Review Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2013, Volume 1, Issue 5
ISSN : 2321–3647(online)

Advance approach on the Impurity Profile of Pharmaceutical Dosage forms: A Review

Jitendra S Patel[1]*, Chirantan N Patoliya[1], Punit M Patel[1], Sandip Dholakia[1], Madhabhai M. Patel[1]
1.Shankersinh Vaghela Bapu Institute of Pharmacy, Vasan, Gandhinagar

ABSTRACT

As per ICH guideline impurity may be defined as any component of drug product that is not the drug substance or an excipient. Now a day apart from purity profile there are an increasing essentiality of impurity profile by regulatory agency. Different regulatory agencies like ICH,USFDA, TGA, etc. work on control and identification of impurities in pharmaceutical dosage forms. To identify and characterize impurities are essential for establishing the biological safety of an pharmaceutical dosage forms. The various pharmacopoeias like IP, BP, USP, etc. were allowed certain limits of impurities in pharmaceutical dosage forms. This review article mainly focuses on various methods available to identify and characterize impurities in pharmaceutical dosage forms. Among all, the most specific techniques are NMR, MASS, GC-MS, LC-MS, HPLC-DAD-MS, LC-MS-MS, HPLC-DAD-NMR-MASS, Capillary electrophoresis, Mass and Flash chromatography.

Key words: Impurities, identification, HPLC-DAD-MS.

*Corresponding Author Email: jiturx@gmail.com
Received 22 July 2013, Accepted 14 August 2013

Please cite this article in press as: Patel JS *et al.*, Advance approach on the Impurity Profile of Pharmaceutical Dosage forms: A Review American Journal of Pharmacy& Health Research 2013.

INTRODUCTION

The impurity profile is a description of identified and characterize impurities in pharmaceutical dosage forms. The impurity may be developed either during formulation or in the final product upon ageing or contact with packaging of the various impurities that can be found in drug product.[1]

Any component of the drug product that is not the chemical entity defined the drug substance or an excipient in the drug product (ICH Q6A: Specification).It is good to give more important to these detrimental impurities. This is true in case of solid dosage forms where the limited mobility restricts the reactivity of larger molecules. For most drugs, the reactive species consist of water, electrophiles, metals, and peroxide. The presence of unwanted chemical affect the efficacy and safety of pharmaceutical dosage forms. Some impurities cause toxicological effects. Now a day, Impurity profiling receiving attention of regulatory agency. The different pharmacopoeia like IP, BP, USP, and other are allowed certain limits of impurities in pharmaceutical dosage forms.

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[2,3] and journal[4] reviews also addresses this topic.

Regulatory Guidelines on Impurities in an Active Pharmaceutical Ingredient[5]

For the reason of safety and efficacy monitoring of impurities in drug products needed. However monitoring impurities and controlling these impurities mean different things to different people.

The United States Food and Drug Administration (US FDA) have permitted the guidance prepared under the guidance of the International Conference of harmonization(ICH). The ICH guideline for impurities in pharmaceuticals was developed with joint efforts of regulators and industry representatives from the European Union (EU), Japan and United States and it has helped to ensure that different regions have consistent interpretation and implementation of regulations.

The various regulatory guidelines regarding impurities are as follows:

1. ICH guidelines “stability testing of new drug substances and products"- Q1A[6]
2. ICH guidelines “Impurities in New Drug Substances”- Q3A[7]
3. ICH guidelines “Impurities in New Drug Products”- Q3B[8]
4. ICH guidelines “Impurities: Guidelines for residual solvents”- Q3C[9]

5. US-FDA guidelines "NDAs -Impurities in New Drug Substances"[10]

6. US-FDA guidelines "ANDAs – Impurities in New Drug Substances"[11]

7. Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority(TGA), Australia[12]

