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Open Access Review Article

A Review on Multi Approaches for Impurity Isolation and its Characterization

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ABSTRACT

International Conference on Harmonization (ICH) has formulated different guideline regarding the control of impurities. In this review, the impurity sources, classification, isolation, detection and characterization methods are described. The some impurities are unavoidable and will be present in trace amounts hence ICH guidelines frame the different policies and establish the specification limits, isolation and characterization is necessary for evaluation and control of impurities. The other regulatory bodies and drug development authorities look up to these guidelines for launching a quality drug into the market. Validation of analytical process for impurity identification is performed to establish the impurity profile of any drug substance. Hence the major focus of this review article is on isolation and characterization of impurities using various technics, sources and classifications, establishment of impurity profile and analytical approaches to establish its profile. We also could apply the QbD to providing statistical documentary evidences.

Keywords: Impurity profile, Impurities isolation methods, chromatographic separation, Impurity Characterization, ICH, QbD.

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Introduction:

The active pharmaceutical ingredients (APIs) manufacturing industries are the base of all kind of pharmaceutical manufacturing industries to provide the sufficient or specified quality of APIs. The major challenge for bulk drug industries is to produce the final drug of required quality and purity, economically. That's almost impossible to obtain absolutely pure materials, as impurities get incorporated into them either during manufacture, purification or during storage. So, to identify the nature of all the related substances or impurities we have to develop method for Isolation, Separation, characterization and quantification of impurities in drug compounds (Gabhe, *et al.*, 2011). It is a very critical task in pharmaceutical product development for quality and safety. Chromatographic isolation and separation of impurities can be a challenging process for the analytical laboratory. Generally ssuitable analytical instrumentation and multiple chromatographic methods are required (Prafulla Kumar Sahu, *et al.* 2015). Conventional preparative HPLC approaches result in large (often aqueous) fraction volumes, instability of the collected fractions. Supercritical Fluid Chromatography (SFC) helpfull to eliminate these problems. In comparison with reversed-phase and normal phase HPLC, SFC offers rapid method development and high efficiency of preparative separations through increased loading capacity, faster linear solvent velocity, and drastically reduced solvent removal effort. Also this facilitate stepwise protocols that would be impractical using other chromatography (Ashraf-Khorassani *et al.*, 2005). Various analytical methodologies were employed for the separation and determination of related components in pharmaceuticals. Impurity profile describes the identified and unidentified impurities present in a new drug substance and drug products. Impurity profiling is the common name of a group of analytical activities, the aim of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drug substances and pharmaceutical formulations (ICH Agency, 2006). The evolution of chemical knowledge and the emergence of increasingly sensitive and selective analytical methods have been continuously stimulating the interest in the determination of drug purity and the impurities themselves in both, natural and synthetic products. This is in line with the policy of the pharmaceutical industry, which has always demanded that the API should be as pure as

possible to achieve efficacy and safety of drug. Two main factors determining the safety of drug therapy are:

1. Pharmacological and toxicological profile of drug substances: the relationship of beneficial and adverse effects of the drug.

2. The impact of impurity present in drug substance: Analytical chemist play important role in monitoring and controlling impurities in drug substances.

In pharma industry the rapid isolation separation, detection, identification, elucidation, and characterization of synthetic or process impurities or degradants is an intense and comprehensive undertaking. Regulatory agencies including the USFDA and ICH mandate dose dependent thresholds for Reporting, Identification, and Qualification of impurities. For example, in the development of a formulated drug substance, the FDA requires that all impurities introduced in the proposed process above 0.1 by area percent need to be isolated and structurally characterized (the requirement can be even lower for drugs with larger doses). Impurities from synthesis, manufacture, or storage are all of interest. Furthermore, in order to develop a robust drug product, degradation products must be characterized with the intent of minimizing their presence. Thus preserving the shelf life of the formulated drug product (Agency, 2006; ICH, 2006a, 2006b; Gabhe, Desai and Patel, 2011).

Glossary

Intermediate: The compounds formed in the process of synthesis for the desired product are called intermediates or reaction intermediates. They are defined as products that have undergone a partial processing and are used as raw material in a successive productive step.

Penultimate intermediate: As the name suggests, this is the compound found in the synthesis chain before the production of the desired compound. Sometimes confusion arises when the desired material is a salt of a free base or acid. In our opinion, it is inappropriate to label the free base or acid as the penultimate intermediate if the drug substance is a salt.

By-product: The unintentional compounds that arise during the reaction are commonly called by-products. Not all by-products can be quantified easily; hence, they present a thorny problem to the analytical chemist. A by-product can be useful and marketable or it can be considered waste.

Transformation product: This relates to an expected and non-expected product that may be formed in the reaction. Transformation products are very similar to by-products, except the term tends to connote that more is known about the reaction products than transformation products.

Interaction product: This term is slightly more comprehensive and more difficult to evaluate than by-products and transformation products in that it considers interactions occurring among various chemicals involved in reaction.

Related product: As mentioned, impurity is a word that is not well liked. So a related product actually is similar to an impurity, but active pharmaceuticals use the term related products instead, thus playing down the negativity frequently attached to the term impurity. These products can have similar chemical structure and might have standardized biological activity; however, this by itself does not provide any guarantee of effect.

Degradation product: The compounds produced due to decomposition of the material of interest or active ingredients often are referred to as degradation products (Singh. *et. al,.* 2017).

Foreign substance: This is the material that may be present due to contamination or adulteration, not as outcomes of synthesis.

