DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION ANTIHYPERTENSIVE AGENT IN PHARMACEUTICAL DOSAGE FORM

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***Abstract*- Medicines are a key part of the healthcare system. Numerous medicines are introduced into the world-market and also, that is increasing every year. Due to rapid growth of the pharmaceutical industry during the last several years, numbers of pharmaceutical formulations enter as a part of the health care system and thus, there has been rapid progress in the field of pharmaceutical analysis. Developing analytical methods for newly introduced pharmaceutical formulation is a matter of most importance because drug or drug combination may not be official in any pharmacopoeias and thus, no analytical method for quantification is available. To check the quality standards of the medicine various analytical methods are used.**

Keywords: **Chest pain, HPLC, sustained released.**

INTRODUCTION

Analytical Chemistry is defined as "The science and the art of determining the composition of materials in terms of the elements or compounds contained." This branch of chemistry, which deals with both theoretical, practical science and is practiced in a large number of laboratories in many diverse ways. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. In analytical chemistry, it is of prime importance to gain information about the qualitative and quantitative composition of substances and chemical species that is to find out what substance is composed and exactly how much. In quantitative analysis the question is how much is present. The research work in this thesis is based on this criterion. Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Analytical methods which are a measure of quality of the drugs play a very comprehensive role in drug development and follow up activities. It assures that a drug product meets the established standard, is stable and will continue to meet purported quality throughout its shelf life. These methods should be selective and sensitive to monitor the known and unknown impurities and have to be written in a format such that they can be reproduced over a period of time and from laboratory to laboratory, i.e., these methods should be validated.

The major objective of studying the stability of a drug is to determine the shelf-life of the drug, to identify the degradation products, and to establish the degradation pathways.

International Conference on Harmonization (ICH) guidelines Q3A (R2) and Q3B (R2) address issues relevant to the regulation of impurities in the drug substance and drug product, respectively. It is mentioned that specification of the drug substance should include a list of impurities. Stability studies, chemical development studies, routine batch analysis, and scientific appraisal of potential by-products from synthetic steps and degradation pathways can be used to predict those impurities which are likely to be present in the drug substance. It is also mentioned that forced degradation studies should be carried out on the drug substance at temperatures in 10°C increments above the accelerated temperatures, extremes of pH, and under oxidative and photolytic conditions so as to establish the inherent stability characteristics and degradation pathways.

CHROMATOGRAPHY

''Chromatography is defined as a procedure by which solutes are separated by a dynamic differential migration process in a system consisting of two phases, one of which moves continuously in a given direction and which the individual substances exhibits mobilities by reasons of difference in adsorption, partition, solubility, vapor pressure, molecular size or ionic charge density.''

The most common chromatographic methods are -

- Planar Chromatography: It includes Paper Chromatography, Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC)
- Column Chromatography: It includes High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), etc

12 Nobel prizes were awarded alone for work in chromatography during 1937 to 1972

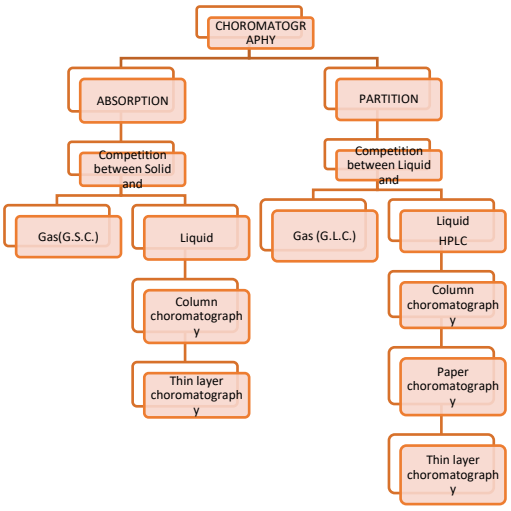

Fig 01: Flow chart of types of chromatographic method.

High Performance Liquid Chromatography (HPLC) [9, 10]

High Performance Liquid Chromatography (HPLC) was developed in the late 1960s and early 1970s. Today it is widely applied for separation and purification in a variety of areas including pharmaceuticals, biotechnology, environmental, polymer and food industries.

High Performance Liquid Chromatography (HPLC) was known as high pressure liquid chromatography. It is a form of column chromatography in which the stationary phase consist of small particle (3-50 um) packing contained in a column with a small bore (2-5mm),one end of which is attached to source of pressurized liquid eluent (Mobile phase). The technique offers major improvements in speed, resolving power, detection, quantification, convenience and applicability to new sample types. Modern HPLC techniques became available in 1969, but from 1990s HPLC become most popular instrument for drug analysis which is presently used in pharmaceutical research and development;

TYPES OF CHROMATOGRAPHY

Normal phase chromatography:

Also known Normal phase HPLC (NP-HPLC), 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: Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC operates on the principle of hydrophobic interactions, which result from 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.

