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Impurity Profiling of Pharmaceutical API : A Review

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Abstract:

In the Pharmaceutical field the word impurity refers to any unwanted chemical or extraneous substance that remains in API during synthesis or processes associated with formulation. Considering the significant role of impurity profiling in pharmaceuticals various regulatory authorities mandated it. The present review focus on study of impurity profiling in API using various analytical techniques such as chromatographic (HPLC, CE, GC) and spectroscopic techniques (UV, NMR, FTIR) by isolation and characterization of impurities.Recent advancement in hyphenated technique(LC-MS, GC-MS, and LC-UV) allows study of impurity to threshold 0.1%.

Keywords: Impurity, Impurity profiling, chromatographic techniques, spectroscopic techniques, hyphenated techniques.

1. Introduction:

For a pharmaceutical product great deal associated with its safety, efficacy and purity. To achieve the final quality in the pharmaceutical product, vigorous quality control tools are utilized. The purity profile is important with respect to stability and safety aspect of pharmaceutical product. But with the advancement in analytical techniques to detect impurity and regulatory aspect for biological safety gave rise to impurity profiling.

1. Impurity definition[4]

Impurity can be defined as any substance that co existing with the drug product or formulation component such as starting material, intermediate, or that is formed due to side reactions.

As per ICH impurity can be defined as any component o new drug product that is not new drug substance or excipient drugproduct

Following are various terminology associated with impurity[15]

(a) Starting component
(b) Intermediate product
(c) By product
(d) Degradation product
(e) Transformation products
(f) Penultimate intermediates
(g) Related products

2. Impurity profiling definition[4,5]

Impurity profiling is the process of acquiring and evaluating data that establishes biological safety of individual impurity thus reveling its need and scope in pharmaceutical research. Thus it is both qualitative and quantitative evaluation of impurities in new drug product.

2.1. Initial phase of impurity profiling[15]

Initial phase of impurity started in the year 2008 with number of publication in a year. This trend was continued in further years. Initial period of impurity profiling includes HPLC, capillary electrophoresis, gas - liquid chromatography, thin-layer chromatography, etc.

2.2. Recent development in impurity profiling[3,8]

With advancement and development in analytical techniques such as hyphenated techniques allows impurity profiling around 0.01% threshold values of formulated product. Various hyphenated techniques used for characterization, structural elucidation and simultaneous determination of impurity.

3. Regulation of impurity profiling[5,16]

Now a day's part from purity profiling major concern focused on impurity profiling by regulatory authorities around the globe because of its essentiality in relation to its safety, efficacy and stability of product. This forms locus of its study in pharmaceutical research. Various authorities involved are:

(i) ICH

(ii) US-FDA

(iii)BP

(iv)IP

(v) TGA

(vi)Canadian health agency

(vii) MHRA

4. Classification of impurities[2,5,16]

4.1. As per ICH guidelines impurities can classified as

i. Organic impurity (process and drug related)
ii. Inorganic impurity (reagent, ligand, catalyst)
iii. Residual impurity (volatile solvents)

i. Organic impurity[14]

Organic impurities may arise during the manufacturing process and or storage of the drug substance may be structurally identified or unidentified, volatile or non-volatile in nature, and may include;

- Starting material

  In the multi-component, multi-step process untreated stating material remains as a impurity.

  E.g. P-amino phenol in paracetmol production.

- Intermediate material

  Partially treated reactant in the processing is an impurity to finished product eg.

- By product

  These are product formed along with the desired product

  E.g. diacetylated paracetamol.

- Degradation product

  Due to unfavourable condition product undergo degradation in trace amount leads to formation of an impurity.

E.g. degradation products of ketoconzole are 1-keto and 1- hydroxyl analogue of ketoconazole.

ii. **Inorganic impurity**[8]

Inorganic impurities derive from themanufacturing process and excipients.Generally, excipients contain high levels ofheavy metals such as arsenic, bismuth,cadmium, chromium, copper, iron, sodium etc.Sometimes they might present in the productduring processing or they leached from packingmaterial.

