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ISOLATION AND CHARACTERIZATION OF PHARMACEUTICAL PRODUCT CONTAINING IMPURITIES BY USING ADVANCED ANALYTICAL INSTRUMENTS

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ABSTRACT

A change in human health was brought about by the invention of pharmaceuticals. These pharmaceuticals can fulfil their function only if they are free of impurities and are given in a sufficient quantity.[1] Impurity profiling requires the recognition, structural elucidation and quantitative determination of the bulk drug materials and pharmaceutical formulations of impurities and degradation items.[2] Isolation, detection and quantification of impurities enable us in different ways to achieve a pure material with less toxicity and protection in drug therapy.[3] Impurities are a critical issue for the pharmaceutical industry at present. Guidelines on the control of impurities were formulated by the International Harmonization Conference (ICH). With specific examples, this review describes different types and origins of impurities and degradation routes.[4] In this analysis, an overview of the various forms and sources of impurities i.e. organic inorganic impurities, residual solvents is provided in relation to the ICH guidelines and the routes of degradation, including detailed examples.[5] A variety of chromatographic and spectroscopic methods are used to detect impurities, either individually or in conjunction with other techniques.[6] This review provides useful information on impurities, their classification and the origins of impurities, and discusses various isolation (Liquid-solid methods of extraction, Liquid-liquid methods of extraction Column chromatography, Flash chromatography, TLC,GC, HPLC, etc.) and characterization methods (Nuclear Magnetic Resonance (NMR), IR, Mass Spectroscopy (MS), LC-MS), analytical techniques for the determination of impurities, and the identification of impurities.[3]

KEYWORDS: Impurities, ICH Guidelines, Classification of Impurities, Sources of impurities, Methods of Isolation, Methods of Characterization.

INTRODUCTION

Drug impurities are the unwanted chemical that remains in formulation. These impurities are developed throughout the formulation or upon aging of either APIs or formulated Active Pharmaceutical ingredients to medicine.[3] According to ICH Q3A (R) Impurities in the New Drug Substance and ICH Q3B (R) Impurities in the New Drug Product.[7] Any component of the drug product that is not the chemical compound defined as the drug substance or excipients in the drug product (ICH Q6A: Specifications).[8] Probably impurities come from the Reagents, intermediates and by-products from the chemical reaction of drug substance during manufacturing process ,degradation and excipients interaction products that may form in the drug substance or the drug product.[9] In past few decades much attention is given towards the quality of pharmaceuticals drug product that enters the market. The major challenge for both pharmaceutical industries & bulk drug industries is to produce high quality products.[2] According to Pharmacists Pharma Journal a drug is any chemical compound that may be used on or administered to humans to help diagnose, treat, cure, mitigate, or prevent disease or other abnormal conditions.[10] In the process of development of new drug molecule it starts with the new drug molecule that has therapeutic significance to fight, control, or cure diseases.[1] It is very important to conduct speedy quality control checks in order to maintain the quality and purity of the new drug output from each pharmaceutical industry.[2] By using chemistry, pharmacology, microbiology and biochemistry has set a framework in the drug discovery where new drugs are generated by the imagination of chemists. These new drugs are the outcome of exchange of knowledge between chemists and biologist.[1]

There are a various type of specialized analytical techniques available for the characterization of the purity of new drugs.[11] Concept about how the purity changes with time and it is irresolvable from the developments in analytical chemistry. Modern separation techniques clearly play a effective role in scientific research today because these methods are simultaneously separate and assess the components hence it makes the separation and characterization of impurities easier.[2] These impurities are mostly similar in structure to the drug substance and high performance liquid chromatography (HPLC) continues to be the primary technique used for the determination of impurity in drug substances and drug

products.[9] It is one of the most frequently used analytical techniques and used nearly every chemical application.[12]

Impurity profiling: Impurity profiling is the description, characterization and quantitation of identified and undefined impurities present in the drug substances is known as impurity profile.[13]

