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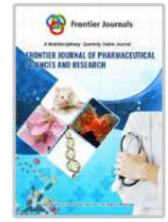

Review Paper

# Advancements in Analytical Method Development and Validation: A Review of Current Trends and Challenges

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ARTICLE INFO

ARTICLE HISTORY

Received 8 July 2025
Received in revised form 28 July 2025
Accepted 30 July 2025
Available online- 10 Aug. 2025

**Key words:**

Analytical method development; Method validation; Quality by Design (QbD); Green analytical chemistry; Chemometrics; Regulatory guidelines

ABSTRACT

Analytical method development and validation are critical processes in pharmaceutical, environmental, food, and clinical laboratories. Recent advancements in analytical technologies, regulatory requirements, and quality assurance approaches have revolutionized these processes. This review highlights the current trends, significant advancements, and persistent challenges in analytical method development and validation. It discusses innovative techniques, the role of Quality by Design (QbD), green analytical chemistry, regulatory perspectives, and common obstacles faced in practical implementation. The article aims to provide researchers and analysts with a comprehensive overview and guide for future research and practice. Furthermore, the review examines the integration of chemometric approaches in to analytical method development and validation, emphasizing their potential to improve accuracy, robustness, and efficiency. The adoption of automated and miniaturized analytical systems, including microfluidics and sensor technologies, is also explored, highlighting their advantages in terms of reduced sample volume, rapid analysis, and portability. Special attention is given to the impact of evolving global regulatory guidelines, such as those by the FDA, EMA, and ICH, on analytical practices. The article underscores the importance of harmonization among international standards and the necessity for laboratories to continually adapt to these evolving criteria.

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## 1. INTRODUCTION

Analytical methods are essential tools for the qualitative and quantitative assessment of chemical compounds in various fields, especially pharmaceuticals. The accuracy, reliability, and reproducibility of these methods underpin product safety, efficacy, and regulatory compliance. With continuous progress in instrumentation and regulatory frameworks, analytical method development and validation have become more systematic and robust. Analytical chemistry is a branch of chemistry that focuses on identifying the components of substances or mixtures (qualitative analysis) and measuring their quantities (quantitative analysis). Qualitative analysis involves determining the identity of components or analytes in a sample, while quantitative analysis involves measuring the precise amounts of these components[1-5].

Analytical data is essential not only in chemistry but also in various other scientific fields like biology and zoology, as well as in arts, including painting and sculpture. It is also important in archaeology, space exploration, and medical diagnostics. Key applications of analytical chemistry include quality control in industries, monitoring environmental pollutants, clinical and biological research, geological assessments, and both fundamental and applied scientific studies[6].

**Analytical Method Development**

Method development involves selecting the most appropriate technique and optimizing parameters to achieve accurate, precise, and reliable results. Traditionally, methods such as chromatography (HPLC, GC), spectroscopy (UV-Vis, IR, NMR), and electrophoresis have been employed. Modern trends include[7-14]

**Hyphenated Techniques**

**Automation and High-throughput Screening**

**Green Analytical Chemistry**

**Quality by Design (QbD)**

**Hyphenated Techniques**

LC-MS, GC-MS, and UPLC-MS/MS for Improved Sensitivity and Selectivity**.** Hyphenated techniques such as LC-MS, GC-MS, and UPLC-MS/MS provide substantial improvements in analytical performance by delivering significantly enhanced signal-to-noise ratios and enabling simultaneous qualitative and quantitative determinations in a single analytical run. These methods offer unparalleled flexibility, accommodating analytes across a wide chemical spectrum — from thermally stable volatiles best suited to GC-MS, to polar, thermally labile, and high-molecular-weight biomolecules ideally analyzed by LC-MS and UPLC-MS/MS — making them indispensable in pharmaceutical analysis, clinical diagnostics, forensic toxicology, metabolomics, environmental monitoring, food safety testing, and biomarker discovery. Their high-throughput capability ensures rapid processing without compromising sensitivity, while structural elucidation is achieved through detailed fragmentation patterns, isotope ratio measurements, and exact mass determinations, especially when paired with high-resolution mass spectrometry (HRMS) platforms such as Orbitrap or time-of-flight (TOF) analyzers offering sub-1 ppm mass accuracy. Ongoing advancements include the adoption of automated and robotic sample preparation workflows for greater reproducibility and throughput, the integration of multi-dimensional separations (e.g., 2D-LC-MS) to tackle ultra-complex mixtures such as crude oil fractions, plant metabolite profiles, and proteomic digests, and the development of ambient ionization techniques (e.g., DESI, DART) for rapid, in-situ analysis with minimal sample preparation, further extending the versatility and applicability of these powerful analytical tools.

