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Impurities Characterization in Pharmaceuticals: A Review

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Swati Patole, Amit Gosar, Tabrez Shaikh*

Indoco Remedies Limited, Analytical research & development department,
Navi-Mumbai, Maharashtra, 400701, India

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ABSTRACT

Impurities are unwanted chemical substances present in the Pharmaceutical drug products and drug substances with no therapeutic benefits or some time potential to harm patient safety if present above a certain limit. The basic need in manufacturing of safe and good quality of drug substances and drug product, control of impurities is very necessary for the pharmaceutical industry. Several regulatory agencies have formulated guidelines for the control of these impurities. The present review article describes the impurities, origin of impurities, classification of impurities, control limit of impurities, guidelines for their control, isolation of impurities and its characterization using various analytical techniques.

INTRODUCTION

Impurities are undesirable chemicals present in the pharmaceuticals arising from normal manufacture. They are not chemicals accidentally or maliciously introduced. Impurities have no therapeutic value and are potentially harmful. Therefore, they need to be identified and controlled.

Interest in the identification of pharmaceutical-related substances dates back to the early 1960s. Methods of separation were simple compared with those employed currently. The analytical techniques included infrared (IR) and ultraviolet (UV) absorption spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry (MS). After the introduction of the commercial spectrometer, fundamental information about molecular level's geometry, bonding, and mechanism of a chemical reaction was known, thus on a practical level, the spectral analysis gave invaluable information to the synthetic chemists needed for characterization of new compounds.

Impurities are generally assumed to be inferior to API because they might not have the same level of pharmacological activity. Presence of impurity in the drug substance compromises the purity of the drug even if impurity present in it contains superior pharmacological or toxicological properties.

Characterization of these related substances [1] in pharmaceutical products is an important feature of the regulatory approval process. If the estimation indicates that the given impurity content is greater than 0.1%, it must be identified and characterized according to all regulatory requirements.

Recently, not only purity profile but also the impurity profile has become essential as per various regulatory requirements. A number of articles [2-4] have stated guidelines and designed approaches for isolation [5] and identification of process-related impurities and degradation products [6], using Mass spectrometry (MS), Nuclear Magnetic Resonance (NMR), High Performance Liquid Chromatography (HPLC), Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) [7], and Tandem Mass Spectrometry for pharmaceutical substances.

The present article reveals different impurities [8] found in the API's, methods for identifying them and the possible measures to deal with the interferences caused by them. Highly

sophisticated instrumentation, such as MS attached to a GC or HPLC [9], is inevitable tools in the identification of minor components (drugs, impurities, degradation products, metabolites) in various matrices. For characterization of impurities, different techniques [10] are used; which will be discussed further.

DEFINITIONS

Impurity

Food and drug administration (FDA) describe an impurity as, "Any component present in the drug substance or drug product that is not the desired product, a product-related substance, or an excipient including buffer component. It may be either process-or-product related". The expert working group of the international conference on Harmonization (ICH) has defined impurity is "Any compound of the medicinal product which is not the chemical entity defined as the active substance or as an excipient in the product".

Characterization

Characterization is the process of establishing structure elucidation of a chemical compound by using different analytical techniques to demonstrate its suitability or use.

Drug Substance

Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product.

Drug Product

A finished dosage form, for example, a tablet, capsule or solution that contains an active pharmaceutical ingredient, generally, but not necessarily, in association with inactive ingredients.

GUIDELINES FOR THE CONTROL OF IMPURITIES AND ITS CHARACTERIZATION

ICH guidelines on impurities in New Drug Substances have the following requirements: The studies conducted to characterize the structure of actual impurities present in the new drug

substance at a level greater than 0.1% should be described. Similarly, ICH Guidelines on Impurities in New drug Products require that the degradation products observed in the stability studies conducted at recommended storage conditions be identified when present at a level greater than the identification thresholds (1% for a maximum daily dose of <1 mg to 0.1% for a maximum daily dose of >2g).

