



The Role of High-Performance Liquid Chromatography for Compound Analysis

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ABSTRACT

High Performance Liquid Chromatography (HPLC) is one of the analytical methods widely used for the separation, identification, and quantification of compounds in various types of samples. This literature review aims to examine the role of HPLC in the analysis of active compounds, contaminants, residues, and chemical components in the fields of pharmacy, food, cosmetics, and natural materials. This review analyzed fourteen scientific articles published from 2019 to 2026 that were selected based on their relevance to the fundamental principles, methodological development, validation, and diverse applications of HPLC. The review was conducted by analyzing articles indexed in reputable scientific journals that provided complete methodological and analytical information. The review results show that HPLC has advantages in terms of sensitivity, selectivity, accuracy, and the ability to separate compounds in complex matrices. Some applications found include the analysis of hydroquinone in cosmetics, determination of niacinamide in chicken meat, β -carotene in carrots, tetracycline residues in tilapia fish, differentiation of bovine and porcine gelatin, and quantification of curcumin and piperine in microparticle formulations. Based on the study results, HPLC has been proven to be an important and reliable analytical method, especially when accompanied by appropriate chromatographic condition optimization and method validation. Despite its excellent analytical performance, challenges related to solvent consumption, operational costs, and method optimization remain important considerations for future development.

Keywords: high performance liquid chromatography, method validation, compound analysis, identification.

I. INTRODUCTION

Chromatography is a separation technique used to identify and determine the chemical components in a mixture. One of the most widely used forms of chromatography in modern analysis is High-Performance Liquid Chromatography (HPLC). This method works based on the differences in interactions between the analyte and the stationary and mobile phases, resulting in each compound exiting the column at a different retention time. HPLC is widely used because it can analyze non-volatile compounds, has high sensitivity, and can be applied to pharmaceutical, food, cosmetic, environmental, and natural product samples (Ali, 2022).

The main components in an HPLC system, include the solvent reservoir, pump, injector, column, detector, and data acquisition system. The column is crucial because the separation process occurs within it. Separation success is influenced by the column type, mobile phase composition, flow rate, detection wavelength, temperature, and the chemical properties of the compounds being analyzed. In reverse-phase HPLC, the stationary phase is generally nonpolar, such as a C18 column, while the mobile phase is more polar. This system

is widely used because it is suitable for a variety of organic analytes, both in simple and complex matrices.

The development of HPLC is not limited to instrument use but also encompasses the development of stationary phases, or stationary phases. Stationary phases can be designed based on principles of affinity, electrostatic forces, or differences in molecular size. These developments aim to improve selectivity, stability, separation capability, and column reusability. Therefore, understanding the separation mechanism is crucial so that HPLC methods can be tailored to the type of analyte being analyzed (Rusli, 2021).

The use of HPLC is also increasingly widespread in product safety analysis. In the cosmetics sector, HPLC is used to detect hydroquinone, which is prohibited in cosmetic preparations because it can cause harmful effects on the skin. Research on liquid cosmetics shows that HPLC can be used to confirm the presence or absence of hydroquinone peaks at specific retention times (Fertiasari *et al.*, 2023). Furthermore, another study on high-dose hand and body lotions circulating in the marketplace showed that HPLC was able to detect the presence of hydroquinone in all tested samples, making this method important for cosmetic safety monitoring (Nurmasithoh *et al.*, 2025).

In the food industry, HPLC is widely used to determine nutritional compounds and chemical contaminants. Examples include the determination of niacinamide in broiler chicken breast, β -carotene in carrots, and the detection of tetracycline residues in tilapia. These analyses demonstrate that HPLC can be used for both nutrient quantification and antibiotic contamination monitoring (Lubis *et al.*, 2026; Mangunsong *et al.*, 2019; Pawestri *et al.*, 2019).

Based on this description, this review is important to understand how HPLC is applied in various analytical fields. This review also compares the objectives, methods, chromatographic conditions, results, and advantages and limitations of each study.

II. METHODS

This review uses a literature review method, reviewing fourteen scientific articles discussing High-Performance Liquid Chromatography (HPLC/HPLC). The articles reviewed consist of review articles and research articles discussing HPLC principles, stationary phase development, method validation, and the application of HPLC to the analysis of cosmetics, food, pharmaceuticals, natural ingredients, and halal products. The articles used include publications from 2019–2026, as well as one research article from 2021 and several articles from 2022–2025 relevant to HPLC applications.

