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Impurity Profiling Of Pharmaceuticals

Ami B Bhoi[1*], Prof.Mitali Dalwadi[2], Dr.U.M Upadhyay[3].
1)7th Semester B.Pharm, Sigma Institute of Pharmacy, Bakrol, Ajwa, Vadodara- 390019 (Gujarat, India).
2)Professor, Sigma Institute of Pharmacy, Bakrol, Ajwa, Vadodara- 390019 (Gujarat, India).
3)Principal, Sigma Institute of Pharmacy, Bakrol, Ajwa, Vadodara- 390019 (Gujarat, India).

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ABSTRACT: In Active pharmaceutical Ingredient's (API's) many regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity necessities and the identification of impurities. Qualification of the impurities is the technique of obtaining and evaluating statistics that establishes biological safety of an individual impurity; thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research.

Identification of impurities is executed via range of Chromatographic and Spectroscopic techniques, both alone or in combination with different techniques. There are different methods for detecting and characterizing impurities with TLC, HPLC, HPTLC, SFC etc. Conventional Liquid Chromatography, particularly, HPLC has been exploited broadly in field of impurity profiling; the wide range of detectors, and stationary phases along with its sensitivity and cost-effective separation have attributed to its assorted applications. Among the various Planar Chromatographic Methods; TLC is the most commonly used separation technique, for isolation of impurities; due to its ease of operation and low cost in compared to HPLC. An advancement of thin layer chromatography HPTLC, is a every day approach for the impurity isolation.

Headspace GC is one of the most preferred techniques for identification of residual solvents. The creation of hyphenated strategies has revolutionized impurity profiling, by not only separation but structural identification of impurities as well. Among all hyphenated techniques, the most exploited techniques, for impurity profiling of drugs are LC-MS-MS, LC-NMR, LC-NMR-MS, GC-MS and LC-MS.

Key words: Impurity, Impurity profiling, Chromatography, Spectroscopy, Spectrometry, Validation.

I. AIM AND OBJECTIVES:

According to the definition of ICH (International Conference on Harmonisation) impurity profile of a drug material is "A description of the identified and unidentified impurities, present in a new drug substance". Impurity profiling is viewed to be the common name of analytical activities, the goal of which is the detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations. The importance of drug impurity profiling is that it affords records which can immediately make contributions to the safety of drug remedy by way of minimizing the impurity-related detrimental outcomes of drug materials and the preparations made thereof. In latest years the value of assay techniques for characterising the quality of bulk drug components has decreased considerably. At the equal time the value of impurity profiling is always increasing. This can be illustrated by the fact that no book was devoted to this topic until 1999, but since then no less than four books have been published dealing solely with impurity profiling of drugs.

Impurity profiling is of utmost importance in all phases of synthetic drug research and manufacturing from the gramscale preparation of new compounds for pharmacological screening up to the scaling up procedure and finally the production of bulk drugs. The latter aspect should be emphasised, considering the reality that even minor changes in the manufacturing technology, source of starting materials, conditions of purification and storage can greatly influence the impurity profile. Its importance in the research and production of pharmaceutical formulations is additionally immense. The pharmaceutical technologist must have a clear picture of the impurity profile of the bulk-drug material used for the development of formulations in order to be in a position to differentiate between synthesis-related

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 477-491 www.ijprajournal.com ISSN: 2249-7781

impurities and degradation products. In this way, it is possible to develop a stability-indicating analytical technique essential in the course of the development of a drug formulation.

II. INTRODUCTION

The bulk drug enterprise kinds base of all pharmaceutical industries as it is the source of Active Pharmaceutical Ingredients (APIs) of specific quality. Over the closing few decades much attention is paid towards the quality of pharmaceuticals that enter the market. The important mission for each bulk drug industries and pharmaceutical industries is to produce quality products. It is necessary to conduct vigorous quality manipulate tests in order to keep the quality and purity of output from each industry. Purity of active pharmaceutical ingredient depends on countless factors such as raw materials, their approach of manufacture and the kind of crystallization and purification process[1].

The impurity profile is a description of recognized and represent impurities in pharmaceutical dosage forms. The impurity can also be developed either throughout formulation or in the final product upon getting older or contact with packaging of the a number of impurities that can be determined in drug product[3]. API's to medicines. The presence of these undesirable chemical compounds even in small amounts may also affect the efficacy and safety of the pharmaceutical products. Impurity profiling, is now getting receiving vital integral attention from regulatory authorities. Various pharmacopoeias, such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly incorporating limits to allowable levels of impurities present in the API's or formulations[2].

A variety of recent articles [4-6] have described a designed approach and guidance for separating and identifying process-related impurities and degradation products using Mass Spectrometry (MS), Nuclear Magnetic Resonance (NMR), High Performance Liquid Chromatography (HPLC), Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS), and tandem mass spectrometry for pharmaceutical substances[2].

- The main reasons for the increasing interest of drug manufacturers and registration authorities in the impurity profiles of bulk drug substances are as follows[1] :

a) In the route of the improvement of a new drug or a new technology for manufacturing an existing drug it is essential to be aware of the structure of the impurities: by way of capacity of possessing the information synthetic organic chemists are often able to alternate the reaction conditions in such a way that the formation of the impurity can be avoided or its quantity decreased to an acceptable level.

b) Having recommended structures for the impurities, they can be synthesized and thus provide final evidence for their structures previously determined by spectroscopic methods.

c) The material synthesized can be used as an 'impurity standard' at some stage in development of a selective technique for the quantitative determination of the impurity and the use of this technique as part of the quality control testing of each batch.

d) In case of major impurities the synthesized or isolated material can be subjected to toxicological studies thus significantly contributing to the safety of drug therapy.

e) For drug authorities the impurity profile of a drug substance is a good fingerprint to point out the level and constancy of the manufacturing process of the bulk drug substance.

