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# IMPURITY PROFILING OF DRUGS: A RE-VIEW

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**Abstract**:

Something that is impure or makes something else impure is considered to be impure. The definition of an impure substance is as follows: a substance of interest that has been combined with or impregnated with a foreign or typically inferior substance. From the point of view of its intended use, the drug substance is impure even if it contains a different substance that has superior pharmacological or toxicological properties. The impurity may appear during formulation or after both raw APIs and formed APIs have aged in pharmaceutical products. Even a little amount of these undesirable compounds can have an impact on the safety and effectiveness of medicinal goods. The impurities are not always worse than the pure materials. In order to identify small components (drugs, contaminants, degradation products, and metabolites) in varied matrices, very sophisticated instrumentation, such as mass spectra metres connected to a Gas Chromatography or HPLC, is a must. The impurities found in APIs are discussed in this article, along with methods for recognising them and potential solutions for dealing with the interferences they cause in pharmaceutical analysis.

**Keywords:** API, Impurity Profiling, Forced Degradation, Sources of Impurities, ICH Guidelines.

## Introduction:

Pharmaceutical impurities are undesired substances that remain with Active Pharmaceutical Ingredients (APIs), occur during formulation, or appear as medications age [1-4]. Impurity profiling is not precisely defined. Impurity profiling provides information on the impurities found in the substance under investigation. Additionally, it makes an educated guess as to how much various contaminants actually exist in the drug [5]. An impurity profile describes the known and unknown contaminants that are present in a typical batch of API made using a certain controlled production process [6–8].The impurity profiling is investigated with the goals of determining the precise relationship between two or more samples, determining drug distribution patterns, for identifying the source of drug samples, as well as for monitoring the process for drug manufacture. Because impurities may have teratogenic, mutagenic, or carcinogenic effects, there may be serious health repercussions [9]. To fully elucidate the chemical structure of an unidentified pharmaceutical impurity existing in either drug substances or drug products over a specific threshold, identification of pharmaceutical impurities is a crucial analytical activity in the drug development process [10]. Impurities are chemicals found in a product that are neither Active Pharmaceutical Ingredients (API) themselves nor the excipients used to create it, according to the International Conference on Harmonisation (ICH) rules [11]. Nevertheless, according to IP, an impurity is any component of a drug substance intended for pharmaceutical use or a drug product that is not the chemical entity that characterises the substance or, in the case of a drug product, is not an excipient. An impurity is any component that coexists with the primary medication, including precursors, intermediates, and by-products of any side effects. An impurity profile is a description of the known and unknown contaminants found in pharmaceutical products [12].

### Classification As Per Ich:

Impurities in pharmacological substances created through chemical synthesis can be roughly categorised into the following three categories, according to ICH guidelines:

A. Organic Impurities (Process and drug-related)
B. Inorganic Impurities (Reagent, ligands, catalysts)

### A. Organic Impurities:

These contaminants develop during the drug substance's manufacturing and/or storage processes. The following sub-impurities are among them.

I. Initiating Components or Intermediate Impure Components If sufficient care is not taken throughout each step of the multistep production of the therapeutic product, these types of contaminants will appear in practically every API. Even though the final goods are always cleaned with solvents, if the producers are not particularly vigilant about impurities, there is a potential that the final products will still contain traces of the unreacted beginning elements.

II. By-products It is extremely uncommon in synthetic organic chemistry to obtain a single end product with a complete yield; there is always a potential of receiving unwanted by-products in addition to the desired end product.

III. Products of Degradation During the production of bulk pharmaceuticals, the ultimate product might potentially degrade and produce impurities. This generally happens as a result of incorrect formulation storage [13,14,15].

