CHAPTER 2
PRINCIPLE OF PHARMACEUTICAL ANALYSIS

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1. INTRODUCTION TO PHARMACEUTICAL ANALYSIS

Pharmaceutical analysis is a go in different directions of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the ingredients of a solution or mixture, or determination of structure of chemical compounds. The substance may be a single compound or a mixture of combination and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, microorganisms, minerals and various synthetic by products.[1]

The test to be analyzed is called as analyse and on the basis of size of test compounds, they can be classified as macro(0.1 g or more), semi micro (0.01 g to 0.1 g), micro(0.001 g to 0.01 g), sub micro (0.0001 g to 0.001 g), ultra micro (below 10-4 g), trace analysis(100 to 10000 ppm). Among all, the semi micro analysis is widely used.[2]

Pharmaceutical analysis plays a crucial role in drug development quality control and regulatory compliance. It involves qualitative and quantitative methods to ensure drug safety, efficacy and purity.[3]

Type of Pharmaceutical analysis

Main two types of pharmaceutical analysis

- Qualitative Analysis
- Quantitative Analysis

Qualitative Analysis

Analysis is done on the base of quality of substance for example identification of elements, identification of functional, group identification test etc.

Quantitative analysis

Quantitative analysis determines the amount of substance for example titrations, gravimetric analysis, limit test of Pharmaceutical substance etc.

In pharmaceutical industry, Pharmaceutical methods are used for the ensuring the quality, safety and efficacy of drug

The basic principle of pharmaceutical analysis is to ensure the products should be free from impurities or within the specified limit. For the purpose different chemical method and instrumental method have been the developed.[4]

2. PRINCIPLES;

Pharmaceutical analysis principle focus on the answering the:

- Quality of drug
- Safety of drug
- Efficacy of drug

By identifying, purifying, quantifying, and separating substances. It involves a range of analytical technique including chromatography Spectroscopy (UV, IR, NMR, and MASS) to analyze raw material, active pharmaceutical ingredient. The goal is to determine the identity, Purity content and stability of Pharmaceutical substance.

Here is a detailed look at the key principle of Pharmaceutical substance-

Identification: It includes determination of identification of Pharmaceutical substance including structure of chemical and determination of purity.

Quantification: It includes measuring of amount of substance present in the sample.

Purification: Separation of the Pharmaceutical substance from impurity.

Stability: The substance should be stored in hygienic condition in a well closed container away from light and moisture.

Quality Control of Pharmaceutical Subsistence: The drugs (Pharmaceutical substance) should meet quality standards during manufacturing process.
Pharmaceutical analysis is used to industrial like the Pharmaceutical, food, cosmetic and for diseases diagnosis.[5]

- **Accuracy And Precision in Pharmaceutical Analysis**
- **Accuracy:** Accuracy is defined as an accuracy results is the one which matches very nearly with true of a measured. Accuracy is inversely proportional to the error i.e. the greater the accuracy, smaller is the error.[6] Accuracy is classified into three types; Point accuracy; it is the accuracy of the instrument only at the particular point on this scale. It does not give any information about the general accuracy of the instrument. Accuracy as percentage of true value; percentage is true value is hen the accuracy of the instrument is determined by identifying the measured value regarding their true value. Accuracy as percentage of scale range; percentage of scale range determines the accuracy of a measurement.[7]
- **Precision:** Precision may be defined as the concordance of a series of measurement of the same quantity. The mean deviation or the relative mean deviation is a measure of precision. The closeness of two or more measurement to

each other is known as the precision of a substance. If you weigh a given substance five times and get 3.2 kg each time, then your measurement is very precise but not necessarily accurate. Precision is independent of accuracy. The below examples will tell you about how you can be precise but not accurate and vice versa. Precision is sometimes separated onto.[8]

Specificity and Selectivity in Pharmaceutical Analysis

Both **specificity** and **selectivity** are key concepts in analytical chemistry, particularly when it comes to pharmaceutical analysis, where it is crucial to accurately identify and quantify active pharmaceutical ingredients (APIs) without interference from other substances.[9]

- **Specificity:** Specificity refers to the capability of an analytical technique to detect and quantify only the intended analyte without being affected by other substances in the sample, such as excipients, impurities, or degradation products. It ensures that the method can reliably distinguish between the target compound and other potentially similar substances. For example, in the case of a drug formulation, the method should ideally isolate the active pharmaceutical ingredient (API) from inactive components (such as binders or fillers) and any degradation products that may be present.[10]

Key factors influencing specificity:

