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A CRITICAL STUDY FOR DEVELOPMENT OF HPLC METHODS FOR MONITORING OF PROCESS IMPURITIES IN DRUG SUBSTANCES

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ABSTRACT

HPLC is currently used to determine the vast majority of drug-related impurity measurements. It provided the requisite sensitivity for highly automated trace level assessments. HPLC is applicable to all drug classes because to the large range of stationary phases and operation modes. By employing ordinary UV detectors, it is usually possible to routinely meet the detection limits of 0.1% or less for drug-related contaminants by HPLC. HPLC was widely employed during the review period to assess and regulate the purity of chemical components used in the production of bulk medicines. Today, high-performance liquid chromatography (HPLC) is a common name for high-pressure HPLC. Chromatographers interchangeably use both of these words, which can both be shortened to HPLC. The use of a range of detectors, including fluorescence, electrometric, mass spectrometry, and others, has greatly expanded the applicability of this practical technology for pharmaceutical chemists.

KEY WORDS: Development, High Performance Liquid Chromatography, Monitoring of Process, Impurities, Drug Substances.

INTRODUCTION

There are several methods that have been suggested for determining the impurity profiles of synthetic pharmaceuticals. These strategies entailed anticipating potential contaminants throughout the synthesis process, isolating them, and identifying them using the appropriate analytical procedures. These, however, are only applicable to materials synthesised through particular routes. Any modifications to the synthetic method should be carefully examined because they can result in a different impurity profile. As they have for more than 20 years, methods relying on some type of HPLC separation continue to outnumber all other assays. Most of these involve UV detection and are isocratic reversed-phase techniques, which are rather straightforward. The employment of more selective detective systems, such as diode array and fluorescence detectors or mass spectrometers, or

separation mode, however, is sometimes used to create more creative ways. As electro-spray ionisation and atmospheric pressure ionisation interfaces improved at larger throughput volumes, coupled LC-MS applications have grown in popularity. Impurities in bulk medicinal compounds and their formulations can be determined using a variety of HPLC methods.

CHROMATOGRAPHY

Chromatographic methods have been employed for centuries to separate components like plant-based dyes. Chromatography was discovered by the Russian botanist Tswett. He separated leaf pigments using a solid polar stationary phase in 1903. Kuhn and Lederer, as well as Reichstein and Van Euw, did not use this procedure for the separation of natural products until the 1930s. For their work in 1941 in which they introduced liquid-liquid partition chromatography, Martin and Synge received the Noble Prize. The idea of theoretical plates was used by Martin and Synge to calculate chromatographic efficiency. The two Greek terms Chroma, which means colour, and Graphein, which means to write, are where the term "Chromatography" comes from.

CHROMATOGRAPHY IN THE PHARMACEUTICAL WORLD

Chromatography is a key analytical technology used in all phases of drug discovery, development, and production in the modern pharmaceutical industry.

Drug development and drug discovery are the two main processes involved in the production of novel chemical entities (NCEs). The objective of the drug discovery programme is to quickly screen a large number of compounds, which produces lead compounds, which are subsequently narrowed down through focused synthesis and selective screening (lead optimisation). Drug development's primary goals are to fully characterise potential active ingredients through drug metabolism, preclinical and clinical testing, and clinical studies. Rugged analytical HPLC separation methods have been developed throughout this drug discovery and development paradigm, and at each stage of development, analyses of numerous samples are carried out in order to effectively control and monitor the quality of the potential drug candidates, excipients, and finished products. Throughout this drug development life cycle, the development of efficient and quick methods is of the utmost importance. To appreciate the many factors that are optimised during quick and efficient HPLC method creation and optimisation, one must have a basic understanding of HPLC principles and theory.

DEFINITION OF CHROMATOGRAPHY

A physical method of separation known as chromatography distributes the components to be separated between two phases, one of which is stationary and the other flows in a specific direction.

