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PHARMACEUTICAL IMPURITY PROFILING: TECHNIQUES, PROCEDURES, AND REGULATORY GUIDELINES

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ABSTRACT

The pharmaceutical impurity profiling is an important aspect of drug development and quality assurance and the drug development procedure guarantees the safety, effectiveness, and regulatory compliance of pharmaceutical products. This is done through identification, quantification and control of impurities that may develop in the process of synthesizing, formulating or storing the drug substances and products. The different methods of analysis applied in order to detect and identify impurities include chromatographic, spectroscopic and mass spectrometric analysis with its emphasis laid on the genotoxic compounds, solvating residues, and degradation products. Also, the tools of artificial intelligence (AI) and Process Analytical Technology (PAT) are becoming increasingly important in improving impurity finds and monitoring. The regulation agencies such as FDA, EMA and ICH offer strict regulations in such a way that the level of impurities is maintained within acceptable levels so that patient health is not compromised. The review covers the main techniques, procedures and regulatory frameworks applied in the pharmaceutical impurity profiling and the significance of high-quality standards in drug development.

KEY WORDS: Drug impurities, impurity profiles, analysis, regulations, quality control.

INTRODUCTION

What Are Impurities?

Adulteration of pharmaceutical products are undesirable substances that may occur in raw materials, intermediates or finished drug product. These contaminants can be as a result of a chemical reaction, degradation or contamination during processing, storage and packaging. [1] Some of the common examples of impurities can be organic, inorganic and residual solvents, which may adversely affect the quality of the drug. The presence and measurement of these impurities are important to guarantee the safety, efficacy and regulation in the final product.[2]

Significance of Impurity Profiling in the Development of Drugs

The impurity profiling is a highly significant component of the drug development that involves the identification and the quantity of the impurities that are present in drug substances and products. This forms an important step in the way the purity of the drug is considered and the same counts on whether it will address the requirements of the regulations. Profiling of impurities

helps in exploration of origin, nature and possible hazards of impurities and is at least one of the primary reasons why it is possible to formulate safe and effective pharmaceutical preparations. It is also used to streamline the manufacturing processes and it assists in developing a powerful quality control processes [3,4].

Implication on Effectiveness, Safety, and Regulatory Adherence

Pollution of a drug product can also undermine its efficacy as well as safety to a great extent. It is a possibility that the desired therapeutic property is being altered or adverse effects are being induced by agglutination is the reason why it is important to ascertain the effect of impurities as part of the drug developmental process. [5] Moreover, agencies such as FDA and EMA insist on rigorous testing of impurities as a means of ensuring that medication is of the desired standards of safety.

Incompetence in solving impurity issues can lead to regulatory delay, recalls or a complete withdrawal of products and so proper impurity profiling can be very useful in ensuring compliance and safeguarding the health of the

population.[6].

Classification of Impurities Based on Origin

The impurities of pharmaceutical products can be categorized according to the source of impurities, such as organic, inorganic, and residual solvents. The impurities under each category have their own issues with drug safety and quality control, and each category has its own way of identifying, quantifying and managing the impurities during drug development.[7]

Organic Contaminants (Starting Materials, By-products)

Organic impurities are generally formed out of the starting materials, intermediate or by-products formed in the production of the drug. These contaminants may be in the form of raw materials that were not used up, intermediate products or chemical by-products that were generated during the process. The organic impurities may be of diverse structures and different degrees of toxicity, this fact makes it necessary to identify and manage the levels of the impurities in order to achieve the safety and effectiveness of the end product.[8,9]

Reagents, Metals, Salts, etc.

The inorganic impurities occur due to reagents, catalysts, or metals that are used in making the drug, and salts which may be left behind after the drug is made. These contaminants may be added by using metals-based reagents or the contamination of the materials during handling of the material. Inorganic impurities might be harmful in nature such as toxicity and may pose an effect on stability and bioavailability of the drug.[10]

Residual Solvents (Class 1, 2, 3)

The residual solvents are chemicals that are used in the process of manufacturing to dissolve or extract or purify the drug. They could be left in minute quantities in the end product. The International Conference on Harmonisation (ICH) distinguishes solvents depending on their toxicity. Class 1 solvents are very toxic and should be avoided and Class 2 and 3 solvents are said to be not so harmful and they should be controlled carefully so that they do not exceed the reasonable limit.[2,11]

On the basis of Manufacturing Stage.

