A Review on a Resend Trends in Impurity Profiling of Pharmaceutical:

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Abstract:

Purity profiling is the process of gathering and analysing information to determine the biological safety of a specific impurity, hence highlighting its importance and range in pharmaceutical research. In the field of pharmaceuticals, impurity has no precise meaning. Identification, structural elucidation, and quantitative determination of impurities and degradation products in bulk medicinal materials and pharmaceutical formulations are all included in impurity profiling. Since unrecognized, possibly poisonous impurities are dangerous to health and should be found and determined by selective procedures in order to increase the safety of drug therapy, impurity profiling has become more significant in contemporary pharmaceutical analysis. Impurities are typically described using words like residual solvents, by products, transformation products, degradation products, interaction products, and related products. Impurity identification is carried out using different chromatographic methods in addition to passing the CGMP, QC, QA, and water activity tests, a pharmaceutical ingredient also needs to satisfy the requirements for a new impurity. It is important to separate and characterize impurities in order to collect and evaluate data that determines biological safety, which emphasizes the need for and promise of drug impurity profiling in pharmaceutical research. The detection and regulatory evaluation of organic impurities is a very challenging task because to the numerous sources of organic impurities, including microbiological contamination, API breakdown products, and traces of intermediates.

Various regulatory authorities such as the International Conference on Harmonization (ICH), the United States Food and Drug administration (FDA), and the Canadian Drug and Health Agency (CDHA) are emphasizing on the purity requirements and the identification of impurities in Active Pharmaceutical Ingredients (APIs). The various sources of impurity in pharmaceutical products are reagents, heavy metals, ligands, catalysts, other materials like filter aids, charcoal, and the like, degraded end products obtained during after manufacturing of bulk drugs from hydrolysis, photolytic cleavage, oxidative degradation, decarboxylation, enantiomer impurity, and so on. The different pharmacopoeias such as the British Pharmacopoeia, United State Pharmacopoeia, and Indian Pharmacopoeia are slowly incorporating limits to allowable levels of impurities present in APIs or formulations. Various methods are used to isolate and characterize impurities in pharmaceuticals, such as, capillary electrophoresis, electron paramagnetic resonance, gas–liquid chromatography, gravimetric analysis, high performance liquid chromatography, solid-phase extraction methods, liquid–liquid extraction method, Ultraviolet Spectrometry, infrared spectroscopy, supercritical fluid extraction column chromatography, mass spectrometry, Nuclear magnetic resonance (NMR) spectroscopy, and RAMAN spectroscopy. Among all hyphenated techniques, the most exploited techniques for impurity profiling of drugs are Liquid Chromatography (LC)-Mass Spectroscopy (MS), LC-NMR, LC-NMR-MS, GC-MS, and LC-MS are most commonly utilized, highlighting the essential role and vast scope of impurity profiling in pharmaceutical research.

Keywords: Characterization, chromatography, identification, impurities, NMR, mass spectrometry

Introduction

Pharmaceutical impurities are unintended chemicals that may be present in drug substances (APIs), drug products, or intermediates during the manufacturing process. These can originate from raw materials, manufacturing processes, degradation during storage, or packaging interactions. Even trace levels of certain impurities, such as genotoxic or elemental impurities, can significantly impact the safety, efficacy, and stability of pharmaceutical products.

Impurity profiling is the science of detecting, identifying, characterizing, and quantifying these undesired chemicals using validated analytical techniques. It plays a crucial role in:

- Ensuring compliance with Good Manufacturing Practices (GMP).
- Meeting the stringent quality standards set by regulatory bodies like the USFDA, EMA, and WHO.
- Supporting the qualification of drug substances and products as per guidelines like ICH Q3A–Q3D and M7.

