International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.13 | Issue 4 | Oct - Dec -2024

www.ijpar.com

DOI : https://doi.org/10.61096/ijpar.v13.iss4.2024.793-799

ISSN: 2320-2831

Review

Mini Review Article: Advanced Techniques in Impurity Profiling and Analysis of Pharmaceuticals

Athul T*, Anuja M P, Anusree J, NihaFathima P, Thameema Fairoos M S, Sarif Niroush Konari

Department of Pharmaceutical chemistry, JDT Islam College of Pharmacy, Calicut – 673012, Kerala, INDIA. corresponding author mail: athulpunnyam@gmail.com. Mobile number: 8921498195

*AuthorforCorrespondence: Athul T
Email: athulpunnyam@gmail.com

Published on:19 Nov 2024

Published by:

Dr Sriram Publications

Abstract

Rigorous impurity profiling is crucial to maintaining the safety and efficacy of pharmaceuticals, as even trace levels of impurities can impact drug quality. This review explores the systematic classification, identification, and quantification of impurities in drug substances. It emphasizes the use of advanced analytical methodologies, including both UV-based multicomponent analysis and cutting-edge techniques for identifying elemental and non-elemental impurities. Notably, sophisticated instrumentation such as Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Atomic Absorption Spectroscopy (AAS), Laser-Induced Breakdown Spectroscopy (LIBS), Energy-Dispersive X-ray Fluorescence (ED-XRF), Neutron Activation Analysis (NAA), Glow Discharge Mass Spectrometry (GD-MS), and Thermal Ionization Mass Spectrometry (TIMS) are employed to accurately measure impurities at ultra-trace levels. The necessity of stringent impurity testing is highlighted by recent case studies involving drug recalls, including the withdrawal of valsartan and the recall of metformin due to the detection of carcinogenic impurities. Such incidents highlight the potential risks of inadequate impurity monitoring. This review aims to provide a holistic perspective on impurity profiling by integrating various analytical methodologies, ensuring a more thorough and reliable approach to pharmaceutical quality control. By uniting these advanced techniques, the review outlines a proactive framework that supports regulatory compliance and fosters drug safety, ultimately enhancing public health outcomes.

Keywords: Pharmaceutical impurities, UV multicomponent analysis, ICP-MS, ICP-OES, AAS, TIMS, LIBS, EDXRF,Neutron activation analysis,GDMS, Valsartan, Metformin, NDMA.

INTRODUCTION

Impurities in drug substances can adversely affect the safety, efficacy, and quality of pharmaceuticals. According to International Conference on Harmonization (ICH) Guidelines, impurities are any components of a drug substance that are not the intended chemical entity and can impact the active ingredient's purity. This review explores both the classification of impurities and advanced analytical techniques for their detection, including UV-based multicomponent analysis methods and elemental analysis techniques. The objective is to provide a holistic view of impurity profiling, emphasizing the integration of various methodologies for effective pharmaceutical quality control.[2]

CLASSIFICATION OF IMPURITIES [1] [3]

Understanding the different types of impurities is essential for effective pharmaceutical analysis:

- Organic Impurities: Includes by-products, degradation products, and starting materials.
- Inorganic Impurities: Non-organic substances such as heavy metals, inorganic salts, reagents, and catalysts.
- Residual Solvents: Organic or inorganic solvents used during manufacturing that may remain in trace amounts.
- Elemental Impurities: Metals that may come from manufacturing equipment or raw materials, categorized based on toxicity and permitted daily exposure (e.g., Class 1: As, Cd, Hg; Class 2A: Co, Ni, V; Class 2B: Ag, Au, Pt; Class 3: Ba, Cr, Cu).
- Polymorphic Forms: Different crystalline forms of a drug substance affecting its stability and bioavailability.
- Enantiomeric Impurities: Stereoisomers of the drug substance with differing pharmacological effects.

The study focuses on two primary analytical approaches for impurity profiling: UV-based multicomponent analysis methods and techniques for identifying elemental impurities.

