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Review Article

A Brief Review on "Advancements in High-Performance Liquid Chromatography: Innovations and Future Trends"

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ABSTRACT

High-Performance Liquid Chromatography (HPLC) remains a cornerstone in analytical chemistry, facilitating the separation, identification, and quantification of compounds in diverse matrices. This review article provides a comprehensive overview of the recent advancements in HPLC instrumentation, method development, and validation techniques. Key developments include the emergence of Ultra-High-Performance Liquid Chromatography (UHPLC), two-dimensional HPLC (2D HPLC) and enhanced automation systems, which significantly improve analytical efficiency and sensitivity. Innovations in method development are highlighted, focusing on the application of chemometric tools, green chemistry principles and novel column technologies. Advances in mobile phase optimization, including the use of ionic liquids and gradient elution techniques, are discussed for their contributions to achieving superior separations. Enhanced detection methods, such as mass spectrometry (MS) and fluorescence detection are reviewed for their role in increasing the sensitivity and selectivity of HPLC analyses. This article also explores current validation protocols, addressing the challenges and solutions in method validation. Emerging trends, such as microfluidic HPLC and the integration of artificial intelligence (AI) in method optimization are examined to provide insights into the future directions of HPLC. Through this review, we aim to inform and guide researchers and practitioners in the field of analytical chemistry, highlighting the impact of these advancements on the precision, efficiency, and environmental sustainability of HPLC analyses.

INTRODUCTION

High-Performance Liquid Chromatography (HPLC) has established itself as an indispensable analytical technique in the field of chemistry, providing unparalleled precision and versatility in the separation, identification, and quantification of compounds. Since its inception, HPLC has

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undergone continuous evolution, adapting to the growing demands of various industries, including pharmaceuticals, environmental monitoring, food and beverage, and biotechnological research [1, 2, 4, 5].

HPLC operates on the principle of liquid chromatography, where a liquid mobile phase carries the sample through a column packed with a stationary phase. The interactions between the analytes, the stationary phase, and the mobile phase result in the differential migration of compounds, allowing for their effective separation [3]. The technique's high resolving power and sensitivity make it ideal for analyzing complex mixtures and trace components, addressing the needs of modern analytical laboratories [2, 7].

The versatility of HPLC is further enhanced by its compatibility with various detection methods, such as UV-Vis spectroscopy, fluorescence detection, and mass spectrometry. These detection techniques enable the accurate and selective identification of analytes, even at low concentrations [26, 27, 28, 24]. Moreover, advancements in column technology, mobile phase composition, and instrumentation have significantly improved the efficiency, speed, and environmental sustainability of HPLC analyses [8, 9, 10, 12, 19, 20].

In recent years, the development and validation of HPLC methods have seen remarkable progress. Innovations such as Ultra-High-Performance Liquid Chromatography (UHPLC) [8], two-dimensional HPLC (2D HPLC) [9, 10], and automated systems [33] have revolutionized the field, offering enhanced resolution, sensitivity, and throughput. Method development has also benefited from the application of chemometric tools [16], green chemistry principles [17, 18], and novel stationary phases, leading to more robust and eco-friendly analytical protocols.

This review article aims to provide a comprehensive overview of the latest advancements in HPLC instrumentation, method development, and validation techniques. By examining recent trends and emerging technologies, we seek to inform and guide researchers and practitioners in the field of analytical chemistry, highlighting the impact of these innovations on the precision, efficiency, and sustainability of HPLC analyses.

2. Recent Advancements

2.1 Instrumentation

Recent advancements in HPLC instrumentation have significantly improved the performance and capabilities of the technique:

- **Ultra-High-Performance Liquid Chromatography (UHPLC):** UHPLC systems operate at pressures up to 15,000 psi, compared to traditional HPLC systems that typically operate at 6,000 psi. The use of smaller particle sizes (sub-2 micron) in UHPLC columns allows for higher resolution and faster analysis times. This results in sharper peaks and better separation efficiency, enabling the analysis of complex samples in shorter timeframes [8].
- **Two-Dimensional HPLC (2D HPLC):** 2D HPLC combines two different separation mechanisms in a sequential manner, providing greater peak capacity and improved resolution for complex samples. The first dimension typically involves a strong separation mechanism like ion exchange or size exclusion, while the second dimension uses a different separation mechanism like reversed-phase or normal-phase chromatography. This approach allows for the separation of analytes

that are difficult to resolve using a single dimension [9, 10].