**LC-MS (Liquid Chromatography–Mass Spectrometry)**

Integrates the high-resolution separation of LC with the molecular detection power of MS. Particularly suited for non-volatile, polar, thermally labile, or high-molecular-weight compounds that cannot be analyzed by GC. Widely applied in pharmaceutical impurity profiling, biomarker discovery, metabolomics, and proteomics. Electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) interfaces allow gentle ionization for fragile molecules, preserving structural integrity.

**GC-MS (Gas Chromatography–Mass Spectrometry)**

Combines the efficient volatility-based separation of GC with the mass-based identification capabilities of MS. Optimal for volatile and semi-volatile compounds, including pesticides, hydrocarbons, flavor compounds, and environmental pollutants. The use of electron impact (EI) ionization produces reproducible fragmentation patterns, enabling comparison against extensive spectral libraries (e.g., NIST), which accelerates compound identification.

**UPLC-MS/MS (Ultra-Performance Liquid Chromatography–Tandem Mass Spectrometry)**

Utilizes high-pressure pumps and sub-2 µm stationary phase particles to achieve faster separations, sharper peaks, and higher chromatographic resolution than conventional HPLC. Tandem MS (MS/MS) enables targeted quantitation and structural elucidation via precursor ion selection and controlled fragmentation in collision cells. This results in extremely low limits of detection (LOD), exceptional selectivity, and robust quantification even in complex biological or environmental matrices.

**Automation and High-throughput Screening**

Automation and High-Throughput Screening: Use of Robotics and Miniaturized Platforms. Automation and high-throughput screening (HTS) leverage advanced robotics, precision liquid-handling systems, and miniaturized assay platforms to accelerate experimental workflows while ensuring exceptional reproducibility. Robotic arms and gantry-based systems can perform repetitive tasks — including sample liquating, reagent dispensing, plate sealing, incubation, and transfer to detection instruments — with minimal human intervention, thereby reducing human error and contamination risks. Miniaturization strategies, such as the use of high-density microliter plates (384-, 1536-, or 3456-well formats) and microfluidic lab-on-a-chip devices, significantly lower reagent consumption and assay costs while enabling simultaneous processing of tens of thousands of reactions in a single day. Integration with multi-mode detection technologies — including fluorescence polarization, time-resolved fluorescence, luminescence, absorbance, surface Plasmon resonance (SPR), and mass spectrometry — broadens assay capabilities and increases sensitivity.

Automated scheduling software coordinates instrument workflows, while Laboratory Information Management Systems (LIMS) ensure secure sample tracking, data integration, and compliance with regulatory standards such as GLP and 21 CFR Part 11. Artificial intelligence and machine learning algorithms are increasingly employed for hit identification, assay optimization, and predictive modeling, transforming raw high-throughput data into actionable insights. In drug discovery, HTS enables the rapid screening of millions of small molecules against biological targets, while in molecular biology and synthetic biology, automated systems facilitate large-scale gene editing, synthetic construct assembly, and high-throughput sequencing sample preparation.

Emerging innovations include acoustic droplet ejection (ADE) for contactless liquid transfer, reducing cross-contamination; integration of organ-on-a-chip systems for physiologically relevant screening; combination of HTS with high-content imaging for simultaneous phenotypic and morphological profiling; and cloud-connected robotic platforms enabling remote experiment setup, monitoring, and data analysis. These developments are pushing HTS toward ultra-high-throughput capabilities and increasingly data-rich outputs, enabling faster, more reliable decision-making in research, diagnostics, and industrial biotechnology.

