

Subject: Analytical Chemistry / Instrumentation

Production of Courseware

 -Content for Post Graduate Courses**Paper No. : 03** Chromatographic Techniques**Module : 03** Chromatographic Techniques- GC, HPLC, SCFC

Principal Investigator: Dr Nutan Kaushik, Senior Fellow,
The Energy and Resources Institute (TERI), New Delhi

Co-Principal Investigator: Dr Mohammad Amir, Professor of Pharm. Chemistry,
Jamia Hamdard University, New Delhi

Paper Coordinator: Dr Abhilasha Shourie, Professor & HOD- Biotechnology, Manav
Rachna International Institute of Research and Studies, Faridabad

Content Writer: Dr. Nidhi Didwania, Associate Professor, Biotechnology, Manav
Rachna International Institute of Research and Studies, Faridabad

Content Reviewer: Dr Abhilasha Shourie, Professor & HOD- Biotechnology, Manav
Rachna International Institute of Research and Studies, Faridabad

Description of Module	
Subject Name	Analytical Chemistry / Instrumentation
Paper Name	Chromatographic Techniques
Module Name/Title	Chromatographic Techniques- GC, HPLC, SCFC
Module Id	03
Pre-requisites	Knowledge of Fundamental Chemistry
Objectives	<ol style="list-style-type: none"> 1. To understand the fundamental concepts, principle, method, applications, advantages and disadvantages of Gas Chromatography. 2. To learn the fundamental concepts, principle, method, applications, advantages and disadvantages of High Performance Liquid Chromatography. 3. To understand the fundamental concepts, principle, method, applications, advantages and disadvantages of Super critical Fluid Chromatography.
Keywords	Gas Chromatography, High Performance Liquid Chromatography, Super critical Fluid Chromatography, partition coefficient, Quantitative Analysis, Qualitative Analysis, Chromatogram, mobile phase, stationary phase

List of Contents:

- Introduction to Gas Chromatography
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- Concept, principle and method of High Performance Liquid Chromatography.
- Applications of High Performance Liquid Chromatography
- Advantages and disadvantages of High Performance Liquid Chromatography

- Introduction to Super critical Fluid Chromatography
- Concept, principle and method of Super critical Fluid Chromatography.
- Applications of Super critical Fluid Chromatography
- Advantages and disadvantages of Super critical Fluid Chromatography

Chromatography is utilized in analytical chemistry for segregating and evaluating the compounds from a sample mixture. Chromatography in general has two phases viz. mobile and stationary phase. Depending upon the type of material used as stationary phase and mobile phase, chromatography can be of various types like ion exchange chromatography, thin layer chromatography, affinity chromatography, adsorption chromatography, gas chromatography etc. Planar Chromatography for example Thin layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) utilizes a flat (planar) stationary phase for separation as opposed to column chromatography (e.g. GC, HPLC).

GAS CHROMATOGRAPHY

Gas Chromatography is used for separating and analyzing the volatile compounds that can be vaporized without decomposition. Here the volatile mixture is separated by using a gaseous mobile phase. Another term given to Gas Chromatography is “Vapor-Phase Chromatography (VPC)” or Gas-Liquid Partition Chromatography (GLPC)”. An inert gas such as helium, hydrogen or nitrogen is the carrier gas which acts as a mobile phase. Helium happens to be the frequently used carrier gas as 90% of the instruments use it, however for better separation hydrogen is the preferred gas. Stationary phase can be either a microscopic liquid layer or

layer of polymer on the inert solid support, inside a column made up of metal or glass. The liquid material used for stationary phase should have high boiling point like that of silicone grease or wax. To obtain better results and for maximum column efficiency, there should be optimum flow rate of mobile gas phase.

General scheme of operating Gas Chromatography involves following steps

- a. Vaporizing of sample
- b. Injection of sample into the column
- c. Inert gaseous mobile phase is passed through the column
- d. Separation of the components on the basis of partition coefficient.
- e. Detector converts analytes into an electrical signal for measurement.