**B. Inorganic Impurities:**

Impurities are also obtained from the manufacturing processes which are used in bulk drug formulation. They are normally known and identified.

a. Reagents, Ligands, and Catalysts, Section A These pollutants only sometimes appear in nature. If during manufacturing procedure is not followed properly will create a problem.
b. Heavy Metals Water is generally used 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.
c. Additional Materials (Charcoal, Filter Aids) In factories that produce bulk medications, filters or filtering aids like centrifuge bags are frequently employed. In many cases, activated carbon is also used, which serves as another source of impurities.. Therefore to avoid the contamination, regular monitoring of fibers and black particles in the bulk drugs is essential[16,17,18]

**ICH Guideline for Impurity Profiling:**

It is now getting an important critical attention from regulatory authorities. The International Conference on Harmonization has published various guidelines on impurities in drug substances and drug products as well as residual solvents.

1) Q1A : "stability testing of new drug substances and products"
2) Q3A (R2) : "Impurities in New Drug Substances"
3) Q3B (R2) : "Impurities in New Drug Products"
4) Q3C (R5) : "Impurities: Guidelines for Residual Solvents"[19]

**Sources of Impurity:**

API and medication preparations contain contaminants from a variety of sources. It includes crystallization related, stereochemistry, residual solvents, and synthetic intermediate and by- products related impurities. Additionally, it covers formulation, impurities that develop during storage, method-related, component interactions, and typical degradation related to functional groups. [20]

**Crystallization related impurity:**

Many medications are found in their crystalline solid forms as polymorphs, solvates, or hydrates. Due to stability and simplicity of handling during various stages of drug development, crystallisation is a legitimate explanation [21]. In the pharmaceutical business, crystallisation is a significant technological process for

particle creation and it also plays a critical role in determining the stability and drug release characteristics of the final dosage forms. In order to analyse the proportion of crystalline forms during the course of the drug's shelf life, FDA therefore needs developed and verified methodologies [22]. Based on the knowledge that the type of structure that a given molecule adopts during crystallisation may have a significant impact on that system's solid state capabilities, the pharmaceutical industry has been forced to take a keen interest in polymorphism and solvatomorphism [23].

**Stereochemistry related impurity:**

A drug's activity in a biological system depends on the spatial arrangement of atoms in the drug molecule, which is known as stereochemistry [24]. Finding stereochemistry-related molecules, or those with similar chemical structures but different spatial orientations, is of utmost importance because they might be regarded as API impurities [25]. Enantiomers are a broad term for chiral compounds. The antipode is regarded as an impurity when chiral medications are supplied as the pure enantiomer. Nowadays, it is thought that the single enantiomeric version of chiral drugs is a better chemical entity, with a higher pharmacological profile, an elevated therapeutic index, and a more favourable adverse reaction profile [26].

**Impurities originating from the starting material of the synthesis:**

This includes the appearance of the isomeric 4-trifluoromethyl impurity in 3-trifluoromethyl--ethylbenzhydrol (flumecinol), which is a result of the presence of 4-trifluoromethyl bromobenzene impurity in 3-trifluoromethyl bromobenzene, which is the starting material of synthesis [27].

**Formulation related impurities:**

Excipients, which are ingredients utilised to produce a pharmacological substance, can contribute a lot of contaminants to a drug product. During the formulation process, a drug substance is exposed to a number of circumstances that may cause it to degrade or have other unfavourable effects. The main component and excipient can occasionally interact to create an undesired product with a lower bioavailability. For all intents and purposes, the interaction result is regarded as an impurity [28]. Hydrolysis and solvolysis are two processes that can naturally degrade solutions and suspensions. Due to degradation and impurities that resulted in subpar fluocinonide topical solution USP, 0.05%, in 60-mL bottles, was recalled in the United States. Liquid dosage formulations are typically extremely prone to microbial contamination and deterioration [29].

**Impurity arising during storage:**

Numerous contaminants can develop when medication items are stored. Stability studies must be conducted in order to forecast, assess, and guarantee the safety of drug products [30].

