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A Review on Impurity Profiling

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ABSTRACT

Any compounds that coexist with the parent medicine or result from side reactions are considered impurities. These substances can include beginning ingredients or intermediates. There is increasing interest in the contaminants found in APIs. Currently, a number of regulatory bodies require both purity and impurity profiles. A general term for a collection of analytical working groups that include defining, measuring, and describing known and unknown impurities found in a novel medicinal substance is impurity profiling. A innovative method for finding, classifying, organizing, and measuring organic and inorganic contaminants as well as leftover solvents in medications is called impurity profiling. Many regulatory agencies, including the USFDA, UKMHRA, ICH, and CDSCO in India, have developed policies for impurity control and restriction with an emphasis on impurity profiling. Different categories are used to categorize impurities according to their origin, content, and biological safety. The final pharmaceutical product's efficacy and safety may be impacted by the presence of these undesirable compounds or substances. The sources of impurities, their classification, and the different analytical techniques for impurity identification and quantification are the main topics of this paper. Impurities are frequently defined using terms like residual solvent, by-product, transformation product, decomposition product, reaction product, and related products.

INTRODUCTION

The various impurities found in drug products can be attributed to the drug substance or inert ingredients used in their formulation, but they can also be introduced into the product during the formulation process or through contact with the packaging.

"Any ingredient or component of the drug product that is not an excipient or the chemical entity designated as the drug substance." (ICH Q6A: Technical Data). It is critical to give these harmful contaminants more thought. The majority of these contaminants are often tiny molecules. This is particularly true for solid dose forms, because bigger molecules' reactivity is constrained by their limited mobility. Water (which can hydrolyze some drugs or affect the performance of the dosage form), small electrophiles (such as derivatives of aldehydes and carboxylic acids), peroxides (which can oxide some drugs), and metals (which can catalyze oxidation and other drug degradation pathways) make up the reactive species for the majority of drugs. Certain impurites can also result in toxicological issues. The safety and effectiveness of pharmaceutical medicines may be impacted by the presence of these undesirable substances, even in trace amounts. Regulatory bodies are now paying close attention to impurity profiling, which is the identification and amount of impurities in medications. Limits to the permissible quantities of impurities present in active pharmaceutical ingredients (APIs) or formulations are gradually being incorporated by the various pharmacopoeias, including the British, USP, Indian, and others. The identification, quantification, and characterization of the numerous chemicals under investigation in drug development pose a considerable analytical problem in and of itself.

IMPURITIES

Impurities are any component of the drug product that is not the drug substance and excipients in the drug product. A pharmaceutical impurity is the unwanted chemicals that remain with the APIs. These impurities may influence efficacy and safety of pharmaceuticals. Impurities can be classified as:

1.Organic impurities 2.Inorganic impurities 3.Residual solvents

CLASSIFICATION OF IMPURITIES:

1. **Organic Impurities**

- These are unwanted organic compounds present in the final product that are not the intended active ingredient(s).
- They can arise from various sources during synthesis, purification, storage, or degradation of the main component.
- Organic impurities can be further categorized based on their origin:

- **Process-related impurities:** Formed during the manufacturing process (e.g., starting materials, reagents, byproducts).
- **Drug-related impurities:** Impurities structurally related to the active ingredient (e.g., isomers, degradation products).

2. Inorganic Impurities:

- These are unwanted inorganic compounds present in the final product.
- They can come from various sources like:
  - Reagents, catalysts, and ligands used in the manufacturing process.
  - Leaching from containers and processing equipment.
  - Contamination from raw materials (e.g., residual metals).
- Examples of inorganic impurities include:
  - Heavy metals (e.g., arsenic, lead, mercury)
  - Inorganic salts
  - Filter aids

3. Residual Solvents:

- These are leftover solvents used during the manufacturing process that haven't been completely removed.
- Some solvents can be toxic or have other undesirable effects, so their levels are strictly controlled.

