

Scientific Approaches for Impurity Profiling in New Pharmaceutical substances and its products-An Overview

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ABSTRACT

The control of impurities in Formulated products and Active Pharmaceutical ingredient's were regulated by various regulatory authorities like US-FDA, ICH, MHRA, TGA etc. As per International Conference on Harmonization guidelines, the Impurity may be defined as any component of new drug product that is not the drug substance or an excipient in drug product. Nowadays apart from purity profile there was an increasing essentiality of impurity profile by regulatory agencies. Hence the Qualification of impurities which is essential for establishing the biological safety of an individual impurity. Thus it reveals the need and scope of impurity profiling of drugs in Pharmaceutical research.

Keywords: Impurities, ICH, Active pharmaceutical ingredient.

INTRODUCTION

The impurity profile is a description of Identified and unidentified impurities. The impurity may be developed either during formulation or in the final product upon ageing. The Various instrumental approaches for isolating and identifying the process related impurities and degradation products are Mass spectroscopy (MS), Nuclear magnetic spectroscopy (NMR), High performance liquid chromatography (HPLC) etc., has been established to review a summary of the problems and the various possibilities offered by modern analytical chemistry. Recent books^{1,2} and journal reviews^{3,4} also addresses this topic.

SOURCES OF IMPURITIES⁵

The type and amount of impurity present in the chemicals or pharmaceutical substances depends upon following factors:

A) Raw materials used in Manufacture.

- B) Method or process employed in manufacture.
- C) Reagents/solvents/Reaction vessels.
- D) Atmospheric contaminants
- E) Particulate contamination
- F) Cross contamination
- G) Microbial contamination
- H) Packing errors
- I) Due to impact of heat, light, oxidants on drug Product.
- J) Change in P^H.
- K) Presence of trace metals which may catalyse and accelerate the reaction.

There was enormous development in the identification and separation techniques of impurities. The chromatographic and spectroscopic techniques are widely used. The various methods employed are UV, TLC, LC, HPTLC etc., but HPLC has been predominantly used for impurity profiling with their wide range of detectors sensitivity and number of choices of Stationary and Mobile Phases.

Economically TLC is the most commonly used one and its advancement is HPTLC technique has also been used for impurity separation. In spite of all above techniques the hyphenated techniques like LC-MS-MS, GC-MS, LC-MS etc., has revolutionized impurity profiling⁶. So the presence of impurity even in small amount may influence the efficacy and safety of pharmaceutical products. Hence the different Pharmacopoeias like British Pharmacopoeia (BP), United States Pharmacopoeia (USP), Indian Pharmacopoeia (IP) etc., are including limits to allowable levels of impurities present in Drug substances and drug products.

A number of articles⁷⁻⁹ have stated guidelines and designed approaches for isolation and identification of process-related impurities and degradation products, using Mass spectrometry (MS), Nuclear Magnetic Resonance (NMR), High Performance Liquid Chromatography (HPLC), Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) and Tandem Mass Spectrometry (MS/MS) for pharmaceutical substances. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has also published guidelines for validation of methods for analysing impurities in new drug substances, products, residual solvents and microbiological impurities¹⁰⁻¹³.

The impurities of a synthetic product can be:

Residues of intermediates, by-products, starting materials and their impurities

Degradation products of a desired product, intermediates, by-products, starting materials and their impurities

Products of reactions among all above species.

Theoretically possible (potential) impurities lead to the following two types of impurities^{14,15}

Primary impurities

The impurities from formation of a desired product, such as by-products, residues of starting materials and intermediates.

Secondary impurities

The residues of impurities of starting materials, degradants of impurities, products of reactions of and among impurities etc.

The primary impurities are given more importance by regulatory authorities but secondary impurities are usually present at very low levels like ppm or ppb and it is highly useful for forensic reasons when looking for specific (characteristic) impurities for a particular synthetic route, often used in order to establish patent infringement. Reactions at the impurity (trace) level are less known and are not systematically studied. The best example for the impurities bis(2-ethyl hexyl) phthalate which is released from plastic packaging and process equipment material with Amlodipine besylate to form Pthalimido derivative is classified as secondary impurity.

Impurities have been named differently or classified as follows;

a) Common names

- By-products
- Degradation products
- Interaction products
- Intermediates
- Penultimate intermediates
- Related products
- Transformation products

b) United State Pharmacopoeia

The United States Pharmacopoeia (USP) classifies impurities in various sections;

- Impurities in Official Articles
- Ordinary Impurities
- Organic Volatile Impurities

c) ICH

The International Conference on Harmonization addresses the questions relating to impurities as follows.

Q1A(R) Stability testing of new drug substance and products.

Q3A(R) Impurities in drug substance
 Q3B Impurities in drug products.
 Q3C Impurities residual solvent.

