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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC): PRINCIPLE, APPLICATIONS, AND THE ROLE OF ARTIFICIAL INTELLIGENCE – A REVIEW

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ABSTRACT

High-Performance Liquid Chromatography (HPLC) is a versatile and powerful analytical technique widely employed to separate, detect, and quantify components of complex mixtures with high precision and accuracy. Rooted in the fundamental principles of chromatography, HPLC operates by exploiting the differential interactions of analytes with stationary and mobile phases, enabling efficient resolution of diverse chemical compounds. This review presents a comprehensive overview of HPLC, elaborating on its fundamental principles, also emphasizes HPLC's extensive applications across multiple fields, particularly in pharmaceuticals for drug analysis, in food safety for contaminant detection, Protein purification and in environmental monitoring for pollutant assessment. Additionally, it discusses recent advancements that have enhanced HPLC performance, including innovations in column design, detection technologies, and automation, as well as the integration of Artificial intelligence (AI) for improved analytical efficiency. The review concludes by highlighting current limitations and offering a holistic perspective on HPLC's continued significance in research and industrial applications.

Keywords: Chromatography, HPLC, Advances, Artificial intelligence, Detector

INTRODUCTION

Chromatography is an analytical method for separating the components of a mixture. It operates on the principle that, as the sample and mobile phase pass across a stationary phase, the constituents are resolved according to their differing affinities for the two phases (**Mahadevarao *et al.*, 2024).** The term *chromatography* originates from the Greek words *chroma* ("color") and *graphein* ("to write"). High-Performance Liquid Chromatography (HPLC), also referred to as High-Pressure Liquid Chromatography, is derived from this concept **(Vitnor *et al.*, 2022).** HPLC is an advanced form of column chromatography widely utilized in biochemistry and analytical chemistry to separate, detect, and measure active compounds. It is regarded as the most prevalent technique for the analysis, isolation, and quantification of drugs **(Rehan & Uzgare, 2026).** HPLC functions by separating mixture components according to their interactions with the stationary and mobile phases **(Hussein, 2025).** HPLC's performance improves when combined with detectors such as UV, fluorescence, electrochemical, or mass spectrometry. Customizing stationary phases (C18, ion-exchange, size-exclusion) and mobile phases (aqueous or organic) allows precise separation of chosen analytes (**Keyfi *et al.*,2024).** These chromatographic techniques provide outstanding separation efficiency, excellent resolution, and accurate quantification of complex mixtures. Since its introduction, HPLC has undergone remarkable advancements, including enhancements in column design, detection technologies, and automation (**Madhuri *et al.*, 2024).** HPLC includes several techniques: reversed-phase, normal-phase, size-exclusion, ion-exchange, and affinity chromatography. Reversed-phase HPLC is the most commonly used, suitable for separating non-polar and slightly polar substances. Normal-phase HPLC focuses on polar or moderately polar compounds. Ion-exchange HPLC separates molecules according to their charge, size-exclusion HPLC classifies them by molecular size, and affinity chromatography isolates components through specific binding interactions **(Kumar,2023).** Ultra-High-Performance Liquid Chromatography (UHPLC) has emerged as a significant advancement in this field, providing higher resolution, faster analysis times, and improved sensitivity compared to conventional HPLC. The development and application of UHPLC have further enhanced the capability of chromatographic techniques in addressing complex analytical challenges across pharmaceutical, food, and environmental sectors **(Hussain, 2024; Kumar,2023; Sonti, 2023) .**
This review examines studies published between 1997 to 2026, drawing on data from Scopus, ResearchGate, Google Scholar, and Directory of Open Access Journals (DOAJ). Its primary objective is to provide a comprehensive overview of HPLC analytical techniques, emphasizing their applications, recent advancements, enhancement with AI and limitations. The review also aims to summarize the functional principles of these techniques, highlighting their contributions to research and industrial applications in biotechnology, pharmaceuticals, and related fields.