IDENTIFICATION OF ELEMENTAL IMPURITIES

INDUCTIVELY COUPLED PLASMA MASS SPECTROSCOPY (ICP-MS) [4] [6] [37] [46]

ICP-MS quantifies elemental concentrations with precision by ionizing the sample using high-temperature plasma and measuring the mass-to-charge ratio Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a technique in mass spectrometry that employs an inductively coupled plasma to ionize samples. It efficiently atomizes the material, producing atomic and small polyatomic ions, which are subsequently detected. ICP-MS is widely recognized for its ability to analyse metals and certain non-metals in liquid samples at extremely low concentrations. Additionally, it can distinguish between different isotopes of the same element, making it valuable for isotopic labelling studies.When compared to atomic absorption spectroscopy, ICP-MS offers faster analysis, improved precision, and enhanced sensitivity. However, despite its advantages, ICP-MS tends to introduce more interfering species than other mass spectrometric techniques like Thermal Ionization Mass Spectrometry (TIMS) and Glow Discharge

MASS SPECTROMETRY (GD-MS)

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY (ICP/OES)[5] [30] [32] [39] [50]

The principle of Inductively Coupled Plasma/Optical Emission Spectrometry (ICP/OES) is based on the spontaneous emission of photons from atoms and ions excited in radiofrequency-induced plasma. In this technique, a liquid sample is introduced into an argon plasma, where it is dried, vaporized, and subjected to high temperatures (up to 10,000 K) that cause excitation and ionization of the sample atoms. These atoms emit light at specific wavelengths when they return to their ground states, and this emission is characteristic of the elements in the sample. A spectrometer detects and measures the emitted photons, which correspond to the presence and concentration of specific elements in the sample. ICP/OES allows simultaneous detection of multiple elements with high sensitivity and accuracy, making it a widely used method for trace element analysis across various applications, from environmental to industrial fields.

ATOMIC ABSORBTION SPECTROSCOPY (AAS) [7] [8] [31] [45]

Atomic absorption spectroscopy detects the light absorbed by free atoms in their gaseous state. Each element absorbs light at a specific wavelength, and the amount of absorbed light indicates the elements concentration. The principle of Atomic Absorption Spectroscopy (AAS) is based on the absorption of light by free, ground-state atoms in the gaseous state. When a light beam at a specific wavelength, which corresponds to the energy difference between the ground state and an excited state of the target element, passes through a sample containing the element of interest, the atoms absorb part of the light. This absorption correlates to the element's concentration in the sample and is governed by Beer's Law, which states that absorbance is directly proportional to concentration. This technique typically involves using a monochromatic radiation source, like a hollow cathode

lamp, that emits light specific to each element being measured. The selectivity of AAS is critical because each element absorbs light at a unique wavelength, allowing for targeted analysis in complex matrices.

LASER INDUCED BREAKDOWN SPECTROSCOPY.[9] [10] [11]

Laser Induced Breakdown Spectroscopy (LIBS) works by focusing a short, intense laser pulse onto a material, creating a plasma. The plasma, composed of ionized atoms from the material, emits light as it cools. The emitted light contains spectral lines unique to the elements present, which are analyzed using a spectrometer. This allows for the identification and quantification of the elemental composition of the sample, relying on the assumption that the plasma is The intensity of these spectral lines is proportional to the concentration of the elements, allowing both qualitative (identification of elements) and quantitative (measurement of concentration) analysis. LIBS is particularly advantageous for elemental analysis due to its multi-elemental detection capability, fast response time, and minimal sample preparation. However, challenges like self-absorption and matrix effects can complicate quantification and must be addressed to ensure accurate measurements in local thermodynamic equilibrium (LTE).