Class 1: Solvents to be Avoided

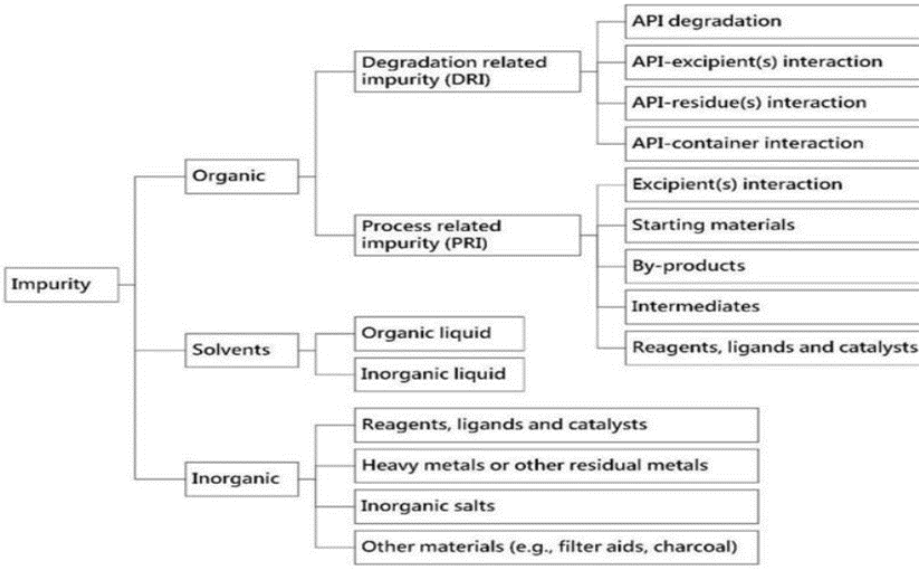
- Description: Known human carcinogens, strongly suspected human carcinogens, and environmental hazards.
- Risk Assessment: Due to their high risk, these solvents should not be used in the manufacturing process wherever possible.
- Examples: Benzene, 1,4-Dioxane, N-Nitroso dimethylamine

Class 2: Solvents to be Limited

- Description: Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity (e.g., neurotoxicity, teratogenicity). Solvents suspected of other significant but reversible toxicities.
- Risk Assessment: Their use is limited based on acceptable daily exposure (PDE) and a thorough risk assessment.
- Examples: Acetonitrile, Dichloromethane, Dimethylformamide, Toluene

Class 3: Solvents with Low Toxic Potential

- Description: Solvents with no known human health hazards at levels normally found in pharmaceuticals. Data on long-term toxicity or carcinogenicity might be limited.
- Risk Assessment: Generally allowed at levels typically used in manufacturing processes. However, control strategies are still important to ensure they remain within acceptable limits.
- Examples: Acetone, Ethanol, Hexane, Isopropanol



CLASSIFICATION OF IMPURITIES

RATIONALE FOR CONTROL AND REPORTING OF DEGRADATION PRODUCTS

The degradation products observed during stability studies should be summarised. Summary based on:

- Potential degradation pathway,
- Impurities arising from interaction with excipients and/or
- The immediate container /closure system Laboratory studies to detect degradation products.

Summary should include test results of batches:

- Manufactured during development process

Representative of proposed commercial process. A rational should be provided for exclusion, of those impurities which are not degradation products. Comparison of impurity profiles of

- Representative batches of proposed commercial process
- With those used in development.

Degradation products observed in stability studies at recommended storage conditions should be identified when present at levels > identification threshold.

If identification is not feasible, laboratory studies should be reported.

Degradation products of ≤ the threshold would not need to be identified Conventional rounding rules, results presented with same number of decimals as given in the limit.

Thresholds for identification of degradation products in new drug products:

Maximum Daily Dose	Threshold
< 1 mg	1% or 5 µg TDI[1],whichever is lower
1 mg - 10 mg	0.5% or 20 µg TDI[1],whichever is lower
> 10 mg - 2 g	0.2% or 2 mg TDI[1], whichever is lower
> 2 g	0.1%

ELEMENTAL IMPURITIES

The elemental impurities in the drug and the drug products such as As, Cd, Cu, Pb, Hg, V and Pt may arise from several sources; they may be added intentionally in synthesis, or may be present as a contaminant, e.g., through interaction with manufacturing equipment,containers and closures.

