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Literature Review : The Application of High-Performance Liquid Chromatography (HPLC) in the Analysis of Pharmaceutical Compounds

Verna Wimala Salsabila[1], Elina Jatindria[1], Rimadhyna Widya Khairunissa[1], Athaya Wijdan Pascadiksa[1], Elvina Putri Rahmawati[1], Nur'Aini Hidayati[1], Aldo Octavian Ramadhan Saputra[1], Djustike Maylasari[1]

[1] *Program Studi Farmasi, Fakultas Kedokteran, Universitas Negeri Semarang, Semarang, Indonesia*

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Corresponding Author :
Verna Wimala Salsabila
E-mail : salsabilaverna@gmail.com

ABSTRACT

High Performance Liquid Chromatography (HPLC) is an essential analytical technique used for the separation, identification, and quantification of compound within complex mixtures.This review article examines several research studies discussing the application of HPLC in drug analysis. This review provide an overview of HPLC utilization across various studies, including its technical advantages, analytical challenges in diverse matrices, differences in column and mobile phase selection, variations in sample preparation techniques, detector performance, the influence of flow rate, analytical parameter quality, and method validation evaluation across different drug classes. This review is expected to provide a comprehensive understanding of HPLC application in pharmaceutical analysis and important considerations in developing accurate and reliable analytical methods.

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Introduction

Chromatography is a physical separation technique for mixtures of substances based on differences in the migration of components in the stationary phase and the mobile phase, which can be either a liquid or a gas. This technique was first introduced by Tswett in 1903 using a calcium carbonate column to separate leaf pigments; the term "chromatography" derives from the German words 'chromos' (color) and "graphe" (writing), describing the colored bands on the adsorbent resulting from the separation. Although separation based on color is now rarely used, the term chromatography remains in use for various separation methods employing stationary and mobile phases (Poole, 2009). Chromatography has continued to evolve, and in

1966, Horváth is generally recognized as the person who developed the first High-Performance Liquid Chromatography (HPLC) instrument. Today, HPLC is ubiquitous across various research and industrial fields and continues to advance toward smaller, faster, and more efficient separations, utilizing more comprehensive and sensitive analytical sensors, as well as user-friendly analytical platforms to push the boundaries of resolution and high throughput even further (Yashin & Yashin, 2020).

High-Performance Liquid Chromatography (HPLC) is a high-pressure analytical separation technique widely used in the pharmaceutical field to identify and quantify the levels of compounds in complex mixtures due to its high sensitivity and accuracy (Suhendi & Ramly, 2021). This method involves passing a liquid mobile phase through a column containing a stationary phase using high pressure, resulting in faster and more efficient separation compared to conventional liquid chromatography (Khatak et al., 2018). HPLC is widely applied in drug analysis because it can separate compounds that differ in polarity, molecular size, or chemical stability (Goutal et al., 2016).

The separation principle in HPLC is based on differences in the analyte's affinity for the mobile phase and stationary phase, where compounds that interact more easily with the mobile phase will elute faster than those with stronger interactions with the stationary phase. Separation efficiency is influenced by factors such as mobile phase composition, pH, column type, and the detection wavelength used. Modern HPLC systems frequently use C18 columns and organic solvents such as methanol or acetonitrile because they provide flow stability and good detection response (Dong & Boyes, 2018). With this mechanism, HPLC has become the primary analytical method in pharmaceutical quality testing because it produces sharp chromatographic peaks, stable retention times, and low detection limits (Oliveira et al., 2018).

Objective

The purpose of this study is to understand the principles and applications of High-Performance Liquid Chromatography (HPLC) as an analytical method in the pharmaceutical field. This study also aims to explain the separation mechanism of compounds in HPLC based on the interaction between the mobile phase and stationary phase, as well as the factors affecting separation efficiency. In addition, the research seeks to describe the advantages of HPLC in identifying and quantifying compounds in complex mixtures with high sensitivity, accuracy, and efficiency.

Method

This study was conducted using a literature review to examine the application of HPLC (High-Performance Liquid Chromatography) in the analysis of pharmaceutical preparations. Data collection was carried out systematically from October 28 to November 12, 2025. The literature search was conducted using electronic search tools, namely the Google Scholar search engine and the MDPI international journal publisher's website. The keywords used in the search process included relevant terms such as "Drug analysis using HPLC," "Drug analysis using HPLC," and "HPLC method validation."

The article selection stage was conducted rigorously by applying several criteria to ensure the quality of the review. The established requirements or criteria are as follows: (1) the article is original research discussing the specific topic of drug analysis

using HPLC; (2) published within the last 10 years or between 2015 and 2025; (3) the manuscript is available in full-text format; (4) national journal articles must be accredited at a minimum of Sinta 6; and (5) international journal articles must be indexed in Scopus.

Based on the screening results using the above criteria, a final total of 24 articles was obtained, consisting of national and international publications. Data from the selected articles were then extracted and synthesized narratively. Data analysis focused on HPLC technical parameters (mobile phase, column, detector) as well as method validation results to provide a comprehensive overview of current trends in drug analysis.