The sample which is to be analyzed is in gaseous phase, it interacts with the stationary phase which is coated on to the column walls. Every compound has a different retention time thereby each compound elutes out at different time. The components are segregated by a series of partitions between stationary liquid phase and moving gas phase of sample held in tube (column) of small diameter. The basic principle in Gas chromatography is that it exploits the differences in partition coefficients of volatilized sample between mobile gaseous phase and stationary liquid phase as the samples pass through the column. The use of this technique is confined to analytes that are volatile and thermally stable. In this process of separation, highly volatile analytes elute out first thereby marking the inverse proportionality of partition coefficient and volatility of analyte. As the sample gas stream comes out from the column, the detector monitors the separated components of the gaseous sample and signals are interpreted after data acquisition. Depending on the application, analytical or preparative, the sample size can be as small as pg to tens of grammes. The technique is highly sensitive and has high speed of resolutions. It not only determines what type of chemical is present in the mixture but also tells the quantity of each chemical in the mixture. Therefore it is used for analyzing complex mixtures and considered better than paper chromatography or thin-layer.

CLASSIFICATION/TYPES OF GAS CHROMATOGRAPHY

On the basis of mobile phase and stationary phase used, Gas Chromatography can be broadly classified into two:

- a. **Gas-Liquid Chromatography (GLC)**, the name of the technique refers to the mobile phase (gas) and stationary phase (liquid) respectively. GLC is often referred to as gas chromatography which is hinged on the principle of partition co-efficient.

The non-volatile liquid is coated on the inert solid powder (solid support) as a thin layer or along the inner wall of capillary tubing. This liquid film helps in partitioning of the sample components between stationary phase (liquid layer) and mobile phase (carrier gas). Inert support basically increases the surface area of liquid film to facilitate better interaction with the sample components. The solid support used in GLC includes materials like glass powder, diatomaceous earths, powdered Teflon, crushed firebricks, carbon black etc while the liquid layer applied over solid support should generally have low volatility and high decomposition temperatures e.g. polyethylene glycol, dimethyl silicone, diethylene glycol succinate (DEGS) etc. In comparison to packed columns, open tubular columns have thin layer of liquid coated on the wall or have their walls coated with inert solid support bearing liquid film. Length of these capillary columns range from 30 m to 100 meters with internal diameter of 0.1 mm – 0.53 mm. Capillary columns achieve better resolution even at lower concentrations than packed columns and also have shorter run times.

- b. **Gas-Solid Chromatography (GSC)**, the name of the technique refers to the mobile phase (gas) and stationary phase (solid) respectively. Here the retention of the sample components are due to the physical adsorption. The basic principle of Gas solid chromatography is physical adsorption. The solid stationary phase consisting of an active adsorbent powder is filled in the separating tube/column. In general, the column length is up to 10m with internal diameters ranging from 2-4mm. The columns are packed with porous material such as activated carbon, silica or alumina powder etc. The stationary phase selectively adsorb and desorb the volatile components. Solid state gas chromatography was developed by Fritz Prior in 1947. Apart from separating low molecular gases such as H₂, CO₂, CO, oxides of N etc., it is also used for separation of geometrical isomers.

Advantages of Gas Liquid Chromatography over Gas Solid Chromatography

- Shorter analysis time and better resolution between the peaks.
- GLC can be used for both, quantitative and qualitative analysis.
- Large concentration range of samples can be evaluated in GLC.
- Wide range of stationary phase i.e. liquid coating is available for large number of separations.

Limitations of Gas Chromatography

First and the foremost limitation of gas chromatography is that the substance which is being analyzed must be volatile so that there is a distribution of a definite fraction of it in the gaseous phase. Volatility of organic substance having molecular weight more than 500 is rarely adequate. Although high temperature (up to 300°C) increases the volatility but can result in decomposition of the substance.

