INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

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Review Article

Detection Of Impurities: A Review On Advance In Impurities Detection And Characterization In Pharmaceuticals By Analytical Techniques

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ARTICLE INFO

Received: 15 Dec 2023
Accepted: 19 Dec 2023
Published: 28 Dec 2023

Keywords:
Impurities, Hyphenated Techniques, Active Pharmaceutical Ingredients, Chromatography, Analytical Technique, Detection.

DOI:
10.5281/zenodo.10433875

ABSTRACT

The detection and characterization of impurities in pharmaceuticals are pivotal aspects of ensuring the safety, efficacy, and quality of drug products. This review provides a comprehensive overview of the analytical techniques employed in the identification and quantification of impurities at various stages of pharmaceutical development, manufacturing, and quality control. Chromatographic methods, including High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC), are foundational for their ability to separate complex mixtures. Mass spectrometry (MS) and Nuclear Magnetic Resonance (NMR) spectroscopy offer high sensitivity and structural insights crucial for impurity characterization. The integration of these techniques, such as in Liquid Chromatography-Mass Spectrometry (LC-MS) and Liquid Chromatography-Nuclear Magnetic Resonance (LC-NMR), provides a synergistic approach to impurity analysis. Advanced hyphenated techniques enhance specificity and accuracy, allowing for real-time monitoring and dynamic adjustments during analysis. Challenges in chiral separation, degradation product analysis, and method validation are addressed through the sophistication of these methodologies. The review underscores the significance of data analysis and validation processes, ensuring the reliability of results in compliance with stringent regulatory standards. Overall, the continuous evolution and integration of analytical techniques play a pivotal role in shaping the future of pharmaceutical research, development, and quality assurance, emphasizing the commitment to producing pharmaceuticals of the highest quality and safety standards.

INTRODUCTION

An impurity in a drug substance, as defined by the International Conference on Harmonisation (ICH) Guidelines, refers to any component present in the drug substance that is not the desired active pharmaceutical ingredient (API). Impurities can include by-products, degradation products, or other substances arising from the manufacturing

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

process or as contaminants [1]. An impurity in a drug substance is indeed any component that is not the intended chemical entity defined as the active pharmaceutical ingredient (API), and it can impact the purity of the active ingredient or drug substance [2]. Indeed, according to pharmaceutical standards and regulatory guidelines, any extraneous material present in the drug substance must be considered an impurity, regardless of its inert nature or superior pharmacological properties. This innovative approach, arising from the integration of a separation technique and a spectroscopic detection technique, is now commonly referred to as a "hyphenated technique." These techniques play a crucial role in analytical chemistry, providing enhanced capabilities for the identification and quantification of compounds in complex samples [3]. Hyphenated separation techniques involve combining two or more methods to separate chemicals from solutions and detect them. Typically, one of the techniques involved is a form of chromatography. These hyphenated techniques find extensive application in the fields of chemistry and biochemistry. The term "hyphenated" emphasizes the coupling of different analytical methods, and a slash is sometimes used instead of a hyphen, particularly if one of the methods already contains a hyphen in its name. The combinations of these techniques result in "hybrid" or "hyphenated" methods. There are numerous examples of hyphenated techniques in use today, and ongoing research and development continue to introduce new hybrid methods. Examples include gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), gas chromatography-infrared spectroscopy (GC-IR), liquid chromatography-nuclear magnetic resonance spectroscopy (LC-NMR), liquid chromatography-infrared spectroscopy (LC-IR), capillary electrophoresis-mass spectrometry (CE-MS), inductively coupled plasma-mass spectrometry (ICP-MS), and others.The synergy achieved by hyphenated techniques combines the strengths of both chromatographic and spectral methods, allowing for enhanced analysis and characterization of complex samples [4-7]. Chromatography is a separation technique that allows for the isolation of pure or nearly pure fractions of chemical components within a mixture. It exploits the differences in the distribution of components between a stationary phase and a mobile phase. This process results in distinct bands or peaks representing individual components, making it easier to isolate and analyze them. On the other hand, spectroscopy is a technique that provides selective information for identification by analyzing the interaction of molecules with electromagnetic radiation. It can yield details about the molecular structure, functional groups, and chemical bonds present in a sample. Spectroscopy is often used for identification by comparing experimental spectra with standards or library spectra, allowing for the recognition of specific compounds based on their unique spectral patterns. When combined, chromatography and spectroscopy in hyphenated techniques offer a powerful analytical approach, as they capitalize on the separation capabilities of chromatography and the information-rich nature of spectroscopy for a more comprehensive analysis of complex mixtures [8].

History

The history of impurity detection techniques traces back to classical analytical chemistry methods. In the early stages, simple chemical tests were employed to identify impurities in substances. As technology advanced, gravimetric and titrimetric methods became popular. In the mid-20th century, chromatographic techniques, such as paper chromatography and thin-layer chromatography, emerged for separating and identifying impurities. Gas Chromatography (GC) gained prominence in

the 1950s, allowing for improved separation and detection.The late 20th century saw the rise of High-Performance Liquid Chromatography (HPLC), offering enhanced sensitivity and efficiency. Mass Spectrometry (MS) integration with chromatography further improved specificity and accuracy in impurity detection. In recent decades, advancements in Nuclear Magnetic Resonance (NMR) spectroscopy, LC-MS (Liquid Chromatography-Mass Spectrometry), and other sophisticated analytical methods have provided comprehensive insights into impurity profiles. These technologies contribute to stringent quality control in industries like pharmaceuticals, ensuring the safety and efficacy of products.

