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REVIEW ARTICLE

Impurity profiling Techniques for Pharmaceuticals – A Review

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ABSTRACT

Impurity profiling is essential for ensuring the quality, efficacy and safety of pharmaceutical products. This review discusses various aspects of impurity profiling, including sources of impurities, separation techniques, and analytical methods used for identification and characterization. Common sources of impurities include starting materials, side reactions, degradation products, and manufacturing processes. Separation techniques like solid phase extraction, liquid-liquid extraction, and chromatographic methods are used to isolate impurities. Analytical techniques such as UV-VIS spectroscopy, NMR, mass spectrometry, and hyphenated techniques like LC-MS and GC-MS are employed for structural elucidation of impurities. The review highlights the advantages of hyphenated techniques in providing comprehensive impurity profiles. Regulatory guidelines require identification and control of impurities above specified thresholds. Acceptance criteria for impurities are established based on stability studies, manufacturing processes, and batch data. The review emphasizes the importance of impurity profiling in drug development and quality control. Advanced analytical methods enable detection of trace impurities and provide structural information crucial for assessing their impact on drug safety and efficacy. Overall, impurity profiling plays a vital role in pharmaceutical analysis and regulatory compliance.

KEYWORDS: API, Excipients, Impurity, HPLC, ICH, Pharmaceutical Product.

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INTRODUCTION

The product includes active pharmaceutical ingredients (API) and excipients. Upon absorption into the body's systemic circulation, the API generates pharmacological effects. Under certain circumstances, the active ingredient or other substances may not be entirely pure and could contain additional components from different sources like synthesis, excipients, residual solvents, or degradation products. Unwanted components, aside from API and excipients, are often known as impurities. Various definitions for impurity and impurity profiling have been clearly outlined in multiple reviews and guidelines. Impurities are substances generated during the synthesis process, either as intermediates or as byproducts of intermediate reactions or undesired chemical reactions. [1]

Identifying and characterizing impurities in pharmaceutical products or pharmacological substances is known as impurity profiling, which involves utilizing appropriate analytical procedures. Identifying and isolating unknown impurities is a systematic process aimed at determining their structure. This method is essential for identifying and measuring contaminants in the therapeutic material.

Identifying and describing impurities is essential to verify their compliance with safety standards and prevent potential harm to human health. It is crucial to synthesize top-notch medicinal products, highlighting the growing significance of impurity reporting. Impurity profiling requires highly sensitive, selective, and efficient analytical methods to monitor and control trace impurities effectively. There is an increasing demand for a technique that is extremely accurate, precise, and sensitive for profiling impurities in the current era. Impurities may be present in minute quantities in drug substances, posing challenges in their isolation and identification with less sensitive and accurate methods. Moreover,

impurities often arise as derivatives or degradation products of the parent drug molecule. Thus, employing hyphenated analytical methods is essential for impurity detection. Various contemporary techniques are available for analyzing impurities to aid in their identification, characterization, and structural comprehension.

Impurities in pharmaceutical products can affect the dissolution and solubility of drug components, potentially influencing their systemic circulation. Thus, it affects patient safety and alters the biopharmaceutical behavior of the drug substance. Impurity profiling is essential for maintaining the quality, efficacy, and safety of pharmaceutical products.

Prior to characterizing the impurity with spectroscopic techniques, it is essential to separate a suitable quantity of the impurity using proper methods. As per the guidelines established by the International Council for Harmonization (ICH), impurities above 0.1% need to be identified and characterized. Various techniques exist for analyzing impurities and elucidating their structure in various active pharmaceutical ingredients (APIs) [1].

CLASSIFICATION OF IMPURITIES

Organic Impurities

Organic impurities may develop during the synthesis process or storage of drug substances. When synthesizing a drug substance, various chemical reactions come into play, and impurities from the raw materials can impact the purity of the final product. Residual solvents are organic volatile impurities found in drug substances or excipients used in drug formulations. Moreover, impurities from the intermediate or starting material might remain in the drug substance if not effectively eliminated during the various synthesis steps. At times, impurities can form during bulk drug manufacturing due to the degradation of final products. These impurities may stem from various factors such as storage, synthesis process, and drug product formulation [2].

Inorganic Impurities

Inorganic impurities are commonly identified and measured through pharmacopeia or suitable standards and can originate from excipients and production methods. Impurities are acquired in drug substances and products during the manufacturing process.

