



## Impurity Profile of Pharmaceuticals Ingredient: A Review

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### ABSTRACT

Various regulatory authorities like ICH, USFDA, Canadian Drug and Health Agency are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredient's (API's). Qualification of the impurities is the process of acquiring and evaluating data that establishes biological safety of an individual impurity; thus, revealing the need and scope of impurity profiling of drugs in pharmaceutical research. Impurities in pharmaceuticals are the surplus chemicals that stay behind with the active pharmaceutical ingredients or develop during formulation or upon aging of both active content and formulated active ingredients to medicines. The efficacy and safety of pharmaceutical product is affected by presence of unwanted traces of impurities. Impurity profiling deals with detection, identification/structure elucidation and quantitative determination of organic and inorganic impurities as well as residual solvents in bulk drugs and pharmaceutical formulations. Impurities in pharmaceuticals are the surplus chemicals that stay behind with the active pharmaceutical ingredients or develop during formulation or upon aging of both active content and formulated active ingredients to medicines. The efficacy and safety of pharmaceutical product is affected by presence of unwanted traces of impurities. The advent of hyphenated techniques has revolutionized impurity profiling, by not only separation but structural identification of impurities as well. The present review covers various aspects related to the analytical method development for impurity profiling of an active pharmaceutical ingredient.

**KEYWORDS:** Degradation Product, Impurity, ICH Guideline, Characterization Tech.

### INTRODUCTION

There is an ever increasing interest in impurities present in API's. Recently, not only purity profile but also impurity profile has become essential as per various regulatory requirements. 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. A good illustration of this definition may be identification of impurity in API's like 1-(1, 2, 3, 5, 6, 7-hexahydro-s-indacen-4-yl)-3,4-dihydro-1-methyl ethyl-furan-2-sulphonylurea using Multidisciplinary approach<sup>1</sup>. 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 Pharmacopoeia (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 analysing impurities in new drug substances, products, residual solvents and microbiological impurities<sup>1-2</sup>.

### Rationale for the reporting and control of degradation products

The applicant should summarize the degradation products observed during manufacture and/or stability studies of the new drug product. This summary should be based on sound scientific appraisal of potential degradation pathways in the new drug product and impurities arising from the interaction with excipients and/or the immediate container closure system. In addition, the applicant should summarize any laboratory studies conducted to detect degradation products in the new drug product. This summary should also include test results of batches manufactured during the development process and batches representative of the proposed commercial process. A rationale should be provided for exclusion of those impurities that are not degradation products (e.g., process

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impurities from the drug substance and impurities arising from excipients). The impurity profiles of the batches representative of the proposed commercial process should be compared with the profiles of batches used in development and any differences discussed. Any degradation product observed in stability studies conducted at the recommended storage condition should be identified when present at a level greater than (>) the identification thresholds. When identification of a degradation product is not feasible, a summary of the laboratory studies demonstrating the unsuccessful efforts to identify it should be included in the registration application. Degradation products present at a level of not more than ( $\leq$ ) the identification threshold generally would not need to be identified. However, analytical procedures should be developed for those degradation products that are suspected to be unusually potent, producing toxic or significant pharmacological effects at levels not more than ( $\leq$ ) the identification threshold. In unusual circumstances, technical factors (e.g., manufacturing capability, a low drug substance to excipient ratio, or the use of excipients that are crude products of animal or plant origin) can be considered as part of the justification for selection of alternative thresholds based upon manufacturing experience with the proposed commercial process.

#### Regulatory guidelines on impurities in an active pharmaceutical ingredient:

Ethical, economic and competitive reasons as well as those of safety and efficacy support the need to monitor impurities in drug products. However monitoring impurities and controlling these impurities mean different things to different people or to the same people at different times, even those in the pharmaceutical sciences and industry. A unified terminology is necessary to assure that everyone uses the same vocabulary when addressing questions related to impurities<sup>3,4</sup>.

