#### **A Review on Supercritical Fluid Chromatography.**

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#### **Abstract**

This paper shows some of the different supercritical fluids available for use in SFC, and discusses the advantages that SFC has over HPLC and GC and how these can provide benefits to the industries which adopt this technique. High performance specifications and unique functionality of chromatographic techniques. This leads to the origin of Supercritical Fluid Chromatography (SFC). It is a rapidly expanding analytical technique. The main feature that differentiates SFC from other chromatographic techniques is the replacement of either the liquid or gas mobile phase with a supercritical fluid mobile phase. It is considered a hybrid of GC and LC technique. It has a unique characteristic of analyzing thermo labile or non-volatile substances. The present article reviews the fundamentals, instrumentation and varied applications of supercritical fluid chromatography in the analytical arena. The different setups available for SFC and how they compare along with the mobile phases and solid phases which are currently used are highlighted within the paper.

#### **Key Words**

Supercritical fluid chromatography, mobile phase, modifier, critical temperature, critical pressure, mass spectrometry.

#### **Introduction**

Supercritical Fluid Chromatography (SFC) is one of the most recent chromatographic techniques used in the modern era of science and technology. It is a revolutionary separation technique. The first suggestion of supercritical fluid chromatography (SFC) was put forward in 1958 demonstrated the first experiments on capillary SFC in 1982 and the first commercial capillary column SFC instrument was introduced in 1985.<sup>[\[1\]](file:///C:\Documents%20and%20Settings\admin\Desktop\sfc_files\sfc.htm%23ref1)</sup> Supercritical Fluid Chromatography may be

defined as a technique that separate components of a compound or mixture by using a mobile phase (supercritical fluid) which is above and relatively close to its critical temperature and pressure. In this type of chromatography, the use of a supercritical fluid as the mobile phase makes it different from other chromatographic techniques like gas chromatography (GC) and high performance liquid chromatography (HPLC). It is a normal phase chromatography. It can be considered as hybrid of gas and liquid chromatography because when the mobile phase is below its critical

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temperature and above its critical pressure, it acts as a liquid, and when the mobile phase is above its critical temperature and below its critical pressure, it acts as a gas.<sup>[\[2\]](file:///C:\Documents%20and%20Settings\admin\Desktop\sfc_files\sfc.htm%23ref2)</sup> Thus, it has some features like liquid and some features like gas. It has the gaseous property of being able to penetrate anything, and the liquid property of being able to dissolve materials into their components. As GC cannot be used for nonvolatile compounds and LC cannot be employed for compounds with those functional groups that cannot be detected by either spectroscopic or electrochemical detectors used in LC, at this time SFC helps in separation and determination of groups of compounds. A supercritical fluid is a substance that has properties intermediate between a liquid and a gas.

## **Principle**

The concept of supercritical fluid can be easily understood by a phase diagram for a pure substance shown in. A temperature above which a substance can no longer exist as a liquid, no matter how much pressure is applied is called supercritical temperature and a pressure above which the substance can no longer exist as a gas no matter how high the temperature is raised is called supercritical pressure.<sup>[\[3\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref4)</sup> A liquid can be converted to supercritical fluid by increasing pressure at constant temperature. Thus, supercritical fluid can be obtained by heating above the critical temperature and compressing above the critical pressure. $^{[4]}$  $^{[4]}$  $^{[4]}$  Table-1indicates that the SCFs have

following properties intermediate between those of a substance in gaseous and liquid state.<sup>[\[5\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref6)</sup> SFC is based on the principle of density of the supercritical fluid which corresponds to solvating power. As the pressure in the system is increased, the supercritical fluid density increases and correspondingly its solvating power increases.

