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RECENT APPROCHES FOR IMPURITY PROFILING IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

In the pharmaceutical world, an impurity is considered as any other organic material, besides the drug substance, or ingredients, arise out of synthesis or unwanted chemicals that remains with API's. The impurity may be developed either during formulation, or upon aging of both API's and formulated API's in medicines. The control of impurities in Formulated products and Active Pharmaceutical ingredients were regulated by various regulatory authorities like US-FDA, ICH, MHRA, TGA etc. Nowadays apart from purity profile there was an increasing essentiality of impurity profile by regulatory agencies. Hence Qualification of impurities which is essential for establishing the biological safety of an individual impurity. Thus it reveals the need and scope of impurity profiling of drugs in Pharmaceutical research. Various methods are used to isolate and characterize impurities in pharmaceuticals, such as, capillary electrophoresis, electron paramagnetic resonance, gas–liquid chromatography, gravimetric analysis, high performance liquid chromatography, solid-phase extraction methods, liquid–liquid extraction method, Ultraviolet Spectrometry, infrared spectroscopy, supercritical fluid extraction column chromatography, mass spectrometry, Nuclear magnetic resonance (NMR) spectroscopy, and RAMAN spectroscopy. Among all hyphenated techniques, the most exploited techniques for impurity profiling of drugs are Liquid Chromatography (LC)-Mass Spectroscopy (MS), LC-NMR, LC-NMR-MS, GC-MS, and LC-MS. This reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

Keywords: Impurity profiling, HPLC, Spectroscopy, ICH guidelines.

INTRODUCTION

A general scheme is set for the estimation of the impurity of drug substances by the rational use of chromatographic, spectroscopic and analytical techniques. The various parameters to be fulfilled in an impurity profile of drug substances are discussed. Impurity is defined as any substance coexisting with the original drug, such as starting material or intermediates or that is formed, due to any side reactions. The presence of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (i.e., the identity as well as the quantity of impurity in the pharmaceuticals), is now gaining critical attention from regulatory authorities. The different Pharmacopoeias, such as the British Pharmacopoeia (BP), United States Pharmacopeia (USP), and Indian Pharmacopoeia (IP) are slowly incorporating limits to allowable levels of impurities present in the API's or formulations. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has also published guidelines for validation of methods for analyzing impurities in new drug substances, products, residual solvents and microbiological impurities [1].

A number of articles [2-4] have stated guidelines and designed approaches for isolation and identification of process-related impurities and degradation products, using Mass spectrometry (MS), Nuclear Magnetic Resonance (NMR), High Performance Liquid Chromatography

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(HPLC), Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS), and Tandem Mass Spectrometry for pharmaceutical substances. Present article reveals different impurities found in the API's, methods for identifying them and the possible measures to deal with the interferences caused by them. Impurity profile is the description of identified and unidentified impurities present in new drug substances.

TYPES OF IMPURITY

Organic medicinal substances are contaminated in exactly the same manner as inorganic substance during their manufacturing processes. Since the organic substances belong to a very wide range of chemical groups and at the same time the contaminating impurities being of varied nature the task of detecting the impurities becomes a difficult job. Therefore, the contaminating impurities for organic medicinal compounds can be classified into –

(1) Inorganic impurities.
(2) Organic impurities.
(3) Contamination by chemical intermediate (ICH 2000).

Impurities closely related to the product and coming from the chemical or from the biosynthetic route itself, Impurities formed due to spontaneous decomposition of the drug during the storage or on exposure to extreme conditions, or the precursors which may be present in the final product as impurities. Impurities present in excess of 0.1% should be identified and quantified by selective methods. The suggested structures of the impurities can be synthesized and will provide the final evidence for their structures, previously determined by spectroscopic methods. Therefore it is essential to know the structure of these impurities in the bulk drug in order to alter the reaction condition and to reduce the quantity of impurity to an acceptable level. Isolation, identification and quantification of impurities help us in various ways, to obtain a pure substance with less toxicity and, safety in drug therapy.

