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RECENT APPROCHES OF "IMPURITY PROFILING" IN PHARMACEUTICAL ANALYSIS: A REVIEW

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ABSTRACT

Impurity is something that is impure or makes something else impure. An impure substance may be defined as follows: 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. The impurity may be developed either during formulation, or upon aging of both API's and formulated API's in medicines. The presence of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the pharmaceutical products. The impurities are not necessarily always inferior. Highly sophisticated instrumentation, such as mass spectra meters attached to a Gas Chromatography or HPLC, are inevitable tools in the identification of minor components (drugs, impurities, degradation products, metabolites) in various matrices. Present article reveals different impurities found in the API's, methods for identifying them and the possible measures to deal with the interferences caused by them in pharmaceutical analysis.

INTRODUCTION: The purity of a drug product is in turn determined on the basis of the percentage of the labelled amount of API found in it by a suitable analytical method. The presence of some impurities may not deleteriously impact on drug quality if they have therapeutic efficacy that is similar to or greater than the drug substance itself. Nevertheless, drug substances can be considered as compromised with respect to purity even if it contains an impurity with superior pharmacological or toxicological property.

Consequently, in order to ensure that an accurate amount of the drug substance is being administered to the patient, drug substance purity must be assessed independently from these undesirable extraneous materials (e.g., inert, toxic, or pharmacologically Superior impurities).

Impurity profiling is the common name of a group of analytical activities, 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. 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 API's or formulations.

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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).

Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research. International Conference on Harmonization (ICH) has published guidelines on impurities in new drug substances, products and residual solvents.

There is a good significant demand for the impurity-reference standards along with the API reference standards from both regulatory authorities and pharmaceutical companies. The estimation of impurity profiles in drug substances and related materials has become one of the most important fields of activity in contemporary pharmaceutical analysis. In general, all impurities present in excess of 0.1% should be identified, for the following reasons [1-3]:

(1) On the basis of the information thus obtained synthetic organic chemists are often able to avoid the formation of the impurity in question or to develop a purification method to decrease its quantity to a tolerable level.

(2) Following the structural identification of an unavoidable impurity, it may be synthesized to provide a sufficient amount for:

a. Final proof of its structure;
b. Its use as an "impurity standard";
c. Its use in toxicological studies.

ICH Q3A covers drug substances and Q3B covers drug products. These guidelines define what investigations and documentation should be made in investigating impurities and degradation products seen in stability studies at recommended storage conditions. In general, according to ICH guidelines on impurities in new drug products, 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). According to ICH, the maximum daily dose qualification threshold is considered as follows [4-7]:

≤ 2g/day 0.1 % or 1 mg per day intake (whichever is lower) ≥ 2g/day 0.05%

Sources/Types of impurity in Medicine: The pharmaceutical preparation should be free from toxic and other impurities. Pharmacopoeia prescribes limits for harmful compound present in substances.

Impurities commonly found in Medicinal preparations:

- Activity depressing impurities.
- Due to coloring or flavoring substances, *e.g.,* Sodium Salicylate.
- Humidity.
- Decrease shelf life.
- Physical and chemical properties.
- Impurities due to which substances become incompatible.

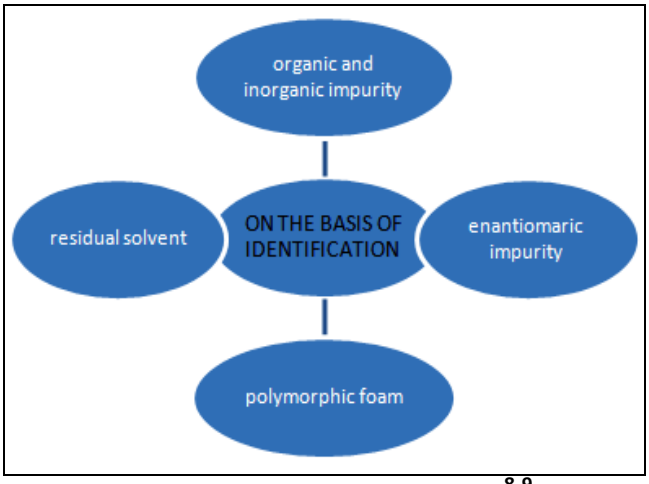


FIG: CLASSIFICATION OF IMPURITY [8-9]