Toxic impurity: Toxic impurities might affect the biological activity, even at very low concentrations. They require identification by qualitative or quantitative means.

Concomitant component: Bulk pharmaceutical chemicals may contain concomitant components, which are geometric and optical isomers and antibiotics that are mixtures.

Ordinary impurity: An impurity having enough potency to have biological activity even at trace level is called an ordinary impurity.

Organic volatile impurity: A solvent that may remain in the drug substance should be considered as an organic volatile impurity (OVI).

Threshold: A boundary beyond which radically different state of affaire exists.

Identified Impurity: An impurity for which a structural characterisation has been achieved.

Unidentified Degradation Product: A degradation product for which a structural characterisation has not been achieved and that is defined solely by qualitative analytical properties (e.g., chromatographic retention time).

Unspecified Degradation Product: A degradation product that is limited by a general acceptance criterion, but not individually listed with its own specific acceptance criterion, in the new drug product specification.

Penultimate Intermediate: It is the last compound in the synthesis chain prior to the production of the final desired (Venkatesan and Valliappan, *et. al* 2014).

Process impurity: An impurity generated during drug substance manufacturing. Process related impurities may include starting materials, byproducts, intermediates, reagents, ligands, and catalyst.

Identification Threshold: A limit above (>) which an impurity should be identified.

Qualification: The process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified.

Qualification Threshold: A limit above (>) which an impurity should be qualified.

Reporting Threshold: A limit above (>) which an impurity should be reported. Reporting threshold is the same as reporting level in Q2B (ICH Agency, 2006).

Regulatory guidelines on impurity:

The United States Food and Drug Administration (FDA) inscribe International Conference on Harmonization guidance of Technical Requirements for Registration of Pharmaceuticals for Human Use. The food and drug administration assigned responsibility to ensure the safety and efficacy of drugs. The various regulatory agencies frame the 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 of Medicines, Therapeutic Governance

Authority (TGA), Australia (Venkatesan and Valliappan, 2014).

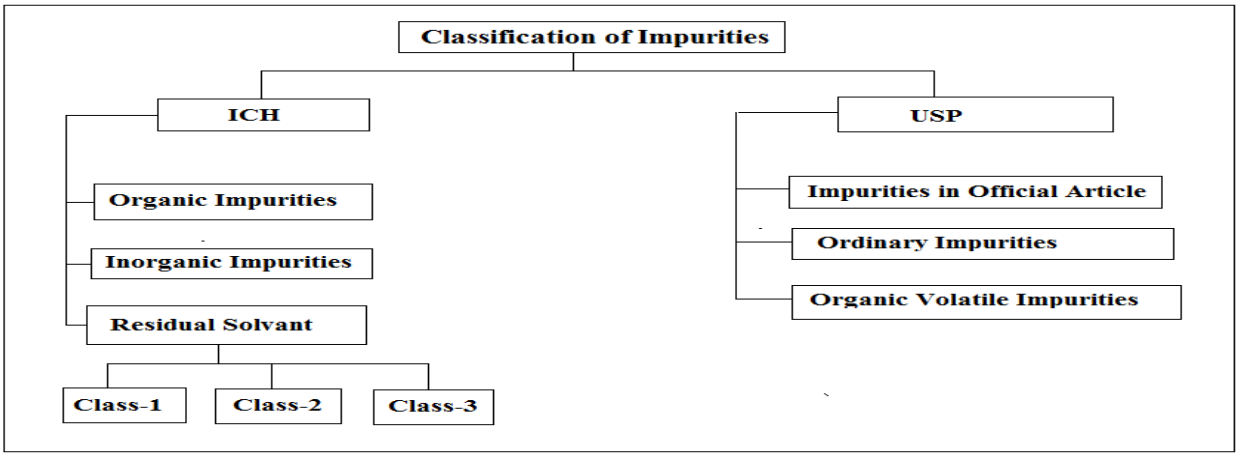

Figure 1: Classification of Impurities:

According ICH guidelines Q3A, Q3B and Q3C and USP Impurities classification

A. According ICH guidelines Q3A, Q3B and Q3C

Organic impurities

These can be formed during the manufacturing process or during storage of new drug substances. They can be identified/unidentified, volatile/non-volatile. This includes starting materials, intermediates, by-products, DPs, reagents, ligands and catalysts.

Inorganic impurities

These can arise during manufacturing process; normally they are known and identified. This includes: reagents, ligands, catalysts, heavy metals, other residual metals, inorganic salts and other materials like filter aids, charcoal. Examples, Cadmium, manganese, sodium etc.

Residual solvents

These are generally inorganic/organic liquids that are used during synthesis of drug substances as a vehicle for preparation of solution or suspension. Appropriate control of residual solvent is necessary since they have known toxicity. ICH Q3C guideline provides the limits of residual solvent based on existing safety and toxicity data. These were classified in three categories:

Class 1 (The most toxic and/or environmentally hazardous):

These are highly toxic in nature and are limited to 28 ppm, for environmentally hazardous chemical like trichloroethane the limit of 1500 ppm is applied. During manufacturing of pharmaceuticals Class 1 solvents should be avoided. But if their presence is unavoidable, the definite concentration limit is applied, regardless of the actual patient intake dose. Examples, Benzene, carbon tetrachloride, 1, 1, dichloromethane,

Class 2 (Considered a lesser risk):

These should be limited in their usage. Two different 1approaches were described in guideline for setting limits of class 2 solvents. The first approach is used when PDE (permitted daily dose) cannot be estimated; concentration limits are calculated on the basis of daily intake of theoretical product mass of 10g. The second approach is used when dose is known; the PDE and/or dose value can be used to determine the permissible concentration. Examples, Acetonitrile, Chloroform, 1, 2,-Dioxane, Pyridine, Toluene.