Applications of Gas Chromatography

Gas chromatography has evolved since its inception in 1950s. It being highly sensitive in nature, have various applications in the field of industrial, pharmaceutical and chemical & biotechnology analytical laboratories. Due to its wide applicability, large number of compounds is analyzed both qualitatively and quantitatively with the help of GC. Due to its high sensitivity nature, it is used to separate and analyze the mixtures of compounds at very low concentrations. Applicability of GC ranges from testing the purity of a particular substance, preparing pure compounds from a mixture or separating different components, to identifying a compound. It can also be used for compounds having boiling points lower than 300°C. Due to very high sensitivity and speed of resolutions, GC has been used for analyzing complex mixtures. GC happens to be a standard analytical method in research and development sector. Many industries such as petrochemical, food and drug industries are using GC in their quality control department to look for purity and screening of contaminants in the product. GC has found is applicability in food flavor/fragrance and environmental sector also.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Introduction

The acronym HPLC was coined by the late Prof. Csaba Horvath in 1970 to Pittcon paper, due to the fact that to develop the flow required for liquid chromatography in packed column huge pressure was utilized. In the beginning, pumps had the pressure capability of 500 psi. Therefore it was originally referred to as High-Pressure Liquid Chromatography. Then there was a tremendous leap in technology in early 1970s. These new HPLC instruments could develop up to 6,000 psi of pressure, and incorporated the use of improved injectors, detectors, and columns. Later on due to continued advances in the working, the name was modified to high performance liquid chromatography but the acronym HPLC endured to be the same. It is also known as High Speed Liquid Chromatography and High Resolution Liquid Chromatography.

HPLC (High Performance Liquid Chromatography) HPLC is a type of column chromatography where a sample mixture is pumped at very high pressure into a solvent (the

mobile phase) and then passed through a column possessing the packing material (stationary phase).

Scope of HPLC

HPLC is best suitable for separation of ionic species, macromolecules, labile products and many other less stable and high molecular weight compounds. The four basic separation modes of HPLC are Ion exchange, Absorption, Partition, and Size exclusion. Furthermore the greater variety of stationary phases widens the scope of liquid chromatography usefulness. The scope of HPLC application widens further by incorporating recycling, pre or post column derivatization, solvent and gradient programming, column switching devices, specific detectors and by using ancillary (tandem) techniques.

HPLC is widely used in different area such as pharmaceuticals, chemical and food industries and environmental monitoring, etc. Both ground and soil water are polluted by complex mixtures of organic chemicals, constituting compounds with actual distinct polarities commencing in an ample range of concentrations. Thus environmental pollution by a huge array of chemicals exhibits a challenge to analyze them. Identifications of unknown compounds/pollutants are much more difficult. HPLC is widely used in monitoring the different contaminants. Polar and non volatile compounds may be analyzed by HPLC. With HPLC, the products may be tested and detection of the raw ingredients used to make them can be analyzed both qualitatively and quantitatively. The products are tested by HPLC before allowing them into market. There are lots of gains by HPLC approach in analytical and industrial fields. It aids in quantitative evaluation of impurities and deteriorated products in pharmaceutical formulations and immense drug materials. It also helps in structure elucidation of the samples.

Types of Analysis

Qualitative Analysis

HPLC is applied for the identification of individual components in the sample to be analyzed and its retention time is the prime important criteria for identification. The time used for elution of that peculiar compound through the column just after injection is termed as retention time. The identification of compounds depends on a number of parameters like chemical structure, the detector used the molecular weight etc.

Quantitative Analysis

HPLC benefits in the measurement of a compound concentration (amount) in a sample. To carry out quantitative analysis of the compound of interest, first of all a sample with known amount is injected and estimation of the peak height or peak area is completed. Thus, for the interpretation of a chromatogram (quantification) the two main paths are as follows:

1. To determine the height of peak of a chromatogram from the base
2. To determine peak area of a chromatogram.
3. The peak height and area as well as the sample amount has a sequential type of relation between them.