Identification of impurities below 0.1 % level, is not taken into account to be necessary, except for potential impurities are expected to be unusually potent or toxic, as per ICH guidelines. Impurities above the ICH identification threshold need to be identified and individually specified in the specifications. The limits must be qualified as safe. The limits should realistically reflect batch and stability data. The limit for any unspecified impurity should be at the ICH identification threshold. According to the ICH, the maximum daily dose identification threshold to be considered is as follows.

Table no 1: Impurities threshold in drug

Threshold	**Maximum drug daily dose**	
	Less than 2 g/day	**More than 2 g/day**
Reporting	0.05 %	0.03 %
Identification	0.10 %	0.05 %
Qualification	0.15 %	0.05 %

Need for the isolation and characterization of impurities in drug substances by drug manufacturers and drug regulatory authorities is as follows

- It is important to know the structure of an impurity during the development of a new drug or modification in the process of an existing drug so that the reaction conditions can be changed to avoid the formation of impurity or to reduce the impurity level at the acceptable level.

- By using suggested structures of the impurities, they can be synthesized to provide the final evidence for their structures which are previously determined by spectroscopic methods.

- The synthesized impurities can be used as an “impurity standard” during the development of an analytical method for the quantitative determination of the impurity.

➢ To contribute to the safety of the drug, the synthesized or isolated impurity can be used for toxicological studies.

Table no. 2: Regulatory guideline related to impurities

Regulatory	Guideline details
ICH (USA, EU, and Japan)	Stability Testing of New Drug Substances and Products Q1A(R2) Feb 2003
	Impurities in New Drug Substances Q3A(R2) Oct 2006
	Impurities in New Drug Products Q3B(R2) Jun 2006
	Impurities: Guideline for Residual Solvents Q3C(R7) Oct 2018
	Guideline for Elemental Impurities Q3D(R1) Mar 2019
	Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. M7 (R1)
US-FDA guidelines	"NDAs -Impurities in New Drug Substances" Nov 1999
	"ANDAs – Impurities in New Drug Substances" Nov 1999
	Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (draft) Dec 2008
Australian regulatory guideline	For prescription medicines, (TGA), Australia 1989
	Guidelines for Prescription Medicines; Appendix 18: Impurities in Active Pharmaceutical Ingredients and Finished Products Jun 2004
EP	General Chapter 5.10, Control of Impurities in substances for pharmaceutical use Jan 2017
EMA (Europe)	Assessment of Quality of Medicinal Products Containing Existing/Known Active Substances EMEA/CHMP/CVMP/QWP/450653/2006 Jul 2007
	Control of Impurities of Pharmacopoeial Substances CPMP/QWP/1529/04
	Guideline on the Limits of Genotoxic Impurities CPMP/SWP/5199/02 & EMEA/CHMP/QWP/251344/2006 Jun 2006
	Guidelines on Specification Limits for Residues of Metal Catalysts CPMP/SWP/QWP/4446/00. Jan 2007
	Guideline on Setting Specifications for Related Impurities in Antibiotics (draft) EMA/CHMP/CVMP/QWP/199250/2009 Jul 2010

Need for monitoring of impurities in drug products is supported by virtuous, monetary and competitive reasons as well as those of safety and efficacy. The United States Food and Drug

Administration (US FDA) has endorsed the guidance prepared under the guidance of the International Conference of Harmonization (ICH). The ICH guideline for impurities in pharmaceuticals was developed by the joint efforts of regulators and industry representatives from the European Union (EU), Japan and the United States and it has helped to ensure that different regions have consistent requirements for the data that should be submitted to various regulatory agencies. The guidelines not only help the sponsors of New Drug Applications (NDA) or Abbreviated New Drug Application (ANDA) with the type of information that should be submitted with their applications but also assist the FDA reviewers and field investigators in their consistent interpretation and implementation of regulations. The various regulatory guidelines regarding impurities are tabulated in the Table-2. In ICH guidelines impurities are addressed from two perspectives.