### **Advantages**

Supercritical fluid chromatography has several main advantages over conventional chromatographic techniques (GC and HPLC). The biggest advantage that SFC has over HPLC lies within the differences in the mobile phases. Supercritical fluids are less viscous, possess a higher diffusivity than liquids under HPLC conditions and allow lower pressure drops along an analytical column. This provides not only the ability to increase column lengths, but also allows for faster flow rates. These factors in turn affect capacity ratios, selectivities and theoretical plate heights. It has been reported that 200,000 theoretical plates have been achieved by using eleven analytical (4.6mm i.d.) columns in series. Additionally, SFC can be set up for sub ambient temperatures, which has been key in many chiral separations. [6]

### **Super Critical fluids: Fundamentals and Properties**

Supercritical fluid may be defined from a phase diagram for a pure substance, in which the regions corresponding to solid, liquid and gaseous state are clear. A substance such as  $CO<sub>2</sub>$  can exist in solid, liquid and gaseous phases under various combinations of temperature and pressure. For every substance there is a temperature above which it can no longer exist as a liquid, no matter how much pressure is applied. Likewise, there is a pressure above which the substance can no longer exist as a gas no matter how high the temperature is raised. These points are called critical temperature and critical pressure respectively<sup>[7]</sup> and are the defining boundaries on a phase diagram for a pure substance. At this point, the liquid and vapour have the same density and the fluid cannot be liquefied by increasing the pressure. Above this point, where no phase change occurs, the substance acts as a supercritical fluid. So SCF can be described as a fluid obtained by heating above the critical temperature and compressing above the critical pressure **[**8**] .** There is a continuous transition from liquid to SCF by increasing temperature at constant pressure or from gas to SCF by increasing pressure at constant temperature. The term, compressed liquid is used frequently to describe a supercritical fluid, a near critical fluid, an expanded liquid or a highly compressed gas.<sup>[9]</sup>

### **Important Properties of Super Critical fluids**[10]

SCFs have high densities (0.2-  $0.5$ gm/cm<sup>3</sup>) due to which they have a remarkable ability to dissolve large, non-volatile molecules, for example,  $SC - CO_2$  readily dissolves n-alkanes containing 5 to 30 carbon atoms, di-nalkyl phthalates with dialkyl group containing 4-16 carbon atoms and compounds with many rings. Solvation strength of SCF is directly related to the fluid density. Thus solubility of solid can be manipulated by making slight changes in temperatures and pressures. Certain important processes are based upon the high solubility of organic species in SC -CO  $_2$  for example; it has been employed for extracting caffeine from coffee beans to get decaffeinated coffee and for extracting nicotine from cigarette tobacco. A second important property of SCFs is that dissolved analytes can be easily recovered by simply allowing the solutions to equilibrate with the atmosphere at low temperatures, for example an analyte dissolved in the SC- CO  $_2$  can be recovered by simply reducing the pressure and allowing to evaporate under ambient laboratory conditions. This property is particularly useful with thermally unstable analytes. Another advantage of many SCFs is that they are inexpensive, innocuous, ecofriendly and non-toxic. With SCFs at hand, there is no need of any organic solvents. Finally SCFs have the advantage of higher diffusion constants and lower viscosities relative to liquid solvents. The low viscosity means that pressure drop across the column for a given flow rate is greatly reduced. The greater diffusibility means longer column length can be used. Higher diffusion coefficient means higher analysis speed that increases in the order HPLC, SFC and GC. These advantages are important in both, chromatography and extractions with

several polycyclic and aromatic

SCFs. SCFs are finding applications in fractionation of low vapour pressure oils, in several reactions in different areas of biochemistry, polymer chemistry, environmental sciences as well as food, polymer and material industries. **[**10]

#### **Instrumentation**

SFC apparatus can be of two types 1) HPLC like apparatus that consists of two reciprocating pumps, a packed analytical column placed, an oven, an optical detector, 2) GC like apparatus which consists of a syringe pump, a capillary column, oven, a restrictor and a flame ionization detector. Before the supercritical fluid enters the analytical column, it is brought into the supercritical region by heating it above its supercritical temperature. Then it is passed through an injection valve where the sample mixture is introduced into the supercritical fluid and then into the analytical column. The fluid must be maintained supercritical as it passes through the column and into the detector by a pressure restrictor. A thermostated oven is required to provide precise temperature control of the mobile phase and a restrictor is used to maintain the pressure in the column at a desired level and to convert the eluent from SCF to a gas for transfer to detector.  $[14]$  In SFC, the mobile phase is initially pumped as a liquid and is brought into the supercritical region by heating it above its supercritical temperature before it enters the analytical column. It passes through an injection valve where the sample is introduced into the supercritical stream and then into the

analytical column. It is maintained supercritical as it passes through the column into the detector by a pressure restrictor placed either after the detector or at the end of the column.

