"Advanced Analytical techniques and regulatory perspectives of impurity profiling of APIS"

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Abstract

The review gives brief introduction about process and product related impurities and emphasizes on the development of novel analytical methods for their determination. It describes application of modern analytical techniques, particularly the UPLC, LC-MS, HRMS, GC-MS and HPTLC. In addition to that the application of nuclear magnetic resonance (NMR) spectroscopy was also discussed for characterization of impurities and degradation products. The significance of quality, efficacy and safety of drug substance/products including the source of impurities, kinds of impurities; adverse effects by the presence of impurities, quality control of impurities, necessity for development of impurity profiling methods, identification of impurities and regulatory aspects were discussed. Other important aspects that were described forced degradation studies and development of stability indicating assay methods.

Keypoint's: UPLC, LC-MS, HPTLC, NMR, GC-MS, perspective of impurity

Introduction:

The pharmaceutical industry is an integral part of world's economy today. The industry has been made and will continue make a large impact on human life. One of the key areas of focus for pharmaceutical industries is R & D sector in order to develop new drug molecules. It spends ~1000 million dollars and takes 10-15 years to develop a new drug molecule [1]. During the ten years period from 2005 to 2014, the FDA approved 268 therapeutic new molecular entities. Hence, the control on the quality, safety and efficacy of these medicines is very important factor to the regulatory authorities.

The present review topic was selected based on the increasing needs of the pharmaceutical industry in developing suitable analytical methods. Among the several other available techniques, the modern analytical techniques such as
UPLC, LC-MS, LC-Q-TOF, GC- MS, HPTLC and LC-NMR were discussed.

Modern analytical techniques for pharmaceutical analysis:

- **High-Performance Liquid Chromatography (HPLC)**

  HPLC is most widely used analytical technique because it is non-destructive and applied to thermally liable compounds also (unlike GC). A large variety of unique column packing (stationary phase) and wide choice of detection techniques are available to provide a wide range of selectivity for separation. Reverse phase liquid chromatography (RPLC) is more widely

used because of its broad selectivity, reproducibility, compatibility with pharmaceutical samples, and its suitability for MS detection. In most of the cases, reversed phase (C_{18}, C_8 etc) columns and UV and PDA detectors are preferred for HPLC analysis. PDA detection also useful in checking purity of chromatographic peaks.

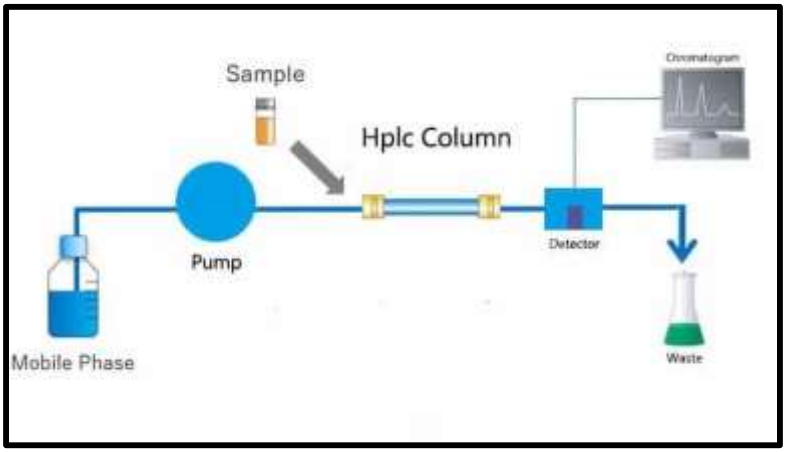

Fig. HPLC

❖ Ultra Performance liquid chromatography (UPLC)

