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IMPURITIES IN PHARMACEUTICALS: A REVIEW

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ABSTRACT

The pharmaceutical industry is required by the Food, Drug and Cosmetic Act to establish the identity and purity of all marketed drug products. The United States Food and Drug Administration (FDA) and other regulatory bodies around the world require that impurities in drug substance and drug product when present at threshold levels recommended by the International Conference on Harmonization (ICH) to be isolated and characterized. The identification of process-related impurities and degradation products is tedious, it provides an understanding of various sources of impurities and degradation products, the process and methodologies involved in separation, isolation, and characterization of impurities. Chiral impurities are also discussed from the standpoint of their origin, analytical methodology and regulatory perspective for controlling them.

KEYWORDS: Isolation, Characterization of impurities, analytical techniques.

INTRODUCTION

Pharmaceutical analysts play a major role in isolation and characterization of impurities. The success in this endeavor requires a broad knowledge of a variety of fields of chemistry and excellent interactions with experts in various other disciplines.

Impurity

any entity of the drug substance or drug product that is not the chemical entity defined as the drug substance, an excipient, or other additives to the drug product.

Degradation product

a molecule resulting from a change in the drug substance brought about over time. For the purpose of stability testing of the products in this guidance, such changes could occur as a result of processing or storage (e.g., deamidation, oxidation, aggregation, and proteolysis).[1]

Need to Identify Impurities

Impurities are generally assumed to be inferior to API because they might not have the same level of pharmacologic activity. However, they are not necessarily always inferior. From the standpoint of its usage, the drug substance is compromised in terms of purity even if it contains another material with superior pharmacologic or toxicologic properties. At first pass this may not be readily apparent; however, on further thought it will become clear that if we are to ensure that the accurate amount of the drug substance is being administered to the patient, we must assess its purity independent of the extraneous materials. Therefore, any extraneous material present in the drug substance or active ingredient must be considered an impurity even if it is totally inert or has superior pharmacologic properties, so that an appropriate evaluation of its content in the drug product can be made. The control of low-level impurities is of great importance when a drug is taken in large quantities; for example, the use of methotrexate (10–20 g) to treat neoplasia. Penicillin and cephalosporin have been known to sustain facile cleavage of the β-lactam bond in aqueous solution. This is particularly interesting since some studies on penicillin have shown that their lack of stability may influence possible reactions involved in penicillin allergy. Special attention should be paid to the detection of DNA in all finished biotechnology products because DNA can be incorporated in the human genome and become a potential oncogene.[2]

Designations of Impurities

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

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

1. By-product.
2. Degradation product.
3. Interaction product.
4. Intermediate.
5. Penultimate intermediate.
6. Related product.
7. Transformation product.

Some of these terms indicate potential sources of impurities; e.g., intermediates; others tend to downplay the negativity, as exemplified by the use of the term ‘‘related product.’’

By-products

The unplanned compounds generated in the reaction to produce API are generally called by-products. Because it might not be possible to theorize all of them, they present a significant challenge to the analytical chemist.

Degradation products

The compounds produced as a result of decomposition of the material of interest or API is often called degradation products. It is necessary to be concerned with these products as well as those brought about by degradation of other compounds that may also be present as impurities in the drug substance.

Interaction products

This term is slightly more inclusive and more difficult to evaluate than the two previously described, i.e., by-products and degradation products, in that it takes into account interactions that could possibly occur between various involved chemicals—purposely or inadvertently.

Intermediates

The planned compounds produced during synthesis of the desired substance are called intermediates, especially if they have been isolated and characterized. The most important requirements are isolation and characterization, i.e., they cannot be just potential reaction products that may be produced theoretically. The theorized products are best designated as potential intermediates.

Penultimate intermediate

As the name implies, this is the last compound in the synthetic chain just preceding the production of the ultimate desired compound. Confusion sometimes occurs when the desired material is a salt of a free base or acid. It is not appropriate to label the free acid or base as the penultimate intermediate if the drug substance is a salt.

Related products

As suggested previously, the term ‘‘related products’’ tends to imply that the impurity is similar to the drug substance and it thus tends to downplay the negativity frequently attached to the term ‘‘impurity.’’ These products may have similar chemical structures and potentially similar biological activities; however, we know that the structure alone does not provide any surety about biological activity.