According to ICH guidelines, impurities in the drug substance produced by chemical synthesis can broadly be classified into following three categories;

- Organic Impurities (Process and Drug related)
- Inorganic Impurities
- Residual Solvents

Organic impurities may arise during the manufacturing process and or storage of the drug substance may be identified or unidentified, volatile or non-volatile, and may include;

- Starting materials
- intermediates
- By-products
- Degradation products
- Reagents, ligands and catalysts.

Inorganic impurities can result from the manufacturing process. They are normally known and identified and include:

- Reagents, ligands and catalysts
- Heavy Metals or other residual metals
- Inorganic salts
- Other materials
- (filter aids, charcoal)

Residual solvents are organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The residual solvents¹² are classified as follows:

Class 1 solvents: Solvents to be avoided in pharmaceutical products Known human carcinogens, strongly suspected human carcinogens and environmental hazards.

Table I: Solvents to be avoided in pharmaceutical products

Solvent	Concentration limit(ppm)	concern
Benzene	2	Carcinogen
CCl ₄	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethane	8	Toxic
1,1,1-Trichloroethane	1500	Environmental hazard

Class 2 solvents: Solvents to be limited in pharmaceutical products.

Non-genotoxic animal carcinogens or possible causative agents of other

irreversible toxicity such as neurotoxicity or teratogenicity. solvents suspected of other significant but reversible toxicities.

Table II: Solvents to be limited in pharmaceutical products

Solvent	Permitted Daily Exposure ^a (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

a-The maximum acceptable intake per day of residual solvent in pharmaceutical products.

Class 3 solvents: Solvents with low toxic potential

Solvents with low toxic potential to man; no health -based exposure limit is needed. These solvents are less toxic in acute or

short term studies and negative in genotoxic studies. The amount of these residual solvents of 50mg or less would be acceptable. Examples for this class of

solvents are Acetic acid, Acetone, Anisole, 1-Butanol, 2-Butanol etc.

Class 4 solvents: Solvents for which No adequate toxicological data was found.

The solvents of this class may be of interesting to manufacturers of excipients,

drug substances or drug products. But there was no adequate toxicological data on which to base a Permitted Daily Exposure was found. Examples for this class of solvents are 1,1-Diethoxy propane, 1,1-Dimethoxy propane, 2,2-Dimethoxy propane, Isooctane etc.,

ICH limits for impurities

Table III: Thresholds for impurities in New Drug substances¹⁰

Maximum daily dose ^a	Reporting Threshold ^{b,c}	Identification Threshold ^c	Qualification Threshold ^c
≤ 1 g	0.05%	0.10% or 1.0 mg per day intake (whichever is lower).	0.15% or 1.0 mg per day intake (whichever is lower).
> 2g	0.03%	0.05%	0.05%

a-The amount of drug administered per day.

b-Higher reporting thresholds should be scientifically justified.

c-Lower thresholds can be appropriate if the impurity is unusually toxic.

The new drug substance specifications should include the limits for

i) Organic Impurities

- Each specific identified impurity
- Each specific unidentified impurity at or above 0.1%
- Any unspecific impurity, with limit of not more than 0.1%.
- Total impurities

ii) Residual solvents

iii) Inorganic impurities

The pharmacopoeias also specifying the qualitative, quantitative or semi quantitative tests for limiting known impurities in certain drugs.

Registration application for the new drug product¹¹

The documentation for New Drug Product should include the following;

- a) Batch identity, strength and size.
- b) Date of Manufacture
- c) Site of Manufacture
- d) Manufacturing Process
- e) Immediate container closure
- f) Degradation product content, individual and total content
- g) Use of Batch includes clinical studies, stability studies
- h) Reference to analytical procedure used
- i) Batch number of drug substance used in the New Drug Product
- j) Storage conditions for Stability studies.

Degradation Products specification for a new drug product

- Specified Degradation Product includes Identified and Un- identified product.
- Un Specified Degradation Product
- Total Degradation product.

According to ICH guidelines, Specified degradation product is a degradation product which is individually listed and limited with a specific acceptance criterion in New drug product specification, Whereas Unspecified degradation product is limited with a specific acceptance criterion but not individually listed in New drug product specification. Similarly Identified Specified Degradation Product is a degradation product for which a structural characterization has been achieved Whereas Un- identified Specified Degradation Product is a degradation product for which a structural characterization has not been achieved and that is solely defined by Qualitative analytical properties. Hence according to ICH guidelines the Threshold limits for impurities are described in Table 4 and 5 for New Drug Substance and New Drug Products.

Table IV: Thresholds for reporting impurities¹⁰

Maximum Daily Dose ^a	Reporting Thresholds	Identification Threshold	Qualification Thresholds
≤ 2 g/day	0.05%	0.1.% or 1.0 mg/day (which is lower)	0.15% or 1.0mg/day (which is lower)
> 2g /day	0.03%	0.05%	0.05%

Reporting impurity content of batches.