HPLC Principle and Instrumentation

The fundamental principle of HPLC separation is adsorption (**Sonti, 2023).** In HPLC, sample components are separated through their differential partitioning between a mobile phase and a stationary phase. The mobile phase, made up of one or more solvents, moves through a column under pressure, while the stationary phase, often silica packed in the column, interacts with the sample, retaining each compound to varying degrees and producing separation. Molecules with greater affinity for the stationary phase progress more slowly, while those with lower affinity pass through more rapidly, since each compound exhibits a unique relative affinity **(Sonti, 2023; Ahmed,2024).** An HPLC system is composed of several key sub-systems, including solvent reservoirs with valves and filters, a high-pressure pump, connectors, an injector, detectors, and a data acquisition and processing unit **(Ahmed,2024).** Figure 1 shows the main parts of the HPLC system, including the solvent reservoir, pump, injector, column, detector, and data system. Solvent reservoir: Modern HPLC instruments are equipped with three or more glass reservoirs, each more of solvent. The solvents flow into a mixing chamber to form a uniform solution, which is then pushed through the column by a high-pressure pump **(Sonti, 2023).**
High Pressure pump: It maintains steady flow and pressure in the mobile phase, essential for consistent separation **(Singh *et al.*, 2024).**
Injectors: The sample is introduced at the head of the column with minimal disturbance to the column packing. Only a small amount of sample should be injected, as overloading can cause band broadening. Common types of HPLC injectors include septum (or syringe) injectors, stop-flow injectors, and Rheodyne injectors **(Stubbs *et al.*, 1990).**
Column: The column is considered the "heart" of an HPLC system because it is the site where the separation of sample components occurs. It is filled with stationary phase material, specifically chosen for the type of analysis being conducted. As the sample passes through, different components interact uniquely with the stationary phase, resulting in their separation. The column's design, including its length, diameter, and packing material, plays a crucial role in

determining the efficiency, resolution, and overall performance of the HPLC analysis **(Singh *et al.*, 2024; Ahmed 2024).**

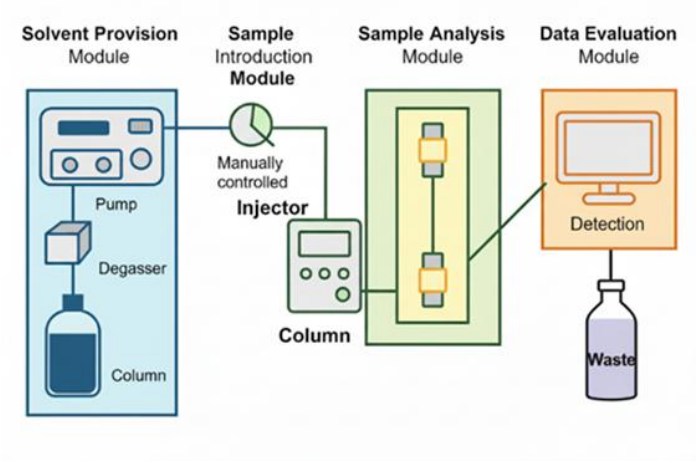

Figure 1 Instrumentation of HPLC

Detector: The detector identifies and measures the separated components as they exit the HPLC column. It provides both qualitative and quantitative information about the sample. Commonly used detectors include UV-Visible (UV-Vis) detectors, fluorescence detectors, and mass spectrometers, each chosen based on the specific properties of the analytes and the requirements of the analysis **(Madhuri *et al*., 2024; Sonti., 2023).**

Data Evaluation System: The data system is specialized software that processes and analyzes the information obtained from the HPLC detector. It provides both qualitative and quantitative details about the components present in the sample, allowing accurate interpretation and reporting of the results **(Madhuri *et al*., 2024; Sonti., 2023).**

Broad Horizons: HPLC Applications

HPLC is widely used across pharmaceuticals, biotechnology, forensic science, agrochemicals, and environmental analysis for detecting, quantifying, and purifying compounds with high accuracy, efficiency, and reproducibility. Its rapid, cost-effective, and versatile nature makes it a gold standard in analytical applications **(Sonti, 2023).**

Pharmaceutical Applications of HPLC

Figure 2 indicates Application of HPLC in Pharmaceuticals.

