ISSN: 0975-766X
CODEN: IJPTFI
Review Article

Available through Online
www.ijptonline.com

PHARMACEUTICAL IMPURITIES -SIGNIFICANCE OF IMPURITY PROFILING

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Received on 06-01-2012 *Accepted on 16-01-2012*

Abstract

Tracking impurities is a crucial task for all stages of development of drug substances and drug products in order to assure quality. Monitoring of impurities is mandatory since they affect QSE of product and at times they may be mutagenic or carcinogenic or teratogenic.It is acknowledged that presence of trace amounts of impurities is inevitable, and impurity specifications need to be established. ICH instructs workable guidelines to drug developers and regional agencies on how to evaluate and control impurities in drug substances and drug products. Orthogonal analytical approaches for impurity investigations are done to provide a complete understanding of drug substance impurity profile. The review outlines types, potential sources of impurities, impurity profiling, isolation and characterization using analytical techniques.

Keywords: Impurities, International Conference on Harmonization, Formulation, Profiling, Isolation, Selective analytical methods.

1. Introduction

An impurity according to Webster's dictionary is something that is impure or makes something else impure.ICH defines impurities as "substances in the API that are not the API itself". For pharmaceutical products, impurities are defined as "substances in the product that are not the API itself or the excipients used to manufacture it". Impurities affect quality, safety, and efficacy (QSE) of the product, which causes serious health hazards[1] and sometimes they can be mutagenic, teratogenic or carcinogenic. The source of impurities in API's are starting materials[2,3] by-products

and residual solvents used during its synthesis; degradation products formed on aging or during process; contaminants from packaging material[4.]

In generics the source and route of formation of impurities have to be carefully monitored [5,6.] Presence of impurities in trace amounts in drug product or drug substance is inevitable, and so their levels should be carefully controlled[7] and monitored which requires impurity profiling (Identification, Isolation, and characterization).As drug safety receives more and more attention from the media and the public, impurity profile of pharmaceuticals is of increasing importance. This topic is addressed by several books [8-11] and journal reviews[12-23] and guidelines are specified by official bodies[24] and legislation (pharmacopeia and ICH guidelines) .ICH has published guidelines for analysis of impurities in new drug substances[25] new drug products[26]residual solvents[27] and microbiological impurities [28,29.]

From pharmaceutical companies and regulatory authorities there is a significant demand for impurity-reference standards; accordingly a company named Mikromol GmbH (luckenwalde, Germany) deals with only impurity-reference standards, has started marketing impurities found in pharmaceuticals through Promochem Group (Wesel, Germany).When a company files Investigational New Drug Application(IND) or Abbreviated New Drug Application (ANDA), there is much emphasis on purity requirements; the limits and threshold values of impurities should comply with those as specified in various regulatory authorities. Isolation and identification of impurities is done using HPLC, MS, NMR, FT-ICR-MS and specifically for identification of residual solvents 'GC' is most preferred technology. For determination of inorganic impurities ICP-MS [18] is most widely used. Impurity profiling has achieved more significance in drug developing and processing. [16,30]

2. Classification

Impurities in drug substance can be classified into following categories [25.]

2.1 Organic impurities

Starting materials

Intermediates

Process-related impurities

Degradation products.

2.2 Inorganic impurities

Salts

Ligands

Catalysts

Heavy metals or other residual metals

2.3 Residual solvents

As per ICH guidelines, organic and inorganic solvents used in manufacturing of drug substances classified into four types : [27,31,32]

Class-I (to be avoided): class-I solvents and their permissible concentration limits given in table-4. Because of their unacceptable toxicity they are not used, if their use is unavoidable, it should be restricted.

Class-II: (should be limited): usage should be limited because of their inherent toxicity.Class- II solvents with their daily permissible exposure is listed in table-5.

Class-III: They have less toxic potential and possess lower risk when compared to class I or II and permitted daily exposure (PDE) of 50 mg or more.

Some solvents are: acetic acid, anisole, butyl acetate, butanol, methyl acetate, isopropyl acetate, pentene, dimethyl sulfoxide, ethyl acetate, ethanol, formic acid, isobutyl ketone, 1-pentanol, heptane, methyl ethyl ketone, 2-propanol, methyl isobutyl ketone.

