IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
January 2017 Vol.:8, Issue:2

An Overview on Different Chromatographic Techniques

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

[1]Prasanna Deepak Madane, [2]Prasanna Mahendra Sapkal, [2]Mrunal Nandkishor palshikar

1 Yashavantrao Chavan institute of Science, Satara

2 Govt. college of pharmacy, Karad, India.

Submission: 7 January 2017
Accepted: 12 January 2017
Published: 25 January 2017

Keywords: Chromatographic method, Hyphenated gas chromatography, Ultra Performance Liquid Chromatography, flash chromatography etc.

ABSTRACT

Chromatography technique for separation of the component or solute or a mixture on the basis of relative amount of each solute distribution between a moving fluid stream, called mobile phase and stationary phase. Chromatography is one of several separation techniques defined as differential migration from a narrow initial zone. As separation method, chromatography has a number of advantages over older techniques such as crystallization, solvent extraction and distillation. The purpose of review is to present different chromatographic techniques and also flash chromatography, ultra performance liquid chromatography. They are rapid form of preparative column chromatography- preparative liquid chromatography based on air pressure driven, optimized for rapid separation of an organic compound.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

The word chromatography means “color writing” which is a way that a chemist can test Liquid mixture. While studying the coloring material in plant life, a Russian botanist invented chromatography in 1903. His name M. S. Tsweet (1).

Chromatography is usually introduced as technique for separating and identifying the components in a mixture. The basic principle is that components in a mixture have different tendencies to absorb onto a surface or dissolve in a solvent. It is a powerful method in industry, where it is used on a large scale to separate and purify the intermediate and product in various syntheses (2).

Chromatography is based on differential migration. The solutes in a mobile phase go through a stationary phase. Solute with a greater affinity for the mobile phase will spend more time in this phase than the solute that prefers the stationary phase. As the solute moves through the stationary phase they separate. This is called chromatographic technique (1).

BASIC PRINCIPLE:

All chromatographic methods require one static part (the stationary phase) and one moving part (in mobile phase). The technique depends on one of the following phenomenon: adsorption; partition; ion exchange; or molecular exclusion.

Adsorption: Adsorption chromatography was developed first. It has a solid stationary phase and a liquid or gaseous mobile phase. Each solute has its own equilibrium between adsorption onto the surface of the solid and solubility in the solvent, the least soluble or best adsorbed ones travel more slowly(2). The result is a separation into bands containing different solutes.

The solvent that is put into a column is called eluent, and the liquid that flows out of the end of the column is called eluate (3).

Partition: In partition chromatography, the stationary phase is a non-volatile liquid which helps as a thin layer (or film) on the surface of an inert solid. The mixture to be separated is carried by a gas or a liquid as mobile phase (2). The solute distributes themselves between the moving and the stationary phase, with more soluble component in mobile phase reaching the end of the chromatography column first (3).

Ion exchange: Ion exchange chromatography is similar to partition chromatography is that it has a coated solid as the stationary phase. The coating is referred to as a resin and has ion covalently bonded to it and ion of the opposite charge are electrostatically bound to the surface. When the mobile phase is eluted through the resin the electrostatically bound ion are released as other ions are bounded preferentially (2).

Molecular exclusion: Molecular exclusion differs from another type of chromatography in that no equilibrium state is established between the solute and the stationary phase. Instead, the mixture passes as a gas or a liquid through a porous gel. The pore size is designed to allow the large solute particle to pass through uninhibited. The small particle, however, permeates the gel and is slowed down so the smaller the particles, the longer it takes for them to get through the column. Thus separation is according to particle size (2).

Chromatographic technique:

Paper chromatography

Paper chromatography is one of the, most common type of chromatography. It uses a strip of paper as stationary phase. Capillary action is used to pull solvent up through the paper and separate the solvent(1).