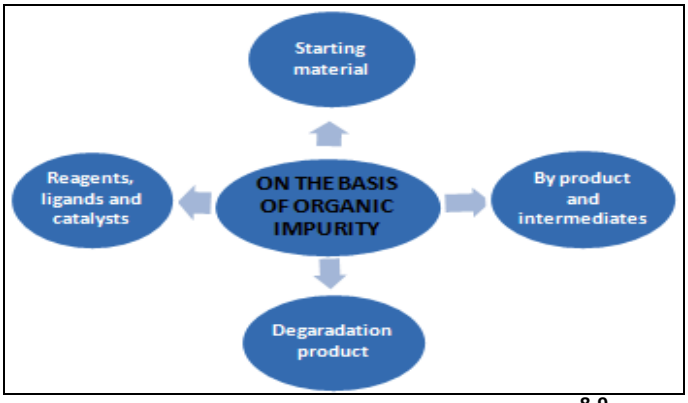


FIG: CLASSIFICATION OF ORGANIC IMPURITY [8-9]

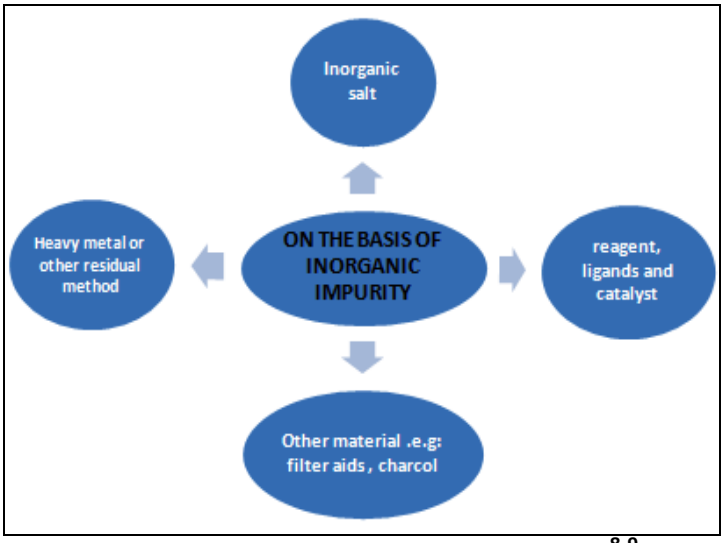


FIG: CLASSIFICATION OF INORGANIC IMPURITY [8-9]

Raw Materials employed in the Manufacturing of the Pharmaceutical Substance: Pharmaceutical substances are either isolated from natural sources or synthesized from chemical starting materials. The natural sources include mineral sources, plants, animals and microbes. It is essential to verify the identity of the source material and to establish its quality otherwise impurities associated with the raw materials may be carried through the manufacturing process to contaminate the final product. In nature minerals rarely occurs in a reasonably pure from.

Almost always mixtures of closely related substances occur together *e.g.,* aluminum ores are usually accompanied by alkali and alkaline earth compounds, barium and magnesium impurities are found in calcium minerals, zinc accompanies magnesium or iron compounds, lead and heavy metals are found as impurities in many sulphides, among the acid radicals or anions, bromides and iodides are often found as impurities in chlorides, bismuth salts contains silver copper and lead as impurities. Rock salt used for the preparation of sodium chloride is contaminated with small amounts of calcium and magnesium chlorides, so that sodium chloride prepared from rock salt will definitely contain traces of calcium and magnesium compounds impurities.

1. **Method of Manufacture:** The process or method of manufacture may introduce new impurities into the final product arising due to contamination by reagents, catalysts and solvents employed at various stages of the manufacturing process. The new impurities may also arise from the reaction vessels and reaction intermediates.

   a. **Reagents employed in the manufacturing process:** Calcium carbonate contains 'soluble alkali' as impurity which arises from the sodium carbonate (Na2CO3) employed in the process. Calcium carbonate is prepared by the interaction of a soluble calcium salt with a soluble carbonate. Therefore, the final product (CaCO3) is liable to contain small amount of 'soluble alkali' as impurities which were not removed by the washing process.

$$CaCl_2 + Na_2CO_3 \rightarrow CaCO_3 \downarrow + 2\ NaCl$$

Anions like Cl^- and $SO4^{-2}$ are common impurities in many substances because of the use of hydrochloric acid and sulphuric acid respectively in processing. Barium ion may be an impurity in hydrogen peroxide so, hydrogen peroxide employed as reagent in the manufacturing process can contaminate the final product.

   b. **Regents used to eliminate other impurities:** Barium is used in the preparation of potassium bromide to remove sulphate which in turn arises from the bromine used in the process. It is likely that potassium bromide will now be contaminated by traces of barium.

   c. **Solvents:** Most of the pharmaceutical substances are prepared in solvated crystalline form. Small amounts of solvents employed in preparation, and purification of reaction intermediates or the final product may also result in the contamination of the pharmaceutical substances. Water is the cheapest solvent available and is used quite frequently in the preparation of inorganic pharmaceuticals. Water can be the major source of impurities as different types of water containing different types and amount of impurities are available.