The prevalent types of inorganic impurities include:

Chemical substances used in reactions

Residual metals such as heavy metals

Inorganic salts

Filter aids, charcoal, and other substances

Residual Solvents:

The volatile organic chemicals found in pharmaceuticals are residual solvents, which are substances that are potentially undesirable. They exist in minimal quantities and must be eliminated. Even small amounts of unwanted chemicals can impact the effectiveness and safety of pharmaceutical products. Residual solvents can also alter the properties of drug substances such as crystalline structure, dissolution rate, and colour. Gas chromatography is utilized to identify residual solvents in a sample. Impurities exceeding 0.1% must be identified and quantified using specific methods to decrease their levels to an acceptable range.

The remaining solvents, according to the ICH guidelines, are:

Class 1: Due to their intolerable toxicity, class 1 solvents should not be used in the production of any drug substances, and their use should be strictly limited. Benzene, 1,2-dichloroethane, 1,1-dichloroethene, 1,1,1 trichloroethane, carbon tetrachloride, and a few other solvents.

Class 2: Due to their inherent toxicity, pharmaceutical products should limit the use of solvents.

Class 3: solvents are less dangerous to humans because of their low toxicity potential. There is no need to provide an explanation for the acceptable daily maximum of 50 mg of residual solvents, which is equivalent to 5000 ppm or 0.5% under option I. The manufacturer is responsible for providing adequate toxicological data to support the levels of solvents used in pharmaceutical products.

Sources of impurities

Impurities in starting materials and intermediates

Drug substances are built from chemical building blocks. Unreacted starting materials and intermediates, especially those in the final steps of synthesis, may survive the synthetic and purification process and appear as impurities later. In the final step of Tipranavir synthesis, aniline is the intermediate. Because aniline and the final product have similar structures, it is difficult to completely remove it during purification. In the drug, it's around 0.1% [2].

Synthesis side products

All chemical reactions are not 100% selective; drug synthesis often involves side reactions. Side reaction byproducts are common drug process impurities. Side reactions like incomplete reaction, overreaction, isomerization, dimerization, rearrangement, or unwanted reactions of starting materials or intermediates with chemical reagents or catalysts can produce byproducts [2].

Over-reaction products

In many cases, the synthesis's previous steps are not selective enough, so the reagents attack the intermediate somewhere else. Decannulation of the 17-OH group concludes the synthesis of nandrolonedecanoate. During overreaction, the reagents attack the 4-ene-3 oxo group, resulting in an enol ester impurity (3, 17β-dihydroxyestra-3, 5-diene disdecanoate) [2].

Side-effect products

Synthetic chemists are familiar with some of the common side reactions that cause trace-level impurities, but impurity profiling must identify others. Peptide synthesis side reactions often form diketopiperazine derivatives [2].

DRUG SUBSTANCE DEGRADATION

Degradation of the end product during bulk drug manufacturing can cause impurities. Common impurities in medicines are degradation products from storage, dosage form formulation, or aging. ICH defines degradation product as a molecule caused by chemical changes in the substance due to time, light, temperature, pH, water, or reaction with the excipient and/or intermediate container closure system [2].

Enantiomeric impurities

The majority of therapeutic chiral drugs used as pure enantiomers are natural products. The high enantioselectivity of their biosynthesis precludes enantiomeric impurities. Synthetic racemates, which are usually sold, are impurities if the pure enantiomer is given. Its presence may be due to incomplete syntheses enantioselectivity or racemate enantiomer resolution. ICH guidelines exclude enantiomeric impurities, but pharmacopeias consider them normal. A single enantiomeric form of the chiral drug may have a better pharmacological profile, therapeutic index, and reaction profile. The pharmacokinetic profiles of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are similar, suggesting that a single isomer has no advantages. Levofloxacin, levalbuterol, and esomeprazole are popular single isomer drugs [2].

Reagents, ligands, and catalysts: APIs rarely contain these chemicals, but they may be impurities. Drug synthesis reagents, ligands, and catalysts can be trace impurities in final products.

Impurities in pharmaceutical drug substances

Impurities are foreign particles that reduce a substance's purity.

Many pharmaceuticals contain various impurities.

A foreign particle that causes harmful or toxic reactions in excess. Lead, heavy metals, arsenic, etc.