USP DEFINITION OF CHROMATOGRAPHY

Chromatography is a process whereby solutes are separated by dynamic differential migration in a system made up of two or more phases, one of which moves continuously in a specific direction, and in which the various substances exhibit different mobilities due to differences in adsorption, partition, solubility, vapour pressure, molecular size, or ionic charge density. Analytical techniques can be used to identify or quantify the specific compounds that were thusly separated.

HISTORY OF CHROMATOGRAPHY

Chromatography advanced quickly during the 1930s and 1940s, with multiple concurrent developments of the earlier work leading to the various chromatographic techniques we use today. Below is a brief note on the historical developments of the key techniques.

- **PAPER CHROMATOGRAPHY**

One of the earliest recognised types of chromatography was paper chromatography, an invention credited to Martin and colleagues. They needed an adsorbent that could retain water more effectively than silica Gel for their work on partition column chromatography. This led to the successful application of cellulose over 20 amino acids utilising a two-dimensional method and the spot-finding agent ninhydrin. Although it is still employed as a screening method, thin layer chromatography has surpassed it due to its higher separating efficiencies.

- **THIN LAYER CHROMATOGRAPHY**

The first Thin Layer Chromatography separations, which used gelatin layers to separate strong acids and enzymes in malt extract, were described by the Dutch biologist Beyerinck in 1889 and Wijsman in 1898. TLC as we know it today was first used to analyse pharmaceutical tinctures, such as extracts of cinnamon, belladonna, and foxglove, in 1938 by Izmailov and Sharaiber. They did this by sprinkling samples onto a thin layer of alumina adsorbent on a glass plate and adding spots of solvent to produce circular chromatograms.

- **ION EXCHANGE CHROMATOGRAPHY**

Taylor and Urey initially described the use of ion exchange chromatography in 1938 when they used zeolite resins to separate the isotopes of lithium and potassium. High performance ion chromatography, sometimes known as LC or HPLC, was created as a result of the 1980s application of high-performance liquid chromatography methods to IEC. Samples with ppm levels of anions or cations can be separated in minutes because to high efficiency ion exchange columns and sensitive conductivity detectors. Ion exchange is a procedure in which an electrolyte solution is brought into contact with an ion exchange resin and active ions (ionic species) on the resin are replaced by ions (from the analyte solution) of similar charge.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

One of the analytical methods that is most frequently employed in the pharmaceutical sector is high performance liquid chromatography (HPLC). A separation technique involving mass transfer between the stationary and mobile phases is a chromatographic procedure. The components of a combination can be separated via HPLC using a liquid mobile phase. A liquid or a solid phase can be the stationary phase. These elements are first dissolved in a solvent, and then they are compelled to pass under intense pressure through a chromatographic column. The mixture separates into its component parts in the column. The degree of resolution is crucial and is influenced by how much the stationary phase and solute components interact. The packing material in the column that is immovable is referred to as the stationary phase. Through various solvent and stationary phase selections, the solute's interaction with mobile and stationary phases can be controlled. Since it can separate a variety of chemical mixtures with ease, HPLC gains a high level of versatility not found in other chromatographic systems.

THEORY

A dynamic adsorption process is HPLC. While passing through the porous packing beads, the analyte molecules have a tendency to interact with the surface adsorption sites.

The various adsorption forces may be incorporated into the retention process depending on the HPLC mode: In reversed-phase (RP) separations, the predominant interactions are hydrophobic (non-specific). In normal phase (NP) mode, dipole-dipole (polar) interactions predominate. In ion-exchange chromatography, retention is caused by ionic interactions. These interactions are all antagonistic. Eluent molecules and analyte molecules compete for adsorption sites. Because of this, the stronger analyte molecules engage the surface. The analyte will be kept on the surface for a longer period of time the weaker the eluent contact.

TYPES OF HPLC

Adsorption chromatography is based on repeated adsorption-desorption stages, where the stationary phase is an adsorbent (such as silica gel or any other silica-based packing).

b. Ion-exchange chromatography: The stationary bed has a surface that is electrically charged in the opposite direction from the ions in the sample. Ionic or ionizable samples are virtually often employed with this approach. The sample will be more strongly attracted to the ionic surface and take longer to elute the stronger its charge. The mobile phase is an aqueous buffer, and the elution period is controlled by the pH and ionic strength.