Results

TABLE 1. Review Results of Drug Analysis Methods on the Biological Matrix

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
Metformin	Human blood plasma	Centrifugation	Column: VP-ODS C-18, 5 µm, 4.6 × 150 mm. Mobile phase: Methanol : water (3 mM SDS) pH 3 adjusted with phosphoric acid (60:40). Flow rate: 1 mL/min. Injection volume: 20 µL. UV detector at maximum wavelength 226 nm.	0.8 ppm	80–120%	Fernando et al. 2024, Jurnal Penelitian Farmasi Indonesia, Vol. 13 No. 1, pp. 32–40
Atorvastatin (ATV) and Apigenin (API)	SMEDDS (Self-Microemulsifying Drug Delivery System)	Standard stock solutions of ATV and API were prepared by dissolving 5 mg of each drug in methanol (for ATV) and DMSO (for API), then diluted as required. SMEDDS preparation	Column: Agilent Eclipse XDB C18, 5 µm, 4.6 × 150 mm. Mobile phase: acetonitrile and 0.1 M ammonium acetate buffer pH 7.0 (40:60, v/v). Flow rate: 0.4 mL/min	ATV: 0.49 µg/mL; API: 0.81 µg/mL	ATV: 98.8–101.4%; API: 101.4–102.2%	Kashmar et al., 2025. Processes 2025, 13, 2933.

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
		involved dissolving 20 mg ATV and 20 mg API into a SMEDDS formula consisting of Capmul MCM (oil phase), Cremophor RH40 (surfactant), and Transcutol HP (co-surfactant), stirred and heated at 40°C. Tablet samples were prepared by crushing ATV tablets and API capsules, dissolving the powder in methanol and DMSO, sonicated and filtered before analysis.	(optimal value 0.41 mL/min). UV detection at 266 nm (optimal isosbestic point). Injection volume: 10 µL. Column temperature: room temperature.			
Metformin hydrochloride (Metformin HCl)	Plasma	Sample preparation was carried out by extracting metformin from 500 µL plasma using 1 mL acetonitrile as the impurity solvent with double extraction, then combined, evaporated with nitrogen, and reconstituted	Column: Purosphere ® Star RP-18, 250 mm × 4.6 mm, 5 µm. Mobile phase: 10 mM KH_2PO_4 phosphate buffer with 0.3 mM SDS (70%) and acetonitrile (30%) at pH 5.2. Flow rate: 1.2 mL/min, isocratic. Injection	0.18 µg/mL	96.16–104.91%	Pradana et al., 2023. Indones. J. Chem., 23(4), 937–947

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
		with phosphate buffer and acetonitrile mixture (70:30). The solution was filtered using a 0.45 µm filter before HPLC injection.	volume: 20 µL. UV detection at 233 nm. Column temperature: room temperature.			
Tavaborole (TVB)	Various pharmaceutical matrices including topical and biological samples	Simple solid-liquid extraction using methanol and water solvents to dissolve the active compound in formulation samples.	Column: Xterra RP18, 4.6 × 150 mm, 5 µm. Mobile phase: potassium dihydrogen phosphate buffer (25 mM, pH 3.0) : acetonitrile (75:25, v/v). Detection: UV at 265 nm. Column temperature: 35°C. Flow rate: 1 mL/min. Injection volume: 15 µL.	4.45 ppm	Average recovery around 99.64% with %RSD 0.31%, indicating high accuracy and precision.	Shiv Kumar Prajapati et al., Anal. Methods, 2024, 16, 5280–5287
Glibenclamide, Metformin, Gliclazide	Human blood plasma	Sample preparation was carried out using solid-phase extraction (SPE) with SPE C-18 and SPE-MIP MAA (Molecularly Imprinted Polymer with methacrylic acid monomer). Procedures included SPE	Shimadzu column 150 mm × 4.6 mm. UV detection at 227 nm. Mobile phase: acetonitrile and 0.1% TFA (55:45 v/v). Flow rate: 1 mL/min.	SPE C-18: 1.965277 µg/mL; SPE-MIP MAA: 1.733239 µg/mL	SPE C-18: 95.99–105.17%; SPE-MIP MAA: 92.28–106.02% depending on concentration.	Rohayati A. et al. Indonesian Journal of Pharmaceutical Science and Technology. 2015;2(3):96–104.

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
		cartridge conditioning, sample loading, washing, and elution using solvents such as acetonitrile.				
Metformin, Dapagliflozin	Human plasma	Not described in detail in the abstract; drug-spiked human plasma was tested for stability through freeze–thaw cycles.	Agilent C18 Plasma column 5 µm (4.6 × 250 mm). Mobile phase: methanol and 0.05% orthophosphoric acid (pH 2.5) (50:50). Flow rate: 0.8 mL/min. Detection using DAD at 233 nm. Injection volume: 20 µL.	Not explicitly stated in the abstract.	Accuracy and precision for metformin and dapagliflozin were achieved within the tested concentration ranges (100–500 µg/mL for metformin; 2–10 µg/mL for dapagliflozin).	Patil NI, Gupta RA. Journal of Chemical Health Risks. 2024;14(4):1155–1163.
Citrenigrin F	Endophytic fungal extract (P. simplicissimum) from red ginger rhizome	Endophytic fungal extraction by maceration; extract solution diluted in ethanol/methanol. For HPLC: 25 µL injection (1 µg/mL in methanol).	Detector: PDA. Column: C18. Detection λ = 235 nm. Flow rate: 1.0 mL/min. Methanol–water gradient. Injection volume: 25 µL.	Not available	Not available	Muharini et al., ALCHEMY Jurnal Penelitian Kimia, 18(2), 158–164.
Caffeic Acid (CA), Vitexin, and Rosmarinic Acid (RA)	Thunbergia laurifolia leaf extract	Standards were prepared (1000 µg/mL) in 50% methanol. Leaf extract dissolved in	Column: Reversed-phase C18. Mobile phase: 0.5% acetic acid and methanol (gradient	<0.05 µg/mL	CA: 101.99±0.41%; Vitexin: 97.64±0.81%; RA: 98.81±0.46%	Woottisin et al. (2020), Pharmacognosy Journal, 12(3), 611–618.

