

IMPURITY PROFILING IN PHARMACEUTICALS: A REVIEW**Dr. Sayyed Hussain¹, Dr. Amit Gosar² and Tabrez Shaikh^{1,2*}**

¹Sir Sayyed College of Arts, Commerce and Science, P.G. Department of Chemistry,
Roshangate, Aurangabad, 431001 Maharashtra, India.

²Indoco Remedies Limited, Analytical Research & Development Department, Navimumbai,
400701, Maharashtra, India.

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Corresponding Author*Tabrez Shaikh**

Indoco Remedies Limited,
Analytical Research &
Development Department,
Navimumbai, 400701,
Maharashtra, India.

ABSTRACT

Impurities in the drug are the components which are responsible for the change in the quality of drug with respect to the safety and efficacy. Regulatory authorities such as the International Conference on Harmonization (ICH), the United States Food and Drug Administration (FDA), and the European Directorate for the quality of Medicines (EDQM) emphasis on the requirements of the pure drugs and identification of their impurities. There are various sources of impurity in pharmaceutical products such as starting material, reagent, catalyst, intermediate, solvent and degradation product formed during storage of the drug. Impurities are classified into various categories depending upon their origin, composition type, and biological safety. There are different isolation technique of impurities from the drug. The various analytical methods are used to qualify and characterize the impurities. This reveals that there is always needs and the scope for the impurity profiling of drugs in drug development.

KEYWORD: Impurities, definition, classification, regulatory and profiling.

INTRODUCTION

Medicine is the branch of science that involves the identification, treatment, and prevention of the disease. It is used from the ancient time to combat the various diseases. Earlier, the drugs were extracted from the plant that may have the active compound to combat the specific disease. As the years past, drugs were also derived from the animals, microorganisms, and by the chemical synthesis, thus increasing the efficacy of the drug by removing the unwanted parts from the drugs.

The drug consists of mainly the two components, one is an active ingredient that is the chemical compound in the drug that makes the drug work on the disease, while another part called as an inactive ingredient consists of excipients, binder, colour, and flavours. The active and inactive ingredients are purposely added to the drug. Besides these, there is another component that gets incorporated into drug during the manufacturing of drug or on the storage of the drug, which is called as an impurity.

The origin, control, and measurement of impurities in drug substance are very important to understand for the production of high-quality drug substances and this is done by the impurity profiling. Thus, impurity profiling is an analytical activity that consists of detection, identification and quantitative determination of impurities in the drug substances. Thus, impurities are critically associated with the quality of the drug substances and drug product which have potential to affect the safety and efficacy of drug substance and drug product present even in trace amounts.

The analysis of impurities is a very intensive task which involves method development, impurity synthesis, isolation, and various analytical approaches to determine the univocal identification of the impurity of interest.

Thus, there is always a great need for the development of new analytical methods for quality evaluation of new emerging drugs. Impurity profiling requires a high-resolution chromatography system capable of reliably and reproducibly separating and detecting all of the known and unknown impurities of the active compounds. Various methods need to be developed for determination of impurities in raw material, intermediate, finished product samples. These methods should be stability indicating and well validated with parameters defined in the International Council for Harmonisation (ICH) guidelines and pharmacopoeias.^[1-5]

Impurities in Pharmaceuticals

Definition of impurities: Impurities are defined by the various official pharmacopoeias, groups, bodies, and ICH as follows.^[6-9] United States Pharmacopoeia (USP) general chapter <1086> 'Impurities in drug substance and drug product' defined impurity as follows.

'Impurity is any component of a drug substance that is not the chemical entity define as the drug substance and in addition, for a drug product, any component that is not a formulation ingredient.'

European Pharmacopoeia (EP) general chapter 5.10 'Impurities in substances for pharmaceutical use' define the impurity as follows.

'Impurity is any component of a substance for a pharmaceutical use that is not the chemical entity defined as the substances.'

International Council for Harmonisation (ICH) defines impurity as follows.

'Impurity is any component of the drug substance that is not the chemical entity defined as the drug substance'.

Food and drug administration (FDA) describe the impurity in drug substances and drug product as follows.

