Different methods of chromatography - analysis

1. General Introduction

Health is of prime importance to a human beings and wants to get cured in the least possible time whenever they falls ill. This desire and necessity has resulted in the use of a large number of synthetic organic compounds as medicines despite the fact that usually side effects are associated with the use of these drugs. In recent times, practice of giving a number of drugs together has very much increased. Due to drug interaction, the levels of the active drugs together has very much increased. Due to drugs interaction, the levels of the active drug may be too high for a longer time to cause side effects. Further, the reduction/ oxidation products of these medicines, which are produced during the metabolism may also be responsible for their side effects. It is therefore necessary to develop sensitive trace analytical methods for the analysis of the drugs by using UV Spectrophotometer, most sophisticated and advanced chromatographic techniques like UPLC, GC, HPLC etc.

Use of pharmaceutical preparations to make their determination, a matter of for most importance. Due to the great variability of the materials, which are to be analyzed; skillful sampling, preliminary clean-up procedure and selection of a appropriate method in the assay is necessary.

With the introduction of new and more potent drugs every year, the pharmaceutical analyst is called upon to devise new analytical methods like chromatographic techniques; with ever increasing sensitivity, specificity and simplicity for new drugs .

HISTORY OF CHROMATOGRAPHY [1]

The study of chromatography happening in eighteenth century when with a immense significance the nature of inorganic compounds was considered on filter paper by Runge.

In 1898 Day in USA forced crude petroleum throughout a column of limestone and fuller’s earth.

The chromatographic theory was discovered first by a Russian botanist , MichaelTswett (1906) who make use of a glass column of calcium carbonate for separation of chlorophyll pigments from plant by using petroleum ether.

The major development occurred around 1930 when Lederer and co-workers in 1931 separated xanthine and lutein on a column of calcium carbonate powder.

In 1935 , Adams and Holmes observed some synthetic ion exchange resins capable of exchanging ions and thus ion exchange chromatography came in to existence.

In 1944, Martin, Consden , Gorden replaced silica gel columns by strips of filter paper and developed Paper chromatography.

Thin layer chromatography though discovered first by Lzmailer and Shraiber , was further developed

By Stahl and co-workers using silica gel on glass plates.

Amongest the newest and most effective chromatographic technique for analyzing complex mixtures is Gas chromatography. It was introduced by Martin and James in 1952.

INTRODUCTION OF CHROMATOGRAPHY [1]

A variety of methods are available for the separation of components from the mixture. They are mainly divided in to two types.

1. Chemical methods
2. Physical methods.

The physical methods include fractional distillation, extraction, counter-current distribution; crystallization etc. These methods are effective in separation, purication and identification of many compounds . how ever difficulty arises in case of compounds where individual components have similar physical and chemical properties i. e mixture of liquids having very close boiling points etc.

Chromatographic methods corresponds to the most handy and potent technique for these problems.

These chromatographic methods are used for the partition of components of a composite mixture. Because of the quickness and efficiency of this methods, it can be used in all fields particulalarly in chemistry, biology, medicine, dyes, forensic departments and clinical studies.

The term Chromatography derived from Greek words Kromatos means colour and Graphos –means written.

Tswett defined chromatography as the technique in which the components of a combination are separated on an adsorbent column in a flowing system. As per IUPAC Chromatography is defined as a method used mainly for the division of the components of a sample , in which the components are

disseminated between two phases, one of which is stationary whereas the other moves. The stationary phase might be a solid or a liquid support on a solid or a gel, and might be packed in a column , spread as a layer or disseminated as a film. The mobile phase possibly will be gaseous or liquid.

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TYPES OF CHROMATOGRAPHY:

Chromatographic methods can be classified on the basis of stationary and mobile phases used, depending on the stationary and the mobile phase used, separation occurs because of a combination of two or more factors such as extent of adsorption, rate of migration and capillary action etc...