Classification of elemental impurities:

Class 1:

- Elements: Arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb)
- Rationale: These elements are highly toxic and have limited or no use in pharmaceutical manufacturing. Their presence is typically from commonly used materials (e.g., mined excipients).

Class 2:

- Class 2A: Beryllium (Be), Thallium (Tl)
- Class 2B: Ag, Au, Os, Pd, Pt, Rh, Ru, Se
- Rationale: Class 2 elements are generally considered as route-dependent human toxicants. Class 2A elements are more toxic than Class 2B elements.

Class 3:

- Elements: Antimony (Sb), Barium (Ba), Chromium (Cr), Copper (Cu), Lithium (Li), Nickel (Ni), Tin (Sn)
- Rationale: Class 3 elements have a lower inherent toxicity than Class 1 and 2 elements. However, they still require careful consideration based on their potential for occurrence and route of administration.

Class 4:

- Elements: Aluminum (Al), Boron (B), Iron (Fe), Zinc (Zn), Potassium (K), Calcium (Ca), Sodium (Na), Manganese (Mn), Magnesium (Mg), Tungsten (W)
- Rationale: Class 4 elements have very low inherent toxicity and are often present in drug products at low levels due to their natural abundance. They are typically not a major concern unless present at exceptionally high levels.

SOURCES OF ELEMENTAL IMPURITIES AND IDENTIFICATION PROCEDURES

1. Crystallization related impurities

The polymorphism is the term used to denote crystal systems where a substance can exists in numerous crystal packing arrangement, all of which has same elemental composition. It is also possible to possess crystal system where the substance exists in numerous crystals packing arrangement, each of which has a different elemental composition; this phenomenon is known as solvatomorphism.

2. Stereochemistry related compounds

It is of paramount importance to seem for stereochemistry related compounds i.e. those compounds that have similar chemical structure but different spatial orientation these compounds will be considered impurities within the API.

3. Impurities arising during storage

A variety of impurities can originate from during storage condition or shipment of the drug products. The impurities can come from glass, rubber stoppers and plastic packaging materials. Metal oxide like NaO2, SiO2, CaO2, MgO are the main components leached from the glass.

4. Mutual interaction amongst ingredients

Most of the vitamins are extremely labile and because of ageing they generate problems of instability in many dosage forms, particularly in liquid dosage forms. A vitamin on degradation doesn't give toxic impurities; on the opposite hand, the potency of active ingredients lowers pharmacopeial specifications.

5. Residual solvents

These are organic volatile chemicals used during manufacturing processor generating during the production. They have toxic or environmentally hazardous properties; their complete removal can be very difficult. The gas chromatography is employed for detection of residual solvents because they're mostly volatile in nature.

6. Synthetic Intermediates and by products

The impurities in a pharmaceutical compound or a new chemical entity originate mainly during the synthetic process from raw materials, solvents, and intermediate and by products. The raw materials are normally used to manufactured the drug substance that having minor purity. Similarly, solvents utilized in the synthesis are containing the variety of impurities which will change the range from a trace level to significant amounts that may react with various chemicals utilized in the synthesis to produce the impurities.

7. Formulation related impurities

The number of impurities in a drug product can arise out of inert ingredients used to formulate a drug substance. In the process of formulation, a drug substance is subjected to a variety of conditions that may result in its degradation or other deleterious reaction. The solutions and suspensions are potentially susceptible to degradation due to hydrolysis. The water utilized in the formulation cannot only contribute its own impurities; it may also provide a ripe situation for hydrolysis and catalysis. The similar reactions are possible in the other solvents that may be used.

8. Functional group related impurities

The ester hydrolysis can be seen in a few drugs viz aspirin, benzocaine, cefotaxime, ethyl paraben, and cefpodoxime proxetil. The oxidative degradation of the drugs that have hydroxyl group directly bonded to an aromatic ring (viz phenol derivatives like catecholamine and morphine), some drugs like hydrocortisone; methotrexate, and, conjugated dienes (viz vitamin A and unsaturated free fatty acids), heterocyclic aromatic rings, nitroso and nitrite derivatives, and aldehydes (especially flavone rings) are all susceptible to oxidative degradation.

