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REVIEW ARTICLE: IMPURITY PROFILING OF PHARMACEUTICAL DRUG SUBSTANCE AND DRUG PRODUCTS

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ABSTRACT

Impurity profiling is very important parameter of pharmaceutical industries. It is used to determine the quality of the pharmaceutical products. Impurities in pharmaceuticals are the surplus chemicals that stay behind with the active pharmaceutical ingredients (or) develop during formulation (or) upon aging of both active content and formulated active ingredients to medicines. The efficacy and safety of pharmaceutical product is affected by presence of unwanted traces of impurities. Impurity profiling plays an important role in the detection, identification, structural elucidation and quantitative determination of organic & inorganic impurities as well as residual solvents in bulk drug and pharmaceutical formulations. Various regulatory authorities like ICH, USFDA, Canadian drug & health agency are emphasizing the identification of impurities in active pharmaceutical ingredients. Identification of impurities is done by variety of chromatographic, spectroscopic and hyphenated techniques used either alone (or) in combination with other techniques.

Definition of impurity

Impurities are undesirable chemicals that are present in active pharmaceutical ingredients (or) develop during manufacturing (or) storage of drug substance / drug product. That decrease

the quality, safety and efficacy of substance/product but may (or) may not make it unfit for its intended use.

As per the international conference on harmonization (ICH) guidelines, "impurities are undesirable chemicals (or) substance present in the pharmaceutical products". That does not have any pharmacological activity.

Definition of impurity profiling

Impurity profiling is the group of analytical activities. Impurity profiling includes detection, identification (or) structural elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations.

(Or) The description, characterization and quantitation of the identified and unidentified impurities present in new drug substances are known as impurity profiling.

INTRODUCTION OF IMPURITIES

In the pharmaceutical industries, impurity is considered as organic material (or) unwanted chemicals remain with the active pharmaceutical ingredient. In general, most of the impurities are present in very small molecules. Impurities most commonly occur in solid dosage forms because of limited mobility. Impurities can reinforce (or) diminish the pharmacological efficacy of the active pharmaceutical ingredient. Some of the impurities may cause toxicological problems. The occurrence of undesirable chemicals, even in very minute amount may influence the safety and efficacy of the product. In olden days, there was very difficult to identify and separate the the impurities present in pharmaceutical products and to maintain the quality and purity of products. These are the major challenges for bulk drug industries. Pharmaceutical impurities can arise from many sources such are starting materials and their contaminants, reagents, catalyst, solvents, intermediates, excipients and their contaminants. But now a days, modern separation methods clearly play an important role in the scientific research because these methods can operate simultaneously separate and quantify the components. Hence, the process of separation and characterization of undesirable chemicals are very simple and easier. The quantity of impurities more than 0.1%, it should be identify and quantify by suitable methods. The structure of impurity is compared with structure of previously determined impurity, then minimize quantity of impurities. Now

a days,not only purity profile but also impurity profiling has become mandatory according to various regulatory authorities.

Disignation of impurities

Various terms are used by regulatory bodies and ICH to describe the impurities such are:

Common names	USP terminology	ICH terminology
1. Starting material 2. Intermediates 3.pneultimate intermediate(final intermediate) 4.by-products 5. Transformation products (similar to by-products) 6. Interaction products 7. Related products (similar to drug substance) 8. Degradation products	1. Impurities in official articles 2. Ordinary impurities 3. Organic volatile impurities	1. Organic impurities 2. Inorganic impurities 3. Residual solvents

Impurity profiling

Abuja (1998) and gorog (2000), Singh et Al. (2012) have published the books consist various aspects of impurities including regulatory requirements, sources and types of impurities, isolation, characterization and monitoring of impurities present in the pharmaceutical products. Many pharmacopoeias such are Indian pharmacopoeia (IP), British pharmacopoeia (BP), European pharmacopoeia (EU), USP etc are given some acceptable limits to allowable levels of impurities present in API and formulations. This acceptable limits does not effect the quality of the product and does not show any harmful effects (or) adverse effects. Impurity profiling is used to identify the how much amount of impurities (undesirable substance) are present in drug substance based on the profiling data, minimize the quantity of impurities and prevent the most of the health complications like carcinogenic effect, teratogenic effect or mutagenic effect. Now a days,the impurity profiling of bulk drug substance given more interest to the manufacturers and regulatory authorities.

Regulatory bodies involved in impurity profiling

Impurity profiling can be done by various regulatory authorities such are ICH, CDSCO, EMA, USFDA and Canadian drug & health agency for the isolation of impurities in active pharmaceutical ingredients.