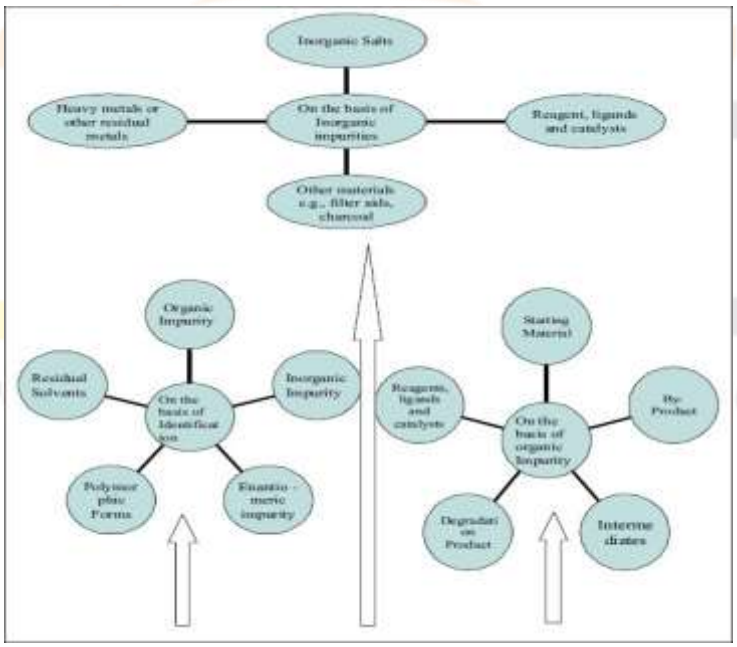
With the increasing complexity of pharmaceutical molecules especially in the era of biologics, peptides, and complex generics traditional impurity detection techniques (like simple UV detection or TLC) are no longer sufficient. Regulatory expectations have evolved, requiring advanced, sensitive, and specific analytical tools that can identify impurities down to parts per billion (ppb) or even lower. Additionally, the demand for green, efficient, and real-time monitoring methods has accelerated the adoption of new technologies and strategies in this field.

Regulatory guidelines, especially those issued by the International Council for Harmonisation (ICH), have categorized impurities and laid down strict reporting, identification, and qualification thresholds. For example:

- ICH Q3A (R2) pertains to impurities in new drug substances.
- CH Q3B (R2) deals with impurities in new drug products.
- ICH Q3D focuses on elemental impurities and sets limits based on permitted daily exposure (PDE).
- ICH M7 (R1) addresses mutagenic and genotoxic impurities, introducing the Threshold of Toxicological Concern (TTC) as a risk-based approach.

To meet these regulatory requirements and mitigate patient risk, pharmaceutical industries are integrating cutting-edge technologies such as:

- High-resolution analytical platforms like LC-MS/MS, UPLC, and NMR.
- Spectroscopy-based PAT tools for real-time impurity tracking.
- Chemometric and AI/ML models for predictive impurity analysis.
- Eco-friendly green chemistry approaches to minimize solvent waste and hazardous reagent usage.



Even a little amount of these undesirable compounds can have an impact on the safety and effectiveness of medicinal goods. Regulatory agencies are now paying critical attention to impurity profiling, which involves the identification and quantification of impurities in medications. Limits to permissible amounts of impurities present in the APIs or formulations are gradually being incorporated by the various Pharmacopoeias, including

the British Pharmacopoeia (BP), United States Pharmacopoeia (USP), and Indian Pharmacopoeia (IP).Guidelines for validating techniques for analyzing impurities in new drug substances, products, residual solvents, and microbiological impurities have also been published by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). [1-3]

Definition of Impurity:

Impurities are compounds found in a product that are neither Active Pharmaceutical Ingredients (API) themselves nor the excipients used to create it, according to the International Conference on Harmonization (ICH) rules. While IP has defined impurity as any pharmacological substance's component for a drug product that is not the chemical entity that is used in a pharmaceutical setting or explains the ingredient, or excipients in the case of a drug product, but not in the case of a drug product Impurity is simply any substance that coexists with the target ingredient. Initial drug, such as the precursors, intermediaries, or produced; as a result of any adverse effects. An impurity profile is a description of both known and unknown impurities. Contaminants in pharmaceutical products. The following are the primary causes for the rising interest of drug producers and drug registration agencies in the impurity profiles of bulk drug compounds:

•It is crucial to understand the structures of the contaminants during the creation of a new drug or a new manufacturing method for an already-marketed drug: Synthetic organic chemists frequently have the knowledge necessary to alter the conditions of reactions in such that the impurity's production can be prevented or its quantity can be decreased to a reasonable level.

• The impurities can be manufactured after having recommended structures for them, providing concrete proof of the structures that were previously established using spectroscopic techniques.

• When creating a selective procedure for the quantitative analysis, the material created might be utilized as an "impurity standard." identifying the impurity and applying using this technique as a quality control measure testing of every batch.

• In the event of significant contaminants, the synthetic or solitary materials could be exposed to Thus, toxicological research significantly contributes to the security of pharmacological therapy.