As well, impurities may be divided according to the manufacturing process step they are added at. The main factors to consider during the drug development are process-related impurities, degradation products as well as formulation-related impurities, and each one of them occurs at various stages of the production cycle.

Process-Related Impurities

Process impurities are found in the active pharmaceutical ingredient (API) production and purification phase. The impurities may be as a result of the reagents, solvents or catalysts that are used in the process, or the incomplete reaction. It is important to carefully observe the impurities associated with the process so that the final drug product can have quality and that the impurities do not have an impact on the quality and safety of the product.[12,13]

Degradation Products

Degradation products are generated when the drug substance decomposes due to environmental conditions e.g. heat, light or humidity. Examples of these impurities are also formed by the chemical instability or interaction with the excipients during the formulation. Observation of degradation products is also significant since they do not only lead to any changes in the stability of the drug but can also pose some safety concerns once they are toxic or adversely impact on the drug efficacy.[14]

Formulation-Related Impurities

Formation-sensitive impurities: These impurities form as a result of excipients or during the invention of the drug into its finished form of dosage, e.g. tablets, capsules or injections. Such impurities may consist of excipient or interaction contaminants between the drug substance and formulation components. These impurities should be detected and managed so that the drug formulation would be safe, effective and consistent.[15]

Nitrosamine Impurities and Genotoxic.

Such impurities as genotoxic and nitrosamine are becoming more prominent in the safety of pharmaceuticals because of the possible occurrence of cancer or genetic mutations. It has been the increased attention of the regulatory bodies towards these impurities as they have serious health hazards, and pharmaceutical manufacturers have to pay special attention to their presence and reduction of the level in the drug products.[16]

Significance in New Regulatory Focus.

Over the past few years, regulatory attention on the impurities of genotoxic substances and nitrosamine has increased because of their potential to lead to severe health outcomes, including cancer and genetic mutations. Detection, risk assessment, and control of such impurities of drug products have been given guidelines by regulatory bodies such as the FDA and EMA.[17] Because of this, organizations are forced to implement tougher testing measures and improved risk management policies in order to guarantee the safety of the people.

Significant Thresholds and Risk Management

Genotoxic and nitrosamine impurities acceptable limits are established after considering the risks assessment that consider the toxicological profile of the substances. Strict maximum levels of these impurities are usually established by regulatory agencies, and many of them demand that the level of such impurities should be minimized to the lowest possible value. [16,18] Risk assessment models are applied to assess the possible carcinogenic or mutagenic risk of these impurities and are assisting in ensuring that drugs are safe enough to make it to the market.

Guidelines on Regulatory Profiling of Impurities

Regulatory policies are important in the definition of the safety, efficacy, and quality of pharmaceutical products according to the requirements. These guidelines have a major component of impurity profiling since the impurities may affect the safety and efficacy of drug substances and products. Different institutions such as the International Conference on Harmonization (ICH). The US FDA, EMA, WHO, and pharmacopoeia organizations have certain regulations and standards to deal with impurities during the drug development and production process.[19]

International Conference on Harmonization (ICH)

International Conference on Harmonization (ICH) constructs the guidelines that will be used by the pharmaceutical sector to align the regulatory needs of nations. The ICH guidelines play a significant role in profiling of impurities that are a lot to do with identification, control, and testing of impurities in drug substances and products. These regulations are very important in global consistency of the quality of drugs, most of the regulatory agencies in the world including the FDA and the EMA adopt such regulations.[20]

ICH Q3A (New Drug Substances Impurities)

ICH Q3A provides a guideline about the impurities which could be present on new drug substances. The guideline puts a focus on the acceptable level of impurities, how the impurities can be identified and measured, as well as how the impurities can be controlled during the manufacturing process. This is also an attempt to ensure that new drug substances are safe to use, by attempting to counter potential toxicity risk of impurities.[21,22] This is a guideline to all emerging active pharmaceutical ingredients (APIs).

ICH Q3B (Impurities in New Drug Products)

ICH Q3B describes the needs of impurities in new drug products, but on the basis of the final dosage form. It also underscores the need to regulate impurities in the drug products to make sure that the products do not affect the safety, efficacy, or stability of the product.

Specifications of acceptable levels of impurities also depend on their toxicity and the possible effects on the quality of the drug product, and this guideline includes these specifications.

