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A VERSATILE, USER FRIENDLY CHROMATOGRAPHY

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ABSTRACT

This article describes the problems associated with usage of large quantities of solvents in preparative chromatography and highlights the importance of usage of green solvents. It emphasizes the usage of supercritical fluid as green solvent in supercritical fluid chromatography (SFC) which **is** more versatile, more cost-efficient, user friendly, with higher throughput, better resolution and faster analysis than the other general liquid chromatographic methods. The instrumentation that is required for supercritical fluid chromatography (SFC) is versatile because of its multi-detector compatibility. Due to this, supercritical fluid chromatography (SFC) has formed a niche in the pharmaceutical industry. The present article also reviews the fundamentals, instrumentation and varied applications of supercritical fluid chromatography (SFC) in the analytical area.

Key words: Preparative chromatography, Green chemistry, Green solvents, Supercritical fluid chromatography (SFC)

INTRODUCTION:

Chromatography [1], a method of separating and identifying the components of a complex mixture by differential movement through a two-phase system, in which the movement is effected by a flow of a liquid or a gas (mobile phase) which percolates through an adsorbent (stationary phase) or a second liquid phase. Preparative chromatography is a powerful technique for the isolation and purification of a variety of chemicals including pharmaceutical compounds, natural products and biological molecules. Since preparative HPLC is a rather expensive technique, solvent consuming and causing environmental hazards its application is limited.

Commonly used solvents: [2-4]

Acetonitrile**,** n-Hexane**,** Methanol**,** 2-Propanol, Benzene, 1-Butanol, 2-Butoxyethanol, Carbon tetrachloride, 1-Chlorobutane, Chloroform,2-Chloropropane.

The focus is on minimizing the hazard and maximizing the efficiency of any chemical choice. Green chemistry, also called

sustainable chemistry, is a philosophy of chemical research and engineering that encourages the design of products and processes that minimize the use and generation of hazardous substances. In the 1960s, klesper proposed the use of supercritical fluid carbon dioxide, which is a green solvent for eluting a chromatographic column and developed the first superficial critical fluid chromatographic equipment. This developed a new window for preparative chromatography. Supercritical fluid is any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist. It can effuse through solids like a gas, and dissolve materials like a liquid. In addition, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties of a supercritical fluid to be "fine-tuned". Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Carbon dioxide and water are the most commonly used supercritical fluids, being used for decaffeination and power generation, respectively. The solvent power of supercritical Carbon dioxide is relatively weak and is strongly linked to its density, but it can be increased by addition of polar solvent such as methanol or acetonitrile. Because of the lower viscosity and higher diffusivity of supercritical fluids compared to common solvents, a higher mobile phase velocity can be used in the column leading to high though put than that of liquid chromatography. Carbon dioxide can be easily removed from the product by decreasing the pressure of the collected fractions. This eliminates the problem of removal of organic solvent removal encountered with the organic eluents. Carbon dioxide being a natural ingredient of the ecosystem is a green physiologically compatible solvent.

Comparison of SFC with Other Types of Chromatography: [5]

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^{10} 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 .

Due to the thermally unstable or non- – volatile nature of many nitrogen and / or sulfur containing compounds, they cannot be analyzed by GC. Even if HPLC is applicable to analyze these compounds, it generates a large number of organic solvents, which need to be ultimately disposed. The disposal cost of organic solvents typically ranges from $5 to $10 per gallon and is constantly rising due to the strict environmental regulations. With the desire for environmentally conscious technology, the use of organic chemicals as used in HPLC could be reduced with the use of SFC. Because SFC generally uses carbon dioxide, collected as a byproduct of other chemical reactions or is collected directly from the atmosphere, it contributes no new chemicals to the environment.

Like GC, SFC is inherently faster than HPLC, because of its lower viscosity and higher diffusion rates. It is well documented that SFC provides a combination of 3-5 times increase in the speed of analysis and a decrease in the analysis cost through saving in organic solvent. Unlike GC or HPLC where the mobile phase dominates the type of detector to be used, SFC utilizes mobile phase, which can be either liquid like or gas like. Therefore both GC and HPLC detectors are applicable to SFC. This multidetector compatibility makes SFC a technique of unparallel success in the analysis of thermally liable species and/or relatively high molecular weight compounds.

