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Impurity Profile of Bulk Drugs and Pharmaceutical Preparations

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ABSTRACT

To assure the quality of drugs, impurities must be monitored carefully. It is important to understand what constitutes an impurity and to identify potential sources of such impurities. Selective analytical methods need to be developed to monitor them. It is generally desirable to profile impurities to provide a yardstick for comparative purposes. New impurities may be observed as changes are made in the synthesis, formulation, or production procedures, albeit for improving them. At times it is necessary to isolate and characterize an impurity when hyphenated methods do not yield the structure or when confirmation is necessary with an authentic material. Availability of an authentic material can also allow toxicological studies and provide a standard for routine monitoring of the drug product.

Keywords: Impurity profiling, degradation products, hyphenated techniques, ICH guidelines, pharmaceutical analysis.

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INTRODUCTION

The definition of the impurity profile of a new drug material is given in the guidelines of ICH (International Conference on Harmonization), which was formed in 1990 with the aim of harmonizing the efforts of registration agencies and pharmaceutical manufacturers organizations to improve the quality of drugs and the safety and efficacy of drug therapy. The definition is as follows: "A description of the identified and unidentified impurities, present in a new drug substance".

As for impurity profiling, it is the common name of analytical activities with the aim of detecting, identifying or elucidating the structure and quantitatively determining organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations.

The safety of drug therapy is closely related to the quality of drugs. The requirements with respect to the active ingredient content of bulk-drug materials in the various pharmacopoeias are usually in the range 98–99%. In the overwhelming majority of the pharmacopoeial monographs, the active ingredient content is determined by selective (usually high performance liquid chromatography (HPLC)) or non-selective (usually titrimetric or ultraviolet (UV) spectrophotometry) methods. In recent decades, the importance of these in characterizing the quality of bulk-drug materials has decreased considerably because, at the same time thanks to the development of chromatographic and spectroscopic methods and their combinations identification and selective, quantitative determination of individual impurities became possible. This is why impurity profiling has become the most important activity in assuring the high quality of drugs.

The aim is to minimize the adverse effects of drug materials and the preparations made thereof. After establishing the pharmacological-toxicological profile of a drug substance, pharmacologists, clinicians and drug-registration authorities consider its beneficial and adverse effects to the human organism and, on the basis of the benefit/risk ratio thus obtained, make the decision with respect to the possibility of introducing it into therapy. In principle, the adverse effects can originate from two sources: It is well known that, in addition to their beneficial effects, all drug materials have adverse (side) effects, which can be considered inherent properties and cannot be influenced by the quality of the drug material; and, if the impurities of a drug material (the difference between 100% and the 98–99% mentioned above) are physiologically highly active (toxic) materials, in principle, they could contribute to the side-effect profile of the drug.

Since the impurity profile of a drug material depends on the synthesis route and other factors, this could make the side-effect profile irreproducible, adversely influencing the safety of drug therapy. By estimating the impurity profile of a drug material and setting strict limits for the impurities, this danger can be minimized[1]

MATERIALS AND METHODS

Identification and qualification thresholds of impurities

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

Q1A (R) stability testing of new drug substances and products

Q3A (R) impurities in drug substances

Q3B (R) impurities in drug products

Q3C impurities: residual solvents

Q6A specifications: test procedures and acceptance criteria for new drug substances and new drug products; chemical substances ICH guidelines for the identification and qualification threshold of impurities and degradation products are provided in Table 1.

Table 1:Thresholds for reporting impurities

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

As can be seen from the data in Table 2, ICH treats the degradation products slightly differently than impurities even though for all intents and purposes the degradation products are impurities.

Table 2:Threshold for reporting degradation products in a new drug product

Maximum daily dose	Threshold
1g	0.1%
>1g	0.05%

SOURCES OF IMPURITIES

Discussed below are three important sources of impurities.