ICH M7 (Determination and Management of DNA Reactive Impurities)

ICH M7 is specifically concerned with the evaluation and management of reactive impurities with DNA, or in other words, the so-called genotoxic impurities. Of special concern are these impurities because they cause cancer or mutations in genes. The guideline defines the approach to the evaluation of the risk created by these impurities, allocating the acceptable limits, and explaining the approach of controlling the presence of these impurities in drug products. The M7 recommendation is part of the patient safety concept, especially in the long-term drug treatment[4,23].

Recommendations of Regulatory Bodies

The US FDA, the European Medicines Agency (EMA), the World Health Organization (WHO), and the Central Drugs Standard Control Organization (CDSCO) also offer certain guidance on impurity profiling of the pharmaceutical. These agencies guarantee that pharmaceutical products are of high safety and effectiveness through setting regulatory limits with regard to identification, qualification and control of impurities.[24]

US FDA, EMA, WHO, CDSCO

The regulations and guidelines of impurity profiling in the US FDA, EMA, WHO, and CDSCO are different. The FDA and EMA tend to follow the ICH guidelines, whereas the WHO offers recommendations that can be applied to the world, in particular, in developing nations. CDSCO is a Indian regulatory body that governs pharmaceutical industry; the organization has certain impurity testing criteria that is in line with the international standards. These regulatory authorities carry out the process of ensuring that pharmaceutical products are safe, of quality and at their effective level and then they are approved to be used. [25]

Disclosure of Reporting, Identification, and Qualification

There are regulatory limits which determine the acceptable limits of different impurities of pharmaceutical products. These limits help manufacturers to determine when and how to disclose impurities as well as when to recognize and to disclose particular impurities based on their toxicity, structure and likely effect on drug safety.[4,26] The regulatory bodies have varying levels of thresholds based on the nature of the impurity (e.g., genotoxic or non-genotoxic) and its content in the end

product. These limits are essential in providing compliance and patient safety.

Pharmacopoeial Standards

Different pharmacopoeias, including the USP (United States Pharmacopeia), EP (European Pharmacopoeia), and IP (Indian Pharmacopoeia) have pharmacopoeial standards that give finer guidelines on impurity profiling. These standards define the allowed maximum limits of impurities in drug substances and products, the testing procedures of impurities detection, measurement and control [27]. The pharmacopoeial requirements are important factors in establishing the quality and safety of pharmaceuticals.

USP <476>, EP, IP Guidelines

USP, European Pharmacopoeia (EP) and Indian Pharmacopoeia (IP) provide information concerning the test of impurities of pharmaceutical substances and products. USP <476> directly addresses quality and purity of the substance of the drugs, giving the methods of determining and quantifying the impurities. The EP and IP also determine standards of impurity testing that are in line with the best practices across the rest of the world, and therefore, safety and efficacy standards of the drugs employed in the respective regions are safe and effective.[28,29]

Monographs and Specification Limits

The important control tools in controlling impurities include pharmacopoeial guidelines, monographs and specification limits. Monographs declare standards of the specific drug substances including acceptable amounts of impurities, test methods and validation. These limits are significant in both quality control and as regulatory compliance when pharmaceutical products are released and in the manufacture of the products themselves, based on their levels of toxicity and the effects they may have on the safety and efficacy of the drug.

Analytical Techniques for Impurity Detection and Quantification

Chromatographic Techniques

An important point in the impurity profiling is the utilization of chromatographic techniques as it has high sensitivity and resolution in separating and identifying the impurities of drug substances and products. The methods enable the detection of organic and inorganic impurities and therefore are important tools in the pharmaceutical industry in the procedure of guaranteeing the quality and purity of medicines.[31]

Organic Impurities HPLC and UPLC

High-Performance Liquid Chromatography (HPLC) and Ultra-Performance Liquid Chromatography

(UPLC) are common in the separation and analysis of organic impurities of pharmaceutical products. The use of HPLC that has the capability of separating complex mixtures makes it suitable in the quantification of organic impurities such as starting materials, by-products and degradation products. [32]UPLC which is the evolution of HPLC has lower analysis rates and a high resolution and is therefore appropriate in identifying traces of impurities in drug substances and products at trace levels.

GC for Residual Solvents

Gas Chromatography (GC) is an extremely strong analytical method applied to identify the presence of solvents left in the pharmaceutical products. The residual solvents are volatile compounds that are left behind after the synthesizing or purification procedure, and they may be disastrous to health when high concentrations are found. [33]The use of GC is especially good in detecting and measuring volatile organic solvents to ensure that the quantity is not exceeded by the regulation and that the end result of the medication is safe to use.