Various types of water which are available are;

   (i) **Tap water**: Containing impurities of Ca2+, Mg2+, Na+, Cl–, CO3–2 and SO4-2intrace amounts. The use of tap water on large scale

will lead to the contamination of the final product with these impurities because the impurities will remain in the product even after washings.

(ii) **Softened water**: It is almost free from divalent cations (Ca2+, Mg2+) but contains more of Na+ and Cl– ions as impurities because of the usual chemical water softening process. Therefore, the final products obtained using softened water as solvent will not haveCa2+ and Mg2+ impurities but still contain Na+ and Cl– impurities.

(iii) **De-mineralized water**: It is prepared by means of ion-exchange and is free from Na+, Ca2+, Mg2+, Cl–, SO4–2 and CO–2 etc. It may have Pyrogen, bacterias and organic impurities. So, it is a better solvent than tap water or softened water but the economic.

(iv) Factors discourage its use on large scale.

(v) **Distilled water**: It is free from all organic and inorganic impurities and is there for the best as a solvent but it is quite expensive. As it is free from all impurities, it does not pass on any impurities to the final products.

d. **Reaction vessels:** The reaction vessels employed in the manufacturing process may be metallic such as copper, iron, cast iron, galvanized iron, silver, aluminum, nickel, zinc and lead. Glass and silica are also used in the construction of the chemical plants but these days many of these are replaced by stainless steel and variety of other alloys. Some solvents and reagents employed in the process may react with the metals of reaction vessels, leading to their corrosion and passing traces of metal impurities into the solution, contaminating the final product.

Similarly, glass vessels may give traces of alkali to the solvent. Lead (Pb) may be found as impurity in commercial sulphuric acid which has been manufactured by lead chamber process. Also, substances prepared by same electrolytic process, may contain electrode material as an undesirable impurity e.g., antimony, bismuth etc.

e. **Intermediates:** Sometimes, an intermediate substance produced during the manufacturing process may contaminate the final product e.g., Sodium bromide is prepared by reaction of sodium hydroxide and bromine in slight excess.

$$6NaOH + 3Br_2 \rightarrow NaBrO_3 + 5NaBr + 3H_2O$$

The sodium bromate an intermediate product is reduced to sodium bromide by heating the residue (obtained by evaporating the solution to dryness) with charcoal.

$$NaBrO_3 + 3C \rightarrow NaBr + 3CO$$

Sodium bromate Sodium bromide If sodium bromate is not completely converted to the sodium bromide then it is likely to be present as an impurity.

f. **Atmospheric contamination during the Manufacturing Process:** Atmosphere may contain dust (aluminum oxide, sulphur, silica, soot etc.) and some gases like carbon dioxide, sulphur dioxide, arsine and hydrogen sulphide. These may contaminate the final product during the manufacturing process. Some substances which are susceptible to action by atmospheric carbon dioxide and water may get contaminated with them during their preparation e.g., sodium hydroxide readily absorbs atmospheric carbon dioxide when exposed to atmosphere.

$$2NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$

Calcium hydroxide solutions can absorb carbon dioxide from the atmosphere to for calcium carbonate.

$$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$$

g. **Manufacturing Hazards:** If the manufacturer is able to control and check impurities from the all above mentioned sources there exists certain manufacturing hazards which can lead to product contamination. The various manufacturing hazards can lead to:

i. **Contamination from the Particulate Matter**: The unwanted particulate matter can arise by a number of ways, such as accidental inclusion of dirt or glass, porcelain, plastic or metallic

fragments from sieves, granulating, tabletting and filling machines and the product container. The particulate contamination mainly arises from the wear and tear of the equipments. It may also arise from the bulk materials used in the formulation or from dirty or improperly maintained equipments *e.g.,* metal particles found in eye ointments packed in metal tubes made up of tin and aluminum.

ii. **Cross-contamination of the Product**: This manufacturing hazard has to be considered in the preparation of solid dosage forms. Cross-contamination of product can occur by air-born dust arising out of handling of powders, granules and tablets in bulk. Cross-contamination is dangerous particularly in case of steroidal and other synthetic hormones and therefore, it should be carefully controlled.

iii. **Contamination by Microbes**: Many products, like liquid preparations and creams intended for topical applications are liable to contamination by microbes from the atmosphere during manufacturing. For all products intended for parenteral administration and ophthalmic preparations, sterility testing is done and it provides an adequate control for microbial contamination in such preparations. Microbial contamination can be controlled by adding suitable antimicrobial and antifungal agents.