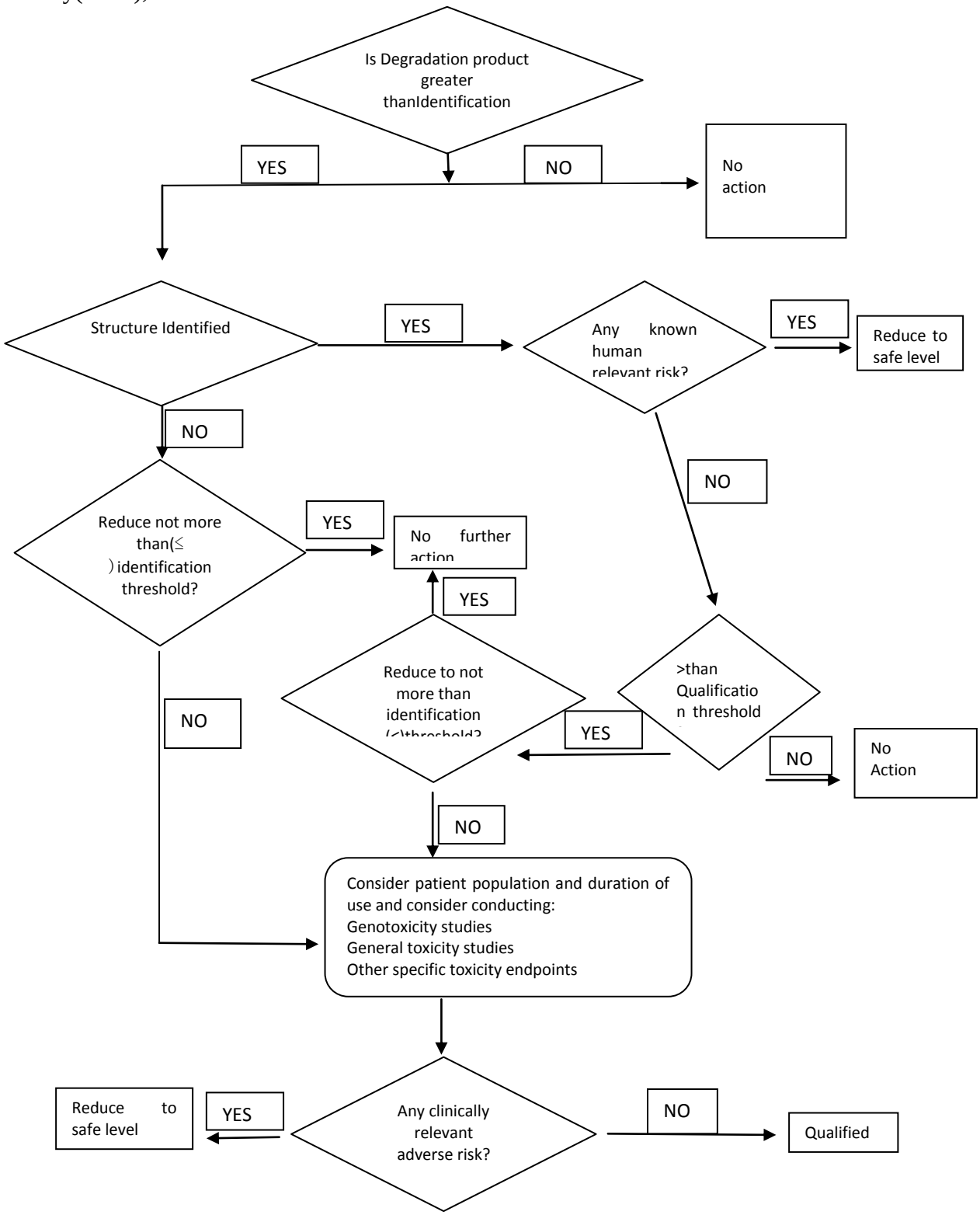


Figure 1: ICH decision tree for Identification and Qualification of Impurities[2,13]

Sources and Types of Impurities: [1,5,14,15,16]

There are many sources for impurities in pharmaceutical dosage forms. Impurities come from starting material, by-products, residual solvent, degradants formed and long term storage. Impurities can be formed heat, light, changes in pH of dosage form interaction with packaging component. Impurities can originate mainly from sources that are given below :

- Crystallization Related Impurities
- Stereochemistry Related Impurities
- Residual Solvent
- Synthetic Intermediate And By-Product
- Formulation Related Impurities
- Impurities Arising During Storage
- Method Related Impurity
- Mutual Interaction
- Functional Groups Related Degradation
- Environment Related Impurities

Impurities have been named differently or classified as follows;[1]

a) Common names

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

b) United State Pharmacopeia

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 questionsrelating 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;

1. Organic Impurities (Process and Drug related)
2. Inorganic Impurities
3. Residual Solvents

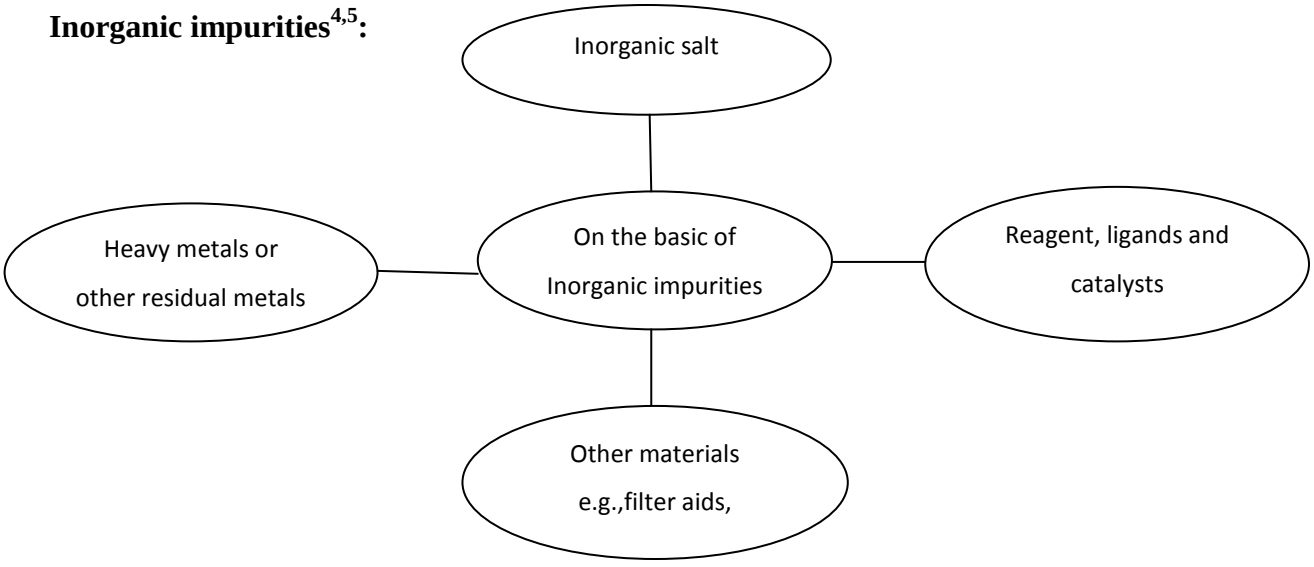
Organic impurities[4,5,17,18]

Organic impurities may arise during the manufacturing process and/or storage of the pharmaceutical dosage forms. They may be identified or unidentified, volatile or non-volatile including starting materials, by-products, intermediates, degradation products, reagents, ligands and catalysts. Starting materials or intermediates are the most common impurities found in ever API unless a proper care is taken in every step involved in throughout the multi-step synthesis.

