SUPERCRITICAL FLUID CHROMATOGRAPHY: A MODERN DAY ANALYTICAL TOOL AND ITS APPLICATIONS

K.Ambika Krishna,* Anupama Koneru, M. Sri Satya, P. Anila

Sultan-ul-uloom College of Pharmacy, Hyderabad, India.

Summary

Supercritical Fluid Chromatography (SFC) is a form of normal phase chromatography that is used for the analysis and purification of low to moderate molecular weight, thermally labile molecules. SFC can be utilized for estimations of concentrations of drugs and pharmaceuticals in pg/ml and lower, separation of natural compounds, chiral compounds, analysis of pesticide residues on foods, etc.

Key Words: Chromatography, supercritical, eco-friendly

***Author for correspondence**
K. Ambica Krishna
Sultan-ul-uloom College of Pharmacy
Road No. 3, Banjara Hills
Hyderabad-500 034
Andhra Pradesh
India
Email: Krishna_0189@yahoo.com

Introduction

Supercritical Fluid Chromatography (SFC) is a form of normal phase chromatography 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.

History:

Hanny and Hogarth in 1879 first demonstrated solubility of substances in Super Critical Fluids. Lovelock in 1958 first put forward the idea of supercritical fluid chromatography. The first commercial packed column of SFC was made available in 1981. The first commercial capillary column SFC instrument was introduced in 1985.

Super Critical fluids:

Supercritical fluid may be defined from a phase diagram [Fig. 1], in which the regions corresponding to solid, liquid and gaseous state are clear.[1] For every substance there is a temperature above which it can no longer exist as a liquid, no matter how much pressure is applied.

Properties of Super critical fluids:

SCFs have high densities (0.2-0.5gm/cm^3). They have a remarkable ability to dissolve large, non-volatile molecules, e.g., alkanes containing 5 to 30 carbon atoms, polycyclic and aromatic compounds. Dissolved analytes can be easily recovered. They are Inexpensive, Innocuous, Eco-friendly and non-toxic. They have higher diffusion constants and lower viscosities.[2] The two supercritical fluids of particular interest are- **Carbon dioxide and water.**

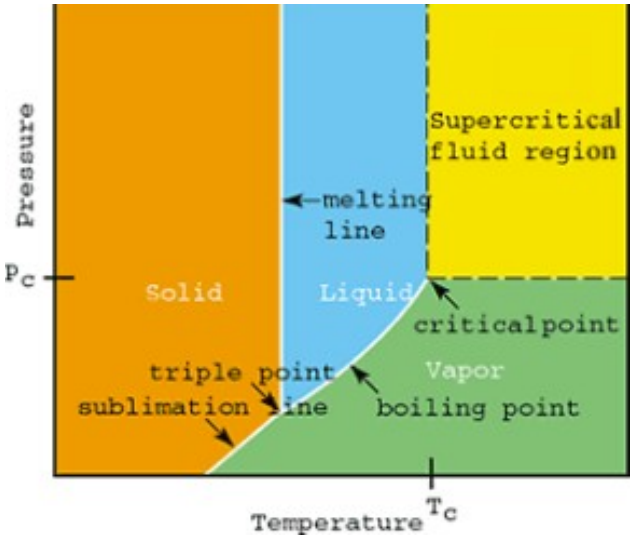

Fig.1. Phase Diagram

***Carbon dioxide*:**

It is a non-flammable, nontoxic and eco-friendly solvent having low critical temperature of 304K.It has a moderate critical pressure of 73bar.It is miscible with variety of organic solvents. It is readily recovered. It diffuses faster than conventional liquid solvents.[1]

Water:

It has a critical temperature of 647^{o}K and critical pressure of 220 bar. 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. Its dielectric constant changes from about 78 at room temperature and atmospheric pressure to roughly 6 at critical conditions.

Instrumentation:

The instrumentation of SFC is similar to instrumentation for HPLC [Fig, 2]. 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.[3]

