physical Properties of Supercritical Fluids

SF show some common features with gases and some with liquids. This enables us to take advantage of a correct combination of the properties.

3.1. 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.

3.2. 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.

3.3. 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.

4. Supercritical Fluid Chromatography (SFC)

Just like SF are combining beneficial properties of liquids and gases, SFC also bring the advantages and strong aspects of HPLC and GC together. 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. This technique can be coupled with MS or FT-IR easily.

SFC surpasses GC when easily decomposable substances are analyzed. These materials can be used with SFC because of its ability to work with lower temperature than GC.

There are three major quality for column chromatographies; selectivity, efficiency and 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. With advanced sides, SFC is both an alternative and a complementary technique for the field.

4.1. 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.

On the other hand, 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.

A microprocessor mechanism is placed in the instrument for SFC. This unit collects data for pressure, oven temperature, detector performance etc. to control the related pieces of the instrument.

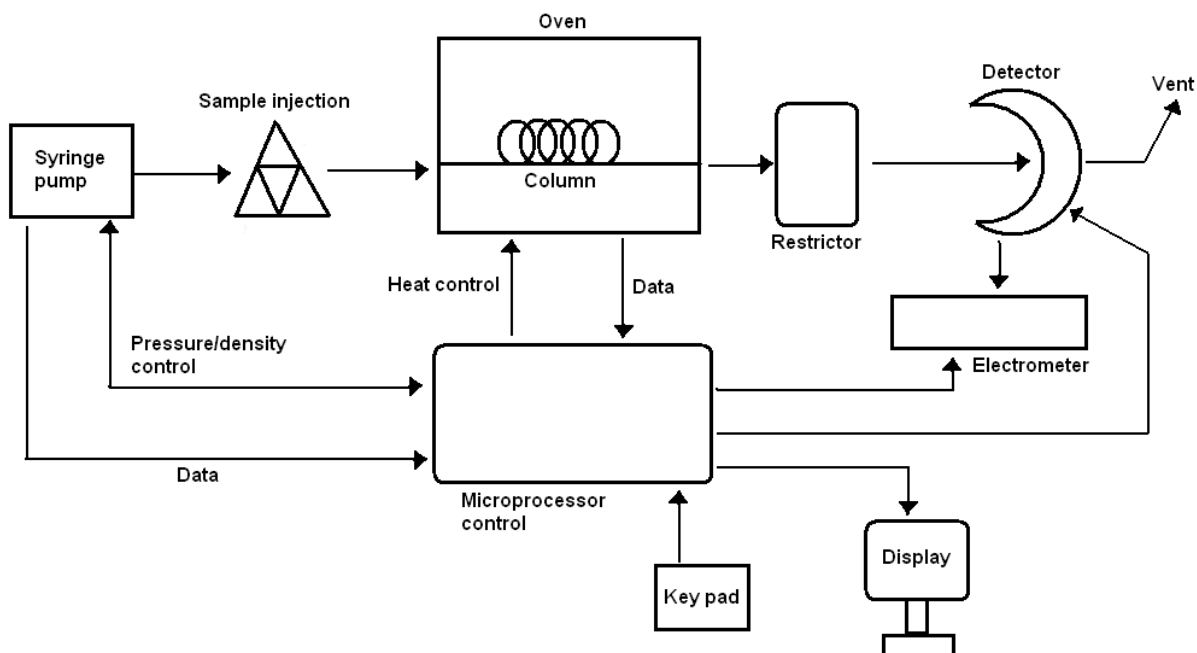


Figure 2. Scheme of a supercritical fluid chromatography instrument. Adapted from Douglas A. Skoog and James J. Leary., *Principles of Instrumental Analysis.*, The United States of America, 1992.

4.1.1. Stationary phases

SFC columns are pretty similar to HPLC columns in terms of coating materials. Open-tubular columns and packed columns are the two common types used in SFC. 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 μ m. The length of that columns can be in the range of 10-20 m.

4.1.2. 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, N_2O , dichlorodifluoromethane, diethyl ether, ammonia, tetrahydrofuran can be used.

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

	Critical Temperature ($^{\circ}C$)	Critical Pressure (bar)
Carbon dioxide	31.1	72
N_2O	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
Dichlorodifluoromethane	111.7	109.8

4.1.3. Detectors

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.

Also, 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 detectors.

4.2. 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.

Thanks to 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.

In addition, 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.

4.3. 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.

5. Supercritical Fluid Extraction (SFE)

Unique physical properties of supercritical fluids; having density, diffusivity and viscosity values between liquids and gases, enables supercritical fluid extraction to be used for the extraction processes which cannot be done by liquids because of their high density and low diffusivity and by gases due to their inadequate density in order to extract and carry the components out.

Complicated structures which involve many components should be subject to an extraction process before they are separated via a chromatographic method in order to have a simpler structure. An ideal extraction procedure should be fast, simple and inexpensive. Also, sample loss or decomposition should not be experienced at the end of the extraction. Following an effective extraction, there should be an effective gathering for each component to measure them quantitatively. Amount of useless materials coming from the extraction mechanism should stay low and be easily desposable; waste should not be harmful for environment. Unfortunately, traditional extraction methods cannot host these qualities perfectly. On the other hand, SFE has some advantages in comparison with the previous techniques.

The extraction speed is dependant on the viscosity and diffusivity of the mobile phase. With a low viscosity and high diffusivity, the component which is aimed to be extracted can pass through the mobile phase easily. The higher diffusivity and lower viscosity of supercritical fluids than regular extraction liquids help the components to be extracted faster than other techniques. Thus, an extraction process lasts 10-60 minutes with SFE while it takes hours or even days with classical methods.