- Reagents, ligands, and catalysts
- Heavy metals
- Other materials (e.g. filter aids, charcoal)

iii. **Residual impurities**[2,6]

Residual solvents are organic or inorganic liquids used during the manufacturing process. It is very difficult to remove these solvents completely by the workup process. Some solvents that are known to cause toxicity should be avoided in the manufacturing of bulk drugs. Depending upon the possible risk to human health, residual solvents are divided in three classes such as class 1, class 2, class 3, class 4.

- Class 1 solvents:

  Solvents to be avoided in pharmaceutical products Known human carcinogens, strongly suspected human carcinogens and environmental hazards.
- Class 2 solvents: Solvents to be limited in pharmaceutical products

  Non-genotoxic animal carcinogens or possible causative agents of otherirreversible toxicity such as neurotoxicity or teratogenicity. Solvents suspected of other significant but reversible toxicities.
- Class 3 solvents: Solvents with low toxic potential Solvents with low toxic potential to man; no health -based exposure limit is needed. These solvents are less toxic in acute or short term studies and negative in genotoxic studies. The amount of these residual solvents of 50mg or less would be acceptable. Examples

for this class of solvents are Acetic acid, Acetone, Anisole, 1-Butanol, 2-Butanol etc.

- Class 4 solvents: Solvents for which No adequate toxicological data was found.

The solvents of this class may be of interesting to manufacturers of excipients, drug substances or drug products. But there was no adequate toxicological data on which to base

a Permitted Daily Exposure was found. Examples for this class of solvents are 1,1-Diethoxy propane,1,1-Dimethoxy propane,2,2Dimethoxy propane, Isooctane etc.

b. Classification As per USP

i. Impurities in official articles

ii. Ordinary impurities

iii. Organic volatile impurities

5. Rational approach for impurity profiling in API [6]

ICH guidelines, 'Impurities in New Drug Substances' (Q3A) states "The applicant should summarize the actual and potential impurities most likely to arise during synthesis, purification and storage of the new drug substance. This should be based on sound scientific knowledge of the chemical reactions involved in the synthesis, impurities associated with raw materials and possible degradation products. Also the applicant should summarize the laboratory studies conducted to detect impurities in new drug substances. This summary should include results from batches from the development process as well as batches from commercial process. Also the studies conducted to characterize the structures of the impurities present above the identification threshold should be described. When identification of impurity is not possible,a summary of laboratory studies demonstratingthe unsuccessful effort should be reported. Theidentification of impurities present at the levelless than the identification threshold is notgenerally considered necessary. But analyticalmethodology needs to be developed for theimpurities that are expected to have unusualtoxic pharmacological effects."

Table1: Threshold for impurity profiling [2]

Maximum daily dose[a]	Reporting Threshold [bc]	Identification threshold[c]	Qualification threshold
≤ 2 gm/day	0.05%	0.1% or 1 mg/day intake	0.15% or 1mg/day intake
>2gm/day	0.03%	0.05%	0.05%

a=amount of drug substance administered per day.

b=higher reporting threshold should be scientifically justified

c=lower threshold can be appropriate if the impurity is unusually toxic

6. analytical methods for impurity profiling[7,9,10]

An impurity profile is a description of the identified and unidentified impurities present in a new drug substance (Source: Guidance for Industry, Q3A Impurities in New Drug Substances). Impurity profiling processes usually begin with the detection of Impurities, followed by their isolation and characterization. For all three types of impurities, it is critical to develop a robust method during process development that can eventually be validated and transferred to QA/QC.

To better detect, identify, quantify, and characterize the impurities present in drug substances and products, pharmaceutical scientists rely on fast analytical tools with high sensitivity and specificity. Major analytical tools for impurity analysis include spectroscopy, chromatography, and various combinations of both, i.e. tandem techniques. The appropriate technique is selected based on the nature of the impurity and the level of information required from the analysis. There are various complex analytical problems in pharmaceutical development that require the use of more than one analytical technique for their solution.

Analytical technique such as LC/UV, LC/MS, GC/MS that are provide the way to detection of impurities in material or in drug with a time efficient manner. So that provide a vital important role in the impurity profiling in pharmaceutical product to elucidation of unknown impurities.

Impurities detection in pharmaceutical agent or in drug has generally by two ways

1) Quantitative estimation of impurity in pharmaceutical drug
2) Qualitative estimation of impurity in pharmaceutical drug

But the following are the various analytical techniques are used in estimation of unknown impurities.