TLC for Rapid Screening

Thin-Layer Chromatography (TLC) is an easy and quick method of initial screening of impurities on pharmaceutical samples. It is usually applicable to quality control laboratories to identify known impurities in quick fashion. Although TLC is not as sensitive and resolution-operating as HPLC or GC, it is an easy and inexpensive technique that could be useful in the initial analysis of impurities, especially with raw material analysis or drug development.[34,35]

Spectroscopic Techniques

Due to the detailed information that spectroscopic techniques can provide on chemical structure of impurities, unknown substances can be identified, as well as the identity of known impurities can be determined. They are especially effective in study of structures and ensuring the existence of certain functional groups in drug compounds.[36]

The UV, IR and NMR to Structural Elucidation

The structural explanation of impurities in drug substances is necessary due to ultraviolet (UV) spectroscopy, Infrared (IR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy. The determination of the functional groups and the realization of the presence of particular chemical bonds is typically done by means of the UV and IR spectroscopy and the NMR provides an individual with the detailed information about the atomic surrounding and structure of the molecules of the impurities. Such measures are needed to determine the identity of impurities and

identify the way they could impact drug safety and efficacy. [37, 38]

Mass Spectrometry (LC-MS/MS, GC-MS)

The Mass Spectrometry (MS) systems including Liquid Chromatography-Mass Spectrometry (LC-MS/MS) and Gas Chromatography-Mass Spectrometry (GC-MS) are powerful systems of identification and quantification of impurities in pharmaceutical products. LC-MS/MS is an amalgamation of the separation capacity of liquid chromatography, sensitivity, and specificity of mass spectrometry, and is suitable in the identification of low-levels of organic impurities. GC-MS is used in volatile impurities namely solvents that are left behind and sensitive and accurate in revealing and determining the amount of impurities.[39,40]

Elemental Impurity Testing

Contaminants of elements including heavy metals are a serious issue in pharmaceutical products, as they are toxic. Elemental impurities testing is a method used to make sure that drugs have no harmful amounts of metals that may result in health hazards. Norms like ICH Q3D are standards of permitted levels of elemental impurities of pharmaceutical products.[41]

Heavy Metals ICP-MS and AAS

ICP-MS and Atomic Absorption Spectroscopy (AAS) are highly popular methods of tracing and measuring heavy metals in pharmaceutical products. ICP-MS is an extremely sensitive method of trace metal detection and AAS is typically employed in routine testing of a particular element. These are essential methods of maintaining that heavy metals like lead, arsenic, and cadmium are not exceeded in the level of toxicity in drug substances and products.

Compliance with ICH Q3D

ICH Q3D offers principles of the tolerable amount of elemental impurities in drug products and substances. The adherence to these requirements is obligatory to get the regulatory approval and manufacturers are required to prove that their products do not exceed the recommended limits within the elemental impurities through rigorous tests. Applying such methods as ICP-MS and AAS allows the pharmaceutical product to be safe and meet the regulations of global standards.[42,43]

New and Hyphenated Techniques

New and hyphenated analytical methods are leading to improvements in profiling of impurities, and allow the detection of unknown impurities to be more sensitive and more precise. Such techniques are also known as multi-analytical techniques because they involve the combination of several analytical methods to

give full data, thus being crucial to the current development of drugs and taking care of all possible impurities that might have been neglected.

LC-QTOF, LC-NMR, and LC-HRMS

The High-Resolution Mass Spectrometry (LC-HRMS), Nuclear Magnetic Resonance (LC-NMR) and Liquid Chromatography with Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF) are the advanced hyphenated methods that can yield detailed information about the molecular structure and identity of impurities. LC-QTOF is sensitive in the detection of unknown impurities and complemented by the LC-NMR and LC-HRMS techniques in the structural elucidation and accurate definition of the molecular identity. [44]The techniques are extremely useful in solving multi-impurity profiles as well as determining the purity of pharmaceuticals.

Unknown Impurity Identification Applications Uses

More recent and hyphenated techniques, such as LC-QTOF, LC-NMR and LC-HRMS are particularly practical in the determination of unidentified impurities. The sophisticated mixtures can be completely characterized with the assistance of these techniques, including the trace amounts of unrecognized substances which, with the help of the conventional techniques, would have remained unnoticeable, can be identified. [45]The complex mixtures could be studied in more detail, not to mention the fact that with the assistance of chromatographic separation and high-resolution mass spectrometry and NMR, the pharmaceutical companies have the opportunity to detect and eliminate any potential threat of unknown impurities that the drug products may contain.

