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REVIEW-IMPURITY PROFILING

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ABSTRACT

The control of pharmaceutical impurities is currently a critical issue to the pharmaceutical industry. The International Conference on Harmonization (ICH) has formulated a workable guideline regarding the control of impurities. In this review, a description of different types and origins of impurities in relation to ICH guidelines and, degradation routes, including specific examples, are presented. A well accepted fact is that some impurities are unavoidable and will be present in trace amounts hence ICH comes into picture and through its guidelines and policies establishes the specification limits, evaluation and control of impurities. The regulatory bodies and drug development authorities look up to these guidelines for launching a quality drug into the market. Validation of analytical process for impurity identification is performed to establish the impurity profile of any drug substance. Hence the major focus of this review article is on characterization of impurities, its sources, establishment of impurity profile and analytical approaches to establish its profile. The article further discusses measures regarding the control of impurities.

KEYWORDS: Impurities, International Conference on Harmonization, Formulation, Profiling, Isolation.

INTRODUCTION

Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceuti-cal ingredients (APIs), or develop during formula-tion, 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. Impurity profiling (ie, the identity as well as the quantity of impurity in the pharmaceuticals), is now getting receiving important critical attention from regulatory authorities. The different pharmacopoe-ias, such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly in-corporating limits to allowable levels of impurities present in the APIs or formulations.

A number of recent articles[1-5] have described a designed approach and guidance for isolating and identifying process-related impurities and degrada-tion products using mass spectrometry, Nuclear Magnetic Resonance (NMR), high-performance liquid chromatography (HPLC), Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), and tandem mass spectrometry for pharmaceutical substances.

In general, according to ICH guidelines on impuri-ties in new drug products[6], identification of im-purities below the 0.1% level is not considered to be necessary unless the potential impurities are ex-pected to be unusually potent or toxic. In all cases, impurities should be qualified. If data are not avail-able to qualify the proposed specification level of an impurity, studies to obtain such data may be needed (when the usual qualification threshold limits given below are exceeded). According to ICH, the maxi-mum daily dose qualification threshold is consid-ered as follows:

≤ 2g/day 0. 1 % or 1 mg per day intake (whichever is lower)

≥ 2g/day 0.05%

This paper reviews the impurities found in the pharmaceuticals-identifying different sources, as well as providing examples and demonstrating pos-sible measures to take care of the impurities in the pharmaceuticals.

Impurity profile

Impurity profile describes the identified and unidentified impurities present in a new drug substance. Impurity profiling is the common name of a group of analytical activities, the aim of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations. It helps identifying the impurity present in pharmaceutical formulation by analytical technique or methods. Numbers of impurities present in formulation such by-products, degradation products, interaction products, intermediates, penultimate intermediates, related products, Transformation product. According to

USP impurities have various sections which are Impurities in Official Articles, Ordinary Impurities, and Organic Volatile Impurities. According to ICH impurities occurred or produced by chemical syntheses which are organic impurities (Process and Drug related), Inorganic Impurities, and Residual Solvents.[7] There are various sources of impurity like heavy metals, ligands, catalysts other materials like degraded end products obtained during or after manufacturing of bulk drug or products. The expert working group of the international conference on harmonization of technical requirements for registration of pharmaceuticals for human use commonly known ICH has "defined impurity is any compound of the medicinal product which is not the chemical entity defined as the active substance or as an excipient in the product".

Critical Factors Affects the Quality of Bulk Drugs
1. Crystallization
2. Washing the wet cake
3. Drying
4. Appropriate packaging

Classification[8-10]

1) Dosage form related

i. Mutual interaction amongst Ingredients
ii. Functional group- related typical Degradation

SOURCES OF IMPURITIES IN MEDICINES

Medicines are the formulated forms of active phar-maceutical ingredients. There are 2 types of impuri-ties in medicines: (1) Impurities associated with ac-tive pharmaceutical ingredients and (2) Impurities that are created during formulation and or with ag-ing or that are related to the formulated forms.