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, selectivity 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

Super Critical Fluids: Fundamentals and properties [6-10]

Supercritical fluid may be defined from a phase diagram for a pure substance (Fig.1), in which the regions corresponding to solid, liquid and gaseous state are clear. A substance such as CO_2 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 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.

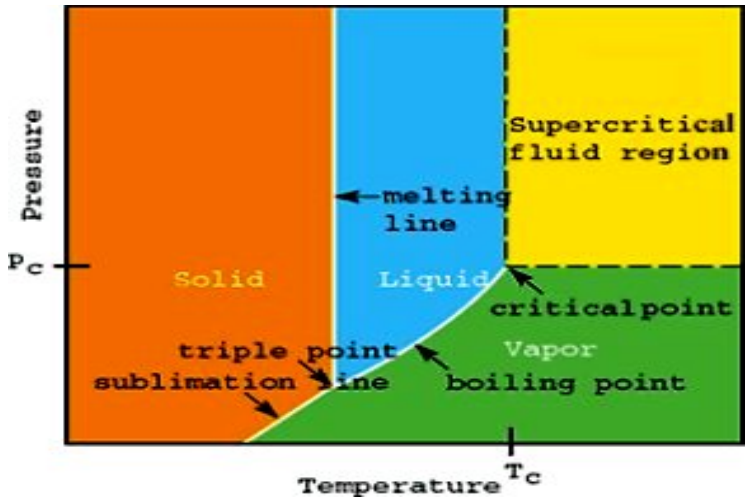

Fig. 1: Phase Diagram for Pure Substance

Important Properties of Super Critical Fluids:

SCFs have high densities (0.2-0.5gm/cm ^{3}) 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-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 -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 evaporating 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. 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.

Table I: SCFs have densities, viscosities and other properties that are intermediate between those of a substance in gaseous and liquid state

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

Table II: Critical properties of some commonly used SCFs

FLUID	CRITICAL TEMPERATURE (K)	CRITICAL PRESSURE (BAR)
Carbon dioxide	304.1	73.8
Water	647.3	221.2
Cyclohexane	553.5	40.7

The two supercritical fluids of particular interest are carbon dioxide and water.

Carbon dioxide: It is a non-flammable, nontoxic and ecofriendly solvent with low critical temperature of 304K and moderate critical pressure of 73bar.It is miscible with variety of organic solvents and is readily recovered after processing. As it's a small and linear molecule, it diffuses faster than conventional liquid solvents. It is often used to replace freons and certain organic solvents.

Water: It has a critical temperature of 647K and critical pressure of 220bar due to its high polarity. The character of water at supercritical conditions changes from one that supports only ionic species at ambient conditions to one that dissolves paraffins, aromatics, gases and salts. Due to this unique property, research has been carried out on supercritical water for reaction and separation processes to treat toxic wastewater. Control of reactions that depend on the dielectric constant of a medium is also possible in supercritical water as its dielectric constant changes from about 78 at room temperature and atmospheric pressure to roughly 6 at critical conditions.

Supercritical Fluid Chromatography

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 relatively recent chromatographic technique and there is a large amount of research currently underway both in SFC method development and in hardware development. Preparative SFC is quite similar to preparative HPLC. However, for preparative supercritical fluid chromatography, the eluent is a mixture of supercritical carbon dioxide and generally up to 20% (sometimes up to 40%) of organic co-solvent (usually an alcohol). SFC process incorporates a cycle of the eluent around its critical point. First liquid CO_2, is

compressed into the desired pressure and adjusted to the required solvent power and separation selectivity. The mixture to be separated is injected into the compressed eluent just before the column inlet. Then the compressed and heated eluent elutes the mixture through a chromatographic column maintained at the same temperature as eluent. this temperature should be near the critical temperature, for which supercritical fluid exhibits its high tuneable properties. The eluent leaving the column is then decompressed below its critical pressure and the supercritical solvent is transformed in to a gas phase. The gaseous carbon dioxide can then be cleaned, condensed and recycled..Achieving suitable selectivity with pure co2 requires too high pressures, so CO_2 is mixed with co solvent modifier.Correct co solvent selection will enhance solvent power and selectivity of separations.

