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Review Article

Supercritical Fluid Chromatography- A Hybrid of GC and LC

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Abstract High performance specifications and unique functionality of chromatographic techniques is a demand of pharmaceutical industry and research. 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. High diffusion coefficient and low viscosity of supercritical fluids is responsible for high speed analysis, high efficiency and high sensitivity. Low mobile-phase flow rate, density programming and compatability with GC and LC detectors make SFC a versatile chromatographic technique in analytical research and development. It has a unique characteristic of analyzing thermo labile or non-volatile substances. This review highlights the role of supercritical fluid chromatography in the separation of polymers, thermally labile pesticides, fatty acids, metal chelates and organometallic compounds, chiral and achiral molecules, identification and analysis of polar samples, explosives, drugs of abuse and application of SFC in forensic science (fingerprinting).

Keywords: 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 by Lovelock. Novotny and Lee et al. demonstrated the first experiments on capillary SFC in 1982 and the first commercial capillary column SFC instrument was introduced in 1985. [1] 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 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.[2] 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. It has good solvating power and high diffusivity, which make it a good choice as a mobile phase in chromatography. Supercritical fluid chromatography offer potential applications in drug identification in clinical and forensic toxicology. In SFC, carbon dioxide is maintained at its supercritical state (above its critical temperature and pressure), above which it cannot be liquefied even with further increase in applied pressure. It has low viscosity, approximate that of a gas, and high diffusivity, between those of a gas and a liquid which provides favorable column efficiency. It may be performed by using either packed or open tubular capillary columns and with GC and LC detectors. Its coupling with gas and liquid chromatography alongwith mass spectrometry is useful in drug analysis; development and discovery.[3] In SFC, proper selection of pressure, temperature, pressure reduction ratio, and density, co-solvent and solvent gradients are the factors that must be considered for optimized separation and analysis. (Figure 1)

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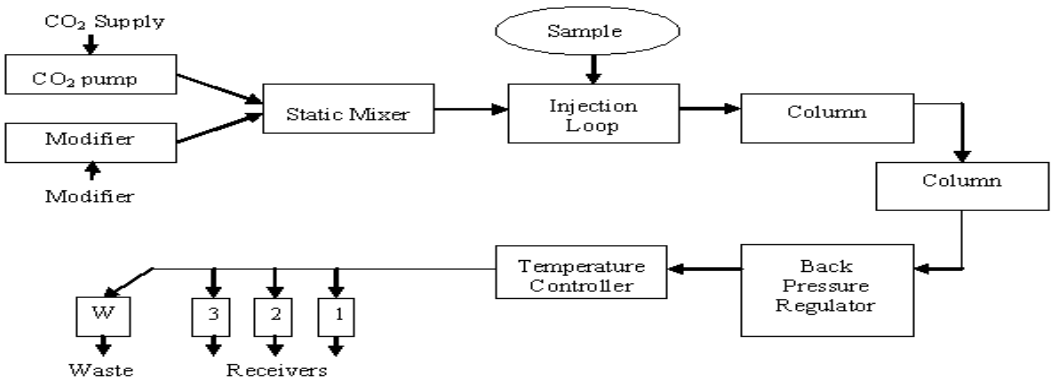

Figure 1: Schematic diagram of SFC unit

Principle

The concept of supercritical fluid can be easily understood by a phase diagram for a pure substance shown in Figure 2. 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. [4] 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. [5] Table 1 indicates that the SCFs have following properties intermediate between those of a substance in gaseous and liquid state**.** [6] 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. Thus, as the density of the supercritical fluid mobile phase is increased, components retained in the column get eluted.