Synthesis-related impurities

Impurities in a drug substance or a new chemical entity (NCE) originate mainly during the synthetic process from raw materials, solvents, intermediates, and by-products. The raw materials are generally manufactured to much lower purity requirements than a drug substance. Hence, it is easy to understand why they can contain a number of components that can in turn

affect the purity of the drug substance. Similarly, solvents used in the synthesis are likely to contain a number of impurities that may range from trace levels to significant amounts that can react with various chemicals used in the synthesis to produce other impurities. Intermediates are also not generally held to the purity level of the drug substance hence the remarks made for the raw materials apply. It is not reasonably possible to theorize all by-products; as a result, any such products that may be produced in the synthesis would be hard to monitor. The "pot reactions," i.e., when the intermediates are not isolated, are convenient, economical, and timesaving; however, they raise havoc in terms of the generation of impurities because a number of reactions can occur simultaneously. Incidentally, this problem of numerous reactions occurring simultaneously can be also encountered in single reactions where intermediate is isolated.

The final intermediate is generally controlled in the pharmaceutical synthesis by conducting regulatory impurity testing. This typically entails residual solvents (that are not used in further downstream processing) or process impurities (in cases where they conclusively demonstrate that these moieties are not also degradation products). It is important to remember that this step is the last major source of potential impurities; therefore, it is very desirable that the methods used for analysis at this stage be rigorous. It should be remembered that base-to salt or acid-to-salt conversions could also generate new impurities. Furthermore, thermally labile compounds can undergo decomposition if any further processing involves heating.

Formulation-related impurities

A number of impurities in a drug product can arise out of interactions with excipients used to formulate a drug product. Furthermore, in the process of formulation, a drug substance is subjected to a variety of conditions that can lead to its degradation or other deleterious reactions. For example, if heat is used for drying or for other reasons, it can facilitate degradation of thermally labile drug substances.

Solutions and suspensions are potentially prone to degradation that is due to hydrolysis or solvolysis (see kinetic studies discussed below). These reactions can also occur in the dosage form in a solid state, such as in the case of capsules and tablets, when water or another solvent has been used for granulation. Not only can the water used in the formulation contribute its own impurities, it can also provide a ripe situation for hydrolysis and metal catalysis. Similar reactions are possible in other solvents that may be used.

Oxidation is possible for easily oxidized materials if no precautions are taken. Similarly, light-sensitive materials can undergo photochemical reactions.

Degradation-related impurities

A number of impurities can be produced because of API degradation or other interactions on storage. Therefore, it is very important to conduct stability studies to predict, evaluate, and ensure drug product safety. Stability studies include evaluation of stability of API, pre-formulation studies to evaluate compatibility of API with the excipients to determine its stability in the formulation matrix, accelerated stability evaluations of the test or final drug product, stability evaluation via kinetic studies and projection of expiration date, routine stability studies of drug products in marketed, sample or dispensed package under various conditions of temperature light, and humidity.

The stability studies under various exaggerated conditions of temperature, humidity, and light can help us determine what potential impurities can be produced by degradation reactions. It is important to establish a viable stability program to evaluate impurities. A good stability program integrates well the scientific considerations with regulatory requirements. The importance of kinetic studies in monitoring and evaluating impurities is discussed below.

Kinetic studies

Most of the degradation reactions of pharmaceuticals occur at finite rates and are chemical in nature. These reactions are affected by conditions such as solvent, concentration of reactants, temperature, pH of the medium, radiation energy, and the presence of catalysts. The order of the reaction is described by the manner in which the reaction rate depends on the concentration of reactant. The degradation of most pharmaceuticals can be classified as zero order, first order, or pseudo-first order, even though they may degrade by complicated mechanisms, and the true expression may be of higher order or be complex and no integer.

An understanding of the limitations of experimentally obtained heat of activation values is critical in stability predictions. For example, the apparent heat of activation of a pH value where two or more mechanisms of degradation are involved is not necessarily constant with temperature. Also, the ion product of water, pKw, is temperature-dependent, and $-\Delta Ha$ is approximately 12 kcal, a frequently overlooked factor that must be considered when calculating hydroxide concentration. Therefore, it is necessary to obtain the heat of activation for all bimolecular rate constants involved in a rate–pH profile to predict degradation rates at all pH values for various temperatures.