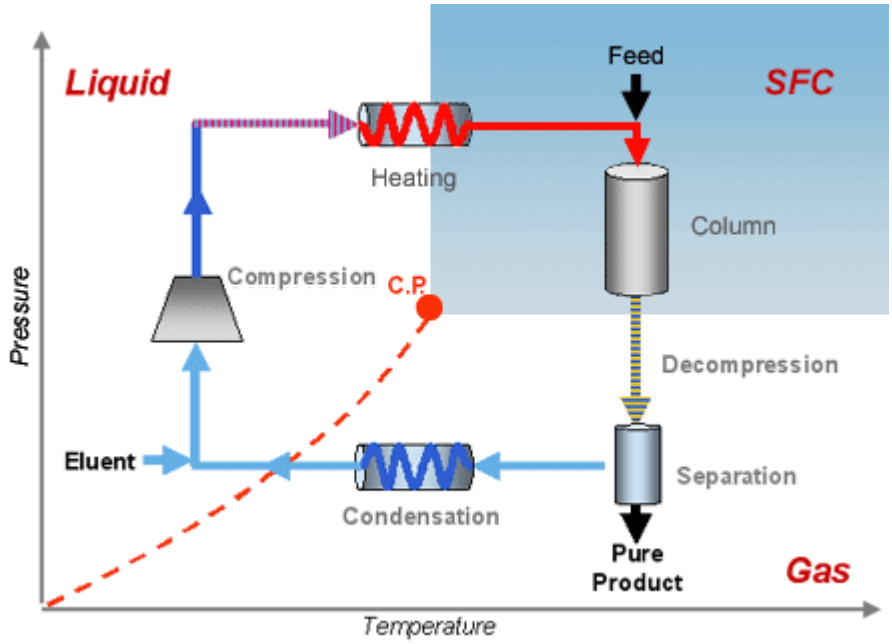

Fig 2: Principle involved in S.F.C

Resolutions using SFC are 3 to 5 times faster than with HPLC. In addition, as the CO_2 evaporates, the isolation of the compound of interest is also very quick. In the lab, this technique uses 3 to 20 times less organic solvent than for HPLC. Moreover, in well engineered preparative SFC systems, the CO_2 is recycled, further improving the process economics and reducing its environmental impact, making it **a** green separation process in the lab and in the pilot plant. SFC is a very quick chromatography technique and, thus, ideal in early development to produce grams to multi-kg amounts of desired products.

SFC 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 [7].

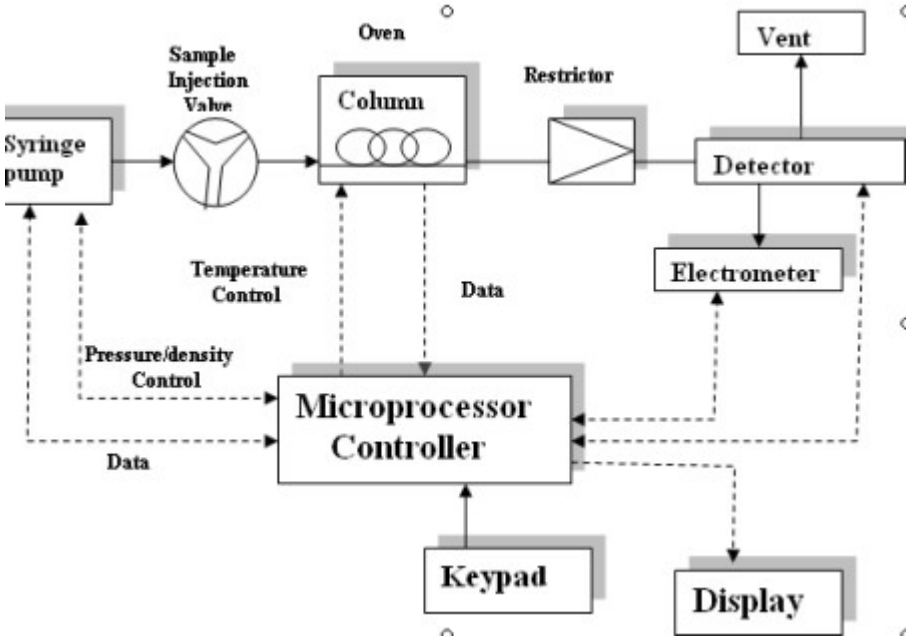

Fig.3: Flow Diagram of Construction of SFC Instrument

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 contract 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 SFCsyringe 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 alumna, silica or polystyrene or stationary phases insoluble in SC -CO_2 . More recent packed column work has involved bonded non-extractable stationary phases such as octadecylsilyl (C_{18}) 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 consists 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.