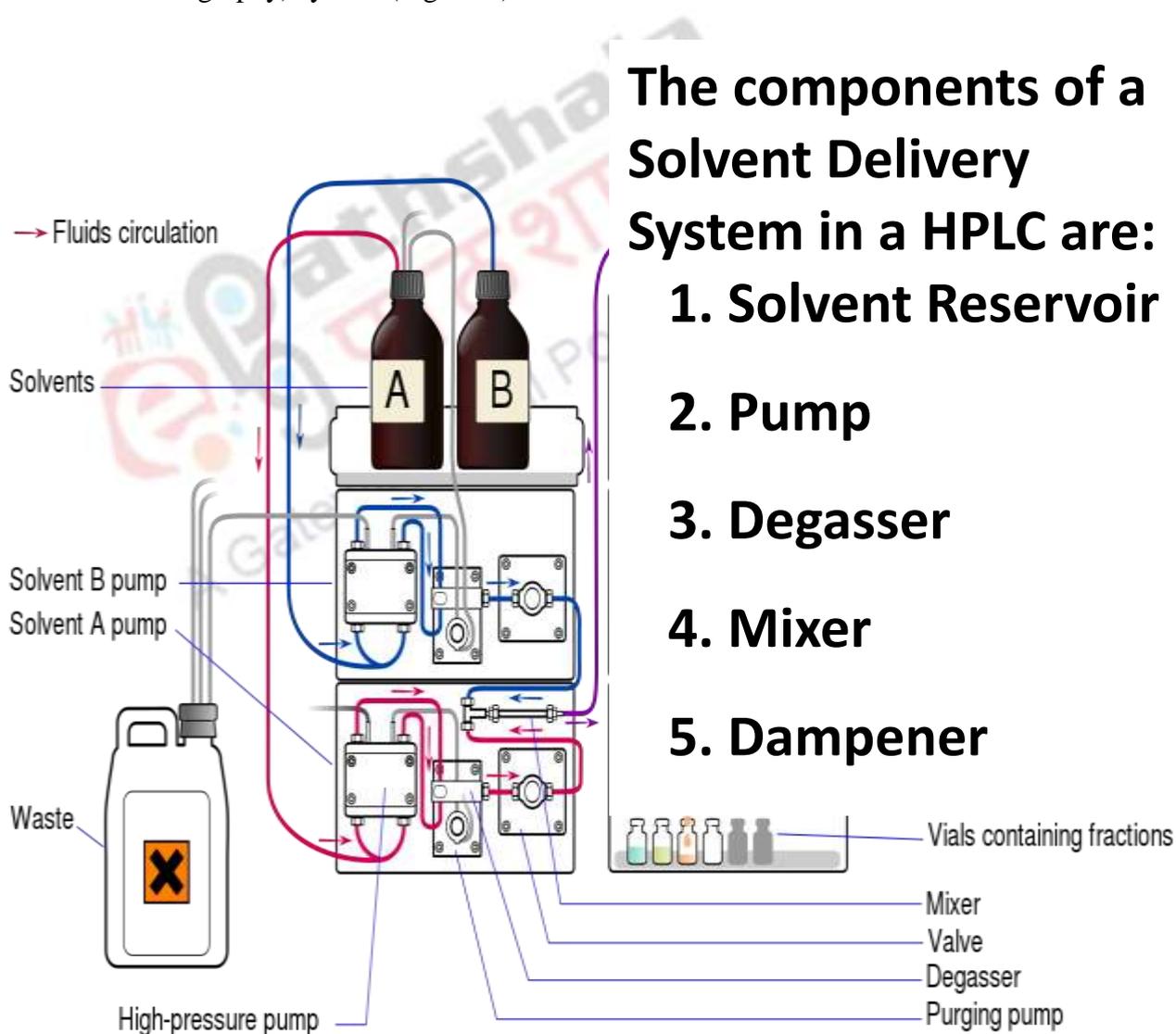
Preparation of Pure Compound(s)

The peaks in chromatograph are collected at the way out of the detector, the analytes are concentrated by evaporating the solvent and thus a pure substance can be prepared very easily by HPLC technique (For instance, synthesis of organic compounds, toxicology studies, clinical studies, etc.). This technique is also known as preparative chromatography.

Trace analysis

The detection of trace compounds is a prominent task in various studies like biological, toxicology, pharmaceutical and environmental. A trace compound is of very low concentration generally less than 1% by weight. It is often in parts per million (ppm) or lower but is of a great interest to the analyst. Separations with high resolution and detectors with high sensitivity are used in HPLC as the trace elements are very difficult to separate or detect.

The diagram below shows the constituents of a basic HPLC (High-performance liquid chromatography) system (Figure-1).