Although the end products are always washed with solvents, there are chances of remaining residual unreacted starting materials unless the manufacturers are very careful about the impurities. In synthetic organic chemistry, getting a single end product with 100% yield is very rare; there is always a chance of formation of by-products. For example, in the case of Paracetamol bulk, diacetylated paracetamol may form as a bi product.

Impurities can also be formed by degradation of the end product during manufacturing of APIs. However, degradation products resulting from storage or formulation to different dosage forms or ageing are other common organic impurities in APIs.

Inorganic impurities[4,5]:



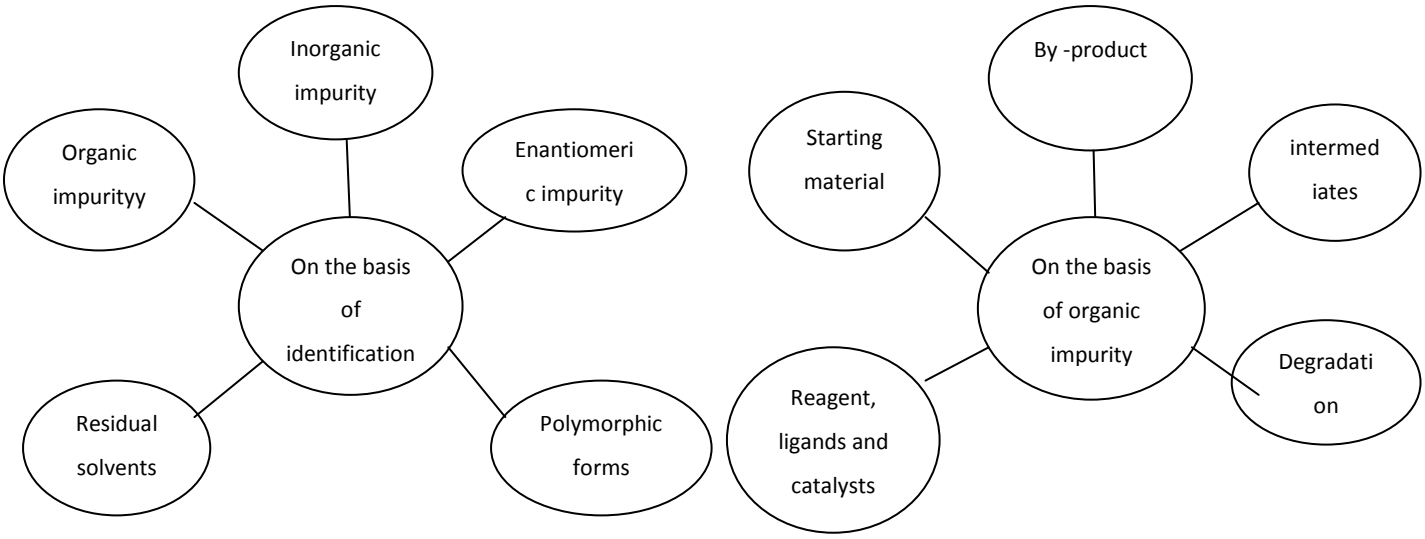


Figure 2: various types of impurities

Inorganic impurities may also arise from manufacturing processes used for bulk drugs. They are normally known and identified and include the following:

Reagents, ligands and catalysts-

The chances of presence of these impurities are rare. However, in some processes, these could create a problem unless the manufacturer takes proper care during production.

Heavy metals-

The main sources of heavy metals are the water used in the processes and the reactors (if stainless steel reactors are used), where acidification or acid hydrolysis takes place. These impurities of heavy metals can easily be avoided using demineralized water and glass-linked reactors.

Other materials (filter aids, charcoal)-

The filters or filtering aids such as centrifuge bags are routinely used in bulk drug manufacturing plants and in many cases activated carbon is also used. The regular monitoring of fibers and black particles in the bulk drugs is essential to avoid these contaminants.

Residual Solvents[4,19,20,21]

Residual solvents are potentially undesirable substances. They either modify the properties of certain compounds or may be hazardous to human health. The residual solvents also affect physicochemical properties of the bulk drug substances such as crystallinity of bulk drug, which in turn may affect the dissolution properties, odor and color changes in finished products. As per the ICH guidelines, the solvents used in the manufacturing of drug classified in to four types

Class I solvents:

Class I solvents and their permissible concentration limits given in the table 1. These solvents not

employed in the manufacture of drug substances, because of their unacceptable toxicity or their deleterious effects.

