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Review Article

IMPURITY PROFILING OF ACTIVE PHARMACEUTICAL INGREDIENTS AND FINISHED DRUG PRODUCTS

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ABSTRACT

Pharmaceuticals impurities are the unwanted chemicals that remain or are generated during the formulation of medicines. Impurity profiling helps in detection, identification and quantification of various types of impurities as well as residual solvents in bulk drugs and in pharmaceutical formulations. It is a best way to characterize quality and stability of bulk drugs and pharmaceutical formulations. Due to rapid development of the analytical methodology it is imperative to review problems related to impurities present in the drug substances and drug products with their solutions. Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agencies are emphasizing on the purity requirements and on identification of impurities in active pharmaceutical ingredients as presence of impurities even in small amounts may influence the efficacy and safety of the pharmaceutical products. Thus enlightening the need of impurity profiling of drug substances in pharmaceutical research this review focuses on various analytical methods for identification as well as quantification of impurities present in the pharmaceuticals.

Keywords: Bulk drugs, Impurities, Formulation, Analytical method development.

INTRODUCTION

IMPURITY

Pharmaceuticals impurities are the unwanted chemicals that remain with the active pharmaceutical ingredients (APIs) or are developed during formulation or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products[1].

IMPURITY PROFILING

It gives an account of impurities present in the bulk and finished drug. It helps in identifying and quantifying the impurities present in drug substance (API) or pharmaceutical formulation. It gives maximum possible types of impurities present in drug substance (API) and in pharmaceutical formulations[1].

CLASSIFICATION OF IMPURITIES

Impurities are classified on the basis of pharmacopeia and guidelines:

As per United State Pharmacopoeia

The United States Pharmacopoeia (USP) classifies impurities in two sections-ordinary impurities and organic volatile impurities.

Ordinary Impurities

Ordinary impurities are found in bulk pharmaceutical chemicals that are innocuous by virtue of having no significance on biological activity of the drug substance. These impurities may arise out of the synthesis, preparation or degradation of chemical.

Organic Volatile Impurities

Organic volatile chemicals are produced in the manufacture of drug substances or excipients or in

the preparation of drug products, they are volatile in nature and by themselves get removed out at time of storage or processing[2].

As per ICH Guidelines

The ICH guideline classifies impurities in three sections- organic, inorganic and process based impurities.

Organic Impurities

Such type of impurities arises during manufacturing process and/or during storage of the drug substance. These include starting or intermediate impurities, by-products impurities, degraded products impurities and enantiomeric impurities.[2,3]

Starting Materials or Intermediates Impurities

These are common type of impurities which are found in almost every API unless a proper care is taken in every step involved throughout the multi-step synthesis of drug product. Although the end products are always washed with solvents but there are chances of having the residual of unreacted starting materials unless the manufacturers are very careful about the impurities. Eg. in paracetamol bulk, there is a limit test for p-aminophenol, which could be a starting material for some manufacturer or be an intermediate for another.[3]

By-Products Impurities

In synthetic organic chemistry, getting a single end product with 100% yield is very rare as there is always a chance of having some by-products along with desired end product. Eg. in case of paracetamol bulk, diacetylated paracetamol may form as a by-product.

Degraded Products Impurities

Impurities can also be formed by degradation of the end product during manufacturing of bulk drugs. Such types of impurities are common in the medicines as they result from improper storage of formulation. The degradation of penicillins and cephalosporins is a well-known example of degradation products[4]. The presence of a ß-lactam ring as well as that of an α-amino group in the C6/C7 side chain plays a critical role in their degradation.[5]

In general, an individual API may contain all of the above mentioned types of organic impurities at various levels ranging from negligible to significant amount. As the organic impurities are the most common product related impurity as well as the process related impurity, it is the responsibility of both the manufacturers of APIs and formulators to take care of these impurities according to ICH guidelines or compendia.

Inorganic Impurities

Inorganic impurities are also obtained from the manufacturing processes which are used in bulk drugs formulation. They are normally known and identified. It includes impurities like heavy metal impurities, other material impurities (filter aids, charcoal) and residual solvent impurities.