Table 1: Comparison of properties of SFC with GC and LC [6]

Property	Gas (STP)	SCF	Liquid
Density (g/cm^3)	$(0.6\text{-}2) \times 10^{-3}$	0.2-0.5	0.6-2
Diffusion coefficient (cm^2/s)	$(1\text{-}4) \times 10^{-1}$	$10^{-3} \times 10^{-4}$	$(0.2\text{-}2) \times 10^{-5}$
Viscosity ($G\ Cm^{-1}\ s^{-1}$)	$(1\text{-}4) \times 10^{-4}$	$(1\text{-}3) \times 10^{-4}$	$(0.2\text{-}3) \times 10^{-2}$

Gradient Elution Methods

SFC involves three types of gradient elution methods: (a) temperature gradient method like GC, (b) modifier programming method like LC and (c) pressure programming method that is unique to SFC. The temperature gradient method consists of positive and negative temperature programming method. The positive temperature programming method elevates temperature versus time and it is applicable for volatile solutes. The negative temperature programming lowers temperature versus time and it is applicable for non-volatile solute. Both the negative and positive programming methods can be effective for separation of moderately volatile solutes. In the modifier programming method, the content of organic modifier in the mobile phase is varied as a function of time which resembles to binary gradient elution in HPLC. In the isothermal mode, the higher the pressure the higher is the density of the mobile phase which increases the mobile phase's strength. This is pressure programming in SFC.

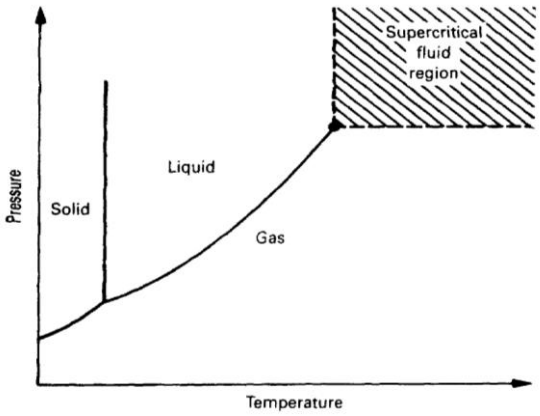

Figure 2: Phase Diagram for pure Substance

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. [7] As a result, unique bands are formed based on the amount of interaction between the individual analytes and the stationary phase in the column. When these bands leave the column, the components of sample mixture get identified and quantified by a detector. [8]

Mobile Phase

The mobile phase used in SFC consists of either high pressure liquid or supercritical carbon dioxide. Supercritical carbon dioxide offers higher solute diffusivity compared with the inert carrier gas conventionally used in gas chromatography and has a lower viscosity than the liquid solvents used in HPLC. The most widely used mobile phase in SFC is carbon dioxide because it is inert, nontoxic, nonflammable and ecofriendly solvent with low critical temperature of 304K and moderate critical pressure of 73bar. Thus, it can exist in solid, liquid and gaseous state at various combinations of temperature and pressure. Its property of miscibility in a variety of organic solvents and easy recovery makes it superior over other supercritical fluids. It diffuses faster than conventional liquid solvents due to its small and linear structure and viscosity similar to that of gas that allows high flow rates and rapid equilibration with stationary phase. It can also replace freons and other organic solvents. [9] Moreover, it has low cost and low interference with chromatographic detectors and it is compatible with FID. Due to its high polarity, water can also be used as a supercritical fluid. It has a critical temperature of 647K and critical pressure of 220bar. It has a unique property of dissolving paraffins, aromatics, gases and salts at supercritical conditions. Thus, this makes it beneficial for treatment of toxic wastewater and other organic and inorganic wastes. As its dielectric constant changes from about 78 at room temperature and atmospheric pressure to 6 at critical conditions, several reactions can be controlled. Finally solubility of sample in supercritical fluid at operating conditions, safety, flammability, phase behavior and cost of fluid are the other factors that determines the type of supercritical fluid used in chromatography. [10] Other commonly used mobile phases include nitrous oxide, ethylene, propylene, propane, n-pentane, ethanol, ammonia, [11] and xenon. Nitrous oxide and ammonia are more-polar mobile phases and they are compatible with primary alkyl amines. As xenon is transparent from ultraviolet to nuclear magnetic resonance regions, it is suitable for SFC-FTIR. [12]

