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REVIEW ON ICH GUIDLINE IN IMPURITY PROFILING

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❖ ABSTRACT –

In every Active Pharmaceutical Ingredient, Impurity is present. In pharmaceutical industry, Purity profile is important factor as well as Impurity profile is important and mandatory according to Regulatory authority. In the pharmaceutical world, an impurity is considered as any other inorganic or organic material, or residual solvents other than the drug substances present in drug and also arise out of synthesis or unwanted chemicals that remains with APIs. The quality of drug product is highly affected by impurity present in drug. There are different types of impurities such as organic impurity, Inorganic impurity, residual solvent starting materials, intermediates, by product and degradation product etc. The International Conference on Harmonisation (ICH) guidelines, state the definitions of the impurities in new drug substances.In this article, we have discussed the types of possible impurities and their sources. We have also listed out the impurity isolation techniques and analytical techniques for the identification, quantification and characterization of impurities.

❖ **KEYWORDS** – Impurity profile, ICH, Spectrophotometry, Chromatography, Isolations.

❖ INTRODUCTON –

In general term impurity means the unwanted or undesired compound or component in desired product. 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. The identification and qualification of impurities in Active Pharmaceutical Ingredients (APIs) and pharmaceutical products, is a very important step performed at many levels of the drug discovery and beyond. Impurity is a substance which exists with original drug that is starting

material or intermediates or the substances which are formed during any side reactions, during the manufacturing process of the drug.

The United States food and drug administrating and other regulatory bodies around the world require that impurities in drug substance present at the threshold levels recommended by the International Conference on Harmonization (ICH) be isolated and characterized.

Regulatory authorities are also emphasizing on not only the purity profile but on impurity profiling (identification, isolation and characterization of impurity) for the licensing purpose and regulatory related issues for particular drug substance and drug product. Different pharmacopoeias like British Pharmacopoeia (BP), European Pharmacopoeia (EP), Indian Pharmacopoeia (IP), Japanese Pharmacopoeia (JP) and United States Pharmacopoeia (USP) are also revising their monographs for the drug substances and drug products every year by introducing the limits for the different types of impurities.

The sources of impurities are the major concern for any drug manufacturer. Some time it has been observed that in the final stage of the production is badly affected by the introduction of a single unknown impurity and due to that unknown impurity; whole batch can get rejected as per the quality criteria. This impurity profile can be explained using multidisciplinary approach including the manufacturing or aging of both active pharmaceutical ingredient (API) and formulation. According to International Conference on Harmonization (ICH) guidelines identifying and characterizing all impurities that are present at a level of 0.10% or more are recommended [7, 10, 3, 8, 12].

ICH Guidelines for impurity profiling [11]-

It is now getting an important critical attention from regulatory authorities. The International Conference on Harmonization has published various guidelines on impurities in drug substances and drug products as well as residual solvents.

1) Q1A-"stability testing of new drug substances and products"

2) Q3A (R2) - "Impurities in New Drug Substances"

3) Q3B (R2) - "Impurities in New Drug Products"

4) Q3C (R5) - "Impurities: Guidelines for Residual Solvents"

Regulatory Guidelines on impurity [8] -

International Conference on Harmonization guidance of Technical Requirements for Registration of Pharmaceuticals for Human Use is inscribed by The United States Food and Drug Administration (FDA).

The FDA has the assigned responsibility of ensuring the safety and efficacy of drugs. The various regulatory guidelines regarding impurities are as follows:

1. ICH guidelines —stability testing of new drug substances and products"- Q1A

2. ICH guidelines —Impurities in New Drug Substances‖- Q3A

3. ICH guidelines —Impurities in New Drug Products‖- Q3B

4. ICH guidelines —Impurities: Guidelines for residual solvents‖- Q3C

5. US-FDA guidelines —NDAs -Impurities in New Drug Substances‖

6. US-FDA guidelines ―ANDAs – Impurities in New Drug Substances‖

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

Qualification of Impurities [8, 11] –

The impurity profile of drug substance may vary for processes like scale-up changes, synthetic route change and changes made to key intermediates. New Molecular Entities (NMEs) limits are classified and restricted by the ICH. Qualification process helps to acquire and evaluate data that establishes the biological safety of an individual impurity.

Limits of impurity in New drug substance -

Maximum daily dose[x]	Reporting Threshold[y,z]	Identification Threshold[z]	Qualificaton Threshold
< 2g/day	0.05%	0.1% or 1 mg per day intake(whichever is lower)	0.15% or 1 mg per day intake(whichever is lower)
> 2g/dsy	0.03%	0.05%	0.05%

x. The amount of drug substance administered per day.

y. Higher reporting thresholds should be scientifically justified.

