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A Review on Impurity Profile in Pharmaceutical Substances.

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Review Article

ABSTRACT

Impurity is defined as any substance coexisting with the original drug, such as starting material or intermediates or that is formed, due to any side reactions. Impurity can be of three types: Impurities closely related to the product and coming from the chemical or from the biosynthetic route itself, Impurities formed due to spontaneous decomposition of the drug during the storage or on exposure to extreme conditions, or the precursors which may be present in the final product as impurities. Impurities present in excess of 0.1% should be identified and quantified by selective methods. The suggested structures of the impurities can be synthesized and will provide the final evidence for their structures, previously determined by spectroscopic methods. Therefore it is essential to know the structure of these impurities in the bulk drug in order to alter the reaction condition and to reduce the quantity of impurity to an acceptable level. Isolation, identification and quantification of impurities help us in various ways, to obtain a pure substance with less toxicity and, safety in drug therapy. This review covers the study of impurities present in new drug substances.

INTRODUCTION

A general scheme is set for the estimation of the impurity of bulk drug substances by the rational use of chromatographic, spectroscopic and analytical techniques. The various parameters to be fulfilled in an impurity profile of drug substances are discussed. Impurity is defined as any substance coexisting with the original drug, such as starting material or intermediates or that is formed, due to any side reactions. The presence of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now gaining critical attention from regulatory authorities. The different Pharmacopoeias, such as the British Pharmacopoeia (BP), United States Pharmacopeia (USP), and Indian Pharmacopoeia (IP) are slowly incorporating limits to allowable levels of impurities present in the API's or formulations. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has also published guidelines for validation of methods for analysing impurities in new drug substances, products, residual solvents and microbiological impurities[1,2,3,4].

Types of Impurity

Organic medicinal substances are contaminated in exactly the same manner as inorganic substance during their manufacturing processes. Since the organic substances belong to a very wide range of chemical groups and at the same time the contaminating impurities being of varied nature the task of detecting the impurities becomes a difficult job. Therefore, the contaminating impurities for organic medicinal compounds can be classified into -

- Inorganic impurities.
- Organic impurities.
- Contamination by chemical intermediates [5],

Impurities closely related to the product and coming from the chemical or from the biosynthetic route itself, Impurities formed due to spontaneous decomposition of the drug during the storage or on exposure to extreme conditions, or the precursors which may be present in the final product as impurities.

Impurities present in excess of 0.1% should be identified and quantified by selective methods. The suggested structures of the impurities can be synthesized and will provide the final evidence for their structures, previously determined by spectroscopic methods. Therefore it is essential to know the structure of these impurities in the bulk drug in order to alter the reaction condition and to reduce the quantity of impurity to an acceptable level. Isolation, identification and quantification of impurities help us in various ways, to obtain a pure substance with less toxicity and, safety in drug therapy.

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, CGMP, TGA, and MCA 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.

Organic Impurities

The actual and potential impurities most likely to arise during the synthesis, purification, and storage of the drug substance should be summarized ,based on sound scientific appraisal of the chemical reactions involved in the synthesis, impurities associated with raw materials that could contribute to the impurity profile of the drug substance. The laboratory studies conducted to detect impurities in the drug substance, which include test results of materials manufactured during the development process and batches from the commercial processes. The impurity profile of the drug lots, intended for marketing should be compared with those used in development. The spectroscopic studies (NMR, IR, MS etc.) conducted to characterize the structure of actual impurities present in the drug substance above an apparent level of 0.1% (e.g., calculated using the response factor of the drug substance) should be described. All recurring impurities above an apparent level of 0.1% in batches manufactured by the proposed commercial process should be identified of these studies.

Inorganic Impurities

Inorganic impurities are normally detected and quantified using Pharmacopeial or other appropriate standards. Carryover of catalysts to the drug substance should be evaluated during development.