Impurities: Any material that affects the purity of the material of interest. Impurities in pharmaceuticals are unwanted chemicals that even in small amount may influence the efficacy and safety of the pharmaceutical products.[14]

ICH Guidelines

ICH guidelines on impurities are as follows: 1. ICH guidelines Q3A- makes recommendations on Impurities in new drug substances and drug products.[15]
2. ICH guidelines Q3B - Impurities in new medicinal products. 3. ICH guidelines Q3C - Impurities: residual solvents. 4. ICH guidelinesQ3D - Guidelines for Elemental Impurities. 5. ICH Guidelines Q6A- Specifications.[16]

As per ICH guidelines on impurities in new drug substance and new drug product, presence of impurities below the level of 0.1% level is not necessary if the potential impurities expected to be unusually potent or toxic.[1] According to ICH, the maximum daily dose qualification threshold is presume as follows, ≤2 g/day 0.1% or 1 mg per day intake (whichever is lower) ≥ 2g/day 0.05% when such impurity profile received by regulatory authorities ,different pharmacopoeias such as British pharmacopoeia (BP),united state pharmacopoeia(USP), Indian pharmacopoeia(IP),European pharmacopoeia (EP)are pay the attention level of impurities present in the new drug substances or APIs and Formulations.[4,16]

Table 1: ICH Guidelines for Impurity Detection and Certification In drugs and formulations for bulk.[17]

Dose	Threshold for	
	Identification (%)	Qualification (%)
<1mg	1.0	1.0
1-10mg	0.5	1.0
10-100mg	0.2	0.5
100mg-2gm	0.1	0.2
>2gm	0.1	0.1

Classification of Impurities

1] As per ICH

Impurities can be classified into the following categories

A) Organic impurities: Organic impurities can arise during the synthetic process or storage of the new drug substance. They can be identified, volatile or non-volatile, and include:

a) Starting materials
b) By-products
c) Intermediates
d) Degradation products
e) Reagents, ligands and catalysts

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

a) Reagents, ligands and catalysts
b) Heavy metals or other residual metals
c) Inorganic salts.
d) Other materials (e.g. Filter aids, charcoal).[16]

C) Residual solvents

- Class 1
- Class 2
- Class 3

A) Organic impurities

a) Starting material /intermediate products

Starting materials (mostly from isomeric impurities) and intermediates (incomplete reaction) are chemical building blocks used to form the final form of a drug substance. If it remain unreacted or when obtained with the final product due to improper removal during manufacturing process these are considered as impurities.[18]

For example: In the synthesis of paracetamol drug the starting material 4-aminophenol is used. This starting material is present as an impurity the final product having a toxic effect on the liver.[19]

b) By-Products

In the synthesis of new drug product in organic chemistry, getting a 100% yield is very rare; there is always a chance of having by-products. By-products from the competing reaction are among the most common process impurities in drugs. By-products can be formed through a variety of parallel reaction, such as incomplete reaction, Over reaction, isomerisation Dimerization, rearrangement, unwanted reactions between starting materials or intermediates with chemical reagents or catalysts.[8]

