



Evolution of LC-MS in pharmaceutical analysis: From historical development to modern application - A comprehensive review

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Abstract

Pharmaceutical analysis is a vital branch of analytical chemistry ensuring the safety, quality, and efficacy of active pharmaceutical ingredients and formulations as per ICH guidelines. It encompasses qualitative and quantitative evaluation using classical, spectroscopic, chromatographic, electrochemical, and biological methods. However, analysis of complex pharmaceutical, biological, environmental, and forensic matrices requires enhanced sensitivity, selectivity, and structural information, leading to the development of hyphenated techniques.

Hyphenated techniques combine a separation technique with a detection technique to provide more reliable data. This review focuses on liquid chromatography-mass spectrometry, a powerful hyphenated platform that synergizes high-performance liquid chromatography separation with mass spectrometric detection.

The development of LC-MS began in the late 1960s with liquid-introduction interfaces, progressed through direct liquid introduction in 1979, and was revolutionized with electrospray ionization in 1984 and its interface in 1985. Modern instrumentation comprises solvent delivery system, autosampler, chromatographic column, ionization source such as electrospray and atmospheric pressure chemical ionization, mass analyser such as quadrupole, time-of-flight and Orbitrap, and data system. Advanced variants include LC-MS/MS, LC-NMR, CE-MS, LC-MS-NMR, GC-MS/MS, and multidimensional LC×LC and GC×GC, which improve resolution of complex mixtures.

Currently, LC-MS is indispensable in drug substance and product analysis, drug discovery and absorption, distribution, metabolism, and excretion studies, pharmacokinetic and bioanalytical studies in plasma and urine, impurity profiling, stability and degradation studies, metabolomics, proteomics, lipidomic, biopharmaceutical analysis, biomarker discovery, food safety, environmental and forensic applications.

Recently, artificial intelligence and machine learning have been integrated with LC-MS for automated data preprocessing, peak detection, retention time prediction, spectral annotation, non-targeted analysis, biomarker discovery, and quality-control anomaly detection. While promising, AI integration requires validated datasets, interpretability, and regulatory acceptance.

Keywords: Pharmaceutical analysis, LC-MS, hyphenated techniques, Artificial Intelligence, bioanalysis

Introduction

Pharmaceutical Analysis

Pharmaceutical Analysis is a branch of analytical chemistry that focuses on the identification, purification and structural characterization of active pharmaceutical ingredients (APIs) and excipients ^[1].

It is essential for ensuring the safety, quality and efficacy of medicines throughout the stages of drug development, manufacturing, storage and shelf life while complying with international regulatory standards such as ICH ^[2].

Pharmaceutical analysis is the branch of pharmaceutical science that focuses on the detection, identification, qualification

and purification of chemical substances. It involves the application of analytical technique to separate the components of complex mixtures, determine the composition pH samples and characterize the chemical structure of compounds ^[3].

Pharmaceutical analysis plays a vital role in ensuring the quality, safety and efficacy of the medicines it is based on the principles of chemistry, physics, microbiology, nuclear science and electronics ^[4].

It mainly involves qualitative analysis (identification) and quantitative analysis (estimation) of drugs. Drugs and pharmaceutical substances may be organic; inorganic is obtained for other natural or synthetic sources ^[5].

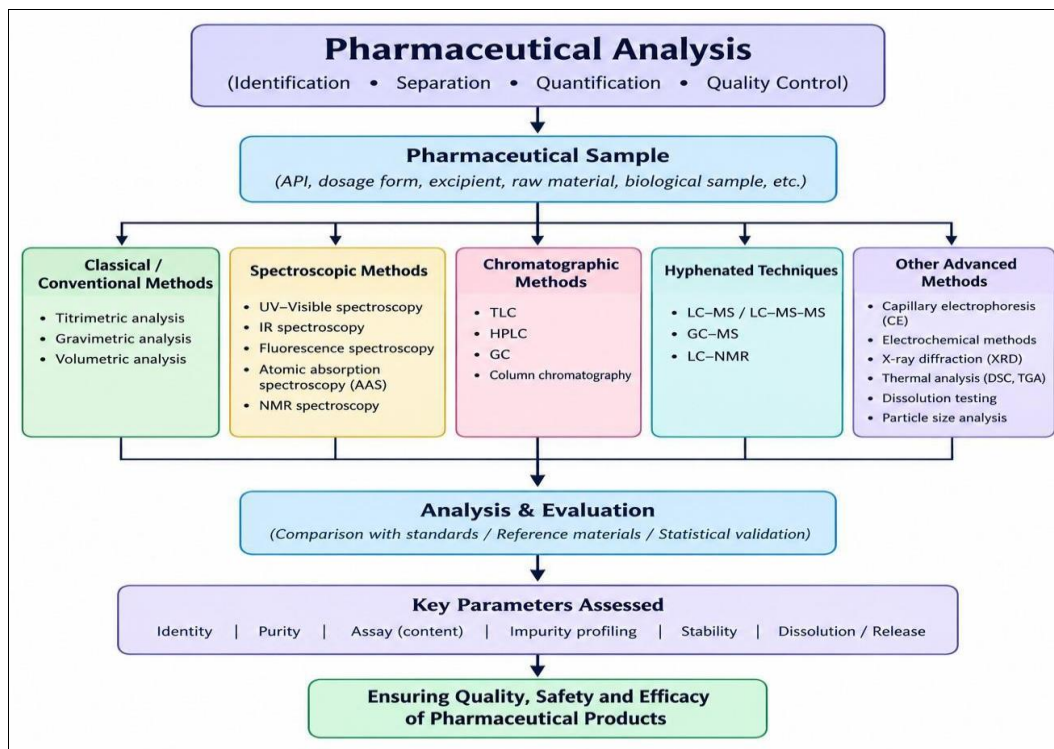


Fig 1: Classification of Pharmaceutical Analytical Methods for Analysis

Major Branches of Pharmaceutical Analysis

Classical and chemical methods

- Titrimetric / volumetry
- Gravimetry

Spectroscopic methods

- Infrared (IR) spectroscopy
- NMR & Mass spectrometry (MS)
- HPLC (High-performance liquid chromatography)
- GC (Gas chromatography)

Electrochemical Methods

- Potentiometry
- Cyclic voltammetry
- Biological Methods [6]

Chromatography

Chromatography was invented by the “Russian Italian botanist” “Mikhail Tsvet” in 1903. Chromatography is an

analytical separation technique widely used to separate, identify, and purify individual components present in complex chemical and biological mixtures [7]. It achieves this by passing the mixture (mobile phase) through a medium (stationary phase), allowing the components to travel at different speed based on the distinct physical or chemical properties [8].