2. **Instability of the Product:**

a. Chemical Instability: Impurities can also arise during storage because of chemical instability of the pharmaceutical substance. Many pharmaceutically important substances undergo chemical decomposition when storage conditions are inadequate. This chemical decomposition is often catalyzed by light, traces of acid or alkali, traces of metallic impurities, air oxidation, carbon dioxide and water vapours. The nature of the decomposition can easily be predicted from the knowledge of chemical properties of the substance. All such decompositions can be minimized or avoided by using proper storage procedures and conditions.

The photosensitive substances should be protected from light by storing them in darkened glass or metal containers thereby inhibiting photochemical decomposition. Materials susceptible to oxidation by air or attack by moisture should be stored in sealed containers and if necessary the air from the containers can be displaced by an inert gas such as Nitrogen. Oxidation can also be prevented by adding suitable antioxidants which are capable of undergoing oxidation at the expense of the substances.

b. Changes in Physical Properties: Pharmaceuticals may undergo changes in physical properties during storage. There can be changes in crystal size and shape, sedimentation, agglomeration and caking of the suspended particles. These physical changes are not always avoidable and may result in significant changes in the physical appearance, pharmaceutical and therapeutic effects of the product.

Particle size and consequently surface area is a critical factor in determining the bioavailability of the low solubility drug such as griseofulvin. Physical changes such as sedimentation and claying in case of multi dose suspension may constitute a safety hazard leading to the possibility of under dosage and later to over dosage of the drugs. Similarly increase in the globule size of the injectable emulsions on storage may lead to fat embolism.

c. Reaction with container material: The possibility of reaction between the container material and the contents cannot be ruled out as it constituents a safety hazard. Preparations susceptible to reaction with metal surfaces *e.g.,* salicylic acid ointment must not be packed in metal tubes. Solutions of substances which are alkali-sensitive *e.g.,* atropine sulphate injection must be packed in glass ampoules which comply with the test of hydrolytic resistance therefore such preparations must not be packed in containers made from soda glass. Plastic containers and closures must be carefully evaluated because of their tendency to give undesirable additives, such as plasticizers,

particularly in the presence of non-aqueous solvents. Plastic containers intended for injectables should be sufficiently translucent to allow visual inspection of the contents and if they are having higher than 500 ml capacity, they must also comply with the test limiting animal toxicity in the cat, ether-soluble extractive and metal additives with special reference to barium and heavy metals like lead, tin and cadmium. Rubber closures are more susceptible to absorb medicaments, antioxidants and bactericides from solution, unless they are appropriately pretreated by immersion in solutions of the concerned compounds.

d. Temperature: The rate of chemical decomposition and physical changes of stored products depends upon the temperature. The susceptible substances may have temperature storage requirements assigned to them in order to protect them against undesirable decomposition [10-12].

3. **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* [27].

4. **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 [28]. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin) lavalbuterol (R-albuterol), and esomeprazole (S- omeprazole).

5. **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 [29].

   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* N, Ndimethylformamide (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 the purity 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 [30].

6. **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 [31], and MDMA samples, produced impurities in intermediates via reductive amination route [32].

7. **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 solvolysis [33]. Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was recalled in the United States because of degradation/impurities leading to sub- potency [34].

   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 consumer-use 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 [35].

8. **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 [27].

9. **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 [36].

   The conditions of the autoclave method (i.e., 123 + 2^{o}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.

10. **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 [37].

    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.

11. **Functional group-related Typical Degradation:** Ester hydrolysis can be explained with a few drugs *viz* aspirin, benzocaine, cefotaxime, ethyl paraben [37] and cefpodoxime proxetil [38]. Hydrolysis is the common phenomenon for ester type of drugs, especially in liquid dosage forms *viz* benzyl-penicillin, 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 degredation pathway in 0.1 mol L-1 hydrochloric acid and sodium hydroxide at 80^{o}C were studied [39].

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. Ergometrine [40], nifedipine [41], nitroprusside, riboflavin and pheno-thiazines 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.

