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Column Chromatography - an important tool in standardization of herbal medical product: A review

Jitendra Bajaj*, Bhaskar Kumar Gupta

School of Pharmacy & Research, People's University Bhopal

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ABSTRACT

Chromatography is a method of separation of a sample into its components. The mixture is dissolved in a fluid solvent gas or liquid called the mobile phase, which carries it through a system a column, a capillary tube, a plate, or a sheet on which a material called the stationary phase is fixed. Chromatography techniques like Column Chromatography, hplc, gas chromatography, paper chromatography, tlc, etc. The study signifies the appliance of chromatography at various stages of drug discovery and development.

*Corresponding Author

Dr. Jitendra Bajaj

Email: jitendrabajajpharma@gmail.com

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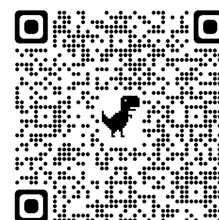

Introduction

Column chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube and allowing mobile phase to pass through it. Based on the nature of stationary phase, i.e. whether it is solid or liquid, it is called as column adsorption chromatography or column partition chromatography. Column adsorption chromatography is widely used technique.

Stationary Phase: The stationary phase is one of the two phases forming a chromatographic system. It may be a solid, a gel or a liquid. If a liquid, it may be distributed on a solid. This solid may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (Bonded Phase) or immobilized onto it (Immobilized Phase)

Mobile Phase: A fluid that percolates through or along the stationary bed, in a definite direction. It may be a liquid (Liquid Chromatography) or a gas (Gas Chromatography) or a supercritical fluid (Supercritical-Fluid Chromatography). In gas chromatography the carrier gas may be used for the mobile phase.

Principle

The individual components move with different rates depending upon their relative affinities. The compound with lesser affinity toward the stationary phase (adsorbent) moves faster and hence it is eluted out of the column first. The one with greater affinity toward the stationary phase (adsorbent) moves slower down the column and hence it is eluted later. Thus the compounds are separated. The type of interaction between the stationary phase and solute is reversible in nature.

Adsorption chromatography: The separation is based mainly on differences between the adsorption affinities of the sample components for the surface of an active solid.

Partition chromatography: Separation is based mainly on differences between the solubility of the sample components in the stationary phase (gas chromatography), or on differences between the solubility of the components in the mobile and stationary phases (liquid chromatography).

Types of column chromatography

Normal phase column chromatography

Reverse phase column chromatography

Table 1. Comparison of normal phase and reverse phase mode

	Normal phase	**Reverse phase**
Stationary phase	Polar	Nonpolar
Mobile phase	Nonpolar	Polar
Compound eluted first	Nonpolar	Polar
Compound eluted last	Polar	Nonpolar
Example of stationary phase	Silica gel and alumina	ODS (C_{18}), C_8, C_4

Column packing materials

The selection of suitable column packing materials is made on the basis of the chromatographic process. The most widely used column-packing material for adsorption chromatography are as follows :

1) Aluminium oxide: Aluminium oxide for chromatography is a white to off white, fine-grained powder, highly porous. Its surface is more polar than that of silica gel. Alumina may be obtained in basic (pH-10), neutral (pH -7) and acidic (pH-4) forms, and it is important to ensure that the correct type is employed because of catalytically induce reactions which each may cause with particular

functional compounds. For example, basic alumina may lead to hydrolysis of esters, acidic alumina may lead to dehydration of alcohols (particularly tertiary alcohols) or may cause isomerisation of carbon-carbon double bonds: in these circumstances neutral alumina is to be recommended. The activity of all three forms of alumina, which is broadly regarded a relating both to the magnitude of the attractive forces between the surface groups on the adsorbent and the molecule being adsorbed, and to the number of sites at which such attraction takes place, is classified into five grades (the brockmann scale). Grade I is the most active (i.e. it retains polar compounds most strongly), and is obtained by heating the alumina at about 300-400^{0} C for several hours. Successively less active grade, II-V, are then obtained by the addition of appropriate amounts of water (II, 3-4%; III, 5-7%; IV, 9-11%; V, 15-19%).

2) Silica gel: Silica gel is highly porous, granular, amorphous, and highest capacity adsorbent available today. The internal structure is composed of vast network of inter-connected microscopic pores, which attract & hold water, alcohols, hydrocarbons, and other chemicals. The capacity towards adsorption is 40% of their own weight of water. The unique property being truly reversible. Silica gel is chemically inert, non-toxic, nondeliquesecent, dimensionally stable and non-corrosive. Silica gel (pH-7) may also be graded according to the amount of water added to the most active grade, obtained by heating for several hours at temperature not exceeding 300 ^{0}C, these are II (5%), III (15%), IV (25%), and V (38%).

Various grades available: -

Silica gel non-indicating white

Silica gel indicating blue

Silica gel H

Silica gel G

Silica gel GF254

Silica gel HF254

Silica gel CC column

Grades of silica gel on the basis of particle size

Silica gel 60 –120

Silica gel 100 –200

Silica gel 200 - 400

Less frequently employed adsorbents include magnesium silicate, magnesium oxide, magnesium carbonate, calcium carbonate, barium carbonate, calcium hydroxide, calcium sulphate, lactose, starch, cellulose and fuller's earth.