The various regulatory guidelines regarding impurities are as follows:

1. ICH guidelines "stability testing of new drug substances and products"- Q1A
2. ICH guidelines "Impurities in New Drug Substances"- Q3A
3. ICH guidelines "Impurities in New Drug Products"- Q3B
4. ICH guidelines "Impurities: Guidelines for residual solvents"- Q3C
5. US-FDA guidelines "NDAs -Impurities in New Drug Substances"
6. US-FDA guidelines "ANDAs – Impurities in New Drug Substances"
7. Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia.

#### Common terms of impurities<sup>5-8</sup>

Following terms are used by various regulatory bodies and ICH to describe the impurities

- A. Intermediate
- B. Penultimate intermediate
- C. By-products
- D. Transformation products
- E. Interaction products
- F. Related products
- G. Degradation products

- A. Intermediate:** The compounds produced during synthesis of the desired material or as a part of the route of synthesis.
- B. Penultimate Intermediate:** It is the last compound in the synthesis chain prior to the production of the final desired compound.
- C. By-products:** The compound produced in the reaction other than the required intermediates. They can occur through a variety of side reactions, such as overreaction, incomplete reaction, demonization and rearrangement, unwanted reactions between starting materials or intermediates with chemical reagents or catalysts.
- D. Transformation Products:** They are related to theorized and nontheorized products that can occur in a reaction. They are similar to by-products except that more is known about these reaction products.
- E. Interaction Products:** These products formed either intentionally or unintentionally interaction between various chemicals involved.
- F. Related Products:** These are chemically similar to drug substance and may even possess biological activity.
- G. Degradation Products:** They are formed by the decomposition of active ingredient or other material of interest by the effect of external factors like heat, light and moisture.

#### Sources of impurities

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

- a. Crystallization-related impurities,
- b. Stereochemistry-related impurities,
- c. Residual solvents,
- d. Synthetic intermediates and by-products,
- e. Formulation-related impurities,
- f. Impurities arising during storage,
- g. Method related impurity,
- h. Mutual interaction amongst ingredients,
- i. Functional group-related typical degradation
- j. Process-related drug substance
- k. Degradation drug substance or drug

Table 1. Sources of impurities

Impurity type	example
Crystallization-related impurities	<i>Polymorphism</i>
	<i>Solvatomorphism</i>
Stereochemistry-related impurities	levofloxacin (S-ofloxacin), lavalbuterol (R-albuterol), and esomeprazole (S- omeprazole).
Residual solvents	Example: Benzene, carbon tetrachloride, Dichloro methane etc
Synthetic intermediates and by-products	impurity profiling of ecstasy tablets by GC-MS and MDMA samples, produced impurities in intermediates via reductive animation route
Formulation-related impurities	Microbial contamination may occur during the shelf life and subsequent consumer-use of a multiple-dose product, either due to
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
Method related impurity	1-(2, 6-dichlorophenyl) indolin-2
Mutual interaction amongst ingredients	nicotinamide, pyridoxine, riboflavin, and thiamine
Functional group-related typical degradation	aspirin, benzocaine, cefotaxime
Process-related drug substance	- Organic - Starting material - Intermediate - By-product - Impurity in starting material
Degradation drug product	Organic product - Degradation products
Degradation drug substance or drug	Organic - Excipient interaction

### A. 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*<sup>9</sup>.

### B. 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. The prominent single isomer drugs, which are being marketed, include levofloxacin (S-ofloxacin), lavalbuterol (R-albuterol), and esomeprazole (S-omeprazole).

### C. 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<sup>10</sup>. Especially, solvents in Classification of residual solvent.

**Class 1 solvents (Solvents to be avoided):** Known human carcinogens, strongly suspected human carcinogens, and environmental hazards. Example: Benzene, carbon tetrachloride, Dichloro methane etc

**Class 2 solvents (Solvents to be limited):** Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. Solvents suspected of other significant but reversible toxicities. **Examples:** Acetonitrile, chlorobanzen, chloroform etc.

**Class 3 solvents (Solvents with low toxic potential):** Solvents with low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents have PDEs of 50 mg or more per day. **Example:** Acetone, acetic acid, heptanes etc.

**Class I, viz benzene (2ppm 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, Ndimethylformamide (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 the purity 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 <sup>16</sup>.