Class-IV: Adequate toxicological data is not available. Class- IV solvents are 1, 1-dimethoxy propane, 1,1-diethoxy propane, 2,2-dimethoxy propane, isooctane, methyl isopropyl ketone, isopropyl ether ,petroleum ether, methyl tetra hydro furan, trichloro acetic acid.

2.4 Other materials

Charcoal

Filter aids

3. Source of impurities

Impurity sources are broadly classified into three categories

- Synthesis related impurities

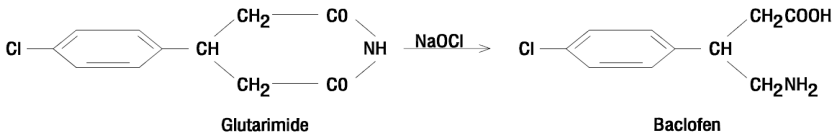
- Impurities related to formulation
- Impurities formed on aging

3.1 Synthesis related impurities: Impurities in pharmaceutical compounds or a new chemical entity (NCE) arises mainly during the synthetic process from raw materials, intermediates, solvents, by-products.

3.1.1 Organic impurities

Organic impurities[33,34] may be identified or unidentified and arise during synthesis of drug substance or during its storage.

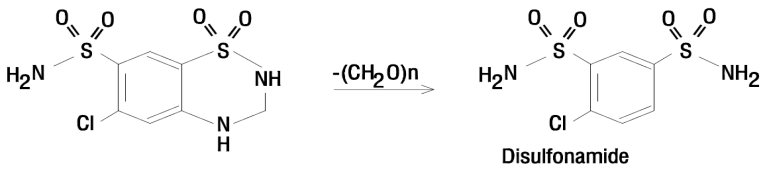
Starting materials or intermediates: In a multi step synthesis, although proper precautions are taken in every step, these are the most common impurities. Although utmost care is taken by washing end products with the solvents, there are always chances of presence of unreacted starting materials. In the synthesis of Amlodipine Besylate traces of 4-(2-chlorophenyl)-3-ethoxy carbonyl-5-methoxycarbonyl-6-methyl-2-[(2-pthalimidoethoxy)methyl]P-1-4-dihydroxypyridine is present as impurity[35.] In Baclofen synthesis, *p*-chloro phenyl glutaric acid is a potential impurity, which is formed as a result of interaction of β-(*p*-chlorophenyl) glutarimide with sodium hydroxide/sodium hypochlorite, at room temperature .



Glutarimide Baclofen

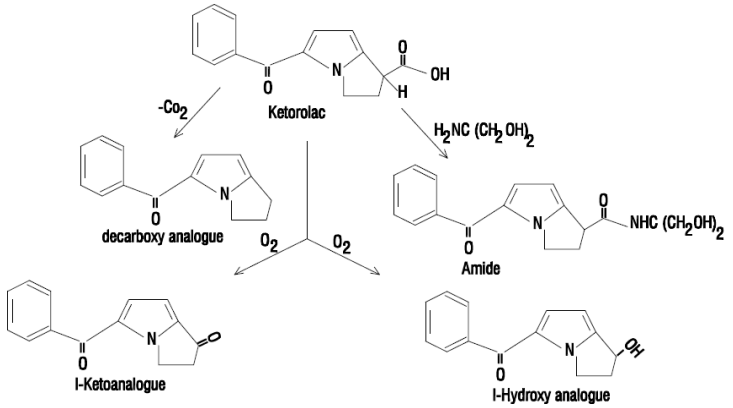
Degradation Products: Degradation of end products often results in formation of impurities in bulk drug manufacturing. Impurities arise from synthesis or due to storage or aging [36.]The presence of a β-lactam ring and amino group in the c_6/c_7 side chain plays a critical role in formation of degradation related impurities in penicillin's and cephalosporin's [37.]