Impurity is any component present in the drug substance or drug product that is not the desired product, a product-related substance, or an excipient including buffer components. It may be either process-or-product related. The common definition of impurity is any substance coexisting with drug substance, such as starting material, reagents, catalyst, raw material or intermediates arising from synthesis or develop during storage or shipment is called as Impurity.

Source of impurities

There are various sources where impurities get generated in drug substances. The major sources of impurities are intermediate and by-products which may be carried into the API as impurities. A starting material used in the synthesis of drug substances, solvents used in the synthesis and purification of API, catalyst and reagent used in the process are also the potential source of impurities in API. Beside these impurities, degradation product generated on storage and shipment or on exposure to light, air oxidation and hydrolysis contribute to the generation of impurities in API. Impurities present in starting material may sometimes be a potential carried to the API. If drug substance required is a specific isomer then stereoisomers of raw material and intermediate also contribute to the generation of chiral impurities in API. Following Fig 1.1 shows generation and its source at various stages of drug synthesis.^[10]

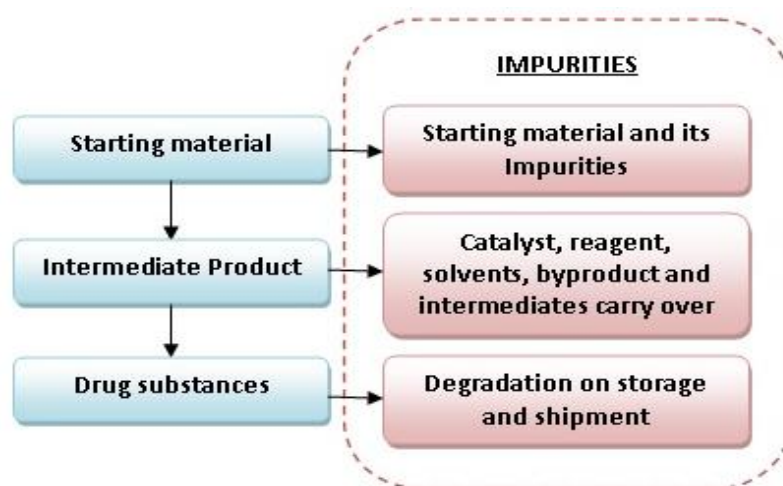


Fig. 1.1: Sources of impurities in drug substance.

Classification of impurities

Impurities are classified into various categories depending upon their origin, composition type, and biological safety. ICH has classified the impurities in drug substances in three main major categories such as Organic impurities, Inorganic impurities and residual solvent.^[11]

Organic impurities

These impurities may arise during the manufacturing process and/or storage of the drug substance (degradation product). The synthetic process-related impurities can be derived from starting materials, intermediates, reagents, ligands, and catalysts used in the chemical synthesis, as well as by-products from the side-reactions or over-reaction of the chemical synthesis. Degradation impurities are formed in drug substance by end product degradation during manufacturing or derived from the chemical degradation or storage under improper or stress conditions. They may be identified or unidentified, volatile or non-volatile chemical substances. In the synthesis of chiral drug substances, chiral impurities may get generated and carried forward to final drug substances. These impurities may have low efficacy as compared to drug substances and should be eliminated or controlled in raw material, intermediate or final stage.

Inorganic impurities

As various inorganic raw materials are used in the synthesis of drug substance, there is the very possibility of carryover of metallic and nonmetallic inorganic impurities carryover into drug substances. These impurities are classified as follows. Raw materials used in the synthesis of drug substances such as acids, alkalies, alkaline earth compound, reagents;

catalysts and inorganic salts contribute to inorganic impurities. Metal residue and heavy metal impurities are generally derived from water and metal catalyst used in the synthesis process of drug substances. Besides this, these impurities can be generated from leaching of equipment such as a reactor, micron filter, transfer line, centrifuge and dryer used in the process. Residual metallic impurities do not provide any therapeutic benefit and can be avoided using distilled water and glass-lined reactors.

Residual solvent: Organic volatile chemicals generally solvents in pharmaceuticals are called as residual solvent. These are used or produced in the manufacture of the drug substances. These solvents do not have any therapeutic benefit and in some case they are toxic, therefore, require to be removed from the drug substance. These solvents are not completely expelled out by practical manufacturing techniques, but its traces remain. Some solvent that is known to cause toxicity should be avoided in the production of drug substances. Residual solvents were evaluated for their possible risk to human health and based on this they are placed into one of three classes.^[12]

Class 1 solvents: These solvents should be avoided due to known carcinogens to human and environmental hazards. E.g. Benzene, 1,2-Dichloroethane, 1,1-Dichloroethene and carbon tetrachloride.