Class 3 (The lowest risk category):

These have low toxic potential and are limited to 5000 ppm (0.5% w/w). Examples, Acetic acid, acetone, ethanol, butanol, isopropyl alcohol.

Sources of Impurities (Venkata *et.al.*, 2016).

Others-Enantiomeric impurities:

In optically active drug there could be an isomer with enantiomeric impurities. Many drugs are chiral and are often supplied as mixtures of enantiomers rather than single enantiomers (Prafulla Kumar Sahu, 2015).

B. According USP Impurities classified as

a. Ordinary impurities: Ordinary impurities are found in bulk pharmaceutical chemicals that are innocuous by virtue of having no significance on the biological activity of the drug substance. These impurities may arise out of the synthesis, preparation or degradation of the chemical.

b. Organic volatile impurities: Organic volatile chemicals are produced in the manufacture of drug substances or excipients or in the preparation of drug products; they are volatile in nature and by themselves get removed out at time of storage or processing (Bari *et al.*, 2007,) (Saibaba, Kumar and Ramu, 2016).

Sources of impurities:

Synthesis Related Impurities: During synthetic process, impurities in new drug substance or new chemical entity (NCE) mainly originate from raw materials, intermediates, by products and solvents. Generally in pharmaceutical synthesis, the purity of final intermediate is controlled by performing regulatory purity/impurity testing. Example, p-Chlorophenyl glutaric acid is a synthesis impurity of Baclofen.

Formulation Related Impurities: The excipients used to formulate drug products, can generate potential impurities. In addition during the process of formulation the drug is subjected to variety of conditions like heat, shear etc, that can contribute undesirable reactions and forms Degradation products. Hydrolysis or solvolysis generally takes place in solutions and suspensions that leads to degradation. Vortex mixing are sometimes used during process of formulation that is considered high risk operations which can cause impurity formation. Example, 1-(2, 6-Dichlorophenyl) Indoline is formulation impurity of Diclofenac.

Degradation Related Impurities: Number of impurities can be generated due to degradation of API and/or other interactions on storage. Therefore, it is important to conduct stability studies in order to predict, evaluate and ensure drug product self-life. Stability studies include evaluation of API stability, pre-formulation studies in order to evaluate compatibility of API with the excipients to evaluate its stability in the formulation matrix, accelerated stability testing of the drug product, kinetic studies and determination of expiration date, stability studies of drug products in market. The stability studies were conducted under various exaggerated conditions of humidity, light and temperature that helps to determine which type of impurities can be generated by degradation reactions. Example, Disulfonamide is a degradation Impurity of Hydrochlorothiazide.

Stereochemistry-related impurities: Stereochemistry related compounds are 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. Example, R- omeprazole is an impurity of esomeprazole.

Reagent, Ligands and Catalysts: These impurities are pretty rare. Proper care during the manufacturing process avoids the chance of these kinds of impurities.

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 (Ayre *et al.*, 2011).

Other Materials (Filter Aids, Charcoal): The filters or filtering aids are routinely used in the bulk drugs manufacturing plants and sometimes activated carbon is also used which acts as a source of impurity. For that reason regular monitoring of fibres and black particles are needed to avoid the contamination (Venkata Pavan Kumar G. Prameela Rani, Pooja and Anil Kumar, 2016).

Crystallization-Related Impurities: Polymorphism is the term used to denote crystal systems where a substance can exist in different crystal packing arrangements, all of which have the same elemental composition. It is also possible to have crystal systems where the substance exists in different crystal packing arrangements, each of which has a different elemental composition; this phenomenon is known as solvatomorphism. Based on the realization that the nature of the 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 by regulatory authorities to take a strong interest in polymorphism and solvatomorphism. The nature of the crystal structure of a given material can influence the following properties given in Table No.1. (Bari *et al.*, 2007)

Table 1: List of properties that influences the crystal structure of a impurities(Bari *et al.*, 2015).

Sr. No.	Name of Properties	Sr. No.	Name of Properties
1	Conductivity	11	Dissolution rate
2	Diffusivity	12	Density
3	Solubility	13	Latent heat of fusion
4	Hygroscopicity	14	Heat of solution
5	Crystal hardness	15	Rates of reactions
6	Crystal shape and colour	16	Phase diagrams
7	Volume	17	Refractive index
8	Crystal hardness	18	Heat capacity
9	Enthalpy of transitions	19	Melting or sublimation properties
10	Surface tension	20	Polymorphism

Factors affecting on formulation related impurities:

A. Environment related:

a. Exposed to adverse temperature: Substance which are labile to heat or in tropical temperature lead to degradation of active constitute and formation of impurity occurs. E.g. Vitamins are heat sensitive and its degradation lead to loss in potency.

b. Exposed to light: Photosensitive material when exposed to light / UV light undergo degradation which forms impurity.

c. Humidity: It can be detrimental to bulk powder and formulation containing solid dosage form.

B. Formation of impurities on ageing:

a. Mutual interaction: Interaction between ingredients involved in formulation lead to mutual interaction which causes impurity formation.

