

# Supercritical Fluid Chromatography

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**Abstract:** *History of Supercritical Fluid Chromatography In 1822, the French physicist Charles Cannard de La Tour placed a liquid and a flint ball in a Papin rifle pressure vessel and heated the liquid with a sealed cannon. 1) When the container was shaken, the bullet broke through the air-liquid interface, and the sound of the bullet scattering was heard. But when I heated the container to a temperature well above the boiling point of the liquid, the noise disappeared. He hypothesized that this was because the liquid and gas densities in the vessel became equal, effectively forming a single phase. This represents the first discovery of what is now called supercritical states and critical points. After this discovery, supercritical fluids became the subject of fundamental physicochemical studies to determine the state change of matter and its properties under supercritical conditions. However, practical applications were not immediately born. Then, in 1879, Hannay and Hogarth reported that supercritical fluids had excellent solvent properties<sup>2</sup>, and the second wave of research began.*

**Keywords:** Fluid Chromatography

## I. INTRODUCTION

Matter can be solid, liquid, or gas depending on the conditions of temperature and pressure. For example, water is a liquid at room temperature and atmospheric pressure, but at atmospheric pressure it becomes vapor (gas) at 100 degrees Celsius and ice (solid) at 0 degrees Celsius. As shown in Figure 1, when water is placed in a closed container and a vacuum is applied, some of the water evaporates and the rest remains in the liquid phase. Once the water vapor pressure reaches a certain value, the evaporation rate equals the condensation rate. This is called saturated water vapor pressure and is temperature dependent. Supercritical liquid chromatography. A supercritical fluid is a state of matter that lies between a gas and an intermediate state Fluids of their properties. This state occurs when it is a gaseous or liquid solvent exposed to certain temperature and pressure conditions critical point. Temperature And this pint pressure known as Critical temperature and Critical pressure and are Characteristic of the solvent. Beyond this point, the solvent neither gas nor liquid, but has the property both phases. If this supercritical fluids are more gas or liquid depends on it pressure and temperature 2. Supercritical fluid extraction Supercritical fluids have several advantages Extraction (SFE): a. SFE is generally faster. Speed of mass transfer between sample matrix and extract determined by species diffusion rate Viscosity of liquids and liquids – the greater The lower the diffusion rate and viscosity, the Mass transfer speed increases. B. The solution strength <sup>7</sup>of the supercritical fluid can be changed by changing it Low pressure, low temperature. C. Many supercritical fluids are gases at ambient conditions. i.e. Some supercritical fluids are cheap, inert, and non-toxic. 3. Supercritical Liquid Chromatography (SFC) a. supercritical fluid. B. The use of supercritical liquid mobile phases in chromatography is First proposed by J. Lovelock in 1958. First usage report A chromatographic system was performed by Klesper et al. in 1962. Who used For the separation of heat-labile porphyrins. C. SFC is important because it allows separation. Identification of inconvenient connections processed by GC or LC. These compounds are (1) non-volatile or thermally unstable, making GC inapplicable; (2) Contains no functional groups that render detection impossible A spectroscopic or electrochemical technique used in LC. B. SFs have higher densities than gas, so that mobile phase has a greater chance of interacting with the solute than that in GC (i.e., carrier gas). This makes the mobile phase important in determining the retention of solutes on the system and give more flexibility in optimizing the separation. For example, retention of solutes in SFC can be changed by using a different column (i.e. different stationary phases) as in GC, or by changing the mobile phase strength as in LC. C. One major advantage of SFC is its ability to use detector available for either GC or LC, such as FID, UV-Vis, and Fluorescence detectors. This gives it a wide range of both universal and selective detections for use in either analytical or preparative-scale work. D. Depending on which supercritical fluid is used, it is also possible to use SFC at lower T than GC. This makes it more

useful in the separation of thermally unstable compounds. E. The stationary phases used in SFC can be similar to those in LC as well as GC. Either packed or open-tubular columns may be used. Because of these advantages, SFC is commonly viewed as a technique which is complementary to both LC and GC. The formation of a supercritical fluid is the result of a dynamic equilibrium. When a material is heated to its specific critical temperature in a closed system, 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. In order to understand the definition of SF better, a simple phase diagram can be 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. 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; the triple point (TP).