Transformation products

This is a relatively commonplace term that relates to theorized and non-theorized products that may be produced in the reaction. Transformation products are comparable to by-products, except that this term tends to imply that more is known about the reaction products.[3-6]

ICH Terminology

According to ICH guidelines, impurities can be broadly classified into the following three categories for the drug substance produced by chemical synthesis, Organic impurities (starting materials, process-related products, intermediates, and degradation products).

Inorganic impurities (salts, catalysts, ligands, and heavy metals or other residual metals) Residual solvents (organic and inorganic liquids used during production and/or recrystallization).

The International Conference on Harmonization addresses the questions relating to impurities as follows:

1. Q1A(R) Stability testing of new drug substances and products.
2. Q3A(R) Impurities in drug substances.
3. Q3B Impurities in drug products.
4. Q3C Impurities: residual solvents.

5. Q6A Specifications: test procedures and acceptance criteria for new drug substances and new drug products: chemical substances it should be noted that none of the terminologies given above adequately highlights polymorphic, and chiral impurities.

Regulatory Requirements

Ethical, economic, and competitive reasons, as well as those of safety and efficacy, support the need to monitor impurities in drug products. However, monitoring impurities and controlling these impurities mean different things to different people or to the same people at different times, even those in the pharmaceutical sciences and industry. A unified terminology is necessary to assure that everyone uses the same vocabulary when addressing questions related to impurities. In this context, the leadership provided by ICH is very helpful. A number of requirements have an effect on monitoring impurities. For example, a country's pharmacopeia or the one accepted by that country often provides the primary guidance as to how impurities are to be monitored and regulated. In a majority of countries these pharmacopeias are run under the auspices of the government. If a product is considered a pharmacopeial item, it must meet the compendial requirements. The United States Food and Drug Administration (USFDA) has endorsed the guidance prepared under the auspices of the ICH. The guidance, developed with the joint efforts of regulators and industry representatives from the European Union, Japan, and the United States, has helped ensure that the different regions have consistent requirements for the data that should be submitted to the various regulatory agencies. The guidelines not only aid the sponsors of New Drug Applications (NDA) or Abbreviated New Drug Applications (ANDA) with the type of information that should be submitted with their applications, but also assist the FDA reviewers and field investigators in their consistent interpretation and implementation of regulations.[7,9-12]

Sources of Impurities

It is clear that impurities can originate from various sources. The most obvious source of impurities is the synthesis, where intermediates and by-products may be carried into the API as impurities or become a source of other impurities resulting from them. Any impurity that may be present in the starting material has the potential to be carried into the active ingredient of interest. Furthermore, the impurities that relate to inert ingredients (excipients) and solvents used during synthesis must also be considered. Impurities can be produced during various drug product formulation steps. These impurities have the possibility of being present

in the final drug product. Potential reaction products relating to these impurities must also be evaluated.[13]

Crystallization-Related Impurities

Polymorphism is the term used to denote crystal systems where a substance can exist in different crystal packing arrangements, all of which have the same elemental composition. It is also possible to have crystal systems where the substance exists in different crystal packing arrangements, each of which has a different elemental composition; this phenomenon is known as solvatomorphism. Based on the realization that the nature of the structure adopted by a given compound upon crystallization could exert a profound effect on the solid-state properties of that system, the pharmaceutical industry is required by regulatory authorities to take a strong interest in polymorphism and solvatomorphism. The nature of the crystal structure of a given material can influence the following properties:

1. Conductivity.
2. Crystal hardness.
3. Crystal shape and color.
4. Density.
5. Diffusivity.
6. Dissolution rate.
7. Electrolytic conductivity.
8. Enthalpy of transitions.
9. Heat capacity.
10. Heat of solution.
11. Hygroscopicity.
12. Latent heat of fusion.
13. Melting or sublimation properties.
14. Phase diagrams.
15. Rates of reactions.
16. Refractive index.
17. Solubility.
18. Surface tension.
19. Viscosity.
20. Volume.

Stereochemistry-Related Impurities

It is of paramount importance to look for stereochemistry-related compounds, i.e., those compounds that have similar chemical structure but different spatial orientation. These compounds can be considered impurities in the API. The simplest case of chirality can be seen in a molecule that has one or more tetrahedral carbons with four different substituents such that its mirror image is not superimposable. Chiral molecules may also occur for a number of other reasons and must be factored into any evaluation of impurities. Stereoisomerism is possible in molecules that have any of the following characteristics:

1. One or more center of chirality.
2. Helicity.
3. Planar chirality.
4. Axial chirality.
5. Torsional chirality.
6. Topological asymmetry.