Sources of Impurities

Impurities within the drug substance can originate from diverse sources and stages in the synthetic process. In the course of synthesis, intermediates and by-products may either be introduced into the drug substance as impurities or serve as a precursor for generating additional impurities. The impurities existing in the initial materials can also be transported into the drug substance. As per the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, impurities are categorized into organic impurities, inorganic impurities, and residual solvents. Organic impurities may stem from starting materials, by-products, synthetic intermediates, and degradation products. Inorganic impurities typically result from the manufacturing process and are commonly recognized and identified as reagents, ligands, inorganic salts, heavy metals, catalysts, filter aids, charcoal, etc. Residual solvents constitute impurities introduced with the solvents used in the synthesis process. Impurities can originate mainly from sources that are given below

- Starting materials and intermediates
- Impurities in the starting materials
- Reagents, ligands, and catalysts
- By-products of the synthesis
- Products of over-reaction
- Products of side reactions
- Impurities originating from degradation of the drug substance.

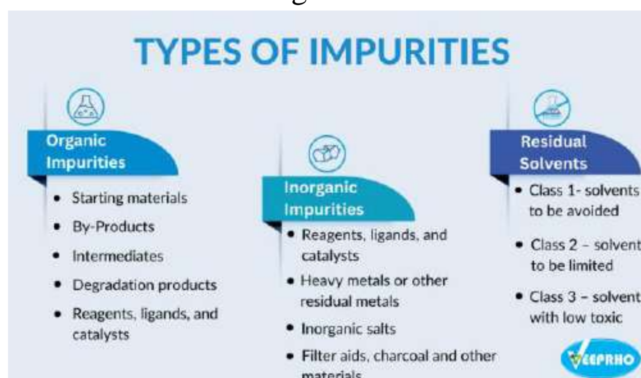

Fig 1: Types of Impurities

Need to Detect Impurities

Impurities are commonly perceived as inferior to the Active Pharmaceutical Ingredient (API) due to potential variations in pharmacologic activity. However, it's crucial to acknowledge that this assumption doesn't hold true universally. From a practical standpoint, the drug substance's purity is compromised even if it incorporates another material with superior pharmacologic or toxicologic properties. While this might not be immediately apparent, a more in-depth consideration reveals the need to assess the drug substance's purity independently of extraneous materials to ensure precise administration. As such, any extraneous material present in the drug substance or active ingredient must be deemed an impurity, irrespective of its inert nature or superior pharmacologic properties. This consideration is essential for accurately evaluating its content in the drug product. Ensuring control over low-level impurities becomes especially vital when administering drugs in substantial quantities, as exemplified by the extensive use of methotrexate (10–20 g) in treating neoplasia. Compounds such as penicillin and cephalosporin are known for facile cleavage of the β-lactam bond in aqueous solution, raising concerns about their stability.

This aspect becomes particularly intriguing in the context of penicillin allergy studies, where the lack of stability may influence potential reactions. Special attention is warranted in detecting DNA in all finished biotechnology products, as its incorporation into the human genome could pose the risk of becoming a potential oncogene. Consequently, a comprehensive understanding and control of impurities, regardless of their characteristics, are imperative for ensuring the safety and efficacy of pharmaceutical products [9].

Table 1: Analytical Method Used To Detect And Identify The Structure Of Impurity In Active Pharmaceutical Ingredients

Sr No	Drug	Impurities	Method to detect impurities	Method to identify impurities	References
1	Ezetimibe	A. 2-(4-hydroxybenzyl)-N,5-bis(4-fluorophenyl) Pentanamide B. 1-(4-fluorophenyl)-3(3-(4-fluorophenyl)propyl)- 4-(4hydroxyphenyl)azetidin-2-one	HPLC (Waters Alliance 2695 separation module)	MS, NMR, FTIR, TLC	10
2	Halobetasol propionate	A. Diflorasone diacetate B. Diflorason acetate 17 propionate C. Halobetasol D. Diflorasone acetate 21 propionate E. Diflorasone17 propionate21 mesylate F. Clobetasol propionate	UPLC (ACQUITY UPLC System)	LC–MS/MS	11
3	Icatibant	4,4(5,5(1E,1E)-3,3(4,4methylenebis(thiophene4,2-diyl))bis(2-carboxyprop-1-ene-3,1-diyl) bis(2-butyl-1H-imidazole-5,1-diyl))bis(methylene) dibenzoic acid	Agilent 1260 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped	NMR, MS-MS	12
4	IIIM-290 (preclinical candidate)	A. Rohitukine, B. IIIM-290-NO	HPLC (LC-6AD HPLC system	NMR, FTIR, and ESI–MS	13
5	Tolterodine tartrate	A. N-(3-(2-hydroxy-5 methylphenyl)- 3-phenylpropyl)-N,N-diisopropyl hydroxylammonium trifluoro acetate salt. B. 3-(2-hydroxy-5-methyl phenyl)-N-isopropyl-3- phenyl propane-1-amine oxide	HPLC (Waters ACQUITYT UPLC system)	MS, NMR	14
6	Olanzapine	A. 2-methyl-4-(4 methylpiperazin-1-yl)-10-((methylthio)methyl)-thieno[2,3-b][1,5]	HPLC(Shimadu LC20AD)	UV,FT-IR, LCMS/ TOF, NMR and X-ray	15