➢ **Classification of impurity[11]:**

Impurities can be classified into the following categories:

1. Organic impurities (process- and drug-related)
2. Inorganic impurities
3. Residual solvents.

1) Organic impurities can show up all through the manufacturing procedure and/or storage of the new drug substance. They can be identified or unidentified, volatile or non-volatile, and include:

- Starting materials
- By-products
- Intermediates
- Degradation products
- Reagents,
- from the manufacturing process. They are normally known and identified and include: Ligands and catalysts

2) Inorganic impurities can result

- Reagents, ligands and catalysts
- Heavy metals or other residual metals
- Inorganic salts
- Other materials (e.g., filter aids, charcoal)

Solvents are inorganic or organic liquids used as vehicles for the preparation of solutions or suspensions in the synthesis of a new drug substance. Since these are commonly of

International Journal of Pharmaceutical Research and Applications
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acknowledged toxicity, the selection of appropriate controls is without problems completed (see ICH Guideline Q3C on Residual Solvents).

Excluded from this document are: (1) extraneous contaminants that should not occur in new drug substances and are greater correctly addressed as Good Manufacturing Practice (GMP) issues, (2) polymorphic forms, and (3) enantiomaric impurities.

3) Residual solvents[14]:

Residual solvents are organic or inorganic liquids used at some point of the manufacturing process. It is very difficult to remove these solvents totally via the work up procedure. Some solvents that are recognised to reason toxicity should be avoided in the manufacturing of bulk drugs. Depending upon the viable danger to human health, residual solvents are divided in three classes.

• Class I: Solvents like benzene (2 ppm limit) and carbon tetrachloride (4 ppm limit) need to be averted.

• Class II: Methylene chloride (600 ppm limit), methanol (3000 ppm limit), pyridine (200 ppm limit), toluene (890 ppm limit) and acetonitrile (410 ppm limit) are the most commonly used solvents.

• Class III: Acetic acid, acetone, isopropyl alcohol, butanol, ethanol and ethyl acetate have permitted day by day exposures of 50 mg or much less per day.

Figure 1:-Classification of Impurity[12]. Fig-[3]

Figure 2:- Classification of Organic impurity[12].

Figure 3:-Classification of Inorganic impurity[12]

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 477-491 www.ijprajournal.com ISSN: 2249-7781

- **Regulatory guidelines on impurities in an active pharmaceutical ingredients[8-10].**

i. ICH guidelines "Stability Testing of New Drug Substances and Products"- Q1A.
ii. ICH guidelines "Impurities in New Drug Substances"- Q3A.
iii. ICH guidelines "Impurities in New Drug Products"- Q3B.
iv. ICH guidelines "Impurities: Guidelines for Residual Solvents"- Q3C.
v. USFDA guidelines "NDAs- Impurities in New Drug Substances".
vi. USFDA guidelines "ANDAs- Impurities in New Drug Substances."
vii. Australian Regulatory Guideline for Prescription of Medicines, Therapeutic Governance Authority (TGA), Australia.

Table 1: International guidelines outlining the regulatory requirements for the control of impurities in drug substances and drug product[15].

Regulatory agency	Guidelines	Issue date
ICH (USA, Europe and Japan)	Q3A (R2) Impurities in new drug substances	25th October 2006
	Q3B (R2) Impurities in new drug products	2nd June 2006
	Q3C (R6) Impurities guideline for residual solvents	20th October 2016
	Q3D Guideline for elemental impurities	16th December 2014
	M7(R1) Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk	31st March 2017
EMA (Europe)	EMA/CHMP/CVMP/QWP/450653/2006 Assessment of the quality of medicinal products containing existing/known active substances	9th February 2009
	CPMP/QWP/1529/04 Control of impurities of pharmaceutical substances	22nd April 2004
	CPMP/SWP5199/02 and EMEA/CHMP/ QWP/251344/2006 Guidelines on the limit of genotoxic impurities	28th June 2006
	EMEA/CHMP/SWP/4446/2000 Guideline on the specification limits for residues of metal catalyst or metal reagent	21st February 2008
	EMA/CHMP/CVMP/QWP/199250/2009 Guideline on setting specification for related impurities in antibiotics	30th June 2013
US-FDA	NDAs: Impurities in new drug substances	February 2000
	ANDAs: Impurities in new drug substances	June 2009
	ANDAs: Impurities in new drug products	November 2010
	Elemental impurities in drug products (draft)	June2016
	Genotoxic and carcinogenic impurities in drug substances and products: Recommended approach (draft)	December 2008
Health Canada	Impurities in existing drug substances and products	6th September 2005
TGA (Australia)	Guidance 18: Impurities in drug substances and drug products	9th August 2013

Table 2 : Reporting, identification and qualification thresholds for the impurities in pharmaceuticals[12].

