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IMPURITY PROFILING: OVERVIEW ON IMPURITY PROFILING AND REPORTING METHODOLOGIES ADOPTED BY UNITED STATES AND EUROPE

Shashank Rawat[1]* and Vijay Kumar[2]

[1]Regulatory Affairs Officer, Ind Swift Laboratories Ltd, Panchkula-134102.

[2]Drug Regulatory Affairs, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara-144 001.

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***Corresponding Author**
Shashank Rawat
Regulatory Affairs Officer,
Ind Swift Laboratories Ltd,
Panchkula-134102.

ABSTRACT

Impurities are not acceptable in drug formulation. It is considered as unwanted chemicals or organic material which remains with Active Pharmaceutical Ingredient (API's). The impurity is produced either during formulation or ageing of both API's and finished dosage form. The existence of these undesired chemicals may influence the safety and the efficacy of the pharmaceutical finished products. In this review article we have discussed about the methodologies adopted by United States and Europe for Impurity profiling given by their respective regulatory authorities. Impurity profiling, calculation and methodologies used to represent impurities in dossier regulation related documents.

KEYWORDS: Impurity, Residual Solvent, API, SFC.

INTRODUCTION

The impurity profile has become essential as per various regulatory requirements. For the pharmaceutical industries impurity is considered as organic material or unwanted chemicals which remain with Active Pharmaceutical Ingredient (API's). The impurity be developed either during formulation or ageing of both API's and Formulation. The presence of these unwanted chemicals may influence the efficacy and the safety of the pharmaceutical products. Impurity profiling gaining more attention from regulatory authorities. The impurity profiling is the description of identified and unidentified impurities present in new drug

substance. Some impurities have been named as per International Conference on Harmonization (ICH) such as.[1]

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

Impurity: "It is defined as any substance coexisting with the original drug, such as starting material, intermediate, and any side reactions".

OR

It is the unwanted chemicals substance which influences the safety and efficacy of the pharmaceutical products.[2]

Profiling: It is the process of the identification through which we get the characteristic of the substance.[3]

Impurity Profiling: It is the process of evaluating the data that establish biological safety of an individual impurity. For maintaining the stability or efficacy of the API we do the profiling of impurities. It helps in identifying and quantifying the impurities in API. It gives maximum possible types of impurities present in drug substance (API) and in pharmaceutical formulations. The impurity control in the pharmaceutical product is the main goal of the drug development and for controlling the impurity, there are various regulatory guidelines which monitored the impurities in API.[4]

Classification of impurities in API

As per the ICH guidelines, the impurities are classified in three types as below·

1. Organic impurities (process and drug related).
2. Inorganic impurities.
3. Residual solvents.

Organic impurities: These impurities are classified as starting material, By-product, degradation product, reagents and chiral impurities.

a) Starting materials: The impurities from the starting material or by product are found in every drug substance, so for that proper care should be done to remove that impurities before it effect the end product. For e.g. In the synthesis of baclofen, the last step is carried out with gutarimide, after which on reaction with sodium hydroxide solution at room temperature it yield a potential impurity i.e. p-chloro phenyl gluteric acid.

b) By-products: There is always a chance of having by-products. Because they can be formed through avariety of side reactions, such as incomplete reaction, over reaction, isomerization, dimerization, rearrangement or unwanted reaction between starting materials or intermediate with the chemical reagents or catalysts. For e.g. In the case of Paracetamol bulk production, diacetylated Paracetamol may form as a by-product.

Inorganic impurities: Inorganic impurities are derive from the manufacturing processand excipients. Inorganic impurities mainly include water, salts from buffers, reagents, ligands, catalysts, heavy metals, or other residual metals and also the inorganic compounds used in the processing such as filter aids and charcoal.