- **Automated Systems:** The integration of automation in HPLC systems has greatly enhanced their efficiency and reliability. Automated sample preparation and injection systems reduce human error and increase throughput. Advanced software controls enable precise and reproducible control of all aspects of the analysis, including solvent delivery, column temperature, and detection parameters. This automation minimizes the need for manual intervention and ensures consistent results [33].

2.2 Method Development

Innovative approaches in method development have led to more robust and efficient HPLC methods:

- **Chemometric Tools:** The application of chemometric tools such as multivariate analysis and design of experiments (DoE) has revolutionized method development in HPLC. These tools allow for the systematic optimization of method parameters, such as mobile phase composition, flow rate, and column temperature. By analyzing multiple variables simultaneously, chemometric tools identify the optimal conditions that maximize separation efficiency and minimize analysis time [16].

- **Green Chemistry:** There is a growing emphasis on implementing environmentally friendly solvents and methods in HPLC to reduce the environmental impact. Green solvents, such as water, ethanol, and supercritical fluids, are being increasingly used as alternatives to traditional organic solvents. Additionally, the development of miniaturized and microfluidic HPLC systems reduces solvent consumption and waste generation, contributing to more sustainable analytical practices [17, 18].

- **Column Technologies:** Advances in column technologies have played a crucial role in enhancing the performance of HPLC. Novel stationary phases, such as core-shell particles, provide higher efficiency and faster separations compared to traditional fully porous particles. Mixed-mode columns, which combine different separation mechanisms (e.g., reversed-phase and ion exchange), offer increased selectivity and resolution for complex samples. These innovative column technologies enable the separation of a wider range of analytes with improved efficiency [19, 20].

2.3 Mobile Phase Optimization

Optimization of the mobile phase is crucial for achieving desired separations:

- **Ionic Liquids:** The use of ionic liquids as mobile phase additives has gained attention in HPLC due to their unique properties. Ionic liquids are salts that are liquid at room temperature and possess high thermal stability, low volatility, and tunable solubility. When added to the mobile phase, ionic liquids can enhance separation efficiency and selectivity by modifying the interactions between analytes and the stationary phase [21].

- **pH Modifiers:** Innovative pH modifiers and buffers have been developed to improve peak shape and resolution in HPLC. These modifiers help maintain a consistent pH in the mobile phase, preventing the degradation or ionization of analytes. The use of pH gradients in gradient elution techniques has also been explored to achieve better separation of complex mixtures by altering the ionization state of analytes during the analysis [22].

- **Gradient Elution:** Advanced gradient elution techniques have been developed to optimize separations in HPLC. These techniques involve varying the composition of the mobile phase over time to achieve better resolution and peak shape. Gradient elution allows for the separation of analytes with a wide range of polarities and improves the overall efficiency of the analysis. Recent advancements in software and hardware have enabled precise control of gradient profiles, leading to more reproducible and efficient separations [23].

2.4 Detection Techniques

Enhanced detection methods have significantly improved the sensitivity and selectivity of HPLC analyses:

- **Mass Spectrometry (MS):** The coupling of HPLC with mass spectrometry (HPLC-MS) has revolutionized the field of analytical chemistry. MS provides high-sensitivity detection and structural elucidation of analytes. Recent advancements in MS, such as high-resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS), have further improved the accuracy and specificity of HPLC-MS analyses. These techniques allow for the identification and quantification of trace analytes in complex matrices [24, 25].
- **Fluorescence Detection:** Fluorescence detection has seen significant advancements in recent years, with the development of improved fluorescent tags and detectors. These advancements enable trace analysis of analytes with high sensitivity and selectivity. Fluorescence detection is particularly useful for the analysis of compounds that naturally fluoresce or can be derivatized with fluorescent labels [26].
- **Electrochemical Detection:** Advances in electrochemical detectors have expanded their application in HPLC. Electrochemical detection offers high sensitivity and selectivity for analytes that can undergo redox reactions. Recent developments include the use of nanomaterials and microelectrodes to enhance detection capabilities. These advancements have made electrochemical detection a valuable tool for the analysis of pharmaceuticals, environmental pollutants, and biomolecules [27, 28].