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. [13] 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, ethanol and isopropyl alcohol are widely used as modifiers. Chloroform, tetrahydrofuran and acetonitrile can also be used as modifiers. Further, it can also be used to improve separation efficiency by blocking some of the highly active sites on the stationary phase. They are widely used in in packed column SFC. 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. Using the fully automated system, the optimal chiral separation of several compounds can be obtained unattended within 24 hr. Program-controlled columns and modifier selectors provide better results. [14] In case of polar solutes, it improves retention and efficiency while for highly retained nonpolar solutes, it increases apparent column efficiency, especially for the C-18 column. [15] Molecular fractions of triacylglycerols can be separated by SFC on micro-packed silver ion columns with a mobile phase of carbon dioxide with isopropanol and acetonitrile. [16]

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 autosampler. It can safely discharge carbon dioxide in the sample loop and continuously inject samples. The injection method can be selected as either a fixed injection volume that fills the entire sample loop or a variable injection volume that introduces the sample in a portion of the sample loop. The variable injection volume method provides full injection with zero sample loss. LC injection valve is commonly used for packed SFC and pneumatic valve is used in capillary SFC as small sample volumes must be quickly injected into the column. A thermostated column oven is required for precise temperature control of the mobile phase to achieve stable extraction efficiency and peak retention times. The ovens used in SFC are generally conventional GC or LC ovens.

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. Due to this some analytes remain longer in the column and thus it allows the separation of the mixture. There are different types of stationary phases available with varying compositions and polarities like absorbents such as alumna, silica

or polystyrene or stationary phases insoluble in SC -CO2. 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 μm. 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.

Microprocessor

One or more microprocessors are present in SFC instruments to control pumping pressures, oven temperature and detector performance.

Detectors

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 detector components must be capable of withstanding the high pressures of SFC. As SFC is compatible with both HPLC and GC detectors, thus optical detectors, flame detectors and spectroscopic detectors can be used. 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). Mass spectrometry and Fourier transform infrared spectrometry can also be used effectively with SFC. The major advantages of capillary SFC-FID are high efficiency separation and possible universal detection of drugs lacking functional groups detected by absorbance or fluorescence.

Advantages of SFC

High efficiency and sensitivity, low mobile-phase flow rate, density programming, and wide range of both GC and LC detectors makes SFC a unique and versatile chromatographic technique in analytical research and development. Moreover, it involves analysis of polar drugs and metabolites without derivatization and it has low cost. As the diffusion of solutes in supercritical fluids is about ten times greater than that in liquids (and about three times less than in gases), SFC separations can be done faster than HPLC separations. This results in a decrease in resistance to mass transfer in the column and allows for fast high resolution separations. SFC separations can be performed without the use of organic solvents as supercritical fluids have higher solubility and thus it provide more column loading. Moreover, they are inexpensive, innocuous, ecofriendly and non-toxic. SFC utilizes carbon dioxide which is available freely in environment and can also be collected as a by-product of other chemical reactions. Therefore, no new hazardous chemical is introduced to the environment. Capillary SFC can provide high resolution chromatography than gas chromatography (GC) at much lower temperatures. This leads to faster analysis of thermolabile compounds as compared to other techniques. It is more cost-efficient, user friendly, with higher throughput, better resolution and faster analysis times than other liquid chromatographic methods. It is a versatile technique because of its multi-detector compatibility. SCFs have high densities (0.2-0.5gm/cm 3) due to which they can dissolve large, non-volatile molecules like n-alkanes containing 5 to 30 carbon atoms, di-n-alkyl phthalates with dialkyl group containing 4-16 carbon atoms and several polycyclic and aromatic compounds with many rings. Solvation strength of SCF is directly related to the fluid density. The high solubility of organic species in SC -CO_2 is used for extracting caffeine from coffee beans to get decaffeinated coffee and for extracting nicotine from cigarette tobacco. Thermally unstable analytes can be easily recovered by allowing the solutions to equilibrate with the atmosphere at low temperatures, like an analyte dissolved in the SC-CO_2 can be recovered by reducing the pressure and allowing evaporating under ambient laboratory conditions. As SCFs have higher diffusion constants, their analysis speed is high and their lower viscosity than other liquid solvents reflects that pressure drop across the column for a given flow rate is greatly reduced. Long columns can be used because of their high diffusibility in this type of chromatography. Therefore, SFC plays an important role in chromatography as well as an extraction technique.