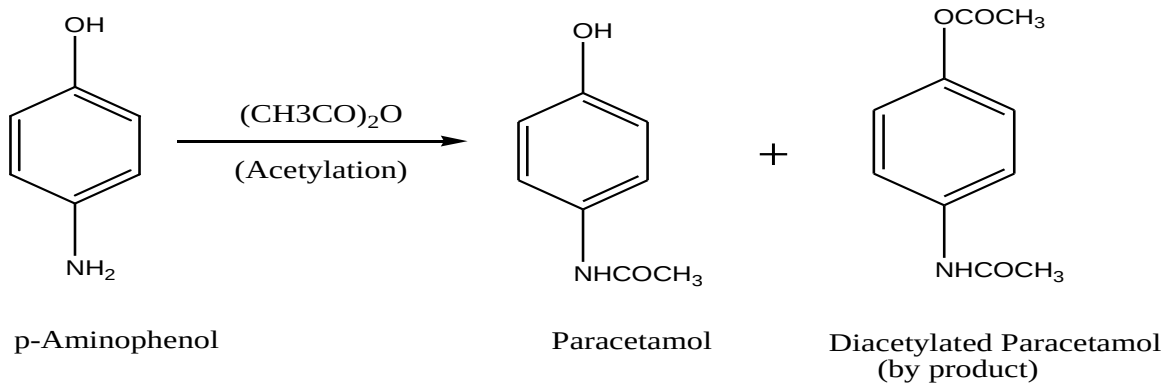
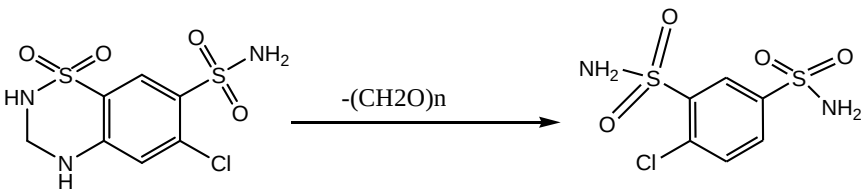


Figure 1: Diacetylated Paracetamol is produced in Paracetamol synthesis as a by product.[18]

c) Degradation Products

Impurities can also be formed by the breakdown in a chemical reaction involving the collisions of the molecules of the end product during manufacturing of bulk drugs. The chances of degradation of products from storage or manufacturing to different dosage forms or aging are also common impurities in the medicines.[8]

Carry out the accelerated stress testing on the drug product to determine significant degradation products.[11] Any chemical change happens due to overheating or changing in condition of solution, such as change in PH, etc. Or reaction end product with container or closure or excipients used in making product.[19] A well-known example of degradation products the degradation of penicillin's and cephalosporin The presence of a ß-lactam ring as well as that of an α-amino group in the C6/C7 side chain plays a critical role in their degradation.[8]



Hydrochlorothiazide Disulfonamide degradtion product

Figure 2: For example, deterioration of hydrochlorothiazide to the starting material i.e. about disulfonamide.[18]

d) Reagents, ligands and catalysts

Such types of impurities are very rarely found. For e.g. in the synthesis of Mazipredone, pyridine is used as catalysts react with the an intermediate to form pyridinium impurity.[18] chemical reagents, ligands and catalysts used in the synthesis of a drug substance can be brought over to the end products as vestige level impurities.[8]

B) Inorganic impurities

Such type of impurity obtained from manufacturing process. They are generally identified and known in nature. It contain impurities like heavy metal impurities, residual solvent impurities and other material impurities such as filter aids.[18]

a) Reagents, ligands, and catalysts: There is very rare chance of having such type of impurities therefore, in some processes, these could create a problem until the manufacturers take proper care during production.

b) Heavy metals The major sources of heavy metals are the water used in the manufacturing process and the reactors are used(if they are stainless steel). it performs the acid hydrolysis or acidification reaction take place. Such type of impurities of heavy metal can efficiently be avoided by using demineralised water and the glass-lined reactors.[8]

c) Other materials: Various types of filtered aids are used for the bulk drug manufacturing plant and in many cases, activated carbon is also used. The regular monitoring of fibres and black particles in the bulk drugs is important to avoid such contaminations**.** (e.g., filter aids, charcoal etc.).[8]

C) Residual solvents

Are potentially unwanted substances .residual solvents have either capacity to modify the properties of the specific compound or they may be hazardous to human health .they can also

affect the physiochemical properties of compound such as crystallinity, in this way it can affect the dissolution parameter such as odour ,colour change in the final product.[4]

Organic volatile chemicals these are used or produced during the manufacturing process of drug product.[20] Residual solvents are classified according to the risk assessment to human health to three main classes:

a) Class 1 solvents: [solvents to be avoided]

Class 1 solvents are unacceptable because of known human carcinogens, strongly suspected human carcinogens, and environmental hazards. these solvents are not used in the manufacturing process of drug product.[14]

Table 2: Class 1 solvents.[14]