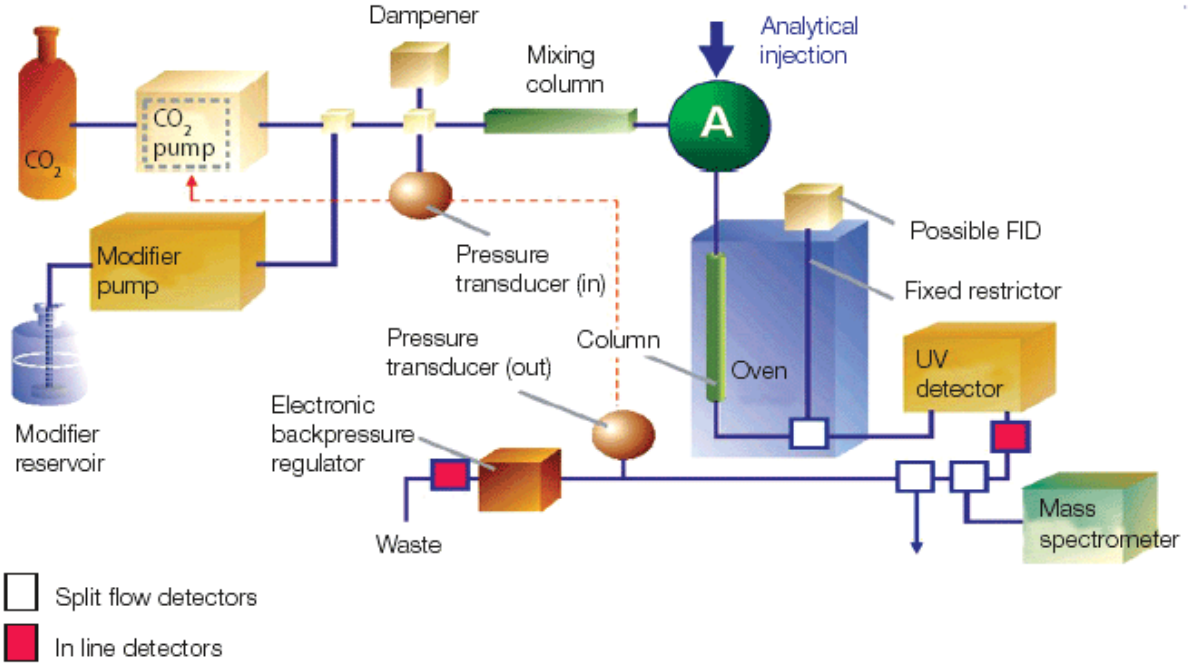

Fig 2. Instrumentation of Super critical Fluid Chromatography

Pumps:

Pump used in SFC is determined by the column type. For packed columns- reciprocating pumps are used. Capillary SFC- syringe pumps are used. Pressure is more important than flow control in SFC. There must be pulse less flow of fluid.

Injector:

Injection of a sample in SFC is done 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. For capillary column SFC small sample volumes must be quickly injected into the column, therefore pneumatically driven valves are used.

Oven:

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

Columns:

Two types of analytical columns are used in SFC, packed and capillary. Earlier absorbents such as alumina, silica or polystyrene were used.[1] Recent packed columns involves bonded non-extractable stationary phases such as octadecylsilyl (C_{18}) or aminopropyl bonded silica.

Restrictor or Back-Pressure Device:

This device is used to maintain desired pressure in the column by a pressure- adjustable diaphragm. The same column-outlet pressure is maintained irrespective of the mobile phase pump flow rate. It keeps the mobile phase supercritical throughout the separation. It 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.

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 is compatible with both HPLC and GC detectors. Conventional gas-phase detectors such as flame ionization detectors and flame photometric detectors can be employed and Liquid-phase detectors like refractive index detectors, ultraviolet-visible spectrophotometric detectors and light scattering detectors have been employed for SFC.

Applications:

SFC has been applied to wide variety of materials including natural products. Some of the important applications are as follows.

Natural Products:

SFC has been employed in determination of chlorophyll and its derivatives, arytenoids, tocopherols, vitamins and phenolic compounds.[4] SFC has been successfully utilized for the separation and estimation of caffeine from tea.

Pesticides:

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

Drugs:

SFC is employed in the analysis of drugs, viz., Phenothiazines, antipscychotics, betablockers, felodipine, isosorbide mononitrate, isosorbide dinitrate, nimodipine, amlodipine, pentoxifylline, lovastatin, atropine, tolnaftate, oestrogens, mefenamic acid, fenbufen, indomethacin mixtures, etc.[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.

SFC has been applied to separation of a large number of enantiomers, diasterioisomers and geometrical isomers like achiral and chiral analysis of temazepam and its metabolites, diasterioisomers of Du P105- a novel oxazolidinone antibacterial agent, chiral separation of 1,3 dioxolane derivatives, diasterioisomers of 2-bromomethyl-2- [(2,4-dichlorophenyl)-1,3-dioxolan-4-yl] methyl benzoate, enantiomers of ibuprofen, chiral antifungal agents, enantiomeric separation of aminoalcohols, triadimefon and triadimenol enantiomers and diasterioisomers, albendazole sulfoxide enantiomers, chiral separation of drugs based on macrocyclic antibiotics, separation of cis and trans beta carotene enantiomers and resolution of D- and L- alpha amino acid derivatives , enantiomeric separation of six triazole pesticides: cyproconazole, propiconazole, diniconazole, hexaconazole, tebuconazole, and tetraconazole, enetiomeric seperation of racemic mixtures of five acidic drugs namely dichlorprop, ketoprofen, warfarin, coumachlor and thalidomide using macrocyclic antibiotic chiral stationary phases (CSPs).[3,]

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.

SFC-MS in Pharmaceuticals:

In pharmaceutical industry, analyte concentrations in the pg/ml or lower range are a common.[6,7] In order to detect the realistic concentration levels, a detector with highest sensitivity, broadest selectivity and best resolution must be used. Currently, the detector that fits all of these criteria is the mass spectrometer.

Conclusions

In conclusion SFC can be utilized for estimations of concentrations of drugs and pharmaceuticals in pg/ml and lower, separation of natural compounds, chiral compounds, analysis of pesticide residues on foods, etc. Thus is developed fast as modern day analytical tool.

References

1. Kumar S K, Johnston K P. Modeling the solubility of solids in supercritical fluids with density as the independent variable. J Supercrit Fluids, 1988;1:15-19.
2. Supercritical Fluid Technology for Drug Product Development. Eds. York P, Kompella UB, Shekunov BY, Marcel Dekker, New York, 2004.
3. Practical Supercritical Fluid Chromatography And Extraction Ed. Marcel Caude, Didier Thiebaut, Harwood Academic publishers, 1999; 219-282.
4. Poudrier JK. SFC Boosts Drug Discovery and Other Processes. Today's Chemist at Work, Jan. 1998.
5. McHugh M, Krukonis V. Supercritical Fluid Extraction, 2nd ed., Butterworth-Heinemann, Boston, 1994.
6. Subramaniam B, Rajewski RA, Snavely K. Pharmaceutical Processing with Supercritical Carbon Dioxide. J Pharm Sci, 1997;86(8):885-890.
7. Klesper E. Chromatography with supercritical fluids. In: Extraction with Supercritical Gases. eds, Schneider GM, Stahl E, Wilke G. Verlag Chemie, Weinheim, 1980;115-139.