ENERGY DISPERSIVE X RAY FLUORESCENCE[12] [13] [43]

XRF analytical methods quantify the relative concentration of each element in a sample by utilizing atomic properties like absorption and fluorescence.EDXRF involves irradiating a sample with X-rays, which excite the atoms in the sample, causing them to emit characteristic secondary X-rays as they return to a lower energy state. These emitted X-rays are then detected and measured. The energy and intensity of emitted X-rays correlate with the elements in the sample, enabling the determination of its elemental composition. EDXRF is a non-destructive technique, requiring minimal sample preparation, and can simultaneously measure multiple elements, including trace impurities.

NEUTRON ACTIVATION ANALYSIS. [14] [15] [42]

Neutron Activation Analysis (NAA) operates on the principle of irradiating a sample with neutrons, which causes certain elements within the sample to capture these neutrons and form radioactive isotopes. As these isotopes decay, they emit gamma rays with specific energies unique to each element. By measuring these gamma rays, NAA can identify and quantify multiple elements in the sample simultaneously, even at very low concentrations. This method is non-destructive and requires minimal sample preparation, making it highly effective for trace metal analysis in various fields, including biomedical research.

GLOW DISCHARGE MASS SPECTROMETRY. [16] [17] [18]

Glow Discharge Mass Spectrometry (GDMS) is a technique for detecting and quantifying trace elements in solid samples. It works by creating a plasma through the application of a direct current or radio frequency between a cathode (sample) and an anode in a low-pressure argon environment. Argon ions bombard the sample, leading to the sputtering, atomization, and ionization of atoms. These ions are then separated by their mass-to-charge ratio and detected by a mass spectrometer. GDMS excels in reducing matrix effects, allowing for accurate quantification without matrix-matched standards. It is particularly useful for trace element analysis and depth profiling of layered materials, offering high sensitivity and precision. The technique is widely used in industries like metallurgy and semiconductor manufacturing due to its ability to analyze samples with minimal interference.

THERMAL IONIZATION MASS SPECTROSCOPY[19] [20]

In Thermal Ionization Mass Spectrometry (TIMS), the process begins by placing the sample on a filament, usually made of a metal like rhenium or tantalum, which is then heated to high temperatures. This heat provides the energy needed for atoms in the sample to overcome their ionization energy, resulting in the formation of ions. TIMS primarily focuses on creating positive ions by thermal ionization, as this is more efficient for elements with relatively low first ionization energies (below approximately 7.5 eV). However, for certain elements that do not ionize easily in their neutral state, oxide or other molecular ions can be generated to enhance ionization efficiency. The ions are then directed into the mass spectrometer, where they are separated based on their mass-to-charge ratio, allowing for precise isotope ratio analysis. One limitation of TIMS is that elements with high ionization potentials or high volatility are not as easily ionized. However, the introduction of additives like silica gel can help extend the technique to some elements with higher ionization potentials (e.g., zinc with 9.4 eV). This method is highly valued for its precision in isotope ratio measurement, which has applications in various fields, including geochronology, environmental science, and nuclear research.

UV-BASED MULTICOMPONENT ANALYSIS METHODS [40] [47] [48]

Several advanced spectrophotometric techniques are used to enhance the accuracy of analytical measurements, especially in complex mixtures and for detecting impurities:

DIFFERENCE SPECTROSCOPY[21] [23] [54] [55]

Difference Spectrophotometry involves measuring the absorbance differences between solutions of the analyte in different chemical forms. By comparing these forms, it becomes easier to identify impurities that might otherwise go undetected in traditional measurements. This method is particularly useful for distinguishing between the analyte and impurities that show overlapping spectra.

DERIVATIVE SPECTROSCOPY[22] [25]

Derivative Spectrophotometry enhances spectral resolution by calculating the derivatives of absorbance spectra. By transforming the original spectra into their first, second, or higher-order derivatives, this method improves the detection of subtle spectral features, which can help in identifying low-concentration impurities that would be difficult to resolve with normal spectrophotometry. The technique is especially useful in separating overlapping spectral peaks, thus improving precision in quantifying each component.

