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Comprehensive Overview of Impurity Profiling in Pharmaceutical Products: Regulations, Characterization, and Analytical Techniques

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Abstract: ***This article provides a thorough exploration of the critical role of impurity profiling in the pharmaceutical industry. Impurities, unwanted chemical substances present alongside active pharmaceutical ingredients APIs, can compromise the safety and efficacy of drugs. Various regulatory bodies, including ICH, FDA, and CDHA, have established stringent purity requirements to ensure the identification and control of impurities in both formulated products and APIs. The classification of impurities into organic, inorganic, and residual solvents is outlined, and their permissible limits are emphasized. The concept of impurity profiling, encompassing identification, structural elucidation, and quantitative determination, is elucidated, underscoring its significance in drug design, quality assurance, and safety assessment. The article also delves into separation techniques such as Accelerated Solvent Extraction and Gas Chromatography, as well as spectroscopy methods like NMR, mass spectrometry, and hyphenated techniques including LC - MS and LC - NMR. This comprehensive overview underscores the pivotal role of impurity profiling in ensuring pharmaceutical product quality and patient safety.***

Keywords: Impurities, impurity profiling, pharmaceutical products, regulatory authorities, analytical techniques

1. Introduction

The therapeutically active product comprising of active pharmaceutical ingredients (API) and excipients, the API is responsible for producing pharmacological effects. However, in some cases, the active ingredient or excipients may not be completely pure and may contain some other component that can come from a variety of sources in the medicinal product, such as synthesis, an excipient, a residual solvent, or a degradation product. Impurities are those undesirable substances that are present in addition to API and excipients (1, 2). Organic material or undesirable compounds that are still present with active pharmaceutical ingredients (APIs) are regarded as impurities in the pharmaceutical sector. The impurities are developed either during formulation or ageing of both API's and Formulation. The efficacy and safety of pharmaceutical products may be impacted by the presence of these undesirable substances (3). The quality of drugs that are introduced to the market has received a lot of attention during the past few decades. Producing quality products is a key difficulty for both the pharmaceutical and bulk medication sectors. To ensure the quality and purity of the product from each industry, rigorous quality control inspections must be carried out. The type of crystallization and purifying technique, as well as the raw materials used in their production, all affect the active medicinal ingredient's purity. The purity and superiority of the pharmaceutical product are jeopardized by even a small amount of impurity, which can endanger the patient's life. As a result of numerous regulatory requirements, the impurity profile has become crucial. Organic material or undesirable compounds that are still present with active pharmaceutical ingredients (APIs) are regarded as impurities in the pharmaceutical sector. The impurity can appear either while formulating new APIs or when formulating older ones. Regulatory agencies are paying more attention to impurity profiling. (4 - 7)

The description of known and unknown impurities found in new medicinal substances is known as impurity profiling. According to the International Conference on Harmonization (ICH), some impurities are named as follows: (8 - 15)

- Byproducts
- Degradation products
- Interaction products
- Intermediates
- Related products
- Transformation products.

a) **By - products**: Compounds created during processes which are not necessary intermediates. They occur as a result of unintended reactions between starting materials or intermediates, and unintended reactions with chemical reagents or catalysts.
b) **Degradation Products**: These are created when an active ingredient or other material becomes degraded due to the influence of outside elements including heat, light, and moisture.
c) **Interaction Products**: These products are the result of purposeful or inadvertent chemical interactions.
d) **Intermediates**: The substances created during the synthesis of the desired material or as part of the synthesis process.
e) **Related products**: These have biological activity and are chemically related to drug substances.
f) **Transformation products**: They are associated with both theoretical and improbable products that can result from a reaction. They are comparable to by - products, but more is known about reaction products.

Types of Impurity

According to USP [16, 17]

The United States Pharmacopoeia (USP) classifies impurities in various sections

a) Impurities in Official Articles
b) **Ordinary Impurities**: This can be discovered in bulk pharmaceutical chemicals, which are safe because they have no significance on the biological activity of the drug substance. These contaminants may result from chemical synthesis, preparation, or degradation.
c) **Organic Volatile Impurities**: When excipients or drug products are manufactured organic volatile chemicals are produced as well. These chemicals are volatile by nature and are removed on their own during processing or storage.