The review process involved reading the abstract, introduction, methods, results, and conclusions of each journal. The data obtained was then organized based on the article title, research objectives, methods used, main results, and the article's relevance to the topic of high-performance liquid chromatography. The review results were analyzed then narratively to explain the development and use of HPLC in various sample types

III. RESULTS

Based on a review of fourteen articles, HPLC is used for a variety of analytical, from understanding basic principles, developing stationary phases, validating methods, to testing specific compounds in real-world samples. A summary of the journal review results is presented in the following table.

Table 1. Summary of journal review results

No.	Author/Year	Article Title	Objective	Method	Key Results
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1	Ali, 2022	<i>High-Performance Liquid Chromatography (HPLC): A Review</i>	Explains the principles, components, types, and applications of HPLC	Literature review	HPLC is widely used in pharmaceutical, food, forensic, manufacturing, and biomonitoring; the main components include reservoirs, pumps, injectors, columns, detectors, and data systems.
2	Rusli, Putri, & Alni, 2022	<i>Recent Developments of Liquid Chromatography Stationary Phases for Compound Separation</i>	Reviewing the development of the stationary phase of liquid chromatography	Literature review	Stationary phases can be developed based on affinity, electrostatics, and molecular size to increase the selectivity of compound separation.
3	Nurmasithoh, Kurniawati, & Putra, 2025	Analysis of Hydroquinone and Mercury Content in High-Dose Handbody Products	Analyzing hydroquinone and mercury in handbody products on the marketplace	HPLC for hydroquinone and color test for mercury	All samples were positive for mercury and hydroquinone; indicating the need for online cosmetics oversight.
4	Lubis et al., 2026	Optimization and Validation of HPLC Method for Determination of Niacinamide Levels in Broiler Chicken Breast Meat	Determining niacinamide levels in raw and boiled chicken	HPLC column C18, mobile phase phosphoric acid:methanol 90:10	The niacinamide content of raw chicken was 8.9 mg/100 g and boiled chicken was 6.5 mg/100 g; the method was valid with r=0.99 and RSD 0.8%.
5	Mangunsong et al., 2019	Determination of β -Carotene in Carrots by U-HPLC	Determining the β -carotene content in carrots	U-HPLC C18 column, mobile phase chloroform–methanol 95:5	The β -carotene content reached 92.5% with a retention time of 1,903 minutes.
6	Pawestri et al., 2019	Detection of Tetracycline Residues in Tilapia Meat	Detecting tetracycline residues in tilapia in Yogyakarta	Shimadzu HPLC/KCKT, samples of 61 fish from 16 markets	A total of 24 samples were positive for tetracycline and 19 samples exceeded the BMR; 31% of samples contained residues above the maximum limit.
7	Zilhada, Syafiqoh, & Betha, 2022	Differentiation of Bovine and Porcine Gelatin in Hard Capsule Shells	Differentiating between bovine and porcine gelatin on the capsule shell	KCKT combined with PCA	HPLC is able to separate the amino acids that make up gelatin, and PCA can classify gelatin in simulated capsules.
8	Fertiasari, Leni, & Kristiandi, 2023	Analysis of Hydroquinone in Liquid Cosmetics Using HPLC	Analyzing the hydroquinone content in liquid cosmetics	HPLC/HPLC with hydroquinone standard	The hydroquinone standard appeared at a retention time of 6,132 minutes, while the liquid cosmetic sample was negative for hydroquinone.
9	Setyaningsih et al., 2021	<i>Isocratic HPLC for Simultaneous</i>	Developing a RP-HPLC method for	Isocratic RP-HPLC, mobile	Valid, selective, and accurate method;