Quantitative determination of these impurities could be used as a method for the quality control and validation of drug substances. Regulatory authorities such as US FDA, CGMP, TGA, and MCA insist on the impurity profiling of drugs. Impurities in new drug substances can be addressed from two perspectives,

- The chemical aspect which includes classification and identification of impurities, report generation, listing of impurities in specifications, and a brief discussion of analytical procedures,
- The safety aspect which includes specific guidance for quantifying impurities, present, substantially at lower levels, in a drug substance used in clinical studies.

1) Organic Impurities

The actual and potential impurities most likely to arise during the synthesis, purification, and storage of the drug substance should be summarized ,based on sound scientific appraisal of the chemical reactions involved in the synthesis, impurities associated with raw materials that could contribute to the impurity profile of the drug substance. The laboratory studies conducted to detect impurities in the drug substance, which include test results of materials manufactured during the development process and batches from the commercial processes. The impurity profile of the drug lots, intended for marketing should be compared with those used in development. The spectroscopic studies (NMR, IR, MS etc.) conducted to characterize the structure of actual impurities present in the drug substance above an apparent level of 0.1% (e.g., calculated using the response factor of the drug substance) should be described. All recurring impurities above an apparent level of 0.1% in batches manufactured by the proposed commercial process should be identified.

2) Inorganic Impurities

Inorganic impurities are normally detected and quantified using Pharmacopeial or other appropriate standards. Carryover of catalysts to the drug substance should be evaluated during development.

3) Residual Solvents

The control of residues of solvents used in the manufacturing process for the drug substance should be discussed. Acceptance criteria should be based on Pharmacopeial standards, or ICH guidelines or known safety data, depends on the dose, duration of treatment, and route of administration.

SOURCES OF IMPURITIES

From the preceding discussion, it is clear that impurities can originate from several sources; such as;

1. Crystallization-related impurities
2. Stereochemistry-related impurities
3. Residual solvents
4. Synthetic intermediates and by-products
5. Formulation-related impurities
6. Impurities arising during storage
7. Method related impurity
8. Mutual interaction amongst ingredients
9. Functional group-related typical degradation [5]

1. Crystallization-related impurities

Based on the realization that the nature of structure adopted by a given compound upon crystallization could exert a profound effect on the solid-state properties of that system, the pharmaceutical industry is required to take a strong interest in polymorphism and solvatomorphism as per the regulations laid down by the regulatory authorities. Polymorphism is the term used to indicate crystal system where substances can exist in different crystal packing arrangements, all of which have the same elemental composition. Whereas, when the substance exists in different crystal packing arrangements,

with a different elemental composition; the phenomenon is known as Solvatomorphism [6].

2. Stereochemistry-related impurities

It is of paramount importance to look for stereochemistry related compounds; that is, those compounds that have similar chemical structure but different spatial orientation, these compounds can be considered as impurities in the API's. Chiral molecules are frequently called enantiomers. The single enantiomeric form of chiral drug is now considered as an improved chemical entity that may offer a better pharmacological profile and an increased therapeutic index with a more favourable adverse reaction profile. However, the pharmacokinetic profile of levofloxacin (S-isomeric form) and ofloxacin (R-isomeric form) are comparable, suggesting the lack of advantages of single isomer in this regard [7]. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin), lavalbuterol (R-albuterol), and esomeprazole (S-omeprazole).

3. Residual solvents

Residual solvents are organic volatile chemicals used during the manufacturing process or generated during the production. 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. Especially, solvents in Class I, *viz* benzene (2 ppm limit), carbon tetrachloride (4 ppm limit), methylene chloride (600 ppm), methanol (3000 ppm, pyridine (200 ppm), toluene (890 ppm) should be avoided. In Class II, *viz* N,Ndimethyl formamide (880 ppm), acetonitrile (410 ppm). Class III solvents, *viz* acetic acid, ethanol, acetone have permitted daily exposure of 50 mg or less per day, as per the ICH guidelines. A selective gas chromatography (GC) method has been developed to determine thepurity of acetone, dichloromethane, methanol and toluene. Using this method, the main contaminants of each organic solvent can be quantified. Moreover, the developed method allows the simultaneous determination of ethanol, isopropanol, chloroform, benzene, acetone, dichloromethane, methanol and toluene with propionitrile as the internal standard [8].