Table : 1 - Class I Residual Solvents

Residual solvent	Concentration limit(ppm)
Benzene	2 (Carcinogenic)
Carbon tetrachloride	4 (Toxic)
1,1 Dichloro ethane	8 (Toxic)
1,2 Dichloro ethane	5 (Toxic)
1,1,1 trichloro ethane	1500 (Environmental hazard)

Class II Solvents:

Class II solvents usage should be limited in pharmaceutical products because of their inherent toxicity lists class II solvents with their daily permissible exposure as follows:

Table :2 - Class II Solvents with their Permissible Daily Exposure Limits

Sr No	Solvents	Permissible daily conc. exposure(mg/day)	Limit (ppm)
1	Cyclohexane	38.8	3380
2	Acetonitrile	4.1	410
3	Hexane	2.9	290
4	Chloroform	0.6	600
5	Methanol	30.0	3000
6	Pyridine	2.0	200

Class III Solvents:

These are less toxic and possess lower risk to human health than class I or class II solvents. Some of the solvents are; Acetic acid, anisole, butanol, 2-butanol, isopropyl acetate, methylacetate, butylacetate, ter-butyl methyl ether.

Class IV Solvents:

Class IV solvents, adequate toxicological data is not available. The manufacturers should justify the residual levels for these solvents in pharmaceutical products. The solvents under class IV are 1, 1-diethoxy propane, 1-1-dimethoxy propane, 2-2- dimethoxy propane, methyl isopropyl ketone, isooctane, isopropyl ether, methyl tetrahydrofuran, petroleum ether, trichloroacetic acid.

Formulation related impurities[5,20,21]:

Apart from bulk drug related-impurities the formulated form of API may contain impurities that are formed in various ways.

Method related impurities:

Some impurities are generated during the formulation process either due to exposure to heat, light, change of pH, solvents etc. (e.g. Formation of impurity 1-(2,6- dichlorophenyl)-indolin-2 one on autoclaving of Diclofenac sodium).

Environment related impurities:

Due to exposures to adverse temperatures (e.g. Vitamins as drug substances are very heat sensitive and degradation frequently leads to loss of potency in vitamin products, especially in liquid formulations)

Due to exposure of light specially UV light (e.g. Ergometrine as well as methylergometrine is unstable under tropical conditions such as light and heat)

Humidity (Humidity is considered detrimental for hygroscopic products e.g. Aspirin and Ranitidine)

Table : 3 - The lists of compounds that affected by light or catalyst[20]

Sr.No.	API/Drug	Light/Catalyst
1	Epinephrine	Sodium metabisulfite
2	Penicillin	Sodium bisulfite
3	Phenothiazine	Light
4	DihydroergotamineMesylate	Light
5	Ergometrine	Light
6	Nifedipine	Light

Dosage form factors related impurities

Formation of impurities on ageing[5,20]:

Table 4 : Impurities on ageing and conditions

		Thermolytic	Hydrolytic	Photolytic
1.	**Solid state**	55, 70, 85_C, Low humidity (e.g., <30% RH or no humidity control); 4–6 weeks	55, 70, 85_C, High humidity (e.g., >70% RH); 4–6 weeks	5–10_the ICH min. confirmatory exposure; (1) thin layer of powder; (2) thin film (optional)
2.	**Solution**		Aqueous solutions or slurries, pH 1–13, RT-70_C, 2–4 Weeks	Aqueous solutions or slurries, 5–10 the ICH minimum confirmatory exposure; At pH's above and below Relevant pK's.

Those formed due to mutual interaction between ingredients[5,20]

Most often, vitamins are highly prone to instability on aging in different dosage forms. i.e., degradation of vitamins such as folic acid, thiamine and cyanocobalamines does not yield toxic impurities but lose their potency well below compendial specifications. Degradation of Thiamine in the presence of Nicotinamide in formulations containing Vitamin B complex.

Functional group related typicaldegradationimpurities[20,22,26]

a) Hydrolysis:

A reaction in which water is the reactant causing precipitation. Well-known examples of such reactions in pharmaceutical compounds are esters and amides. Many drugs are derivatives of

carboxylic acids or contain functional groups based on the moiety.

Eg. Esters, amides, lactones, lactams, imides and carbamates, which are susceptible to acid base Hydrolysis.

Ester hydrolysis e.g. Formation of Salicylic acid impurity from aspirin. Hydrolysis e.g. Bezylpenicillin, Chlordiazepoxide

Oxidation:

There are three major pathways,

(1) Autoxidation or radical-mediated oxidation,

(2) Peroxide-mediated, and

(3) Photo chemically induced.

Drugs which prone to oxidation are hydrocortisone, adinazolam, catecholamine, conjugated-dienes (Vitamin–A), heterocyclic aromatic rings, nitroso and nitrite derivatives. In pharmaceuticals, the most common for oxidative decomposition is auto-oxidation through a free radical chain process.

E.g. Auto-oxidation of ascorbic acid studies reveals that cupric ion known to oxidize ascorbic acid rapidly to dehydroascorbic acid and potassium cyanide.

Photolysis:

Photolytic cleavage on aging includes examples of pharmaceutical drugs or products that are prone to degradation onexposure to UV-light. During manufacturing process as solid or solution, packaging or on storage, drugs like ergometrine, nifedipine, nitropruside, riboflavin and phenothiazines are liable to photo oxidation.

Decarboxylation:

Some of the carboxylicacids such as *p*-amino salicylic acid shown loss of carbon dioxide from carboxyl group when heated. For instance, photo reaction of rufloxacin tablet enteric coated with cellulose acetate phthalate (CAP) and sub-coating with calcium carbonate cause hydrolysis of CAP liberating acetic acid, which on reacting with calcium carbonate produced carbon dioxide, aby-product that blew off the cap from 24 the bottle after cap was loosened.