This is probably the first, and simplest, type of chromatography that people meet. A drop of a solution of a mixture of dye or inks is placed on piece of chromatography paper and allow to dry. The mixture separates as solvent front advances past the mixture. Filter paper and blotting paper are frequently substituted for chromatography paper if precision is not required. Separation is not efficient if the atmosphere is saturated in solvent vapor (2).

Paper chromatography works by the partition of solute between water in the paper fibers (stationary phase) and the solvent (mobile phase). Common solvents that are used include pentane, propanone, and ethanol. Mixture of solvent are also used, including aqueous solutions, and solvent system with a range of polarities can be made. A mixture useful for separating dye on smarties is a 3:1:1 mixture (by volume) of butan-1-ol: 0.880 ammonia solution (3).

As each solute distribute itself (equilibrates) between the stationary and the mobile phase, the distance a solute move is always the same fraction of the distance moved by the solvent. This

fraction is variously called the retardation factor or the retention ratio and is given the symbol R or R_f (3).

Retention ratio = distance moved by solute / distance travelled by solvent = R_f

Applications:

- The separation of amino acid by using ninhydrin as detecting agent
- Structural analysis
- Separation of inorganic cations or complexes

Thin layer chromatography (TLC)

Thin layer chromatography uses an absorbent material on flat glass or plastic plates. This is a simple and rapid method to check the purity of an organic compound. It is based to detect pesticide or insecticide residues in food. It is also used to forensics to analyze the dye composition of fiber (1).

Thin layer chromatography is similar to paper chromatography, but the stationary phase is a thin layer of a solid such as alumina or silica supported on an inert base such as glass, aluminum foil or insoluble plastics. The mixture is spotted at the bottom of TLC plate and allowed to dry. The plate is placed in a closed vessel containing solvent (mobile phase) so that liquid level is below the spot (2).

TLC has an advantage over paper chromatography in that its results are reproducible, and that separation is very efficient because of the much smaller particle size of stationary phase(3).

The solvent ascends the plate by capillary action, the liquid filling the spaces between the solid particles. The technique is usually done in a closed vessel to ensure that the atmosphere is saturated with solvent vapor and evaporation from the plate is minimized before the run is complete. The plate is removed when the solvent front approaches the top of plate and the position of the solvent front recorded before it is dried that is this allow the R_f value to be calculated(3).

Applications:

- The checking of purity of sample
- The identification of organic compounds
- The separation of inorganic ions (3)
- Progress of chemical reaction
- Estimation of biomolecules
- Bio- assay (10)

Gas chromatography (GC)

Gas chromatography is used in airport to detect bombs and is used in forensics in many different ways. It is used to analyze fiber on a person body and also analyze blood found at a crime scene. In gas, chromatography helium is used to move a gaseous mixture through a column of absorbent material (1).

This technique uses a gas as the mobile phase, and the stationary phase can either be a solid or a nonvolatile liquid. If a solid stationary phase is used the technique is described as gas-solid adsorption chromatography, and if stationary phase is liquid it is called gas-liquid partition chromatography.

For separation or identification the sample must be either a gas or have an appreciable vapor pressure at temperature of column- it does not have to be room temperature. The sample is injected through a sealing disc into a rubber heated chamber where it is vaporized if necessary. Although the sample must all go into the column as a gas, once it is the temperature can be below the boiling point of the fraction as long as they have appreciable vapor pressure inside the column. This ensures that all solute passes through the column over the reasonable time span the injector oven is usually 50-100^{0}C hotter than the start of column (2). The sample is taken from the column by an inert gas such as helium gas or nitrogen which must be dry to avoid interference from water molecules (2).

Hyphenated gas chromatography:

Hyphenated gas chromatography refers not only the coupling of a gas chromatography to information rich detector includes mass and infrared spectrometers, where automated sample preparation system include static headspace (HS), dynamic headspace (PT), large volume injection(LVI), and solid-phase microextraction (SPME). Hyphenated of gas chromatography approaches also include coupling of two gas chromatograph and is commonly referred to as multidimensional chromatography.