### **Mobile Phase and Stationery Phase**

In SFC the density of the mobile phase is about 200-500 times greater than that in gas chromatography. Compounds with high molecular weights are not usually detectable in gas chromatography, however with the density of the mobile phase being greater they can therefore be chromatographed. [**16**] A wide range of compounds has been tested for use as SFC mobile phases (Table 1). However, a variety of these required special conditions, and would therefore not be suitable. This resulted in carbon dioxide (CO2) being used as it is easily obtainable, low cost and safe <sup>[17],</sup> along with the critical temperature being 31oC and critical pressure being 73.8 atm. <sup>[18]</sup> A problem with CO2 as a mobile phase in a packed column is that if CO2 mobilizes a species then there is a possibility that the compound will be irreversibly adsorbed onto the column. This is because of the high activity of most sorbents; the problem can be avoided by the use of modifiers. This however, does not happen in capillary SFC as inert fused silica open-tubular columns are used.  $[18]$  Stationary phases that have been studied include, 'octadecylsiloxane-bonded silica (ODS), cyanopropylsiloxane-bonded silica, divinylbenzene-ODS, polydimethylsiloxane and porous graphitic carbon (PGC)'. <sup>[19]</sup> Some

examples of achiral polar stationary phases include silica, cyano-propyl (CN) and 2-ethyl-pyridine (2-EP) which was specifically designed for  ${\rm SFC}$   $^{[20]}$ .

## **Modifier**

The problem with carbon dioxide is that it is unable to elute very polar or ionic analytes or compounds which can be overcome by adding a small portion of a second fluid called a modifier fluid**. [**21**]** A modifier fluid is used to improve the solvating ability of the supercritical fluid and it also increases selectivity of the separation. For example, methanol, CHCl3 ethanol and isopropyl alcohol are widely used as modifiers. Modifiers increase the column efficiency for highly retained nonpolar solutes and they improve both retention and efficiency for polar solutes. Separation of components depends on the type of modifier and the modifier content. Temperature and pressure have less influence.

### **Pumps, Injectors and Ovens**

A pump is required for stable transfer of liquid carbon dioxide. The choice of pump used in SFC depends on the type of column used. The incoming carbon dioxide and pump heads must be kept cold in order to maintain the carbon dioxide in a liquid state where it can be effectively metered at specified flow rate. Reciprocating pumps are used for packed columns and syringe pumps are used for capillary SFC. The function of reciprocating pump is to allow easy mixing of the mobile phase or introduction of modifier fluids and the

function of syringe pump are to provide consistent pressure for a mobile phase. A modifier delivery pump is also present that performs stable transfer of modifiers. In SFC, supercritical fluid is injected by switching of the content of a sample loop into the carrier fluid at the column entrance by using auto sampler. It can safely discharge carbon dioxide in the sample loop and continuously inject samples.

### **Columns**

The analytical column contains a highly viscous liquid i.e. a stationary phase into which the analytes are adsorbed and then released according to their chemical nature. The recent packed columns consist of bonded non-extractable stationary phases such as octadecylsilyl (C-18) or aminopropyl bonded silica. In SFC, two types of analytical columns are used i.e. packed like HPLC and capillary like GC. Packed columns are made up of stainless steel and contain small deactivated particles to which the stationary phases adhere. They are 0.03-0.25m long and their internal diameter is 2.0-4.6mm. Capillary columns are made up of fused silica and the stationary phase is bonded to the wall of the column. They are open tubular columns of narrow internal diameter of 0.025-0.1mm and they are 1-35 m long. The film thickness in capillary columns ranges from 0.1-3 ΅m. C-18, phenyl, and cyano columns are used with both nonpolar and polar solutes

## **Pressure Restrictor or Back Pressure Regulator**

Pressure restrictor or back pressure regulator maintains desired pressure in the column to achieve extraction and chromatograms with high reproducibility. The pressure restrictor has an important role as it keeps the mobile phase supercritical throughout the separation and must be heated to prevent clogging. It can be variable or fixed. As SFC utilizes carbon dioxide as the mobile phase; thus the entire chromatographic flow path must be pressurized.