➢ Chemistry aspects include classification and identification of impurities, report generation, setting specifications, and a detail discussion of analytical procedures.

➢ Safety aspects include specific guidelines for qualifying impurities that were not present in batches of new drug substances and products used in safety and clinical studies and /or impurity levels substantially higher than in those batches.

SOURCE OF IMPURITY

Impurities in drug substance can emerge from various sources and phases of the synthetic process. During synthesis, intermediates and by-products may be carried into the drug substance as impurities or become a source of other impurities resulting from them. Impurities present in starting material may be carried into drug substance. According to the ICH, impurities are classified as organic impurities, inorganic impurities, and residual solvents. Organic impurities may arise from starting materials, by-products, synthetic intermediates and degradation products. Inorganic impurities may be derived from the manufacturing process and are normally known and identified as reagents, ligands, inorganic salts, heavy metals, catalysts, filter aids, and charcoal, etc. Residual solvents are the impurities introduced with solvents. Impurities can originate mainly from sources that are given below.

- Starting materials and intermediates
- Impurities in the starting materials

- Reagents, ligands, and catalysts
- By-products of the synthesis
- Products of over-reaction
- Products of side reactions
- Impurities originating from degradation of the drug substance.

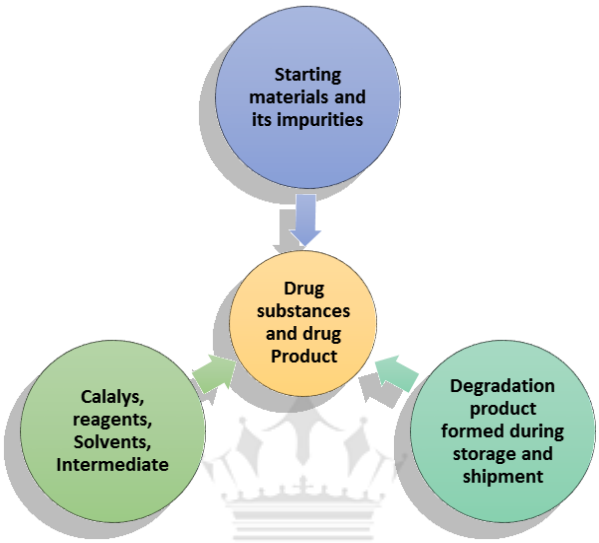

Figure no 1: Sources of impurities in drug substance and drug product

CLASSIFICATION OF IMPURITIES

Impurities are classified into various categories depending upon their origin, composition type, and biological safety. ICH has classified the impurities in drug substances in three main major categories such as Organic impurities, Inorganic impurities, and residual solvent.

- ➢ Organic Impurities (Process and drug-related)
- ➢ Inorganic Impurities
- ➢ Residual Solvents

ORGANIC IMPURITIES

These impurities may arise during the manufacturing process or generate during the storage of the drug substance. These impurities may have low potency as compared to drug

substances and should be eliminated or controlled in the raw material, intermediate or final stage. They may be identified or unidentified, volatile or non-volatile, and include:

- Starting materials
- By-products
- Intermediates
- Degradation products
- Reagents, ligands, and catalysts

Inorganic impurities

These impurities are derived from the manufacturing process. Residual metallic impurities do not provide any therapeutic benefit and can be avoided using distilled water and glass-lined reactors. They are normally known and identified and include:

- Reagents, ligands, and catalysts
- Heavy metals
- Inorganic Salts
- Other materials (filter aids, charcoal)

Residual solvents

These are organic solvents used during the manufacturing process. Since these are generally of known toxicity, the selection of appropriate controls is easily accomplished. Based on possible risk to human health, residual solvents are classified in three classes.

Class 1 solvents

These solvents should be avoided due to known carcinogens to human and environmental hazards. E.g. 1,1-Dichloroethene, Benzene, 1,2-Dichloroethane, and carbon tetrachloride.