Solvents	Class 1 solvents Concentration in ppm	Adverse effect
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Tricholroethane	1500	Environmental hazard

b) Class 2 solvents [solvents to limited]

These are very limited use in the pharmaceutical products due to their carcinogenic potential (non-genotoxicity), neurotoxicity or teratogenicity.[21]

Table 3: Class 2 solvents in pharmaceutical products.[21]

Solvent	Permissible daily exposure(mg/day)	Concentration (ppm)
Acetonitrile	4.1	410
Chloroform	0.6	60
Cyclohexane	38.8	3880
1,2 –Dichloroethene	18.7	1870
Dichloromethane	6.0	600
1,2- Dimethoxyethane	1.0	100
N,N-Dimethylacetanide	10.9	1090
N,N- Dimethylformamide	8.8	880
1,4- Dioxane	3.8	380
2- Etoxyethanol	1.6	160
Ethyleneglycol	6.2	620
Formamide	2.2	220
Hexane	2.9	290
Methanol	30.0	3000
2-Methoxyetanol	0.5	50
Methylbutylketone	45	50

Methylcyclohexane	11.8	1180
N-Methylpyrrolidone	5.3	4840
Nitromethane	0.5	50
Pyridine	2.0	200
Sulpholane	1.6	160
Tetralin	1.0	100
Toulene	8.9	890
1,1,2- Trichloroethene	0.8	80
Xylene	21.7	2170

c) Class 3 solvents [solvents with low toxic potential]

Class 3 solvents with low toxic potential to human; if these are present in drug product at normally accepted levels class 3 solvents have PDEs of 50mg or more per day.[14]

Table 4: Class 3 solvents.[21]

Acetic acid	1-propanol
Acetone	2-propanol
1-butanol	Heptanes
2-butanol	Isobutyl acetate
Butyl acetate	Isopropyl acetate
tert-butylmethyl ether	Methyl acetate
Dimethyl sulfpxide	3-methyl-1-butanol
Ethanol	Methylethyl ketone
Ethyl acetate	2-methyl-1-propanol
Ethyl ether	pentane
Ethyl formate	Propyl acetate
formic acid	triethylamine

Sources of impurities

The type and amount of impurity present in the chemicals or pharmaceutical substances depend upon several factors like those listed below:

a) Crystallization related impurities

b) Stereochemistry related impurities

c) Residual solvents

d) Chemical process related impurities

e) Manufacturing related impurities.

f) Impurities arising during storage

g) Method related impurity

h) Mutual interaction amongst ingredients

i) Functional group related typical degradation.[6]

Sources of impurities

a) Crystallization related impurities

In the crystallization process solution and solute interaction take place at molecular level start the formation of cluster and nucleation process particularly affects the crystal structure and morphology. Polymorphism means crystal substance can present in different crystal packaging, all of which have the same elemental composition.[22]

b) Stereochemistry related impurities

It is of foremost significance to search for stereochemistry related mixes; that is, Those compounds that have similar chemical structure but different 3-Dimensional structure, these compounds can be considered as impurities in the API's. Chiral molecules are frequently called enantiomers. The single enantiomeric type of chiral drug is presently considered as a better compound atom that may offer a superior pharmacological profile and an expanded helpful record with a more reasonable unfavorable response profile. In any case, the pharmacokinetic profile of levofloxacin (S-isomeric structure) and ofloxacin (R-isomeric structure) are tantamount, recommending the absence of preferences of single isomer in such manner.[6]

c) Residual solvents

Water is ordinarily present in drug items. Accordingly, water is by a long shot the most regularly discovered unpredictable contamination in drug items, and more often than not it isn't viewed as a debasement. It is predominant both in drug substances and excipients and is utilized in measurement structure arrangements also. Dampness substance can be significant when a measurement structure is bundled with the end goal that equilibration with the climate doesn't happen. Under these conditions, the dampness brought into the framework through the excipients can be adequate to achieve hydrolysis. Moreover, water from the climate can influence drug items and can regularly be impeding to their compound dependability or dose structure execution.[6]