• The impurity profile of a drug substance is a reliable fingerprint for drug regulators to identify the consistency and scale of the production process of the bulk drug substance [4].

Pharmacopoeial and Regulatory Guidelines and Status on Impurity Profiling:

The impurity profiling of the medications was not given significant emphasis in earlier editions of several pharmacopoeias. However, in more recent editions, emphasis has been placed on impurity profiling for numerous medications and inclusion in the monograph. Limits to permissible levels of contaminants contained in API and formulations have been established by IP, BP, and USP. Guidelines for validating techniques for analyzing impurities in new drug substances, products, residual solvents, and microbial impurities have also been published by the International Conference on Harmonization (ICH) Guideline for Technical Requirements for Registration of Pharmaceuticals for Human Use.[5]

According to USP, improvements in analytical chemistry are inseparable from changes in the idea of purity over time. If an item that was once thought to be pure can now be classified as having inorganic, organic, isomeric, or polymeric components that are deemed impurities. Setting pharmacopoeial standards can be difficult because completed medications might have either high or low purity levels. Where a preparation degrades with time when time is a factor, the same analytical techniques that indicate stability also indicate purity. [6]

Impurities, according to BP, are separated into two subtypes called "Qualified 'Detectable impurities' and other 'Impurities'. These are the eligible impurities. formerly recognized as qualified viz. Impurities by competent authorities They are present as natural metabolites, along with other "Detectable Impurities," These were not found in any samples of the chemicals taken during the process of elaboration of the monograph or that happens in concentrations less than 0.1% but has been demonstrated being constrained by testing The pharmacopoeia's monographs have been created to guarantee the consumers' access to drug substances and

products of a minimum acceptable standard. Numerous monographs have looked into similar substance tests to reduce contaminants and degradation by products. Although ensuring the identity, strength, purity, and quality of official products is one of the Pharmacopoeia's main goals, it is not practical to include a test for every impurity, contaminant, or even adulterant that might be present in each monograph. [7]

Criterion	For drug substance	For drug product
Each identified specified impurity	0.50%	-
Each unidentified impurity	0.30%	-
Total impurity	1.00%	-
Each identified specific degradation product	-	1.00%
Each unidentified degradation product	-	0.50%

Table no.1: Acceptance criteria for impurities

CLASSIFICATION:

The ICH guideline classifies impurities in three sections- organic, inorganic and residual solvents given in below:

(1) Organic impurities:

To detect organic impurities present in the drug substance, the laboratory studies are conducted, which include test results of materials during production and manufacturing in development process. The impurity profile of the drug which will use for marketing should be compared with those used in development.

(2) Inorganic impurities:

Like organic impurities, Inorganic impurities are also detected from the manufacturing processes which are used in bulk drugs formulation. They are normally known and identified like heavy metal impurities, residual solvent impurities, filter aids, charcoal etc.

(3) Residual Solvents:

Residual solvents are inorganic or organic liquids used as vehicles for the preparation of suspensions or solutions in the manufacturing of new drug products. The control of residual solvents used in the manufacturing process for the drug substance is necessary. [8]

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR IMPURITY PROFILE:

The impurities can be identified, minimized by various methods like reference standard method, spectroscopic method, separation method, characterization method and isolation method. [9-17]

1. Reference Standard Method:

It is the benchmarks for assessment of drug safety for patient consumption. These methods provide clarity to the overall life cycle as well as qualification in the development and control of new drugs. A reference standard includes evaluation of process and product performance. These standards are required for starting materials, APIs, excipients, impurities, degradation products and process intermediates in pharmaceutical dosage forms.

2. Spectroscopic Method:

The UV, IR, MS, NMR and Raman spectroscopic methods are used for analysing various types of impurities.

a) **UV-Spectrophotometry**:

UV-Spectroscopy is a technique mainly used to determine purity and concentration of substance. According to Beer-Lambert law, the absorbance of a solution is directly proportional to the concentration of the substance

in the solution and the path length. Therefore for a fixed wavelength and path length the UV-Spectroscopy can be used to determine the concentration as well as purity of the absorber in a solution. (18)

b) Infrared Spectroscopy:

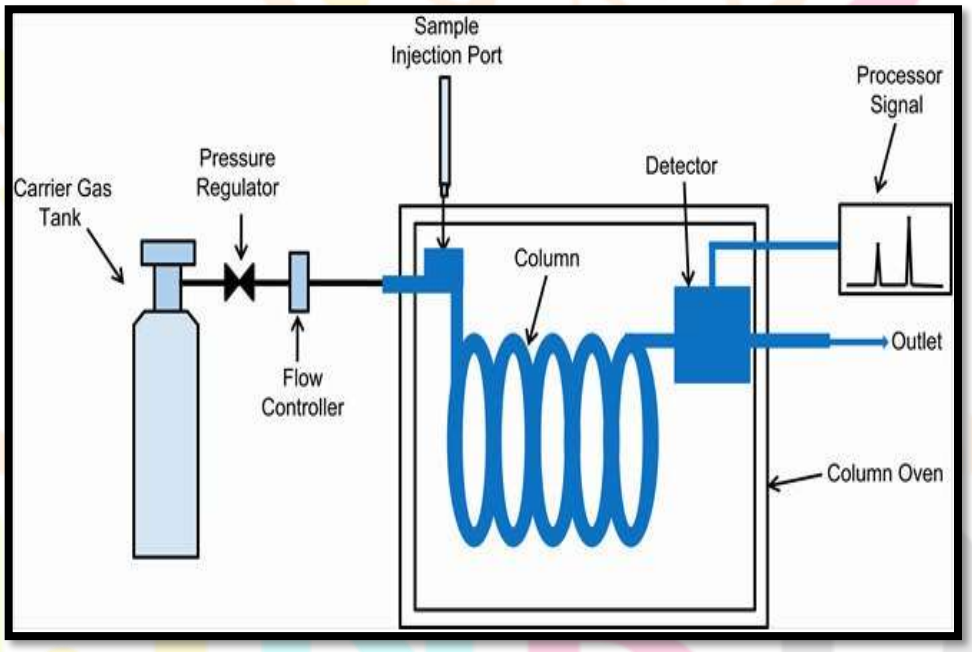
Infrared spectroscopy is the type of absorption spectroscopy that deals with the infrared region of the electromagnetic spectrum. It is used to identify compounds and investigate sample compositions by using infrared spectrophotometer instrument. The IR electromagnetic spectrum is usually divided into three regions i.e. near, mid and far- infrared used to study the fundamental vibrations and associated rotational-vibrational structure present in samples. [19]

3. Separation Method:

Capillary Electrophoresis (CE), Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), High Performance Liquid Chromatography (HPLC) has mainly used for impurity separation as well as degradation products.

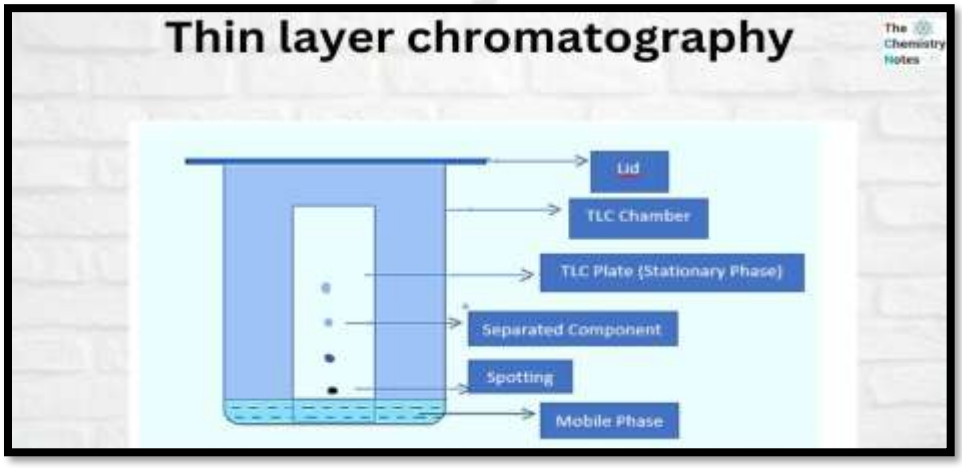
A) Gas Chromatography:

Gas chromatography (GC) is mainly used to identifying a compound, separation, test the purity and analyse compounds in analytical chemistry with obtaining pure compounds. In that chromatography the substance can be vaporized without decomposition in to the mixture of compounds. (20)



B) Thin Layer Chromatography (TLC):

Thin layer chromatography is performed on a sheet of various types of materials like plastic foil, aluminium foil as well as on glass materials. This is coated with silica gel or aluminium oxide in a thin layer. That layer



of adsorbent is called the stationary phase. A solvent mixture i.e. mobile phase is drawn up the plate via capillary action after the sample has been applied on the plate. At Different rates, concentrations and compositions the separation is achieved. Thin layer chromatography mainly applicable to identify, determine the components that are present in plants, monitoring of organic reaction, detection of pesticides or insecticides in food and water as well as unknown compounds analysis.