Class 2 solvents: These solvents are to be limited as they are non-genotoxic, but suppose to be neurotoxic and teratogenic.

Class 3 solvents: These solvents have the low toxic potential to the humans with PDEs of 50 mg or more per day. E.g. Acetic acid, Ethanol and Methanol. The solvent for which no adequate toxicity data is found, these solvents are to be limited in drug substance based on justification. E.g. Trifluoroacetic acid, Trichloroacetic acid and Isooctane based on their permitted daily exposure (PDE).

Genotoxic impurities: Based on toxicological risk assessment Genotoxic impurities are those compounds which have the potential to damage DNA at any level of exposure and that such damage may lead to the formation of a tumour.^[13-15]

Classification of impurities as genotoxic means that there is positive result established in vitro or in vivo genotoxicity tests. Below listed functional groups of the compounds are known to be involved in reactions to the DNA that could be used as structural alerts Fig. 1.2.

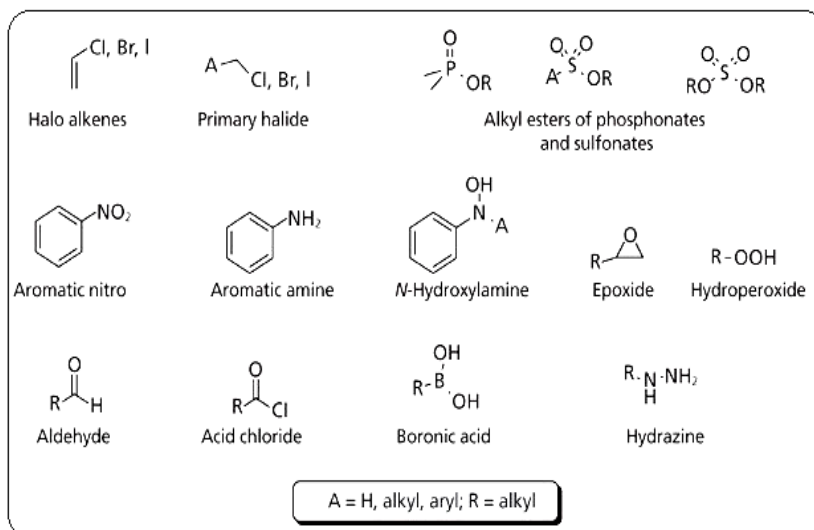


Fig.1.2: Structure alerting functional groups in impurities.

These genotoxic impurities are further categorized into five classes depending upon their risk assessment these are as follows.

Class 1: These impurities have established mutagenic and carcinogenic data and known to be the most serious risk with the need to eliminate them by modifying the process. If this is not possible, these impurities are to be limited at “Threshold of Toxicological Concern (TTC)” as a last resort.

Class 2: These impurities have the established mutagenic data, but their potential to cause carcinogen is not known. Hence, these impurities need to be controlled using TTC approach.

Class 3: These impurities are having alert structures unrelated to the structure of the drug substances and of unknown genotoxic potential. Based on functional groups within their molecule, they can be linked to genotoxic. The toxicity of these impurities is identified based on the structure-activity relationship (SAR).

Class 4: These impurities are having structures similar to the structure of drug substances and additionally contain functional or moiety that has potentially alert shared with the parent structure.

Class 5: These impurities have no alert structures, and evidence indicates the absence of genotoxicity. These compounds are to be treated as normal impurities and controlled according to the ICH guidelines.