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

❖ SOURCES OF IMPURITIES IN MEDICINES [15] –

Medicines are the formulated forms of active pharmaceutical ingredients. There are 2 types of impurities in medicines:

(1) Impurities associated with active pharmaceutical ingredients and

(2) Impurities that are created during formulation and or with aging or that are related to the formulated forms.

Impurities associated in with APIs -

According to ICH guidelines, impurities associated with APIs are classified into the following categories:

- Organic impurities (Process and Drug-related)
- Inorganic impurities
- Residual solvents

ORGANIC IMPURITIES [8, 15, 11] –

These types of impurities form during the manufacturing process or during storage of the drug substance. The sub- types of these impurities are given below.

- Starting Materials or Intermediate Impurities –

During multistep synthesis process there are high chances of impurities formed as by products, intermediates are produced. So, special care is needed. It results in unreacted starting material in the final product. The impurities that arise from starting materials or intermediates is found in every API unless proper care is not taken in every step involved in the multi-step synthesis. Although the end product are always washed with solvents, there is always chance that the residual unreached starting material remain, except the manufactures are very careful about the impurities. In Paracetamol bulk, there is a limit test for p-aminophenol, which could be a starting material for some one manufacturer or be an intermediate for other.

- By-products –

In organic chemistry 100% pure product is not generally formed as there is always a chance of having byproducts. By products can be formed through variety of side reactions, such as incomplete reaction, rearrangement, dimerization, over reaction, isomerization or unwanted reactions between starting materials. For example diacetylated paracetamol may forms as a by-product In the case of paracetamol production.

- Degradation products –

Impurities can also be formed by degradation of the end product during manufacturing of bulk drugs. However, degradation products resulting from storage or formulation to different dosage forms or aging are common impurities in the medicines. The degradation of penicillins and cephalosporins is a wellknown example of degradation products.

INORGANIC IMPURITIES [8, 15] –

Inorganic impurities are also obtained from the manufacturing processes which are used in bulk drug formulation. They are normally known and identified. Inorganic impurities are normally detected and quantified using different pharmacopeia or other appropriate standards.

- Reagents, ligands, and catalysts - The chances of having these impurities are rare: however, in some processes, these could create a problem unless the manufacturers take proper care during production.
- Heavy Metals - Water is essential during manufacturing process and it is the main source of heavy metals, like Ar, Cd, Cr, Na, Mg, Mn, etc. These can be avoided by the use of demineralization plant, reverse osmosis technique that produces mineral free water.
- Other materials (eg, filter aids, charcoal) - The filters or filtering aids such as centrifuge bags are routinely used in the bulk drugs 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 contaminations.

RESIDUAL SOLVENT [8, 15, 11] –

Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. It is very difficult to remove these solvents completely by the work-up process; however, efforts should be taken to the extent possible to meet the safety data. Some solvents that are known to cause toxicity should be avoided in the production of bulk drugs. Depending on the possible risk to human health, residual solvents are divided into three classes,

1] Class I solvents -These solvents are either avoided or restricted to a limit in the manufacture of excipients and drug substances because of their unacceptable toxicity or their deleterious effects. These are generally carcinogens.

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 ethene	5 (Toxic)
1,1,1 trichloro ethane	1500 (Environmental hazard)

2] Class II solvents - As Class II solvents are inherently toxic, their usage should be limited in pharmaceutical Industry. These are generally Non-genotoxic, animal carcinogens and possible neurotoxicants.

Class II Solvents with Their Permissible Daily Exposure Limits –

Solvent	Permissible daily exposure (mg/day)	Concentration limit (ppm)
Acetonitrile	4.1	410
Chlorobenzene	3.6	360
Chloroform	0.6	60
Cyclohexane	38.8	3880

3] Class III Solvents - As they are less toxic and possess lower risk to human health than class I or class II solvents, they do not have any serious health hazard. According to several data‘s, long term toxicity is generally not reported. The amount of these residual solvents of 50 mg or less would be acceptable. E.g. for this class of solvents are Acetic acid, Acetone, Anisole, 1-Butanol.

Formulation-related impurities [8] –

Drug substance varies with conditions that lead to its degradation or other chemical reactions. Solutions and suspensions are prone to degradation due to hydrolysis. Water used in formulation contribute to not only its impurity but also provide stimulation for process like hydrolysis and catalysis.