- Chemical properties of the analyte and other constituents.
- Analytical technique (e.g., UV spectrophotometers, chromatography)
- Instrumentals parameters (e.g., detection wavelength, stationary phase and mobile phase in chromatography)[11]

Selectivity: Selectivity, although closely associated with specificity, is a more comprehensive concept. It denotes an analytical method's capability to discriminate the analyte from other substances, even if they possess similar structures or exist in substantial concentrations. In pharmaceutical analysis, selectivity ensures that the method can differentiate between various compounds, even when they exhibit comparable chemical or physical attributes, such as in complex mixtures or formulations. For example, a selectivity assessment may be necessary to confirm that a method can distinguish between the active pharmaceutical ingredient and closely related metabolites or impurities, which may have comparable retention times in chromatography.[12]

Key factors influencing selectivity:

- Chromatographic parameters (e.g., mobile phase composition, column temperature)
- Spectroscopic characteristics (e.g., absorption wavelengths, fluorescence signals)
- Diverse chemical interactions in the technique (e.g., ionization processes in mass spectrometry).[13]

Example in Practice

- Let's consider an analysis of a tablet containing an antibiotic. If a method is **specific**, it will only measure the concentration of the antibiotic without being influenced by excipients like starch, lactose, or any potential by-products. If it's **selective**, it will be able to separate and quantify the antibiotic even if similar antibiotics or impurities are present in the sample. Both principles are crucial when developing analytical methods that are both precise and reliable in assessing the quality and safety of pharmaceutical products.[14]

Sensitivity: Analytical chemistry is detecting and determining compounds in small amounts of samples (microanalysis),

determining very low concentrations or small amounts in larger samples (trace analysis), or are of determining low concentrations in small samples. Progress in analytical chemistry might be measured by shifting the detection limit towards lower values.
Uncertainties in the lower limits to the detection of elements and compounds arise because of the presence of uncertainties (errors or noise) in the measured analytical result.[15]

Repeatability and Reproducibility:
Repeatability and reproducibility are both measures of how closely repeated measurements agree, but they differ in the conditions under which they are taken. Repeatability and reproducibility are both measures of how closely repeated measurements agree, but they differ in the conditions under which they are taken. Repeatability refers to the closeness of measurements taken under the same conditions by the same person or instrument, while reproducibility refers to the closeness of measurements taken under different conditions, possibly by different people or instruments.

Repeatability; the variation arising hen the condition are kept identical and repeated measurement are taken during a short time period. This refers to the consistency of results when a measurement is repeated under the same conditions, using the same equipment, and ideally by the same person. For example, if you measure the weight of an object multiple times using the same scale, the repeatability would be the closeness of those measurements.

Reproducibility; the variation arising using the same measurement process among different instrument and operators, and over longer time periods. This refers to the consistency of results when a measurement is repeated under different

conditions, potentially by different people, using different equipment, or at different locations. For example, if you measure the same object's weight in different laboratories using different scales, the reproducibility would be the closeness of that measurements.[16]

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The Limit of Detection (LOD) is the lowest concentration of an analyte that can be reliably detected, while the Limit of Quantification (LOQ) is the lowest concentration that can be reliably detected and quantified with acceptable accuracy and precision. LOQ is typically higher than LOD.

Limit of Detection (LOD)

- The LOD represents the lowest concentration of an analyte that can be distinguished from background noise or a blank sample with a certain level of confidence.
- It indicates that a signal is present, but the exact concentration may not be determined with high accuracy.
- LOD is often expressed as a multiple of the standard deviation of the blank or a similar measure of noise.
- A common approach is to use a signal-to-noise ratio of 3:1 or 3.3:1 to determine LOD.

Limit of Quantification (LOQ):

- The LOQ is the lowest concentration of an analyte that can be quantified with acceptable accuracy and precision.
- It means that the analyte can not only be detected but also measured with sufficient reliability for quantitative analysis.
- LOQ typically requires a calibration curve with a slope that can be reasonably determined at the LOQ level.
- A common approach is to use a signal-to-noise ratio of 10:1 to determine LOQ.[16]

Analytical technique: From the stages of drug development to marketing and post marketing, analytical techniques play a great role, be it understanding the physical and chemical stability of the drug, impact on the selection and design of the dosage form, assessing the stability of the drug molecules, quantitation of the impurities and identification of those impurities which are above the established threshold essential to evaluate the toxicity profiles of these impurities to distinguish these from that of the API, when applicable and assessing the content of drug in the marketed products. The analysis of drug and its metabolite which may be either quantitative or qualitative is extensively applied in the pharmacokinetic studies. This review highlights the role of various analytical techniques and their corresponding analytical methods in the analysis of pharmaceuticals.[17]
Using different method example chromatography, Spectroscopy to analyze pharmaceutical substances or drugs.

