REVIEW ARTICLE
IMPURITY PROFILING OF DRUGS TOWARDS SAFETY AND EFFICACY: THEORY AND PRACTICE

*KIRAN R. DHANGAR, RAKESH B. JAGTAP, SANJAY J. SURANA AND ATUL A. SHIRKHEDKAR**

Department of Pharmaceutical Chemistry, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur Dist: Dhule (MS) 425 405 India

ABSTRACT

Last decade has witnessed enormous changes in the studies of impurity profiling of drugs which can be reflected from pharmacopoeia and regulatory guidelines. The present review article is an attempt to provide comprehensive knowledge about various aspects and details about the impurity profiling in context with regulatory guidelines. Article also focused on isolation, separation and characterization techniques of impurities. It gives preliminary idea about applicability of virtual software used for studies on safety limit for impurities. The comprehensive information related to residual solvents, residual metals and genotoxicity studies of isolated impurities have also been incorporated into present article.

Keywords: Impurity Profiling; Regulatory guidelines; Residual solvent; Genotoxicity.

1. INTRODUCTION

Thrust for development of new drug molecules providing knowledge about impurities and its ill effects on human health has gained immense importance. Last decade has witnessed an enormous interest in impurity profiling of drugs in pharmaceutical field. The occurrence of these undesirable chemicals, even in trifling quantity may influence the efficacy and safety of the pharmaceutical products. Many Pharmacopoeias *viz* United States Pharmacopeia (USP) [1], Indian Pharmacopoeia (IP) [2], and British Pharmacopoeia (BP) [3], European Pharmacopoeia (EU) [4] have designed monographs to ensure minimum acceptable quality of drug substances and drug products for users. The monograph included in pharmacopoeia on impurity profiling is getting critical attention from regulatory authorities [5, 6].

The impurity profiling is studied with objectives to establish specific link between two or more samples, ascertaining drug distribution pattern, for identification of source of drug sample and also for monitoring the process for drug manufacturing. The health implications of impurities can be significant because of their potential teratogenic, mutagenic, or carcinogenic effects [7]. Identification of pharmaceutical impurities is a critical analytical activity in the drug development process whose goal is to fully elucidate the chemical structure of unknown pharmaceutical impurity present in either drug substances or drug products above a particular threshold [8].

1.1 Definition of impurity and impurity profiling

As per the International Conference on Harmonization (ICH) guidelines "Impurities are substances in the product that are not the Active Pharmaceutical Ingredients (API) itself or the excipient used to manufacture it" [9].

While, IP has defined impurity as any component of drug substances for pharmaceutical use or of a drug product that is not the chemical entity that defines the substance or in the case of a drug product not an excipients in the product [2].

In short, impurity can be defined as any substance coexisting with the original drug, such as starting material or intermediates or formed; due to any side reactions. Impurity profile is a description of the identified and unidentified impurities present in drug products [9].

1.2 Pharmacopoeial and Regulatory Guidelines and status on Impurity profiling

In the previous editions of various Pharmacopoeias, much of the stress was not given on the impurity profiling of the drugs. But, their recent editions have emphasized on impurity profiling of many of the drugs and included in the monograph. IP, BP, and USP have includedlimit to allowable levels of impurities present in API and formulations. ICH guideline for technical requirement for registration of pharmaceutical for human use has also published guidelines for validation of methods for analyzing impurities in new drug substances, product, residual solvents and microbial impurities [9].

As per USP, the concepts of purity changes with time and are inseparable from developments in the analytical chemistry. If a material previously considered being pure can be redefined into new terms of purity and impurity, inorganic, organic, isomeric, or polymeric components considered as impurities. Purity or impurity measurements on finished preparations present a challenge to pharmacopoeial standard setting. Where degradation of a preparation over time is at issue, the same analytical methods that are stability-indicating are also purity indicating [1].

According to BP, impurities are divided into two sub types entitled 'Qualified impurities' and other 'Detectable impurities'. The Qualified impurities are those previously accepted by competent authority, as being qualified *viz* Impurities which occur as natural metabolites and Other 'Detectable Impurities' are those that have not been detected in any samples of the substances during elaboration of the monograph or that occurs in amounts below 0.1% but have been shown to be limited by tests [3]. The monographs of the pharmacopoeia have been designed to ensure the minimum acceptable quality of drug substances and drug products for users. Tests for related substances have been explored in many monographs to limit impurities and degradation products. Although, one of the primary objective of the Pharmacopoeia is to guarantee the identity, strength, purity and quality of official articles, it is not possible to include in each monograph a test for every impurity or contaminant or even an adulterant that might be present [2].

The acceptance criterion of impurity is given in **TABLE 1.** Ethical, economic and competitive reasons as well as those of safety and efficacy support need to monitor impurities in drug products. However, monitoring impurities and controlling these impurities mean different things to different people or to the same people at different times, even those in the pharmaceutical sciences and industry [10]. A combined terminology is necessary to assure that everyone uses the same vocabulary when addressing questions related to impurities. The United States Food and Drug Administration (US-FDA) have approved the guidance prepared under the guidance of the ICH [11]. The ICH guideline for impurities in pharmaceuticals was developed with joint efforts of regulators and industry representatives from the European Union (EU) [12], Japan [13] and United States [14]and it has helped to ensure that different regions have reliable requirements for the data that should be submitted to various regulatory agencies. The guidelines not only assist the sponsors of New Drug Applications (NDA) or Abbreviated New Drug Application (ANDA) with the type of information that should be submitted with their applications, but also support the FDA reviewers and field investigators in their consistent interpretation and implementation of regulations [15]. The various regulatory guidelines regarding impurities, **TABLE 2.**

Table 1: Acceptance criteria for Impurities (As per Indian Pharmacopoeia).

Criterion	For Drug Substances	For Drug Products
❖ Each identified specified impurity	0.5 %	-
❖ Each unidentified impurity	0.3%	-
❖ Total impurity	1.0%	-
❖ Each identified specific degradation product	-	1.0%
❖ Each unidentified degradation product	-	0.5%
❖ Total degradation product	-	2.0%

e-mail: shirkhedkar@gmail.com

Table 2: Regulatory guidelines.

Guideline	Depiction
Q1A	ICH guidelines "stability testing of new drug substances and products"
Q3A	ICH guidelines "Impurities in New Drug Substances"
Q3B	ICH guidelines "Impurities in New Drug Products"
Q3C	ICH guidelines "Impurities: Guidelines for residual solvents"
US-FDA	"NDAs -Impurities in New Drug Substances"
US-FDA	"ANDAs – Impurities in New Drug Substances"
Australian regulatory guideline	Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia.

2. Current Outlook on Impurity Profiling [16-41]

The magnitude of topic on an impurity profiling can be learnt from the review articles and research papers published on the topic; to our knowledge more than 26 review articles on various facet of impurity profiling have been published by different authors. Literature studies revealed many review articles published in different journals on the impurity profiling of active pharmaceutical ingredients are depicted in **TABLE 3.**

Table 3: Current outlook on impurity profiling.

