Recent Approaches of Impurity Profiling In Pharmaceutical Analysis
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ABSTRACT

There is an ever-increasing interest in impurities present in APIs. Now days, not only purity profile but also impurity profile has become mandatory according to various regulatory authorities like USFDA, CDSCO, MHRA, TGA. In the pharmaceutical world, an impurity is considered as any other inorganic or organic material, or residual solvents other than the drug substances or ingredients. Impurity profiling includes identification, structure elucidation, quantitative determination of impurities, degradation products in bulk drug materials and pharmaceutical formulations.

Identification of impurities is done by variety of Chromatographic and Spectroscopic techniques, either alone or in combination with other techniques. There are different methods for detecting and characterizing impurities with TLC, HPLC, HPTLC, etc. The present review covers various aspects related to the analytical method development for impurity profiling of Active Pharmaceutical ingredient and pharmaceutical products and the possible measures to deal with the interferences caused by them in pharmaceutical analysis.

KEYWORDS:
Impurity profiling, Gas Chromatography, HPLC, Hyphenated Methods, ICH guidelines

1.INTRODUCTION

Impurities in pharmaceuticals are unwanted chemicals, that even in small amounts may influence the efficacy and safety of the pharmaceutical products.

Impurity Profiling:
Impurity profiling is the common name of a group of analytical activities, the aim of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations. The different pharmacopoeias

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Such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP) are slowly incorporating limits to allowable levels of impurities present in the API’s or formulations. Quantification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research. International Conference on Harmonization (ICH) has published guidelines on impurities in new drug substances, products and residual solvents.

There is a good significant demand for the impurity-reference standards along with the API reference standards from both regulatory authorities and pharmaceutical companies. The estimation of impurity profiles in drug substances and related materials has become one of the most important fields of activity in contemporary pharmaceutical analysis. In general, all impurities present more than 0.1% should be identified for the following reasons. On the basis of the information thus obtained synthetic organic chemists are often able to avoid...
The formation of the impurity or developed a purification method to decrease its quantity to a tolerable level.\textsuperscript{1-4} For structural identification of an unavoidable impurity, it may be synthesized to provide a sufficient amount for:

- Final proof of its structure;
- Its use as an “impurity standard”;
- Its use in toxicological studies.

**The various regulatory guidelines regarding impurities are as follows:**

- ICH guidelines “stability testing of new drug substances and products”- Q1A
- ICH guidelines “Impurities in New Drug Substances”-Q3A
- ICH guidelines “Impurities in New Drug Products”- Q3B
- ICH guidelines “Impurities: Guidelines for residual solvents”- Q3C
- US-FDA guidelines “NDAs - Impurities in New Drug Substances”
- US-FDA guidelines “ANDAs – Impurities in New Drug Substances”
- Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia.

These guidelines define what investigations and documentation should be made in investigating impurities and degradation products seen in stability studies at recommended storage conditions.

**Table 1. Threshold Specifications**

<table>
<thead>
<tr>
<th>Max. daily dose</th>
<th>Reporting Threshold</th>
<th>Identification Threshold</th>
<th>Qualification Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2g/day</td>
<td>0.05%</td>
<td>0.10% or 1.0 mg per day intake (whichever is lower)</td>
<td>0.15% or 1.0 mg per day intake (whichever is lower)</td>
</tr>
<tr>
<td>&gt; 2g/day</td>
<td>0.03%</td>
<td>0.05%</td>
<td>0.05%</td>
</tr>
</tbody>
</table>

Impurities commonly found in Medicinal preparations are\textsuperscript{5-8}

1. Activity depressing impurities.
2. Due to colouring or flavouring substances, e.g., Sodium Salicylate.
3. Humidity.
4. Residual solvents.
5. Impurities due to which substances become incompatible.

**Figure 1:** Flow diagram of Impurity profile

**Figure 2:** Classification of impurity

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Anions like Cl⁻ and SO₄²⁻ are common impurities in many substances because of the use of hydrochloric acid and sulphuric acid respectively in processing. Barium ion may be an impurity in hydrogen peroxide so, hydrogen peroxide employed as reagent in the manufacturing process can contaminate the final product.

**Regents used to eliminate other impurities:**
Barium is used in the preparation of potassium bromide to remove sulphate which in turn arises from the bromine used in the process. It is noticed that potassium bromide will now be contaminated by traces of barium.

**Solvents:** Most of the pharmaceutical substances are prepared in solvated crystalline form. Small amounts of solvents employed in preparation, and purification of reaction intermediates or the final product may also result in the contamination of the pharmaceutical substances. Water is the cheapest solvent available and is used quite frequently in the preparation of inorganic pharmaceuticals. Water can be the major source of impurities as different types of water containing different types and amount of impurities.