**D. 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 and MDMA samples, produced impurities in intermediates via reductive animation route.

**E. 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 solvolysis. Fluocinonide Topical Solution USP, 0.05%, in 60-mL bottles, was recalled in the United States because of degradation/impurities leading to sub- potency [20]. 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<sup>13</sup>.

**Table 2. Drug Formulated With Trehalose**

Drug	Manufacturer	API Class	Formulation Type
Adcetris	Seattle Genetics	ADC	Lyophilized Powder
Avastin	Genentech/ Roche	mAb	Solution
Blinicyto	Amgen	Bispecific / mAb	Lyophilized Powder
Gazyva	Genentech/Roche	mAb	Solution
Herceptin	Genentech/Roche	mAb	Lyophilized Powder

**F. 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<sup>6</sup>.

**G. 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<sup>14</sup>. The conditions of the autoclave method (i.e., 123 + 2°C) enforce the intermolecular 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.

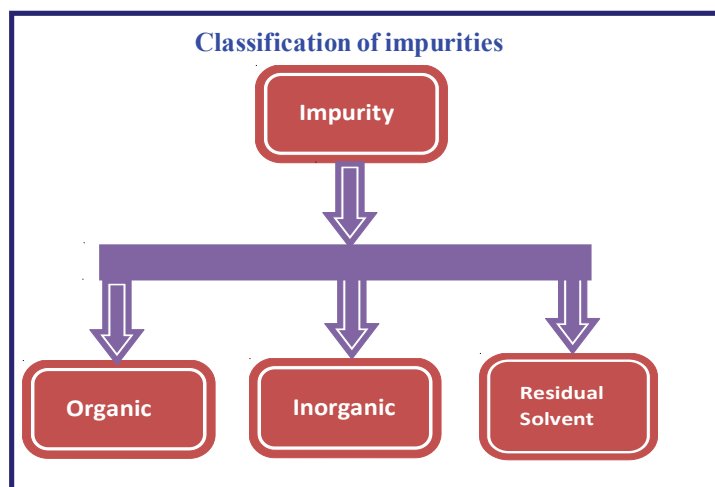
**H. Mutual interaction amongst ingredients**

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 Pharmacopoeia specifications.

**I. Functional group-related typical degradation**

Ester hydrolysis can be explained with a few drugs viz aspirin, benzocaine, cefotaxime, Ethyl paraben<sup>23</sup> and cefpodoxime proxetil<sup>25</sup>.

**Classification of impurities:**



**Figure 1. Classification of Impurity**

Impurities have been named differently or classified as per the ICH<sup>11</sup> as follows;

**a) Common names**

- By-products
- Degradation products
- Interaction products
- Intermediates
- Penultimate intermediates
- Related products
- Transformation products

**b) United State Pharmacopeia**

The United States Pharmacopoeia (USP) classifies impurities in various sections;

- Impurities in Official Articles
- Ordinary Impurities
- Organic Volatile Impurities

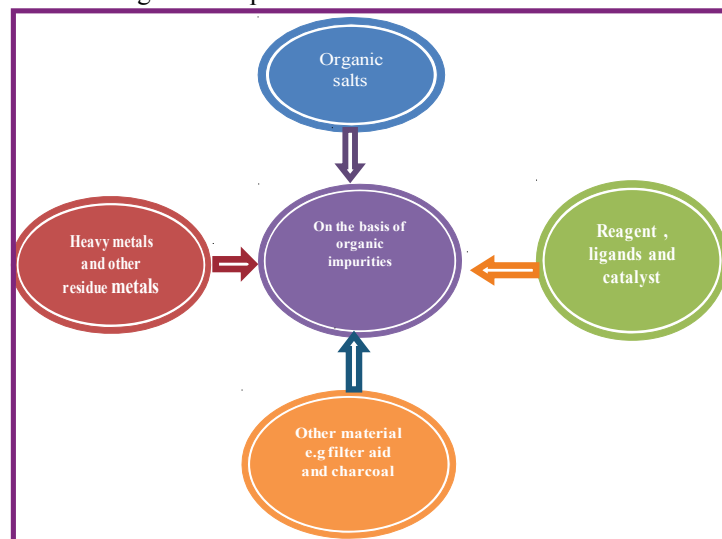
**c) ICH Terminology**

According to ICH guidelines, impurities in the drug substance produced by chemical synthesis can broadly be classified into following three categories;