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. [17] Oligomeric polymer mixtures or complex mixtures of oleophilic components can be readily solubilized in supercritical fluids. Unlike GC, by changing the mobile phase the selectivity can be varied in SFC. [7] Moreover, GC is unable to analyze thermally unstable or non-volatile 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. [17] Due to higher diffusion rates and low vis-

cosity, SFC provides 3-5 times increase in the speed of analysis and a decrease in the cost of analysis by saving organic solvent. [18] 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 thermolabile 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. Due to this, capacity ratios, selectivities and theoretical plate heights are affected. In addition, SFC can be operated at sub ambient temperatures, which makes it useful in many chiral separations.

Applications

Due to its unique and versatile characteristics, SFC is widely used in various fields of research and development in pharmaceutical industry. Table 2 includes the list of various SFC instruments available in the market.

S.No.	SFC instrument (Model)	Uses
1.	Method Station II, Thar Technologies, Inc.	Screening of multiple compounds with multiple columns and multiple mobile phase compositions
2.	Analytical SFC System, JASCO	Separation and sample preparation applications, replaces normal phase chromatography for environmental analysis procedures
3.	Preparative SFC System, JASCO	Separation and purification from hundreds of milligrams to several grams, allows use of a wide range of detectors including UV-Vis, multi-channel and circular dichorism
4.	Anal SFC Semi-Prep Investigator II, Thar Technologies, Inc.	Isolate and collect less than 1 gram of specific compounds.
5.	SFC–MS Resolution II, Thar Technologies, Inc.	Robust and fast chromatographic separations and mass spectral data without software interruptions and downtime.
6.	SFC PetroAnalyzer, Thar Technologies, Inc.	Petroleum industry, such as refineries for gasoline, diesel, and jet fuels.
7.	SFC Assurance, Thar Technologies, Inc.	Manual injection of single compound, higher productivity, faster equilibrium, less labour, and typically 90% less solvent.
8.	Prep SFC-MS 30, Thar Technologies, Inc.	Under super-optimal conditions, a kg/kg adsorbents/day (kkd) throughput achieved that can deliver a total flow of 400 ml/min.
9.	Prep SFC–MS Prep 100, Thar Technologies, Inc.	Optimizes the purification run to reduce the run time, reduce the solvent usage and enhance the quality of the collected fraction.
10.	Prep SFC 80, Thar Technologies, Inc.	High pressure separators for quantitative recovery of purified products, such as enantiomers, complex synthetic chemicals and natural products, chiral and achiral separations, optimized for separations 50 grams or less, and can be configured with up to 12 fraction collectors.
11.	Prep SFC 200, Thar Technologies, Inc.	Optimized for purifications of 25 grams/ hour or less, and can be configured with 4 or 6 fraction collectors.
12.	Prep SFC 350, Thar Technologies, Inc.	Optimized for purifications of 1 KG or less, and can be configured with 4 or 6 fraction collectors
13.	Series 4000 SFC, Selerity Technologies	Analysis of both nonvolatile and volatile analytes, including thermally labile analytes, provides SFC columns & accessories
14.	Deven Supercriticals Pvt. Ltd.	Design, manufacture and supply wide range of SCF processing equipment along with complete technical support for process / product development, process optimization and efficient operation of the plant.