Residual Solvents

The control of residues of solvents used in the manufacturing process for the drug substance should be discussed. Acceptance criteria should be based on Pharmacopeial standards, or ICH guidelines or known safety data, depends on the dose, duration of treatment, and route of administration.

Sources of Pharmaceutical Impurity

Impurities associated in with APIs Organic impurities, Inorganic impurities, Solvent residues Impurities related to formulation Impurity forms during formulation, Formation of impurities on aging. Medicines are the formulated forms of active pharmaceutical ingredients. There are 2 types of impurities in medicines: (1) Impurities associated in with active pharmaceutical ingredients and (2) Impurities that form are created during formulation and or with aging or that are related to the formulated forms.

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 degradation 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 C_6/C_7 side chain plays a critical role in their degradation [6].

- **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 levels varying from negligible to significant. A detailed investigation of impurities in semi-synthetic penicillin was performed both by the manufacturers and the

different research groups. A review paper on penicillins and cephalosporins describes methods of isolation, detection, and quantification of degradation products, and antigenic polymeric by-products. Studies show the presence of traces of ampicillin polymers and hydrolyzed products in the API [7]. .It has also been found that the presence of certain chemicals such as triethylamine has a degradative effect on the product. Ampicillin trihydrate samples having triethylamine content of 2000 ppm to 4000 ppm (determined by visual color method developed by Gist- Brocades, Delft, Holland)(7) were found to be stable under accelerated stability testing. However, the product showed appreciable degradation when triethylamine content became 7000 ppm. Recent pharmacopoeia [8] included the limit tests for the traces of impurities present in ampicillin and amoxycillin bulk raw materials. The residual solvents associated with these APIs have also been determined [7].

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 compendia.

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 improved chemical entity that may offer a better pharmacological profile and an increased therapeutic index with a more favorable adverse reaction profile.9 However, the pharmacokinetic profile of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are comparable, suggesting the lack of advantages of single isomer in this regard [9]. In any case, cost benefits as well as the patient's compliance need to be considered in selecting drugs. For the manufacturers of single enantiomeric drug (eutomer), the undesirable stereoisomers in drug control are considered in the same manner as other organic impurities. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin), levalbuterol (R-albuterol), esomeprazole (S-omeprazole). 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 acidification or acid hydrolysis takes place. These impurities 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.

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 possible 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 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 (200ppm), 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 [10] for limits should be strictly followed.

Apart from bulk drug-related impurities, the formulated form of API may contain impurities that form in various ways.

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 [11]. It was the condition of the autoclave method (ie, 123 + 2°C) that enforced the intramolecular cyclic reaction of diclofenac sodium forming the indolinone derivative and sodium hydroxide. The formation of this impurity has been found to depend on the initial pH of the formulation. The concentration of the impurity in the resultant 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 temperatures. For example, vitamins as drug substances are very heat-sensitive and degradation frequently leads to loss of potency in vitamin products, especially in liquid formulations.

Light–especially UV light: Several studies have reported that ergometrine as well as methyl ergometrine injection is unstable under tropical conditions such as light and heat, and a very low level of active ingredient was found in many field samples [12,13,14]. in only 50% of the marketed samples of ergometrine injections tested14 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 dosage form factors that influence drug stability force 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 [15]. In general, liquid dosage forms are very much susceptible 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.

APPLICATIONS

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, miscellaneous [16].

Some Food Products contain Organic Impurities [17,18,919,20,21]:

A recent interest of the food chemistry devoted to the safeguard of human health concerns synthetic dyes which are commonly added to a great number of foodstuffs and largely preferred to natural colors, essentially because of their greater stability along the production industrial process [17].