Hyphenated gas chromatography is the versatile tool in pharmaceutical science with wide range of applications such as determination of volatile oil, separation of enantiomeric volatile component in essential oil using solid-phase microextraction or gas chromatography, determination of trace component in water using large volume injection it improve precision and provide for more effective use of laboratory personnel, particularly for industrial routine analysis. It also helps to process the high number of samples, necessary to get the many data for method validation to certify an analytical method (4).

Liquid chromatography

Liquid chromatography is used in word to test water sample to look for pollution in lake and river. It is used to analyze metal ions and organic compound in solution. Liquid chromatography uses liquids which may incorporate hydrophilic, insoluble molecule (1).

Liquid chromatography is similar to gas chromatography but uses a liquid instead of gaseous mobile phase. The stationary phase usually an inert solid such as silica gel, alumina, or cellulose supported in glass column. The adsorbing property of silica and alumina are reduced if they absorb water, but reduction is reversed by heating to 200-400^{o}C. Silica is slightly acidic and strongly adsorb acidic solutes.

A wide range of solvents is used in this technique, including hydrocarbons, aromatic compounds, alcohols, ketones and esters. A mixture of solvent can also be used (2).

Applications:

- The determination of homogeneity of chemical substance.
- The concentration of substance from dilute solution such as those obtained when

natural product is extracted with large volumes of solvent from the roots and leaves of trees, plants etc.

- The separation of geometrical isomers, diastereomers, racemates and tautomers(3).

High performance thin layer chromatography (HPTLC):

High performance thin layer chromatography (HPTLC) is a method that can be used for screening lichen substance. It is as simple to use as standard TLC but has many advantages: it is more sensitive, it is possible to run more sample in a shorter period of time, and amount of sample use is much smaller (5). It is a method of analysis in which the flow of solvent or gas promotes the separation of substance by differential migration in a porous adsorption medium.

Some features of HPTLC:

1. Simultaneous processing of sample and standard- better analytical precision and accuracy less need for internal standard
2. Several analysts work simultaneously
3. Lower analysis time and less cost per analysis
4. Simple sample preparation- handle sample of divergent nature
5. No prior treatment for solvent like filtration and degassing
6. Low mobile phase consumption per sample
7. Low maintenance cost
8. Visual detection possible-open system (5).

Supercritical fluid chromatography (SFC):

Supercritical fluid chromatography (SFC) is 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. SPF utilizes carbon dioxide as mobile phase, therefore the entire chromatographic path must be

pressurized. Because of supercritical phase represent a state in which liquid and gas properties coverage, supercritical fluid chromatography is sometimes called "convergence chromatography."(6)

Supercritical fluid chromatography is one of the most important column chromatography methods after gas chromatography and high performance liquid chromatography. SFC useful properties of gas and liquid phase. The characteristic properties of supercritical fluid are density, diffusivity, and viscosity etc.

Applications of SFC:

- There are applications for food, environmental and pharmaceutical product.
- Pesticides, herbicides, polymers, explosives and fossil fuel are other classes to be used.
- SFC is dominantly used for non-polar compounds because of the weakness of carbon dioxide, which is the most common supercritical fluid mobile phase, in term of dissolving polar solutes effectively.
- SFC can take place in petroleum industry with the application on total aromatic content analysis or other hydrocarbon separations (6).

Ultra Performance Liquid Chromatography:

UPLC refers to Ultra Performance Liquid Chromatography. It improves in three areas: chromatographic resolution, speed and sensitivity analysis. It uses fine particle and save time and reduce solvent consumption UPLC is come from HPLC (7).