Chemical activity is reduced by impurities, which may not be toxic. Hard soap with too much water.

Impurities that make the active ingredient incompatible or reduce its properties [2]

Sources of Impurities in Pharmaceuticals

Many factors affect the types and amounts of impurities in chemicals and pharmaceuticals [2], including:

i. Raw materials utilized in production
ii. Chemicals utilized in the production process
iii. Manufacturing method/process
iv. Chemical processes utilized in manufacturing
v. Pollution in the atmosphere caused by the manufacturing process.
vi. Intermediate goods during production
vii. Manufacturing defects
viii. Hazards in manufacturing

a) Particulate contamination refers to the presence of impurities such as dirt, dust, glass, porcelain, or plastic fragments in a product.

A. Active ingredients in the product and their pharmacopeial limits can impact the manufacturing process and lead to process errors.
B. Cross-contamination can occur when powders, granules, and tablets are handled in bulk, generating airborne dust that can contaminate the product.
C. Microbial contamination is a common issue for liquid preparations and creams, making them vulnerable to bacterial and fungal growth.
D. Packing errors such as incorrect labelling or disposal of excess labels can pose a packing hazard.
E. Insufficient storage conditions

ix. Product decomposition during storage.
x. Unintentional replacement or intentional contamination with counterfeit or ineffective substances.

Separation Techniques

Solid Phase Extraction Technique

Solid phase extraction (SPE) is a technique that is becoming more and more beneficial for sample preparation. SPE can prevent numerous issues associated with liquid-liquid extraction, including the use of expensive, breakable specialty glassware, the disposal of large volumes of organic solvents, less-than-quantitative recoveries, and incomplete phase separation. SPE is more efficient than liquid extraction, as it is rapid, automated, and produces quantitative extractions that are simple to perform. Laboratory time and solvent consumption are diminished. SPE is frequently employed to prepare liquid samples and extract semi-volatile or non-volatile analytes. However, it can also be employed with solids that have been pre-extracted into solvents. SPE products are exceptional for the extraction, concentration, and cleaning of samples. They are available in a diverse range of diameters, chemistries, and adsorbents. It is crucial to choose the most appropriate product for each application and sample [3].

Liquid – Liquid Extraction Methods

Liquid–liquid extraction, which is also called solvent extraction and partitioning, is a way to separate chemicals based on how well they dissolve in two different liquids that don't mix, usually water and an organic solvent. It takes something out of one liquid phase and puts it into another liquid phase. A separating funnel is used in chemical labs to do liquid–liquid extraction, which is a basic method. As part of the workup, this kind of process is often done after a chemical reaction [3].

Accelerated Solvent Extraction Technique

The accelerated solvent extraction technique is a specialized method that relies on the use of solvents to extract chemically active components from solid matrices by penetrating their pores. It has been widely used in different pharmaceutical sectors. Commonly used for extracting natural chemical constituents from herbal plant materials. The application is not limited to extracting natural constituents, but it is also used for profiling impurities in drug substances. This method has been widely used to screen microorganisms, dietary supplements, insecticide residue, examine environmental samples, and detect organic contaminants. This technique offers numerous advantages compared to conventional methods such as soxhlet extraction, maceration, purification, turbo-extraction, and sonication.

This technique employs high temperatures and pressures to enhance the extraction process. Increasing the temperature will speed up the extraction process, resulting in a lower viscosity of the sample. This, in turn, promotes the movement of the liquid into the sample. Under high pressure, the solvent will be pushed into the pores of the sample medium, making the extraction process more efficient [4].

Supercritical Fluid (SCF)

Supercritical fluid chromatography (SFC) is a technique that utilizes a supercritical fluid as the mobile phase, which is generated when a chemical is heated above its critical temperature. SFC is a hybrid technique that integrates the superior features of both gas and liquid chromatography. Supercritical fluid chromatography fundamentally relies on the density of the supercritical fluid, which correlates with its solvating capacity. As system pressure rises, the density of the supercritical fluid increases, hence enhancing its solvating capacity. Consequently, the components retained in the column are eluted. Supercritical fluid chromatography (SFC) has advanced significantly in recent years, especially in the domain of enantiomer separations [4].

