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Review Article

A REVIEW ON SPECTROSCOPY AND HPLC METHOD DEVELOPMENT

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Development and validation of analytical method play an essential role in the discovery, development and manufacturing of pharmaceuticals. Every year, number of drugs entered into the market; hence it is mandatory to develop newer analytical methods for such drugs. After the development, it becomes necessary to validate the new analytical method. Method development is the process which proves that the analytical method is acceptable for use. Analytical method and its validation gives information about various stages and parameters like accuracy, precision, linearity, Limit of Detection, Limit of Quantification, specificity, range and robustness. Analytical method development should be done as per regulatory guidelines such as ICH guidelines. This article was pre- pared with an aim to review analytical method development.

Keywords: Analytical Method, UV-VIS spectroscopy, HPLC , Validation, ICH Guideline

INTRODUCTION

Analytical chemistry is a branch of chemistry which deals with identification of components (qualitative) and determination of quantity of components (quantitative) of substances or samples or mixture. There are two types of analysis, one is qualitative analysis and another one is quantitative analysis. In qualitative analysis, there is identification of components or analyte of mixture or sample is carried out. In quantitative analysis, there is determination of amount of components or analyte of mixture or sample is carried out. Analytical data is required not only in chemistry but also in other sciences like biology, zoology, arts such as painting and sculpture, archaeology, space exploration and clinical diagnosis. Important areas of application of analytical chemistry are quality control in manufacturing industries, monitoring and control of pollutants, clinical and biological studies, geological assays, fundamental and applied research.[1-2]

Analytical Method

Analytical method includes use of a specified technique and detailed-stepwise instructions which are used in qualitative, quantitative or structural analysis of a sample for one or more analytes.

Analytical methods are mainly classified into two types: Classical methods and Instrumental methods. A method in which the signal is proportional to the absolute amount of analyte is called classical method. A method in which the

signal is proportional to the analytes concentration is called instrumental method.[3]

Classical methods are divided into 3 main types are:

a) Separation of analyte,

b) Qualitative analysis and

c) Quantitative analysis.

Separation of analyte includes extraction, distillation, precipitation and filtration. Qualitative analysis includes boiling point, freezing point, colour, odour, density, reactivity and refractive index. Quantitative analysis includes gravimetric analysis and volumetric analysis.

Instrumental methods are divided into four main types are:

a) spectroscopic methods,

b) electrochemical methods,

c) chromatographic methods and

d) other techniques.

Spectroscopic methods include ultraviolet-visible spectroscopy, infrared spectroscopy, Raman spectroscopy, atomic absorption spectroscopy and atomic emission spectroscopy, x-ray spectroscopy and nuclear magnetic spectroscopy.

Electrochemical methods include Potentiometry, Coulometry and Voltametry.

Chromatographic methods include column chromatography, paper chromatography, thin layer chromatography, high performance liquid chromatography, gas chromatography and modern methods (LC-MS, GC-MS, LC-MS-MS, GC-MS-MS, LC-NMR and GC-NMR).

Other techniques include x-ray methods, radioactivity, mass spectrometry, optical methods (Refractometer, optical rotation) and thermal methods (Thermogravimetry, differential thermal analysis and differential scanning calorimetry).[4-5]

Spectroscopy

Spectroscopy is the study of interaction of electromagnetic radiation with matter. These interactions involve absorption and emission of radiation (energy) by the matter. Spectroscopy is two types, absorption spectroscopy and emission spectroscopy. The study of electromagnetic radiation absorbed by the sample, in the form of spectra is called absorption spectroscopy (UV-visible, IR, NMR, microwave and Radiowave spectroscopy). The study of electromagnetic radiation emitted by the sample, in the form of spectra is called emission spectroscopy (flame photometry and fluorimetry). Spectroscopy is useful for the study of atomic and molecular structure and used in the analysis of a wide range of samples. Atomic spectroscopy is the study of interaction of electromagnetic radiation with atoms, changes in energy takes place at atomic level (e.g. atomic absorption spectroscopy and flame photometry). Molecular spectroscopy is the study of interaction of electromagnetic radiation with

molecules, changes in energy takes place at molecular level (e.g. ultraviolet and infrared spectroscopy).[6-7]

UV-VIS spectroscopy[8]

In UV-visible spectroscopy, the amount of light absorbed at each wave- length of UV and visible region of electromagnetic spectrum is measured. This absorption spectroscopy uses electromagnetic radiations between 200 nm to 800 nm and is divided into the ultraviolet (UV, 200-400 nm) and visible (VIS, 400-800 nm) regions.

