Impurity Profiling by Hyphenated Techniques: Review

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ABSTRACT: One of the most vital areas of operations in modern pharmaceutical analysis is the identification, elucidation of structure, and quantitative assessment of impurities and degradation products in bulk pharmaceuticals and pharmaceutical formulations. Affected by impurities, the product's efficacy, stability, and quality might all be impaired. The potency and efficacy of pharmaceutical products can be influenced by even trace amounts of these undesirable compounds. Given the apparent adverse effect that impurities have on the quality of pharmaceuticals, pharmaceutical product impurity controls are a primary concern for pharmaceutical development. The study relies on other hyphenated approaches that are typically applied for monitoring of impurities, including LC-MS, GC-MS, LC-NMR, CE-MS, and ICP-MS.

KEYWORDS: Impurity profiling, ICH Guidelines, Hyphenated techniques

INTRODUCTION:

Pharmaceutical impurities are undesired compounds that are either created during the formulation process, persist with the active pharmaceutical ingredients (APIs), or evolve over time as the APIs and their finished product age. The current regulatory guidance on impurities acknowledges this, and for drug products with a dosage of less than 2 g/day, impurity identification is set at levels of 0.1 percent and above (ICH Q3B(R2), 2006)[7]. Any substance that is not the chemical entity specified as the active pharmaceutical ingredient (API) is referred to as having an impurity profile, according to the ICH (International Conferences on Harmonization) criteria. According to ICH Q3B(R2), 2006, the limit for impurities is 0.1 percent and above.

Regulatory Framework for Controlling Impurities:

The regulatory guidelines of ICH are as follows

Table1: Different guidelines given in ICH regarding impurities are: [2,3]

Q3A(R2)	Impurities in New Drug Substances
Q3B(R2)	Impurities in New Drug Product
Q3C(R8)	Guideline for Residual Solvents
Q3C(R9)Maintenance EWG	Maintenance of the Guideline for Residual Solvents
Q3D(R1)	Guideline for Elemental Impurities
Q3D(R2)MaintenanceEWG	Revision of Q3D(R1) for cutaneous and transdermal products
Q3DTraining	Implementation of Guideline for Elemental Impurities
Q3E EWG	Impurity: Assessment and Control of Extractables and Leachable for Pharmaceuticals and Biologicals

- ✓ US-FDA guidelines “NDAs- Impurities in New Drug Substances”
- ✓ US-FDA guidelines “ANDAs – Impurities in New Drug Substances”
- ✓ Australian regulatory guideline for prescription medicines, Therapeutic Governance Authority (TGA), Australia

Table.2. Thresholds for Impurities as per ICH guidelines. [5]

Max. Daily dose	Reporting threshold	Identification threshold	Qualification threshold
<2g/day	0.05%	0.10% or 1.0mg/day intake (whichever is lower)	0.15 or 1.0mg/day intake (whichever is lower)
>2g/day	0.03%	0.05%	0.05%

The contributors and routes formation of impurity in patented medications are distinctive cases, and they are much the same as in the reference drug product. Which consist of by-products,raw resources and residual solvents from the synthesis of the API; degradants formed during the method and during extended storage; residues from packaging materials and other drug substances developed in the same facility.

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

1. Intermediate
2. Penultimate Intermediate
3. By-products
4. Transformation Products
5. Interaction Products
6. Related Products

1. **Intermediate** - Compounds developed during the synthesis of the required product or as a leftover of the synthesis's process.
2. **Penultimate Intermediate** - It's the last compound produced in the chain of synthesis just before final compound that had been strived afterwards.
3. **Byproducts** - The stuff created during the reaction apart from the needed intermediates.
4. **Transformation Products** - Such products relate to both postulated and non-theorized events that could also occur. They resemble by-products, although there is more data given about these reaction products.
5. **Interaction Products** – Deliberately or accidentally interactions between the different substances involved led directly to these products.
6. **Related Products** - These get some chemical characteristics with drug substances and might have biological activity.
7. **Related Products**- They occur as a consequence of the degradation of the active ingredient or other materials underneath the presence of moisture, heat, and light of the outside world.