Maximum Daily dose	Reporting threshold	Identification threshold	Qualification threshold
≤2 g/d	0.05%	0.10% or 1.0 mg/d intake (whichever is maximum)	0.15% or 1.0 mg/day intake (whichever is maximum)
>2 g/d	0.03%	0.05%	0.05%

International Journal of Pharmaceutical Research and Applications
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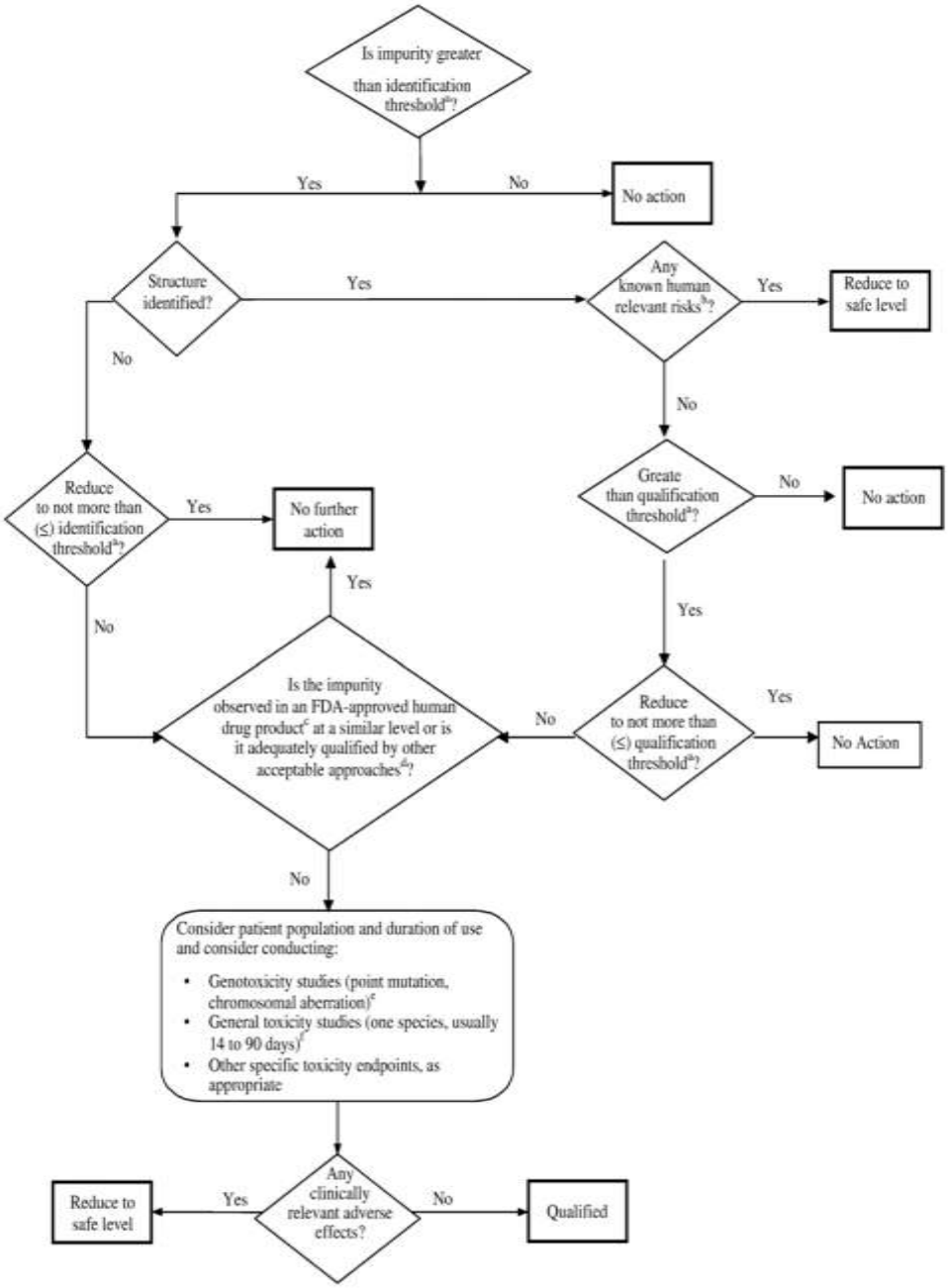

Figure 4: Identification and qualification of impurities in drug substance and drug product[13].

III. FORMULATION ASPECT & METHOD OF PREPARATION

➢ **Selective Analytical Methodologies[13]**

- New drug development requires significant and reliable analytical statistics to be produced at wide variety stages of the development.

a) Sample set selection for analytical method development.

b) Screening of chromatographic conditions and phases, commonly the use of the linear solvent strength model of gradient elution.

c) Optimization of the approach to fine tune parameters related to ruggedness and robustness.

- The impurities can be identified predominantly through following methods;

I. Reference standard method
II. Spectroscopic method

International Journal of Pharmaceutical Research and Applications
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III. Separation method
IV. Isolation method
V. Characterization method

1. Reference standard method :

The key goal of this is to supply clarity to the overall life cycle qualification and governance of reference standard used in development and manipulate of new drug. Reference standards serve as the basis of evaluation of each method and product performance and are the benchmarks for evaluation of drug safety for patient consumption. These standard are needed, not only for the active ingredients in dosage forms but also for impurities, degradation products, starting materials, process intermediates, and excipient.

2. Spectroscopic methods :

The following spectroscopic methods can be used;
A. Ultraviolet (UV)
B. Infrared (IR)
C. Nuclear magnetic resonance (NMR)
D. Mass spectrometry (MS)

3. Separation methods:

The following separation methods can be used;
a) Thin-layer chromatography (TLC)
b) Gas chromatography (GC)
c) High-pressure liquid chromatography (HPLC)
d) Capillary electrophoresis (CE)
e) Supercritical fluid chromatography (SFC)

4. Isolation method:

It is frequently essential to isolate impurities. But if the instrumental techniques are used isolation of impurities is averted as it directly characterizes the impurities. Generally chromatographic and non-chromatographic technique are used for isolation of impurities prior its characterization.

A list of methods that can be used for isolation of impurities is given below.