Analytical Results of all batches used for Clinical, Safety, Stability testing, Representative of proposed commercial process. Impurities should be designated by Code number ,an appropriate descriptor like Retention time.Listing of impurities in specifications should include List of impurities, Stability studies data, Chemical development studies data, Routine Batch analysis data, Degradation product data.

• **Qualification of degradation products**

According to ICH guidelines Qualification is defined as the process of acquiring and evaluating data that establishes the biological safety of an individual degradation product or a given degradation profile at the level(s) specified.Hence for any degradation product present in a new drug product

should be adequately tested in safety and clinical studies to be considered for Qualification of degradation products.

An impurity is considered as qualified based on the acceptance of one or more of the following conditions [16]

a)When the observed level and proposed acceptance criterion for the impurity do not exceed the level observed in an FDA approved human drug product.b)When the impurity is a significant metabolite of the drug substance.c)When the observed level and predetermined accepted level for the impurity is adequately justified in the scientific literature.d)When the observed level and predetermined accepted level for the impurity do not exceed the level that has been adequately evaluated in comparative invitro genotoxicity studies.Decision tree for identification and Qualification of a Degradation product¹¹ below.

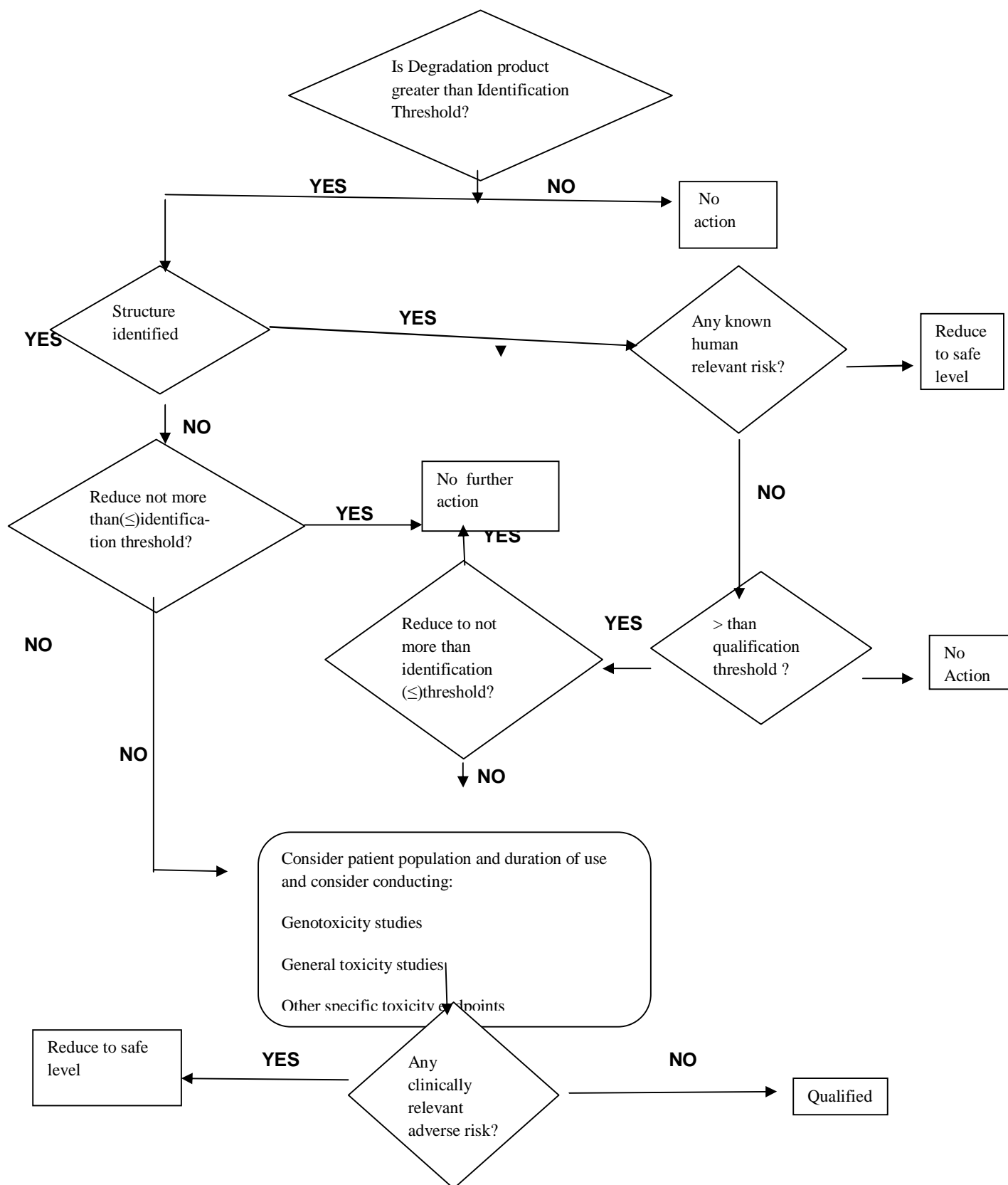


Fig. 1: Decision tree for identification and Qualification of a Degradation product