		benzodiazepine B. 10-(3-(1H-benzo[d]imidazol-2-yl)-5-methylthiophen-2-yl)-2-methyl-4-(4- methylpiperazin-1-yl)-thieno[2,3-b][1,5] benzodiazepine		diffraction analysis	
7	Isoproterenol hydrochloride	A. isoproterenone or (1-(3,4-dihydroxyphenyl)-2(isopropylamino) ethanone hydrochloride) B. 4-[2-(propan-2-ylamino)ethyl]benzene-1,2-diol	HPLC (Shimadu LC20AD)	UV,FT-IR, LCMS/ TOF, NMR and X-ray diffraction analysis	16
8	Meprobamate	Carbamic acid 2-carbamoyloxymethyl-2-methylpent-3-enyl ester	SFC(Waters Acquity UPC)	LC–MS	17
9	Metoprolol tartrate	C27H45NO13-adduct of lactose and Metoprolol formed by Maillard reaction	HPLC (Waters Model Alliance 2695 Separation Module)	NMR, IR	18
10	Dapoxetine	A. 1-(2E) cinnamyloxynaphthalene B. 1-(2Z)-cinnamyloxynaphthalene	TLC	IR, NMR, and MS	19
11	Rosuvastatin	A. Anti-isomer impurity: (3R,5R)-7- [4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl) amino]pyrimidin-5-yl]- 3,5-dihydroxy-6(E)-heptenoic acid. B. Lactone impurity: N-{4-(4-fluoro-phenyl)-5- [2-(4-hydroxy-6-oxo-tetrahydro-pyran-2-yl)- vinyl]-6-isopropyl-pyrimidin-2-yl}-N-methylmethanesulfonamide.	UPLC (Waters Acquity system)	UPLC-MS	20
12	Simvastatin	7-[7-(2,2-dimethyl-butyryloxy)-2,6- dimethyl-1,2,6,7,8,8a-hexahydro-naphthalen-1- yl]-3-hydroxy5-hydroxymethyl-heptanoic acid	HPLC(Waters Acquity system)	MS/MS	21
13	Amlodipine Maleate	5-ethyl-7-methyl-6-(2-chlorophenyl)-8-methyl3,4,6,7-tetrahydro-2H-1,4-benzoxazine-5,7- dicarboxylate	HPLC(Agilent 1100 series)	LC-MS/MS	22
14	Ofloxacin	A. des carboxy ofloxacin B. ofloxacin-N-oxide C. N-des methyl ofloxacin D. 9-methyl piperizine E. Edifluoro pyrido benzoxazine carboxylic acid	HPLC(Water Breeze)	NMR, IR	23

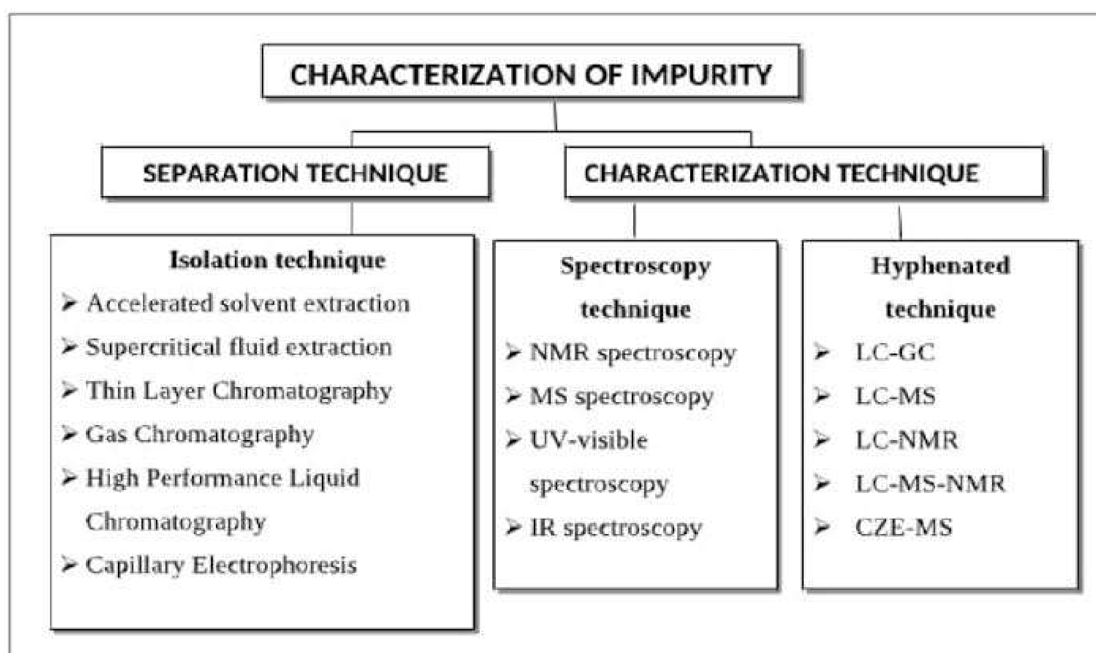

Fig 2: Characterization of Impurity

Impurity detection methods

Reliable and meaningful analytical data is needed in order to evaluate drug products at every stage of synthesis. The impurities can be identified by the following methods.

- Isolation methods.
- Separation methods.
- Characterization methods.
- Isolation method

Approximate estimations of potential impurities in a synthetic process are typically based on the assumption that the impurities would share some structural relationship with the compound of interest. Once hypothesized, suspected, or reverse-engineered impurities are synthesized, the subsequent step involves their isolation and monitoring throughout the actual synthetic process. Chromatographic techniques are commonly employed for the isolation of impurities. If instrumental methods have the capability to directly characterize impurities, it becomes possible to bypass the isolation step.

I. Extraction

- **Liquid-solid extraction:**

The selection of a solvent capable of dissolving the impurity of interest is a crucial step in the extraction process. In cases where a compound contains multiple types of impurities, an organic solvent blend is often employed for extraction. These solvents are chosen for their tendency to volatilize at low temperatures, which aids in concentrating the impurity. Common solvents utilized in liquid-solid extraction include toluene, methanol, water, cyclohexane, and others.

- **Soxhlet extraction:**

This technique is used for extracting compound of interest from crude drug products, etc, It utilizes a small volume of solvent which is repeatedly siphoned through a product to produce a concentrated extract. Natural compounds are isolated by this method, for instance isolation of Curcumin from rhizomes of Turmeric. In impurity profiling, Soxhlet extraction finds use, when the desired compound has limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent, then simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that

instead of using many portions of warm solvent being passed through the sample, just one batch of solvent is recycled [24] .