Heavy Metals Impurities

The main source of heavy metals is water which is generally used in different manufacturing processes, where acidification or acid hydrolysis takes place. These impurities of heavy metals can easily be avoided by using demineralized water and glass-lined reactors.

Other Materials (Filter Aids, Charcoal) Impurities

The filters or filtering aids such as centrifuge bags are routinely used in the bulk drugs manufacturing plants and in many cases, activated carbon is also used which also act as a source of impurity. Therefore regular monitoring of fibers and black particles in the bulk drugs is essential so as to avoid their contaminations[6].

Residual Solvent Impurities

It is very difficult to remove these solvents completely by the work-up process.[7,8] However, efforts should be taken to the extent possible so as to meet the safety data. Some solvents that are known to cause toxicity should be avoided in the production of bulk drugs. Depending on the possible risk to human health, residual solvents are divided into 3 classes.

Solvents of class I included benzene (Class I, 2 ppm limit) and carbon tetrachloride (Class I, 4 ppm limit). These are to be avoiding because of their carcinogenic, toxicity effects.

On the other hand, solvents of class II includes methylene chloride (600 ppm), methanol (3000 ppm), pyridine (200 ppm), toluene (890 ppm), N, N-dimethylformamide (880 ppm) and acetonitrile (410 ppm).These are most commonly used solvents.

Solvents of class III includes acetic acid, acetone, isopropyl alcohol, butanol, ethanol and ethyl acetate have permitted daily exposures of 50 mg or less per day. In this regard, ICH guidelines for limits should be strictly followed.

Process Based Impurities

Apart from bulk drug-related impurities, the formulated form of API may contain impurities that are formed in various ways during the processing of the drug like impurity obtained due to method defect, impurity obtained due to environmental defect, impurity obtained due to factor defect, impurities formed due to mutual interaction among ingredients and impurities formed due to functional group reaction degradation.

Impurity Obtained Due To Method Defect

Impurity related to method may be caused by improper manufacturing processes which don't follow the optimized conditions like pressure, temperature during processing.[9] Eg. 1-(2, 6-diclorophenyl) indolin-2-one is formed as impurity in the production of a parenteral dosage form of diclofenac sodium[10], if it is terminally sterilized by autoclave. It was the condition of the autoclave method (i.e., 123 + 2°C) that enforced the intramolecular cyclic reaction of diclofenac sodium forming the indolinone derivative and sodium hydroxide. Formation of such impurity also depends on the initial pH of the formulation.[11]

Impurity Obtained Due To Environmental Defect

The primary environmental factors that can reduce stability include the following: Exposures to adverse temperatures**,** there are many APIs that are labile to heat or tropical temperatures. Eg. vitamins as drug substances are very heat-sensitive and get degradated frequently leading to loss of potency in vitamin products, especially in liquid formulations. Light-especially UV light causes initiating a large number of systems that are photolyzed; and causes formation of free radicals as end products.[12] Several studies have reported that ergometrine as well as methyl ergometrine injections are unstable under tropical conditions such as light and heat.

Impurity Obtained Due To Factor Defect

Although the pharmaceutical companies perform pre-formulation studies, including stability studies before marketing the products, sometimes the dosage form factors influence the drug stability and forces the company to recall the product. Fluocinonide Topical Solution USP, 0.05%, (Teva Pharmaceuticals USA, Inc., Sellersville, Pennsylvania) in 60-ml bottles was recalled in the United States because of degradation/impurities leading to sub-potency. In general, liquid dosage forms are very much susceptible to both degradation and microbiological contaminations. In this regard, water content, pH of the solution/suspension, compatibility of anions and cations, mutual interactions of ingredients and the primary container are also some critical factors. Microbiological growth resulting from the growth of bacteria, fungi and yeast in a humid and warm environment may result in oral liquid products that are unusable and unsafe for human consumption.