Common techniques in pharmaceutical analysis
Common techniques in pharmaceutical analysis include chromatography (like HPLC and GC), spectroscopy (like UV-Vis and IR), and titrimetric methods. These techniques are used to identify, quantify, and purify substances in pharmaceutical preparations.

Chromatography:
This separation technique is widely used in pharmaceutical analysis to isolate and identify different components of a mixture. High-performance liquid chromatography (HPLC) is a sophisticated technique that separates complex mixtures, while gas chromatography (GC) is used for volatile organic compounds. Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) are also employed.

- **Spectroscopy:**
  Spectroscopy involves analyzing the interaction of electromagnetic radiation with a substance. UV-Vis spectrophotometry is a common technique for qualitative and quantitative analysis, relying on the absorption of UV and visible light. Fourier transform infrared spectroscopy (FTIR) and atomic absorption spectroscopy (AAS) are other examples.
- **Titrimetric Methods:**
  Titration is a volumetric analysis technique where a substance of known concentration (titrant) is added to a solution of unknown concentration (analyte) to determine the concentration of the analyte. Acid-base titrations are a common type, used in various industrial and pharmaceutical settings.
- **Other Techniques:**
  Besides the above, other techniques used in pharmaceutical analysis include mass spectrometry, electrochemical methods like Potentiometry, and hyphenated techniques that combine different methods.
- **Hyphenated Techniques:**
  These techniques combine two or more analytical methods to enhance their capabilities. For example, GC-MS (gas chromatography coupled with mass spectrometry) combines the separation ability of GC with the identification and quantification capabilities of mass spectrometry.[18]

Titrimetric techniques

Origin of the titrimetric method of analysis goes back to somewhere in the middle of the 18th century. It was the year 1835 when Gay–Lussac invented the volumetric method which subsequently leads to the origin of term titration. Although the assay method is very old yet there are signs of some

modernization, i.e., spreading of non-aqueous titration method, expanding the field of application of titrimetric methods to (very) weak acids and bases as well as potentiometric end point detection improving the precision of the methods. With the development of functional group analysis procedures titrimetric methods have been shown to be beneficial in kinetic measurements which are in turn applied to establish reaction rates. There are many advantages associated with these methods which include saving time and labor, high precision and the fact that there is no need of using reference standards. In the past titrimetric methods have been used for the determination of captopril, albendozole and gabapentin in commercial dosage forms.[19]

Chromatographic techniques

Thin layer chromatography

Although an old technique yet it finds a lot of application in the field of pharmaceutical analysis. In thin layer chromatography, a solid phase, the adsorbent, is coated onto a solid support as a thin layer usually on a glass, plastic, or aluminum support. Several factors determine the efficiency of this type of chromatographic separation. First the adsorbent should show extreme selectivity toward the substances being separated so as to the dissimilarities in the rate of elution be large. For the separation of any given mixture, some adsorbents may be too strongly adsorbing or too weakly adsorbing.

Thin layer chromatography is a popular technique for the analysis of a wide variety of organic and inorganic materials, because of its distinctive advantages such as minimal sample clean-up, wide choice of mobile phases, flexibility in sample distinction, high sample loading capacity and low cost. TLC is a powerful tool for screening unknown materials in bulk drugs.

Principle of TLC

Thin Layer Chromatography (TLC) separates mixtures based on the principle of differential migration between a stationary phase and a mobile phase. The stationary phase is a thin layer of adsorbent, like silica gel or alumina, coated on a plate, while the mobile phase is a liquid solvent. Mixtures are spotted onto the plate, and as the solvent moves up, different components of the mixture travel different distances based on their affinity for the stationary and mobile phases, resulting in separation.[20]

High performance thin layer chromatography

With the advancement of the technique, high performance thin layer chromatography (HPTLC) emerged as an important instrument in drug analysis. HPTLC is a fast separation technique and flexible enough to analyze a wide variety of samples. This technique is advantageous in many means as it is simple to handle and requires a short analysis time to analyze the complex or the crude sample cleanup. HPTLC evaluates the entire chromatogram with a variety of parameters without time limits.[21]