Ultra Performance liquid chromatography (UPLC) is the successor technology to HPLC techniques since the 1970s. UPLC provides similar analytical efficiency as that of HPLC, but operates at much higher pressures. The most of HPLC columns contains particles size in between 2.5 to 5 microns. While UPLC columns was developed based upon sub 2-micron porous particles. These require a higher pressure ~15,000 psi in order to obtain high flow rates as compared to particles in HPLC column (< 6000 psi). Due to the small size of the particles, the diffusion path between the stationary phase and analytes is shorter and the efficiency is higher. Conceptually, the sensitivity of the UPLC detection has 2-3 times higher than HPLC detection, depending on the detection technique used. Due to significant advances in instrumentation and column technology were made to achieve prominent increases in speed, resolution and sensitivity in UPLC. Hence, UPLC make them ideally suited for use with mass spectrometry and it is an important driving force in today's pharmaceutical industry

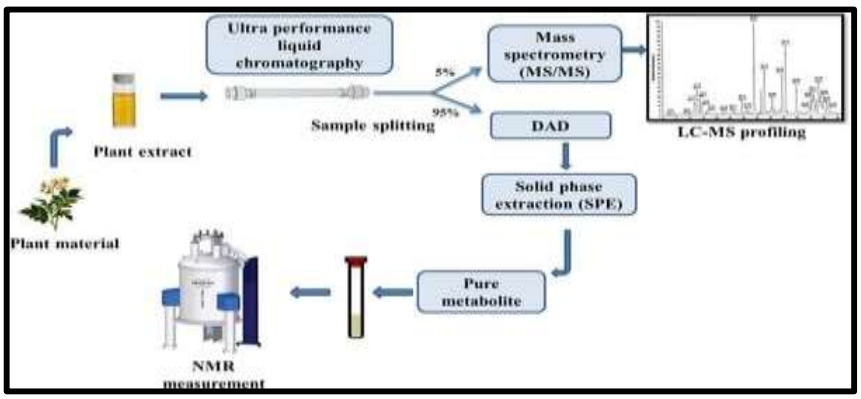

Fig: Ultra performance Liquid chromatography

❖ Liquid chromatography-mass spectrometry (LC-MS)

Liquid chromatography-mass spectrometry (LC-MS) is a powerful tool for identification and structural characterization of organic molecules in various matrices. It generates mass spectral data that can produce valuable information about the molecular weight, identity, quantity, purity and structure of a sample. It can analyze compounds that have lack of suitable chromophore, which is not possible by LC-UV/PDA for analysis. Hence, it can be considered as a universal detector for analysis of pharmaceutical samples. It can also to identify components in unresolved chromatographic peaks and reducing the need for desired separation. Thus LC-MS found a place in every drug development activity right from research to toxicology studies.

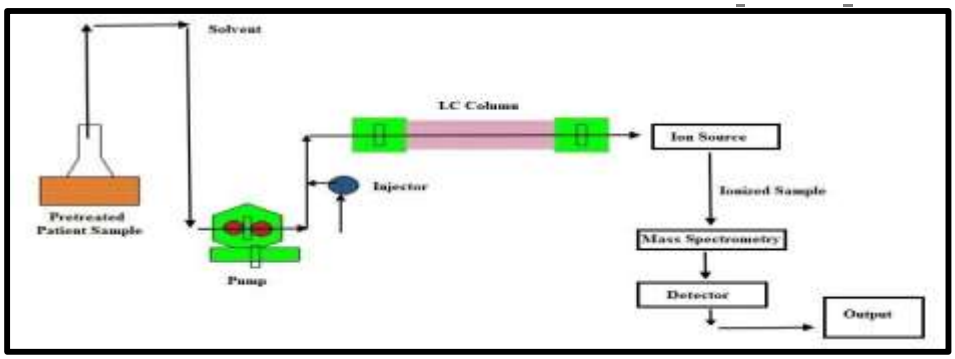

Fig: Liquid chromatography- Mass Spectrometry

❖ High-Resolution Mass Spectrometry (HRMS)