d) Synthetic intermediates and by-products

Debasements in drug mixes or another substance element (NCE) can begin during the engineered cycle from crude materials, intermediates or potentially side-effects. For instance, contamination profiling of rapture tablets by GC-MS and MDMA tests, created pollutants in intermediates by means of reductive amination course.[6]

e) Manufacturing related impurities

Numerous pollutions in a medication item can start from excipients used to plan a medication substance. Also, a medication substance is exposed to an assortment of conditions during the time spent plan that can cause its corruption or have other unfortunate responses. In the event that the source is from excipients, fluctuation from part to parcel may make a minimal item, inadmissible for dependability. Arrangements and suspensions are inalienably inclined to corruption because of hydrolysis or solvolysis Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was reviewed in the US due to corruption/pollutants prompting sub-strength.[6]

f) Impurities arising during storage

During the storage (shelf life) or shipment of drug products, a number of impurities can occur. In order to predict, assess and ensure drug product safety, it is necessary to perform stability studies. However, depending on their discipline in the pharmaceutical sciences and industry, stability may have various implications for different individuals. In order to cover the what, how and why of stability, a number of concepts are currently used: kinetic analysis, compatibility study, stability assessment, Stability-indicating assay, expiry date, obsolescence, shelf life, storage legend, pre-formulation analysis, failure of the batch to comply with requirements, microbiological stability, active ingredient stability, formulation stability, package stability, package stability, package stability, package stability, and market stability. Both of these are factors in a drug product's stability, and it is important to ensure that everybody recognises the value of stability studies.[23]

g) Method related impurity

Different activities during the formulation process any undesirable products may be produced from a drug product. . To be determined, certain impurities are needed If they are greater than the boundaries set by the regulatory authorities. In the manufacture of parenteral dosage forms of Diclofenac, for example, As terminal sterilisation is performed, sodium, Autoclaving enforces the intra-molecular (i.e. 123 + 2 ° C) Diclofenac sodium cyclic reaction which forms a 1-(2, 6- indolin-2-one dichlorophenyl) indolin-2-one and sodium hydroxide.[14]

h) Related to the climate

Major environmental variables that can decrease Stability contain the following information:

Adverse temperature conditions

There are several APIs for heat or tropical temperatures that are labile. For, Vitamins, for example, are very hot as drug substances- Sensitivity and degradation also contribute to the loss of Potency in vitamin products, in particular in liquids Formulations.[5]

Light-especially UV light

Many studies have reported that the injection of ergometrine as well as methyl ergometrine is unstable under tropical conditions, such as light and heat, and several field samples have found a very low level of active ingredient. The amount of the active ingredient complied with the BP / USP limit of 90 percent to 110 percent of the specified content in only 50% of the marketed samples of ergometrine injections tested. The custom-made ergometrine injection (0.2 mg / mL) showed almost complete degradation If held in direct sunlight for 42 hours.[8]

Humidity

Hygroscopic compounds are a key destabilising element. Bulk powder and formulated drug formulations are degraded by humidity, such as Aspirin and Ranitidine tablets.[18]

i) Mutual interaction amongst ingredients

Most vitamins are very unstable and create a problem of instability in various dosage forms during ageing, especially in liquid dosage forms. Vitamin degradation does not give rise to harmful impurities; however, the potency of active ingredients falls below the requirements of Pharmacopoeia. The presence of nicotinamide in a formulation that contains nicotinamide due to mutual interaction four vitamins (nicotinamide, pyridoxine, riboflavin, and thiamine) can cause thiamine to degrade to below the sub-standard amount of vitamin B-complex injections over a one year shelf life. Marketed vitamin B-complex injection samples have been found to have a pH range of 2.8-4.0. A custom-made simple distilled-water formulation and a standard formulated vehicle like disodium edetate and benzyl alcohol were examined and similar mutual interactions were observed causing degradation.[6]

j) Functional group-related typical degradation

Ester hydrolysis

Examples of ester hydrolysis include the following: Aspirin, Benzocaine, etc.