C. Functional Group Related Impurities

a. Ester hydrolysis: Drugs like aspirin, benzocaine, cefoxime, cocaine, ethyl paraben undergo ester hydrolysis.

b. Hydrolysis: Commonly drugs like benzyl penicillin, barbital, chloramphenicol undergo hydrolysis.

c. Oxidative degradation: Drugs like hydrocortisone, methotrexate, heterocyclic aromatic ring, nitroso/nitrile derivative.

d. Photolytic cleavage: Product exposed to light while manufacturing or storage in hospital pending use or by consumer pending use.

e. Decarboxylation: some dissolved carboxylic acid such as P-Amino salicylic acid Loose CO2 when it heated(Pawale, *et. al.*, 2012).

D. Packaging material Related Impurities: Impurities formed from packaging material like closures and containers. For most drugs, water, metals, peroxides derived from the packaging material can lead to formation or introduction of impurities. Various impurities such as polypropylene, zinc stearate (stabilizer in PVC), styrene, and diethylhexylpthalate (DEHP) etc. can leach in from rubber stopper and plastic material; whereas oxides like NO2, SiO2, CaO and MgO can be released from glassy surfaces (Singh. *et. al,.* 2017).

Isolation:

Isolation and characterization of impurity is depends upon the nature of the impurity that means structure of impurity, its physicochemical properties and availability. It is often necessary to isolate impurities because the instrumental methods that were mentioned earlier for directly characterizing impurities without isolating them are not available or when the authentic material is needed for further confirmation of the structure or its toxicity. Isolation and removal of the compound of interest from the other compounds present in a mixture. Further purification is achieved based on the compounds intended use. The first two sections, solid-phase extraction and liquid-liquid extraction deal with liquid samples. The sections on supercritical fluid extraction and accelerated solvent extraction focus mainly on solid samples while the centrifugation and filtration sections handle suspensions. Isolation methods include both chromatographic and non-chromatographic methods. Simple methods should be tried first, as they can lead to considerable savings in time and can produce a larger quantity of materials with greater ease (Singh *et al.*, 2017). For isolation of a given compound from a complex mixture, the chromatographic methods utilized for separation of impurities in analytical determinations are the methods of first choice that are suitably modified for the purpose of isolation of impurities where an appropriate fraction is collected. For isolation of impurity Following methods are commonly used for the isolation as below (figure no.2).

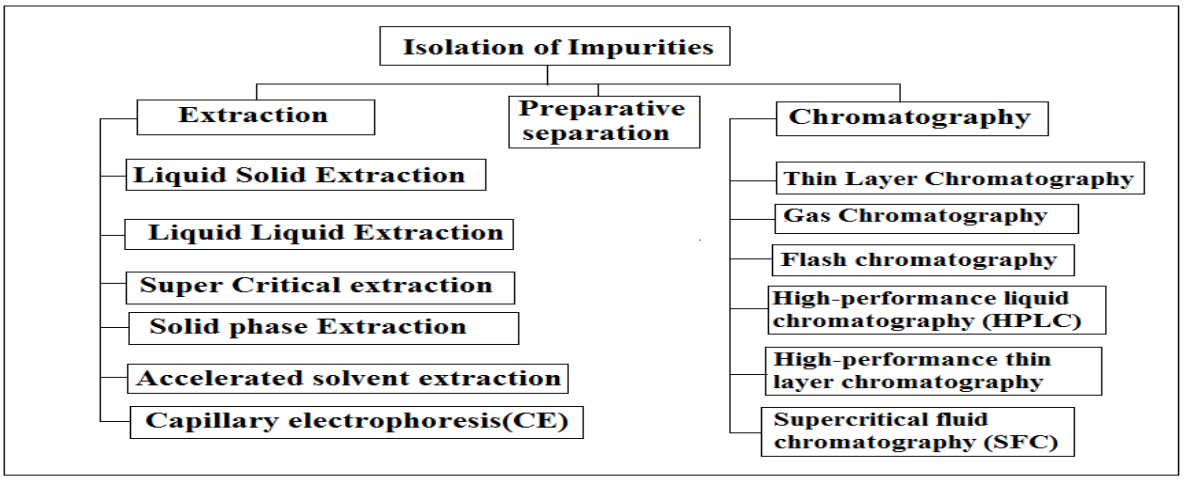

Figure 2: Isolation of impurities (Alsante *et al.*, 2001)

Solid-phase extraction methods: The conventional process of solid-phase extraction involves passing a liquid sample over a solid surface in order to separate target analytes from the sample matrix. The attractive interactions of the analyte with the solid surface must be greater than the attractive interactions of the analyte with the sample matrix in order for retention and extraction to occur. The technique is based on the same principles as chromatographic separations (partitioning, adsorption, or ion exchange).

Liquid-liquid extraction methods (LLE): This simply entails extraction of one liquid with another generally one of those liquid is aqueous and other is organic. The primary requirement is that these liquids to be immiscible. This procedure is very useful when the liquid into which the material of interest is being extracted is easy to volatilize, thus permitting concentration of the material. Hence the choice of solvents must be made with that consideration in mind. Liquid–liquid extraction is a very good sample clean up technique for nonpolar or moderately polar analytes that can be deionized in aqueous solution by simple pH adjustment. From a practical perspective, the best recoveries are obtained when an excess of organic solvent (3- to 10-fold excess) or multiple-step extractions are used. Multiple step extractions have recently fallen into disfavour for routine use because they are time-consuming and labour-intensive. In this type of extraction process, a solute is distributed between two immiscible solvents. The extraction is controlled by distribution or partition co-efficient which defines the ratio of concentration of the solute in two solvents a and b.

$$K_d = C_a / C_b$$

K_d is the distribution co-efficient or partition coefficient. The distribution co-efficient related to a single species and does not include possible products of side reactions.

