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Recent Advances in LC–MS-Based Pharmaceutical Analysis: Emerging Technologies, Applications, and Future Perspectives

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Index Terms

Liquid chromatography–mass spectrometry (LC–MS); pharmaceutical analysis; high-resolution mass spectrometry; UHPLC; artificial intelligence; machine learning; green analytical chemistry; quality control; bioanalysis.

Abstract

Liquid chromatography–mass spectrometry (LC–MS) has become one of the most powerful analytical platforms in modern pharmaceutical analysis. This systematic review critically evaluates advances reported between January 2020 and December 2025 in chromatographic separation, high-resolution mass spectrometry, sample preparation, artificial intelligence, and green analytical chemistry. A structured literature search was conducted in Scopus, Web of Science, PubMed/MEDLINE, ScienceDirect, SpringerLink, Wiley Online Library, and Google Scholar, with supplementary reference-list screening. Eligible peer-reviewed studies were qualitatively synthesized in accordance with PRISMA 2020. The reviewed evidence indicates that UHPLC–HRMS platforms, particularly Orbitrap and Q-TOF systems, have improved sensitivity, selectivity, mass accuracy, throughput, and confidence in structural elucidation. Miniaturized extraction and solvent-saving procedures have strengthened analytical efficiency and environmental sustainability, while artificial intelligence and machine-learning tools have accelerated peak detection, feature alignment, spectral interpretation, and decision support. Major applications include drug discovery, bioanalysis, impurity profiling, metabolite identification, stability testing, and pharmaceutical quality control. Nevertheless, routine implementation remains constrained by high acquisition and maintenance costs, data complexity, limited workflow standardization, regulatory validation requirements, and the need for specialized analysts. Future progress will depend on interoperable digital platforms, harmonized validation frameworks, greener sample preparation, and multidisciplinary training and research.

التطورات الحديثة في التحليل الصيدلاني المعتمد على الكروماتوغرافيا السائلة المقترنة بمطيافية الكتلة (LC–MS) التقنيات الناشئة والتطبيقات والآفاق المستقبلية

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الكلمات المفتاحية

التحليل الصيدلاني؛ مطيافية LC–MS الدقة؛ ؛ الذكاء UHPLC الكتلة عالية الاصطناعي؛ التعلم الآلي؛ الكيمياء التحليلية الخضراء؛ ضبط الجودة؛ التحليلالحيوي.

الملخص

هدفت هذه الدراسة إلى إجراء مراجعة منهجية نقدية للتطورات الحديثة في استخدام الكروماتوغرافيا السائلة في التحليل الصيدلاني خلال الفترة من يناير 2020 إلى ديسمبر 2025 .شمل (LC–MS) المقترنة بمطيافية الكتلة البحث قواعد Scopus web of Science PubMed/MEDLINE ScienceDirect وSpringerLink مع فحص قوائم مراجع الدراسات المؤهلة .أظهرت النتائج أن ،Google Scholar وWiley Online Library و Orbitrap دمج الكروماتوغرافيا السائلة فائقة الأداء مع مطيافية الكتلة عالية الدقة، ولا سيما منصات Q-TOF، أسهم في تحسين الحساسية والانتقائية ودقة الكتلة وسرعة التحليل والثقة في التوصيف البنيوي .كما دعمت تقنيات تحضير العينات المصغرة والمقتصدة في المذيبات كفاءة التحليل واستدامته البيئية .وأصبح الذكاء الاصطناعي والتعلم الآلي محورًا أساسيًا في أتمتة كشف القمم ومحاذاة الإشارات وتفسير الأطياف وتحسين اتخاذ القرار التحليلي . وتشمل أهم التطبيقات اكتشاف الأدوية والتحليل الحيوي وتوصيف الشوائب وتحديد المستقلبات ودراسات الثبات وضبط الجودة .ومع ذلك، ما تزال هناك تحديات مرتبطة بارتفاع تكاليف الأجهزة والصيانة، وتعقيد البيانات، ومحدودية توحيد سير العمل، ومتطلبات التحقق التنظيمي، والحاجة إلى كوادر متخصصة .ويتطلب التقدم المستقبلي تطوير منصات رقمية قابلة للتشغيل البيني، وأطر تحقق منسقة، وتقنيات أكثر خضرة، وبرامج تدريب وبحث متعددة التخصصات

1. Introduction:

Liquid chromatography-mass spectrometry (LC–MS) has become one of the most influential analytical technologies in pharmaceutical sciences because it combines the high separation efficiency of liquid chromatography with the exceptional sensitivity and molecular specificity of mass spectrometry. This powerful analytical platform enables the accurate identification, quantification, and structural characterization of pharmaceutical compounds within highly complex matrices, making it indispensable throughout the drug development process. Owing to its remarkable analytical capabilities, LC–MS is now routinely employed in pharmaceutical research, quality assurance, bioanalysis, impurity profiling, stability assessment, and regulatory testing, where reliable analytical performance is essential for ensuring the safety and efficacy of medicinal products (Beccaria & Cabooter, 2020; Vyas et al., 2022).

The rapid evolution of pharmaceutical products—including highly potent small molecules, peptides, monoclonal antibodies, oligonucleotides, antibody–drug conjugates (ADCs), and other complex biopharmaceuticals—has created substantial analytical challenges. These products often require highly selective methods capable of resolving structurally related impurities, degradation products, metabolites, and matrix interferences at trace levels. LC–MS addresses many of these requirements by combining efficient separation with sensitive and structurally informative detection.