**Green Analytical Chemistry**

Green Analytical Chemistry: Minimizing Solvent Usage and Environmental Impact, Green Analytical Chemistry (GAC) emphasizes the development of analytical methodologies that maintain accuracy, precision, and sensitivity while reducing environmental hazards and resource consumption. Central strategies include minimizing or eliminating toxic and volatile organic solvents by adopting solvent-free or solvent-minimized sample preparation techniques such as solid-phase microextraction (SPME), dispersive liquid–liquid microextraction (DLLME), stir-bar sorptive extraction (SBSE), headspace gas analysis, and pressurized fluid extraction with greener solvents. Greener solvent alternatives include water, bio-based solvents (e.g., ethanol from renewable sources), ionic liquids, and deep eutectic solvents (DES), as well as supercritical fluids like $CO_2$, which can be easily recovered and recycled.

Miniaturized analytical platforms — such as micro-LC, nano-LC, capillary electrophoresis (CE), and lab-on-a-chip systems — reduce solvent usage by orders of magnitude while also decreasing energy consumption and waste production. Direct analysis techniques, including direct analysis in real time (DART-MS), portable X-ray fluorescence (pXRF), and handheld Raman or infrared spectroscopy, eliminate the need for elaborate sample preparation altogether. Energy efficiency is further enhanced through low-temperature separations, ambient ionization techniques, and instrumentation designed with energy-saving standby modes.

Waste reduction is achieved via method consolidation (multi-analyte/multi-residue methods), recyclable column materials, and the adoption of biodegradable polymers in consumables. Automation and microfluidics help optimize reagent delivery at the microliter or nanoliter scale, avoiding overuse. Analytical chemists are increasingly applying life cycle assessment (LCA) and "greenness metrics" (e.g., Analytical Eco-Scale, GAPI — Green Analytical Procedure Index) to objectively evaluate and improve the environmental performance of analytical protocols.

Recent advances include renewable energy-powered laboratories, AI-assisted method development for reduced solvent and energy use, 3D-printed microfluidic devices from biodegradable polymers, and integration of real-time monitoring systems that prevent unnecessary sampling and transportation. Regulatory bodies are also incorporating GAC principles into method validation guidelines for pharmaceuticals, environmental testing, and food safety, making sustainability a formal component of analytical quality standards.

**Quality by Design (QbD)**

Quality by Design (QbD): Systematic, Risk-Based Approaches to Method Development. Quality by Design (QbD) is a proactive, structured framework for analytical method development that integrates scientific understanding with risk management to ensure consistent performance and regulatory compliance. It starts with establishing the Analytical Target Profile (ATP) — a precise definition of the method's intended purpose, target analytes, required performance characteristics (accuracy, precision, specificity, sensitivity, linearity), and acceptance criteria. Critical Method Attributes (CMAs) and Critical Method Parameters (CMPs) are systematically identified using structured risk assessment tools such as Failure Mode and Effects Analysis (FMEA), Ishikawa (fishbone) diagrams, Hazard Analysis and Critical Control Points (HACCP), and Cause-and-Effect matrices. The relationship between CMPs and method performance is explored through Design of Experiments (DoE), employing factorial, central composite, or Box–Behnken designs to establish a Design Space — the multidimensional region within which method quality is assured.

QbD emphasizes method understanding over empirical trial-and-error, using statistical modeling, Monte Carlo simulations, and predictive analytics to optimize robustness. In addition to method validation, QbD incorporates control strategies such as in-process monitoring, statistical process control (SPC) charts, and capability analysis to detect variability early and ensure ongoing compliance with the ATP. It aligns with ICH Q8(R2), Q9, Q10, and Q14 guidelines, which advocate a lifecycle approach to analytical procedures, enabling post-approval flexibility as long as operations remain within the approved design space.

The QbD framework finds applications across pharmaceutical development, biopharmaceutical characterization, stability-indicating method development, impurity profiling, bioanalytical assays, and medical device testing. In combination with Process Analytical Technology (PAT), QbD supports real-time release testing (RTRT), adaptive manufacturing controls, and continuous process verification. Emerging innovations include the integration of digital twins for simulating analytical performance under varied conditions, machine learning for rapid prediction of method robustness, and cloud-based QbD platforms that facilitate global collaboration and regulatory documentation. This approach not only reduces development time and costs but also enhances method reliability, fosters regulatory trust, and ensures that analytical procedures remain fit-for-purpose throughout their lifecycle.

**Analytical Method Validation: Principles and Parameters**

Method validation ensures that an analytical procedure consistently produces reliable results. Key validation parameters include[15-25]

**Specificity/Selectivity**

**Linearity and Range**

**Accuracy**

**Precision (Repeatability and Reproducibility)**

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

**Robustness and Ruggedness**

**System Suitability Testing**

Regulatory bodies such as ICH, USP, and EMA provide detailed guidelines for method validation.

