Recent and Advance study on Impurity Profiling: A Review

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Abstract-The main aim of pharmaceutical product development is to cure and prevention of disease with achieve safety and efficacy. In every Active Pharmaceutical Ingredient, Impurity is present. In pharmaceutical industry, Purity profile is important factor as well as Impurity profile is important and mandatory according to Regulatory authority. In the pharmaceutical world, an impurity is considered as any other inorganic or organic material, or residual solvents other than the drug substances present in drug and also arise out of synthesis or unwanted chemicals that remains with APIs. The quality of drug product is highly affected by impurity present in drug. There are different types of impurities such as organic impurity, Inorganic impurity, residual solvent starting materials, intermediates, by product and degradation product etc. The International Conference on Harmonisation (ICH) guidelines, state the definitions of the impurities in new drug substances. In this article, we have discussed the types of possible impurities and their sources. We have also listed out the impurity isolation techniques and analytical techniques for the identification, quantification and characterization of impurities.

Key Words: Ingredient, Impurity, product development, pharmacopoeias, regulatory bodies.

I.INTRODUCTION

Impurities in pharmaceuticals are the unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during formulation, or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (ie, the identity as well as the quantity of impurity in the pharmaceuticals), is now getting receiving important critical attention from regulatory authorities. The different pharmacopoeias, such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly incorporating limits to allowable levels of impurities present in the APIs or formulations.[1]

Also, the International Conference on Harmonization (ICH) has published guidelines on impurities in new drug substances, products[2], and residual solvents[3]. In addition, Ahuja[4] and Gorog[5] have published books covering different aspects of impurities, including the governmental regulations and guidelines and the identification and monitoring of impurities found in drug products. There is a significant demand for the impurity-reference standards along with and the API reference standards to for both regulatory authorities and pharmaceutical companies. Interestingly, a company dealing with only impurity-reference standards, named Mikromol GmbH (Luckenwalde, Germany), has started marketing impurities found in pharmaceuticals through Promochem Group (Wesel, Germany).

A number of recent articles[6-8] have described a designed approach and guidance for isolating and identifying process-related impurities and degradation products using mass spectrometry, Nuclear Magnetic Resonance (NMR), high-performance liquid chromatography (HPLC), Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), and tandem mass spectrometry for pharmaceutical substances.

In general, according to ICH guidelines on impurities in new drug products [2], identification of impurities below the 0.1% level is not considered to be necessary unless the potential impurities are expected to be unusually potent or toxic. In all cases, impurities should be qualified. If data are not available to qualify the proposed specification level of an Impurity, studies to obtain such data may be needed (when the usual

qualification threshold limits given below are exceeded).

II. ROLE OF MODERN ANALYTICAL TECHNIQUES FOR PHARMACEUTICAL ANALYSIS

Until the beginning of 20th century, medicines were produced and marketed with no control over purity and safety. At that time quality of the most of drugs were poor and patented with dubious value. For example, thalidomide is a sedative drug introduced in the early 1960s.

This caused serious malformation in newborn babies of women who consumed during early days of pregnancy. Thereafter, it was identified that the s-enantiomer of thalidomide possessed teratogenic action and also has no power for the desired sedative property[9] Thereupon, the Food, Drug, and Cosmetic act (FD&C) was revised requiring advance proof of safety and several other controls for new drugs.

The best example to explain the necessity for development impurity profiling methods is aspirin. In 1970s, its quality was tested by titrimetric assay using United States, British and Indian pharmacopoeia[10]. A colour test was included in pharmacopeia, used for detection of free salicylic acid as a degradation product. When HPLC become popular in pharmaceutical analysis, in addition to salicylic acid, three more impurities[11-12] were found in bulk drug substances as shown in Figure 1. It was found that these impurities are reacting with protein amino functions that are responsible for allergic reactions[13]. Hence, it is clear that sophisticated equipments are necessary for pharmaceutical analysis especially in impurity profiling. Out of all analytical techniques, the chromatographic techniques such as HPLC, UPLC and LC-MS have gained prime importance.

III. 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 –

(1) Inorganic impurities.

(2) Organic impurities.