The formulation related impurities can be classified as follows:

i. Method related ii. Environmental related

The primary environmental factors that can reduce stability can be sub classified as,

i. Exposures to adverse temperatures -

ii. Light - especially UV light

iii. Humidity

Dosage form related -

i. Mutual interaction amongst ingredients

ii. Functional group- related typical degradation -

a) Ester hydrolysis

b) Hydrolysis

c) Oxidative degradation

d) Photolytic cleavage

e) Decarboxylation

❖ **Analytical method development [8] -**

Meaningful and reliable analytical data is needed to produce new drug various stages of the development.

a) Sample set selection for analytical method development

b) Screening of Chromatographic conditions and Phases, typically using the linear solvent- strength model of gradient elution.

c) Optimization of the method to fine-tune parameters related to ruggedness and robustness

The impurities can be identified predominantly by following methods:

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

Isolation and identification of impurities in active pharmaceutical ingredients [12] -

An impurity profile is a description of the identified and unidentified impurities present in a new drug substance (Source: Guidance for Industry, Q3A Impurities in New Drug Substances). Impurity profiling processes usually begin with the detection of impurities, followed by their isolation and characterization. For all three types of impurities, it is critical to develop a robust method during process development that can eventually be validated and transferred to QA/QC. Developing reliable methods for impurities regulated at very low levels, such as genotoxic impurities, adds further challenges to this process. To better detect, identify, quantify, and characterize the impurities present in drug substances and products, pharmaceutical scientists rely on fast analytical tools with high sensitivity and specificity. Major analytical tools for impurity analysis include spectroscopy, chromatography, and various combinations of both, i.e. tandem techniques. The appropriate technique is selected based on the nature of the impurity and the level of information required from the analysis. There are various complex analytical problems in pharmaceutical development that require the use of more than one analytical technique for their solution. Analytical techniques such as LC/UV, LC/MS, GC/MS, CE/MS, and LC/UV provide the orthogonal detection and complementary information that can address these challenges in a time efficient manner. As a result, they play a vital role in impurity profiling of pharmaceuticals from identification to the final structure elucidation of unknown impurities.

ICH limits for impurities [2] -

According to ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level, is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic.

According to ICH, the maximum daily dose qualification threshold to be considered is as follows; < 2g/day 0.1 % or 1 mg per day intake (whichever is lower) >2g/day 0.05%.

A] Spectroscopic Methods –

1) UV- Visible spectroscopy
2) Infrared spectroscopy
3) Nuclear Magnetic Resonance spectroscopy
4) Mass spectroscopy
5) Atomic Absorption Spectroscopy

B] Chromatographic Methods –

1) High Performance Liquid Chromatography (HPLC)

2) High – Performance Thin – Layer Chromatography (HPTLC)

3) Gas Chromatography (GC)

4) Thin Layer Chromatography (TLC)

5) Column Chromatography (CC)

6) Flash Chromatography (FC)

7) Supercritical Fluid Chromatography (SFC)

8) Capillary Electrophoresis (CE)

C] Extraction Methods –

1) Solid phase Extraction

2) Liquid – Liquid Extraction

3) Supercritical Fluid Extraction

D] Hyphenated Methods –

1) Liquid Chromatography and Mass Spectrometry (LC/MS)

2) Liquid Chromatography and Ultraviolet Spectrometry (LC/UV)

3) GC-MS

4) LC–NMR

5) LC–DAD–MS

6) LC-NMR-MS

- UV – Visible Spectroscopy [7, 10, 9] –

UV at a single wavelength provides minimal selectivity of analysis; however, with the availability of diode array detectors (DAD), it is now possible to get sufficient simultaneous information at various wavelengths to ensure greater selectivity.

It is desirable to obtain a UV spectrum in a solvent that has a window in the region of the wavelength, because this wavelength would be most desirable for the purpose of quantization.

UV-VIS spectroscopy is based on the absorption of visible and ultraviolet (UV) radiation in the wavelength range of 200-800 nm and causes excitation of electrons from HOMO to LUMO, in both atoms and molecules, to higher energy states e.g., benzene absorbs in the 260 nm region. Electrons in the bond within molecule become excited to occupy a higher quantum state by absorbing energy passing through solution. Every molecule has its own absorption maxima. As there is an increase in the degree of conjugation, spectrum will shift towards red region e.g. naphthalene absorbs light up to 300 nm and anthracene absorbs to about 400 nm. UVVIS spectroscopy is one of the easiest methods to determine purity of drug substance. UV-VIS spectroscopy can only be used with those samples where component or some of its derivatives are spectrophotometrically active. UV-VIS spectroscopy is also used as stability-indicating method for characterization of impurities or degradation products formed. Number of methods are available in literature for analysis of drug substance by using UV-VIS spectroscopy as stability indicating method e.g. tinidazole. The technique is very cost effective, quick, little sample preparation, UV is a rapid means of analysis, and can provide very high precision and accuracy. It is useful for a wide variety of chemicals, and it is non-destructive. It can be used both quantitatively and qualitatively on pure substances. UV has limited use in analyzing mixtures, due to addition of absorbance. It requires special equipment (a UV light source and UV-transparent sample holders, for example), and it is not selective for compounds if they absorb at the same wavelength.