Liquid-solid extraction

To simplest form, a solvent is selected that would dissolve the impurity of interest but not the solid matrix. If compound contains more than one impurity means, in that case desirable to use an organic solvent for extraction because of its unique properties. It is generally easier to volatilize the organic solvent at low temperatures in order to concentrate the impurity.

T**able 2:** List of solvents for Liquid-Solid extraction.

Solvents	Boiling Point	Dielectric constant
n-Hexane	190	1.9
Cyclohexane	81	2
Methanol	65	32.7
Dimethyl formamide	153	36.7
Acetonitrile	82	37.5
Water	100	80

Supercritical fluid extraction: It can be argued that the first supercritical fluid extractions (SFE) were performed in 1879 when Hannay and Hogarth investigated the solvating capabilities of ethanol. However, it took roughly 100 years ago supercritical fluids made any significant impact on industrial processes. The removal of caffeine from coffee beans was reported in the 1970s and led to a large increase in the number of published articles focusing on large-scale extractions using supercritical carbon dioxide. Supercritical fluid extraction (SFE), with Aid of carbon dioxide as the solvent, was tested for its ability to remove common reactive impurities from several pharmaceutical such as extraction of the small molecule impurities, formic acid and formaldehyde etc. The development of commercial supercritical fluid chromatographs and extraction systems escorted a slew of publications about analytical scale work in the 1980s (Deshpande *et al.*, 2012). In general, the use of supercritical fluids for extraction was touted as having several advantages over traditional extraction techniques such as LLE. These included lower solvent cost, better selectivity through pressure and temperature modification, and better solvating power because of fluid properties. A complete understanding of SFE and its relation to other extraction methods cannot be made without some knowledge of the basic properties of supercritical fluids and the basic principles of analytical SFE instrumentation. The purpose of this section is to give an introduction to the use of supercritical fluids in analytical-scale extractions while focusing on the application of SFE to pharmaceutical analysis (Ashraf-Khorassani *et al.*, 2005).

Table 3: List of solvents for SFE

Solvent	**Pressure(ATM)**	**Temperature**
n-Pentane	33.3	196.6
Carbondioxide	72.9	
Ammonia	111.3	132.3

Column Chromatography: This technique is commonly used for the separation of pharmaceutical compounds in preparative chemistry. The separation of quantities ranging from micrograms to kilograms, which depends on the size of the column. Detection of the eluent is generally performed by UV- spectrophotometry, either continuously by using a flow cell or periodically by monitoring the collected fractions from a given sample that alerts the emergence of UV- active components. Commonly silica gel or alumina is used in classic adsorption chromatography. Ion exchange resins to chemically modified polydextran gels used primarily for the analysis of biological samples. For liquid-liquid partition chromatography columns, inert carrier such as celite or kieselguhr is impregnated with an aqueous buffer or another polar solvent such as dimethyl formamide or dimethyl sulfoxide and elution is carried out with non-polar solvents.

Thin Layer Chromatography: It is a valuable technique for isolation and purification of compounds. All the modes of chromatography including adsorption, partition, ion exchange and gel filtration can be utilized. In addition choosing a sorbent and an eluent for performing TLC it is necessary to select a suitable method for applying a sample to the plate. Silica gel plates with or without fluorescent indicator are frequently used for most application. Detection is frequently performed by UV eg. 366nm or Iodine vapours can help to detect most of the organic substance. To elute the material from the plates, the simplest method is scraping the sorbent containing the material of interest and it is extracted with a suitable solvent, followed by filtration or centrifugation. The solvent is removed to collect the desired substance. If aluminium plates are used means cut the sample and eluted (Singh. *et. al,.* 2017).

Gas Chromatography: It is very useful for isolation and characterization of volatile components or those components that can be made volatile by derivatization technique and the detector used should be non-destructive. Now GC is more appropriate to be used in combination with mass spectrometry (GC/MS) for characterization of impurities (Singh. *et. al,.* 2017). [14]

Process Chemistry

Process Chemists are responsible for developing the synthetic route for development candidates. They are expected to collaborate with the other groups below to facilitate the identification and characterization of impurities or to refine their synthetic route design to eliminate or minimize the presence of these impurities. Critical factors affecting the quality of bulk drugs are;

1. Crystallization

2. Washing the wet cake

3. Drying

4. Appropriate packaging

1. Crystallization: The size of crystals sometimes determines the quality, especially the stability, of bulk drugs. Large-size crystals can entrap a minute amount of chemicals from the mother liquor, which ultimately causes the degradation of the drug. Thus, the manufacturers of bulk drugs should take care to produce finer crystals while isolating the products.

2. Washing the wet cake Washing the wet cake or powder in the centrifuge should be thorough to re-move unwanted chemicals including residual sol-vents.

3. Drying- Use of a vacuum dryer or a fluid-bed dryer is always preferable to a tray dryer in the pharmaceutical bulk drug industry. The high thermal efficiencies, reduction of drying time, and more uniform drying are helpful in drying sensitive drug substances. However, if a tray dryer is used then initial airflow at ambient conditions should be considered before exposing the materials to a relatively higher fixed temperature.