Chromatography is a separation technique in which two mutually immiscible phases are brought into contact one phase remains stationary (stationary phase) while the other moves (mobile phase). The separation of components occurs due to their different interactions with these two phases [9]. Chromatography is a separation technique used to separate the components of a mixture into individual components based on their equilibrium distribution between a stationary phase and mobile phase [10]. It has undergone explosive growth over the last 30 years. The term Chromatography is derived from the Greek words 'chromates' meaning Colour and 'graph's' meaning to write [11].

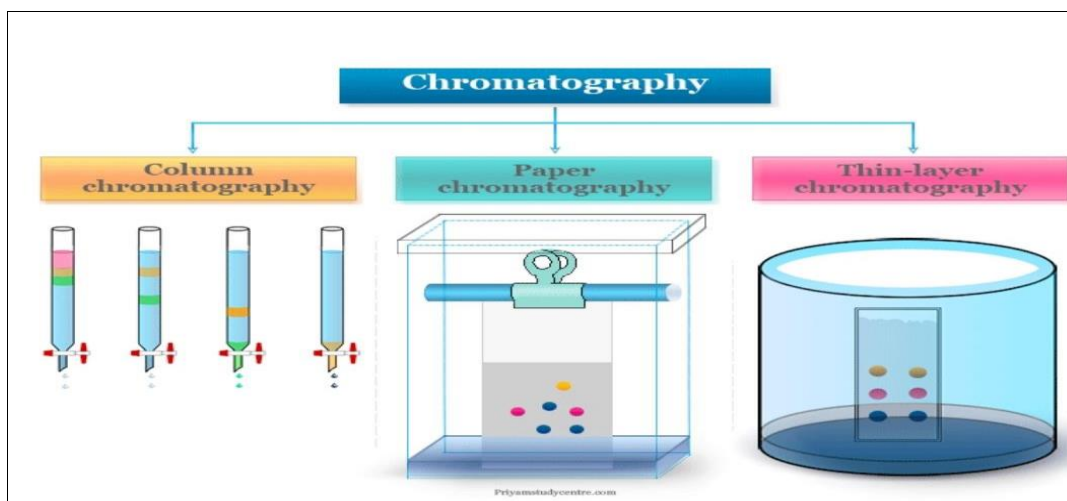


Fig 2: Types of Chromatography Techniques

Different Methods

- Adsorption chromatography
Ex: Column chromatography
- Thin layer chromatography (TLC)
- Partition chromatography
Ex: Paper chromatography
- Gas liquid chromatography (GLC)
- Ion-Exchange chromatography
- Affinity chromatography
- Gas chromatography (GC)
- High-performance liquid chromatography (HPLC) ^[12]

Advantages

- Chromatography provides high separation efficiency making it suitable for analysing complex mixtures.
- It offers high sensitivity and selectivity, enabling the detection of even trace amounts of compounds.
- The technique ensures accurate qualitative and quantitative analysis of various substances.
- Only a small volume is required to perform chromatographic analysis effectively.
- Chromatography is highly suitable for the purification and isolation of individual compounds from mixtures.
- It can be used to analyze a broad range of sample types in various analytical fields.

Disadvantages

- High cost of sophisticated instruments.
- Required trained persons for operation.
- Time consuming sample preparation in some methods.
- Uses expensive and sometimes hazardous solvents.
- Regular maintenance and calibration are required.
- Some techniques are limited to specific types of compounds.
- Instrumental methods require a stable laboratory environment ^[13].

Spectrometry

Spectrometry is an analytical method used to characterize chemical compounds and measure their concentrations by examining how they interact with electromagnetic radiation ^[14]. It has widespread applications in pharmaceutical analysis, chemistry, biotechnology, environmental science and clinical diagnosis ^[15]. Its excellent sensitivity, precision, and analytical performance make spectrometry an important technique in contemporary scientific investigations and quality control processes. Spectrometry is one of the most widely applied analytical techniques in pharmaceutical analysis for the identification and qualification of active pharmaceutical ingredients (APIs), impurities, degradation products and excipients. The technique is recognized for its excellent sensitivity, accuracy, rapid analysis and capability to detect analytes present at trace level ^[16].

The continuous advancement of spectrometry technology has established spectrometry as an indispensable technique in pharmaceutical analysis. It is extensively used in drug development, quality control, stability testing and regulatory compliance to ensure the safety, efficacy and quality of pharmaceutical products ^[17].

Different Methods of Spectrometry

- Mass spectrometry (MS)
- Ultraviolet-visible spectrometry (UV-VIS)
- Infrared spectrometry (IR)
- Nuclear magnetic resonance (NMR)

- Atomic absorption (AAS) ^[18]

Advantages

- Spectrometry has high sensitivity and specificity making it possible to detect and measure compounds even at very low concentration.
- Spectrometry enables rapid and reliable analysis, making it suitable for routine testing and pharmaceutical investigations.
- Many spectrometric methods are non-destructive so the sample can be used for further testing.
- It helps determine the structural characteristics, chemical compositions and purity of pharmaceutical substances.
- Only a small amount of sample is required making the technique efficient and cost-effective ^[19].