4. Synthetic intermediates and by-products

Impurities in pharmaceutical compounds or a new chemical entity (NCE) can originate during the synthetic process from raw materials, intermediates and/or by-products. For example, impurity profiling of ecstasy tablets by GC-MS [9], and MDMA samples, produced impurities in intermediates via reductive amination route [10].

5. Formulation-related impurities

Many impurities in a drug product can originate from excipients used to formulate a drug substance. In addition, a drug substance is subjected to a variety of conditions in the process of formulation that can cause its degradation or have other undesirable reactions. If the source is from an excipient, variability from lot to lot may make a marginal product, unacceptable for reliability. Solutions and suspensions are inherently prone to degradation due to hydrolysis or solvoly [11]. Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was recalled in the United States because of degradation/impurities leading to sub- potency. In general, liquid dosage forms are susceptible to both degradation and microbiological contamination. In this regard, water content, pH of the solution/suspension, compatibility of anions and cations, mutual interactions of ingredients, and the primary container are critical factors.

Microbiological growth resulting from the growth of bacteria, fungi, and yeast in a humid and warm environment may results in unsuitability of an oral liquid product for safe human consumption. Microbial contamination may occur during the shelf life and subsequent consumer-use of a multiple-dose product, either due to inappropriate use of certain preservatives in the preparations, or because of the semi-permeable nature of primary containers [12].

6. Impurities arising during storage

A number of impurities can originate during storage or shipment of drug products. It is Essential to carry out stability studies to predict, evaluate, and ensure drug product safety.

7. Method related impurity

A known impurity, 1-(2, 6-dichlorophenyl) indolin-2-one is formed in the production of a parenteral dosage form of diclofenac sodium, if it is terminally sterilized by autoclave [13]. TheConditions of the autoclave method (i.e., 123 + 2°C) enforce the intramolecular cyclic reaction of diclofenac sodium forming an indolinone derivative and sodium hydroxide. The formation of this impurity has been found to depend on initial pH of the formulation.

8. Mutual interaction amongst ingredients

Most vitamins are very labile and on aging they create a problem of instability in different dosage forms, especially in liquid dosage forms. Degradation of vitamins does not give toxic impurities; however, potency of active ingredients drops below Pharmacopoeial specifications. Because of mutual interaction, the presence of nicotinamide in a formulation containing four vitamins (nicotinamide, pyridoxine, riboflavin, and thiamine) can cause the degradation of thiamine to a sub-standard level within a one year shelf life of vitamin B-complex injections [14]. The marketed samples of vitamin B-complex injections were found to have a pH range of 2.8 - 4.0. A custom-made formulation with simple distilled-

water and a typical formulated vehicle including disodium edetate and benzyl alcohol were investigated and similar mutual interactions causing degradation were observed.

9. Functional group-related typical degradation

Ester hydrolysis can be explained with a few drugs *viz* aspirin, benzocaine, cefotaxime, ethyl paraben [15] and cefpodoxime proxetil [16]. Hydrolysis is the common phenomenon for ester type of drugs, especially in liquid dosage forms *viz* benzylpenicillin, oxazepam and lincomycin. Oxidative degradation of drugs like hydrocortisone, methotrexate, hydroxyl group directly bonded to an aromatic ring (*viz* phenol derivatives such as catecholamines and morphine), conjugated dienes (*viz* vitamin A and unsaturated free fatty acids), heterocyclic aromatic rings, nitroso and nitrite derivatives, and aldehydes (especially flavorings) are all susceptible to oxidative degradation. In mazipredone, the hydrolytic and oxidative degradation pathway in 0.1 mol L-1 hydrochloric acid and sodium hydroxide at 800C were studied [17]. Photolytic cleavage includes example of pharmaceutical products that are exposed to light while being manufactured as solid or solution, packaged, or when being stored in pharmacy shops or hospitals for use by consumers. Ergometrine [18], nifedipine [19]. nitroprusside, riboflavin and phenothiazines are very liable to photo-oxidation. In susceptible compounds, photochemical energy creates free radical intermediates, which can perpetuate chain reactions. Most compounds will degrade as solutions when exposed to high-energy UV exposures. Fluroquinolone antibiotics are also found to be susceptible to photolytic cleavage [20]. In ciprofloxacin eye drop preparation (0.3%), sunlight induces photo cleavage reaction producing ethylenediamine analog of ciprofloxacin [21]. Decarboxylation of some dissolved carboxylic acids, such as p-amino salicylic acid; shows the loss of carbon dioxide from the carboxyl group when heated. An example of decarboxylation is the photoreaction of rufloxacin [22]. As seen earlier, impurities in drug products can come from the drug or from excipients or can be brought into the system through an in process step by contact with the packaging material.