4. Appropriate packaging Light- sensitive pharmaceutical products need light protective packaging. An accelerated stability testing conducted using marketed ampoules of ergometrine wrapped with either black carbon paper or aluminium foil produced negligible degradation against direct sunlight. Similar use of opaque containers for ciprofloxacin eye drop preparation can protect the active ingredient from photo degradation to some extent compared with transparent containers (Saibaba *et.al.*, 2016).

Strategy for Method Development

A. Method development strategy should have the following details.

1. Physico chemical data
2. Ionization constant
3. Solubility
4. Water absorption
5. Distribution co-efficient
6. Optical rotation
7. Crystal form (Venkata *et.al.*, 2016)

B. Impurities can be analyzed by the following instruments:

1. Ultra Violet Spectroscopy
2. IR Spectroscopy
3. NMR Spectroscopy
4. Mass Spectrometry
5. Gas Chromatography
6. HPLC (Prafulla Kumar Sahu, 2015).

Need to Identify Impurities: Impurities are generally assumed to be inferior to API because they might not have the same level of pharmacologic activity. However, they are not necessarily always inferior. From the standpoint of its usage, the drug substance is compromised in terms of purity even if it contains another material with superior pharmacologic or toxicologic properties.

The control of low- level impurities is of great importance when a drug is taken in large quantities; for example, the use of methotrexate (10–20 g) to treat neoplasia. Penicillin and cephalosporin have been known to sustain facile cleavage of the β-lactam bond in aqueous solution. This is particularly interesting since some studies on penicillin have shown that their lack of stability may influence possible reactions involved in penicillin allergy. Special attention should be paid to the detection of DNA in all finished biotechnology products because DNA can be incorporated in the human genome and become a potential oncogene.

Analytical Research and Development: Pharmaceutical Development groups are ultimately responsible for the Chemistry, Manufacturing, and Controls sections of Investigational New Drug (IND) applications and New Drug Application (NDA) submissions. In general the various groups involved in this process from a synthetic chemistry and data collection and analysis. This group is basically responsible for the development of Drug Substance, Drug Product and specifications. This group will work combined with the process chemistry and structure characterization groups in concert to enable impurity profiling and chemical stability evaluation with degradant identification. The impurity profile method is developed by Analytical R&D and this method is used to help identify all impurities through each stage of the synthetic route and to help the characterization group decide which impurities require structure elucidation and characterization. In this group, a significant amount of time, technology, and knowledge is also invested in method development, tests of robustness, and validation (Vijayalakshmi R, 2012).

Method for impurity detection and characterization After the impurity isolation and identification, it must be characterised following technics are generally utilised as shown figure-3.

Highly sophisticated instrumentation, such as Ion Chromatography, Capillary Electrophoresis, HPLC, TLC and HPTLC, are inevitable tools in the detection of minor components (drugs, impurities, degradation products, metabolites) in various matrices.

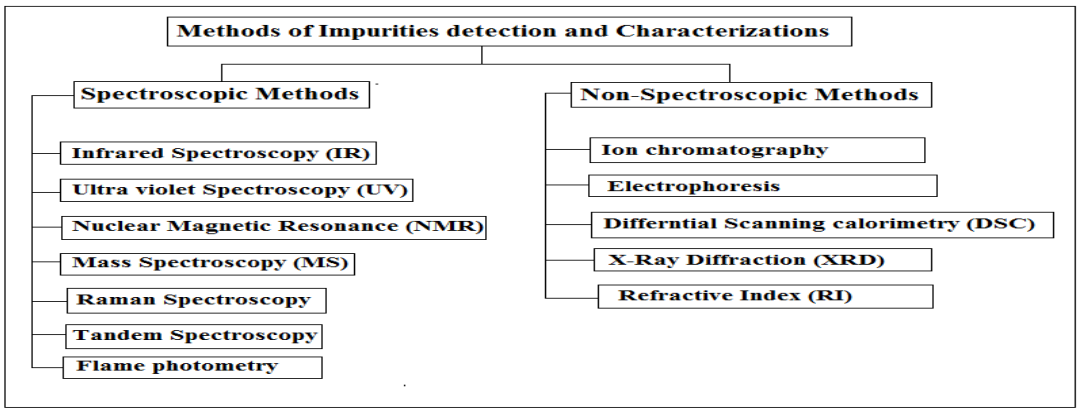

Figure 3: Methods of Impurities detection and Characterizations

1. Infrared Spectroscopy (IR): IR-Spectroscopy Provides the information in the form of wave numbers which is indicate the presence of particular functional groups. Routinely used identification assay for various intermediates and final drug substances and also as quantitative technique for solution phase study. The IR traditional technique are used for characterization including identification and structural elucidation of molecules, solid state characterization such as polymorphism (Devid E.B. *et. al.,* 2002).

2. Nuclear Magnetic Resonance (NMR): NMR provides information about specific bonding between peak area and number of nuclei responsible for peak. Most important application of NMR is identification and structure elucidation of molecules. Analysis of multicomponent mixture is possible using NMR technique. The ability of NMR- based diffusion coefficient determination to distinguish between monomeric and dimeric substances was validated using a standard mixture of authentic materials containing both monomers and dimers (Ingale *et al.*, 2011).

3. Mass Spectroscopy (MS): Mass spectroscopy is a most relevant and sophisticated 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. Now a days mass spectroscopy connected with various hyphenated techniques like GC-MS, LC-MS,LCMS-MS HPLC- DAD-MS, HPLCDAD-NMR-MS, Tandem Mass spectroscopy and capillary electrophoresis-Mass spectroscopy (Saibaba *et.al.*, 2016).