Impurities associated in with APIs

According to ICH guidelines[11], impurities associ-ated with APIs are classified into the following categories:
• Organic impurities (Process and Drug-related)
• Inorganic impurities
• Residual solvents
iii. Ester hydrolysis
iv. Hydrolysis
v. Oxidative degradation

2. USP Classification

According to united state pharmacopoeia impurities classify as
i. Impurities in official articles
ii. Ordinary impurities
iii. Organic volatile impurities

3. ICH Classification2

i. Organic impurities
ii. Inorganic impurities
iii. Residual solvents

Impurities coming from

- Interaction between the primary packaging and drug product
- At the time of contact between processing materials and storage bags, closure, filters, tubing material etc.
- Impurities also introduce during storage of compound
- Impurities also coming from label, ink, overwrap, cardboard, boxes etc.
- Impurities can be present at the synthesis of product (called genotoxic impurities) that is solvent, residues, catalysts, reaction product in synthesis etc.

Method for impurity detection[12-14]

1. Isolation and characterization
2. Column chromatography
3. Gas chromatography
4. Flash chromatography
5. TLC
6. GC
7. HPLC
8. HPTLC
9. Capillary electrophoresis (CE)

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1. Isolation

Isolation of impurity is necessary for accurate monitor, but it is only necessary if instrumental method is not available as it directly characterizes the impurity. Which method used for isolation and characterization of impurity is depends upon the nature of the impurity that means structure of impurity, its physicochemical properties and availability. For isolation of impurity methods used are, Chromatographic and Non – Chromatographic.

Following methods are commonly used for the isolation, they are
i. Extraction
ii. Column chromatography
iii. Preparative separation
iv. Extraction:
v. Liquid –solid extraction
vi. Liquid-liquid extraction

Organic impurities

Organic impurities may arise during the manufac-turing process and/or storage of the drug substance. They may be identified or unidentified, volatile or non-volatile, and include the following:

Starting materials or intermediates

These are the most common impurities found in every API unless a proper care is taken in every step involved throughout the multi-step synthesis. Although the end products are always washed with solvents, there are always chances of having the residual unreacted starting materials may remain unless the manufacturers are very careful about the impurities. In paracetamol bulk, there is a limit test for p-aminophenol, which could be a starting material for some one manufacturer or be an intermediate for an-other.

By-products

In synthetic organic chemistry, getting a single end product with 100% yield is very rare; there is always a chance of having by-products. In the case of paracetamol bulk, diacetylated paracetamol (Figure 1) may form as a by-product.

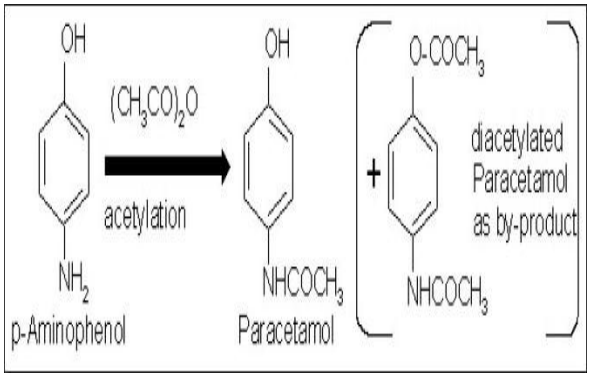

Figure: 1. Production of Paracetamol from intermediate, p-Aminophenol[15].

Degradation products

Impurities can also be formed by degradation of the end product during manufacturing of bulk drugs. However, degradation products resulting from storage or formulation to different dosage forms or aging are common impurities in the medicines. The deg-radation of penicillins and cephalosporins is a well-known example of degradation products. The presence of a ß-lactam ring as well as that of an a-amino group in the C6/C7 side chain plays a critical role in their degra-dation.[16]

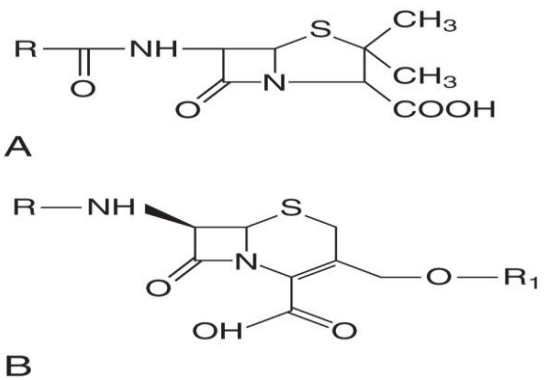

Figure: 2. General Structures of (I) Penicillins and (II) Cephalosporins.