The use of artificial color in food has a long history. In the 18th and 19th centuries, both "unnatural" color and vegetable extracts were used in food and drink. Sweets, for example, were colored with lead chromate, mercuric sulfide, lead oxide and copper arsenite. Legislation and the newly developed chemically synthesized dyes eliminated the use of these metallic compounds. The synthetic dyes were much brighter, cheaper, more uniform, more stable (in their reactions to high processing temperatures, acids, carbon dioxide, storage and light) and more potent (i.e., less could be used to gain the same effect) than anything seen before, and offered a wider range of shades, so they had great advantages over natural dyes as well. As the use of new dyes became more popular, their toxic properties also became apparent. Since that time, there has been an increase in synthetic dye use, but we have also become even more aware of toxicity [18].

Many beverages contain ascorbic acid (vitamin C) and sodium benzoate. When benzoate salts and Vitamin C come in contact with high levels of light and/or heat, there is a strong chance that a chemical reaction will occur. Benzene is the product of this process [19]. Benzene formation may occur at part per billion (micrograms per kilo) levels in some beverage formulations(20). Sodium benzoate is a permitted food preservative that may be added to many food products to ensure the microbiological safety of the food. Ascorbic acid is also an approved food additive (antioxidant) which may be added to drinks. It also occurs naturally in fruit and fruit juices. Ascorbic acid reacts with metals (copper, iron) found in water to form hydroxyl radicals, which react with benzoic acid to form low levels of benzene. Benzene levels are likely to be higher in beverages where benzoic acid and ascorbic acid are deliberately added to ensure microbial safety [21].

General Scheme for Drug Impurity Profiling [22, 23, 24]

The procedure of impurity profiling, begins with the detection of the impurities using the thin-layer chromatogram, high-performance liquid chromatogram or gas chromatogram. Procurement of standard impurity samples from the synthetic organic chemists which include, last intermediate of the synthesis, products of predictable side reaction, degradation products if any etc In the case 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 [22,23]. 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 matching of the synthesized material with the impurity in question is carried out as outlined above [24].The possibilities of spectroscopic techniques in drug impurity profiling without chromatographic separation are also worth 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 [24].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. Detection of new or higher levels of a GTI in the FDC product compared with the single-entity API product, or an initial GTI assessment that uncovers potential GTIs in a marketed product, may result in additional regulatory scrutiny of that product, particularly if a GTI assessment has not been previously conducted on that product. This can lead to additional process controls or even suspension of distribution for a period of time while the outstanding issues are resolved.