Chiral impurities: These are organic impurities present in drug substance which is having a structure similar to drug substances, but the only difference in spatial orientation around a chiral carbon atom in the molecule. For e.g. Besifloxacin Hydrochloride is R-isomer while its Impurity-A is S-isomer. Drug substances which have optical isomers are having these types of impurities. These impurities have different therapeutic and pharmacological profile compared to drug substance and in some case, they may be toxic. For e.g. Thalidomide drug is having one chiral centre with two isomers, R-isomer is sedative and S-isomer is a teratogenic impurity which causes a birth defect. Therefore, these impurities should be controlled in the drug substances.^[16-17]

Extractables and Leachables impurities

Extractables impurities are compounds that can get extracted from elastomeric components, plastic components or coatings of the container and closure system when in the presence of an appropriate solvent while Leachables are compounds that leach from elastomeric, or plastic components or coatings of the container and closure system as a result of direct contact with the drug product formulation. These impurities are generally in the drug product.^[18]

Other Impurities

Addition to above impurities, unwanted foreign substances or particulate matter arising from glass, porcelain, filter aids such as hyflo, activated carbon, fibre filters may also contribute to impurities. Regular monitoring of fibres and black particles in the bulk drugs substances is essential to avoid their contamination. These impurities can be eliminated by good manufacturing practices.

Impurity profiling

Impurity profiling is the group of activity carried out for the detection, identification, and quantitation of impurities in drug substances. This also involves the process of acquiring and evaluating data that establishing the biological safety of individual impurity. Generally, impurity profiling starts with identifying new, unknown impurity in drug substance if it is less than reporting threshold, then it is controlled under single unspecified impurity. If impurity exceeds the reporting threshold, then primarily sample is subjected to identification by primary screening by any spectroscopic technique, if this reveals the structure of impurity and its biological safety is established or known then it is controlled at qualification level. If the structure is not identified, then sample enriched with an impurity is subjected to isolation

of impurity. After isolation of impurity, the structure is elucidated and impurity is taken for qualification based on its qualification, its limit is decided and analytical method is developed for its detection. The developed analytical method is taken for validation and thus impurity is controlled in drug substances.^[19-20]

Identification of impurities: It is one of the activities of Impurity profiling, where the goal is to identify the chemical structures of impurities present in the drug substances or observed in the stability studies above a particular threshold. Knowledge of the chemical structure of impurity and its formation mechanism is very important to assess its toxicological implications thus improving the synthetic chemical processes to reduce or eliminate the impurity. Identification of pharmaceutical impurities can be done by various spectroscopic techniques, such as Ultraviolet (UV), Infrared (IR) Mass spectrometry (MS) and Nuclear magnetic resonance (NMR) while its quantitation can be done by chromatographic technique such as High performance liquid chromatography (HPLC), Gas chromatography (GC), Supercritical fluid chromatography (SFC) and Thin layer chromatography (TLC/HPTLC). ICH guidelines indicate that all the impurity present in drug substance should be identified if present at or above a certain limit which is called as identification threshold. This limit depends on a daily dose of the drug substance Table 1.1.^[21]

Qualification of impurities

Qualification is the process of acquiring and evaluating data that establishes the biological safety of an individual impurity. The levels of any impurities present in a drug substance that has been adequately tested for safety or clinical studies would be considered qualified Table 1.1. Impurities that are also significant metabolites of drug substance present in animal and/or human studies are generally considered qualified. In many cases, studies performed to qualify an impurity will depend not only on the daily dose intake but also on the patient population, route of administration and duration of drug administration. It is preferable to decrease the level of impurity below the threshold, rather than providing additional studies. Based on adequate safety data available in the scientific literature, the impurity can be qualified. Fig. 1.3 gives the decision tree for consideration of safety studies for to qualify an impurity. Evaluation of impurity using Quantitative Structure-Activity Relationships (QSAR) models may also be an acceptable way for evaluating the safety of impurities in addition to in vitro genotoxicity studies.^[22]