According to ICH guidelines

According to ICH guidelines, impurities in drug substance produced by chemical synthesis can be broadly classified into following three categories

1) Organic Impurities (Process and drug - related)
2) Inorganic Impurities (Reagent, ligands, catalysts)
3) Residual Solvents (Volatile solvents)

Organic impurities: They are the most common impurities found in every API unless proper care is taken throughout the multistep synthesis. They may be identified or unidentified. They may be volatile or non - volatile. It can be any of following.

a) Starting Material
b) By product
c) Intermediates
d) reagents

Inorganic impurities: They may also derive from the manufacturing processes used for bulk drugs. They are normally known & identified & include the Reagents, Ligands, Catalysts, Heavy Metals, Filter aids, Charcoals etc. Pharmacopeia or other relevant standards are typically used to identify and measure inorganic contaminants. During development, the carryover of catalysts to the drug substance should be assessed.

Residual solvents: Organic volatile compounds utilised in manufacturing processes or produced during production are residual solvents. During production of the drugs, certain solvents that are known to be toxic should be avoided. Depending on the possible risk to humans, residual solvents are divided into 3 classes, Class 1: Human carcinogens.
Class 2: Non genotoxic.
Class 3: Lower risk to human health.

Genotoxic impurities: These are the contaminants that alter the genetic code and damage DNA.
Example: Alkylation

2. Impurity Profiling Significance

It's critical to check for contaminants in the drug product throughout manufacturing in order to ensure the high standard of quality of drug goods that are released onto the market. The standard impurity spectrum is more superior and pure as compared to the online spectrum that was produced during the earlier impurity profiling investigation and is needed for registration of the drug master file. Toxicological research and impurity determination standards both employ synthetic impurities. The presence of an impurity in a pharmaceutical product can alter how easily a medication constituent dissolves and is dissolved, which can have an impact on systemic circulation. As a result, it alters the medication substance's biopharmaceutical behaviour as well as patient safety. Impurity profiling is therefore crucial for ensuring the effectiveness, safety, and quality of drugs. (18 - 20).

3. Factors Affecting Impurity [21 - 23]:

- **Stereochemistry:** Compounds with a same chemical structure but a different spatial orientation are referred to as stereochemistry - related compounds, and they might be regarded as contaminants in APIs.
- **Synthetic Intermediates & By Product:** Impurities in new chemical entities (NCEs) or medicinal compounds can develop during the synthetic process from starting materials, intermediates, and/or byproducts.
- **Residual Solvents:** Residual solvents are organic volatile compounds produced or employed as a carrier, dissolving medium, or for granulation during the manufacturing process. When making bulk drugs, certain solvents that are known to be toxic should be avoided. To assess the purity of acetone, dichloromethane, methanol, and toluene, a selective gas chromatography (GC) method has been created. The primary pollutants of each organic solvent can be quantified using this technique.
- **Formulation Related Impurities:** Excipients used to create a medicinal substance can be the source of a large number of contaminants in the drug product. A drug material is also put to a number of circumstances during the formulation process that may cause it to degrade or have other unfavourable effects. Due to hydrolysis, solutions and suspensions are intrinsically vulnerable to deterioration. In general, liquid dosage formulations are vulnerable to microbial contamination and deterioration.

A warm, humid atmosphere that encourages the growth of bacteria, fungus, and yeast can cause an oral liquid product to become unfit for safe human consumption.

- **Impurities during Storage**: Numerous impurities can develop while drug products are being stored or transported. Stability studies must be conducted in order to anticipate, assess, and guarantee the safety of pharmacological products.
- **Method Related Impurities**: Due to deviation in pH and column temperature, impurities may develop.
- **Environmental:** Effect of humidity, temperature, light may also develop impurities.
- **Mutual Interaction:** The majority of vitamins are relatively labile, and as they age, they become unstable in various dose forms, particularly liquid dosage forms. While vitamin degradation does not produce toxic impurities, the potency of the active ingredients falls short of pharmacopoeial requirements.
- **Functional group:** Several medications, including aspirin, benzocaine, cefotaxime, ethyl paraben, and cefpodoximeproxetil, undergoes ester hydrolysis. For

ester - type medications, particularly those with liquid dosage forms like benzylpenicillin, oxazepam, and lincomycin, hydrolysis is a frequent occurrence. Drugs that are susceptible to oxidative degradation include hydrocortisone, methotrexate, hydroxyl groups directly bonded to aromatic rings (viz. phenol derivatives like catecholamines and morphine), conjugated dienes (viz. vitamin A and unsaturated free fatty acids), heterocyclic aromatic rings, nitroso and nitrite derivatives, and aldehydes (especially flavourings).