Packaging Material:

Impurities result also from packaging materials i.e., containers and closures. For most drugs the reactive species for impurities consists of; Water – hydrolysis of active ingredient. Small electrophiles Aldehydes and carboxylic acid derivatives.

Enantiomericimpurities[22]

The single enantiomeric form of a chiral drug is now considered as an improved chemical entity

that may offer a better pharmacological profile and an increased therapeutic index with a more favorable adverse reaction profile. However, the pharmacokinetic profile of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are comparable, suggesting the lack of advantages of single isomer in this regard. IN any case, cost benefits as well as the patient's compliance need to be considered in selecting drugs. For the manufacturers of single enantiomeric drug (eutomer), the undesirable stereoisomers in drug control are considered in the same manner as other organic impurities. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin), levalbuterol (R-albuterol), and esomeprazole (S-omeprazole).

Crystallization related impurity[2,14]

First goal in pharmaceutical manufacturing is to produce phase-pure drug substance and remains in that state as long as bulk material. It is another goal in formulation of drug substance so that it remains in same phase-pure state during manufacturing and any storage. Solid properties of system may affected by nature of structure adopted by compound on crystallization.

ANALYTICAL METHOD DEVELOPMENT[20]

In new drug development, impurity profiling (characterization and isolation) plays a vital role. Regulatory bodies such as US FDA, ICH mandates to estimate the impurity present above 0.1% level. ICH provided guidance document for evaluate and analytical validation of impurities. New drug development requires meaningful and reliable analytical data to be produced as

- Sample set selection for analytical method development.
- Screening of Chromatographic conditions and Phases, typically using the linear solvent strength model of gradient elution.
- Optimization of the method to fine-tune parameters related to ruggedness and robustness.

Stages of Analytical Method Development

- Qualification of Impurities
- Identification of Impurity
- Isolation of Impurity
- Characterization of Impurity

Qualification of Impurities[20,23]

Qualification

- Establishing the biological safety of an individual impurity or a given impurity profile at levels specified.
- Any impurity tested in safety or clinical studies considered qualified.

- Impurities which are metabolites present in animal or human studies are qualified.

Thresholds

Higher or lower threshold limits based on scientific rationale including drug class effects and clinical experience.

- Adverse reaction in patients (lower)
- Patient population higher
- Drug class effects higher
- Clinical experience higher

Decision Tree for Identification and Qualification[23] which is given in ICH Q3A guideline describes considerations for the qualification of impurities when thresholds are exceeded.

Table5 : Qualification Threshold for API(3)

Max. Daily Dose	Reporting Threshold	Identification Threshold	Qualification Threshold
≤ 2g/day	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/day	0.03%	0.05%	0.05%

Identification of Impurity

The impurities can be identified predominantly by following methods;

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

Reference standard method[1,24]

The key 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[5,12]

The UV, IR, MS, NMR and Raman spectroscopic methods are routinely being used for characterizing impurities.

Separation methods[1,2,24]

The separation method includes chromatographic techniques like Capillary Electrophoresis (CE),

Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), High Performance Liquid Chromatography (HPLC) , Gel permeation etc are regularly being used for separation of impurities and degradation products.

Isolation methods[1,24]

It is often necessary to isolate impurities. But if the instrumental methods are used, isolation of impurities is avoided as it directly characterizes the impurities. Generally, chromatographic and non chromatographic techniques are used for isolation of impurities prior to its characterization. In loratidine, impurity found was ofloratidine. Other examples include celecoxib and amikacin. A list of methods that can be used for isolation of impurities are solid-phase extraction methods, liquid-liquid extraction methods, accelerated solvent extraction methods, supercritical fluid extraction, column chromatography, flash chromatography, capillary electrophoresis (CE), gas chromatography (GC), thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC).

Solid-phase extraction methods[1,25,26]

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. With SPE, many of the problems associated with liquid-liquid extraction can be prevented, such as incomplete phase separation, less than quantitative recoveries, use of expensive, breakable specially glassware, and disposal of large quantities of organic solvent. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantitative 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. 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 pH.

Liquid-liquid extraction methods[1,26]

In this type of extraction, two immiscible liquids was selected. Usually, one phase is aqueous (hydrophilic) and the other is a(hydrophobic) organic solvent. Liquid-liquid extraction is a basic

technique in chemical laboratories, where it is performed using a separating funnel. In that[31-34] the solute is distributed between two immiscible solvents .The extraction was based on Distribution Co-efficient or Partition Co-efficient (Kd), which is the ratio of concentration of solute in two different solvents a and b.

$$Kd = C_a/C_b.$$

Accelerated solvent extraction methods[1,26]

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. ASE offers a lower cost per sample than other technique, reducing solvent consumption by up to 90%.

Column chromatography[1,26,27-30]

Column chromatography is use to purify individual chemical compound from mixture of compound. Column chromatography is generally used as a purification technique. In column chromatography 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).The mixture to be analyzed by column chromatography is applied to th e 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. At the end of the column they elute one at a time, during the entire chromatography process the eluent is collected in a series of fraction. The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compound, for example, by analytical chromatography, UV absorption or fluorescence. Colored compound (or fluorescent compounds, with the aid of an UV lamp) can be seen through the glass wall as moving bands. 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[1,26]

Flash Chromatography is a rapid form of preparative column chromatography based on optimized 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." Flash chromatography utilizes 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. By Flash chromatography decreasing the time needed to purify the sample[31]. 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[1,26]

Thin-Layer Chromatography[27-30] is a simple and inexpensive technique that is often used to separate mixtures and 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 R_f values with reference. A number of enhancements can be made to the original method, to automated the different steps, to increase the resolution achieved with TLC, and is referred to as HPTLC or high performance TLC.