For most drugs, the reactive species consist of;

• Water- that can hydrolyze some drugs or affect the dosage form performance

• Small electrophiles- like aldehydes and carboxylic acid derivatives

• Peroxides- that can oxidize some drugs

• Metals- which can catalyze oxidation of drugs and the degradation pathway

• Leachable or Extractables - can come from glass, rubber stoppers, and plastic

Packaging materials: Metal oxides such as NaO2, SiO2, CaO, MgO, are the major components leached/extracted from glass [23]. Generally most synthetic materials contain leachable oligomers/monomers, vulcanizing agents, accelerators, plasticizers, and antioxidants [24]. Some examples of leachable/extractables from synthetic materials include styrene from polystyrene, [25] diethyl hexylphalate (DEHP, plasticizer in PVC), [26] dioctyltin isooctyl mercaptoacetate (stabilizer for PVC), [27] zinc stearate (stabilizer in PVC and polypropylene), [28] 2-mercaptobenzothiazole (accelerator in rubber stopper and furfural from rayon).

According to ICH guidelines the Threshold limits for impurities are described in Table 1 for New Drug Substance and New Drug Products.

ANALYTICAL METHODS FOR IDENTIFICATION OF IMPURITIES:

The impurities can be identified by following different methods like

a) Reference standard method
b) Spectroscopic method
c) Separation method
d) Isolation method
e) Characterization method

a) Reference standard method

The main objective of this method is to provide clarity to the overall life cycle, qualification and governance of reference standards used in development and control of new drugs. Since the Reference standards provides the basic information for evaluating process and product performance of drug substances, drug products, impurities, degradation products, starting materials, process intermediates, and excipients.

b) Spectroscopic methods

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

c). Separation methods

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

c) Isolation methods

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

1. Solid-phase extraction methods
2. Liquid-liquid extraction methods
3. Accelerated solvent extraction methods
4. Column chromatography
5. Flash chromatography
6. TLC
7. GC
8. HPLC

9. HPTLC
10. Capillary electrophoresis (CE)
11. Supercritical fluid chromatography (SFC).

1. Solid-phase extraction methods

Solid-phase extraction [29] is an extraction method that uses a solid phase and a liquid phase to isolate the impurity of interest from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantitate the amount of analytes in the sample. SPE uses the affinity of solutes dissolved or suspended in a liquid which act as a mobile phase for a solid through which the sample is passed which act as the stationary phase to separate a mixture into desired and undesired components. The result is that either the desired analytes of interest or undesired impurities in the sample are retained on the stationary phase. The common solvents used in SPE are described in Table-2.When the sample passes through the stationary phase, the analytes in the sample will interact and retain on the sorbent but the solvent, salts and other impurities pass through the cartridge. After the sample is loaded, the cartridge is washed with buffer or solvent to remove further impurities. Then, the analyte is eluted with a non-polar solvent or a buffer of the appropriate PH.

2. Liquid-liquid extraction methods

In this type of extraction, two immiscible liquids were selected. Usually, one phase is aqueous (hydrophilic) and the other is a (hydrophobic) organic solvent. In that [29-32] the solute is distributed between two immiscible solvents .The extraction was based on Distribution Co-efficient or Partition Co-efficient (Kd), which is the ratio of concentration of solute in two different solvents a and b.
Kd = Ca/Cb.