The principle of UV-Visible spectroscopy is based on the absorption of ultraviolet light or visible light by sample or chemical substance which results in the production of different spectra. When a molecule absorbs UV radiation, the electron present in that molecule undergo excitation, this causes transition of electron within a molecule from a lower level to a higher electronic energy level and the ultraviolet emission spectra arise from the reverse type of transition. Most commonly used solvents in UV spectroscopy are water, methanol, ethanol, ether, chloroform, carbon tetrachloride, cyclohexane and dichloroethane. Applications of UV spectroscopy are detection of functional groups, detection of conjugation, detection of geometrical isomers and detection of impurities.

Instrumentation of UV-Visible spectroscopy

A. Radiation sources: Most commonly used radiation sources are tungstan lamp, hydrogen discharge lamp, deuterium lamp, xenon discharge lamp and mercury arc (Figure 1).

B. Wavelength selector: The monochromator is used to disperse the radiation according to the wavelength. The basic elements of a monochromator are an entrance slit, a dispersing element and an exit slit.

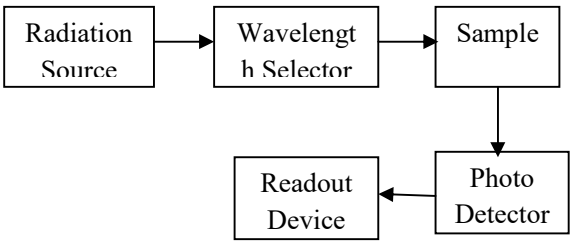

Figure 1: UV-Visible spectroscopy

C. Sample cell: In UV-Visible spectroscopy sample containers are used to hold liquid sample are called as cells or cuvettes. Cuvettes are made from quartz.

D. Photo detector: Most commonly used detectors in UV spectrophotometer are barrier layer cell, photocell and photomultiplier tube.

E. Readout device: The output from the detector is suitably amplified and then displayed on a readout device.

Chromatography

Chromatography is a physicochemical method for separation of mixture of compounds. Chromatography is a method of separation of mixture of compounds into individual

components between two phases, a stationary phase and a mobile phase.[9]

Chromatography is classified as follows:

Based on interaction of solute to stationary phase

- Adsorption chromatography
- Partition chromatography
- Ion exchange chromatography
- Molecular exclusion chromatography

Based on chromatographic bed shape

- Column chromatography
- Planar chromatography
- Paper chromatography
- Thin layer chromatography
- Displacement chromatography

Techniques by physical state of mobile phase

- Gas chromatography
- Liquid chromatography
- Affinity chromatography

HPLC

HPLC stands for high performance liquid chromatography or high-pressure liquid chromatography. HPLC can separate, identify and quantify the compounds present in any sample which can be dissolved in liquid.[10]

The main principle of liquid chromatography is adsorption. It is a chromatographic technique in which mobile phase is liquid. Sample is in the form of liquid solution. Sample is injected into a column of a porous material (stationary phase) and a liquid phase (mobile phase). Sample move through the column with mobile phase by high pressure delivered by a pump. Sample components travel according to their affinity towards the stationary phase. The component which has more affinity towards the stationary phase travels slower. The component which has less affinity towards the stationary phase travels faster. The components are separated from each other. The most common solvents used for HPLC are n-hexane, methylene chloride, chloroform, methyl-t-butyl ether, Tetrahydrofuran (THF), Isopropanol (IPA), Acetonitrile (MeCN or CAN), Methanol (MeOH) and water. Fundamental chromatographic parameters are efficiency (number of theoretical plates), retention factor, selectivity, resolution and pressure.