Fluroquinolone antibiotics are also found to be susceptible to photolytic cleavage [42]. In ciprofloxacin eye drop preparation (0.3%), sunlight induces photocleavage reaction producing ethylenediamine analog of ciprofloxacin [43]. Decarboxylation of some dissolved carboxylic acids, such as p-aminosalycylic acid; shows the loss of carbon dioxide from the carboxyl group when heated. An example of decarboxylation is the photoreaction of rufloxacin [44].

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 packaging material. For most drugs, the reactive species consist of;

- Water- that can hydrolyze some drugs or affect the dosage form performance
- Small electrophiles-like aldehyde and carboxylic acid derivatives
- Peroxides- that can oxidize some drugs
- Metals- which can catalyze oxidation of drugs and the degradation pathway
- Leachable or Extractables- can come from glass, rubber stoppers, and plastic packaging materials.

Metal oxides such as NaO_2, SiO_2, CaO, MgO, are the major components leached/extracted from glass [45].

Generally most synthetic materials contain leachable oligomers/monomers, vulcanizing agents, accelerators, plasticizers, and antioxidants [46].

Some examples of leachable/extractables from synthetic materials include styrene from polystyrene, [47] ethylhexylphalate (DEHP, plasticizer in PVC), [48] dioctyltin isooctylmercapto-acetate (stabilizer for PVC), [49] zinc stearate (stabilizer in PVC and polypropylene), [50] 2-mercaptobenzothiazole (accelerator in rubber stopper), [51] and furfural from rayon [52]. These impurities are needed to be analyzed by using different analytical methods.

CLASSIFICATION OF SOLVENTS ON THE BASIS OF THEIR LIMIT IN PARTS PER MILLION (PPM)

CATEGORY	NAME OF THE SOLVENT/LIMIT	UNIT/SPECIFICATION
Class 1	Benzene (2ppm), carbon tetra chloride (4ppm), methyl chloride (600ppm), methanol (3000ppm), pyridine (200ppm), ethanol	More than this should be avoided
Class 2	N, N-dimethylformamide (800ppm), Acetonitrile (410)	More than this should be avoided
Class 3	Acetic acid, ethanol, acetone (50mg)	Have permitted daily exposure of 50mg or less per day as per ICH guidelines

CLASSIFICATION OF METALS ON THE BASIS OF THEIR SAFETY CONCERN

Category	Example
Class -1 (metal of significant safety concern)	Ir (Iridium), pt (platinum), Rh (rubidium), Mo (molybdenum), V (vanadium), Cr (chromium), Ni (nickel).
Class -2 (Metal with low safety concern)	Cu (copper), Mn (manganese)
Class -3 (Metal with minimal safety concern)	Fe (iron) and Zn (zinc).

CLASSIFICATION OF Q-GUIDELINE ON THE BASIS OF IMPURITY

Section	Impurities	Sub-section
Q3A(R2)	Impurities in new drug substance	Q3A(R)
Q3B(R2)	Impurities in new drug products	Q3B(R)
	Impurities: guidelines for residual solvents	Q3C
	Impurities: guidelines for residual solvents	Q3C(M)
Q3C(R4)	(Maintenance)	
	PDE for tetrahydrofuran [in Q3c(R3)]	Q3C(M)
	PDE for N-methylpyrrolidone [in Q3c(R3)]	

THRESHOLDS FOR REPORTING IMPURITIES

Maximum dose	Reporting threshold	Identification threshold	Qualification threshold
Less or equal to 2gm/day	0.05%	0.1% or 1mg/day (Which is lower)	0.15%or 1mg/day (Which is lower)
>2gm/day	0.03%	0.05%	0.05%

THRESHOLD FOR REPORTING DEGARADATION PRODUCTS IN NEW DRUGS PRODUCTS

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

Selective Analytical Methodologies:

- Development of a new drug mandates that meaningful and reliable analytical data be generated at various steps of the new drug development. Ensuring the safety of a new pharmaceutical compound or drug requires that it meet the established purity standards as a chemical entity or when admixed with animal feeds for toxicity studies or pharmaceutical excipients for human use.

- These requirements demand that the analytical methodology that is used be sensitive enough to measure low levels of impurities. This has led to analytical methods that are suitable for determination of trace/ultratace levels, i.e., sub-microgram quantities of various chemical entities. A variety of methods are available for monitoring impurities. The primary criterion is the ability to differentiate between the compounds of interest.

- New drug development requires meaningful and reliable analytical data to be produced at various stages of the development.

a) Sample set selection for analytical method development.

b) Screening of chromatographic conditions and phases, typically using the linear solvent-strength model of gradient elution.

c) Optimization of the method to fine tune parameters related to ruggedness and robustness.