Table V: Thresholds for Degradation products in New Drug Products¹¹

Reporting Thresholds	
Maximum Daily Dose ^a	Threshold ^{b,c}
≤ 1 g	0.1%
≥ 1g	0.05%
Identification Thresholds	
Maximum Daily Dose ^a	Threshold ^{b,c}
< 1 mg	1.0% or 5µg TDI, whichever is lower
1mg-10 mg	0.5% or 20µg TDI, whichever is lower
> 10mg-2g	0.2% or 2mg TDI, whichever is lower
> 2g	0.10%
Qualification Thresholds	
Maximum Daily Dose ^a	Threshold ^{b,c}
< 10mg	1.0% or 50µg TDI, whichever is lower
10mg-100 mg	0.5% or 200µg TDI, whichever is lower
> 100mg-2g	0.2% or 3mg TDI, whichever is lower
> 2g	0.15%

a-The amount of drug 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 VI: Shows the list of drugs and its corresponding impurities

DRUG	IMPURITIES
Aceclofenac	a) Impurity A 2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid. Aceclofenac EP b) Impurity B Methyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetate Aceclofenac c) Impurity C Ethyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetate Aceclofenac d) Impurity D Methyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate Aceclofenac EP e) Impurity E Ethyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate f) Impurity F Benzyl [2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetate g) Impurity G [2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetic acid h) Impurity H [2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxy]acetyl]oxy]acetic acid i) Impurity I 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one.
Acebutolol HCl	a) Impurity A 3'-Acetyl-4'-(2,3-epoxypropoxy)-butyraniide b) Impurity B N-[3-acetyl-4-[(2RS)-2-hydroxy-3-[(1-methylethyl) amino] propoxy] phenyl] acetamide . c) Impurity C N-(3-Acetyl-4-hydroxyphenyl)butanamide d) Impurity D 1-[5-amino-2-[(2RS)-2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]ethanone.
Acebrophylline	a) Impurity A (2-amino-3,5-dibromophenyl)methanol b) Impurity B trans-4-(6,8-dibromo-1,4-dihydroquinazolin-3(2H)yl)cyclohexanol c) Impurity C trans-4-[[E]-2-amino-3,5-dibromobenzylidene]amino]cyclohexanol d) Impurity D cis-4-[[E]-2-amino-3,5-dibromobenzyl]amino]cyclohexanol e) Impurity E 2-amino-3,5-dibromobenzaldehyde.
	Impurity A

Bisoprolol Fumarate	(RS)-1-(4-Hydroxymethylphenoxy)-3-isopropylaminopropan-2-ol Impurity B (RS)-1-Isopropylamino-3-[4-(2-propoxyethoxymethyl) phenoxy] propan-2-ol Impurity C (RS)-1-[4-[4-(2-Hydroxy-3-isopropopylaminopropoxy)-benzyl]phenoxy]- 3-isopropylaminopropan-2-ol Impurity G (2RS)-1-[4-(((2-Isopropoxy-ethoxy)methoxy)methyl)phenoxy]-3- (isopropyl-amino)-2-propanol Impurity J (2RS)-3-[4-((2-Isopropoxyethoxy)methyl)phenoxy]-1,2-propanediol Impurity K 2-Isopropoxyethyl 4-(((2RS)-2-Hydroxy-3-(isopropylamino)propyl)-oxy]benzoate Impurity L 4-(((2RS)-2-Hydroxy-3-(isopropylamino)-propyl)oxy]benzaldehyde Hydrochloride Impurity M 4-[(2-Isopropoxyethoxy)methyl]phenol Impurity N (2RS)-1-[4-((2-Ethoxyethoxy)methyl)phenoxy]-3-(isopropylamino)-2-propanol Impurity Q (2RS)-1-Isopropylamino-3-[4-(2-methoxyethoxy)methyl]-phenoxy-2-propanol Impurity S 4-Hydroxybenzaldehyde Alcohol Impurity 4-Hydroxybenzylic Alcohol Epoxide Impurity 2-[4-(2-Isopropoxyethoxymethyl)phenoxy]methyl]oxirane.
Captopril	Captopril EP Impurity A (2S,2'S)-1,1'-[disulphanediylbis[(2S)-2-methyl-1-oxopropane-3,1-diy]l]-bis[pyrrolidine-2-carboxylic] acid Captopril Acid Impurity -3-mercapto-2-methylpropanoic acid Captopril Acid Amine Salt -3-mercapto-2-methylpropanoic acid 1,2-diphenylethylamine salt Captopril S-Methyl Metabolite -(2S)-1-[(2S)-2-Methyl-3-methylsulphanylpropanoyl]pyrrolidine-2-carboxylic acid Captopril S-Methyl Sulfoxide -(2S)-1-[(2S)-2-Methyl-3-methylsulfinylpropanoyl]pyrrolidine-2-carboxylic acid.
Ciprofloxacin	Ciprofloxacin EP Impurity A 7-Chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid . Ciprofloxacin EP Impurity B . 1-Cyclopropyl-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid hydrochloride Ciprofloxacin EP Impurity C 7-[(2-Aminoethyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid Ciprofloxacin EP Impurity D 7-Chloro-1-cyclopropyl-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid Ciprofloxacin EP Impurity E 1-Cyclopropyl-6-fluoro-7-(piperazin-1-yl)quinolin-4(1H)-one.
Clopidogrel Bisulfate	Clopidogrel USP Related Compound A [(+)-(S)-(o-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetic acid hydrochloride Clopidogrel USP Related Compound B Methyl (±)-(o-chlorophenyl)-4,5-dihydrothieno[2,3-c]pyridine-6(7H)-acetate hydrochloride. Clopidogrel USP Related Compound C Methyl (-)-(R)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate hydrogen sulfate Clopidogrel Bisulfate Racemate Clopidogrel Acid Racemate Clopidogrel Acid R-Isomer Clopidogrel Cyano Racemate (RS)-(o-Chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetonitrile Clopidogrel TTP Impurity 4,5,6,7-Tetrahydrothieno[3,2-c] pyridine hydrochloride ("TTP").