- IR Spectroscopy [7, 10, 9] –

This method provides specific information on some functional groups, present in drugs. Infrared spectrophotometry provides specific information on some functional groups that may allow quantification and selectivity. However, low level detectability is frequently a problem that may require more involved approaches to circumvent the problem.

Sample is subjected to electromagnetic radiation ranging between 500 cm-1 and 4000 cm-1 which influence bonds present in molecule and generate stretching or bending in molecule due to absorption of energy of specific wavelength. Wavelengths absorbed are characteristic for various kinds of bonds which help in determining structure of samples. Solid and semi-solid samples can be characterized by IR spectroscopy. IR spectroscopy provides a complex but unique fingerprint of any molecule which helps in analyzing drug samples and determining presence of impurities in drugs. It can also be used for determining presence of polymorphs of drugs. Photoacoustic spectroscopy in infrared region is a helpful technique to characterize impurities in pharmaceutical products, for example characterization of 12tungstophosphoric acid and related salts using photoacoustic spectroscopy in infrared region. It is cost effective and fast compared to things like NMR. It also works for a wide variety of samples and can detect compounds very strongly, whereas similar techniques like Raman spectroscopy are weaker. Disadvantages of the techniques are that sample preparation is time consuming and that it can't give information as detailed as other techniques such as NMR. It is a destructive analysis method and therefore precious or scarce sample should be analysed by a non-destructive method such as Raman. It is qualitative rather than quantitative and there are a lot of compounds which are not IR active and therefore can't be detected.

- Mass Spectroscopy [2, 16, 9] –

It has an increasingly significant impact on the pharmaceutical development process over the past several decades. Advances in the design and efficiency of the interfaces, that directly connect separation techniques with Mass Spectrometers have afforded new opportunities for monitoring, characterizing, and quantification of drug-related substances in active pharmaceutical ingredients and pharmaceutical formulations.

Mass spectroscopy is a most accurate method for determining the molecular mass of the compound and its elemental composition. Mass spectroscopy is used to prove identity of two compound, establish the structure of new compound, give exact molecular mass, give molecular formula and most important for structure elucidation. Mass spectroscopy has the advantages like small sample size is required, it is fast, differentiates isotopes, can be combined with GC and LC to run mixtures, or can be run in tandem for proteins it can even give elemental composition but it doesn't directly give structural information, needs pure compounds, difficult with non-volatile compounds.

- Nuclear Magnetic Resonance Spectroscopy (NMR) [9, 12, 11] –

NMR is a powerful analytical tool that enables the study of compounds both in solution and in the solid state. It has wide applicability because it provides specific information about bonding and stereochemistry within a molecule, which is particularly important in the structural characterization of drug impurities and degradant often present only in extremely limited quantities. The nondestructive, non-invasive nature of NMR spectroscopy makes it a valuable tool for the characterization of impurities and degradant present at very low levels. NMR can also provide quantitative output, an important aspect of impurity profiling.

The ability of NMR base diffusion coefficient determination to distinguish between monomeric and dimeric substances was validated using a standard mixture of authentic mixture which contains both monomers and dimers. However, NMR is been used as less traditional method compared to other analytical techniques. Conventionally sample requirements for NMR is in order of 10 mg, as compared with MS, which requires less than 1 mg

The versatility of nuclear magnetic resonance (NMR) spectroscopy has made it an inevitable tool for determination of chemical structure. NMR can detect very fine structural components, works for organic and inorganic compounds, qualitative as well as quantitative determination is possible, and technique is versatile but the main drawbacks of this technique are; it is very expensive, time consuming and spectra take long time to interpret. Along with one dimensional NMR spectroscopy which is used to study chemical bonding, advanced two or multi-dimensional NMR spectroscopy such as Correlation Spectroscopy (COSY), Nuclear Overhausser Enhancement Spectroscopy (NOESY), and Heteronuclear Correlation Spectroscopy (HETCOR) are also used for detection of impurity. NMR frequencies from 60 MHz to 1 GHz have been used for its application in research studies.

- Capillary Electrophoresis (CE) [12] -

The determination of drug-related impurities is currently the most important task for CE within pharmaceutical analysis because it achieves high separation efficiencies compared to other chromatographic techniques. CE can be employed when HPLC techniques are not able to adequately measure impurities, especially in the case of very polar compounds. A detection limit of 0.1 % is widely accepted as a minimum requirement for a related impurities determination method and this can be achieved using CE. In addition, CE is very useful for the separation of closely related compounds, such as diastereomers and enantiomers.

- Fourier – Transform Infrared Spectroscopy (FTIR) [12] -

FTIR is very helpful for identifying and confirming the structure of an impurity or degradant because it provides a complex fingerprint that is specific to a particular compound. An FTIR spectrum of an organic molecule is determined by the functional groups present. The technique helps to identify the structure and measure the concentration of the compound under investigation. Changes in the structure can be correlated with the help of an FTIR spectrum of a patent drug compared to that of the impurity or degradant.