Disadvantages

- Spectrometric instruments are expensive to buy and maintain.
- Operating spectrometric instruments and accurately interpreting the analytical data require trained and experienced personnel.
- Certain analytical procedures involve lengthy sample preparations before the actual measurements can be performed.
- Regular calibration and maintenance are necessary to obtain accurate results.
- Matrix effects and spectral interferences can affect the accuracy and precision of the analysis ^[20].

Why Need Hyphenated Techniques

- Hyphenated analytical techniques combine separation and detection methods to improve sensitivity, selectivity, accuracy, and the speed of analysis ^[21].
- Development in technology, chemistry and physics have contributed to the development of novel chemical and analytical techniques while enhancing existing methods ^[22].
- These techniques are particularly useful for the analysis of complex pharmaceutical, biological, environmental and food samples ^[23].
- Hyphenated techniques are needed because they combine two different analytical techniques, usually a separation technique with a detection or identification technique. This combination provides more reliable and detailed information for the analysis of complex samples.
- Hyphenated techniques in which a chromatographic technique is coupled with a spectroscopic or spectrometric technique for the analysis of various biological, chemical, and toxicology samples. Over time, this analytical technique has been continuously improved and is now widely applied to detect and analyze various biological and toxicological substances, including proteins, immunological compounds, poisons, adulterants, toxins, dyes, and even explosives. Such techniques are also commonly used in forensic science ^[24].

History of LC-MS

- The scientific foundation of LC-MS can be traced to early attempts to combine liquid phase separation with mass spectrometric detection during the late 1960s and early 1970s ^[25].

- During the 1970s, several approaches were investigated to transfer analytes from the liquid chromatograph into the mass spectrometer ^[26].
- An important milestone occurred in the last 1970s, when early commercial LC- MS interfaces began to appear.
- According to historical accounts, Hewlett – Packard developed direct – liquid – introduction approach around 1979 ^[27].
- The practical development of electrospray for mass spectrometry gained significant importance during the 1980s ^[28].
- An important development in mass spectrometry took place in 1984, when John B. Fenn and Masamichi Yamashita published experimental research highlighting the use of electrospray ionization technique for mass spectrometric analysis.
- White House and co-workers reported an important LC-MS electrospray interface in 1985. Subsequently, Brainerd, Covey and Henion reported the ion- spray interface in 1987. The last 1980s represented a major turning point in LC- MS technology ^[29].
- During the 1990s, LC- MS became increasingly powerful with the wider adoption of tandem mass spectrometry. (LC-MS/MS). By the mid-1990s, LC-MS had become particularly important in bio analytical application.
- During the 2000s and 2010s LC-MS became increasingly integrated into pharmaceutical, clinical, biological, environmental, and food analysis laboratories ^[30].
- Advances in machine learning and computational power enabled broader applications in LC- MS data processing, metabolomics compound identification in during the 2020s.
- chromatography-mass spectrometry (LC-MS) is a powerful analytical technique that has revolutionized the fields of chemistry and biology. The development of LC-MS dates to the 1950's, when researchers first combined liquid chromatography with mass spectrometry to enhance the separation, identification, and detection of chemical compounds.
- As technology continues to evolve, LC-MS is expected to undergo further innovation that will expand the boundaries of analytical chemistry and enable the identification of compounds with even greater precision.
- Mass spectrometry originated in the early 20th century following the invention of the first mass spectrometer by J.J. Thomson. It is a powerful analytical technique used to identify, and quantify molecules based on their mass-to-charge(M/Z) ratio. Owing to its high accuracy and sensitivity, mass spectrometry has become an indispensable tool in proteomics, metabolomics and drug discovery.
- The hyphenation of liquid chromatography (LC) with mass spectrometry (MS) has produced an extremely powerful range of analytical tools for the qualitative and quantitative analysis of complex samples ^[31].
- The development of LC-MS has profoundly influenced biological and analytical sciences, ushering in a new era of advanced analytical methodologies.
- Liquid chromatography -mass spectrometry (LC-MS) is an analytical chemistry technique that combines the

physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS) coupled chromatography MS systems are widely used in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid chromatography separates mixtures containing multiple components, mass spectrometry provides spectral information that helps identify or confirm the suspected identity of each separated component.

Advanced Hyphenated Techniques

Advanced hyphenated techniques combine two or more compatible separation and detection methods into a single analytical system, enabling comprehensive characterization of complex samples. These techniques combine the high separation efficiency of chromatographic or electrophoretic methods with the sensitive detection and structural identification capabilities of mass spectrometry or spectroscopy. Such integration improves the reliability of qualitative and quantitative analysis and reduces the need for repeated sample handling ^[32].

1. LC–MS/MS (Liquid Chromatography–Tandem Mass Spectrometry)

LC–MS/MS combines liquid chromatographic separation with two stages of mass spectrometric analysis. The first mass analyser selects ions according to their mass-to-charge ratio, while the selected ions undergo fragmentation and the resulting product ions are analysed in the second stage. This provides highly specific structural and quantitative information. LC–MS/MS is extensively used for drug identification, impurity profiling, metabolite determination, pharmacokinetic studies and bioanalysis ^[33].

2. LC–NMR (Liquid Chromatography–Nuclear Magnetic Resonance)

LC–NMR couples liquid chromatography with nuclear magnetic resonance spectroscopy. Chromatography enables the separation of individual components from a mixture, while nuclear magnetic resonance (NMR) spectroscopy helps in elucidate the molecular structures of the isolated compounds. It is particularly useful for the characterization of unknown compounds, degradation products and natural-product constituents. Although LC–NMR provides valuable structural information, its application may be limited by relatively lower sensitivity and high instrumental cost compared with LC–MS ^[34].