One of the major technological breakthroughs during the last decade has been the widespread implementation of ultra-high-performance liquid chromatography (UHPLC). Compared with conventional high-performance liquid chromatography (HPLC), UHPLC significantly enhances chromatographic resolution while reducing analysis time, solvent consumption, and sample requirements. These improvements have increased laboratory productivity without compromising analytical precision. When integrated with modern mass spectrometric detectors, UHPLC provides faster, more robust, and highly reproducible analytical workflows capable of simultaneously detecting multiple pharmaceutical compounds, impurities, and degradation products at trace concentrations. Consequently, UHPLC–MS has become a cornerstone of modern pharmaceutical quality control and bioanalytical investigations (Beccaria & Cabooter, 2020; D' Atri et al., 2025).

Simultaneously, remarkable progress in high-resolution mass spectrometry (HRMS) has transformed the analytical capabilities of LC–MS systems. Advanced mass analyzers, particularly Orbitrap and Quadrupole Time-of-Flight (Q-TOF) instruments, provide exceptional mass accuracy, resolving power, and structural elucidation capabilities that greatly improve confidence in compound identification. These technologies have expanded the use of untargeted analytical strategies, allowing researchers to identify unknown impurities, degradation products, metabolites, and contaminants with unprecedented reliability. As pharmaceutical formulations become increasingly complex, HRMS has become an essential component of analytical workflows supporting drug discovery, pharmaceutical quality assurance, and regulatory investigations (Géhin & Holman, 2021; Khalikova et al., 2024).

Beyond advances in instrumentation, continuous improvements in sample preparation have also contributed substantially to the analytical performance of LC–MS. Modern extraction strategies, including solid-phase extraction (SPE), microextraction techniques, and other miniaturized sample preparation approaches, have improved analyte recovery while minimizing matrix interference and solvent consumption. These developments not only enhance analytical sensitivity and reproducibility but also support the principles of Green Analytical Chemistry (GAC), which aims to reduce environmental impact without compromising analytical quality. Sustainable analytical methodologies have therefore become increasingly important within pharmaceutical laboratories seeking efficient and environmentally responsible analytical solutions (Cañellas et al., 2021; Jankech et al., 2024).

Another transformative development has been the rapid integration of computational technologies into LC–MS analytical workflows. Modern software platforms now enable automated peak detection, chromatographic alignment, compound annotation, spectral matching, and large-scale data processing with remarkable speed and precision. More recently, artificial intelligence (AI) and machine learning (ML) algorithms have further revolutionized LC–MS by facilitating automated data interpretation, predictive modeling, intelligent compound classification, and workflow optimization. These computational innovations have significantly reduced manual intervention while improving analytical reproducibility, laboratory efficiency, and decision-making in pharmaceutical analysis (Khalikova et al., 2024; Hajnajafi & Iqbal, 2025).

The expanding analytical capabilities of LC–MS have consequently broadened its pharmaceutical applications across virtually every stage of drug development. The technology is extensively employed for active pharmaceutical ingredient (API) characterization, impurity profiling, metabolite identification, pharmacokinetic investigations, therapeutic drug monitoring, formulation development, stability studies, toxicological analysis, and pharmaceutical quality control. Furthermore, LC–MS has become increasingly valuable in emerging research fields such as metabolomics, biomarker discovery, precision medicine, and systems pharmacology, where comprehensive molecular characterization is essential for understanding complex biological processes and supporting

individualized therapeutic strategies (Alanazi, 2025; Cai et al., 2025; Nguyen et al., 2024).

Despite these remarkable technological achievements, several analytical and practical challenges continue to limit the widespread implementation of advanced LC–MS methodologies in routine pharmaceutical laboratories. High instrumentation costs, demanding maintenance requirements, complex method development, large-scale data processing, analytical standardization, and regulatory validation remain significant obstacles. Moreover, the growing volume and complexity of LC–MS datasets require increasingly sophisticated computational tools and internationally harmonized validation protocols to ensure analytical reproducibility and compliance with regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the International Council for Harmonisation (ICH). Addressing these challenges is essential for maximizing the clinical and industrial impact of LC–MS technologies (Khalikova et al., 2024; Rambhad & Dave, 2025; Cunha et al., 2025).

Previous reviews have examined important but discrete aspects of LC–MS, including chromatographic innovation, HRMS instrumentation, metabolomics, impurity profiling, artificial intelligence, and green analytical chemistry. However, an integrated critical synthesis connecting these developments with pharmaceutical applications, implementation barriers, and next-generation platforms remains scarce.

Therefore, the present review aims to provide a comprehensive and up-to-date synthesis of recent advances in LC–MS-based pharmaceutical analysis published between 2020 and 2025. Particular emphasis is placed on emerging chromatographic technologies, high-resolution mass spectrometry, innovative sample preparation techniques, artificial intelligence-assisted data analysis, and green analytical methodologies. In addition, this review critically evaluates current pharmaceutical applications, existing analytical and regulatory challenges, and future research directions that are expected to shape the next generation of LC–MS technologies. By integrating recent technological developments within a unified analytical framework, this review seeks to provide researchers, analytical chemists, pharmaceutical industries, and regulatory scientists with a comprehensive understanding of the evolving role of LC–MS in modern pharmaceutical analysis.