ICH guidelines

1. ICH stand for international council for harmonization of technical requirements for pharmaceuticals for human uses is unique in both regulatory authorities and pharmaceutical industry for the discussion of scientific and technical aspects of pharmaceutical drug registration.
2. ICH guidelines to ensure the safe, effective and high quality medicines are developed and registered.
3. ICH guidelines have been promote the public health & prevent unnecessary duplication of clinical trials in human and minimize the use of animal testing without safety & effectiveness.
4. ICH guidelines are divided into four categories such are

- Quality guidelines
- Safety guidelines
- Efficacy guidelines
- Multidisciplinary guidelines

Classification of quality (Q) guidelines on the basis of impurities:

S. No	Section	Impurities	Sub-section
1	Q3A(R2)	Impurities in new drug substances	Q3A(R)
2	Q3B(R2)	Impurities in new drug products	Q3B(R)
3	Q3C(R5)	Impurities: guideline for residual solvents Impurities: guideline for residual solvents (maintenance) PDE for tetrahydrofuran (in Q3C(R3)) PDE for N-methyl pyrrolidine (in Q3C(R3))	Q3C Q3C(M) Q3C(M)

Other regulatory guidelines

1. Usfda

1. USFDA stand for United State of food and drug administration.
2. USFDA authority is very broad and oldest comprehensive protection agency.
3. USFDA regulates the different varieties such are

- Foods including food additives, dietary supplements and other food products.
- For drugs,it regulates the prescription &non-prescription drugs.
- It regulates medical devices, tobacco products, veterinary products and cosmetics.
- For biologics,it regulates the vaccines, blood and blood products.

4. This guideline mainly responsible for protecting the public health by assuring safety and efficacy of various products and also regulating the manufacturing, marketing & distribution of tobacco products to prevent misusage of tobacco.

Guidelines	Depiction
US-FDA	""NDA"s- impurities in new drug substance"
US-FDA	""ANDA"s- impurities in NDS"
Australian regulatory guideline	Australian regulatory guideline for prescription medicines, therapeutic governance authority (TGA) Australia.

2. EMA (European medicines agency)

1. This agency is responsible for the protection and promotion of public and animal health through the evaluation and supervision of medicines for human and veterinary usage.
2. It is a decentralised agency of European Union.it is located at London.
3. It also responsible for scientific evaluation of the medicines. These are developed by pharmaceutical companies.
4. EMA is responsible for coordinating the safety morning (or) pharmacovigilance system for medicines monitoring through the European network.
5. EMA agency under the various works are

1. Providing the report to pharmacovigilance activities.
2. Fee developing guidelines & setting standards.
3. To monitoring the pharmaceutical industries along with their pharmacovigilance obligations.
4. Finally informing to all the public about drug safety.

Sources of impurities

1. In pharmaceutical drug development, impurities can be arise in two main areas.

1) Bulk drug substance synthesis development
2) Drug product formulation development

2. During the process of drug substance synthesis development, impurities are generated from synthesis process (or) process of degradation.
3. In drug product formulation development, impurities can be generated from degradation products.
4. Impurities may also arise from physical contamination and improper storage conditions.
5. There are diverse sources of impurities.it includes

i. Raw materials at initial stage of synthesis.
ii. Reagents used for reaction. Eg:- catalyst
iii. Impurities can be arise from side reactions during synthesis.
iv. Impurities can be produced intermediately during synthesis of required compound (drug substance).
v. Therapeutically active compound.
vi. Impurities originated from thermolytic, photolytic, hydrolytic degradation of drug substance.
vii. Impurities formed due to excipient incompatibility,wet granulation, compression and impurities under category F.

Classification of impurities

According to ICH: Based on the source, impurities are classified into three categories. Such are

1. Organic impurities: it consists of two categories.

A. Degradation related impurities (DRI):

a. API-degradation
b. API- excipient interaction
c. API-residual interaction
d. API-container interaction

B. Process related impurities (PRI):

a. Excipient interaction
b. Starting materials
c. By-products
d. Intermediates
e. Reagents, ligands & catalyst

2. Residual solvents:

a. Organic volatile liquid
b. Inorganic volatile liquid

3. Inorganic impurities:

a. Reagents, ligands, catalyst
b. Heavy metals/ other residual metals
c. Inorganic salts
d. Other materials (Eg: filter aids, charcoal)

Ich limits of impurities and degradation products

1. According to ICH guidelines, identification of impurities below 0.1% level is not considered to be necessary.
2. According to ICH, the maximum daily dose qualification threshold is <2g/day,0.1% (or) >2g/day,0.05%.

