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A Review on Impurity Profiling of Pharmaceuticals

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ABSTRACT

Impurity plays a serious role in pharmaceuticals; thus, identification of impurity is incredibly vital within the pharmaceutical analysis which incorporates identification, structure elucidation and quantitative determination of impurities and degradation product in bulk drug materials and pharmaceutical formulations. . Impurity is any substance coexistent with the initial drug like beginning material or intermediates or that is formed due to any side reaction. API's present within the formulations contain these reasonably unwanted impurities, which affect purity of the API's. Therefore, with % purity, impurity identification is additionally required to be dole out of all the API's. The presence of those unwanted chemicals, even in touch, could influence the effectiveness and safety of the pharmaceutical product. Thus, Impurity profiling helps us in various ways to get a pure substance with less toxicity and safety in drug medical aid. Nowadays, it's a compulsory demand in varied pharmacopeias to grasp the impurities present in API's and finished drug product. Thus, impurity profiling will act as a top quality management tool. The present review covers the valuable information regarding the impurities, its classification, and sources of impurities and varied techniques of isolation and characterization, analytical techniques for the determination, qualification of impurities are described. Guidelines and limit for impurity present within the pharmaceutical and impurity profiling as per ICH is mentioned.

KEYWORDS: Impurity Profiling, Impurity, Intermediates, Degradation Product, Gas Chromatography, HPLC.

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INTRODUCTION:

Various drug formulations that are meant to be administered within the body for providing one or more desired pharmacological actions contain both active and inactive ingredients. The active pharmaceutical ingredient, i.e. API is chargeable for therapeutic efficacy and inactive ingredients don't have pharmacological activity. Generally, APIs present within the formulations don't seem to be fully pure, they contain some impurity. As medicines are meant for saving lives and even minute quantities of impurities are unacceptable. Thus, it's invariably necessary to hold out impurity profiling.

IMPURITY: Impurity is any unwanted material that affects the purity of material of interest i.e. active pharmaceutical ingredient or drug substance. Associate impurity is taken into account as the other organic material, besides the drug substance, or ingredients, arising out of synthesis or unwanted chemicals that remain with APIs.

IMPURITY PROFILING: Impurity profiling of a substance under investigation is description of most attainable sorts of identified or unidentified impurities present in any sample of APIs created by a particular controlled production method. It's the simplest way to characterize the standard and stability of bulk medicine and pharmaceutical formulations. It helps in identifying and quantifying the impurities present in drug substance (API) or pharmaceutical formulation.

LIMITS FOR IMPURITIES:

According to the ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level is not measured to be necessary unless otherwise potential impurities are expected to be unusually potent or toxic. ICH has given following limits for impurities:

When the dose is less than 2 gm/day impurity present should be less than 0.1 % or 1 mg/day intake, whichever is lesser.

When the dose is more than 2 gm/day impurity present should be less than 0.05 % of intake.

REGULATORY GUIDELINES ON IMPURITY:

The United States Food and Drug Administration (FDA) inscribe International Conference on Harmonization guidance of Technical Requirements for Registration of Pharmaceuticals for Human Use. The FDA has the assigned responsibility of ensuring the safety and efficacy of drugs. The various regulatory guidelines regarding impurities are as follows:

1. ICH guidelines "Stability Testing of New Drug Substances and Products"- Q1A.

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2. ICH Guidelines "Impurities in New Drug Substances"- Q3A.

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

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

5. US-FDA Guidelines "NDAs- Impurities in New Drug Substances".

6. US-FDA Guidelines "ANDAs- Impurities in New Drug Substances".

7. Australian Regulatory Guideline for Prescription of Medicines, Therapeutic Governance Authority (TGA), Australia

IMPORTANT TERMS USED TO DESCRIBE IMPURITIES:

1. By-Products: These are the compound made within the reaction apart from the specified intermediates .The by- product can be formed due to side reactions, or the unwanted reactions which will occur between intermediates or beginning materials or intermediates which can react with a catalyst or any chemical reagents used.

2. Intermediates: These are the carry forward compounds formed during multi-step synthesis process.

3. Degradation Products: they are produced as a result of decay of the API is usually referred to as degradation products or degradants.

