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A REVIEW ON IMPURITY PROFILING IN DRUG DEVELOPMENT

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ABSTRACT

Impurity is something that is impure. A substance of interest mixed or impregnated with an extraneous or usually inferior substance, from the standpoint of its usage, the drug substance is compromised in terms of purity even if it contains another material with superior pharmacological or toxicological properties. Impurity profiling has gained importance in modern pharmaceutical analysis due to the fact that unidentified, potentially toxic impurities are hazardous to health and in order to increase the safety of drug therapy, impurities should be identified and determined by selective methods. Terms such as residual solvents, by product, transformation products, degradation products, interaction products and related products are frequently used to define impurities. Identification of impurities is done by variety of Chromatographic and Spectroscopic techniques, either alone or in combination with other techniques. There are different methods for detecting and characterizing impurities with TLC, HPLC, HPTLC, etc. Impurity profiling study has been in the limelight in the recent pharmaceutical scenario and its importance is increasing day-by-day. The present review covers various aspects related to the analytical method development for impurity profiling of Active Pharmaceutical ingredient and pharmaceutical products.

Key words: Gas chromatography, HPLC, ICH guidlines

1. INTRODUCTION

According to the definition of ICH {International Conference On Harmonization} impurity profile of a drug material is "A description of the identified and unidentified impurities, present in a new drug substance "OR "An impurity is considered as any other inorganic or organic material, or residual solvent other than the drug substances, or ingredients, that arise out of synthesis or unwanted chemicals that remain with APIs" **[1]**. The aim of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations. Impurity profiling is of utmost importance in all phases of synthetic drug research and production from the gram scale preparation of new compounds for pharmacological screening up to the scaling up procedure and finally the production of bulk drugs. Impurities in formulated product and APIs are regulated by various regulatory authorities such as ICH, USFDA, Canadian Drug and Health Agency that are emphasizing the purity requirement and the identification of impurities in APIs **[2]**.

2. Regulatory guidelines on impurities in an API and/or in formulation[2]

Monitoring and controlling of impurities implies different things. Therefore simple terminology should be used to address questions related to impurities. The United States food and drug administration (US-FDA) has endorsed the guidelines prepared by International Conference on Harmonization (ICH). The ICH guidelines for impurities were developed with joint efforts of various regulators such as European Union (EU), Japan and United States and they help in ensuring consistent requirement of data that should be submitted to various regulatory agencies. ICH guidelines "Stability Testing of New Drug Substances and Products"-Q1A.

- ICH Guidelines "Impurities in New Drug Substances"-Q3A.
- ICH Guidelines "Impurities in New Drug Products"-Q3B.
- ICH Guidelines "Impurities: Guidelines for Residual Solvents"-Q3C.
- US-FDA Guidelines "NDAs-Impurities in New Drug Substances".
- US-FDA Guidelines "ANDAs-Impurities in New Drug Substances".
- Australian Regulatory Guideline for Prescription of Medicines, Therapeutic Governance Authority (TGA), Australia.

3. Classification of impurities

Impurities can be classified as organic impurities (process and drug related), inorganic impurities and residual solvent. Organic impurities may arise during the manufacturing process or storage of the new drug substance which includes starting material, by product, intermediates, degradation product, regents, ligands and catalysts. Inorganic impurities include reagents ligands and catalysts, heavy metals or other residual metals, inorganic salts, filter aids, charcoal etc **[3]**.

Classification of impurities can be shortly explained in **Figure 1**.

