"A REVIEW ON IMPURITY PROFILLING"

A Dissertation Submitted To The

Dr. BABASAHEB AMBEDKAR TECHNICAL EDUCATION,LONERE.

In Partial Fulfillment Of The Eight Semester Of

BACHELOR OF PHARMACY

Submitted By

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Under the guidance of

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CERTIFICATE

This is to certify that, the work presented in this titled **"A REVIEW ON IMPURITY PROFILLING''** for the submission in the partial fulfillment of **"Bache lor Of Pharmacy"** affiliated to, Dr. Babasaheb Ambedkar Technical Education Lonere at the ***ADITYA INSTITUTE PHARMACEUTICAL BEED*** by **Mr. RATHOD LAXMAN NARAYAN** PRN.NO.**1925551823052** to my satisfaction, this project is ready forsubmission.

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MR.GARAD R.S.

DECLARATION

I hereby declare that this project entitled **"A REVIEW ON IMPURITY PROFILLING"** submitted to Dr. Babasaheb Ambedkar Technical Education ,Lonere review work carried out by me at, ***ADITYA INSTITUTE PHARMACEUTICAL BEED*** under the guidance of **MR.GARAD R.S.**

Mr. RATHOD LAXMAN NARAYAN

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Mr. RATHOD LAXMAN NARAYAN

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1. ABSTRACT:

The description, characterization and quantisation of the identified and unidentified impurities present in new drug substances is known as impurity profile. Impurity is defined as any substance coexisting with the original drug, such as starting material or intermediates or that is formed, due to any side reactions. Impurity can be of three types: Impurities closely related to the product and coming from the chemical or from the biosynthetic route itself, Impurities formed due to spontaneous decomposition of the drug during the storage or on exposure to extreme conditions, or the precursors which may be present in the final product as impurities. 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.

AIM AND OBJECTVES:

1. To study the importance of impurity profiling in drug product and API's
2. To classify the impurity according to regulatory bodies.
3. To develop new method for impurity profiling, selection, identification.
4. To control the quality of product and it's safe to human use.
5. Identification and quantification of trace-level impurities in drug substancesand drug products.
6. Simultaneous identification, characterization, comparison including tracelevel impurities using analytical method.
7. The main aim is to prevent degradation, contamination of drug product.

PLAN OF WORK:

- Title: A Review on Impurity Profiling.
- To study the Aim and Objectives of thesis.
- Literature Survey.
- Method for impurity profiling.
- Prevention of impurity & Application of impurity profiling.

2. INTRODUCTION:

Pharmaceuticals impurities are the unwanted chemicals that remain or are generated during the formulation of medicines. Impurity profiling helps in detection, identification and quantification of various types of impurities as well as residual

solvents in bulk drugs and in pharmaceutical formulations. It is a best way to characterize quality and stability of bulk drugs and pharmaceutical formulations. Due to rapid development of the analytical methodology it is imperative to review problems related to impurities present in the drug substances and drug products with their solutions. Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agencies are emphasizing on the purity requirements and on identification of impurities in active pharmaceutical ingredients as presence of impurities even in small amounts may influence the efficacy and safety of the pharmaceutical products. Thus enlightening the need of impurity profiling of drug substances in pharmaceutical research this review focuses on various analytical methods for identification as well as quantification of impurities present in the pharmaceuticals.The bulk drug industry forms base of all pharmaceutical industries as it is the source of active pharmaceutical ingredients (APIs) of specific quality. Over the last few decades much attention is paid towards the quality of pharmaceuticals that enter the market. The major challenge for both bulk drug industries and pharmaceutical industries is to produce quality products.

For a pharmaceutical product great deal associated with its safety, efficacy and purity.Toachieve the final quality in the pharmaceutical product, vigorous quality control tools areutilized. The purity profile is important with respect to stability and safety aspect ofpharmaceutical product. But with the advancement in analytical techniques to detect impurityand regulatory aspect for biological safety gave rise to impurity profiling.

IMPURITY DEFINITION:

Impurity can be defined as any substance formulation component such as starting material, intermediate, or that is formed due to side reactions.

As per ICH impurity can be defined as any component new drug product that is not newdrug substance or excipient drug product. Following are various terminology

associated with impurity.

(a) Starting component

(b) Intermediate product

(c) By product

(d) Degradation product

(e) Transformation products

(f) Penultimate intermediates

(g) Related products

IMPURITY PROFILING DEFINITION:

Impurity profiling is the process of acquiring and evaluating data that establishes biologicalsafety of individual impurity thus reveling its need and scope in pharmaceutical research.Impurity Profiling is both qualitative and quantitative evaluation of impurities in new drug product.

Initial phase of impurity profiling:

Initial phase of impurity started in the year 2008 with number of publication in a year. Thistrend was continued in furtheryear. Initial period of impurity profiling includes HPLC,liquid chromatography, thin-layer chromatography, capillary electrophoresis etc.