Reagents, ligands, and catalysts

These chemicals are less commonly found in APIs; however, in some cases they may pose a problem as impurities.

In general, an individual API may contain all of the above-mentioned types of organic impurities at lev-els varying from negligible to significant.

A detailed investigation of impurities in semi-syn-thetic penicillin was performed both by the manu-facturers and the different research groups. A re-view paper on penicillins and cephalosporins[17] describes methods of isolation, detection, and quan-tification of degradation products, and antigenic polymeric by-products. Studies show the presence of traces of ampicillin polymers and hydrolyzed products in the API.[18] It has also been found that the presence of certain chemicals such as triethyl-amine has a degradative effect on the product. Am-picillin trihydrate samples having triethylamine content of 2000 ppm to 4000 ppm[19] were found to be stable under accelerated stability testing. However, the product showed appreciable degradation when triethylamine content became 7000 ppm. Recent pharmacopoeia[20] included the limit tests for the traces of impu-rities present in ampicillin and amoxycillin bulk raw materials. The residual solvents associated with these APIs have also been determined.[21]

As the organic impurities are the most common product-as well as process-related impurities, it is the responsibility of both the manufacturers of APIs and the users (ie, formulators) to take care of these impurities according to ICH guidelines or compen-dia.

In addition, for an optically active single isomer drug there could be enantiomeric impurities present in the API.

Enantiomeric impurities

The single enantiomeric form of a chiral drug is now considered as an im-proved chemical entity that may offer a better pharmacological profile and an increased therapeu-tic index with a more favorable adverse reaction profile.[22] However, the pharmacokinetic profile of levofloxacin (S-isomeric form) and oflox-acin (R-isomeric form) are comparable, suggesting the lack of advantages of single isomer in this re-gard.[23] In any case, cost benefits as well as the patient's compliance need to be considered in se-lecting drugs. For the manufacturers of single enan-tiomeric drug (eutomer), the undesirable stereoi-somers in drug control are considered in the same manner as other organic impurities. The prominent single isomer drugs, which are being marketed, in-clude levofloxacin (S-ofloxacin), levalbuterol (R-albuterol), esomeprazole (S-omeprazole).

Inorganic impurities

Inorganic impurities may also derive from the manufacturing processes used for bulk drugs. They are

normally known and identified and include the following:

Reagents, ligands, and catalysts
The chances of having these impurities are rare: however, in some processes, these could create a problem unless the manufacturers take proper care during production.

Heavy metals
The main sources of heavy metals are the water used in the processes and the reactors (if stainless steel reactors are used), where acidifi-cation or acid hydrolysis takes place. These impuri- ties of heavy metals can easily be avoided using demineralized water and glass-lined reactors.

Other materials (eg, filter aids, charcoal)
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. The regular monitoring of fibers and black particles in the bulk drugs is essential to avoid these contaminations.

Solvent residues
Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. It is very difficult to remove these solvents completely by the work-up process; however, efforts should be taken to the extent pos-sible 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 possi-ble risk to human health, residual solvents are di-vided into 3 classes.[24] Solvents such as benzene (Class I, 2 ppm limit) and carbon tetrachloride (Class I, 4 ppm limit) are to be avoided. On the other hand, the most commonly used solvents such as methylene chloride (600 ppm), methanol (3000 ppm), pyridine (200 ppm), toluene (890 ppm), N,N-dimethylformamide (880 ppm), and acetonitrile (410 ppm) are of Class II. Class III solvents (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[25] for limits should be strictly followed.

Impurities related to formulation
Apart from bulk drug-related impurities, the for-mulated form of API may contain impurities that form in various ways.