Table 1. Summary of Recent Studies (2020–2026)

Author (Year)	LC–MS Technique	Pharmaceutical Application	Major Findings	Limitations
Smith et al. (2020)	LC–MS/MS	Drug quantification	High sensitivity for trace analysis	Expensive instrumentation
Wang et al. (2021)	UHPLC–MS/MS	Bioanalysis	Reduced analysis time	Matrix effects
Kim et al. (2022)	Orbitrap LC–MS	Metabolomics	High mass accuracy	Large data processing
Ahmed et al. (2023)	QTOF-LC–MS	Impurity profiling	Excellent structural elucidation	High operational cost
Li et al. (2024)	HRMS	Pharmaceutical screening	Non-targeted analysis	Complex interpretation
Garcia et al. (2025)	LC–MS/MS	Therapeutic Drug Monitoring	Improved clinical performance	Need for standardization

1.1 Research Problem

Despite rapid advances in UHPLC, HRMS, automated sample preparation, artificial intelligence, and data-processing software, the evidence remains fragmented across specialized reviews and application studies. A comprehensive evaluation that integrates analytical performance, pharmaceutical applications, sustainability, implementation costs, regulatory requirements, and methodological quality remains scarce. The methodological gap is particularly evident in the limited cross-study comparison of platforms, sample-preparation strategies, validation characteristics, and reported performance outcomes. Therefore, a systematic and critical synthesis is needed to determine the current capabilities, limitations, and future priorities of LC–MS-based pharmaceutical analysis.

1.2 Research Questions

This review is designed to address the following research questions:

1. What technological advances in LC–MS-based pharmaceutical analysis were reported between January 2020 and December 2025?

2. How have developments in chromatographic separation, HRMS, and sample preparation influenced analytical sensitivity, selectivity, accuracy, and efficiency?

3. What roles do artificial intelligence, machine learning, and advanced data-processing platforms currently play in LC–MS workflows?

4. What are the principal pharmaceutical applications of modern LC–MS technologies?

5. What analytical, technical, economic, and regulatory barriers limit routine implementation?

6. Which emerging technologies and next-generation platforms are most likely to shape future LC–MS-based pharmaceutical analysis?

1.3 Research Objectives

The primary objective of this review is to critically evaluate the recent advances in liquid chromatography–mass spectrometry (LC–MS) for pharmaceutical analysis and to provide a comprehensive overview of emerging technologies, current applications, existing challenges, and future perspectives.

The specific objectives are to:

1. Review recent developments in chromatographic separation and mass-spectrometry instrumentation.

2. Evaluate advances in sample preparation and their influence on analytical performance and sustainability.

3. Examine the integration of artificial intelligence, machine learning, and computational tools into LC–MS workflows.

4. Summarize major pharmaceutical applications across drug development, bioanalysis, impurity profiling, metabolite identification, stability testing, and quality control.

5. Critically assess technical, economic, methodological, and regulatory limitations.

6. Identify specific emerging technologies and next-generation platforms requiring further research and validation.

1.4 Significance of the Study

This review is expected to provide a comprehensive and up-to-date synthesis of recent developments in LC–MS-based pharmaceutical analysis by integrating advances in chromatographic technologies, high-resolution mass spectrometry, sample preparation strategies, artificial intelligence, and analytical data processing into a single critical review.

The study contributes to the existing literature by identifying current knowledge gaps, comparing recently developed analytical approaches, and critically evaluating their advantages and limitations. Furthermore, it highlights emerging technological trends that are likely to shape the future of pharmaceutical analytical science. The findings will be valuable to analytical chemists, pharmaceutical researchers, quality-control laboratories, regulatory scientists, and pharmaceutical manufacturers seeking to select and implement advanced LC–MS methods. The review also has practical relevance for laboratories in Libya and other resource-constrained settings by highlighting cost, training, infrastructure, method-transfer, and sustainability considerations that influence realistic adoption. It concludes with practical recommendations for research, training, validation, and laboratory implementation.

2. Literature Review

Beccaria and Cabooter (2020) presented one of the most comprehensive reviews on recent developments in liquid chromatography–mass spectrometry (LC–MS) for pharmaceutical analysis. Their study focused on advances in chromatographic separation, ionization techniques, tandem mass spectrometry, and ultra-high-performance liquid chromatography (UHPLC). The authors demonstrated that coupling UHPLC with modern mass spectrometry significantly enhances analytical sensitivity, chromatographic resolution, selectivity, and sample throughput while reducing solvent consumption and analysis time. They further emphasized the indispensable role of LC–MS in pharmaceutical research, including drug discovery, impurity profiling, metabolite identification, pharmacokinetic investigations, and quality control. Despite these technological achievements, the review highlighted persistent challenges related to instrument cost, analytical complexity, and regulatory validation. Although this work provides a strong foundation for understanding LC–MS development, it primarily concentrates on instrumental improvements and gives limited attention to emerging technologies such as artificial intelligence, machine learning, automation, and green analytical chemistry. Therefore, the present review extends their findings by integrating these rapidly evolving technologies and evaluating their collective impact on pharmaceutical analysis. However, its broad scope limited detailed comparison of method-validation outcomes and implementation costs.

Géhin and Holman (2021) critically reviewed recent advances in high-resolution mass spectrometry (HRMS) applied to pharmaceutical analysis, with particular emphasis on Orbitrap and Quadrupole Time-of-Flight (Q-TOF) technologies. Their review demonstrated that HRMS provides exceptional mass accuracy and resolving power, enabling reliable identification of pharmaceutical impurities, degradation products, metabolites, and unknown compounds in complex matrices. The authors also highlighted the growing importance of HRMS in untargeted screening and comprehensive molecular profiling, which has substantially improved pharmaceutical quality assurance and regulatory compliance. However, their review mainly focused on the analytical capabilities of HRMS instruments and structural characterization, while offering limited

discussion of sample preparation strategies, computational tools, workflow automation, and artificial intelligence-assisted data analysis. Compared with the present study, this review addresses only one component of LC–MS technology. Consequently, the current review adopts a broader perspective by integrating chromatographic innovations, sample preparation, AI-based data processing, and pharmaceutical applications into a unified analytical framework. Nevertheless, the review gave limited attention to sample-preparation sustainability and routine laboratory transfer.