Thin layer Chromatography

Thin layer chromatography (TLC) is a chromatographic method employed for the separation of mixtures. Thin layer chromatography is conducted on a substrate of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, typically silica gel, aluminium oxide, or cellulose. The adsorbent layer is referred to as the stationary phase. Upon application of the sample onto the plate, a solvent or solvent combination, referred to as the mobile phase, ascends the plate through capillary action. Separation is accomplished as various analyte migrate up the TLC plate at distinct rates. Thin layer chromatography is often utilized to identify the constituents present in plants. It is utilized for monitoring organic reactions, analysing ceramides and fatty acids, detecting pesticides or insecticides in food and water, examining the dye composition of fibers in forensics, identifying compounds in substances, and assessing the radiochemical purity of radiopharmaceuticals [Figure 4]. Several improvements can be implemented to the original method to automate various procedures; better the resolution attained with TLC, and facilitate more precise quantification. This technique is known as HPTLC, or 'high-performance thin-layer chromatography [5].

Gas chromatography

Gas–liquid chromatography (GLC), often referred to as gas chromatography (GC), is the most common type of chromatography employed in analytical chemistry for the separation as well as analysis of compounds that can be vaporized without undergoing decomposition. Common applications of gas chromatography (GC) encompass assessing the purity of a specific substance and isolating the various constituents of a mixture, including the quantification of their relative proportions. In certain circumstances, gas chromatography may assist in the identification of a compound. In preparative chromatography, gas chromatography (GC) can be employed to isolate pure compounds from a mixture [5].

HPLC

The High-Performance Liquid Chromatography (HPLC) is an automated separation technique characterized by improved sensitivity, selectivity, and resolution capabilities. The technique is rapid and efficient for isolating contaminants from the pharmaceutical substance and evaluating its purity. Reverse phase HPLC technique is extensively employed for the identification of pollutants in biological materials. A UV detector can provide high-quality UV spectra when high-performance liquid chromatography (HPLC) is employed as the separation technique. This approach facilitates straightforward sample preparation and minimizes errors [5].

Capillary Electrophoresis

Capillary electrophoresis is an effective technique for impurity profiling. The primary advantage of CE compared to other separation techniques is its superior peak efficiency. The separation capability enhances since CE can be utilized in multiple applications. The identical capillary can analyse analyte varying in size from minuscule ions to larger protein molecules, resulting in enhanced separation efficiency. Most extraction methods utilize an organic solvent; however, capillary electrophoresis is the sole technique that predominantly employs an aqueous buffer. The global deficiency of organic solvent, especially acetonitrile, can be rectified. The implementation of circular economy practices reduces waste production and is ecologically sustainable. Sample preparations must occur within a capillary for analyte derivatization procedures [6].

UV-VIS spectroscopy

In the absence of chromatographic separation, the application of UV-VIS spectroscopy as a method for the detection and structure elucidation of impurities in pharmaceuticals is of very little significance. All contaminants that absorb specifically in the ultraviolet area above 200 nm are the only ones that can benefit from this approach. Identifying pure drug compounds can be accomplished with the help of a technology known as UV-Visible. Substances that include chromophores have the ability to absorb a certain wavelength of ultraviolet or visible light, and this absorption is directly proportional to the concentration of the sample [6].

NMR

NMR's capability to furnish insights into the unique bonding structure and stereochemistry of pharmaceutical compounds has rendered it a potent analytical tool for structural elucidation. The capability of NMR-based diffusion coefficient determination to differentiate between monomeric and dimeric compounds was confirmed using a standard combination of real materials comprising both monomers and dimers. Regrettably, NMR has conventionally been regarded as a less sensitive tool in comparison to other analytical methods. Standard sample needs for NMR are approximately 10 mg, whereas MS necessitates less than 1 mg [7].

Mass spectrometry

Mass spectrometry (MS) is an analytical technique for describing matter through the measurement of atomic or molecular masses of distinct species. Existent in a specimen. This technique obliterates the compound; yet, due to the significant sensitivity only a minimal quantity is required for the process.