Hydrolysis

Hydrolysis is a prevalent trend for Ester drug type, particularly in the liquid dosage phase, Examples nclude barbital, benzyl penicillin, Lincomycin, chloramphenicol, chlordiazepoxide, and Oxazepam.[5]

Oxidative degradation

Methotrexate, hydrocortisone, a group of hydroxyls directly bound to an Aromatic ring (e.g., derivative of phenols such as Morphine and catecholamine), conjugated dienes (e.g., Heterocyclic, vitamin A and unsaturated free fatty ac-ids), The aromatic rings, derivatives of nitroso and nitrite, and Aldehydes (e.g., flavourings) are all resistant to oxidative activity.[5]

Photolytic cleavage

The results of pharmaceuticals are exposed to light when being formed as a solid or a solid packaged solution, held in pharmacy shops or hospitals Pending use, or retained by pending use by the user. Nifedipine, nitroprusside, riboflavin, and ergometrine Phenothiazines for photo-oxidation are very labile.

In Photo-chemical energy produces susceptible molecules, Free radical intermediates that are capable of maintaining the chain about responses. Many com-pounds would degrade as alternatives upon exposure to UV exposure with high energy.

Fluoroquinolones antibiotics are found to be prone to photolytic cleavage. Sunlight causes a photo cleavage reaction that creates ethylenediamine analogue of ciprofloxacin in the preparation of ciprofloxacin eye drops (0.3 percent).[5]

Decarboxylation

Certain carboxylic acids which have been dissolved, losing carbon dioxide, such as p-amino salicylic acid, from when heated, to the carboxyl group, and decarboxylation photoreaction of rufloxacinin occurred.[5]

k) Impurities obtained from Container / Closer / Packaging

Leachables are impurities of drug products leached from the container / closer / container /Components for packaging. In a variety of drugs, leachables can be found Materials, including orally inhaled and nasal drug products (OINDP), Injectables, solid dosage forms, etc.; both organic and Inorganic chemical entities.

Polymeric material oligomers, or additives, cross-link / curing the release of antioxidants, plasticizers, pigments, lubricants and mould Agents, etc., that are used to produce the container / closer / container Materials for packaging. Labels, inks and adhesives associated with the Container / closer / packaging systems of drug products can also leach impurities. To the product of the drug. Identification of leachables can be a major consideration for some dosage forms, analytical challenge. In an MDI, for example, the components of rubber and plastic metering The valve, which is primarily in direct, constant contact with the formulation, is A propellant such as chlorofluorocarbon (CFC) or a more ozone-friendly propellant HFA, which are all good organic solvents (hydrofluoroalkane).[7]

Separation method

Several techniques have also been developed, such as spectrophotometry or electrophoresis. Chromatographic approaches, specifically GC and LC, using either traditional detectors such as UV detection, or coupling with mass spectrometry, are the most common methods for determining these analyte.[24]

Methods of Isolation

Isolating impurities is also important. But if the use of analytical methods is used, Impurity isolation is discouraged since the impurities are specifically characterised. Generally, prior to characterization, chromatographic and non-chromatographic methods are used to separate impurities. The word 'chromatographic reactor' refers to the use of an analytical column as both a flow-through reactor and a separation medium for the reactant(s) and product(s) at the same time. The solution-phase hydrolysis kinetics of the A prepitant prodrug, by means of an HPLC chromatographic reactor approach. A list of methods that can be used for impurity isolation is given below.[6]

1. Liquid-solid methods of extraction
2. Liquid-liquid methods of extraction
3. Column chromatography
4. Flash chromatography
5. TLC
6. GC
7. HPLC
8. HPTLC 9)Capillary electrophoresis (CE)
9. Supercritical fluid chromatography (SFC).[6]

1) Liquid-solid methods of extraction

A solvent is chosen that will remove the impurity of interest. For extraction, an organic solvent blend is used where a substance contains more than one kind of impurity. At low temperatures, these solvents begin to volatilize, promoting impurity concentrations. Toluene, methanol, water, and cyclohexane are examples of common solvents used in liquid-solid extraction**.**