### **Detectors and microprocessor**

A detector is required for evaluating optical characteristics under a supercritical state. The mobile phase composition, column type and flow rate must be considered while selecting a detector.. The widely used detectors in SFC are refractive index detectors, ultraviolet-visible spectrophotometric detectors, flame ionization detector (FID), flame photometric detectors and evaporative light scattering (ELS). One or more microprocessors are present in SFC instruments to control pumping pressures, oven temperature and detector performance.

### **Comparison of SFC with GC and LC**

As SFC is a combination of gas and liquid chromatography, its properties are intermediate between gases and liquids. It is faster like GC than LC because of high flow rate due to low viscosity. Diffusion rates in SCFs are intermediate between gases and liquids. Thus, band broadening is greater in SCFs but less, than in gases.

Therefore, SFCs have intermediate diffusivities and viscosities which make it faster techniques compared to others.[22] Oligo-meric polymer mixtures or complex mixtures of oleophilic components can be readily solubilized in supercritical fluids.Moreover, GC is unable to analyze thermally unstable or nonvolatile substances and in this case if HPLC is used, it produces a large number of organic solvents. The disposal cost of these organic solvents is very high. SFC solves this problem as it utilizes carbon dioxide, collected as a byproduct of other chemical reactions or is collected directly from the atmosphere. Further, it do not contribute any new chemical to the environment.<sup>[22]</sup> Due to higher diffusion rates and low viscosity, SFC provides 3-5 times increase in the speed of analysis and a decrease in the cost of analysis by saving organic solvent.<sup>[23]</sup> As it is a hybrid of GC and LC technique, it can utilize both GC and LC detectors. Multidetector compatibility is a unique characteristic of SFC that makes it superior over all other chromatographic techniques and it proves to be beneficial for successful analysis of thermo labile and high molecular weight compounds. Moreover, it has many advantages over conventional chromatographic techniques. Long columns can also be used in SFC due to low viscosity and higher diffusion rates which is responsible for lower pressure drop along the analytical column.

## **Applications**

## **1. Separation of polymers**

It is difficult to separate large molecular weight compounds, large biomolecules and polymers by HPLC but as SFC has combined features of GC and LC techniques, it is capable of their separation at low temperature. SFC is used for the analysis of fluorinated polymers like Polymethyl-333-trifluoropropylsiloxane which is difficult due to their insolubility with common solvent for HPLC analysis and their nonvolitility for GC analysis. [\[24\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref19) Polynuclear aromatic hydrocarbons in automobile exhaust, $^{[25]}$  $^{[25]}$  $^{[25]}$  polyole-finic antioxidants /light stabilizers<sup>[\[26\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref21)</sup> and polyethoxylated alkylphenols $^{[27]}$  $^{[27]}$  $^{[27]}$  are analysed successfully by using SFC. Various dimethyl polysiloxane oligomers<sup>[\[28\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref23)</sup> and polycyclics aromatic hydrocarbon extracted from carbon black using fluorescence detection<sup>[\[29\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref24)</sup> can be separated. Silanised polyglycerols can also be analysed.  $[30]$ 

## **2. Separation of thermally labile pesticides**

The most important application of SFC is the separation of thermally labile pesticides without resorting to sample derivatization. As GC has limitation that it can only be used for the separation of volatile and thermostable compounds, analysis and purification of low to moderate molecular weight, thermally labile molecules and non-volatile compounds is done by SFC. Various pesticides belonging to different classes, triazines (ametryne, atrazine), carba-mates (carbofuran) and sulfonylureas (chlorsulfuron, metsulfuron methyl and benzsulfuron

methyl) are detected and quantified in soil by packed-column supercritical fluid chromatography interfaced with atmospheric pressure chemical ionization mass spectrometry SFC- $APCI-MS$ <sup>[\[31\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref26)</sup>