The impurities can be identified by following different methods like a) Reference standard method b) Spectroscopic method c) Separation method d) Isolation method e) Characterization method

Reference standard method

The main objective of this method is to provide clarity to the overall life cycle, qualification and governance of reference standards used in development and control of new drugs. Since the Reference standards provides the basic information for evaluating

process and product performance of drug substances, drug products, impurities, degradation products, starting materials, process intermediates, and excipients.

Spectroscopic methods

The UV, IR, MS, NMR and Raman spectroscopic methods are widely used¹

Separation methods

The separation method includes chromatographic techniques like TLC, HPTLC, HPLC, Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), Electrophoresis techniques like Capillary electrophoresis, Gel permeation etc.¹

Isolation methods

Mostly the chromatographic techniques are used for isolation of impurities along with non-chromatographic techniques are also rarely used. The following methods are widely used:

- a) Solid-phase extraction methods
- b) Liquid-liquid extraction methods
- c) Accelerated solvent extraction methods
- d) Column chromatography
- e) Flash chromatography
- f) TLC
- g) GC
- h) HPLC
- i) HPTLC
- j) Capillary electrophoresis (CE)
- k) Supercritical fluid chromatography (SFC).

Solid-phase extraction methods¹⁷

Solid-phase extraction (SPE) is an extraction method that uses a solid phase and a liquid phase to isolate the impurity of interest from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantitate the amount of analyte(s) in the sample. SPE uses the affinity of solutes dissolved or suspended in a liquid which act as a mobile phase for a solid through which the sample is passed which act as the stationary phase to separate a mixture into desired and undesired components. The result is that either the desired analytes of interest or undesired impurities in the sample are retained on the stationary phase. The common solvents used in SPE are described in Table-7. When the sample passes through the stationary phase, the analytes in the sample will interact and retain on the sorbent but the solvent, salts and other impurities pass through the cartridge. After the sample is loaded, the cartridge is washed with buffer or solvent to remove further impurities. Then, the analyte is eluted with a non-polar solvent or a buffer of the appropriate P^H .

TABLE VII: Characteristics of solvents commonly used in Solid phase extraction method

Polarity			Solvent	Miscible in Water
	Hexane	NO		
	Isooctane	NO		
	Carbon tetrachloride	NO		
	Chloroform	NO		
	Methylene chloride	NO		
	Tetrahydrofuran	YES		
	Diethyl ether	NO		
	Ethyl acetate	POORLY		
	Acetone	YES		
	Acetonitrile	YES		
	Isopropanol	YES		
	Methanol	YES		
	Water	YES		
	Acetic acid	YES		

Liquid-liquid extraction methods

In this type of extraction, two immiscible liquids was selected. Usually, one phase is aqueous (hydrophilic) and the other is a (hydrophobic) organic solvent. In that¹⁸⁻²¹ the solute is distributed between two immiscible solvents. The extraction was based on Distribution Co-efficient or Partition Co-efficient (K_d), which is the ratio of concentration of solute in two different solvents. a and b.

$$K_d = C_a/C_b.$$

Accelerated solvent extraction methods

Accelerated solvent extraction (ASE) is a fully automated technique that uses common solvents to rapidly extract solid and semisolid samples. ASE operates at temperatures above the normal boiling point of most solvents, using pressure to keep the solvents in liquid form during the extraction process. Typically, ASE methods are completed in 15–25 min, while consuming only 15–50 mL of solvent.