**Development of impurity profiling methods using modern analytical techniques:**

The pharmaceutical industry's growing demand for the development of appropriate analytical procedures led to the selection of the current review topic. Modern analytical techniques like UPLC, LC-MS, LC-Q-TOF, GCMS, HPTLC, and LC-NMR were reviewed among the many other accessible techniques. In addition to that the source of impurities, kinds of impurities; control of impurities, identification of impurities, regulatory aspects, degradation products and stability indicating assay methods (SIAMs) were discussed.[31]

**High-Performance Liquid Chromatography (HPLC):**

HPLC is most widely used analytical technique because it is non-destructive and applied to thermally liable compounds also (unlike GC). To give a wide range of selectivity for separation, a large variety of distinctive column packing (stationary phase) and a wide selection of detecting techniques are available. Because of its broad selectivity, repeatability, compatibility with pharmaceutical materials, and suitability for MS detection, reverse phase liquid chromatography (RPLC) is more frequently utilised [32]. Reversed phase (C18, C8, etc.) columns, UV detectors, and PDA detectors are frequently chosen for HPLC analysis. PDA detection is effective for examining the chromatographic peak purity [33].

**Ultra Performance liquid chromatography (UPLC):**

Since the 1970s, ultra performance liquid chromatography (UPLC) has replaced HPLC procedures. The analytical performance of UPLC is comparable to that of HPLC, however it operates at much greater pressures. Most HPLC columns include particles with a size of 2.5 to 5 microns or smaller. While sub-2 micron porous particles were the basis for the development of UPLC columns. These need a greater pressure of approximately 15,000 psi to offer higher flow rates than the HPLC column's particles (6000 psi). The efficiency is higher and the diffusion path between the stationary phase and analytes is shorter because of the small size of the particles. Depending on the detection method employed, the sensitivity of UPLC detection is conceptually 2-3 times greater than that of HPLC detection. Significant improvements in instrumentation and column technology have enabled UPLC to make notable improvements in speed, resolution, and sensitivity. Therefore, UPLC makes them excellent for use with mass spectrometry and is a major driving force in the pharmaceutical sector today [34,35].

**Liquid chromatography-mass spectrometry (LC-MS):**

Liquid chromatography-mass spectrometry (LC-MS) is a powerful tool for identification and structural characterization of organic molecules in various matrices. It produces mass spectrum data that can yield important details about a sample's molecular weight, identity, quantity, purity, and structure. It can analyse substances for which there is no adequate chromophore, which LC-UV/PDA cannot do. This makes it a suitable detector for all types of pharmaceutical sample analysis. Additionally, it can be used to locate components in chromatographic peaks that cannot be resolved, hence minimising the requirement for desired separation. As a result, LC-MS has a position in all phases of drug development, from research to toxicological studies [36].

**High-Resolution Mass Spectrometry (HRMS):**

The discipline of high-resolution MS (HRMS) is expanding quickly in several areas of contemporary analytical science. It offers details about a compound's molecular structure, elemental makeup, and weight at the molecular level. In order to get more fragmented ions, tandem mass spectrometry (MS/MS) investigations can also be carried out using it. The fragmented ions are assembled for the purpose of predicting the structure of molecules after functional groups or moieties in the ions have been identified. Up to four decimal places, HRMS can reliably calculate m/z values. In addition to offering precise mass measurements,

TOF analyzers may also determine a compound's most likely molecular formula. Lists of these possibilities are particularly helpful when analysing the spectrum of an unidentified substance because a given nominal mass can correspond to a number of molecular formulas. Composition tables are available for this purpose, and a handy programme for computing all H, C, N, and O combinations that result in a given nominal mass is also accessible. HRMS is particularly helpful in metabolomics since it can calculate unknown species' isotopic ratios as well as their elemental composition. Instruments used for HRMS have resolutions between 10,000 and millions [37].

**High-performance thin layer chromatography (HPTLC):**

HPTLC is a modern adaptation of TLC with better and advanced separation efficiency and detection limits. The only chromatographic technique that permits the presentation of the data as an image is HPTLC. Other benefits include ease of use, sample analysis in parallel, low cost, quick findings, and the ability to detect various components. The HPTLC approach could greatly lessen the difficulties associated with disposing of harmful organic effluents and minimise the danger of exposure. With the advent of stationary phases and densitometers as detection tools, HPTLC is still making headway in the field of pharmaceutical analysis. HPTLC is one of the most widely applied methods for the analysis in pharmaceutical industries, forensic chemistry, clinical chemistry, biochemistry, food and drug analysis, cosmetology, environmental analysis, and other areas [38-40].