Method Development and Validation Stability-Indicating Methods

The stability indicating techniques are necessary in the assessment of stability of pharmaceutical products in a variety of environmental conditions. These methods are used in determining the products of degradation and the influence of the different factors such as the light, heat or moisture on the drug substance. By testing the stress as well as separating the impurities these methods are employed in order to ensure that the drug is effective and safe throughout the shelf life.[46,47]

Stress Testing and Forced Degradation

Stress testing and forced degradation studies are conducted to determine how a drug substance or product would behave under the most extreme environments. These tests expose the drug to stress conditions that include high temperature, humidity, light, or oxidative conditions purposely to cause degradation. It is aimed at finding the possible degradation products that can be

formed and the stability of the drug in time. Through such tests, manufacturers are able to know more about the behavior of the drug in real world settings and set right storage conditions and time of expiration.[48]

Separation of impurity and maximum purity

Separations of impurities and the highest purity are essential concerns of stability-revealing techniques. Separation of impurities guarantees that any degradation product developed during stability testing is appropriately detected and quantified whereas peak purity guarantees that any analytical peaks detected during chromatographic techniques are that of the target compound only. Through measuring these parameters, pharmaceutical companies will be able to make sure that degradation products do not affect the therapeutic effect and safety of a drug.[6,49] This enables the proper detection of impurities and it is also able to keep the product within the stipulated quality requirements during the shelf life.

Parameters of validation (As per ICH Q2 (R2))

Validation parameters play a very crucial role in maintaining accuracy of analytical methods applied in impurity profiling, reliability, and appropriateness of the purpose applied. The guidelines on the validation of the analytical methods, such as assessing the specificity, limits of detection (LOD), limits of quantification (LOQ), and other important performance characteristics, are provided in the international conference on harmonisation (ICH) Q2 (R2). The correct validation will guarantee that the methods used to analyze the data are consistent and reproducible which are critical in compliance with regulations and quality assurance.[15,45,50]

Specificity, LOD, LOQ

Specificity, the ability of an analytical process, is the ability to detect the target compound of interest, without interference with other chemical substances present such as impurities or degradation products. LOD and LOQ are the important parameters, as they define the sensitivity of the method. LOD and LOQ are the lowest achievable concentration of an analyte, both are measurable with certainty and the lowest concentration which can be reliably measured with reasonable accuracy and precision, respectively. [51] Both LOD and LOQ are important parameters to ensure that the measurements of the pharmaceutical products regarding the low concentration of impurities are at a level that can be measured reliably and with a reasonable accuracy and precision respectively

Accuracy, Precision, Robustness, Linearity.

The linearity, accuracy, precision, and the robustness are the critical validation parameters that

define the performance and reliability of the analytical methods. Linearity is a component that defines the capability of the analytical procedure in use to identify and identify various levels of the analyte. The difference between the actual value and the measured value is called accuracy whereas the similarity of outcome in the same conditions is called precision. The concept of robustness is applied to assess whether the methodology used does not vary when the tackling of the experiment conditions change slightly. [52,53] Validity of the parameters is a concept that helps in ensuring that the analytical process is good, can be replicated and gives reliable results without which approval by the regulation and quality control cannot be effective.

Case Studies in Impurity Profiling

Genotoxic APIs Impurities

Genotoxic impurities are chemicals, which can cause genetic mutation or cancer and are very dangerous to the health of the patient. These contaminants are very especially important to the active pharmaceutical ingredients (APIs) since they can not only impact the drug safety but also its regulatory approval. The development and production of drugs require the identification and management of genotoxic impurities to avoid the long-term health effects.[34,54] To make sure that the drug products are safe to human consumption, regulatory agencies demand that these impurities be tested and risk assessed to a high degree. Risk-based analysis and application of acceptable analytical methods are essential in the occurrence of genotoxic impurities at low levels in APIs.

Sartans recall because of Nitrosamine Contamination

The nitrosamine family of chemical compounds, which are already known to be carcinogenic, became the focus of attention in the world because they were contaminated in sartans (angiotensin II receptor antagonist) and other medicines. Certain manufacturing processes have been associated with the contamination of sartans with nitrosamines, thus the importance of close attention paid in the process of production of drug substances.[55] Authorities such as FDA and EMA have established tolerable levels of nitrosamine contamination and have provided manufacturers with directions to test and eradicate nitrosamine impurities. Drugs that contain nitrosamines are hazardous to human health and thus, their identification and elimination is a critical component of quality assurance and regulatory compliance of drug manufacturing.