Over the past decade, HPLC has largely supplanted spectroscopic methods and gas chromatography (GC) for the qualitative and quantitative analysis of pharmaceuticals. Initially considered complementary to GC, HPLC is now preferred due to its flexible mobile phase options, adjustable polarity, and diverse stationary phases, which enable superior separation and resolution of complex drug mixtures **(Nikolin *et al.*,2004).**

Several recent studies illustrate the versatility and precision of HPLC across different pharmaceutical applications. For instance, **Alqahtani *et al*., (2025)** developed a green HPLC method for analyzing four cephalosporins in pharmaceuticals and water, achieving rapid separation under 6 minutes with validated accuracy, precision, and environmental friendliness (AGREE 0.75, BAGI 77.5). Similarly, **Raut & Shaji (2024),** reported a validated RP-HPLC method for tetrahydrocurcumin (THC), demonstrating excellent linearity ($R^2 = 0.9998$) and precision, suitable for routine quality control. Comparative analysis of drug impurities, as shown by **Matmour *et al.*, (2022)** in ciprofloxacin hydrochloride samples, highlights HPLC's superior sensitivity over TLC in detecting trace impurities and ensuring compliance with pharmacopoeial standards.

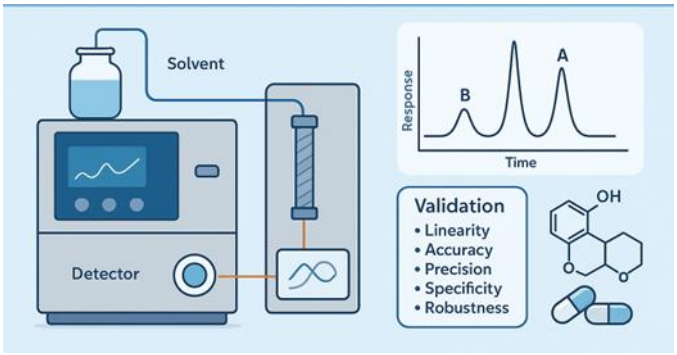

Figure 2 Application of HPLC in Pharmaceuticals

HPLC's adaptability extends to multi-drug analysis, as demonstrated by **Nassar *et al*., (2025)** who developed a rapid RP-HPLC method for simultaneous quantification of five COVID-19 antivirals. The method achieved high precision and accuracy, with excellent greenness scores (AGREE 0.70, BAGI 82.5, CACI 79), underscoring both analytical reliability and environmental consideration. Similarly, **Godela *et al.*, (2025) validated** a rapid RP-HPLC approach for Fosamprenavir and its impurities, confirming HPLC's efficiency in impurity profiling and routine quality control. The combination of HPLC with atmospheric pressure ionization tandem mass spectrometry (HPLC-API/MS/MS), as discussed by **Korfmacher *et al.*, (1997**) further expands its capabilities for metabolite identification and plasma drug quantification, offering critical insights during drug discovery and development.

Innovations in HPLC methodology, such as the use of relative molar sensitivity (RMS) by **Ohtsuki *et al.*, (2025)** enable accurate clinical drug monitoring without requiring identical reference standards, demonstrating the technique's flexibility for both research and clinical applications. **Bhardwaj *et al.*, (2020)** further emphasize a structured approach to method development, integrating drug chemistry, chromatographic optimization, and stress testing to ensure robustness and specificity. Collectively, these studies underscore HPLC's pivotal role in pharmaceutical analysis, highlighting its comparative advantages in sensitivity, speed, environmental compatibility, and adaptability across diverse analytical contexts.