The UPLC is based on the principle of use of stationary phase consisting of particle less than 2 µm (while HPLC column is typically filled with particles of 3 to 5 µm). The underlying principle of this evolution is governed by the van deemter equation, which is an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency). The Van Deemter curve, governed by an equation with three components shows that the usable flow range for good efficiency with small diameter particle is much greater than for larger diameters(8).

$$H=A+B/v+Cv$$

Where A, B and C are constant and v is the linear velocity, the carrier gas flow rate. The A term is independent of velocity and represents "eddy" mixing. It is smallest when the packet column particles are small and uniform. The B term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rate and so this term is divided by v. The c term is due to kinetic resistance to equilibrium in the separation process. The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again. The greater the flow of gas, the more a molecule on the packing tends to lag behind molecules in the mobile phase. Thus this term is proportional to v. Therefore it is possible to increase throughput and thus the speed of analysis without affecting the chromatographic performance. The advent of UPLC has demanded the development of a new instrumental system for liquid chromatography, which can take advantage of the separation performance (by reducing dead volumes) and consistent with the pressure (about 8000 to 15000 PSI, compared with 2500 to 5000 PSI in HPLC). Efficiency is proportional to column length and inversely proportional to the particle size 18. Therefore, the column can be sorted by the same factor as the particle size without loss the resolution. Efficiency is three times greater with 1.7 µm particles compared to 5 µm particles and two times greater compared to 3.5 µm particles. Resolution is 70% higher than with 5 µm particles and 40% higher than with 3.5 µm particles. High speed is obtained because column length with 1.7 µm particle can be reduced by factor of 3 compared to 5 µm particles for the same efficiency and flow rate can be three times higher. The application of UPLC resulted in the detection of additional drug metabolism, superior separation and improved spectral quality (9).

APPLICATIONS OF UPLC

- Analysis of amino acids
- Analyzing of natural products and traditional herbal medicines
- Analysis of levofloxacin in human plasma. Identification of metabolite.
- Study of metabonomics/metabolomics
- ADME Screening
- Bioanalysis/bioequivalence studies

- Dissolution testing
- Forced degradation studies (7)

Ion exchange chromatography:

Ion exchange chromatography is a versatile, high resolution chromatography technique to purify the protein from a complex mixture. In addition, this chromatography has a high loading capacity to handle large sample volume and the chromatography operation is very simple (10).

Ion exchange chromatography uses an ion-exchange resin as the stationary phase. The mechanism of separation is based on ion-exchange equilibria. When a chromatography format is chosen for the analysis of an ionic compound, ion exchange is generally considered after attempt at developing reversed phase (11).

Ion exchange chromatography is based on attraction between oppositely charged particle. In ion exchange chromatography analyte is retained on column on the basis of ionic interaction. The stationary phase display ionic functional group R-X which interact with analyte ion of opposite charge.

On the basis of this ion exchange chromatography is divided into

a) Cationic ion exchange chromatography

b) Anionic ion exchange chromatography (12).

Flash chromatography

Column chromatography an extremely consuming stage in any lab and can quickly become a bottleneck for any processing lab. This leads to the development of novel preparative chromatography in which mobile phase flows down by positive air pressure called as Flash chromatography (13).

Column chromatography has found his place in many laboratories for preparative purpose as well as for reaction control in organic syntheses. The importance of column chromatography is mainly due to following factors are given below.

- Simple packing procedure.
- Low operating pressure.
- Low expense for instrumentation (14).

Flash chromatography is basically an air pressure driven hybrid of medium pressure and short column chromatography which has been optimized for particularly rapid separation (13).

It is classified into two types:

1. **LPLC-** Low Pressure Liquid Chromatography (LPLC) system which operates around 50-75 psi.

2. **MPLC-** Medium Pressure Liquid Chromatography (MPLC) system which operates above 150 psi (13).

Principle

➢ The principle of that the eluent is, under gas pressure (normally nitrogen or compressed air) rapidly pushed through a short glass column with large inner diameter. The glass column is packed with an adsorbent of defined particle size.

➢ The most used stationary phase is silica gel 40-63 µm, but obviously packing with another particle size can be used as well. Particle smallest than 25µm should only be used with very low viscosity mobile phase because otherwise, the flow rate would be very low.

➢ Normally gel beds about 15cm high with working pressures of 1.5 - 2.0 bars. Originally only unmodified silica was used as the stationary phase so that only normal phase chromatography was possible. In that meantime, however, and parallel to HPLC, reversed phase materials are used more frequently in flash chromatography (13).

