



International Journal of Pharmaceutical Research & Analysis

e-ISSN: 2249 – 7781
Print ISSN: 2249 – 779X

www.ijpra.com

SUPER CRITICAL FLUID CHROMATOGRAPHY: AN OVERVIEW

Ranjeet Kumar V*, SaiMalakondaiah D, Ajitha A, UmaMaheshwaraRao V

Department of Pharmaceutical Analysis and Quality Assurance,
CMR College of Pharmacy, Kandlakoya(V), MedchalRoad, Hyderabad – 501 401, A.P, India.

ABSTRACT

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

Keywords: Critical pressure, Critical temperature, Diffusivity, Supercritical fluid.

INTRODUCTION

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

Supercritical fluids (SF) have densities and dissolving capacities similar to those of certain liquids, but

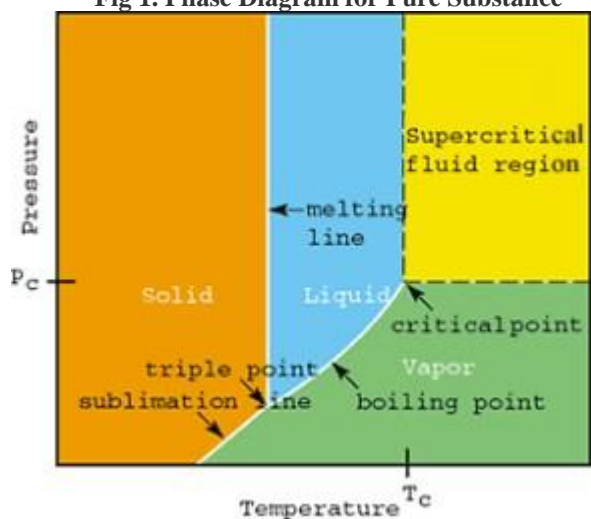
lower viscosities and better diffusion properties. Accordingly, SF used as mobile phases in chromatography should act both as substance carriers like the mobile phases in gas chromatography (GC) and also dissolve these substances like the solvents in liquid chromatography (HPLC). This chromatographic variant is known as supercritical fluid chromatography (SFC) [1].

Supercritical fluids 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 CO₂ 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

Corresponding Author:-V.Ranjeet Kumar Email:- vranjeetkumar@gmail.com

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 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. 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.

Fig 1. Phase Diagram for Pure Substance



Important properties of super critical fluids

SCFs have high densities (0.2-0.5gm/cm³) due to which they have a remarkable ability to dissolve large, non-volatile molecules, for example, SC - CO₂ readily dissolves 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. 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 -CO₂, 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- CO₂ 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 [2].

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 SCFs.

Physical Properties of Supercritical Fluids

Density

Density characteristic of a supercritical fluid is between a gas and liquids near liquid. In the supercritical region, density of a supercritical fluid increases when pressure rises at a constant temperature. When pressure is constant, density of the material decreases with increasing temperature. Dissolving effect of a supercritical fluid is dependent on its density value. Also, supercritical fluids are better carriers than gases thanks to their high density. Therefore, density is an essential parameter for analytical techniques using supercritical fluids as

Diffusivity

Diffusivity of a supercritical fluid can be 100 times more than a liquid and 1,000-10,000 times less than a gas. Because supercritical fluids have more diffusivity than a liquid, a solute can show better diffusivity in a supercritical fluid than in a liquid. Diffusivity is parallel with temperature and contrary with pressure. Increasing pressure affects supercritical fluid molecules to become closer to each other and decreases diffusivity in the material. The greater diffusivity gives supercritical fluids the chance to be faster carriers for analytical applications. Hence, supercritical fluids play an important role for chromatography and extraction methods [3].