Optimization of the Process to minimize deficiencies

Optimization of the processes is very important in order to minimize impurities in drug products. Improving the manufacturing process, pharmaceutical firms can reduce the amount of impurities introduced in

the process, increase the final product purity and the general safety of the drug. Such techniques as optimization of reactions, use of high-quality raw materials and effective purification are used to manage the quantities of impurities throughout the production process. [56,57] Furthermore, the constant monitoring of the processes and their validation are necessary in order to make sure that the drug manufacturing process does not get out of control. The optimization of these processes is the key to the safety and effectiveness of the drug as well as contributes to fulfilling regulatory obligations and reducing the delays in approving the products that increase the costs of these products significantly.

Risk Assessment and Control Strategies

Permitted Daily Exposure (PDE) and Threshold of Toxicological Concern (TTC)

The main terms in the evaluation of these two are Permitted Daily Exposure (PDE) and Threshold of Toxicological Concern (TTC), which are used to evaluate the safety of impurities in pharmaceutical products. PDE is the limit of the number of grams of an impurity that can be safely used on a daily basis with no adverse effects. It is applied in setting safety limits of impurities in drug products. [58,59] TTC, conversely, is a risk-based methodology to establish safety limits of chemical substances in which sufficient toxicity data are not available. Regulatory agencies both use PDE and TTC in assuring that the concentration of impurities in the drug products is within safe ranges to prevent unnecessary health hazards to patients. These principles inform the formulation of acceptable levels of impurities in drug substances particularly in cases where one has little information regarding a given impurity. [3,60]

Control Strategies in QbD Approach

Quality by Design (QbD) is a process of pharmaceutical development that focuses on quality design in the product at an early stage. Control strategies form part of the QbD approach that entails the identification of critical quality attributes (CQAs) and setting up controls on processes that affect such attributes. As far as the impurity profiling is concerned, the control strategies are designed so as to reduce the occurrence of impurities during the manufacturing stage so that the product is safe, effective, and meets the regulatory criteria. [61,62] These plans can involve optimization of processes, risk estimations and constant observation. The principles of QbD allow pharmaceutical companies to make the manufacturing process more robust and consistent and minimize variability in impurity levels and increase the product quality.

Analytical Quality by Design (AQbD) Role

Analytical Quality by Design (AQbD) is an extension of the QbD concept to the procedure of the drug development and production. AQbD is concerned with the design and optimization of methods of analysis to make them both robust and reliable and to fulfill the regulatory and quality requirements. Within the framework of impurity profiling, AQbD is very important in making sure that the methods of analysis are able to identify and measure impurities highly precise and accurate. [63] Applying to principles of AQbD, the pharmaceutical companies are able to anticipate, proactively, the possible sources of analytical variability, optimise analytical operation, and enhance the dependability of impurity detection. The strategy improves the general quality control procedure and the safety and effectiveness of drug products to patients can be ensured. [64,65]

Challenges in Impurity Profiling

Identification of Unknown or Trace-Level Impurities

The challenge in the analysis of pharmaceuticals is the identification of unknown or trace-level impurities. These contaminants, even in minute amounts, could be formed during the synthesis, storage and degradation of the drug substance. High-resolution mass spectrometry (HRMS), liquid chromatography-mass spectrometry (LC-MS/MS) and nuclear magnetic resonance (NMR) are advanced methods of analysis frequently used to identify and determine such trace impurities. The correct identification is of utmost value in determining the possible risks to patient safety and adherence to the regulatory standards. Identification of unknown impurities is one of the major tasks in impurity profiling because even small traces of some substances may affect the safety, efficacy and stability of the final product. [66]

Complex Formulations and Excipients Interference

Active pharmaceutical ingredients (APIs) in complex pharmaceutical formulations may react with excipients to produce new impurities or make changes to the stability of the drug product. The interactions usually make impurity profiling difficult, as excipients might interfere with analysis techniques and mask, or complicate, the analysis of impurities. [14,67] It is important to note that excipients can induce impurities to drug preparations, complicating the analysis of impurities. Such strategies as excipient compatibility testing, sophisticated analytical procedures and application of control strategies in the development of the drug product can be used to counter these issues and assure that the drug product contains no deleterious amounts of impurities

Regulatory Scrutiny and Documentation Requirements

The regulatory bodies like FDA, EMA and ICH have strict requirement in impurity profiling especially in the identification, quantification and control of drug product impurities. The documentation requirement covers the detailed characterization of impurities, identification of the critical impurities, methods of test, and the limit of each impurity. The manufacturers also

have to supply information on the safety and any possible toxicological risks of impurities, and the means used to limit their occurrence. The purity profile of drugs is regulated to make sure that the drugs are safe to be used by members of the society. Failure to adhere to these regulatory requirements may lead to drug approval delays, market recall and recall. A successful process of approval of the product requires proper documentation and compliance with the regulations.