## **3. Separation of fatty acids, triglycerides and lipids**

Geometric isomers of fatty acids can be separated by open tubular columns in SFC along with quantification of triglycerides.[\[32\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref31) Analysis of mono-, di- and triglyceride mixtures in several pharmaceutical excipients can be performed using capillary SFC with carbon dioxide as mobile phase and flame ionization detection.[\[33\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref32) The fatty acids like fatty acid methyl esters, (FAMEs) or free fatty acids, (FFAs) can be separated by supercritical fluid chromatography (SFC) using pure  $CO2$ .<sup>[\[34\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref33)</sup> Capillary-SFC is used to analyse various natural and processed fish oil triglyceride mixtures. As a result, free fatty acids, squalene, a-tocopherol, cholesterol, wax esters, cholesterol esters, di-and triglycerides get separated. This analysis is not possible by gas chromatography or high-performance liquid chromatography methods without prior treatment of the fish oil, thus making SFC superior for this application.<sup>[\[35\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref34)</sup> Carbon dioxide is effectively used for the separation of lipids of high molecular weight like triacylglycerols without thermal cracking like GC or relatively long elution time like HPLC. Paraffin wax, free fatty acids, mono-, di-and triacylglycerols and detergents like Triton  $X-100$  can be separated<sup>[\[36\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref35)</sup> without thermal cracking using pressure programming and a high

degree of quantification is also observed. Separation, identification and quantification of triacylglycerols can be easily performed using SFC-MS.[\[37\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref36) Various phospholipids, glycosphingolipids, archae-bacterial lipids can also be analyzed.<sup>[\[38\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref37)</sup>

### **4. Fossil fuels**

On-line coupling of supercritical fluid chromatography-gas chromatographymass spectrometry helps in the determination of diaromatic and polyaromatic groups of di-esel-range petroleum fractions. The SFC-GC system is beneficial because the SFC solvent (CO2) can be easily eliminated and has no disturbing effect on the GC separation and detection and thus mass detection.<sup>[\[39\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref38)</sup> Fraction collection is more convenient in SFC because the primary mobile phase evaporates leaving only the analyte and a small volume of polar co-solvent. SFC-MS is used to analyze diesel fuels, diesel fuels sediments, coal derived solids and liquids etc.  $[40]$ 

#### **5. Forensic Science**

SFC is found to have a beneficial application in the field of forensic science. It is used in the identification and analysis of explosives containing nitroaromatics, nitrate esters, nitramines and drugs of abuse like amphetamines, cocaine and other stimulants, barbiturates, benzodiazepines, cannabis products, opiate drugs and lysergic acid diethylamide and related compounds.  $[41]$  Fingerprinting is also a major application of SFC in forensic science. It can be used for both time-of-deathrelated drug analysis and for obtaining

information relating to long term drug abuse.[\[42\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref42)

### **6. Chiral and achiral separations**

The first demonstration of a chiral separation by SFC took place in 1985. SFC is widely used in chiral separations because of easier and faster method development, high efficiency, superior and rapid separations of a wide variety of analytes, extended-temperature capability, analytical and preparativescale equipment improvements and a selection of detection options.  $[43-44]$  $[43-44]$  A number of chiral molecules can be analysed using SFC due to its high column efficiency at normal flow rates.[4[5-46\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref46) SFC is used for rapid enantiomer resolution of large numbers of compounds in a very less time by using methanol and isopropanol used as modifiers. The compounds which are not completely separated by reverse-phase or normal phase chromatography are successfully separated by SFC due to its unique properties. As it offers a higher success rate, performance and throughput for chiral separations of new compounds, it contributes in drug development and drug discovery.<sup>[\[47\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref47)</sup> Negligible interferences from achiral impurities, enantiomeric excess determined with much lower detection limits than UV and much shorter analysis times compared to other separation techniques makes SFC– MS superior.