Author(s)	**Description**	**Year**	**References**
❖ Saranjit Singh and Monika Bakshi	Guidelines for stability of drugs	2000	16
❖ Jiben Roy	Sources of impurities	2002	17
❖ SilkeKlick et.al	Stress testing guidelines	2005	18
❖ Satinder Ahuja	Terminology, sources and isolation, characterization technique	2006	19
❖ Nafisur Rahman	Importance of impurity profiling in pharmaceuticals	2006	20
❖ David Jacobson-kram and Timothy Mc-Govern	Regulatory guideline related to toxicity of impurity	2006	21
❖ John Kovaleski	Impurities in generic pharmaceuticals	2006	22
❖ Sanjay S. Bari et al	Focused on various types, sources and analytical method development and characterization	2007	23
❖ Sendhilkumar Poornachary	Effect of impurities on crystal growth process	2007	24
❖ Andrew Worth et.al	Software used for genotoxicity and carcinogenicity	2010	25
❖ Henry Hatakka	Crystallization related impurities	2010	26
❖ Derek I. Robinson	Control of genotoxicity impurity in API	2010	27
❖ A.Ayre	Focused on guidelines given by ICH and sources of impurities	2011	28
❖ SS. Pawale et.al	Focused on qualification of impurities	2012	29
❖ Ranjit Singh and Rehman	Mechanism of formation and characterization of generated impurities during development	2012	30
❖ M.Blessy et.al	Forced degradation and stability of drug	2013	31
❖ Santosh Kumar S.	Give attention towards the analytical method for identification of impurity	2014	32
❖ P. Vyankatasan and K. Valliapp	Aspects related to the analytical method development for impurity profiling	2014	33
❖ Y. Jiang et al	Guidelines and strategies of the international conference on harmonization (ICH) and its member states to overcome existing impurity control problem for antibiotics in china	2015	34
❖ S. Zaza et al	Recent advances in the separation and determination of impurities in pharmaceutical products	2015	35
❖ V. Desfontaine et al	Super critical fluid chromatography in pharmaceutical analysis	2015	36
❖ P.P. Patil and V.S. Kasture	Quality guidelines and applications of impurity profiling for pharmaceutical	2015	37
❖ B. Ramachandra	Development of Impurity Profiling Methods using Modern Analytical Techniques	2016	38
❖ A. C. Kogawa, R.N. Herida, Salgado	Impurities and forced degradation studies: A Review	2016	39
❖ S.V. Saibaba, M.Satish Kumar eta al	Pharmaceutical Impurities and their Characterization: A Review	2016	40
❖ R. Solank et al	Impurity profiling of Active Pharmaceutical Ingredients and Finished drug products was recently reviewed and emphasis has been given on the comparison of the regulatory requirements of different countries.	2017	41

2.1 Classification of impurities as per USP and ICH

Impurities are classified into various types based on their 'common names', ICH Terminology and USP. As per the common names impurities are named as by-product; degradation products; intermediates; Penultimate intermediates; related products and transformation product. As per USP, the impurities are named as impurities in official articles; ordinary impurities and organic impurities. ICH has termed impurities as organic impurities; inorganic impurities and residual solvents.

2.2 Types of Impurities and Sources

Impurities can be broadly divided into four type, they are;

- Process related drug substance
- Process related drug product
- Degradation drug substance or drug product and
- Degradation drug product

There is a variation in sources for impurities in each type. The details about the types and sources of impurities are depicted in **FIGURE 1.**

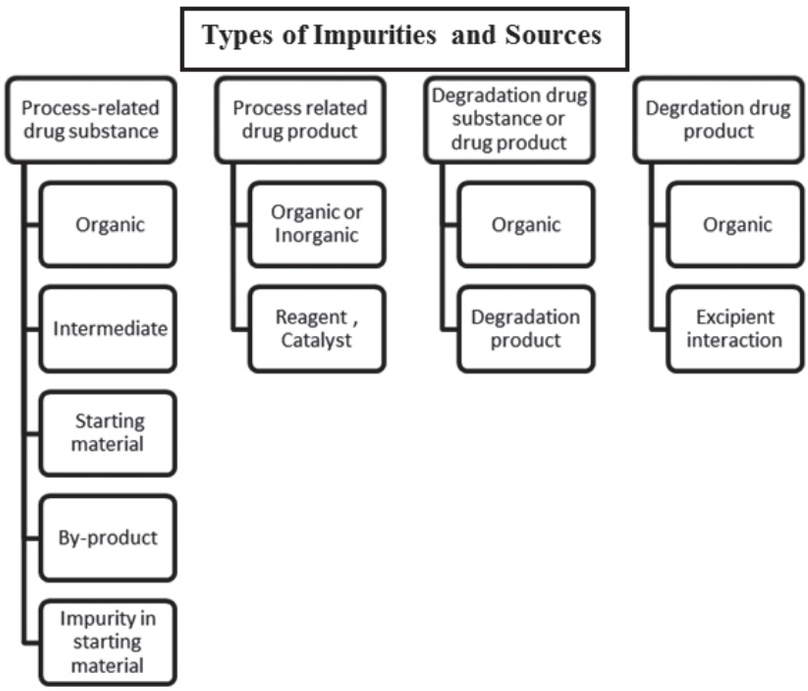


Figure 1: Types of Impurities and Sources.

3. Sources of impurity

There are diverse sources of impurities in API and drug products. It includes crystallization related, stereochemistry, residual solvents, and synthetic intermediate and by- products related impurities [42]. Further, it also includes formulation, impurity arising during storage, method related, mutual interaction amongst ingredients and functional group related typical degradation.

3.1 Crystallization related impurity

Many drugs exist in crystalline solid states and can exit in the form of polymorphs, solvates or hydrates. The valid reason for formation of crystalline is due stability and ease of handling during various stages of drug development [43]. Crystallization is a major technological process for particle formation in pharmaceutical industry and, in addition, plays acrucial role in defining the stability and drug release properties of the final dosage forms. Therefore, FDA needs development and validated methods for analysis of the proportion of crystalline forms throughout the drug's shelf life [44] .

Based on the understanding that the nature of structure adopted by a given compound upon crystallization could exert a deep effect on the solid-state properties of that system; therefore, pharmaceutical industry has required taking a deep interest in polymorphism and solvatomorphism [45].

In crystallization process, the reproducibility of solid-state attributes of the crystalline product is important issue. Whenever, there is a batch-to-batch variation in the crystal habit or polymorphs, a crucial issue may well be the presence or absence of typical "impurities" in the material used to obtain the crystalline products, further possible changes in the operating conditions. Given sucha situation, it is not only important to identify the sources of the impurities, but also understand the mechanism underlying their role on the crystal growth process *viz*effect of impurities on the growth of glycine crystals in aqueous solutions has been studied. Polymorphic transition pathway for many such as chloramphenicol palmitate, cephalexin and indomethacin has been established [24, 46].