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.

Modifiers [11-14]

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 %) 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 and1-propanol. For highly retained nonpolar solutes, modifiers increase the column efficiency. For polar solutes, they improve retention and efficiency.

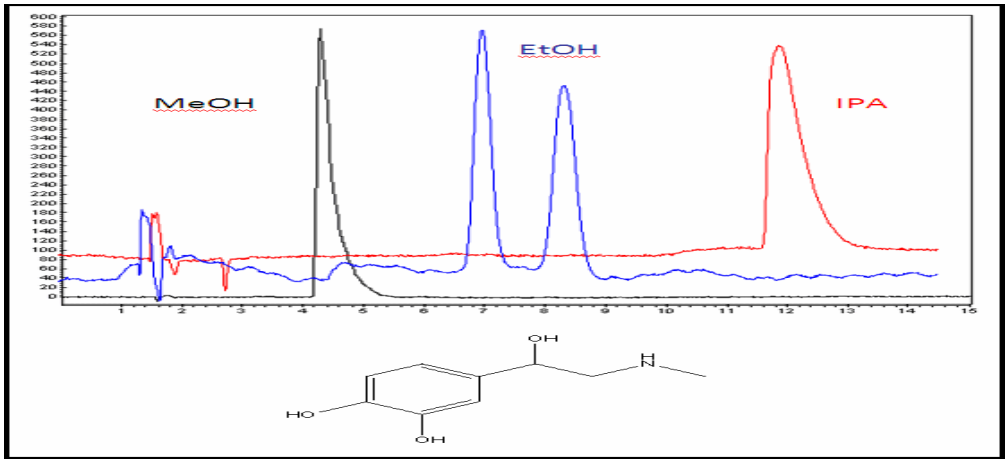

Fig 4: Separation of racemic epinephrine on a Chiralcel AD-H column with three modifiers and 0.1% ethane sulfonic acid (ESA) as the additive.

Exclusive CO_2 recycling devices:

CO_2 recycling after solute separation is standard design for preparative SFC systems. If not, CO_2 consumption would easily exceed 10 or even 20 kg of liquefied gas per hour for a preparative SFC system equipped with a 50 mm I.D. column. Systems are equipped with a robust and efficient CO_2 recycling device that reduces the required quantities of fresh CO_2 by 10 to 20 times. Except for analytical and small preparative instruments, CO_2 should be recycled; otherwise carbon dioxide consumption would easily exceed 10 or even 20 kg of liquefied gas per hour for a preparative SFC system equipped with a 50 mm I.D. column. Furthermore, selectivity is governed also by CO_2 polar modifier and predictions of the most effective modifier must be determined by mostly trial and error as illustrated in Figure 5 where one of three low molecular weight alcohols has a unique dramatic effect on resolution of an enantiomeric pair.