- **Liquid-liquid extraction:**

This process entails the extraction of one liquid by another, where one is aqueous and the other is organic, and both liquids are mutually immiscible [25] .

Benefits of extraction technique over another technique : The accelerated solvent extraction technique is a rapid and efficient method for extracting chemical constituents from solid samples. This technique offers faster extraction times, resulting in a more efficient process. With a reduced volume of solvent consumption, it contributes to a decrease in environmental pollution. The increased extraction yield, coupled with lower overall extraction costs, makes it a cost-effective approach. Additionally, the technique is known for its reproducibility, ensuring consistent results across multiple extractions.

Limitations:

Thermolabile compounds are vulnerable to degradation when exposed to elevated temperatures. Exposure to high temperatures can lead to the degradation of these compounds [26].

II. Thin Layer Chromatography

Thin-layer chromatography (TLC) is a widely used technique for analyzing a diverse range of organic and inorganic materials, thanks to its distinctive advantages. These include minimal sample clean-up requirements, a broad selection of mobile phases, flexibility in sample separation, high sample loading capacity, and cost-effectiveness. TLC serves as a powerful tool for screening unknown materials, especially in the context of bulk drugs [27] . TLC offers a relatively high level of confidence in separating all potential components of a drug. The technique's high specificity has been effectively harnessed for quantitative analytical purposes through spot elution followed by spectrophotometric measurement. Additionally, TLC has found application in the determination of certain steroids [28]. pioglitazone [29],celecoxib [30] and noscapine [31]. Thin-layer chromatography (TLC) assumes a pivotal role in the initial phases of drug development, especially when information regarding impurities and degradation products in the drug substance and drug product is insufficient. Numerous pharmaceutical impurities have been successfully identified and quantified using TLC [32-33]. Thin-layer chromatography (TLC) is a valuable separation technique based on the principle of adsorption. Silica gel plates are commonly preferred for conducting separations. Detection is often carried out using UV light. To elute the desired material, the adsorbent from the plates is scraped off and then extracted with suitable solvents. TLC finds diverse applications, including the determination of components present in plants. It is also utilized for analyzing the dye composition of fibers in forensics, as saying the radiochemical purity of radiopharmaceuticals, monitoring organic reactions, analyzing ceramides and fatty acids, detecting pesticides or insecticides in food and water, and identifying compounds in a given substance. As an example, the separation of the dehydro-apixaben impurity from a mixture of other compounds can be achieved using TLC. Limitations: TLC results may lack complete reproducibility due to its open structure, influenced by factors like environmental changes and analyst variation. It provides qualitative data only, with no straightforward quantification. Being a non-automated technique, manual errors can affect accuracy. Recent advancements, including high precision densitometers and automatic multiple developments (AMD), address these limitations, enabling quantitative assessments and improving method selectivity.

III. Gas chromatography:

Gas chromatography (GC) is valuable for isolating and characterizing volatile impurities or compounds that can be volatilized through derivatization. In the production of Doxorubicin hydrochloride, acetone and ethanol impurities were identified using gas chromatography. GC is a potent separation technique for detecting volatile organic compounds. Its combination of separation and on-line detection enables precise quantitative determination of complex mixtures, even detecting traces down to parts per trillion in specific cases. Gas liquid chromatography is widely used in the pharmaceutical industry for the analysis of pharmaceutical products [34]. Gas chromatography faces limitations in handling high-molecular-mass products like polypeptides or thermally unstable antibiotics. Its primary constraint lies in the non-volatility of drug substances, necessitating derivatization. Despite these challenges, recent applications of gas chromatography include drug assays, such as in the case of isotretinoin [35], cocaine [36] and employed in the determination of residual solvents in betamethasone valerate [37]. Gas chromatography serves as a crucial tool for analyzing impurities in pharmaceuticals. In recent years, GC has been employed to estimate process-related impurities in pharmaceuticals, with residual solvents listed as impurities by the International Conference of Harmonization being analyzed using various detectors in GC [38-43].

Limitations:

It cannot be practice for the study of the non-volatile and thermally unstable compound. GC is a unique separation technique where ultraviolet (UV) spectra cannot be taken. A direct injection mode of sample pre-treatment decreases the sensitivity.

IV. High Performance Liquid Chromatography

High-performance liquid chromatography (HPLC), also known as high-pressure liquid chromatography, is a column chromatography technique extensively employed in biochemistry and analytical chemistry. It separates, identifies, and quantifies compounds based on their unique polarities and interactions with the column's stationary phase. HPLC utilizes various stationary phases, typically hydrophobic saturated carbon chains, along with a pump that moves the mobile phase(s) and analyte through the column. A detector provides a characteristic retention time for the analyte, and in some cases, additional information like UV/Vis spectroscopic data. Analyte retention time depends on its interactions with the stationary phase, the solvent ratio/composition, and the mobile phase flow rate [44] High-Performance Liquid Chromatography (HPLC) stands out as an automated separation method renowned for its exceptional sensitivity, selectivity, and resolution capabilities. This swift and efficient technique is especially valuable for assessing purity and isolating impurities within pharmaceutical compounds. Reverse phase HPLC is widely utilized in scrutinizing impurities present in biological materials. The incorporation of a UV detector in HPLC, when employed for separation purposes, yields superior-quality UV spectra. The system is characterized by user-friendly operation, facilitating straightforward sample preparation and reducing potential errors in the analytical process[45].

Limitations:

In High-Performance Liquid Chromatography (HPLC), the detection of a compound relies on specific structural features, including a UV chromophore, fluorescence element, or electrochemical activity, to achieve optimal detection sensitivity. For drug substances lacking these structural elements, derivatization of the drug substance is performed to facilitate analysis [46].