Viscosity

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

Supercritical fluid chromatography

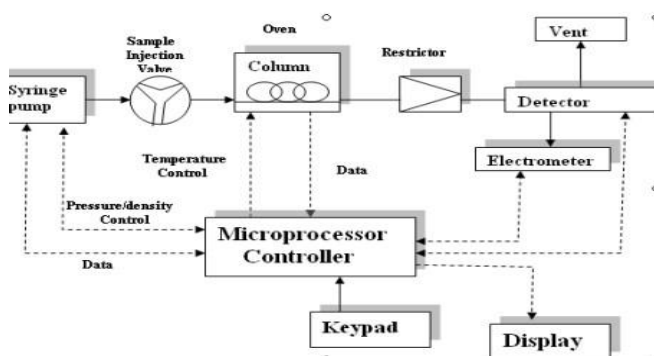
Chromatography is an analytical technique used for the separation of complex chemical mixtures into individual components. In SFC, the sample is carried through a separating column by a supercritical fluid where the mixture is divided into unique bands based on the amount of interaction between the individual analytes and the stationary phase in the column. As these bands leave the column, their identities and quantities are determined by a detector.

SFC is a hybrid of gas and liquid chromatography because when the mobile phase is below its critical temperature and above its critical pressure, it acts as a liquid, so the technique is liquid chromatography (LC) and when the mobile phase is above its critical temperature and below its critical pressure, it acts as a gas so the technique is gas chromatography (GC). Thus SFC combines some of the best features of each, LC as well as GC. SFC is important because it permits separation and determination of group of compounds that are not conveniently handled by either GC or LC. For example, GC is inapplicable for nonvolatile or thermally unstable compounds. Similarly, LC cannot be employed for compounds with those functional groups that cannot be detected by either spectroscopic or electrochemical detectors used in LC. SFC is a relatively recent chromatographic technique and there is a large amount of research currently underway both in SFC method development and in hardware development [4].

Instrumentation

The instrumentation of SFC is similar in most regards to instrumentation for HPLC because the pressure and temperature required for creating supercritical fluid from several gases or liquids lie well within the operating limits of HPLC equipment. However, there are two main differences between the two. First, a thermostated oven similar to that of GC, is required to provide precise temperature control of the mobile phase and second, a restrictor or a back pressure device 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.

Fig 2. Flow Diagram of Construction of SFC Instrument



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.

Pumps

In contrast to HPLC pumping system, pressure rather than flow control is necessary and pulseless operation is more critical. In general, the type of high-pressure pump used in SFC is determined by the column type. For packed columns, reciprocating pumps are generally used while for capillary SFC, syringe pumps are most commonly employed. Reciprocating pumps allow easier mixing of the mobile phase or introduction of modifier fluids. Syringe pumps provide consistent pressure for a neat mobile phase.

Injector

Injection in SFC is usually achieved by switching of the content of a sample loop into the carrier fluid at the column entrance by means of a suitable valve. For packed column SFC, a conventional HPLC injection system is adequate, but for the capillary column SFC, the sample volume depends on column diameters and small sample volumes must be quickly injected into the column, therefore pneumatically driven valves are used.

Oven

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

Columns

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

Restrictor or Back-Pressure Device

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

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

Microprocessor

The commercial instruments for SFC are ordinarily equipped with one or more microprocessors to control such variables as pumping pressures, oven temperature and detector performance.

Detector

SFC utilizes mobile phases, which can either be liquid like or gas like. Therefore it is compatible with both HPLC and GC detectors. Conventional gas-phase detectors such as flame ionization detectors and flame photometric detectors, liquid-phase detectors like refractive index detectors, ultraviolet-visible spectrophotometric detectors and light scattering detectors have been employed for SFC. Mass spectrometry and fourier transform infrared spectrometry can also be used effectively with SFC. The choice of detectors will depend upon the mobile phase composition, column type, flow rate and ability to withstand the high pressures of SFC.

Effect of Pressure

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

Mobile Phase

There are a number of possible fluids, which may be used in SFC as a mobile phase. However, based on its low cost, low interference with chromatographic detectors and good physical properties (nontoxic, nonflammable, low critical values) CO_2 is the most used mobile phase for SFC. It is an excellent solvent for a variety of nonpolar organic molecules. In addition, it transmits in the UV. It permits a wide selection of temperatures and pressures without exceeding the operating limits of modern HPLC equipments [6].