Chiral molecules are frequently called enantiomers. Enantiomers are optical isomers that have the same chemical structure but different spatial arrangement, which leads to different optical rotation. Therefore, the undesired optical isomer is considered a chiral impurity of the API. Furthermore, it is important to remember that the number of chiral impurities increases with the increasing number of asymmetric carbon atoms in a molecule.

Residual Solvents

Water is commonly present in drug products. As a result, water is by far the most commonly found volatile impurity in drug products, and most of the time it is not even considered an impurity. It is prevalent both in drug substances and excipients and is used in dosage form preparations as well. Moisture content can be important when a dosage form is packaged such that equilibration with the environment does not occur. Under these conditions, the moisture brought into the system through the excipients can be sufficient to bring about hydrolysis. In addition, water from the environment can affect drug products and can very often be detrimental to their chemical stability or dosage form performance.[14-17]

Synthetic Intermediates and By-products

In addition to the residual solvents, polymorphic, solvatomorphic, and chiral impurities mentioned previously, impurities in a pharmaceutical compound or a new chemical entity (NCE) can originate during the synthetic process from raw materials, intermediates and/or

by-products. Raw materials are usually produced to lesser purity requirements than a drug substance.

Therefore, it is easy to understand why they could include a number of components that in turn could have an impact on the purity of the drug substance. The solvents used in synthesis are also likely to involve a number of impurities that may extend from trace levels to critical quantities that can react with various chemicals used in the synthesis, to give rise to other impurities.

Impurities Arising During Storage

A number of impurities can originate during storage (shelf life) or shipment of drug products. It is essential to carry out stability studies to predict, evaluate, and ensure drug product safety. Stability, however, can have different meanings to different people, based on their discipline in the pharmaceutical sciences and industry. A variety of terms are currently used to encompass the what, how and why of stability: kinetic study, compatibility study, stability evaluation, stability-indicating assay, expiration dating, outdating, shelf life, storage legend, preformulation study, failure of a batch to meet specifications, microbiological stability, stability of the active ingredient, stability of the formulation, stability in the marketed package, stability in the sample package, stability in the dispensing package, and stability in the hands of the consumer. All of these are considerations in stability of a drug product, and it is important to ensure that everyone understands the importance of stability studies.[18,19]

Degradation Kinetics

The majority of the degradation reactions of pharmaceuticals takes place at finite rates and are chemical in nature. Solvent, concentration of reactants, temperature, pH of the medium, radiation energy and the presence of catalysts are important factors that affect these reactions. The order of the reaction is characterized by the manner in which the reaction rate depends on the reactant concentration. The degradation of most pharmaceuticals is classified as zero order, first order or pseudo-first order, although the compounds may degrade by complicated mechanisms, and the true expression may be of higher order or be complex and non-integer. To ensure better stability predictions, an understanding of the limitations of experimentally obtained heat of activation values is critical.[21]

Analytical Method Development

New drug development requires that meaningful and reliable analytical data be produced at various stages of the development. Assuring the safety of a new pharmaceutical compound or drug substance demands that the new drug substance meet the established purity standards as a chemical entity or when admixed with animal feeds for toxicity studies or when formulated with or without pharmaceutical excipients for human use. Furthermore, it should exhibit excellent stability throughout its shelf life. These requirements mandate that the analytical method (s) employed for this purpose should be sufficiently sensitive to measure low levels of impurities. This has resulted in development of analytical techniques that are appropriate for measurement of trace/ultra-trace levels, i.e., sub-microgram quantities of a variety of chemical entities.[9]

The Role of Reference Standards

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 Methods

The following spectroscopic measurement techniques have been used for characterizing impurities; most of these are very useful as detectors for chromatographic methods:

1. Ultraviolet (UV).
2. Infrared (IR).
3. Raman spectroscopy.
4. Mass spectrometry (MS).
5. Nuclear magnetic resonance (NMR).