**Specificity/Selectivity**

Specificity, also known as selectivity, is the ability of an analytical method to accurately identify and quantify the analyte of interest in the presence of other components such as impurities, degradants, excipients, co-eluting compounds, or background matrix elements. A highly specific method ensures there is no interference at the analyte's detection wavelength, mass-to-charge ratio, or retention time. Demonstration of specificity often involves analyzing blank matrices, spiked samples, stressed samples (for degradation products), and reference standards to confirm that the analyte signal is free from overlap or distortion. In chromatographic methods, peak purity assessment using diode array detection (DAD) or mass spectrometry can further verify specificity.

**Linearity and Range**

Linearity is the method's ability to produce results that are directly proportional to analyte concentration within a specified working range. It is evaluated by preparing calibration standards across a range of concentrations and determining the correlation coefficient (r), slope, and intercept of the calibration curve, typically aiming for $r \geq 0.999$ in pharmaceutical analysis. The range is defined as the interval between the lowest and highest analyte concentrations that can be measured with acceptable accuracy, precision, and linearity. Both parameters confirm that the method will provide valid results over the intended application window, whether for trace-level quantification or high-concentration assay work.

**Accuracy**

Accuracy reflects the closeness of agreement between the test results and a true or accepted reference value, representing the method's trueness. It is usually expressed as percent recovery by comparing results from samples of known concentration (reference standards or spiked samples) to the actual value. Accuracy should be tested across the method's working range, and acceptance criteria (e.g., 98–102% recovery for assay methods) are often dictated by regulatory guidelines such as ICH Q2(R2). In biological assays, accuracy may be assessed using certified reference materials or standard addition techniques to account for matrix effects.

**Precision (Repeatability and Reproducibility)**

Precision indicates the closeness of agreement among independent test results obtained under prescribed conditions. Repeatability (intra-assay precision) measures variation under the same operating conditions over a short interval, using the same analyst, instrument, and reagents. Intermediate precision considers variations such as different days, analysts, or equipment within the same laboratory, while reproducibility assesses inter-laboratory variability. Precision is typically expressed as the relative standard deviation (RSD) or coefficient of variation (CV), with acceptable limits depending on the type of analysis (e.g., ≤2% for potency assays).

**Limit of Detection (LOD) and Limit of Quantification (LOQ)** LOD is the lowest concentration of analyte that can be detected but not necessarily quantified, typically defined by a signal-to-noise ratio (S/N) of 3:1. LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy, often corresponding to an S/N of 10:1. These parameters are critical for trace analysis, impurity profiling, and residue monitoring. Determination methods include signal-to-noise calculations, standard deviation of the response and slope of the calibration curve, or visual evaluation. Establishing reliable LOD and LOQ ensures method suitability for regulatory requirements, such as impurity limits in pharmaceuticals or contaminant levels in environmental samples.

**Robustness and Ruggedness**

Robustness is the method's ability to remain unaffected by small, deliberate changes in parameters like pH, column temperature, flow rate, mobile phase composition, or detection wavelength, reflecting its reliability under routine usage. Robustness testing identifies critical parameters that need tight control to maintain method performance. Ruggedness extends this concept to variability across different conditions, such as changes in laboratories, analysts, instruments, or reagent lots. Demonstrating ruggedness ensures that the method can be reliably transferred between sites without compromising data quality, which is essential for multi-site manufacturing or global regulatory submissions.

**System Suitability Testing**

System suitability testing (SST) verifies that the analytical system is performing adequately before and during sample analysis. Parameters such as resolution between critical peaks, theoretical plate number (efficiency), tailing factor (peak symmetry), capacity factor, retention time reproducibility, and baseline noise are assessed against predefined acceptance limits. SST ensures the system is capable of producing valid and reproducible results, and failure of SST criteria usually requires investigation and corrective action before proceeding with analysis. In regulated environments, SST results are documented as part of the analytical run to comply with cGMP and GLP standards.