4. Interaction Products: they're difficult to evaluate than by-products and degradation products, these are formed due to interactions of assorted chemicals involved within the synthesis of the API.

5. Penultimate Intermediate: it's the last compound within the synthesis chain before the production of the final desired compound.

6. Transformation Products: they're associated with theorized and non theorized products which will occur in a reaction. They're kind of like by-products except that more is known regarding these reaction product.

7. Related Products: These are chemically similar to drug substance. However, the structure alone doesn't give any surety regarding biological activity.

SOURCES OF IMPURITIES:

Impurities in formulations can originate from a number of sources. Sources of impurities involve,

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APIs related impurities: It can be due to stereochemistry, crystallization, functional group of APIs.

Stereochemistry: Stereochemistry of APIs can be a cause of impurities. Generally, single enantiomeric form of any API is taken into account as an improved chemical entity that may offer a better pharmacological efficacy and an increased therapeutic index than other enantiomers or racemic mixture. E.g. ofloxacin is marketed as S-conformation (levofloxacin).

Crystallization: Crystallization is a cause of generation of impurities in the APIs. A crystalline drug can exist as more than one polymorph or pseudomorph. Polymorphs differ from each other in their physical properties as solubility, crystal shape, density, melting point, vapour pressure, optical and electrical properties. Polymorphism can influence biopharmaceutical behaviour of the drug. E.g. bioavailability of more soluble B form of chloramphenicol palmitate was better after oral administration as compared to less soluble A form or their mixture.

Functional Group: Various functional groups are responsible for degradation of a chemical entity as these undergo some reactions which are responsible for generation of degradation products or impurities. Various drugs containing ester moiety such as aspirin, benzocaine, cefotaxime, ethyl paraben, etc. are very susceptible to hydrolysis.

Process Related Impurities: These are the impurities which are intermediates and by-products formed during production process.

Chemicals, Reagents and Catalysts: The chemicals, reagents and catalysts used for the synthesis of APIs that may remain in finished product. In some cases they may cause a problem as impurities. Presence of such impurities has degradation effect on products.

Residual Solvents: These are organic chemicals which are volatile in nature and used or generated during production process. Those solvents that are identified to cause toxicity are avoided. Solvents are classified into three classes.

- ***Class 1 (Solvent to be Avoided):*** Solvents of class 1 are usually unacceptable because of their toxicity or harmful environmental effects. However, if their use is inescapable, then their levels should be below the limits specified by regulatory authority. Some common class 1 solvents are shown in Table 1.

Table 1.	**Class 1 Solvents**	
Solvent	**Concentration (ppm)**	**Adverse Effect**
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

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- ***Class 2 (Solvents to Be Limited)***: Use of solvents as per class 2 is extremely restricted in pharmaceutical products due to their inherent toxicity. Permitted Daily Exposure (PDE) of these solvents is given to nearest 0.1 mg/day, and concentrations are given to nearest 10 ppm. Some common class 2 solvents are shown in Table 2.

Table 2.
Class 2 Solvents

Solvent	**Permitted Daily Exposures (mg/day)**	**Concentration Limit (ppm)**
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
Formamide	2.2	220
1,4-Dioxane	3.8	380

- ***Class 3 (Solvents with Low Toxic Potential)***: Those solvents which are less toxic and of lower risk to human health are considered under class 3 solvents. Class 3 solvents are considered non-hazardous to human health, if these are present in pharmaceutical products at normally accepted levels. It is considered that amounts of these residual solvents of 50 mg per day or less would be acceptable without justification. Some common class 3 solvents are shown in Table 3.

Table 3.
Class 3 Solvents

Acetic acid	Cumene
Acetone	Dimethylsulfoxide
Anisole	Ethanol
1-Butanol	Ethyl acetate
2-Butanol	Ethyl ether
Butyl acetate	Ethyl formate
Isopropyl acetate	Formic acid

***Synthetic Intermediate Products*:** These are the most common impurities found in each APIs and finished product unless a proper care is taken in every step concerned throughout multi-step synthesis. Synthetic intermediate formed during synthesis of pharmaceutical compounds can be present in final product, e.g. Methylene dioxy methamphetamine (MDMA), used in tablets produce intermediate impurities via reductive amination.