High performance Liquid chromatography (HPLC)[1,26]

Normal-phase HPLC[27-30] 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 themobile 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 RMe2SiCl, where R is a straight chain alkyl group such as$C_{18}H_{37}$ 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; there by 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. The detector may also provide other characteristic information i.e., UV / Visible spectroscopic data for the analyte if so equipped.

Supercritical fluid chromatography (SCF)[1,26]

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. SFC typically utilizes carbon dioxide as the mobile phase; therefore, the entire chromatography flow path must be pressurized.

Gas chromatography (GC)[1,26]

Gas chromatography[27-30] 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, 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[32].

Capillary Electrophoresis (CE)[1,26]

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 candidate for CE separation is ions. The basic instrumental set-up consists of a high voltage power supply (0 to 30 kV), a fused silica (SiO_2) capillary, two buffer reservoirs ,two electrodes, and an on column detector.

Electron Paramagnetic Resonance[26]

Electron Paramagnetic Resonance (EPR) or electron spin resonance (ESR) spectroscopy is a technique for studying the chemical species that have one or more unpaired electrons, such as organic and inorganic free radicals or inorganic complex processing a transition metal ion. The basic physical concepts of EPR are analogous to those of NMR, but it is electron spins that are excited here instead of spins of the atomic nuclei. As the most stable molecules have all their electrons paired, the EPR technique is less widely used than NMR. However, this limitation is less widely used than NMR. EPR technique is one of great specificity, as ordinary chemical solvents and matrices do not give to EPR spectra.

Characterization methods[1,24,26]

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[1,26]

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 multi component mixtures, Elemental analysis etc., The best example for the NMR study of the impurity state in heavily doped Si:P[33] 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[34] 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.

Cryogenic NMR probe technology [35]

Cryogenic NMR probe technology is used for identification of low level impurities in pharmaceutical dosage forms. In this technique, due to design consideration necessary to cool the internal components to temperature for the coil in range of 10-25K and preamps housed in probe body to temperature near 50K without destroying those components during thermal cycling. This technique is high sensitive, detect low concentration << 1% at the main component.

Mass spectroscopy[1,26]

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.

HPLC-DAD-MS[1,26]

This HPLC-DAD and LC-ESI-MS technique have been used for the analysis of doxycycline and its related impurities like metacycline and 6-epidoxycycline[56]**.**The mobile phase of oxalic acid (0.02 M; pH 2.5)-acetonitrile 82:18 (v/v) was used.

LC-MS[1]

In case of LC-MS a similar to GC-MS, though rather more difficult problem arises in the removal of liquid carrier from an HPLC eluent before samples are passed in to the MS source. The normal eluent flow rates of 0.5-2.0ml min^{-1} cannot be handled by the MS pumping system. 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 -methoxy-1,6-dimethylergoline-8-methanol 5-bromonicotinic acid ester (Nicergoline) and its related substances[37]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.

LC-MS-MS[1]

In this type of technique, the Characterization and quantitative determination of four impurities in piperaquine phosphate by gradient reverse phase HPLC and LC/MS/MS was developed[38] 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/MS[39] method.

Combined LC-MS / LC-SPE-cryo NMR method / LC-UV

This technique use mother liquor as a batter source of impurity.

Stability indicating HPLC-DAD / UV-ESI / MS method

This technique use for impurity profiling of the anti-malarial drug Iumefantrine

HPLC-DAD-NMR-MS[1]

The LC-DAD-MS/SPE-NMR Hyphenation techniques have been used in the Identification of Isobaric Iridoid Glycoside Regioisomers as minor constituents from *Harpagophytumprocumbens* of Pharmaceutically Used Plant Extracts[40]. Hence by using of this technique provides the spectral data needed for structure elucidation.

Tandem Mass spectrometry[1]

The tandem mass spectrometry (MS/MS) scanning modes are product ion, precursor ion, and 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)[1]

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 subsequently 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.

GC-MS[1,26]

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[57] by using of two distinct soft ionization techniques, the atmospheric pressure ionization with electrospray source (API–ESI) and the chemical ionization (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.

Applications of Impurity Profiling[5,20,26,41]

Numerous applications have been sought in the areas of drug designing and in monitoring quality, stability, and safety of pharmaceutical compounds, whether produced synthetically, extracted from natural products or produced by recombinant methods. . The applications include alkaloids, amines, amino acids, analgesics, antibacterial, anticonvulsants, antidepressant, tranquilizers, antineoplastic agents, local anesthetics, macromolecules, steroids, miscellaneous[42].