### **7. Surfactants**

Separation of the oligomers in a sample of the nonionic surfactant Triton X100 has been reported where the detection was by measuring the total ion current produced by the

chemical ionization mass spectrometer.**[48]**

## **Applications in pharmaceutical industry**

SFC is used for high throughput screening and purifications of pharmaceuticals.[\[49\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref51) It has become a technique for solving problems that are difficult to be monitored by other GC and LC techniques. Various aliphatic and aromatic monohydroxamic acids can be separated by SFC using methanol modified CO2 on a diol column.<sup>[\[50\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref61)</sup> Using supercritical fluids CO2 and water, fine particles like micro and nano-particles can be formed because chemical and physical properties of solvent can be varied with temperature or pressure that ultimately affect the degree of super-saturation and nucleation.<sup>[\[51\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref62)</sup> Dexamethasone and betaniethasone, pred-nisolone, and cortisone and hydrocortisone can be resolved by using a methylpolysiloxane open tubular capillary column and SF CO2 as the mobile phase. Phencycli-dine, methaqualone, methadone, propoxyphene, erythromycin, atenolol, and oxytetracyclin and many other drugs are analyzed by SFC. Biodegradable particle formation for drug and gene delivery using supercritical fluid and dense gas is a remarkable application of SFC that makes it important in pharmaceuticals.[\[52\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref64)

# **Capillary SFC coupled with mass spectrometry**

A new development in supercritical fluid chromatography is the combination of capillary supercritical fluid chromatography with mass

spectrometry for faster analysis and high-resolution chromatographic separations with increased chromatographic efficiency, and more precise quantitation of sample mixtures. Rapid flow injection and solute elution, compatibility of solvent with mobile phase, less sample carryover and cycle time makes SFC-MS superior than LC-MS.<sup>[\[53\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref65)</sup> here, the analysis of nonvolatile compounds is possible due to selectivity and sensitivity of mass spectrometric detection.<sup>[\[54\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref66)</sup> A coupled technique of supercritical fluid chromatography and gas chromatography can be used for to analyse volatiles of cloudberry oil extracted with supercritical carbon dioxide. Here, capillary supercritical fluid chromatography is used for the pre-separation of the oil and for the introduction of the volatile fraction into gas chromatography. This leads to identification of 69 components using chemical and electron impact ionization mass spectrometry.<sup>[\[55\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref67)</sup> Online coupling of a supercritical fluid chromatography with gas chromatography– mass spectrometry (GC– MS) is performed using a custom-made cryo-trap cell for the determination of poly-cyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls in sediment reference material samples, and in spiked sea-water samples.<sup>[\[56\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref68)</sup>

**SFC coupled with other techniques**

A coupled supercritical fluid extraction (SFE)-supercritical fluid chromatography (SFC) technique helps in quantitative analysis of additives in various polyethylene and polypropylene polymers[\[57\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref72) and separation of coal-derived products.<sup>[\[58\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref73)</sup>

When coupled with proton high-field nuclear magnetic resonance spectroscopy, it is used for the separation of phthalates under supercritical conditions with carbon dioxide as eluent. The advantage of carbon dioxide is that the whole spectral range of the 1H NMR spectra can be observed and no solvent suppression techniques are necessary to obtain the NMR spectra.<sup>[\[59\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref74)</sup> Capillary SFC coupled with FTIR helps in qualitative analysis of chemical additives in polymers.<sup>[\[60\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref75)</sup> SFC-FT-IR is also used in separation of various sulphanilamides.[61[-62\]](http://www.cysonline.org/article.asp?issn=4444-4443;year=2010;volume=1;issue=2;spage=12;epage=22;aulast=Sethi#ref78)

#### **Conclusion**

SFC acts as a versatile and dynamic intermediate technique of GC and LC. As the mobile phase widely used is carbon-dioxide, no chemical waste is produced. High resolution and high efficiency with improved recovery and reproducibility is achieved due to low mobile-phase flow rate, density programming and detector compatibility that proves its potential. Separation of chiral compounds is a significant application of SFC. Moreover, it can analyze thermo labile or non-volatile compounds which are important advantages of SFC over GC and it can perform separations faster than HPLC without using organic solvents. It is well suited for the analysis of polar drugs and metabolites. It is used in fingerprinting, analysis of drugs of abuse and explosives as well. In addition, it is involved in fractionation of low vapour pressure oils, in several reactions in different areas of biochemistry, polymer chemistry,

environmental sciences as well as food, polymer and material industries.

