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# Pharmaceutical Impurity Analysis Of Raw Material And Final Product By Using Analytical Techniques

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**ABSTRACT:** Isolation and identification of unknown components and impurities: The evaluation of pharmaceutical raw materials and finished products for impurities and degradation products is an essential part of the drug development and manufacturing testing process. Additionally, toxicological information must be obtained on any drug-related impurity that is present at a concentration of greater than 0.1% of that of the active pharmaceutical ingredient (API). In pharmaceutical QC and manufacturing, impurity analysis has traditionally been performed by HPLC with UV, PDA, or MS detection. As it is essential to detect and measure all of the impurities in the sample, it is necessary to have a high resolution separation process. This usually involves long analysis times resulting in low throughput. As candidate pharmaceutical compounds become more potent and are dosed at lower and lower levels, ever more sensitive assays are needed to detect and measure impurities. The low throughput of HPLC can become the rate-limiting step in product release testing or process evaluation. Since much of the process of impurity identification involves the coupling of LC to sophisticated MS, any reduction in analysis time will result in a more efficient use of these significant investments. Analytical technology advances such as UPLC and UPC offer significant improvements in throughput and sensitivity, with benefits to the process of product release and identification of drug-related impurities. The most characteristic feature of the development in the methodology of pharmaceutical and biomedical analysis during the past 25 years is that HPLC became undoubtedly the most important analytical method for identification and quantification of drugs, either in their active pharmaceutical ingredient or in their formulations during the process of their discovery, development and manufacturing.

## I. INTRODUCTION

The evaluation of pharmaceutical raw materials and finished products for impurities and degradation products is an essential part of the drug development and manufacturing testing process. Additionally, toxicological information must be obtained on any drug-related impurity that is present at a concentration of greater than 0.1% of that of the active pharmaceutical ingredient (API). In pharmaceutical QC and manufacturing, impurity analysis has traditionally been performed by HPLC with UV, PDA, or MS detection. As it is essential to detect and measure all of the impurities in the sample, it is necessary to have a high resolution separation process. [1] This usually involves long analysis times resulting in low throughput. As candidate pharmaceutical compounds become more potent and are dosed at lower and lower levels, ever more sensitive assays are needed to detect and measure impurities. The low throughput of HPLC can become the rate limiting step in product release testing or process evaluation. Since much of the process of impurity identification involves the coupling of LC to sophisticated MS, any reduction in analysis time will result in a more efficient use of these significant investments. Analytical technology advances such as UPLC and UPC offer significant improvements in throughput and sensitivity, with benefits to the process of product release and identification of drug-related impurities. The most characteristic feature of the development in the methodology of pharmaceutical and biomedical analysis during the past 25 years is that HPLC became undoubtedly the most important analytical method for identification and quantification of drugs, either in their active pharmaceutical ingredient or in their formulations during the process of their discovery, development and manufacturing.[2]

An impurity profile is a description of the identified and unidentified impurities present in a drug substance (Source: Guidance for Industry, Q3A Impurities in New Drug Substances). Impurity profiling processes usually begin with the detection of impurities, followed by their isolation and characterization. For all three types of impurities, it is critical to develop a robust method during process development that can eventually be validated and transferred for use in



QA/QC. Developing reliable methods for impurities regulated at very low levels, such as genotoxic impurities, adds further challenges to this process. To better detect, identify, quantify, and characterize the impurities present in drug substances and products, pharmaceutical scientists rely on robust analytical tools with high sensitivity and specificity. Major analytical tools for impurity analysis include spectroscopy, chromatography, mass spectrometry, and various combinations of these, that is, tandem techniques. The appropriate technique is selected based on the nature of the impurity and the level of information required from the analysis. There are various complex analytical problems in pharmaceutical development that require the use of more than one analytical technique for their solution. Analytical techniques such as LC/UV, LC/MS, GC/MS, CE/MS, and LC/UV provide the orthogonal detection and complementary information that can address these challenges in a time-efficient manner. As a result, they play a vital role in impurity profiling of pharmaceuticals from identification to the final structure elucidation of unknown impurities.[3] Since impurities in drug substances are usually present at very low quantities, detailed analysis is only possible after isolation of the impurities. This is a major challenge in pharmaceutical laboratories.