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. [19] Polynuclear aromatic hydrocarbons in automobile exhaust, [20] polyolefinic antioxidants /light stabilizers [21] and polyethoxylated alkylphenols [22] are analysed successfully by using SFC. Various dimethyl polysiloxane oligomers [23] and polycyclics aromatic hydrocarbon extracted from carbon black using fluorescence detection [24] can be separated. Silanised polyglycerols can also be analysed. [25]

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), carbamates (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 ionisation mass spectrometry (pSFC-APCI-MS). [26]
Direct coupling of a capillary supercritical fluid chromatograph to a bench top electron ionization (EI) mass spectrometer (SFC-EI-MS) using a GC-MS interface, aldicarb, diuron, methiocarb, alachlor, bendiocarb, and carbaryl plus other analytes are analysed with good chromatographic integrity and sensitivity. [27] Supercritical fluid extraction and chromatography has been used for the analysis of pesticides and their metabolites in foods and drinks. [28]

Separation of metal chelates and organometallic compounds

SFC is a versatile technique for the separation of metal chelates, transition metals and heavy metals. Organometallic compounds of lead, mercury, and tin are separated by SFC. Lanthanide and actinide complexes are also separated using SFC. Solubility of organometallic compounds can be easily determined using SFC. [29] Solubility of nickel(II), copper(II), and chromium(III) hexafluoroacetylacetone and chromium(III) acetylacetone chelates is measured in supercritical CO_2. The metal chelates like metal diethyldithiocarbamate (DDC) and metal bis(trifluoroethyl) dithiocarbamate (FDDC) have also been extracted from aqueous environment using pure supercritical CO_2. Direct extraction of Ni+2 and Cu+2 from an aqueous matrix is also possible via in-situ chelation using diethyldithiocarbamate and bis(trifluoroethyl) dithiocarb -amate as the ligands. [30]

Separation of fatty acids, triglycerides and lipids

Geometric isomers of fatty acids can be separated by open tubular columns in SFC alongwith quantification of triglycerides. [31] 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. [32] The fatty acids like fatty acid methyl esters, (FAMEs) or free fatty acids, (FFAs) can be separated by supercritical fluid chromatography (SFC) using pure CO_2. [33] Capillary-SFC is used to analyse various natural and processed fish oil triglyceride mixtures. As a result, free fatty acids, squalene, α-tocopherol, cholesterol, wax esters, cholesteryl 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. [34] 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 [35] 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. [36] Various phospholipids, glycosphingolipids, archaebacterial lipids can also be analyzed. [37]

Fossil fuels

On-line coupling of supercritical fluid chromatography–gas chromatography–mass spectrometry helps in the determination of diaromatic and polyaromatic groups of diesel-range petroleum fractions. The SFC–GC system is beneficial because the SFC solvent (CO_2) can be easily eliminated and has no disturbing effect on the GC separation and detection and thus mass detection. [38] 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. [39] Using nonpolar carbon dioxide as the mobile phase, high resolution separations of the polycyclic aromatic hydrocarbon fractions of marine diesel fuel samples can be achieved. [40]

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-death-related drug analysis and for obtaining information relating to long term drug abuse. [42]

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 preparative-scale equipment improvements and a selection of detection options. [43, 44] A number of chiral molecules can be analysed using SFC due to its high column efficiency at normal flow rates. [45, 46] 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. [47] 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. A new concept of rapid chiral method development using sample pooling and supercritical fluid chromatography–mass spectrometry (SFC–MS) on four chiral stationary phases, namely Chiralpak AD and AS, and Chiralcel OJ and OD, and eight different modifier concentrations (5 to 40% methanol–0.2% isopropylamine) has also been reported. [48] Moreover, the higher diffusivity and lower viscosity of supercritical and near-critical fluids leads to faster analysis with improved resolution for chiral separations. [49] A packed column supercritical fluid chromatography (SFC) method for the separation of ibuprofen enantiomers on a chiral stationary phase and CO_2 with modifier as mobile phase has been developed. [50] The use of SFC in achiral separations is a novel approach. Here, several achiral methods have been optimised and batches of compounds purified using a retention time mapping strategy. It allows fast analytical purity analysis without compromising the ability to scale up to the preparative system, leading to drug discovery. [51]