The components of a Solvent Delivery System in a HPLC are: Solvent Reservoir, Pump, Degasser, Mixer and Dampener

Solvent reservoirs are provided to store the mobile phase. These are usually made of glass. The mobile phase may be a liquid of single composition or a mixture of different solvents. If it is a mixture, it may consist of polar and non-polar solvents mixed in variable proportions, depending on the composition of the sample. Therefore the solvent delivery system in HPLC consists of a single reservoir or multiple solvent reservoirs. To facilitate the solvent flow under high pressure, valves are provided. These valves also allow rapid refilling of the solvent reservoir.

Mixing of Solvents in HPLC

- The solvent delivery system must be capable of mixing solvents homogeneously and vary polarity of mobile phase gradually. In an isocratic mode the solvent composition is kept constant throughout the run and the analyte elutes out when a fixed mobile phase volume has transited through the column. However, in a gradient elution, the mobile phase composition is required to change over the course of chromatographic run.
- Also the viscosity of the mobile phase may change due to change in its composition and the pressure may be required to adjust to maintain the required volumetric flow rate. The pump that delivers the mobile phase, can also be used to mix or blend the solvents.

The two basic types of injectors are – Manual Injection Systems, and Automatic Injection System

Manual sample injectors used in HPLC, transfer the sample from a syringe to a constant volume sample loop which is further connected to the stream of high pressure mobile phase through a valve which allows the sample inside the column. The valve is a six -port rotary injection valve, specially designed for this purpose.

Automatic injection systems consist of auto samplers that draw samples mechanically.

Today's HPLC requires very special apparatus which includes the following.

1. Extremely precise gradient mixers.
2. High pressure pumps in HPLC having a constant flow.
3. Unique high accuracy, low dispersion, HPLC sample valves.
4. Very high efficiency HPLC columns with inert packing materials.
5. High sensitivity low dispersion HPLC detectors.
6. High speed data acquisition systems.
7. Low dispersion connecting tubes for valve to column and column to detector

Applications of HPLC

HPLC is used to separate and analyze thermally-unstable or non-volatile compounds.

Common non-volatile compounds are:

- Pharmaceutical products such as acetaminophen (Tylenol), aspirin or ibuprofen etc
- Sodium chloride and potassium phosphate (salts)
- Blood protein / egg white (protein components)
- Polystyrene, polyethylene (polymers)
- Asphalt / motor oil (hydrocarbons)
- Herbal medicines, plant extracts (natural products)
- TNT, enzymes (thermally unstable compounds)
- HPLC can be enforced to any of the fields as environmental, pharmaceuticals, food, forensic, nutraceuticals, industrial and cosmetics,

Advantages of HPLC

- High sensitivity : HPLC can evaluate samples of nanogram and picogram concentrations
- High Precision and resolution: Detect very similar molecules.
- Highest accuracy: Identification of individual components of highly complex mixtures.
- Extremely quick and efficient: The process can be completed in very less time approximately 10 to 30 minutes.
- Highly reproducible
- Highly versatile
- Largely automated: Minimal training is required to run basic HPLC
- Administers security features, data management, forms report and instrument validation.
- Powerful and dynamically adaptable
- Manages all the areas of analysis and increases the productivity from sample to instrument as well as from segregation to reporting of results.
- Continuous monitoring of the column effluent
- Adsorption, partition, ion exchange and exclusion column separations are done very efficiently.
- No sample pre treatment is required for the analysis of both aqueous and non aqueous samples.
- For specific analysis, a high degree of selectivity can be provided as a variety of solvents and column packing's are available for HPLC.
- Multiple components can be determined in a single analysis.
- HPLC is more versatile than GLC in some aspects. It is not defined to volatile and thermally stable solute and there is a much extensive choice of mobile and stationary phases in HPLC.

Disadvantages of HPLC

- HPLC is expensive as it requires large quantities of organics of high cost.

- Existing HPLC methods are relatively easy to use but as there are series of different columns, modules and mobile phases, so it can be convoluted to establish new methods or to troubleshoot the problems
- Some compounds are irreversibly adsorbed and so cannot be detected by HPLC. Thus HPLC have low sensitivity for such type of compounds. For example gas chromatography is better used for separation of volatile substances.