3. Method Validation

Method validation is a critical process in HPLC that ensures the reliability, accuracy, and reproducibility of analytical methods. This section provides a detailed overview of current validation protocols, challenges, and case studies in method validation for HPLC.

3.1 Validation Protocols

Current protocols for HPLC method validation are guided by regulatory bodies such as the International Council for Harmonisation (ICH), the U.S. Food and Drug Administration (FDA), and the European Medicines Agency (EMA). These protocols outline specific parameters that must be evaluated to ensure the method's reliability and accuracy. Key parameters include:

- **Accuracy**: Accuracy measures how close the test results are to the true value. It is evaluated by analyzing known quantities of the analyte and comparing the measured values to the true values. Accuracy is usually expressed as a percentage of the true value [1, 2, 5].

- **Precision**: Precision assesses the reproducibility of the method under various conditions. It is evaluated through repeatability (intra-day precision) and intermediate precision (inter-day precision). Precision is expressed as the relative standard deviation (RSD) or coefficient of variation (CV) of the replicate measurements [1, 3, 4].

- **Specificity**: Specificity, also known as selectivity, evaluates the method's ability to accurately measure the analyte in the presence of other components, such as impurities, degradants, and matrix components. Specificity is demonstrated by analyzing samples containing potential interfering substances and showing that the analyte can be accurately measured without interference [1, 4, 5].

- **Linearity and Range**: Linearity assesses the method's ability to produce results that are directly proportional to the concentration of the analyte within a given range. The linearity is evaluated by preparing standard solutions at different concentrations and plotting the response versus concentration. The range is the interval between the upper and lower concentration levels where the method demonstrates acceptable accuracy, precision, and linearity [1, 5].

- **Limit of Detection (LOD) and Limit of Quantification (LOQ)**: LOD is the lowest concentration of the analyte that can be detected but not necessarily quantified. LOQ is the lowest concentration that can be quantified with acceptable accuracy and precision. LOD and LOQ are determined by analyzing samples with decreasing concentrations of the analyte and identifying the concentration levels where the analyte can be detected or quantified with acceptable criteria [1, 5].

- **Robustness**: Robustness evaluates the method's ability to remain unaffected by small, deliberate variations in method parameters, such as changes in pH, temperature, flow rate, and column type. It is assessed by varying these parameters within a certain range and evaluating the impact on method performance [1, 2, 5, 52].

3.2 Challenges

Despite advancements in HPLC method validation, several challenges persist:

- **Matrix Effects**: Complex sample matrices can interfere with the accurate measurement of the analyte. Matrix effects occur when co-eluting substances affect the analyte's response, leading to inaccuracies. Addressing matrix effects requires careful selection of sample preparation techniques, such as solid-phase extraction (SPE) and liquid-liquid extraction (LLE), to minimize interference [3, 5].

- **Regulatory Compliance**: Meeting stringent regulatory requirements for method validation can be challenging. Regulatory guidelines specify detailed criteria for each validation parameter, and ensuring compliance requires thorough documentation and adherence to standardized procedures. Regular updates to regulatory guidelines necessitate continuous monitoring and adaptation of validation practices [1, 2].

- **Cost and Time**: Comprehensive method validation can be time-consuming and expensive. The need to perform extensive experiments to evaluate all validation parameters, including accuracy, precision, specificity, and robustness, can lead to increased costs and extended timelines. Balancing the need for thorough validation with resource constraints is a common challenge [1, 2, 5]

4. Emerging Trends

4.1 Advanced Column Technologies

Column technology continues to evolve, pushing the boundaries of what HPLC can achieve:

- **Core-Shell Particles**: Core-shell particles consist of a solid core surrounded by a porous shell. This design reduces the diffusion path and enhances mass transfer, resulting in higher efficiency and sharper peaks compared to fully porous particles. Core-shell columns are widely used in both HPLC and UHPLC for their excellent performance [19, 20].

- **Sub-2 Micron Particles**: Columns packed with sub-2 micron particles offer higher resolution and faster separations. These columns are designed to withstand the high pressures required for UHPLC, allowing for shorter analysis times and improved peak capacity. They are particularly beneficial for complex sample analyses and high-throughput applications [8].