- Reagent, ligand and catalyst: The chances of these impurities are rare but these impurities could create a problem so proper care has to be taken during production. For e.g. chloride.
- Heavy metals: The main sources of the heavy metals are, the reactors where acidification and acid hydrolysis takes place. The impurities which are arising due to the heavy metals, that can easily be avoided by demineralized water and by using glass lined reactors. For e.g. water.
- Filter aids and charcoal: These are the centrifuge bags which are routinely used in the bulk drugs manufacturing plants. For e.g. activated carbon.

Residual solvents: These are the undesirable substance. They modify the properties ofcertain compounds or may be hazardous for the human health. These solvents also affect thephysicochemical properties of the bulk drug such as crystallinity of the bulk drug.

As per ICH guidelines, the solvents are classified into three categories:

1. Class 1 solvent: These solvents are not employed in the manufacture of drug substances, excipients and formulations because of their unacceptable toxicity or their deleterious effects Table.1.
2. Class 2 solvent: these solvents have limited use in the pharmaceutical products because of their inherent toxicity Table.2.

3. Class 3 solvents: these are the less toxic and possess lower risk to the human health. These solvents do not have any serious hazardous Table.3.

Table 1: Class 1 Solvents to be avoided in pharmaceutical products.[5]

Solvent	Concentration limit(ppm)	Concern
Benzene	2	Carcinogen
CCl4	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethane	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Table 2: Class 2 solvents to be limited in pharmaceutical products.

Solvent	PDE(mg/day)	Concentration limit(ppm)
Acetonitrile	4.1	410
Chloroform	0.6	60
Cyclohexane	38.8	3880
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000

Table 3: Solvents for which no adequate toxicological data was found.[5]

1,1-Diethoxypropane	Methyl isopropyl ketone
1,1-Dimethoxymethane	Methyltetrahydrofuran
2,2-Dimethoxypropane	Petroleum ether
Isooctane	Trichloroacetic acid
Isopropyl ether	Trifluoroacetic acid

Impurities present in formulation

Number of impurities in a drug product can arise from ingredients which are used for the product formulation. In the process of formulation there are varieties of conditions that can lead degradation or other deleterious reaction. In the formulation use of water cannot only contribute its own impurities but it is also provide a ripe situation for the hydrolysis and catalysis.[6]

Impurity forms during formulation are.

a) Method related.

b) Environmental related.

The primary environmental factors that can reduce stability include the following.

- Exposures to adverse temperatures.
- Light-especially UV light.
- Humidity.

c) Dosage form related impurities.

A) Mutual interaction amongst ingredients.

B) Functional group- related typical degradation.

- Ester hydrolysis
- Hydrolysis
- Oxidative degradation
- Photolytic cleavage
- Decarboxylation

Method related impurity: This is the impurity which is related from method. For e.g. in the production of the parental dosage form of diclofenac sodium, Indolin-2-one is the impurity formed during the sterilization by autoclave. During the autoclave condition, the formation of indolinone derivative and sodium hydroxide takes place due to the intramolecular cyclic reaction of the diclofenac sodium.[6]

Environmental related impurities: Environment related factors are the factors which that reduce the stability of the drug substance such as.

- Exposure to adverse temperature: Many of the drug substance are heat sensitive in nature. For e.g. vitamins are very heat sensitive in nature due to which they undergo degradation in liquid formulation that cause the decrease in potency of vitamin.
- Light especially UV light: This light makes unstable to the drug substance. For e.g. Ergometrine and methyl ergometrine injection is unstable or shows the complete degradation when kept in the direct sunlight.
- Humidity: It affect the hygroscopic products, which are sensitive in the humid environment. It affect the bulk powder and solid dosage form for e.g. Aspirin and Ranitidine.

Dosage form related impurities: Sometimes the dosage form factors that influence the stability of the drug. In general, liquid dosage forms are much susceptible to both degradation

and microbial contamination. In which, water content, pH of the solution, compatibility of the anions and cations, mutual interaction of the ingredients, and the primary containers are the critical factors for the impurities.

a) Mutual interaction amongst ingredients.