Impurity forms during formulation
a) Method related
A known impurity, 1-(2,6-diclorophenyl)indolin-2-one is formed in the production of a parenteral dosage form of diclofenac sodium if it is terminally sterilized by autoclave [26]. It was the condition of the autoclave method (ie, 123 + 2°C) that enforced the intramolecular cyclic reaction of diclofenac so-dium forming the indolinone derivative and sodium hydroxide. The formation of this impurity has been found to depend on the initial pH of the formula-tion. The concentration of the impurity in the resul-tant product in the ampoule exceeds the limit of the raw material in the BP.

b) environmental related
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 tem-peratures. For example, vitamins as drug substances are very heat -sensitive and degradation frequently leads to loss of potency in vitamin products, espe-cially in liquid formulations.

Light-especially UV light
Several studies have reported that ergometrine as well as methyl er-gometrine injection is unstable under tropical con-ditions such as light and heat, and a very low level of active ingredient was found in many field sam-ples.[27-30] In only 50% of the marketed samples of ergometrine injections tested[31] did the level of active ingredient comply with the BP/USP limit of 90% to 110% of the stated content. The custom-made injection of ergometrine (0.2 mg/mL) showed almost complete degradation when kept 42 hours in direct sunlight.

Humidity
For hygroscopic products, humidity is considered detrimental to both bulk powder and formulated solid dosage forms. Aspirin and ranitidine are classical examples.

c) Dosage form factors related
Although the pharmaceutical companies perform pre-formulation studies, including a stability study, before marketing the products, sometimes the dos-age form factors that influence drug stability force the company to recall the product. Fluocinonide Topical Solution USP, 0.05%, (Teva Pharmaceuti-cals USA, Inc., Sellersville, Pennsylvania) in 60-mL bottles, was recalled in the United States because of degradation/impurities leading to sub-potency.[32] In general, liquid dosage forms are very much sus-ceptible to both degradation and microbiological contamination. In this regard, water content, pH of the solution/suspension, compatibility of anions and cations, mutual interactions of ingredients, and the primary container are 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 for human consumption. Microbial contaminations may occur during the shelf life and subsequent consumer-use of a multiple-dose prod-uct due to inappropriate use of certain preservatives in the preparations[33], or because of the semi-permeable nature of primary containers.

Formation of impurities on aging

a. Mutual interaction amongst ingredients

Most vitamins are very labile and on aging they pose a problem of instability in different dosage forms[34], especially in liquid dosage forms. Deg-radation of vitamins such as folic acid, pantothenic acid, cyanocobalamin, and thiamine do not give toxic impurities; however, potency of active ingre-dients 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-com-plex injections.[35] The marketed samples of vita-min B-complex injections were found to have a pH in the range of 2.8-4.0. The custom-made formula-tion 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 was observed.

b. Functional group- related typical degradation

Ester hydrolysis -Examples included the follow-ing: Aspirin, benzocaine, cefotaxime, cocaine echothiophate, ethyl paraben, cefpodoxime proxetil.[36]

Hydrolysis

Hydrolysis is a common phenomenon for the ester type of drugs, especially in liquid dos-age forms. Examples include benzylpenicillin, bar-bitol, chloramphenicol, chlordiazepoxide, lincomy-cin, and oxazepam.[37]

Oxidative degradation

Hydrocortisone, meth-otrexate, 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 ac-ids), heterocyclic aromatic rings, nitroso and nitrite derivatives, and aldehydes (eg, flavorings) are all susceptible to oxidative degradation.

Photolytic cleavage

Pharmaceutical products are exposed to light while being manufactured as a solid or solution, packaged, held in pharmacy shops or hospitals pending use, or held by the consumer pending use.

Ergometrine, nifedipine, nitroprusside, riboflavin, and phenothiazines are very labile to photo-oxidation. In susceptible compounds, photo-chemical energy creates free radical intermediates, which can perpetuate chain reactions. Most com-pounds will degrade as solutions when exposed to high energy UV exposure. Fluoroquinolones antibi-otics are found to be susceptible to photolytic cleav-age.[38]

In ciprofloxacin eye drops preparation (0.3%), sunlight induces photocleavage reaction producing ethylenediamine analog of ciprofloxacin.