2) Liquid-solid methods of extraction

This includes the extraction of one liquid with another, one of which is aqueous and the other organic, both of which are mutually immiscible.[18]

3) Flash chromatography

All major techniques for the purification of organic compounds are distillation, re-crystallization, and extraction. The process used most often in modern organic science however, is 'flash' chromatography. The sample to be purified is placed on top of a column containing a certain solid support, often silica gel, in conventional column chromatography. The remaining column is then filled with a solvent (or a solvent mixture), which then passes through the solid. The rest of the column is then filled with a solvent (or a combination of solvents) which, under the force of gravity, then runs through the solid support. At different speeds, the different components to be separated pass through the column and are then collected separately when they emerge from the bottom of the column. The rate at which the solvent percolates through the column, unfortunately, is sluggish. However, air pressure is used in flash chromatography to speed up the solvent's flow, significantly reducing the time needed to purify the sample.[8]

4) Column chromatography

It can be used for the quantitative separation of impurities ranging from milligrams to kilograms. By occasionally monitoring the collected fractions from a given sample, UV-spectrophotometry is used for identification of the eluent. Example- It is possible to isolate the Mirabegron impurity (associated with more than one impurity) by the column process.[18]

5) Gas chromatography (GC)

It is useful for isolating and characterising volatile impurities or compounds that can be volatilized through derivatization. For example, acetone and ethanol were found as impurities by gas chromatography in the manufacture of Doxorubicin hydrochloride.[18]

6) Thin Layer Chromatography

Chromatography of thin layers is a common technique for the Test of a large range of organic and inorganic products, Due to its distinctive benefits, such as the minimum sample, Clean-up, large choice of mobile phases, sample versatility Distinction, high potential for sample loading and low cost. The TLC It is a effective method for screening unidentified bulk products Drugs. It offers a comparatively high standard of the degree of assertion that all likely drug components are Different are separated. The elevated specificity of TLC was exploited Using spot elution followed for quantitative analytical purposes by calculating Spectrophotometric. Different Pharmaceutical impurities have been found and Using TLC Determined.[1]

7) High Performance Liquid Chromatography (HPLC)

One of the most common and mature analytical techniques is HPLC. The separation method is by far the most commonly used. It's got over the past 40-plus, they have been used in laboratories worldwide Years of Medicinal Chemistry, Pharmaceutical Sciences, Analyses of food and the environment, synthetic chemistry, etc. The process of chromatographic mode or separation, it relies on the overall interactive connections between the stationary, mobile and analytical stages. Particle- packed columns with either fully porous or absolutely porous columns The newly-developed particles and monolithic core-shell Columns in conventional or miniaturised HPLC are used.[25] HPLC coupled with UV detection is used for impurity analysis, which is found in pharmaceutical quality control laboratories. UV spectrometry is a perfect instrument for drug impurity or degradation detection, based on maximum absorption. Due to its high selectivity, this technique is one of the most important and thorough analytical methods available for impurity profiling, especially for routine analyses where standards are available.[10]

Methods of Characterization

1) Nuclear Magnetic Resonance (NMR)
2) Infrared (IR)
3) Mass Spectroscopy (MS)
4) LC-MS

1) Nuclear Magnetic Resonance

It can provide knowledge on molecular structure and stereochemistry of molecule. You can easily evaluate multicomponent mixtures,[18] NMR research in order to assign For this large

number of DPs, the structures. The Compounds Preparative chromatography and one of the IMPs have been insulated**,** Characterized from precise mass and 1D/2D NMR data as a dimer.[26] Unfortunately, relative to other analytical methods, NMR has historically been response-limited.[23] Forced degradation is a very significant factor for the identification of degradation materials helpful method. By stressing the drug under different circumstances, not only does one stress the drug under different circumstances, Obtain details on the mechanism of the creation of the unknown, however often, for isolation and detection, an enriched sample. If the degradation Chemistry (plausible structure, formation mechanism, etc.) is set up. In order to further enrich it, a preparatory forced degradation should be performed. For isolation, the sample.[7]