C) High Performance Liquid Chromatography (HPLC):

HPLC mainly used for separation of compounds. It is based onto the adsorption and partition principle of chromatography. In this the normal phase and reverse phases are used with various types of suitable detectors like refractive index detector, PDA detector, fluorescence detectors, electrochemical detectors, electrical conductivity detectors, light scattering detectors, evaporative light scattering detectors, Corona Charged Aerosol Detector (CAD), Nano Quantity Aerosol Detector (NQAD), etc. HPLC provide an accurate, precise and robust method for quantitative analysis for pharmaceutical products as well as impurities analysis because it has speed, high resolution, sensitivity, reproducibility automation. HPLC mainly used for qualitative, quantitative analysis of degradation products like Stability indicating method for simultaneous determine the compounds present in diprosalic lotion like salicylic acid, betamethasone propionate and their other related compounds by using HPLC instrumental method. [21-27]

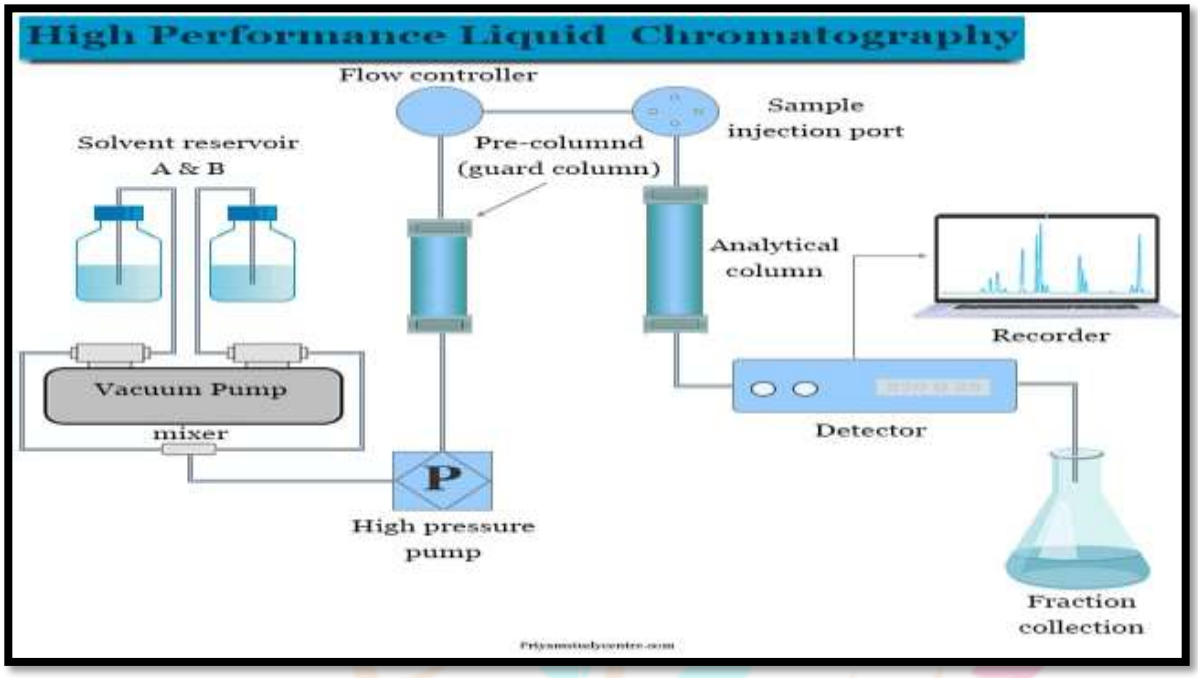
Advantages of HPLC:

- Speed
- High resolution
- Sensitivity
- Reproducibility of +/- 1%
- Accuracy
- Automation.

Disadvantages of HPLC:

- Costly
- Complexity
- Low sensitivity for some compounds
- Irreversibly adsorbed compounds not detected

• Co-elution (two compounds escaping from the tubing at once) difficult to detect



4. Characterization Method:

In this method LC-MS or GC-MS are used mainly in the identification of metabolites, drugs, impurities as well as degradation products as a minor component. Furthermore the NMR and MS are used to characterize them after identification of these minor components.