It is incumbent upon the chemist to perform some kinetic studies to predict stability of a drug substance and to evaluate degradation products. However, it is also important to recognize the limitations of such predictions.

SELECTIVE ANALYTICAL METHODOLOGIES

Development of a new drug mandates that meaningful and reliable analytical data be generated at various steps of the new drug development. Ensuring the safety of a new pharmaceutical compound or drug requires that it meet the established purity standards as a chemical entity or when admixed with animal feeds for toxicity studies or pharmaceutical excipients for human use. Furthermore, it should exhibit excellent stability throughout its shelf life. These requirements demand that the analytical methodology that is used be sensitive enough to measure low levels of impurities. This has led to analytical methods that are suitable for determination of trace/ultra trace levels, i.e., sub-microgram quantities of various chemical entities.

A variety of methods are available for monitoring impurities. The primary criterion is the ability to differentiate between the compounds of interest. This requirement reduces the availability of methods primarily to spectroscopic and separation methods or a combination thereof.

Spectroscopic methods

The following spectroscopic methods can be used:

- Ultraviolet (UV)
- Infrared (IR)
- Nuclear magnetic resonance (NMR)
- Mass spectrometry (MS)

UV Spectrophotometry

UV at a single wavelength provides minimal selectivity of analysis; however, with the availability of diode array detectors (DAD), it is now possible to get sufficient simultaneous information at various wavelengths to ensure greater selectivity.

Infrared spectrophotometry

Infrared spectrophotometry provides specific information on some functional groups that may allow quantification and selectivity. However, low-level delectability is frequently a problem that may require more involved approaches to circumvent the problem.

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectroscopy provides fairly detailed structural information on a molecule and is a very useful method for characterization of impurities; however, it has limited use as a quantitative method because of cost and time considerations.

Mass spectrometry

Mass spectrometry provides excellent structural information, and, based on the resolution of the instrument, it may provide an effective tool for differentiating molecules with small differences

in molecular weight. However, it has limited use as a quantitative technique because of cost and time considerations.

In summary, IR, NMR, and MS are excellent techniques for characterization of impurities that have been isolated by any of the techniques discussed above. UV has been found to bee specially useful for analyzing most samples with high-pressure liquid chromatography. This combination is commonly used in pharmaceutical analysis.

Separation methods

The following separation methods can be used:

- Thin-layer chromatography (TLC)
- Gas chromatography (GC)
- High-pressure liquid chromatography (HPLC)
- Capillary electrophoresis (CE)
- Supercritical fluid chromatography (SFC)

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 techniques are chromatographic methods. CE is an electrophoretic method that is frequently lumped with the chromatographic methods because it shares many of the common requirements of chromatography. However, it is not strictly a two-phase separation system — a primary requirement in chromatography. Hyphenated methods such as GC–MS, LC–MS, GC–LC–MS, LC–MS–MS, etc. are all used.

A broad range of compounds can be resolved using TLC by utilizing a variety of different plates and mobile phases. The primary difficulties related to this method are limited resolution, detection, and ease of quantification. The greatest advantages are the ease of use and low cost.

Gas chromatography is a very useful technique for quantification. It can provide the desired resolution, selectivity, and ease of quantification. However, the primary limitation is that the sample must be volatile or has to be made volatile by Derivatization. This technique is very useful for organic volatile impurities.

High-pressure liquid chromatography is frequently casually referred to as high-performance liquid chromatography today. Both of these terms can be abbreviated as HPLC, and they are used interchangeably by chromatographers. This is a useful technique with applications that have been significantly extended for the pharmaceutical chemist by the use of a variety of detectors such as fluorescence, electrometric, MS, etc.

Capillary electrophoresis is a useful technique when very low quantities of samples are available

and high resolution is required. The primary difficulty is assuring reproducibility of the injected samples.

Supercritical fluid chromatography offers some of the advantages of GC in terms of detection and HPLC in terms of separations, in that volatility of the sample is not of paramount importance. This technique is still evolving, and its greatest application has been found in the extraction of samples.