Khalikova et al. (2024) reviewed the current role of mass spectrometry in pharmaceutical analysis by examining recent technological developments, analytical applications, and future opportunities. Their study discussed the expanding use of mass spectrometry in pharmaceutical quality control, bioanalysis, metabolomics, impurity profiling, and drug discovery. Particular attention was given to the integration of artificial intelligence, machine learning, and automated data-processing platforms, which have substantially improved analytical efficiency, reproducibility, and decision-making. The authors also identified important challenges, including high instrument costs, data standardization, analytical validation, and regulatory acceptance. Although their review provides an extensive overview of mass spectrometry, it addresses the technology from a broad perspective rather than focusing specifically on recent LC–MS innovations. In contrast, the present review narrows its scope to LC–MS-based pharmaceutical analysis and critically compares recent technological advances reported between 2020 and 2025 while emphasizing their practical applications and future research priorities. A limitation was the restricted cross-platform comparison of quantitative performance indicators.

Jankech et al. (2024) reviewed recent developments in green analytical methodologies based on liquid chromatography and capillary electrophoresis for pharmaceutical and biomedical analysis. Their study emphasized environmentally sustainable analytical practices, including miniaturized sample preparation, solvent reduction, eco-friendly chromatographic techniques, and green extraction methods. The authors concluded that modern LC–MS methodologies can achieve excellent analytical performance while significantly reducing environmental impact, thereby supporting the principles of Green Analytical Chemistry (GAC). Nevertheless, the review primarily focused on analytical sustainability and paid comparatively less attention to pharmaceutical quality control, high-resolution mass spectrometry, artificial intelligence integration, and advanced bioanalytical workflows. Compared with their work, the present review expands the discussion beyond sustainability by integrating technological innovation, digital transformation, automation, and regulatory aspects into a comprehensive evaluation of LC–MS applications in pharmaceutical analysis. However, the review focused primarily on greenness and offered limited discussion of HRMS data complexity.

Alanazi (2025) presented a recent review highlighting emerging applications of liquid chromatography–mass spectrometry in biological and pharmaceutical sciences. The study examined the expanding role of LC–MS in biomarker discovery, precision medicine, metabolomics, clinical diagnostics, toxicology, and pharmaceutical development. It also discussed recent improvements in chromatographic separation, high-resolution mass spectrometry, and intelligent data analysis, demonstrating their contribution to enhancing analytical sensitivity, molecular characterization, and laboratory efficiency. Furthermore, the review predicted that future LC–MS research would increasingly depend on automation, cloud-based analytical platforms, and artificial intelligence to support personalized medicine and high-throughput pharmaceutical research. However, the primary emphasis was placed on biomedical and clinical applications rather than routine pharmaceutical quality control, impurity profiling, and regulatory implementation. Therefore, the present review complements this work by providing a more focused and critical evaluation of LC–MS technologies in pharmaceutical analysis, with particular emphasis on analytical validation, quality assurance, and future technological trends. Its wide application focus reduced the depth of pharmaceutical quality-control analysis.

Vyas et al. (2022) provided a comprehensive overview of the fundamental principles of liquid chromatography–mass spectrometry (LC–MS), emphasizing its analytical workflow, ionization techniques, and broad applications in pharmaceutical, forensic, environmental, and food analyses. The authors highlighted that the integration of chromatographic separation with mass spectrometric detection significantly improves analytical sensitivity, specificity, and molecular identification compared with conventional chromatographic techniques. Their review also discussed various LC–MS interfaces and demonstrated the versatility of the technique in qualitative and quantitative pharmaceutical analysis. However, the review primarily focused on the theoretical foundations and general applications of LC–MS, with limited discussion of recent technological innovations, artificial intelligence (AI), automation, and regulatory considerations that are increasingly influencing modern pharmaceutical analysis. As an introductory review, it provided limited critical assessment of recent regulatory and computational developments.

Expanding beyond the general analytical principles, Rambhad and Dave (2025) concentrated specifically on the application of LC–MS for impurity profiling in pharmaceutical substances and finished drug products. Their review demonstrated that recent advances in tandem mass spectrometry and high-resolution LC–MS platforms have substantially improved the identification

of trace impurities, degradation products, and process-related contaminants while facilitating compliance with ICH and FDA regulatory guidelines. Furthermore, the authors emphasized the importance of LC–MS in ensuring pharmaceutical quality and patient safety. Nevertheless, the review was largely restricted to impurity analysis and did not comprehensively address other pharmaceutical applications such as metabolomics, bioanalysis, pharmacokinetic investigations, or emerging computational approaches that are rapidly transforming pharmaceutical analytical science. However, evidence on long-term cost-effectiveness and interlaboratory reproducibility remained limited.

Cai et al. (2025) extended the scope of LC–MS applications by exploring its emerging role in modern drug research. Their review highlighted the increasing use of chromatography–mass spectrometry in biomarker discovery, metabolomics, precision medicine, ADME studies, toxicological assessment, and drug development. In addition, the authors discussed how improvements in high-resolution mass spectrometry, intelligent data processing, and machine-learning algorithms have enhanced analytical efficiency and enabled more comprehensive molecular characterization. The study further predicted that future pharmaceutical research would increasingly rely on AI-assisted analytical platforms and high-throughput automation to accelerate drug discovery and personalized medicine. The breadth of applications was valuable, although platform-specific validation evidence was not systematically compared.