In the synthesis of hydrochlorthiazide it is degraded to disulfonamide [17]

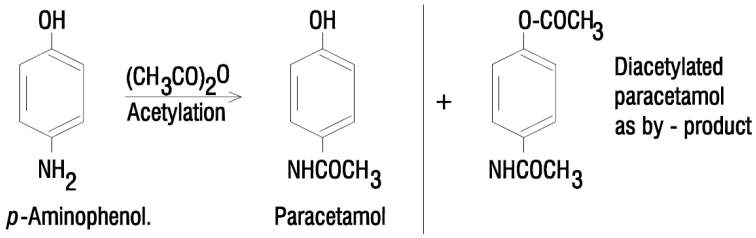


Hydrochlorothiazide Disulfonamide Degradation product

Degradation pathway of ketorolac in solid and solution state



By products:-It is seldom possible to get a single end product with 100% yield in synthetic organic chemistry, as there is always a chance of having by-products. By products can be formed due to variety of side reactions like incomplete reaction, over reaction, dimerization, isomerization, or due to unwanted reactions between starting materials or intermediates with catalysts or chemical reagents [38.]



Diacetylated paracetamol may form as by product in paracetamol bulk production [39]

3.1.2. Inorganic impurities

Inorganic impurities may derive from manufacturing process and excipients used in production of desired formulation. These impurities can be known and identified and include the following: Reagents, Ligands and Catalysts: These are present from negligible to significant amounts in API; in some cases they may create a problem unless manufacturers take proper precautions duringproduction. Heavy metals: The main sources of heavy metals are water used in process and the reactors (if they are made of stainless steel).These can be avoided by using demineralised water and glass-linedreactors.

Usually, excipients contain high levels of heavy metals such as Arsenic, Copper, Bismuth, Sodium, Chromium, Cadmium, Mercury, Iron and Lead.

3.1.3. Residual solvents

As per ICH guidelines, the solvents used in manufacturing of drug substances classified into four types. Solvents with their permissible daily exposure limits are present in table 4 and 5 are according to ICH guidelines.

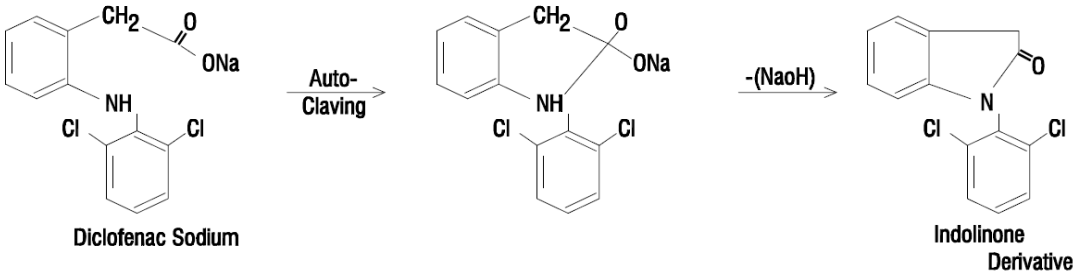
3.2 Impurities related to formulation

The formulated form of API may contain impurities that are resulted due to drug excipient interaction.

3.2.1 Method related impurities

In production of parenteral dosage form of Diclofenac sodium, 1-(2, 6-dicholrophenyl) indolin-2-one is formed as impurity. This impurity formation depend on initial pH of preparation and sterilization condition i.e. autoclaving at 123 ± 2^0 c enforces intramolecular cyclic reaction of

diclofenac sodium forming sodium hydroxide and indolinone derivative[40.]



3.2.2. Dosage form related impurities:-

In case of dosage forms like solutions, the impurities can be significantly noticed; as they are very much susceptible to both degradation and microbiological contamination. Due to various factors like pH, environment or leaching, precipitation of main ingredient may occur. e.g. pH alteration of lidocaine HCl solution in presence of 5% dextrose in saline or normal saline solution. Precipitation of imipramine HCl with sodium bisulfite is reported [38].Microbiological contamination may occur in liquid dosage forms due to growth of fungi, yeast and bacteria or due to inappropriate use of certain preservatives in multi-dose containers [41.] Dosage form factors influenced Teva

pharmaceuticals, USA, Inc., Sellersville, Pennsylvania to recall 60 ml bottles of 0.05% Flucinonide topical Solution USP, from the market due to degradation impurities leading to sub potency [17,42.]