Ultraviolet spectrophotometry (UV) at a single wavelength furnishes minimum selectivity of analysis; however, with the current accessibility of diode array detectors, it is conceivable to obtain sufficient simultaneous information at various wavelengths to assure greater reliability. Infrared spectrophotometry (IR) affords specific information on some functional groups that offer selectivity and allow quantification. However, low-level detectability is difficult. This requires more complex approaches, which are generally a deterrent to pharmaceutical analysts. Raman spectroscopy is based on the measurement of scattered electromagnetic radiation resulting from the irradiation of matter. Specifically, when a

material is irradiated with a strong monochromatic light source, a small amount of radiation is inelastically scattered at a wavelength different from the original incoming wavelength. It is this difference in vibrational energy between the scattered beam and incident beam that is measured. Raman spectroscopy is considered complementary to IR spectroscopy, as the two techniques provide a complete vibrational picture of a material. Raman spectroscopy is not as widely used for identification purposes as IR spectroscopy because of the relative complexity and the cost of instrumentation. Mass spectrometry (MS) provides excellent structural information, and, based on the resolution of the instrument, it may be an effective tool for differentiating molecules with small differences in molecular weight. However, it has finite uses as a quantitative procedure. Nuclear magnetic resonance spectroscopy (NMR) provides reasonably detailed structural information on a molecule and is an extremely useful method for characterization of impurities.[22]

Separation Methods

The following methods can be used for separation of impurities and degradation products:

1. Capillary electrophoresis (CE).
2. Chiral separations.
3. Gas chromatography (GC).
4. High-pressure liquid chromatography (HPLC).
5. Supercritical fluid chromatography (SFC).
6. Thin-layer chromatography (TLC).

The nature and complexity of the separation problem determines which method should be used. The primary goal of a good separation method is resolution of all impurities of interest. A brief account of the above listed methods is given here to provide a quick review of their potential use. Except for CE, all these methods are chromatographic methods. CE is an electrophoretic method that is frequently lumped with the chromatographic methods because it shares with chromatography many of the common requirements. However, it is not strictly a two-phase separation system— a primary requisite for chromatography. Capillary electro chromatography meets this requirement. Capillary electrophoresis is an effective technique in situations where very low quantities of samples are available and high resolution is essential. Its relatively lower reproducibility is the principal difficulty of this procedure. Gas chromatography is an extremely useful technique for quantification. It can afford the desired resolution, selectivity, and ease of quantification. The chief limitation, however, is that the

sample must be volatile or must be made volatile by derivatization. This technique is very practical for organic volatile impurities. High-pressure liquid chromatography is often referred to as high performance liquid chromatography today. Both terms can be abbreviated as HPLC, and the terms are used interchangeably by chromatographers. The applications of this very effective technique have been significantly expanded for the pharmaceutical chemist by the use of a variety of detectors such as fluorescence, electrometric, MS, and so forth. Supercritical fluid chromatography (SFC) offers some of the advantages of GC in terms of detection and of HPLC in terms of separations, in that volatility of the sample is not of paramount importance. The greatest application of this technique has been found in the extraction of samples. SFC is generally performed in the normal phase (NP) mode, and often NP-TLC or NP-HPLC methods can be readily adapted to SFC methods. SFC generally provides an orthogonal separation method to traditional reversed phase HPLC. Because of the similarity to HPLC in the chromatographic measurement process, this technique can be used to accurately quantify nonpolar impurities of the sample of interest. Thin-layer chromatography coupled with densitometric detection is a highly sensitive method for quick assessment of the purity of various compounds including reference standards. High-performance TLC (HPTLC) is an improved version of TLC that uses stationary phases of decreased thickness and lower particle size, providing improved resolution over shorter elution distances. TLC can resolve a large range of compounds by employing a variety of different plates and mobile phases. Limited resolution, detection, and ease of quantification are the main problems associated with this method. The foremost advantages are ease of use and low cost.[9,19,23, 26]

Isolation Methods

It is often necessary to isolate impurities because the instrumental methods that were mentioned earlier for directly characterizing impurities without isolating them are not available or when the authentic material is needed for further confirmation of the structure or its toxicity. Isolation entails removal of the compound of interest from the other compounds present in a mixture. Further purification is achieved based on the compound's intended use. Isolation methods include both chromatographic and non-chromatographic methods. Simple methods should be tried first, as they can lead to considerable savings in time and can produce a larger quantity of materials with greater ease. For isolation of a given compound from a complex mixture, the chromatographic methods utilized for separation of impurities in analytical determinations are the methods of first choice that are suitably modified for the

purpose of isolation of impurities where an appropriate fraction is collected. A list of the methods that can be used for isolation of impurities is given below.