Recent development in impurity profiling:

With advancement and development in analytical techniques such as hyphenated techniquesallows impurity profiling around 0.01% threshold values of formulated product. Varioushyphenated techniques used for characterization, structural elucidation and simultaneousdetermination of impurity.

Regulation of impurity profiling:

Now a day's part from purity profiling major concern focused on impurity profiling

byregulatory authorities around the globe because of its essentiality in relation to its safety,efficacy and stability of product.

Various authorities involved are:

(i) ICH

(ii) US-FDA

(iii)BP (iv)IP

(v) TGA

(vi)Canada health agency.

(vii) MHRA

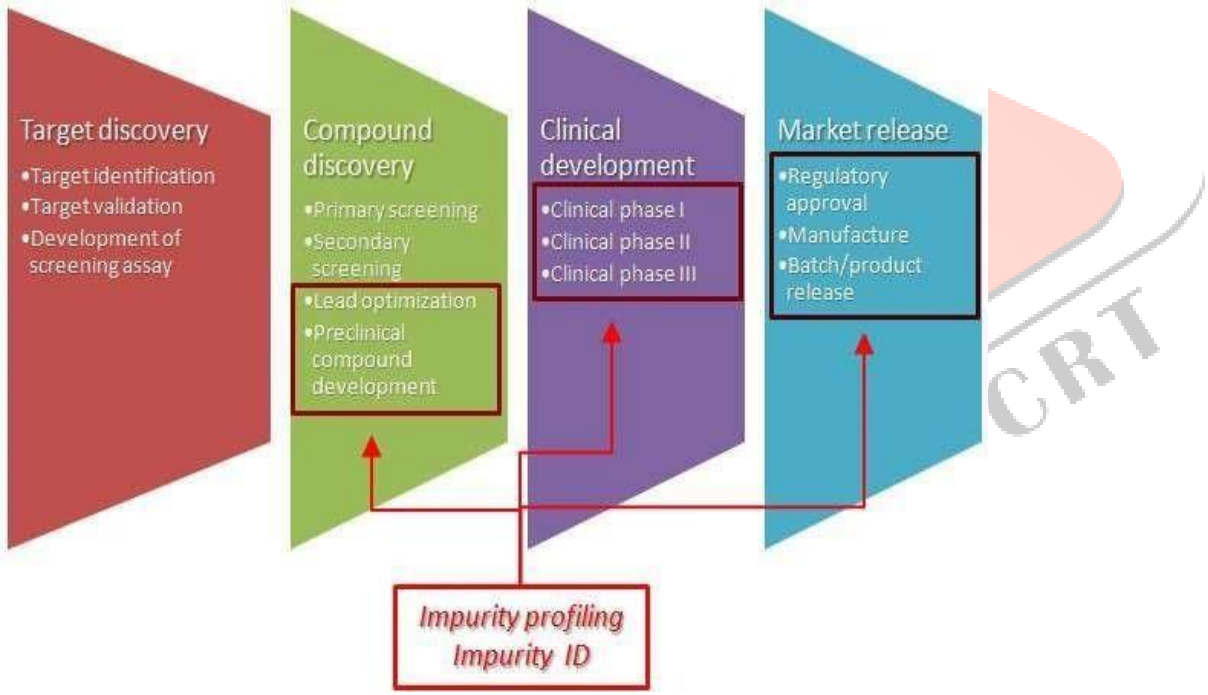

Figure 1. Drug impurity profiling / identification activities are carried out all alongthe drug discovery pipeline and beyond.

The monitoring and understanding of occurrence of impurities need to be addressed at a very early stage of development of a small molecule. It will indeed have a significant impact on the researched activity, production process, formulation, or even on the drug delivery. It is sometimes not economic or technically feasible to remove all impurities

during the production process, so theyneed to be monitored. Introducing a compound to the market will always be

accompanied with an impurityprofileto guarantee that the product's quality matches with the specification filed at the regulatory authorities (e.g. FDA, EMEA).

Rationale for Reporting of Impurities in Active Pharmaceutical Ingredient:

The setting of limits for allowable impurities in bulk drugs is a complex process which depends on number of factors like toxicology of impurities related to drug, route of administration, daily dose, target population, source of drug substance and duration of therapy. The basis behind setting limits on level of impurities is that impurities in drug substance must be controlled to ensure the safety and efficacy and quality of API throughout its development and use as a product, as some of these impurities might possess certain undesirable toxicological potential.

ICH guidelines, 'Impurities in New Drug Substances' (Q3A) states "The applicant should summarize the actual and potential impurities most likely to arise during synthesis, purification and storage of thenew drug substance. This should be based on sound scientific knowledge of the chemical reactions involved in the synthesis, impurities associated\ with raw materials and possible degradation products. Also the applicant should summarize the laboratory studies conducted to detect impurities in new drug substances. This summary should include results from batches from the development process as well as batches from commercial process. Also the studies conducted to characterize the structures of the impurities present above the identification threshold should be described. When identification of impurity is not possible, a summary of laboratory studies demonstrating the unsuccessful effort should be reported. The identification of impurities present at the level less than the identification threshold is not generally considered necessary. But analytical methodology needs to be developed for the impurities that are expected to have unusual toxic pharmacological effects."