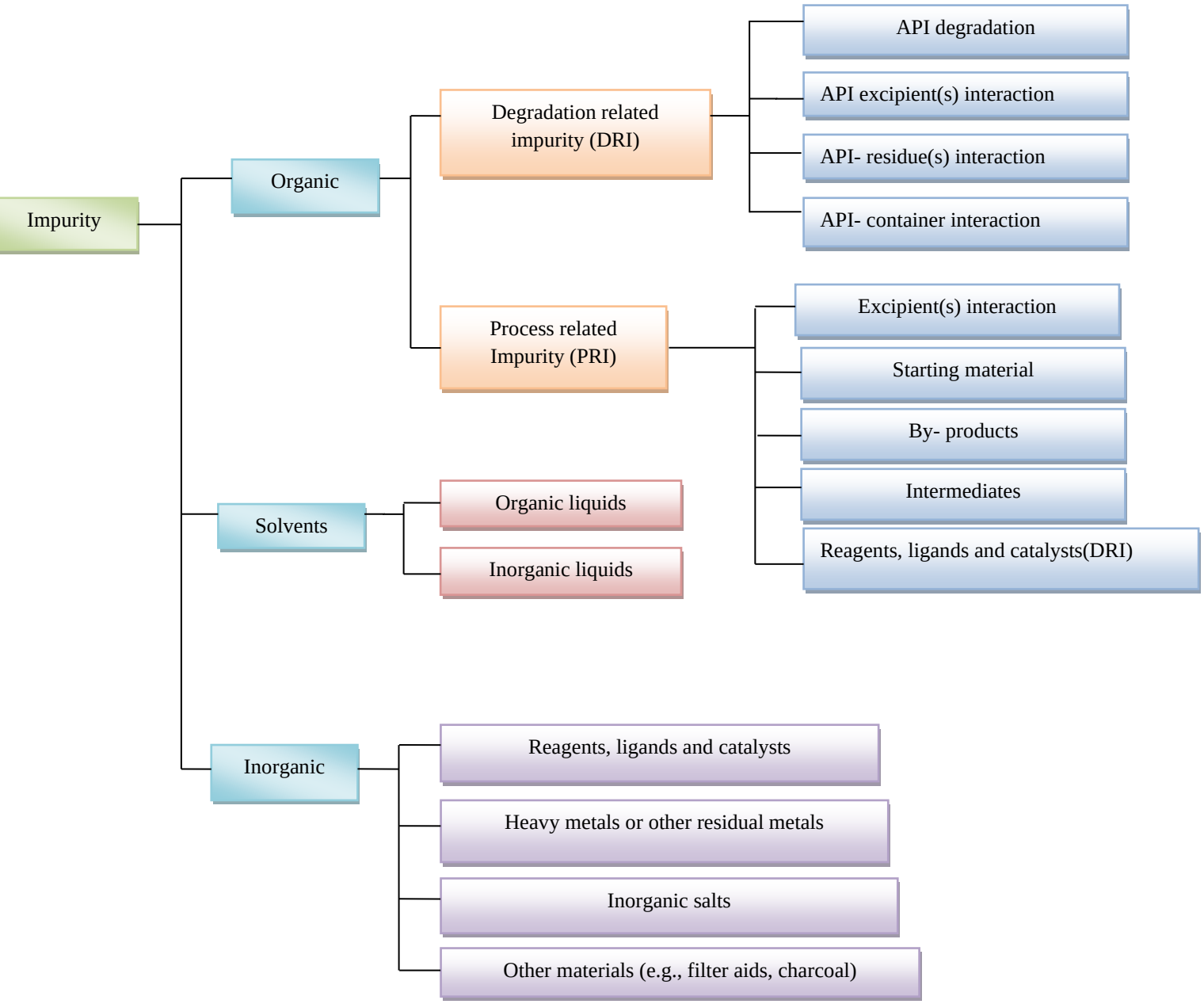

Figure 1: Sources of Impurities

Residual solvents are the remains from the solvents that have been utilized to manufacture pharmacological entities, or excipients, or even those waiting to be incorporated into the finished product. Residual solvents can be categorized as far as how severe they are:

Class I: It is preferable to resist from the use of the class's solvents. Benzene and carbon tetrachloride are two examples.
Class II: It is essential to control the employment about certain kinds of solvents. Such are acetonitrile, chloroform, and hexane.
Class III: Solvents in this class have less severe effects. Examples are formic acid, acetone, and acetic acid.
Class IV: Those solvents may not have the requisite toxicological knowledge. Examples include trifluoroacetic acid, petroleum ether, and isopropyl ether.

Elemental impurities may be classified as:[7,30]

• Class I elements: The elements of this class have minimal or no use in the manufacture of medicinal products. However, if present they have lethal effects on human beings. Ex: As, Cd, Pb, and Hg.
• Class II elements: The elements of this class have route dependent toxicity.
• Class 2A: The elements of this class have a relatively high risk of existence in the medicinal product. Ex: Co, V, and Ni.
• Class 2B: The elements of this class have a low possibility of a presence in the medicinal product. Ex: Ag, Rh, Au, Ru, and Pd.
• Class III elements: The elements of this class have comparatively low lethality by enteral route of administration, but may be toxic in case of inhalation and parenteral route.
• Other elements: The elements of this class have low elementary toxicity and/or difference in provincial regulations.

