Asian Journal of Pharmaceutical Education and Research
Vol -14, Issue-3, July- September 2025
ISSN:2278 7496

REVIEW ARTICLE Impact Factor: 7.014

A REVIEW ON ANALYSIS OF IMPURITY PROFILING IN PHARMACEUTICALS

Akshay S. Shendge[1*], Ramdas B. Darade[1], Amol Gayke[2], Vikram Sarukh[2]

[1]JES's SND College of Pharmacy, Yeola, Nashik, Maharashtra, India
[2]JES's SND College of Pharmacy, Yeola, Nashik, Maharashtra, India

*Corresponding Author's E mail:akshay17shendge@gmail.com
Received 17 March 2025; Revised 21March. 2025; Accepted 16 April. 2025, Available online 15 July. 2025

Cite this article as: Shendge AS, Darade RB, Gayke A and Sarukh V. A review on analysis of impurity profiling in pharmaceuticals. Asian Journal of Pharmaceutical Education and Research. 2025; 14(3):1-14.

https://dx.doi.org/10.38164/AJPER/14.2.2025.1-14

ABSTRACT

This review provides a critical analysis of impurity profiling within the pharmaceutical domain, underscoring its pivotal function in the assurance of drug safety and therapeutic effectiveness. Impurities, constituted by any unintended components within pharmaceutical substances or products, are recognized as potentially arising from diverse phases of the drug lifecycle, encompassing synthetic processes, manufacturing procedures, and storage conditions. The identification and quantification of these impurities, through impurity profiling, are posited as indispensable for the preservation of product quality, compliance with regulatory frameworks (as defined by ICH guidelines), and the optimization of manufacturing paradigms. The imperative for impurity profiling is derived from its direct influence on the safety, efficacy, quality, and stability attributes of pharmaceutical products. A comprehensive discussion of organic, inorganic, and residual solvent impurities is presented, elucidating their respective origins and formation mechanisms. Analytical methodologies, including chromatographic techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and thin-layer chromatography (TLC), alongside spectroscopic technique such as mass spectrometry (MS), nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and ultraviolet-visible spectroscopy (UV-Vis), are emphasized for their efficacy in the detection and characterization of impurities. Moreover, the review accentuates the significance of impurity control through the implementation of robust process development, rigorous analytical monitoring, and adherence to established specifications, thereby ensuring patient safety and the reliability of pharmaceutical products.

Keywords: *Mycobacterium tuberculosis*, anti-mycobacterial activity, mechanisms, resistance, synthetic methods, SARs.

INTRODUCTION

IP defines an impurity as any component of pharmaceutically used drug substances or drug products that is not the chemical entity that constitutes the substance or, in the case of a drug product, is not an excipient in the product.[1]

Pharmaceutical impurities are any substances present in a drug product that are not the intended active pharmaceutical ingredient (API) or an intentionally added excipient. These impurities can originate from various sources throughout the drug lifecycle, including starting materials used in API synthesis, the manufacturing process itself, formulation and packaging, and environmental factors.[2,3]

Since it provides active pharmaceutical ingredients (APIs) of a particular grade, the bulk drug sector serves as the foundation for all pharmaceutical industries. The quality of drugs that are introduced to the market has received a lot of attention in recent decades. Producing high-quality goods is the main obstacle facing the pharmaceutical and bulk medication sectors. To preserve the quality and purity of the product from each industry, strict quality control inspections must be carried out. The type of crystallization and purifying technique, raw materials, and manufacturing procedure all affect how pure an active medicinal ingredient is. The idea of purity evolves over time and is inextricably linked to advancements in analytical chemistry. [4]

IMPURITY PROFILING

The term "impurity profiling" doesn't have a precise definition, but it describes the impurities that are present in the substance being examined and determines the amount of specific contaminants that are actually present in the medication. The impurity profile of a material under investigation is an inventory or explanation of the broadest range of known or unknown contaminants that could be present in every API sample made using a particular controlled production method. The impurity profile must include both qualitative and quantitative information about contaminants.[5]