Class 2 solvents

These solvents are to be limited as they are non-genotoxic, but supposed to be neurotoxic and teratogenic. E.g. Acetonitrile, chloroform, Cyclohexane, etc.

Class 3 solvents

These solvents have low toxic potential to humans with PDEs of 50 mg or more per day. E.g. Acetic acid, Ethanol and Methanol. The solvents for which no adequate toxicity data is found, these solvents are to be limited in drug substance based on justification. E.g. Isooctane, Trifluoroacetic acid and Trichloroacetic acid based on their permitted daily exposure (PDE).

GENOTOXIC IMPURITIES

Based on toxicological risk assessment Genotoxic impurities [10-13] are those compounds which have the potential to damage DNA at any level of exposure and that such damage may lead to the formation of a tumor. These genotoxic impurities are further categorized into five classes depending upon their risk assessment as per ICH M7 guidelines as follows.

Class 1 -These impurities have established mutagenic and carcinogenic data and are known to be the most serious risk and need to eliminate them by modifying the process. If this is not possible, these impurities are to be limited at "Threshold of Toxicological Concern (TTC)" as the last option.

Class 2 -These impurities have the well-established mutagenic data, but their potential to cause carcinogen is not known. Hence, these impurities need to be controlled using the TTC approach.

Class 3 - These impurities are having alert structures unrelated to the structure of the drug substances and unknown genotoxic potential. Based on functional groups within their molecule, they can be classified as genotoxic. The toxicity of these impurities is identified based on the structure-activity relationship (SAR).

Class 4 - These impurities are having structures similar to the structure of drug substances and additionally contain functional or moiety that has potentially alert shared with the parent structure and consider to be non-genotoxic.

Class 5 **-** These impurities have no alert structures, and evidence indicates the absence of genotoxicity. These compounds are to be treated as normal impurities and controlled according to the ICH guidelines.

CHIRAL IMPURITIES

These are the organic impurities present in drug substances which are having optical isomers in their molecules. Chiral compounds that are supposed to exist as single enantiomers may contain an inactive enantiomer with a structure similar to drug substances, but the difference in spatial orientation around a chiral carbon atom in the molecule is called as a chiral impurity. e.g.: Rasagiline Mesylate is R-isomer while its chiral Impurity is S-isomer.

Extractables impurities

Compounds that are removed from the glass, elastomeric, plastic components or coating of the container closure system in the presence of an appropriate solvent(s) under laboratory conditions. Extractables are potential leachables.

Leachables impurities

Compounds that migrate from glass elastomeric, plastic components or coatings of the container closure system as a result of contact with formulation under normal condition of use. Leachables are typically a subset of extractable. Extractables and Leachables impurities are generally present in drug products.

Other Impurities

The filters or filtering aids such as hyflo are routinely used in the bulk drugs manufacturing plants and sometimes activated carbon, fiber filters are also used which acts as a source of impurity. For that reason, regular monitoring of fibers and black particles are needed to avoid the contamination.

SEPARATION AND ISOLATION OF IMPURITIES

Usually, it is required to isolate the impurities as the use of only instrumental methods does not characterize the impurity. Generally, the chromatographic techniques are used for isolation of impurities along with classical techniques before its characterization. If instrumental methods are used, isolation of impurities is avoided, as it directly characterizes

the impurities. Often the analysis of complex materials requires, as a preliminary step that is, separation of the analyte or analytes from a sample matrix. The following methods can be used for the separation of impurities from drug substances and drug products.