Genotoxic impurities are DNA reactive substances can injure DNA, when present at low levels leading to the transformation of DNA, and therefore, likely cause cancer. They can be classified into two types,

• **Mutagenic carcinogens:** These are usually found by bacterial reverse mutation (mutagenicity)
assay. Ex: DCBC is a mutagenic impurity present in MLN9708 proteasomes.
• **Non-mutagenic genotoxicants:** These have threshold mechanism, and at low levels, they do not pose a carcinogenic risk in humans. Ex: 2-bromo-2-chloro-1,1-difluoroethylene is a genotoxic degradation product of Halothane.

STRUCTURAL CHARACTERIZATION USING HYPHENATED TECHNIQUES:

A hyphenated approach is the coupling of two distinct analytical methods with the use of anthe proper interface. Various methods that combine separation-separation, identification-identification, and separation-identification are referred described as "hyphenated techniques."A hyphenated technique will involve a combination of the separation approach and an online spectroscopic detection technology. A slash is commonly used in place of a hyphen when one of the methods' titles already has one.[14]

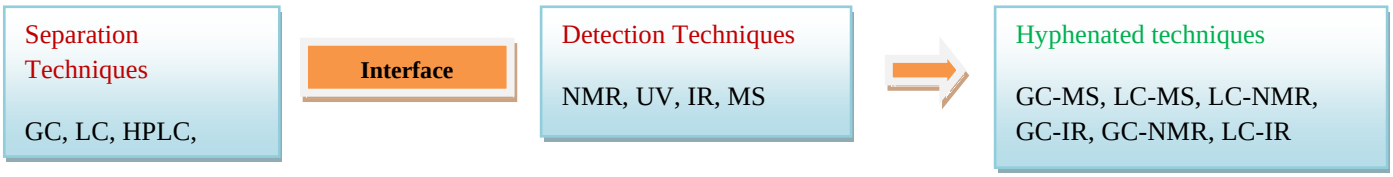

Figure2:Summary of hyphenated techniques [14, 17]

A. LC-MS (LIQUID CHROMATOGRAPHY – MASS SPECTROSCOPY) [21, 22, 23]

In LC-MS, the accuracy of LC separation is combined with an MS's ability to recognize an analyte while simultaneously detecting it. Since the mass spectrometer is a "compound specific detector," it may be able to gain insight on the molecular composition of an analyte. The use of LC-MS for pharmaceutical analysis has grown tremendously concurrently with this advancement in LC-MS technology. LC-MS is now recognized by the pharmaceutical sector as a standard tool.

Recent developments in the LC-MS method have been remarkable. LC-MS is primarily utilized in the analytical development of pharmaceutical products to check products for impurities and identify any that are discovered. This technique enables the detection of all contaminants above 0.1 percent down to a detection limit of a few hundred ppm. Soft ionization techniques, which predominantly yield molecular ion species with relatively few fragment ions, make up the majority of the ionization techniques used in LC-MS. The two most often used interfaces are air pressure chemical ionization (APCI) and electrospray ionization (ESI) (APCI). These ionization technologies can be employed to a variety of mass spectrometers, such as quadrupole, ion trap, and time of flight (TOF) analyzers.

B. GC-MS (GAS CHROMATIGRAPHY-MASS SPECTROSCOPY) [13,24]

The analysis of compounds by GC-MS is simple for those that are sufficiently volatile, small, and stable at high temperatures. Electron Impact (EI), in addition to Chemical Ionization, is a method of ionizationutilized in GC-MS (CI). Based on a probable fragment pattern, the mass spectra acquired aid in the determination of structure. Structure can be established by comparing library spectra to fragmentation patterns with various relative abundances. Compounds that are suitably volatile, tiny, and stable at high temperatures can be easily analyzed by GC-MS. A method of ionization used in GC-MS is electron impact (EI), in addition to chemical ionization (CI). The mass spectra obtained aid in the identification of structure based on a likely fragment pattern. Comparing library spectra to fragmentation patterns with different relative abundances might help identify structure.

Injecting a sample into a heated system caused it to evaporate. Then, an inert gaseous mobile phase eluted via a column and was identified. Carrying the sample through the column is the carrier gas, a gaseous, inert mobile phase. The gas metering valves and pressure regulators control the flow. The MS ion source operates at 10-5 Torr, while the GC operates at atmospheric pressure—a pressure differential of 108 times. Transferring the GC peak components to the MS ion source and extracting the carrier gas are both involved.