From a different perspective, D'Atri et al. (2025) examined the evolving role of liquid chromatography in pharmaceutical analysis. Rather than focusing exclusively on mass spectrometry, the review critically evaluated recent developments in chromatographic separation technologies, including ultra-high-performance liquid chromatography (UHPLC), multidimensional liquid chromatography (2D-LC), and novel stationary-phase materials. The authors demonstrated that these innovations significantly improve chromatographic resolution, analytical speed, and separation efficiency for increasingly complex pharmaceutical compounds, particularly biopharmaceuticals. However, the review devoted comparatively less attention to recent developments in mass spectrometry instrumentation, AI-driven data interpretation, and integrated LC–MS analytical workflows. Its chromatographic emphasis provided less detailed evaluation of mass-analyzer performance and AI-assisted interpretation.

Similarly, Patidar and Kamble (2025) presented a broad review of LC–MS as a hyphenated analytical technique, describing its operating principles, instrumentation, and diverse applications across pharmaceutical, biomedical, forensic, environmental, and food sciences. The authors emphasized that LC–MS remains one of the most powerful analytical platforms because of its exceptional sensitivity, selectivity, and versatility. Despite providing an accessible overview, the review remained largely descriptive and did not critically evaluate recent innovations such as artificial intelligence, sustainable analytical methodologies, automation, regulatory harmonization, or high-resolution analytical platforms that are becoming central to contemporary pharmaceutical analysis. The review was informative at a general level but offered limited methodological appraisal of the cited evidence.

Mandal et al. (2024) conducted a comprehensive review of recent developments in non-targeted liquid chromatography–mass spectrometry (LC–MS) analysis, with particular emphasis on analytical workflows, sample preparation, and high-resolution detection techniques. The authors highlighted that recent improvements in LC–MS instrumentation and bioinformatics have significantly enhanced the identification of unknown pharmaceutical compounds and complex metabolites. They also pointed out that although non-targeted LC–MS provides broader chemical coverage and greater analytical sensitivity, challenges remain regarding data standardization, computational processing, and result reproducibility. Their findings suggest that future research should focus on harmonizing analytical protocols and improving automated data interpretation. Its focus on non-targeted analysis restricted direct applicability to validated routine pharmaceutical assays.

Nguyen et al. (2024) reviewed recent advances in mass spectrometry-based metabolomics and lipidomics, emphasizing the contribution of LC–MS to pharmaceutical and clinical investigations. The study demonstrated that modern high-resolution LC–MS platforms have substantially improved the detection and quantification of metabolites, thereby facilitating biomarker discovery and precision medicine. Furthermore, the authors emphasized that continuous improvements in computational data analysis have strengthened the reliability of metabolomic profiling and expanded its applications in drug development and therapeutic monitoring. However, clinical translation and harmonization of reporting standards were not examined in sufficient depth.

In their review, Hajnajafi and Iqbal (2025) examined the complete workflow of mass spectrometry-based metabolomics, covering sample preparation, chromatographic separation, mass spectrometric detection, and data analysis. The authors emphasized the growing role of artificial intelligence and machine learning in simplifying complex datasets and improving metabolite identification. They concluded that integrating intelligent computational tools with LC–MS technology has significantly accelerated pharmaceutical research while enhancing analytical accuracy and efficiency. The review covered the complete workflow, but pharmaceutical regulatory validation was not a central focus.

Cunha et al. (2025) explored the current analytical challenges associated with biopharmaceutical

analysis, focusing on the expanding role of LC–MS in ensuring product quality, regulatory compliance, and analytical reliability. The review discussed recent technological innovations, including automated analytical systems and artificial intelligence, and explained how these developments have improved pharmaceutical characterization and quality assessment. Despite these advances, the authors identified instrument cost, regulatory requirements, and analytical complexity as continuing obstacles to routine implementation. A key limitation was the limited availability of comparable performance and implementation data across biopharmaceutical platforms.

Cañellas et al. (2021) investigated the analytical coverage of the metabolome using LC–MS, with particular attention to the influence of sample preparation procedures and chromatographic separation on analytical performance. Their review demonstrated that selecting appropriate extraction methods and chromatographic conditions greatly improves metabolite detection, analytical sensitivity, and overall data quality. The authors concluded that optimizing these preliminary analytical steps is essential for obtaining reliable LC–MS results in pharmaceutical and biomedical research. The tutorial clarified coverage limitations, although its focus was not specifically on pharmaceutical quality-control workflows.

2.1 Research Gap

Although recent studies have significantly advanced the application of liquid chromatography-mass spectrometry (LC–MS) in pharmaceutical analysis, most have addressed isolated aspects of the technology rather than providing an integrated evaluation of the complete analytical workflow. For example, Mandal et al. (2024) focused on non-targeted LC–MS analysis and bioinformatics challenges, whereas Nguyen et al. (2024) and Hajnajafi and Iqbal (2025) emphasized metabolomics, lipidomics, and AI-assisted data interpretation. Similarly, Cañellas et al. (2021) concentrated on sample preparation and chromatographic optimization, while Cunha et al. (2025) highlighted analytical and regulatory challenges in biopharmaceutical analysis. Despite these valuable contributions, there remains a lack of a comprehensive review that critically integrates recent advances in chromatographic separation, high-resolution mass spectrometry, sample preparation, artificial intelligence, automated data processing, pharmaceutical quality control, impurity profiling, regulatory requirements, and green analytical chemistry within a single framework. Furthermore, limited attention has been given to comparing how these emerging technologies collectively influence analytical performance, laboratory efficiency, sustainability, and future pharmaceutical applications. Therefore, a comprehensive and up-to-date synthesis of these technological developments is still needed to identify current knowledge gaps and provide practical directions for future LC–MS-based pharmaceutical analysis.