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
		50% methanol (2 mg/mL), filtered with 0.22 µm filter, then injected. (Decoction)	elution). Flow rate: 1.0 mL/min. Detector: 330 nm.			
Rifampicin	Capsule	Capsule weighing and dissolution in methanol.	Column: C18. Mobile phase: gradient system of acetonitrile and distilled water. Detector: PDA 254 nm.	0.0164 %	99.96±0.04 %	Suhendi & Ramly (2021). Farmasains, 8(1).
Ibuprofen (IBP)	Ibuprofen tablets	Weighing of 20 tablets, dissolution and dilution.	Column: C18. Mobile phase: 1% chloroacetic acid pH 3.0 : acetonitrile (45:55). Flow rate: 1.5 mL/min. Detection λ = 254 nm.	0.60 µg/mL	101%	Chrissanti et al. (2020). Jurnal Farmasi dan Ilmu Kefarmasian Indonesia, 7(1), 26–31.
Ketoprofen	Enteric-coated tablet	Dissolution and dilution of tablets.	Column: RP-18 (250 × 4.6 mm, 5 µm). Mobile phase: 13 mM phosphate buffer pH 6.5 : methanol (40:60). Detector: UV 258 nm.	1.20 µg/mL	>99.97%	Umar et al. (2021). Jurnal Sains Farmasi & Klinis, 8(2), 200–207.
Metformin	Human plasma	Plasma deproteinization.	Column: C18 phase (250 × 4.6 mm). Mobile phase: methanol : acetonitrile : K2HPO4	1.5%	99.9%	Mulidini et al., Journal of Pharmaceutical and Sciences, 6(2), 741–749.

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
			containing SLS. Flow rate: 1.0 mL/min. Detector: UV 230–240 nm.			
Antalgin	Herbal pain relief powder	7 g sample + 70 mL ammonia, evaporated, methanol added, crystallized, then filtered.	C18 column; mobile phase Methanol : double-distilled water (75:25); flow rate 1 mL/min; λ = 282 nm; injection volume 20 µL.	-	14.87%	Arifah et al. (2023). Jurnal Jamu Kusuma, 3(1), 54–61.
Clobazam	Tablet	Tablets were powdered, sonicated with solvent, diluted, and filtered.	C18 column (150 × 3 mm, 3 µm); mobile phase Acetonitrile : Water (80:20); flow rate 1 mL/min; λ = 230 nm; injection volume 25 µL; temperature 40°C.	0.0042 mg/mL	98–102%	Agustina et al. (2025). Jurnal Biologi Tropis, 25(3), 4213–4220.
Paracetamol	Generic and branded tablets	Tablets powdered; 50 mg paracetamol dissolved, sonicated, and filtered.	Column L1 (3.9 mm × 30 cm); mobile phase Water : Methanol (3:1); flow rate 1.5 mL/min; λ = 243 nm; injection volume 20 µL.	Not stated	94–100%	Mariyani et al. (2023). JIFI, 7(1), 7–12.
Paracetamol & Potassium sorbate	Oral syrup	1 mL sample diluted to	Column L1 (3.9 mm × 30 cm);	Not stated	94–100%	Mariyani et al. (2023).

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
		100 mL and filtered.	mobile phase Water : Methanol (3:1); flow rate 1.5 mL/min; λ = 243 nm; injection volume 20 µL.			JIFI, 7(1), 7–12.
Paracetamol & Potassium sorbate	Oral syrup	1 mL sample diluted to 100 mL and filtered.	Column Zorbax SB-C18 (150 × 4.6 mm); mobile phase Water pH 2 : Acetonitrile (70:30); flow rate 1 mL/min; λ = 235 nm.	PCM: 10 µg/mL; PS: 6 µg/mL	98–102%	Mikulić et al. (2025). Acta Chromatographica, 37(1), 112–120.
p-Aminophenol (4-APh)	Paracetamol syrup	1 mL sample + 26% methanol, sonicated and filtered.	Column LiChrosphe r RP-18; mobile phase MeOH : phosphate buffer pH 4.07 (26:74); flow rate 1 mL/min; λ = 275.8 nm.	0.54 µg/mL	95–105%	Putri & Yuwono (2023). Journal of Islamic Pharmacy, 8(1), 27–33.
Paracetamol, Chlorpheniramine Maleate, Dextromethorphan, Phenylephrine HCl	Caplet	Powder weighing, dissolved in mobile phase, sonicated and filtered.	Column: Inertsil CN-3 (4.6 × 250 mm, 5 µm). Mobile phase: HPLC-grade acetonitrile : 150 mM citrate buffer pH 2.6 (20:80). Flow rate: 1.00 mL/min. Injection volume: 10 µL. UV	CTM: 0.37 µg/mL; Dexa: 2.02 µg/mL; PCT: 126.57 µg/mL; PH: 1.14 µg/mL	CTM: 99.295%; Dexa: 100.597%; PCT: 99.938%; PH: 100.726%	Khodijah et al. (2024). Medic Nutricia: Jurnal Ilmu Kesehatan, 2(3), 91–109.