Mutual interaction among the ingredient is also the major problem which cause the instability in the drug product. Most of the vitamins are very liable and on storing they pose a problem of instability in different dosage form but especially in the liquid dosage form. For e.g. during the formulation, one formulation is in simple distilled water and other is in typical formulated vehicle that include disodium edetate and benzyl alcohol both have similar mutual interaction causing the degradation of the product.

b) Functional group related typical degradation.

Ester hydrolysis: It is the reaction when the ester reacts with the water to produce ethanoic acid and ethanol.

Hydrolysis: It is a reaction which occurs due to water. Mainly this is a reaction of breaking bond in a molecule by using water. For e.g. sodium acetate is a salt which get hydrolyze by adding water and then separate in to the sodium ions and acetate ions.

Oxidative degradation: It is the degradation in which the cleavage of c=c with the introduction of new carbon and oxygen bond. For e.g. hydrocortisone, methotrexate, conjugated dienes, nitrite derivatives and aldehydes all are susceptible to the oxidative degradation.

Photolytic cleavage: it occurs due to the direct exposure of the sunlight. For e.g. in the preparation of ciprofloxacin eye drop, sunlight induce the photocleavage reaction producing ethylenediamine analog of ciprofloxacin.

Decarboxylation: In this case when we heat the p-aminosalicylic acid then it lose the carbon dioxide from the carboxyl group. Decarboxylation also occurred in the case of photoreaction of rufloxacin.[1]

Impurities present in raw material

It is the impurities present in the raw material which contaminate the final product. These impurities are Arsenic, lead heavy metals etc all are present in the raw materials. For e.g.

Rock salts contain small amount of calcium and magnesium chloride, so the sample of Nacl is likely to contain traces of calcium and magnesium as a impurity. Sodium compounds are prepared from Nacl due to low cost and easy availability, in which Cl is present as an impurity.

Identification of impurities[1]

The impurities can be identified by the following methods.

- Reference standard method
- Spectroscopic method
- Separation method
- Isolation method

Reference standard method: It is the method in which reference standard prepared for use as the standard in an assay, identification, or purity test. By this method we can evaluate both the process and product performance. It is not only gives the information for the active ingredient in dosage form but also for the impurities, degradation product, starting materials, and excipients.

Spectroscopic method: The UV, IR, MS, NMR, and Raman spectroscopic methods are routinely being used for characterizing the impurities.

a) Ultraviolet spectrometry: It is a physical technique of the spectroscopy that uses light. It is determine the concentration of the absorber in a solution. By rapidly change in the absorbance we can determine the impurity.
b) Infrared spectroscopy: It is used in the research to identify samples, do quantitative analysis, or detect impurities. It can be used on solid, liquid or gaseous samples and also does not destroy the sample in the process.
c) Mass spectrometers: It provides the accurate mass measurements of a sample molecules, sample identification, and quantitation of the samples. It can be used with GC-MS and LC-MS. It is a combine feature of gas and liquid chromatography.
d) NMR Spectroscopy: It is very sophisticated system which is based on the use of nuclear magnetic resonance technology. It is used to test atomic and molecular properties of the sample. It is very sensitive in nature.[1]
e) Raman spectroscopy: It is used to study vibrational, rotational and other low frequency modes in a system. It is fairly good sensitivity and detect the process related impurities.