DERIVATIVE RATIO SPECTRA METHOD[24] [26]

The Derivative Ratio Spectra Method builds upon the principles of derivative spectrophotometry by employing absorbance ratio spectra. By using the ratios of the absorbance at different wavelengths, this technique removes constant interference, allowing for a clearer analysis of the individual components in a mixture. This is particularly useful when dealing with complex matrices where baseline shifts or other interferences might obscure accurate detection.

Q ABSORBANCE RATIO METHOD [21] [23] [25] [55]

The Q-Absorbance Ratio Method provides a more targeted approach to determining the concentration of components in a mixture. By calculating absorbance ratios at specific wavelengths where the components have known absorbances, this method ensures accurate quantification even in cases where the components have overlapping spectra. It is widely used in pharmaceutical analysis to precisely measure active ingredients in the presence of excipients.

ISOBESTIC POINT METHOD[22][24]

Lastly, the Isobestic Point Method is a powerful tool for analyzing mixtures with overlapping spectra. The isobestic point is a specific wavelength where two or more substances have the same absorptivity, meaning their combined spectra intersect. By measuring at this point, it becomes possible to determine the concentrations of individual components in a mixture, making this method highly effective in situations where spectral overlap occurs, such as in the analysis of drug degradation or reaction kinetics.

Together, these methods expand the capabilities of traditional spectrophotometry, improving the detection and quantification of components in complex mixtures and enhancing the ability to identify impurities.

DISCUSSIONS

In terms of UV-based multicomponent analysis, the Simultaneous Equation Method proves effective for analyzing mixtures with distinct absorbance wavelengths, while Difference Spectrophotometry enhances specificity by altering chemical forms to detect trace impurities. Derivative Spectrophotometry is particularly useful for highlighting minor spectral features, aiding in the detection of low-concentration impurities. The Derivative Ratio Spectra Method resolves complex mixtures by eliminating constant interference, and the Q-Absorbance Ratio Method offers accurate quantification through absorbance ratios. The Isobestic Point Method is effective for the simultaneous determination of substances with spectral overlap.

For elemental impurity detection, ICP-MS is highly sensitive and precise, making it suitable for trace elemental analysis. ICP-OES provides effective multi-element analysis and offers a comprehensive impurity profile. AAS is accurate for determining specific elemental concentrations based on light absorption.

CASE STUDIES[27] [28] [29]

Recent case studies emphasize the critical need for stringent impurity testing. In the case of Valsartan, the detection of the carcinogenic impurity N-Nitrosodimethylamine (NDMA) led to a product recall, highlighting the importance of rigorous impurity profiling. EMA is reviewing valsartan medicines due to the presence of NDMA, aprobable carcinogen found in the active substance from Zhejiang Huahai Pharmaceuticals. The estimated risk is one extra case of cancer for every5,000 patients taking the highest dose daily for 7 years. . EMA is collaborating internationally and will provide updates on its website as the review progresses.

Similarly, NDMA contamination in Metformin Hydrochloride Extended-Release Tablets prompted a recall, further underscoring the necessity for continuous monitoring of pharmaceutical impurities. Lupin Pharmaceuticals Inc. has issued a voluntary recall of Metformin Hydrochloride Extended-Release Tablets of 500mg and 1000mg due to the detection of N-Nitrosodimethylamine (NDMA) impurity.