V. Capillary electrophoresis (CE):

Capillary electrophoresis (CE) stands as a highly valuable technique for impurity profiling, especially when dealing with complex impurity structures that may be structurally analogous to the core drug substance. The key advantage of CE lies in its high peak efficiency, surpassing other separation techniques. CE can be operated in various modes, contributing to an enhanced separation capability [47].

Limitations:

Due to the low optical path length of the UV detector, capillary electrophoresis has a low sensitivity. There is overheating of the sample, therefore limited to the use of low voltage. In terms of instrumentation, a few options available. As compared to the liquid chromatography technique, CE is more complicated.

- **Separation method**

I. Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful and intricately theoretical analytical tool. It involves deducing the chemical environment of specific nuclei based on information obtained about them. NMR is a phenomenon exhibited by magnetic nuclei in a magnetic field when subjected to electromagnetic (EM) pulses. These pulses cause nuclei to absorb and subsequently radiate energy at a specific resonance frequency, dependent on factors like magnetic field strength. NMR phenomena are extensively utilized in scientific techniques for studying molecular physics, crystals, and non-crystalline materials through NMR spectroscopy. Additionally, NMR plays a routine role in advanced medical imaging, notably in magnetic resonance imaging (MRI). Stable nuclides with an odd number of protons and/or neutrons possess intrinsic magnetic properties and angular momentum, referred to as spin > 0, making them suitable for NMR studies. Commonly studied nuclei include H and C, though high-field NMR spectroscopy examines nuclei from isotopes of various elements. An essential feature of NMR is that the resonance frequency of a substance is directly proportional to the applied magnetic field strength. This characteristic is exploited in imaging techniques, where the resolution depends on the field gradient. Efforts are made to enhance resolution by developing more powerful magnets, often using superconductors. NMR effectiveness is further improved with hyperpolarization and advanced multi-frequency techniques, including two-dimensional, three-dimensional, and higher-dimensional approaches. Nuclear Magnetic Resonance phenomena also find application in low-field NMR, NMR spectroscopy, and MRI within Earth's magnetic field, known as Earth's field NMR [48].

Limitations:

The sensitivity is a critical problem in the effective application of NMR spectroscopy. While the unequivocal determination of trace quantity of analyte or impurities is of key significance concerning a figure of essential industrialized welfare, such as in-market approval application, quality control and quality assurance of the formulations, regulatory features and protection of the patent right. After the separation of impurity by using the regular TLC plate and analytical HPLC column, it is impossible to acquire the NMR spectra. The preparative scale separation requires isolating enough quantity of impurity for NMR spectroscopy. Another disadvantage of NMR spectroscopy is that it requires a long time to understand the spectra and the NMR instrument is very expensive.

II. Mass spectrometry (MS)

Mass spectrometry is characterized by high reproducibility, specificity, and exceptional sensitivity, making it a powerful tool for trace compound analysis and structural elucidation. The technique is particularly valuable for identifying biomolecules or protein molecules in biological samples. The study of high molecular mass, non-

volatile, and thermally susceptible compounds is made possible through the implementation of soft ionization techniques. In mass spectrometry, the parent molecule undergoes ionization, producing ions or fragments that travel to the instrument's analyzer compartment. Here, the ions are resolved based on their mass-to-charge ratio. The resulting mass spectrum provides valuable data regarding the molecular composition of the parent drug compound. Mass spectrometry is frequently coupled with various chromatographic techniques, creating a hyphenated approach that finds wide application in determining impurity structures [49].

Limitations:

The method is restricted to the differentiation of the isomers with the same molecular weight. The differentiation between the stereoisomer and positional isomers can be made possible by the prior separation of the sample by chromatographic technique followed by
mass spectrometry analysis.

III. Ultraviolet (UV)

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near infrared ranges. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV / VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration.

Limitations:

The drug structure needs to incorporate a UV chromophore to absorb UV radiation effectively. The UV cut-off is a crucial parameter when selecting a solvent for preparing a stock solution. It is essential that the absorption maxima of the drug substance and the solvent used for the stock solution preparation are not identical. This ensures proper distinction and accurate measurement in UV spectroscopy [50].

IV. Infrared (IR)

Infrared (IR) spectroscopy : The drug material will absorb a specific wavelength when exposed to electromagnetic energy, a specific bond present in the structure will absorb at a characteristic's wavelength. Thus, this technique can be active to detect the sample structure by identifying the functional group prevailing in the sample. A lot of time is consumed in the sample preparation in the chromatographic technique, even though it has many advantages such as the better resolution of the impurities even in the multi-component sample. A new advance in the impurity profiling method is the use of a spectroscopy method coupled with chemometric as a replacement for the chromatographic method. The Fourier transform infrared spectroscopy (FT-IR) technique is a fast, less expensive technique, but for separation and determination of impurity, this technique cannot be applied directly. Hence chemometric technique is requiring chiefly multivariate regression for impurity profiling. The chemometric technique seems to be crucial in the extraction of information from complex data sets. The combination of IR with chemometrics will simplify and improve the quality control method of the drug substance in the manufacturing process [51].

Limitations:

Sample preparation is very time-intense. It can't give detailed information as nuclear magnetic resonance (NMR) spectrometry. The method is destructive; the sample cannot be reused for further analysis. For detection in the IR range, require IR active sample.

❖ **Hyphenated technique**

I. LC-MS

LC-MS is a chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry .A typical

automated LC-MS system consists of double three-way diverter in-line with an Auto sampler, LC system, the Mass spectrometer. The diverter generally operates as an automatic switching valve to divert undesired portions of elute from the LC system to waste before the sample enters the MS. The ionization techniques used in LC-MS are generally soft ionization techniques that mainly display the molecular ion species with only a few fragment ions [52]. The information obtained from a single LC-MS run, is not sufficient for confirmation of identity of compound [53]. Nevertheless, this problem has now been resolved by the introduction of tandem mass spectrometry (MS-MS), which provides fragments through collision-induced dissociation of the molecular ions produced. Use of LC-MS-MS is increasing speedily day by day [54]. Hyphenated techniques, such as High-Performance Liquid Chromatography (HPLC) coupled with UV and mass spectrometry (LC-UV-MS), have proven to be exceptionally valuable. This combination, when used alongside biological screening, allows for a rapid survey of natural products.