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By Product: When carrying out a synthesis, it is very difficult to get a single end product with 100 % yield. Probability of generation of undesired products is very high, e.g. in the synthesis of paracetamol in bulk, diacetylated paracetamol may form as by-product.

Method Conditions Related Impurities: Numerous operations carried out during formulation process of a drug product may generate some unwanted products or impurities. These impurities are required to be determined if they are more than the limits given by regulatory authorities. e.g. in the production of parenteral dosage form of diclofenac sodium, when terminal sterilization is carried out, autoclaving (i.e., 123 + 2 °C) enforces the intra-molecular cyclic reaction of diclofenac sodium forming a 1-(2, 6- dichlorophenyl) indolin-2-one and sodium hydroxide.

Stability Related Impurities: Each pharmaceutical entity whether it is an API or any formulation has a specific shelf-life. Throughout their shelf-life period a drug is subjected to various storage and shipment conditions of temperature, humidity, and exposed to light. Other factors including components of a dosage form, ageing, storage condition etc., are also responsible for degradation of APIs and result in the formation of degradation products or impurities which are undesired.

Degradation or Transformation of APIs: A pharmaceutical product is subjected to various stress conditions during shelf life period like temperature, humidity, photo-degradation, etc. e.g. degradation of penicillin and cephalosporin is a well-known example of degradation products.

Mutual Interactions between APIs: Impurities can form due to interaction of APIs in a multi-component drug product. Vitamins are one of the major examples of drugs that undergo degradation during their shelf-life but their degradation does not give toxic impurities.

Types Of Impurities:

There are two types of impurities in medicines:

1) Impurities associated with active pharmaceutical ingredients (APIs).

2) Impurities that are formed during formulation and or with ageing or that are related to the formulated forms.

According to ICH guidelines, Impurities associated with APIs are classified into the following categories:

• Organic impurities (Process and Drug related)

• Inorganic impurities

• Residual solvents

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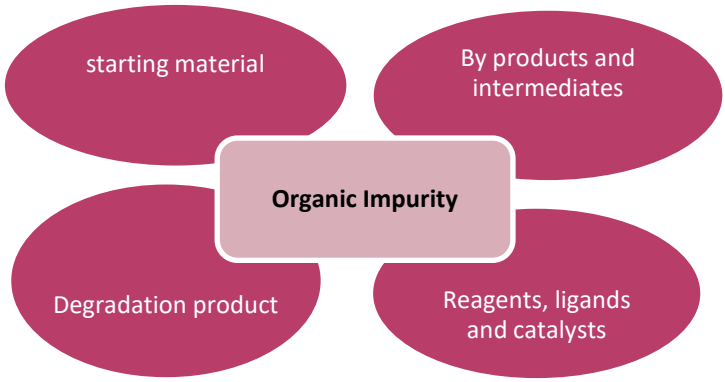
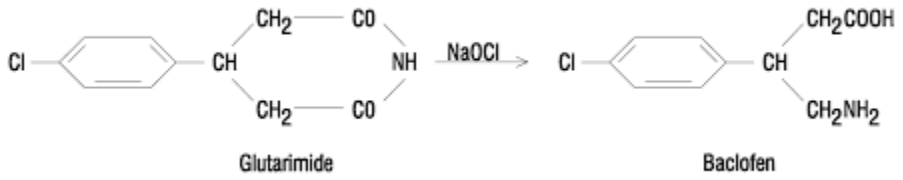
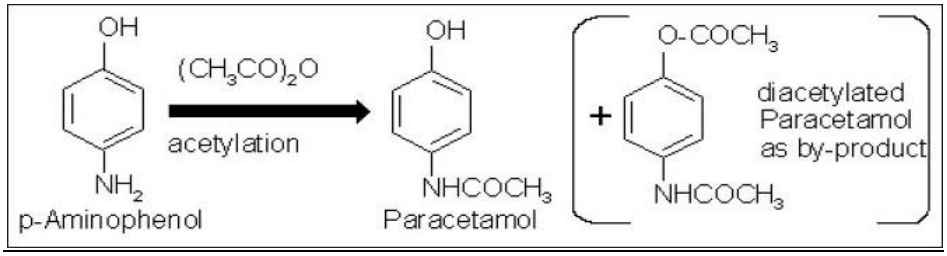
Organic Impurity: These impurities may arise during the manufacturing process or storage of the new drug substances, which includes starting materials, by-products, intermediates, degradation products, intermediates, reagents, ligands, and catalysts. They are volatile or non volatile substances which can arise from contamination of one enantiomeric form with another or racemisation. This could result in undesired biological activity.