Impurities have a clear negative influence on the nucleation and growth rate kinetics of the semi synthetic antibiotic ampicillin crystallization. The reported impurities such as phenylglycine and 6- aminopenicillanic acid are the building blocks of ampicillin. Hence, present in ampicillin manufacturing process and also in degraded products studied [47]. The crystal growth of the L-alanine surface is observed and to be promoted by L-valine impurity with higher impurity concentration [48]. Ramified crystals of 5- Nitro acetyl salicylic acid are more susceptible to hydrolysis thanor column shaped crystals [49]. The stability of drugs in their amorphous form is generally lower than that of drugs in their crystalline form because of higher free energy level of the amorphous state [50].

Recent studies have highlighted that even small changes in crystallization conditions *viz* super saturation, temperature, cooling rate, and impurities can produce significant changes in the crystal properties. These effects have been recognized as the major batch-to-batch and source variation problems leading to inconsistency of the product properties. A very common example in this is of Ritonaviran anti-retroviral. Only one crystal form was ever identified in the drug development process [51]. Olanzapine crystallizes in more than 25 crystalline forms, of which Form II has been designated the most stable form and is used in the dosage form [52,53].

Usually, metastable polymorphic form is accidently produced due to temperature, mechanical treatment, and moisture during processing and storage of the drug product [54]. For example Salmeterolxinafoate is reported to occur in two crystalline polymorphic forms, out of which form I is more stable than Form II (metastable polymorph) under ambient conditions [55].

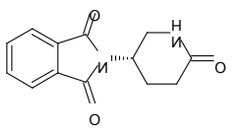
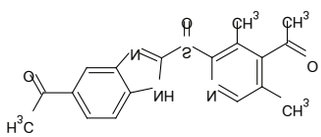
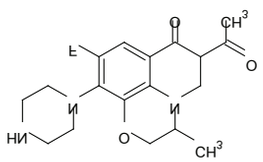
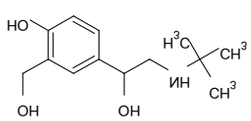
3.2 Stereochemistry related impurity

Stereochemistry is the three-dimensional aspects of a molecule; the action of drug in the biological system depends on the spatial arrangement of atoms in the drug molecule [56].It is of chiefmagnitude to look for stereochemistry

related compounds; that is, those compounds that have analogous chemical structure but different spatial orientation; these compounds can be considered as impurities in the API's [57]. Chiral molecules are generally called enantiomers. In case of chiral drug administered as the pure enantiomer the antipode is considered as impurity. The single enantiomeric form of chiral drug is nowadays considered as an improved chemical entity that may tender a better pharmacological profile and an increased therapeutic index with a more favorable adverse reaction profile [58].

There are two isomers of Thalidomide. The (R)-(+) Thalidomide having sedative and hypnotic action on the other hand (s)-(-) Thalidomide shows mutagenic activity [59]. Though, the pharmacokinetic profile of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are similar, illustrating the deficiency 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) [60, 61]. The significance of stereo chemical purity of formoterol, a selective Beta2-adrenoreceptoragonist is reported [62]. The active isomer of drug and their structure, **TABLE 4.**

Table 4: Active isomer of drug.

Drug	Structure of active isomer	Active isomer
Thalidomide		R - (+) Thalidomide
Esomeprazole		S - Omeprazole
Levofloxacin		S - Ofloxacin
Lavalbuterol		R - Albuterol

3.3 Residual Solvents

Residual solvents are defined as organic volatile impurities that may remain in active Pharmaceutical substances, excipient or medicinal products after processing. During the manufacturing processes, the solvents are not completely removed. The solvents may be used to improve the yield in the synthesis of active pharmaceutical substances besides imparting characteristics of crystal form, purity and solubility. Residual solvents do not have any therapeutic effect. Therefore, efforts should be made to remove them to the extent possible to meet the specification prescribed [2].Gas-chromatography method has been developed to study the impurites present in acetone, dichloromethane, methanol and toluene. By using this technique, the major contaminants of each organic solvent can be quantified [63, 64].

The residual solvents are classified into three types on the basis of possible risk on human health. They are mainly **Class-1**(Solvent to be avoided), **Class-2** (Should be limited in drug substance) and **Class-3** (less toxic and low health hazard to humans). The **Class-1**type of the solvents is hazardous, known human carcinogenic, strongly suspected carcinogenic and also causing environmental hazards. The **class-2**, types of solvents are nongenotoxic animal carcinogen or possible causative agents of other irreversible toxicity *viz* neurotoxicity or teratogenicity. These types of solvents are suspected of other significant but reversible toxicities. The **Class-3** solvents are with low toxic potential, low toxic potential to humans and no health based exposure limit is needed. The detailed accounts on the solvent list, its class and concentration limit in parts per million (ppm) is furnished in**TABLE 5** [1].

Table 5: Residual Solvents, its class and limits.

Name of solvent	Class –I	Class-II	Class-III	Concentration limit (ppm)
Acetic acid	-	-	+	A*
Acetone	-	-	+	A*
Acetonitrile	-	+	-	410
Anisole	-	-	+	A*
Benzene	+	-	-	2
1-Butanol	-	-	+	A
2-Butanol	-	-	+	A*
Butyl acetate	-	-	+	A*
Tert-Butylmethyl ether	-	-	+	A*
Carbon tetrachloride	+	-	-	4
Chlorobenzene	-	+	-	360
Chloroform	-	+	-	60
Cumene	-	-	+	A*
Cyclohexane	-	-	+	3880
1,2-Dichloroethane	+	-	-	1870
1,1-Dichloroethane	+	-	-	8
1,2-Dichoroethene	-	+	-	5
Dichloromethane	-	+	-	600
1,2-Dimethoxyethane	-	+	-	100
N,N-Dimethylacetamide	-	+	-	1090
N,N-Dimethylformamide	-	+	-	880
Dimethyl sulphoxide	-	-	+	A*
1.4-Dioxane	-	+	-	380
Ethanol	-	-	+	A*
2-Ethoxyethanol	-	+	-	160
Ethyl acetate	-	-	+	A*
Ethylene glycol	-	+	-	620
Ethyl ether	-	-	+	A*
Ethyl formate	-	-	+	A*
Formamide	-	+	-	220
Formic acid	-	-	+	A*
Heptane	-	-	+	A*
Hexane	-	+	-	290
Isobutyl acetate	-	-	+	A*
Methanol	-	+	-	3000
2-Methoxyethanol	-	+	-	50
Methyl acetate	-	-	+	A*
3-Methyl-1-butanol	-	-	+	A*

Continue table 5				
Name of solvent	**Class –I**	**Class-II**	**Class-III**	**Concentration limit (ppm)**
Methylbutylketone	-	+	-	50
Methylcyclohexane	-	+	-	1180
Methylethylketone	-	+	-	A*
Methylisobutylketone	-	-	+	A*
2-methyl-1-propanol	-	-	+	A*
N-Methylpyrrolidone	-	+	-	530
Nitromethane	-	-	+	50
1-Pentanol	-	-	+	A*
1-Propanol	-	-	+	A*
2-Propanol	-	-	+	A*
Propyl acetate	-	-	+	A*
Pyridine	-	+	-	200
Sulfolane	-	+	-	160
Tetrahydrofuran	-	+	-	720
Tetralin	-	+	-	100
Toluene	-	+	-	890
1,1,1-Trichloroethane	+	-	-	1500
1,1,2-Trichloroethane	-	+	-	80
Xylene	-	+	-	2170

A* the concentration limit of 5000 ppm would be acceptable.