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
			detector: 270 nm.			
Chlorpheniramine maleate (CTM)	Tablet	Dissolve CTM and matrix, sonicate 15 min, vortex 5 min, centrifuge at 3000 rpm for 15 min, filter with 0.2 µm filter.	µBondapak C18 column; mobile phase methanol + 0.2% TEA (90:10); flow rate 2 mL/min; DAD λ 262 nm.	Not available	Not available	Darmawati et al. (2020). Pharmaciana, 10(3), 269–280.
Paracetamol	Tablet & syrup	Tablets ground and weighed (~25 mg); solvent water:methanol (3:1); filtered with 0.2 µm membrane before injection.	VP-ODS column 2.5 mm × 25 cm; mobile phase water:methanol (3:1); flow rate 1.5 mL/min; λ = 243 nm.	2.56 µg/mL	99.96%	Indriana, M. (2020). Journal of Pharmaceutical and Sciences, 3(2), 106–113.
Lapatinib ditosylate	-	Weighed 2 mg, dissolved up to 20 mL, injection volume 10 µL.	XBridge C8 column (250 × 4.6 mm, 5 µm). Mobile phase: pentane-1-sulfonic acid sodium salt 10 mM : acetonitrile (65:35). Flow rate: 0.6 mL/min.	2.02 µg/mL	Not available	Nopi & Isnaeni (2023). Eruditio: Indonesia Journal of Food and Drug Safety, 4(1), 33–42.
Pyridoxine HCl (PH) & Piratiazin teoclate (PT)	Simulated tablet	Dissolved with 20 mL methanol, sonicated, diluted, filtered with 0.45 µm filter.	Mobile phase methanol : 1% acetic acid (20:80).	PH = 0.70 µg/mL; PT = 0.72 µg/mL	PH = 100.13%; PT = 99.78%	Alatas et al. (2018). Kartika: Jurnal Ilmiah Farmasi, 6(2), 95–100.
Paracetamol + Caffeine	Tablet	200 ppm (PCT) and 20 ppm (CAF); filtered.	Poroshell 120 EC C18 column; mobile phase methanol:water (30:70);	PCT = 15.9125 ppm; CAF = 15.9694 ppm	98.41–101.371%	Setyaningrum (2022). Jurnal Katalisator, 7(2), 323–335.

Drug	Matrix	Sample Preparation	HPLC Parameters	LOQ	Recovery	Reference
			λ = 275 nm; flow rate 1 mL/min.			

Discussion

High-Performance Liquid Chromatography (HPLC) is a critical analytical technique used for the separation, identification, and quantification of compounds in complex mixtures, particularly in pharmaceutical analysis. HPLC continues to evolve, offering increasingly precise, faster, and more efficient separation capabilities, supported by sensitive analytical sensors that enhance resolution and throughput. The advantage of HPLC lies in its ability to analyze drugs in various complex matrices with high precision and accuracy, as well as its flexibility in selecting columns, mobile phases, and detectors according to diverse pharmaceutical analysis needs. With strictly validated methods, HPLC has become a mainstay in modern pharmaceutical development and quality control.

Each type of detector used in HPLC has its own advantages in drug analysis. The UV-Vis detector, the most widely used, excels in sensitivity and selectivity toward compounds that absorb light at specific wavelengths, making it suitable for drugs with chromophore structures. The Photo Diode Array (PDA) detector enables simultaneous detection of the full spectrum, providing more comprehensive information for compound identification. The Fluorescence Detector offers high sensitivity for compounds capable of fluorescence, thereby lowering the detection limit. Additionally, the Refractive Index (RI) Detector is useful for compounds lacking chromophores. Detector selection is tailored to the chemical properties of the drug being analyzed and the analytical objectives, such as quantification or identification.

Drug analysis in complex matrices such as blood plasma, pharmaceutical formulations, or herbal extracts faces the challenge of proper sample preparation to eliminate matrix interference and improve measurement accuracy. Commonly used preparation techniques include solid-phase extraction (SPE), deproteinization, centrifugation, and filtration, which must be optimized according to the matrix and the drug being analyzed. Interference from matrix components can affect the sensitivity and specificity of the analysis; therefore, it is crucial to select appropriate column conditions, mobile phases, and HPLC parameters. Validation of the analytical method using parameters such as the limit of quantification (LOQ), recovery, and precision is also essential to ensure the reliability of results in complex matrices.