		<i>Quantification of Curcumin and Piperine</i>	the quantification of curcumin and piperine	phase acetonitrile–methanol–water	curcumin and piperine can be analyzed simultaneously with good recovery.
10	Gangnus & Burckhardt, 2021	<i>Targeted LC-MS/MS Platform for the Comprehensive Determination of Peptides in the Kallikrein-Kinin System</i>	Analysis of peptides and the kallikrein-kinin enzyme system	HPLC-based LC-MS/MS	Capable of detecting peptides at pg / mL levels.
11	Ahmada et al., 2021	<i>In Silico Method Development for the Reversed-Phase Liquid Chromatography Separation of Proteins</i>	Developing protein separation using RP-LC	RP-HPLC with computer modeling	Modeling successfully predicted optimal conditions for protein separation.
12	Zagst et al., 2022	<i>Combination of Strong Anion Exchange Liquid Chromatography with Microchip Capillary Electrophoresis</i>	Separation of complex protein mixtures	SAX-HPLC and CE	Two-dimensional methods improve the resolution of complex proteins.
13	Reinmuth-Selzle et al., 2022	<i>Determination of the Protein Content of Complex Samples</i>	Determining protein levels in complex matrices	LC-UV and AAA	The LC-220 provides accurate results on complex samples.
14	Dadouch et al., 2021	<i>In-Capillary Digestion-Reduction-Separation: A Smart Tool for Middle-Up Analysis of mAb</i>	Monoclonal antibody analysis	HPLC and capillary electrophoresis	Effective methods for mAb characterization.

IV. DISCUSSION

High-Performance Liquid Chromatography (HPLC) is an instrumental analytical method widely used in various fields due to its high sensitivity, selectivity, and accuracy in separating complex compounds. Based on a review of fourteen journals, discussions on the use of HPLC can be grouped into several categories: basic principles and development of HPLC methods, cosmetic analysis, food analysis and food safety, pharmaceutical analysis and herbal formulations, and biomolecular analysis and halal product authentication

Basic Principles and Development of HPLC/HPLC Methods

HPLC is a liquid chromatography method that works based on the differences in analytical interactions with the stationary phase and the mobile phase. Ali (2022) explains that HPLC is performed by injecting a liquid sample into a stream of mobile phase that is flowed under high pressure through a column containing the stationary phase. The different levels of interaction of each compound with the stationary phase cause each component to have a different retention time, allowing the compounds to be separated effectively. HPLC is also

widely used in the pharmaceutical, food, environmental, biotechnology, and industrial sectors because it can analyze non-volatile compounds with high sensitivity. The reverse-phase HPLC system is the most frequently used system because the stationary phase is nonpolar while the mobile phase is polar, making it suitable for separating various organic compounds. In addition, the success of the separation is influenced by the type of column, particle size, mobile phase, flow rate, temperature, and system pressure (Ali, 2022).

The development of the HPLC method is also supported by the development of the stationary phase. Rusli, Putri, and Alni (2022) explain that the stationary phase is developed based on affinity interactions, electrostatic interactions, and molecular size to increase selectivity and separation efficiency. This development allows HPLC to be used for the analysis of complex compounds such as proteins, biomolecules, and small organic compounds with improved sensitivity. The main components of an HPLC instrument include a solvent reservoir, pump, injector, column, detector, and data acquisition system. The column is the most crucial component, as it is where the separation process occurs. The pump delivers the mobile phase at high pressure, ensuring rapid and efficient separation (Ali, 2022).

Ahmada et al. (2021) developed an *in silico development method* for optimizing protein separation using reversed-phase liquid chromatography. This research was conducted because proteins are complex biomolecules that easily undergo structural changes during the chromatography process. The study used proteins with a molecular weight of 12–670 kDa and utilized mathematical modeling to predict optimal separation conditions. The mobile phase used contains a catotropic agent to minimize protein conformational changes during the separation process. The research results showed that computer simulations could predict optimal protein separation conditions with a low error rate compared to actual experimental results. The use of a cathotropic agent increased protein recovery and maintained protein structure stability during analysis. This research demonstrated that integrating computational technology with HPLC can accelerate method optimization while reducing solvent consumption and experimental time.

Cosmetic Analysis Using HPLC/HPLC

In cosmetics, HPLC is used for product safety analysis, particularly in detecting hydroquinone. Hydroquinone is a phenolic compound commonly used as a skin whitening agent, but its use is restricted due to its potential side effects such as irritation, ochronosis, and skin damage with long-term use.

Fertiasari, Leni, and Kristiandi (2023) explained that the working principle of HPLC in hydroquinone analysis is carried out by separating components based on their polarity level and detected using a UV detector that produces a chromatogram. In this study, liquid cosmetic samples were prepared using methanol, then centrifuged and filtered using a 0.45 µm membrane filter before being analyzed using HPLC. The results showed that the hydroquinone standard had a retention time of 6,132 minutes with a peak area of 506730 µL/minute. However, in the tested liquid cosmetic samples, no peak was found at that retention time, so they were declared negative for containing hydroquinone (Fertiasari et al., 2023).