CONCLUSION

The guideline for impurity level provides the quality criteria for manufacturers. This review provides depth knowledge on importance of impurity profile for new drug Substance and New Drug Product with various techniques of isolation and characterization of impurities. The key aspect is that the impurity profiling of a new chemical entity must be shown to be qualified. With a qualification threshold of 0.1%, or lower for high dose compounds, the pharmaceutical analyst must give careful thought to their analytical technology. Especially in the development phases it may be necessary to utilize methods with high selectivity, including hyphenated techniques. Beginning with limit tests for impurities, this field of impurity identification and quantitation has progressed. Isolation and characterization of impurities is required for acquiring and evaluating data that establishes biological safety which reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

REFERENCES

1. Vijayalaxami R, Kumaravel S, Anbazhagan S. 'Scientific approaches for impurity profiling in new pharmaceutical substance and its product-An Overview, International Journal Of Pharmaceutical And Chemical Science.vol-1, jan-mar 2012; 386-403.
2. S. Ahuja. Impurities Evaluation of Pharmaceuticals, Marcel Dekker, Inc. New York, 2006.
3. Gorog S. Identification and Determination of impurities in Drugs. Elsevier science publishing company, Amsterdam. 2000;142.
4. Roy J. Pharmaceutical Impurities–a mini review. AAPS PharmSciTech. 2002;1-8.
5. Ayer A, Varpe D, Nayak R. Impurity profiling of pharmaceuticals. International Journal For Pharmaceutical And Allied Research. vol-1, 2011;76-90.
6. ICH Harmonized Triplicate Guideline: Stability Testing of New Drug Substances and Products Q1A (R2), ICH Steering Committee, Step 4 of ICH process, 6th Feb, 2003.
7. ICH Harmonized Triplicate Guideline: Impurities in New Drug Substances Q3A (R2), ICH Steering Committee, Step 4 of ICH process, 25th Oct. 2006.
8. ICH Harmonized Triplicate Guideline: Impurities in New Drug Products Q3B (R2), ICH Steering Committee, Step 4 of ICH process, 2nd June 2006.
9. ICH Harmonized Triplicate Guideline: Guideline for Residual Solvents Q3C (R3), ICH Steering Committee, Step 4 of ICH process, Nov 2005.
10. US-FDA Guidelines, NDAs: Impurities in New Drug Substances.
11. US-FDA Guidelines, ANDAs: Impurities in New Drug Substances.
12. Australian Regulatory Guidelines for Prescription Medicines - Impurities in Active Pharmaceutical Ingredients and Finished Products, Therapeutic Governance Authority (TGA), Australia.
13. Ahuja S, Scypinski S. Handbook of Modern Pharmaceutical Analysis, Vol.3, Separation Science and Technology, Academic press, USA. 2001;85-172.
14. Sapra A, Kakkar S, Narasimhan B. Source of impurity. International Journal Of Pharmacy.vol-3, Jan 2012;57-59.
15. S. Ahuja. Assuring quality of drugs by monitoring impurities, Advanced drug delivery reviews. Jan-2007;3-11.
16. Chatwal GR. Pharmaceutical inorganic chemistry.Vol.1, Himalaya publishing house, NewDelhi, 1991;31-68.

17. www.pharmainfo.net/exclusive/reviews/impurity_profile:_a_review/
18. www.pharmainfo.net/exclusive/reviews/impurity_profile_of_active_pharmaceutical_ingredient_:_a_review/
19. Ahuja S, Alsante KM. Handbook of Isolation and Characterization of Impurities in Pharmaceuticals. Vol. 5, Separation Science and Technology.Academic press,USA 2003;1-20,166-286.
20. ICH Harmonized Triplicate Guideline: Stability Testing: Photostability Testing of New Drug Substances and Products Q1B (R2), ICH Steering Committee, Step 4 of ICH process, November 1996.
21. Rao NR, Mani Kiran SS, Prasanthi NL. Indian J. Pharm. Educ. Res. 2010;302-306.
22. Deshmukh RS, Umarkar AR, Bavaskar SR. Impurity profile in pharmaceutical substance. International Journal Of Pharmacy And Biological Science. vol-1, oct-dec-2011;382-392.
23. International Conference on Harmonization (2000) Draft Revised Guidance On Impurities In New Drug Substances. Federal Register Q3A(R) 65 (140): 45085.
24. Solanki R. Impurity profiling of active pharmaceutical ingredient and finished drug products. International Journal Of Drug Research And Technology, vol-2, 2012;231-238.
25. ZwirFerenc A, Biziuk M. Solid Phase Extraction Techniques-Trends ,opportunities and applications. Polish Journal of Environmental studies. 2006;677-690.
26. Pilaniya K., Chandrawashi HK, Pilaniya U. Recent trends in the impurity profile of pharmaceuticals.,Journal Of Adavanced Pharmaceutical Technology And Research ,vol-1, jul-sep 2010; 302-310.
27. Beckett AH and Stenlake JB. Practical Pharmaceutical Chemistry,4thedition University press,cambridge,1997;85-468.
28. Willard HH, Meritt LL, Dean JA, Settle FA. Instrumental methods of analysis,7th edition CBS Publishers and Distributors,NewDelhi,1986.
29. Sharma BK. Instrumental methods of chemical analysis,18th edition, Krishna Prakashan Media Pvt Ltd, Merut,2000;c 20-212..
30. Chatwal GR, Sham A. Instrumental methods of chemical analysis, 5th edition, Himalaya publishing house, NewDelhi,2002.
31. Still WC, Khan M, MitraA. ,Flash chromatography. J Org Chem. 1978;43:2923-5

