A Brief Study About Analytical Techniques In Pharmaceutical Analysis (Chromatography & Spectroscopy):A Review Article

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ABSTRACT:-

Now a days, pharmaceutical products brought a great revolt in human health. These pharmaceuticals products would show their impact on individuals only if they are free from impurities & administered in an adequate quantity. Pharmaceutical products may attain impurities at various levels of their development or manufacture, storage and transportation which make the pharmaceutical product risky to be administered. Thus, at different stages of their development the impurities must be detected and they must be quantified in which analytical techniques play important roles. This review highlights the spectroscopic techniques like UV - Visible, IR, NMR and chromatographic techniques like HPLC, TLC with their corresponding methods with applications, principles and instrumentations that have been applied in analysis of various pharmaceutical products. In the conclusion we will find out the most widely used and advanced analytical technique with greater advantages and efficiency in pharmaceuticals analysis. [1]

KEYWORDS: - Analytical techniques, Pharmaceuticals, Spectroscopy, Chromatography.

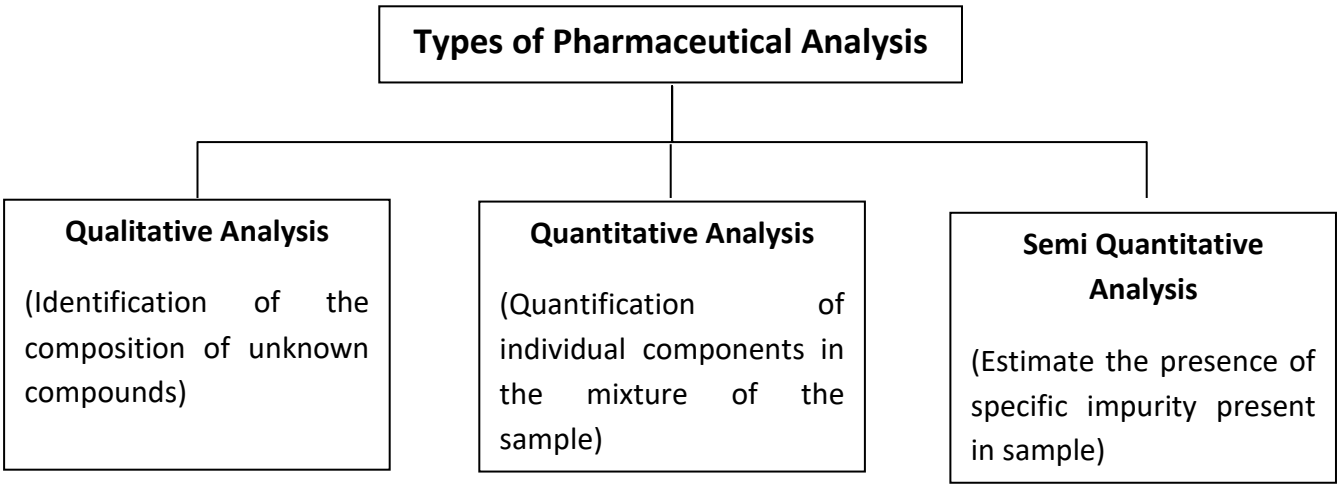
1. INTRODUCTION: -

The process of development of drug starts with the innovation of a drug molecule that has showed therapeutic effects to control diagnosis or to cure disease. The drug molecule which shows some therapeutic effect is known as Active Pharmaceutical Ingredient (API). The analytical investigation of bulk drug material, Intermediate drug products and sample containing drugs and their metabolites is very important. For checking the quality of product various analytical techniques like titrimetry, spectrometry and electro analytical methods are used. [2]

Pharmaceutical Analysis: -

Pharmaceutical analysis is the branch of chemistry, which involves the analysis (testing) of compounds at qualitative & qualitative level. It involves the analytical procedures which are used to determine the purity, safety, and quality, separation of the components of drugs and determination of structure of chemical compounds. [2, 3]

Table: 1



1.1 Analytical Techniques:-

Analytical techniques are the process that allow us to know qualitatively and/or quantitatively the composition of any material and chemical state in which it is located or identify / qualify the substances. There is variety of analytical techniques such as: -Chromatographic, Electrochemical, Titrimetric, Spectroscopic, Electrophoresis and their corresponding methods that have been applied for the analysis of pharmaceuticals. [3]

Spectroscopic and chromatographic method of analysis in brief with their application in pharmaceutical analysis.