3.2.3 Environment related impurities

Light: It causes degradation of photosensitive formulation. Sunlight having about 8000 foot-candles destructs vitamin B_{12} to about 34% in 24 hours [43.] Ergometrine and Methylergometrine injection is unstable under tropical conditions such as heat and light, and in many field samples very low level of active ingredient was found [44-46.]

Temperature: Certain classes of compounds such as vitamins and antibiotics are heat labile, so extreme care should be taken to prevent these formulations from degradation.

Humidity: In case of hygroscopic compounds (both bulk powder and formulated solid dosage form) humidity is a key factor. High humid conditions causes loss of potency of aspirin and Ranitidine [30.]

3.3 Impurities formed on aging

3.3.1 Mutual interaction among ingredients

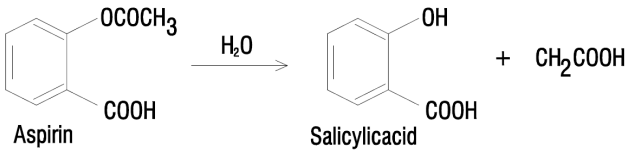
Most vitamins especially in liquid dosage forms are very labile and on aging they create a problem of instability 43. In case of multi-vitamin formulation containing four vitamins (nicotinamide, thiamine, riboflavin, pyridoxine) within 1-yearshelf life degradation of thiamine occurs to a sub-standard level due to mutual interactions [47.]

3.3.2 Functional group-related degradation

Hydrolysis: Amides, esters, lactams, lactones, carbamates and imides are more susceptible to acid-base hydrolysis.e.g. Atropine, barbiturates, benzyl penicillin, chloramphenicol, chlordiazepoxide, lincomycin [38.]

Ester hydrolysis:-Ester hydrolysis results in impurities formation and were observed in aspirin, benzocaine, cocaine, cefotaxime, echothiophate, ethylparaben [38,]cefpodoxime proxetil.[48]

Ester hydrolysis of Aspirin

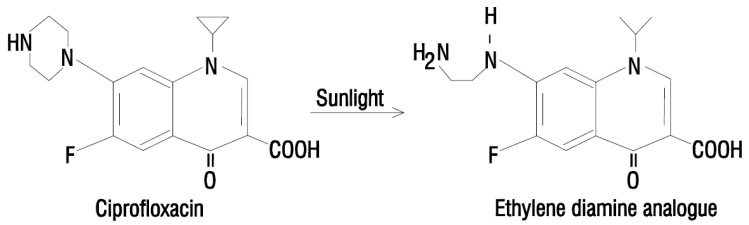


Oxidation:- The most common form of oxidation in pharmaceuticals is auto oxidation through a free radical chain process e.g. Studies on auto oxidation of ascorbic acid reveals that cupric ion known to oxidize ascorbic acid rapidly to dehydroascorbic acid and potassium cyanide which results in cleavage of chain due to formation of copper complexes. In Mazipredone, the results of oxidative and hydrolytic degradation pathway in 0.1M/lit. Hydrochloric acid and sodium hydroxide at 80^0 c were investigated [49.]

Decarboxylation:-Photo reaction of enteric coated rufloxacin tablet which is coated with cellulose acetate phthalate (CAP) and sub-coating with calcium carbonate causes hydrolysis of CAP liberating acetic acid, which yields carbon dioxide on reaction with calcium carbonate. Loss of carbon dioxide occurs when carboxylic acids such as *p*-amino salicylic acid was heated.

Photolysis: - When being manufactured, packaged, held in pharmacopeias or held by hospital or consumer pending use pharmaceutical products are exposed to light. Fluroquinolone antibiotics were susceptible to photolytic cleavage [50-53.] Riboflavin, Nitroprusside, ergometrine [46,] phenothiazines, and nifedipine [54] are liable to photo oxidation, in these compounds photochemical energy creates free radical intermediates, which perpetuate chain reactions.

Sunlight induces photo cleavage reaction of 0.3% ciprofloxacin eye drops preparation producing ethylenediamine analogue of ciprofloxacin [50.]