Impurities Formed Due To Mutual Interaction Among Ingredients

Most vitamins are very labile and on ageing they pose a problem of instability in different dosage forms, especially in liquid dosage forms. Degradation of vitamins such as folic acid, pantothenic acid, cyanocobalamin and thiamine do not give toxic impurities. However, potency of active ingredients drops below pharmacopoeial specifications. Because of mutual interaction the

presence of nicotinamide in a formulation containing 4 vitamins (nicotinamide, pyridoxine, riboflavin, and thiamine) causes the degradation of thiamine to a sub-standard level within a 1-year shelf-life of vitamin B-complex injections. The marketed samples of vitamin B-complex injections were found to have a pH in the range of 2.8-4.0. The custom made formulation in a simple distilled-water vehicle and in a typical formulated vehicle that included disodium editate and benzyl alcohol was also investigated and similar mutual interaction causing degradation were also observed.

Impurities Formed Due To Functional Group Reaction Degradation

Degradation products of drugs are considered to be transformation products of the drug substance formed due to the effect of heat, solvents (including high and low pH), oxidising agents, other chemical reagents, humidity and light.[12,13]

Hydrolysis

Hydrolysis is a common phenomenon for ester and amide type of drugs, especially in liquid dosage forms. Certain drugs which undergo hydrolysis are benzylpenicillin, barbitol, chloramphenicol, chlordiazepoxide, lincomycin and oxazepam.[13]

Oxidation

The oxidative decomposition of pharmaceutical compounds is responsible for the instability of a considerable number of pharmaceutical preparations. These reactions are mediated either by free radicals or by molecular oxygen. Drugs which undergo oxidative degradation are hydrocortisone, methotrexate, adinazolam, hydroxyl group directly bonded to an aromatic ring (eg, phenol derivatives such as catecholamines and morphine), conjugated dienes (eg, vitamin A and unsaturated free fatty acids), heterocyclic aromatic rings, nitroso and nitrite derivatives and aldehydes (eg, flavorings).

Decarboxylation

Some dissolved carboxylic acids such as p-amino salicylic acid, lose carbon dioxide from the carboxyl group when heated. Decarboxylation also occurred in the case of photoreaction of rufloxacin.

Photolysis

Pharmaceutical products are bared to light while being held improperly in pharmacy shops or hospitals, or when held by the consumer for imminent use. Drugs which undergo photolytic cleavage are ergometrine, nifedipine, nitroprusside, riboflavin and phenothiazines are very labile to photo-oxidation. In vulnerable compounds, photochemical energy creates free radical intermediates, which can accomplish by chain reactions. Most compounds degrade as solutions when bared to high energy UV exposure. Fluoroquinolones antibiotics are found to be susceptible to photolytic cleavage. In ciprofloxacin eye drops preparation (0.3%), sunlight induces photo cleavage reaction producing ethylenediamine analog of ciprofloxacin.

GUIDELINES FOR IMPURITY PROFILE

It is now getting important critical attention from regulatory authorities. The different pharmacopoeias, such as the British Pharmacopoeia (BP), the United States Pharmacopoeia (USP) and the Indian Pharmacopoeia (IP) are slowly incorporating limits to allowable levels of impurities present in the APIs or formulations. Also, the International Conference on Harmonization (ICH) has published certain guidelines on impurities in drug substances, products and residual solvents. There is a significant demand for the impurity reference standards and the API reference standards for both regulatory authorities and pharmaceutical companies. According to ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic. Limits for impurities in drug substances are shown in table1 while limits for impurities in degraded products of drugs are shown in table 2.

Specifications should be set for identified and unidentified impurities expected to be present in the drug substances and drug products over the period of intended use and under recommended storage conditions. These impurities are known as specified impurities and they should be individually listed in the specifications. Stability studies, chemical development studies and routine batch analyses can be used to establish impurities likely to occur in the commercial new drug substances and new drug products. A general specification limit of not more than 0.1% for any unspecified impurity should also be included. A rationale for why impurities were included or excluded from the specifications for the drug substance and drug products should be provided. Limits for impurities should be set no higher than the level which can be justified by safety data and unless safety data indicate otherwise, no lower than the level achievable by the manufacturing process and the analytical capability.

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR IMPURITY PROFILE[14-22]

The impurities can be identified predominately by methods like reference standard method, spectroscopic method, separation method, isolation method and characterization method.

Reference Standard Method

The key objective of this is to provide clarity to the overall life cycle, qualification and governance of reference standards are used in the development and control of new drugs. Reference standards serve as the basis of evaluation of both process and product performance and are the benchmarks for assessment of drug safety for patient consumption. These standards are needed not only for the active ingredients in dosage forms but also for impurities, degradation products, starting materials, process intermediates and excipients.