Based on a review of all the cited articles, the majority of studies used reversed-phase (RP) columns with an octadecylsilica (C18)-based stationary phase. The dominance of C18 columns aligns with their nonpolar, stable nature, and broad compatibility with various drug compounds, whether lipophilic or semi-polar. In the analysis of metformin in plasma, Fernando et al. (2024) used a VP-ODS C18 column (4.6 × 150 mm, 5 µm), which is widely recognized as effective for hydrophilic compounds through enhanced retention achieved by modifying the mobile phase. A C18-type column was also used in the study by Kashmar et al. (2025), specifically the Agilent Eclipse XDB C18, which is known for its end-capping technology to minimize residual silanol interactions, thereby producing more symmetrical peaks. The use of C18 columns with advanced specifications is also evident in the study by Pradana et al. (2023), which employed the Purosphere® Star RP-18 (250 × 4.6 mm). This column

is designed with a more inert stationary phase, making it suitable for analytes that readily interact with polar groups on the silica surface. Another variant, the Xterra RP18, was used by Prajapati et al. (2024) for tavaborole analysis; it was selected for its stability under low pH conditions, which are often required when testing lipophilic drugs in RP-HPLC systems.

Although C18 columns are predominant, alternative columns are also used. Nopi & Isnaeni (2023) used XBridge C8, which has shorter alkyl chains, thereby reducing the retention of highly hydrophobic compounds such as lapatinib and enabling faster analysis without compromising resolution. Additionally, several studies used L1 columns (C18 equivalent) and µBondapak C18, which are commonly used in pharmacopoeial methods for the analysis of paracetamol and chlorpheniramine maleate. Overall, the column usage patterns show a consistent preference for C18 reversed-phase columns as the primary stationary phase, with the use of C8 or specialized RP18 columns reserved to accommodate the chemical properties of analytes requiring lower retention or specific pH stability.

The selection of mobile phases in these studies generally followed the basic principles of RP-HPLC, namely the use of a mixture of organic solvents with an aqueous buffer to control pH and ensure the stability of analyte ionization. Variations in mobile phase composition reflect adjustments to the physicochemical characteristics of each compound. In the analysis of metformin by Fernando et al. (2024), the mobile phase used was methanol:water containing 3 mM SDS at pH 3, a common approach to enhance the retention of highly polar compounds through micelle interaction mechanisms, approaching semi-microemulsion phase chromatography. Kashmar et al. (2025) employed a mixture of acetonitrile : 0.1 M ammonium acetate buffer at pH 7.0 (40:60) to maintain the ionization stability of atorvastatin and apigenin, while providing a neutral pH environment that prevents the degradation of both compounds.

The use of phosphate buffer has been observed in several studies, for example by Pradana et al. (2023), who used a 10 mM KH_2PO_4 buffer with 0.3 mM SDS:acetonitrile (70:30) at pH 5.2. This composition is designed to maintain the stability of metformin, which tends to strongly interact with the aqueous mobile phase, while preserving peak symmetry in an isocratic system. A similar composition was used by Prajapati et al. (2024), namely a phosphate buffer at pH 3:acetonitrile (75:25), which is suitable for the optimal analysis of tavaborole at acidic pH. Gradient systems are primarily used in studies involving analytes with significant differences in polarity, such as the study by Sarılmışer et al. (2017) on a mixture of ibuprofen, pseudoephedrine HCl, and chlorpheniramine maleate. The use of a gradient mobile phase between a pH 2.5 buffer and methanol allows each component to elute at proportional retention times, improving resolution without excessively prolonging the analysis time. Some other studies still use simple compositions such as water : methanol (3:1) for paracetamol. Because paracetamol has moderate polarity and good stability in the neutral pH range, a buffer-free system is still capable of providing separation that meets method validation requirements.

Overall, the mobile phase compositions across all studies demonstrate the application of analytical principles based on pH suitability, the log P of the analyte, and the requirements of the separation system. The use of phosphate buffer, ammonium acetate, or surfactants such as SDS reflects targeted adjustments to the chemical stability of the analyte and improvements in chromatogram quality.

The HPLC analysis process begins with sample preparation, which varies across different journals. In this context, sample preparation is highly dependent on the matrix used in the study. Generally, sample preparation for HPLC analysis involves weighing a specific amount of the active substance to be analyzed and dissolving it in an appropriate solvent. The appropriate solvent is selected based on the solubility characteristics of the matrix and considerations regarding the mobile phase. After dissolution, the sample solution is sonicated to remove any air bubbles that may still be present in the sample (degassing).

Additionally, other methods for sample preparation are described in the literature, particularly when using human plasma as the matrix. Deproteinization is performed, as in the study by Mulidini et al. (2023), where deproteinization involves the removal of proteins from the biological matrix. Subsequently, repeated extractions are conducted to increase the amount of extracted analytes. In other journals, the SPE (Solid Phase Extraction) method is also used to separate the desired compounds from interfering compounds. There is also the process of isolating plant extract samples performed using the maceration method with a solvent over a specific period. Thus, in general, sample preparation is carried out according to the matrix to be analyzed.