**Various types of water which are available are:**
- **Tap water:** Containing impurities of Ca²⁺, Mg²⁺, Na⁺, Cl⁻, CO₃⁻², and SO₄²⁻ in trace amounts. The use of tap water on large scale will lead to the contamination of the final product with these impurities because the impurities will remain in the product even after washing.
- **Softened water:** It is almost free from divalent cations (Ca²⁺, Mg²⁺) but contains more of Na⁺ and Cl⁻ ions as impurities. Therefore, the final products obtained using softened water as solvent will not have Ca²⁺ and Mg²⁺ impurities but still contain Na⁺ and Cl⁻ impurities.
- **De-mineralized water:** It is prepared by means of ion-exchange and is free from Na⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄²⁻, and CO₃⁻² etc. It may have Pyrogen, bacteria and organic impurities. So, it is a better solvent than tap water and softened water and it is economic.

**Distilled water:** It is free from all organic and inorganic impurities and is the best solvent, but it is quite expensive. As it is free from all impurities, it does not pass-on any impurities to the final products.

**Reaction vessels:** The reaction vessels employed in the manufacturing process may be metallic such as copper, iron, cast iron, galvanized iron, silver, aluminium, nickel, zinc and lead. Glass and silica are also used in the construction of the chemical plants but these days many of these are replaced by stainless steel and variety of other alloys. Some solvents and reagents employed in the process may react with the metals of reaction vessels, leading to their corrosion and passing traces of metal impurities into the solution, contaminating the final product.
**Intermediates:** Sometimes, an intermediate substance produced during the manufacturing process may contaminate the final product e.g., Sodium bromide is prepared by reaction of sodium hydroxide and bromine in slight excess. 

\[ 6 \text{NaOH} + 3\text{Br}_2 \rightarrow \text{NaBrO}_3 + 5\text{NaBr} + 3\text{H}_2\text{O} \]

The sodium bromate an intermediate product is reduced to sodium bromide by heating the residue (obtained by evaporating the solution to dryness) with charcoal.

\[ \text{NaBrO}_3 + 3\text{C} \rightarrow \text{NaBr} + 3\text{CO} \]

If sodium bromate is not completely converted to the sodium bromide then it is likely to be present as an impurity.

**Manufacturing Hazards:** If the manufacturer is able to control and check impurities from the all above mentioned sources also still there exists certain manufacturing hazards which can lead to product contamination. The various manufacturing hazards can lead to:

- **Contamination from the Particulate Matter:** The unwanted particulate matter can arise by several ways, such as accidental inclusion of dirt or glass, porcelain, plastic or metallic fragments from sieves, granulating, tabletting and filling machines and the product container. The particulate contamination mainly arises from the wear and tear of the equipments. It may also arise from the bulk materials used in the formulation or from dirty or improperly maintained equipments e.g., metal particles found in eye ointments packed in metal tubes made up of tin and aluminium.

- **Cross-contamination of the Product:** This manufacturing hazard must be considered in the preparation of solid dosage forms. Cross-contamination of product can occur due to air-born dust arising out of handling of powders, granules and tablets in bulk. Cross-contamination is dangerous particularly in case of steroidal and other synthetic hormones and therefore, it should be carefully controlled.

- **Contamination by Microbes:** Many products, like liquid preparations and creams intended for topical applications are liable to contamination by microbes from the atmosphere during manufacturing. For all products intended for parenteral administration and ophthalmic preparations sterility testing is done, and it provides an adequate control for microbial contamination in such preparations. Microbial contamination can be controlled by adding suitable antimicrobial and antifungal agents.

- **Instability of the Product:**

**Chemical Instability:** Impurities can also arise during storage because of chemical instability of the pharmaceutical substance. Many pharmaceutically important substances undergo chemical decomposition when storage conditions are inadequate. This chemical decomposition is often catalysed by light, traces of acid or alkali, traces of metallic impurities, air oxidation, carbon dioxide and water vapours. The nature of the decomposition can easily be predicted from the knowledge of chemical properties of the substance. All such decompositions can be minimized or avoided by using proper storage procedures and conditions.

**Changes in Physical Properties:** Pharmaceuticals may undergo changes in physical properties during storage. There can be changes in crystal size and shape, sedimentation, agglomeration and caking of the suspended particles. These physical changes are not always avoidable and may result in significant changes in the physical appearance, pharmaceutical and therapeutic effects of the product.

- **Reaction with container material:** The possibility of reaction between the container material and the contents cannot be ruled out as it constitutes a safety hazard. Preparations susceptible to reaction with metal surfaces e.g., salicylic acid ointment must not be packed in metal tubes. Solutions of substances which are alkali-sensitive e.g., atropine sulphate injection must be packed in glass ampoules which comply with the test of hydrolytic resistance therefore such preparations must not be packed in containers made from soda glass.