- Solid-phase extraction method
- Liquid-liquid extraction method
- Accelerated solvent extraction method
- Supercritical fluid extraction
- Column chromatography
- Flash chromatography
- TLC
- GC
- HPLC
- HPTLC
- Capillary electrophoresis(CE)
- Supercritical fluid chromatography (SFC)

5. Hyphenated methods/ characterized method:

The following hyphenated methods can be used effectively to monitor impurities.

- GC–MS
- LC–MS
- LC–DAD–MS
- LC–NMR
- LC-MS-MS
- HPLC-DAD-MS
- HPLC-DAD-NMR-MS

A. Ultraviolet (UV)[28]:

Response of analyte depends on the chromophore present in the molecule. This spectral technique helpful to find out the absorption maxima of the particular compound, impurity or degradant and can serve as an identification test. When main drug and its impurity or degradant absorb considerably at the same wavelength which is same or close to parent drug in such cases it is very tough to distinguish the drug and impurity only on the basis of absorption maxima. Low selectivity evaluation is the major disadvantage of UV spectroscopy.

B. Infrared spectrophotometry (IR)[12]:

Infrared spectrophotometry provides precise information on some functional groups that might also allow quantification and selectivity. However, low level delectability is often a problem that may also require extra involved approaches to circumvent the problem.

C. Nuclear magnetic resonance spectroscopy (NMR)[28]:

NMR is greater integral spectroscopic technique than IR. Mostly hydrogen and carbon nuclei are studied with the help of NMR. It gives information about variety of magnetically distinct atoms of the type being studied. Combination of IR and NMR data is frequently adequate to determine the complete structure of a molecule.

NMR is a effective and theoretically complicated analytical tool that permits the find out about of compounds in both solution or in the solid state and serves equally in quantitative as in structural analysis.

The capacity of NMR to provide the data regarding the particular bonding structure and stereochemistry within a molecule has created huge applicability.

Recent technological trends in the field of magnetic resonance have allowed significant

International Journal of Pharmaceutical Research and Applications
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improvement in sensitivity levels. This turns into mainly vital in the structural characterization of drug impurities and degradants, which are often present only in extremely limited quantities.

The non-destructive, non-invasive nature of NMR spectroscopy makes it a valuable tool for the characterization of low-levels impurities and degradants. In addition, NMR can be considered close to a "universal detector".

In NMR, quantitation is accurate over a dynamic range of nominally 4 orders of magnitude, even though it is not as particular as that of other analytical equipment mainly at low ranges however it has important place in impurity profiling.

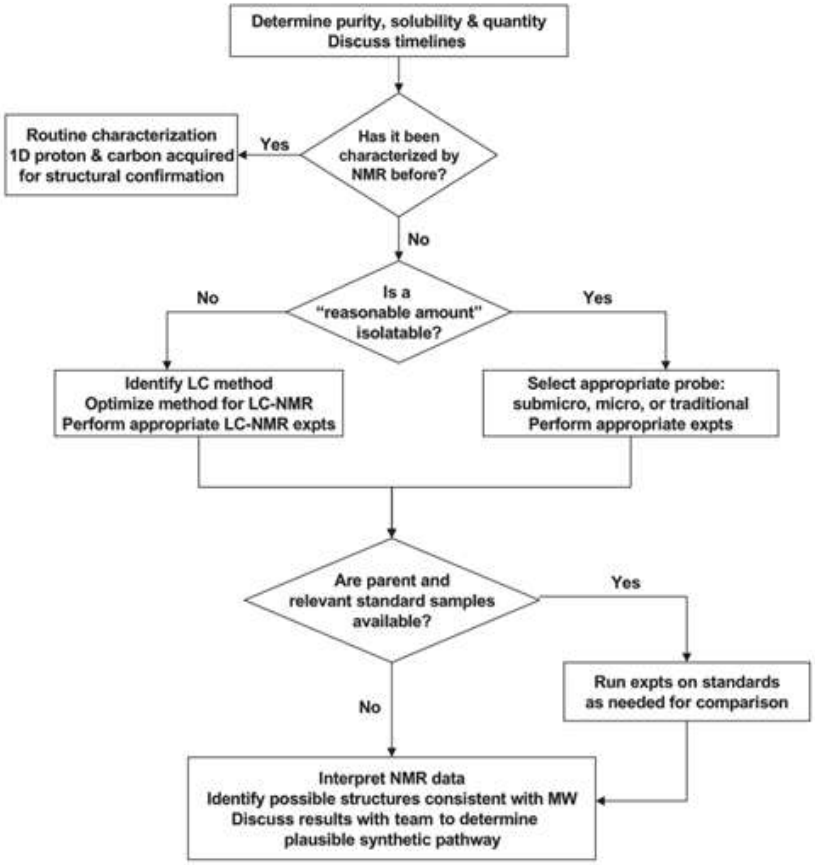

Figure 5: Flow chart for structural characterization of impurities and degradants by NMR. 1D = One dimentional ; MW = Molecular weight[29]:

D. Mass spectrometry (MS)[28] :

MS is an analytical method of characterizing matter, based on the determination of atomic or molecular masses of individual species present in a sample. This method destroys the compound, however owing to the great sensitivity of the technique, solely a tiny quantity is required.

MS is a effective analytical technique due to the fact it can provide precious structural information with a high degree of specificity. MS can be used to confirm, identify or to characterize impurity. MS is extensively used technique to obtain most-justifiable data. MS, alone and in combinations with other analytical instruments, is beneficial to elucidate unknown structure of impurities even in trace level concentrations. It is structurally sensitive analytical technique, gives the statistics of molecular mass and probable fragmentation pattern of the specific compound of interest. It is not a universal detector; otherwise it is quantitatively sensitive technique. The distinctive mass spectrum or fragmentation pattern for each molecule makes it a definitive and effective device for identifying unknown impurities or degradation products. The structure that is received from mass spectral studies is often not complete. Depending on the resolution of the instrument it is possible to differentiate molecules with small differences in the molecular weights even though the mass spectroscopy is effective method for structure

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 477-491 www.ijprajournal.com ISSN: 2249-7781

elucidation, MS should be constantly used in combination with other spectroscopic or separation techniques for confirmation of structure of an impurity.