The impurities can be identified predominantly by following methods [13-16];

- Reference standard method
- Spectroscopic method
- Separation method
- Isolation method
- Characterization method

i. **Reference standard method:** The key objective of this is to provide clarity to the overall life cycle qualification and governance of reference standard used in development and control of new drug. Reference standards serve as the basis of evaluation of both process and product performance and are the benchmarks for assessment of drug safety for patient consumption. These standard are needed, not only for the active ingredients in dosage forms but also for impurities, degradation products, starting materials, process intermediates, and excipient.

ii. **Spectroscopic methods** [17]**:** The following spectroscopic methods can be used;

A. Ultraviolet (UV)

B. Infrared (IR)

C. Nuclear magnetic resonance (NMR)

D. Mass spectrometry (MS)

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

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

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

D. Mass Spectrometry: Mass spectrometry provides excellent structural information, and, based on the resolution of the instrument; it may provide an effective tool for differentiating with small differences in molecular weight. However, it has limited use as a quantitative technique because of cost and time considerations.

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

iii. **Separation methods:** The following separation methods can be used [18-20]:

a) Thin-layer chromatography (TLC)

b) Gas chromatography (GC)

c) High-pressure liquid chromatography (HPLC)

d) Capillary electrophoresis (CE)

e) Supercritical fluid chromatography (SFC)

A brief account of the above-listed methods is given here to provide a quick review of their potential use. Except for CE, all these techniques are chromatographic methods. CE is an electrophoretic method that is frequently lumped with the chromatographic methods because it shares many of the common requirements of chromatography. However, it is not strictly a two phase separation system — a primary requirement in chromatography. Hyphenated methods such as GC–MS, LC–MS, GC–LC–MS, LC–MS–MS, etc.

- A broad range of compounds can be resolved using TLC by utilizing a variety of different plates and mobile phases. The primary difficulties related to this method are limited resolution, detection, and ease of quantification. The greatest advantages are the ease of use and low cost. Gas chromatography is a very useful technique for quantification. It can provide the desired resolution, selectivity, and ease of quantification. However, the primary limitation is that the sample must be volatile or has to be made volatile by derivatization. This technique is very useful for organic volatile impurities.

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

Capillary electrophoresis is a useful technique when very low quantities of samples are available and high resolution is required. The primary difficulty is assuring reproducibility of the injected samples.

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

iv. Isolation method: It is often necessary to isolate impurities. But if the instrumental methods are used isolation of impurities is avoided as it directly characterizes the impurities. Generally chromatographic and non-chromatographic technique are used for isolation of impurities prior its characterization. The term 'chromatographic reactor' refers to the use of any analytical scale column as both a flow through reactor, and simultaneously, as separation medium for the reactant(s) and products. By using HPLC, chromatographic reactor approach, the solution phase hydrolysis kinetics of the Aprepitant prodrug, fosaprepitant dimeglumine, were investigated. In loratidin [21], impurity found was of loratidine [22]; other examples include celecoxib [23] and amikacin [24].

A list of methods that can be used for isolation of impurities is given below.

- Solid-phase extraction method
- Liquid-liquid extraction method
- Accelerated solvent extraction method
- Supercritical fluid extraction
- Column chromatography
- Flash chromatography
- TLC
- GC
- HPLC
- HPTLC
- Capillary electrophoresis(CE)
- Supercritical fluid chromatography (SFC)

v. Hyphenated methods /characterized method: The following hyphenated methods can be used effectively to monitor impurities.

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

An example of reverse-phase LC-MS analysis in gradient elution with two distinct soft ionization techniques is the Atmospheric pressure ionization with electrospray source (API-ESI) and the chemical ionization of d-allethrine [25].

The popularity of LC-MS-MS systems for complex mixture analysis of thermally labile and biologically relevant molecules, *viz* mosapride, is largely attributed to the "soft" nature of atmospheric pressure chemical ionization (APCI), and atmospheric pressure ionization (APPI) [26].

HPLC-DAD-MS (HPLC coupled with a diode array UV detector and a mass spectrometer, and such other techniques are almost routinely used.