4. GC-MS: To identify different substances within a test sample gas chromatography-mass spectrometry (GC- MS) method used, that combines the features of gas- liquid chromatography and mass spectroscopy. In this method gas chromatography separate volatile and semi- volatile compounds with great resolution. Mass spectroscopy can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. Sample vaporized by injection into a heated system, eluted through a column by inert gaseous mobile phase and detected. The sample is transported through the column by the flow of an inert, gaseous mobile phase, the carrier gas. Flow is regulated by the pressure regulators and gas metering valves. GC operates at atmospheric pressure and the MS ion source at 10-5 Torr.108 fold pressure difference. The carrier gas must be removed and GC peak components transferred to the MS ion source (Kulkarni, 2018).

5. LC-MS: LC/MS is a hyphenated technique, combining the separation power of HPLC, with the detection power of mass spectrometry. LC/MS became really popular with the introduction of the thermospray interface and the particle beam interface. This is same as GC-MS but removal of liquid carrier from an HPLC eluent before samples are passed in to the MS source. For handle normal eluent flow rate 0.5-2.0ml min-1 which is not handled by MS pumping system moving belt inlet systems, jet separators and vacuum nebulizers are used (Saibaba, Kumar and Ramu, 2016). HPLC-DAD-MS (HPLC coupled with a diode array UV detector and a mass spectrometer, and such other techniques are almost routinely used. In GC-MS of methamphetamine and in LC-MS of risperidone, and cetrizine tablets a number of other chromatographic and spectroscopic configurations are found to be perfectly suitable for initial characterization of the impurities.

The single method most of time fails to provide the necessary selectivity then the coupling of chromatographic techniques such as HPLC-TLC and HPLC-CE, or coupling of chromatographic separations with information rich spectroscopic methods such as HPLC-MS or HPLC-NMR may need to be contemplated, but hopefully only as a development tool rather than a tool for routine QC use. Also the some Hyphenated techniques are crucially plays an important role in the segment of Impurity characterization. Within a short time these methods provides most relevant data to the chemist(Ingale *et al.*, 2011; Saibaba, Kumar and Ramu, 2016).

Hyphenated Methods:

LC-MS-MS: The popularity of LC-MS-MS systems for complex mixture analysis of thermally labile and biologically relevant molecules viz. mosapride, is largely attributed to the soft nature of atmospheric pressure chemical ionization (APCI), and atmospheric pressure ionization (APPI).

HPLC-DAD-MS: HPLC-DAD-MS (HPLC coupled with a diode array UV detector and a mass spectrometer, and such other techniques are almost routinely used.

HPLC-DAD-NMR-MS: NMR has now been added to this combination to provide HPLC-DAD-NMR-MS capabilities in a commercial instrument (Ingale *et al.*, 2011; Venkatesan and Valliappan, 2014).

Control of organic impurities: Control of the organic impurities in new drug substances is based on the maximum daily dose and total daily intake (TDI) of the impurities. Table provides the ICH threshold for control of the organic impurities in new drug substances and new drug products (figure 4).

Drug substance impurities thresholds	
Maximum Daily Dose [a]	**Reporting threshold** [b, c]
≤ 2g/day	0.05%
≥ 2g/day	0.03%
Maximum Daily Dose [a]	**Identification threshold** [b, c]
≤ 2g/day	0.1% or 1mg/ day intake, whichever is lower
≥ 2g/day	0.05%
Maximum daily dose [a]	**Qualification threshold** [b, c]
≤ 2g/day	0.15% or 1mg/ day intake, whichever is lower
≥ 2g/day	0.05%

Thresholds for degradation products in drug products	
Maximum Daily Dose [a]	**Reporting threshold** [d, e]
≤1 g	0.10%
>1 g	0.05%
Maximum Daily Dose [a]	**Identification threshold** [d, e]
<1 mg	1.0% or 5 µg TDI, whichever is lower
1 mg–10 mg	0.5% or 20 µg TDI, whichever is lower
>10 mg–2 g	0.2% or 2 mg TDI, whichever is lower
>2 g	0.10%
Maximum daily dose [a]	**Qualification threshold** [d, e]
<10 mg	1.0% or 50 µg TDI, whichever is lower
10 mg–100 mg	0.5% or 200 µg TDI, whichever is lower
>100 mg–2 g	0. 2% or 3 mg TDI, whichever is lower
>2 g	0.15%

a- The amount of drug substance administered per day.
b- Higher reporting threshold should be scientifically justified.
c- Lower threshold can be appropriate if the impurities are unusually toxic.
d- Thresholds for degradation products are expressed either as a percentage of the drug substance or as total daily intake (TDI) of the degradation product. Lower thresholds can be appropriate if the degradation product is unusually toxic.
e- Higher thresholds should be scientifically justified

Figure 4: Organic impurity threshold in new drug substances and drug products based on ICH.

Depending on whether the maximum daily dose is higher or lower than 2g, organic impurities in a new drug substance at (or greater than) 0.05% or 0.1% requires identification. Based on the maximum daily dose, the identification thresholds for organic impurities in new drug products are divided into four groups to give more consideration to low dose drug products. For most of the new drug products, the maximum daily dose is between 10mg–2g/day. Therefore,

any impurities at 0.2% or greater would have to be identified (Agency, 2006; ICH, 2006b, 2006a).