- Organic Impurities (Process and Drug related)
- Inorganic Impurities
- Residual Solvents

Organic impurities may arise during the manufacturing process and or storage of the drug substance may be identified or unidentified, volatile or non-volatile, and may include;

- Starting materials or intermediates
- By-products
- Degradation products

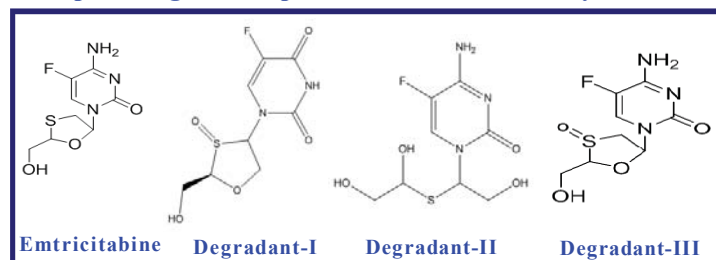


**Figure 2. Classification of Impurity on the Basis of Organic Impurities**

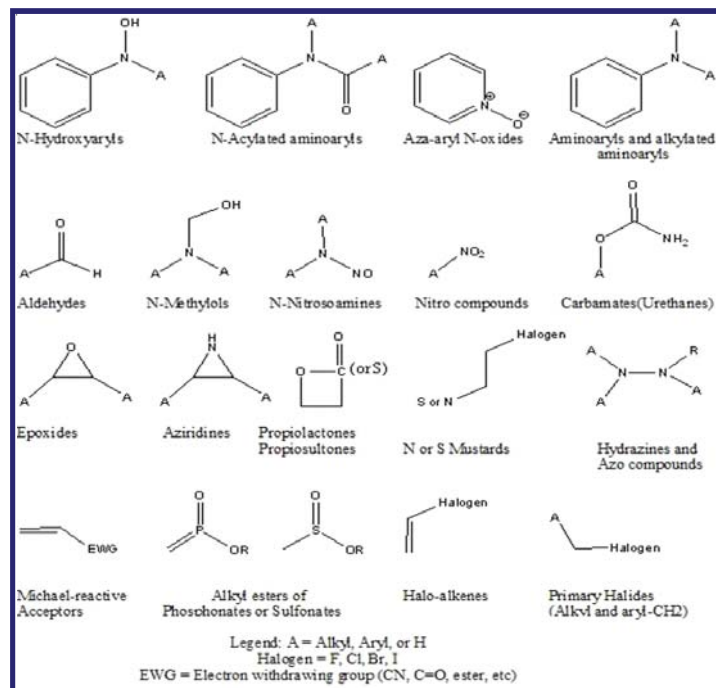
**ICH limits for impurities**

According to ICH guidelines on impurities in new drug products, identification of impurities below 0.1% level, is not considered to be necessary, unless potential impurities are expected to be unusually potent or toxic. According to ICH, the maximum daily dose qualification threshold to be considered is as follows; < 2g/day 0.1 % or 1 mg per day intake (whichever is lower) >2g/day 0.05%

**Example of degradation product observed in stability studies**



**Figure-3: Chemical structure of emtricitabine and its major degradation products**



**Figure 4: Structural alerts for Pharmaceutical impurities.**

**Analytical method development**

New drug development requires meaningful and reliable analytical data to be produced at various stages of the development<sup>17-20</sup>.

- Sample set selection for analytical method development.
- Screening of Chromatographic conditions and Phases, typically using the linear solvent- strength model of gradient elution.
- Optimization of the method to fine-tune parameters related to ruggedness and robustness

The impurities can be identified predominantly by following methods;

- Reference standard method
- Spectroscopic method
- Separation method
- Isolation method
- Characterization method
- HPTLC
- Capillary electrophoresis (CE)
- Supercritical fluid chromatography (SFC)

#### Reference standard method

The key objective of this is to provide clarity to the overall life cycle, qualification and governance of reference standards used in development and control of new drugs. standards serve as the basis of evaluation of both process and product performance and are the benchmarks for assessment of drug safety for patient consumption. These standards are needed, not only for the active ingredients in dosage forms but also for impurities, degradation products, starting materials, process intermediates, and excipients.