In the pharmaceutical industry, the process of determining and measuring the different impurities that are present in an Active Pharmaceutical Ingredient (API) is known as impurity profiling. One During the production process, these impurities may originate from a number of sources, including starting materials, solvents, reagents, chemical reaction byproducts, product degradation, as well as contaminants from the environment.[6]

NEED OF IMPURITY PROFILING

Impurity Profiling: The production process of any formulation must include an analysis of the contaminants found in the raw materials used for formulation, as these contaminants may impact the solubility of APIs. The presence of these undesirable compounds or substances may also affect a drug's safety parameters by causing unfavourable drug responses or toxicities in the body, which compromises both the safety and effectiveness of APIs.[7]

Safety: Even at low concentrations, impurities may have toxicological consequences. To protect patient safety, profiling aids in the detection and management of harmful contaminants.[8]

Efficacy: Impurities can interfere with the therapeutic activity of the drug substance, reducing its efficacy. Profiling helps maintain the drug's potency and effectiveness.[9]

Quality: From batch to batch, impurity profiling guarantees the medication product's uniformity and quality. This is essential to preserving patient confidence and product dependability.[10]

Regulatory compliance: As part of the registration and approval procedures for drugs, regulatory bodies such as the FDA and EMA want comprehensive impurity profiles. Profiling guarantees adherence to regulatory requirements and aids in proving the medication product's quality and safety.[11]

Process optimization: By identifying the root cause of impurities in the process of manufacturing, impurity profiling can assist minimize the production of impurities and optimize the process.[12]

Stability: Profiling aids in determining how stable a medication product is over time and in various storage settings. The right shelf life and storage conditions are determined using this information.[13]

Intellectual property: The drug substance itself and the manufacturing process's intellectual property rights can be safeguarded by using impurity profiles.

TYPES AND SOURCES OF IMPURITIES

- **Organic Impurities:**

Starting materials, by-products, intermediates, degradation products, reagents, ligands, and catalysts are examples of organic impurities, which are undesirable substances that can occur during the synthesis, purification, or storage of the drug substance. Their presence can affect the drug product's safety and efficacy, so identifying and controlling them is essential for pharmaceutical quality assurance.

- Starting material or intermediates - Starting materials, which result from improper transition during the synthesis process, are a prevalent kind of organic contaminant in medicines.
- By-products - It is quite rare to obtain a single end product with a 100% yield in synthetic organic chemistry; by-products are always possible. One of the most prevalent process contaminants in medications is by products from adverse reactions.[14,15]
- Products of overreaction - Some of the frequently occurring side reactions (which are unavoidable in drug synthesis) are well- known to the synthetic chemist; other which lead to trace level impurities have to be detected and elucidated during impurity profiling.[15]

- Degradation products - Degradation of the final product during the production of bulk medications might potentially result in impurities. Nevertheless, aging and degradation products from storage and synthesis to various dosage forms are also frequent contaminants in medications.[16,17]
- Reagents, ligands and catalysts – The final products may contain trace levels of contaminants from chemical reagents, ligands, and catalysts utilized in the production of a medicinal ingredient. For instance, chloromethyl tetrahydro-pyran-4-yl ester of carbonic acid.[18]
- Impurities originated from reaction solvents - Impurities may also originate from impurity in the solvents.

- **Inorganic Impurities**
  - Reagents, ligands and catalysts - Although these contaminants are uncommon, they might cause issues in particular processes if manufacturers don't take the right precautions throughout manufacturing.[19]
  - Heavy metals - The primary sources of heavy metals are the water used in the processes, the reactors (if stainless steel reactors are utilized), and the areas where acidification or acid hydrolysis occurs. Glass-lined reactors and demineralized water are simple ways to prevent these heavy metal contaminants.[19]
  - Other materials - Bulk drug production facilities frequently utilize filters or filtering aids like centrifuge bags, and activated carbon is frequently used as well. To prevent these contaminations, fibres and black particles in bulk medications must be regularly monitored. For instance, charcoal and filter helps.[19]