Column chromatography

In column chromatography¹⁸⁻²¹ the stationary phase is a solid adsorbent which is placed in a vertical glass (usually) column and the mobile phase used is a liquid which is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique. The mixture to be analyzed by column chromatography is applied to the top of the column. The liquid solvent (the eluent) is passed through the column by gravitational force or by the application of air pressure. An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. Column chromatography is

separated into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called **gravity column chromatography**. If the solvent is forced down the column by positive air pressure, it is called **flash chromatography**.

Flash chromatography

Flash Chromatography is a rapid form of preparative column chromatography based on optimised pre-packed columns through which is pumped solvent at a high flow rate. It is a simple and economical approach to Preparative LC. It is "an air pressure driven hybrid of medium and short column chromatography optimized for rapid separation." This approach was pioneered by W.C. Still at Columbia University. Flash chromatography utilises a plastic column filled with some form of solid support, usually silica gel, with the sample to be separated placed on top of this support. The rest of the column is filled with an isocratic or gradient solvent which, with the help of pressure, enables the sample to run through the column and become separated. Flash chromatography used air pressure initially, but today pumps are used to speed up the separation. This technique is considered a low to medium-pressure technique and may be scaled up for separations from a few mg to many tens or hundreds of grams.

Thin layer chromatography

Thin-Layer Chromatography¹⁸⁻²¹ is a simple and inexpensive technique that is often used to judge the purity of a synthesized compound or to indicate the extent of progress of a chemical reaction. In this technique, a small quantity of a solution of the mixture to be analyzed is deposited as a small spot on a TLC plate, which consists of a thin layer of silica gel (SiO_2) or alumina (Al_2O_3) coated on a glass or plastic sheet. The plate constitutes the stationary phase. The sheet is then placed in a chamber

containing a small amount of solvent, which is the mobile phase. The solvent gradually moves up the plate via capillary action, and it carries the deposited substances along with it at different rates. The desired result is that each component of the deposited mixture is moved a different distance up the plate by the solvent. The components then appear as a series of spots at different locations up

the plate. Substances can be identified from their so-called R_f values with reference to suitable adsorbents and eluting solvents as listed in Table-8 and 9. The order in the table is approximate, since it depends upon the substance being adsorbed, and the solvent used for.

Table VIII: Chromatographic adsorbents

ADSORBENT	ADSORBENT NAME	CHEMICAL FORMULA
Most Strongly Adsorbent	Alumina	Al ₂ O ₃
	Charcoal	C
	Florasil	
Least Strongly Adsorbent	MgO/SiO ₂ (anhydrous)	SiO ₂
	Silica gel	

If the substances in the mixture differ greatly in adsorbability, it will be much easier to separate them. Often, when this is so, a succession of solvents of increasing eluting power is used. One substance may be eluted easily

while the other stays at the top of the column, and then the other can be eluted with a solvent of greater eluting power. Table 10 indicates an approximate order of adsorbability by functional group.

Table IX: Eluting solvents for chromatography

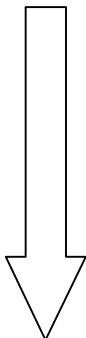

Elutingpower		Eluting solvents	
Least Eluting Power (alumina as adsorbent)  Greatest Eluting Power (alumina as adsorbent)	I N C R E A S I N G E L U T I N G P O W E R	Petroleum ether (hexane; pentane)	
		Carbon tetrachloride	
		Benzene	
		Dichloromethane	
		Ether (anhydrous)	
		Ethyl acetate (anhydrous)	
		Acetone (anhydrous)	
		Ethanol	
		Water	
		Pyridine	

Table X: Adsorbability of organic compounds by functional group

		Least Strongly Adsorbed	
	Most Strongly Adsorbed	Saturated hydrocarbons; alkyl halides	
		Unsaturated hydrocarbons; alkenyl halides	
		Aromatic hydrocarbons; aryl halides	
		Polyhalogenated hydrocarbons	
		Ethers	
		Esters	
		Aldehydes and ketones	
		Alcohols	
		Acids and bases (amines)	

Gas chromatography (GC)

Gas chromatography¹⁸⁻²¹ is an analytical technique for separating compounds based primarily on their volatilities. Gas chromatography provides both qualitative and quantitative information for individual compounds present in a sample. Compounds move through a GC column as gases with their Linear velocity and flow rates were summarized in Table-

11, because the compounds are normally gases or they can be heated and vaporized into a gaseous state. The compounds partition between a stationary phase, which can be either solid or liquid, and a mobile phase (gas). The differential partitioning into the stationary phase allows the compounds to be separated in time and space.