- Liquid-liquid extraction methods
- Solid-phase extraction methods
- Accelerated Solvent Extraction Methods
- Supercritical fluid extraction
- Column chromatography
- Flash chromatography
- Thin-layer chromatography (TLC)
- Gas chromatography (GC)
- High-pressure liquid chromatography (HPLC)
- Capillary electrophoresis (CE)
- Supercritical fluid chromatography (SFC)

IDENTIFICATION AND STRUCTURE ELUCIDATION OF IMPURITIES

Impurity structural elucidation or impurity profiling [14-15] (determination and characterization of impurities associated with drugs or drug products) is increasingly viewed as a valuable and essential part of quality requirements. The characterization of impurities generally requires the collective efforts of synthetic organic chemists, pharmaceutical scientists responsible for formulation development, and analytical scientists. The analytical chemists utilize the latest separation techniques (e.g., HPLC, GC, CE, etc.) and structure elucidation methods (e.g., NMR, IR and Raman spectroscopy, X-ray crystallography, MS, etc.) in combination with the insight provided by physical organic chemists versed in the degradation behavior of various classes of therapeutic compounds. The online capability of both MS and more recently NMR spectroscopy, make them renowned techniques in providing preliminary information about the related substances profile of a drug substance or

product obtained using HPLC separation. Subsequent changes in either synthetic route or composition of the formulation are tracked, using the initial profile as a comparative phoresis (CE), ion chromatography (IC), thin-layer chromatography (TLC), etc. are used for classes of compounds where they offer significant or unique advantage, but HPLC remains the most-often used separation technique in pharmaceutical research and development [16-18].