- **Others**
  - Residual Solvents
  - Enantiomeric impurities - Additionally, the API may contain enantiomeric impurities for a single isomer medication that is optically active.[20]

IDENTIFICATION METHODS OF IMPURITY IN PHARMACEUTICALS

A variety of analytical methods are used to find contaminants in medications.[21]

These consist of hyphenated methods (e.g., LC-MS, GC-MS), spectroscopy (e.g., UV, IR, NMR, MS), and chromatography (e.g., HPLC, GC). Chromatography uses the physical and chemical characteristics of the contaminants to separate the drug substance from them.[22]

Spectroscopic techniques, such as infrared (IR), nuclear magnetic resonance (NMR), and ultraviolet-visible (UV-Vis) spectroscopy, can provide valuable insights into the molecular structure of impurities. Hyphenated methods, such as liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), combine the separation capabilities of chromatographic techniques with the sensitive detection and structural elucidation provided by mass spectrometry, offering a robust approach for impurity analysis.[23]

Chromatography:

Chromatography is typically used in chemical analysis, even though it is essentially a separation method. To a limited degree, however, it is also employed for preparatory purposes, especially for the separation of relatively tiny quantities of materials with relatively high intrinsic value. Chromatography is arguably the most potent and adaptable method that an analyst may use today. It can simultaneously offer a quantitative estimate of each ingredient and split a mixture into its constituent parts in a single step. Samples might be solid, liquid, or gaseous, and their complexity can vary from a straightforward combination of two enantiomers to a multi-component mixture with a large variety of chemical species. Additionally, the study can be performed on a basic, low-cost thin layer plate at one extreme or on a highly expensive and sophisticated apparatus at the other.[24]

Separate contaminants from the primary therapeutic ingredient: Depending on their physical and chemical characteristics, several chromatography techniques, including Thin-Layer Chromatography (TLC), Gas Chromatography (GC), and High-Performance Liquid Chromatography (HPLC), can distinguish the therapeutic material from contaminants.[25,26]

Determine and describe impurities: Chromatography can be used in conjunction with other analytical methods, such as Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS), to determine the characteristics and chemical makeup of impurities.

Quantify impurities: Chromatography can accurately measure the amount of each impurity present in the drug substance, allowing for precise control and monitoring.[27]

Track the drug product's stability: Chromatography may be used to evaluate the development of degradation products over time and in various storage settings, giving important information for figuring out the drug product's shelf life.[28]

High-Performance Liquid Chromatography (HPLC): With a high resolution and separation capacity for both qualitative and quantitative analysis, high-performance liquid chromatography (HPLC), also referred to as high-pressure liquid chromatography, is a column chromatographic technique used in pharmaceuticals to separate a mixture of compounds with the goal of identifying, quantifying, and purifying individual components present in the mixture. HPLC can be applied to both volatile and non-volatile compounds.[29]

Gas Chromatography (GC): Gas is used as the mobile phase and either a solid or a liquid is used as the stationary phase in gas chromatography, which is also known as gas solid chromatography (GSC) or gas liquid chromatography (GLC). In gas chromatography, a moving gas phase is passed over a stationary sorbent to separate the mixture components; the only difference between the two is that in the former, a moving gas is used as the mobile phase, while in the latter, it is a liquid. The stationary phase stays the same, i.e. a solid or a liquid.[30]

Thin-Layer Chromatography (TLC): Adsorption is the separating principle. One or more chemicals are detected on a thin layer of adsorbent on a chromatographic plate. The solvent from the mobile phase flows through the TLC plate due to capillary action, which opposes gravity. The components' affinity for the stationary phase determines how they are separated on a thin layer chromatographic plate. The components that have a higher affinity for the stationary phase move more slowly, whereas the components that have a lower affinity move more quickly.[31]

Spectroscopy:

Spectroscopy is a scientific technique used to study the interaction between matter and electromagnetic radiation. It involves analysing the spectrum of electromagnetic radiation emitted, absorbed, or scattered by a substance. By examining this spectrum, scientists can gain valuable information about the structure, composition, and properties of the matter under investigation.[32]

Spectroscopy is essential for identifying contaminants in medicines since it offers comprehensive details on the chemical makeup and structure of the medicinal material as well as possible contaminants.[33]

Mass Spectrometry (MS)

In the analytical chemistry field of mass spectrometry, chemical species in gas-phase ionic form may be recognized and described based on their mass and the quantity of elementary charges they contain.