Specifications for Impurities:

The specifications for a new drug substance should include limits for impurities.

Stability studies, chemical development studies and routine batch analysis can be used to predict those impurities likely to occur in the commercial product. A rationale for the inclusion or exclusion of impurities in the specifications should be presented. This rationale should include a discussion of the impurity profiles observed in the safety and clinical development batches, together with a consideration of the impurity profile of material manufactured by the proposed commercial process.

Reporting of Impurities:

All impurities above (>) reporting threshold should be reported

Identification of Impurities:

All impurities above (>) identification threshold are supposed to be identified. These include development of a suitable technique for isolation of desired impurities and their identification/characterization using various spectroscopic techniques to know the chemical structure of these impurities, and to suggest a possible synthetic route for formation of these impurities.

Qualification of Impurities:

The profile of impurities in a new drug substance may change for a variety of reasons, such as process scale-up changes, synthetic route change and changes made to key intermediates. ICH decision tree help to classify quality and select limits for New Molecular Entities (NMEs). If an impurity exceeds the qualificationthreshold listed in Table 1, studies are needed to qualify that impurity in drug substances. 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) specified.

3. SOURCES OF IMPURITIES

- The pharmaceutical preparation should be free from toxic and otherimpurities.
- Pharmacopoeia prescribes limits for harmful compound present in substances.

4.1 Impurities commonly found in Medicinal preparations:

1. Activity depressing impurities.

2. Due to colouring or flavouring substances, *e.g.,* Sodium Salicylate.

3. Humidity.

4. Decrease shelf life.

5. Physical and chemical properties.

6. Impurities due to which substances become incompatible.

It is clear that impurities can originate from several sources; such as;

1. Crystallization-related impurities.

2. Stereochemistry-related impurities.

3. Residual solvents.

4. Synthetic intermediates and by-products.

5. Formulation-related impurities.

6. Impurities arising during storage.

7. Method related impurity.

8. Mutual interaction amongst ingredients.

9. Functional group-related typical degradation.

1. Crystallization-related impurities:

Based on the realization that the nature of 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 to take a strong interest in polymorphism and solvatomorphism as per the regulations laid down by the regulatory authorities. Polymorphism is the term used to indicate crystal system where substances can exist in different crystal packing arrangements, all of which have the same elemental composition. Whereas, when the substance exists in different crystal packing

arrangements, with a different elemental composition; the phenomenon is known as Solvatomorphism.

Stereochemistry-related impurities:

It is of paramount importance to look for stereochemistry related compounds; that is, those compounds that have similar chemical structure but different spatial orientation, these compounds can be considered as impurities in the API's. Chiral molecules are frequently called enantiomers. The single enantiomeric form of chiral drug is now considered as an improved chemical entity that may offer a better pharmacological profile and an increased therapeutic index with a more favourable adverse reaction profile. However, the pharmacokinetic profile of levofloxacin (S- isomeric form) and ofloxacin (R-isomeric form) are comparable, suggesting the lack of advantages of single isomer in this regard. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin), lavalbuterol (R- albuterol), and esomeprazole (S-omeprazole).

2. Residual solvents:

Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. Some solvents that are known to cause toxicity should be avoided in the production of bulk drugs. Depending on the possible risk to human health, residual solvents are divided into three classes. Especially, solvents in Class I, *viz*benzene (2 ppm limit), carbon tetrachloride (4 ppm limit), methylene chloride (600 ppm), methanol (3000 ppm, pyridine (200 ppm), toluene (890 ppm) should be avoided. In Class II, *viz*NNdimethylformamide (880 ppm), acetonitrile (410 ppm). Class III solvents, *viz*acetic acid, ethanol, acetone have permitted daily exposure of 50 mg or less per day, as per the ICH guidelines. A selective gas chromatography (GC) method has been developed to determine thepurity of acetone, dichloromethane, methanol and toluene. Using this method, the main contaminants of each organic solvent can be quantified. Moreover, the developed method allows the simultaneous determination of ethanol, isopropanol, chloroform, benzene, acetone, dichloromethane, methanol and toluene with propionitrile as the internal standard.

3. Synthetic intermediates and by-products:

Impurities in pharmaceutical compounds or a new chemical entity (NCE) can originate during the synthetic process from raw materials, intermediates and/or by-products. For example, impurity profiling of ecstasy tablets by GC-MS, and MDMA samples, produced impurities in intermediates via reductive amination route.