2) Infra-Red (IR)

The sample is exposed to electromagnetic radiation ranging from Between 500 cm-1 and 4000 cm-1 which affect the present bonds in molecules and in molecules create stretching or bending Due to the absorption of particular wavelength radiation. The absorbed wavelengths are typical of different kinds of waves. Bonds that assist in the determination of sample structures. Strong, Solid IR spectroscopy can characterise samples and semi-solid samples. IR spectroscopy provides any molecule with a complex but distinctive fingerprint that assists in analysing drug samples and evaluating the existence of impurities in drugs. Compared to things like NMR, it is cost efficient and fast. It also works for a wide range of samples and can very strongly detect compounds, although related methods are weaker, such as Raman spectroscopy.[14]

3) Mass Spectroscopy (MS)

The most precise method of determining the molecular mass of the compound and its elemental composition is mass spectroscopy. Mass spectroscopy is used to prove the identity of two molecules, to determine the structure of the new compound, to provide precise molecular mass, to provide molecular formula and, most importantly, to elucidate the structure. Mass spectroscopy associated with various hyphenated techniques such as GC-MS, LC-MS, LCMS-MS HPLC-DAD-MS, HPLCDAD-NMR-MS, Tandem Mass Spectroscopy and Capillary Electrophoresis-Mass Spectroscopy.[5]

4) LC-MS

Using the HPLC procedures described in the following paragraph, LC-MS experiments were carried out on either a Micro mass Instruments Quattro-LC triple quadruple system or a

Micromass QTOF 1 tandem quadruple flight time system. Ionization with electro spray was used. After the UV-Vis detector, but before the mass spectrometer, a Valco flow splitter was inserted into the HPLC solvent flow direction. Even though the mega flow electro spray interface on the mass spectrometer is rated to handle larger flow rates, when a lower flow rate is presented, we observe more satisfactory full spectral efficiency. Flow was split after separation and UV-Vis detection, passing the smaller portion of the flow to the mass spectrometer and the larger portion of the flow to waste, in order to perform chromatographic separations comparable to those achieved by analytical HPLC but to conform to the flow constraint of the LC-MS interface.[27] Atmospheric pressure ionisation with electro spray source (API-ESI) and the chemical ionisation of d-allethrine are an example of reverse-phase LC-MS analysis in gradient elution with two distinct soft ionisation techniques.[20]

Table 5: The list of drugs and associated impurities is as follows.[8,20]

Drug	Impurity	Analysis Method
Amphotericin B	Tetraenes	Ultra violet spectroscopy
Atropine Sulphate	Apo atropine	Ultra violet spectroscopy
Cloxacillin	N,N dimethyl aniline	Gas chromatography
Dextrose	5-Hydroxy Methyl furfural	Ultra violet spectroscopy
Doxorubicin Hydrochloride	Acetone and ethanol	Gas chromatography
Ethambutol Hydrochloride	2-amino butanol	Thin layer chromatography
Mercaptopurine	Hypoxanthine	Ultra violet spectroscopy

CONCLUSION

An above overview we conclude that all pharmaceutical formulation and drug required to be a prescribed quality and purity as per ICH guidelines. The above-mentioned drug examples such as paracetamol (degraded into deacetylate paracetamol), hydrochlorothiazide (degraded into disulfonamide derivative). Likewise, other drugs also degraded. The degraded product in the pure drug is considered to be as an impurity in a pure drug. To achieve prescribed quality& purity of drug, we need to analyse and isolate the particular compound by using the various analytical techniques, such as Liquid-solid methods of extraction, Liquid-liquid methods of extraction, Column chromatography, Flash chromatography, TLC, GC, HPLC, HPTLC, Capillary electrophoresis (CE), Supercritical fluid chromatography (SFC). Some analytical techniques have used to characterization of drug Nuclear Magnetic Resonance (NMR), Infrared (IR), Mass Spectroscopy (MS), LC-MS.

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