Thresholds for reporting impurities

Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
Less (or) equal to 2g/day	0.05%	0.10% (or) 1.0mg/day (Whichever is lower)	0.15% (or) 1.0mg/day (Whichever is lower)
>2g/day	0.03%	0.05%	0.05%

Threshold for reporting degradation products

Maximum daily dose	Reporting threshold	Identification threshold	Qualification threshold
≤1 mg		1.0% (or) 5µg TDI (Whichever is lower)	0.15% (or) 1.0 mg/day (Whichever is lower)
1 mg - 10 mg		0.5% (or) 20µg TDI (Whichever is lower)	0.05%
10 mg - 100 mg			0.5% (or) 200µg TDI (Whichever is lower)
< 10 mg			1.0% (or) 50µg TDI (Whichever is lower)
> 10 mg - 2 g		0.2% (or) 2mg TDI (Whichever is lower)	
>100 mg - 2 g			0.2% (or) 3mg TDI (Whichever is lower)
≤ 1 g	0.1%		
> 1 g	0.05%		
> 2 g		0.1%	
> 2 g			0.15%

Analytical methods of impurities

- Analytical methods are most aboundantly needed to isolate & characterize the impurities and monitor them accurately.
- These methods are used for the identification, determination, quantitation and separation of impurities in new drug substance (or) new drug products.

- Analytical methods gives specific limits depending upon the quality & purity of the new product. If the product exceed specific limits,it can be rectified by repeating the procedure and gathering more information. Then finally identify the error and rectify them.
- This methods gives safety and quality assurance to the new products for human and animal consumption.
- Quality is an important factor in every product/service.but it plays Vital role in pharmaceutical products.
- Analytical methods are also involved in the validation of products.it gives more accuracy and precision of compounds.
- Nowadays, hyphenated techniques are also used for analysis because of this techniques can reduce the time consumption and to produce more accuracy.
- Standard analytical limits of specific products/ substance are given by regulatory authorities such are ICH, USFDA, EMA etc.
- Standard analytical procedures and limits of every product (or) substance are not present in the pharmacopoeias. Hence,we can perform the analytical testing methods several times and gathering the data obtained from testing results. Then finally fix the specific limit for specific product.
- Impurity can be identified predominantly by following methods.

1. Reference standard method
2. Spectroscopic method
3. Separation method
4. Isolation method and
5. Characterization method

1. Reference standard method

i. In this method, reference standards were prepared for the comparison of results of new developed products.
ii. This can be used for the assessment of safety of new drugs for patient consumption.
iii. Reference standards are used for identification, quantitation and separation of impurities in newly developed drug.
iv. This method not only used for active ingredients but also used for impurities, degradation products, starting materials and excipients.

2. Spectroscopic methods

A. UV-Visible spectroscopy

Uv-visible spectroscopy is widely used technique for determination of impurities present in the sample (solution). This method can determine presence of impurities by representing as additional peaks. Then it will be compared with standard raw materials. This technique measures the absorbance of impurities at specific wavelength and then finally detected.

B. Infrared spectroscopy

This method is most commonly used. it can detect the impurities present even in per mill range(0.1-10%). It provides the information about specific functional groups of pharmaceutical products. It can detect the structure and quantitation of impurities present in pharmaceutical products because every compound has a unique spectral fingerprint.

FT-IR

Now a days, FT-IR method is very useful. it it can functional to resolve the presence (or) absence of chemically related impurities present in the raw materials. It is especially used on analysing the organic compounds determined by structure and functional groups of compound. Functional group of compound can determine the structure and concentration of compound.

Agilent Cary 630 FT-IR gives more precision results for both qualitative and quantitative analysis of pharmaceutical products. In this, solid and liquid samples are used. Cary 630 FT-IR can quick and easy to measure the contaminants such as ethylene glycol and diethylene glycol in glycerol.

C. NMR

It is very sophisticated system.it require nuclear magnetic resonance technology. It provides information regarding the structural characterization of compound like bonding structure and stereochemistry of molecules of pharmaceuticals. It can distinguish between monomeric and dimeric substances compare with standard mixture of authentic materials contain monomers and dimers. Sample requirement of NMR is 10mg as compared with mass spectroscopy (it requires <1%). This method is less sensitive in nature. It has limited use because of cost and time consideration.

D. Mass spectroscopy

It provides very good structural information. It has a significant impact on pharmaceutical development. It provide accurate measurement of identification, quantitation of impurities in samples. It has limited use because of cost and time consideration. Based on resolution of instrument, it may differentiate with small difference in molecular weight. In NMR, advanced design and efficient interfaces are used in separation techniques of mass spectroscopy for monitoring, characterization and quantification of drug related substances in API and formulations.

E. Raman spectroscopy

It is sensitive to impurities in pharmaceuticals. It is a convenient method for analysing the impurities in solid state crystals.

3. Isolation methods

A. HPLC

HPLC system consists two types i.e normal phase HPLC system and reverse phase HPLC system. In reverse phase HPLC system, mobile phase is polar in nature, eg: water, methanol, acetonitrile and stationary phase is non-polar in nature, eg: C8, C18, phenyl etc. It is used for the analysis of polar compounds (amines, alcohol, acids etc.). This method is widely used in the pharmaceutical industries compared to normal phase. It gives more accurate results and sophisticated method.