Instrumentation of HPLC

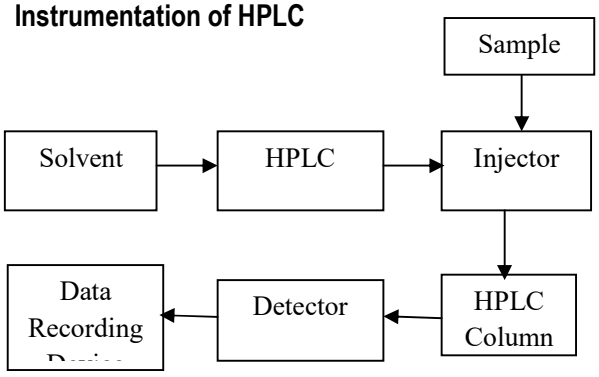

Figure 2: HPLC system

Applications of HPLC are chemical separation, purification and identification. Other applications of HPLC include pharmaceutical applications, environmental applications, forensics, clinical,

food and flavour[11-12] (Figure 2).

Components of the HPLC system:

Solvent reservoir, mixing system and degassing system

High pressure pump

Sample injector

Column

Detector

Data recording system

Solvent reservoir, mixing system and degassing system: Solvent reservoir stores the solvent (mobile phase). These are glass or stain-less-steel containers. The most common type of solvent reservoir is glass bottle. In addition to delivery of mobile phase, the pump must mix solvents with high accuracy and high precision. Two types of mixing unit are low pressure mixing and high pressure mixing. Degassing system removes en- trapped air bubbles from the solvent. Degassing is done by degasser techniques are ultra-sonication and filtration.[13-14]

High pressure pump: The role of pump is to force a liquid and give a specific flow rate. Flow rate is expressed in millilitres per minute (ml/min). Normal flow rate is 1-2 ml/min. Pump pressure range is 6000-9000 psi (400-600 bar). Commonly used pump types are constant pressure pump, syringe type pump and reciprocating piston pump.[15]

Sample injector: The liquid sample is introduced into the mobile phase by sample injector. Sample valve come between the pump and the column. An injector (auto sampler) is able to inject the sample into the continuous flowing mobile phase stream that carries the sample into the HPLC column. Typical sample volumes are 5-20 microliters (µl). Two types of injector are manual injector and automatic injector.[16]

Column: Column is a place where actual separation of components takes place. Column is made up of stainless steel. It is 5-25 cm long and 2-4.6 cm internal diameter.[16]

Detector: The detector can detect the individual component that elute from the column and convert the data into an electrical signal. Types of detector used are of two types, specific detectors and bulk property detectors. Specific detectors include UV-VIS detector, photo diode array detector, fluorescence detector and mass spectrometric detector. Bulk property detectors include refractive index detector, electrochemical detector and light scattering detector.

Data recording system: The output is recorded as a series of peaks and the area under the peak can be calculated automatically by the computer linked to the display.

Analytical method development

Analytical method development is the activity of selecting an accurate assay procedure to find out the composition of a formulation.

Development of analytical method is the process which is used to prove that an analytical method is suitable for use in laboratory. Analytical methods must be used inside GMP and GLP environments and should be developed by using the given protocols and acceptance criteria in the ICH guidelines Q2 (R1).[17-18]

The requirements for method development are as follows:

Qualified analysts

Instruments-qualified and calibrated

Documented methods

Reliable reference standards

Sample selection and integrity

Change control

Analytical method development is useful for:

New process and reactions

New molecule development

Active ingredients (Macro analysis)

Residues (Micro analysis)

Impurity profiling

Degradation studies

Herbal products

CONCLUSION:

This article gives an idea that how to develop a method, what is importance of Analytical method, how to perform its process and its parameters to prove that the method is suitable for its intended use. The primary objectives of development of analytical methods are for identification, purification and eventually to qualification any necessary drug etc. The development of analytical methods helps in understanding the critical process parameters and to reduce their effects on precision and accuracy.

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