4. Formulation-related impurities:

Many impurities in a drug product can originate from excipients used to formulate a drug substance. In addition, a drug substance is subjected to a variety of conditions in the process of formulation that can cause its degradation or have other undesirable reactions. If the source is from an excipient, variability from lot to lot may make a marginal product, unacceptable for reliability. Solutions and suspensions are inherently prone to degradation due to hydrolysis or solvoly. Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was recalled in the United States because of degradation/impurities leading to sub- potency. In general, liquid dosage forms are susceptible to both degradation and microbiological contamination. In this regard, water content, pH of the solution/suspension, compatibility of anions and cations, mutual interactions of ingredients, and the primary container are critical factors.

Microbiological growth resulting from the growth of bacteria, fungi, and yeast in a humid and warm environment may results in unsuitability of an oral liquid product for safe human consumption. Microbial contamination may occur during the shelf life and subsequent consumeruse of a multiple-dose product, either due to inappropriate use of certain preservatives in the preparations, or because of the semi- permeable nature of primary containers.

5. Impurities arising during storage:

A number of impurities can originate during storage or shipment of drug products. It is Essential to carry out stability studies to predict, evaluate, and ensure drug product safety.

7. Method related impurity:

A known impurity, 1-(2, 6-dichlorophenyl) indolin-2-one is formed in the production of a parenteral dosage form of diclofenac sodium, if it is terminally sterilized by autoclave. The Conditions of the autoclave method (i.e., 123 + 2°C) enforce the intramolecular cyclic reaction of diclofenac sodium forming an indolinone derivative and sodium hydroxide. The formation of this impurity has been found to depend on initial pH of the formulation.

8. Mutual interaction amongst ingredients:

Most vitamins are very labile and on aging they create a problem of instability in different dosage forms, especially in liquid dosage forms. Degradation of vitamins does not give toxic impurities; however, potency of active ingredients drops below Pharmacopoeial specifications. Because of mutual interaction, the presence of nicotinamide in a formulation containing four vitamins (nicotinamide, pyridoxine, riboflavin, and thiamine) can cause the degradation of thiamine to a sub- standard level within a one year shelf life of vitamin B-complex injections. The marketed samples of vitamin B-complex injections were found to have a pH range of 2.8 - 4.0. A custom-made formulation with simple distilled- water and a typical formulated vehicle including disodium edetate and benzyl alcohol were investigated and similar mutual interactions causing degradation were observed.

9. Functional group-related typical degradation:

Ester hydrolysis can be explained with a few drugs *viz*aspirin, benzocaine, cefotaxime, ethyl paraben. andcefpodoximeproxetil. Hydrolysis is the common phenomenon for ester type of drugs, especially in liquid dosage forms *viz*benzylpenicillin, oxazepam and lincomycin. Oxidative degradation of drugs like hydrocortisone, methotrexate, hydroxyl group directly bonded to an aromatic ring (*viz*phenol derivatives such as catecholamines and morphine), conjugated dienes (*viz*vitamin A and unsaturated free fatty acids), heterocyclic aromatic rings, nitroso and nitrite derivatives, and aldehydes (especially flavorings) are all susceptible to oxidative degradation. In mazipredone, the hydrolytic and oxidative degradation pathway in 0.1 mol L-1 hydrochloric acid and sodium hydroxide at 800C were studied. Photolytic cleavage includes example of pharmaceutical products that are exposed to light while

being manufactured as solid or solution, packaged, or when being stored in pharmacy shops or hospitals for use by consumers. riboflavin and phenothiazines are very liable to photo-oxidation. In susceptible compounds, photochemical energy creates free radical intermediates, which can perpetuate chain reactions. Most compounds will degrade as solutions when exposed to high-energy UV exposures. Decarboxylation of some dissolved carboxylic acids, such as p- amino salicylic acid; shows the loss of carbon dioxide from the carboxyl group when heated. An example of decarboxylation is the photoreaction of rufloxacin. As seen earlier, impurities in drug products can come from the drug or from excipients or can be brought into the system through an in process step by contact with the packagingmaterial.

For most drugs, the reactive species consist of;

• Water- that can hydrolyze some drugs or affect the dosage form performance

• Small electrophiles- like aldehydes and carboxylic acid derivatives

• Peroxides- that can oxidize some drugs

• Metals- which can catalyze oxidation of drugs and the degradation pathway

4. CLASSIFICATION OF IMPURITIES

As per ICH guidelines impurities can classified as i. Organic impurity (process and drug related) ii. Inorganic impurity (reagent, ligand, catalyst) iii.Residual impurity (volatile solvents).

i. Organic impurity

Organic impurities may arise during the manufacturing process and or storage of the drug substance may be structurally identified or unidentified, volatileor non-volatile in nature.

1.Starting material:

In the multi-component, multi-step process untreated starting material remains asimpurity. E.g. P-amino phenol in paracetmol production.

2.Intermediate material:

Partially treated reactantant in the processing is an impurity to finished product.

3.By product:

These are product formed along with the desiredproduct. E.g. diacetylatedparacetamol.