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**Fig. 1:** Flow Diagram of Construction of SFC Instrument.

<b>Property</b>	Gas (STP)	<b>SCF</b>	Liquid
Density $(g/cm^3)$	$(0.6-2)$ x 10 <sup>-3</sup>	$0.2 - 0.5$	$0.6 - 2$
Diffusion coefficient	$(1-4)$ x 10	$10^{-3}$ x 10 <sup>-4</sup>	$(0.2-2) \times 10^{-5}$
$\rm \left( cm\ ^{2}/s\right)$			
Viscosity (G Cm $^{-1}$ s $^{-1}$ )	$(1-4) \times 10^{-7}$	$(1-3) \times 10^{-4}$	$(0.2-3)$ x 10 <sup>-2</sup>

**Table 1:** Comparison of properties of SFC with GC and LC.

**Table2:** SCFs have densities, viscosities and other properties that are intermediate between those of a substance in gaseous and liquid state  $[11]$ 



Table 3: Critical properties of some commonly used SCFs <sup>[12-13]</sup>



<b>SFC</b> Instrument (Model)	uses	
Method Station II, Thar Technologies, Inc.	multiple compounds Screening of with	
	multiple columns and multiple mobile phase	
	compositions	
Analytical SFC system, JASCO	Sepeartion and sample preparation	
	applications, replaces normal phase	
	chromatography for environmental analysis	
	procedures	
Preparative SFC system , JASCO	Seperation and purification from hundreds of	
	milligram to several grams, allow use of a	
	wide range of detectors including UV-Vis,	
	multi-channel and circular dichroism	
Anal SFC Semi-prep Investigator II, Thar	Isolate and collect less than 1 gram of specific	
technologies, Inc.	compounds.	
<b>SFC-MS</b> Resolution ,Thar $\mathbf{I}$	Robust and fast chromatographic separation	
Technologies, Inc.	and mass spectral data without software	
	interruptions and downtime.	
Thar <b>SFC</b> Petro Analyzer,	Pertroleum industry, such as refineries for	
Technologies, Inc.	gasoline, diesel and jet fuels.	
SFC Assurance, Thar Technologies, Inc.	Manual injection of single compound, higher	
	productivity, faster equilibrium, less labor,	
	and typically90% less solvent.	
Prep SFC-MS30, Thar Technologies, Inc.	Under super optimal conditions, a kg/kg	
	adsorbents/day(kkd)throughput achieved that	
	can deliver a total flow of 400ml/min.	
Prep SFC-MS Prep 100, Thar technologies,	Optimizes the purification run to reduce the	
	run time, reduce the solvent usage and	
	enhance the quality of collected fraction.	
Prep SFC 80, Thar Technologies, Inc.	High pressure seperators for quantitative	
	recovery of purified products, such as	
	enantiomers, complex synthetic chemicals and	
	natural products, chiral and achiral	
	seperations, optimized for seperations	
	50grams or less, and can be configured with	
	up to 12 fraction collectors.	
Prep SFC 200, Thar Technologies, Inc.	Optimized for purifications of 25grams/hour	
	or less, can be configured with 4 or6 fractional	
	collectors.	
Prep SFC 350, Thar Technologies, Inc.	Optimized for purification of 1kg or less, and	
	can be configured with 4 or 6 fraction	
	collectors	
Series 4000 SFC, Selerity Technologies	Analysis of both nonvolatile and volatile	
	analytes, including thermally labile analytes	
	provides SFC columns &accessories.	

**Table 4:** Different types of instruments used in S.F.C.