Flow rate is one of the key parameters in HPLC analysis. Flow rate directly affects retention time, resolution, peak shape, and system pressure. Based on 24 journal articles, it is evident that researchers adjust the flow rate according to the characteristics of the sample matrix being analyzed. In the analysis of atorvastatin (ATV) and apigenin (API), for example, flow rates ranging from 0.4–0.41 mL/minute were used, and these conditions were shown to provide better separation because the slower flow allows the analytes to interact longer with the stationary phase, thereby improving resolution. Low flow rates, as in these two studies, are suitable for analyzing highly complex compounds or for analyzing two compounds with closely spaced retention times.

In most other journals, a flow rate of 1.0 mL/min is used, as seen in samples of metformin in human plasma and ketoprofen in tablets. This flow rate is chosen because it provides a balanced result between resolution, analysis time, and system pressure. Sharp, symmetrical chromatographic peaks with stable retention times are obtained. This moderate flow rate also prevents excessive pressure on the column, particularly for biological samples with more complex matrices that have the potential to increase backpressure. Some other journals indicate that a higher flow rate, around 1.5 mL/min, is used. Researchers likely employ this to accelerate analysis time. However, increasing the flow rate to improve time efficiency does not necessarily mean that the results obtained will be better. In general, a high flow rate tends to reduce resolution because the interaction time between the analyte and the stationary phase is shorter, so the separation that occurs may be less than optimal if the compounds have similar chemical properties.

Overall, the determination of the flow rate in each study is based on the ultimate goal to be achieved. A lower flow rate can improve resolution because the sample has more time to interact with the stationary phase. However, a flow rate that is too low can also prolong the analysis time, resulting in broader chromatogram peaks. Conversely, a high flow rate can shorten retention times, allowing the analysis to be completed in a shorter time. However, using an excessively high flow rate can reduce resolution and produce less sharp chromatogram peaks. Additionally, an increase in system pressure may occur, which affects the service life of the HPLC column.

HPLC method validation is a process to ensure that the analytical procedure meets standards for reliability, accuracy, precision, and aligns with the intended objectives. A GC-MS method is considered properly validated if all parameters specified in analytical guidelines, such as ICH Q2(R1) or the Pharmacopoeia, are met. Key parameters that must be met during method validation include accuracy, linearity, precision, and the limits of detection (LOD) and quantification (LOQ). Accuracy is the method's ability to produce values close to the true concentration, typically expressed as recovery percentage and considered acceptable if within the range of 98–102% or 80–120%, depending on the analyte concentration. Several journals state that the accuracy of KCKT methods falls within an acceptable accuracy range.

Next is precision, which indicates the consistency of results between repeated measurements. A method is considered precise if the %RSD value is below 2%. Based on the 24 journals mentioned above, samples of ketoprofen, ibuprofen, and various other analytes exhibited precision <2%, indicating stable analytical results with good precision. Linearity is also a critical requirement, where the detector response must be proportional to the analyte concentration and yield a correlation coefficient (r^2) of at least 0.999. Additionally, the method must have a valid working range, as well as sufficiently low LOD and LOQ values to ensure compounds can be detected and measured accurately at low concentrations.

Data from the 24 journals collected indicate that the GC/HPLC methods used in the analysis of various drugs demonstrate robust validation performance, particularly regarding specificity, quantitative sensitivity, and extraction accuracy. These findings demonstrate that the optimization of chromatographic conditions—such as column selection, mobile phase, flow rate, and wavelength—is critical to the method's success. This indicates that the data obtained reflect a direct scientific relationship between the physicochemical properties of the drug and the validation parameters achieved.

The method's specificity proved to be excellent, particularly for drugs with simple matrices such as ibuprofen, which was analyzed using 258 nm detection with a methanol–water mobile phase. The analyte peak appeared as a single, symmetrical peak with no significant interference, indicating that the aromatic group of ibuprofen has high UV absorbance, making it easy to separate from tablet excipients. The strong UV absorption at the aromatic ring makes this method highly selective because other components do not exhibit peaks at this wavelength (Maulina et al., 2024).

In a much more complex plasma matrix, such as a mixture of metformin, glibenclamide, and gliclazide, the method still demonstrated excellent specificity at 233 nm. All three compounds were successfully separated without overlap despite the plasma containing proteins, lipids, and other metabolites. The presence of a minor peak before the main peak in metformin did not interfere with quantification, as evidenced by a recovery of 104.91%, so it can be scientifically concluded that this minor peak is not an active interferent.

This high specificity can be explained by the scientific mechanism of analyte interaction with the stationary phase on the C18 column. Lipophilic drugs like glibenclamide strongly interact with the stationary phase, resulting in more stable separation, while polar drugs like metformin are separated through control of the mobile phase pH and the water-methanol composition. This mechanism aligns with chromatographic retention theory, which states that optimal separation occurs when the polarity of the mobile phase and the analyte molecule are in a specific equilibrium.

The Limit of Quantification (LOQ) is the lowest concentration of an analyte that can still be quantified accurately and precisely by the analytical method, where the analyte signal has a signal-to-noise (S/N) ratio of 10:1, allowing it to be clearly distinguished from the instrument's baseline noise (Sangeetha & Mahalakshmi, 2025). HPLC can achieve very low LOQs, particularly for ibuprofen, which has an LOQ of 1.20 µg/mL with a recovery rate >99.97%. Scientifically, this occurs because ibuprofen possesses an aromatic group that strongly absorbs at high wavelengths, thereby generating a strong signal even at low concentrations. Beer–Lambert's law states that the higher the molar absorption coefficient, the lower the minimum detectable concentration.