4.2 Hyphenated Techniques

Combining HPLC with other analytical techniques enhances its capabilities:

- **HPLC-MS**: The coupling of HPLC with mass spectrometry (MS) provides high-sensitivity detection and structural elucidation of analytes. High-resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS) are commonly used to identify and quantify trace compounds in complex matrices. HPLC-MS is invaluable in pharmaceutical research, environmental analysis, and metabolomics [24, 25].

- **HPLC-NMR**: HPLC coupled with nuclear magnetic resonance (NMR) spectroscopy allows for detailed structural analysis of compounds. This hyphenated technique is useful for the identification of unknown compounds and the characterization of complex mixtures. HPLC-NMR is often used in natural product research and drug discovery [3].

4.3 Multidimensional Chromatography

Multidimensional chromatography (MDC) enhances the separation power of HPLC:

- **2D HPLC**: Two-dimensional HPLC involves the use of two different separation mechanisms in a sequential manner. The first dimension often employs a strong separation mechanism like ion exchange or size exclusion, while the second dimension uses a different mechanism like reversed-phase chromatography. This approach significantly increases peak capacity and resolution, making it ideal for complex sample analyses such as proteomics and metabolomics [9, 10].

- **Comprehensive 2D-LC (LC×LC)**: Comprehensive two-dimensional liquid chromatography (LC×LC) provides even higher separation power by combining two orthogonal separation techniques. Each fraction from the first dimension is further separated in the second dimension, resulting in a comprehensive separation profile. LC×LC is particularly useful for analyzing complex samples with a wide range of analytes [11, 13].

4.4 Green HPLC

The focus on environmentally friendly practices in HPLC is growing:

- **Green Solvents**: The use of green solvents, such as water, ethanol, and supercritical fluids, is increasing in HPLC. These solvents are less harmful to the environment and reduce the overall environmental impact of the analytical process. Green solvents are especially important in industries with stringent environmental regulations [17, 18].

- **Miniaturized Systems**: Micro-HPLC and nano-HPLC systems consume less solvent and generate less waste compared to traditional HPLC systems. These miniaturized systems are more sustainable and cost-effective, making them attractive for laboratories aiming to reduce their environmental footprint [30, 36].

- **Solvent Recycling**: Solvent recycling systems allow for the reuse of solvents in HPLC, reducing solvent consumption and waste. These systems are equipped with advanced purification technologies to ensure the recycled solvent meets the required purity standards for reuse [38].

4.5 Automated Method Development

Automation is transforming HPLC method development:

- **Automated Optimization**: Automated systems use algorithms and artificial intelligence (AI) to optimize method parameters, such as mobile phase composition, flow rate, and column temperature. This approach reduces the time and effort required for method development and ensures consistent and reproducible results [43].

- **High-Throughput Screening**: Automated platforms enable high-throughput screening of multiple samples and method conditions. These platforms can perform parallel analyses, significantly increasing throughput and efficiency. High-throughput screening is particularly valuable in pharmaceutical research and quality control [44].

4.6 Micro- and Nano-HPLC

Miniaturized HPLC systems offer unique advantages:

- **Micro-HPLC**: Micro-HPLC systems use capillary columns and microfluidic devices to perform separations with minimal sample and solvent volumes. These systems offer high efficiency, reduced analysis time, and lower operational costs. Micro-HPLC is ideal for applications requiring high sensitivity and low sample availability, such as environmental monitoring and clinical diagnostics [35, 36].

- **Nano-HPLC**: Nano-HPLC systems further miniaturize the separation process, using nano-scale columns and flow rates. These systems provide ultra-high sensitivity and are suitable for the analysis of minute sample volumes. Nano-HPLC is commonly used in proteomics, metabolomics, and single-cell analysis [29, 30].

4.7 Chiral and Enantioselective HPLC

Separation of enantiomers is crucial in various fields:

- **Chiral Stationary Phases**: Advances in chiral stationary phases have improved the resolution and selectivity of enantioselective HPLC. These phases are designed to interact differently with each enantiomer, allowing for their separation. Chiral HPLC is essential in the pharmaceutical industry, where enantiomers can have different therapeutic effects and safety profiles [31, 32].

- **Derivatization Techniques**: Derivatization techniques enhance the detection and separation of enantiomers. By attaching a chiral tag to the analytes, their interactions with the chiral stationary phase are modified, improving resolution. These techniques are valuable in the analysis of chiral drugs and natural products [10].