3. Accelerated solvent extraction methods

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

4. Column chromatography

In column chromatography [33-36] the stationary phase is a solid adsorbent which is placed in a vertical glass (usually) column and the mobile phase used is a liquid which is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique. The mixture to be analyzed by column chromatrography is applied to the top of the column. The liquid solvent (the eluent) is passed through the column by gravitational force or by the application of air pressure. An equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. Column chromatography is separated into two categories, depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity, or percolation, it is called gravity column chromatography. If the solvent is forced down the column by positive air pressure, it is called flash chromatography.

5. Flash chromatography

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

6. Thin layer chromatography

Thin-Layer Chromatography is a simple and inexpensive technique that is often used to judge the purity of a synthesized compound or to indicate the extent of progress of a chemical reaction. In this technique, a small quantity of a solution of the mixture to be analyzed is deposited as a small spot on a TLC plate, which consists of a thin layer of silica gel (SiO_2) or alumina (Al_2O_3) coated on a glass or plastic sheet. The plate constitutes the stationary phase. The sheet is then placed in a chamber containing a small amount of solvent, which is the mobile phase. The solvent gradually moves up the plate via capillary action, and it carries the deposited substances along with it at different rates. The desired result is that each component of the deposited mixture is moved a different distance up the plate by the solvent. The components then appear as a series of spots at different locations up the plate. Substances can be identified from their so-called R_f values with reference to suitable adsorbents and eluting solvents as listed in Table-3 and 4. The order in the table is approximate, since it depends upon the substance being adsorbed, and the solvent used for.

If the substances in the mixture differ greatly in adsorbability, it will be much easier to separate them. Often, when this is so, a succession of solvents of increasing eluting power is used. One substance may be eluted easily while the other stays at the top of the column, and then the other can be eluted with a solvent of greater eluting power. Table 5 indicates an approximate order of adsorbability by functional group.

7. Gas chromatography (GC)

Gas chromatography [33-36] is an analytical technique for separating compounds based primarily on their volatilities. Gas chromatography provides both qualitative and quantitative information for individual compounds present in a sample. Compounds move through a GC column as gases with their Linear velocity and flow rates were summarized in Table-6, because the compounds are normally gases or they can be heated and vaporized into a gaseous state. The compounds partition between a stationary phase, which can be either solid or liquid, and a mobile phase (gas). The differential partitioning into the stationary phase allows the compounds to be separated in time and space.

8. High performance Liquid chromatography (HPLC)

Normal-phase HPLC separates analytes based on adsorption to a stationary surface chemistry and by polarity. NP-HPLC uses a polar stationary phase and a non-polar, non-aqueous mobile phase, which effectively separates the analytes that are readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity, and the interaction between the polar analyte and the polar stationary phase (relative to the mobile phase) increases the elution time. The interaction strength depends not only on the functional groups in the analyte molecule, but also on steric factors. Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily. The retention time can be increased by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger. Similarly, the decreasing of retention time by adding more organic solvent to the eluent can be done.

9. High performance thin layer chromatography (HPTLC)

Similar to other chromatographic methods HPTLC is also based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved. Once separation occurs individual components are visualized as spots at respective level of travel on the plate. Their nature or character are identified by means of suitable detection techniques.

10. Capillary Electrophoresis (CE)

Capillary Electrophoresis (CE) is a separation technique based on the differential transportation velocities of charged species in an electric field through a conductive medium. Primary candidates for CE separation are ions. The basic instrumental set-up consists of a high voltage power supply (0 to 30 kV), a fused silica (SiO_2) capillary, two buffer reservoirs ,two electrodes, and an on column detector.