High-resolution MS (HRMS) is rapidly advancing into many fields of modern analytical sciences. It provides information related to the molecular weight, elemental composition, and molecular structure of a compound. It can also be used to perform tandem mass spectrometry (MS/MS) experiments to obtain more fragmented ions. After identification of functional groups or moieties in the fragmented ions are used to assemble for the prediction of structure of a molecule. HRMS can determine m/z values accurately up to four decimal place TOF analyzer not only provides accurate mass measurements but also establish probable molecular formula of an unknown compound. Since a given nominal mass may correspond to several molecular formulas, lists of such possibilities are especially useful when evaluating the spectrum of an unknown compound

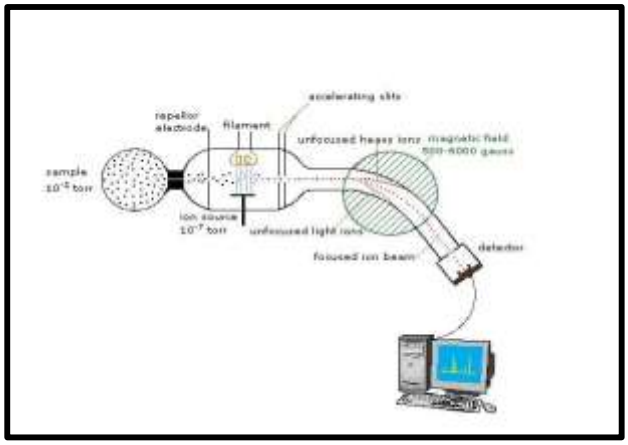

Fig: High Resolution Mass Spectrometry

❖ Gas Chromatography–Mass Spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) is a hyphenated analytical technique that is used to separate volatile, semi volatile compounds, residual solvents and thermally stable compounds. The separation mechanism depends on the column dimensions, type of carrier gas, column temperature and the chemical properties of the analyte such as vapour pressure and polarity etc. The analytes should have significant vapour pressure between 30°C and 300°C for better separation. When mixture of vaporized analytes carried through the GC column with the help of heated carrier gas, due to difference in the boiling points the separation occurs in column. Then the separated components enter into the MS through an interphase. This is followed by ionization, mass analysis and detection of m/z ratios of ions generated from each analyte taken place. Two widely used Ionization techniques in GC-MS are the electron impact ionization (EI) and chemical ionization (CI) in either positive or negative modes.

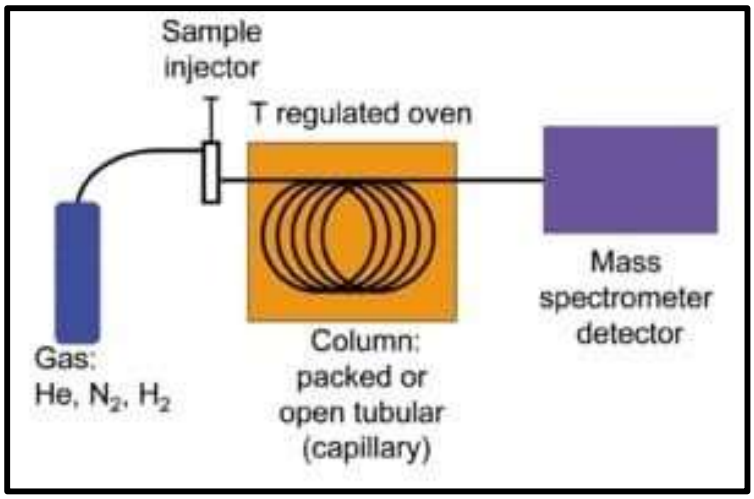

Fig : Gas Chromatography–Mass Spectrometry

The main advantage of GC-MS is that the mass spectra show a specific pattern corresponding to compounds. Therefore, the comparison of a measured spectrum to a database an effective identification method. GC-MS is also an important tool for identification, characterization of drugs and drug metabolites, stability testing, analysis of impurities in pharmaceuticals, analysis of pesticides and herbicides, quantization of pollutants in drinking and waste water, oils in creams, ointments, lotions