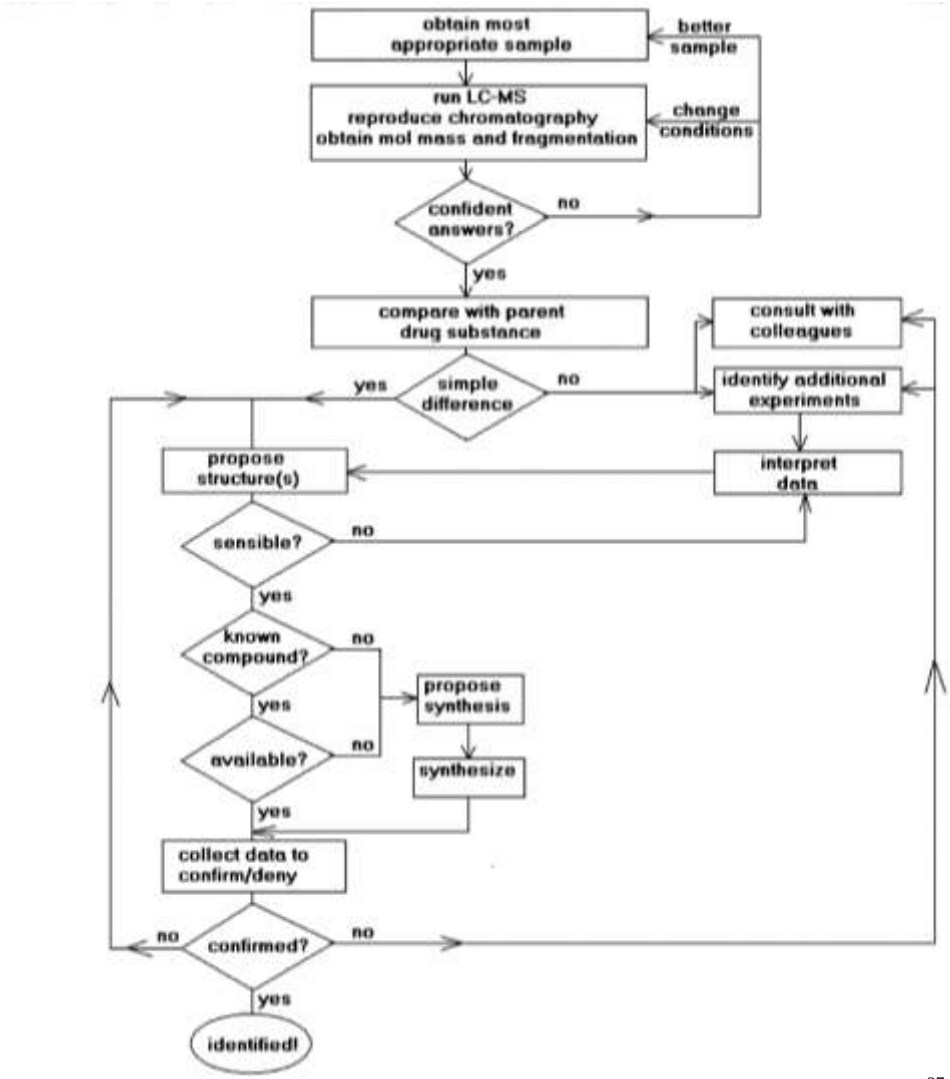

Figure6: Mass spectrometry process flow chart for identification of impurities[27]:

E. Thin-layer chromatography (TLC)[3]:

Thin-Layer Chromatography is a easy and less expensive approach that is often used to separate mixtures and decide the purity of a synthesized compound or to point out the extent of progress of a chemical reaction. In this technique, a small extent of a solution of the combination 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 positioned in a chamber containing a small quantity of solvent, which is the mobile phase. The solvent progressively moves up the plate via capillary action, and it carries the deposited materials 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 with the aid of the solvent. The components then show up as a series of spots at different areas up the plate. Substances can be identified from their Rf values with reference. A number of enhancements can be made to the original method, to automatic the one of a kind steps, to increase the resolution done with TLC, and is referred to as HPTLC or high performance TLC.

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 477-491 www.ijprajournal.com ISSN: 2249-7781

F. Gas chromatography (GC)[3] :

Gas chromatography is an analytical technique for separating compounds based primarily on their volatilities. Gas chromatography offers both qualitative and quantitative information for individual compounds present in a sample. Compounds go through a GC column as gases with their linear velocity and flow rates, because the compounds are commonly 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.

G. High-pressure liquid chromatography (HPLC)[28] :

It is also called as liquid chromatography (LC). HPLC is the best ever analytical technique for impurity profiling of drugs. Its simplicity, high velocity and broad range of sensitivity makes it perfect for analysis of many drugs in each different dosage forms and various biological mediums. HPLC differ from other types of liquid chromatography with regards to packing material of small, uniform particles .The small size of particles supply excessive column efficiencies that also results in high pressure drop across the columns and therefore desired flow rates are achieved by applying use of greater pressure. HPLC affords a number of benefits during analysis, such as excessive resolving power, speed of separation, reproducible effects and automation of analytical procedure and data handling. Higher resolution lets in ideal separation of impurities from the drug substance and a range of detection modes approves correct measurement of impurities. With current tendencies in the detection systems of HPLC newer strategies can be utilized depending on requirement such as, Evaporating Light Scattering Detection (ELSD) and Refractive Index (RI) widely used for compounds with poor or no chromophore.