IMPURITIES PRESENT AND METHOD OF ANALYSIS IN MARKETED DRUGS

Sr. no	Drugs	Impurities	Method
1.	Amphotericin B	Teteaenes	UV spectroscopy
2.	Atropine sulphate	Apo atropine	UV spectroscopy
3.	Cloxacillin	N, N -dimethyl	Gas chromatography
4.	Dextrose	5- hydroxy methyl furfural	UV spectroscopy
5.	Doxorubicin hydrochloride	Acetone and Ethanol	Gas chromatography
6.	Ethambutol hydrochloride	2 -amino butanol	TLC
7.	Fluorescence sodium	Dimethyl formamide	Gas chromatography
8.	Framicetinsulphate	Neamine	TLC
9.	Morphin	6- mono acetylmorphin	HPLC

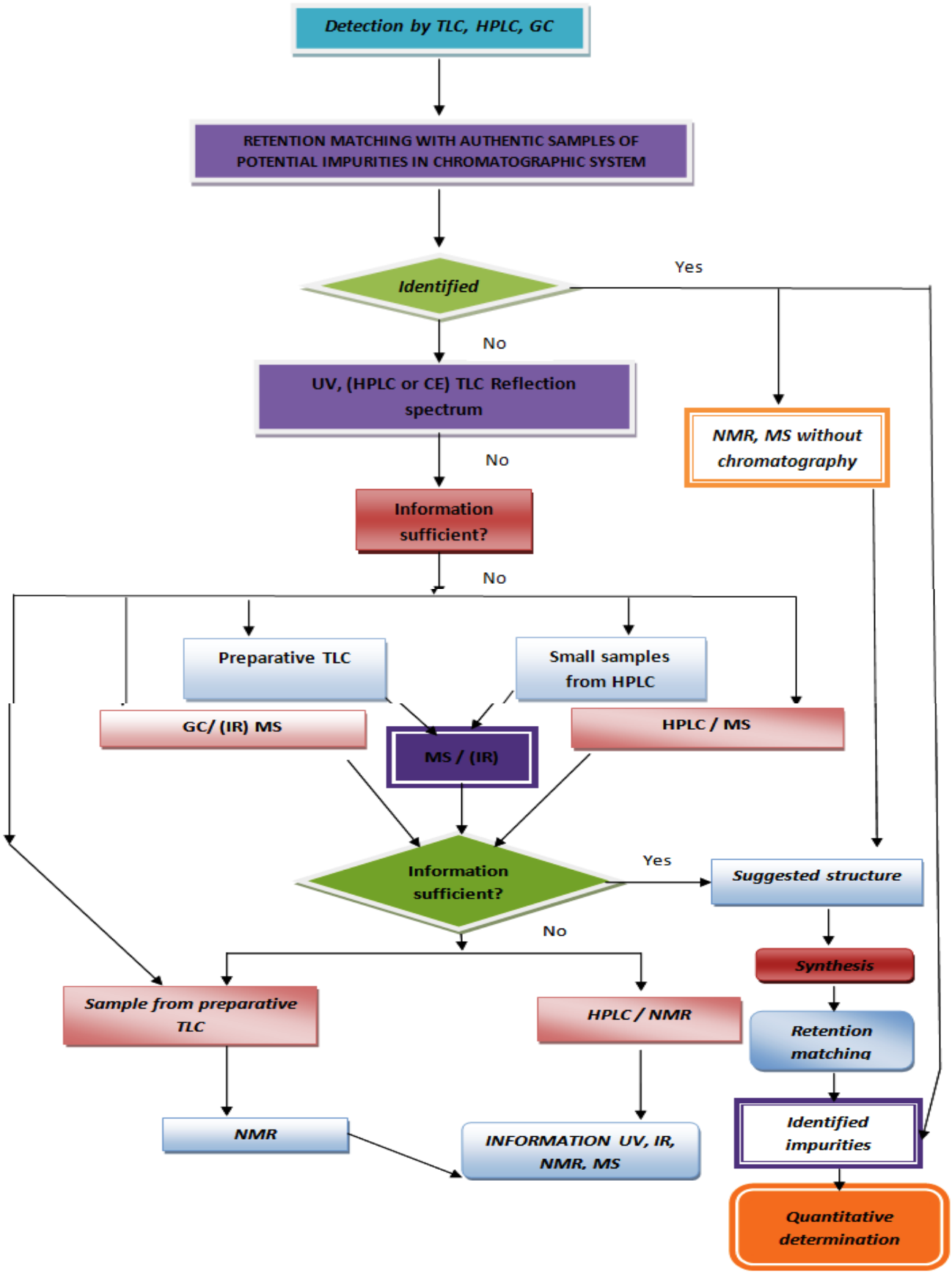
10.	10-hydroxymorphin	10- oxomorphin	HPLC
11.	Mercaptopurin	Hypoxanthin ,2,5-bis[(N' cyano –N'' – methyl)guinidinoethylthiomethyl]-4-methylimidazol	UV spectroscopy
12.	Norgestrei	3,17a-diethyl-13 ethyl-3,5-gonadiene-17-ol spectroscopy	TLC, HPLC and UV
13.	Cimitidine	1,8-bis[(N' cyano-N''-methyl)guinidino]-3,6-dithiaoctane	HPLC
14.	Celecoxib	[5-(4-methylphenyl)-3-trifluromethyl-1H-pyrazole],4-[5-(2'-methylphenyl)-3-(trifluromethyl-1H-pyrazole-1-yl]-benzenesulphonamide,and4-[4-(4'-methyl phenyl)-3-(trifluromethyl)-1-Hpyrazole-1-yl]-benzenesulphonamide	HPLC, LC, LC-MS-MS
15.	Ethynodioldiacetate	17 a-ethinylestr-4-ene-3a,17-diol-3-acetate-17-(3'-acetoxy-2'-butenoate)17 a-ethinylestr-4-ene-3a,17-diol-3-acetate-17-(3-oxo-butanoate)	HPLC
16.	Methamphetamine	1,2-dimethyl-3-phnylaziride,ephedrine,methylephedrine,N-formylmethamphetamine,N-acetylmethamphitamine,,N-formylphedrine,N-acetylephedrine,N,Oacetylephedrine,methametamine dimmer	HPLC
17.	Repaglinide	4-carboxymethyl-2-ethoxybenzoic acid,4-cyclohexylaminocarbamoylmethyl-2ethoxy-benzoic acid,1-cyclohexyl-3-[3-methyl-1-2-(piperidine-1-ylphenyl)-butyl]-urea,1,3-dicyclohexyl urea	GC
18.	Morphine	6-monoacetylmorphine	HPLC
19.	Morphine sulphate	5-(hydroxymethyl)2-furfural	HPLC
20.	10-hydroxymorphine	10-oxomorphine	HPLC