- **Temperature:** The rate of chemical decomposition and physical changes of stored products depends upon the temperature. The susceptible substances may have temperature storage requirements assigned to them in order to protect them against undesirable decomposition.

- **Crystallization-related impurities:** Based on the realization that the nature of structure adopted by a given compound upon crystallization could exert a profound effect on the solid-state properties of that system, the pharmaceutical industry is required to take a strong interest in polymorphism and solvatomorphism as per the regulations laid down by the regulatory authorities.

- **Polymerization-related impurities:** Polymorphism is the term used to indicate crystal system where substances can exist in different crystal packing arrangements, all of which have the same elemental composition. Whereas, when the substance exists in different crystal packing arrangements, with a different elemental composition; the phenomenon is known as Solvatomorphism.

- **Stereochemistry-related impurities:** It is of paramount importance to look for stereochemistry related compounds, that is those compounds that have similar chemical structure but different spatial orientation, these compounds can be considered as impurities in the API’s. Chiral molecules are frequently called as enantiomers.

**Residual solvents:** Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. Some solvents that are known to cause toxicity should be avoided in the production of bulk drugs. Depending on the possible risk to human health, residual solvents are divided into three classes.
Especially, solvents in Class I; viz benzene (2 ppm limit), carbon tetrachloride (4 ppm limit) should be avoided. In Class II; viz methylene chloride (600 ppm), methanol (3000 ppm, pyridine (200 ppm), toluene (890 ppm), N, N-dimethylformamide (880 ppm), acetonitrile (410 ppm), should be limited. Class III solvents; viz acetic acid, ethanol, acetone have permitted daily exposure of 50 mg or less per day, as per the ICH guidelines.

A selective gas chromatography (GC) method has been developed to determine the purity of acetone, dichloromethane, methanol and toluene. Using this method, the main contaminants of each organic solvent can be quantified.

3. SYNTHETIC INTERMEDIATES AND BY-PRODUCTS:

Impurities in pharmaceutical compounds or a new chemical entity (NCE) can originate during the synthetic process from raw materials, intermediates and/or by-products.

Formulation-related impurities: Many impurities in a drug product can originate from excipients used to formulate a drug product. In addition, a drug substance is subjected to a variety of conditions in the process of formulation that can cause its degradation or have other undesirable reactions. If the source is from an excipient, variability from lot to lot may make a marginal product, unacceptable for reliability. Solutions and suspensions are inherently prone to degradation due to hydrolysis or solvolysis. Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was recalled in the United States because of degradation/impurities leading to decreased potency.

In general, liquid dosage forms are susceptible to both degradation and microbial contamination. In this regard, water content, pH of the solution/suspension, compatibility of anions and cations, mutual interactions of ingredients, and the primary container are critical factors. Microbial growth resulting from the growth of bacteria, fungi, and yeast in a humid and warm environment may result in unsuitability of an oral liquid product for safe human consumption.

Impurities arising during storage: A number of impurities can originate during storage or shipment of drug products. It is essential to carry out stability studies to predict, evaluate, and ensure drug product safety.

Method Related Impurity: A known impurity, 1-(2, 6-dichlorophenyl) indolin-2-one is formed in the production of a parenteral dosage form of diclofenac sodium, if it is terminally sterilized by autoclave.

Selective Analytical Methodologies\textsuperscript{[11-15]}

Development of a new drug requires generation of meaningful and reliable analytical data at various steps of the new drug development. Ensuring the safety of a new pharmaceutical compound or drug requires that it meet the established purity standards as a chemical entity or when admixed with animal feeds for toxicity studies or pharmaceutical excipients for human use.

These requirements demand that the analytical methodology that is used be sensitive enough to measure low levels of impurities. This has led to analytical methods that are suitable for determination of trace/ultra-trace levels, i.e., sub-microgram quantities of various chemical entities. A variety of methods are available for monitoring impurities. The primary criterion is the ability to differentiate between the compounds of interest.

New drug development requires meaningful and reliable analytical data to be produced at various stages of the development.

Sample set selection for analytical method development.

Screening of chromatographic conditions and phases, typically using the linear solvent-strength model of gradient elution.

Optimization of the method to tune parameters related to ruggedness and robustness.

The impurities can be identified predominantly by following methods

Reference standard method

Spectroscopic method

Separation method

Isolation method

Characterization method

Reference standard method: The key objective of this is to provide clarity to the overall life cycle qualification and governance of reference standard used in development and control of new drug. Reference standards serve as the basis of evaluation of both process and product performance and are the benchmarks for assessment of drug safety for patient consumption. These standards are needed, not only for the active ingredients in dosage forms but also for impurities, degradation products, starting materials, process intermediates, and excipient.