3.4 Synthetic intermediates and by products

Impurities in pharmaceutical compounds or a new chemical entity can originate during the synthetic process from raw materials, intermediates and or by-products. The raw materials are relatively manufactured to much lower quality requirement than a drug substance. Hence, it is easy to understand why they can contain a number of components that can turn affect the purity of drug substances. During synthesis of product having chances to generate impurities, because number of reactions can occur concurrently. Be remembered that base to salt or acid to salt conversion could also generate new impurities [57]. For example, In the synthesis of ethynodioldiacetate in final step is diacetylation of ethynodiol, during reaction reactivity of secondary 3-hydroxy group is much higher than that of tertiary 17-hydroxyl a impurity is formed (ethynodiol-3-acetate). In the synthesis of pipecuronium bromide (2β, 16β-bis-(4-dimethylpiperazino)-3α,17β-diacetoxy-5α-androstane dibromide) is diacetylation of 3α,17β-hydroxy derivative and impurity is formed 17β-monoacetyl derivative [65]. Impurity profiling experiment on ecstasy tablets by GC-MS, and MDMA (3, 4-methylenedioxy-methamphetamine) samples showed impurities in intermediates *via* reductive amination route [66-68].

3.4.1 Impurities originating from the starting material of the synthesis

Presence of impurities in the starting materials of the drug synthesis can also be sources of impurities in the drug materials which includes appearance of the isomeric 4-trifluoromethyl impurity in 3-trifluoromethyl-α-ethylbenzhydrol (flumecinol) is a consequence of the presence of 4-trifluoromethyl bromobenzene impurity in 3-trifluoromethyl bromobenzene which is starting material of synthesis [69].

3.5 Formulation related impurities

Many impurities in a drug product can obtain from excipient used to formulate a drug substance. A drug substance is exposed to a variety of conditions in the process of formulation that can cause its degradation or have other unwanted reactions.

The excipient can sometimes interact with the main ingredient to produce an undesirable product that does not have the same bioavailability. The interaction product for all practical purpose is considered asimpurity [70]. Solutions and suspensions are intrinsically prone to degradation due to hydrolysis or solvolysis. Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was recalled in the United States due to degradation/impurities leading to sub potency. In general, liquid dosage forms are quite susceptible to both degradation and microbiological contamination [71].

3.6 Impurity arising during storage

A number of impurities can originate during storage of drug products. It is essential to carry out stability studies to predict, evaluate, and ensure drug product safety [72].

3.7 Method related impurities

A known impurity, 1-(2, 6-dichlorophenyl) indolin-2-one is generated in the manufacturing 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 intra-molecular 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 [65] [73].

3.8 Mutual interaction amongst ingredients

In vitamins, mutualinteraction amongst ingredients is very labile and on aging they generate a problem of instability in different dosage forms, particularly in liquid dosage forms. Degradation of vitamins does not give toxic impurities; but, potency of active ingredients drops below Pharmacopoeial limits. 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 [74]. 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, benzyl alcohol were explored and similar mutual interactions causing degradation were observed [75].

3.9 Functional group related degradation

There are many ways by which functional group related degradation takes place *viz*hydrolysis, ester hydrolysis, and oxidation and photo cleavage. In the ester type of drugs in liquid dosage form undergoes hydrolysis. The most common examples include procaine, chloramphenicol, atropine, methyl phenindate, benzyl penicillin, oxazepam and lincomycin [76].

Cocaine undergoes hydrolysis to produce benzylecogonine methyl ester [77]

Lactones, cyclic esters pilocarpine undergoes hydrolysis due to ring opening [78]

Ester hydrolysis is one of the prominent mechanisms for the degradation of drugs. It can depicted using some drugs *viz*Aspirin [79],cephotaxime [80], ethyl paraben, benzocaine [81].

Photolytic cleavage

It has been observed that several times pharmaceutical products are exposed to light in many conditions such as manufactured as solids or solutions and packaged. Also,occasionally it is exposed to light in pharmacy shops or hospitals pending use, or held by the consumer pending use [82].

Oxidative degradation

The oxidative degradation is generallytakes place in the organic compounds possessing hydroxyl group, conjugated dienes, heterocyclic aromatic rings, nitroso and nitrile derivatives and also in aldehydes [83]. A variety of organic medicinally vital compounds containing hydroxyl group *viz* Catecholamine, Morphine, Ergometrine, Nifedipine, Nitroprusside, Phenothiazine are very liable to photo-oxidation undergoes oxidation [84]. Some compounds such as Vitamin A and Unsaturated Free acid under conjugated dienes class demonstrate oxidation when exposed to light. Compound like hydrocortisone, Methotrexate also illustrate oxidation when exposed to light [85].Epinephrine undergoes oxidation to form more color compound known as adrenochrome [86].

The oxidative degradation of drug substance involves and electron transfer mechanism to form reactive anions and cation; for example amines, sulfide, and phenolsare susceptible to electron transfer oxidation to give n-oxide, hydroxylamine sulfones and sulfoxide [87].

The functional group with labile hydrogen like benzoyl carbon, allylic carbon andtert carbon or alpha position with respective hetero atom is susceptible to oxidation to form hydrogen peroxides or ketones [88].

Inorganic Impurities

Inorganic impurities include the impurities as residues of metal catalysts.

3.9.1 Impurity originating from catalysts

The employment of homogeneous catalysts may lead to create rarely occurring impurities in which catalyst is incorporated *viz* Tosylation of prednisolone at 21 position catalyzed by pyridine in synthesis of Mazipredone. Impurity in intermediate prednisolone-21-tosylate was found to be quaternary 21-pyridinium derivative [89].

3.9.2 Specific limits for Residues of Metal Catalyst

As per the European Medicine guidelines, maximum acceptable limits of residues in drug substance and excipients have been recommended. Theguidelines have clearly stated objectivesgiving emphasis on safety of the patient. Therefore, maximum acceptable metal residues arising from the use of metals as catalysts or reagents in the synthesis of drug substances and excipients are given. Basically, there is no therapeutic benefit from residual metals [90].

Metals are assess for their potential risk to human health andgrouped as **Class 1** metal (metal of Significant safety concern), **Class 2** (Metal with low safety concern) and **Class 3** (Metal with minimal safety concern). The classification of residues of metal catalyst on the basis of their safety concern is given in **TABLE 6** [91].

Table 6: Classification of residues of metal catalyst on the basis of their safety concern.