(3) Contamination by chemical intermediates[14], 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.

IV. LIMITS FOR IMPURITIES

According to ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level, is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic.

According to ICH, the maximum daily dose qualification threshold to be considered is as follows as shown in table no.2-5;< 2g/day 0.1 % or 1 mg per day intake (whichever is lower) >2g/day 0.05%[46]

V. QUALIFICATION OF IMPURITIES[16]

Qualification is the process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) being considered. When appropriate, we recommend that applicants provide a rationale for establishing impurity acceptance criteria that includes safety considerations.

An impurity is considered qualified when it meets one or more of the following conditions:

• When the observed level and proposed acceptance criterion for the impurity do not exceed the level observed in an FDA approved human drug product.

• When the impurity is a significant metabolite of the drug substance.

• When the observed level and the proposed acceptance criterion for the impurity are adequately justified by the scientific literature.

• When the observed level and proposed acceptance criterion for the impurity do not exceed the level that has been adequately evaluated in comparative in vitro genotoxicity studies.

VI. RESEARCH STUDIES DESCRIBES IDENTIFICATION OF IMPURITY

First Case Study on Identification of impurities: Due to the increasing number of drug cases, as well as the widening globalization of illicit drugs, law enforcement agencies worldwide have adopted the strategy of profiling of drug impurities. Detailed impurity information has been reported on the methamphetamine drugs seized in countries such as the European Commission, Japan, Thailand, Korea, the Philippines and Australia, where methamphetamine abuse is one of the most serious drug issues. The information obtained can be used to establish drug trafficking patterns and distribution networks, and to identify methods used in the manufacture of illicit drugs. Methamphetamine hydrochloride is currently one of the most widely used illicit drugs in the China. However, in the open literatures there has been little information available on impurity characteristics or profiling of methamphetamine drug seizures in China. A total of 48 methamphetamine hydrochloride samples from eight seizures were analyzed using gas chromatography–mass spectrometry (GC–MS) and a flame ionization detector (GC–FID). Eight seizures of Methamphetamine hydrochloride from BPSB captured between 2006 and 2007 were analyzed. Typically the seizures were crystals and had a purity of more than 95%. Each of seizures weighed over 400g and belonged to a bag. The contents of each selected bag (seizure) were divided into six samples. Thus, a total of 48 samples were obtained. 10g were weighed out from each sample and crushed. Fifty milligrams were taken for analysis. The each sample was analyzed three times to determine the variability within each seizure and whether the samples from the same bag (seizure) belong to the same batch. The present method offers superior separation of impurities in methamphetamine hydrochloride crystals using chromatographic techniques. The 17 peaks selected

were characteristic and diagnostic for the classification and comparison of chromatograms. The Euclidean distance of 17 relative peak areas after logarithmic transformation was effective for the evaluation of similarity and/or dissimilarity of impurity profiles. The preliminary work shows that it is very useful for getting intelligence from methamphetamine impurity profiling. Information about the impurities in methamphetamine allowed identification of the drug synthetic routes. In the drugs manufactured via the ephedrine route where the marker compounds, the aziridines or naphthalenes, were present distinctively. [17]

Second Case study on Quantification of active pharmaceutical ingredient and impurities in sildenafil citrate:

Consumers can obtain prescription drugs *via* the Internet without any difficulty and professional oversight. The accessibility of prescription drugs produced outside of the United States, most notably sildenafil citrate (innovator product, Viagra®), has been made much easier by the Internet. Clinicians and policymakers are more concern to product quality and patient safety. The US Food and Drug Administration (FDA) has issued warnings to potential buyers that the safety of drugs purchased from the Internet cannot be guaranteed and may present a health risk to consumers from substandard products.