Sr. No	Type of Impurities	Technologies used
1	organic	FTIR, Preparative LC, LC-UV, LC-MS, NMR, SFC
2	Inorganic/elemental	ICP-OES, ICP-MS
3	Residual solvent	GC and GC-MS

FTIR

FTIR is very helpful for identifying and confirming the structure of an impurity or degradant because it provides a complex fingerprint that is specific to a particular Compound. An FTIR spectrum of an organic molecule is determined by the functional groups present. The technique helps to identify the structure and measure the concentration of the compound under investigation. Changes in the structure can be correlated with the help of an FTIR spectrum of a patent drug compared to that of the impurity or degradant.

LC-UV

A number of impurity analysis methods found in pharmaceutical quality control (QC) laboratories use high-performance liquid chromatography (HPLC) coupled with UV detection (HPLC/UV methods). UV spectrometry helps identify impurity or degradants in drug substances based on absorption maxima. This technique is one of the most important and versatile analytical methods available for impurity profiling today due to its high selectivity. Especially for routine analysis where standards are available.

HPLC generally does the resolution and separation the specific compound from the mixture and in impurity related concept it separate the pure drug and their related

impurity. For the detection purpose the UV detector are preferred in liquid chromatography.

Newer , stationary phase system are available, which operate in several modes, such as ion pairing, increased hydrophobic interaction and available pH, allowing a varieties of sample to be analyzed concurrently based upon their unique properties. High resolution is particularly helpful when using LC/UV analysis, for impurities detection, because all impurities can be identified with less chances of errors.

LC-MS:

LC-MS is a powerful analytical tool that is routinely used in pharmaceutical development to test and identify product impurities. The detection limit of a few hundred ppm is readily achievable, ensuring the identification of all the impurities present at concentrations greater than 0.1 %. MS-based methods generally provide additional robustness and ruggedness compared to techniques such as UV alone, due to their high specificity and sensitivity. While single quadrupole mass spectrometers work well as analytical tools for the confirmation of known impurities and the preliminary structural assessment of unknown impurities, highly sensitive Q-TOF mass spectrometers provide higher resolution and mass accuracy that enables the unambiguous identification of unknown trace impurities, making them very useful for genotoxic impurity analysis[17]. MS-based methods are often selected for the impurity profiling of APIs during process development.Triple-quadrupole (QQQ) LC-MS-MS systems have become a standard platform for the quantitative analysis of organic impurities in pharmaceutical analytical laboratories.

Capillaries electrophoresis:

The determination of drug-related impurities is currently the most important task for CE within pharmaceutical analysis because it achieves high separation efficienciescompared to other chromatographic techniques. CE can be employed when HPLC techniques are not able to adequately measure impurities, especially in the case of very polar compounds. A detection limit of 0.1 % is widely accepted as a minimum

requirement for a related impurities determination method and this can be achieved using CE. In addition, CE is very useful for the separation of closely related compounds, such as diastereomers and enantiomers.

Supercritical Fluid Chromatography (SFC):

SFC, which uses supercritical CO_2 as mobile phase, is another orthogonal technique that can be used for impurity detection because it offers HPLC-level sensitivity with reduced organic solvent usage. SFC also offers the advantage of chiral impurity analysis enabling the determination of enantiomeric excess at very low impurity levels.

ICP-OES

ICP-OES provides parts per billion (ppb) detection limits for most regulated elements in pharmaceutical products, easily meeting the specified limits in cases where direct sample analysis or small dilution factors are appropriate. It also provides extended dynamic range, robust plasma, and one-step measurement of major, minor, and trace elements. Therefore, ICP-OES addresses the needs of a wide range of users, including those seeking a cost effective solution for the direct analysis of elemental impurities in bulk raw materials and pharmaceutical products.

NMR

The ability of NMR (Nuclear Magnetic Resonance) to provide information regarding the specific bonding structure and stereochemistry of molecules of pharmaceutical interest has made it a powerful analytical instrument for structural elucidation. The ability of NMR- based diffusion coefficient determination to distinguish between monomeric and dimeric substances was validated using a standard mixture of authentic materials containing both monomers and dimmers. Unfortunately, NMR has traditionally been used as a less sensitive method compared to other analytical techniques.