Principle of HPTLC

High-Performance Thin Layer Chromatography operates on the fundamental principle of differential migration of compounds between a stationary phase and a mobile phase [10]. The separation occurs on a flat surface of modified sorbent material, typically silica gel, with precisely controlled particle size and pore dimensions [11]. The enhanced resolution in HPTLC compared to conventional TLC stems from the use of finer particle sizes (5-7 μm) and more uniform layer thickness (100-200 μm) [12]. The migration of analytes follows complex physicochemical interactions, including adsorption, partition, and capillary action, contributing to the separation efficiency. HPLC High-Performance Liquid Chromatography operates

through the interaction of analytes between a liquid mobile phase and a solid stationary phase under high pressure. The separation mechanism relies on various molecular interactions, including hydrophobic interactions in reverse-phase chromatography, polar interactions in normal-phase chromatography, and ionic interactions in ion-exchange chromatography. The efficiency of separation is governed by theoretical plates, resolution factors, and capacity factors, which are influenced by operational parameters such as mobile phase composition, flow rate, and column characteristics.[22]

High-performance liquid chromatography (HPLC)

HPLC is an advanced form of liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to recognize better the role of individual molecules. It was in the year 1980, HPLC methods appeared for the first time for the assay of bulk drug materials. The specificity of the HPLC method is excellent and simultaneously sufficient precision is also attainable.

During the survey of the literature it was observed that among the chromatographic techniques HPLC has been the most widely used system. In liquid chromatography the choice of detection approach is critical to guarantee that all the components are detected. One of the widely used detectors in HPLC is UV detector which is capable of monitoring several wavelengths concurrently; this is possible only by applying a multiple wavelength scanning program. If present in adequate quantity, UV detector assures all the UV-absorbing components are detected.[23]

Principle of HPLC

HPLC is a form of liquid chromatography, where separation (or partition) occurs between a mobile phase (the solvent) and a

stationary phase (the column packing). It is the ability with which the sample constituents will distribute themselves between the two phases that will affect the separation.

Gas chromatography

Moving ahead with another chromatographic technique, gas chromatography is a powerful separation technique for detection of volatile organic compounds. Combining separation and on-line detection allows accurate quantitative determination of complex mixtures, including traces of compounds down to parts per trillions in some specific cases. Gas liquid chromatography commands a substantial role in the analysis of pharmaceutical product. The creation of high-molecular mass products such as polypeptides, or thermally unstable antibiotics confines the scope of this technique.[24]

Spectroscopic techniques

Spectrophotometer

Another important group of methods which find an important place in pharmacopoeias are spectrophotometric methods based on natural UV absorption and chemical reactions. Spectrophotometers are the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. The advantages of these methods are low time and labor consumption.

The colorimetric methods are usually based on the following aspects:

- Complex-formation reaction.
- Oxidation-reduction process.
- A catalytic effect.

It is important to mention that colorimetric methods are regularly used for the assay of bulk materials. For example, the blue

tetrazolium assay is used for the determination of corticosteroid drug formulations. Derivative spectroscopy uses first or upper derivatives of absorbance with respect to wavelength for qualitative investigation and estimation. The concept of derivatizing spectral data was first offered in the 1950s, when it was shown to have many advantages.[24]

Near infrared spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) is a rapid and non-destructive procedure that provides multi component analysis of almost any matrix. In recent years, NIR spectroscopy has gained a wide appreciation within the pharmaceutical industry for raw material testing, product quality control and process monitoring. The growing pharmaceutical interest in NIR spectroscopy is probably a direct consequence of its major advantages over other analytical techniques, namely, an easy sample preparation without any pretreatments, the probability of separating the sample measurement position by use of fiber optic probes, and the expectation of chemical and physical sample parameters from one single spectrum. The major pharmacopoeias have generally adopted NIR techniques.

Nuclear magnetic resonance spectroscopy (NMR)

Since the first report appeared in 1996 describing the use of NMR spectroscopy to screen for the drug molecules, the field of NMR based screening has proceeded promptly. Over the last few years, a variety of state-of-the art approaches have been presented and found a widespread application in both pharmaceutical and academic research. Recently NMR finds its application in quantitative analysis in order to determine the impurity of the drug, characterization of the composition of the drug products and in quantization of drugs in pharmaceutical formulations and biological fluids.

Fluorimetry and phosphorimetry

The pharmaceutical industries continuously look for the sensitive analytical techniques using the micro samples. Fluorescence spectrometry is one of the techniques that serve the purpose of high sensitivity without the loss of specificity or precision. A gradual increase in the number of articles on the application of fluorimetry and phosphorimetry in quantitative analysis of various drugs in dosage forms and biological fluids has been noticed in the recent past.