Application of HPLC in Forensic Science

Recent advances have introduced new technologies to forensic investigations, with chromatography emerging as a key analytical tool. Techniques such as GC, paper chromatography, HPLC, and TLC are widely applied due to their accuracy, cost-effectiveness, and operational simplicity **(Bhoj *et al.*, 2020).** Among these, HPLC has become particularly important for detecting explosives and specific drugs, demonstrating its versatility and adaptability in forensic science **(Young *et al.*, 2022; Serol *et al.*, 2023).** By enabling the analysis of volatile compounds, gunpowder residues, and toxic substances, HPLC provides crucial insights into materials used in criminal activities and forensic evidence.

Several studies illustrate HPLC's capabilities in forensic applications. **Sánchez-Sellero *et al*.,(2024)** developed and validated a method for measuring lamotrigine in human plasma, suitable for therapeutic monitoring and forensic cases such as accidental overdose or suicide. The method achieved excellent linearity ($r = 0.993$), high recovery (>98.9%), and low limits of detection, proving robust for routine forensic toxicological analysis. Pragst, (2008) highlighted HPLC's advantages over gas chromatography for drugs, pesticides, and organic poisons, particularly for thermally unstable or non-volatile compounds, emphasizing continuous improvements in sensitivity, separation power, and applicability to complex matrices.

In environmental forensic studies, HPLC also proves valuable. **Lee *et al.*** showed its utility in profiling non-volatile compounds in soils, while noting that UPLC offered greater sensitivity and efficiency under optimized conditions. **Ünsal & Erkan (2022)** applied RP-HPLC for reliable separation and quantification of organic explosives, achieving accurate detection of TNT and RDX in seized samples within 18 minutes, demonstrating HPLC's effectiveness for rapid forensic screening.

Collectively, these studies highlight HPLC's broad applicability in forensic science, illustrating not only its sensitivity and accuracy but also its comparative advantages over other chromatographic methods for analyzing complex, non-volatile, or thermally labile compounds. This underscores its central role in modern forensic investigations and its potential for continued methodological innovation.

HPLC in Food Analysis

HPLC is a widely used and highly reproducible technique for food analysis, allowing both qualitative and quantitative assessment of nutrients, bioactive compounds, and contaminants **(Yiasmin *et al.*, 2021).** Compared to traditional methods, it offers superior sensitivity and selectivity, though it often requires careful method optimization and validation to ensure accuracy across diverse food matrices **(Nie *et al*., 2019).**

For instance, **Esteki *et al.*, (2019)** employed HPLC with chemometric processing to develop a "food fingerprinting" approach, enabling authentication and detection of adulteration, while Kowalski *et al*., (demonstrated a simple HPLC screening method for 1,3-dichloropropane-2-ol in soy sauce, which is less resource-intensive than GC-MS but may lack the same resolution for complex samples. **Gaspar *et al*., (2009)** successfully quantified furfuraldehydes and patulin in foods, showing HPLC's effectiveness in rapid quality assessment, although method development remains critical for ensuring selectivity and recovery.

Recent innovations aim to improve both sustainability and analytical performance. **Karageorgou *et al.*, (2025)** reviewed green HPLC approaches, including eco-friendly solvents and energy-efficient instruments, but adoption remains limited by cost and accessibility. Studies by **Varzakas & Kiokias (2016); Ollilainen (1999), and Prabahar, (2012)** highlight HPLC's ability to resolve complex bioactive profiles such as carotenoids, vitamin B6 vitamers, and flavonoids, demonstrating

its versatility, yet also underscoring the need for specialized expertise and rigorous method validation for reliable results.
Overall, HPLC remains an essential tool in food analysis, offering unmatched reproducibility and versatility, but its effective application depends on careful method development, appropriate detection strategies, and awareness of limitations in complex matrices.

Application of HPLC in Peptide and Protein purification

Peptides and proteins, composed of long chains of L-amino acids, perform essential biological functions, making their characterization and purification critical in research and pharmaceutical applications. HPLC has become a central tool in both analytical and preparative workflows due to its reproducibility, versatility, and compatibility with downstream techniques such as mass spectrometry (**Jadaun *et al.*, 2017; Carr *et al.*, 2014).** Figure 3 shows the process of peptide and protein purification using HPLC.