11. Supercritical fluid chromatography (SCF)

A pure supercritical fluid (SCF) is any compound at a temperature and pressure above the critical values (above critical point). Above the critical temperature of a compound the pure, gaseous component cannot be liquefied regardless of the pressure applied. The critical pressure is the vapor pressure of the gas at the critical temperature. In the supercritical environment only one phase exists. The fluid, as it is termed, is neither a gas nor a liquid and is best described as intermediate to the two extremes. This phase retains solvent power approximating liquids as well as the transport properties common to gases. A comparison of typical values for density, viscosity, diffusivity of gases, liquids, SCF and critical conditions for various solvents are presented in Table 7 and 8.

e) Characterization methods

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

NMR

A unique aspect of NMR spectra is the direct proportionality between peak areas and the number of nuclei responsible for the peak. The most important chemical application of proton NMR spectroscopy have been to the identification and structure elucidation of organic, metal-organic and biochemical molecules, Analysis of multicomponent mixtures, Elemental analysis etc., The best example for the NMR study of the impurity state in heavily doped Si:P [37] over a wide temperature range (100–500 K). The results shows that free carriers in Si:P are in dynamic exchange with residual impurity states

at concentrations as high as 1019 $^{cm-3}$ and at temperatures well above room temperature.An another example for impurity study of dilute Vanadium in copper [38] to measure the Knight shift of the 51V impurity resonance and analyzed it in terms of a nonmagnetic virtual bound state. The research concludes that vanadium impurities in copper are magnetically similar to cobalt impurities.

Mass spectroscopy

Mass spectroscopy has wide applications in structural elucidation of organic and biological molecules, detection and identification of of species separated by chromatography and capillary electrophoresis. since the interpretation of the resulting complex spectrum is often impossible, the Chemists have developed methods in which mass spectrometers are coupled with various hyphenated techniques like GC-MS, LC-MS, LCMS-MS, HPLC-DAD-MS, HPLCDAD-NMR-MS, Tandem Mass spectrometry, Capillary electrophoresis-Mass spectrometry.

GC-MS

GC-MS has become one of the most powerful tools available to the chemists for the analysis of complex mixtures. The spectra which are collected from the chromatographic technique are stored in a computer for subsequent processing. In the case of GC-MS, GC coupled to a Mass spectrometer through an interface that enriches the concentration of the sample in the carrier gas by taking advantage of the higher diffusivity of the carrier gas. Scanning times are rapid so that several MS can be obtained during the elution of a single peak from the GC unit. The major technical difficulty was to find an efficient gas separator or interface for GC/MS. The best example for this GC-MS technique is the impurity profiling of synthetic pesticide d-allethrin[42] by using of two distinct soft ionization techniques, the atmospheric pressure ionisation with electrospray source (API–ESI) and the chemical ionisation (APCI). An another research work of determination of impurity like cyclohexone,N-methyl pyrrolidone,Atlox 3406-F(an agricultural dispersant) in Triflorine a hexachlorinated ,an fungicide using electrospray ionization of GC-MS technique.

LC-MS

In case of LC-MS a similar to GC-MS, though rather more difficult problem arises in the removal of liquid carrier from an HPLC eluent before samples are passed in to the MS source.The normal eluent flow rates of 0.5-2.0ml min-1 cannot be handled by the MS pumping system. Hence moving belt Inlet systems, jet separators and vacuum nebulizers are all techniques that are used to remove solvent and pass analytes in to the source. The best example for this technique is in the investigation of 10 - methoxy- 1, 6-dimethylergoline-8-methanol 5-bromonicotinic acid ester (Nicergoline) and its related substances[43] was performed by using ammonium acetate and methanol mixture as the mobile phase. It was characterized by HPLC/API-MS in terms of their molecular weight.

HPLC-DAD-MS

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

LC-MS-MS

In this type of technique,the Characterization and quantitative determination of four impurities in piperaquine phosphate by gradient reverse phase HPLC and LC/MS/MS was developed [39] and validated as per ICH guidelines. Another example, for determinations of Low content of Methyl Methanesulfonate and Ethyl Methanesulfonate Impurities as they were potential genotoxic impurities (PGIs) in Emtricitabine, an Active Pharmaceutical Ingredient using LC/MS/MS [40] method.