- High Performance Liquid Chromatography (HPLC) [9] –

HPLC is a versatile method of analysis as it is not limited to volatile or stable sample and separation is based on the fact that certain compounds have different migration rates on a particular stationary and mobile phase. Separation of components by utilizing HPLC method with any suitable detector like refractive index detector, PDA detector, fluorescence detectors, electrochemical detectors, electrical conductivity detectors, light scattering detectors, evaporative light scattering detectors, Corona Charged Aerosol Detector (CAD), Nano Quantity Aerosol Detector (NQAD), etc. provide an accurate, precise and robust method for quantitative analysis for pharmaceutical products as well as impurities. HPLC also involves monitoring of stability of pure drug substance and in case of drug formulations. It can be applied for quantification of degradation products e.g. stability indicating method for simultaneous determination of salicylic acid.

Various advantages of HPLC are: (i) speed (minutes), (ii) high resolution, (iii) sensitivity, (iv) Reproducibility of +/- 1%, (v) accuracy, (vi) automation. While, there are some disadvantages of HPLC such as cost, complexity, low sensitivity for some compounds, irreversibly adsorbed compounds not detected, co-elution (two compounds escaping from the tubing at once) difficult to detect.

- Gas Chromatography (GC) [9] –

GC is used as a technique for qualitative and quantitative estimation of APIs, particularly with regards to detection of impurities which are volatile and thermo-stable in nature. It can be used as a limit test for solvent residue and other volatile impurities in drug substances. It is also utilized for characterization of raw materials used in synthesis of drug molecules. GC has advantages like (i) shorter run times; (ii) greater sample throughput; (iii) cheaper columns; (iv) higher signal to noise ratio. But on the other hand, it has some disadvantages like careful attention required when working on the instrument. Gas chromatography can only be used in cases where the substances can be vaporized without decomposing and where they can be vaporized at a reasonable temperature (i.e. not so hot that it destroys the column packing). The samples analysed are limited to those that are volatile or can be made volatile (reaction to form a volatile derivative). The samples must be thermally stable to prevent degradation when heated. It cannot be used to prepare samples for further analysis once separated. Problems can be encountered when injecting the sample: It is difficult to measure and inject such small samples (approx 0.3 µl) accurately without evaporation of the sample, for example. The rubber seal through which sample is injected may leak leading to loss of the sample. Small pieces of the rubber septum may be adsorbed onto the column giving 'ghost peaks'. Sample may be injected directly into the heated part of injector so vaporization may not occur. GC is capable of same quantitative accuracy and precision as HPLC, particularly when used in conjunction with an internal standard.

- Column Chromatography [9] –

Column chromatography is based on principle of partition chromatography and works under influence of mobile phase involving separation of components of the sample during its passage through stationary phase. On the basis of their affinity towards mobile phase various components elute out at different rates from column under gravity which leads to efficient separation. Unfortunately, rate at which the solvent percolates through the column is very slow. Biggest advantage of column chromatography is that it can usually be scaled to the project in hand. This is especially useful if one is trying to separate and purify a reaction mixture preparing an intermediate in a sequence of reactions. The corresponding disadvantage is column may take a long time to properly prepare and use.

- Thin Layer Chromatography (TLC) [9] –

TLC is the technique used for the identification of various components up to trace amounts. This technique has been used for developing stability-indicating analytical method. Its disadvantages are variability, non-quantative most easy, simple, and simultaneous determination is possible. It can be used as a quantitative technique, in conjunction with densitometric detection i.e. high performance thin layer chromatography (HPTLC) for compounds which are difficult to analyze by other chromatographic method because of the absence of chromophore. The detection using TLC is based upon the chemical reaction between the components and detection reagent. TLC is very much used during initial degradation and stress studies to study the number of degradation products formed. HPTLC is more sensitive and faster compare to conventional TLC technique. HPTLC is better in many regards than TLC, such as (i) it requires very less amount of sample; (ii) more than ten spots can be quantified simultaneously; (iii) easily attached with various detectors (iv) give 3D images of all the spots which is very useful for quantitative estimation; (v) separation time is reduced compare to TLC. Various stability indicating methods have been published using HPTLC technique. Drugs such as Telmisartan and Ramipril in tablets, Prasugrel, Drotaverine and Aceclofenac in tablets.

- Flash Chromatography [9] -

Flash chromatography is a good alternative to slow and often inefficient gravity fed-chromatography. Flash chromatography is air pressure driven hybrid of medium pressure and short column chromatography. It is used to speed up the flow of solvent, which dramatically decreases the time needed to purify sample. Flash chromatography includes use of small silica gel particles (250-400 mesh size) and pressurized to drive solvent through surface of stationary phase.