ICP-MS

ICP-MS is a powerful and sensitive technique that delivers a reliable trace-level analysis of all 16 elements whose limits are defined in USP<232>. The low detection

limits of ICP-MS ensure that all regulated elements in drug substances or drug products can easily be determined using the new method, at or below regulated levels, and even when large sample dilutions are required. ICP-MS can also be used in combination with a variety of separation techniques, such as HPLC, GC, and CE, providing several options for separation (or speciation) of the different chemical forms of the elements, and depending upon the nature of sample. ICP-MS achieves low detection limits for almost all elements.

Gas Chromatography (GC):

In combination with flame ionization detection (FID), GC is the standard choice for the analysis of volatile organic impurities, such as residual solvents. The gas chromatography headspace method is used worldwide for residual solvent analysis in quality control laboratories because it closely follows ICH Q3C guidelines. Sample preparation and introduction is via a static headspace which facilitates the selective introduction of volatile solvents without contamination by mostly non-volatile drug substance or drug products. Therefore, the use of an FID detector helps preferentially identify and quantify residual solvents. More recently, the combination of gas chromatography and mass spectroscopy (GC-MS) has been successfully used for confirmation and identification purposes, highlighting the flexibility of this technology.

7. Application of impurity profiling in API[17]

Table: 3 impurity profiling in anti-tubercular API

API	Impurities found	Method details
Isoniazid	Impurity I: 1-nicotinyl-2- lactosyl hydrazine	HPLC with a 10 µm cyanopropyl stationary phase and a mobile phase consisting of a mixture of pH 3.5 10 Mm acetate buffer and acetonitrile (95/5, v/v). Flow rate and detection wavelength not specified.

Rifampicin	Hydrazones: Rifampicin quinine and 25-desacetyl rifampicin	HPTLC with a silica gel 60 TLC plate (Merck) with a Chloroform/methanol/water (80/20/2.5, v/v/v) mobile phase. Examined using Scanner II (Camag) at 330nm for 25-desacetyl rifampicin and 490 nm for rifampicin quinone.
Rifampicin	Hydrazones: rifampicin quinone	HPLC with 10 µm silyl and 10µm nitrile stationary phases (Micro Pak Si-10 and MicroPak CN, respectively) and anisocratic mobile phase consisting of a mixture of chloroform and methanol of varying proportions. Flow rate 0.2–0.7 ml/min; detection at 334 nm.
Rifampicin	Hydrazones: rifampicin quinone, 25-desacetyl-21-acetyl-rifampicin, 25-desacetyl-23-acetyl-rifampicin	HPLC with direct injection (DI) onto a 3 µm ODS stationary phase (Hypersil ODS) at 25 □C and an isocratic mobile phase consisting of a mixture of pH 7.4, 50 mM phosphate buffer and acetonitrile (64/36, v/v). Flow rate 1.4 ml/min; detection at 240 nm. Alternatively, a 10 µm ODS stationary phase (Hypersil ODS)
Rifampicin, Isoniazid, Pyrazinamide FDC	Hydrazones: rifampicin quinone, desacetyl rifampicin, isonicotinyl hydrazone	HPLC with a 5 µm L1 ODS stationary phase at 25 □C and a gradient mobile phase consisting of varying mixtures of mobile phase A (pH 6.8 phosphate buffer/acetonitrile, 96/4, v/v) and mobile phase B (pH 6.8 phosphate buffer/acetonitrile, 45/55, v/v or 55/45, v/v). Flow rate 1.5 or 1.0 ml/min; detection at 238 nm. Three L1 columns were evaluated: 1: Zorbax XDB, 2: Shim-pak CLC ODS and 3. Nucleosil EC 120-5.

8. Conclusion

This article gives a perspective on impurities in API. Impurity profile of pharmaceuticals is receiving an increasing importance and drug safety receives more and more attention from literature. 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 in API. Now a day, it is

mandatory requirement in various pharmacopoeias to know the impurities present in APIs and finished drug products. Thus impurity profiling can act as a Quality Control tool. It can provide crucial data regarding the toxicity, safety, various limits of detection and limits of quantization of several organic and inorganic impurities usually accompany with APIs and finished Products. There is strong requirement to have unique specifications/standards with regard to impurities. This reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

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