## II. PHYSICAL PROPERTIES OF SUPERCRITICAL FLUIDS

As mentioned above, SF shares some common features with both gases and liquids. This enables us to take advantage of a correct combination of the properties.

- **Density-:** Density characteristic of a supercritical fluid is between that of a gas and a liquid, but closer to that of a liquid. In the supercritical region, density of a supercritical fluid increases with increased pressure (at constant temperature). When pressure is constant, density of the material decreases with increasing temperature. The dissolving effect of supercritical fluid is dependent on its density value. Supercritical fluids are also better carriers than gases thanks to their higher density. Therefore, density is an essential parameter for analytical techniques using supercritical fluids as solvents.
- **Diffusivity-:** Diffusivity of a supercritical fluid can be 100 x that of a liquid and 1/1000 to 1/10000x less than a gas. Because supercritical fluids have more diffusivity than a liquid, it stands to reason 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. Viscosity:- Viscosity for a supercritical fluid is almost the same as a gas, being approximately 1/10 of that of a liquid. Thus, supercritical fluids are less resistant than liquids towards components flowing through. The viscosity of supercritical fluids is also distinguished from that of liquids in that temperature has a little effect on liquid viscosity, where it can dramatically influence supercritical fluid viscosity. These properties of viscosity, diffusivity, and density 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. Super Fluid Chromatography (SFC) Just like supercritical fluids combine the benefits of liquids and gases, SFC bring the advantages and strong aspects of HPLC and GC together. SFC can be more advantageous than HPLC and GC when compounds which decompose at high temperatures with GC and do not have functional groups to be detected by HPLC detection systems are analyzed.

There are three major qualities for column chromatographies:

- Selectivity.
- Efficiency.
- Sensitivity.

Generally, HPLC has better selectivity than SFC owing to changeable mobile phases (especially during a particular experimental run) and a wide range of stationary phases. Although SFC does not have the selectivity of HPLC, it has good quality in terms of

Sensitivity and efficiency. SFC enables change of some properties during the chromatographic process. This tuning ability allows the optimization of the analysis. Also, SFC has a broader range of detectors than HPLC. SFC surpasses

GC for the analysis of easily decomposable substances; these materials can be used with SFC due to its ability to work with lower temperatures than GC

### 2.1 Advantages of Working with SFC

The physical properties of supercritical fluids between liquids and gases enables the SFC technique to combine with the best aspects of HPLC and GC, as lower viscosity of supercritical fluids makes SFC a faster method than HPLC. Lower viscosity leads to 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 compounds which decompose at high temperatures or materials which have low vapor pressure/volatility such as 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 observed in HPLC due to the higher diffusivity of supercritical fluids over traditional liquids mobile phases. This results in better distribution into the mobile phase and better separation.

### 2.2 Applications of SFC

The applications of SFC range from food to environmental to pharmaceutical industries. In this manner, pesticides, herbicides, polymers, explosives and fossil fuels are all classes of compounds that can be analyzed. SFC can be used to analyze a wide variety of drug compounds such as antibiotics, prostaglandins, steroids, taxol, vitamins, barbiturates, non-steroidal anti-inflammatory agents, etc. Chiral separations can be performed for many pharmaceutical compounds. SFC is dominantly used for non-polar compounds because of the low efficiency of carbon dioxide, which is the most common supercritical fluid mobile phase, for dissolving polar solutes. SFC is used in the petroleum industry for the determination of total aromatic content analysis as well as other hydrocarbon separations.