## II. DISCUSSION

LC/MS is a highly sensitive and specific analytical tool that is routinely used in pharmaceutical development to detect, identify, and quantify product impurities. A detection limit of a few hundred ppm is readily achievable, ensuring the identification of impurities present at concentrations greater than 0.1 %. Mass spectrometry-based methods generally provide additional sensitivity and specificity compared to techniques such as UV alone. While single quadrupole mass spectrometers are well suited to the confirmation of known impurities and the preliminary structural assessment of unknown impurities, highly sensitive Q-TOF mass spectrometers provide high-resolution accurate mass information that enables the unambiguous identification of unknown trace impurities.[4] This makes them particularly useful for genotoxic impurity analysis. In combination with flame ionization detection (FID), GC is the standard choice for the analysis of volatile organic impurities, such as residual solvents. The gas chromatography headspace method is used worldwide for residual solvent analysis in quality control laboratories because it closely follows ICH Q3C guidelines. Sample preparation and introduction is through a static headspace, which facilitates the selective introduction of volatile solvents without contamination by mostly nonvolatile drug substances or drug products. Therefore, the use of an FID detector helps preferentially identify and quantify residual solvents. The combination of gas chromatography and mass spectrometry (GC/MS) can be used for confirmation and identification purposes, highlighting the flexibility of this technology.

The determination of drug-related impurities is one application of CE in pharmaceutical analysis because it achieves high separation efficiencies compared to other chromatographic techniques. CE can be used when HPLC techniques are not able to adequately measure impurities, especially very polar compounds. A reporting threshold of 0.05 % is widely accepted as a minimum requirement for a related impurities determination method, and this can be achieved using CE. In addition, CE is very useful for the separation of closely related compounds such as diastereomers and enantiomers. An example of the value of CE in impurities analysis is demonstrated using heparin (a polymeric anticoagulant). In this case, standard LC failed to distinguish drug lots associated with adverse events, while CE was easily able to identify an unknown impurity.[5]

Harmonized USP and ICH elemental impurities methods (ICH Q3D/Q2(R1) and USP/) require that an appropriate, specific, quantitative analytical procedure is used to determine the level of elemental impurities. USP specifies that an instrument-based method is used, and the reference methods use ICP-MS or ICP-OES. With both methods, sample analysis can be accomplished in three ways:

- Directly (unsolvated)
- Following sample preparation by solubilization in an aqueous or organic solvent, or
- After acid digestion using a closed-vessel microwave system ICP-OES provides parts per billion (ppb) detection limits for most regulated elements in pharmaceutical products, easily meeting the specified limits for oral dosage forms in cases where direct sample analysis or small dilution factors are appropriate.

### III. RESULTS

#### Analytical techniques

##### Titration

Origin of the titrimetric method of analysis goes back to some-where 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 as-say 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 endpoint 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.[6]

##### 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 maybe 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.

##### 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. Moreover, there is simultaneous but independent development of multiple samples and standards on each plate, leading to an increased reliability of results. HPTLC has been used to quantitate drugs as ethinyl estradiol and cyproterone, alfuzosin and tramadol and pentazocine .[7]

##### 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 . As seen this has become the principal method in USP XXVII and to a lesser extent but one of the most widely used methods also .The specificity of the HPLC method is excellent and simultaneously sufficient precision is also attainable. However, it has to be stated that the astonishing specificity, precision and accuracy are attainable only if wide-ranging system suitability tests are carried out before the HPLC analysis.

##### 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. [8]

##### Spectrophotometry

Another important group of methods which find an important place in pharmacopoeias are spectrophotometric methods based on natural UV absorption and chemical reactions . Spectrophotometry is 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 precision of these methods is also excellent. The use of UV-Vis spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years . The colorimetric methods are usually based on the following aspects:-



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

#### **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.[9]

#### **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 quantitation of drugs in pharmaceutical formulations and biological fluids. Many reviews on the application of NMR in pharmaceuticals have been published.

#### **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 electroanalytical methods are available for quantification of pharmaceuticals. An amberliteXAD-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.[10]

#### **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. Several drugs have been determined by using the CAR technique with photometric and fluorimetric detection. The usage of catalysts to accelerate analytical reactions is feasible with both reaction rate and equilibrium estimations.[9]

#### **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.

#### 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 record the changes in absorbance, electrode potential, or other physical methods

#### 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. Recently are view of HPLC with UV or MS/MS' detection is presented for the analysis of meloxicam in biological samples and pharmaceutical formulations. Liquid chromatography-electrospray ionization-mass spectrometry method for the qualitative and quantitative determination of metabolites after oral administration of Rhizome coptidis and Zuojinwan preparation in rat urine has been developed the same analytical technique was used for the simultaneous determination of L-ascorbic acid and acetylsalicylic acid in aspirin C effervescent. [10]

#### IV. CONCLUSION

The main aim of the pharmaceutical drugs is to serve the human to make them free from potential illness or prevention of the disease. For the medicine to serve its intended purpose they should be free from impurity or other interference which might harm humans. This review is aimed at focusing the role of various analytical instruments in the assay of pharmaceuticals and giving a thorough literature survey of the instrumentation involved in pharmaceutical analysis. The review also highlights the advancement of the techniques beginning from the older titrimetric method and reaching the advanced hyphenated technique stages.

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