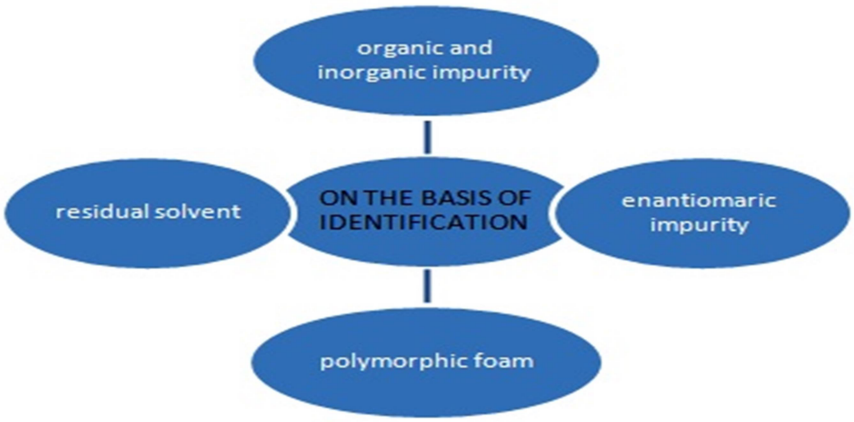

Figure 1: Classification of Impurity

1. ORGANIC IMPURITIES.

a. Starting material or Intermediate impurity
b. By-products.
c. Degradation product impurities
d. Synthesis related impurities
e. Formulation related impurities
   - Environment related.
   - Formation of impurities on ageing
   - Functional group related impurities

2. INORGANIC IMPURITIES.

1. A. Reagents, Ligands and Catalysts
2. Heavy metals
3. Other materials.

3. RESIDUAL SOLVENTS

4. FORMULATION RELATED IMPURITIES {IMPURITIES IN DRUG PRODUCTS}

1. Method related
2. Environment related
3. Dosage form related

1. ORGANIC IMPURITIES:-

These types of impurities arise during the manufacturing process and/or during storage of the drug substance. These include following sub-impurities**.** In Starting Materials or Intermediate Impurities These types of impurities

occur in almost every API unless a proper care is taken in every step during the multistep synthesis of drug product. Although the end products are always washed with solvents but there are chances of having the residual of unreacted starting materials unless the manufacturers are very careful about the impurities. In By-products Insynthetic organic chemistry, getting a single end product with complete yield is very rare; there is always a chance of having by products along with desired end products. And Degradation Products is Impurities can also be formed by degradation of the end product during manufacturing of bulk drugs. This mainly occur due to improper storage of formulation **(Figure 2) [3, 4]**.

Factors Affecting On Formulation Related Impurities

a. Environment related

a. Exposed to adverse temperature: Substance which are labile to heat or in tropical temperature lead to degradation of active constitute and formation of impurity occurs. E.g. Vitamins are heat sensitive and its degradation lead to loss in potency.

b. Exposed to light: Photosensitive material when exposed to light / UV light undergo degradation which forms impurity.

c. Humidity: It can be detrimental to bulk powder and formulation containing solid dosage form

B. Formation of impurities on ageing: Mutual interaction: Interaction between ingredients involved in formulation leads to mutual interaction which causes impurity formation.

C. Functional Group Related Impurities

Ester hydrolysis, Hydrolysis, Oxidative degradation, Photolytic cleavage, Decarboxylation.

2. INORGANIC IMPURITIES

Inorganic impurities are also obtained from the manufacturing processes which are used in bulk drug formulation. They are normally known and identified in Reagent, Ligands and Catalysts Rare chances of occurrence of these impurities. If during manufacturing procedure is not followed properly will create a problem. Heavy Metals Water is generally used in different manufacturing processes which act as the main source of heavy metals, like Ar, Cd, Cr, Na, Mg, Mn, etc., where acidification or acid hydrolysis takes place. By using demineralized water and glass-lined reactors heavy metal impurities can be easily avoided. Other Materials (Filter Aids, Charcoal) **(Figure 3) [5, 6]**.

Figure 2: Classification of Organic Impurity [2]