Table 2. Research gap matrix for recent LC–MS reviews.

Review area	Frequently covered	Commonly underreported	Contribution of the present review
Instrumentation	UHPLC and HRMS developments	Total ownership cost and transferability	Integrates performance with implementation constraints
Sample preparation	SPE and microextraction advances	Cross-study greenness and robustness comparisons	Links analytical efficiency with sustainability
AI and computation	Automated feature processing	Explainability, validation, and governance	Evaluates technical and regulatory readiness
Applications	Drug discovery, metabolomics, impurities	Routine QC and resource-limited settings	Connects applications with realistic adoption
Regulation	General need for standardization	Alignment with current validation frameworks	Provides specific harmonization priorities
Future platforms	High-resolution and non-targeted analysis	Long-term cost-effectiveness and workforce needs	Defines measurable research and training priorities

3. Methodology

3.1 Review Design

This study was conducted as a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines. The review aimed to comprehensively evaluate recent advances in liquid chromatography–mass spectrometry (LC–MS) technologies for pharmaceutical analysis, with particular emphasis on emerging analytical platforms, applications, current challenges, and future perspectives. Since this review synthesized previously published studies, ethical approval and informed consent were not required.

PRISMA 2020 Flow Diagram
Identification
Records identified from databases and supplementary searching (n = 90)
|
Duplicate records removed (n = 3)
|
Records screened by title and abstract (n = 87)
|
Records excluded (n = 17)
|
Full-text reports assessed for eligibility (n = 70)

Full-text reports excluded, with reasons (n = 50)
Studies included in qualitative synthesis (n = 20)

3.2 Information Sources

A comprehensive literature search was performed across international scientific databases to identify relevant studies published between January 2020 and December 2025. The databases included:

- Scopus
- Web of Science Core Collection
- PubMed/MEDLINE
- ScienceDirect
- SpringerLink
- Wiley Online Library
- Google Scholar (supplementary searching only because of its limited filtering and reproducibility features)

To ensure completeness, the reference lists of eligible articles were manually screened to identify additional relevant publications.

3.3 Search Strategy

The search strategy combined controlled vocabulary and free-text terms using Boolean operators (AND, OR). A representative PubMed search string was: (("liquid chromatography mass spectrometry" OR "LC–MS" OR "LC-HRMS" OR "UHPLC-HRMS") AND (pharmaceutical* OR drug* OR bioanalysis OR "quality control" OR impurity OR metabolite) AND (advance* OR application* OR technolog* OR artificial intelligence OR machine learning OR green)). Search syntax was adapted to the indexing requirements of each database.

The complete database-specific search strategies, dates of searching, and numbers retrieved should be retained in the review records and supplied as supplementary material or made available upon request.

3.4 Eligibility Criteria

Studies were selected according to predefined inclusion and exclusion criteria.

Inclusion Criteria

- Original peer-reviewed research articles.
- Articles published between January 2020 and December 2025.
- No language restriction was applied during initial screening; however, only English-language full texts were included in the final synthesis.
- Studies involving LC–MS technologies in pharmaceutical analysis.
- Studies evaluating analytical performance, pharmaceutical applications, technological developments, or clinical applications.

Exclusion Criteria

- Conference abstracts.
- Editorials and commentaries.
- Letters to the editor.
- Book chapters.
- Patents.
- Non-English publications.
- Studies unrelated to pharmaceutical LC–MS applications.
- Articles lacking sufficient experimental information.

3.5 Study Selection

All retrieved records were exported to reference-management software for duplicate removal. Reviewer A and Reviewer B independently screened titles and abstracts against the predefined eligibility criteria. Potentially relevant studies underwent full-text assessment, and disagreements were resolved through discussion and consensus. Where sufficient screening records are available, inter-reviewer agreement should be reported using Cohen’s kappa coefficient.

The study selection process was documented using the PRISMA 2020 Flow Diagram.

3.6 Data Extraction

Data extraction was independently performed using a standardized form. The following information was collected from each eligible study:

- Author(s)
- Publication year
- Country
- LC–MS platform
- Chromatographic conditions
- Mass analyzer
- Pharmaceutical application
- Analytical performance
- Major findings
- Study limitations, funding source, and declared conflicts of interest

3.7 Quality Assessment

The methodological quality of included studies was evaluated using the JBI critical-appraisal tool appropriate to each study design. Reviewer A and Reviewer B independently assessed methodological clarity, analytical validity, reproducibility, data interpretation, and reporting quality. Appraisal outcomes were used to inform interpretation rather than to conceal uncertainty.

Studies judged to have critically low methodological quality were excluded from synthesis, and the number and reasons for exclusion should be reported in the study-selection record.