3. CE–MS (Capillary Electrophoresis–Mass Spectrometry):

CE–MS combines the efficient separation capability of capillary electrophoresis with the sensitive and selective detection of mass spectrometry. It is suitable for analysing ionic, polar and charged compounds and requires only a small sample volume. The technique has applications in pharmaceutical analysis, metabolomics, peptide analysis and chiral separations.

4. LC–MS–NMR

LC–MS–NMR is a multi-hyphenated system that combines chromatographic separation with mass spectrometric and NMR detection. The three techniques provide complementary information such as retention behaviour,

molecular mass, fragmentation pattern and detailed structural characteristics. Therefore, this approach can improve the identification of unknown compounds in complex pharmaceutical and biological samples and is especially useful in metabolite and natural product research [35].

5. GC-MS/MS (Gas Chromatography-Tandem Mass Spectrometry)

GC-MS/MS provides enhanced selectivity compared with conventional GC-MS by using multiple stages of mass selection and fragmentation. It is particularly useful for volatile and thermally stable compounds, trace-level impurities, residual solvents and analysis of complex samples. Nevertheless, non-volatile substances and compounds that are sensitive to heat may need chemical modifications through derivatization before they can be analyzed.

6. Two-dimensional chromatographic techniques

Advanced multidimensional techniques such as LC×LC and GC×GC

employ two different separation mechanisms to increase the resolving power of chromatography. They are particularly beneficial when samples contain many chemically similar components that cannot be adequately separated by conventional one-dimensional chromatography. These techniques are increasingly useful in impurity profiling, metabolomics and complex pharmaceutical formulations.

Instrumentation of LC-MS

Liquid chromatography-mass spectrometry (LC-MS) is an analytical technique that combines the separation capability of liquid chromatography with the molecular mass and structural information provided by mass spectrometry. The instrumentation of LC-MS consists mainly of a solvent delivery system, sample injector, chromatographic column, ionization interface/source, mass analyser, detector and data processing system. In LC-MS, liquid chromatography separates the constituents of a sample, after which the mass spectrometer ionizes the analytes and distinguishes the generated ions based on their mass-to-charge ratio (m/z) before detecting them [36].

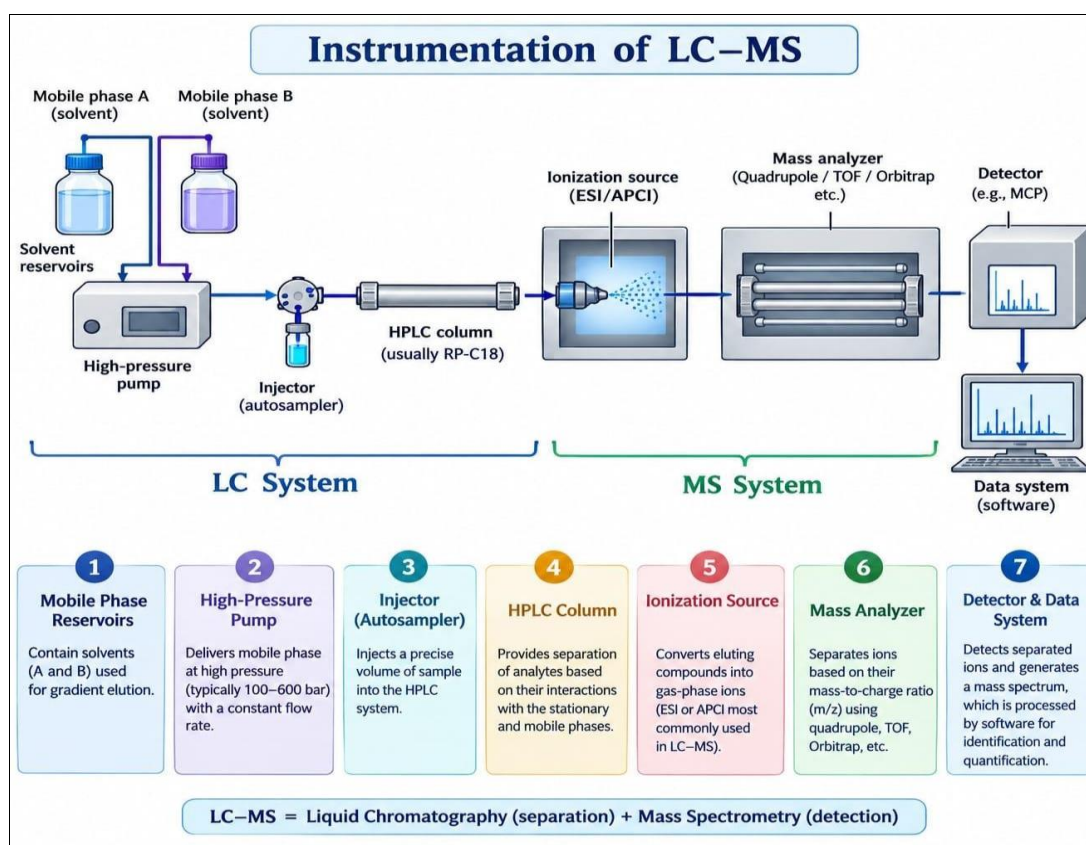


Fig 3: Instrumentation of LC-MS

Solvent Delivery System

The solvent delivery system supplies the mobile phase to the chromatographic column at a controlled flow rate and pressure. High-pressure pumps are generally used to provide a constant and reproducible flow of the mobile phase.

The mobile phase may be delivered under isocratic or gradient conditions depending on the analytical requirement. Proper control of flow rate is important because it affects chromatographic separation and the efficiency of ionization in the mass spectrometer [37].

1. Sample Injector

The injector introduces a measured volume of sample into the mobile-phase stream. Manual or automated autosampler systems may be used. The injected sample is carried by the mobile phase into the chromatographic column, where its individual components are separated according to their physicochemical properties [38].