Ciprofloxacin Ethylene diamine analogue

4. Impurity profiling

The step impurity profiling[55] aims at detection, structural elucidation, identification and quantitative determination of impurities. Each impurity must be investigated with respect to both chemistry and safety aspects. The former include identification (structural characterization), reporting and quantitation (by suitable analytical procedures), where as the later include a process of acquiring and evaluating data concerning biological safety of an impurity (qualification). Driving forces for studying impurity profile are

- Quality considerations
- Regulatory (FDA) requirements

4.1 Samples to be profiled

Active ingredient

Process check (synthesis or formulation)

Final product

4.2 Impurity profile components

Impurity profile refers to description of identified and unidentified existing impurities in chemical drug substance[56]. It should show all impurities in single format and includes

Synthesis related impurities

Formulation-related impurities

Interaction products

Degradation products

Normally, more than one analytical system is applied for confirmation of an IP.[57-60]

Efforts are mainly focused on organic impurities profiling, where as inorganic impurities and residual solvents are easily detected and their toxicity is known.

5. Isolation and characterization

In order to monitor impurities accurately it is necessary to isolate and characterize them. Isolation can be done by using following methods .But application of any method depends on nature of impurity that is its structure, physicochemical properties.

Liquid-solid Extraction:-A solvent is selected which would dissolve impurity of interest but not solid matrix. If compound contains more than one impurity, then it is advisable to use organic solvent because of its unique properties .After extraction organic solvent is volatilized to concentrate the impurity. Some commonly used solvents with b.p. are presented in Table-1.

Table-1: Commonly used solvents.

Solvent	Boiling Point
n-hexane	190
Cyclohexane	81
Carbon tetrachloride	77
Ethanol	78
Methanol	65
DMF	153

Soxhlet extraction:-It is mostly used for extraction of natural products. The material is placed in extractor, adequately heated to ensure volatilization of solvent vapors, and these vapors are condensed **.**Advantage of this method is that it allows utilization of small volume of solvent to produce concentrated extract.

Supercritical fluid extraction (SFE):-It provides excellent means of isolating impurities in a short period of time. It provides idealized means of extracting materials due to lower viscosity, high solute diffusivity and excellent solvating properties. Carbon dioxide is most commonly used in this method.

Steam distillation: - It is used for extracting volatile components from natural materials.

Liquid-liquid extraction:-It involves extraction of one liquid with another of the two liquids one is aqueous and other is organic and both are immiscible. Solute is distributed between two immiscible solvents; the extraction is controlled by partition co-efficient.

Column chromatography:-It is commonly used in preparative chemistry for separation of pharmaceutical compounds. Separation depends on size of column(quantities ranges from micro grams to kilograms). In adsorption chromatography silica gel or alumina is used. For analysis of biological samples, ion exchange resins are used. Elution is characterized out with non polar solvents in liquid-liquid partition chromatography.

Gas chromatography:-It is used for isolation and characterization of volatile components. If components are non-volatile they are made volatile by derivatization technique. For characterization of impurities, it is more apt to use GC in combination with Mass spectrometry.

Thin layer chromatography:-It is a valuable technique for isolation and purification of compounds. Silica gel plates are frequently used for most applications. Material is eluted from the plates by scraping the sorbent containing material of interest and later it is extracted suitable solvent, followed by filtration or centrifugation.

Characterization of impurities [61] :By the following means characterization of impurities is generally achieved;

- Matching retention data
- IR
- UV
- MS
- NMR

Once impurity was detected in a drug substance, it becomes necessary to estimate its content. In quantification of impurities, initial estimations are done against parent compound because in most of cases there is lack of availability of authentic sample of impurity. If it is estimated that the content of given impurity is greater than 0.1%, then it must be characterized as per ICH and FDA requirements.

6. Analytical methodology

The primary criteria of analytical methodology [22,62] is to differentiate the compounds of interest and impurities. New drug development mandates generation of meaningful and reliable analytical data at various steps [63].The safety data of a new pharmaceutical compound or drug ensures that it met the established purity standards as a chemical entity or when admixed with pharmaceutical excipients. The formulation must be stable throughout its shelf life .All these requirements lead to development of analytical methods that are suitable for determination trace/ultra trace levels [64-67.]