**Current Trends in Analytical Method Development and Validation**[26-31]

**Quality by Design (QbD)**

QbD has emerged as a significant trend in analytical method development, emphasizing deep method understanding, structured risk assessment, and the establishment of a scientifically justified design space. By identifying critical method parameters (CMPs) and critical method attributes (CMAs) early through tools such as Design of Experiments (DoE) and Failure Mode and Effects Analysis (FMEA), QbD enables the development of methods that are inherently robust, flexible, and compliant with global regulatory standards such as ICH Q8(R2), Q9, Q10, and Q14. This approach reduces revalidation needs, facilitates method transferability across laboratories, and supports lifecycle management. Recent advancements include the integration of machine learning algorithms for predictive method optimization, the use of digital twins to simulate performance under varied conditions, and cloud-based QbD platforms that allow global collaboration and regulatory documentation sharing.

**Digitalization and Data Integrity**

The integration of digital technologies, advanced software, and automated systems into analytical laboratories is transforming how methods are developed, validated, and maintained. Digitalization supports every stage of the process — from experimental design and instrument control to data acquisition, processing, and regulatory submission — ensuring compliance with ALCOA+ principles (Attributable, Legible, Contemporaneous, Original, Accurate, plus Complete, Consistent, Enduring, and Available). Tools such as Laboratory Information Management Systems (LIMS), Electronic Laboratory Notebooks (ELNs), and chromatography data systems (CDS) enable secure, traceable, and efficient workflows. Artificial intelligence and chemometric modeling provide deeper data insights, pattern recognition, and predictive analytics for method performance. Cloud-based data storage and collaborative platforms facilitate global access, real-time monitoring, and remote audits, strengthening compliance with regulations such as 21 CFR Part 11 and EU Annex 11.

**Advances in Instrumentation**

Recent technological innovations in analytical instrumentation are enabling faster, more sensitive, and more selective analyses. Ultra-performance liquid chromatography (UPLC) and supercritical fluid chromatography (SFC) deliver high-resolution separations in shorter run times, increasing throughput without sacrificing quality. Coupling these with tandem mass spectrometry (LC-MS/MS, UPLC-MS/MS) achieves ultra-trace detection in complex matrices. High-resolution mass spectrometry (HRMS) platforms such as Orbitrap and time-of-flight (TOF) systems offer sub-ppm mass accuracy for confident identification and structural elucidation. In spectroscopy, advances in hyperspectral imaging, miniaturized nuclear magnetic resonance (NMR), Fourier-transform infrared (FTIR) with attenuated total reflectance (ATR), and portable Raman devices are expanding field and in-process applications. Integration with microfluidics, automated sample handling, and AI-assisted instrument control is paving the way for fully autonomous, high-efficiency analytical workflows that require minimal operator intervention.

**Challenges in Method Development and Validation[32-35]**

Despite significant advancements in analytical science, several challenges continue to affect the efficiency, reliability, and regulatory compliance of method development and validation processes:

**Matrix Effects:** Complex sample matrices such as biological fluids, environmental extracts, food products, or industrial process streams can cause ion suppression/enhancement, co-elution, or spectral overlap, significantly affecting accuracy and precision. These effects are particularly problematic in LC-MS/MS, GC-MS, and other hyphenated techniques.

**Method Transfer and Harmonization:** Achieving reproducibility across different laboratories, instruments, and analysts remains a key challenge. Variations in equipment calibration, environmental conditions, column batches, and operator techniques can lead to inconsistencies. Robust method transfer protocols, cross-validation studies, and harmonized SOPs are essential but resource-intensive.

**Regulatory Changes and Compliance:** Rapidly evolving international guidelines (e.g., ICH Q14 for analytical procedure development, USP <1220>, EU Annex 1 updates) require constant updates to analytical methods, validation protocols, and documentation. Adapting to these changes while avoiding operational disruptions is an ongoing challenge.

**Resource Constraints:** Limited budgets, compressed timelines, and shortages of skilled analytical chemists can slow development and compromise method quality. This is especially challenging for highly regulated industries where both speed and rigor are essential.

**Data Management and Integrity:** Ensuring secure, traceable, and compliant data handling in line with ALCOA+ principles, 21 CFR Part 11, and EU Annex 11 requires robust LIMS, ELNs, and CDS systems. Transitioning from legacy paper-based processes to fully digital workflows often involves significant investment, training, and cultural change.