Natural product applications

Capillary supercritical fluid chromatography (SFC) is widely used for the separation of complex mixtures of natural products having nonpolar to moderately polar components with a molecular weight range of 100-1000 Daltons. It is used to separate components from nonvolatile sample matrix, reaction products from higher molecular weight starting materials and the deformulation of commercial products like oleophilic compounds of natural origin. When coupled with micro-scale SFE, it also permits the characterization of micro samples, such as portions of single seeds and extractables from single, live insects, [52] which proves its efficiency in solving many problems related to agricultural chemistry. All *trans* alpha- and beta-carotene can be separated from their respective *cis*-isomers with capillary SFC. Beta-Carotene *cis*-isomers are separated with a SB-cyanopropyl-25-polymethylsiloxane column and alpha-carotene isomers are separated with two SB-cyanopropyl-50-polymethylsiloxane columns using carbon dioxide with 1% ethanol as the SFC mobile phase. [53] Many solutes can also be analysed at trace levels. Various compounds and complex mixtures can be analysed using supercritical fluids as mobile phase and mass for detection. [54] Analysis of nicotine and other alkaloids in tobacco can be done at picogram level and subnanogram by using supercritical fluid chromatography–ion mobility detector (SFC–IMD) that provides high sensitivity, nicotine specific detection and lower instrumentation cost. [55] Packed columns are used for anlysis of polar solutes and drugs [56, 57] like carbohydrates [58], sucrose polyesters etc. [59]

Applications in pharmaceutical industry

SFC is used for high throughput screening and purifications of pharmaceuticals. [51] It has become a technique for solving problems that are difficult to be monitored by other GC and LC techniques. With pure supercritical CO_2, it is difficult to analyze polar samples so polar modifiers are added to supercritical CO_2 for their separation like the separation of vitamins is possible by supercritical fluid chromatography using water-modified carbon dioxide as the mobile phase. [60] Various aliphatic and aromatic mono-hydroxamic acids can be separated by SFC using methanol modified CO_2 on a diol column. [61] Using supercritical fluids CO_2 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 supersaturation and nucleation. [62] Various stereoisomers (enantiomers and geometrical isomers) of furan derivatives which are important intermediates for the synthesis of physiologically active natural products can be separated. Thermally unstable furan derivatives can also be separated. [63] Dexamethasone and betaniethasone, prednisolone, and cortisone and hydrocortisone can be resolved by using a methylpolysiloxane open tubular capillary column and SF CO_2 as the mobile phase. Phencyclidine, 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. [64]

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, compatability of solvent with mobile phase, less sample carryover and cycle time makes SFC-MS superior than LC-MS. [65] Here, the analysis of nonvolatile compounds is possible due to selectivity and sensitivity of mass spectrometric detection. [66] 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. [67] On-line 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 polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls in sediment reference material samples, and in spiked sea-water samples. [68] A capillary (SFC) combined with a triple-quadrupole mass spectrometer (MS) via a liquid chromatography-atmospheric pressure chemical ionization (LC-APCI) interface, is used for the analysis of berry oil triacylglycerols. [69] Analysis of high molecular weight substances like oligosaccharides can be done on a double-focusing magnetic sector instrument by supercritical fluid chromatography/mass spectrometry. [70] When coupled with electrospray ionization mass spectrometry, it shows greater potential for the analysis of polar molecules. [71]

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 [72] and separation of coal-derived products. [73] 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.[74] Capillary SFC coupled with FTIR helps in qualitative analysis of chemical additives in polymers. [75] SFC-FT-IR is also used in separation of various sulphanilamides. [76, 77]

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 thermolabile 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.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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