Modifiers

CO_2 is not a very good solvent for high molecular weight, ionic and polar analytes. This can be overcome by adding a small portion of a second fluid called modifier fluid. This is generally an organic solvent, which is completely miscible with carbon dioxide (alcohols, cyclic

ethers) but can almost be any liquid including water. Therefore in some applications methanol is introduced in small concentrations (1-20 mol%) to modify solvation power of CO_2 . Including chemical additives like acids and bases in the modifier can further enhance the solubility. Modifiers can also enhance selectivity of separation and improve separation efficiency by blocking some of the highly active sites on the stationary phase. Small amount (3.5%) of methanol to CO_2 increases solubility of cholesterol. If an analyte is only soluble in an aqueous solution, it is probably a poor candidate for SFC. Apart from methanol other solvents are also used as modifiers like acetonitrile, ethanol and 1-propanol. For highly retained nonpolar solutes, modifiers increase the column efficiency [7].

Comparison of SFC with Other Types of Chromatography

SFC combines some of the characteristics of gas and liquid chromatography, as several physical properties of SCF are intermediate between gases and liquids. Like GC, SFC is inherently faster than LC because the lower viscosity makes use of higher flow rates. Diffusion rates in SCFs are intermediate between gases and liquids. As a consequence, band broadening is greater in SCFs but less, than in gases. Thus, the intermediate diffusivities and viscosities of SCFs result in faster separation than is achieved in LC, accompanied by lower zone broadening than is encountered in GC. The mobile phases play different role in GC, LC and SCF. In GC, the mobile phase causes the zone movement. In LC, the mobile phase transports the solute molecule and also interacts with them thus influencing the selectivity. When a molecule dissolves in supercritical medium, the process resembles volatilization but at much lower temperature than that of GC. Thus, at a given temperature the vapor pressure for a large molecule in SCF may be 10 to 100 greater than in the absence of that fluid. As a consequence, high molecular weight compounds, thermally unstable species, polymers and large biological molecules can be eluted from a column at a reasonably low temperature. The biggest advantage that SFC holds over GC is the ability to separate thermally labile compounds. This is appreciated in the pharmaceutical fields since roughly 20% of all drugs candidates fall in this category. Unlike GC, by changing the mobile phase the selectivity can be varied in SFC [8].

Advantages of SFC

- 1) The physical properties of supercritical fluids between liquids and gases enables the SFC technique to combine some of the strong aspects of HPLC and GC. Lower viscosity of supercritical fluids makes SFC a definitely faster method than HPLC.
- 2) Lower viscosity leads high flow speed for the mobile phase.

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

Disadvantages

- 1) There have been a few technical issues that have limited adoption of SFC technology,
- 2) First of which is the high pressure operating conditions. High-pressure vessels are expensive and bulky, and special materials are often needed to avoid dissolving gaskets and O-rings in the supercritical fluid.
- 3) A second drawback is difficulty in maintaining pressure (backpressure regulation). Whereas liquids are nearly incompressible, so their densities are constant regardless of pressure, supercritical fluids are highly compressible and their physical properties change with pressure - such as
- 4) the pressure drop across a packed-bed column. Currently, automated backpressure regulators can maintain a constant pressure in the column even if flow rate varies, mitigating this problem.
- 5) A third drawback is difficulty in gas/liquid separation during collection of product. Upon depressurization [9].

APPLICATIONS

By now SFC has been applied to wide variety of materials including natural products, drugs, foods, pesticides, herbicides, surfactants, polymers and polymer additives, fossil fuels, petroleum, explosives and propellants. Some of the important applications are as follows.