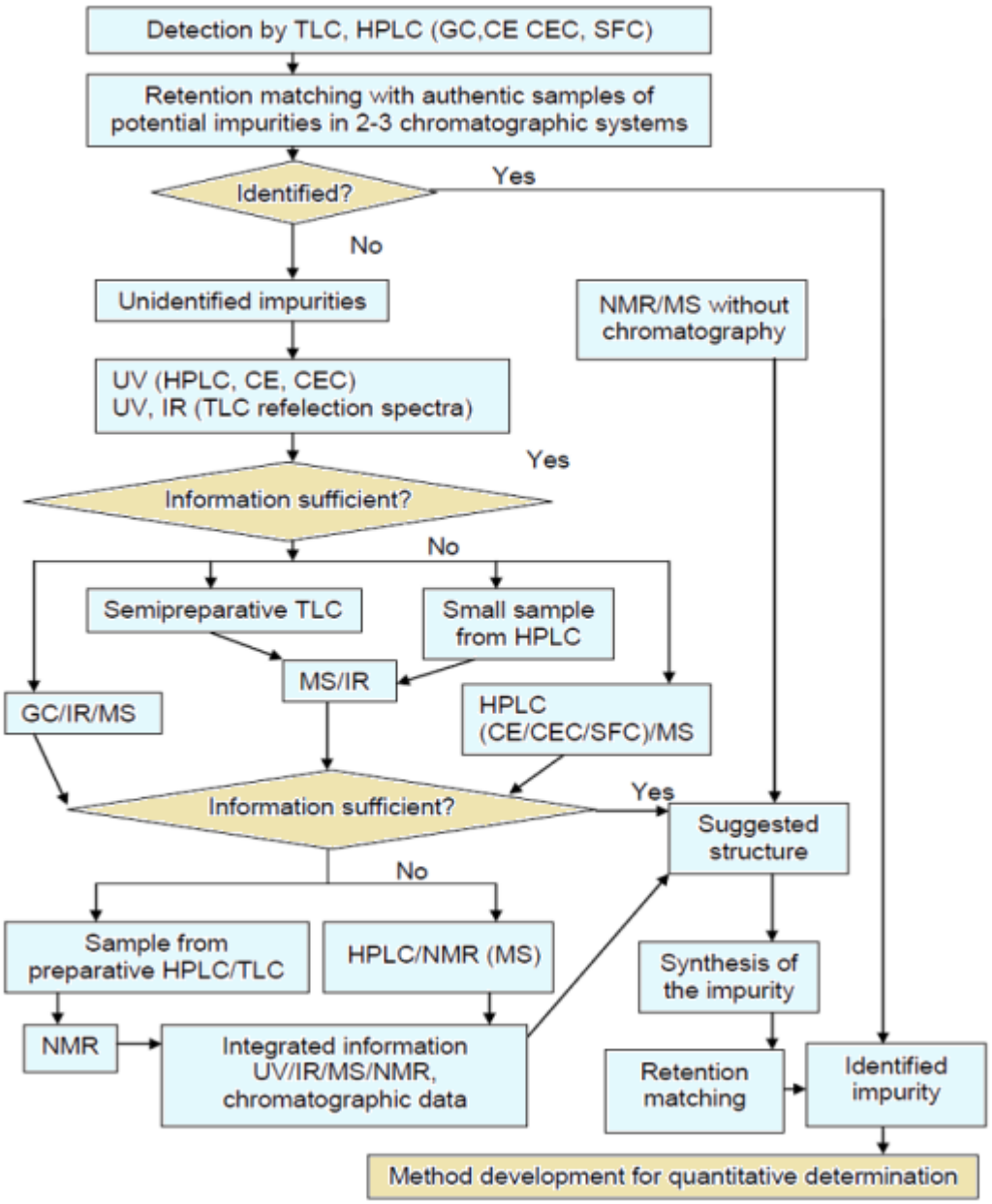

Figure no 2: Schematic flowchart for detection, identification, structure elucidation

CONVENTIONAL APPROACH

The conventional approach of identification and structure elucidation of unknown impurities and DPs involves separation, impurity or degradant enrichment and isolation or synthesis which is followed by spectral analysis. The separation is usually carried out by High-Performance Liquid Chromatography (HPLC), Ultra High Performance Liquid Chromatography (UPLC/UHPLC), Thin Layer Liquid Chromatography (TLC), High Performance Thin Layer Liquid Chromatography (HPTLC), Hydrophilic Interaction Liquid Chromatography (HILIC), Gas Chromatography (GC), Capillary Electrophoresis (CE), Super Critical Fluid Chromatography (SFC) and/or any other relevant separation technique. The detection is usually carried out by UV or other detectors like fluorescence, Evaporative Light Scattering Detector (ELSD), Chemiluminescent Nitrogen Detector (CLND), Corona CAD (C-CAD, Corona Charged Aerosol Detectors) have also been utilized advantageously in impurity profiling and degradation study. But usually, UV/PDA detectors are employed, which also provides purity of each peak. Simultaneous orthogonal techniques can also be used for ex. HPLC and CE; RPLC, MEKC, GLC, and SFC; HILIC and LC; CE and CEC; SCF and LC. Due to differential selectivity, these techniques ensure separation of a large number of impurities and DPs.

The presence of particular impurities and DPs can be checked by matching retention times of unknown analytes with their standards by developed method, usually done through spiking. Impurity or degradation peak that is above the identification threshold (as specified in ICH guidelines Q3A and Q3B) are marked. For identification and structural elucidation of such impurity or DPs, it is usual practice to enrich and isolate them using an appropriate tool (Fig.-2) For unknown DPs different stress conditions like acid, base, neutral hydrolysis, oxidation, and light are used and optimized to obtain sufficient amounts of the desired DPs. The structure of purified impurity or DPs is deduced from the spectral data. Some investigations at this stage may also include single crystal analysis of the pure crystalline product. The prediction of the structure is then followed by the actual synthesis of impurity or DPs, then followed by spectral matching by spiking to confirm the presence or absence of the identified and isolated compound.

GRAVIMETRIC ANALYSIS

Gravimetric analysis describes a set of methods in analytical chemistry for the quantitative determination of an analyte based on the mass of a solid. A simple example is the measurement of solids suspended in a water sample: A known volume of water is filtered, and the collected solids are weighed. In most cases, the analyte must first be converted to a solid by precipitation, with an appropriate reagent. The precipitate can then be collected by filtration, washed, dried to remove traces of moisture from the solution, and weighed. The amount of analyte in the original sample can then be calculated from the mass of the precipitate and its chemical composition.

UV SPECTROMETRY

Ultraviolet (UV) spectroscopy is a physical technique of the optical spectroscopy that uses light in the visible, ultraviolet, and near-infrared ranges. The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV / VIS spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how rapidly the absorbance changes with concentration.