Selection of solvent

Eluotropic series given below serve as guide for sequential solvent selection, most these solvents have sufficient low boiling points to permit ready recovery of eluted material.

Table 2. Elutropic series

SOLVENT	POLARITY INDEX
Hexane	0
Toluene	2.4
Diethyle ether	2.8
Dichloromethane	3.1
Butanol	3.9
Chloroform	4.1
Ethyl acetate	4.4
Acetone	5.1
Methanol	5.1
Ethanol	5.2
Acetonitrile	5.8
Acetic acid	6.2
Water	9.0

The polarity of the solvent, which is passed through the column, affects the relative rates at which compounds move through the column. Polar solvents can more effectively compete with the polar molecules of a mixture for the

polar sites on the adsorbent surface and will also better solvate the polar constituents. Highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds will elute from the column.

Proper choice of an eluting solvent is thus crucial to the successful application of column chromatography as a separation technique. TLC is generally used to determine the mobile phase for a column chromatography separation. Often a series of increasingly polar solvent systems are used to elute a column. A non-polar solvent is first used to elute a less-polar compound. Once the less-polar compound is off the column, a more-polar solvent is added to the column to elute the more-polar compound.

It is always advisable to use good quality of solvents and for column chromatography we should use analytical grade solvents, or double distilled solvents.

Column characteristic

The material of the column is mostly good quality neutral glass since solvents, acids or alkalies should not affect it. An ordinary burette can also be used as column for separation. The column dimensions are important for effective separations. The length-diameter ratio ranges from 10:1 to 30:1. For more efficiency the length:diameter ratio can be 100:1. The length of column depends upon.

Affinity of compounds towards the adsorbent used.

Number of compounds to be separated.

Type of adsorbent used.

Quantity of the sample.

Packing of column

The column packing is one of the critical step to decide the effective elution of compound from column. The bottom portion of column is packed with cotton wool or glass wool or asbestos pad, above which column of adsorbent is packed. After packing the column with adsorbent and sample, pure sea sand or glass beads is kept on the top of sample so that the sample layer is not disturbed during the introduction of mobile phase. Disturbance in the layer of sample will lead to irregular bands in separation. There are two packing techniques.

1. **Dry packing technique**: - In this technique, the required quantity of adsorbent is packed in the column in the dry form and then the mobile phase is allowed to flow through the column till equilibrium is reached. The demerit with this technique is that air bubbles are entrapped between the solvent and the stationary phase and the column may not be uniformly packed. Cracks appear in the adsorbent present in the column. Hence the uniformity in flow characteristics and clear band of the separated component may not be obtained.

2**. Wet packing technique**: - This is the ideal technique to pack the column. There are two ways to pack the column either we prepare slurry of adsorbent and poured it into the column or first we fill the column with mobile phase to three fourth height and then add required quantity of adsorbent with frequent tapping on the wall of column in order to settle down the adsorbent uniformly. Left the column for one to two hours for removal of air bubbles and for effective packing.

Introduction of sample

The sample which is usually mixture of components is first dissolved in minimum quantity of the mobile phase used for packing the column and then add equal amount of adsorbent (same which is used to pack the column) in order to get the components adsorb on the adsorbent and make it dry completely under vaccum in rotavapor. Now transferred the adsorbed sample on the top portion of the column and allow it to settle down completely on bed of adsorbent.

Elution technique

Elution means running the mobile phase through column with successive separation of individual components in different frac-

tions (equal volume 20, 50, 100 or 250ml) collected throughout the process. There are two techniques to elute out the components from mixture.

1. Isocratic elution technique: - In this elution technique, the same solvent composition or solvent of same polarity is used throughout the process of separation.

2. Gradient elution technique: - In this elution technique, solvents of gradually increasing polarity or increasing elution strength are used during the process of separation. Initially low polar solvent is used followed by gradually increasing the polarity to a more polar solvent.

Elution

The best technique is to recover the components by a process called as elution. The components are called as eluate; the solvent called as eluent and the process of separating the components from the column is called as elution. Recovery is done by collecting as different fractions of mobile phase of equal volume like 10ml, 20ml, etc. They can also collected time wise i.e. a fraction every 10 or 20 minutes etc. the recovered fractions are detected by using T.L.C technique, then similar fractions are pulled up so that the bulk of the compound of each type is obtained in pure form. If a fraction, still contains several components, it can be separated by using another column.

Detection of components

The detection of coloured components can be done visually. Different coloured bands are seen moving down the column, which can be collected separately and make it confirm by applying T.L.C simultaneously of each fraction against extract or mixture. But for colourless compounds, T.L.C is detected under UV lamp at 254 and 366 nm and for non-UV active compounds; T.L.C is sprayed with suitable spraying reagent and heat in oven for development of spot.

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