Natural Products

Lipophilic-amphiphilic compounds with properties between volatiles and hydrophilic compounds often create problems in connection with their isolation and analytical determination resulting in an analytical gray area, But SFC has been found to give relatively fast and simple procedures for determination of oil constituents such as chlorophyll and its derivatives, carotenoids, tocopherols vitamins and phenolics which may be

important for the oil quality. Thereby it gives a tool to determine the origin of oil and improved possibilities of determination of relations between oil constituents and physical as well as biochemical properties of oil. Separation of bile salts and common free bile acids like ursodeoxycholic acid and chenodeoxycholic acid in pharmaceutical preparations has been reported using phenyl bonded silica column and SFC-CO₂ modified with methanol. SFC has been successfully utilized for the separation of underivatized triterpene acids, estimation of caffeine from tea and conjugated bile acids. Capillary-SFC has been used for analysis of panaxadiol/panaxatriol in ginseng and its preparations, vegetable carotenoids and pyrrolizidine alkaloids.

Pesticides

Supercritical fluid extraction and chromatography has been used for the analysis of pesticide residues in canned foods, fruits and vegetables wherein pyrethroids, herbicides, fungicides and carbamates have been tested.

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.

Lipids

As SFC operates at low to moderate temperature, it is most suited for the analysis of high molecular weight lipids like triacylglycerols. Even though HPLC methods give excellent resolutions, the elution times are relatively long and quantitative detection becomes a problem. With GC there is a possibility of thermal cracking of stationary phase or of the sample. Separation of paraffin wax, free fatty acid, mono-di and tri acyl glycerol detergents like Triton X-100 has been achieved using a capillary column coated with a nonpolar stationary phase at a temperature between 60°-120° at which no thermal damage to lipids is observed.

SFC has also been applied to analyze phospholipids after conversion to diacyl glycerol derivatives. Separation of fatty acid methyl esters, biosynthetic polyunsaturated fatty acids (PUFA), nonsaponifiable lipids, cholesterol and its esters in human serum and food samples, mono-, di- and triglycerides in pharmaceutical excipients has been carried out by SFC successfully. SFC has also been applied to analysis of archaeobacterial lipids and glycosphingolipids. A number of review articles have appeared in recent years on this topic and should be consulted for more detailed information.

Polymers

SFC provides elution of high molecular weight compounds, polymers and large biological molecules from

a column at a reasonably low temperature. Separation of the series of dimethyl polysiloxane oligomers and polycyclic aromatic hydrocarbon extracted from carbon black using fluorescence detection has been reported. SFC has also been applied to analysis of polyethoxylated alkylphenols, polyolefinic antioxidants /light stabilizers and polynuclear aromatic hydrocarbons in automobile exhaust [10].

Drugs

Modern drug substances are commonly nonvolatile and thermally or chemically labile therefore analysis by HPLC is common over GC. In SFC the conditions are mild and no volatilization is required so it is possible to handle such drug substances by SFC. Separation of various categories of drugs like antidepressants, phenothiazine antipsychotics, beta blockers, felodipine, a new dihydropyridine drug-clevidipine, methylated betacyclodextrins, vasodilators like isosorbide mononitrate, isosorbide dinitrate, cyclandelate, nimodipine, amlodipine, pentifylline, pentoxifylline, lovastatin, pyrethrins, isosorbide -5- mononitrate and related compounds in bulk

substances and tablets, atropine, indol-3-yl methyl oligomers and ascorbigens, tolnaftate and related impurities have been carried out by SFC. Separation and trace estimation of benzidine and its macromolecular adducts, fusarium mycotoxins, oestrogens, combinations of various nonsteroidal anti-inflammatory drugs like flufenamic acid, mefenamic acid, fenbufen, indomethacin mixtures, flufenamic acid, mefenamic acid, acetyl salicylic acid, ketoprofen and fenbufen mixtures and mixtures of ibuprofen, fenoprofen, naproxen, ketoprofen and fenbufen by SFC has been reported. SFC has also been applied for estimation of prostaglandins, determination of mefloquine in blood, anticancer drugs like cyclophosphamide, diaziquone, mitomycin C, thiotepa, sorbitantriolate in metered-dose inhalers, steroids, [14C] propranolol, underivatized 2,4-dichlorophenoxy acetic acid, determination of urinary metabolites of styrene, high speed screening of combinatorial libraries, determination of phenylbutazone and its major metabolite oxyphenbutazone in serum, phenylbutazone in dosage forms and sulphadoxin in blood plasma, mesoprostol from tablets, simultaneous SFC of ibuprofen and methocarbomal in solid dosage form [11].