Table XI: Recommended linear velocities and flow rates of Carrier gases

Diameter (mm)	Linear Velocity (cm/sec)		Flow Rate (ml/min)	
	Helium	Hydrogen	Helium	Hydrogen
0.18	30-45	45-60	0.5-0.7	0.7-0.9
0.25	30-45	45-60	0.9-1.3	1.3-1.8
0.32	30-45	45-60	1.4-2.2	2.2-2.9
0.53	30-45	45-60	4.0-6.0	6.0-7.9

High performance Liquid chromatography (HPLC)

Normal-phase HPLC¹⁸⁻²¹ separates analytes based on adsorption to a stationary surface chemistry and by polarity. NP-HPLC uses a polar stationary phase and a non-polar, non-aqueous mobile phase, which effectively separates the analytes that are readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength depends not only on the functional groups in the analyte molecule, but also on steric factors.

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl , where R is a straight chain alkyl group such as $\text{C}_{18}\text{H}_{37}$ or C_8H_{17} . With these

stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily. The retention time can be increased by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger. Similarly, the decreasing of retention time by adding more organic solvent to the eluent can be done.

High performance thin layer chromatography (HPTLC)

Similar to other chromatographic methods HPTLC is also based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved. Once separation occurs individual

components are visualized as spots at respective level of travel on the plate. Their nature or character are identified by means of suitable detection techniques.

Capillary Electrophoresis (CE)

Capillary Electrophoresis (CE) is a separation technique based on the differential transportation velocities of charged species in an electric field through a conductive medium. Primary candidates for CE separation are ions. The basic instrumental set-up consists of a high voltage power supply (0 to 30 kV), a fused silica (SiO₂) capillary, two buffer reservoirs, two electrodes, and an on-column detector.

Supercritical fluid chromatography (SCF)

A pure supercritical fluid (SCF) is any compound at a temperature and pressure above the critical values (above critical point). Above the critical temperature of a compound the pure, gaseous component cannot be liquefied regardless of the pressure applied. The critical pressure is the vapor pressure of the gas at the critical temperature. In the supercritical environment only one phase exists. The fluid, as it is termed, is neither a gas nor a liquid and is best described as intermediate to the two extremes. This phase retains solvent power approximating liquids as well as the transport properties common to gases. A comparison of typical values for density, viscosity, diffusivity of gases, liquids, SCF and critical conditions for various solvents are presented in Table 12 and 13.

Table XII: Comparison values for Gas, SCF and Liquid

Property	Density (kg/m ³)	Viscosity(Cp)	Diffusivity (mm ² /s)
Gas	1	0.01	1-10
SCF	100-800	0.05-0.1	0.01-0.1
Liquid	1000	0.5-1.0	0.001

Table XIII: Critical Conditions for Various Supercritical Solvents

Supercritical Solvents	Critical Temperature (k)	Critical pressure (bar)
Carbon dioxide	304.1	73.8
Ethane	305.4	48.8
Ethylene	282.4	50.4
Propane	369.8	42.5
Propylene	364.9	46.0
Trifluoromethane	299.3	48.6
Chlorotrifluoromethane	302.0	38.7
Trichlorofluoromethane	471.2	44.1
Ammonia	405.5	113.5
Water	647.3	221.2
Cyclohexane	553.5	40.7
n-Pentane	469.7	33.7
Toluene	591.8	41.0

Characterization methods

The different techniques of highly sophisticated instruments like NMR, Mass spectroscopy, HPLC etc., are highly used in the identification of drugs, impurities, degradation products, metabolites in various matrices. For characterization of impurities the following various techniques are used;

NMR

A unique aspect of NMR spectra is the direct proportionality between peak areas and the number of nuclei responsible for the peak. The most important chemical application of proton NMR spectroscopy have been to the identification and structure elucidation of organic, metal-organic and biochemical molecules, Analysis of multicomponent mixtures, Elemental analysis etc., The best example for the NMR study of the impurity state in heavily doped Si:P [22] over a wide temperature range (100–500 K). The results shows that free carriers in Si:P are in dynamic exchange with residual impurity states at concentrations as high as 10^{19} cm^{-3} and at temperatures well above room temperature. An another example for impurity study of dilute Vanadium in copper [23] to measure the Knight shift of the ^{51}V impurity resonance and analyzed it in terms of a nonmagnetic virtual bound state. The research concludes that vanadium impurities in copper are magnetically similar to cobalt impurities.

Mass spectroscopy

Mass spectroscopy has wide applications in structural elucidation of organic and biological molecules, detection and identification of species separated by chromatography and capillary

electrophoresis. since the interpretation of the resulting complex spectrum is often impossible, the

Chemists have developed methods in which mass spectrometers are coupled with various hyphenated techniques like GC-MS, LC-MS, LC-MS-MS, HPLC-DAD-MS, HPLC-DAD-NMR-MS, Tandem Mass spectrometry, Capillary electrophoresis-Mass spectrometry.