In Flash Chromatography some adsorbents which are mainly used in flash chromatography:

1. Silica: slightly acidic medium. Best for ordinary compounds, good separation is achieved.

2. Florisil: Mild, neutral medium, 200 mesh can be effective for easy separation. Less than 200 mesh best for purification by filtration. Some compounds stick on florisil, test first.

3. Alumina: Basic or neutral medium. Can be effective for easy separations, and purification of amine.

Reverse phase silica: The most polar compounds elute fastest, the most nonpolar slowest.

Solvent systems

Flash column chromatography is usually carried out with a mixture of two solvents, with polar and nonpolar components (15).

One-component solvent system

1. Hydrocarbons: pentane, petroleum ether, hexanes
2. Ether and dichloromethane (very similar polarity)
3. Ethyl acetate

Two-components solvent system

1. Ether/Petroleum Ether, Ether/Hexane, and Ether/Pentane
2. Ethyl Acetate/Hexane
3. Methanol/Dichloromethane
4. 10% Ammonia in Methanol Solution/Dichloromethane (16).

CONCLUSION

Chromatographic techniques have become very important in industry for purification and separation of intermediates in multi-stage syntheses. We concluded that, in term of scientific advance, one of the major innovations in the past few years has been the development of efficient column capable of separating specific chiral compound from mixture. Many chromatography techniques increase productivity in both chemistry and instrumentation providing more information per unit of work as it gives increased resolution, speed and sensitivity. The time spent optimizing new methods can also be greatly reduced.

REFERENCES

1) chromatography, Available on:
https://www.soinc.org/sites/default/files/uploaded_files/forensics/For_Chromatography3.pdf
2) chromatography, unilever, Available on:
http://media.rsc.org/Modern%20chemical%20techniques/MCT5%20Chromatography.pdf
3) Chromatography, available on: https://satyapsingh.files.wordpress.com/2012/09/chromatography-and-distillation.pdf
4) Kaushalendra k. chaturvedi, Pune, India, hyphenated gas chromatography, Dec 2010, Available on : http://www.globalresearchonline.net/journalcontents/volume5issue3/article-004.pdf
5) Vikram kumar, Udaipur, HPTLC, Mar 2014, Available on: https://urpjournals.com/tocjnls/6_14v4i2_1.pdf
6) Preeti Gopaliya, Rajasthan, Supercritical fluid chromatography, May 2014, Available on: http://www.rroij.com/open-access/a-review-article-on-supercritical-fluid chromatography.pdf
7) Patil.v.p., Aurangabad, Ultra performance liquid chromatography, June 2011, Available on: http://www.irjponline.com/admin/php/uploads/vol-2_issue-6/8.pdf
8) Mac nair JE, patel KD and jorgensonjal; ultrahigh-pressure reversed phase capillary liquid chromatography, in packed capillary columns. Anal che. 1997,69:983-989.
9) Wu N, lippert JA and leemac; practical aspect of ultra-high pressure capillary liquid chromatography, j chromatogr .A. 2001;A 911:1-12.
10) Chromatographic technique, NPTEL, Available on: http://nptel.ac.in/courses/102103044/pdf/mod5.pdf
11) Politecnico di Milano, Italy, Gas and liquid chromatography, available on: http://www.eolss.net/sample-chapters/c06/e6-11-02-02.pdf
12) Rathore.k.s.,Udaipur,Ion exchange chromatography, 2 Jan 2012, Available on:
http://www.ijarpb.com/pdf/vol_02/07.pdf
13) G.swati, Kandlakoya, Flash chromatography, Aug 2015, Available on:
www.wjpps.com/download/article/1438354107.pdf
14)Anonymous. www.chem.rochester.edu/how to flash.html.
15)Anonymous. www.sorbeadindia.com.
16) Hetal Chaudhari, Baroda, Flash chromatography, Feb 2012, Available on:
http://www.ijpdt.com/File_Folder/80-84.pdf