SUPERCRITICAL FLUID CHROMATOGRAPHY

Column chromatography includes Supercritical fluid chromatography as a method forth with gas chromatography (GC) and high-performance liquid chromatography (HPLC). The mobile phase in supercritical fluid chromatography is a Supercritical fluid. A substance is called a supercritical fluid at temperatures and pressures above its critical temperature and pressure (critical point). The temperature above which a clear liquid phase does not exist is its critical temperature is. The critical pressure is the vapor pressure at its critical temperature. Only gases, liquids, solids, and plasmas are recognized as separate state of matter and supercriticals are not included in the list as a separate state of matter. The supercritical region is a kind of transitional region, where a liquid can be converted to a solid, and vice versa, without any phase transition by changing pressure and temperature.

The characteristic properties of supercritical fluids are density, diffusivity and viscosity. Supercriticals fluid tracts vary with temperature & pressure.

Usually supercritical Carbon dioxide is used as the mobile phase because the supercritical stage is achieved at comparatively mild temperatures and pressures in CO₂. The supercritical fluids have properties that are intermediate between Gas and Liquid and hence Supercritical fluid chromatography can combine the beneficial features of HPLC and GC.

Instrumentation for SFC

The composition of HPLC device is very similar to Super Fluid Chromatography. Commonly stationary phases and column types are also very similar in HPLC and SFC. But some of the discrepancies are always there. For supercritical fluids, temperature is analytical and therefore in SFC a device to maintain heat is required just same as gas chromatography. Pressure is also one of the essential aspects for supercritical fluids and therefore needs to be maintained at the suitable optimum level. A restrictor for controlling pressure is present in SFC. To regulate the relevant pieces of the instrument, a microprocessor assemble data for oven temperature, pressure, and detector performance.

Mobile phase

In SFC, mobile phase used is made up of a variety of materials. like inorganic solvents, ethers, hydrocarbons, halides, alcohols, acetonitrile, acetone, pyridine, etc. Carbon dioxide is the utmost familiar supercritical fluid which is applied in this chromatography due to many reasons. The critical temperature and pressure of carbon dioxide are very simple to reach. The carbon dioxide for non-polar molecules is a good solvent and also inert towards ultraviolet light. It is not-toxic, low-cost and easy to get. Ethane, n-butane, N₂O, dichlorodifluoromethane, diethyl ether, ammonia and tetrahydrofuran can be utilized other than carbon dioxide as mobile phase.

Ideal properties of SFC mobile phases

- Non-polar compounds with little to modest critical properties are needed generally
 - eg. CO₂, N₂O, pentane
- Segregations in normal phase
 - non-polar mobile phase and low polarity stationary phase
- Elution = function of molecular weight & polarity

The critical parameters of an ideal mobile phase in SFC should be easily achieved using the available instrumentation capabilities. It should have the following desirable properties:

1. High Solvent strength
2. High diffusion
3. Inertness
4. Compatibility with Detectors

Additives in SFC

The range of solutes that can be analyzed by supercritical chromatography has been extended significantly by the use of a mixture of mobile phases i.e. carbon dioxide, a low concentration of additive which is a much more polar substance and methanol as a modifier. Elution of highly polar compounds such as aliphatic amines and polyfunctional acids cannot be achieved only with binary mixtures of CO₂ and modifier. The inclusion of small quantities of a third substance known as additive is often required that results in efficient elution. Additives are stronger members belonging to same functional group. For eg. trifluoroacetic acid for elution of other acids, isopropylamine for eluting amines and acids/ bases for elution of bases or acids.

Stationary phases

The columns in supercritical fluid chromatography are very similar to columns in HPLC in the form of materials used for coating. The two utmost general types of columns used in SFC are open-tubular and packed. The open-tubular type has similarities to HPLC fused-silica columns and therefore is preferred. A cross-linked siloxane material is used to internally coat this type of column and this column is a stationary phase in the chromatography. The thickness of the coating may range from 0.05 μm to 1.0 μm . The length of the column may also vary from 10 to 20 m.

Columns for SFC

Open tubular which have been derived from GC columns.

- Higher efficiency
- Lower pressure drop

Packed columns which have been obtained from HPLC

- Analysis is faster
- Flow rates are higher
- Sample capacity is more

Detectors

A wide range of detectors are available in Supercritical Fluid Chromatography as compared to HPLC. The use of Flame ionization detector (FID) started in gas chromatography technique and is also very well utilized in SFC. Since FID has a property of high sensitivity, it is applied to the qualitative analysis in SFC. As compared to HPLC it is simple to connect SFC with UV-visible spectrometer, mass spectrometer, IR spectrometer. Detectors like fluorescence emission spectrometer or thermionic detectors frequently used in HPLC can also be attached to SFC.