The dissolving effect of a supercritical fluid can be arranged by temperature and pressure. Liquids are not affected by temperature and pressure changes this much. Therefore, SFE has the potential to be optimized to provide a better dissolving capacity.

In classical methods, heating is required to get rid of the extraction liquid; however, this step makes the temperature-sensitive materials decomposed. For SFE, when the critical pressure is removed, a supercritical fluid transforms to gas phase. Because supercritical fluid solvents are chemically inert, harmless and inexpensive; they can be released to atmosphere without leaving any waste. This way, extracted components can be obtained much more easily and sample loss is minimized as well.

5.1. Instrumentation for SFE

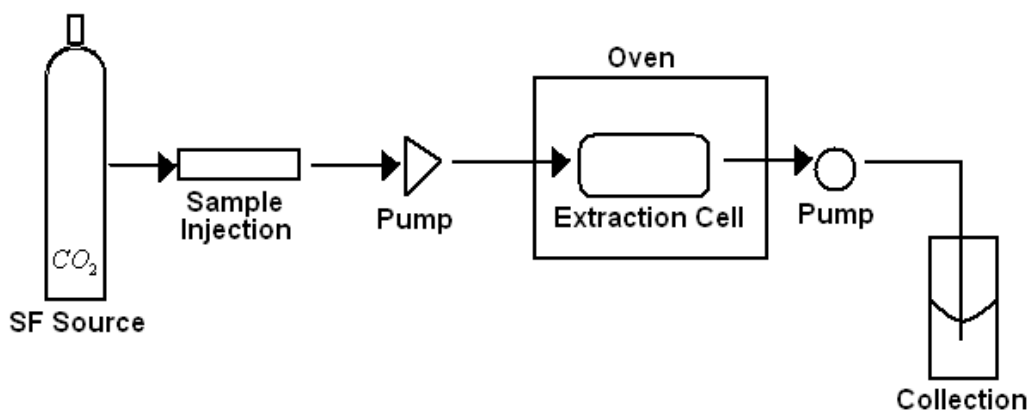


Figure 3. Scheme of an idealized supercritical fluid extraction instrument.

The necessary apparatus for a SFE setup is simple. This mechanism is basically composed of a reservoir of supercritical fluid, a pressure tuning injection unit, two pumps

to take the components in the mobile phase in and to send them out, and a collection chamber.

There are two principles to run the instrument; static extraction and dynamic extraction. In dynamic extraction, the second pump sending the materials out to the collection chamber is always open during the extraction process. Thus, mobile phase reaches extraction cell and extracts components in order to take them out consistently. In static extraction, there are two steps; mobile phase fills the extraction cell and interacts with the sample; then, the second pump is opened and the extracted substances are taken out at once.

While mobile phase is chosen for SFE, some parameters are taken into consideration such as polarity and solubility of sample in mobile phase. Carbon dioxide is the most common mobile phase for SFE. It has a capability to dissolve non-polar materials like alkanes. For semi-polar compounds, such as polycyclic aromatic hydrocarbons, aldehydes, esters, alcohols etc., carbon dioxide can be solely used. However, for the compounds which have polar characteristic, carbon dioxide must be modified by addition of polar solvents like methanol. These extra solvents can be taken into the system through a separate injection pump.

5.2. Extraction Modes

There are two modes in terms of collecting and detecting the components; Off-line and On-line extraction. Off-line extraction is done by taking the mobile phase out with the extracted components and directing them towards the collection chamber. At this point, supercritical fluid phase is evaporated and released to atmosphere and the components are captured in a solution or a convenient adsorption surface. Then, the extracted fragments are processed and prepared for a separation method. This extra manipulation step between extractor and chromatography instrument can cause errors.

On-line method is more sensitive because it directly transfers all extracted materials to a separation unit, mostly a chromatography instrument, without taking them out of the mobile phase. In this extraction/detection type, there is no extra sample preparation after extraction for separation process. This minimizes the errors coming from manipulation steps. Also, sample loss does not happen and sensitivity increases.

5.3. Applications of SFE

If a classification is done, some applications can be mentioned as environmental applications, pharmaceutical applications, biochemical applications, polymeric applications, industrial applications, natural product applications and food applications. SFE can be applied to a broad range of materials through the applications expressed above such as polymers, oils and lipids, carbonhydrates, pesticides, organic pollutants, volatile toxins, polyaromatic hydrocarbons, biomolecules, foods, flavors, pharmaceutical metabolites, explosives, organometalics etc.

In environmental applications, some materials which are analyzed are oils and fats, pesticides, alkanes, organic pollutants, volatile toxins, herbicides, nicotine, phenanthrene, fatty acids, aromatic surfactants in samples from clay to petroleum waste, from soil to river sediments.

In food analyses; caffeine, peroxides, oils, acids, cholesterol etc. are extracted materials from samples such as coffee, olive oil, lemon, cereals, wheat, potatoes and dog feed.

Through industrial applications, the extracted materials vary from additives to different oligomers, from petroleum fractions to stabilizers. Samples analyzed are plastics, PVC, paper, wood etc.

Drug metabolites, enzymes, steroids etc. are extracted from plasma, urine, serum or animal tissues in biochemical applications.

6. Conclusion

Supercritical fluid chromatography and supercritical fluid extraction 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. Both SFC and SFE demonstrate their versatility by the wide array of applications in many distinct domains in an advantageous way compared to their predecessors.