1.1.1 Spectroscopy:

Spectroscopy is defined as the branch of science which studies the interactions of matters with light or electromagnetic radiations. In this method electromagnetic waves of particular wavelength or range of wavelength are use to identify the qualitative and quantitative analysis of matter. [2, 4]

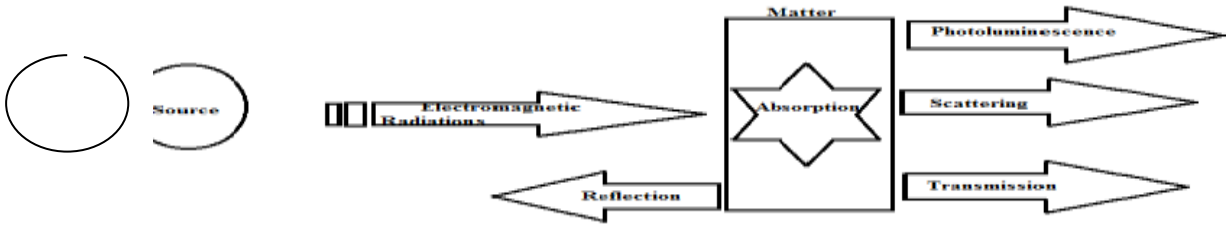
Principle: Spectroscopy study is based on the interaction of electromagnetic radiations with matter. When a beam of electromagnetic radiation fall of sample (atoms/ molecule) it either absorbed, reflected, transmitted, scattered. As a result spectrum is obtained which is analysised.

Spectrum: - A plot between the wavelength of electromagnetic radiation and frequency of light as a function of response.

Spectrophotometer: - It is device which is used in the spectroscopy for the measurement of spectrum.

Figure: 1

Principle of Spectroscopy



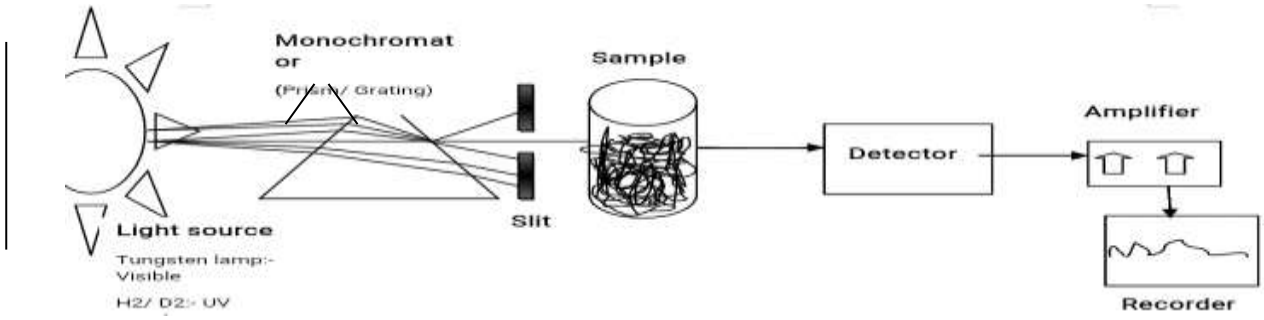
The main advantage of UV visible spectroscopy methods are that they take less time and have low labor consumption. The precision of this method is excellent. The use of UV-Visible spectrophotometer especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years. [2, 3]

1.1.1(a) UV-Visible spectroscopy:

Principle: It is the measurement of the attenuation of a beam of light after reflection from a sample surface. The ultraviolet absorption spectra arise from transition of electron with in a molecule from lower to higher level. The easily accessible part of this region (wavelength of 200-800nm) shows absorption only if conjugated pie electrons are present. [4]

Figure: 2

Instrumentation of UV-Visible Spectroscopy



Applications:

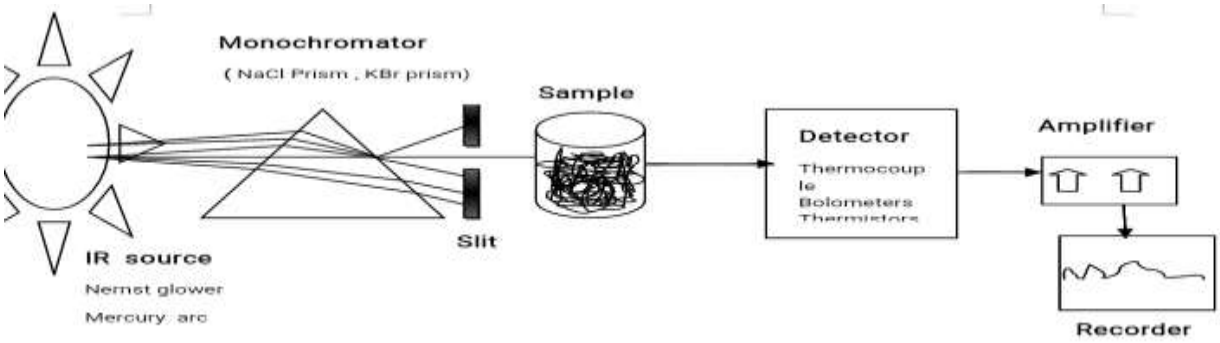
- Quantitative analysis of pharmaceutical substances.
- Detection of functional group presence in sample.
- Qualitative analysis.
- Detection of impurities in colored / organic samples.

1.1.1(b) Infra Red Spectroscopy (IR):

Principle: It is based on the principle of absorption. Absorption of lower energy radiations causes vibe rational and rotational excitation of groups of atoms within the molecules. Because of their characteristics absorption, identification of functional group is easily accomplished. [5, 6]

Figure: 3

Instrumentation of Infra Red Spectroscopy



Applications:

- Identification of functional group and structure elucidation.
- Identification of drug substance.
- Study of polymers.
- Ratio of cis-trans isomers in a mixture of compounds.
- Study of hydrogen bonding-whether it is of intermolecular or intermolecular type.

1.1.1(c) Mass Spectroscopy:

Mass spectrometer is an instrumental technique in which sample is converted to rapidly moving positive ions by electron bombardment and charged particles are separated according to their masses. [1, 3]

Principle: It works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their masses to charge ratios.

Applications:

- Phyto chemical analysis: Mass spectroscopy is widely employed in phyto chemical analysis due to capability to identify and measure metabolites having low molecular weight at very low concentration.
- Detection of impurities: Impurities present can be detected by the additional peaks, highest value of mass peaks than compounds itself.
- Structure elucidation: Mass spectroscopy has major use in structure elucidation of compounds.
- Clinical studies: Implementation of mass spectroscopy in clinical laboratory resulted in significant advancements.

1.1.1(d) Nuclear magnetic resonance spectroscopy (NMR):

Principle: NMR is based on the absorption of electromagnetic radiations in radio frequency region 4-900MHz by nuclei of the atom. It is a powerful analytical technique that gives us information about the number & types of atoms in a molecule. Absorption in the low energy radio frequency part of the spectrum causes excitation of nuclear spin state. [4]

Applications:

- Structure elucidation of organic compounds.
- Determination of optical purity.
- Study of molecular interactions.
- Investigation of dynamic properties.

1.1.2 Chromatography:

Chromatography is a physical method for the separation of mixture components. The mixture (sample) is dissolved in a fluid called mobile phase, which flow through the stationary phase. Chromatography can be analytical or preparative. The main purpose of preparative chromatography is to identify & separate the different components present in mixture, it is a form of purification. Analytical chromatography done to measure the proportions of analytes in a mixture. Chromatography is the separation of a mixture into individual components using stationary phase& mobile phase. Stationary phase mainly solid or liquid supported on a solid or gel surface. It can't be gas. Mobile phase can be liquid or gas but can't be solid. [1, 7]

- **Principle of Chromatography:** Chromatography techniques mainly based up the principles of adsorption & partition. In chromatography separation among the components of mixture occur between the two phase of different polarity & strength out of which one is mobile phase(gas/liquid) in which mixture dissolved while other is immobile , immiscible stationary phase(solid).
- **Classification of Chromatography:**
  1. **Based on the principle of separation :**
  - ✓ **Adsorption chromatography:** Based on the principle of adsorption, in this type of chromatography stationary phase is solid while mobile phase can be liquid/gas.
    Example: Thin layer chromatography (TLC), Column chromatography, High performance liquid chromatography (HPLC), Gas- liquid chromatography.
  - ✓ **Partition chromatography:** Based on the principle of partition for separation in this stationary phase is liquid over solid/gas surface, while mobile phase can be liquid/gas.
    Example: Gas- liquid chromatography, Paper partition chromatography, Column partition chromatography.