2. Chromatographic Column

The chromatographic column contains the stationary phase responsible for separating different components of the

sample. Reversed-phase columns, particularly C18 columns, are commonly used in LC–MS analysis. Separation occurs because analytes interact differently with the stationary and mobile phases. The separated compounds subsequently enter the mass spectrometer through the interface ^[39].

3. Ionization Source/Interface

The LC effluent must be converted into gas-phase ions before entering the mass analyzer. The ionization source is therefore an important part of LC–MS instrumentation. The commonly used atmospheric-pressure ionization techniques are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) ^[40].

Electrospray Ionization (ESI): In ESI, the liquid from the LC column passes through a fine capillary maintained at high voltage. The electric field produces charged droplets. Solvent evaporation and subsequent droplet desolation result in the formation of gas-phase ions, which are transferred into the mass spectrometer. ESI is particularly useful for polar, ionic, thermally labile and high-molecular-weight compounds ^[41].

Atmospheric-Pressure Chemical Ionization (APCI)

In APCI, the LC effluent is vaporized with the help of a heated region and ionization occurs through a corona discharge. APCI is commonly suitable for relatively small and moderately polar compounds. Both ESI and APCI are atmospheric-pressure ionization techniques widely used for coupling LC with MS ^[42].

4. Mass Analyzer

The mass analyser separates ions according to their mass-to-charge ratio (m/z). Different types of mass analysers are available, including quadrupole, ion trap, time-of-flight (TOF), magnetic sector and Fourier-transform ion cyclotron resonance (FT-ICR) analysed ^[43].

Applications of LC–MS in the Current Scenario

Liquid chromatography–mass spectrometry (LC–MS) has progressively developed into a highly versatile analytical platform and currently occupies an important position in pharmaceutical, biomedical, biological, environmental, and food-related research. The combination of chromatographic separation with mass-based detection enables the analysis of chemically diverse compounds with high sensitivity, selectivity, and structural information. In the present scenario, technological developments such as high-resolution mass spectrometry, tandem mass spectrometry, improved ionization techniques, and advanced data-processing approaches have further expanded the analytical capabilities of LC–MS. Consequently, the technique is now applied across different stages of pharmaceutical research, ranging from drug discovery and development to quality assessment and biological investigations ^[44].

1. Pharmaceutical Drug Analysis

One of the major contemporary applications of LC–MS is the analysis of pharmaceutical drug substances and drug products. LC–MS can provide both chromatographic separation and mass related information, allowing pharmaceutical compounds to be identified, characterized, and quantified even when they are present in complex samples. Moreover, LC–MS and LC–MS/MS methods are useful for investigating active pharmaceutical ingredients,

related substances, degradation products, and other components associated with pharmaceutical formulations. Therefore, the technique supports analytical method development and characterization throughout different stages of pharmaceutical development.

2. Drug Discovery and Development

Furthermore, LC–MS has become an important analytical tool in modern drug-discovery workflows. During the early stages of drug research, it can be employed for compound identification, purity assessment, metabolite investigation, and structural characterization. In addition, chromatography–MS approaches can support studies related to drug absorption, distribution, metabolism, and excretion (ADME). By providing sensitive detection and molecular information, LC–MS assists researchers in evaluating the behaviour of drug candidates and selecting compounds for further development ^[45].

3. Pharmacokinetic and Bioanalytical Studies

Another important application of LC–MS is the quantitative analysis of drugs and metabolites in biological matrices such as plasma, serum, urine, and tissue samples. LC–MS/MS is particularly useful when analytes occur at low concentrations because tandem mass spectrometry provides highly selective detection. Consequently, LC–MS-based methods are widely applied in pharmacokinetic investigations to determine drug concentration–time profiles and to evaluate absorption, distribution, metabolism, and elimination. These applications provide important analytical support during preclinical and clinical drug-development studies.

4. Impurity Profiling

In pharmaceutical analysis, the detection and characterization of impurities are essential for ensuring the quality and safety of drug substances and products. LC–MS has become particularly valuable for impurity profiling because modern instruments can provide accurate-mass measurements together with fragmentation information. Thus, known as well as previously unidentified impurities can be investigated more effectively. In addition, LC–MS/MS can provide structural information that assists in determining the possible identity and origin of impurity related components.

5. Stability and Degradation Studies

LC–MS is also increasingly used for evaluating the stability of pharmaceutical compounds. During storage or exposure to stress conditions, drug substances may undergo chemical transformation and produce degradation products. LC–MS can separate the parent compound from degradation related components and subsequently provide mass and fragmentation information for their characterization. Therefore, LC–MS can contribute to the investigation of degradation pathways and the development of stability-indicating analytical procedures.

6. Metabolomics

Moreover, LC–MS has become a key technology in metabolomics, where large numbers of metabolites are analysed in biological samples. Its ability to detect a broad range of compounds with different chemical properties makes it suitable for investigating complex metabolic profiles. LC–MS-based metabolomics is applied to the study

of biological pathways, disease associated metabolic alterations, drug responses, and potential biomarkers. The continued development of high-resolution instruments has further improved the ability to detect and characterize low-abundance metabolites ^[46].

7. Proteomics and lipidomic

In addition to metabolomics, LC-MS is extensively incorporated into proteomic and lipidomic investigations. Proteomic applications involve the identification and characterization of proteins and peptides, whereas lipidomic studies focus on the comprehensive analysis of lipid molecules. Because these biological systems contain numerous structurally related compounds, the combined separation and mass-detection capabilities of LC-MS provide an effective analytical strategy. Consequently, LC-MS contributes to a better understanding of biological mechanisms and disease-related molecular changes.

8. Biopharmaceutical Analysis

The application of LC-MS has also expanded significantly into the analysis of biopharmaceuticals, including therapeutic proteins and monoclonal antibodies. Such molecules possess complex structures and may undergo modifications that influence their quality, safety, and biological activity. LC-MS can provide qualitative and quantitative information regarding protein composition and structural modifications, including post-translational modifications such as glycosylation and phosphorylation. Therefore, LC-MS can support the characterization and quality assessment of modern biological medicines.