Table 1: Impurity Classification Based on Origin.

Category	Description	Examples	Safety Concerns
Organic Impurities	Impurities from raw materials, intermediates, or by-products	Unreacted starting materials, by-products	Toxicity, potential effect on efficacy
Inorganic Impurities	Result from reagents, catalysts, or metal contaminants	Reagents, metals, salts	Toxicity, effect on stability
Residual Solvents	Used to dissolve or purify drugs but may remain in the final product	Class 1, 2, and 3 solvents	Toxicity (Class 1), must be controlled

Table 2: Regulatory Guidelines for Impurity Profiling.

Guideline	Agency	Focus	Key Standards
ICH Q3A	ICH	Impurities in new drug substances	Identifying and controlling impurities in APIs
ICH Q3B	ICH	Impurities in new drug products	Regulating impurity levels in finished products
ICH M7	ICH	Genotoxic impurities	Evaluating and managing genotoxic impurities

Table 3: Analytical Techniques for Impurity Detection.

Technique	Application	Impurities Detected	Advantages
HPLC & UPLC	Separation of organic impurities	Organic impurities, degradation products	High sensitivity, good for complex mixtures
GC	Residual solvent detection	Volatile solvents	Highly sensitive to volatile compounds
Mass Spectrometry	Identification of unknown impurities	Organic and inorganic impurities	High specificity and sensitivity, effective for trace impurities

Figure 1: Method Development and Validation

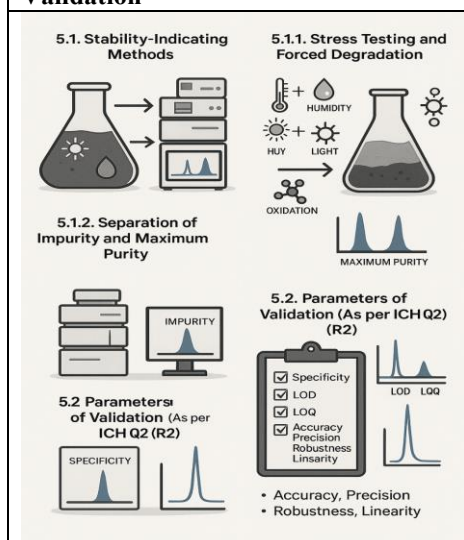


Figure 2: Case Studies in Impurity Profiling

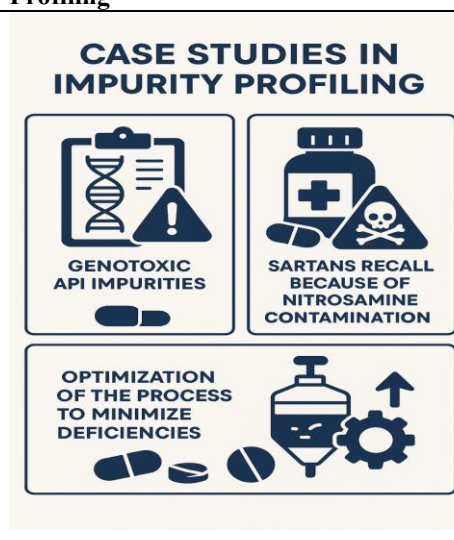
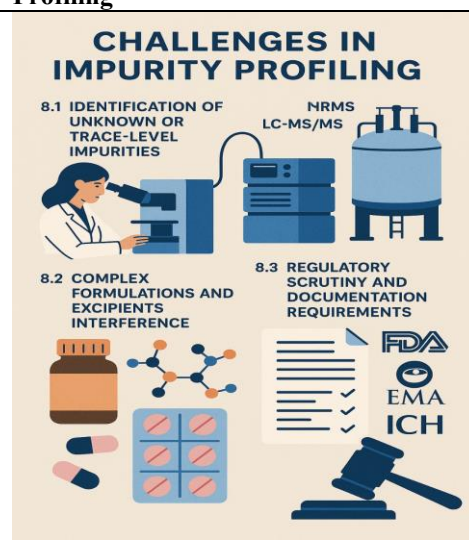


Figure 3: Challenges in Impurity Profiling



Future Perspectives

Artificial Intelligence and Software-based Spectral Interpretation.