Spectroscopic methods: The following spectroscopic methods can be used;

- Ultraviolet (UV)
- Infrared (IR)
- Nuclear magnetic resonance (NMR)
- Mass spectrometry (MS)

Ultraviolet (UV): UV at a single wavelength provides minimal selectivity of analysis; however, with the availability of diode array detectors (DAD), it is now possible to get sufficient simultaneous information at various wavelengths to ensure greater selectivity.

Infrared Spectrophotometry: Infrared Spectrophotometry provides specific information on some functional groups that may allow quantification and selectivity. However, low level detectability is frequently a problem that may require more involved approaches to circumvent the problem.
Nuclear Magnetic Resonance Spectroscopy:
Nuclear magnetic resonance spectroscopy provides structural information of a molecule and is a very useful method for characterization of impurities, however, it has limited use as a quantitative method because of cost and time considerations.

Mass Spectrometry: Mass spectrometry provides excellent structural information and based on the resolution of the instrument it may provide an effective tool for differentiating with small differences in molecular weight. However, it has limited use as a quantitative technique because of cost and time considerations.

In summary, IR, NMR, and MS are excellent techniques for characterization of impurities that have been isolated by any of the techniques discussed above. UV has been found to be especially useful for analysing most samples with high-pressure liquid chromatography. This combination is commonly used in pharmaceutical analysis.

Separation methods: The following separation methods can be used,
- Thin-layer chromatography (TLC)
- Gas chromatography (GC)
- High-pressure liquid chromatography (HPLC)
- Capillary electrophoresis (CE)
- Supercritical fluid chromatography (SFC)

Except CE all these techniques are chromatographic methods. CE is an electrophoretic method that is frequently lumped with the chromatographic methods because it shares many of the common requirements of chromatography. However, it is not strictly a two-phase separation system — a primary requirement in chromatography.

Isolation method: It is often necessary to isolate impurities. But if the instrumental methods are used, isolation of impurities are avoided as it directly characterizes the impurities.

I. Solid-phase extraction method
II. Liquid-liquid extraction method
III. Accelerated solvent extraction method / Supercritical fluid extraction
IV. Column chromatography
V. Flash chromatography
VI. Supercritical fluid chromatography (SFC)

Hyphenated methods / characterization methods:
The following hyphenated methods can be used effectively to monitor impurities.
- HPLC–DAD, NMR–MS

Analytical procedures:[16-20]

4.METHOD DEVELOPMENT:
Method development usually requires the choice of columns, mobile phase, detectors, and method of quantization etc. Development of new method is required when the,

Existing method may be inaccurate, artefact, contamination prone, or they may be unreliable (have poor accuracy or precision).

Existing method may be too expensive, time consuming or energy intensive, or they may not be easily automated.

Existing methods may not provide adequate sensitivity or analyte selectivity in samples of interest.

Newer instrumentation techniques may be evolved which can provide opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision and better returns on investments.

Validation of Analytical Methods:
Validation is the process of establishing a documentary evidence to a developed method. The validation process involves confirmation or establishing a developed method by laboratory studies, procedures, systems, which can give accurate and reproducible result for an intended analytical application in a proven and established range. The performance characteristics of the method should meet the requirements of the intended analytical applications and the process can provide documented evidence that the system or procedure do what it is intended for in a systematic, precise and reliable manner. According to ICH, typical analytical performance characteristics that should be considered in the validation of all the types of methods are Specificity, accuracy, precision, Linearity, sensitivity, Range, LOD, LOQ, Robustness and ruggedness.

5.APPLICATIONS:
Numerous applications have been sought in the areas of drug designing and in monitoring quality, stability and safety of pharmaceutical compounds, whether produced synthetically, extracted from natural products or produced by recombinant methods. The applications include different classes of drugs namely alkaloids, amines, amino acids, analgesics, antibacterial, anticonvulsants, anti-depressant, tranquilizers, antineoplastic agents, local anaesthetics, macromolecules, steroids and miscellaneous.[21-24]
Identify significant impurities

Determine origin of impurities and method for elimination or reduction

Establish a control system for impurities involving:
1) Processing/manufacturing conditions
2) Suitable analytical methods/specifications

Identify potential degradation product through stress testing and actual degradation products through stability studies.

Understand degradation pathway and methods to minimize degradation.

Establish a control system for impurities involving:
1) Processing/manufacturing conditions
2) Suitable analytical methods/specifications
3) Long term storage conditions including packaging
4) Formulation.

**Table 2: Goals of Impurity Investigations**
CONCLUSION
This review provides a perspective on Isolation and characterization of impurities. Isolation and characterization of impurities is mandatory for acquiring and evaluating data that establishes biological safety, efficacy which reveals the need and scope for impurity profiling of drugs in pharmaceutical research.

Conflicts Of Interest
The authors do not have any conflict of interest.

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