- *Starting Materials or Intermediate Impurities:* If proper care is not taken in each step during the multistep synthesis of drug, these impurities are present in almost every API. We can see this during the synthesis of Baclofen, pchloro phenyl glutaric acid is an impurity which is formed in the last step carried when beta (Chlorophenyl) glutarimide on reacting with NaOH at room temperature.



- *By-Products*: In the process of drug synthesis, getting a single end product with 100 % yield is very difficult, as the undesired product is always formed, e.g. during the synthesis of Paracetamol, by-product may be formed that is diacetylated paracetamol. Synthesis of Paracetamol from P-Aminophenol by acetylation is explained in fig below.



- *Degradation Products:* During the manufacturing of bulk drugs impurities can be formed by degradation of the end product. Degradation during improper storage or due to aging

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of drug is also a major source of degradation. The degradation of Penicillins and Cephalosporins by acid or base is a very good example of degradation products.

- *Synthesis Related Impurities*: During the process of synthesis, from raw material, solvent, intermediate, by product the completely new chemical entity are being generated. In the process of synthesis, if any impurity is present in trace or may be an insignificant amount in any of substance involved in the reaction, then it will finally result in the production of final product which is contaminated with one or more unwanted materials that can be called as impurity.

Inorganic Impurity: Inorganic impurities are also obtained from the manufacturing processes that are employed in bulk drug formulation. They are usually known and identified. The various inorganic impurities include:

- *Reagent, Ligands and Catalysts:* Rare probabilities of prevalence of those impurities. If during manufacturing procedure is not followed properly will create a problem.
- *Heavy Metals:* Water is usually employed in different manufacturing processes which act as the main source of heavy metals, like Ar, Cd, Cr, Na, Mg, Mn, etc., where acidification or acid hydrolysis takes place. By using demineralized water and glass-lined reactors heavy metal impurities can be easily avoided.
- *Other Materials (Filter Aids, Charcoal):* The filters or filtering aids like centrifuge bags are routinely used in the bulk drugs manufacturing plants and in several cases, activated carbon is also used which also act as a source of impurity. Therefore to avoid the contamination, regular observation of fibers and black particles in the bulk drugs is essential.

Residual Solvents: They are volatile chemicals which are used during the process of manufacturing. Most these solvents are toxic and also cause environment hazard, and their complete removal is also very difficult. Residual solvents can be divided into 3 classes based upon the risks they'll probably cause to human health. Class 1 Solvents are human carcinogens, which can cause environmental hazards. They should be avoided in pharmaceutical product. Class 2 Solvents are Non-genotoxic animal carcinogens are found to be responsible for irreversible toxicity such as neurotoxicity or teratogenicity in case of pregnant women. Class 3 Solvents are less toxic to human health are considered under class 3 solvents, and if the presence of that substances in human body is normal. However, class 3 solvents don't seem to be having any long-term toxicity or carcinogenicity. (Examples of these 3 classes are given in table 1, table 2, and table 3).

Analytical Methods of Impurity Profiling:

The impurities can be identified predominantly by following methods,

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- Reference standard method
- Spectroscopic methods
- Separation methods
- Isolation method
- Characterization method

Reference Standard Method:

We know that the reference standards enable us to understand the essential data for evaluating and observing the performance of bulk drug, by- products, impurities, degradation products, excipients, raw materials, intermediates. Main purpose of this method is to quantify and to control reference standards which are used in the process of development and control of new drugs. Reference standards serve as the basis of evaluation of both process and product performance. These standards are required, not just for the active ingredients in dosage forms however conjointly for impurities, degradation products, starting materials, process intermediates, and excipients.