4.Degradation product:

Due to unfavourable condition product undergo degradation in trace amount formation of an impurity.E.g. degradation products of ketoconazole are 1-keto & 1- hydroxyl analogue of ketoconazole.

ii. Inorganic impurity:

Inorganic impurities derive from the manufacturing process and excipients. Generally, excipients contain high levels ofheavy metals such as arsenic, bismuth, cadmium, chromium, copper, iron, sodium etc.Sometimes they might present in the productduring processing or they leached from packingmaterial.

1.Reagents, ligands, and catalysts

2.Heavy metals

3.Other materials (e.g. filter aids, charcoal)

Residual impurities

Residual solvents are organic or inorganic liquids used during the manufacturing process. It is very difficult to remove these solvents completely bythe workup process. Some solvents that are know manufacturing of bulk drugs.

Depending upon the possible risk to human health, residual solvents are divided inthree classes such as class 1, class 2, class 3, class 4.

1. Class 1 solvents:

Solvents to be strongly suspected human carcinogens and environmental hazards.

2. Class 2 solvents:

Solvents to be limited in pharmaceutical productsNon-genotoxic animal carcinogens

or possible causative agents otherirreversible toxicity such asneurotoxicity or teratogenicity. Solvents suspected of other significant butreversible toxicities.

3. **Class 3 solvents**:

Solvents with low toxic potentialpotential to man; no health less toxic in acute or short term studies and negative in genotoxic studies. The amount of these residualsolvents of 50mg or less would be acceptable.eg: Acetic acid & Acetone.

4. **Class 4 solvents:**

The solvents of this class may be of interesting to manufacturers of excipients drug substances or drug products. But there was no adequate toxicological data on which to base a permitted daily exposure was found.eg: Isooctane

5. IMPURITY PROFILING METHODS

Impurities can be analyzed by the following instruments:

1. Ultra Violet Spectroscopy
2. IR Spectroscopy
3. NMR Spectroscopy
4. Mass Spectrometry
5. Gas Chromatography
6. HPLC

1. NMR:

NMR spectroscopy was also employed for the structural elucidation of previously unknown impurities coming from new isolationprocesses. The traditional procedures for isolating vincristine andvinblastine from Catharanthusroseus are relatively inefficient.Recent efforts have resulted in a more effective manufacturing process at the expense of the appearance of new impurities. These andother known impurities were unambiguously identified by NMRspectroscopy, combined with MS technique after preparative chromatographic isolation of impurities. An 800 MHz NMRspectrometer

equipped with a1H{13C/15N} Triple Resonance13CEnhanced Salt Tolerant Cold Probe was employed, where 2D experiments could be run overnight with 10 mg samples.1H–1H, direct1H–13C, long-range1H–13C scalar and dipolar spin–spin connectivities were established from a combination of 1D (1H,13C) and 2D(gHSQCAD, zTOCSY, gHMBCAD, NOESY and ROESY) NMR
experiments.

Applying LC-SPE-cryoNMR method, we identified 1H-NMR resonances characteristic of anomeric protons (see **Figure**) The use of SPE also allowed us to get a better insight into the chemical exchange process that was taking place, as theSPE step helped to slow down the exchange [impurity 1 ↔ impurity. Indeed, usingdirect transfer from SPE to NMR, we were able to observe the conversion before thefinal equilibrium could be reached.

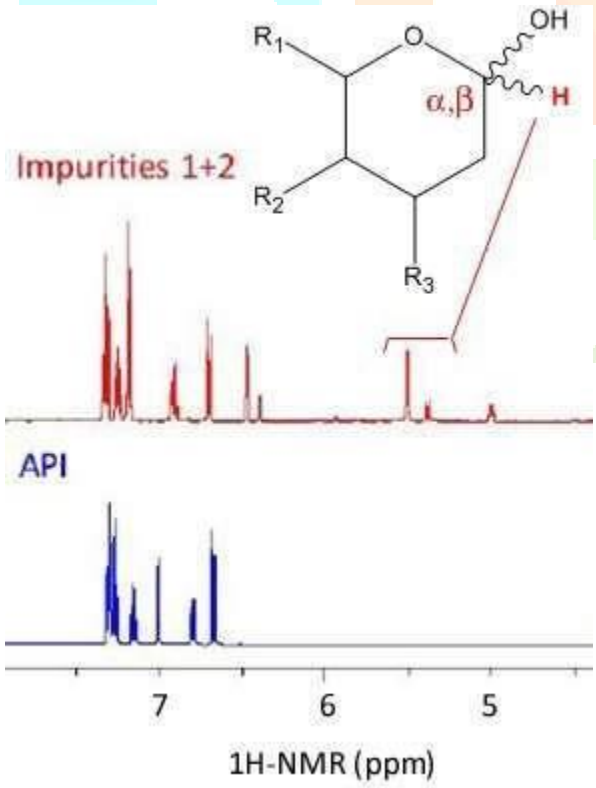

Figure: 1H-NMR spectra of the API and of a mixture of the 2 API-related impurities. 2 new NMR resonances are observed in the region of hemi-acetalic function, relating to the 2 hemiacetal forms.