Size exclusion chromatography:

Size exclusion chromatography (SEC), 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.

Ion exchange chromatography:

In Ion exchange chromatography, retention is 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.

Applications of Chromatography

Chromatography plays a vital role in the chemical industry for the testing of water samples for purity.

The testing of air samples for their purity is also accomplished by chromatographic techniques in the chemical industry**.**

Advantages Of Chromatography:

- All different kinds of complex mixtures can be separated by column chromatography.
- The mobile phase has a wide range.

- There is no limit for quantity as any amount of mixture can be separated by this technique.
- It is a robust method.
- The separated analytes can be reused.
- This process can be automated.
- Disadvantages Of Column Chromatography
- It is a time-consuming process for the separation of compounds.
- It is expensive as higher quantities of solvents are required.
- The automated process becomes complicated and therefore costly.
- It has a low separation power.

General protocol

Forced degradation factors necessary include acid and base hydrolysis, thermal degradation, photolysis, and oxidation and may include freeze–thaw cycles and shear.

Various degradation conditions:

Hydrolysis: Over a wide range of pH most common degradation, chemical reactions are Hydrolysis The decomposition of a chemical compound by reaction with water is called Hydrolysis. In acidic and basic hydrolysis the catalysis of ionisable functional groups present in the molecule occurs. Forced degradation of a drug substance occurs when the drug interacts with acid and base. It produces primary degradants in the desirable range.

Depending on the stability of the drug substance the class and concentrations of acid or base taken should be decided. For acid hydrolysis hydrochloric acid or sulphuric acids (0.1-1 M) considered to be most suitable whereas sodium hydroxide or potassium hydroxides (0.1-1M) for base hydrolysis are suggested. Co-solvents can be used if compounds are poorly soluble in water. Forced degradation started at room temperature and further temperature increased if there is no degradation.

Oxidation conditions: For oxidative forced degradation, hydrogen peroxide is broadly used. Apart from this as metal ions, oxygen, and radical initiators: azobi-isobutyro-nitrile, AIBN can also be used. Drug structure will allow selecting concentration and condition of oxidizing agents. An electron transfer mechanism occurs in the oxidative degradation of drug substance.

Photolytic conditions:

The light exposure does not affect the drug substance for this purpose photo stability is conducted. Photo stability studies are performed to produce primary degradants of drug substance by exposure to UV or fluorescent conditions. In ICH guidelines some recommended conditions for photo stability testing are described.

Thermal conditions:

Thermal degradation (e.g., dry heat and wet heat) should be carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions.

Samples of solid-state drug substances and drug products should be exposed to dry and wet heat. Liquid drug products should be exposed to dry heat. For a shorter period studies may be conducted at higher temperatures. Through the Arrhenius equation the effect of temperature on thermal degradation of a substance can be studied.

Humidity:

Humidity is one of the effective factors in establishing the potential degradants in the finished product and active pharmaceutical ingredient. Normally 90% humidity for the duration of one week shall be recommended for the establishment of forced degradation samples.

CONCLUSION

In present study for routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. Diltiazem is official in IP, BP and USP.

MS using ESI and other ionization methods can be applied to a much wider range of biological molecules than GC-MS and will thus find greater application in clinical biochemistry. Direct injection methods can determine many analyses with high through-put when highly specific tandem MS is used for detection. LC-MS provides superior specificity and sensitivity compared to direct injection methods. When combined with stable isotope dilution, LC-MS can be used to develop highly accurate and reproducible assays. Modern mass spectrometers are highly sensitive and LC-MS assays are now viable replacements for many immunoassays. Although start-up costs are relatively high, reagent costs are low if prepared in-house, and there may be considerable long-term cost savings when an existing commercial assay can be replaced by LC-MS. LC-MS strategies are also emerging for sensitive, quantitative, multiplexed assays of plasma peptides and proteins. As the sensitivity of MS continues to improve, LC-MS assays will be of increasing interest as potential replacements for existing immunoassays.

A very few analytical methods appeared in the literature for the determination of Diltiazem includes HPLC, HPTLC and UV-Visible spectrophotometric methods. In view of the above fact, some simple analytical methods were planned to develop with sensitivity, accuracy, precision and economical. HPLC methods were validated as and results of linearity, precision, accuracy, Specificity, System suitability and robustness pass the limit. The HPLC method is more sensitive, accurate and precise compared to the previously reported method. There was no any interference of excipients in the recovery study. The low value of %RSD, molar extinction coefficient (L mol-1 cm-1) suggested that the developed methods are sensitive. The proposed high-performance liquid chromatographic method has also been evaluated over the accuracy, precision and robustness and proved to be convenient and effective for the quality control of Diltiazem. Developed method was found simple and cost effective for the quality control of Diltiazem.

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