Different types of chromatographic techniques as follows

a. Adsorption chromatography

b. Partition chromatography

c. Paper chromatography

d. Thin layer chromatography

e. Gas-liquid chromatography

f. Gas-solid chromatography

g. Ion exchange chromatography

A Adsorption Chromatography:

The principle underlying the separation of the compounds is adsorption at the solid –liquid interface, for successful separation , the compounds of a mixture must show different degrees of affinity for the solid support and the interaction between adsorbent and component must be reversible, as the adsorbent is washed with fresh solvent the various components will therefore move down the column until, ultimately, they are arranged in order of their affinity for the adsorbent , those with least affinity move

Paper chromatography:

Paper partition chromatography was developed by consden et al,

In this paper partition chromatography paper is used as the support or adsorbent but partition probably plays a greater part than adsorption in the separation of components of the mixtures

In this chromatography substances are distributed between two liquids ie one is the stationary liquid (generally water)which is detained in the fibers of the paper and is called as stationary phase, the other is the touching liquid or rising solvent and is called moving phase,

The components of the mixture to be separated at different rates and appear as spots at different points on the paper

The moment of components on the paper depends on the amount and nature of the stationary phase compared with the amount of mobile phase in the same part of the paper and also on the partition coefficient

The rate of movement of mobile phase at the solvent front tends to be faster than at the position of the component on the paper , it is better to defined as R_f

RF= DISTANCE traveled by centre of component /Distance travelled by solvent front

Types of paper chromatography

1 Descending chromatography

Is defined as while the development of the paper is made by permiting the solvent to travel down the paper

Advantage:

1. The development can be continued indefinitely even though the solvents run off at the other end of the paper

2. Ascending chromatography

once the improvement of the paper is done by permitted the solvent to move up the paper it is recognized as ascending technique

3. Ascending – Descending chromatography

In this procedure the upper fraction of the ascending chromatography can be folded over a glass rod permit the ascending expansion to change over into the descending after crossing the glass rod

4. Radial Paper chromatography

This is also known as circular paper chromatography, this constructs utilize of radial development

5. Two dimensional chromatography

In this a square or rectangular paper is utilized the sample is applied to one of the spot of the paper. The second development is performed at right angle to the direction of the first run

This type of chromatography can be conceded out with identical solvent systems in the both the directions or by two solvent systems

Importance of paper chromatography

It is used for analyzing the polar compounds like amino acids, sugars and natural products,

It also has been applied for the separation of many organic and biochemical products

Thin layer chromatography:

Thin layer chromatography is similar to paper chromatography except that a thin (025 mm) layer of some inert material such as AI2O3, MgO or Si o2 is used as the substrate instead of paper

The process of thin layer chromatography was first established by izmailor and shraiber in 1938

Thin layer chromatography offers a faster and more efficient separation than paper chromatography and majority of paper chromatographic separations have now been superseded by thin layer chromatography procedures

Thin layer chromatography has many advantages when compared to the other techniques like paper and column chromatography

They are

1. It requires very little time for separation
2. Spraying with corrosive agents for credentials is also tolerable which is not achievable in paper chromatography as cellulose gets destroyed
3. The method is used for partition , adsorption , ion exchange chromatography as there is huge range of adsorbents obtainable
4. This technique can be apply to preparative separation with the aid of thicker layer of adsorbents

Thin layer chromatography has been included under both adsorption and partition chromatography , in this the separation is carried on a glass or plastic plate which is coated with a thin uniform layer of finely divided inert adsorbent such as silica gel or alumina

The plates are activated, the solution of the sample in a volatile solvent is applied by using a capillary tube or a micropipette to a spot keeping 1-2 cm from the bottom of TLC Plate , the position of the sample spot is indicated by making a origin line on the plate with the lead pencil

When the blemish has dried, the plate is positioned vertically in a suitable tank with it’s lower edge immersed in selected mobile phase

The solvent rises by capillary action, resolving the sample mixture into separate spots at the end of the run the solvent is tolerable to vanish from the plate and the separated spots are situated and recognized by various physical and chemical methods

Preparation of chromate plates

With the help of pouring, dipping, spraying and spreading methods the chromatoplates are prepared, with help of qualitative and quantitative methods the TLC plate evaluated

Ion exchange chromatography

Separation of ionic substances may be carried out in glass columns similar to those described for adsorption and partition chromatography the chromatography medium – stationary phase is an ion exchange resin which is a polymer containing fixed charged groups and replaceable counter ions of the opposite charge, when a sample containing organic or inorganic ions is passed down the column the ions of the same charge as the counter ions displace the counter ions into the mobile phase and are retained on the column cationic and anionic exchange resins have positively and negatively charged counter ions respectively , and retard the migration of the sample cations and anions respectively ,

Ion exchange chromatography:

Separation of Ionic substances may be carried out in glass columns similar to those describes.

Ion exchange resins:

Modern resins are based on cross linked polystyrene prepared in bead form by the copolymerization of styrene & divinyl benzene (DVB)

Most commonly useful resins are prepared with approximately 8% DVB.