#### Spectroscopic methods

The UV, IR, MS, NMR and Raman spectroscopic methods are routinely being used for characterizing impurities<sup>21</sup>.

#### Separation methods

The Capillary electrophoresis (CE), Chiral Separations, Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), TLC, HPTLC, HPLC are regularly being used for separation of impurities and degradation products<sup>21</sup>.

#### Isolation methods

often necessary to isolate impurities. But if the instrumental methods are used, isolation of impurities is avoided as it directly characterizes the impurities.

Generally, chromatographic and non-chromatographic techniques are used for isolation of impurities prior its characterization. The term 'chromatographic reactor' refers to the use of an analytical-scale column as both a flow-through reactor, and simultaneously, as separation medium for the reactant(s) and product(s). By using an HPLC, chromatographic reactor approach, the solution-phase hydrolysis kinetics of the Aprepitant (Emend<sup>TM</sup>) prodrug, fosaprepitant dimeglumine, were investigated<sup>22</sup>. In loratidine, impurity found was ofloratidine<sup>22</sup>. other examples include and amikacin<sup>23</sup>. A list of methods that can be used for isolation of impurities is given below.

- Solid-phase extraction methods
- Liquid-liquid extraction methods
- Accelerated solvent extraction methods
- Supercritical fluid extraction
- Column chromatography
- Flash chromatography
- TLC
- GC
- HPLC

#### Characterization methods

Highly sophisticated instrumentation, such as MS attached to a GC or HPLC, are inevitable tools in the identification of minor components (drugs, impurities, degradation products, metabolites) in various matrices. For characterization of impurities, different techniques are used; which are as follows;

##### 1. NMR

The ability of NMR to provide information regarding the specific bonding structure and stereochemistry of molecules of pharmaceutical interest has made it a powerful analytical instrument for structural elucidation. 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<sup>23</sup>. Unfortunately, NMR has traditionally been used as a less sensitive method compared to other analytical techniques. Conventional sample requirements for NMR are on the order of 10 mg, as compared with MS, which requires less than 1 mg.

##### 2. MS

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

#### Hyphenated Methods:

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

An example of reverse-phase LC-MS analysis in gradient elution with two distinct soft ionization techniques is the Atmospheric pressure ionization with electrospray source (API-ESI) and the chemical ionization of d-allethrine<sup>24</sup>.

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)<sup>25</sup>. HPLC-DAD-MS (HPLC coupled with a diode array UV detector and a mass.

**Analytical challenges to current methods and potential new methods**

While the threshold for identification and qualification of organic impurities is set at 0.1% for the majority of compounds, it is important to recognize that the implication is that a Limit of Quantification (LOQ) of approximately 0.05% will be required: For a compound that is 98% pure, the 2% impurities could be composed of between 10 and 20 components at a level of scrutiny of 0.05%. In future, it may become essential to increase selectivity through the use of gradient separation, both in HPLC and TLC, or through the use of alternative technologies. However, gradient HPLC is the more usual technique, If single methods fail to provide the necessary selectivity, orthogonal 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. The future may see the significantly increased use of spectroscopic techniques for impurity measurement. NMR has shown values for stereo isomers and for process related impurity, but still does not quite show the sensitivity required. Near Infrared spectroscopy is rapidly increasing in use and can detect impurities, although more demonstrations of true validation for low levels of impurities are required.<sup>17</sup> One single method that is showing great promise in pharmaceutical analysis is Capillary Electrophoresis (CE). With its much increased efficiency and great variety of separation modes it may provide sufficient peak capacity, and indeed CE is finding increasing favour for pharmaceutical analysis. CE also adds speed to selectivity, and many of the concerns over the robustness and transferability of CE separations have been dispelled recently through a number of collaborative studies. Additionally, while enantiomers

are outside the scope of the current ICH Guidelines, there is no doubt that, when they are potential impurities, their level(s) must be controlled. CE-MECC can provide the necessary detectability to control enantiomers to the 0.1% level<sup>18</sup>.