## III. SUPERCRITICAL FLUID EXTRACTION (SFE)

The unique physical properties of supercritical fluids, having values for 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 due to their high density and low diffusivity and by gases due to their inadequate density in order to extract and carry the components out. Complicated mixtures containing many components should be subject to an extraction process before they are separated via chromatography. An ideal extraction procedure should be fast, simple, and inexpensive. In addition, sample loss or decomposition should not be experienced at the end of the extraction. Following extraction, there should be a quantitative collection of each component. Ideally, the amount of unwanted materials coming from the extraction should be kept to a minimum and be easily disposable; the waste should not be harmful for environment. Unfortunately, traditional extraction methods often do not meet these requirements. In this regard, SFE has several advantages in comparison with traditional techniques. The extraction speed is dependent on the viscosity and diffusivity of the mobile phase. With a low viscosity and high diffusivity, the component which is to be extracted can pass through the mobile phase easily. The higher diffusivity and lower viscosity of supercritical fluids, as compared to regular extraction liquids, help the components to be extracted faster than other techniques. Thus, an extraction process can take just 10-60 minutes with SFE, while it would take hours or even days with classical methods. The dissolving efficiency of a supercritical fluid can be altered by temperature and pressure. In contrast, liquids are not affected by temperature and pressure changes as 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 causes the temperature-sensitive materials to decompose. 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. Through this, extracted components can be obtained much more easily and sample loss is minimized.

## IV. INSTRUMENTATION

Instrumentation for SFC can be obtained commercially or adapting system used for either LC and GC. B. The main difference of a SFC than a LC or GC system is the need to control both temperature and pressure of mobile phase.

This must be done to keep the mobile phase as a Supercritical fluid. Control of the pressure (density) of the supercritical fluid can also be used to vary strength of mobile phase during the gradient elution in SFC ..

#### 4.1 Instrumentation of SFE

The necessary apparatus for a SFE setup is simple. Figure depicts the basic elements of a SFE instrument, which is 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 of the extraction cell), and a collection chamber

There are two principle modes to run the instrument:

- Static extraction.
- Dynamic extraction.

In dynamic extraction, the second pump sending the materials out to the collection chamber is always open during the extraction process. Thus, the mobile phase reaches the extraction cell and extracts components in order to take them out consistently.

In the static extraction experiment, there are two distinct steps in the process:

1. The mobile phase fills the extraction cell and interacts with the sample.
2. The second pump is opened and the extracted substances are taken out at once.

In order to choose the mobile phase for SFE, parameters taken into consideration include the polarity and solubility of the samples in the 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 used as a single component mobile phase. However, for compounds which have polar characteristic, supercritical carbon dioxide must be modified by addition of polar solvents like methanol (CH<sub>3</sub>OH). These extra solvents can be introduced into the system through a separate injection pump.

#### Extraction Modes

There are two modes in terms of collecting and detecting the components:

- Off-line extraction.
- 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. The 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. Additionally, sample loss does not occur and sensitivity increases.

#### Applications of SFE

SFE can be applied to a broad range of materials such as polymers, oils and lipids, carbohydrates, pesticides, organic pollutants, volatile toxins, polyaromatic hydrocarbons, biomolecules, foods, flavors, pharmaceutical metabolites, explosives, and Organometallics, etc. Common industrial applications include the pharmaceutical and biochemical industry, the polymer industry, industrial synthesis and extraction, natural product chemistry, and the food industry. Examples of materials analyzed in environmental applications: 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 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, and from petroleum fractions to stabilizers. Samples analyzed are plastics, PVC, paper, wood etc. Drug Metabolites, enzymes, steroids are extracted from plasma, urine, serum or animal tissues in biochemical applications.

### V. CONCLUSION

While it may have its drawbacks, SFC remains an untapped resource in the ways of chromatography. The advantages to using supercritical fluids as mobile phases demonstrate how resolution can be increased without sacrificing time or increasing column length. Nonetheless, it is still a well-utilized resource in the organic, biomedical, and pharmaceutical industries. SFC shows promise as a reliable way of separating and analyzing mixtures.

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