Size exclusion chromatography: The sample is simply screened or filtered based on its solvated molecular size, and the column is filled with material with carefully controlled pore sizes. Smaller molecules enter the packing particles' pores and elute later whereas larger ones quickly wash through the column. This method is also known as gel permeation chromatography or gel filtration. Regarding the first kind, normal and reversed-phase chromatography are the two defined modes based on the relative polarity of the two phases. In normal phase chromatography, the mobile phase (such as n-hexane) is nonpolar while the stationary bed (such as silica gel) is extremely polar in nature. As a result, more polar samples are kept on the polar surface of the column packing than less polar ones. Its opposite is reversed-phase chromatography. Unlike the mobile phase, which is a polar liquid like acetonitrile or combinations of water and methanol, the stationary bed is nonpolar in nature. Here, the material will be retained for a longer period of time the more nonpolar it is. Nearly 90% of all chromatographic applications involve reverse phase chromatography. In all types of HPLC, eluent polarity is crucial. Elution comes in two flavours: gradient and isocratic. The first kind pumps a continuous eluent composition across the column for the duration of the analysis. Eluent content (and strength) is gradually modified during the second type of run.

When compared to the traditional LC technique, HPLC is distinguished by:

1. Excellent resolution
2. Columns with a tiny diameter (4.6 mm), made of stainless steel, glass, or titanium
3. Very tiny (3, 5 and 10 m) particle column packing
4. Comparatively high inlet pressures and regulated mobile phase flow
5. Continuous flow detectors that can detect very small amounts and handle low flow rates
6. Quick analysis

Modern liquid chromatography was initially given the moniker "high pressure liquid chromatography" or HPLC because pressure was chosen as the key requirement. However, this phrase was unfortunate because it seems to suggest that the increased performance is primarily the result of the intense pressure. However, this is untrue. High pressure column slurry packing techniques, accurate low volume sample injectors, sensitive low volume detectors, and effective pumping systems are only a few of the elements that contribute to high performance. In fact, high performance is the end consequence of numerous factors. Naturally, pressure is required to allow for a specific mobile phase flow rate.

STATIONARY PHASES

HPLC separations depend on the kinds of adsorption sites and are dependent on surface interactions. Small, stiff, porous particles with a high surface area are the modern HPLC adsorbents.

- The primary adsorbent variables are:
- Particle size ranges from 3 to 10 m.
- Narrowest particle size dispersion achievable, typically within 10% of the mean.

An adsorbent surface chemistry is represented by the list's final parameter. Adsorbents can be either normal phase (-OH, -NH2), reversed phase (C5, C8, C 18 CN, NH2), anion (CH2NR3 +OH-), or cation (R-SO3-H+) exchangers, depending on the kind of ligand connected to the surface.

- **MOBILE PHASES**

One of the factors affecting the separation in HPLC is the type and makeup of the eluent. Despite the wide range of solvents employed in HPLC, there are a number of common characteristics, including:

1. Purity
2. Compatibility with detectors
3. The sample's solubility
4. A thin viscosity
5. Chemical inertness

In reversed-phase mode, eluents are typically a mixture of water and some polar organic solvents like acetonitrile or methanol. Solvents are primarily nonpolar in normal phase mode. Size-exclusion Special requirements apply to

HPLC. SEC eluents must dissolve polymers, but their ability to prevent sample molecules from interacting with packing material surfaces is even more crucial.

INSTRUMENTATION OF HPLC

A pump, injector, column, detector, and data system are components of HPLC apparatus. The column where separation takes place is the brain of the system. The mobile phase must be pumped through the column at high pressure because the stationary phase is made up of porous micrometer-sized particles. The solute is injected onto the top of the column to start the chromatographic process. As the analyte and mobile phase are pumped through the column, the components separate. Each component eventually emerges from the column as a peak (or narrow band) on the recorder. Depending on the detector being utilised, it may be necessary to detect the eluting components selectively or universally. A chromatogram, which is displayed on a chart recorder or computer screen, shows the detector's reaction to each component. Computer, integrator, and other data processing tools are routinely used to gather, store, and analyse the chromatographic data .