Hyphenated NMR techniquesLC–NMR is one of the most powerful techniques for

the characterization of complex mixtures, since it combines a very efficientseparation technique and the most important structure elucidationmethod. However, peak isolation can be also performed by liquidor gas chromatography (GC), as well as by other methods such ascapillary electrophoresis (CE). These methods are very different,particularly with respect to sensitivity.Only recently has LC–NMR coupling become efficient enough hand hyphenation allowed the long- expected gain of maximal on line information about individual constituents of a mixture,with due speed and efficiency. Details of the instrumentation andpotential are given in many specific booksits generalapplications in pharmaceutical analysis have also been detailed.Analogous hyphenations such as GC–NMR,CZE–NMR, CEC–NMR and CE–NMR havealso been explored. However, in the on-line mode none of themhave been used to solve problems in the area of pharmaceuticalimpurities and currently this application seems still unfeasible,mainly due to sensitivity problems. To date, CE–NMR is not a well established method; however, development of smaller volume NMR probes and continuous enhancements of NMR sensitivitywill probably transform CE–NMR in the near future into a valuable technique for biopharmaceutical analysis.

2. Mass spectroscopy:

It has 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 quantification of drugrelated substances in active pharmaceutical ingredients and pharmaceutical formulations. If single method fails to provide the necessary selectivity, orthogonal coupling of chromatographic techniques such as HPLC-TLC and HPLCCE, or coupling of chromatographic separations with information rich spectroscopic methods such as NHPLC-MS or HPLC-NMR may need to be contemplated, but hopefully only as a development tool rather than a tool for routine QC use.

Hyphenated Methods:

• LC-MS-MS

• HPLC-DAD-MS

• HPLC-DAD-NMR-MS

• GC-MS

• LC-MS

A common goal for investigation of both process and product degradation-related impurities is to determine which of the many potential impurities are, in fact, produced in the manufacturing process and which occur under a given set of storage conditions.

3. Gas Chromatography:

It is very useful for isolation and characterization of volatile components or those components that can be made volatile by derivatization technique and the detectorused should be non destructive. Now GC is more apt to be used in combination with mass spectrometry (GC/MS) for characterization of impurities.

Hyphenated techniques:

1. LC-MS-MS
2. HPLC-DAD-MS
3. HPLC-DAD-NMR-MS
4. GC-MS
5. LC-MS

Hyphenated techniques are first line of defense in impurity determination.Hyphenated techniques are those techniques, where two or more analytical techniques are combined. The various hyphenated techniques used for impurity characterization are LC-MS, LCNMR, LC-MSNMR, LC-MS-MS, GC-IR and GC-MS.

The two most commonly used hyphenated techniques for impurity profiling are LC- MS and LCMS-NMR. In these techniques chromatographic techniques are coupled with a spectroscopic detector. Thus impurity structure determination can be performed in real

time during chromatographic separation and both isolation and characterization is performed in one single step. The use of hyphenated techniques for impurity determination is on rise due to easy availability of bench-top instrumentation and their distinct advantages like versatility, sensitivity, possibility of profiling sub structural analysis and rapid selective quantitative determination of targeted compound even in mixtures. The only limitation of hyphenated techniques is the heavy cost of instrumentation due to which their use is not common and spread worldwide like GC, HPLC, MS or NMR systems. As on today these sophisticated techniques are mainly used for the purpose of monitoring, characterization and identification of impurities but they can be used for other analytical purposes.

4.HPLC:

HPLC separation →If the method is non MS compatible

Develop equivalent MS compatible LC method

↓

If the method is MS compatible

LC/MS analysis using
Agilent 6540 Q -TOF with
full MS scan followed
by auto MS/MS

Figure: Software assisted workflow for impurity identification and profiling of pharmaceuticals.

6. SOURCES OF CONTROL OF IMPURITIES :

1. Washing
2. Drying
3. Re-crystallization
4. Sublimation.

1. **Washing:** When water soluble substance has to be washed away and water insoluble substances is needed.Chalk from native CaCO3, water soluble substances are washed with water and dried and is required to have NLT 97% CaCO3 on dry basis while precipitate CaCO3 as water insoluble subs. is required to have NLT 98.5% of CaCO3 after drying in a manner which is similar to that used for prepared chalk.
2. **Drying:**Generally dried in air Anhydrous chemicals—Vacuum drying has been an impurifiedunit operation and needs care and precaution so that chemicals may not deteriorate due to oxidation, caking or mould growth.
3. **Re-crystallization of solid substances from water:**Re-crystallization is most common method of purifying soluble salts. With a few exceptions, the solubility of salt in a solvent get ↑ with ↑ in temperature and hence a saturated. Solution is allowed to cool slowly after which crystals of a greater purity could be obtained.
4. **Sublimation:**Applicable to very few substances *e.g.*, AS2O3, I, HgCl2, sublimed sulphur. The organic compounds purified by this process are camphor and benzoic acid.