3.8 Data Synthesis

A quantitative meta-analysis was not performed because of substantial heterogeneity in LC–MS platforms, chromatographic conditions, sample-preparation procedures, analytes, validation designs, and reported outcome measures. Findings were therefore synthesized using a qualitative narrative and thematic approach. A summary of included studies is provided in Table 1. Studies were grouped according to:

- LC–MS instrumentation
- Pharmaceutical applications
- Analytical performance
- AI-assisted analytical approaches
- Green analytical chemistry

Author/year	Review focus	Main contribution	Key limitation
Beccaria & Cabooter, 2020	LC–MS in pharmaceutical analysis	Integrated overview of instrumentation and applications	Limited recent AI and implementation-cost analysis
Géhin & Holman, 2021	HRMS in pharmaceuticals	Detailed assessment of accurate-mass platforms	Limited sustainability and transfer analysis
Cañellas et al., 2021	Metabolome coverage	Linked preparation and chromatography to coverage	Not focused on routine pharmaceutical QC
Vyas et al., 2022	LC–MS principles and applications	Accessible overview of workflow and ionization	Limited critical methodological appraisal
Jankech et al., 2024	Green LC and CE	Emphasized environmental performance	Limited HRMS data-complexity discussion
Khalikova et al., 2024	Role of MS in pharmaceutical analysis	Broad current appraisal of MS applications	Limited cross-platform quantitative comparison
Mandal et al., 2024	Non-targeted LC–MS	Mapped non-targeted workflows and challenges	Restricted direct relevance to validated routine assays
Nguyen et al., 2024	Targeted metabolomics/lipidomics	Highlighted clinical research applications	Limited reporting-standard harmonization
Alanazi, 2025	Biological and applied LC–MS	Summarized emerging application areas	Broad scope limited pharmaceutical QC depth
D'Atri et al., 2025	Evolving role of LC	Detailed chromatographic trends	Less emphasis on mass-analyzer and AI performance
Cai et al., 2025	Chromatography–MS in drug research	Connected technology with drug-research applications	Limited systematic validation comparison
Cunha et al., 2025	Biopharmaceutical analysis	Identified quality and regulatory challenges	Limited comparable implementation data
Hajnajafi & Iqbal, 2025	MS-based metabolomics workflow	Covered end-to-end analytical workflow	Regulatory validation not central
Patidar & Kamble, 2025	General LC–MS review	Broad instrumentation and application summary	Limited methodological appraisal
Rambhad & Dave, 2025	Impurity profiling	Focused analysis of pharmaceutical impurities	Limited cost-effectiveness and interlaboratory evidence

- Emerging technologies
- Current limitations
- Future research directions

Table 3. Summary and critical appraisal of the reviewed literature.

Technique	Main advantage	Key limitation	Green contribution
Solid-phase extraction (SPE)	Selective cleanup and concentration	Cartridge cost and method optimization	Can reduce solvent use when miniaturized
Microextraction	Low sample and solvent volume	Sensitivity to operating conditions	Strong solvent and waste reduction
QuEChERS	Rapid multi-analyte preparation	Matrix effects may persist	Low solvent use and high throughput
Protein precipitation	Simple and inexpensive	Limited cleanup for complex matrices	Low energy and short preparation time
Online/automated extraction	Reduced handling and improved reproducibility	Higher equipment and maintenance cost	Lower repeat analyses and waste

4. Results and Discussion

The systematic analysis of literature published between January 2020 and December 2025 indicates that LC–MS has undergone substantial technological development, particularly through the integration of UHPLC with HRMS. Across the reviewed evidence, these developments were associated with improved chromatographic resolution, shorter run times, enhanced mass accuracy, greater selectivity, and stronger structural elucidation. Direct numerical comparison of limits of detection, limits of quantification, recovery, and precision was not feasible because analytical targets, matrices, instruments, and validation protocols differed substantially across studies.

These findings strongly support those reported by Beccaria and Cabooter (2020), who demonstrated that coupling UHPLC with advanced MS technologies significantly enhances chromatographic resolution and analytical efficiency. Likewise, Géhin and Holman (2021) emphasized that HRMS platforms provide unprecedented mass accuracy, allowing confident structural elucidation of unknown compounds. The consistency among these independent reviews suggests that improvements in LC–MS performance are primarily driven by complementary advances in chromatographic separation and mass spectrometric detection rather than by isolated instrumental modifications alone.

A major theme was the integration of artificial intelligence, machine learning, and advanced computational platforms into LC–MS workflows. Compared with manual peak integration and conventional rule-based interpretation, modern algorithms support automated feature detection, retention-time alignment, spectral deconvolution, compound annotation, pattern recognition, and prioritization of analytical signals. Nevertheless, relatively few studies have evaluated long-term robustness, model transferability, explainability, cybersecurity, and regulatory acceptability in routine pharmaceutical laboratories.

The review further revealed that recent developments in sample preparation have contributed substantially to analytical performance. Miniaturized extraction techniques, solid-phase extraction (SPE), and microextraction methods not only improve analyte recovery and minimize matrix interference but also reduce solvent consumption, thereby supporting the principles of Green Analytical Chemistry (GAC). These findings closely correspond with those of Jankech et al. (2024), who emphasized environmentally sustainable chromatographic methodologies, and Cañellas et al. (2021), who demonstrated that optimized sample preparation remains one of the most influential factors affecting LC–MS sensitivity and reproducibility. Nevertheless, while sustainable analytical approaches have become increasingly attractive, relatively few studies have comprehensively evaluated their long-term implementation in routine pharmaceutical quality-control laboratories, indicating an important research gap.

Despite clear analytical benefits, routine implementation remains restricted by acquisition and maintenance costs, requirements for specialized analysts, complex data governance, limited interoperability, workflow standardization, and regulatory validation. These barriers are particularly important for laboratories with constrained infrastructure and training resources. Future implementation research should therefore include prospective cost-benefit analyses, interlaboratory transfer studies, standardized data formats, and competency-based training outcomes.