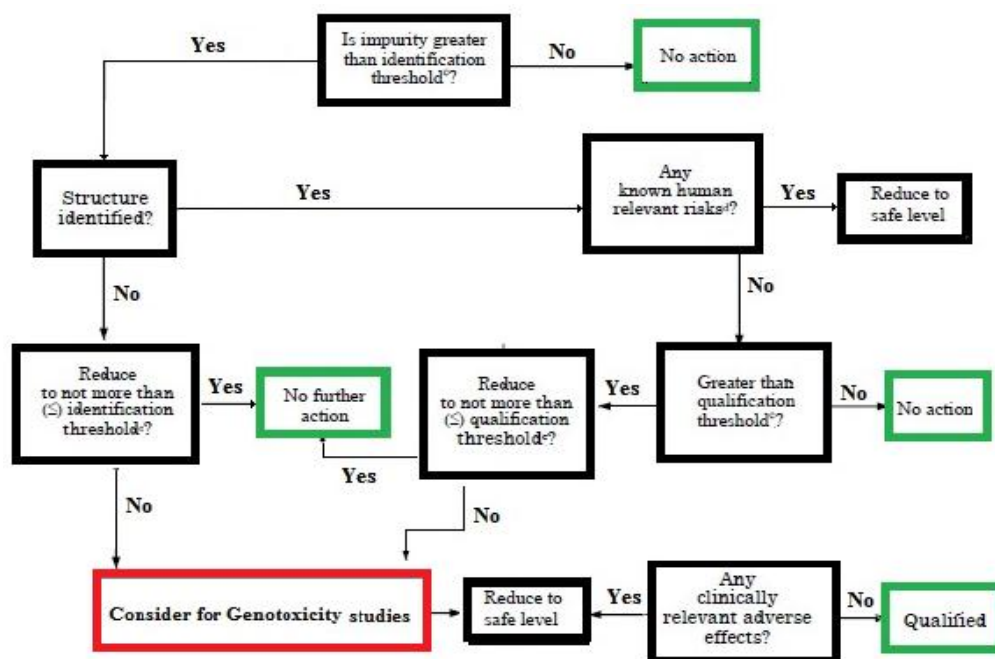


Fig. 1.3: Impurities decision tree for impurity qualification.

Reporting of impurities: A limit above which impurity should be reported is called as reporting threshold Table 1.1. All quantitative results should be reported in numerical value and not terms such as “complies”, “pass” etc. Impurities should be abbreviated with alphabetic or numeric code such as Impurity-A. The higher reporting threshold for impurity should be justified. Impurities greater than the reporting threshold should be summed and reported as total impurities. Below 1.0%, the results should be reported to two decimal places (e.g. 0.12%); at and above 1.0% it should be reported in one decimal (e.g., 1.3%). Reporting of impurity should always be rounded. For last digit greater or equal to 5, the retained digit is increased by 1 and for last digit less than 5 the succeeding number is retained. E.g. for 0.992% it will be rounded to 0.99%, while for 0.117% it will be rounded to 0.12%. Impurities should be reported in the same unit as given in specification limit unit.^[23]

Table. 1.1: Impurities threshold in drug.

Threshold	Maximum drug daily dose	
	Less than 2 g/day	More than 2 g/day
Reporting	0.05 %	0.03 %
Identification	0.10 %	0.05 %
Qualification	0.15 %	0.05 %

Quantification of impurities

According to ICH Guideline, each impurity must be investigated with respect to both chemistry and safety aspects. The former include identification (structural Characterization),

reporting and quantitation using suitable analytical procedures, while the latter includes a process of acquiring and evaluating data concerning the biological safety of an impurity (qualification). Individually listed impurities, limited with specific acceptance criteria, are referred to as specified and they can be either identified or unidentified.^[24-25]

Unspecified impurities are limited by a general acceptance criterion. A decision tree for the identification and qualification along with the corresponding thresholds, which are dependent on the maximum permitted daily dose (MDD), is given by ICH. Summing up, the following list of organic impurities must be presented in the specification of a synthetic drug substance

- Each specified identified or unidentified impurity
- Any unspecified impurity
- Total impurities

Isolation of impurities

Mostly the chromatographic techniques are used for isolation of impurities along with classical techniques. It is often necessary to isolate impurities because the only instrumental methods do not characterize the impurity. 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.

The following methods have been used for isolation of impurities.^[26-28]

- Solid-phase extraction
- Column chromatography
- Flash chromatography
- Supercritical fluid chromatography
- Thin-layer chromatography
- Capillary electrophoresis
- Preparative high-pressure liquid chromatography
- Accelerated solvent extraction
- Liquid-liquid extraction

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. In chromatographic method desired impurity peak or band is separated, concentrated, and isolated.