9. Degradation related impurities

The Impurities can also be formed by degradation of the end product during manufacturing of the bulk drugs. The degradation of penicillin and cephalosporin's are well-known examples of degradation products. The presence of a β-lactam ring, likewise a α- amino group in the C6/C7 side chain plays a critical role in their degradation.

10. Method related impurities

The diclofenac sodium in the parenteral formulation containing an impurity; 1-2, 6 dichlorophenyl indole. In diclofenac sodium indolinone derivatives could also be formed due to condition of autoclave (i.e., 123±20ºC) as it induced intramolecular reaction.

ICH GUIDELINES FOR IMPURITIES

Q1A(R) – Stability testing of new drug substances and products. Q3A- Impurities in new drug substances.

Q3B- Impurities in new drug products. Q3C-Guidelines for residual solvents. Q3D- Guidelines for elemental impurities.

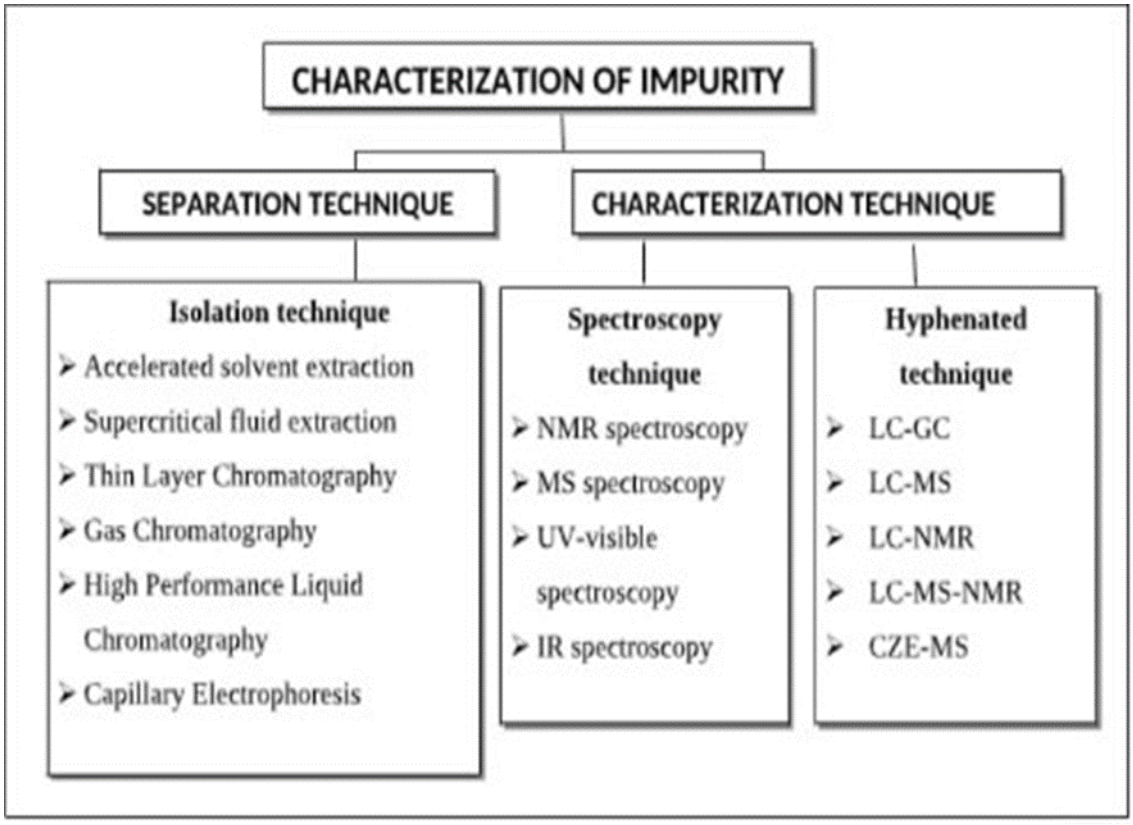
WHO GUIDELINES FOR IMPURITIES

WHO Provides guidelines for impurity profiling primarily to ensure quality safety and efficacy of pharmaceutical products. These guidelines outline principles and procedures for identifying, characterising, and controlling impurities in pharmaceutical substances and products.it includes:

WHO TRS 943 (Annex 3): This document focuses on chemical reference substances. It mentions that impurities with similar properties to the main component might not be a major concern, but even trace amounts of impurities with significantly different properties can render a substance unsuitable as a reference. This indirectly highlights the importance of understanding and controlling impurities.

WHO Quality Assurance and Safety of Medicines Programme: This program focuses on ensuring the quality, safety, and efficacy of medicines. They might have resources related to impurity control within this broader context.

IDENTIFICATION AND CHARACTERIZATION OF IMPURITY



TECHNIQUES FOR IMPURITY PROFILING

A) Reference standard method:

The main purpose of this method is to quantify and to control reference standards which are used in the process of the development and control of a new drugs.

B) Spectroscopic methods

a) Ultraviolet-visible: The UV-VIS spectroscopy is based on the absorption of the visible and ultraviolet (UV) radiation in the wavelength range of 200-800 nm.The valence electrons absorbs the energy in UV-visible from ground state to excited state . The transition depends upon the nature of electrons.

b) Infrared spectroscopy: The sample is subjected to electromagnetic radiations which are within the range of 600 cm-1 and 4000 cm-1. This radiation effects the bonds present in the molecule and then it stretches or causes bending in molecule due to absorption of energy of a specific wavelength. It provides though little complex but significant or unique fingerprint of any molecule which can be used for analysis of samples and thus determining the impurities present.

c) Raman spectroscopy: It is a spectroscopic technique used to study vibrational, rotational, and other low frequency modes in a system. It relies on the Raman scattering of the monochromatic light usually from a laser, in the visible, near infrared, or near ultraviolet range.

C) Mass spectrometry (MS)

The technique, where charged species (ions) are separated and detected according to their mass to charge ratio (m/z) is known as Mass spectrometry (MS). The MS is specific highly selective and sensitive methods for molecular analysis provides insights to the structure of the analyte. It is also used for monitoring, characterizing and quantification of the drug related substances in an API.

D) Nuclear magnetic resonance (NMR)

The NMR plays a vital role in identifying low level impurities in bulk drug materials with or without chromatographic isolation. The structural elucidation of impurities in the drug materials mostly involve 1H and 13C experiments, the information obtained from these experiments is sufficient to ascertain the structure of the unknown impurity in the drug material.

E) Separation methods

a) Capillary electrophoresis (CE): The high separation efficiencies compared to other chromatographic techniques is achieved by the CE for determination of the drug-related impurities. In case, the HPLC technique fails to adequately measure impurities, the CE can be employed, especially for very the polar material compounds.

b) Gas chromatography (GC): The GC technique involves vaporization of the sample and subsequent injection in to the gas chromatographic column. The sample is passed through the column by means of gas flow. The solvent used is an inert gas and the stationary. phase is a liquid film coated on a support of used silica or a packed sorbent. The sample in the vapour form moves through the column by adsorption and partition phenomenon.

c) High pressure liquid chromatography (HPLC): The HPLC is especially used for identifying, quantifying and purifying the impurities and each component of a substance. The HPLC is used to elucidate structures and quantitatively determine impurities and degradation products in bulk drug substances and pharmaceutical formulations.

d) Thin layer chromatography (TLC): The TLC is the technique used for the identification of various components up to a trace amounts. This technique has been used for developing stability-indicating analytical method. The TLC is very much used during the initial degradation and stress studies to study the number of degradation products formed.

e) Supercritical fluid chromatography (SFC): The SFC is considered a normal phase technique because it utilizes the relatively nonpolar, "liquid" carbon dioxide as the bulk of the mobile phase that is used for the analysis and purification of the low to moderate molecular weight, thermally labile molecules. It is often used for the separation of chiral compounds. The SFC primarily uses supercritical CO2 as an eluent.