SUMMARY

Ensuring the safety and efficacy of pharmaceutical products is critical, and one of the essential steps in this process is the rigorous profiling and analysis of impurities. Impurities, as defined by the International Conference on Harmonization (ICH) guidelines, refer to any component of a drug substance that is not the intended chemical entity. These impurities can negatively affect the purity, safety, and efficacy of pharmaceuticals. This review explores advanced techniques used in impurity profiling, focusing on both the classification of impurities and the analytical methods for their detection. The importance of stringent impurity testing is underscored by recent case studies, such as the withdrawal of Valsartan and the recall of Metformin, both of which were found to contain carcinogenic impurities like N-Nitrosodimethylamine (NDMA).Impurities in pharmaceuticals can be classified into various types, including organic impurities (by-products, degradation products, and starting materials), inorganic impurities (such as heavy metals and inorganic salts), residual solvents, elemental impurities, polymorphic forms, and enantiomeric impurities. These different types of impurities can originate from the manufacturing process, degradation over time, or interactions with raw materials. Effective impurity profiling and analysis require advanced techniques to accurately detect and quantify these impurities, ensuring the drug's quality and safety. One of the key methods used in impurity analysis is UV-based multicomponent analysis. This involves techniques like the Simultaneous Equation Method (Vierordt's Method), which utilizes differential absorbance at distinct wavelengths to quantify components in a mixture, and Difference Spectrophotometry, which measures absorbance differences between the analyte in different chemical forms. Derivative Spectrophotometry, which calculates the derivative of absorbance spectra to enhance resolution, and the Derivative Ratio Spectra Method, which uses absorbance ratios to remove interference, are also valuable techniques. The Q-Absorbance Ratio Method and the Isobestic Point Method, both of which rely on absorbance ratios, offer accurate quantification of components in pharmaceutical mixtures.

In addition to UV-based methods, the detection of elemental impurities is critical for ensuring pharmaceutical safety. Advanced techniques such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), which measures ions based on their mass-to-charge ratio, and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), which analyzes light emitted from excited atoms, are highly effective in detecting trace elements. Atomic Absorption Spectroscopy (AAS), which measures light absorption by free atoms, is another important tool for determining specific elemental concentrations. The importance of advanced impurity analysis techniques is highlighted by recent case studies in the pharmaceutical industry. The detection of NDMA, a carcinogenic impurity, led to the recall of several batches of Valsartan and Metformin, emphasizing the need for rigorous impurity testing. These incidents demonstrate the critical role of advanced analytical methods in identifying harmful impurities and ensuring the safety of pharmaceutical products.

CONCLUSION

In conclusion, impurity profiling and analysis are vital components of pharmaceutical quality control. The use of advanced techniques such as UV-based multicomponent analysis and elemental impurity detection methods ensures accurate impurity profiling, helping pharmaceutical companies comply with regulatory requirements and safeguard patient safety. As the pharmaceutical industry continues to evolve, ongoing advancements in analytical methods and regulatory practices will be crucial in addressing emerging challenges in impurity analysis.

Acknowledgement

The authors would like to express their sincere gratitude to JDT Islam College of Pharmacy and the orphanage management for providing the necessary research facilities. Special thanks are extended to Dr. Anjana John, Principal, for her continuous encouragement and unwavering support, which were instrumental in providing essential facilities throughout the study duration.

REFERENCES

1. International Conference on Harmonisation. ICH harmonised guideline for elemental impurities: Q3D (RI). 2019 Mar.
2. Ahuja S. Impurities evaluation of pharmaceuticals. New York: Marcel Dekker; 1998.
3. Food and Drug Administration. FDA recall list for valsartan-containing products. National Center for Biotechnology Information. Available from: [NCBI website]
4. Heumann KG. Int J Mass Spectrom Ion Processes. 1992;118:575.
5. Boss CB, Fredeen KJ. Concept, instrumentation and techniques in inductively coupled plasma optical emission spectrometry. 2nd ed. Norwalk: Perkin-Elmer; 1997.