4.8 Comprehensive Data Analysis

Advanced data analysis tools are enhancing HPLC:

- **Chemometrics**: Chemometric techniques, such as principal component analysis (PCA) and partial least squares (PLS), are used to analyze complex HPLC data. These tools identify patterns, correlations, and trends in large datasets, providing valuable insights into the analytical process. Chemometrics is particularly useful in method development and quality control [15, 16].

- **Machine Learning**: Machine learning algorithms are being applied to optimize HPLC methods and predict separation outcomes. These algorithms can analyze historical data and identify the optimal conditions for a given analysis. Machine learning is revolutionizing HPLC by improving efficiency, accuracy, and reproducibility [34, 42].

5. Future Directions

The field of High-Performance Liquid Chromatography (HPLC) is continuously evolving, driven by advancements in technology and the increasing demands for more efficient, sensitive, and environmentally friendly analytical methods. This section explores the potential future directions in HPLC, highlighting areas of research and innovation that are expected to shape the future of the technique.

5.1 Miniaturization and Microfluidics

The trend toward miniaturization and the development of microfluidic HPLC systems is expected to continue:

- **Microfluidic Devices:** Microfluidic HPLC systems leverage the principles of fluid dynamics at the microscale, enabling precise control of fluid flow and reducing sample and solvent volumes. These devices offer high efficiency and rapid analysis times, making them ideal for applications requiring high-throughput and low sample consumption. Future research may focus on integrating microfluidic HPLC with other analytical techniques, such as mass spectrometry and electrophoresis, to create multifunctional lab-on-a-chip systems [35, 36].

- **Portable HPLC Systems:** The development of portable HPLC systems is anticipated to expand the applicability of HPLC beyond traditional laboratory settings. These compact and lightweight systems can be used for on-site analysis in environmental monitoring, clinical diagnostics, and food safety testing. Advances in battery technology and miniaturized components will play a crucial role in making portable HPLC a reality [37, 38].

5.2 Advanced Detection Techniques

The future of HPLC detection techniques lies in enhancing sensitivity, selectivity, and versatility:

- **Enhanced Mass Spectrometry:** Continued advancements in mass spectrometry, including the development of high-resolution and tandem mass spectrometers, will further enhance the capabilities of HPLC-MS [24].
- **Photonic Detection Methods:** Emerging photonic detection methods, such as surface-enhanced Raman scattering (SERS) and photothermal spectroscopy, hold promise for highly sensitive and selective detection of analytes. These techniques utilize the interaction of light with matter to provide unique spectral fingerprints of analytes, enabling their identification and quantification at trace levels [26, 41].
- **Quantum Dot Fluorescence:** Quantum dots are semiconductor nanocrystals that exhibit unique fluorescence properties. Their use as fluorescent labels in HPLC detection is expected to grow, providing high sensitivity and multiplexing capabilities. Future research may focus on developing quantum dot-based detection methods for a wide range of analytes [40, 41].

5.3 Data Integration and Artificial Intelligence

The integration of advanced data analysis tools and artificial intelligence (AI) into HPLC workflows is expected to revolutionize the field:

- **Machine Learning Algorithms:** Machine learning algorithms can analyze large datasets, identify patterns, and optimize method parameters. Future research may focus on developing AI-driven platforms that can predict separation outcomes, troubleshoot method issues, and automate method development processes. These platforms will enhance the efficiency, accuracy, and reproducibility of HPLC analyses [34, 43].
- **Big Data Analytics:** The integration of big data analytics into HPLC workflows will enable the comprehensive analysis of complex datasets. Future research may explore the use of big data techniques to identify trends, correlations, and anomalies in HPLC data. This approach will provide valuable insights into the behavior of analytes and the performance of HPLC methods [44].
- **Cloud-Based Data Management:** Cloud-based platforms for data storage, management, and sharing are expected to become more prevalent in HPLC. These platforms will facilitate collaboration among researchers, enable real-time data access, and ensure the secure storage of large datasets. Future developments may focus on enhancing data security and integrating advanced data analytics tools into cloud-based systems [45, 46].