Spectroscopic Methods: The following spectroscopic methods can be used;

- ***Ultraviolet (UV):*** UV at single wavelength doesn't provide sufficient information. To ensure greater selectivity and to get maximum information about molecule diode array detectors are used nowadays. It's currently attainable to get sufficient simultaneous information at various wavelengths to ensure greater selectivity.
- ***Infrared (IR):*** Infrared spectrophotometry provides specific information on some functional groups that may allow quantification and selectivity. This radiation effects the bonds present in the molecule and then it stretches or causes bending in molecule because of absorption of energy of a particular wavelength. The wavelength at which they are absorbed gives us information about different types of bonds which can be used for knowing the structure of samples.
- ***Nuclear magnetic resonance (NMR) :*** Nuclear magnetic resonance spectroscopy provides the necessary information regarding the specific bond, various structure and also the stereochemistry of desired chemical entity. It provides fairly structural information on 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 (MS):*** For several years mass spectroscopy, has been an essential tool for characterization of impurities present in desire drug products. ; it may provide an effective tool for differentiating with small differences in molecular weight, it can differentiate isotopes also.

Separation Methods:

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- ***Thin-Layer Chromatographic (TLC) Method:*** This is the most commonly used method. A broad range of compounds are often resolved using TLC by utilizing a variety of different plates and mobile phases. The greatest advantages are the ease of use and low cost. It's capability to analyze multiple samples simultaneously is excellent.
- ***Gas Chromatography (GC)***: Gas chromatography is a very useful technique for quantification. It can provide the desired resolution, selectivity, and ease of quantification. GC technique involves vaporization of the sample and subsequent injection into the gas chromatographic column. The sample is passed through the column by means of gas flow. The components in the sample mixture are separated by means of their individual affinity to involve in the adsorption and desorption processes.
- ***High-pressure Liquid Chromatography (HPLC):*** HPLC is basically an improved version of column chromatography. Instead of a solvent being allowed to pass through a column by means of gravitation, the solvent is pumped through the column under high pressures up to 5000 psi. Thus, the separation on column takes place much faster and in a more reproducible manner.
- ***Capillary Electrophoresis (CE):*** Capillary electrophoresis is a useful technique when very low quantities of samples are available and high resolution is required. Capillary electrophoresis involves the introduction of a solution containing a mixture of components into a narrow capillary zone and induces to move through the zone by means of applied potential. The components in the mixture pass through the capillary zone with different rates of velocities or migration based on the individual mobility of components under the influence of electric field. Thus, the mixture of components is then separated into different discrete zones of individual components after a certain time period.
- ***Supercritical Fluid Chromatography:*** It has some benefits as compared to GC and HPLC in terms of detection and separations respectively. This technique is still evolving, and its greatest application has been found in the extraction of samples.

Isolation Method:

Isolation of impurity is very essential, if we use instrumental method for this then, isolation of impurities won't occur as this directly characterizes the impurities. Generally chromatographic and non-chromatographic technique are used for isolation of impurities prior its characterization. The term 'chromatographic reactor' refers to the use of any analytical scale column as both a flow through reactor, and simultaneously, as separation medium for the reactant(s) and products. A list of methods that can be used for isolation of impurities is given below.

• Solid-phase extraction method.

• Liquid-liquid extraction method.

• Accelerated solvent extraction method

Characterization Methods:

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This makes use of highly sophisticated instrument such as attachment of Mass spectroscopy with gas chromatography or HPLC. They are an essential tool in identifying minor components like impurities, degradation products, and metabolites in various matrices. They are also known as HYPHENATED METHOD. Following are the various hyphenated method used;

- GC-MS
- LC-MS
- LC-DAD-MS
- LC-NMR
- LC-DAD-NMR-MS
- LC-MS-MS

This method is receiving great ever attention as they are a means for solving various analytical problems.

Conclusion: Impurity profile of pharmaceuticals is receiving an increasing importance as it increases drug safety. By estimating and discarding the impurities in the drug materials we can establish the security of drug products, and therefore the danger can be minimized. This article provides the valuable information about the impurities types and its classification, various techniques of isolation and characterization, analytical techniques for the determination, qualification of impurities. Guidelines and limit for impurity present in the pharmaceutical and impurity profiling as per ICH is discussed. In conclusion, Impurity profiling is beneficial in deciding safety parameters for drugs.

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