Separation method: The following separation methods are as follows.

a) **Thin-layer chromatography:** It is a chromatography technique which is used to separate the mixtures. It is performed on the sheet of the glass, plastic, and aluminum foil which is coated with the adsorbent material such as silica gel, aluminum oxide and cellulose. After the sample has been applied on the plate then a solvent mixture is drawn up by the capillary action. After some time the mixture gets separate.

b) **Gas chromatography:** It is a common type of chromatography which is used for separating and analyzing the compounds. Mainly it is of two types' i.e Gas-Liquid chromatography and Gas chromatography. Most of the time gas chromatography is used for the testing of purity or separating the different components of the mixtures. It is helpful in the preparation of the pure compounds from the mixtures.

c) **High-pressure liquid chromatography:** It is a column chromatography used to separate, identify, and quantify the compounds. HPLC have different type's stationary phases and a pump that moves the mobile phase and also there is a detector to provide the characteristic information about the compound such as API and its impurities. It is helpful to check the quality of the API starting material and also signify the unknown impurities.

d) **Capillary electrophoresis:** It is also known as capillary zone electrophoresis. It is used to separate the ionic species by their charge and size in the small capillary filled inside with an electrolyte. It is based on the different separation principles and also used for the quality control of the pharmaceutical products.

e) **Supercritical fluid chromatography (SFC):** It is the chromatography in which we separate one component from other component by using the super critical fluid. Carbon dioxide is used as a supercritical fluid where ethanol or methanol used as a co-solvent. In this we provide the critical temperature of 31^{o}c with critical pressure of 72 bars.

Isolation method: It is necessary to isolate the impurities. Generally chromatographic and non-chromatographic techniques are used for the isolation of the impurities. There are various methods by which we can isolate the impurities such as.

a) **Solid-phase extraction method:** It is the method which is used to trace the organic compound as well as remove the interfering compound to obtain a clear extract. Mainly this technique is used for the extraction and purification of the compounds. Main use of this method is to clean up the sample before use for the chromatographic technique to quantify the analyte in the sample. This technique is widely applied for the isolation of analytes from a liquid matrix.

b) **Liquid-liquid extraction method:** It is also known as solvent extraction and partitioning. It is a method which is used to separate compounds based on their relative solubility's in two different immiscible liquids usually water and organic solvents. The method is perform in the separating funnel. Commonly solvents used for liquid-liquid extraction are ethyl acetate, methylene chloride and hexanes.

c) **Accelerated solvent extraction method:** It is the better technique used for the extraction of solid and semi-solid samples. All the process done at the elevated temperature and pressure to get the fast and efficient removal of analysts from the samples. It perform the experiment in less time with using smaller quantity of solvent.

Regulatory bodies involved in impurity profiling

There are various regulatory authorities like ICH, USFDA, EMA, CDSCO and Canadian drug and health agency for the identification of impurities in active pharmaceutical ingredients. Such regulatory authorities are discussed below.

ICH: It stand for International Conference on Harmonization. It is for the registration of pharmaceuticals for human use. ICH is unique in bringing together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspect of drug registration. ICH's mission is to achieve greater harmonization to ensure safe, effective, and high quality medicines are developed and registered in the most efficient manner. These activities have been under taken to promote the public health, prevent unnecessary duplication of clinical trials in humans and minimize the use of animal testing without compromising safety and effectiveness. ICH divides its guidelines into four categories such as.[7]

Quality guidelines: For achieving quality they conduct stability studies, relevant threshold for impurity testing and more flexible approach to the pharmaceutical quality based on good manufacturing practice.

Safety guidelines: ICH produced comprehensive guidelines for achieving the safety and reduce the potential risk such as carcinogenicity, genotoxicity and reprotoxicity.

Efficacy guidelines: For the efficacy it concern with the design, conduct, safety and reporting of clinical trials. And it also covers the novel types of medicines derived from biotechnological process. The main aim is to produce better targeted medicines.