2. **Based on the nature of stationary & mobile phase:**

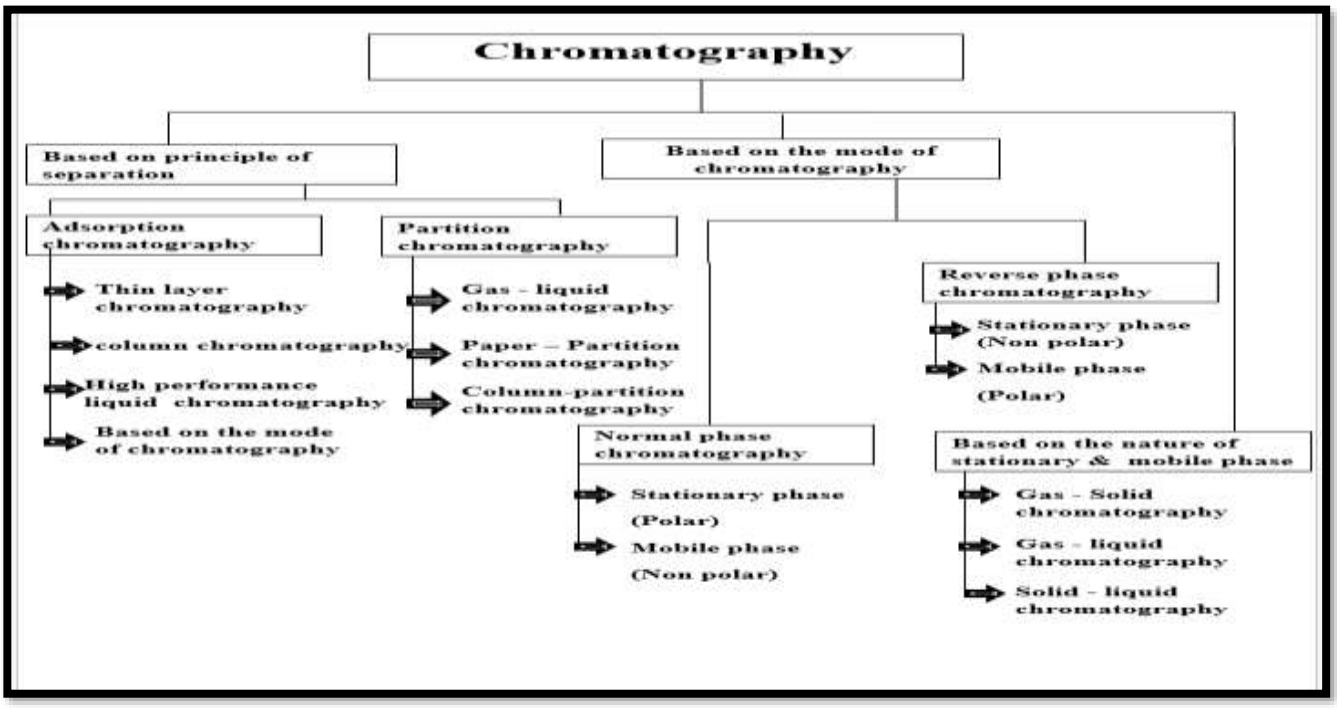
- ✓ Gas- Solid chromatography
- ✓ Gas – liquid chromatography
- ✓ Solid – liquid chromatography

3. **Based on the mode of chromatography:**

- ✓ Normal phase chromatography: Mobile phase : Polar ; Stationary phase : Non Polar
- ✓ Reverse phase chromatography: Mobile phase : Polar ; Stationary phase : Non Polar.[7]

Table: 2

Types of Chromatography



1.1.2 (a) Column Chromatography:

It is a type of absorptions chromatography in which column is used for the separation of components from mixture.

Principle:

Column chromatography is based on the principle of separation by adsorption .In column chromatography stationary phase is solid, while mobile phase is liquid & both are of reversible nature [7]. When a mixture is dissolved in mobile phase & introduced in column, then the individual components moves with different rates depending upon their relative affinities. The compound with lesser affinity towards stationary phase moves faster, hence elute out first from the column and vice versa.

Requirements:

Stationary phase: - Solid (Silica gel), 100-200 mesh size, 60-200 micron partical size.

Mobile phase: - Liquid (petroleum ether, Acetone, ether, toluene, esters, chloroform etc.)[7]

Applications:

- Separation of mixture of compounds.
- Removal of impurities or purification process.
- Isolation of metabolites from biological fluids.
- Estimation of drugs in formulation or crude extracts.
- Isolation of active constituents.