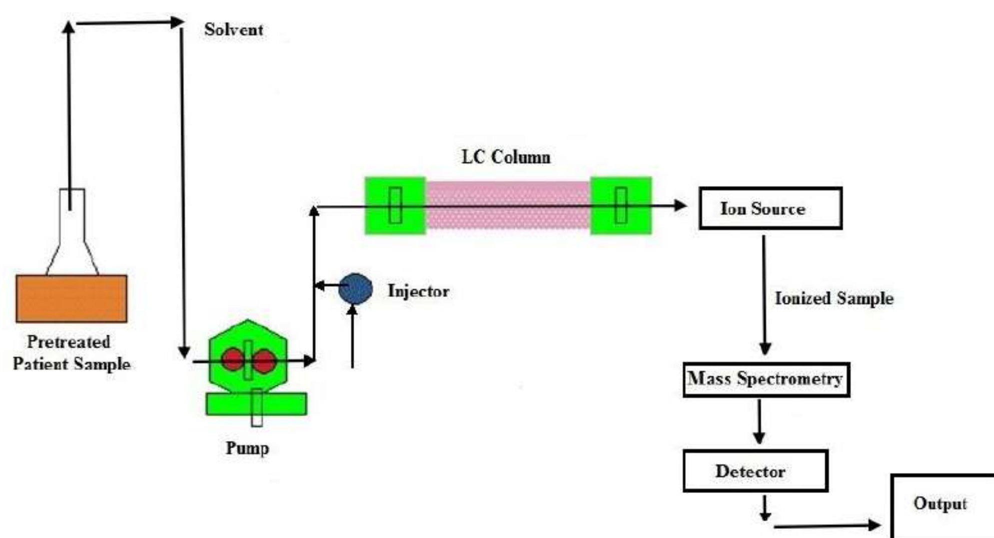

Fig 3: LC-MS

Limitations:

The first-generation instrument employs a soft ionization method, providing molecular mass records only. In LC-MS, there's a recurring issue of excipient interference. The drug product in solution form often contaminates the capillary tip. Confirming the final structure of the compound may be incomplete without an NMR spectroscopy study.Optimizing the method involves considerations such as mobile phase composition, flow rate, and additives like buffers or ion pairs. Additionally, factors related to ionization techniques, such as spray voltage, nebulizer temperature, cone voltage in MS, gas nature, and pressure, play crucial roles and need careful consideration for successful implementation.

II. LC-GC

LC-GC, a powerful practice, combines the wide separation mechanism of Liquid Chromatography (LC) with the high efficiency of Gas Chromatography (GC). It is particularly suitable for pure samples demanding high sensitivity and selectivity. LC-GC excels in exploiting high sample capacity and is extensively used in offline mode. However, operating in offline mode does present drawbacks, including time-consuming analyses, exhaustive operational requirements, and lower reproducibility. These factors should be considered when choosing the analytical approach [55].

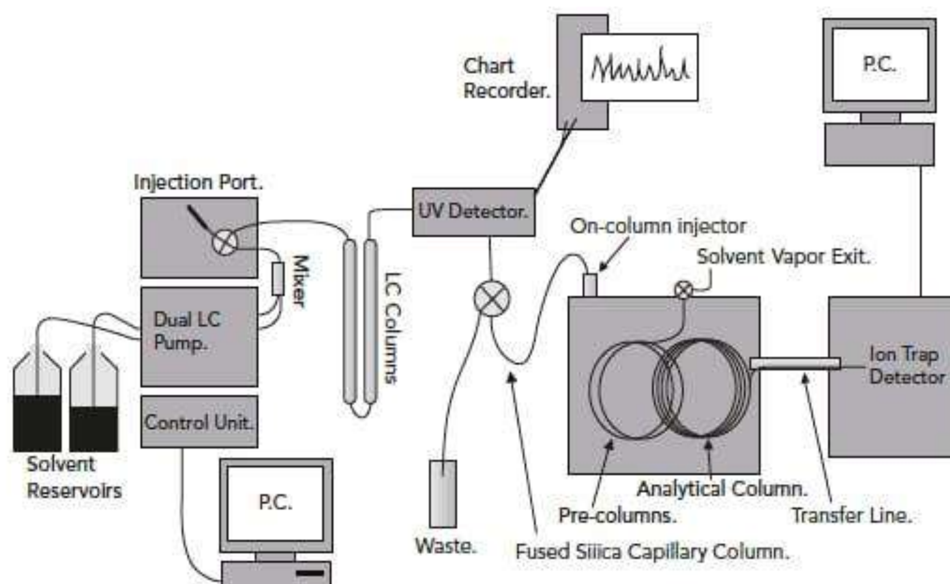

Fig 4: LC-GC

Limitations:

LC-GC technique requires a complex instrument that used at different interface. Require trained users for careful optimization of various parameters.