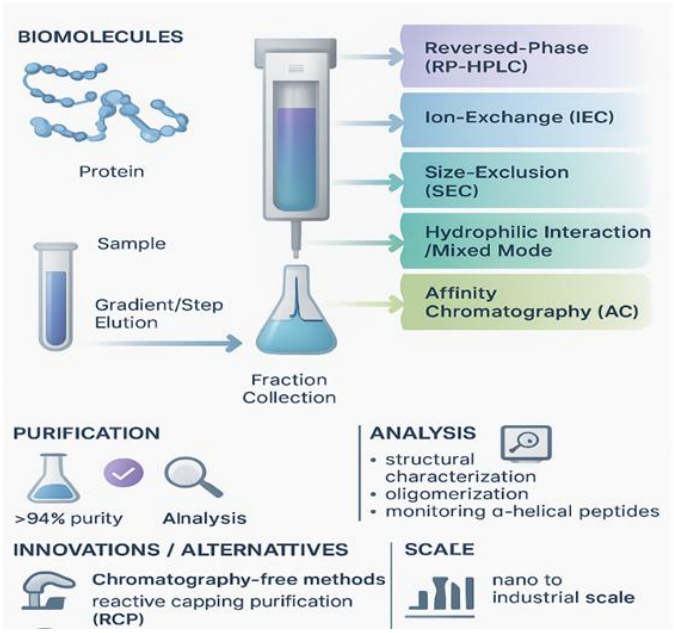

Figure 3 Peptide and Protein purification

HPLC remains one of the most powerful and adaptable techniques for peptide and protein isolation, purification, and characterization. **Mant *et al.*, (2007)** demonstrated its versatility across major chromatographic modes size-exclusion, ion-exchange, and reversed-phase while also introducing a mixed-mode hydrophilic interaction/cation-exchange method for complex peptide analysis. These methods not only enable structural and oligomerization studies but also support preparative-scale purification, with complementary use of capillary electrophoresis enhancing resolution for peptide mixtures.
Mills *et al.*,(2009*)* developed a scalable one-step RP-HPLC protocol for purifying recombinant proteins directly from *E. coli* lysates, achieving over 94% purity and recovery. Similarly, **Josic & Kovac (2010)** emphasized RP-HPLC's scalability from nano to industrial levels and its compatibility with mass spectrometry for ultra-trace detection, providing detailed guidance on gradient elution, desalting, and column maintenance.
Recent innovations have improved automation and efficiency. **Riek *et al*., (2018)** upgraded the standard Äkta Explorer HPLC system for automated multidimensional purification, reducing processing time by 85% through a four-column scheme and integrated cleaning-in-place (CIP) cycles. In the biopharmaceutical sector, **Mahdi *et al.*, (2025)** reinforced HPLC's critical role in ensuring product purity, stability, and characterization of monoclonal antibodies and recombinant proteins. Despite challenges such as matrix interference and peak overlap, advancements in UHPLC and HPLC–MS hybrids have significantly enhanced resolution and throughput.
Beyond conventional pharmaceutical applications, HPLC has also been adapted for authentication and niche biological studies**. Gan *et al*., (2019)** integrated RP-HPLC with chemometric tools such as HCA and PCA to authenticate *Ranae Oviductus*, establishing reproducible chromatographic fingerprints that differentiate genuine from counterfeit products. Innovative alternatives such as the reactive capping purification (RCP) approach proposed by **Wunderlich *et al*., (2023**) eliminate chromatography steps altogether while maintaining purification efficiency, whereas **Boughanmi *et al.*, (2024)** simplified venom peptide purification into a one-step chromatographic process, minimizing sample loss and processing time.
Collectively, these studies underscore HPLC's continuing evolution from traditional analytical and preparative separations to automated, green, and hybrid techniques. Its adaptability across diverse biomolecular systems highlights its enduring importance in peptide and protein research, though continuous improvements in automation, sustainability, and detection integration remain essential for future advancement.