Nuclear magnetic resonance spectroscopy (NMR)

NMR spectroscopy exploits the magnetic properties of certain atomic nuclei. It determines the physicochemical properties of atoms or molecules in which they are contained. Structural elucidation of impurities in drug substance/product mostly involves the application of ^{1}H and ^{13}C NMR spectroscopy. In addition, the two dimensional experiments such as double quantum filtered correlation spectroscopy (DFC-COSY), and hetero nuclear single quantum coherence (HSQC) are also useful in structure elucidation of organic molecules

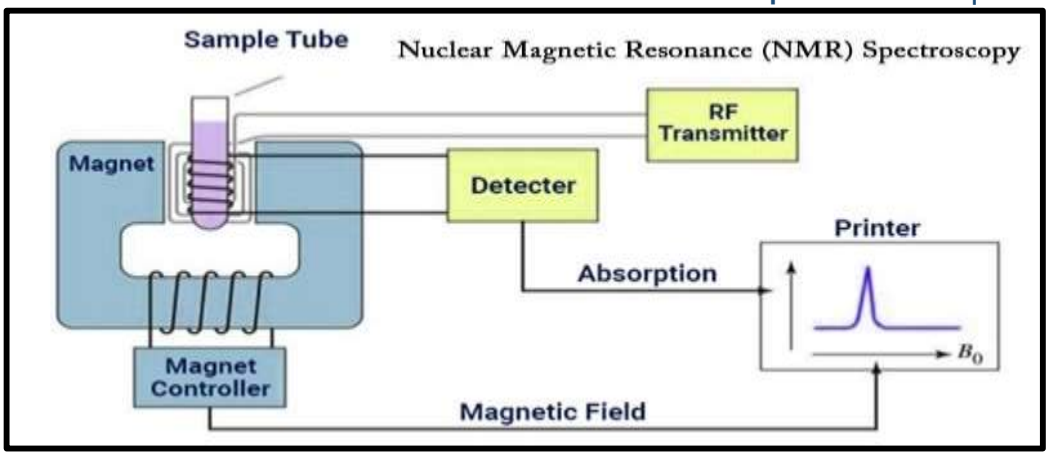

Fig : Nuclear magnetic resonance spectroscopy

Regulatory Perspective of impurity profiling of APIS

From a regulatory perspective, "impurity profiling of APIs" refers to the mandatory process of identifying, characterizing, and quantifying any unwanted substances (impurities) present in an Active Pharmaceutical Ingredient (API), which is crucial for ensuring patient safety and is strictly regulated by agencies like the FDA and ICH, requiring manufacturers to establish detailed impurity profiles for their APIs, including identification of potential impurities, their structural elucidation, and setting acceptable limits for their presence in the final drug product.

Key aspects of regulatory perspectives on impurity profiling:

1] ICH Guidelines:
The International Conference on Harmonization (ICH) has established comprehensive guidelines (Q3A, Q3B, Q3C) that outline the requirements for impurity profiling, including methods for identification, quantification, and setting appropriate limits for impurities in APIs.

2] Impurity Classification:
Regulatory agencies categorize impurities into different types like process-related impurities (from synthesis), degradation products (from storage conditions), and residual solvents (from manufacturing process), each requiring specific control measures.

3] Limit Setting:
For each identified impurity, a specific acceptable limit is set based on its potential toxicity and the available safety data, considering factors like the impurity's structure, known toxicity, and the drug's therapeutic index

4] Analytical Method Validation:
Regulatory bodies expect manufacturers to develop and validate robust analytical methods capable of detecting and quantifying impurities at their specified limits, including specificity, sensitivity, and accuracy.

5] Documentation Requirements:
Detailed documentation is necessary, including the rationale for impurity limits, analytical methods used, impurity identification data, and justification for any changes made to the impurity profile during drug development.

Importance of Impurity Profiling:
Patient Safety:
By identifying and controlling impurities, potential adverse effects related to the presence of unknown or poorly characterized contaminants can be minimized.