**Nuclear magnetic resonance spectroscopy (NMR):**

NMR spectroscopy exploits the magnetic properties of certain atomic nuclei. It establishes the physico-chemical characteristics of the molecules or atoms that they are contained in. One of the main applications of 1H and 13C NMR spectroscopy in the structural elucidation of contaminants in pharmacological substance/product. Additionally, two-dimensional studies like hetero nuclear single quantum coherence (HSQC) and double quantum filtered correlation spectroscopy (DFC-COSY) are helpful in determining the structures of organic molecules [41].

**The impurities can be identified predominantly by following methods :**

a) Reference standard method
b) Spectroscopic method
c) Isolation method
d) Characterization method

**a) Reference standard method:**

Clarifying the full life cycle certification and governance of the reference standard used in the development and management of new drugs is the main goal of this. Reference standards are the benchmarks for determining if a drug is safe for patients to consume and are used as the foundation for evaluating process and product performance. These standards are required for starting materials, process intermediates, degradation products, excipients, contaminants, and excipients in addition to the active substances in dosage forms[42–45].

**b) Spectroscopic methods :**

The following spectroscopic methods can be used;

i. Ultraviolet (UV)
ii. Infrared (IR)
iii. Nuclear magnetic resonance (NMR)
iv. Mass spectrometry (MS)

**i. Ultraviolet (UV):**

UV at a single wavelength offers only a very limited degree of analytical selectivity; but, with the advent of diode array detectors (DAD), it is now possible to obtain enough information simultaneously at different wavelengths to provide more selectivity.

**ii. Infrared Spectrophotometry:**

Infrared spectrophotometry provides specific information on some functional groups that may allow quantification and selectivity. Low level delectability, however, is frequently a problem that may call for more extensive solutions to address the issue.

**iii. Nuclear Magnetic Resonance Spectroscopy:**

Nuclear magnetic resonance spectroscopy is a highly helpful technique for characterising impurities and offers good structural information on molecules, but its application as a quantitative technique is constrained due to costs and time constraints.

**iv. 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, due to time and money constraints, it is only occasionally used as a quantitative technique [46].

**c) Isolation method:**

It is often necessary to isolate impurities. However, since using instrumental methods directly characterises the impurities, isolation of impurities is avoided. Generally, contaminants are isolated before being characterised using chromatographic and non-chromatographic techniques. The employment of any analytical scale column as a flow through reactor and a medium for reactant and product separation simultaneously is referred to as a "chromatographic reactor."[47].

**d) Characterized method:**

The following hyphenated methods can be used effectively to monitor impurities.

- GC–MS
- LC–MS
- LC–DAD–MS
- LC–NMR

- LC-MS-MS
- HPLC-DAD-MS
- HPLC-DAD-NMR-MS[48].

## Applications:

Numerous applications have been sought in the fields of drug design and monitoring the quality, stability, and safety of pharmaceutical substances, whether they are manufactured synthetically, derived from natural materials, or produced via recombinant techniques. Drugs from several classes, including as alkaloids, amines, amino acids, analgesics, antibacterials, anticonvulsants, antidepressants, tranquillizers, antineoplastic agents, local anaesthetics, macromolecules, steroids, and other substances, are used in a variety of applications.

## Conclusion:

The present review article provides a perspective on impurities in drug substance and drug products. Impurity profile of pharmaceuticals is receiving an increasing importance and drug safety receives more and more attention from the public and from the media. This article offers helpful information on the different types of impurities and their classification, different ways for isolating and characterising them, analytical techniques for determining their qualifications, and important aspects to take into account while preparing bulk pharmaceuticals. Isolation and characterization of impurities is required for acquiring and evaluating data that establishes biological safety which reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

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