CHAPTER - 1

INTRODUCTION

1.1. INTRODUCTION

To know about the composition and structure of matter, Analytical chemistry, a branch of science, is used, by acquiring, practicing and conveying information. It is not confined to definite compounds or reactions and it deals with the study of the natural and artificial materials. Geometrical features like molecular morphologies and species identity are constituted in the properties of analytical chemistry[1]. The development of its various concepts and theories include safety and quality of food, pharmaceuticals and water, environmental monitoring, biomedical applications and also to support the legal processes (forensics) and diagnose diseases, etc., Analytical chemists play a vital role to support this[2].

To identify and measure the chemical species in a sample, analytical chemists use different techniques. By comparison of the known substance to a similar substance (whose concentration is known), which is called a standard reference material, and almost every technique will be carried out[3].

In general, the drugs may be new or partially modified in structure of the existing ones with combinations releasing into the market annually. Frequently, from its introduction into the market to the inclusion of pharmacopoeias, it is being delayed, so there is a lack of analytical methods for these drugs, and for such drugs, this can lead to the development of newer analytical methods.

In the literature, for the drugs, no appropriate analytical methods are available. Excipients cause interference in drug formulation; hence no suitable methods are available for drug analysis. The use of some expensive reagents and solvents leads to the convolution of extraction and separation procedures, which may not be trustworthy[4].

1.2 CHROMATOGRAPHY- AN OVERVIEW:

To resolve a multi-component mixture into its individual components, chromatography, is a new, well-known and a primary tool of separation and it can be applied both quantitatively and qualitatively. Despite, some other methods like IR spectroscopy, Nuclear Magnetic resonance spectroscopy or Mass spectroscopy etc. are required for the final identification and confirmation.

Tswett. M, in 1806, in Warsaw, invented a new technique, while separating the plant pigments by a column of calcium carbonate, which acted as an adsorbent and the different substances get adsorbed to different extent and this give rise to the different colored bands, at different positions on the column. In Greek, chroma means color and graphos means writing. Hence, he termed the system of colored bands as the chromatogram and the method as chromatography[5].

To separate coloured as well as colorless substances, recent advances have been made there after. Thus, in general, a sample moves over a stationary phase through the mobile phase in chromatography. Chromatography is one of the best and most likely used analytical techniques, now-a-days and in foreseeable future. It is the cornerstone of molecular analytical chemistry. Recent advancements of chromatography have been introduced by A.P.J. Martin and R.L.M. Synge in 1941, made them noble prize winners[6].

1.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):

HPLC is used to figure out the amount of specific compound in a solution. It supports reliable quantitative range to allow the determination of substances in a single run. This method is considered to be rapid, accurate, precise and specific and offers the ease of

automation. It is because methods using HPLC have more advantages over the conventional methods [7].

1.4 PRINCIPLE OF HPLC:

A mixture of sample is dissociated into components for its identification, quantification and purification by HPLC, due to the differences in their relative affinities for the mobile phase and stationary phase used. Especially, RP-HPLC, relies on the principle of hydropohobic interactions, as the more non-polar the material is, longer it will be retained. Due to their low affinities and polar nature, most of the drugs elute at a faster rate through the column and so they are separated and detected easily.

The optimization of laboratory resources is ensured by the effective method development, while methods meet the objectives required at each stage of drug development. To approve the drug, at certain stages, method development is required by the regulatory agencies [8-11].

1.5 METHOD DEVELOPMENT OF DRUGS:

The analyte is enough to know the information of the compound. One can select the most suitable HPLC method development by the physical and chemical characteristics and by a vast literature review. Information regarding the sample can be achieved by molecular weight, structure, functionality, Pka value, UV-spectra and solubility of the compound. By knowing whether the pure compound is organic soluble or water soluble, one can select the best mobile phase and column for HPLC method development. In many laboratories, typical detectors like Mass spectroscopy, UV-Visible detectors are used as they can detect a wide variety of compounds [12-15].

Analytical methods are intended to establish the identity, purity, physical characteristics and potency of the drugs. To support drug testing against specifications that arise during manufacturing and quality release operators, as well as during long-term stability studies and for safety and characterization studies or evolution of drug performance, methods are developed.

Before the final method optimization, all individual components should be investigated during the preliminary method development. By this, it is easy to evaluate the method performance in each component and streamline the final method optimization. Resolving power, specificity and speed are the major attributes of method development. By combining different factors like composition of solvent, type of stationary phase, mobile phase, P^H and buffers, selectivity can be achieved. For the chromatographic separation, changing solvents and stationary phases, proper range of P^H are the most suitable approaches. Better chromatographic resolution can be achieved by the P^H ranging from 1-12 and the development of the method depends upon column efficiency, selectivity and retention time. For chromatographic separation, mobile phase composition or strength plays a vital role. The most commonly used solvents in RP-HPLC are acetonitrile, methyl alcohol and Tetra hydro furan (having the wavelength of 190, 205 & 212nm). By the selection of right column temperature and changing the mobile phase, the separation of many samples can be enhanced [16-19].

1.6 METHOD VALIDATION OF DRUGS:

According to ISO definition, validation is defined as “Verification, where the specified requirements are adequate for an intended use.” Method validation can be used for qualitative, semi-quantitative or quantitative methods. The scientific soundness of the measurement or characterization and also to varying extents throughout the regulatory

submission process, the validation of analytical method is required. The method development includes the measurement of the correct substance, in correct amount and in appropriate range. The goal of method validation is to identify the critical parameters and to establish the acceptance criteria of system suitability [20-21].

The effort done in method development and optimization leads to the effective development of HPLC method and its final performance. For the method development of samples in chromatographic separation, method validation is very important[22].

1.7 ICH GUIDELINES:

For analytical measures and method authentication, U.S FDA has given some manufacturing directions. The ICH guidance also provides clear text on the method validation of drug analysis. The USP has precise strategies for method validation for compound assessment. USP describes eight steps for endorsement. They are, Linearity, Accuracy, Precision, Selectivity and Specificity, Limit of quantification, Limit of detection, Robustness and Ruggedness [23].