Implementation of quality by design approach

Quality by Design (QbD) is "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" and has the aim of improving product quality and of increasing regulatory flexibility. Impurity level is a critical quality attribute for a drug substance or a drug product because levels higher than the toxicologically qualified amount could affect the safety and efficacy of the product (Savkare A. D, 2016).

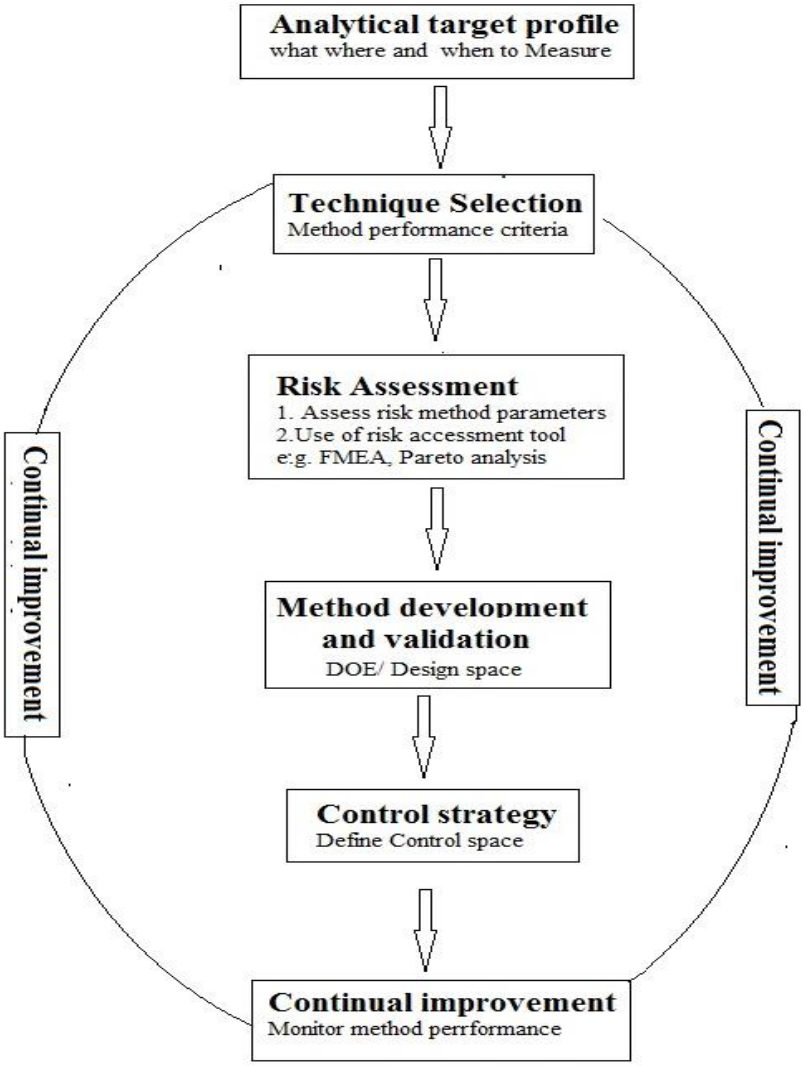

Figure 5: Schematic representation of Strategies for applying QbD for Impurity profiling (Palve R. *et. al*., 2018)

Applications: (Venkata *et.al*., 2016).

Drug	Impurity	Method
Amphotericin B	Tetraenes	HPLC with UV
Atopine sulphate	Apo atopine	HPLC with UV
Cloxacilin	N,N, dimethylaniline	HPLC with GC
Doxirubicine hydrochloride	Acetone &Ethanol	HPLC with GC
Dextrose	5-hydroxy methyl fulfural	HPLC with UV
Ethambytal Hydrochloride	2 amino butanol	TLC
Flurescene sodium	Dimethyl formaamide	HPLC with GC
Farmyctin Sulphate	Neamine	TLC
Marcptopurine	Hypoxinthine	HPLC with UV
Metformin	Cynoguanidine	HPLC with UV
Topiramate	Topiramate RC-A	HPLC with RI
Topiramate	Sulpha mate	HPLC with IC
Artemether	Artemisinin	TLC
Hyoscine butyl bromide	Impurity G	HPTLC
Lansoprazole	Sulfide Impurity	HPLC With (UV)

Conclusion

A high quality of drug substances and products helps to provide a consumer protection, hence the manufacturing companies focussed on isolation, separation, identification and characterization of impurities during the developmental stages. Identification of impurities establishes an overall profile of a drug and drug product which includes its toxicity and safety limits, limits of quantization and detection. Identification and isolation of impurities should start right from using API till the finished dosage form of a drug. There should be well established and standard specifications in the guidelines for overall control and isolation of impurities which should serve as a guiding specification to all the regulatory agencies pharmaceutical drugs substances and drugs products manufacturers. Validation of methods to identify and characterize impurities is a good approach and is currently being in practice for evaluation of impurities. The isolation, identification of impurities and impurity profiling is carried out using various techniques such as chromatographic separation techniques generally used in combination with sophisticated instruments like HPLC, UPLC, GC, and SFC. Also some hyphenated techniques like LC-MS-MS, HPLC-DAD-MS, and HPLC-DAD-NMR-MS. These are very helpful and advantageous techniques for characterization of impurity profiling. To providing documentary evidence the quality by design approach is very effective and promising tool for reducing the trials. QbD provides or generate statistical data which is help full for further reference purpose.

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