F) Isolation methods

These methods are mandatory to separate the impurities for their structural identification. Generally, the chromatographic and nonchromatographic methods are utilized for isolation of impurities before characterization. A list of methods that may use for the isolation of impurities is given below.

Solid phase extraction methods

a) Liquid- Liquid extraction methods

b) Accelerated solvent extraction methods

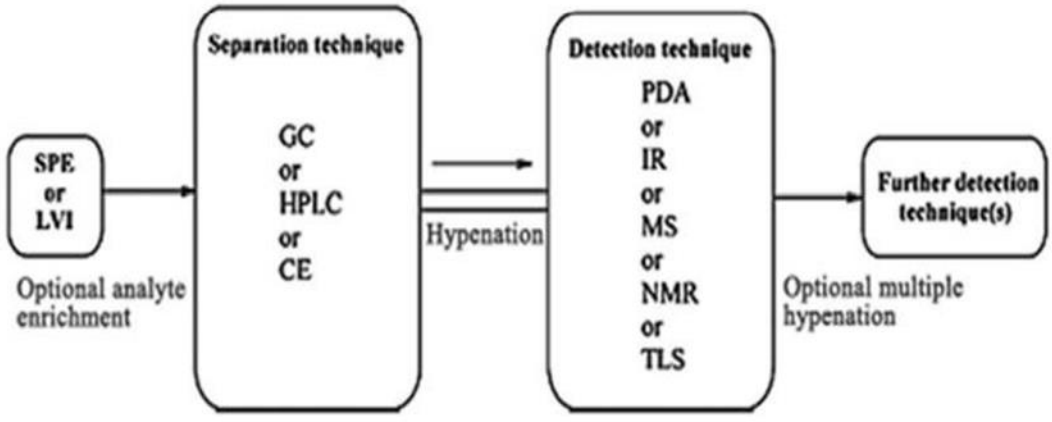
c) Column chromatography

d) Flash Chromatography

HYPHENATED METHODS

A hyphenated technique is combination or coupling of two different analytical techniques with the help of proper interface. Mainly chromatographic techniques are combined with spectroscopic techniques. The term hyphenated techniques ranges from the combination of separation- separation, separation-identification & identification - identification techniques. The hyphenation of these techniques leads to better analysis of the components. Hyphenated techniques show specificity and sensitivity.

Chromatography - Produces pure or nearly pure fractions of chemical components in a Mixture and Spectroscopy – Produces selective information for identification using standards or library spectra. “The coupling of a separation technique and an on-line spectroscopic detection technology will lead to hyphenated technique.



Schematic presentation of Hyphenation of chromatographic and spectrometric techniques.

Types of hyphenated techniques

I. Double hyphenated techniques.

II. Triple hyphenated techniques.

Double hyphenated techniques

1. GC-MS
2. LC-MS
3. LC-NMR
4. EC-MS
5. CE-MS
6. GC-IR
7. GC-NMR
8. GC-AES

Triple hyphenated techniques

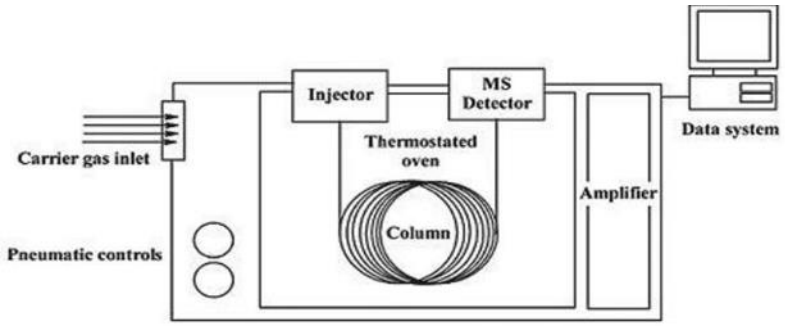
1. LC-MS-MS
2. GC-MS-MS
3. GC-GC-MS

Advantages of hyphenated techniques

1. Fast and accurate analysis
2. Higher degree of automation
3. Higher sample throughput
4. Better reproducibility
5. Reduction of contamination due to its closed system
6. Separation and quantification achieved at same time.