In contrast to the research, Nurmasithoh, Kurniawati, and Putra (2025) found that all high dose handbody samples obtained from the marketplace were positive for hydroquinone and mercury. Hydroquinone analysis was carried out using HPLC with a C18 column, a 254 nm UV detector, a mobile phase of methanol: water (70:30) added with 0.1% acetic acid, and a flow rate of 1.0 mL/min. Sample preparation was carried out by extraction using methanol then filtered using a 0.45 µm syringe filter membrane before being injected into the HPLC system. Hydroquinone levels were determined based on the peak area of the chromatogram and compared with a standard calibration curve (Nurmasithoh et al., 2025).

Both studies showed that HPLC has high sensitivity and selectivity in detecting hydroquinone in complex cosmetic matrices, making this method very important in cosmetic safety monitoring.

Food Analysis and Food Safety Using HPLC/HPLC

In the food sector, HPLC is used for analysis of nutritional content and Detection of chemical contaminants. Lubis et al. (2026) optimized and validated the HPLC method to determine niacinamide levels in raw and boiled broiler chicken breast. Niacinamide is a vitamin B3 derivative that plays an important role in energy metabolism and skin health. The method utilized a C18 column with a mobile phase of phosphoric acid: methanol (90:10), a flow rate of 0.30 mL/min, and UV detection at a wavelength of 260 nm. The optimization results showed that niacinamide eluted at a retention time of 6.6 minutes with a symmetrical peak shape and good resolution.

The validation of the method showed linearity with a correlation value of 0.99, precision with an RSD value of 0.8%, recovery of 110%, LoD of 0.0066 ppm, and LoQ of 0.0223 ppm. The results showed that the niacinamide level in raw chicken meat was 8.9 mg/100 g while in boiled chicken it was 6.5 mg/100 g. This indicates that the boiling process can reduce niacinamide levels due to its water-soluble and heat-sensitive nature.

In addition to vitamin analysis, HPLC is also used for the analysis of β -carotene in carrots and tetracycline residues in tilapia. Research by Mangunsong et al. (2019) demonstrated the use of U-HPLC in the analysis of β -carotene in carrots. β -carotene is an antioxidant compound that is beneficial for health, so determining its levels is important in the field of functional foods. The study used a C18 column and a chloroform–methanol mobile phase, with the results of a β -carotene content reaching 92.5% and a retention time of 1,903 minutes. These results indicate that HPLC is not only useful for the detection of hazardous compounds, but also for determining the levels of natural bioactive compounds.

In addition to nutritional analysis, HPLC is also used in food safety monitoring for antibiotic residues. Pawestri et al. (2019) examined tetracycline residues in 61 tilapia samples from 16 traditional markets in Yogyakarta City using Shimadzu HPLC/HPLC. The results showed that 24 samples tested positive for tetracycline residues and 19 samples exceeded the maximum residue limit (MRL). A total of 31% of the samples contained residue above the safe limit, indicating a health risk due to uncontrolled antibiotic use in fish farming.

The analysis shows that HPLC is used not only to determine nutritional compounds but also as a method for monitoring food safety against hazardous chemical contaminants. According to these journals, HPLC is crucial in the food industry because it can provide sensitive, accurate, and selective analysis results for complex biological matrices.

Pharmaceutical Analysis and Herbal Formulation Using HPLC

In the pharmaceutical field, HPLC is the primary method for drug quality control and analysis of active pharmaceutical ingredients. Setyaningsih et al. (2021) developed an isocratic RP-HPLC method for the simultaneous quantification of curcumin and piperine in microparticle formulations made from *Curcuma longa* and *Piper nigrum*. Curcumin is the active compound in turmeric, while piperine increases curcumin bioavailability.

The RP-HPLC method employs a mixture of acetonitrile, methanol, and water as the mobile phase and detection at a wavelength of 353 nm. Validation results demonstrate good linearity, precision, and accuracy, confirming the method's validity for the simultaneous analysis of both active compounds. Furthermore, Ali (2022) explains that HPLC is widely used in the pharmaceutical industry for drug stability analysis, active ingredient quality control, impurity determination, and analytical method validation due to its high sensitivity and

reproducibility. The use of HPLC in the pharmaceutical and herbal formulation fields demonstrates that this method can produce fast, accurate, and efficient analyses, making it crucial in ensuring the quality and safety of pharmaceutical products and natural ingredients.