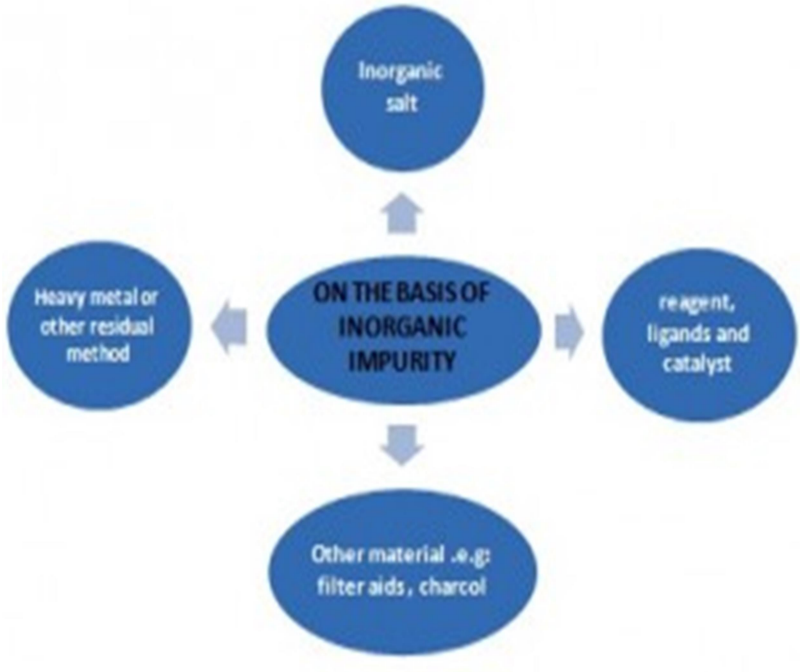

Figure 3: Classification of Inorganic Impurity [2]

3. RESIDUAL SOLVENTS

These are organic or inorganic liquids which are generally used in various manufacturing processes. They may modify properties of certain compounds or can be hazardous to human health. Some liquids exhibit toxic behavior so they need to be eliminated however it is a very tedious task to accomplish, as trace amounts are usually difficult to detect and remove. For the detection of residual solvent, gas chromatography is used because they are mostly volatile in nature **[7]**. Non-volatile solvents are converting to volatile solvents by chemical derivatization. Gas chromatographic techniques are used to determine purity of toluene, acetone, methanol, dichloromethane Residual solvents with their classification and permissible limits are listed below **[8]**. Class: These types of solvents should be limitedly used in pharmaceutical products because of inherent toxicity **(Table 1)**.

4. METHOD TO CONTROL IMPURITY:

Identification of impurities is done by variety of chromatographic and spectroscopic techniques either alone or in combination with other techniques **(Figure 4, 5) [9]**.

1. Electric paramagnetic resonance
2. Single crystal X ray diffractions
3. Complete molecular confidence {cmc}
4. Structural elucidation

Table 1: Class solvents with their permissible daily exposure limits

S. No.	Residual solvent	Permissible daily exposure (mg/day)	Concentration limit (ppm)
1.	Acetonitrile	4.1	410
2.	Chloroform	0.6	60
3.	1,2 Dioxane	3.8	380
4.	Pyridine	2	200

Figure 4: EPR Spectroscopy

Figure 5: Single Crystal X-Ray diffraction

- **Separation methods**

After isolation, the next step is to separate impurities from mixture of compounds into individual components, by various techniques **[10-16]**.

Thin layer chromatography: It is a valuable technique for separation of compounds and works upon the principle of adsorption. Silica gel plates are generally preferred for carrying out separation. Detection is usually performed by UV. To elute the desired material, the adsorbent from plates is scrapped off and then extracted with suitable solvents. TLC is used in determination of components present in plants **[17]**.

NMR: It can provide information regarding molecular structure and stereochemistry of compound. Multicomponent mixtures can be easily analyzed. For example impurities in Benzyl (4-morpholinophenyl) carbamate, Dehydro-apaxiben, Mirabegronare can are analyzed by NMR. A unique aspect of NMR spectra is the direct proportionality between peak areas and the number of nuclei responsible for the peak. The most important chemical application of proton NMR spectroscopy have been to the identification and structure elucidation of organic, metal-organic and biochemical molecules **[18]**.

MS: It is the most accurate technique for determining the molecular mass and elemental composition of the desired compound. It is also used for monitoring, characterizing and quantification of drug related substances in API. If single method fails to provide necessary selectivity, coupling of this technique with GC, HPLC, and LC lead to rich information. For example- Des-fluoro impurities of Sertalin and Iinezolid have been identified and quantified by MS **[19]**.

GC-MS: To identify different substances within a test sample, GC can be coupled with MS to provide valid information that is difficult to solve by one method. In this combination GC separates volatile and semi-volatile components whereas MS provides detailed structural information. Various residual solvents are analyzed by GC such as ethanol, hexane, benzene, carbontetrachloride etc. **[20]**.

LC-MS: In case of LC-MS a similar to GC-MS, though rather more difficult problem arises in the removal of liquid carrier from an HPLC eluent before samples are passed in to the MS source. The normal eluent flow rates of 0.5-2.0ml min-1 cannot be handled by the MS pumping system **[21]**.