In normal phase HPLC system, mobile phase is non-polar (hexane, dichloromethane, ethyl acetate) and stationary phase is polar in nature (silica). It is used for the analysis of non-polar compounds.

HPLC system consist inert mobile phase reservoir. In this different types of pumps are used but nowadays mechanical pumps (receprocating pump) and pneumatic pumps are widely used. Pump can force the mobile phase through HPLC at a precise flow. Injector can introduce the sample into column. Columns are available in different types, shapes and dimensions. Columns are made up of silica (nonpolar organic phase bonded with silica). it is a heart of the chromatograph. Separation occur in the column. Column oven maintain the temperature of column. Detector can detect the different molecules elite from column based on physical and chemical properties. Detector measures the concentration of compound & quantitative analysis of impurities and samples. Control and data processing system, control

the all units of system and receive the signals generated from detector then find out the retention time (qualitative analysis) and concentration of sample (quantitative analysis).

B. Thin layer chromatography

Thin layer chromatography is a chromatographic technique. It is used for the separation of mixture of components. It is most widely used technique, simple, less economic and easy to perform. TLC was performed on a sheet of glass, plastic (or) aluminium. Which is coated with a thin layer of stationary phase phase (i.e. silica gel, cellulose and aluminium oxide). Sample was spotted on the sheet. After sample application,the plate has been placed in the saturated closed chamber. This chamber was Saturated with mobile phase (like hexane, acetone etc). The mobile phase is drawn up the plate via capillary action. As different analytes ascend the TLC plates at different rates, separation is achieved.

Now a days hyphenated techniques are available. Those techniques produce more resolution and good reproducibility when compared to normal techniques.

C. Gas chromatography

GC is used for analysis of organic volatile impurities such as residual solvents present in pharmaceuticals. It is worldwide used technique because it follows ICH Q3 guidelines. This technique provides more resolution and selectivity of compound. Sample must be gaseous state (mobile phase) Eg: helium, hydrogen etc. Stationary phase is coated as a thin layer of polymer/liquid on the inner surface of column. Gaseous compound can interact with column and elution take place at different times is known as retention time. Detectors can separate the mixture of compounds and analyse the purity of compound. Now a days, GC-MS method is used as hyphenated technique can be used for determination and identification of impurities.

D. Capillary electrophoresis

Capillary electrophoresis is easy and simple technique. This technique is used for the analysis compound is available in minute quantity. But It cannot be detected by other techniques. It can provide even in minute range of threshold values. This is a more selective and highly resolution technique. In this technique, three vials are used such are sample vial, source vial and destination vial. Vials are filled with aqueous buffer solution and sample solution placed in sample vial. Place the electrodes in vials like anode in source vial and cathode in destination vial. Capillary tube is placed in sample vial and filled with sample by capillary action, pressure.then replaced into source and destination vials and apply the high

voltage power. The migration of analyte takes place through capillary tube in same direction by electroosmotic flow. Detector can sent the data to computer and analyse the compound. Based on data, seperation of chemical compounds takes place and identify the impurities by their different retention times in an electropherogram.

Hyphenated techniques

LC-MS Spectroscopy

This is a highly sensitive technique and most commonly used to detect, identify and quantify the impurities present in the pharmaceutical products. It is a sophisticated hyphenated technique. It gives more accurate results compared to normal methods.

LC-MS spectroscopy distinguished into two types: HPLC and MS. In HPLC, the procedure of instrumentation is same as above discussed in hplc technique. But the detectors used in LC-MS is mass spectrometers. Other HPLC detectors are either placed in a series or mobile phase can be directly delivered to spectrometer by splitter. In this spectroscopy, some times, secondary detectors are also used such are refractive index detector, fluorimetry detector, UV detector etc.

Mass spectroscopy technique gives provides more sensitivity and specificity compared to other technique. Quadrupole detector in MS more suitable to conformation of know impurities and preliminary structural characterization of unknown impurities. Quadrupole-time of light in MS is highly sensitive and used to determine the traces of unknown impurities.

LC-MS-MS technique

In this technique, triple quadrupole detectors are used. It gives selective and specific results for quantitative analysis of organic impurities in pharmaceuticals. It is a highly sensitive method. This method can be used for bioanalytical studies of mixture of compounds and biological matrices (urine, saliva, blood).

CONCLUSION

This article provides the information about the impurities present in the pharmaceutical products and substances. It gives ICH limits for impurities. This article provides the data about quality, safety, efficacy and genotoxicity of drug substance and products. The main aspect for the profiling of the impurities is to detect the new entity over its threshold limit of

0.1% in the pharmaceutical formulations. Thus the analyst performing the quantification have to utilize the correct methods. Which have high selectivity nature.

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