Mass spectrometry is an exceptionally sensitive technique for analysing trace molecules and elucidating their structure, providing outstanding consistency. Specificity and sensitivity. Mass spectrometry can be utilized to identify any biomolecules or proteinaceous compounds present in a biological specimen, and the application of a soft ionization method can be utilized to analyse high molecular weight, non-volatile substances thermally sensitive compounds. Ionization of the original molecule yields ions or fragments, which are subsequently conveyed to the analyser section of the instrument and determined according to their mass-to-charge ratio. Details regarding the parent pharmaceutical compound the molecular composition will be derived from the mass Spectrum. MS is integrated with several chromatographic techniques. Techniques. This hyphenated approach is often employed to ascertain the composition of contaminants [7].

FT-IR technique

Infrared spectroscopy is a branch of spectroscopy that focuses on the infrared part of the electromagnetic spectrum. It encompasses various approaches, with the predominant method being a type of absorption spectroscopy. Like all spectroscopic techniques, it can be utilized to detect chemicals and analyse sample compositions. An infrared spectrophotometer is a prevalent laboratory tool that employs this technique. The infrared segment of the electromagnetic spectrum is typically categorized into three regions: near-infrared, mid-infrared, and far-infrared, designated based on their proximity to the visible spectrum. The far-infrared region, around 400 – 10 cm (1000 – 30 μm), situated close to the microwave spectrum, possesses low energy and can be utilized for rotational spectroscopy. The mid-infrared range, around 4000 – 400 cm (30 – 2.5 μm), can be utilized to investigate basic vibrations and their corresponding rotational-vibrational structures.

Infrared spectrophotometry offers accurate data regarding certain functional groups, facilitating both quantification and selectivity. Nonetheless, low-level detectability frequently presents an issue that may necessitate more complex strategies to mitigate the problem [8].

Flash chromatography

In column chromatography, the application of positive air pressure to propel the solvent down the column is referred to as flash chromatography. Flash chromatography is an air pressure-driven hybrid of medium pressure and short column chromatography, ideal for rapid separation. Flash chromatography is a technique employed to isolate mixtures of molecules into their distinct components, frequently utilized in the drug development process. Flash chromatography employs a plastic column with a solid support, often silica gel, onto which the sample intended for separation is positioned. The remainder of the column contains fragmentation data, which provides extensive structural information on potential structures. Liquid chromatography employs either isocratic or gradient solvents, utilizing pressure to facilitate the passage of the sample through the column, resulting in separation [9].

Hyphenated Technique

In analytical science, the hyphenated technique is a modern method that integrates two or more procedures through an interface. The hyphenated methodology is essential for establishing the fingerprint of an unidentified impurity, as the reference standard for the impurity may occasionally be inaccessible for characterisation [13].

The hyphenated technique is a contemporary method in analytical research that integrates various approaches through an interface. The criteria for characterizing contaminants are frequently not easily obtainable. In these instances, the hyphenated approach is essential for producing the fingerprint of the unidentified contaminant. The combination of MS with another chromatographic technique improves the signal-to-noise ratio, leading to a more rapid reduction in noise. This results in enhanced selectivity of the analyte due to a greater number of couplings. Currently, due to constrained budgets and sporadic utilization, several research laboratories are unable to finance the installation of hyphenated procedures on-site because of the substantial expenses linked to this technology. The laboratory procedure involves determining the structure of the chromatographically separated fraction, followed by its analysis using mass spectrometry. This process can be arduous and time-intensive. Conducting an analysis on a limited sample size can be difficult in offline mode. Conducting the study in online mode provides a rapid, reliable, and effective method for attaining enhanced sensitivity and examining diverse samples. This approach is expedited, entirely automated, exceptionally sensitive, and remarkably reproducible. Decrease solvent use and mitigate complications related to sample management and ambient air interference. The diverse uses of the LCGC technology are delineated in the reference [9].

LC-MS

LC/MS is a potent analytical instrument commonly employed in pharmaceutical research for the testing and identification of product contaminants. The detection limit of several hundred ppm is easily attainable, guaranteeing the identification of all contaminants at quantities above 0.1%. MS-based methodologies typically offer enhanced resilience and Ruggedness in comparison to procedures like UV alone, owing to their elevated specificity and sensitivity. While single Quadrupole mass spectrometers function effectively as analytical instruments for the verification of identified contaminants and the A preliminary structural examination of unidentified impurities is facilitated by very sensitive Q-TOF mass spectrometers, which offer superior performance. Resolution and mass accuracy that facilitate the unequivocal identification of unknown trace contaminants, rendering them highly beneficial for the analysis of genotoxic impurities. Mass spectrometry-based techniques are frequently employed for the impurity profiling of active pharmaceutical ingredients.