A critical comparison of the reviewed literature also demonstrates differences in research emphasis. Several studies concentrated primarily on individual analytical components, such as HRMS instrumentation (Géhin & Holman, 2021), impurity profiling (Rambhad & Dave, 2025), metabolomics (Nguyen et al., 2024), or chromatographic separation (D'Atri et al., 2025). In contrast, the present review integrates these diverse technological advances into a unified pharmaceutical analytical framework, thereby providing a broader understanding of how chromatographic innovation, intelligent data analysis, sustainable methodologies, and regulatory requirements collectively influence pharmaceutical analysis. This integrated perspective represents an important contribution because modern pharmaceutical laboratories increasingly require multidisciplinary analytical solutions rather than isolated technological improvements.

Overall, LC–MS is evolving from a sensitive analytical instrument into an integrated digital analytical platform combining high-resolution separation, accurate-mass detection, automated interpretation, and sustainability-oriented sample preparation. For example, UHPLC–Q-

TOF may be advantageous for broad screening and non-targeted impurity discovery, whereas UHPLC–Orbitrap platforms can offer very high resolving power and mass accuracy for complex structural characterization. Platform selection should therefore be driven by the analytical question, validation requirements, throughput, available expertise, and total cost of ownership.

4.1 Study Limitations

This study has several limitations. First, the review included peer-reviewed articles published between January 2020 and December 2025; relevant evidence outside this period was not considered. Second, only English-language full texts were included in the final synthesis, which may have introduced language bias. Third, Google Scholar was used only for supplementary searching because of its limited filtering and reproducibility features.

Furthermore, qualitative thematic synthesis was used instead of meta-analysis because of heterogeneity in analytical platforms, chromatographic conditions, sample-preparation procedures, target analytes, matrices, validation designs, and reported outcome measures. Consequently, direct statistical comparison of analytical performance across studies was not feasible.

Another limitation is that the conclusions depend on the methodological quality and reporting accuracy of the included publications, which may introduce publication bias and reporting bias. In addition, the rapid pace of technological advancements in LC–MS, artificial intelligence, and computational data analysis means that new developments emerging after the literature search period may not be reflected in this review.

Finally, although the review comprehensively evaluated recent technological advances and pharmaceutical applications, it did not experimentally validate the reported analytical methods under laboratory conditions. Therefore, the findings should be interpreted as evidence synthesized from published literature rather than direct experimental observations.

Emerging trend	Expected benefit	Current barrier	Research priority
AI-assisted data interpretation	Faster and more consistent review	Validation, explainability, and data governance	Prospective regulated-laboratory validation
Ion mobility–LC–HRMS	Additional gas-phase separation	Cost and workflow complexity	Standardized collision-cross-section libraries
Miniaturized and microflow LC–MS	Higher sensitivity with less solvent	Robustness and throughput constraints	Routine QC feasibility studies
Cloud and interoperable data platforms	Collaborative analysis and scalable computing	Cybersecurity and standardization	FAIR and regulator-ready data architectures
Automated sample preparation	Improved reproducibility and throughput	Capital cost and method transfer	Cost-effectiveness and cross-site studies

5. Conclusions

The present systematic review demonstrates that liquid chromatography–mass spectrometry (LC–MS) has become one of the most influential analytical platforms in modern pharmaceutical analysis. Recent technological developments, particularly the integration of ultra-high-performance liquid chromatography (UHPLC) with high-resolution mass spectrometry (HRMS), have significantly enhanced analytical sensitivity, selectivity, mass accuracy, throughput, and overall analytical reliability.

The review further indicates that recent advances in sample preparation techniques and green analytical methodologies have improved analytical efficiency while reducing solvent consumption and environmental impact. Moreover, the incorporation of artificial intelligence (AI), machine learning (ML), and advanced computational data-processing tools has transformed LC–MS workflows by improving automated compound identification, data interpretation, and analytical decision-making.

Despite these remarkable achievements, widespread routine implementation of advanced LC–MS technologies continues to be limited by several challenges, including high instrumentation costs, complex analytical workflows, data standardization issues, regulatory validation requirements, and the need for highly trained personnel.

Overall, the findings suggest that the future of pharmaceutical analysis will increasingly depend on integrating chromatographic innovation, high-resolution mass spectrometry, intelligent computational technologies, sustainable analytical practices, and internationally harmonized regulatory frameworks. Such integration is expected to enhance pharmaceutical quality control, accelerate drug development, improve laboratory efficiency, and strengthen regulatory compliance.

6. Recommendations

1. Promote evidence-based adoption of UHPLC–HRMS platforms in pharmaceutical quality-control laboratories after needs assessment, method-transfer evaluation, and total-cost-of-ownership analysis.

2. Expand validated AI-assisted workflows using documented, auditable platforms for peak detection, feature alignment, compound annotation, and review by qualified analysts.

3. Implement green analytical chemistry through miniaturized solid-phase extraction, microextraction, QuEChERS where appropriate, shorter columns, reduced flow rates, and safer solvents.

4. Align method development and validation with internationally recognized frameworks, including ICH Q2(R2), ICH Q14, and relevant regulatory bioanalytical-method-validation guidance.

5. Establish regional competency-based training programs in Libya and North Africa covering LC–MS operation, maintenance, troubleshooting, validation, bioinformatics, and data integrity.

6. Establish at least one multidisciplinary LC–MS research and training center linking chromatography, mass spectrometry, artificial intelligence, bioinformatics, and pharmaceutical sciences.

7. Conduct prospective cost-benefit and implementation studies comparing UHPLC–HRMS with conventional HPLC–UV in routine quality-control laboratories, including throughput, maintenance, staffing, and regulatory outcomes.

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