III. LC-NMR

Among the available spectroscopic techniques, NMR is considered one of the least sensitive, yet it provides highly valuable structural information. The online combination of High-Performance Liquid Chromatography (HPLC) and NMR presents a unique opportunity for swiftly gathering detailed structural data from samples, surpassing other hyphenated techniques. The direct coupling of liquid chromatography to NMR using the stop-flow method was first reported in 1978. [56-57]. LC-NMR experiments can be conducted in both continuous-flow and stop-flow modes, addressing a variety of bioanalytical challenges. Systems with varying field strengths, such as 500, 600, and 800 MHz, equipped with 1H, 13C, 2H, 19F, and 31P probes, are employed. Key requirements for on-line LC-NMR, in addition to NMR and HPLC instrumentation, include a continuous-flow probe and a valve positioned before the probe. This setup allows for the recording of either continuous-flow or stopped-flow NMR spectra. [58]. A UV-VIS detector serves as a primary detector for LC operations. Magnetic field strengths exceeding 9.4 T are recommended, with a 1H resonance frequency of 400 MHz for standard HPLC-NMR coupling. Ongoing technical advancements are expected to significantly enhance NMR sensitivity in the future. These advancements involve higher magnetic field strengths and observation frequencies. Additionally, developments in NMR probes and preamplifiers cooled with cryogenic liquids are anticipated to offer lower detection limits and higher sensitivities, surpassing the benefits arising solely from an increase in magnetic field strength [59-61].

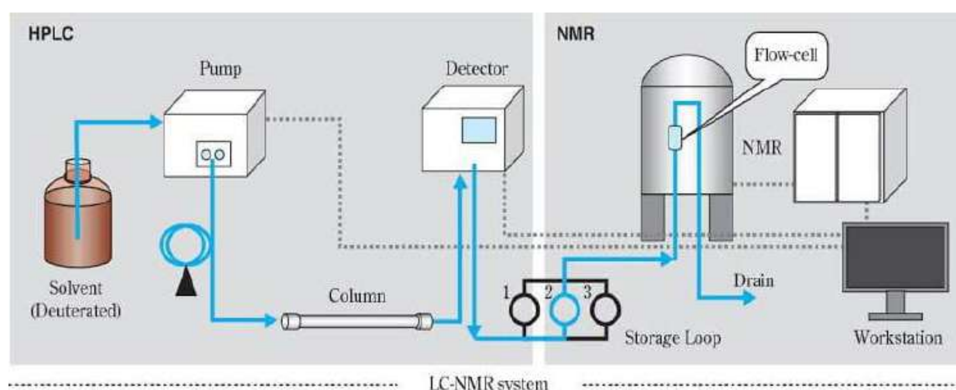

Fig 5: LC-NMR

Limitations:

The sensitivity issues of the NMR are the main limitation in the application of LC-NMR [62]. In LC-NMR, the protonated solvent cannot be used as a mobile phase for HPLC. The protonated solvent shows a resonance signal that dominates 1H-NMR spectra and swamps the moderately weak signal from a small amount of analyte. Thus, to compensate for this issue deuterated solvent can be used, but it is cost expensive, result in peak broadening and Rt shift may be observed. Need solvent suppression technique, for using the deuterated solvent, this result in suppression of analyte proton signals near the suppression solvent line and result in damage of spectral information [63].

IV. CZE-MS

The main reason to couple the capillary zone electrophoresis with the mass spectroscopy technique is that the ideal separation power of the capillary electrophoresis (CE) has been achieved whereas; mass spectra will provide sufficient structural information. There should be a high grade of orthogonality between the methods during the separation of impurity, to accomplish better resolution of separated impurities [64-69].Different techniques for impurity profiling, include coupling of CE with varying MS ionization systems such as electrospray ionization (ESI-MS), atmospheric pressure chemical ionization (APCI-MS), atmospheric pressure photoionization (APPI-MS) and thermos pray ionization (TSI-MS). The ESI-MS and TSI-MS are useful for detecting the ionic compound whereas; the APCI-MS and APPI-MS are not capable of detecting the ionic sample [70-76]. Accordingly, it can be also aid to differentiate between the ionic or non-ionic unknown impurity.

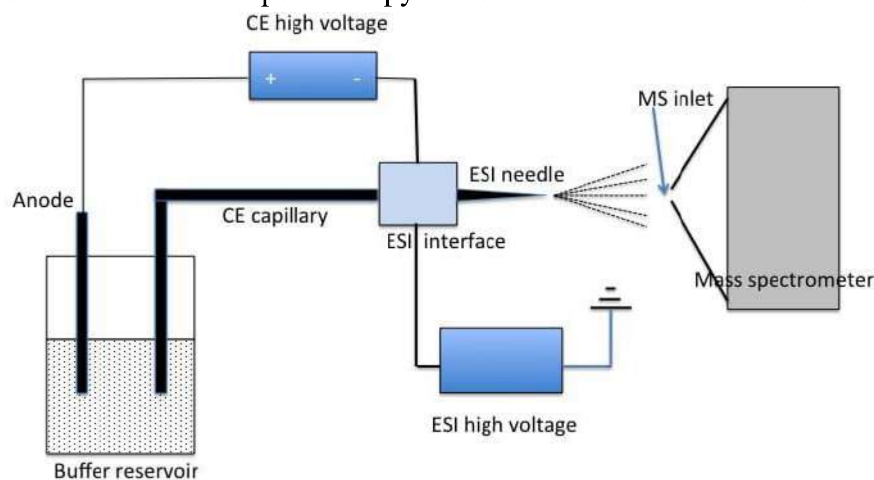

Fig 6: CZE-MS

Limitations:

Capillary electrophoresis is a very complicated technique since it requires the optimization of all the parameters that have an impact on the separation mechanism. The less sensitive technique, therefore cannot detect the trace quantity of impurity.

Applications of Impurity Detection Technique

1. **High-Performance Liquid Chromatography (HPLC):**

Widely used for separating, identifying, and quantifying impurities in pharmaceuticals. HPLC is versatile and can handle a wide range of compounds.

2. **Gas Chromatography (GC):**

Particularly effective for volatile and thermally stable compounds. GC is suitable for analyzing impurities in pharmaceuticals that can be vaporized without decomposition.

3. **Mass Spectrometry (MS):**

Coupling mass spectrometry with chromatographic techniques (such as LC-MS or GC-MS) enables the identification and quantification of impurities based on their mass-to-charge ratios.