The interpretation of spectral data used in pharmaceutical analysis is getting a revolution with the use of Artificial Intelligence (AI) and sophisticated software. Machine learning models, especially the AI algorithms, are finding wider and wider application in analyzing complicated spectral data, which could be acquired using NMR, IR and UV spectroscopy techniques. Patterns are identified with more accuracy and efficiency using these tools, compounds are classified and detecting impurities is more accurate and efficient than the manual interpretation.[68] Automating the spectral analysis process will help to minimize human error and increase the speed of the analysis process as well as the accuracy with which impurities are detected. Prediction of stability of compounds, determination of degradation products as well as ensuring that the pharmaceutical products conformed to regulatory requirements of impurity profiles could also be achieved through the use of AI-driven software solutions.

PAT Tools- Real-Time Monitoring.

Process Analytical Technology (PAT) is applied to real time monitoring and control of pharmaceutical manufacturing processes. PAT tools allow the ongoing analysis of essential process parameters and quality characteristics such as impurity levels in the process of production. In-line spectroscopy, chemometrics, and sensors that are implemented into the manufacturing process help to identify the impurities and lack of desired product quality immediately. PTA tools are used to monitor products in real-time, ensuring that the quality of the products produced stays the same, the efficiency of the processes is increased, and the variation of the batches decreases.[69] PAT tools also lead to enhanced decisions in the process optimization and identifying the possible source of impurities when producing drugs as they ensure that data is available on time.

Sustainable and Green Analytical Methods.

Sustainable and green methods of analytical techniques are becoming widely known in the pharmaceutical analysis as the sector tries to reduce environmental effects. The methods serve to minimise the utilisation of dangerous chemicals, minimise on the use of energy and minimise on the waste being produced. Some examples of this are seen in the use of green solvents in chromatography, micro-scale analysis to minimize sample sizes, quantities of reagents used, and the use of non-toxic, eco-friendly alternatives in analysis. [70]The green chemistry concept is implemented to develop more sustainable approaches that do not only guarantee the precision of the impurity profiling but also supports global

endeavors in terms of sustainability. Introducing green and sustainable analyses in the analysis of impurities will help in protecting the environment as well as ensuring better adherence to the regulation of environmental impact.

CONCLUSION

Pharmaceutical impurity profiling remains a cornerstone of drug safety, efficacy, and regulatory compliance. As pharmaceutical formulations and manufacturing processes become increasingly complex, the identification, quantification, and control of impurities are essential to safeguard patient health. Impurities may arise from raw materials, synthesis by-products, degradation processes, or environmental contaminants, and even trace levels can compromise therapeutic effectiveness or cause adverse effects. Therefore, impurity profiling is not merely a regulatory obligation but a critical component of pharmaceutical quality assurance.

Advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC), Ultra-Performance Liquid Chromatography (UPLC), and Gas Chromatography (GC) play a central role in separating and quantifying organic impurities and residual solvents. More sophisticated tools, including LC-MS/MS, GC-MS, and High-Resolution Mass Spectrometry (HRMS), enable highly sensitive detection and structural characterization of trace and unknown impurities. Spectroscopic methods such as UV, IR, and NMR further support structural elucidation. The integration of artificial intelligence, chemometrics, and machine learning has enhanced spectral interpretation, improving speed, accuracy, and reliability in impurity detection.

The implementation of Process Analytical Technology (PAT) allows real-time monitoring of impurity levels during manufacturing, promoting proactive quality control, reducing waste, and improving operational efficiency. Regulatory compliance remains fundamental, with international guidelines such as ICH Q3A, Q3B, Q3D, and M7 providing frameworks for impurity limits and control strategies. Adherence to these standards is essential to avoid regulatory penalties, product recalls, and reputational damage.

Emerging challenges, particularly in biologics and biosimilars, demand refined analytical approaches to address complex process-related impurities. Simultaneously, sustainability and green chemistry initiatives are influencing impurity profiling practices, encouraging environmentally responsible analytical methods. Looking ahead, digital technologies, predictive modeling, and laboratory automation are expected to further transform impurity management. Overall, robust impurity profiling ensures the continued production of safe, effective, and high-quality pharmaceutical products for global healthcare needs.

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