Metal	Class I	Class II	Class III
Chromium	+	-	-
Copper	-	+	-
Iridinium	+	-	-
Iron	-	-	+
Manganese	-	+	-
Molibidnum	+	-	-
Nickel	+	-	-
Platinum	+	-	-
Rhubedium	+	-	-
Vanadium	+	-	-
Zinc	-	-	+

4. Forced Degradation Studies and Impurity Profiling

Forced degradation studies are anticipated to generate vast amount of data in connection for identification of potential degraded products, pathway for degradation and intrinsic stability of drug molecule [92]. Forced degradation studies are normally carried out by exposing drug to hydrolytic, oxidation, photolytic and thermal conditions [93, 94].The hydrolytic study is carried out under acidic and basic conditions. In Oxidation conditions, hydrogen peroxides are extensively exercised**.** The oxidative degradation of drug substances involves an electron transfer mechanism to form reactive anions and cations [95]. Functional group with labile hydrogen like benzylic carbon, allylic carbon, and tertiary carbon or α-positions with respect to hetero atom is prone to oxidations. Photo stability studies are carried out to create mainly primary degradants of drug substances by exposure to UV or fluorescent conditions. Drug substances are exposed to a minimum of 1.2 million lx and 200 Wh/ m^2light. Some of the functional group *viz* carbonyls, nitro aromatic, N-oxide, alkenes aryl chlorides weak C-H and O-H bonds, sulfides and polyenes are photosensitive [96].

Thermal degradations are studied by exposing drug to dry heat and wet heat.

It may possible to carry out thermal degradation by enhancing the temperature for shorter duration of time. The steps involved in stability-indicating method include sample generation, method development and optimization, method validation [97].

It also includes validation of forced degradation studies carried out with intention;

- To developed and validated a stability-indicating method
- To establish degradation pathway of drug substance and drug products
- Toisolate and identify impurity related to drug substance or excipient
- To understand chemistry of drug molecule
- To generate degradation profile as per ICH condition and
- Toresolve stability related problems.

The stress conditions required for force degradation are shown in **TABLE 7** [93].

Table 7 : Stress Condition.

Parameters	**Acid/Base**	**Oxidative**	**Light**	**Temperature**	**Temperature/Humidity**
Conditions	0.01 to 0.1N	0.3%H_2O_2	1200 Lux h	10ºC to 70ºC	10 º C to 70 º C and 60 to 90 r h
Drug Substance	1-7 days	Few hours to 7 days	>48h	up to 2 weeks	up to 2 weeks
Drug Product	24 to 48h	24 to 48h	>48h	up to 3 weeks	up to 3 weeks

The analysis of five functional groups reports for an approximately 70% of the alerting structures found in the degradants within the record: (1) aldehydes; (2) unsaturated carbonyls; (3) aromatic amines, hydroxylamine and its derived esters; (4) epoxides; and (5) polyaromatic hydrocarbons. The second phase of the analysisinvolved categorizing the major chemical reactions responsible forthe generation of the five most prevalent alertingstructures. Thistwo-step approach led, in turn, to a proposal for the prediction offunctional groups that may have a propensity to degrade toalerting structures not necessarily present in the parent molecule [98-101].

5. Analytical Method for identification of Impurity

It becomes obligatoryto isolate and characterize impurities in order to monitor them accurately, because approximate estimations of impurities are generally made against the material of drug substance and can be uncorrected. These estimations are based on the assumption that impurities are structurally related to the material of interest and thus, have the same detector response. Number of methods will be of significance for isolation and characterization of impurities [102].

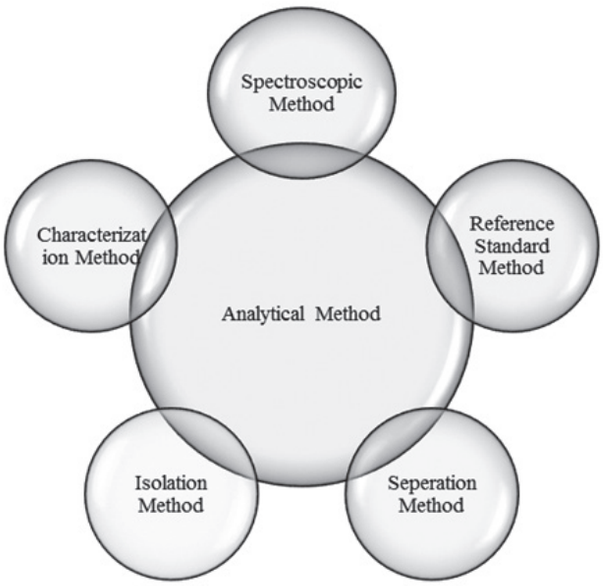
The impurity can be identified various analytical methods as shown in **FIGURE 2**. Many analytical techniques are used for separation of impurities and characterization of it [103- 106].

5.1 Techniques for separation and characterization of impurities [107-109]

A general scheme is set up for the estimation of the impurity profile of

bulk drug substances by the multifaceted use of chromatographic, spectroscopic and hyphenated techniques. Huge number of examples reported are showing the use of chromatographic methods such as Thin-Layer Chromatography (TLC), Gas-Chromatography (GC), analytical and preparative High-Performance Liquid-Chromatography (HPLC), Supercritical Fluid Chromatography (SFC), High-Performance Thin-Layer Chromatography (HPTLC), Spectroscopic methods such as Mass-Spectrometry (MS) , NMR Spectroscopy, UV-Spectroscopy etc.Further, hyphenated techniques *viz*LC-MS-MS, LC-NMR, LC-NMR-MS, GC-MS and LC-MS. have found to be of great significance due to its simplicity and rapid way of analysis.

Figure 2: Analytical Method for identification of Impurities.



5.1.1 High-Performance Liquid-Chromatography

HPLC is one of exploited chromatographic technique most abundantly referred to identify and separation of impurities. Drug compound were identified on the basis of retention time and direct comparison with known standards. For isolation and identification impurities by HPLC requires column, optimized system and suitable mobile phase system. Specialized instruments are used to carry out HPLC in the preparative mode. Most explored analytical method for separation of impurities includes HPLC. The drugs along with their separated impurities by HPLC are shown in **TABLE 8** [110- 145].

Table 8: HPLC method for separation of impurities.

Drug(s)	Solvent/ mobile phase	Impurities	References
Aalicylic acid and betamethasone dipropionate	Methanesulfonicacid:ACN (0.05%)	Salicylic acid related: 7-betamethasonedipropionate	111
Almotriptan malate	Sodium phosphate buffer : ACN (80:20)	3 impurities	104
Alogliptinbenzoate	Gradient System A – 0.1% Perchloric acid (pH adjusted 3.0 with triethylamine) B – Acetonitrile	9 impirities	132
Atomoxetine hydrochloride	Ortho –Phosphoric acid, Octanesulfonicacid :n-Propanol	Phenyl methylaminopropanol and mandelic acid	105
Atorvastatin calcium	ACN:CH_3COOH_4 Buffer gradient	Diastereomer-atorvastatin (DSAT) Desfluoro-atorvastatin (DFAT)	106, 107,137
Bendazac lysine	Acetonitrile: aqueous buffer (1.0 ml glacial acetic acid in 1000 ml Water) (47:53)	2 impurities	133
Bosentan	Gradient system A – Acetonitrile and Ammonium acetate (pH 3.3) (27:73) B – Methanol and ammonium acetate (pH 4.5) (70:30)	4 impurities	136