HPLC: A Tool for Environmental Safety and Agrochemical Evaluation

HPLC plays a vital role in environmental analysis, particularly in identifying and quantifying contaminants such as pesticides, herbicides, heavy metals, and volatile organic compounds (VOCs) in water, soil, and air. Its ability to handle complex matrices with high sensitivity and precision makes it indispensable for environmental monitoring and pollution control **(Hussain, 2025).**
Recent studies have demonstrated significant advancements in HPLC-based environmental applications. **Melo *et al., (*2005)** developed a multiresidue RP-HPLC method for detecting six pesticides in tomatoes using laboratory-made silica-based C18 and NH_2 solid-phase extraction (SPE) sorbents. Their results showed that aminopropyl sorbents outperformed C18 in recovery and precision, while laboratory-made sorbents performed comparably to commercial materials highlighting both cost-effectiveness and analytical reliability. Similarly, **Singh & Dhalani, (2025)** proposed a simplified RP-HPLC method for simultaneous determination of seven pesticide formulations, enabling rapid, accurate, and reproducible quantification suitable for routine monitoring and quality assurance.
In a complementary direction, **Gaber *et al.*, (2011**) introduced an Environmental Assessment Tool (EAT) for evaluating the "greenness" of HPLC methods, integrating health, safety, and environmental metrics into method validation. By quantifying solvent impacts and comparing waste management strategies such as incineration and distillation, this approach aligns chromatographic analysis with sustainability principles. **Adiki *et al.*, (2021)** further improved efficiency through a fast RP-HPLC method for detecting metsulfuron-methyl, chlorantraniliprole, and chlorimuron-ethyl in rice stems, achieving clear separation with short retention times and excellent linearity, supporting its suitability for high-throughput pesticide residue monitoring.
Collectively, these studies reinforce HPLC's central role in environmental chemistry not only as a precise analytical tool but also as a platform for innovation in green, efficient, and cost-effective analysis. Its continued integration with sustainability assessment and advanced detection systems ensures its ongoing relevance in safeguarding environmental and public health.

Advantages and Limitations of HPLC Across Various Fields

Table 1 indicates HPLC advantages and limitations in Various fields

Table 1 HPLC advantages and limitations in Various fields

Name of the Fields	Advantages	Limitations	References
Pharmaceuticals	Accurate, precise, rapid separation; versatile mobile/stationary phases; suitable for impurity profiling; environmentally friendly (green HPLC)	Expensive instruments; trained personnel required; time-consuming sample preparation	**(Alqahtani *et al*., 2025; Raut *et al*., 2021; Bhoj *et al.*, 2020)**
Forensic Science	Sensitive for drugs, explosives, toxins; analyzes non-volatile/thermally labile compounds; suitable for complex matrices	High cost; extensive sample preparation; lower throughput than simple screens	**(Bhoj *et al.*, 2020; Ollilainen, 1999)**
Food Analysis	Quantifies nutrients, bioactive compounds, contaminants; supports food authentication; compatible with chemometrics	Requires careful sample prep; high equipment cost; derivatization may be needed	**(Ünsal, 2022; Nie *et al.*, 2019)**
Peptide & Protein Purification	Preparative and analytical separation; scalable; multiple chromatographic modes; MS-compatible	Stability issues; peak overlap; high solvent use; complex method development	**(Jadaun *et al.*, 2016; Mills *et al.*, 2009; Josic *et al.*, 2010)**
Environment & Agrochemicals	Accurate quantification of pesticides, herbicides, and contaminants; adaptable for multiresidue analysis; supports green assessment tools	Complex matrices; detection limits may require advanced detectors	**(Hussain 2025; Josic *et al.*, 2010; Adiki *et al.*, 2021)**