**Specifications for Impurities<sup>25</sup>:**

The specifications for a new drug substance should include limits for impurities. Stability studies, chemical development studies and routine batch analysis can be used to predict those impurities likely to occur in the commercial product.

A rationale for the inclusion or exclusion of impurities in the specifications should be presented. This rationale should include a discussion of the impurity profiles observed in the safety and clinical development batches, together with a consideration of the impurity profile of material manufactured by the proposed commercial process.

**Reporting of Impurities<sup>4-25</sup>:**

All impurities above (>) reporting threshold should be reported

**Applications of impurity profiling**

Numerous applications have been sought in the areas of drug designing and in monitoring quality, stability, and safety of pharmaceutical compounds, whether produced synthetically, extracted from natural products or produced by recombinant methods.

**The applications include**

Alkaloids, Amines, Amino Acids, Analgesics, Antibacterial, Anticonvulsants, Antidepressant, Tranquilizers, Antineoplastic Agents, Local Anesthetics, Macromolecules, Steroids Etc.

**There are a few examples of impurities reported in the APIs mentioned**

**Table 3: Various impurities reported in API's**

Drug	Impurities	Method	Reference no
Budensonide	Impurities Or Degradation Product	HPLC	29
Cefidininir	Related Substance	HPLC	30
Donepizil	Process related impurities	HPLC	31
Linezolid	Process related impurities	HPLC	32
Loradtidine	Process related impurities	HPLC	33
Repaglinide	Process related impurities	HPLC	34
Rofecoxib	Process related impurities	HPLC	35
Zaleplon	Process related impurities	HPLC	36
Amphoterecin B	Process related impurities	UV Spectroscopy	37
Doxorubicin Hydrochloride	Residual solvent	GC	38
Framycetin Sulphate	Process related impurities	TLC	39
Cimitidine	Process related impurities	HPLC	40
Norgestrel	Related Solvent	TLC, HPLC, UV Spectroscopy	41
Celecoxib	Process related impurities	HPLC, LC-MS-MS	42

### Current good manufacturing practices

Current Good Manufacturing Practices (cGMPs) have become a way of life for those of us in the healthcare industry. With cGMPs, the trend toward paperless will continue. New product FDA submissions will routinely be electronic. QC laboratory management systems (LMS) will be routine with a dramatic reduction in manual transcriptions. The industry has not yet grasped the significance of the FDA's Process Analytical Technology (PAT) initiative. PAT is the clearest view of the future among published FDA guidelines. What future manufacturing and QC testing will look like is written on (and between) the lines of that guideline. The vision of a monitored and controlled manufacturing process that moves significantly away from a batch production system is at the center of PAT. Based on the results of in/on/at line testing, processing decisions will be made without subjective human intervention (i.e. art gives way to science). With the ever shrinking world in this global economy, the quest to harmonize rules, regulations, guidance documents, and pharmacopeias will continue along with the frustration of getting so many different world organizations and national bureaucracies to agree<sup>28</sup>.

### Isolation and identification of impurities in active pharmaceutical ingredients<sup>27</sup>

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

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

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

### c. Liquid Chromatography and Ultraviolet Spectrometry (LC/UV):

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

### d. Liquid Chromatography and Mass Spectrometry (LC/MS):

LC/MS is a powerful analytical tool that is routinely used in pharmaceutical development to test and identify product impurities. The detection limit of a few hundred ppm is readily achievable, ensuring the identification of all the impurities present at concentrations greater than 0.1 %. MS-based methods generally provide additional robustness and ruggedness compared to techniques such as UV alone, due to their high specificity and sensitivity. While single quadrupole mass spectrometers work well as analytical tools for the confirmation of known impurities and the preliminary structural assessment of unknown impurities, highly sensitive Q-TOF mass

spectrometers provide higher resolution and mass accuracy that enables the unambiguous identification of unknown trace impurities, making them very useful for genotoxic impurity analysis. MS-based methods are often selected for the impurity profiling of APIs during process development, while UV-based methods are generally used to test for genotoxic impurities in QC laboratories at manufacturing sites. Triple-quadrupole (QQQ) LC/MS/MS systems have become a standard platform for the quantitative analysis of organic impurities in pharmaceutical analytical laboratories.