Characterisation of Impurities

Impurities and degradation product after isolation are characterized where the structure of impurities is elucidated by the spectroscopic technique such as Nuclear magnetic resonance, Infrared, Ultra-visible and Mass along with elemental analysis. After confirming the impurity structure, it is used as a standard for further quantification of that impurity in a drug substance or drug product.^[29]

Regulatory guidelines

Various regulatory authorities and pharmacopoeia have specified the guideline for impurities in the pharmaceutical.^[30]

ICH (International Council on Harmonisation)

International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a project that brings together the regulatory authorities and pharmaceutical industries of Europe, Japan and the United States to discuss scientific and technical aspects of pharmaceutical product registration.

Following are the guidelines for impurity profiling in ICH

Impurities in New Drug Substances Q3A (R2)

This guideline elaborates the chemistry and safety aspects of impurities, including the listing of impurities in specifications and defines the thresholds for reporting, identification and qualification of drug substances.

Impurities in New Drug Products Q3B (R2)

This guideline addresses only those impurities in new drug products classified as degradation products of the drug substance or reaction products of the drug substance with an excipient and/or immediate container closure system.

Impurities: Guideline for Residual Solvents Q3C (R6)

This guideline recommends the use of less toxic solvents in the manufacture of drug substances and dosage forms and sets pharmaceutical limits for residual solvents (organic volatile impurities) in drug products.

Guideline for Elemental Impurities Q3D: This guideline aims to provide a global policy for limiting metal impurities qualitatively and quantitatively in drug products and ingredients.

Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk M7(R1): This guideline emphasizes considerations of both safety and quality risk management in establishing levels of mutagenic impurities that are expected to pose a negligible carcinogenic risk. It outlines recommendations for assessment and control of mutagenic impurities that reside or are reasonably expected to reside in final drug substance or product, taking into consideration the intended conditions of human use.

The United States Food and Drug Administration (USFDA)

The U.S. Food and Drug Administration (FDA or USFDA) is an agency of the United States Department of Health and Human Services and is responsible for controlling and supervising the safety of foods, dietary supplements, drugs, vaccines, biological medical products, blood products, medical devices, radiation-emitting devices, veterinary products, and cosmetics.

The Guidelines of impurity profiling in USFDA are mentioned below.

Impurities in New Drug Substances Q3A

Impurities in New Drug Products Q3B (R2)

ANDAs: Impurities in Drug Substances

ANDAs: Impurities in Drug Products

Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals To Limit Potential Carcinogenic Risk M7(R1)

Analytical method development: Development is important features in any analytical method of measurement because it is closely related to the quality of the results. As global regulatory requirements have gone more stringent, analytical methods for global products must be able to meet global regulatory requirements. To attain this objective of method reliably they must be a comprehensively designed, validated and correctly implemented in the quality system. It should be fully documented and effectively monitored. The basic task for development is to reduce this high variability by conducting a series of controlled experiments to make this information known and thus predictable. Method development is a continuous process where the goal is to consistently improve the quality of the product.^[30]

Analytical method validation

A very general definition of validation is establishing documented evidence which provides a high degree of assurance that a specific procedure, process, equipment, activity or system will consistently produce a product meeting its predetermined specifications and quality attributes. Validation is an important feature after the development of any analytical method because it is closely related to the quality of the results. All analytical methods, whether qualitative or quantitative are required to be validated. The degree of validation varies for the type of method and its application. For several years now, method validation studies, guidelines and procedures have focused mainly on quantitative methods of analysis. Validation is an imperative activity in the process of impurities profiling where the developed analytical method used for the determination of impurities in drug substances is validated in order to establish that the method is suitable for its aimed purpose. The analytical methods are validated for the following parameters in accordance with ICH Harmonized Tripartite Guidelines.^[31-32]

Forced degradation

The primary purpose of forced degradation is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommended storage conditions, retest periods, and shelf lives to be established. Forced degradation testing helps to identify the impurities which may be formed through the degradation of the drug under stress condition such as acidic, basic or oxidative condition.^[33-34]

CONCLUSION

The present review describes the details regarding profiling of impurities in drug substance and drug product (Pharmaceuticals). Impurity profiling is important in providing the safety and efficacy of the drug. The article also provides the valuable information regarding the impurities types, its classification, various techniques for its isolation and characterization, analytical techniques for the determination, quantification of impurities. The establishment of guidelines from various regulatory agencies and pharmacopoeias for impurity levels in drug substances and products provides the quality criteria for manufacturers.

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