With modest quantities of material, mass spectrometric investigations can yield structure-rich information about organic molecules, including those detected at trace impurity levels. MS offers crucial information on the molecular weights of the chromatographed compounds when combined with an LC separation and when electrospray (ESI) or atmospheric-pressure chemical ionization (APCI) are employed, particularly when high-resolution mode is used. Additionally, it can reveal the analytes' structure (by the fragmentation pattern), particularly if there are only slight alterations in the impurity relative to the medicinal ingredient and some prior knowledge of the molecular skeleton is known.[34]

One of the most effective analytical methods for locating and describing contaminants in medications is mass spectrometry (MS). This is how it operates:[35]

Ionization: Turning the sample—which might contain a combination of the drug material and contaminants—into ions is the initial stage of mass spectrometry. Usually, methods like matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) are used for this.[36,37]

Mass Separation: Next, using a variety of techniques, the ions are divided according to their mass-to-charge ratio (m/z), including: Quadrupole mass analyzers: These filter ions according to their m/z values using electric fields.[38]

The time it takes for ions to travel a specific distance is measured using time-of-flight (TOF) mass analyzers, which show that lighter ions arrive first.[39]

Ion trap mass analyzers: These devices use electric fields to capture ions, which are subsequently released one after the other for mass analysis.[40]

Detection: After the separated ions are found, a mass spectrum is produced using the data, which displays the ions' abundance in relation to their m/z values.[41]

Identification of Impurities: The existence of impurities can be determined by comparing the sample's mass spectrum to reference spectra or databases that are known to exist.
Even minute levels of contaminants may be identified thanks to the distinct mass-to-charge ratios of various molecules, which function as fingerprints.[42]

- **Nuclear Magnetic Resonance (NMR) Spectroscopy:** A strong analytical technique for structural elucidations is NMR. Regretfully, in comparison to other analytical methods, NMR has historically had poor sensitivity. For example, mass spectroscopy takes less than 1 mg of material, but conventional NMR requires about 10 mg. Therefore, in the past, analytical chemists have not always used NMR spectroscopy as their primary method for identifying an unknown molecule.[43]
- **Infrared (IR) Spectroscopy:** One of the most significant analytical methods is infrared spectroscopy, which has the main benefit of being able to analyze almost any material in almost

any condition. By using the appropriate sample procedure, it is possible to investigate solids, liquids, pastes, powders, fibers, and gases.[46]The infrared (12800–10 cm) portion of the electromagnetic spectrum is the focus of infrared (IR) spectroscopy. Infrared signifies beyond red since the word "infra" means "beyond." It includes a variety of methods, the most popular of which being absorption spectroscopy.[44-45]

- **Ultraviolet-Visible (UV-Vis) Spectroscopy:** Electronic spectroscopy, another name for UV-visible spectroscopy, measures the quantity of light absorbed at each visible and UV wavelength in the electromagnetic spectrum. Molecular excitation results from the molecules' absorption of electromagnetic energy. Atomic absorption spectroscopy is based on the energy that ground state atoms absorb while they are in the gaseous state. The intensity of radiation absorbed or emitted by a sample plotted against frequency (V) or wavelength (λ) is known as spectroscopy.[46]

Other Techniques:

- **X-ray Diffraction (XRD):** High-tech materials early microstructure and impurity phase behavior affect or even dictate their physical characteristics and performance. It is crucial to characterize these impurities and their relationships to the surrounding matrix, yet doing so frequently necessitates a destructive approach that runs the danger of misinterpreting the data. By using an X-ray nanoprobe in conjunction with our advancements in high resolution X-ray diffraction computed tomography, materials with tiny heterogeneous microstructure (with a grain size between 10 nm and 10 μm) may be described non-destructively using crystallography.[47]
- **Thermal Analysis:** The International Confederation for Thermal Analysis and Calorimetry (ICTAC) defines “thermal analysis” as a group of techniques in which a physical or chemical property of a sample is monitored against time or temperature while the temperature of the sample, in a specified atmosphere, is programmed.[48,49]

ICH GUIDELINES FOR IMPURITY PROFILING:

- Comprehensive criteria for impurity profiling in pharmaceutical compounds are provided by the International Conference on Harmonization (ICH). The purpose of these recommendations is to guarantee the effectiveness, safety, and quality of pharmaceuticals.[50,51,52]
- The guidelines for identifying and managing contaminants in novel pharmacological compounds are described in ICH Q3A(R2). It sets a 0.1% cutoff point for identifying unidentified contaminants. An impurity must be recognized and described if it surpasses this level, taking into account any possible effects on efficacy and safety.[53]

- Guidelines for contaminants in novel pharmaceutical products are provided by ICH Q3C(R6). It highlights how crucial it is to comprehend the possible origins of contaminants and how they affect the end product's quality. By taking into account variables including the patient demographic, dose, and delivery method, this guideline promotes a risk-based approach to impurity management.
- Specific guidelines for the management of genotoxic contaminants are provided by ICH M7. It describes a tiered method of risk assessment and control that takes into account the exposure levels and possible genotoxicity of contaminants. The goal of this recommendation is to reduce the possibility of genotoxicity in pharmaceuticals.[54]

IMPURITY CONTROL

- Controlling pharmaceutical impurities is essential to the production of drugs in order to guarantee their efficacy and safety.[55] Starting materials, synthesis procedures, and deterioration during storage are some of the causes of impurities.[56] Controlling these contaminants is crucial to preserving product quality and avoiding negative patient consequences.
- Developing and validating robust processes is one method of impurity control. This entails having a solid grasp of the synthetic pathway, spotting any contaminants, and putting the right controls in place. Monitoring the amounts of impurities in the finished product and during the production process requires the use of analytical techniques. One To guarantee accurate and dependable impurity detection and quantification, these techniques must be sensitive, specific, and precise. [57]
- Another key strategy is to establish appropriate specifications and acceptance criteria for impurities. These limits are based on factors such as the toxicity of the impurity, its potential impact on drug efficacy, and the intended use of the pharmaceutical product. Regular monitoring and testing are necessary to ensure that impurity levels remain within acceptable limits.
- Initiatives for ongoing improvement are also essential to sustaining efficient impurity control. This entails routinely reviewing stability data, process parameters, and analytical techniques. Manufacturers may prevent any problems and uphold the highest standards of quality by regularly assessing and improving these factors.
- To sum up, controlling pharmaceutical impurities is a complex process that calls for an all-encompassing strategy. Manufacturing companies can guarantee the safety and effectiveness of their goods, thereby helping patients all around the world, by combining strong process development, exacting analytical techniques, and continuous improvement programs.