HPLC-DAD-NMR-MS

The LC-DAD-MS/SPE-NMR Hyphenation technique has been used in the Identification of Isobaric Iridoid Glycoside Regioisomers as minor constituents from *Harpagophytum procumbens* of Pharmaceutically Used Plant Extracts [41-46]. Hence by using of this technique provides the spectral data needed for structure elucidation.

Tandem Mass spectrometry

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

Capillary electrophoresis-Mass spectrometry (CE-MS)

CE-MS was recently implemented in the method development approach to support impurity profiling of pharmaceutical products. Capillary electrophoresis (CE) is based on a different separation principle and consequently has different selectivity compared to HPLC. CE coupled to a Mass Spectrometer using electrospray ionization (ESI). Recently, atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) have become available for CE/MS. can be helpful for

identification and structural elucidation purposes. The combination of CE and MS has relied on interfaces to allow efficient transfer of analytes on-line from the electrophoretic capillary to the mass spectrometer without sacrificing separation efficiency. CE by its nature is particularly well suited to the separation of polar compounds readily ionizable in solution. Although numerous publications have appeared on CE-MS, this technique is still not widely accepted for routine use. The major limitation of CE is the limited sample volumes that can be analyzed without compromising separation efficiency. Another drawback with CE-MS is that migration times tend to fluctuate with a change of temperature in the environment. The use of non-volatile buffers in CE-MS is generally avoided. Hence various goals of impurity investigations are Process –related impurities, degradation –related impurities, identifying the significant impurities, identifying the degradation product by stress studies and its actual degradation products through stability studies, determine the origin of impurities and to establish a method for eliminating or reducing the impurities, to understand the degradation pathway and to minimize the degradation.

Table 1. Thresholds for reporting impurities

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

a-The amount of drug administered per day.

Table 2. Characteristics of solvents commonly used in Solid phase extraction method

Polarity	**Solvent**	**Miscible in Water**
Non-Polar **Strong RP-SPE** **Weak RP-SPE** ↓ ↑ ↓ **Polar** **Week RP-SPE** **Strong RP-SPE**	Hexane	NO
	Isooctane	NO
	Carbon tetra chloride	NO
	Chloroform	NO
	Methylene chloride	N O
	Tetra hydro furan	YES
	Diethyl ether	NO
	Ethyl acetate	POORLY
	Acetone	YES
	Acetonitrile	YES
	Isopropanol	YES
	Methanol	YES
	Water	YES
	Acetic acid	YES

Table 3. Chromatographic adsorbents

Adsorbent	**Adsorbent**	**Name Chemical Formula**
Most Strongly Adsorbent	Alumina	Al2O3
	Charcoal	C
	Florisil	MgO/SiO_2 (anhydrous)
Least Strongly Adsorbent	Silica gel	SiO2

Table 4. Eluting solvents for chromatography

ELUTING POWER **Least Eluting Power (alumina as adsorbent)** ↓ **Greatest Eluting Power (alumina as adsorbent)**	Eluting solvents ,Petroleum ether (hexane; pentane)
	Carbon tetrachloride
	Benzene
	DichIoromethane
	Ether (anhydrous)
	Ethyl acetate (anhydrous)
	Acetone (anhydrous)
	Ethanol
	Water
	Pyridine

Table 5. Adsorbability of organic compounds by functional group

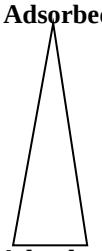
Least Strongly Adsorbed  **Most Strongly Adsorbed**	Saturated hydrocarbons; alkyl halides
	Unsaturated hydrocarbons; aIkenyl halides
	Aromatic hydrocarbons; aryl halides
	Polyhalogenated hydrocarbons
	Ethers
	Esters
	Aldehydes and ketones
	Alcohols
	Acids and bases (amines)

Table 6. Recommended linear velocities and flow rates of Carrier gases

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

Table 7. Comparison values for Gas, SCF and Liquid

Property	Density (kg/m3)	Viscosity(Cp)	Diffusivity (mm2/s)
Gas	1	0.01	1-10
SCF	100-800	0.05-0.1	0.01-0.1
Liquid	1000	0.5-1.0	0.001

Table 8. Critical Conditions for Various Supercritical Solvents

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

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