GC-MS

GC-MS has become one of the most powerful tools available to the chemists for the analysis of complex mixtures. The spectra which are collected from the chromatographic technique are stored in a computer for subsequent processing. In the case of GC-MS, GC coupled to a Mass spectrometer through an interface that enriches the concentration of the sample in the carrier gas by taking advantage of the higher diffusivity of the carrier gas. Scanning times are rapid so that several MS can be obtained during the elution of a single peak from the GC unit. The major technical difficulty was to find an efficient gas separator or interface for GC/MS.

The best example for this GC-MS technique is the impurity profiling of synthetic pesticide d-allethrin [24] by using of two distinct soft ionisation techniques, the atmospheric pressure ionisation with electrospray source (API-ESI) and the chemical ionisation (APCI). An another research work of determination of impurity like cyclohexone, N-methyl pyrrolidone, Atlox 3406-F (an agricultural dispersant) in Triflorine a hexachlorinated, an fungicide using electrospray ionization of GC-MS technique.

LC-MS

In case of LC-MS a similar to GC-MS, though rather more difficult problem arises in the removal of liquid carrier from an HPLC eluent before samples are passed in to the MS source. The normal eluent flow rates of 0.5-2.0 ml min⁻¹ cannot be handled by the MS pumping system. Hence moving belt inlet systems, jet separators and vacuum nebulizers are all techniques that are used to remove solvent and pass analytes in to the source. The best example for this technique is in the investigation of 10 α -methoxy-1,6-dimethylergoline-8-methanol 5-bromonicotinic acid ester (Nicergoline) and its related substances²⁵ was performed by using ammonium acetate and methanol mixture as the mobile phase. It was characterized by HPLC/API-MS in terms of their molecular weight.

HPLC-DAD-MS

This HPLC-DAD and LC-ESI-MS technique have been used for the analysis of doxycycline and its related impurities like metacycline and 6-epidoxycycline²⁶. The mobile phase of oxalic acid (0.02 M; pH 2.5)-acetonitrile 82:18 (v/v) was used.

LC-MS-MS

In this type of technique, the characterization and quantitative determination of four impurities in piperazine phosphate by gradient reverse phase HPLC and LC/MS/MS was developed²⁷ and validated as per ICH guidelines. Another example, for determinations of low content of Methyl Methanesulfonate and Ethyl Methanesulfonate impurities as they were potential genotoxic impurities (PGIs) in Emtricitabine, an Active

Pharmaceutical Ingredient using LC/MS/MS²⁸ method.

HPLC-DAD-NMR-MS

The LC-DAD-MS/SPE-NMR Hyphenation technique have been used in the identification of isobaric Iridoid Glycoside Regioisomers as minor constituents from *Harpagophytum procumbens* of Pharmaceutically Used Plant Extracts²⁹. Hence by using of this technique provides the spectral data needed for structure elucidation.

Tandem Mass spectrometry

The tandem mass spectrometry (MS/MS) scanning modes are product ion, precursor ion, constant neutral loss etc., In addition, the special case of selected reaction monitoring (SRM) is occasionally used to enhance selectivity in quantitative mass spectrometry. MS/MS methods generally involve activation of selected ions, typically by collision with an inert gas, sufficient to induce fragmentation (collision induced dissociation, CID). The precursor ion scan involves selection of the ion of interest, activation of that ion and mass analysis of the product ions. This is a widely used technique and is particularly appropriate for aiding structure determination and for biopolymer sequencing.

Capillary electrophoresis-Mass spectrometry (CE-MS)

CE-MS was recently implemented in the method development approach to support impurity profiling of pharmaceutical products. Capillary electrophoresis (CE) is based on a different separation principle and consequently has different selectivity compared to HPLC. CE coupled to a Mass Spectrometer using electrospray ionization (ESI). Recently, atmospheric pressure

chemical ionization (APCI) and atmospheric pressure photoionization (APPI) have become available for CE/MS. can be helpful for identification and structural elucidation purposes. The combination of CE and MS has relied on interfaces to allow efficient transfer of analytes on-line from the electrophoretic capillary to the mass spectrometer without sacrificing separation efficiency. CE by its nature is particularly well suited to the separation of polar compounds readily ionizable in solution. Although numerous publications have appeared on CE-MS, this technique is still not widely accepted for routine use. The major limitation of CE is the limited sample volumes that can be analyzed without compromising separation efficiency. Another drawback with CE-MS is that migration times tend to fluctuate with a change of temperature in the environment. The use of non-volatile buffers in CE-MS is generally avoided.

Hence various goals of impurity investigations are Process –related impurities, degradation –related impurities, identifying the significant impurities, identifying the degradation product by stress studies and its actual degradation products through stability studies, determine the origin of impurities and to establish a method for eliminating or reducing the impurities, to understand the degradation pathway and to minimize the degradation .

CONCLUSION

The guidelines for impurity level provides the quality criteria for manufacturers. This review provides a depth knowledge on importance of impurity profile for New drug Substance and New Drug Product

with various techniques of isolation and characterization of impurities.

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