6. CONTROL OF IMPURITIES

8.1 Pharmacopoeial Methods: Official monographs for pharmaceutical substances provide description and information in addition to prescribing standards for the product and its storage conditions. An official monograph for a pharmaceutical substance generally includes the following:

1. **Title**: It is the official name of the substance. Sometimes the common names or synonyms are also mentioned.
2. **Chemical formulae:** When the chemical structure of the compound is known, the graphic and molecular formulae and the molecular weight are given following the title. A chemical formula refers to the chemically pure substance and is not an indication of purity of the substance.
3. **Chemical names:**

   Sometimes the IUPAC name of the substance is also given.

4. **Category:**It is indicative of the medical or pharmaceutical application of the substance. It is generally the more common application, representing the main pharmacological action of the substance or its active ingredient and the substance may posses other uses or activities also.
5. **Dose:**The doses mentioned in the pharmacopoeia are for the general guidance andrepresents the average range of quantities regarded suitable for adults when administeredorally.
6. **Description:** It gives information regarding the general physical and organolepticproperties of the substance. It helps in the preliminary evaluation of the integrity of thearticle and should not be considered as the analytical requirements.
7. **Solubility:**The solubility of the substance given in the monograph is primarily forinformation and should not be regarded as standards or test for purity but if a quantitativesolubility test is given under ‘STANDARDS’ th the substance should comply with the givenrequirement. If the exact solubility of the substance is not known, the approximate solubilityof the substance is indicated by the descriptive terms.

Following table gives the meaning ofsuch descriptive terms for substances at 20 to 30°C.

8. **Storage:**It contains information regarding the storage conditions of

pharmaceutical substances so that they can be guarded against possible contamination and deterioration. The precautions need to be taken regarding the effect of atmosphere, moisture, heat and light are also indicated where appropriate in the individual monograph. The temperature conditions related to the storage of pharmaceutical substances are specified in some monographs. The following terms are used in the IP for defining the conditions of temperature.

(*a*) *Cold*: Any temperature not exceeding 8°C and usually between 2°C and 8°C. A refrigerator is a cold place in which the temperature is maintained thermostatically between 2°C and 8°C. **(b) Cool:** Any temperature between 8°C and 25°C. An article directed to be stored in cool place, may, alternatively be stored in a refrigerator, unless otherwise specified in the monograph.

(c) ***Room temperature*:**The temperature prevailing in the working area.

(d) Warm: Any temperature between 30°C and 40°C.

(e) ***Excessive heat*:** Any temperature above 40°C.

(f) ***Protection from freezing*:**The label of container bears this instruction where, in addition to the risk of breaking of the container, freezing results in a loss of strength or potency or in destructive alteration of the characteristics of an article.

(g) ***Storage under non-specific conditions*:** When no specific storage conditions are indicated in the monograph, the storage conditions include protection from moisture, freezing and excessive heat.

9. Standards as determined by the assay: It specifies the quantitative purity of the official compound. If an article does not complies with all the stated requirements it is not of pharmacopoeial quality. These requirements are applicable only to those articles that are intended for medicinal use and not to articles that may be marketed under the same name for other purposes.

10. Identification test: It includes various chemical tests to verify the identity of the substance. They are not absolute proof of identity.

11. Test for purity including limits tests: Different limits for impurities are prescribed for different substances. Test for purity are tests for the presence of

impurities in the substance and fix the limits of tolerance for undesirable impurities.

12. **Assay:** It describes the official method for the quantitative determination of the active ingredient of the pharmaceutical substance and its preparation.

8.2 Identification test:

The purpose of identification test is to ensure the correct labeling of the substances. Identification tests are specific but they are not necessary sufficient in establishing the absolute proof of identity of the substances. If an articles taken from a labeled container do not meet to the requirements of a prescribed identification test indicates that the articles is either mislabeled or substituted. In same monographs, more than one identification tests are given. In such cases, if the articles complies with the either one or the other identification test, in sufficient to verify the identity of the article.

Identification tests are generally based upon the combination of simple chemical test and measurement of the appropriate physical constants. There is considerable overlap between identification tests and the limit tests. Limit tests are designed to ensure that the undesirable impurities are within the prescribed limits. Identification tests whether physical or chemical, provided they are sufficiently specific, can be used as the basis of a quantitative estimation or in the design of specific limit tests. Practically, a single identification test may contribute to identification as well as standardization of the substance.

Chemical tests, used for identification are basically qualitative confirming to the presence of the substance under investigation. They may be far too general or lack specificity but can be considered sufficiently specific when used in conjugation with the other requirement of the monograph.Physical constants such as melting point, boiling point, solubility, weight per ml, refractive index, optical rotation, viscosity etc., have characteristic values for a given substance.

They can be used in identification, checking quality and maintaining standard of purity.