A study was conducted to determine whether generic sildenafil citrate tablets from international markets obtained *via* the Internet are equivalent to the US innovator product regarding major aspects of pharmaceutical quality: potency, accuracy of labelling, and presence and level of impurities. As in case a total of 15 sildenafil citrate tablets were taken for pharmaceutical analysis out of which 14 generic samples from international Internet pharmacy websites and one was innovator product. According to US Pharmacopeial guidelines, tablet samples were tested using high performance liquid chromatography for potency of active pharmaceutical ingredient (API) and levels of impurities (impurities A, B, C, and D). Impurity levels were compared with International Conference on Harmonisation (ICH) limits. As outcome of among 15 samples, 4 samples possessed higher impurity B levels than the ICH qualification threshold, 8 samples possessed higher impurity C levels than the ICH qualification threshold, and 4 samples possessed more than 1% impurity quantity of maximum daily dose (MDD). For API, 6 of the samples failed to fall within the 5% assay limit. Outcomes of study revealed that in that manufacturing standards for sildenafil citrate generic drug products compared with the US innovator product are not equivalent with regards to potency and levels of impurities. They have implications for safety and effectiveness that should be addressed by clinicians to safeguard consumers who choose to purchase sildenafil citrate and foreign-manufactured drugs, *via* the Internet. [18]

VII. THE REGULATORY GUIDELINES FOR IMPURITIES IN THE API

Impurity Monitoring and Control have different meanings. Therefore, simple terminology should be used for questions related to impurities. The US Food and Drug Administration (USFDA) has approved guidelines prepared by the International Conference on Harmonization (ICH). The ICH Guidelines for Impurities have been jointly developed by various regulatory bodies such as the European Union (EU), Japan, and the United States and help ensure consistency in the data requirements that must be submitted to the various regulatory bodies. This guide provides information to sponsors of a new drug application (NDA) or abbreviated new drug application (ANDA) to send with their application, as well as helps FDA reviewers and researchers in a consistent application and interpretation of the rule[19-20-21]. The following are various regulatory requirements of ICH Guideline

1. Stability Testing of New Drug Substances and Products.
2. Impurities in New Drug Substances.
3. Impurities in New Drugs.
4. Recommendations Impurities: Residual Solvent.
5. USFDA Guidelines for Impurities in NDAs in New Medicinal Substances.
6. ANDA Impurities in New Drug Substances.
7. Australian Prescription Drug Regulatory Guide, Treatment Australia (TGA)(17).

VIII. ANALYTICAL METHOD

1.Isolation method

Approximate estimations of probable impurities in a synthetic process are made on the basis of assumption that impurity would be somehow structurally related to compound of interest. After synthesizing hypothesized/suspected/reverse engineered impurities, next step is to isolate and monitor them during the actual synthetic process. Generally, chromatographic techniques are used for isolation of impurities. If instrumental methods can directlycharacterize impurities, then isolation step can be avoided.

Extraction

Liquid-solid extraction: A solvent that would dissolve the impurity of interest is selected. An organic solvent blend is used for extraction where a compound contains more than one type of impurity. These solvents tend to volatilize at low temperature, facilitating concentration of impurity. Examples of common solvents used in liquid-solid extraction include toluene, methanol, water, cyclohexane etc.

 Soxhlet extraction: This technique is used for extracting compound of interest from crude drug products, etc, It utilizes a small volume of solvent which is repeatedly siphoned through a product to produce a concentrated extract. Natural compounds are isolated by this method, for instance isolation of Curcumin from rhizomes of Turmeric. In impurity profiling, Soxhlet extraction finds use, when the desired compound has limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of using many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.[22]

Liquid-liquid extraction: It involves extraction of one liquid with another, in which one is aqueous and the other is organic with both being mutually immiscible.[23]

Gas chromatography: It is useful for isolation and characterization of volatile impurities or compounds that can be volatilized by derivatization. For instance, in production of Doxorubicin hydrochloride, acetone and ethanol were found as impurities by gas chromatography.

2. sepration

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 thesample 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.[24-25-26-27]

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

1. Nuclear Magnetic Resonance (NMR):
NMR provides information about specific bonding between peak area and number of nuclei responsible for peak. Most important application of NMR is identification and structure elucidation of molecules. Analysis of multicomponent mixture.