GC-MS interface: -

There are four types of interfaces available:

a) Jet separator
b) Permselective membrane
c) Molecular effusion
d) Direct introduction

Laniewski*et al*. [25, 26] successfullyused GC-MS together with gas chromatography with atomicemission detection (GC-AED) and on-line GC-FTIR supplementedby off-line NMR for the identification of impuritiesin the pharmaceutical intermediates 1,3-dichloro-5-(difluoromethoxy)benzene and *tert*-butyl 2-fluoro-3-hydroxypropylcarbamate.

C.LC-NMR(LIQUID CHROMATOGRAPHY-NUCLEAR MAGNETIC RESONANCE)[15, 31]

Even though NMR is now the least sensitive spectroscopic technique used, it nonetheless provides the most essential structural information. The online pairing of HPLC and NMR has the capacity to immediately obtain precise structural information from the materials in a way that no other hyphenated technique can. Stop flow techniques for the direct coupling of liquid chromatography (LC) and NMR were first disclosed in 1978. There are several examples in the literature right now, including how to characterize contaminants using LC-NMR technology. Finding an impurity in a synthetic drug precursor is the focus of HPLC-NMR.The importance of HPLC-NMR in the identification/structure elucidation of impurities and degradation products (often coupled with HPLC-MS) is also emerging, even though the majority of applications are from the fields of metabolite and natural product analysis.

D. CE-MS (CAPILLARY ELECTROPHORESIS-MASS SPECTROSCOPY) [12, 27]

CE is a microscale separation technique that separates challenging samples remarkably well. The characteristics of CE include low sample and solvent consumption, rapid analytical times, and great separation efficiency. CE analysis is supplied by a magnetic field. Due of CE's versatility and vast choice of uses, nearly all compounds can be separated with it. When an MS detector is coupled to a CE system in order to gather real-time MS data of the separated chemical, the resulting combination is known as CE-MS. While hyphenating CE to MS, it is challenging to maintain high CE separation efficiency and high MS sensitivity.This requires for an interface that enables application of electrical contact that results in either little or very little band broadening.

ESI is the most widely use ionization technique when performingCE-MS. Similar to LC-MS different types of mass analyzers such as Quadrupole,TOF and Ion Trap analyzers can be used depending upon requirement.The different types of compounds being analyzed by CE-MS are steadily onthe increase, with more and more people starting to use the technique.

E. ICP-MS(Inductively Coupled Plasma Mass Spectrometry) [28, 29]

Plasma is used to separate the ions in a sample using a multi-element strategy and an especially sensitive analytical instrument. A mass spectrometer is used to extract the ions from the plasma, and they are then recognized based on the mass to charge ratio. Pharmaceuticals can contain inorganic pollutants from a diversity of ways and processing steps, including the raw materials, reagents, solvents, catalysts, intermediate reaction vessels, cabling, and other devices utilized in the synthesis of APIs. Depending on the substance, ICPMS can be used with other separation techniques such as HPLC, GC, and CE.

Table. 3.Impurity profiling of drugs using various analytical techniques[18, 19, 20]

DRUG	IMPURITY	PROFILING TECHNIQUE
Clopidogrel	5-[1-(2-175 chlorophenyl)-2-methoxy-2-oxoethyl]-6,7-dihydrothieno[3,2-c]pyridin-5-ium	LC-MS
5-aminosalicylic acid	Unknown impurity N1	LC-MS
Eprosartan	4,42-(5,52-(1E,12E)-3,32-(4,42-methylenebis(thiophene-4,2-diyl)bis(2-carboxyprop-1-ene-3,1-diyl)bis(2-butyl-1H-imidazole-5,1-diyl))bis(methylene)dibenzoic acid	LC-MS
Acetylspiramycin	Unknown impurities	LC-MS
Pantoprazole sodium[20]	5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]thio]-1Hbenzimidazole	LC-MS

Acetylspiramycin	Unknown impurities	LC-MS
Piperacilin[19]	6[6-[[2-[[(4-Ethyl-2,3-dioxo-1-iperazine1-yl)carbonyl]amino]2-phenylacetyl]amino]-3,3- dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptanecarbonyl]amino]3,3-dimethyl-7-oxo-4- thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid	LC-MS
Methamphetamine	Inorganic Impurities:Na, Ba, Pd, I, Br	ICP-MS
Diclofenac	Inorganic Impurities:Cl35/Cl37	ICP-MS
Ketoconazole	cis-ketoconazole	CE-MS
Vestipitant	Biphenyl impurities	LC-NMR

CONCLUSIONS:

The present review revealed that the importance of impurity profiling and various rules that different regulatory organizations established to preserve the safety and quality of pharmaceutical products are discussed above. Hybrid techniques including LC-MS, GC-MS, LC-NMR, CE-MS, and ICPMS have been developed to meet numerous challenging analytical problems in the pharmaceutical industry. These methods offer a speedy resolution to these problems. The sample required for hyphenated techniques is lower than for conventional procedures.

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