Table 8: Continued

Drug(s)	Solvent/ mobile phase	Impurities	References
Cephotaxime	Buffer:Methanol: Water (80:15:05)	5 impurities	119
Clopidogrel	Eluent A:ACN: potassium phosphate buffer (20:80); Eluent B: ACN: potassium phosphate buffer (pH 2.3; 10 mM) (80:20);	5-[1-(2-Chlorophenyl)-2- methoxy-2-oxoethyl]-6,7- dihydrothieno[3,2-c] pyridin-5-ium	108
Dapoxetine	Methanol and Water with 0.1% formic acid	1 impurity	127
Econazole Nitrate	MeOH: H_2O; gradient	4-Chlorobenzyl alcohol and α-(2,4-dichloro-phenyl) -1 H -imidazole-1-ethanol)	109
Efavirenz	Water Acetonitrile and Methanol	5 impurities	128
Ertapenam	A- 0.1 N Buffer in water(pH 8) B- ACN Gradient profile	4 impurities	122
Ezetimibe	A- Orthophosphoric acid in water B- Acetonitrile and water (50:50)	2 impurities	124
Flupirtine maleate	A – 0.02 M Ammonium dihydrogen phosphate buffer (pH 5.5 with phosphoric acid) B – Acetonitrile (60:47)	4 impurities	131
Ibuprofen	ACN:phosphate buffer (25 mM, pH 2.1) (40:60)	3-[4-(2- Methylpropyl)phenyl] propanoic acid	112
Icofungipen	ACN:H_2O (25:75)	(1R,2S)-2-(Cinnamyl amino) -4-methylene cyclopentane carboxylic acid	113
Irbesartan	gradient mixture of solvent A (0.55% v/v ortho-phosphoric acid, pH adjusted to 3.2 with triethyl amine) and B (95:5 v/v mixture of acetonitrile and solvent A)	4 impurities	129
l -Aspartic acid and l –alanine	MeOH:H_2O (50:50)	Succinic acid, citric acid,malic acid, maleic acid, fumaric acid, glycine, glutamic acid	118
Meglumine	A – 0.1 % Formic acid in water B – 0.1 % formic acid in 90:10 Methanol : Acetonitrile	2 impurities	135

Drug	Solvent/ mobile phase	Impurities	References
Nevirapine	Gradient System A – 0.1 % Formic acid B – Acetonitrile with 0.1% Formic acid	4 impurities	134
Omlesartan	Phosphate buffer: Acetonitrile	Omlesartan acid	102
Paclitaxal	H_2O:ACN (52:48)	10 Deacetylbaccatin III, baccatin III, 10-deacet-yl-7-xylosyltaxol C, photo-degradant, taxol C, ceph-alomannine, 10-deacetyl-7-epitaxol, 7-Epi-taxol	110
Phenazopyridine	H_2O:ACN (25:75)	3-Phenyl-5-phenylazo- pyridine-2,6-diamine	114
Pridinol Mesylate	Potassium phosphate buffer :MeOH:2- propanol	3-Piperidino-propiophen- one, hydrochloride, 1-(3,3-diphenylprop-2-en- 1-yl)piperidine	115
Retigabine	Acetonitrile and water	2 impurities	125
Rizatriptan benzoate	Ammonium dihydrogenortho-phosphate (20 mM) + 2 ml TEA (pH 2): ACN; gradient	Rizatriptan-1,2-dimer and Rizatriptan-2,2-dimer	116
Ropinirole HCl	ACN:sodium heptane sulfonate (5 mM) (21.6:78.4) (pH 2)	4-[2- (Dipropylamino)ethyl]- 1 H -indol-2,3-dione	117

Table 8: Continued

Drug	Solvent/ mobile phase	Impurities	References
Sumatriptan succinate	Acetonitrile:Methanol	4 impurity	103
Temozolomide	Graadient system A – water with 0.5% acetic acid B - Acetonitrile	3 impurities	130
Tolperosone	Buffer (0.01 M potassium dihydrogen phosphate pH 8) and ACN gradient system (50:50)	4 impurities	123
trans-resveratrol	A- sodium dihydrogen orthophosphate dehydrate in water B- Acetonitrile	5 impurities	126
Trimethoprim	gradient elution A triethylamine (0.25%) and formic acid (1.1%) in water (pH5.8) elution B Acetonitrile	2 impurities	121
Verenicilline	Ammonium acetate buffer:Methanol (70:30)	4,6,7,8,9,10-hexahydro-1H-6,10-methanopyrazino[2,3-h][3] benzazepine-2,3- dione.	120

5.1.2 Gas-Chromatography

Gas-Chromatography is a very imperative technique for quantitation. It can provide the desired resolution, selectivity, and ease of quantitation. 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. The drugs and their impurities separated by GC are given in **TABLE 9** [146]

Table 9: Gas Chromatography.

Drug	Mobile phase/Solvent	Impurity	References
Cloxacillin	Cyclohexane	N,N dimethyl aniline	3
Doxorubicin hydrochloride	Dioxane	Acetone and Ethanol	2
Fluorescence sodium	Methanol	Dimethyl formamide	2
Methamphetamine	n-Hexane and Phosphate buffer	1,2-dimethyl-3- phenylaziridine, ephedrine, methylephedrine, N- formylmethamphetamine, N- acetylmethamphetamine, N-formylphedrine, N- acetylephedrine,N,O- diacetylephedrine, methametamine dimmer	138

5.1.3 UV-Visible Spectroscopy

The use of UV-VIS spectroscopy as a tool for the identification and structure elucidation of impurities in drugs without chromatographic separation is of very modest relevance. This method is useful only impurities absorbs specifically in the ultraviolet region above 200 nm. UV-Visible is a technique useful in identification of pure drug compounds. Compounds containing chromophores absorbs specific wavelength of ultraviolet or visible light that is directly related to the concentration of the sample. The identification of impurity by UV-Visible spectroscopy methods are shown in **TABLE 10** [147].

Table 10: UV-Visible Spectroscopy method.

Drug	Mobile phase/Solvent	Impurity	References
Amphotericin B	Dimethyl Sulphoxide and methanol	Tetraenes	3
Atropine sulphate	Methanol	Apo atropine	3
Dextrose	Water	5 hydroxyl methyl furfural	2
Mercaptopurine	Dimethyl sulphoxide and 0.1 M HCl	Hypoxanthine	1
Norgestrel	Ethanol	3,17α-diethinyl-13-ethyl-3,5-gonadiene-17-ol	139

5.1.4 FT-IR Spectroscopy

IR is infrequently used technique for analysis of impurities. FT-IR spectrometry can be functional to resolve the presence or absence of chemically related impurities in raw pharmaceutical substances if their chemical structure is known and are found above as assured limits of percentage in the substance. Impurities in statins such as Atorvastatin and Simvastatin are reported to be analyzed by FT-IR spectroscopy [148].

5.1.5 Capillary Electrophoresis

CE is a practical technique when very low quantities of sample are available and high resolution is required. The primary complexity is relatively lower reproducibility. The separation of impurities from drug by capillary electrophoresis given in **TABLE 11** [149-157].

