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Review Article

An Overview on Scientific Approaches for Impurity Profiling In New Pharmaceutical Substances and Products- A Review Article

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ABSTRACT: In the pharmaceutical world, an impurity is considered as any other organic material, besides the drug substance, or ingredients, arise out of synthesis or unwanted chemicals that remains with API's. Impurity profiling is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing its need and scope in pharmaceutical research. The control of impurities is currently a critical issue to the pharmaceutical industry. The control of impurities in Formulated products and Active Pharmaceutical ingredient's were regulated by various regulatory authorities like US-FDA, ICH, MHRA, TGA etc. As per International Conference on Harmonization guidelines, the Impurity may be defined as any component of new drug product that is not the drug substance or an excipient in drug product. Impurity profiling has gained importance in modern pharmaceutical analysis due to the fact that unidentified, potentially toxic impurities are hazardous to health and in order to increase the safety of drug therapy, impurities should be identified and determined by selective methods. The present review covers various aspects related to the analytical methods for impurity profiling of an active pharmaceutical ingredient.

KEYWORDS: pharmaceutical, hazardous, impurities, analytical method, Impurity profiling.

INTRODUCTION

ICH defines impurities as for pharmaceutical products; impurities are substances in the product that are not the API itself or the excipients used to manufacture it. They can lowered or change
the pharmacological efficacy of active pharmaceutical ingredients (API). Sometimes the effect produced by impurities can be teratogenic, mutagenic or carcinogenic.[1] This can be fetal for human health therefore; there is an ever increasing interest in controlling and monitoring impurities present in API/pharmaceutical products. Hence API impurity profiling is required.[2] Quantitative determination of these impurities could be used as a method for the quality control and validation of drug substances. Regulatory authorities such as US FDA (United States Food and Drug Administration), CGMP (Current Good Manufacturing Practice), TGA (Thermo Gravimetric Analysis), and MCA (Ministry of Corporate Affairs) insist on the impurity profiling of drugs. Impurities in new drug substances can be addressed from two perspectives:- (1) The chemical aspect, which includes classification and identification of impurities, report generation, listing of impurities in specifications, and a brief discussion of analytical procedures. (2) The safety aspect, which includes specific guidance for quantifying impurities, present, substantially at lower levels, in a drug substance used in clinical studies.[3]

Impurity profile [4]

There is no precise definition for impurity profile. It gives an account of impurities present in it. Impurity profile is a description of the identified and unidentified impurities present in a typical batch of API (Active Pharmaceutical Ingredient) produced by a specific controlled production process. It includes the identity or some qualitative analytical designation (e.g. retention time), the range of each impurity observed, and type of each identified impurity. Impurity profile of a substance under investigation gives maximum possible types of impurities present in it. It also estimates the actual amount of different kinds of impurities present in it. For each API there should be an impurity profile describing the identified and unidentified impurities present in a typical

batch. The impurity profile is normally dependent upon the process or origin of the API.

Terminology for Impurity

Impurities have been named differently by various groups of scientists who deal with them. Terms that are used by official bodies such as compendia or that have been found acceptable by ICH and various regulatory bodies.

1. Common Names [5]

Various terms that have been commonly used to describe impurities are listed alphabetically below.

- ✓ By-product
- ✓ Degradation product
- ✓ Interaction product
- ✓ Intermediate
- ✓ Penultimate intermediate
- ✓ Related product
- ✓ Transformation product

2. U.S. Pharmacopeia Terminology

The United States Pharmacopeia (USP) discusses impurities in various sections:

- ✓ Impurities in Official Articles
- ✓ Ordinary Impurities
- ✓ Organic Volatile Impurities

3. ICH Terminology [6]

According to ICH guidelines impurities can be broadly classified into the following three Categories for the drug substance produced by chemical synthesis.

- ✓ **Organic impurities:** starting materials, process-related products, intermediates, and degradation products.
- ✓ **Inorganic impurities:** salts, catalysts, ligands, and heavy metals or other residual metals.
- ✓ **Residual solvents:** organic and inorganic liquids used during production.