1. Solid-phase extraction methods.
2. Liquid–liquid extraction methods Accelerated solvent extraction methods.

1. Supercritical fluid extraction.
2. Column chromatography.
3. Flash chromatography.
4. Thin-layer chromatography (TLC).
5. Gas chromatography (GC).
6. High-pressure liquid chromatography (HPLC).
7. Capillary electrophoresis (CE).
8. Supercritical fluid chromatography (SFC).

Analytical chemists generally run a series of different assays (orthogonal assays) to confirm that they have sufficiently characterized the sample and have identified all impurity peaks found in the sample. Furthermore, they ensure that peaks which may co-elute using one technique can be detected with an orthogonal method. Analytical HPLC is the most common technology employed for assay and impurity profiling of pharmaceuticals. When the sample has been analyzed, the impurity may need to be isolated. While techniques such as LC-MS may give an indication of the compound identification from the bulk assay, the definitive proof is always obtained from an independent analysis of the isolated compound.[29]

Characterization Methods

When an impurity has been detected, it becomes necessary to estimate its content. Adequate detectability frequently means that a given component provides a signal at least twice that of background noise or baseline noise. At times, the multiple is set higher for greater assurance of detectability. Initial estimations are generally done against the parent compound because in most cases the authentic sample of impurity is not available. It is important that the authentic sample be used for estimations when it is available. If the estimations indicate that a given impurity content is greater than 0.1%, it must be characterized according to FDA requirements. The ability of NMR to provide information regarding the specific bonding structure and stereochemistry of molecules of pharmaceutical interest has made it a powerful analytical tool for structure elucidation. Unfortunately, NMR has traditionally been

sensitivity-limited compared to other analytical techniques. Conventional sample requirements for NMR are on the order of 10 mg, as compared with mass spectroscopy, for example, which requires less than 1 mg. Therefore, NMR spectroscopy historically has not been the first choice for an analytical chemist when identifying an unknown compound. Mass spectrometry has had an increasingly significant impact on the pharmaceutical development process over the past several decades. Advances in the design and efficiency of the interfaces that directly connect separation techniques with mass spectrometers have afforded new opportunities for monitoring, characterizing, and quantifying drug related substances in active pharmaceutical ingredients and pharmaceutical formulations. Possessing exceptional analytical specificity and sensitivity, mass spectrometry significantly reduces the cycle time of chromatographic method development, validation, and sample analysis. The popularity of LC-MS-MS systems for complex mixture analysis of thermally labile, biologically relevant molecules is largely attributed to the ‘‘soft’’ nature of atmospheric pressure ionization techniques such as electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). Although the determination of chemical identity or molecular structure for related substances in pharmaceutical products has continuously benefited from the availability and evolution of modern instrumentation, fundamental knowledge about solution phase chemistry, ionization, and gas phase processes is still vitally important for achieving success in this endeavor. There is economic pressure to devise instrumentation methods that can deliver many data sets at once, thus shortening the time-to-market of drug products. Coupling of instruments has become easier with expanded capabilities, e.g., HPLC-DAD-MS (HPLC coupled with a diode array UV detector and a mass spectrometer), is almost routinely used. Nuclear magnetic resonance spectrometry has now been added to this combination to provide HPLC-DAD-NMR-MS capabilities in a commercial instrument. Another pressure on pharmaceutical scientists is the promise of biopharmaceuticals and high-potency active pharmaceutical ingredients. These compounds often have complicated impurity and degradation profiles at low absolute concentrations. Fortunately, instrument manufacturers have been quick to attempt to satisfy both these needs. The challenge now to the pharmaceutical scientist is the organization of the many data sets into presentation-quality formats so that scientists and managers can make correct decisions quickly. Software has been developed to speed the collation, analysis, and presentation of the many spectroscopic characterization techniques necessary. Detection limits for mass spectrometers are now approaching the zeptomole level, and NMR spectrometers have recently seen dramatic increases in sensitivity down to the

nanogram level. Hyphenated methods such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) and a number of other chromatographic and spectroscopic configurations are perfectly suitable for initial characterization of the impurities. Of course, these methods are not always applicable, especially when the authentic material is needed for purposes of structure confirmation, synthesis, or toxicity studies.[27, 28, 30]

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