Strong action exchange resins are prepared by sulphonating the free benzene rings.

Strong anion exchange resins includes quaternary ammonium residues are prepared by chloromethylation of the free benzene rings followed by treatment with a tertiary amine salt ex: Trimethylamine amino hydrochloride.

The strength and exchange capacities of ion exchange resins depend on the acidic or basic strength of the fine charged group.

Thus the strongly acidic suphonic acid and strongly basic quaternary ammoinium groups give strong ion exchange resins with a high exchange capacity.

Weaker exchange resins containing the weakly acidic carboxylic acid (COOH) or weakly basic derivatives of ammonia (ex: $NHR_2^+ Cl^-$) generally have a lower exchange capacity.

Applications:

Used for the separation of similar ions

Used for softening the hard water

Purification of organic compounds

Anion exchange chromatography include the assay of total halogenic salts using a resin in the OH^- form.

Anion exchange is also used to separate heamine, and neomycin C from neomycin B t o test for neomycin C in Framycetin sulphate and neomycin sulphate.

Gas Chromatography:

The division of the components of a combination in the gaseous state could be achieved by partition column chromatography using a gaseous mobile phase was first made by martin and synge in 1941.

In gas liquid chromatography: The immobile phase is a thin layer of a non-volatile liquid bound to a solid support and the mobile phase is a gas .

A partition process occurs

In gas-solid chromatography:

Utilizes a solid adsorbent as the stationary phase and an adsorption process takes place

Technique of gas chromatography:

In this technique the sample is introduces in to the moving carrier gas stream and is carried by it through the column.

The column contains either the active solid or a liquid of low vapour pressure held upon an inert solid.

The active solid or non volatile liquid act as stationary phase whereas the carrier gas acts as mobile phase.

The components of mixture sample distribute between two phases.

The solubility or adsorption possessions might vary from component to component and therefore the components are carried along the column at different varies and finally emerge at the outlet of the column in distinct zones separates by the carrier gas.

On rising the vapours of the constituent are detected by suitable detector accompanied by an automatic recording.

Gas chromatographic appearances consists of

Carrier Gas:

Ex: Hydrogen, Helium

Flow regulators and flow meters

Ex: Rota-meter, soap bubble meter

Injection devices:

Columns

1. Depending on its use:-
    1. Analytical Column

    2. Preparative Column
2. Depending on its nature
    1. Packed column (Placed column are described as analytical column)
    2. Open tubular column
    3. Support coated open tubular column
3. Temperature control devices
4. Detectors ex:
    1. Katharometer
    2. Flame ionization factor
    3. Argon ionization factor
    4. Electron capture factor
5. Recording and integrators
6. Applications of GC:
    1. For qualitative analysis
    2. Quantitative analysis
    3. It is used for finishing of impurities present in the samples
    4. It is used for the separation and identification of lipids, carbohydrates, proteins, flavours, preservatives, colorants in food as well as vitamins steroids
    5. It is used for converting the non-volatile compounds in to volatile compounds by derivatization method.
    6. It is used for the determination of solvent residues or solvent if crystallization

HPLC:

The course of action of high performance liquid chromatography was developed in the late 1960s.

The attitude of high performance liquid chromatography is so called because of it’s improved performance when compared to classified column chromatography.

It is also described as high pressure liquid chromatography.

Advantages of HPLC:

There is ease of sample introduction and sample preparation

There is speed of analysis

Te analysis by HPLC is specific accurate and precise.

It is used for the analysis of many polar, ionic substances, metabolic products and thermo-labile as well as non-volatile substances.

Principles of HPLC:

The technique is based on the same modes of separation as classified column chromatography i. e. partition, ion-exchange, adsorption and gel permeation, but it vary from column chromatography in that the mobile phase is pumped through the packed column under high pressure.

Apparatus: the mode of operation of this system is isocratic i. e. one partition solvent or mixture is pumped throughout the analysis.

For some determinations the solvent composition may be altered gradually to give gradient elution.

Pumps: pumps are mandatory to distribute a stable flow of mobile phase at pressures varying from 1 to 550 bar.

They are two types of pumps:

1. Mechanical pumps: if the reciprocating piston type give a pulsating supply of mobile phase
2. Dual piston reciprocating pump produce the two pistons are carefully phased so that simultaneously is filling the other is pumping.

Injection systems:

Injection ports are of two fundamental types

1. Those in which the sample is injected directly in to the column
2. Those in which the sample is deposed prior to the column bay and then swept by a valving action in to the column by mobile phase.