Regulatory Guidelines on Impurities in an Active Pharmaceutical Ingredient:

Ethical, economic and competitive reasons as well as those of safety and efficacy support the need to monitor impurities in drug products. However monitoring impurities and controlling these impurities mean different things to different people or to the same people at different times, even those in the pharmaceutical sciences and industry. A unified terminology is necessary to assure that everyone uses the same vocabulary when addressing questions related to impurities. The United States Food and Drug Administration (US FDA) have endorsed the guidance prepared under the guidance of the International Conference of harmonization (ICH). The ICH guideline for impurities in pharmaceuticals was developed with joint efforts of regulators and industry representatives from the European Union (EU), Japan and United States and it has helped to ensure that different regions have consistent requirements for the data that should be submitted to various regulatory agencies. The guidelines not only aid the sponsors of New Drug Applications (NDA) or Abbreviated New Drug Application (ANDA) with the type of information that should be submitted with their applications, but also assist the FDA reviewers and field investigators in their consistent interpretation and implementation of regulations. The various regulatory guidelines regarding impurities are as follows:

1. ICH guidelines “stability testing of new drug substances and products"- Q1A
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 medicines, Therapeutic Governance Authority (TGA), Australia

Rationale for Reporting of Impurities in Active Pharmaceutical Ingredient:

The setting of limits for allowable impurities in bulk drugs is a complex process which depends on number of factors like toxicology of impurities related to drug, route of administration, daily dose, target population, source of drug substance and duration of therapy. The basis behind setting limits on level of impurities is that impurities in drug substance must be controlled to ensure the safety and efficacy and quality of API throughout its development and use as a product, as some of these impurities might possess certain undesirable toxicological potential. 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 demonstrating the unsuccessful effort should be reported. The identification of impurities present at the level less than the identification threshold is not generally considered necessary. But analytical methodology needs to be developed for the impurities that are expected to have unusual toxic pharmacological effects."

Specifications for Impurities [7]

The specifications for a new drug substance should include limits for impurities. Stability studies, chemical development studies and routine batch analysis can be used to predict those impurities likely to occur in the commercial product. A rationale for the inclusion or exclusion of impurities in the specifications should be presented. This rationale should include a discussion of the impurity profiles observed in the safety and clinical development batches, together with a consideration of the impurity profile of material manufactured by the proposed commercial process.

Reporting of Impurities

All impurities above (>) reporting threshold should be reported

Identification of Impurities

All impurities above (>) identification threshold are supposed to be identified. These include development of a suitable technique for isolation of desired impurities and their identification/characterization using various spectroscopic techniques to know the chemical structure of these impurities, and to suggest a possible synthetic route for formation of these impurities.

Qualification of Impurities

The profile of impurities in a new drug substance may change for a variety of reasons, such as process scale-up changes, synthetic route change and changes made to key intermediates. ICH decision tree help to classify quality and select limits for New Molecular Entities (NMEs). If an impurity exceeds the qualification threshold listed in Table 1, studies are needed to qualify that impurity in drug substances. Qualification is the process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified.

Thresholds

Higher or lower threshold limits based on scientific rationale including drug class effects and clinical experience.

- Adverse reaction in patients (lower)
- Patient population higher
- Drug class effects higher
- Clinical experience higher

Table 3: Thresholds for degradation products in drug substances

Max. Daily Dose(a)	Reporting Threshold (b,c)	Identification Threshold (b,c)	Qualification Threshold (b,c)
≤ 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%

a The amount of drug substance administered per day.

b Higher reporting threshold should be scientifically justified.

c Lower threshold can be appropriate if the impurities are unusually toxic.

Table 4: Thresholds for degradation products in drug products

Maximum daily dose a	Reporting threshold b,c
≤1 g	0.1%
>1 g	0.05%
Maximum daily dose a	**Identification threshold b,c**

<1 mg	1.0% or 5 µg TDI, whichever is lower
1 mg–10 mg	0.5% or 20 µg TDI, whichever is lower
>10 mg–2 g	0.2% or 2 mg TDI, whichever is lower
>2 g	0.10%
Maximum daily dose a	**Qualification threshold b,c**
<10 mg	1.0% or 50 µg TDI, whichever is lower
10 mg–100 mg	0.5% or 200 µg TDI, whichever is lower
>100 mg–2 g	0. 2% or 3 mg TDI, whichever is lower
>2 g	0.15%

a: The amount of drug substance administered per day.