Hyphenated methods

The following hyphenated methods can be used effectively to monitor impurities:

- GC–MS
- LC–MS
- LC–DAD–MS
- LC–NMR
- LC–DAD–NMR–MS
- LC–MS–MS

Of course, these methods are not always available or applicable. In case it is necessary to procure authentic material for purposes of structure confirmation, synthesis or isolation methods should be utilized.

Impurity profiling

Ideally an impurity profile should show all impurities in a single format to allow monitoring of any variation in the profile because of planned or unplanned changes in synthesis, formulation, or stability, etc. The driving forces for studying an impurity profile are

Quality considerations

Regulatory (FDA) requirements

It is the belief of this author that quality considerations should be the driving force for profiling.

Samples to be profiled

Impurity profiling should be done for the following samples:

- Active ingredient
- Process check (synthesis or formulation)
- Final product.

Components seen in a profile

Ideally, an impurity profile should show the following:

- Synthesis-related impurities
- Formulation-related impurities

▪ Degradation products

▪ Interaction products.

Isolating impurities

It is often necessary to isolate impurities because the instrumental methods mentioned above are not available or further confirmation is needed. For example, when hyphenated methods such as LC–MS are not suitable or do not provide unambiguous characterization, it may be necessary to isolate impurities for further confirmation of structure or for conducting toxicity studies. Of course, after the structure has been established, these impurities can be synthesized by a suitable route.

The following methods have been used for isolation of impurities:

▪ Solid-phase extraction

▪ Liquid–liquid extraction

▪ Accelerated solvent extraction

▪ Supercritical fluid extraction

▪ Column chromatography

▪ Flash chromatography

▪ Thin-layer chromatography

▪ Gas chromatography

▪ High-pressure liquid chromatography

▪ Capillary electrophoresis

▪ Supercritical fluid chromatography.

Isolation should be initiated based on simple extraction or partition methods. It may be possible to extract impurities selectively on the basis of acidity, basicity, or neutrality. The extraction process usually involves liquid–liquid extraction, where one phase is an aqueous solution and the other is an organic phase that is nonpolar. By appropriate adjustment of the pH of the aqueous solution, one can extract acidic, basic, or neutral impurities. Further separations can be made by chromatographic methods. Frequently, the isolation methods tend to be the same methods that are used for analysis.

Characterization of impurities

The characterization of impurities is generally achieved by the following means:

▪ Matching retention data

▪ UV

▪ IR

- NMR
- MS

Once an impurity has been detected, it becomes necessary to estimate its content. Detectability frequently means that a given component provides a signal at least twice that of background noise or the baseline. For quantification of impurity, the multiple is set much higher. Initial estimations are generally done against the parent compound because in most cases the authentic sample of impurity is not available. When the authentic sample is available, it is important that it be used for estimations. If the estimations indicate that a given impurity content is greater than 0.1%, it must be characterized as per the FDA and ICH requirements.

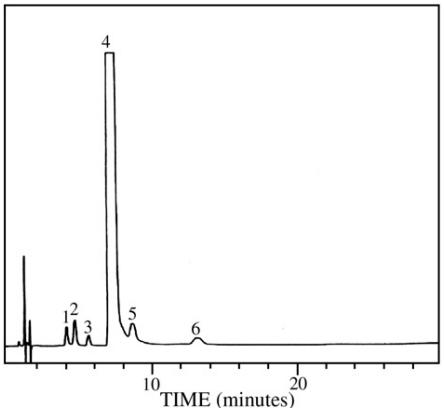
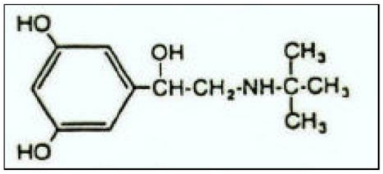


Figure. 1. Resolution of potential degradation products. 1=3,5-dihydroxyacetophenone, 2=3,5-dihydroxybenzaldehyde, 3=2-t-butyl-4,6,8-trihodroxy-tetrahydroisoquinoline, 4=terbutaline, 5=3,5-dihydroxy-ù-t-butylaminoacetophenone, 6=3,5-dihydroxybenzoic acid, ethyl ester.