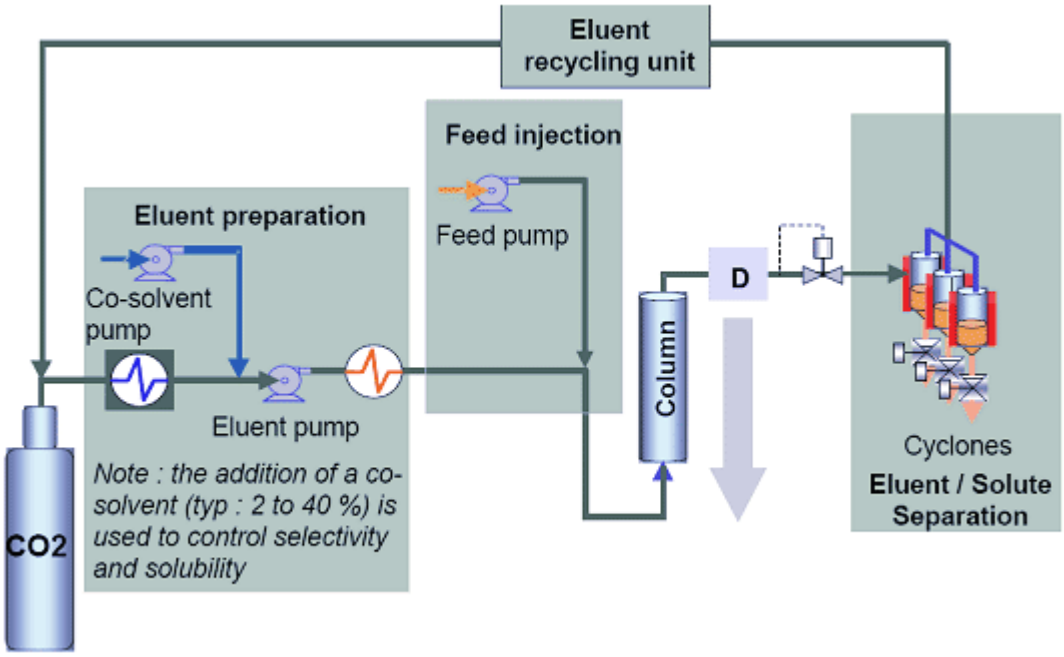

Fig 5: Recycling Device

APPLICATIONS:

Separation of polymers [11]

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 nonvolaitility for GC analysis. Polynuclear aromatic hydrocarbons in automobile exhaust, polyole-finic antioxidants /light stabilizers and polyethoxylated alkylphenols are analysed successfully by using SFC. Various dimethyl polysiloxane oligomers and polycyclics aromatic hydrocarbon extracted from carbon black using fluorescence detection can be separated. Silanised polyglycerols can also be analysed.

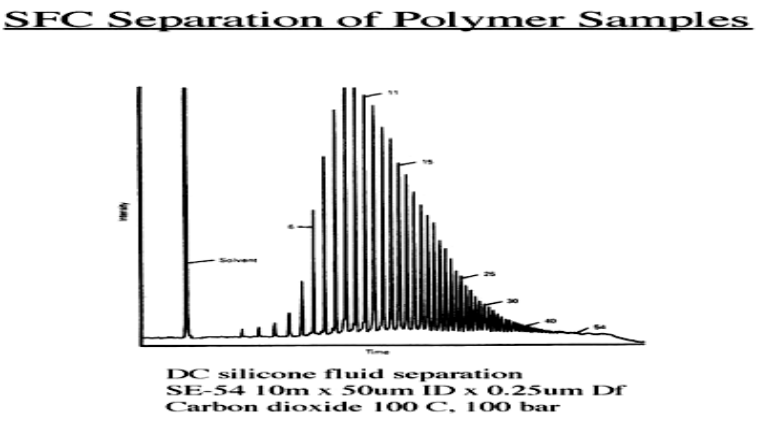

Fig: 6- SFC Separation of Sample

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, met-sulfuron methyl and benzsulfuron methyl) are detected and quantified in soil by packed-column supercritical fluid chromatography interfaced with atmospheric pressure chemical ionisation mass spectrometry .

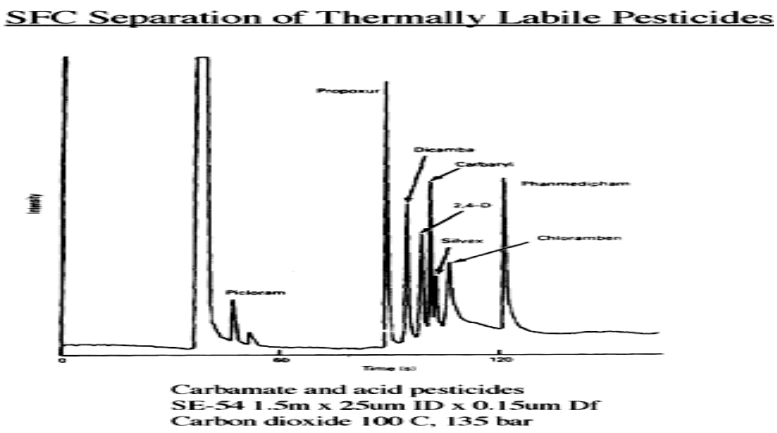

Fig 7: SFC Separation of thermally labile Pesticides

Separation of fatty acids, triglycerides and lipids [16-18]