HPLC-DAD-MS: In this type of technique, the Characterization and quantitative determination of four impurities in piperaquine phosphate by gradient reverse

phase HPLC and LC/MS/MS was developed27 and validated as per ICH guidelines. Another example, for determinations of Low content of Methyl Methanesulfonate and Ethyl Methane-sulfonate Impurities as they were potential genotoxic impurities (PGIs) in Emtricitabine, an Active Pharmaceutical Ingredient using LC/MS/MS28 method **[22]**.

HPLC-DAD-NMR-MS: The LC-DAD-MS/SPE-NMR Hyphenation technique have been used in the Identification of Isobaric Iridoid Glycoside Regioisomers as minor constituents from Harpagophytum procumbens of Pharmaceutically Used Plant Extracts. Hence by using of this technique provides the spectral data needed for structure elucidation. Tandem Mass spectrometry The tandem mass spectrometry (MS/MS) scanning modes are product ion, precursor ion, constant neutral loss etc. **[22]**.

Tandem Mass spectrometry: The tandem mass spectrometry (MS/MS) scanning modes are product ion, precursor ion, constant neutral loss etc., In addition, the special case of selected reaction monitoring (SRM) is occasionally used to enhance selectivity in quantitative mass spectrometry. MS/MS methods generally involve activation of selected ions, typically by collision with an inert gas, sufficient to induce fragmentation (collision induced dissociation, CID) **[23]**.

Capillary electrophoresis-Mass spectrometry (CE-MS): CE-MS was recently implemented in the method development approach to support impurity profiling of pharmaceutical products. Capillary electrophoresis (CE) is based on a different separation principle and consequently has different selectivity compared to HPLC. CE coupled to a Mass Spectrometer using electrospray ionization (ESI). The use of non-volatile buffers in CE-MS is generally avoided. Hence various goals of impurity investigations are Process – related impurities, degradation –related impurities, identifying the significant impurities **[24]**.

Drugs, corresponding impurities and method used for identification are given below in **Table 3**.