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

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

4. LC-MS:
LC/MS is a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectrometry. LC/MS became really popular with the introduction of the thermo spray interface and the particle beam interface. This is same as GC-MS but removal of liquid carrier from an HPLC eluent before samples are passed in to the MS source. For handle normal eluent flow rate 0.5-2.0ml min-1 which is not handled by MS pumping system moving belt inlet systems, jet separators and vacuum nebulizers are used.[28-29-30-31]

4.Reference standard method
The main purpose of this method is to afford clarity on the whole life cycle, qualification and control of reference standards used in development and control of new drugs is very important. As because the reference standards provides the basic information for evaluating process and product performance of drug substances, drug products, impurities, degradation products, starting materials, intermediates, and excipients. [32]

5.Spectroscopic methods
The UV, IR, MS, NMR and Raman spectroscopic methods are abundantly used for the identification of impurities. Now a day’s ICP MS also play a vital role for the identification of impurities. And it haswide choice throughout the different regulatory authority.[33]

IX. IMPLEMENTATION OF QUALITY BY DESIGN APPROACH

Quality by Design (QbD) is "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" and has the aim of improving product quality and of increasing regulatory flexibility. Impurity level is a critical quality attribute for a drug substance or a drug product because levels higher than the toxicologically qualified amount could affect the safety and efficacy of the product. [35]