1.1.2 (b) Thin layer chromatography (TLC) :

Thin layer chromatography can be defined as a method of separation or identification of a mixture of component into individual components by using finely divided adsorbent (silica gel) spread over a glass plate and liquid as mobile phase.

Principle:

It is based on the principle of adsorption chromatography .The mixture of compound are spotted on a TLC plate. The mobile phase solvent flows through via capillary action. The compound moves according to their affinity towards adsorbent. The compound with more affinity towards the adsorbent travel slower vise-versa [9, 10].

Applications:

- Separation of mixture of drugs from chemical or biological origin, plant extract etc.
- Identification of related compounds in drug.
- Detection of decomposition products in drugs.
- Detect the presence of foreign substances in drugs.
- Identification of drugs.[8]

1.1.2 (c) Paper Chromatography:

It is a chromatography technique in which the analysis of unknown substances is carried out mainly by the flow of solvents on specially designed filter paper.

Principle:

The principle of separation in paper chromatography is partition rather than adsorption ,cellulose layer in the filter paper contain moisture which act as stationary phase while organic solvents or buffers are used as a mobile phase.[7,8]

Paper used: Choices of filter paper depend upon thickness, flow rate, purity, technique etc.

What man filter paper of different grade like NO.1, NO.3MM, and NO.17 etc. are used.

Applications:

- Identification of drugs and impurities.
- Separation of mixture having polar and non-polar compounds.
- It is used to control the purities of pharmaceuticals.[7]

1.1.2 (d) Gas Chromatography:

Gas chromatography consists of gas solid and gas liquid chromatography .in both types gas is used as mobile phase and solid/liquid is used as stationary phase. [9]

Principle:

The principle of separation in gas liquid chromatography is partition, where gas is used as mobile phase, stationary phase is liquid coated over solid support. The mixture to be separated is to be converted into vapours and mixed with mobile phase. The components are separated according to their partition coefficients which are more soluble will eluted later, while least soluble elute out first.

Carrier gas: These are used as mobile phase in gas chromatography in which mixture of components to be separated is mixed.e.g. hydrogen, helium, nitrogen, argon.[7,9]

Application:

- Purification of compound can be determined for drugs like clove oil, atropine sulphate, stearic acid.
- Quality control and analysis of drug product like antibiotics, general anesthetics, antivirals etc.
- To determine the level of metabolites in body fluids like blood plasma, serum and urine.[9]

1.1.2 (e) High performance liquid chromatography(HPLC):

It is an advance technique of column chromatography. It is also known as high pressure liquid chromatography. It is an analytical technique used for the separation, quantification and identification of each constituent of mixture.

Principle:

The principle of separation in HPLC is adsorption. The mixture of component is mixed in the liquid solvent which administered into column under high pressure upto 400 atmospheres. The column is filled with solid adsorbent material. The interaction of each sample component will be different which causes difference in the flow rates of each component of mixture. Hence the component of mixture moves according to their relative affinities towards the adsorbent. [7, 14]

Type of HPLC:-Depending on the type of stationary phase used, the HPLC is divided into following types. [14]

- **Normal phase chromatography**: - Stationary phase is polar in nature (silica gel), while mobile phase is non polar in nature (diethyl ether, chloroform).The non polar sample will elute out first& polar samples are retained on column.
- **Reverse Phase HPLC**: - Stationary phase is non polar, while mobile phase is polar in nature. Hence, the polar sample elute out more.
- **Size –exclusion HPLC**: - The column is filled with precisely controlled substrate. Based upon the difference in molecular sizes the separation of components of mixture occurs.
- **Ion- exchange HPLC**: - The surface of the stationary phase is ionically charged which is opposite to the charge of the sample. The mobile phase used is aqueous buffer which will control ionic strength & pH.

Applications:-

- Control the drug stability & quality control.
- Quantity of drug determination from pharmaceutical dosage forms.e.g.- Dopamine in levodopa drug
- Quantity of drug present in body fluids.e.g.- Blood glucose level
- Analysis of natural contaminations e.g. Mercury & phenol in Sea water.[14,15]

Conclusion:

It can be concluded from the entire review that HPLC from chromatography & UV- Visible and Nuclear Magnetic Resonance from Spectroscopy are versatile, reproducible analytical techniques for the estimation of drug products. They have wide applications in different fields in term of quantitative and qualitative estimation of active molecules. [1, 4, 7, 9].

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