b: Thresholds for degradation products are expressed either as a percentage of the drug substance or as total daily intake

(TDI) of the degradation product. Lower thresholds can be appropriate if the degradation product is unusually toxic.

c: Higher thresholds should be scientifically justified

ISOLATION AND CHARACTERIZATION [8]

It is frequently necessary to isolate and characterize impurities in order to monitor them accurately, because approximate estimations of impurities are generally made against the material of interest (i.e. drug substance) and can be incorrect. These estimations are based on the assumption that impurities are structurally related to the material of interest and thus have the same detector response. It is important to test this assumption because impurities frequently have different structures with significantly different detector responses. Most of the time it is difficult to ensure that the assumption stated above is correct. Number of methods can be used for isolation and characterization of impurities. But the application of any method depends on the nature of impurity (i.e.) its structure, physicochemical properties and availability. The following methods are commonly used for the isolation, they are

1. Extraction
2. Column Chromatography
3. Preparative Separations

Extraction

1. Liquid-Solid extraction
2. Liquid-Liquid extraction

LIQUID-SOLID EXTRACTION

To simplest form, a solvent is selected that would dissolve the impurity of interest but not the solid matrix. If compound contains more than one impurity means, in that case desirable to use an organic solvent for extraction because of its unique properties. It is generally easier to volatilize the organic solvent at low temperatures in order to concentrate the impurity. Commonly using various organic solvents are enlisted in Table No. 1 with boiling point and dielectric constant.

Table 1: List of solvents for Liquid-Solid extraction

Solvent	Boiling point	Dielectric constant
n-Hexane	190	1.9
Cyclo hexane	81	2.0
Carbon tetrachloride	77	2.2
Toluene	110	2.4
Ethyl ether	35	4.3
Chloroform	61	4.8
Methylene chloride	40	8.9
Ethanol	78	24.6
Acetonitrile	82	37.5
Dimethyl formamide	153	36.7
Water	100	80
Formamide	210	111
Dimethyl formamide	153	36.7

Soxhlet Extraction

It is a popular method for extracting compounds of interest from solids. eg. Natural products are isolated by reputedextraction with the suitable solvent. The main advantage of this method is that it allows utilization of a small volume of solvent to produce a fairly concentrated extract. The material to be extracted is placed in the Soxhlet extractor, the extraction vessel is heated adequately to ensure volatilization of solvent vapors, which are condensed n the top of the material to be extracted. The condensed solvent percolates through the material and drains back into the extraction vessel to repeat the process.

Steam Distillation

It is yet another method that can be used for extracting volatile components from natural materials and other matrixes of interest.

Supercritical fluid extraction (SFE)

Supercritical fluid extraction provides idealized means of extracting materials, since high solute diffusivity, lower viscosity and excellent solvating properties can be obtained with supercritical fluids, they provide excellent means of isolating impurities and other compounds of interest in a short period of time. The critical pressure, critical temperature and density of a few compounds used for SFE are given in Table No. 2. But carbon dioxide is most commonly used for SFE because of its availability, ease of use and disposition.

Table 2: List of solvents for SFE

Solvent	Pressure (ATM)	Temperature	Density (g/ml)
n-pentane	33.3	196.6	0.232
Carbondioxide	72.9		0.448
Ammonia	111.3	132.3	0.24

LIQUID-LIQUID EXTRACTION [9,10]

This simply entails extraction of one liquid with another generally one of those liquid is aqueous and other is organic. The primary requirement is that these liquids to be immiscible. This procedure is very useful when the liquid into which the material of interest is being extracted is easy to volatilize, thus permitting concentration of the material. Hence the choice of solvents must be made with that consideration in mind. In this type of extraction process, a solute is distributed between two immiscible solvents. The extraction is controlled by distribution or partition co-efficient which defines the ratio of concentration of the solute in two solvents a and b

$$Kd = Ca / Cb$$

Kd is the distribution co-efficient or partition coefficient. The distribution co-efficient related to a single species and does not include possible products of side reactions.