5.4 Green and Sustainable HPLC

The emphasis on environmentally friendly and sustainable practices in HPLC will continue to grow:

- **Eco-Friendly Solvents:** The development of eco-friendly solvents and solvent-free extraction techniques will be a key area of research. Future research may focus on identifying alternative solvents that are less toxic, biodegradable, and derived from renewable sources. These solvents will reduce the environmental impact of HPLC analyses while maintaining high performance [17, 18].

- **Waste Reduction:** Strategies for reducing waste generation in HPLC, such as recycling and reusing solvents, will be explored further. Future research may focus on developing closed-loop systems that minimize solvent consumption and waste production. These systems will contribute to more sustainable and cost-effective HPLC workflows [38].

- **Energy Efficiency:** Improving the energy efficiency of HPLC systems is another important area of research. Future developments may focus on optimizing instrument design, reducing power consumption, and utilizing renewable energy sources. Energy-efficient HPLC systems will contribute to the overall sustainability of analytical laboratories [38].

5.5 Personalized and Precision Medicine

The application of HPLC in personalized and precision medicine is expected to expand:

- **Biomarker Discovery:** HPLC will play a crucial role in the discovery and validation of biomarkers for disease diagnosis, prognosis, and therapy monitoring. Future research may focus on developing HPLC methods for the analysis of novel biomarkers, including proteins, metabolites, and nucleic acids. These methods will provide insights into disease mechanisms and enable the development of targeted therapies [47, 48].

- **Therapeutic Drug Monitoring:** HPLC will be used for therapeutic drug monitoring (TDM) to optimize drug dosing and minimize adverse effects. Future developments may focus on creating HPLC methods for the rapid and accurate quantification of drugs and their metabolites in biological samples. These methods will support personalized treatment strategies and improve patient outcomes [49, 50].

- **Pharmacogenomics:** The integration of HPLC with pharmacogenomic studies will enable the analysis of genetic variations that influence drug response. Future research may explore the use of HPLC for the quantification of genetic biomarkers and the assessment of gene-drug interactions. This approach will contribute to the development of individualized treatment plans based on a patient's genetic profile [51].

5.6 Quality-by-Design (QbD) in HPLC

The implementation of Quality-by-Design (QbD) principles in HPLC method development and validation is expected to grow:

- **Systematic Method Development:** QbD emphasizes a systematic approach to method development, focusing on understanding the relationships between method parameters and analytical performance. Future research may explore the use of QbD tools, such as design of experiments (DoE) and risk assessment, to optimize HPLC methods. This approach will enhance method robustness, reliability, and regulatory compliance [52].

- **Continuous Improvement:** QbD principles promote continuous improvement throughout the method lifecycle. Future developments may focus on creating QbD frameworks that integrate real-time monitoring, data analysis, and feedback mechanisms. These frameworks will enable the continuous optimization and refinement of HPLC methods, ensuring consistent performance and quality [52].

CONCLUSION

In summary, High-Performance Liquid Chromatography (HPLC) remains an indispensable tool in analytical chemistry, providing unparalleled precision and versatility in the separation, identification, and quantification of compounds. Recent advancements in HPLC instrumentation, method development, mobile phase optimization, and detection techniques have significantly enhanced the efficiency, sensitivity, and environmental sustainability of HPLC analyses. The integration of innovative approaches, such as chemometric tools, green chemistry principles, and advanced column technologies, has led to the development of more robust and eco-friendly methods.

Method validation continues to play a critical role in ensuring the reliability and accuracy of HPLC methods, with current protocols and case studies highlighting the importance of addressing challenges such as matrix effects, regulatory compliance, and cost considerations. Emerging trends, including miniaturization, microfluidics, hyphenated techniques, and the use of artificial intelligence, are poised to further revolutionize HPLC, enabling faster, more efficient, and more sustainable analytical workflows.

The future of HPLC looks promising, with ongoing research and innovation driving the development of advanced column technologies, enhanced detection methods, and comprehensive data analysis tools. The application of HPLC in personalized and precision medicine, biomarker discovery, and therapeutic drug monitoring is expected to expand, providing new opportunities for improving patient outcomes and advancing scientific knowledge.

By staying informed about these advancements and embracing new technologies and methodologies, researchers and practitioners can leverage the full potential of HPLC to address complex analytical challenges and contribute to the continuous evolution of the field of analytical chemistry.

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