9. Biomarker Discovery and Personalized Medicine

Furthermore, LC-MS is increasingly relevant to biomarker discovery and personalized medicine. Biological samples may contain numerous molecular features that differ between healthy and disease-associated conditions. LC-MS can generate detailed molecular profiles, while advanced data-analysis approaches can help identify patterns associated with specific biological conditions. In drug research, chromatography-MS is also being investigated for applications related to personalized medicine, where molecular information may contribute to understanding differences in drug response between individuals ^[47].

10. Food Safety and Environmental Analysis

Beyond pharmaceutical and biomedical applications, LC-MS is currently employed in food-safety and environmental investigations. In food analysis, it can be used for detecting and quantifying residues, contaminants, additives, and other chemical components. Similarly, environmental applications include the investigation of trace-level contaminants and emerging pollutants in complex environmental samples. The high sensitivity and selectivity of LC-MS make it suitable for detecting compounds that may occur at very low concentrations.

11. Forensic Applications

Another developing area is forensic science. LC-MS can assist in the detection and identification of drugs, metabolites, toxic substances, and other chemical evidence in biological or forensic samples. The combination of chromatographic separation and mass spectral information can improve the specificity of analysis when multiple

compounds are present. Accordingly, LC-MS based methods have become useful analytical tools in modern forensic investigations.

12. High-Throughput and Advanced Analysis

Finally, recent developments in chromatographic systems, mass analysers, automation, and data processing software have improved the suitability of LC-MS for high-throughput analytical workflows. High-resolution mass spectrometers and tandem MS systems can provide detailed information from complex samples, while improved computational approaches facilitate the processing of large datasets. As a result, modern LC-MS workflows can support faster and more comprehensive analysis in pharmaceutical research, biological sciences, and other analytical fields ^[48].

Application of Artificial Intelligence in LC-MS

The integration of artificial intelligence (AI) and machine learning (ML) with liquid chromatography-mass spectrometry (LC-MS) has emerged as an important advancement in modern analytical science. LC-MS generates highly complex datasets containing chromatographic retention information, mass-to-charge ratios, isotopic patterns, fragmentation spectra, and signal intensities. Because manual processing and interpretation of such multidimensional data can be time-consuming, AI-based computational approaches are increasingly being investigated to improve data processing, pattern recognition, compound identification, and quantitative analysis. Recent research particularly highlights the application of machine learning to LC-MS-based nontargeted analysis, where large numbers of known and unknown chemical features must be extracted and interpreted ^[49].

Automated Data Processing:

One of the important applications of AI in LC-MS is the automated processing of raw analytical data. LC-MS experiments can generate a large number of signals that require baseline correction, noise removal, peak detection, alignment, and normalization before meaningful interpretation. Machine-learning algorithms can assist in these preprocessing steps by recognizing relevant signal patterns and distinguishing analytical features from background noise. Consequently, AI-assisted data processing can reduce manual intervention and improve the efficiency of LC-MS workflows

Peak Detection and Peak Recognition

Furthermore, AI can be applied to the detection and characterization of chromatographic peaks in LC-MS data. Complex samples may contain closely eluting compounds or overlapping signals, making conventional peak recognition difficult. Machine-learning approaches, including neural network-based methods, can recognize characteristic signal patterns and assist in peak selection and integration. Such approaches may therefore improve the consistency of feature extraction, particularly when large numbers of samples are analysed ^[50].

Metabolite and Compound Identification

Another important application is the identification of metabolites and other chemical compounds. LC-MS produces information that can be compared with spectral

libraries, databases, retention time information, and experimentally obtained reference data. AI algorithms can learn relationships between molecular structures and spectral patterns and can subsequently assist in assigning identities to detected compounds. In metabolomics, AI has been investigated for identifying both known metabolites and previously uncharacterized features, thereby supporting the interpretation of complex biological samples^[51].

Prediction of Retention Time

AI and ML models can also be used for predicting chromatographic retention behaviour. Retention time is an important parameter for LC–MS-based compound identification because it provides complementary information to mass spectral data. Machine-learning models can learn relationships between molecular characteristics and chromatographic behaviour from existing datasets. Therefore, predicted retention times can be combined with mass-to-charge ratios and MS/MS fragmentation information to support more reliable compound annotation.

Spectral Interpretation and Annotation

In addition, AI can assist in interpreting complex mass spectra. LC–MS/MS experiments generate fragmentation patterns that contain structural information about molecules. However, interpreting these patterns manually can become difficult when samples contain numerous compounds. Machine-learning and deep-learning approaches can identify relationships between spectral features and molecular structures, thereby assisting spectral annotation and compound characterization. Recent developments in ML for mass spectrometry include applications involving spectral-data preprocessing, peak annotation, molecular-structure prediction, and other computational tasks.

Non-Targeted Analysis

AI is particularly valuable in non-targeted LC–MS analysis, where the objective is to detect and characterize as many chemical features as possible without restricting the analysis to a predefined list of compounds. Such experiments produce large and complex datasets that are challenging to interpret using conventional approaches alone. Machine-learning algorithms can support data preprocessing, feature extraction, classification, compound identification, and interpretation. Consequently, AI-assisted LC–MS can facilitate the investigation of unknown compounds in areas such as metabolomics, pharmaceutical research, food analysis, and environmental analysis.

Biomarker Discovery

Moreover, the combination of LC–MS and AI have important applications in biomarker discovery. LC–MS-based metabolomics can generate large numbers of molecular features from biological samples. AI algorithms can analyse these features and identify patterns that distinguish different biological conditions or sample groups. Machine-learning models such as random forests, support vector machines, and artificial neural networks have been explored for classification, prediction, and biomarker-related analysis. Thus, AI-assisted LC–MS can contribute to the identification of potential biomarkers in biomedical and pharmaceutical research^[52].