4. Separation Techniques

Accelerated solvent extraction: The accelerated solvent extraction technique is distinct, quick, repeatable, efficient, and widely used. It entails the extraction of a chemically active constituent with the aid of a solvent that permeates the pores of the solid matrix in order to extract the desired chemical constituent. It has been used in a number of pharmaceutical fields. Most frequently used in the impurity profiling of drug substances, for the extraction of a natural chemical constituent from herbal plant materials, used to test for organic pollutants, nutritional supplements, insecticide residue, and microorganisms, as well as to examine environmental samples. This method makes use of high pressure and temperature to enhance the extraction process. The increased temperature will improve the kinetics of extraction, which reduces the viscosity of the sample medium and improves the diffusion of the liquid into the medium. The extraction process will be facilitated by high pressure since it will drive the solvent into the sample medium's pores. (24, 25)

Supercritical fluid extraction: A supercritical fluid (SCF) is a material that is at or above its critical temperature and pressure. It possesses both fluid and gaseous properties. The supercritical fluid extraction method possesses both liquid and gas properties, allowing it to diffuse as a gas inside the sample medium and dissolve as a liquid to speed up the mass transfer process. Carbon dioxide is a commonly used supercritical fluid due to its affordability, accessibility, and environmental friendliness. The critical temperature and pressure for CO_2 are 30.9 °C and 73.8 bar, respectively. As it continues to operate at low temperatures, it is used for samples that are thermo labile or easily oxidizable compounds. (26 - 28)

Thin - layer chromatography (TLC): The purity and identification of any substance can be verified using TLC, which is a special and highly trustworthy analytical tool. TLC would require less development time, easier visualisation of the separated component, a quicker separation process, and lower cost. It also requires a smaller sample size and uses less solvent. All contamination can be found because TLC tests a wider polarity range. (29, 30)

Gas chromatography (GC): A special investigative tool for impurity profiling is gas chromatography. Over the specifically used sample pre - treatment, it is capable of separating the volatiles from non - volatile medium. Since no other method can be used to determine a residual solvent, GC is the only way to do so. High separation power, superior selectivity, and a variety of flame ionisation detectors (FID) are all characteristics of GC. The highest sensitivity is provided by the dynamic headspace mode of sample pre - treatment. For examination of the compound that degrades when exposed to higher temperatures, derivatization procedure is necessary. The polar functional group is replaced by the derivatization process to improve its volatility and detectability. Most frequently, derivatizing agents like trimethylsilyl are utilised. (31 - 33)

High - performance liquid chromatography (HPLC): An automated separation method with high levels of sensitivity, selectivity, and resolution power is the HPLC. The method is quick and effective for separating impurities from the drug material and assessing purity. The reverse phase HPLC technology is widely utilised for the determination of contaminants in biological material. UV - detector can generate high - quality UV spectra when HPLC is used as a separation method. In this system, sample preparation is simple and error is also reduced. (34)

Capillary electrophoresis (CE): In terms of impurity profiling, capillary electrophoresis is a very useful technique. The advantage and strength of CE over other separation techniques is its high peak efficiency. The separation capability increases since CE can be used in a variety of ways. The same capillary can be used to analyse analytes ranging in size from tiny ions to bigger protein molecules, and this results in a higher separation efficiency. The majority of extraction procedures use an organic solvent, however CE is the only one that primarily uses an aqueous buffer. As a result, the global shortage of organic solvent, particularly acetonitrile, can be solved. The use of CE lessens waste generation and is environmentally friendly. Sample preparations must take place inside a capillary for analyte derivatization processes. (35 - 37)