REFERENCES

1. Dhangar KR, Jagtap RB, Surana SJ and Shirkhedkar AA. Impurity Profiling of Drugs towards Safety and Efficacy: Theory and Practice.J. Chil. Chem. Soc. 2017; 62 (2):
2. Pilaniya, K, Chandrawanshi HK, Pilaniya U, Manchandani P, Jain P and Singh N. Recent trends in the impurity profile of pharmaceuticals. *Journal of Advanced Pharmaceutical Technology & Research*, 2010; 1(3): 302–310. doi:https://doi.org/10.4103/0110-5558.72422.
3. U.S. food and Drug Administration. Facts About the Current Good Manufacturing Practice (CGMP). *FDA*; 2024. [online] Available at: https://www.fda.gov/drugs/pharmaceutical-quality-resources/facts-about-current-good-manufacturing-practice-cgmp.
4. Venkatesan P and Valliappan K. Impurity Profiling: Theory and Practice. [online] Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=443094676958de9fd388f34e1aa9ccee30e5bc16.
5. Mande SR, Yelmame SS and Borse LB. A Review on Impurity Profiling In Pharmaceutical Substances. Asian Journal of Pharmaceutical Research and Development. 2024; 12(5): 46-51. DOI: https://doi.org/10.22270/ajprd.v12i5.1477
6. Shree Ganesh Remedies Ltd., India. Sources of Impurities in Pharmaceutical Substances. [online] Available at: https://www.ganeshremedies.com/blog/Sources-of-Impurities-in-Pharmaceutical-Substances.
7. Venkatesan P, and Valliappan K. Impurity Profiling: Theory and Practice, Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalainagar, (2014) :254-269.
8. Dhangar KR, Jagtap RB, Surana SJ and Shirkhedkar AA. Review article impurity profiling of drugs towards safety and efficacy: theory and practice. Journal of the chilean chemical society. 2017; 62(2):3543-3557. Available at: https://jcchems.com/index.php/JCCHEMS/article/view/204.
9. Nadikatla A. Impurity Profiling (Sources of Impurities*)*. 2018. [online] Available at: https://www.slideshare.net/slideshow/impurity-profiling-sources-of-impurities/122984657 [Accessed 14 Jan. 2025].
10. Celma C. Impurity Profiling: Characterization of unknown impurities. 2024. [online] Kymos. Available at: https://kymos.com/news/impurity-profiling/.
11. GMP Insiders. Impurities In Pharmaceuticals: Types, Regulations And Strategies. 2023. [online] Available at: https://gmpinsiders.com/impurities-in-pharmaceuticals/.

12. Palve R, Annasaheb G, Mandale K and P. Reshma. A Review of Impurity Profile in Pharmaceutical Substances. International Journal of Pharmacy and Pharmaceutical Research. 2018; 13(1): 89-100.
13. Yasmeen, A., Sofi, G. and Khan, K. (2020). A Review on Impurity Profiling and its Regulatory Aspects- An Important and Necessary Tool in Stability Studies. *Indo Global Journal of Pharmaceutical Sciences*, 10(01), pp.57–68. doi:https://doi.org/10.35652/igjps.2020.10108.
14. Kushwaha P. The organic impurities in pharmaceuticals. Pharma info. Net. 2008; 6(4).
15. Sandor G. Impurity profile in Bulk Drugs and Pharmaceutical. Anal Bioanal. Chem. 2003, 377, 852-862.
16. Krimpen PC, Bennekom WP, Bult A. A Review on Impurity Profile in Pharmaceutical Substances. Pharm Weekbl.1987, 9, 1-23.
17. Roy J, Mohammad G, Banu A, Pharmaceutical impurities-A mini-review. Indian Drugs. 1993; 5(30): 211-218.
18. Qiu F and Norwood DL. Identification of Pharmaceutical Impurities. Journal of liquid chromatography & related technologies. 2007; 30: 877-935.
19. Telgi VS, Gajeli GB, Chougule GK, Thorat YS, Shivsharan US and Kumbhar ST. Significance of impurity profiling: a review. International Journal of Drug Formulation and Research. 2011; 2: 174-195.
20. Riley TN. Steric aspects of drug action. Pharmacist. 1998; 23(3): 40-51.
21. Pilaniya K, Chandrawanshi HK, Pilaniya U, Manchandani P, Jain P and Singh N. Recent trends in the impurity profile of pharmaceuticals. Journal of Advanced Pharmaceutical Technology & Research. 2010; 1(3): 302–310. doi:https://doi.org/10.4103/0110-5558.72422.
22. Dejaegher B and Dumarey M. Method Development for Drug Impurity Profiling: Part 1. 2010;23(4): 218-224. [online] Available at: https://www.chromatographyonline.com/view/method-development-drug-impurity-profiling-part-1
23. Scott RPW. Principles and Practice of Chromatography. Chrom-Ed Book Series ed. [online] library4science. 2003. Available at: http://www.library4science.com/eula.html [Accessed 13 Jan. 2025].
24. Chaudhary A. HPLC for Impurity Analysis-Veeprho. 2024. [online] Available at: https://veeprho.com/high-performance-liquid-chromatography-hplc-for-impurity-analysis
25. Vijayakumar EKS, Dhore DM and Kumar M. HPLC Method for Simultaneous Determination of Impurities and Degradation Products in Zonisamide. Indian Journal of Pharmaceutical Sciences. 2009; 71(5): 521–526. doi:https://doi.org/10.4103/0250-474X.58183.