Spectroscopic Method

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

Separation Method

Capillary Electrophoresis (CE), Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), High Performance Liquid Chromatography (HPLC) are regularly being used for separation of impurities and degradation products.

Isolation Method

It is often necessary to isolate impurities. But if the instrumental methods are used, isolation of impurities is avoided as it directly characterizes the impurities. Generally, chromatographic and non chromatographic techniques are used for isolation of impurities prior to its characterization. The term ‘chromatographic reactor’ refers to the use of an analytical-scale column as both flow-through reactor and simultaneously, as separation medium for the reactant(s) and product(s). By using an HPLC, chromatographic reactor approach, the solution-phase hydrolysis kinetics of the Aprepitant (EmendTM) prodrug, for aprepitant dimeglumine, were investigated. In loratidine, impurity found was ofloratidine. Other examples include celecoxib and amikacin. A list of methods that can be used for isolation of impurities are solid-phase extraction methods, liquid-liquid extraction methods, accelerated solvent extraction methods, supercritical fluid extraction, column chromatography, flash chromatography, capillary electrophoresis (CE), gas chromatography (GC), thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC).

Characterization Method

Highly sophisticated instrumentation, such as GC-MS or LC-MS are inevitable tools used in the identification of minor components (drugs, impurities, degradation products, metabolites) in various matrices. After this identification of minor components and then their characterization can be done using NMR and MS.

The procedure of impurity profiling, begins with the detection of the impurities using the thin-layer chromatography, high-performance liquid chromatography or gas chromatography. Procurement of standard impurity samples from the synthetic organic chemists which includes, last intermediate of the synthesis, products of predictable side reaction and degradation products if any etc.

In the condition of unsuccessful identification with standard samples the most reasonable way to determine the structure of impurity starts with the investigation of the UV spectra, easily obtainable with the aid of the diode-array detector in the case of HPLC and the quantification with the help of densitometer. In exceptional cases, with full knowledge of the synthesis of drug martial, the structure of the impurity can be generated on the basis of NMR spectral data.

If the information obtained from the UV spectrum is not sufficient, the next step in the procedure of impurity profiling is to take the mass spectrum of the impurity. The major disadvantage of this method is the volatility and thermal stability problems of the impurities. The use of derivatization reactions widely used in GC/MS analysis is problematic because the side-products of the derivatization reaction can be confused with the impurities.

The next step in the impurity profiling is the synthesis of the material (impurity standard) with the proposed structure. The retention and spectral matching of the synthesized material with the impurity in question is carried out as outlined above.

The possibilities of spectroscopic techniques in drug impurity profiling without chromatographic separation are also worth mentioning. Spectra obtained by using high-resolution, highly sensitive NMR spectrometers and mass spectrometers with APCI /ESI facilities are suitable to provide a fingerprint picture regarding the purity of the sample.

The validation process involves confirmation or establishing a developed method by laboratory studies, procedures, systems, which can give accurate and reproducible results for an intended analytical application in a proven and established range.

The performance characteristics of the method (accuracy, precision, sensitivity, ruggedness, etc) should meet the requirements of the intended analytical applications and then the process can be used in a reliable manner.

CONCLUSION

Impurity profiling study is very important during the synthesis and manufacturing of drug substances (API) and dosage forms, as it helps in providing crucial data regarding the safety limit, limits of detection, limit of quantification, limit of several organic and inorganic impurities along with their toxicity limit. Thus, by the help of impurity profile study, it become convenient to design such a method and product where in expected impurity cannot interfere.

Table 1: Limits for impurities in drug substance

Drug Substance Impurity	Limits
Each identified specified impurity	Not more than 0.5 per cent
Each unidentified impurity	Not more than 0.3 per cent
Total impurities	Not more than 1.0 per cent

Table 2: Limits for impurities in degradated products of drugs

Degradation Product Impurity	Limits
Each identified degraded product	Not more than 1.0 per cent
Each unidentified degraded product	Not more than 0.5 per cent
Total degraded products	Not more than 2.0 per cent

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