HPLC is widely applied across diverse fields due to its accuracy, sensitivity, and versatility. In pharmaceuticals, it enables precise separations and impurity profiling, though it requires costly instruments and trained personnel **(Alqahtani *et al*., 2025; Raut *et al*., 2021; Bhoj *et al.,* 2020).** Forensic science benefits from its ability to detect drugs, explosives, and toxins in complex matrices, despite high costs and extensive preparation **(Bhoj *et al.,* 2020; Ollilainen, 1999).** In food analysis, HPLC quantifies nutrients and contaminants while supporting authentication, though sample preparation and derivatization may be needed **(Jadaun *et al.,* 2016; Mills *et al.,* 2009; Josic *et al.,* 2010).** It is also essential for peptide and protein purification and environmental/agrochemical analysis, offering scalability and adaptability, albeit with challenges in method complexity and detector requirements (**Hussain 2025; Josic *et al.,* 2010; Adiki *et al.,* 2021).**

Recent Advances in HPLC

HPLC continues to evolve, with recent innovations enhancing speed, sensitivity, and sustainability UHPLC represents a major advancement, employing columns packed with sub-2 µm or core-shell particles and operating at very high pressures (up to 1000 bar), which greatly shortens analysis times while improving resolution and sensitivity compared to conventional HPLC. Advances in column design, such as monolithic and superficially porous (core-shell) columns, as well as hybrid particles with enhanced pH stability, have further improved separation efficiency, selectivity, and column durability. Detector technology has also progressed, with HPLC–mass spectrometry (LC-MS and LC-MS/MS) offering high sensitivity and structural discrimination for trace-level analysis, while diode array detectors (DAD) and evaporative light scattering detectors (ELSD) have expanded the analytical capabilities of HPLC. At the same time, green analytical chemistry approaches are gaining traction, emphasizing reduced solvent use, eco-friendly solvents, and alternative methods such as temperature-responsive liquid chromatography (TRLC) that can utilize water as a mobile phase. Automation and method development, guided by Quality by Design (QbD) principles, are optimizing HPLC workflows for faster, more reliable, and reproducible separations. Additionally, nano-LC systems allow highly sensitive analysis of extremely small sample volumes, making them particularly useful in metabolomics and proteomics research **(Hussain, 2025).**

Modern HPLC: Innovations Enhanced by Artificial Intelligence (AI) and Machine Learning (ML)

Artificial Intelligence (AI) is an emerging discipline that replicates human thinking through computational systems. Its ability to process complex datasets, learn dynamically, and generate accurate predictions makes it a transformative tool for analytical applications **(Prasad *et al.,* 2024).** Artificial intelligence (AI) and machine learning (ML) have seen rapid advancement and are increasingly adopted across various domains due to their high accuracy, speed, and predictive power. Machine learning techniques utilize historical data to detect patterns and make informed predictions. In the field of chromatography, AI and ML have been widely applied, especially in liquid chromatography method development, where they enable faster, more precise, and more efficient achievement of desired analytical outcomes **(Sigh *et al.,* 2023**). Figure 4 shows the enhancement of HPLC through the integration of artificial intelligence (AI).