5.1.6. Hyphenated techniques

The most advent form of analytical techniques includes hyphenated technique such LC-MS, GC-MS, CE-MS [158-168] have also been reported. The separation and characterization of impurities by hyphenated techniques, given in **TABLE 12.**

5.1.7. Supercritical fluid Chromatography

Orthogonal separations using SFC have been explained as recent tool in impurity profiling of pharmaceuticals [169].

The use of SFC as a technique for drug impurity profiling was studied to define starting conditions in method development for drug impurity profiling. A set of dissimilar stationary phases was screened in parallel. The possibility to select a set of dissimilar columns using the retention factors (*k*-values) for a set of 64 drugs were measured on 27 columns [170].

The comparison of ultra-high performance methods in liquid chromatography and supercritical fluid chromatography coupled to electrospray ionization- mass spectrometry for impurity profiling of drug candidate have been reported [171].

Table 11: Capillary Electrophoresis.

Drug	**Impurity**	**References**
Alcuronium	Diallylcaracurine (DAC), Monomeric allyl-Wieland-Gumlich-aldehyde (WAG)	149
Ceftazidimepentahydrate	Anti-isomer of ceftazidime, 7-epimer of ceftazidime, 3-methylidene compound	144
Cephotaxime	6 impurities	146
Cephradine	Cephalexine	145
Fluvoxamine maleate	an addition product (adduct) and fluvoxketone (ketone)	142
Gentamicin Sulphate	Geramine, Paromamine, 2-deoxystreptamine	143
Lincomycin	Lincomycin B	147
Meclophenoxate	N,N-dimethyl ethanolamine	141
Minocycline	4-epiminocycline, 6-deoxy-6-demethyltetracycline, 7-didemethylminocycline, 7-monodemethylminocycline, 9-minocycline	148

Table 12: Hyphenated Techniques in Impurity Profiling of Drugs.

Drug	**Method**	**Mobile phase**	**Impurity**	**References**
Deferesirox	HPLC-UV	Water:Methanol	Deferesirox A and B	150
Dup941	LC-UV-Diode array	Acetonitrile:Water:Trifluoro acetic acid	PC , SL, LS	151
Salicylaldehyde-isonicotinoyl-hydrazone	LC–ESI-MS	Phosphate buffer:Methanol (40:60)	2-Hydroxy-acetophen- one, Isonicotinoylhydrazone, 2-hydroxy-propiophenoneIsonicotinoylhydrazone	152
Trinitrotoluene	GC-MS	Nitrogen gas	2,4-dinitrotoluene	1
Norethisterone	ESI/MS detection	MeOH:H_2O (53:47)	19-Norandrostenedione	153
Capreomycin	LC-MS	CAN and Formic cid	20-N-delysine-20-N-glutamine, 20-N-delysine-36-N-lysine	154
Lumefantrine	HPLC-DAD/UV-ESI/MS	H_2O :CAN	Desbenzylketo derivative	3
Mycophenolatemofetil	LC/DAD LC/MS/MS	ACN:0.015M KH_2PO_4 (28:72 V/V)	Mycophenolic acid	155
Pholcodine	LC-ESI-MS	Conc.Ammonium solution and ACN	Pholcodine A, B, C	156
d-allethrin	GC-FID/MS	Helium	Crysolactone, allethrolone, chrysanthemic acid	157
Saxagliptine	LC-ESI-MS/MS	Gradient System A – aq. Ammonium formate solution B - Methanol	7 impurities	158
Ritonavir	LC-MS/MS	HPLC – Water : Methanol : Acetonitrile (40:20:40) MS – Nitrogen gas	8 impurities	159
Toremifene	LC-MS/MS	HPLC – Methanol and Water (85:15) MS – Nitrogen gas	4 impurities	160

The importance and the challenges of impurity profiling in modern pharmaceutical analysis have been discussed [172]

Evaluation of mobile phase gradient supercritical fluid chromatography for impurity profiling of pharmaceutical compounds have been studied. The use of gradient supercritical fluid chromatography (SFC) for the impurity profiling of pharmaceutical products is not widely practiced. Historically, the limited advancement in SFC instrumentation and the lag in column development have resulted in marginal sensitivity, selectivity and reproducibility when compared with high performance liquid chromatography (HPLC) [173].

The quantitative determination of salbutamol sulphate impurities using achiral supercritical fluid chromatography has been studied [174]

5.1.8 Reported Impurities in various Pharmacopoeias

Various pharmacopoeia such EP, BP, USP and IP have incorporated impurity present in drugs and drug products in their monograph is shown in **TABLE 13.**

6. Efficacy guidelines (ICH)

The work explained by ICH under the Efficacy heading is dealt with the design, conduct, and safety and also preparing reports of clinical trial which includes types of medicines derived from biotechnological processes. The data generated from the pharmacokinetics/pharmacogenomics techniques would create better targeted medicines.

Table 13: Reported impurity in different pharmacopoeia.

Drug	**Reported Impurities**	**Category**	**Pharmacopoeia**
Aceclofenac	9	NSAID	EP 2005
Acetyl Salicylic acid	6	NSAID	EP 2005
Acyclovir	8	Antiviral	EP 2005
Allupurinol	5	Treatment Of Gout	EP 2005
Ampicillin	13	Antibiotic	EP 2005
Betamethasone	10	Anti-Inflammatory	EP 2005
Bromhexine hydrochloride	5	Expectorant	EP 2005
Bacitracin	11	Antibiotic	EP 2005
Baclofen	2	Antispastic Agent	EP 2005
Benserazide hydrochloride	3	Antidyskinetic	EP 2005
Captopril	1	Anti-Hypertensive	EP 2005
Carbamazepine	6	Anticonvulsant	EP 2005
Carmustine	1	Anti-Cancer	EP 2005
Cephalexine monohydrate	6	Antibiotic	EP 2005
Cephixime	6	Antibiotic	EP 2005
Diclofenac sodium	5	NSAID	EP 2005
Digoxin	2	Anti-Arrythmic	EP 2005
DiltiazemHCl	6	Anti-Anginal	EP 2005
Disulfiram	2	Antialcoholic Agent	EP 2005
Domperidone maleate	6	Antiemetic	EP 2005
Ebastine	7	Anti-Allergic	EP 2005
Econazole	3	Anti-Fungal	EP 2005
Ephedrine hydrochloride	2	Bronchodilator	EP 2005
Etoposide	14	Anti-Cancer	EP 2005
Fluoxetine hydrochloride	3	Antidepressant	EP 2005
Flutamide	6	Anti-Androgen	EP 2005
Furosemide	5	Diuretic	EP 2005
Gentamicin	5	Antibiotic	EP 2005
Glibenclimide	2	Anti-diabetics	EP 2005
Haloperidol	6	Antipsychotic	EP 2005
Hydrocortisone	7	Topical	EP 2005
Imipenem	1	Anticonvulsant	EP 2005
Itraconazole	7	Antifungal	EP 2005
Ivermectin	11	Anthelmintic	EP 2005
Lorazepam	2	Anxiolytic	EP 2005
Lovastatin	4	HMG-CoA Reductase Inhibitor	EP 2005
Mefenamic acid	2	Analgesic	EP 2005
Metformine hydrochloride	6	Anti-diabetics	EP 2005
Neomycin sulphate	7	Local Antibacterial	EP 2005
Nicotine	5	Cholinergic Agonist	EP 2005
Acamprosate Calcium	1	Anti-alcoholic Agent	IP 2014
Alfacalcidol	5	Active Metabolite of Vitamin D	IP 2014
AlfuzocinHCl	4	Benign Prostatic Hypertrophy Agent	IP 2014