SFC Separations

- Variation of the composition of mobile phase alters the separation similarly as in HPLC.
- In Supercritical Fluid Chromatography, there is a direct relation between mobile phase density and solvating power of the supercritical fluid.
- Pressure is controlled in the system which restrains density
- Due to highly reactive nature and critical parameters it is very hard to handle highly polar samples

Comparison of High Performance Liquid Chromatography and Supercritical Fluid Chromatography

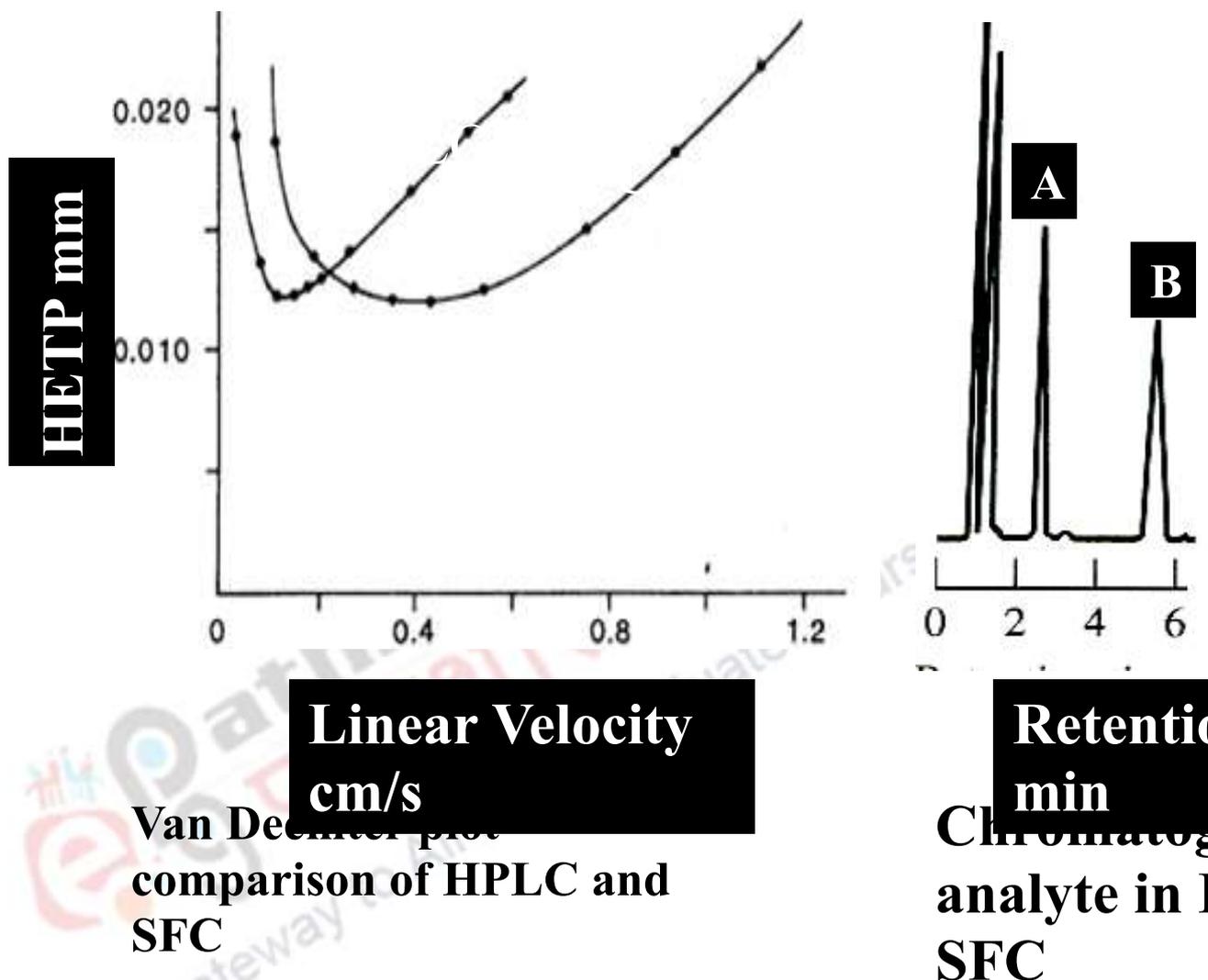


Figure 2: shows typical van Deemter plots of HPLC and SFC. It can be seen that SFC can be operated at 4 times greater flow velocities as compared to HPLC without appreciable increase in plate height. Supercritical fluids have lower density and viscosity than liquids. This facilitates diffusion of solutes and therefore solutes have large diffusion coefficients in supercritical fluids. Therefore, SFC has greater efficiencies at higher linear velocity as compared to HPLC. In the slide you can also see a comparison between the retention obtained in HPLC and SFC. SFC have far less retention time as compared to HPLC. That means it substantially reduces analysis time.

Advantages of SFC

Supercritical fluids have a number of advantages over liquid mobile phases and carrier gasses in various aspects like physical properties, solubility properties and detector compatibility.

Supercritical chromatography is gaining importance due to its numerous advantages such as

High speed analysis: Low viscosity and higher diffusion coefficients of the supercritical fluids allow quick and efficient separations

Low pressure drop across columns: Low viscosity and high diffusion coefficients of supercritical fluids results in lower problems due to pressure drop across the columns

Ability to analyze a wide variety of analytes: SFC can be applied to the analyze of many solutes without derivatization.

Analysis of thermolabile compounds: Due to low operating temperatures as compared to GC; SFC can be applied to thermolabile compounds also.

Use of ecofriendly mobile phase

A wider choice of detectors available for SFC

Comparison between HPLC, GC and SFC