26. Chew YL, Khor MA and Lim YY. Choices of chromatographic methods as stability indicating assays for pharmaceutical products: A review. Heliyon. 2021; 7(3): p.e06553. doi: https://doi.org/10.1016/j.heliyon.2021.e06553
27. Talele GS and Shahare HV. A Textbook of Instrumental Methods of Analysis. Pune: Everest Publishing House. 2023; 209-210.
28. Malathi S, Patil PM and Kumar S. Instrumental Methods of Analysis. Lucknow: Thakur Publication PVT. LTD. 2022; 210-11.
29. Sonawane SS, Pathade PA and Kshirsagar SJ. Instrumental Methods of Analysis. Pune: Technical Publications. 2022; 8.2.
30. www.horiba.com. (n.d.). *Spectroscopy Matters*. [online] Available at: https://www.horiba.com/int/scientific/resources/spectroscopy-matters/.
31. Parmar I, Rathod H and Shaik S. A Review: Recent Trends in Analytical Techniques for Characterization and Structure Elucidation of Impurities in the Drug Substances. Indian Journal of Pharmaceutical Sciences. 2021; 83(3): 402-415. doi:https://doi.org/10.36468/pharmaceutical-sciences.789.
32. Deubner R and Holzgrabe U. Micellar electrokinetic capillary chromatography, high performance liquid chromatography and nuclear magnetic resonance—three orthogonal methods for characterization of critical drugs. Journal of Pharmaceutical and Biomedical Analysis. 2004; 35(3): 459–467. doi:https://doi.org/10.1016/j.jpba.2004.01.015.
33. Deschamps E, Calabrese V, Schmitz I, Hubert-Roux M, Castagnos D and Afonso C. Advances in Ultra-High-Resolution Mass Spectrometry for Pharmaceutical Analysis. *Molecules (Basel, Switzerland)*. 2023; 28(5): 2061. doi:https://doi.org/10.3390/molecules28052061.
34. Premierbiosoft.com. Mass Spectrometry. Introduction, Principle of Mass Spectrometry, Components of Mass Spectrometer, Applications. [online] Available at: https://premierbiosoft.com/tech_notes/mass-spectrometry.html [Accessed 14 Jan. 2025].
35. Proteomics C. (n.d.). Electrospray Ionization. [online] Creative Proteomics. Available at: https://www.creative-proteomics.com/support/electrospray-ionization.html
36. Wikipedia. Quadrupole mass analyzer. [online] Available at: https://en.wikipedia.org/wiki/Quadrupole_mass_analyzer
37. Emissions Analytics. (n.d.). Time-of-Flight mass spectrometry. [online] Available at: https://www.emissionsanalytics.com/time-of-flight-mass-spectrometry
38. Bains B. A Beginner's Guide to Mass Spectrometry: Mass Analyzers - ACD/Labs. 2024. [online] Available at: https://www.acdlabs.com/blog/a-beginners-guide-to-mass-spectrometry-mass-analyzers/.