Table 13: Continued

Alprostadil	6	Erectile Dysfunction Agent	IP 2014
Table 13: Continued			
AzelastinHCl	5	Antihistamine	IP 2014
Azethromycin	7	Antibiotic	IP 2014
BetaxololHCl	5	Beta-Adrenergic Blocker	IP 2014
Bezafibrate	5	Lipid-Lowering Agents	IP 2014
Bicalutimide	3	Anti-androgen	IP 2014
Bupivacaine	6	Anesthetic, Local	IP 2014
Cabergoline	4	Prolactin Secretion Inhibitor	IP 2014
Dobesilate	1	Venotropic Agent	IP 2014
Celiprolol	9	Beta-Adrenergic Blockers	IP 2014
Clobetasol	8	Anti-Inflammatory	IP 2014
Dipivefrine	2	Antiglaucoma	IP 2014
Dobutamine	3	Sympathomimetic	IP 2014
Epinastine	2	Antiallergic Agents	IP 2014
Finasteride	3	Alopecia Agent	IP 2014
Fluoxate	2	Antidepressant	IP 2014
Flumazenil	3	Antidote	IP 2014
Ibuprofen	2	Anti-Inflammatory Drugs	IP 2014
Isotretinoin	5	Anti-acne	IP 2014
Lamivudine	6	Anti-Hepatitis Agents	IP 2014
Leflunomide	2	Immune Suppressant	IP 2014
Mepyramine	4	Antihistamine	IP 2014

ICH gives guideline for genotoxicity includes specifically S2A and S2B [175, 176]. The reason of the guideline is to optimize the standard genetic toxicology battery for prediction of potential human risks, and to provide guidance on interpretation of results, with the ultimate goal of improving risk characterization for carcinogenic effects that have their basis in changes in the genetic material. The threshold for impurities as per ICH guideline is given in **TABLE 14.**

Table 14: Threshold [8].

Maximum daily dose	**Reporting Threshold**	**Identification Threshold**	**Qualification Threshold**
< 2g/day	0.05%	0.1% or 1 mg per day intake	0.15% or 1 mg per day intake
> 2g/day	0.03%	0.05%	0.05%

6.1 Genotoxic impurities and Principle of genotoxicity [177,178]

The possibility for mutagenic effect and also damaging DNA by genotoxic impurities and chemical compounds cannot be denied.The genotoxicity tests are performed as *in vitro* and *in vivo*tests. Usually, these tests are proposed to identify compounds that create genetic damage by several mechanisms [179]. These tests are meant for identification of hazards in concerned with damage to DNA and also its fixation. Damage to DNA can be manifested by several ways *viz* gene mutations, larger scale chromosomal damage or recombination. It can further be studied for heritable effects and in the multi-step process of malignancy. The entire process to understand is complex in which genetic changes might possibly play only a part [180]. There will be a change in chromosomal number which may be linked up with be linked up with tumor genesis.Carcinogenicity can be predicated by genotoxicity test [181].It may possible to identify the potent mutagenic impurities in drug substance when it is checked for conventional mutagenicity investigation. The details regarding the same are explained in ICH S2 guidance on Genotoxicity Testing and Data Interpretation.

The safe doses for chemicals/ solvents can be calculated virtually using various QSAR based software *viz* MDL, DS TOPKAT, Tox boxes, Leadscope toxicity *etc*. The virtual safety doses for Acrylonitrile (7.6 µg/day), 2- Amino-4-nitrophenol (1007 µg/day), Nitrobenzene (31 µg/day) were calculated and matches with the carcinogenic safety dose limits studied in animals. From, this investigation, it can be concluded that the *in silico* determination of structural liabilities for mutagenicity provides a highly sensitive and conservative method for identification of potentially genotoxic impurities [182]. In absence of sound literature on additional genotoxicity testing of impurity should be considered typically using bacterial reverse mutation assay (Ames test) [183- 185]

The recent year's new strategies arise for dealing with genotoxicity impurities or potential genotoxicity impurities arising from drug synthesis have gained considerable focused.These genotoxic impurities may be crept in as starting material, reagent, intermediate catalyst, by-product, and degradation product or isomer *etc* [186].

6.2 Safety profile of impurity

As per the guideline of various pharmacopoeia and ICH, the main objective is to suggest acceptable amounts for impurities in pharmaceuticals for the safety of the patient. The guideline describes levels considered to be toxicologically acceptable for some residual solvents.

The phrase tolerable daily intake (TDI) is referred by the International Program on Chemical Safety (IPCS) to illustrate exposure limits of toxic chemicals. And, the phrase acceptable daily intake (ADI) is used by the World Health Organization (WHO) and other national and international health authorities and institutes. The current phrase permitted daily exposure (PDE) is defined in the present guidance as a pharmaceutically acceptable intake of residual solvents to avoid confusion of differing values for ADI's of the same substance [187].

Genetic toxicology studies are reported in *Salmonella typhimurium*, Chinese hamster ovary cells, Drosophila melanogaster, mouse bone marrow cells and mouse peripheral blood cells. The *in vitro*studies can be conducted

with and without exogenous metabolic activation from induced S9 liver enzymes [188].

6.3 Environmental Regulation of Organic Volatile Solvents

Several of the residual solvents often used in the manufacturing of pharmaceuticals are listed as toxic chemicals in Environmental Health Criteria (EHC) monographs and the Integrated Risk Information System (IRIS) [175,176] [189, 190].The goal of such groups as the International Programme on Chemical Safety (IPCS), the U.S. Environmental Protection Agency (EPA), and the U.S. Food and Drug Administration (FDA) comprise the estimation of acceptable exposure levels [191]. The aim is safe guarding human health and maintenance of environmental integrity against the possible harmful effects of chemicals generating from long-term environmental exposure. The methods implicated in the estimation of maximum safe exposure limits are generally depends on long-term studies [192].

7. DISCUSSION AND CONCLUSIONS

The present review article provides a viewpoint on impurities in drug substance and drug products. It furnishes valuable information about impurities types, its classification, various techniques for isolation and characterization, and also for the determination, qualification of impurities. This knowledge can create ample information about bulk drugs, drug product and guidance for its storage. Further the article furnishes information regarding the virtual safety limits for solvents and chemicals. Preliminary information about the Genotoxity studies, guidelines and principle is more relevant information described. Over all discussion provided above about impurity profiling and several associated issues would be of general and broader interest.

8. Future Prospects

The various regulatory bodies have outlined guidelines with regarding to safety and efficacy of impurities but there is a strong requirement to have unified specification/ standards for regulation of impurities.

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