Quality Assurance:
Impurity profiling ensures the consistent quality of an API throughout the manufacturing process ,

DESIGNATION OF IMPURITIES

A. Common Terms of Impurities

Following terms are used by various regulatory bodies and ICH to describe the impurities

1. Intermediate
2. Penultimate intermediate
3. By-products
4. Transformation products
5. Interaction products
6. Related products
7. Degradation products

1. **Intermediate:** The compounds produced during synthesis of the desired material or as a part of the route of synthesis.
2. **Penultimate Intermediate:** It is the last compound in the synthesis chain prior to the production of the final desired compound.
3. **By-products:** The compound produced in the reaction other than the required intermediates. They can occur through a variety of side reactions, such as overreaction, incomplete reaction, demonization and rearrangement, unwanted reactions between starting materials or intermediates with chemical reagents or catalysts.
4. **Transformation Products:** They are related to theorized and nontheorized products that can occur in a reaction. They are similar to by-products except that more is known about these reaction products.
5. **Interaction Products:** These products formed either intentionally or unintentionally interaction between various chemicals involved.
6. **Related Products:** These are chemically similar to drug substance and may even possess biological activity.
7. **Degradation Products:** They are formed by the decomposition of active ingredient or other material of interest by the effect of external factors

Factors Affecting On Formulation Related Impurities

a. Environment related

I. Exposed to adverse temperature: Substance which are labile to heat or in tropical temperature lead to degradation of active constitute and formation of impurity occurs. E.g. Vitamins are heat sensitive and its degradation lead to loss in potency.

II. Exposed to light: Photosensitive material when exposed to light / UV light undergo degradation which forms impurity.

III. Humidity: It can be detrimental to bulk powder and formulation containing solid dosage form.

b. Formation of impurities on ageing: Mutual interaction: Interaction between ingredients involved in formulation leads to mutual interaction which causes impurity formation.

B. Functional Group Related Impurities

a) Ester hydrolysis: Drugs like aspirin, benzocaine, cefoxime, cocaine, ethyl paraben undergo ester hydrolysis
b) Hydrolysis: Commonly drugs like benzyl penicillin, barbital, and chloramphenicol undergo hydrolysis.
c) Oxidative degradation: Drugs like hydrocortisone, methotrexate, heterocyclic aromatic ring, nitroso/nitrile derivative.
d) Photolytic cleavage: Product exposed to light while manufacturing or storage in hospital pending use or by consumer pending use.
e) Decarboxylation: Some dissolved carboxylic acid such as p-amino salicylic acid loose CO2 when heated.

Conclusion -

This review describes role of modern analytical techniques, particularly UPLC, LC-MS, HPLC , GC-MS and HPTLC. In addition to that the application of nuclear magnetic resonance (LC-NMR) spectroscopy was also discussed for characterization of impurities and degradation products. The significance of quality, efficacy and safety of drug substance/products including the source of impurities, kinds of impurities; adverse effects by the presence of impurities in drug substance/product, control of impurities, necessity for development impurity profiling methods, identification of impurities, regulatory aspects and its quality control were discussed. Another important aspect that was described was stability studies.

And also a perspective on impurities in drug substance and drug product. Impurity profile of pharmaceuticals is receiving an increasing importance and drug safety receives more and more attention from literature. This article provides the valuable information about the impurities types and its classification, various techniques of isolation and characterization, analytical techniques for the determination, qualification of impurities and critical factors to be considered while preparation of the bulk drugs. Now a day, it is mandatory requirement in various pharmacopoeias to know the impurities present in APIs and finished

drug products. Thus impurity profiling can act as a Quality Control tool. It can provide crucial data regarding the toxicity, safety, various limits of detection and limits of quantitation of several organic and inorganic impurities, usually accompany with APIs and finished products. There is strong requirement to have unique specifications/standards with regard to impurities.

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