INFRARED SPECTROSCOPY

Infrared spectroscopy is the subset of spectroscopy that deals with the infrared region of the electromagnetic spectrum. It covers a range of techniques, the most common being a form of absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify compounds and investigate sample compositions. A common laboratory instrument that uses this technique is an infrared spectrophotometer. The infrared portion of the electromagnetic spectrum is usually divided into three regions; the near-, mid- and far-infrared, named according to their relation to the visible spectrum. The far-infrared, approximately 400 – 10 cm^{-1} (1000 – 30 μm), lying adjacent to the microwave region, has low energy and may be used for rotational spectroscopy. The mid-infrared, approximately 4000 – 400 cm^{-1} (30 – 2.5 μm), may be used to study the fundamental vibrations and associated rotational-vibrational structure.

FLUORESCENCE SPECTROSCOPY

Fluorescence spectroscopy is called as fluorometry or spectrofluorometry. It is a type of electromagnetic spectroscopy, which analyzes the fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, which excites the electrons in the molecules of certain compounds and causes them to emit light of lower energy, typically, but not necessarily, visible light. A complementary technique is absorption spectroscopy. Devices that measure fluorescence is called fluorometers or fluorimeters.

HYPHENATED METHODS

There are a few limitations of the conventional approach, these are:

- The process is time-consuming and sometimes become complicated if several impurities and DPs have to be characterized in a single sample.

- If the impurity or DPs formed are present in trace amount and cannot be enriched, the process becomes more tedious.

- If unstable impurity or DP is formed, or if there is a possibility of a secondary reaction during processing, isolation becomes difficult.

Due to these reasons, hyphenated techniques are preferred choice for the identification and characterization from the last few years, especially if impurity or DPs formed are in trace level. Mostly the available hyphenated instruments have LC, GC or CE on the front end connected to MS, NMR or IR on the detection side. These are LC-MS, GC-MS, CE-MS, LC-NMR, CE-NMR, LC-NMR/MS, and LC-IR, etc. Especially, LC-MS instruments are mostly used. Also, combined LC-MS-NMR systems are available.

MASS-BASED HYPHENATED TECHNIQUES

GAS CHROMATOGRAPH-MASS SPECTROMETER (GC-MS)

It was the first hyphenated technique introduced for determination of organic volatile impurities, and residual solvents in a sample and used till today. However, the volatility and thermal stability of analytes are essential for GC-MS. Therefore few kinds of literature exist on the use of GC-MS in the characterization of impurities and DPs.

LIQUID CHROMATOGRAPH-MASS SPECTROMETER (LC-MS)

The most popular hyphenated technique for characterization of impurities and DPs is LC-MS, as it has the potential to give nearly clear structural information about the unknown analyte. Although it was introduced much after GC-MS, several advancements and ranges of this instrument are available commercially. These are LC-MS (Single Quad), LC-MS-MS (Triple Quad), LC-TOF, LC-MS-TOF (Q-TOF, Triple TOFTM), LC-MS-3DTRAP (MSn), LC-MS-2DTRAP (Q-TrapTM), LC-Hybrid Trap TOF Systems (LCMS-IT-TOF®), LC-OrbitrapTM, LC-FTICR (Fourier Transform Ion Cyclotron Resonance). These are either used alone or in combination to get desired information useful for structural characterization.

CAPILLARY ELECTROPHORESIS AND CAPILLARY ELECTROCHROMATOGRAPHY CE-MS

CE (Capillary electrophoresis) and CEC (capillary electrochromatography) are important techniques for separation and identification of impurities and DPs. CEC is a hybrid technique that involves both high efficiency of CE and stationary and mobile phase selectivity of LC. Few kinds of literature are available wherein CE/CEC have been hyphenated with MS for characterization of impurities and DPs but are gradually gaining significance. The technique is usually restricted to the separation of analytes.