1.GC-MS

GC is able to separate the volatile and semi volatile compounds but it unable to identify them whereas MS can identify the compound by giving its structural information at molecular level but it unable to separate them. Therefor the combination of these two techniques is took place shortly after the development of GC. GC-MS was the first technique to be hyphenated and this technique can confirm the organic volatile semi volatile compounds and residual solvents with great resolution. For the analysis of the compound by GC-MS the compound should possess the property such as volatility and thermal stability. These two techniques highly compatible with each other, the sample is in the vapour phase in both the techniques. But there is incompatibility between two techniques is GC is operate at high pressure (760 torr) and in this the carrier gas is present whereas in case of mass spectroscopy it operates at a vaccum10–6 to l0–5 torr.



Schematic presentation of GC-MS

Applications:

1. Quantitation of pollutants in drinking and waste water using official U.S. Environmental Protection Agency (EPA) methods.
2. Quantitation of drug in metabolites and urine is done for the pharmacological and forensic use.

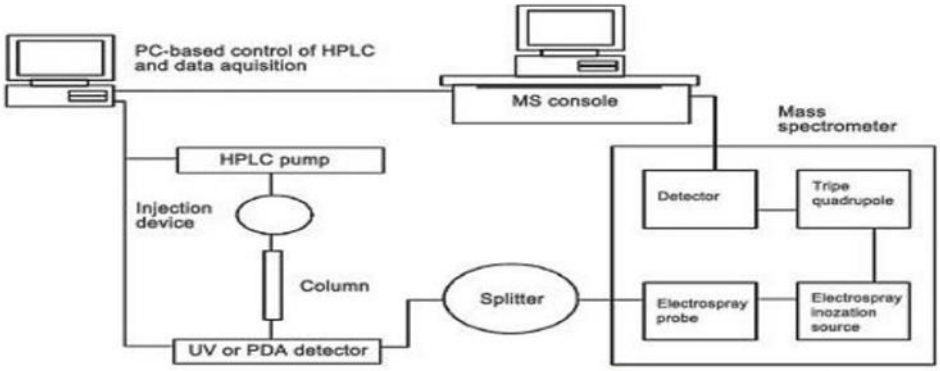
3. Identification of unknown organic compounds in hazardous waste dumps and reaction products by synthetic organic chemistry.
4. Used for drug analysis, pesticide and herbicide detection.

2. LC-MS

Liquid chromatography-mass spectrometry is the technique which performs separation by liquid chromatography and mass analysis with the help of the mass spectrometry. With the

help of HPLC impurities and degradation products can be separated and Mass Spectrometry allows us to obtain the molecular weight and identification of the same. LC-MS is highly

selective and sensitive technique. LC-MS leads to detection and identification of chemicals in presence of other chemicals therefor it is called as specific. The flow rate of HPLC is around 1ml/min which is difficult to accommodate in mass spectrometry vacuum system also the diluent which is used has to be vaporized which leads to damage of the thermally labile compounds by excessive heating. By hyphenation of these two techniques capabilities of both the techniques were improved.



Schematic presentation of LC-MS

Applications:

1. LC-MS used to detect compounds from polyaromatic (non-polar) to peptide and proteins.
2. LC-MS used for compounds identification and purity.
3. Used for determination of pesticides, herbicides & organic pollutant for environmental monitoring.
4. proteome analysis is done by this technique.

CONCLUSION

The need and potential applications of drug impurity profiling in pharmaceutical research are demonstrated by the requirement for impurity isolation and characterisation in the collection and assessment of data pertaining to biological safety. Numerous instrumental analytical techniques have been regularly employed to separate and quantify the contaminants. Furthermore, because there are so many different types of organic impurities—from microbial contamination to API breakdown products—as well as traces of intermediates, identifying and considering them legally is a very difficult task. Even though ICH has a clear plan of action on impurities, much more work needs to be done. Therefore, there is an urgent need for consistent standards and specifications pertaining to contaminants.

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