H. Capillary electrophoresis (CE)[28]:

CE is now becoming a routine analytical approach for the evaluation of pharmaceuticals, as it offers matching data to HPLC, simplicity, ease of operation, low wavelength detection, speed and high efficiency with low consumption of chemicals. CE is turning into a remarkable complementary and alternative approach to LC.

The separation of charged compounds by means of capillary zone electrophoresis (CZE) is primarily based on variations in electrophoretic mobility. The determination of drug related impurities is at present the most vital undertaking of CE within pharmaceutical analysis. An advantage of CE over its different chromatographic techniques is that high separation efficiencies are attainable. The resulting peak sharpness regularly renders a small degree of selectivity to adequate resolution. CE can be employed when HPLC is not capable to measure impurities adequately. A detection limit of 0.1% is broadly accepted as a minimum requirement for a associated impurities determination method which can be achieved by this CE.

CE is essentially beneficial for the separation of closely related compounds such as diastereomers and enantiomers of compounds. In order to separate neutral compounds through CE, a charged surfactant must be added to the CE separation buffer so that the overall mobility of a compound is both a function of charge and phase partitioning between micelles and the aqueous solvent. This type of CE is called Micellar Electrokinetic Chromatography (MEKC). MEKC is more suitable approach for impurity profiling than CZE because the nature and physical properties of unknown impurities are partially known so separation of neutral and charged molecules is possible simultaneously.

I. Supercritical fluid chromatography (SFC)[30] :

SFC is a technique in which mobile phase is supercritical fluid (supercritical fluid is formed whenever a substance is heated above its critical temperature). SFC is a hybrid of gas and liquid chromatography that combines some of excellent aspects of each. Supercritical fluid chromatography is primarily based on the principle of density of supercritical fluid which corresponds to solvating power. As pressure in the system is increased, the supercritical fluid density increases and correspondingly its solvating power increases. Thus as the components retained in the column get eluted. Supercritical fluid chromatography (SFC) has developed rapidly in latest years, particularly in the area of enantio separations.

J. High performance thin layer chromatography (HPTLC)[31] :

HPTLC is additionally primarily based on the principle of separation which relies upon on the relative affinity of compounds towards stationary

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 477-491 www.ijprajournal.com ISSN: 2249-7781

and mobile phase. The compound under the have an impact on of mobile phase (driven by using capillary action) travels over the surface of stationary phase. During this movement the compounds with greater affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved.

K. Column chromatography[31]:

Here the stationary phase is a solid adsorbent which is placed in a vertical glass column and the mobile phase used is a liquid which is delivered to the top and flows down via the column. This is generally used as a purification technique. The mixture to be analyzed is applied to the top of the column. As 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.

L. Flash chromatography[30]:

In column chromatography, if the solvent is forced down the column through positive air pressure, it is known as flash chromatography. Flash chromatography is basically an air pressure driven hybrid of medium pressure and shorter column chromatography which has been optimized for particularly fast separation. Flash chromatography is a method used to separate mixtures of molecules into their individual constituents, often used in the drug discovery process. Flash chromatography utilizes 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 anfragmentation data, respectively, offer rich structural information on candidate structures. Liquid chromatography isocratic or gradient solvent which, with the help of pressure, allows the sample to run through the column and turn out to be separated.

M. LC-MS[30] :

Mass spectrometry coupled with modern high performance liquid chromatography (HPLC) allows trace elements in complex mixtures to be studied directly with no prior preparative purification or fractionation to enrich the impurities. LC-MS has become the fundamental method for the identification of low-level impurities in samples ensuring from synthesis or from degradation of APIs. Full scan and product ion scan analysis, presenting molecular weight information and -Mass spectroscopy (LC-MS) is an analytical technique that couples high resolution chromatographic separation with sensitive and spectroscopic detection.

N. GC-MS[30] :

Gas chromatography – mass spectrometry (GC -MS) is a technique that combines the features of gas - liquid chromatography and mass spectrometry, to become aware of exceptional components inside a test sample. 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 fast so that quite a few MS can be obtained at some point of the elution of a single peak from the GC unit. For example GC-MS technique is used in impurity profiling of synthetic pesticide d-allethrin.

O. HPLC-DAD-MS[30] :

In this technique HPLC coupled with a diode array UV detector and a mass spectrometer, used in characterization of impurities in pharmaceuticals. For instance evaluation of doxycycline and its related impurities like metacycline and 6-epidoxycycline. HPLC-DAD is used for the detection of a wide polarity range of compounds present in water. With this technique, the spectra of all eluting (UV absorbing) organic compounds are acquired. In this study, the combination of both HPLC-DAD and HPLC-Q-TOF MS techniques was used for the detection and identification of an unknown micro-contaminant in water samples.

P. LC-NMR[30]:

LC-NMR is a progressive technique that connects NMR with HPLC online and can provide not only 1-D but also 2-D NMR spectra for the components separated by HPLC. LC-NMR has come into wide use because of improved sensitivity due to higher magnetic fields of superconductive magnet and superior techniques, specially the solvent suppression method. For example, LC-NMR has been applied for the analysis of medicinal metabolites, impurities in medicinal specialties, and metabolites of natural products.