8.3 Test for purity:

'Test for purity' for substances have been prescribed by the pharmacopoeias of the

various countries in order to ensure reasonable freedom from the undesirable impurities. The socalled 'Test for purity' are infact the tests for the presence of impurities in the substance and fix the limits of tolerance for these undesirable impurities. Test for purity are not framed to guard against all the possible impurity rather they provide appropriate limitation of the potential impurities only.

The guiding factor for fixing a limit of tolerance for the various impurities is the amount of impurity that is likely to be harm. Arsenic and lead are quite dangerous even in trace amounts therefore very small limits of tolerance have been fixed for their presence in all pharmaceutical substances. Another factor is the practicability of the commercial method of production of the substance meeting the requirements of a particular standard of purity. It would be useless to fix the limits of tolerance which can only be attained at a very high cost. There are cases in which the limits fixed in the pharmacopoeia were later relaxed because they were found to be too difficult to attain by the available methods of manufacturing. The ultimate objective is that the pharmaceutical substances if not completely free from the undesirable or toxic impurities should be of reasonable good purity ensuring therapeutic safety. The presence of sodium bromide (NaBr) in the more expensive potassium bromide (KBr) is not likely to cause any harm to the patient but at the same time the KBr should be of sufficiently good pharmaceutical quality and purity not containing excess amountof sodium bromide.

Some of the tests which may be undertaken to ascertain the purity of a substance are:

(a) **Clarity of solution:**

The degree of clarity or opalescence of solution is measured by direct comparison with a reference solution having standard opalescence. The comparison is against a black background by viewing vertically downward under diffused light. A solution is considered clear if its clarity is the same as that of water or of the solvent employed in the preparation of the solution being examined.

(b) **Colour of solution:**

In Indian Pharmacopoeia, the colour standards are based on three primary

colorimetric solutions: yellow, red and blue prepared from ferric chloride, cobaltous chloride and cupric sulphate respectively. These primary solutions are mixed in various proportions with or without 1% w/v hydrochloric acid to give five reference colour solutions which are yellow (YS), greenish yellow (GYS), brownish yellow (BYS), brown (BS), and red (RS). The colour of the solution is compared with reference colour solution by viewing vertically downwards through the columns of liquids in diffused light. A solution may be considered as colourless if it has the same appearance as water or as the solvent employed in the preparation of the solution being examined.

(c) **Acidity or alkalinity:**

Pharmaceutical substances prepared using chemical reactions involving acids and alkalies may possess some degree of acidity or alkalinity resulting from improper purification by inadequate washings after their separation. The limits for acid or alkali impurities are fixed for various pharmaceutical substances and the test for acidity and alkalinity is of great help in determiningthe extent of such impurities.

(d) **Loss on ignition:**

It is the loss of weight in % w/w resulting from a volatile partof any test material that is driven off under specified conditions. It is applied to thermostablesubstances which contain thermolabile impurities that decompose and lose a volatile product*e.g.*, zinc carbonate decomposes losing carbon dioxide. The substance is heated, cooled andweighed repetitively until a constant weight is attained. The loss on ignition in this caseshould not be more than 2% w/w.

(e) **Loss on drying:**

It is the loss of weight in % w/w resulting from water andvolatile matter that is lost under specified conditions. The temperature to which the substanceis subjected varies considerably according to the nature of the substance. The temperatureapplied should not be so high as to cause decomposition of the substance but at the sametime it should be sufficiently high to produce the desired

results within a reasonable time.It is usually applied by drying the substance to constant weight at 105°C.

(f) **Moisture content:**

Sometimes the determination of the moisture content of thesubstance is a good measure of the purity of the substance especially in case of crude drugs.

(g) **Ash values:**

The determination of ash values in crude drugs, organic compoundsand certain inorganic compounds provides valuable information regarding the extent ofheavy metals and minerals impurities.

7.0 APPLICATIONS OF IMPURITY PROFILING

1. Drug designing and in monitoring quality, stability, and safety of pharmaceutical compounds, whether produced synthetically, extracted from natural products or produced by recombinant methods.
2. The detection of impurity in drug product like alkaloids, amines, amino acids, analgesics, antibacterials, anticonvulsants, antidepressant, tranquilizers, antineoplastic agents, local anesthetics, macromolecules, steroids etc.

8.4 Assay:

An assay method should be specific for the substance or chemical species being examined. Nevertheless non-specific assay methods are quite commonly employed particularly in acid-base titrations. Many inorganic salts are assayed by simply determining the content of one of the ions present *e.g.*, sodium sulphate to assayed by determining its sulphate content by precipitating the sulphate as barium sulphate. Although non-specific an assay method can be considered as sufficiently specific when used in conjugation with other requirement of the monograph.

8. CONCLUSION

Impurity profile of pharmaceuticals is receiving an increasing importance and drug

safety receives more and more attention from the public and from the media. Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredients (API's). This review 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. The most efficient analytical method for Impurity profiling is HPLC,GC, & then spectroscopy. Nowadays, it is mandatory requirement in various pharmacopoeias to know the impurities present in APS's & drug product.

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