Multidisciplinary guidelines: It is different from quality, safety, and efficacy. It includes ICH medical terminology, and the common technical document.[8]

USFDA: It stand for U.S Food and Drug Administration. Mainly FDA is responsible for protecting the public health by assuring the safety, efficacy and security of human and veterinary drugs, biological products, medical devices, cosmetics and product that emit radiation. It also have a responsibility for regulating the manufacturing, marketing and distribution of tobacco product to prevent the human health and to reduce the tobacco use. It play a significant role in the Nation Counterterrorism. The FDA's fulfills these responsibilities by ensuring the security of the food supply and by fostering the development of medical products for the public health. The regulatory authority is very broad. They regulate foods including dietary supplements, food additives, and other food products. For drug they regulate prescription and non-prescription drugs. For biologics they regulate vaccines, blood and blood products etc. Another they regulate medical devices, cosmetics, veterinary products, tobacco products etc. USFDA is the oldest comprehensive protection agency in the U.S federal government.[9]

The organization of FDA consists

- Office of the Commissioner
- Office of Foods and Veterinary Medicine
- Office of Global Regulatory Operations and Policy
- Office of Medical Products and Tobacco
- Office of Operations[10]

EMA: Mainly it stand for European Medicines Agency. It is a decentralized agency of the European Union, located in London. This is the agency which is responsible for the scientific evaluation of the medicines developed by the pharmaceutical companies. The main responsibilities of the European Medicines Agency is the protection and promotion of the public health and animal health, which is done through the evaluation and supervision of medicines for human and veterinary use. The agency is responsible for the scientific evaluation of application for the European Union market Authorization for the human and the veterinary medicines.

Most of the scientific evaluation work is carried out by its own scientific committees which are made up members of EEA countries, as well as representative of patient, consumer and

healthcare professionals' organizations. There are various related tasks to the development, assessment, and supervision of medicines in the EU which is done by these committees.

The EMA is responsible for the coordinating safety-monitoring or pharmacovigilance system for the medicines. It monitors the safety of medicines through the EU network and up on the information of benefit-risk balance of medicines they can take the action and has changed since it was authorized. This agency works.

- This agency provides the specific report to pharmacovigilance activities for centrally authorized product.
- Fee developing guidelines and setting standards.
- Coordinating to monitoring the pharmaceutical companies with their pharmacovigilance obligations.
- It informing all the public about the safety of the medicines and interact with the representatives of patients and healthcare professionals.[11]

The agency is also responsible for coordinating inspections such as.

- ❖ Good manufacturing practice, (GMP)
- ❖ Good clinical practice, (GCP)
- ❖ Good laboratory practice, (GLP)
- ❖ Pharmacovigilance, (Phv)

The European Medicine Agency does not control[12]

- The price of the medicines.
- Patents on medicines.
- The availability of the medicines.
- Medical devices.
- Homeopathic medicines.
- Herbal supplements.
- Food supplements.
- Cosmetics.
- Advertisement of medicines.

Guidelines given by regulatory authorities for Impurity Profiling

The impurity profiling guidelines are given by the ICH which is common for all the regulated and semi regulated countries. But it is not mandatory to follow the ICH guidelines; different

regulatory agencies can follow their own guidelines also. But for obtaining the good result in impurity profiling it is good to follow the guidelines which are given by the ICH.

ICH Q3AR guideline for the impurity testing in new drug substances. This guideline provides the guidelines for registration application on the content and qualification of impurities in new drug substances produced by chemical synthesis. Specification should include.

Organic impurities

Each specified identified impurity.

Each specified unidentified impurity.

Any unspecified impurity.

Total impurities.

Each specified identified impurity are those impurity which are listed with a specific acceptance criterion in the new drug substance or which is easily identified. Each specified unidentified impurity which is not more than identification threshold Table.3.

Table No 3: Threshold values

Maximum Daily Dose	Reporting Threshold	Identification Threshold	Qualification threshold
<2g/day	0.05%	0.10% or 1.0 mg/day intake (whichever is lower)	0.15% or 1.0 mg/day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

The threshold limit is based on scientific data including drug class effects and clinical experience. These threshold limits are either lower or higher can be. Such as given below.

-Adverse reaction in patients

-Patient population

-Drug class effect

-Clinical experience

The whole study will depend up on the number of factors like.

- Patient population
- Daily does
- Routes and duration of drug administration.[13]

As according to different countries they provide the different guidelines on the impurity profiling. Such are discussed below.