5. Characterization Techniques:

UV - Visible Spectroscopy: In comparison to other analytical techniques, this technique is more affordable, and sample preparation is simple and quick. Require less time for analysis and offer greater precision and accuracy. Give a wide range of options for the choice of chemicals and solvents used in the analysis of the sample. Since there is no destructive effect during analysis, the sample can be recovered and used again for further analysis. (38)

Infrared (IR) spectroscopy: When the drug material is subjected to electromagnetic energy, a particular bond existing in the structure will absorb at a wavelength characteristic of the drug material. Therefore, by determining the functional group that predominates in the sample, this technique can be used to actively detect the sample structure. Even though the chromatographic technique has many benefits, such as better resolution of the impurities even in the multi - component sample, it takes a lot of time to prepare the samples. The employment of a spectroschromatographic method is a new development in the impurity profiling technique. Although the Fourier transform infrared spectroscopy (FT - IR) method is quick and inexpensive, it cannot be used directly to separate and identify impurities. Therefore, multivariate regression is a key component of chemometric technique for impurity

profiling. The chemometric method appears to be essential for obtaining information from large data sets. The combination of IR and chemometrics will streamline and enhance the approach used to check the drug substance's quality during manufacture. (39)

Mass spectrometry (MS): Mass spectrometry is a highly sensitive method for analysing a trace molecule and for illuminating the structure that offers excellent consistency, specificity, and sensitivity. Mass spectroscopy can be used to identify any biomolecules or protein molecules found in a biological sample, and the use of a soft ionisation technique can be used to examine high molecular mass, non - volatile compounds that are thermally susceptible. Ionisation of the parent molecule results in ions or fragments, which are then transported to the analyzer compartment of the instrument and resolved based on their mass to charge ratio. Information about the parent medication compound's molecular composition will be available from the mass spectrum. MS is combined with several chromatographic methods. This hyphenated method is widely used to determine the structure of impurities. (40)

Nuclear magnetic resonance (NMR) spectrometry: The magnetic property of atomic nuclei is provided by NMR spectroscopy, making it an effective technique for determining the structure of unidentified molecules. The main methods used for structural elucidation in NMR spectroscopy are ^{1}H and ^{13}C. Two - dimensional experiments used often for structural elucidation include heteronuclear single quantum coherence (HSQC) and double quantum filtered correlation spectroscopy (DFC - COSY). NMR spectroscopy is the ultimate and decisive method for structural elucidation. Modifying the pulse - field gradient, suppressing the solvent, improving the probe tools, and configuring a high magnetic field are the innovations in this technique that will offer the driving force for the structure elucidation of the unidentified impurity. Insignificant contaminants that are present before or after chromatographic separation can be found using NMR, which is a crucial duty. It is an excellent approach for identifying the configuration and structure elucidation of synthetic and organic compounds, provided that they are available in an acceptable purity and quantity, including molecular mass of no more than 50 kDa. (41 - 44)

6. Hyphenated Techniques

In the realm of analytical science, the hyphenated technique is a more contemporary method in which two or more techniques are combined with the aid of an interface. The hyphenated approach is crucial for creating the fingerprint of an unknown impurity because the standard of impurity is sometimes unavailable for its characterization. (45)

Liquid chromatography - gas chromatography (LC - GC): A very effective technique, LC - GC combines the high efficiency of GC with the wide separation mechanism of LC. It is particularly suitable for pure samples where great sensitivity and selectivity are needed. High sample capacity can be utilised by LC - GC. It is challenging to do an analysis of a small sample size in offline mode due to factors like lengthy analysis, labor - intensive operation, and poor reproducibility. As a result, online analysis is a quick, dependable, and effective tool for increasing sensitivity and analysing multiple sample types. The method is quicker, fully automated, more sensitive, and highly reproducible. Using less solvent will help cut down on issues with manipulating samples or producing artefacts as a result of atmospheric air interference. (46)