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

**f. Supercritical Fluid Chromatography (SFC):** SFC, which uses supercritical CO<sub>2</sub> as mobile phase, is another orthogonal technique that can be used for impurity detection because it offers HPLC-level sensitivity with reduced organic solvent usage. SFC also offers the advantage of chiral impurity analysis enabling the determination of enantiomeric excess at very low impurity levels.

**g. Nuclear Magnetic Resonance Spectroscopy (NMR):** NMR is a powerful analytical tool that enables the study of compounds both in solution and in the solid state. It has wide applicability because it provides specific information about bonding and stereochemistry within a molecule, which is particularly important in the structural characterization of drug impurities and degrading 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 impurities and degradant present at very low levels. NMR can also provide quantitative output, an important aspect of impurity profiling.

**h. Inductively-Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Inductively-Coupled Plasma Mass Spectrometry (ICP-MS):** The new draft elemental impurities procedure (USP) requires that an instrument-based method is used to determine elemental impurities and that the reference methods are based on either ICP-MS or ICP-OES. With both methods, sample analysis can be

accomplished in three ways: directly (unsolvated), following sample preparation by solubilization in an aqueous or organic solvent, or after acid digestion using a closed-vessel microwave system.

**ICP-OES:** ICP-OES provides parts per billion (ppb) detection limits for most regulated elements in pharmaceutical products, easily meeting the specified limits in cases where direct sample analysis or small dilution factors are appropriate. It also provides extended dynamic range, robust plasma, and one-step measurement of major, minor, and trace elements. Therefore, ICP-OES addresses the needs of a wide range of users, including those seeking a cost-effective solution for the direct analysis of elemental impurities in bulk raw materials and pharmaceutical products.

**ICP-MSL:** ICP-MS is a powerful and sensitive technique that delivers a reliable trace-level analysis of all 16 elements whose limits are defined in USP. The low detection limits of ICP-MS ensure that all regulated elements in drug substances or drug products can easily be determined using the new method, at or below regulated levels, and even when large sample dilutions are required. ICP-MS can also be used in combination with a variety of separation techniques, such as HPLC, GC, and CE, providing several options for separation (or speciation) of the different chemical forms of the elements, and depending upon the nature of sample. ICP-MS achieves low detection limits for almost all elements, including those found in the more extensive analyte list proposed in the ICH Q3D, such as Au and Tl.

**Gas Chromatography (GC):** In combination with flame ionization detection (FID), GC is the standard choice for the analysis of volatile organic impurities, such as residual solvents. The gas chromatography headspace method is used worldwide for residual solvent analysis in quality control laboratories because it closely follows ICH Q3C guidelines. Sample preparation and introduction is via a static headspace which facilitates the selective introduction of volatile solvents without contamination by mostly non-volatile drug substance or drug products. Therefore, the use of an FID detector helps preferentially identify and quantify residual solvents. More recently, the combination of gas chromatography and mass spectroscopy (GC/MS) has been successfully used for confirmation and identification purposes, highlighting the flexibility of this technology.

#### CONCLUSION:

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 provides the valuable infor-

mation about the impurities types and its classification, various techniques of isolation and characterization, analytical techniques for the determination, qualification of impurities and critical factors to be considered while preparation of the bulk drugs. Nowadays, it is mandatory requirement in various pharmacopoeias to know the impurities present in API's. Impurity profiling of a substance under investigation gives maximum possible account of impurities present in it. The establishment of guidelines for impurity levels in drug substances and products provides the quality criteria for manufacturers. The key aspect is that the impurity profiling of a new chemical entity must be shown to be qualified. With a Qualification threshold of 0.1%, or lower for high dose compounds, the pharmaceutical Analyst must give careful thought to their analytical technology, especially in the development phases.

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