SFC has a wide variety of stationary phases and changeable mobile phases (especially during a particular experiment), yet it does not have very high selectivity as found in HPLC. SFC has high sensitivity and efficiency which make it an efficient technique for qualitative analysis. Some of the properties can be altered during SFC which helps in allowing the optimization of the experiment. There is a broader range of detectors SFC as compared to HPLC. It is more efficient than GC in evaluation of biodegradable materials as SFC can work with low temperatures that are not possible in GC.

Supercritical Fluid Chromatography Advantages vs High Performance Liquid Chromatography

- Owing to low viscosities in SFC they provide faster analysis as compared to HPLC
- Pressure drop is considerably lower in SFC as compared to HPLC
- Open tubular columns can be used just like GC
- Columns have shorter lengths
- A wider choice of detectors
- Resolution power is much higher

Supercritical Fluid Chromatography Advantages vs Gas Chromatography

- Estimation of analytes having polarity, adsorption property and those that are non-volatile in SFC can be carried out without derivatization.
- Analysis of thermally labile compounds.
- Low density of Mobile phase in GC limits the molecular weight of compounds that can be determined by GC
- SFC can be employed for the analysis of high molecular weight compounds.

Applications of Supercritical Fluid Chromatography

There are numerous applications of SFC in environmental, food, pharmaceutical industries and many more. In this way, a wide range of herbicides, pesticides, explosives, polymers and fossil fuels can be estimated with SFC. SFC can be utilized to assess different drug components such as barbiturates, anti-inflammatory agents, antibiotics, prostaglandins, vitamins, steroids, taxol, non-steroidal etc. Chiral segregations can be implemented for numerous pharmaceutical compounds. The most general supercritical fluid mobile phase, carbon dioxide has very less efficiency for deliquescent polar solutes and therefore SFC is predominantly utilized for non-polar compounds. SFC is commonly used in the petrochemical field for the determination of entire aromatic components and other hydrocarbon segregations.

Environmentally friendly

SFC is considered ecofriendly or green despite using CO₂ as the main component as it is recycled from other industries. In HPLC, the mobile phase is often burned after use which results in generation of new carbon dioxide. By using small concentrations of alcohols in place of acetonitrile the toxic effect can be decreased efficiently. The CO₂ evaporates at the end of the system and expensive disposal is minimized as the volume of toxic liquid waste is greatly reduced.