Gas chromatography - Mass spectrometry (GC - MS): A potent technique for chemical analysis and structural elucidation of a volatile and thermally stable molecule is GC - MS. With the aid of GC and MS, if they are sensitive enough, analyte separations can be accomplished using coupling and spectral data. There are certain an important aspect of it, such as progressive chromatographic resolution, which improves peak capability, only needs one mobile phase for separation, and causes fewer issues with solubility and separation than those that can be achieved using electronic controls like heat programming. A small, significantly less expensive, and well - known instrument in modern pharmaceutical labs is the GC - MS combination. The role of GC - MS in determining the residual solvent is crucial. In addition to completing the impurity profiling, the structural elucidation may also be accomplished. If the peak is linked to solvent interference, it can be identified, revealing the existence of the poisonous and dangerous class I solvent. The main advantage of GC - MS is that it may provide molecular mass evidence using a chemical ionisation method and information on fragmentation using an electron impact (positive and negative charges) ionisation approach to clarify the composite structure. (47 - 49)

High - performance liquid chromatography - Mass spectrometry (HPLC - MS): Due to LC - MS's great selectivity, sensitivity, dynamic range, and robustness, it has been widely used in the field of pharmaceutical research. It is extremely sensitive to even minute amounts of impurities and deteriorating products. The advancement of technology has led to an increase in the use of tandem LC - MS, commonly known as LC - MS/MS. It can be used to quantitatively analyse and reveal the structure of an unknown impurity. Since there is less chance of flow rate and solvent interference at the LC - MS interface, the LC and MS can be skillfully coupled. One of HPLCMS's key advantages is its compatibility with UV photodiode array detectors. HPLC/UV/MS/MS is the method most frequently used to provide all the data necessary for the impurity's structure explanation. By using the LC - MS in the multiple reaction monitoring modes, which will run the experiment (n) times, where (LC - MS^{n}) is the number of MS - MS experiments, the experiment will be run (n) times. For structure elucidation, this method is frequently utilised. (50, 51)

Liquid chromatography - nuclear magnetic resonance spectrometry (LC - NMR): Sensitivity problems exist with LC - NMR; in this context, sensitivity refers to the ability of the NMR spectrometer to collect enough data to enable the clear structure elucidation of the trace intensity in a compound mixture under HPLC analysis. Since the signal - to - noise ratio for cryoprobes is four times higher than that of regular probes, only 0.1 µg of sample is needed for a cryoprobe instead of the usual 0.5 µg. As a result, it cuts

down on both the sample needed for analysis and the amount of time it takes to complete it. The use of LC - NMR would speed up and simplify identification and analysis by eliminating the labour - and time - intensive isolation phase. (52 - 57)

LC - MS/NMR: All pharmaceutical laboratories use directly coupled LC - MS/NMR. LC - MS/NMR is the name given to the coupling of the HPLC with the MS and NMR technologies. In this type of hyphenated technique, the eluent from the HPLC column is divided into two relatively equal portions, with one portion going to the MS (ESI) and the other portion going to the NMR spectrometer because the MS has higher sensitivity than the NMR spectrometer. The connection of the two data types enables a clear correlation between the NMR spectra and a specific trace level analyte. But for a complete structural elucidation, the MS spectra are insufficient. Thus, the coupling of LC, MS, and NMR will provide further details about the drug's structural configuration, functional group, and NMR silent heteroatoms (N, Cl, and O) present in unidentified impurities. It is a fully automated process with a small sample size need. Reduce the amount of time required for analysis while minimising the possibility of sample deterioration and repurposing the sample. (58)

Capillary zone electrophoresis - Mass spectrometry (CZE - MS): The key justification for combining capillary zone electrophoresis with mass spectroscopy is that CE's optimal separation power has been attained, whilst mass spectra will suffice to reveal structural information. To get greater resolution of separated impurities, there should be a high degree of orthogonality between the procedures used during impurity separation. The connection of CE with several MS ionisation systems, including electrospray ionisation (ESI - MS), atmospheric pressure chemical ionisation (APCI - MS), atmospheric pressure photoionization (APPI - MS), and thermospray ionisation (TSI - MS), are different approaches for impurity profiling. While the APCI - MS and APPI - MS are unable to detect the ionic sample, the ESI - MS and TSI - MS are useful for detecting the ionic compound. As a result, it can also help to tell an unknown impurity whether it is ionic or non - ionic. APCI is typically used to detect less polar chemicals and solutions that have not altered. Due to its sensitivity and softness, ESI - MS is primarily used. (59 - 61)

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