39. Bioquest AAT. What are the four stages of mass spectrometry? AAT Bioquest. 2022. [online] www.aatbio.com. Available at: https://www.aatbio.com/resources/faq-frequently-asked-questions/What-are-the-four-stages-of-mass-spectrometry.
40. Tsizin S, Fialkov AB and Amirav A. Analysis of impurities in pharmaceuticals by LC-MS with cold electron ionization. Journal of Mass Spectrometry. 2020; 55(10): 4587. doi:https://doi.org/10.1002/jms.4587.
41. Lohr LL, Jensen AJ and Sharp TR. NMR Characterization of Impurities, Handbook of Isolation and Characterization of Impurities. 2004; 301-302
42. Sonawane SS, Pathade PA and Kshirsagar SJ. Instrumental Methods of Analysis. Pune: Technical Publications. 2022; 3.2-3.3.
43. Malathi S, Patil PM and Kumar S. Instrumental Methods of Analysis. 2022nd ed. Lucknow: Thakur Publication PVT. LTD. 2022; 80-81.
44. Talele GS and Shahare HV. A Textbook of Instrumental Methods of Analysis. Pune: Everest Publishing House, 2023; 02-03.
45. Bonnin A, Wright JP, Tucoulou R and Palancher H. Impurity precipitation in atomized particles evidenced by nano x-ray diffraction computed tomography. 2017. [online] Available at: https://pubs.aip.org/aip/apl/article-abstract/105/8/084103/1078243/Impurity-precipitation-in-atomized-particles?
46. Plante AF, Fernández JM and Leifeld J. Application of thermal analysis techniques in soil science. Geoderma. 2009; 153(1-2), 1–10. doi:10.1016/j.geoderma.2009.08.016
47. Alsante KM, Baertschi SW, Coutant M, Marquez BL, Sharp TR and Zelesky TC. Degradation and Impurity Analysis for Pharmaceutical Drug Candidates. Separation Science and Technology. 2011; 59–169. doi:10.1016/b978-0-12-375680-0.00003-6
48. EMA. ICH Q3A (R2) Impurities in new drug substances - Scientific guideline European Medicines Agency. European Medicines Agency. [online] Available at: https://www.ema.europa.eu/en/ich-q3a-r2-impurities-new-drug-substances-scientific-guideline.
49. Gmp-compliance.org. ICH Q3A(R2) Impurities in New Drug Substances - ECA Academy. 2024. [online] Available at: https://www.gmp-compliance.org/guidelines/gmp-guideline/ich-q3ar2-impurities-in-new-drug-substances.
50. European Medicines Agency (EMA). ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk - Scientific guideline | European Medicines Agency (EMA). 2023. [online] Available at: https://www.ema.europa.eu/en/ich-m7-assessment-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential-carcinogenic-risk-scientific-guideline.

51. Gmp-compliance.org. ICH Q3A(R2) Impurities in New Drug Substances - ECA Academy. 2024. [online] Available at: https://www.gmp-compliance.org/guidelines/gmp-guideline/ich-q3ar2-impurities-in-new-drug-substances.
52. European Medicines Agency (EMA). ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk - Scientific guideline | European Medicines Agency (EMA). 2023. [online] Available at: https://www.ema.europa.eu/en/ich-m7-assessment-control-dna-reactive-mutagenic-impurities-pharmaceuticals-limit-potential-carcinogenic-risk-scientific-guideline.
53. qualitymatters.usp.org. (n.d.). Shedding Light on Impurities in Medicines | Quality Matters | U.S. Pharmacopeia Blog. [online] Available at: https://qualitymatters.usp.org/shedding-light-impurities-medicines.
54. https://symeres.com/impurity-synthesis/#:~:text=These%20impurities%20and%20decomposition%20products,during%20the%20manufacturing%20and%20storage
55. https://www.registech.com/blog/how-to-identify-and-control-drug-impurities-quickly-with-a-holistic-approach/
56. Intertek.com. Pharmaceutical Impurity Testing. 2020. [online] Available at: https://www.intertek.com/pharmaceutical/gmp-cmc laboratory/pharmaceutical-impurity-testing/.
57. Dsouza SJ, Sandeep DS, Charyulu RN, Gowrav MP and Pradeep HK. Impurities in Drug Substance-An Overview of ICH Q3A, Q3C and M7 Guidelines. International Journal of Pharmaceutical Investigation, 2024; 14(2): 299–305. doi:https://doi.org/10.5530/ijpi.14.2.37.