Analysis of Proteins, Enzymes, Biomolecules and Authentication of Halal Products

HPLC is also used in biomolecular analysis and halal product authentication. Zilhadia, Syafiqoh, and Betha (2022) used a combination of HPLC and Principal Component Analysis (PCA) to differentiate bovine and porcine gelatin in hard capsule shells. In this study, HPLC was used to separate the amino acid profiles of gelatin, which were then analyzed using PCA to determine the grouping patterns of bovine and porcine gelatin. The results showed that gelatin from the same animal source had similar amino acid profiles, while gelatin from different sources had different patterns.

Recent developments in HPLC have focused heavily on the separation of proteins, peptides, enzymes, and monoclonal antibodies because these biomolecules have complex structures and are prone to conformational changes. Gangnus and Burckhardt (2021) developed an HPLC-based LC-MS/MS platform for the analysis of peptides in the kallikrein-kinin system, which is associated with blood pressure regulation, inflammation, and COVID-19. This method is capable of detecting endogenous peptides down to pg / mL levels, making it highly sensitive for biological biomarkers.

Zagst et al. (2022) developed a two-dimensional method using strong anion exchange HPLC combined with microchip capillary electrophoresis for the separation of complex protein mixtures. This combination of methods improves separation resolution compared to one-dimensional methods, making it highly useful in proteomic and biotechnological analysis.

Reinmuth-Selzle et al. (2022) used liquid chromatography-UV absorbance to determine protein levels in complex samples such as pollen extracts and airborne particulates. The LC-220 method provides accurate and stable results even in complex matrices, making it suitable for environmental and biological protein analysis.

Dadouch et al. (2021) developed a middle-up analysis method for monoclonal antibodies (mAbs) using a combination of capillary electrophoresis and HPLC. This method integrates reduction, digestion, and separation in a single system, making it more efficient in characterizing monoclonal antibodies used in the biopharmaceutical industry.

The combined use of HPLC and chemometrics shows that HPLC can be used not only for the analysis of simple compounds but also for the identification of complex biomolecules and the authentication of halal products.

Future Trends and Technological Developments of HPLC

Recent advances in HPLC technology have focused on improving separation efficiency, reducing analysis time, and increasing analytical sensitivity. The development of ultra-high-performance liquid chromatography (UHPLC), monolithic columns, core-shell particle technology, and multidimensional chromatography has significantly enhanced chromatographic performance. Furthermore, the integration of HPLC with mass spectrometry (LC-MS/MS) has expanded its applications in proteomics, metabolomics, pharmaceutical analysis, and biomarker discovery.

Another important trend is the implementation of green analytical chemistry principles. Researchers are increasingly developing environmentally friendly mobile phases, reducing solvent consumption, and utilizing miniaturized chromatographic systems. In addition, artificial intelligence and machine learning are beginning to be employed for chromatographic method optimization, peak identification, and automated data interpretation. These

developments indicate that HPLC will continue to play a crucial role in analytical science while becoming more efficient, sustainable, and adaptable to future analytical challenges.

V. CONCLUSION

Based on the results of a review of fourteen journals, High Performance Liquid Chromatography (HPLC) is an analytical method that has high sensitivity, selectivity, and accuracy so it is widely used in various fields such as pharmaceuticals, food, cosmetics, biomolecules, and natural materials. HPLC works based on the interaction of analytes with stationary and mobile phases so that it can separate complex compounds effectively. In the cosmetics field, HPLC is used to detect hydroquinone in cosmetic products. In the food sector, HPLC is used to determine the content of nutrients such as niacinamide and β -carotene and detect antibiotic residues. In the pharmaceutical and herbal formulation fields, HPLC is used for quality control and simultaneous analysis of active ingredients such as curcumin and piperine. In addition, HPLC is also applied in the authentication of halal products through the analysis of biomolecules such as bovine and porcine gelatin. Based on all the journals reviewed, HPLC has been proven to be an effective, reliable, and flexible analytical method for various types of analysis both qualitatively and quantitatively.

Despite its outstanding analytical performance, several challenges remain in the application of HPLC, including high solvent consumption, operational costs, instrument maintenance requirements, and the need for extensive method optimization when analyzing highly complex matrices. Future developments are expected to focus on greener chromatographic approaches, reduced solvent usage, enhanced automation, and integration with advanced detection systems such as mass spectrometry and artificial intelligence-assisted data processing. These advancements may further improve the efficiency, sustainability, and analytical capability of HPLC in modern research and industrial applications.

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