A) Liquid Chromatography -Mass Spectrometry (LC-MS):

Liquid Chromatography -Mass Spectrometry is a powerful analytical tool used to test and identify product impurities in pharmaceutical development process. The detection limit of a few hundred PPM is difficult to achieve with identification of all the impurities present at concentrations greater than 0.1%. MS based methods is high specificity and sensitivity to provide additional robustness and ruggedness as compared to UV techniques. Highly precise, sensitive Q-TOF mass spectrometers provide higher resolution as well as mass accuracy that enablethe identification of unknown trace impurities. And it is mainly used for genotoxic impurity analysis.

B) Gas Chromatography-Mass Spectroscopy (GC-MS):

Gas chromatography-mass spectrometry is powerful tool in test sample to identify trace elements, different unknown substances with drug detections present in it. The GC-MS has also applicable into forensic substance identifications because it identifies the actual presence of a particular substance in a given sample. [28]

5. Isolation Method:

Chromatographic and non-chromatographic techniques are used for isolation of impurities because isolation of impurities becomes prior one. The analytical scale columns involved flow through reactor for separation of medium for the reactant & product under term chromatographic reactor. For example, ofloratidine impurity was found in loratidine. Other examples like Amikacin &Celecoxib also included in this one.

CURRENT MARKETED FORMULATION WHICH CONTAIN IMPURITY:

Indian pharmacopoeia specifies qualitative, quantitative tests for monitoring known impurities present in certain drugs. A few examples of impurities present in drug and identified by using particular method have given in following table. [29]

Sr. No.	Drug Name	Impurity present	Method used
1.	Amphotericin B	Tetraenes	UV-Spectroscopy
2.	Atropine Sulphate	Apo atropine	UV-Spectroscopy
3.	Flurescene	sodium Dimethyl form amide	Gas Chromatography
4.	Cloxacilin	N,N, dimethyl aniline	Gas Chromatography

Table No.2 Drug name impurity present method used

Application of Resent Trends in Impurity Profiling of Pharmaceuticals:

1. Ensuring Drug Safety and Efficacy
   Helps identify and quantify impurities that may impact the safety or therapeutic effectiveness of pharmaceutical products.
2. Regulatory Compliance
   Aids in meeting strict guidelines set by regulatory agencies such as ICH, USFDA, EMA, and CDSCO regarding impurity limits and specifications.
3. Quality Control and Assurance
   Enhances quality assurance practices by providing insights into advanced analytical techniques used to detect impurities.
4. Support in Drug Development
   Assists in designing safer formulations by understanding impurity formation during synthesis, storage, and degradation.
5. Optimization of Manufacturing Process
   Helps identify process-related impurities, enabling optimization of synthesis and purification steps.
6. Improved Analytical Techniques
   Encourages adoption of modern methods like LC-MS/MS, GC-MS, Q-TOF, UHPLC, and NMR for better detection and quantification.
7. Risk Assessment and Management
   Contributes to the evaluation of potential toxicological effects of impurities, supporting risk management strategies.
8. Research and Academic Study Serves as a basis for M.Pharm/PhD-level research, literature reviews, and case studies.

9. Impurity Threshold and Qualification Helps in determining threshold limits for impurities and qualification based on toxicological data.

10. Pharmacopoeial Updates and Monograph Development. [30, 31]

Conclusion

Pharmaceutical substances are required to comply not only with cGMP, Quality Control (QC), Quality Assurance (QA), and water activity evaluations, but must also fulfil the standards set for newly identified impurities. The isolation and characterization of these impurities are crucial steps in collecting and analysing data that ensures their biological safety, thereby emphasizing the importance and growing relevance of impurity profiling in pharmaceutical research. Various instrumental analytical methods are routinely used for the detection, separation, and quantification of impurities. However, identifying and regulating organic impurities remains a significant challenge due to their multiple origins, such as microbial contamination, degradation of active pharmaceutical ingredients (APIs), and residual intermediates from the manufacturing process. Although the International Council for Harmonisation (ICH) provides structured guidelines for impurity management, current efforts are still insufficient. As a result, there is a strong and immediate need

for global harmonization of impurity standards and regulatory requirements to improve drug safety and consistency across the pharmaceutical industry.

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