- Preparative Liquid Chromatography (LC) [12] -

Since the impurities in the drug substance are usually present at very low quantities, detailed analysis is only possible upon isolation of the impurities. However, this is a major challenge in pharmaceutical laboratories. Preparative LC helps isolate impurities (usually from impurity-enriched analytes, such as the solution remaining from the crystallization of APIs) in sufficient quantities to carry out structural analysis, usually using techniques such as FTIR, NMR, LC/MS, or GC/MS.

- Supercritical Fluid Chromatography (SFC) [12, 9] -

SFC, which uses supercritical CO2 as mobile phase, is another orthogonal technique that can be used for impurity detection because it offers HPLC-level sensitivity with reduced organic solvent usage. SFC also offers the advantage of chiral impurity analysis enabling the determination of enantiomeric excess at very low impurity levels.

Supercritical fluid chromatography (SFC) can be used on an analytical scale, where it combines many advantages of high performance liquid chromatography (HPLC) and gas chromatography (GC). It can be used with non-volatile and thermally labile analytes (unlike GC) and can be used with the universal flame ionization detector (unlike HPLC), as well as producing narrower peaks due to rapid diffusion. In practice, advantages offered by SFC have not been sufficient to displace widely used HPLC and GC, except in a few cases such as chiral separations and analysis of high-molecular-weight hydrocarbons.

Hyphenated Techniques [1] –

Hyphenated techniques are first line of defense in impurity determination. Hyphenated techniques are those techniques, where two or more analytical techniques are combined. The various hyphenated techniques used for impurity characterization are LC-MS, LC NMR, LC-MS-NMR, LC-MS-MS, GC-IR and GC-MS. The two most commonly used hyphenated techniques for impurity profiling are LC-MS and LC-MS-NMR. In these techniques chromatographic techniques are coupled with a spectroscopic detector. Thus impurity structure determination can be performed in real time during chromatographic separation and both isolation and characterization is performed in one single step.

The use of hyphenated techniques for impurity determination is on rise due to easy availability of bench-top instrumentation and their distinct advantages like versatility, sensitivity, possibility of profiling sub structural analysis and rapid selective quantitative determination of targeted compound even in mixtures. The only limitation of hyphenated techniques is the heavy cost of instrumentation due to which their use is not common and spread worldwide like GC, HPLC, MS or NMR systems.

1] Liquid Chromatography and Mass Spectrometry (LC/MS) [12] -

LC/MS is a powerful analytical tool that is routinely used in pharmaceutical development to test and identify product impurities. The detection limit of a few hundred ppm is readily achievable, ensuring the identification of all the impurities present at concentrations greater than 0.1 %. MS-based methods generally provide additional robustness and ruggedness compared to techniques such as UV alone, due to their high specificity and sensitivity. While single quadrupole mass spectrometers work well as analytical tools for the confirmation of known impurities and the preliminary structural assessment of unknown impurities, highly sensitive Q-TOF mass spectrometers provide higher resolution and mass accuracy that enables the unambiguous identification of unknown trace impurities, making them very useful for genotoxic impurity analysis. MS-based methods are often selected for the impurity profiling of APIs during process development, while UV-based methods are generally used to test for genotoxic impurities in QC laboratories at manufacturing sites. Triple-quadrupole (QQQ) LC/MS/MS systems have become a standard platform for the quantitative analysis of organic impurities in pharmaceutical analytical laboratories.

2] Liquid Chromatography and Ultraviolet Spectrometry (LC/UV) [12] -

A number of impurity analysis methods found in pharmaceutical quality control (QC) laboratories use high-performance liquid chromatography (HPLC) coupled with UV detection (HPLC/UV methods). UV spectrometry helps identify impurity or degradants in drug substances based on absorption maxima. This technique is one of the most important and versatile analytical methods available for impurity profiling today due to its high selectivity (i.e., ability to quantitatively determine a number of the individual components present in a sample using a single analytical procedure), especially for routine analysis where standards are available. Newer, stationary phase systems are available which operate in several modes, such as ion pairing, increased hydrophobic interactions, and variable pH, allowing a variety of samples to be analyzed concurrently based upon their unique properties. High resolution is particularly helpful when using LC/UV analysis for impurity detection, because all impurities can be identified with less chance of error.