A case study



Structure of Terbutaline

A case study is presented below relating to monitoring impurities in terbutaline sulfate (it is sold as a racemate).

HPLC methods

The first step in this process was to review all potential sources of impurities in terbutaline. Synthesis: starting materials, solvents used, intermediates, theorize potential by-products. Formulation: solvents used, potential interaction products, any potential degradation products. Stability: potential degradation products or reaction products that may be produced because of thermal, hydrolytic, oxidation, or photochemical reactions.

A careful assessment revealed that there could be 11 potential impurities in terbutaline (for chemical names, seelegends in Figure 1 and 2) that must be resolved. HPLC was clearly indicated as the preferred methodology of choice, based on physicochemical properties of terbutaline.

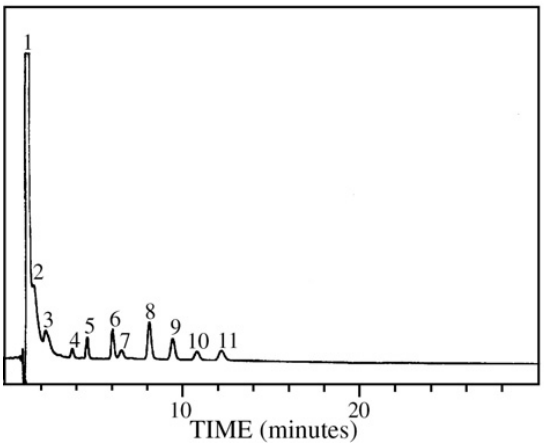


Figure. 2. Resolution of potential dibenzyloxyphenyl impurities.

1=terbutaline, 2=solvent,3=solvent, 4=α-[(t-butylamino)methyl]-3,5-dibenzyloxybenzyl alcohol, 5=α-methyl-3,5-dibenzyloxybenzyl alcohol, 6=3,5-dibenzyloxyacetophenone, 7=α-[(benzyl-t-butylamino)methyl]-3,5-dibenzyloxybenzyl alcohol, 8=3,5-dibenzyloxy-2,6-dibromoacetophenone, 9=3,5-dibenzyloxy-1′-bromoacetophenone, 10=3,5-dibenzyloxy-2,6,α-tribromacetophenone, 11=1′-benzyl-t butylamino-3,5-dibenzyloxyacetophenone.

Achiral impurities

All of the potential impurities were classified into four groups to assist the method development:

- Dihydroxyphenyl compounds with t-butylamino side chain
- Cyclized dihydroxyphenyl compounds with basic N in the ring
- Dibenzyloxyphenyl compounds with no t-butylamino side chain
- Dihydroxyphenyl compounds with no t-butylamino side chain

Two HPLC methods were developed to resolve all achiral impurities with the same C-8 column with 3-µm particle size.

System 1 (suitable for degradation products and less likely synthetic impurities): 0.005 mol 1-octanesulfonic acid in water:tetrahydrofuran:methanol (75:11:14).

System 2 (suitable for dibenzyloxyphenyl compounds; starting material and intermediates): water:tetrahydrofuran: acetonitrile:acetic acid: triethylamine (500:465:35:5:2).

In summary, System 2 was designed primarily for quality control of API. Since no impurities were found in the API with System 2, the quality of drug product for QC and stability studies can

be monitored using System 1 only.

2.7.3 Chiral impurities

The L-isomer of terbutaline is 3000 times more potent as a relaxant of tracheal smooth muscle than the D-isomer.

1. The isomers can be resolved on AGP column with 0.003 M tetrapropyl–ammonium bromide solution adjusted to pH 7.0.

2. Capillary electrophoresis can be used to resolve enantiomers with a background electrolyte that contains β-cyclodextrin or heptakis (2,6-di-O-methyl)-β-cyclodextrin.

CONCLUSION:

To assure quality of drug substances and drug products, it is important to give a careful consideration as to what constitutes impurities for a given case and proceed carefully to design a program to achieve the desired results. It is believed that the discussion included herein would be helpful in developing such a program.

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