Variations in LOQ among drugs follow a clear scientific pattern: drugs with strong chromophores (ibuprofen, ketoprofen) always yield lower LOQs, whereas non-aromatic drugs like metformin have lower sensitivity and thus require λ = 233 nm to enhance absorbance. This aligns with the theory by Jayawardhane et al. (2021), which states that chemical structure is the primary factor determining the sensitivity of chromatographic methods.

Recovery is a validation parameter that describes the efficiency of analyte extraction from the matrix—specifically, the percentage of analyte successfully recovered compared to the actual amount in the sample—thus indicating the method's analytical accuracy (Ali, 2022). Recovery analysis yielded important scientific findings that the method exhibits excellent extraction accuracy, as seen in ibuprofen (recovery >99.97%) and metformin in plasma (104.91%). These figures not only meet the ICH Q2(R1) standards but also indicate that the extraction process, solvent, and filtration are efficient. Recovery >100% for metformin likely stems from the plasma matrix effect, which enhances the UV response through baseline shifts—a phenomenon known as matrix enhancement.

Recovery also shows a striking difference between solid and liquid matrices. In tablets or capsules, recovery is stable due to a homogeneous matrix and minimal interference. However, in syrups, recovery is more variable due to the presence of preservatives such as potassium sorbate, which can absorb UV light at wavelengths similar to the analyte. This aligns with the scientific concept of matrix suppression, where non-target compounds in the liquid matrix can decrease or increase the analyte's signal response. When compared to other studies, the trends in recovery values found in the data are consistent with the report by Fauziah et al. (2024), which states that drugs in solid matrices almost always yield a recovery of >98%, whereas liquid or herbal matrices can yield a recovery of 95–108%. This similarity in results reinforces the notion that the matrix effect is a global factor influencing method validation. (Fauziah et al., 2024).

The effect of drug type on HPLC method validation is highly significant and must be given special attention during the development of analytical methods. Each drug type has distinct chemical and physical properties that influence its interaction with the chromatographic column, mobile phase, and detector (Maulina et al., 2024). These differences in properties also affect the sensitivity and specificity of the method. For example, metformin, which lacks a strong chromophore, requires a lower UV wavelength (233 nm) for more sensitive detection, unlike ibuprofen, which relies on absorption at a higher wavelength of 258 nm. Therefore, parameters such as wavelength, mobile phase pH, and solvent composition must be optimized according to the drug's chemical characteristics to ensure accurate and precise measurements.

Additionally, drug characteristics also determine the effectiveness of sample preparation. Drugs soluble in biological matrices such as plasma require a different extraction process compared to those in solid dosage forms. Drugs in mixtures, such as metformin, glibenclamide, and gliclazide, also require analytical methods capable of specifically separating all three without peak overlap. This indicates that HPLC method validation cannot rely on a single generic, uniform method but must be tailored to the profile of the drug being analyzed. The influence of drug characteristics on method validation underscores the need for a comprehensive combination of validation parameters—such as specificity, linearity, accuracy, precision, recovery, and LOQ—tailored to the type of drug.

Based on the 24 journals reviewed, a strong correlation was found between drug chemical properties and validation performance. Lipophilic drugs (ibuprofen, ketoprofen) exhibit stable retention on C18 columns, resulting in high peak resolution, low LOQ, and recovery approaching 100%. Conversely, hydrophilic drugs like metformin require more optimization to achieve comparable performance. Thus, a method optimal for one drug is not automatically optimal for another; therefore, each method must be designed based on molecular characteristics.

An evaluation of the referenced journals revealed methodological findings. International journals generally report complete validation parameters, including chromatograms, specificity tests, LOQ/LOD, three-level precision, and robustness. Meanwhile, some national journals, although accredited by SINTA and adhering to certain standards, are sometimes inconsistent in reporting complete validation data. In some cases, only basic information such as column conditions or a single recovery value is provided, without mentioning LOQ or visual chromatogram data. This lack of detailed explanation can limit a comprehensive understanding of the reliability of the methods used, thereby compromising the quality of the research and the interpretation of results. These limitations are noted as shortcomings in the critical evaluation of the paper to help readers understand potential biases or data limitations.

Overall, these scientific findings support the research hypothesis that the success of HPLC method validation depends on the balance of interactions between the analyte and the mobile and stationary phases, as well as the complexity of the matrix. The data indicate that the method used meets international scientific criteria, with high specificity, a low LOQ, and excellent recovery. This reinforces modern chromatography theory and aligns with recent studies, making it valid for use in further method development.

Conclusion

According to a review of 24 journals discussing the use of High-Performance Liquid Chromatography (HPLC) in drug analysis, this technique plays a crucial role in the accurate separation, identification, and quantification of drugs in various types of matrices. The success of the HPLC method depends heavily on the compatibility between the physicochemical properties of the drug and the chromatographic conditions used. These factors include column selection, mobile phase composition, detector type, sample preparation methods, and flow rate settings. Compared to non-chromophore polar drugs, aromatic and lipophilic drugs demonstrate better sensitivity and validation performance.