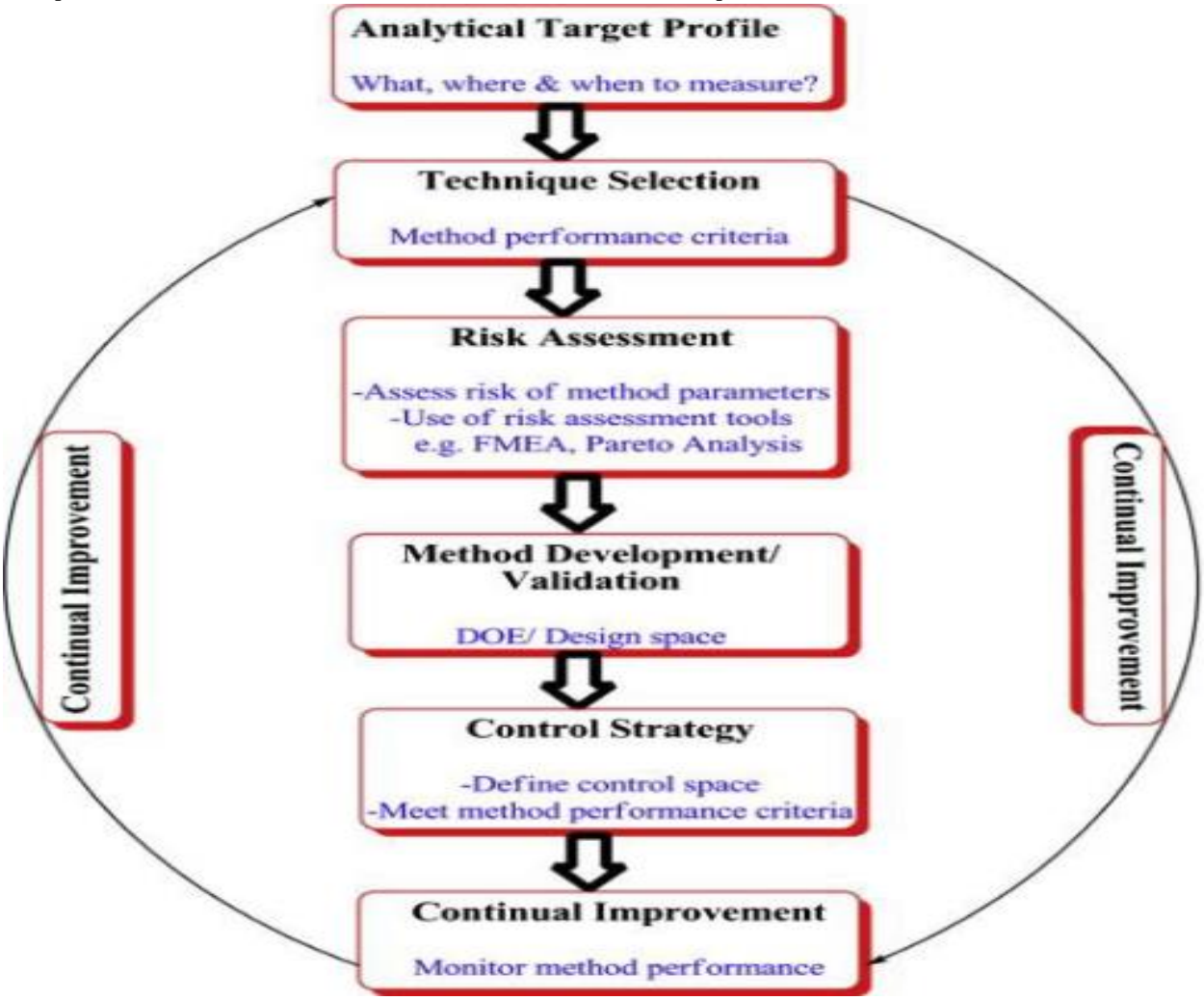

Fig.1 Systematic implementation of QBD approach.

QBD APPROACH IN IMPURITY[34]

For Patients: They receive the product of high quality.

For Regulators: A clear control strategy from the manufacturers provides transparency and added assurance that risk of impurity has been adequately controlled.

Pharmaceutical companies: A clear control strategy is identified which ultimately facilitates the successful launch of the product and various post-approval supplements like scale-ups, tech transfers etc.

X. CURRENT MARKETED FORMULATION WHICH CONTAINS IMPURITY

Indian pharmacopoeia specifies qualitative, quantitative tests for monitoring known impurities present in certain drugs. A few

examples of impurities present in drug and identified by using particular method have given in following table. [36]

Table No.1: Drug names and their impurities with methods of detection.

Sr.no.	Drug name	Impurity present	Method used
1	Amphotericin B	Tetraenes	UV-Spectroscopy
2	Atropine Sulphate	Apo atropina	UV-Spectroscopy
3	Flurescene sodium	Dimethyl Formaamide	Gas chromatogaphy
4	Cloxacilin	N,N,dimethylaniline	Gas chromatogaphy

X. Importance and Application of impurity profiling

Formation of drug is imperfect without identification of impurities. For market approval of drug, identification, quantification and control of impurities in drug are important. Quantitative determination of impurities is useful in validation of drug. Structural elucidation and thereafter synthesized impurity can be used as an impurity standard, which can be used for development of selective analytical methods for quantitative determination of impurities. It is essential to submit impurities to various drug authorities which will use impurities as standard for regulatory analysis. Other applications include understanding degradation pathways for amines, alkaloids, analgesics, steroids, anti-cancer drugs, tranquilizers etc. To establish a control system for impurities so that they cannot interfere with final desired compound. Impurities are essential to obtain market approval.[37]

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 [38-39]

XI FUTURE PROSPECTS

The various regulatory bodies have outlined guidelines with regarding to safety and efficacy of impurities but there is a strong requirement to have unified specification/ standards for regulation of impurities. In the future, impurity profiling is likely to see advancements in analytical techniques, enabling more accurate detection and characterization of impurities in various substances. Additionally, there may be a growing emphasis on real-time monitoring and automation, enhancing efficiency and reducing time in the analysis process. Integration of artificial intelligence could further improve data interpretation and pattern recognition in impurity profiles, contributing to better quality control in industries such as pharmaceuticals and chemicals. As regulatory standards evolve, there may be an increased focus on addressing novel impurities and ensuring the safety of products across diverse applications.

XII CONCLUSION

This review provides a perspective on impurities in drug substance and drug product. Impurity profile of pharmaceuticals is receiving an increasing importance and drug safety receives more and more attention from the public and from the media. This article provides the valuable information about the impurities types and its classification, various techniques of isolation and characterization, analytical techniques for the determination, qualification of impurities and critical factors to be considered while preparation of the bulk drugs. Nowadays, it is mandatory requirement in various pharmacopoeias to know the impurities present in API's. Isolation and characterization of impurities is required for acquiring and evaluating data that establishes biological safety which reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

The conclusion of impurity profiling depends on the specific context or experiment. Generally, it involves summarizing findings related to impurities in a substance, discussing their potential impact, and suggesting measures for purification or quality improvement if necessary. If you provide more details, I can offer a more tailored response.

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