**Maintaining Robustness:** Designing methods that remain unaffected by small but deliberate changes in parameters (pH, temperature, mobile phase composition, flow rate) can be challenging, especially for unstable analytes or trace-level detection.

**Instrumentation and Technology Limitations:** Even with advanced instrumentation, issues such as maintenance downtime, sensitivity drift, detector saturation, or compatibility problems with certain solvents or sample matrices can affect performance and throughput.

**Sample Stability and Handling:** Some analytes degrade rapidly or adsorb onto container surfaces, making stability studies critical but difficult. Poor sample handling can introduce variability before the analysis even begins.

**High-Throughput Pressures:** In industries such as pharmaceuticals, clinical diagnostics, and food safety, demand for rapid analysis can conflict with the need for comprehensive validation, forcing laboratories to balance speed with compliance.

**Interdisciplinary Coordination:** Method development often requires collaboration between analytical chemists, statisticians, engineers, quality assurance, and regulatory affairs. Miscommunication or misaligned priorities can delay progress and introduce errors.

**Environmental and Sustainability Considerations:** Increasing pressure to comply with green chemistry principles requires minimizing solvent use, waste generation, and energy consumption, which can limit method options or necessitate additional optimization.

**Emerging Analytical Complexity:** Novel materials such as nanomaterials, complex biologics, and multi-component formulations introduce unique analytical challenges, often requiring new technologies, unconventional approaches, and extended validation efforts.

**Global Supply Chain Issues:** Delays or shortages of critical consumables, columns, or reference standards can halt method development or validation schedules, especially in multi-site global operations.

**Regulatory Perspectives[36-40]**

International guidelines such as ICH Q2(R1) for method validation, ICH Q14 for analytical procedure development, USP <1225> for validation of compendial procedures, and region-specific guidance from agencies like the EMA and FDA provide standardized frameworks for developing, validating, and maintaining analytical methods. These guidelines define critical parameters such as accuracy, precision, specificity, linearity, robustness, and detection limits, ensuring consistent quality and reliability of analytical results across industries. However, complete harmonization across regulatory bodies remains a challenge due to regional differences in documentation expectations, acceptance criteria, and review processes. Increasingly, the industry is moving toward a science- and risk-based approach, as reflected in ICH Q8–Q14, focusing on analytical lifecycle management, Quality by Design (QbD) principles, and enhanced method understanding rather than solely end-point validation. Regulatory agencies are also emphasizing data integrity, electronic record compliance (e.g., 21 CFR Part 11, EU Annex 11), and traceability to ensure transparency and reproducibility. Collaborative initiatives such as the ICH harmonization program and pharmacopeial harmonization efforts (USP, EP, JP) are helping to reduce duplication of work and promote global alignment.

**Future Prospects[41-45]**

Future directions in analytical method development and validation will be shaped by continued integration of automation, artificial intelligence (AI), and advanced computational modeling to accelerate method optimization and robustness testing. AI-assisted platforms are expected to enable predictive method design, automated risk assessment, and real-time performance monitoring. Greener analytical approaches will become tial to meet the evolving needs of modern industry and public health.

## 2.CONCLUSION

Analytical method development and validation continue to evolve rapidly, driven by advancements in instrumentation, automation, digitalization, and data analytics, alongside increasing regulatory expectations and sustainability imperatives. The adoption of approaches such as Quality by Design (QbD), green analytical chemistry, and lifecycle management frameworks has strengthened method robustness, flexibility, and compliance. While significant progress has been achieved, persistent challenges — including matrix interferences, method transfer variability, regulatory harmonization gaps, and resource limitations — require ongoing innovation and proactive problem-solving. Emerging technologies such as artificial intelligence, machine learning, microfluidics, and real-time analytics promise to accelerate method optimization, reduce environmental impact, and enhance global standardization. To remain effective, analytical science must embrace a collaborative approach, uniting academia, industry, and regulatory bodies in developing forward-looking strategies that ensure analytical methods are not only scientifically sound and regulatory compliant but also agile, cost-effective, and environmentally responsible. Ultimately, sustained research, continuous improvement, and strategic adoption of new technologies will be essential to meet the evolving needs of modern industry and public health.

## 3.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

## 4. ACKNOWLEDGEMENT

The authors are thankful to the Management & Principal of Malla reddy College of Pharmacy, Hyderabad, T.S., India for providing necessary facilities and support to carry out this work.

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