Geometric isomers of fatty acids can be separated by open tubular columns in SFC along with quantification of triglycerides. 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. 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. Capillary-SFC is used to analyze various natural and processed fish oil triglyceride mixtures. As a result, free fatty acids, squalene, 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 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 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. various phospholipids, glycosphingolipids, archae-bacterial lipids can also be analyzed.

Chiral and achiral separations [19-21]

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. A number of chiral molecules can be analysed using SFC due to its high column efficiency at normal flow rates. 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. 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. Moreover, the higher diffusivity and lower viscosity of supercritical and near-critical fluids leads to faster analysis with improved resolution for chiral separations. A packed column supercritical fluid chromatography (SFC) method for the separation of ibuprofen enantiomers on a chiral stationary phase and CO2 with modifier as mobile phase has been developed. 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. SFC [22-30] is used for high throughput screening and purifications of pharmaceuticals. It has become a technique for solving problems that are difficult to be monitored by other GC and LC techniques. With pure supercritical CO2, it is difficult to analyze polar samples so polar modifiers are added to supercritical CO2 for their separation like the separation of vitamins is possible by supercritical fluid chromatography using water-modified carbon dioxide as the mobile phase. Various aliphatic and aromatic mono-hydroxamic acids can be separated by SFC using methanol modified CO2 on a diol column. Using supercritical fluids CO2 and water, fine particles like micro and nanoparticles 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. 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. Dexamethasone and betaniethasone, prednisolone, and cortisone and hydrocortisone can be resolved by using a methylpolysiloxane open tubular capillary column and SF CO2 as the mobile phase. Phencyclidine-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.

Isotope separation by supercritical fluid chromatography:

Lithium isotope separation by supercritical fluid chromatography was investigated using with supercritical carbon dioxide and LiCl methanol solution. Supercritical carbon dioxide is a hopeful solvent of the 21st century and reduces the effects on the environment.

Lithium isotope separation by displacement chromatography was performed with a resin packed column of 0.8 cm inner diameter and 100 cm length at 293 K under the pressure of 10, 12, 15 and 18 kPa. Lithium-7 and lithium-6 were enriched in the solution phase and in the resin phase, respectively. Enrichment factor became minimum at 15 MPa, though the difference between the values under each pressure was not large. Enrichment factor was obtained as 0.002-0.012.

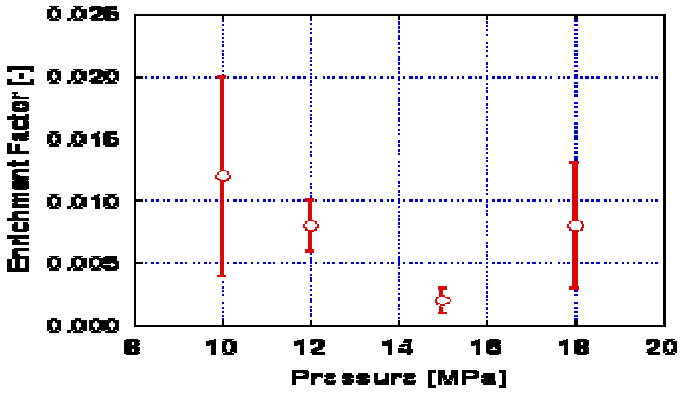

Fig. 8: Correlation between pressure and enrichment factor.

CONCLUSION:

Thus SFC has found a niche in the field of pharmaceutical chemistry and has gained much support in the field of bioanalytical applications. 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. There are too many examples in the literature testifying to the practicality of using SFC to separate specific compounds. The list of compounds separated by SFC is increasing day by day. SFC enjoys many advantages over the existing chromatographic techniques. But the most important contribution that SFC has made is towards separation of chiral compounds. SFC is enjoying great success in meeting the challenges of stereoisomer separation and may have already surpassed HPLC in the ability to provide appreciable selectivity of molecular stereoisomers. 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.

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