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC-MS)

A small number of reports are available on the use of SFC-MS for characterization of impurities and DPs for pharmaceutical substances and products. The technique has its advantage of saving LC solvents but its bench-top instrument was not available commercially for analysis; recently it has been introduced into the market.

NUCLEAR MAGNETIC RESONANCE (NMR) BASED HYPHENATED TECHNIQUES

LIQUID CHROMATOGRAPH-NMR

In 1978 for the first time, the coupling of LC effluent to NMR was reported. To improve the instrument sensitivity and resolution, modern LC-NMR instruments are accompanied by multiple technological advancements, like microprobes, strong field magnets (above 500 MHz), and cryoprobe technology. SPE (Solid phase extraction) units are embedded in

between LC and NMR to overcome the requirement of high volumes of expensive deuterated solvents in the mobile phase. The LC effluent contains low sample concentrations, due to which 13C detection is usually not possible. Also, the insufficient quantity of analyte did not allow acquisition of heteronuclear HSQC and HMBC spectra. Specific NMR pulse sequences are used to obtain clean spectra free from corresponding residual non-deuterated solvents. Usually, supportive information is gathered from LC-NMR for structural confirmation for the components separated on the LC column. Several reports are available on the use of LC-NMR for structural characterization of impurities and DPs. The useful LC-NMR could be collected for a concentration of 0.06%, though most reported studies involve 0.5% and above. Isomers, that generate the same mass and fragmentation pattern, for such compounds LC-NMR data is very useful to confirm their identity. Unlike LC-MS phosphate buffer is preferred choice for LC-NMR, because of the presence of multiple protons in a volatile buffer like formate or acetate.

CAPILLARY ELECTROPHORESIS CHROMATOGRAPH-NMR

If analytes are present in relatively small amounts, hyphenated CE-NMR provides similar advantages as LC-NMR concerning separation, chemical identification, and structural information. Both continuous and stopped flow modes, similar to LC-NMR are used in CE-NMR. The typical problem associated with CE-NMR is the shorter residence time of the sample in NMR due to small sample volume output from CE that affects the detection sensitivity. Although intensive innovative efforts have been made to improve this, only a few publications reported the application of CE-NMR to identification and characterization of trace amount of impurities and DPs.

FOURIER TRANSFORM INFRARED SPECTROMETER BASED HYPHENATED TECHNIQUE

Conventional FT-IR system requires 1–5 mg of sample hence recording becomes difficult when analytes are present or generated in trace quantities or cannot be isolated. LC-IR provides benefits in such cases and has been recently commercialized. Some limitations exist while recording the IR spectrum of impurities or DPs at levels of 0.1% in LC-IR, these include:

- On-line enrichment of analyte is essential.

- Interference of mobile phase components.

- It is difficult to apply chemometrics especially in the case of gradient elution since the background absorption is strongly influenced by the slight variation in mobile phase composition.

- Complete removal/ elimination of the solvents are difficult.

- Analytes should have low volatility than the mobile phase.

- Differential nature i.e. amorphous or crystalline; of analyte post deposition and also post solvent elimination.

Interface constitutes the most critical component in LC-IR due to above-cited reasons. It is available in two types- (i) flow cell (on-line) (ii) solvent elimination (semi on-line). On-line LC-IR has limited use and is restricted to major constituents only due to its poor detection limits, while semi on-line has comparatively better sensitivity and gives improved spectral data. Few literature reports are available on the use of LC-IR in the characterization of impurities and DPs.

CONCLUSION

Isolation and characterization of impurities are mandatory for acquiring and evaluating data that establishes biological safety, which reveals the need and scope for impurity profiling of drugs in pharmaceutical research. To isolate and quantify the impurities, various instrumental analytical techniques have been used routinely. Moreover, the recognition and regulatory contemplation of organic impurities is an extremely complex problem owing to numerous sources ranging from microbial contamination to degradation products of APIs apart from traces of intermediates. Although, ICH has an out lighted course of action about impurities, still much more needs to be done. Hence, there is a strapping need to have unified specifications/standards about impurities.

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