Analytical procedures:

1. **Method Development:** Method development usually requires the choice of columns, mobile phase, detectors, and method of quantization etc. The factors to be considered for the method developments are the following, Existing method may be inaccurate, artefact, or contamination prone, or they may be unreliable (have poor accuracy or precision). Existing method may be too expensive, time consuming or energy intensive, or they may not be easily automated. Existing methods may not provide adequate sensitive or analyte selectivity in samples of interest. Newer instrumentation techniques may have evolved which can provide opportunities for improved methods, including improved analyte identification or detection limits, greater accuracy or precision and better returns on investments.

2. **Validation of Analytical Methods:** The validation process involves confirmation or establishing a developed method by laboratory studies, procedures, systems, which can give accurate and reproducible result for an intended analytical application in a proven and established range. The performance characteristics of the method (accuracy, precision, sensitivity, ruggedness, etc) should meet the requirements of the intended analytical applications and the process can or provide documented evidence that the system or procedure do what it is intended for in a systematic, precise and reliable manner. According to ICH, typical analytical performance characteristics that should be considered in the validation of all the types of methods are:

APPLICATIONS: 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 different classes of drugs namely alkaloids, amines, amino acids, analgesics, antibacterial, anticonvulsants, anti-depressant, tranquilizers, antineoplastic agents, local anaesthetics, macromolecules, steroids and miscellaneous.


Detection by TLC, HPLC, GC
RETENTION MATCHING WITH AUTHENTIC SAMPLES OF POTENTIAL IMPURITIES IN CHROMATOGRAPHIC SYSTEM
Identified
Yes
No
UV, (HPLC or CE) TLC Reflection spectrum
NMR, MS without chromatography
No
Information sufficient?
No
Preparative TLC
Small samples from HPLC
GC/ (IR) MS
HPLC / MS
MS / (IR)
Information sufficient?
Yes
Suggested structure
No
Synthesis
Sample from preparative TLC
HPLC / NMR
Retention matching
NMR
INFORMATION UV, IR, NMR, MS
Identified impurities
Quantitative determination

Goals of Impurity Investigations:

Process-related impurities	Degradation–related impurities
Identify significant impurities	Identify potential degradation product through stress testing and actual degradation products through stability studies.
Determine origin of impurities and method for elimination or reduction	Understand degradation pathway and methods to minimize degradation.
Establish a control system for impurities involving: 1) Processing/manufacturing conditions 2) Suitable analytical methods/ specifications	Establish a control system for impurities involving: 1) Processing/manufacturing conditions 2) Suitable analytical methods/specifications 3) Long term storage conditions including packaging 4) Formulation.

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. Now a day, 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.

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