United States for Food and Drug Administration: USFDA addressed the impurities in new drug substance as by chemical aspects and safety aspects. Mainly they follow the guidelines of ICH for the impurity profiling. Threshold values are shown in Table.4.[13]

Table 4: Threshold values.

Maximum Daily Dose	Reporting Threshold	Identification Threshold	Qualification Threshold3
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
>2g/day	0.03%	0.05%	0.05%

Table No 4a.

Raw" Action Result (%)	Reported Result (%) Reporting threshold =0.05%	Calculated Total Daily Intake (TDI) (mg) of the impurity (rounded result in mg)	Identification (Threshold 0.10% (exceeded?)	Qualification (Threshold 0.15% (exceeded?)
0.044	Not reported	0.2	None	None
0.0963	0.10	0.5	None	None
0.12	0.12	0.6	Yes	None
0.1649	0.16	0.8	Yes	Yes

Table No 4b.

"Raw" Result (%)	Reported Result (%) Reporting threshold =0.05%	Calculated Total Daily Intake (TDI) (mg) of the impurity (rounded result in mg)	Identification (Threshold 0.10% (Exceeded?)	Qualification (Threshold 1.0 mg TDI (Exceeded?)
0.066	0.07	0.6	None	None
0.124	0.12	1.0	Yes	None
0.143	0.14	1.1	Yes	Yes

In USFDA, attachment 1 is similar as ICH where attachment 2 is differs from EMA.

European Medicines Agency: There is draft version for the impurity profiling in case of antibiotics. They developed guidelines for the impurities in antibiotic that are fermented products or semi synthetic substance. Therefore they are not included in the ICH guideline.

EMA will depend on the European pharmacopeia which is also abbreviated as Ph. Eur. This contain monograph which tells about the substance manufactured by fermentation not by the semi-synthesis. The general consideration for the impurity thresholds in antibiotic through fermentation, these are present in this guideline. For the antibiotic drug substances, the impurity profile should be characterized according to the guidance described in ICH 3QA (VICH GL10).

In according with that guidance limits should be set for.

- Each specified identified impurity
- Each specified unidentified impurity
- Any unspecified impurity
- Total impurities

For the different types of antibiotics thresholds are given below.

- Active substance manufactured by semi-synthesis.

For active substance used in veterinary medicines threshold for reporting, identification and qualification are 0.10%, 0.20% and 0.50%, respectively.

- Active substance manufactured by fermentation, single compound.

Reporting threshold: 0.10%

Identification threshold: 0.15%

Qualification threshold: 0.15%

- For active substance used in veterinary medicines.

Reporting threshold:0.10%

Identification threshold: 0.20%

Qualification threshold: 0.20%

- Active substances manufactured by fermentation, family of compounds.

Reporting threshold: 0.10%

Identification threshold: 0.15%

Qualification threshold: 0.50%/0.15%

- For active substance used in veterinary medicines.

Reporting threshold: 0.10%.

Identification threshold: 0.20%.

Qualification threshold: 0.50%.

- Active substance manufactured by semi-synthesis.

Reporting threshold: 0.1%.

Identification threshold: 0.2%.

Qualification threshold: 0.2%.

- Active substance manufactured by fermentation, single compound.

Reporting threshold: 0.15%.

Identification threshold: 0.2%.

Qualification threshold: 0.2%.

- Active substance manufactured by fermentation, family of compound.[14]

Reporting threshold: 0.15%.

Identification threshold: 0.2%.

Qualification threshold: 0.5%/0.2%.

CONCLUSION

From this review we conclude that, it is mandatory requirements in various pharmacopoeias to know the impurities present in API's and Formulation. 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. To isolate and quantify the impurities, various instrumental analytical techniques are routinely been used. We conclude that both United States and Europe having well established guidelines for impurity identification and specified limits for control impurity in API and Formulation.

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