32. Donald LP, Gary ML. Introduction to Organic Laboratory Techniques. Vol.4. Thomsons Brooks;2006;797-817.
33. Ernesta M, Meinties, Jeremy D, William W, Warren Jr. Temperature-dependent NMR study of the impurity state in heavily doped Si:P Phys. Rev. B .2005;1-14.
34. Follstaedt DM,Slichter CP. NMR study of copper with vanadium impurities. Phys Rev. 1977;21–29.
35. http://link.springer.com/chapter/10.1007%2F1-4020-3910-7_129
36. Fiori J, Grassli G. HPLC-DAD and LC ESI-MS analysis of doxycycline and related impurities in doxipan mix, a medicated premix for incorporation in medicated feedstuff. J Pharm Biomed Analysis. 2005;979-985.
37. Banno K, Horimoto S. Separation and quantitation of nicergoline and related substances by highperformance Liquid chromatography/atmospheric pressure ionization mass spectrometry. Chromatographia31:51-54.
38. Dongre VG, Karmusse PP. Characterization and quantitative determination of impurities in piperaquine phosphate by HPLC and LC/MS/MS. J.Pharm Biomed Analysis. 2007;186-195.
39. Christopher S, Markus G. LC-DAD-MS/SPE-NMR Hyphenation. A Tool for the Analysis of Pharmaceutically Used Plant Extracts: Identification of Isobaric Iridoid Glycoside Regioisomers from Harpagophytumprocumbens Anal Chem. 2005;878–88.
40. Kakadiya PR, Chandrashekhar TG. Low Level Determinations of MethylMethanesulfonate and Ethyl Methanesulfonate Impurities in EmtricitabineActive Pharmaceutical Ingredient by LC/MS/MS Using Electrospray Ionization. Analytical Chemistry Insights. 2011;21-28.
41. Bari SB, Kadam BR, Jaiswal YS, Shirkhedkar AA. Impurity profile: Significance in Active Pharmaceutical Ingredient. Eurasian Journal of Analytical Chemistry. Vol- 2, 2007;33,43- 46.
42. Lohr LL, Sharp TR, Alsante KM, Hatajik TD. Isolation and Identification of Process Related Impurities and Degradation Products from Pharmaceutical Drug Candidates. Part II: The Roles of NMR and Mass Spectrometry. American Pharmaceutical Review.
43. JoythirmayeeM,Phanikumar K. Pharmaceuticalimpurities:significance of impurity profiling. International Journal Of Pharmacy And Technology. vol-4, April 2012;1822-1842.

44. Patil P,Dr.Vaidyai. Overview on impurity profiling. International Journal ForPharmaceutical Research Scholar. vol-2, april-2013;54-65.
45. Ingale SJ, Sahu CM, Paliwal RT. Advance approaches for the impurity profiling of pharmaceutical drug. International Journal Of Pharmacy And Life Science, vol-2, July 2011;955-962.
46. ICH Harmonized Triplicate Guideline: Validation of Analytical Procedures: Text and Methodology Q2 (R1), ICH Steering Committee, Step 4 of ICH process, November 2005.
47. Guidance for industry- ANDAs:Impurities in Drug substances, Draft guidance, U.S.Department of Health and Human services, Food and drug Administration, Center for Drug Evaluation and Research.1998
48. Alsante KM, Hatajik TD, Lohr LL, Sharp TR. Isolation and Identification of Process Related Impurities and Degradation Products from Pharmaceutical Drug Candidates. Part1, American Pharmaceutical Review.AAPS Pharmscitech. 2001;1-8.
49. Food and Drug administration for immediate release. Consumer media: 888-Info-FDA.May 6, 1998.
50. http://pharmtech.findpharma.com/pharmtech/Analytical/Organic-Impurities-in-Chemical
51. Van K, PC, Bult, A. Penicillins and cephalosporins: physicochemical properties and analysis in pharmaceutical and biological matrices. **IJPS**. Vol-22, 1987;67-80.
52. Connor KA. Chemical Stability of Pharmaceuticals. Handbook for Pharmacists. John Wiley & Sons, New York, 1986;354-62.
53. http://pharmtech.findpharma.com/pharmtech/Analytical/Inorganic-Impurities-in-Chemical
54. Gazdag M, Babjag M, Brlik J, Maho S, Tuba ZandGorog S.Estimation of profile of drug andrelated materials Part 18. Impurities and degradation product of mazipredone. J Pharm Biomed Anal. 1999;1029-36
55. Ahuja S. Trace and Ultra trace Analysis by HPLC.Willey, New York, 1992;84
56. Ravindra KY. Application of LCMS/ MS for the identification of the polar impurity in mosapride, agastroprokinetic drug. J Pharm Biomed Anal.2003 32:361.
57. Joachim E. The use of hyphenated LC-MS technique for characterization of impurity profilesduring drug development. J Pharm Biomed Anal. 1998;18

58. Babjak M. Estimation of impurity profile of drugs and related materials Part XXI. HPLC/UV/MS study of the impurity profile of ethynodioldiacetate. J Pharm Biomed Anal. 2002;29.
59. Alsante KM, Boutres P, Couturier MA, Fridmann RC, Harwood JW, Horan GJ, Jensen AJ, Liu Q, Lohr LL, Morris R, Raggon JW, Reid GL, Santafianos DP, Sharp TR, Tucker JL, Wilcox GE. Pharmaceutical Impurity Identification: A Case Study Using a Multidisciplinary Approach. Journal of Pharmaceutical Sciences. 2004; 2296.
60. Food and Drug Administration for Immediate Release Consumer Media (1998) 888-Info- FDA. May 6, 1998;45
61. Analytical procedures and methods validation chemistry, manufacturing, and controls documentation- Guidance Document (2000) U.S. Department of Health andHuman Services Food and Drug Administration. Center for Drug Evaluation andResearch (CDER), Center for Biologics Evaluation and Research (CBER).
62. Ms. Meera SH. Impurity Profile: A Review Pharmainfo_net (2005) available at :http://www.pharmainfo.net/exclusive/reviews/impurity_profile: A Review.