6. Carey JM, Caruso JA. Electrothermal vaporization for sample introduction in plasma source spectrometry. Crit Rev Anal Chem. 1992;23(5):397–439.
7. García R, Torres MC, Báez A. Determination of trace elements in total suspended particles at the Southwest of Mexico City from 2003 to 2004. Rev Chem Ecol. 2008;24(2):157-67.
8. García R, Belmont R, Padilla H, Torres MC, Báez A. Trace metals and inorganic ions measurements in rain from Mexico City and a nearby rural area. Rev Chem Ecol. 2009;25(2):71-86.
9. Song K, Lee Y, Sneddon J. Advances in atomic spectroscopy. Amsterdam: Elsevier Science; 2002. p. 6.
10. Noll R. Laser-induced breakdown spectroscopy: fundamentals and applications. Berlin: Springer; 2012.
11. Doucet FR, Lithgow G, Kosierb R, Bouchard P, Sabsabi M. J Anal AtSpectrom. 2011;26:536−41.
12. Abbey S. Studies in "standard samples" of silicate rocks and minerals, 1969-1982. Ottawa: Geological Survey of Canada; 1983. Paper 83-15, p. 114.
13. Andermann G, Kemp JW. Scattered X-rays as internal standards in X-ray emission spectroscopy. Anal Chem. 1958;30:1306-9.
14. Hambidge K. The need for trace element analyses in the medical sciences. Elemental Analysis of Biological Materials. 1980;19–28.
15. Advisory Group of The International Atomic Energy Agency. Quality assurance in biomedical neutron activation analysis. Vienna: ACA; 1982. p. 1–29.
16. Pisonero J, Fernández B, Pereiro R, Bordel N, Sanz-Medel A. Trends Anal Chem. 2006;25:11–8.
17. Nelis T, Paying R. Glow discharge optical emission spectroscopy: a practical guide. Cambridge: The Royal Society of Chemistry; 2003.
18. Hoffmann V, Kasi M, Robinson PK, Venzago C. Anal Bioanal Chem. 2005;381:173–88.
19. Klinger JA, Savickas PJ, Harrison WW. The pulsed glow discharge as an elemental ion source. J Am Soc Mass Spectrum. 1990;1:138–43.
20. Faure G. Principles of isotope geology. 2nd ed. New York: John Wiley & Sons; 1986.
21. Shanker RS. Textbook of pharmaceutical analysis. 4th ed. Tirunelveli: Rx Publication; 2018.
22. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. Mumbai: Himalaya Publishing House; 2009.
23. Skoog DA, Holler JF, Crouch SR. Principles of instrumental analysis. 7th ed. 2018.
24. Shahare HV, Talele GS, Pathade PA, Gedam SS. Advanced instrumentation techniques. Pune: Nirali Prakashan Publications; p. 9.3-9.7.
25. Malathi S, Patil PM, Kumar S. Instrumental methods of analysis. Lucknow: Thakur Publications; p. 46-58.
26. Sharma V, Gupta V. Instrumentation for environmental monitoring. 2016.
27. U.S. Food and Drug Administration. FDA updates and press announcements on angiotensin II receptor blocker (ARB) recalls, including Valsartan, Losartan, and Irbesartan. FDA.
28. U.S. Food and Drug Administration. FDA updates on Metformin extended release NDMA contamination. FDA.
29. International Agency for Research on Cancer. IARC Monographs on the evaluation of carcinogenic risks to humans – N-Nitrosodimethylamine. IARC.
30. Ahuja S, Scypinski S. Handbook of modern pharmaceutical analysis. Amsterdam: Elsevier Science; 2001.
31. Dong MW. Modern HPLC for practicing scientists. Hoboken: John Wiley & Sons; 2006.
32. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th ed. Belmont: Wadsworth Publishing; 1988.
33. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. 3rd ed. Hoboken: John Wiley & Sons; 2010
34. SarifNiroushKonari, Jane T Jacob. Stability Indicating LC Analytical Method Development and Validation for the Simultaneous Estimation of Flucloxacillin and Amoxicillin in Pharmaceutical Dosage Form. J.Taibah Uni. Sci. 2015; 9(2): 167-176..
35. Konari S. and Jacob J. Stability indicating Validated UPLC Technique for the Simultaneous Analysis of Raltegravir and Lamivudine in Pharmaceutical Dosage Forms, HIV and AIDS Review. 2016; 15(4): 161-169.
36. SarifNiroushKonari, Jane T Jacob. Stability indicating Validated RP-HPLC Technique for the Analysis of Multicomponent Anti-Diabetic Drug Combos in Pharmaceutical Dosage Forms. Karbala Int. J. Mod. Sci. 2015; 1(1): 39-48,
37. Helaleh MI, Al-Omair A, Hassoun ME. Development and validation of a method for the determination of pharmaceutical impurities using gas chromatography–mass spectrometry. J Pharm Biomed Anal. 2015;114:180-6.
38. Rizvi SA, Saleem S. Applications of capillary electrophoresis in pharmaceutical analysis: a review. Anal Sci. 2010;26(3):327-35.