Strategy for method development: - Method development strategy should consider the following physico chemical data

- Solubility
- Ionization constant
- Water absorption
- Crystal form

- Distribution coefficient
- Optical rotation

The impurities can be predominantly identified by following methods:-

6.1 Spectroscopic methods

Various spectroscopic methods can be used are

UV Spectrophotometry:-It provides minimal selectivity of analysis at a single wave length. Greater selectivity is ensured with use of diode array detectors (DAD) where sufficient simultaneous information at various wave lengths is possible.

IR Spectrophotomery:-Selectivity of analysis can be achieved as it provides specific information of functional groups.

Nuclear magnetic resonance Spectroscopy:-It provides fairly detailed structural information of a molecule. This method is very useful in characterization of impurities but has limited use due to time and cost factors.

Mass spectrometry: - It provides excellent structural information and is an effective tool for differentiating molecules with small differences in molecular weight. It has limited use because of time and cost factors .It is widely used for detection of inorganic impurities [18.]

6.2 Separation methods

Various chromotagraphic methods [68] can be used are

Thin layer chromatography can be used to elute broad range of impurities using different mobile phase and different plates. Though it is easy to use and cost effective, it has limited resolution detection and ease of quantification.

Gas chromatography is useful for detection of organic volatile impurities. It provides desired resolution, selectivity and ease of quantification.It is very useful in quantification.

High performance liquid chromatography is widely used and has various applications due to use of variety of detectors such as electrometric, Fluorescence, MS etc;

Capillary electrophoresis is used when very low quantities of sample are available and high resolution is required.

Supercritical fluid chromatography is widely used in extraction of samples. It is more advantageous than GC in terms of detection and HPLC in terms of separation .It is still an evoluting technique.

6.3 Hyphenated methods

Various hyphenated techniques are employed for effective monitoring of impurities [69-74]. They include

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

Impurities in d-allethrine [72] are estimated using reverse-phase LC-MS analysis in gradient elution using to ionization techniques that is atmospheric pressure ionization and chemical ionization.

LC-MS-MS is used in complex mixture analysis of thermally labile and biologically relevant molecules viz mosapride is mainly attributed to soft nature of atmospheric ionization (APPI) and atmospheric pressure chemical ionization(APCI) [73.]

Analysis by GC-MS of methamphetamine, LC-MS of risperidone and cetrizine tablets are found to be perfectly suitable for initial characterization of impurities [70,75.]

7. Qualification of impurities

Qualification is a process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity at the levels being considered.When an impurity meets one or more of the following conditions then it is said to be qualified.

- When the impurities observed level and proposed acceptance criterion don't exceed the level observed in an FDA approved human drug product.
- When impurity is a significant metabolite of drug substance.
- When the impurities observed level and proposed acceptance criterion are adequately justified by the scientific literature.

- When the impurities observed level and proposed acceptance criterion don't exceed level that has been adequately evaluated in comparitive invitro genotoxicity studies.

Recommended qualification threshold for drug substances based on maximum daily dose as mentioned in table No.2 and for drug product table No.3 mentioned in ICHQ3A and ICHQ3B .

Table 2: Drug substance impurities thresholds.

Maximum daily dose[a+]	**Reporting threshold[b,c]**	**Identification threshold[b,c]**	**Qualification threshold[b,c]**
≤2g/day	0.05%	0.10% or 1.0mg/day in take (whichever is less)	0.15% (or)1.0mg/day intake(whichever is less)
≥2g/day	0.03%	0.05%	0.05%

a The amount of drug substance administered per day.
b Higher reporting threshold should be scientifically justified.
c Lower threshold can be appropriate if the impurities are unusually toxic.

Table-3: Thresholds for degradation products in drug products.