4. **Nuclear Magnetic Resonance (NMR) Spectroscopy:**

Provides structural information about impurities, aiding in their identification. It's especially valuable for elucidating the chemical structure of organic compounds.

5. **Infrared (IR) Spectroscopy:**

Useful for identifying functional groups in pharmaceuticals, helping to detect impurities based on their characteristic IR spectra.

6. **Ultraviolet-Visible (UV-Vis) Spectroscopy:**

Applied for quantifying impurities with distinct absorbance patterns in the UV-Vis range.

7. **Capillary Electrophoresis (CE):**

Suitable for separating and analyzing charged molecules, making it valuable for detecting impurities in ionic compounds.

8. **Thin-Layer Chromatography (TLC):**

Simple and cost-effective, TLC is used for qualitative analysis and can be a preliminary step in impurity detection.

9. **Identification of Impurities:**

Hyphenated Technique is highly effective in identifying impurities in complex pharmaceutical samples. The combination of liquid chromatography for separation and mass spectrometry for detection allows for the determination of molecular weights and structural information, aiding in impurity identification.

10. **Quantification of Impurities:**

Hyphenated technique facilitates accurate quantification of impurities present in pharmaceutical formulations. The technique can provide precise measurements of impurity levels, helping ensure compliance with regulatory standards.

11. **Characterization of Metabolites:**

In drug metabolism studies, Hyphenated Technique is employed to identify and characterize metabolites formed in the body. Understanding the metabolic profile is essential for assessing the safety and efficacy of pharmaceutical compounds.

12. **Chiral Separation:**

Hyphenated technique can be utilized for chiral separation of enantiomers, helping to identify and quantify chiral impurities in pharmaceuticals. This is crucial as the biological activity of enantiomers can differ significantly.

13. **High Sensitivity and Selectivity:**

Hyphenated Technique offers high sensitivity, allowing the detection of impurities at low concentrations. Additionally, the selectivity of mass spectrometry helps in distinguishing closely related compounds.

14. **Analysis of Degradation Products:**

Hyphenated Technique is valuable for studying the degradation products of pharmaceuticals under various conditions. This aids in understanding the

stability of drug formulations and identifying potential impurities arising from degradation pathways.

15. Method Validation:

Hyphenated Technique methods used for impurity detection can be validated according to regulatory guidelines. This ensures the reliability and accuracy of the analytical results, supporting the quality control processes in pharmaceutical manufacturing.

16. Real-time Monitoring:

Hyphenated Technique enables real-time monitoring of chromatographic peaks, allowing for dynamic adjustments and optimization of analytical conditions. This is particularly useful for detecting impurities during method development and sample analysis.

Significance of Impurities Detection and Characterization

1. **Safety and Efficacy:**

Impurities can affect the safety and efficacy of pharmaceutical products. Identifying and understanding these impurities helps ensure that the final drug product is of high quality and meets regulatory standards.

2. **Regulatory Compliance:**

Regulatory authorities, such as the FDA, require thorough analysis of impurities in pharmaceuticals. Meeting these regulatory standards is essential for obtaining approval to market and sell drugs.

3. **Patient Safety:**

Impurities can pose risks to patient safety. Detecting and characterizing these substances enable manufacturers to mitigate potential health hazards associated with the consumption of contaminated or impure drugs.

4. **Quality Control:**

Continuous monitoring and control of impurities during the manufacturing process are essential for maintaining consistent product quality. This is critical for pharmaceutical companies to produce reliable and effective medications.

5. **Stability and Shelf Life:**

Impurities can impact the stability and shelf life of pharmaceutical products. Understanding the types and levels of impurities helps in designing proper storage conditions and determining product expiration dates.

6. **Analytical Method Development:**

Detecting impurities challenges analytical techniques and methods. Developing robust analytical methods for impurity analysis is essential for accurate and reliable results.

7. **Process Optimization:**

Identifying impurities can provide insights into the manufacturing process. This information can be used to optimize production methods, reduce impurity levels, and enhance overall efficiency.

8. **Product Development:**

In the early stages of drug development, characterizing impurities can aid in refining the drug formulation and production processes, contributing to the eventual success of the pharmaceutical product.

CONCLUSION

The diverse range of analytical methods, including chromatographic, spectroscopic, and mass spectrometric techniques, enables a comprehensive approach to impurity analysis. The application of these techniques allows for the identification, quantification, and characterization of impurities throughout the various stages of drug development, manufacturing, and quality control. High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Liquid Chromatography-Mass Spectrometry (LC-MS), Nuclear Magnetic Resonance (NMR) Spectroscopy, and others provide a toolkit for pharmaceutical scientists to address the complexity of impurity profiles in drug substances and formulations. The ability to detect impurities at trace levels, coupled with advancements in

hyphenated techniques, enhances the precision and sensitivity of analytical methods. This is crucial for meeting stringent regulatory requirements imposed by health authorities globally. The focus on real-time monitoring, method validation, and chiral separation further highlights the sophistication of these techniques in addressing the evolving challenges in pharmaceutical impurity analysis. Overall, the continuous advancement and integration of analytical techniques contribute significantly to the pharmaceutical industry's ability to produce high-quality medications. The diligence in impurity detection not only safeguard patient well-being by minimizing potential health risks but also ensures compliance with regulatory standards, fostering trust in the pharmaceutical sector. As technology continues to evolve, these analytical techniques will undoubtedly play a pivotal role in shaping the future of pharmaceutical development and quality assurance.

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HOW TO CITE: Sagar Pagade*, Sahil Agrawal, Prathmesh Shejal, Pranjal Chougule, Nilesh Chougule, Detection Of Impurities: A Review On Advance In Impurities Detection And Characterization In Pharmaceuticals By Analytical Techniques, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 12, 860-879. https://doi.org/10.5281/zenodo.10433875