Additional possible triggers for a GTI assessment of the FDC product include the choice of formulation or a change in salt or form (e.g. from amorphous to crystalline) of the API or the generic selected for the FDC product compared with the proprietary drug (see Figure 1). While a change in salt may not generally be an automatic trigger for a GTI assessment, some changes – such as a switch to a mesylate form – may raise the level of regulatory concern. Formulation considerations for the FDC product may either augment or minimise the risks of the formation of new GTIs in the FDC product. For example, co-formulation may increase the risk of an Interaction between the APIs in the FDC product, thereby increasing the risk of potential formation of new GTIs or higher amounts of known GTIs. Alternatively, 'layered' tablets (e.g. bi-layer or tri-layer tablets) may minimize the risk by limiting interaction between the APIs in the FDC product [25].
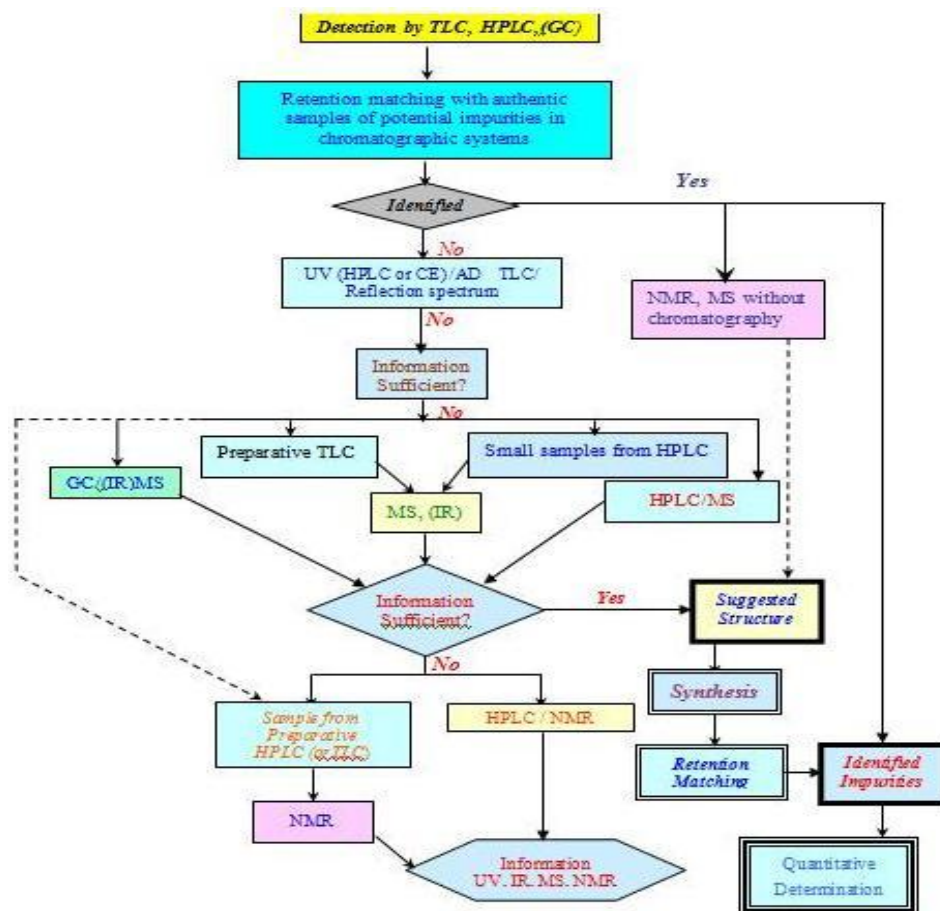

Figure 1: General Scheme for Drug Impurity Profiling

Acceptance Criteria for Impurities

For newly synthesized drug substances, the specification should include acceptance criteria for impurities. Stability studies, chemical development studies, and routine batch analyses 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 specification should include a discussion of the impurity profiles observed in batches under consideration, together with a consideration of the impurity profile of material manufactured by the proposed commercial process. For impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the quantitation or detection limit of the analytical methods should commensurate with the level at which the impurities need to be controlled. Appropriate qualitative analytical descriptive label included in the specification of unidentified impurities. A general acceptance criterion of not more than 0.1 % for any unspecified impurity should be included. Acceptance criteria should be set, based on data generated on actual batches of the drug substance, allowing sufficient latitude to deal with normal manufacturing and analytical variation, and the stability characteristics of the drug substance. Although normal manufacturing variations are expected, significant variation in batch-to-batch impurity levels could indicate that the manufacturing process of the drug substance is not adequately controlled and validated. The acceptance criteria should include limits for organic impurities; each specified identified impurity, each specified unidentified impurity at or above 0.1%, and any unspecified impurity, with a limit of not more than 0.1%, total impurities, residual solvents and inorganic impurities.

Analytical Procedures

Method Development

Method development usually requires the choice of columns, mobile phase, detectors, and method of quantitation etc. The factors to be considered for the method developments are the following, Existing method may be inaccurate, artifact, or contamination prone, or they may be unreliable (have poor accuracy or precision). Existing method may be too expensive, time consuming or energy intensive, or they may not be easily automated. Existing methods may not provide adequate sensitive or analyte selectivity in samples of interest. Newer instrumentation techniques may have evolved which can provide opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision and better returns on investments.

CONCLUSION

Impurity profiling is very important during the synthesis of drug substances and manufacture of dosage forms, as it can provide crucial data regarding the toxicity, safety, various limits of detection, and limits of quantitation, of several organic and inorganic impurities, usually accompany with bulk drugs and finished products. An accurate method development and validation of the procedures make the impurity profiling task easy.

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