Column Chromatography

This technique is commonly used for the separation of pharmaceutical compounds in preparative chemistry. The separation of quantities ranging from micrograms to kilograms, which depends on the size of the column. Detection of the eluent is generally performed by UVspectrophotometry, either continuously by using a flow cell or periodically by monitoring the collected fractions from a given sample that alerts the emergence of UVactive components. Commonly silica gel or alumina is used in classic adsorption chromatography. Ion exchange resins to chemically modified polydextran gels used primarily for the analysis of biological samples. For liquid-liquid partition chromatography columns, inert carrier such as celite or kieselguhr is impregnated with an aqueous buffer or another polar solvent such as dimethyl formamide or dimethyl sulfoxide and elution is carried out with non-polar solvents.

Thin Layer Chromatography

It is a valuable technique for isolation and purification of compounds. All the modes of chromatography including adsorption, partition, ion exchange and gel filtration can be utilized. In addition choosing a sorbent and an eluent for performing TLC it is necessary to select a suitable method for applying a sample to the plate. Silica gel plates with or without fluorescent indicator are frequently used for most application. Detection is frequently performed by UV eg. 366nm or Iodine vapors can help to detect most of the organic substance. To elute the material from the plates, the simplest method is scraping the sorbent containing the material of interest and it is

extracted with a suitable solvent, followed by filtration or centrifugation. The solvent is removed to collect the desired substance. If aluminium plates are used means cut the sample and
eluted.

Gas Chromatography [10]

It is very useful for isolation and characterization of volatile components or those components that can be made volatile by derivatization technique and the detector used should be non destructive. Now GC is more apt to be used in combination with mass spectrometry (GC/MS) for characterization of impurities.

Hyphenated techniques [11]

1. LC-MS-MS
2. HPLC-DAD-MS
3. HPLC-DAD-NMR-MS
4. GC-MS
5. LC-MS

Hyphenated techniques are first line of defense in impurity determination. Hyphenated techniques are those techniques, where two or more analytical techniques are combined. The various hyphenated techniques used for impurity characterization are LC-MS, LCNMR, LC-MS-NMR, LC-MS-MS, GC-IR and GC-MS. The two most commonly used hyphenated techniques for impurity profiling are LC-MS and LC-MS-NMR. In these techniques chromatographic techniques are coupled with a spectroscopic detector. Thus impurity structure determination can be performed in real time during chromatographic separation and both isolation and characterization is performed in one single step. The use of hyphenated techniques for impurity determination is on rise due to easy availability of bench-top instrumentation and their distinct advantages like versatility, sensitivity, possibility of profiling sub structural analysis and rapid selective quantitative determination of targeted compound even in mixtures. The only limitation of hyphenated techniques is the heavy cost of instrumentation due to which their use is not common and spread worldwide like GC, HPLC, MS or NMR systems. As on today these sophisticated techniques are mainly used for the purpose of monitoring, characterization and identification of impurities but they can be used for other analytical purposes as well.

APPLICATIONS OF IMPURITY PROFILING

Numerous applications have been sought in the areas of drug designing and in monitoring quality, stability, and safety of pharmaceutical compounds, whether produced synthetically, extracted from natural products or produced by recombinant methods. The applications include alkaloids, amines, amino acids, analgesics, antibacterials, anticonvulsants, antidepressant, tranquilizers, antineoplastic agents, local anesthetics, macromolecules, steroids etc. There are a few examples of impurities reported in the APIs mentioned in Table 3.

Table 3: various impurities reported in APIs

Drug	Impurities	Method	Ref No.
Budensonide	Impurities or degradation products	**HPLC**	***[12]***
Cefdinir	Related substances	HPLC	***[13]***
Donepezil	Process related impurities	HPLC	***[14]***
Linezolid	Process related impurities	HPLC	***[15]***
Loratidine	Process related impurities	HPLC	***[16]***
Repaglinide	Process related impurities	HPLC	***[17]***
Rofecoxib	Process related impurities	HPLC	***[18]***
AmphotericinB	Process related impurities	UV	***[19]***
Cimetidine	Process related impurities	HPLC	***[20]***

CONCLUSION

This review provides a perspective on impurities in drug substance and drug product. Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredients (API's). The key aspect is that the impurity profiling of a new chemical entity must be shown to be qualified. With a qualification threshold of 0.1%, or lower for high dose compounds, the pharmaceutical analyst must give careful thought to their analytical technology. The above study provides the valuable information about the impurities types and its classification,

various techniques of isolation and characterization, and regulatory guidelines.

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