Pharmacokinetic and Pharmaceutical Applications

In pharmaceutical research, AI-assisted LC–MS can support the analysis of drugs and metabolites in biological matrices.

LC–MS/MS is widely used for quantitative analysis in pharmacokinetic studies because of its sensitivity and selectivity. AI-based approaches can assist in processing and interpreting the resulting complex datasets and may support automated data deconvolution, pattern recognition, and predictive analysis. Recent literature specifically describes the emerging integration of AI with LC–MS/MS in pharmacokinetic research while also identifying challenges related to algorithm validation and regulatory acceptance^[53].

Quality Control and Anomaly Detection

AI can additionally support pharmaceutical quality-control applications by identifying unusual patterns in LC–MS data. When chromatographic or mass-spectral profiles from a sample differ from established patterns, machine-learning models can potentially flag these deviations for further investigation. Such approaches may be useful for detecting unexpected impurities, abnormal samples, or changes in analytical profiles. However, AI-based findings still require appropriate analytical confirmation and scientifically validated procedures before being used for regulated quality-control decisions.

Future Perspectives

Overall, the combination of LC–MS with artificial intelligence represents a developing area with considerable potential for pharmaceutical and analytical applications. AI can assist in several stages of the LC–MS workflow, including data preprocessing, peak detection, retention-time prediction, spectral interpretation, metabolite annotation, non-targeted analysis, biomarker discovery, and predictive modelling. Nevertheless, the reliability of AI-assisted analysis depends strongly on the quality, representativeness, and validation of the training data. Issues such as model interpretability, reproducibility, standardization, validation, and regulatory acceptance remain important considerations. Therefore, AI is best viewed as a complementary analytical tool that can enhance LC–MS workflows rather than completely replace chromatographic and mass spectrometric expertise.

Conclusion

Liquid chromatography–mass spectrometry (LC–MS) is an important analytical technique in pharmaceutical analysis because it provides sensitive and reliable identification and quantification of compounds. It is widely used in drug analysis, impurity profiling, stability studies, pharmacokinetic studies, metabolomics, and the analysis of biological, food, and environmental samples. The combination of liquid chromatography and mass spectrometry helps researchers analyse complex samples and obtain valuable information about chemical compounds. The integration of artificial intelligence (AI) and machine learning (ML) with LC–MS has further improved data processing, peak detection, compound identification, and biomarker discovery. However, proper validation, reliable data, and regulatory acceptance are essential for the effective use of AI-based methods. Overall, LC–MS combined with AI has promising applications in pharmaceutical research and quality control, and further developments may improve the accuracy and efficiency of analytical studies.

References

1. Watson DG. Pharmaceutical analysis: a textbook for pharmacy students and pharmaceutical chemists. 5th ed. Elsevier, 2020.
2. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Latest edition. Ghaziabad: Indian Pharmacopoeia Commission.
3. Chopade VV, Patel PM. Pharmaceutical analysis-I, 2022.
4. Ravi Shankar S. Pharmaceutical analysis. 5th ed.
5. Harris DC. Quantitative chemical analysis. 10th ed. New York: W.H. Freeman, 2020.
6. Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 7th ed. Boston: Cengage Learning, 2018.
7. Milne GL, Morrow JD. Chromatography. In: Clinical and translational science. Elsevier, 2009.
8. Kumari VBC, Patil SM, Ramu R, Shira Hatti PS, Kumar N, Sowmya BP, *et al.* Chromatographic techniques: types, principles, and applications. In: Analytical techniques in biosciences: from basics to applications. Academic Press, 2022, 73-101.
9. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th ed. Wadsworth, 1988.
10. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. 5th rev ed. Himalaya Publishing House, 2008.
11. Sharma BK. Instrumental methods of chemical analysis. Meerut: N.A.S. College.
12. Coşkun O. Separation techniques: Chromatography. North Clin Istanbul, 2016;3(2):156-60. doi:10.14744/nci.2016.32757.
13. Gajji Babu G. Advances in chromatography: contemporary techniques and applications. Separations, 2025. doi:10.1080/28378083.2025.2466624.
14. Niessen WMA, Tinke AP. Liquid chromatography-mass spectrometry: general principles. J Chromatogr A, 1995;703:37-57.
15. Khalikova M, Jireš J, Horáček O, Douša M, Kučera R, Nováková L. What is the role of current mass spectrometry in pharmaceutical analysis? Mass Spectrom Rev, 2024;43:560-609.
16. Adhatrao M. Integrating QbD principles for enhanced method development. J Pharma Insights Res, 2024.
17. Beccaria M, Cabooter D. Current developments in LC-MS for pharmaceutical analysis. Analyst, 2020;145:1129-57.
18. Kanu AB. Recent developments in sample preparation techniques combined with high-performance liquid chromatography: A critical review. Journal of Chromatography A, 2021;1654:462444. doi: 10.1016/j.chroma.2021.462444.
19. Khalikova M, *et al.* Role of mass spectrometry in API identification and impurity profiling. Mass Spectrom Rev, 2024.
20. Adhatrao M, *et al.* Integrating QbD principles for enhanced method development in analytical spectroscopy. J Pharma Insights Res, 2024;2(4):25-30.
21. Harris M. Hyphenated techniques: combining mass spectrometry with separation techniques. J Mass Spectrom Purif Tech, 2024;10:240.
22. Patel P, *et al.* Advanced pharmaceutical analysis: applications of hyphenated techniques. Number Analytics, 2024.
23. Metri S, *et al.* Hyphenated techniques: LC-MS and GC-MS, coupling separation with identification, 2024.
24. Tal'rose VL, *et al.* First attempt to interface liquid chromatography to mass spectrometry. Russ J Phys Chem, 1968.
25. Dole M, *et al.* Molecular beams of macroions. J Chem Phys, 1968;49:2240-7.
26. McLafferty FW. Liquid chromatography-mass spectrometry. J Chromatogr A, 1973.
27. Niessen WMA. Development of LC-MS interfaces: moving belt, direct liquid introduction and thermospray. J Chromatogr A, 2001.
28. Yamashita M, Fenn JB. Electrospray ion source. J Phys Chem, 1984;88:4451-9.
29. Whitehouse CM, Dreyer RN, Yamashita M, Fenn JB. Electrospray interface for liquid chromatographs and mass spectrometers. Anal Chem, 1985;57(3):675-9. doi:10.1021/ac00280a023.
30. Pitt JJ. Principles and applications of liquid chromatography-mass spectrometry in clinical biochemistry. Clin Biochem Rev, 2009;30:19-34.
31. Aureli C, *et al.* Recent advances in LC-MS for pharmaceutical analysis with machine learning integration, 2024.
32. Triple hyphenated techniques: LC-NMR-MS and LC-UV-NMR-MS-ESI [presentation]. KMCH College of Pharmacy.
33. LC-NMR applications in complex mixtures, natural products and drug-related metabolites in biofluids.
34. Bharti SK, Roy R. LC-MS-NMR as a multi-hyphenated technique for natural product analysis and metabolite profiling, 2012.
35. Niessen WMA. Tandem mass spectrometry configurations, including Q-TOF and triple quadrupole systems, in hyphenated techniques. J Chromatogr A, 1998.
36. GC-MS/MS for enhanced selectivity in the analysis of volatile compounds and trace-level impurities. J Pharm Anal, 2024.
37. Pirok BWJ, *et al.* Online comprehensive two-dimensional liquid chromatography (LC×LC) for complex non-volatile samples complementary to LC-MS. J Chromatogr A, 2023.
38. Holčápek M, Jirásko R, Líba M. Recent developments in liquid chromatography-mass spectrometry and related techniques. J Chromatogr A, 2012;1259:3-15. doi: 10.1016/j.chroma.2012.08.072.
39. Niessen WMA. Advances in instrumentation in liquid chromatography-mass spectrometry and related liquid-introduction techniques. J Chromatogr A, 1998;794(1-2):407-35.
40. Holčápek M, Jirásko R, Líba M. Recent developments in liquid chromatography-mass spectrometry and related techniques. J Chromatogr A, 2012;1259:3-15.
41. Donato P, Cacciola F, Tranchida PQ, Dugo P, Mondello L. Mass spectrometry detection in comprehensive liquid chromatography: basic concepts, instrumental aspects, applications and trends. Mass Spectrom Rev, 2012;31(5):523-59.
42. Thomas SN, French D, Jannetto PJ, Rappold BA, Clarke WA. Liquid chromatography-tandem mass