During process development, UV-based approaches are typically employed to assess genotoxic contaminants in quality control. Laboratories located at production facilities. Triple-quadrupole (QQQ)

LC/MS/MS systems have established themselves as a common platform. For the quantitative assessment of organic contaminants in pharmaceutical analytical laboratories.

The combination of LC-MS offers numerous benefits compared to using LC and MS spectrometry separately. Due to its exceptional selectivity, sensitivity, dynamic range, and durability, LC-MS has found widespread use in pharmaceutical research. The sensitivity of this product is exceptional when it comes to detecting even the tiniest traces of impurities and degradation products. In the modern age, the LC-MS/MS, also referred to as tandem LC-MS, has seen a rise in usage due to technological advancements. This technique has a broad range of applications in quantitatively analyzing and determining the structure of unknown impurities.

When the LC is combined with the MS, there is minimal risk of solvent and flow rate interference in the LC-MS interface. One of the key advantages of HPLCMS is its compatibility with a photodiode array UV detector. The most commonly used method for analyzing impurities and providing detailed structural information is HPLC/UV/MS/MS. Using the LC-MS in the multiple reaction monitoring modes allows for the repetition of the experiment a specified number of times. In this case, the number of times the experiment is repeated is determined by the value of n in LC-MSn, which represents the number of MS-MS experiments. This method is widely employed for structure elucidation. [9]

GC-MS

Gas chromatography-mass spectrometry (GC-MS) is a technique that integrates the principles of gas-liquid chromatography and mass spectrometry to identify specific components inside a sample. GCMS involves the coupling of Gas Chromatography (GC) with a Mass Spectrometer via an interface that enhances the concentration of the sample in the carrier gas by utilizing the greater diffusivity of the carrier gas. The scanning times are rapid, allowing for the acquisition of many mass spectra during the elution of a single peak from the gas chromatograph.

Online GC-MS offers a significant advantage by providing simultaneous data on impurities at levels below 0.01%. One major advantage of GC-MS is its ability to provide molecular mass evidence through the use of a chemical ionization method. Additionally, it can offer information on fragmentation by utilizing electron impact ionization techniques, which can be done with both positive and negative charges.

The function of GC-MS in identifying residual solvents is essential. Alongside the completion of impurity profiling, structural elucidation may also be achieved. If the peak is associated with solvent interference, it can be discerned, indicating the presence of the toxic and hazardous class I solvent. The primary benefit of GC-MS is its ability to furnish molecular mass evidence through a chemical ionization method, as well as fragmentation information via an electron impact ionization approach, elucidating the composite structure [10].

LC-NMR

There are issues with sensitivity when it comes to LC-NMR. In this context, sensitivity refers to the capability of the NMR spectrometer to capture sufficient data to enable the clear structure elucidation of the trace intensity in a compound mixture that is being analyzed by HPLC. The ratio of signal to noise in cryoprobes is four times higher than that of conventional probes. As a result, the amount of sample required for a cryoprobe is just 0.1 µg, which is significantly less than the typical requirement of 0.5 µg with regular probes. Not only does this reduce the amount of time required to perform the analysis, but it also reduces the amount of sample that is required for the analysis. The elimination of the labor- and time-intensive isolation phase would be made possible by the utilization of LC-NMR, which would speed up and simplify the identification and analysis processes. Not only can LC-NMR produce 1-D NMR spectra for the components that have been separated by HPLC, but it can also provide 2-D NMR spectra. This is a progressive technology that integrates NMR with HPLC as an online process. Improvements in sensitivity brought about by greater magnetic fields of superconductive magnets and superior procedures, particularly the solvent suppression approach, have led to the widespread adoption of liquid chromatography nuclear magnetic resonance (LC-NMR). As an illustration, LCNMR has been utilized for the purpose of analysing medicinal metabolites, contaminants in various medical specializations, and metabolites of natural items [10].