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

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

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

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

Chiral compounds

Chiral separation by SFC was first documented in 1985. Due to the high efficiency, fast separation, low temperature analysis and applicability to wide variety of detectors, SFC has now become an attractive alternative for chiral drug separation. The success of SFC in the field of bioanalytical chemistry is well documented. A real break through for SFC in the bioanalytical field has been its contribution to chiral separations and in the near future it may surpass HPLC in the ability to provide appreciable selectivity of molecular stereoisomers.

Organometallics

Separation of metal chelates and organometals of thermally labile category, chelates of transition metals,

heavy metals, lanthenides and actinides as well as organometallic compounds of lead, mercury and tin has been carried out by SFC. Determination of solubility of organometallic compounds by SFC is also reported [12].

CONCLUSION

Supercritical fluid chromatography are the techniques which take advantage of supercritical fluids and their unique physical properties to surpass other related methods in both chromatography and extraction fields. Sometimes, they take place as alternative instrumental analytical techniques while, in some other cases, they are used as complementary partners for binary systems. In the overall ranking of chromatographic techniques, it has been judged that SFC falls somewhere between HPLC and GC

as the chromatographic method of choice. The biggest advantage that SFC holds over GC is the ability to separate thermally labile compounds, which is a very significant application in the pharmaceutical field as 20% of all drug candidates fall in this category. With the advent of SFC-MS, even picogram per milliliter concentrations can be detected easily which is not possible with other techniques. The enforcement of strict quality standards has produced a

need for fast, complete and sensitive analysis of drug candidate. SFC can provide the fast and complete analysis and MS can provide universal, sensitive detection. SFC-MS shows great potential in the field of bioanalytical chemistry, but especially in chiral separation and detection. As the science advances, it would be reasonable to foresee the practicality of this analytical technique reach into mainstream of analytical chemistry.

REFERENCES

1. Smith RM. Nomenclature for supercritical chromatography and extraction, IUPAC Recommendations. *Pure Appl. Chem.*, 65(11), 1993, 2397-2403.
2. Christie WW. Supercritical Fluid Chromatography and Lipids, *Lipid Technology*. 2, 1990, 107-109.
3. Dixon DJ and Jhonston KP. Encyclopedia of Separation Technology, Ruthven D, John Wiley, 1997, 1544-1569.
4. Akgerman A and Giridhar M. Fundamentals of Solid Extraction by Supercritical Fluids, In *Supercritical Fluids-Fundamentals for Applications*. Sengers JMH and Kiran E, Eds., Klüwer Academic Publishers, 1994, 669-696.
5. Smith R. *Supercritical Fluid Chromatography*. The Royal Society of Chemistry, London, 1988, 5.
6. Rozzi NL and Singh RK. Supercritical fluids and the food industry. *Comprehensive Reviews in Food Science and Food Safety*, 1, 2002, 33-40.
7. Raymond Scott PW. *Liquid Chromatography for the Analyst* Marcel Dekkar, 1994, 7.
8. Chester TL and Pinkston JD. Supercritical fluid and unified chromatography. *Anal. Chem*, 74(12), 2000, 2801-2811.
9. Chester TL and Pinkston JD. Supercritical fluid and unified chromatography. *Anal. Chem*, 96(16), 2004, 4606-4613.
10. Zou W, Dorsey JG *et al.*, Modifier effects on efficiency in packed-column supercritical fluid chromatography. *Anal. Chem.*, 72(15), 2000, 3620-3626.
11. Rouessac F and Rouessac A. *Chemistry analysis, Modern instrumentation methods and techniques*. 2th ed., Johnwiley& sons Ltd, 2007, 127-133.
12. Hizir SM, Barron RA. *Basic Principle of supercritical fluid chromatography and superfluid extraction*, 2013.