International Journal of Pharmaceutical Research and Applications
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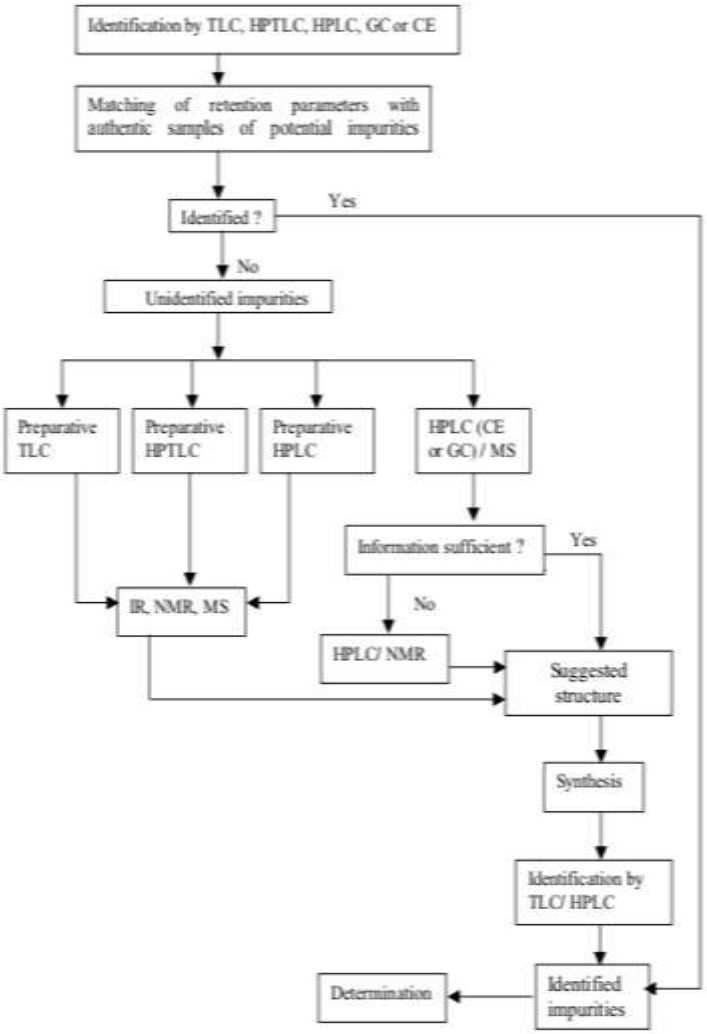

Figure7: Proposed chart for profiling drug impurity[26]:

IV. EVALUATION PARAMETER

➢ **Validation of impurity profiling method[31] :**

The real reason of the validation system is to challenge the method and decide limits of allowed variability for the conditions wanted to run the method. According to USP, 'Validation is the process of presenting documented proof that the method does what it is meant to do'. A properly documented validation plan for checking out the method and acceptance criteria is essential. During impurity profiling, the developed method needs to be validated to meet with the specifications. The parameters of validation include specificity, accuracy, precision, limit of detection, limit of quantitation, linearity, range, robustness and system suitability test.

➢ **Validation of analysis methods during drug development[32]:**

Before new pharmaceutical drug can be marketed, a lengthy three-stage procedure is involved, commonly referred to as Phase I, Phase II, and Phase III. As would be expected, the analytical method validation development also consists of three phases. Phase I consists of preparation of calibration functions for testing the linearity, variance homogeneity, and evaluations of the lower limit of the calibration range. Selectivity is established using stressed samples, and testing for the have an impact on of matrix on the accuracy is performed.

A system precision is performed with six injections. The validation process begun in Phase I is extended during Phase II. In this phase, selectivity is investigated the use of a number of

International Journal of Pharmaceutical Research and Applications
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batches of drugs, accessible impurities, excipients, and samples from stability studies. Accuracy need to be determined the use of at least three levels of concentration, and the intermediate precision and the quantitation limit should be tested. For quality assurance evaluation of the analysis results, control charts can be used, such as the Shewart-charts, the R-charts, or the Cusumcharts. In this phase, the analytical method is refined for routine use. In Phase III, the final dosage formulation has been established and the pivotal clinical trials are being conducted.

Degradation products have been identified, so the method selectivity should be re-evaluated to ensure that all degradants can be detected and quantitated. The analytical methods are totally validated, and appropriate for routine quality assurance and control purposes. The type and frequency of system suitable testing (SST) should be determined, and an excellent publication on SST for chromatography systems is available. If the new operating range was used outside of the existing operating range (changes in column temperature, sample matrix, column or instrument type, etc.), the method should be revalidated. When all of this work is complete, all through the marketing phase, an inter laboratory testing is recommended for standardization of the method. In addition, for reporting a routine analytical result, the result have to consist of its confidence interval 'CI', or with same meaning term, uncertainty 'U' or standard certainty 'STC'. The result have to be mentioned as:

Result$\pm \frac{t \times SD}{\sqrt{N}}$ (CT or U or STC)

In his book, Kromidas described, the value of ''t'' can be replaced with ''k'' (constant),forp=0.05that k=2,orfor p=0.01thatk=3. Kaiser recommended using N=4, so in this case the STC is about three times of the SD (for p=0.01, and F=N-1). In this case, if SD is about 1%, the reported result is (result$\pm$ 3)%.