Decarboxylation

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

Critical factors regarding bulk drugs' quality

During crystallization

The size of crystals some-times determines the quality, especially the stability, of bulk drugs. Large-size crystals can entrap a min-ute amount of chemicals from the mother liquor, which ultimately causes the degradation of the drug. Thus, the manufacturers of bulk drugs should take care to produce finer crystals while isolating the products.

Washing the wet cake

Washing the wet cake or powder in the centrifuge should be thorough to re-move unwanted chemicals including residual sol-vents.

Drying

Use of a vacuum dryer or a fluid-bed dryer is always preferable to a tray dryer in the pharma-ceutical bulk drug industry. The high thermal effi-ciencies, reduction of drying time, and more uni-form drying are helpful in drying sensitive drug substances. However, if a tray dryer is used then initial airflow at ambient conditions should be con-sidered before exposing the materials to a relatively higher fixed temperature.

Appropriate packaging

Light- sensitive pharmaceutical products need light-protective packaging. An accelerated stability test-ing conducted using marketed ampoules of er-gometrine wrapped with either black carbon paper or aluminum foil produced negligible degradation against direct sunlight. Similar use of opaque containers for ciprofloxacin eye-drop preparation can protect the active ingredient from photodegra-dation to some extent compared with transparent containers.[39]

Use of production method based on stability studies

In finalizing the method of preparation, a detailed investigation, including stability studies, should be undertaken. In the case of parenteral preparations, aseptic filtration versus terminal autoclaving method for sterilization should be evaluated before finalizing the method. For diclofenac sodium injec-tions, the aseptic filtration process has been recently recommended as the alternative to the autoclave method that produces impurity.[40]

Measures by pharmacopoeias

It has been observed in the pharmacopoeias that there is an impurity limit shown in the specifica-tions of certain raw materials but not given in the case of products made of those raw materials. Al-though the impurity limit on the drug substances is applicable to the drug products, it would be con- venient for the users if the impurity limits

were also mentioned in each dosage forms. The limits may vary from orals to injectables. Diclofenac sodium is such an example where an impurity limit is not mentioned in the case of injections. ICH recommendation can be incorporated in the pharma-copeias. In addition, a generalized monograph on the impurity issues can be added in the pharmaco-poeia.

Characterization Methods[36-39]

1. NMR
2. Mass spectroscopy
3. LC-MS
4. GC-MS

1. Nuclear Magnetic Resonance (NMR)

NMR provides information about specific bonding between peak area and number of nuclei responsible for peak. Most important application of NMR is identification and structure elucidation of molecules. Analysis of multicomponent mixture.

2. Mass Spectroscopy (MS)

Mass spectroscopy is a most accurate method for determining the molecular mass of the compound and its elemental composition. Mass spectroscopy is used to prove identity of two compound, establish the structure of new compound, give exact molecular mass, give molecular formula and most important for structure elucidation. Now a day's mass spectroscopy connected with various hyphenated techniques like GC-MS, LC-MS,LCMS-MS HPLC-DAD-MS, HPLCDAD-NMR-MS, Tandem Mass spectroscopy and capillary electrophoresis-Mass spectroscopy,.

3. GC-MS

To identify different substances within a test sample gas chromatography-mass spectrometry (GC-MS) method used, that combines the features of gas-liquid chromatography and mass spectroscopy. In this method gas chromatography separate volatile and semi-volatile compounds with great resolution. MS: can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. Sample vaporized by injection into a heated system, eluted through a column by inert gaseous mobile phase and detected. The sample is transported through the column by the flow of an inert, gaseous mobile phase, the carrier gas. Flow is regulated by the pressure regulators and gas metering valves. GC operates at atmospheric pressure and the MS ion source at 10-5 Torr.108 fold pressure difference. The carrier gas must be removed and GC peak components transferred to the MS ion source.

4. LC-MS

LC/MS is a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectrometry. LC/MS became really popular with the introduction of the thermo spray interface and the particle beam interface. This is same as GC-MS but removal of liquid carrier from an HPLC eluent before samples are passed in to the MS source. For handle normal eluent flow rate 0.5-2.0ml min-1 which is not handled by MS pumping system moving belt inlet systems, jet separators and vacuum nebulizers are used

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