- spectrometry for clinical diagnostics. *Nat Rev Methods Primers*,2022;2(1):96. doi:10.1038/s43586-022-00175-x.
43. Lam W, Cai W. The Application of LC-MS in Pharmaceutical Analysis. *Molecules* (Basel, Switzerland),2026;31(15):2624. <https://doi.org/10.3390/molecules31152624>
 44. Alanazi S. Recent Advances in Liquid Chromatography-Mass Spectrometry (LC-MS) Applications in Biological and Applied Sciences. *Anal Sci Adv*,2025;6(1):e70024. doi: 10.1002/ansa.70024. PMID: 40584301; PMCID: PMC12204688.
 45. Rambhad SR, Dave H. A Review on Applications of Liquid Chromatography Coupled With Mass Spectrometry in Impurity Profiling of Drug Substances and Drug Products. *Sep Sci plus.*,2025;8:e70073. <https://doi.org/10.1002/sscp.70073>
 46. Kaddah MMY, El Demellawy MA, Talaat W, *et al.* Comprehensive analytical approaches: The use of LC-MS and IC-MS in modern pharmaceutical and biomedical sciences. *Microchemical Journal*,2025;216:114780. DOI: 10.1016/j.microc.2025.114780.
 47. Cai H, Xing X, Su Y, Yang C. Innovative applications and future perspectives of chromatography-mass spectrometry in drug research. *Frontiers in pharmacology*,2025;16:1529468. <https://doi.org/10.3389/fphar.2025.1529468>
 48. Khalikova M, Jireš J, Horáček O, Douša M, Kučera R, Nováková L. What is the role of current mass spectrometry in pharmaceutical analysis? *Mass Spectrom Rev*,2024;43:560-609. <https://doi.org/10.1002/mas.21858>
 49. Application of machine learning in LC-MS-based non-targeted analysis. *Trends in Analytical Chemistry*, 2025. DOI: 10.1016/j.trac.2025.118243.
 50. Beck AG, Muhoberac M, Randolph CE, Beveridge CH, Wijewardhane PR, Kenttämää HI, *et al.* Recent Developments in Machine Learning for Mass Spectrometry. *ACS measurement science au*,2024;4(3):233–246. <https://doi.org/10.1021/acsmeasuresciau.3c00060>
 51. Liebal UW, Phan ANT, Sudhakar M, Raman K, Blank LM. Machine Learning Applications for Mass Spectrometry-Based Metabolomics. *Metabolites*,2020;10(6):243. <https://doi.org/10.3390/metabo10060243>
 52. Chi J, Shu J, Li M, Mudappathi R, Jin Y, Lewis F, *et al.* Artificial Intelligence in Metabolomics: A Current Review. *Trends in analytical chemistry: TRAC*,2024;178:117852. <https://doi.org/10.1016/j.trac.2024.117852>
 53. Analytical Science Journal Artificial Intelligence in LC-MS/MS Data Analysis for Pharmacokinetic Studies. *Current Pharmaceutical Analysis*, 2026. DOI: 10.2174/0118723128434043260513112516.