Maximum daily dose[a]	**Reporting threshold[b,c]**
≤1g >1g	0.1% 0.05%
Maximum daily dose[a]	**Identification threshold[b,c]**
<1mg 1mg-10mg >10mg-2g >2g	1.0% or 5 µg TDI, whichever is lower 0.5% or 20 µg TDI, whichever is lower 0.2% or 2mg TDI, whichever is lower 0.10%
Maximum daily dose[a]	**Qualification threshold[b,c]**
<10mg 10mg-100mg >100mg-2g >2g	1.0% or 50 µg TDI, whichever is lower 0.5% or 200µg TDI, whichever is lower 0.2% or 3mg TDI, whichever is lower 0.15%

a. The amount of drug substance administered per day.
b. Thresholds for degradation products are expressed either as a percentage of the drug substance or as total daily intake (TDI) of the degradation product. Lower thresholds can be appropriate if the degradation product is unusually toxic.
c. Higher thresholds should be scientifically justified

Table-4: Class I Residual solvents.

Residual solvent	**Concentration limit(ppm)**
Benzene	2 (carcinogenic)
Carbon tetrachloride	4 (toxic)
1,2 dichloro ethane	8 (toxic)
1, 1 dichloro ethane	5 (toxic)
1,1,1 trichloro ethane	1500 (environmental hazard)

Table-5: Few Class II solvents with their Permissible Daily Exposure limits.

Solvent	Permissible daily exposure (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2-Dichloroethane	18.7	1870
Dichloromethane	6.0	600
1,1-Dimethoxyethane	1.0	100
N,N-Dimethyl acetamide	10.9	1090
N,N-Dimethyl formamide	8.8	880
1,2-Dioxane	3.8	380
2-Ethoxyethanol	1.6	160
Ethylene glycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxy ethanol	0.5	50
Methyl butyl ketone	0,5	50
Methyl cyclo hexane	11.8	1180
N-methyl pyrrolidone	48.8	4840
Nitromethane	0.5	50
Pyridine	2.0	200
Sulfolane	1.6	160
Tetralin	1.0	100
Toluene	8.9	890
1,1,2-Trichloro ethane	0.8	80
Xylenes	21.7	2170

8. Various impurities reported in API's

Table-6: Various impurities reported in API's.

Drug	**Impurity**	**Method**	**Reference**
Amphotericin B	Tetraenes	UV spectroscopy	76
Cloxacillin	N,N dimethyl aniline	GC	76
Dextrose	5 hydroxy methyl furfural	UV spectroscopy	77
Doxorubicin hydrochloride	Acetone and ethanol	GC	77
Ethambutol hydrochloride	2 amino butanol	TLC	77
Framycetin sulphate	Neamine	TLC	78
Cimetidine	4-methylimidazole and 1,8-bis[(N'cyano-N''-methyl)guinidino]-3,6-dithiaoctane	HPLC	79
Norgestrel	3,17α-diethinyl-13-ethyl-	TLC,HPLC and	80

	3,5-gonadiene-17-ol	UV Spectroscopy	
Celecoxib	[5-(4-methylphenyl)-3-trifluromethyl-1H-pyrazole],4-[5-(2'-methylphenyl)-3-(trifluromethyl)-1H-pyrazol-1-yl]-benzenesulphonamide ,and 4-[4-(4'-methylphenyl)-3-(trifluromethyl)-1H-pyrazole-1-yl]-banzenesulfonamide	HPLC,LC, LC-MS-MS	81
Ethynodiol diacetate	17 α-ethinyl-estr-4-ene-3β,17-(3'-acetoxy-2'-butenote), 17α-ethinyl-estr-4-ene-3β,17-diol-3-acetate-17-(3-oxo-butanote)	HPLC	82
Methamphetamine	1,2-dimethyl-3-phenylaziridine,ephedrine, methylephedrine, N-formylmethamphetamine, N-acetylmethamphetamine, N-formylphedrine, N-acetylephedrine,N,O-diacetylephedrine, methametamine dimmer	GC	83
Morphine	6-monoacetylmorphine	HPLC	84
Morphine sulphate	5-(hydroxymethyl)-2-furfural,10-hydroxymorphine, 10-oxomorphine	HPCL	85

9. Conclusion

Identification and quantification of impurities play a crucial role to assure quality of drug substances and drug products. For investigation of impurities various analytical methods are used routinely. Though ICH has outlined guidelines, there is much more requirement to have unified specifications with regard to impurities.

10. Acknowledgements

The authors wish to thank management and faculty of Nirmala college of pharmacy,Guntur for encouragement and permission to communicate the manuscript for publication.

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