LC-MS/NMR

Direct coupling of LC-MS/NMR is widely employed in pharmaceutical laboratories. The combination of HPLC with MS and NMR is referred to as LC-MS/NMR. In this technique, the eluent from the HPLC column is divided into two parts: one part is directed to the MS (ESI) for its advanced sensitivity, while the other part is sent to the NMR spectrometer. The combination of these data types enables a clear correlation between NMR spectra and a specific analyte at a trace level. Often, the MS spectra alone do not provide enough information for a complete understanding of the structure. By combining LC, MS, and NMR, you

can obtain extra details about the structural configuration of the drug, the functional groups, and the presence of NMR-silent heteroatoms (N, Cl, O) in unknown impurities.[10]

One of the key advantages of combining capillary zone electrophoresis with mass spectroscopy is the ability to achieve highly effective separation using capillary electrophoresis (CE), while also obtaining valuable structural information from the mass spectra. For better resolution of separated impurities, it is important to ensure a high level of orthogonality between the methods used in the impurity separation process. Various methods for impurity profiling involve combining CE with different MS ionization systems, such as electrospray ionization (ESI-MS), atmospheric pressure chemical ionization (APCI-MS), atmospheric pressure photoionization (APPI-MS), and thermospray ionization (TSI-MS). The ESI-MS and TSI-MS are effective in identifying the ionic compound, while the APCI-MS and APPI-MS cannot detect the ionic sample. Furthermore, it can also help to distinguish between the ionic or non-ionic unknown impurity.

The charge solution's sensitivity decreases in the following order: The order of preference for the different mass spectrometry techniques is ESI-MS, TSI-MS, APPI-MS, and APCIMS. APCI is commonly used for detecting solutions that remain unchanged and for less polar compounds. ESI-MS is commonly used because of its high sensitivity and gentle nature. In a recent study, Dora et al. conducted a comparison between three different methods for impurity profiling in galantamine hydrochloride under stressed conditions. The methods included RP-HPLC, Capillary electrophoresis, and CE-ESI-MS/MS. Significant findings were obtained through a comparative analysis of three methods, revealing the presence of two impurities at a low concentration. This technique combines separation and identification methods, allowing for the analysis of small sample volumes with high resolution and sensitivity. [10]

REFERENCE STANDARD METHOD

The primary objective of this strategy is to measure and regulate reference standards utilized in the creation and control of novel pharmaceuticals. The reference standards provide essential information for assessing and monitoring the performance of bulk drugs, by-products, contaminants, degradation products, excipients, raw materials, and intermediates.

Acceptance Criteria for Impurities

Newly synthesized drug compounds must have specifications that include acceptability criteria for contaminants. Stability studies, chemical development studies, and routine batch testing can anticipate the contaminants likely to manifest in the final product. The justification for including or excluding contaminants in the specification should encompass an analysis of the impurity profiles identified in batches. Impurity profile of some drugs is shown in Table 1**.** Under evaluation, with an assessment of the impurity profile of the material produced by the planned commercial method. For contaminants recognized for their exceptional potency or for inducing harmful or unforeseen pharmacological effects, the quantification or detection threshold of the analytical Methods must be commensurate with the amount of impurity control required. Suitable qualitative analytical descriptive designation incorporated in the specification of undetermined contaminants. The general acceptance threshold for any unidentified impurity should not exceed 0.1% encompassed. Acceptance criteria must be established based on data derived from actual batches of the drug material, permitting adequate flexibility to accommodate typical variations. Variability in manufacture and analysis, as well as the stability properties of the medication material. While standard manufacturing discrepancies are anticipated substantial fluctuations in impurity levels between batches may suggest that the drug substance's manufacturing process is insufficiently controlled, regulated and authenticated. The acceptance criteria must encompass thresholds for organic contaminants, including each detected impurity and each undetermined impurity at or above specified levels. 0.1% for any undefined contaminant, with a cumulative limit of no more than 0.1% for total impurities, residual solvents, and inorganic impurities [11].

