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RP-HPLC Method Development and Validation for Estimation of Acebrophylline

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ABSTRACT
Chromatography, a separation technique, is mostly used in chemical analysis in which High-performance liquid chromatography (HPLC) is an extremely versatile technique where analytes are separated by passage through a column packed with micrometer-sized particles. Theses day reversed-phase chromatography is commonly used separation technique in HPLC. The reasons for this include the simplicity, versatility, and scope of the reversed-phase method as it is able to handle compounds of a diverse polarity and molecular mass. Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character, such as proteins, peptides and nucleic acids, can be separated by reversed phase chromatography with excellent recovery and resolution. This review covers the importance of RP-HPLC in analytical method development and their strategies along with brief knowledge of critical chromatographic parameters need to be optimized for an efficient method development.

Key Words- HPLC, RP-HPLC

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INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) involves the separation of molecules on the basis of hydrophobicity. The separation depends on the hydrophobic binding of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent.[1] The solute mixture is initially applied to the sorbent in the presence of aqueous buffers, and the solutes are eluted by the addition of organic solvent to the mobile phase.[2] Elution can proceed either by isocratic conditions where the concentration of organic solvent is constant, or by gradient elution whereby the amount of organic solvent is increased over a period of time. The solutes are, therefore, eluted in order of increasing molecular hydrophobicity.[3] RP-HPLC is a very powerful technique for the analysis of drugs because of a number of factors that include:

1. The excellent resolution that can be achieved under a wide range of chromatographic conditions for very closely related molecules as well as structurally quite distinct molecules
2. The experimental ease with which chromatographic selectivity can be manipulated through changes in mobile phase characteristics;
3. The generally high recoveries and, hence, high productivity
4. The excellent reproducibility of repetitive separations carried out over a long period of time, which is caused partly by the stability of the sorbent materials under a wide range of mobile phase conditions.[4]

Theory of Reversed Phase Chromatography:

Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution. The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilized hydrophobic ligand, i.e. the stationary phase.[5] The actual nature of the hydrophobic binding interaction itself is a matter of heated debate but the conventional wisdom assumes the binding interaction to be the result of a favorable entropy exact. The initial mobile phase binding conditions used in reversed phase chromatography are primarily aqueous which indicates a

high degree of organized water structure surrounding both the solute molecule and the immobilized ligand. As solute binds to the immobilized hydrophobic ligand, the hydrophobic area exposed to the solvent is minimized. Therefore, the degree of organized water structure is diminished with a corresponding favorable increase in system entropy. In this way, it is advantageous from an energy point of view for the hydrophobic moieties, i.e. solute and ligand, to associate.[6]

TYPES OF HPLC

HPLC generally depend on phase System used in the process. Following types of HPLC generally used in analysis- [7,8]

Normal phase chromatography:

This method separates analytes based on Polarity. NP-HPLC uses a polar stationary Phase and a non-polar mobile phase. The polar analyte interacted with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase increases the elution time.

Reversed phase chromatography:

It has a non-polar stationary phase and an aqueous, moderately polar mobile phase. It operates on the principle of hydrophobic interactions, which result rom repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. [9,10]

Size exclusion chromatography:

It also called as gel permeation chromatography or gel filtration chromatography mainly separates particles on the basis of size. It is **also** useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This technique is widely used for the molecular weight determination of polysaccharides. [11,12]

Ion exchange chromatography:

Retentionis based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. This form of chromatography is widely used in purifying water, Ligand-exchange chromatography, Ion-exchange chromatography of proteins, High-pH anion-exchange chromatography of carbohydrates and oligosaccharides, etc.

Bio-affinity chromatography:

Separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands. [13,14]

INSTRUMENTATION

Injection of the sample:

Septum injectors are available; using which sample solution is injected. Sample can be injected when the mobile phase is flowing or it is stopped. A new advanced rotary valve and loop injector can be used to produce reproducible results.

The detector

There are several ways of detecting when a substance has passed through the column. Generally UV spectroscopy is attached, which detect the specific compounds. Many organic compounds absorb UV light of various wavelengths. The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time.

Interpreting the output from the

Detector:

The output is recorded as a series of peaks, each one representing a compound in the mixture passing through the detector and absorbing UV light. The area under the peak is proportional to the amount of substance, which is passed through detector, and this area can be calculated automatically by the computer linked to the display.[15, 16]

APPLICATION

HPLC includes identification, quantification, and resolution of a compound. Preparative HPLC refers to the process of isolation and purification of compounds. This differs from analytical HPLC, where the focus is to obtain information about the sample compound.

Chemical Separations It is based on the fact that certain compounds have different migration rates given a particular column and mobile phase, the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

Purification: Purification is defined as the process of separating or extracting the target compound from a mixture of compounds or contaminants. Each compound showed a characteristic peak under certain chromatographic conditions. The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any other undesired compound.

Identification assay of compounds are carried using HPLC. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed. [17, 18]

ADVANTAGE

Pharmaceutical applications

- Tablet dissolution study of Pharmaceutical dosages form.
- Shelf-life determinations of Pharmaceutical products
- Identification of active ingredients of Dosage forms
- Pharmaceutical quality control

Environmental applications

Detection of phenolic compounds in Drinking Water Identification of diphenhydramine in sedimented samples Bio-monitoring of pollutant

Forensics

- Quantification of the drug in biological samples.
- Identification of anabolic steroids in serum, urine, sweat, and hair
- Forensic analysis of textile dyes.
- Determination of cocaine and metabolites in blood

Clinical

- Quantification of ions in human urine
- Analysis of antibiotics in blood plasma.
- Estimation of bilirubin and bilivirdin in blood plasma in case of hepatic disorders.
- Detection of endogenous neuropeptides in extracellular fluids of brain.

Food and Flavor

- Ensuring the quality of soft drink and Drinking water.
- Analysis of beer.
- Sugar analysis in fruit juices.
- Analysis of polycyclic compounds in vegetables.
- Trace analysis of military high explosives in agricultural crops.[19,20]

DISADVANTAGES:

1. Appearance of intense chemical background
2. Not suitable for thermally liable compounds
3. Surface effect reduces detection limits [21]

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