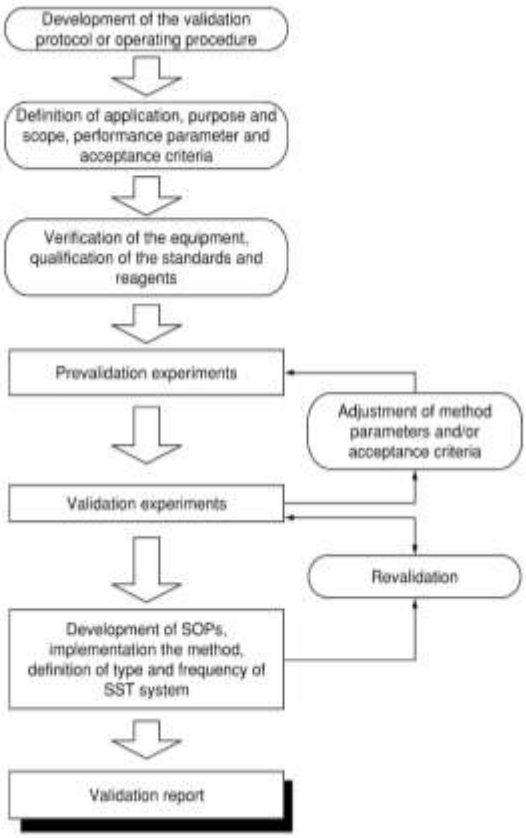

Figure 8: Steps taken during the validation of an analysis method[24] :

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 477-491 www.ijprajournal.com ISSN: 2249-7781

V. SUMMARY, CONCLUSION& FUTURE SCOPE

➢ **Summary :**

Table 3 : Various impurities reported in API's

Sr. No	Drug	Impurity	Method	Reference
1	Amphotericin B	Tetraenes	UV spectroscopy	34
2	Atropine sulphate	Apo atropine	UV spectroscopy	34
3	Cloxacillin	N, N –dimethyl	Gas chromatography	12
4	Dextrose	5- hydroxy methyl furfural	UV spectroscopy	12
5	Doxorubicin hydrochloride	Acetone and Ethanol	GC	34
6	Ethambutol hydrochloride	2 amino butanol	TLC	34
7	Fluorescene sodium	Dimethyl formamide	GC	34
8	Framicetinsulphate	Neamine	TLC	12
9	Morphin	6- mono acetylmorphin	HPLC	12
10	Mercaptopurine	Hypoxanthine	UV	34
11	Norgestrel	3,17α-diethinyl-13-ethyl-3,5gonadiene-17-ol	TLC, HPLC and UV spectroscopy	34
12	Celecoxib	[5-(4-methylphenyl)-3-trifluromethyl-1H-pyrazole],4-[5-(2'methylphenyl)-3-(trifluromethyl-1H-pyrazole-1-yl]benzenesulphonamide,and4-[4-(4'-methyl phenyl)-3(trifluromethyl)-1-Hpyrazole-1-yl]-benzenesulphonamide	HPLC, LC, LC-MS-MS	12
13	Ethynodioldiacetate	17 a-ethinylestr-4-ene-3a,17-diol-3-acetate-17-(3'-acetoxy-2'butenoate)17 a-ethinylestr-4-ene-3a,17-diol-3-acetate-17-(3oxo-butanoate)	HPLC	12
14	Methamphetamine	1,2-dimethyl-3phenylaziridine, ephedrine, methylephedrine, Nformylmethamphetamine, Nacetylmethamphetamine, Nformylphedrine, Nacetylephedrine,N,Odiacetylephedrine, methametamine dimmer	GC	34
15	Repaglinide	4-carboxymethyl-2-ethoxy benzoic acid, 4cyclohexylaminocarbamoylmethyl-2-ethoxy-benzoic acid, 1-cyclohexyl-3-[3-methyl-1-2(piperidin-1-yl-phenyl)-butyl]urea, 1,3-dicyclohexyl urea	HPLC	34
16	Morphine sulphate	5-(hydroxymethyl)2-furfural	HPLC	12
17	Cimitidine	1,8-bis*(N' cyano-N''-methyl)guinidino]-3,6-dithiaoctane	HPLC	12
18	10-hydroxymorphin	10- oxomorphin	HPLC	12

VI. CONCLUSION:

This review gives a perspective on impurities in drug substance and drug product. Impurity profile of pharmaceuticals is receiving an increasing importance and drug safety receives more and more attention from the public and from the media. This article gives the valuable information about the impurities types and its classification, a number of methods of isolation and characterization, analytical techniques for the determination, qualification of impurities and critical factors to be considered while preparation

of the bulk drugs. Now a day, it is mandatory requirement in various pharmacopoeias to comprehend the impurities existing in API's. Isolation and characterization of impurities is required for obtaining and evaluating records that establishes biological safety which reveals the need and scope of impurity profiling of drugs in pharmaceutical research.

Various types of instrumental techniques routinely employed for the impurity profiling of pharmaceuticals such as spectroscopic and chromatographic technique. Selection of method depends upon the nature of sample to be analyzed and types of impurities present.

Hyphenated techniques such as LC-MS, GC-MS, LC-NMR, CE-MS and ICPMS have been developed to solve a variety of complex analytical problems in pharmaceutical industry. These techniques clear up such problems in time efficient manner. Sample requirement for hyphenated technique is less as compare to conventional technique.

VII. SCOPE:

The aim of this review paper is to give an overview on the state-of-art in impurity profiling of drugs often based on papers published in the final five years. Only papers dealing with identification and quantification of related organic impurities have been reviewed: residual solvents and inorganic impurities are outside the scope of this paper. Only related impurities in drugs with "small molecules" (synthetic and natural products) are dealt with: special methods for impurity profiling of biomacromolecules are also outside the scope of the review.

ABBREVATIONS

GC – Gas chromatographty
HPLC – High performance liquid chromatography
UV – Ultra violet spectroscopy
MS – Mass spectrometry
IR – Infrared
NMR - Nuclear magnetic resonance
TLC- Thin-layer chromatography
CE – Capillary electrophoresis
SFC - Supercritical fluid chromatography
HPTLC - High performance thin layer chromatography
CZE - capillary zone electrophoresis (CZE)

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