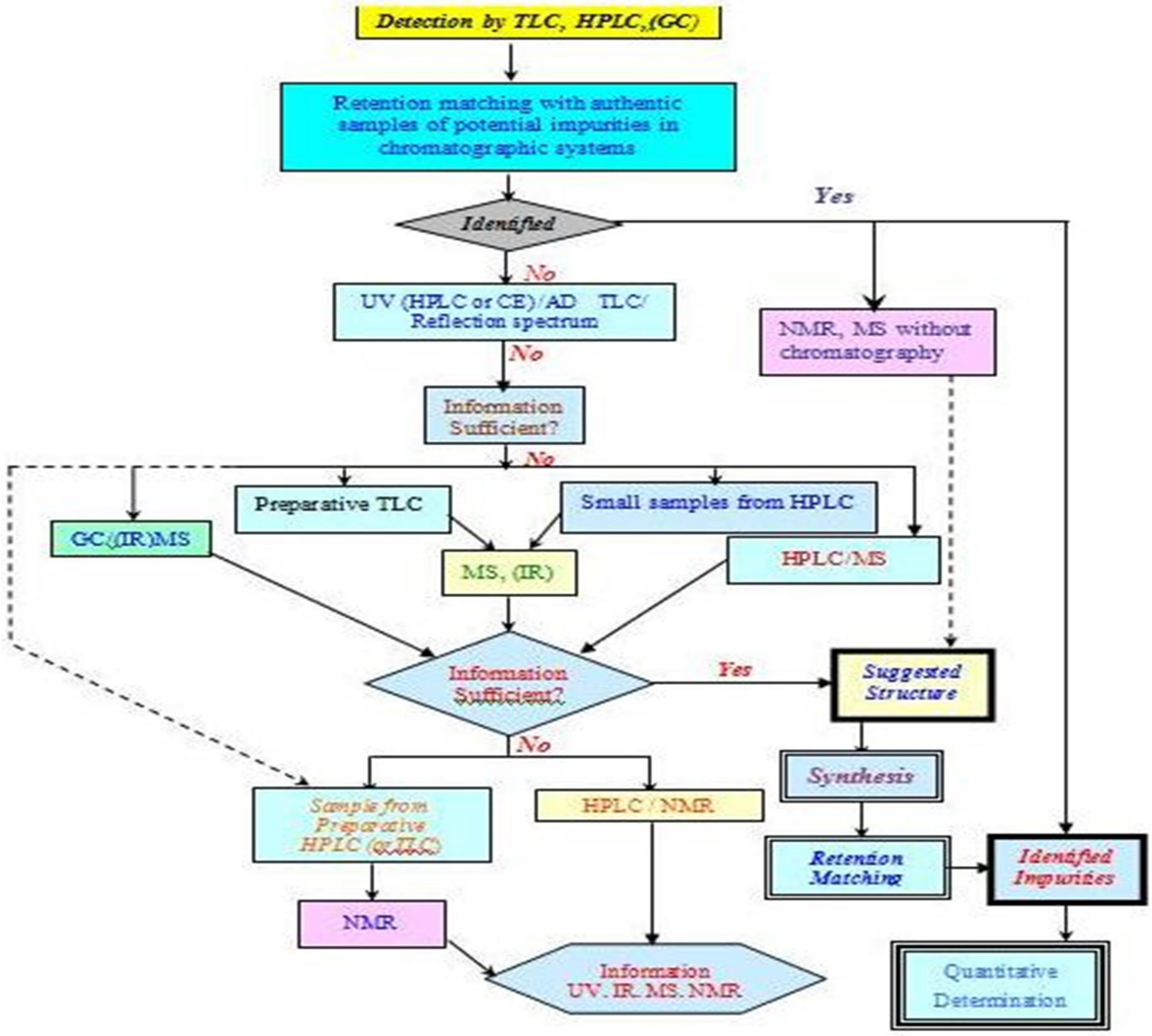

Figure 6: Schematic representation of steps involved in separation of impurities

Table 3: Pharmaceutical products and their effect of chirality

S. No.	Drug	Isomer	Effect
1.	Barbiturates	R-isomer	Convulsant
		S-isomer	Depressant
2.	Thalidomide	R-isomer	Anti-nausea, Sleep inducing
		S-isomer	Teratogenic
3.	Labetalol	R,R-isomer	β-Blocker
		S,R-isomer	α-Blocker
4.	Penicillamine	D-isomer	Anti-arthritic
		L-isomer	Toxic
5.	Warfarin	R-isomer	Toxic
		S-isomer	Anti-coagulant
6.	Ethambutol	R,R-isomer	Blindness
		S,S-isomer	Tuberculostatic
7.	Naproxen	R-isomer	Toxic
		S-isomer	Analgesic

5. APPLICATIONS

Application have been sought in the areas of drug designing and in monitoring quality, stability, safety of pharmaceutical compound, whether produced synthetically, extracted from natural products or produce by recombinant method **[13]**.

The application includes different classes of drugs namely alkaloids, amines, amino acids, analgesics, antibacterial, anticonvulsant, antidepressant, tranquillizers, antineoplastic agent, local anaesthetics, macromolecules, steroids, and miscellaneous **[14]**.

Identification, quantification, and control of impurities in API and the drug product are critical in drug development. Organic impurities are often free radicals from by-products, intermediates, or degradation products. Inorganic impurities include transition metals, reagents, and ligands **[15]**.

Electron Paramagnetic Resonance (EPR) spectroscopy is the only technique for the direct and non-invasive detection, identification and quantification of paramagnetic impurities (organic free radicals and transition metals).

6. CONCLUSION

Impurity profiling is a mandatory requirement in various pharmacopoeias to know the impurities present in the API. Impurity profile of pharmaceuticals have been receiving greater importance recently. It is an essential for regulatory filing of new drug candidates. Impurity profiling and reporting is also compulsory in various pharmacopoeias. Isolation and characterization of impurities are required for acquiring and evaluating data that is used in creating biological safety datasheet of new drug products. Many instrumental methods are regularly used to isolate and quantify impurities. Thus impurity profiling may work as a Quality Control tool.

It may offer fundamental data about safety, toxicity, limits of detection and limits of quantitation of several organic and inorganic impurities, generally accompanying APIs and finished products. This review paper consequently focuses on basic aspects of impurities in drug substances and drug products. Thus, by implementing impurity profiling, it becomes plausible to develop products where expected impurity cannot interfere in the performance of final product. Although different regulatory bodies have provided individual guidelines describing identities and permissible limits of impurities, there is an urgent need for unified specifications/ standards for regulation of impurities.

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