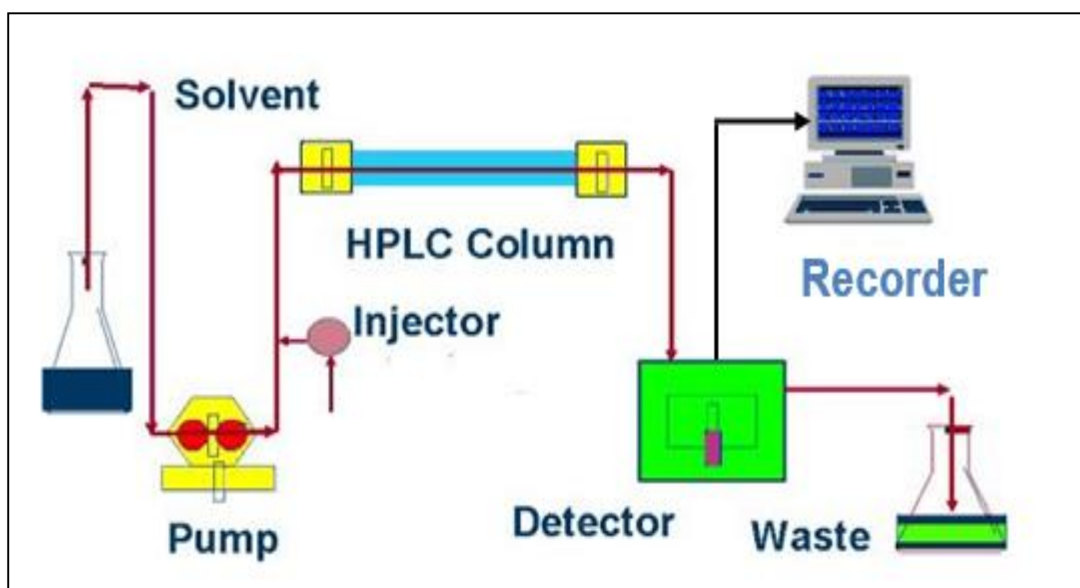

FIGURE - 1: REPRESENTATION OF HPLC

HPLC METHOD DEVELOPMENT GUIDELINES

Modern analytical chemists are scrambling to find better, quicker approaches to build stability indicating methods due to the requirement to reduce method development time and increase accuracy. The method development

chemist can save time and money by following this step-by-step process, which starts with HPLC columns that offer outstanding repeatability, column lifetime, and sensitivity. This strategy adheres to the development process.

CONCLUSION

Any chemical that coexists with the original drug, such as beginning material, intermediates, or that is created as a result of any side effects, is referred to as an impurity. There are three categories of impurities:

1. Closely related impurities to the product that originate from the chemical or the biosynthetic process itself.
2. Impurities developed as a result of the drug's spontaneous disintegration during storage or after exposure to harsh circumstances.
3. The precursors that could be contaminants in the finished product.

Selective procedures should be used to identify and quantify any impurities present in excess of 0.1%. The impurities' proposed structures are synthesizeable and will offer the conclusive proof for those structures, which were previously established by spectroscopic techniques. In order to change the reaction conditions and lower the amount of impurity to an acceptable level, it is crucial to understand the structure of these impurities in the medicinal material. We can acquire a pure material that is less harmful and safe for use in pharmacological therapy by isolating, identifying, and quantifying impurities.

A approach for the quality control and validation of pharmacological ingredients could be the quantitative determination of these contaminants. Drug impurity profiling is required by regulatory agencies including the European Medicines Agency (EMA) and the United States Food and Drug Administration (USFDA). It is possible to approach impurities in novel medicinal compounds from two angles:

1. The chemical component, which includes classifying and identifying impurities, creating reports, including contaminants in specifications, and briefly discussing analytical techniques.
2. The safety component, which provides detailed instructions for assessing contaminants found in batches of new drug material used in clinical research at much lower levels.

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