3] GC-MS [17] –

HPLC based technique such as HPLC- DAD and HPLC-MS can analyze at least 80% of the organic compounds and GC based technique such as GC-FID (Gas Chromatography-Flame Ionization Detector) and GS-MS play major role in analysis of the remaining 20% of the organic compounds. These compounds are either not suitable for HPLC analysis (e.g, volatile compounds) or not suitable for ionization by API techniques (e.g nonpolar compounds. Electron Ionization (EI) is a better technique for non - polar compounds. EI is hard ionization technique, therefore an EI spectrum can contain many fragment ions because of this inherent feature it is usually not necessary to acquire MS/MS data with EI. On other hand, extensive fragmentation in EI often results in the absence of the molecular ion, which adds the difficulty in the determination of molecular weight of the unknown impurity. Therefore Chemical Ionization (CI) and other soft ionization techniques have been developed. The identification and quantification of the residual solvents and other organic volatile impurities is done using GC-FID or GC-MS methods. But the importance of GC-MS in the identification of pharmaceutical impurity has decreased due to routine availability of API based LC-MS.

4] LC-NMR-MS [17] –

Though NMR is arguably the most versatile analytical platform for complex mixture analysis, it is rarely possible to solve the structure of a novel compound/ impurity by NMR alone. Specifically, interfacing liquid chromatography with parallel NMR and mass spectrometry (LC–NMR–MS) gives comprehensive structural data on identification of impurities. Common functional groups such as carboxylic acid, phenol and amino groups are NMR-silent in many

solvents because of proton deuterium exchange. Nitro groups and sulfate conjugates do not contain protons and although these functional groups are not directly detectable in proton NMR spectra, they can be readily detected by mass spectrometry (MS). Conversely, MS data might give molecular weight, fragmentation and molecular formulae that are insufficient to unambiguously assign the molecular structure of an unknown compound. In the most difficult cases closely eluting isobars and isomers are indistinguishable by LC– MS. Parallel on-line NMR and MS detection efficiently provides complementary data and minimizes ambiguities between LC–MS and LC–NMR systems. These integrated LC–NMR–MS systems are highly versatile.

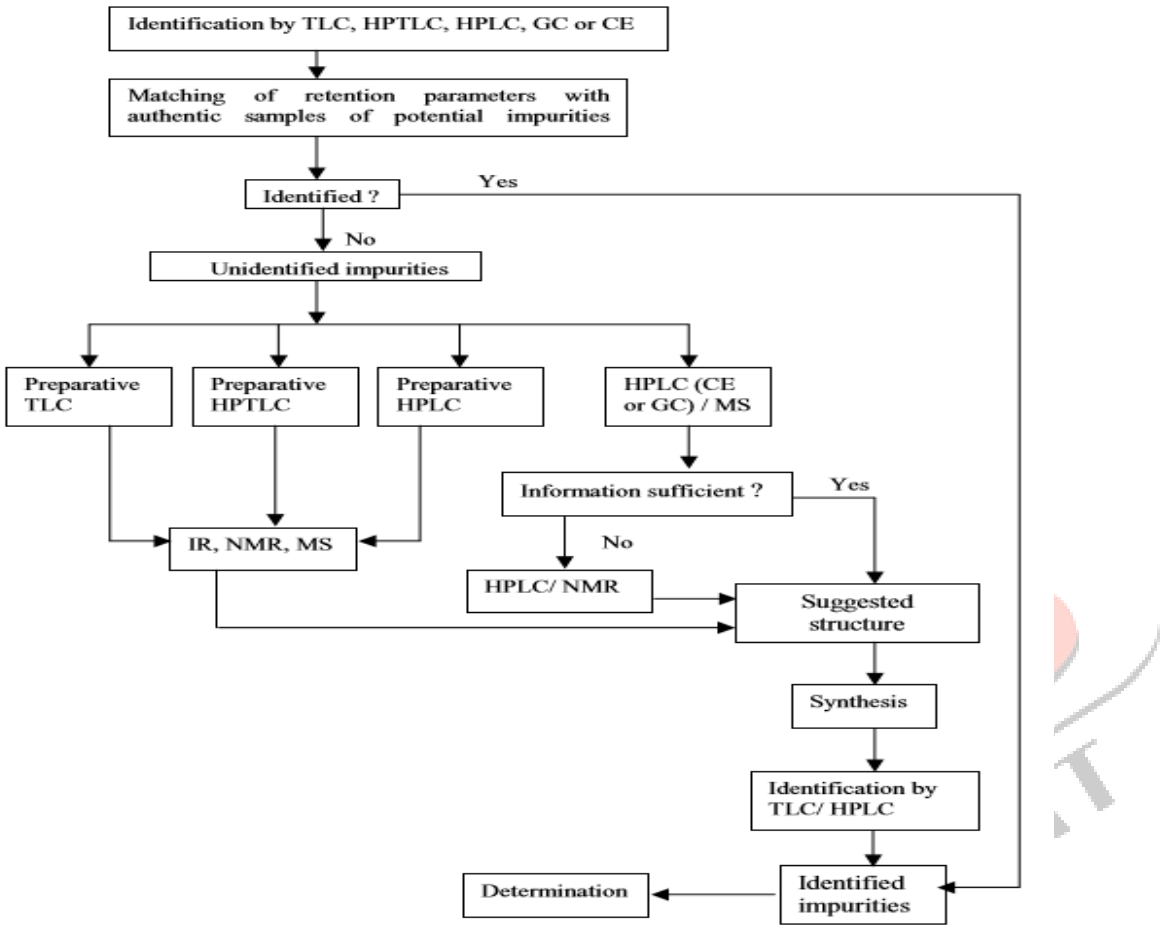

Figure 1: Proposed chart for profiling drug impurity [13]

❖ **Applications –**

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, antibacterials, anticonvulsants, antidepressant, tranquilizers, antineoplastic agents, local anesthetics, macromolecules, steroids, miscellaneous [18, 2].