Electrochemical methods

The application of electrochemical techniques in the analysis of drugs and pharmaceuticals has increased greatly over the last few years. The renewed interest in electrochemical techniques can be attributed in part to more sophisticated instrumentation and to increase the understanding of the technique themselves. Moreover, a large number of electro analytical methods are available for quantification of pharmaceuticals. An amber lite XAD-2 and titanium dioxide nanoparticles modified glassy carbon paste was developed for the determination of imipramine, trimipramine and desipramine. The electrochemical behavior of these drugs was investigated using cyclic voltammetry, chronocoulometry, electrochemical impedance spectroscopy and adsorptive stripping differential pulse voltammetry. The capsaicin modified carbon nanotube modified basal-plane pyrolitic graphite electrode or p-chloranil modified carbon paste electrodes have been developed for the determination of benzocaine and lidocaine.

Kinetic method of analysis

Kinetic method of analysis has been developing since 1950s and yet in modern days it is taking a major resurgence in activity. The repetitive interest in the kinetic methods can be credited to the advancements made in principles, in automated instrumentation,

in understanding the chemical and instrumentation, in data analysis methods and in the analytical application. From the literature it is evident that the kinetic approach to analytical chemistry is rather general with several advantages over traditional equilibrium approach.

Essentially, kinetic methods trust the measurements of concentration changes (detected via signal changes) in a reactant (which may be the analyte itself) with time after the sample and reagents have been mixed manually or mechanically. Going through the literature it can be evident that fixed time and initial rate methods have been used more often for the determination of drugs in pharmaceutical formulations. Automatic techniques for the kinetic methods are generally based on open systems; among the popular techniques are the stopped flow system and the continuous addition of reagent (CAR) technique.

Electrophoretic methods

Another important instrument essential for the analysis of pharmaceuticals is capillary electrophoresis (CE). CE is a relatively new analytical technique based on the separation of charged analytes through a small capillary under the impact of an electric field. In this technique solutes are perceived as peaks as they pass through the detector and the area of individual peak is proportional to their concentration, which allows quantitative estimations. In addition to pharmaceutical studies it finds an application in the analysis of biopolymer analysis and inorganic ions. CE analysis is generally more effective, can be performed on a quicker time scale, requires only a small amount, lesser up to Nano liter injection volumes and in most cases, takes place under aqueous conditions. These four characteristics of CE have proven to be beneficial to many pharmaceutical applications. Several reports have appeared on the application of this technique in the routine drug analysis.

Flow injection and sequential injection analysis

Laboratory automation was introduced in the second half of the XX century. Steward in the U.S. as well as Ruzicka and Hansen in Denmark, created the flow injection analysis (FIA) technique for the automation of chemical procedure. The introduction of this technique approached to transform the conception of automation in chemical analysis by permitting instrumental measurement to be carried out in the absence of physical and chemical equilibria.

The basis of Flow injection analysis (FIA) is injection of a liquid sample into a moving, non-segmented uninterrupted carrier stream of a suitable liquid. The injected sample forms a zone, which is then transported toward a detector that uninterruptedly records the changes in absorbance, electrode potential, or other physical parameter resulting from the passage of the sample material through the flow cell.

Hyphenated techniques

The coupling of a separation technique and on-line separation technique leads to the development of a hyphenated technique. The last two decades saw a remarkable advancement in the hyphenated techniques and its application in pharmaceutical analysis. A variety of hyphenated techniques such as LC-MS, GC-MS, LC-NMR, CE-ICP-MS and CE-MS. have been applied in the analysis of pharmaceuticals. The determination of drugs in biological materials is an important step in drug discovery and drug development. The determination of drugs in biological materials is an important step in drug discovery and drug development. HPLC together with various types of detection such as ultraviolet, fluorescence, and mass spectrometry has become the method of choice for bioanalytical method development. Recreational drug abuse is a growing issue and new substances are detected frequently in clinical and forensic samples. Diphenyl-2-pyrrolidinemethanol is one of these substances and therefore

work has been done to identify it and its metabolites in rat urine using gas chromatography–mass spectrometry and liquid chromatography–high resolution–mass spectrometry.

The method was successfully applied to the determination of methyl, ethyl, propyl, butyl, isopropyl and isobutyl esters of 4-hydroxybenzoic acid. To assess the pharmacokinetics of selective substrates of human cytochrome P450s in mini pigs, caffeine, warfarin, omeprazole, metoprolol and midazolam were administered in combination either through intravenous route or orally. Plasma samples obtained upto 24 h after dosing were analyzed by liquid chromatography–tandem mass spectrometry to estimate typical pharmacokinetic parameters for each analyte.[25]

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