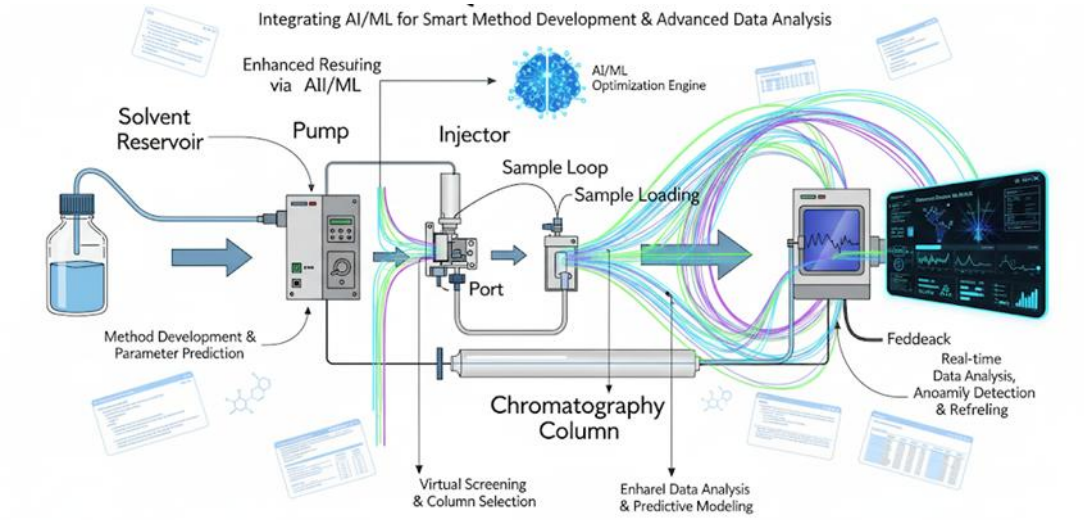

Figure 4 Enhancement of HPLC with AI

Artificial intelligence (AI) is increasingly revolutionizing chromatographic techniques such as high-performance liquid chromatography (HPLC), hydrophilic interaction liquid chromatography (HILIC), and reversed-phase liquid chromatography (RPLC). By leveraging machine learning (ML) and deep learning algorithms, AI enables simultaneous optimization of multiple chromatographic parameters, including resolution, peak capacity, and analysis time. These algorithms can process complex multidimensional datasets, identify hidden trends, and transfer learned insights between related analytical tasks, significantly accelerating method development and improving reproducibility (**Workman Jr, 2024).**

Comparative studies demonstrate the complementary strengths of AI and traditional optimization. **Lotfy *et al.,* (2025**) showed that AI-generated HPLC methods for simultaneous drug analysis offered strong predictive accuracy but required expert refinement to achieve optimal speed and solvent efficiency. Similarly, **Usman *et al.,* (2020) and Abba *et al., (*2020)** found that nonlinear AI models—particularly artificial neural networks (ANN) and adaptive neuro-fuzzy inference systems (ANFIS)—outperformed classical regression models in predicting retention times and response surfaces. These findings highlight AI's ability to enhance predictive precision and reduce experimental iterations during method development.

Osipenko *et al*., (2020) extended AI applications to nano-HPLC, employing gradient boosting models trained on large metabolite datasets. Their approach achieved lower prediction errors and fewer false positives compared with earlier machine learning frameworks, demonstrating AI's scalability to complex, high-resolution chromatographic systems.

Overall, integrating AI and ML into HPLC signifies a paradigm shift from empirical optimization to data-driven intelligence. AI enhances analytical accuracy, speeds up method design, and supports the transition toward more sustainable and automated chromatographic workflows. However, the continued involvement of human expertise remains essential for contextual interpretation, ethical data use, and methodological validation. Future developments are expected to produce hybrid AI–human analytical systems that combine computational power with expert insight, driving the next generation of intelligent, efficient, and environmentally responsible chromatography.

CONCLUSION

HPLC remains an indispensable analytical technique across pharmaceuticals, forensic science, food analysis, environmental monitoring, and peptide/protein purification, enabling precise separation, identification, and quantification of complex mixtures with high accuracy and reproducibility. Advances in column technologies, detectors, and green analytical chemistry approaches have enhanced HPLC's efficiency, selectivity, and environmental sustainability. The integration of Artificial Intelligence (AI) and machine learning (ML) further strengthens HPLC by enabling automated method development, retention time prediction, multi-objective optimization, and robust data analysis, improving workflow speed and analytical reliability. Despite these advancements, challenges remain, including limited real-world AI adoption, the need for standardized and scalable protocols, high solvent consumption in protein and peptide analysis, and underexplored applications in multi-omics and complex biological sample analyses. Addressing these gaps will enhance HPLC's efficiency, sustainability, and predictive capabilities, reinforcing its role as a versatile, innovative, and future-ready analytical platform. In our opinion, continued development and integration of AI-driven strategies, supported by advances in instrumentation and methodology, will be key to overcoming current limitations and unlocking the full potential of HPLC for diverse and complex analytical challenges.

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Author contribution: The review article was conceptualized and written by FS supervised and combined edited by AU.

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