39. Patil KR, Rane RA, Shinde DD. Quality control of pharmaceutical substances: comprehensive impurity profiling by LC-MS/MS. J Anal Bioanal Tech. 2014;5(4):189-94.
40. Hori Y, Fujita K, Uchiyama N, Muto S. Simultaneous determination of pharmaceutical impurities using HPLC–UV method. J LiqChromatogrRelat Technol. 2016;39(12):547-53.
41. Fillet M, Hubert P. Liquid chromatography–tandem mass spectrometry in pharmaceutical analysis. Trends Anal Chem. 2004;23(5):357-65.
42. Yadav AK, Khanna S, Duraipandiyan V. Characterization of pharmaceutical impurities using advanced spectroscopic techniques. SpectrochimActa A MolBiomolSpectrosc. 2018;193:257-65.
43. Nageswara Rao R, Venkateswarlu N. Determination of pharmaceutical impurities using high-resolution mass spectrometry. J Chromatogr Sci. 2008;46(4):362-9.
44. Balzarini J, Neyts J. Identification and analysis of impurities in antiviral drugs. Antiviral Res. 2009;81(3):193-207.
45. Daughtrey EH, Moody JR. Analysis of trace impurities in drugs by atomic absorption spectrometry. J Pharm Sci. 1978;67(5):679-84.
46. Yang Y, Zhang X, Shi Y, Song H, Chang J. Detection and quantification of pharmaceutical impurities by liquid chromatography-mass spectrometry. J Pharm Biomed Anal. 2015;102:278-83.
47. Grover L, Bhandari A, Koul S, Kaloti M. Spectrophotometric techniques in pharmaceutical impurity profiling. Int J Pharm Pharm Sci. 2016;8(4):1-5.
48. Xu C, Zheng Z, Zhao X, Li S, Jin W. Application of Raman spectroscopy in pharmaceutical impurity analysis. SpectrochimActa A MolBiomolSpectrosc. 2020;224:117386.
49. Caruso JA, Montes-Bayón M, Luo P. Elemental speciation for pharmaceutical analysis using inductively coupled plasma mass spectrometry. J Anal At Spectrum. 2001;16(7):734-8.
50. Jain VK, Garg AK, Gupta R. Assessment of pharmaceutical impurities using ICP-OES. Anal Bioanal Chem. 2015;407(9):2511-20.
51. Kaneta T, Furusawa M, Tanaka S. Analysis of trace pharmaceutical impurities by capillary electrophoresis-mass spectrometry. J Chromatography A. 2011;1218(47):8726-32.
52. Lee DC, Webb M, Lin TY. Detection of impurities in pharmaceuticals by infrared spectrophotometry. Vis pectroscopy. 2003;31(1):71-9.
53. Swartz ME, Krull IS. Analytical method development and validation for pharmaceutical impurities. J Pharm Biomed Anal. 2012;54(5):1044-51.
54. Sarif NK, Jacob JT, Prakash V. Stability indicating UV spectrophotometric method for linagliptin and metformin in pharmaceutical dosage form. Pharm Methods. 2017;8(2):121-6
55. Jacob JT, Niroush SN. Spectrophotometric Forced Degradation Studies for Prasugrel and Aspirin in Pharmaceutical Dosage Form. *Inventi Rapid: Pharm Analysis & Quality Assurance.* 2018;2018(4):1-6.