Process-related impurities	Degradation–related impurities
Identify significant impurities	Identify potential degradation product through stress testing and actual degradation products through stability studies.
Determine origin of impurities and method for elimination or reduction	Understand degradation pathway and methods to minimize degradation.
Establish a control system for impurities involving: 1) Processing/manufacturing conditions 2) Suitable analytical methods/ specifications	Establish a control system for impurities involving: 1) Processing/manufacturing conditions 2) Suitable analytical methods/ specifications 3) Long term storage conditions including packaging 4) Formulation.

Figure 2: Goals of impurity investigations [2]

Drug	Impurities	Method
Budensonids	Impurities or degradation product	HPLC
Cefdinir	Related substance	HPLC
Donepezil	Process related impurities	HPLC
Linezolid	Process related impurities	HPLC
Loratidine	Process related impurities	HPLC
Repaglinide	Process related impurities	HPLC
Rofecoxib	Process related impurities	HPLC
Zaleplon	Process related impurities	HPLC
AmphotericinB	Process related impurities	UV spectroscopy
Doxorubicin hydrocholide	Residual solvents	GC
Framycetin sulphate	Process related impurities	TLC
Cimetidine	Process related impurities	HPLC
Norgestrel	Related substance	TLC, HPLC & UV spectroscopy
Celecoxib	Process related impurities	HPLC, LC-MS-MS
Ethynodiol diacetate	Process related impurities	HPLC
Methamphetamine	Process related impurities	GC
Morphine	Process related impurities	HPLC
Morphine sulphate	Related substance	HPLC

Figure: 3 some various impurities reported in APIs [1]

❖ REMEDIES TO PREVENT THE IMPURITIES IN PHARMACEUTICAL PRODUCTS [3] –

Some of the remedies to prevent the impurities in pharmaceutical products are listed below:

- Control of critical factors during the manufacturing of any product; which affect the product.
- Extreme operational care should be taken while handling the equipments, machineries, reactors and other tools that by any mean due to the operational activity, impurity should not be entered into the product.
- The wet cake should be thoroughly washed to remove all unwanted chemical including the residual solvents.
- In the specification, maximum possible impurities should be specified with stringent limits for the better quality products.
- Time to time the specifications of drug substances and drug products should be studied and revised for specific impurity profiling and should be made strict for impurity acceptance criteria.
- During analytical method development and validation study of any drug substance and drug product, the method parameters should be optimized in such a way that the method can resolve maximum number of impurities which will help the synthetic chemist to improve the synthetic process.
- Stability study should be carried out methodically and meticulously for the identification of degradation products and to fix the shelf life of drug substances and drug products.
- Stress study should be performed for any drug substance or drug product to handle the transportation related issues properly.
- Packaging care should be taken for the moisture/light/environment/stress sensitive materials.
- Regulatory authorities should become stricter before giving any license or permission for any product to be sold in any regulated market.
- Before giving any approval related to FDA, for any pharmaceutical product to any company, the authorities should ensure the total compliance of the manufacturing site and product, as this is the matter related to human health and it cannot be taken in very casual way. If some of the listed remedies are implemented seriously and strictly, then the pharmaceutical industries can get rid of this burning issue of impurities at major extent.

❖ CONCLUSION –

This review provides a perspective on impurity profiling in drug substance and drug product. This article provides valuable information regarding the types of impurities and its various techniques for isolation and characterization, various analytical techniques for determination, identification and qualification of impurities and critical factors ha to be considered while the preparation of the bulk drugs[7].

During the analytical method development of any pharmaceutical product, identification of different types of impurities is very important. It provides the crucial data of quality, safety and efficacy of the drug. Different regulatory authorities and ICH have already defined criteria in their guidelines but still even they ate not sufficient to ensure the quality of product by 100% and hence they need to be revised for the further improvement for the better quality of pharmaceutical products. At the time of analytical method validation, different types of impurities must be properly evaluated for the detection and quantization limits. Thus the data of analytical method development and validation will help a lot in impurity profiling and it will make the impurity profiling task easy as impurity profiling is mandatory to establish the quality, safety and efficacy of any pharmaceutical product[3].

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