Table 1: Impurity Profile of Some Drugs

Drugs	Method	Mobile Phase	Impurity	Reference
Telmisartan	LC-MS/MS	Acetonitrile:0.01 Ammonium acetate buffer	2-Methyl-6-nitro aniline	[15]
Saxagliptin	RP-HPLC	Water: Acetonitrile	Degradation related Impurities	[16]
Vildagliptin	HPLC	Water: Acetonitrile	(2S)-1-[2-[(3- hydroxyadamantan-1-yl)imino]acetyl]pyrrolidine-2-carbonitrile	[17]
Linagliptin	HPLC	Methanoic acid : Acetonitrile	Process related Impurities	[18]
Rosiglitazone	RP-HPLC	Acetonitrile	2,4Thiazolidinedione	[19]
Alogliptin	RP-HPLC	Water:acetonitrile: trifluoroacetic acid	Impurity A,B,C,D,E,F,G	[20]
Glibenclamide	HPLC	A mixture of acetonitrile (ACN) and potassium dihydrogen orthophosphate	Sulfonamide impurity	[21]
Miglitol	HPLC	Solution A: 10 mM dipotassium hydrogen orthophosphate adjusted to pH 8.0 using concentrated phosphoric acid Solution B: Acetonitrile	Impurity A (Dialkylated Miglitol), Impurity B (Ido)	[22]
Glipizide	HPLC	ACN: Ammonium acetate	Degradation products	[23]
Glimepiride	HPLC	ACN: Ammonium acetate	Degradation products	[24]
Repaglinide	HPLC	acetonitrile–ammonium acetate buffer	4-carboxymethyl-2-ethoxy-benzoic acid (I), 4-cyclohexylaminocarbamoylmethyl-2-ethoxy-benzoic acid (II), 1-cyclohexyl-3-[3-methyl-1-(2-piperidin-1-yl-phenyl)-butyl]-urea (IV) and 1,3-dicyclohexyl urea (III)	[25]

ANALYTICAL METHOD DEVELOPMENT

The creation of a novel medicine necessitates diverse dependable analytical data at different stages of the process.

The gathering of the sample.

We must evaluate the chromatographic plate using the linear solvent-strength model of gradient elution. Refinement of the methodology to calibrate metrics associated with ruggedness and robustness [12].

ICH limits for impurities

In order to comply with ICH recommendations for impurities in new medicinal products, it is not essential to identify impurities below 0.1% level unless those impurities are anticipated to be exceptionally hazardous or potent. As per the ICH guidelines, the highest daily dosage that needs to be taken into account is as follows: less than 2 grams per day with a concentration of 0.1% or less than 1 mg per day with a concentration of 0.05%.

- Identified impurities - Unidentified impurities above 0.1% - Unspecific impurities within 0.1% limit [12].
- Total impurities

Regulatory Guidelines on Impurities in API'S

For the purpose of safety and efficacy as well as virtuous, social, economic reasons prefer the need to monitor the various impurities in drug products. For different people the monitoring and controlling of impurities may mean different as per individual. These guidelines not just provide the type of information that should be submitted with their application by New Drug Applications (NDA) or Abbreviated New Drug Application (ANDA) but also help the FDA reviewers and field investigators in their implementation of regulations and interpretation of results. The different guidelines related to impurities are as follows:

ICH Guidelines on the stability testing of new drug substances and products - Q1A

ICH Guidelines "Impurities in New Drug Products" - Q3B.

ICH Guidelines on "Impurities in New Drug Products" - Q3A.

ICH Guidelines on "Impurities: Guidelines for Residual Solvents"- Q3C.

US-FDA Guidelines. "ANDAs: New Drug Substance Impurities.

US-FDA Guidelines on New Drug Applications: Impurities in New Drug Substances [13].

CONCLUSION

As per the regulatory guideline, it is necessary to conduct analytical monitoring of impurity in a new drug substance in order to obtain market authorization. Therefore, it is crucial to conduct analytical monitoring of pharmaceutical products to detect even the slightest traces of impurities. Given that APIs are not completely pure, even small amounts of impurities can potentially impact the safety and effectiveness of the final formulation, which in turn may affect patients who consume these products. Standard assay procedures alone do not provide a comprehensive description of impurities, both in terms of their quality and quantity. Various techniques utilized in impurity profiling can greatly aid in the identification and structural elucidation of unknown impurities. The successful implementation of this technique relies heavily on understanding the specific characteristics and origins of impurities found in the drug substance. When it comes to removing impurities, liquid chromatography techniques are commonly used. However, it is important to have a sufficient amount of the isolated fraction in order to conduct further structural analysis offline. In today's modern age, numerous advancements have revolutionized the analysis technique, making it faster and more efficient while reducing the amount of effort required. The online mode of analysis not only saves time, but also provides qualitative and quantitative data for unknown impurities. The hyphenated technique has been widely employed in the field of impurity profiling. Various methods can be used to monitor impurities, either individually or in combination, depending on the specific needs.

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