Method validation across the journal demonstrates that the majority of HPLC procedures meet international standards with high accuracy, specificity, and

precision, low LOQ levels, and good recovery despite variations between matrices. This indicates that HPLC is a reliable technique for drug analysis under various conditions, including in complex plasma matrices.

References

Agustina, R., Mulyadi, D., & Yuningsih, L. M. (2025). Optimization and Validation of Clobazam Content Determination Method in Tablet Preparations Using High Performance Liquid Chromatography (HPLC). *Jurnal Biologi Tropis*, *25*(3), 4213-4220.

Alatas, F., Sujono, H., & Sucipto, W. A. (2018). Pengembangan dan validasi metode kromatografi cair kinerja tinggi (KCKT) untuk estimasi kadar simultan antiemetik piridoksin hidroklorida dan piratiazin teoklat dalam bentuk sediaan tablet. *Kartika: Jurnal Ilmiah Farmasi*, *6*(2), 95-100.

Ali, A. H. (2022). High-performance liquid chromatography (HPLC): A review. Ann. Adv. Chem, 6, 010-020.

Arifah, R. H., Permatasari, D. A. I., & Artini, K. S. (2023). Penggunaan Metode HPLC pada Analisis Jamu Depot yang Mengandung Antalgin. *Jurnal Jamu Kusuma*, *3*(1), 54-61.

Chrissanti, R. D., Darmawati, A., & Yuwono, M. (2020). Optimasi Metode KCKT untuk Penetapan Kadar 4-Isobutilasetofenon dan 2-(4-Isobutirilfenil) Asam Propanoat dalam Tablet Ibuprofen. *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia Vol*, *7*(1), 26.

Darmawati, A., Annuryanti, F., & Primaharinastiti, R. (2020). Determination and stability testing method of chlorpheniramine maleate in the presence of tartrazine using HPLC. *Pharmaciana*, *10*(3), 269-280.

Dong, M. W., & Boyes, B. E. (2018). Modern trends and best practices in mobile-phase selection in reversed-phase chromatography. LC-GC Europe, 31, 572–583.

Fauziah, A. N., Kim, M. J., So, B. R., Son, J. E., & Jung, S. K. (2024). Validation of the High-Performance Liquid Chromatography Method for Determining Quercitrin in Capsicum annuum L. Cultivar Dangjo. Preventive nutrition and food science, 29(4), 504–511. https://doi.org/10.3746/pnf.2024.29.4.504

Fernando, A., Putri, N. K., & Fadhli, H. (2024). Validasi Metode Penetapan Kadar Metformin Dalam Plasma Darah Yang Diberikan Bersamaan Dengan Pemberian Sari Buah Pare (Momordica charantia L.) Secara Kromatografi Cair Kinerja Tinggi (KCKT). *Jurnal Penelitian Farmasi Indonesia*, *13*(1), 32-40.

Goutal, S. et al. (2016). Validation of a simple HPLC-UV method for rifampicin determination in plasma. Journal of Pharmaceutical and Biomedical Analysis, 123, 173–178.

Indriana, M. (2020). Determination of paracetamol levels in tablets and oral solutions by high-performance liquid chromatography (HPLC). *Journal of Pharmaceutical And Sciences*, *3*(2), 106-113.

Kashmar, S. A., Abou Assi, R., Alhijjaj, M., & Chan, S. Y. (2025). Development and Validation of a Simultaneous HPLC Stability-Indicating Method for Atorvastatin and Apigenin in a Novel SMEDDS Formulation Using Quality by Design (QbD) Approach. *Processes*, *13*(9), 2933.

Khatak, S. et al. (2018). Development and validation of RP-HPLC method for antitubercular drugs. Indian Journal of Pharmaceutical Sciences, 80, 996–1002.

Khodijah, S., Sriwidodo, & Roestan, R. (2024). Validasi metode analisis penetapan kadar Paracetamol, Chlorphenamine Maleate, Dextromethorphan HBr, dan Phenylephrine HCl dalam sediaan kaplet secara HPLC. *Medic Nutricia: Jurnal Ilmu Kesehatan, 2*(3), 91–109

Maulina, D., Asya, Z., Cahyo, D., Samira, S., & Mahmudah, W. (2024). Analisis Ibuprofen Menggunakan HPLC dan Pengujian Validasi Metode. Jurnal Ilmiah Wahana Pendidikan, 10(14), 390-423. https://doi.org/10.5281/zenodo.13580099

Mikulić, M., Sazdanić, D., Kladar, N., Radulović, J., Srđenović Čonić, B., & Atanacković Krstonošić, M. (2025). Validation of HPLC-DAD method for analysis of paracetamol and potassium sorbate in liquid oral formulations and its application to forced degradation study. *Acta Chromatographica*, *37*(1), 112-120

Muharini, R., Ersando, E., Triana, Y., Nukila, M., Ulwan, R., Masriani, M., & Proksch, P. (2022). Analisis Profil HPLC-PDA Berkombinasi dengan LC-ESI-MS dan Aktivitas Biologi dari Ekstrak Jamur Endofit, Penicillium simplicissimum, yang Diisolasi dari Rimpang Jahe Merah. *ALCHEMY Jurnal Penelitian Kimia*, *18*(2), 158-164.

Mulidini, M., Nanda, A. Y. D., Hanum, N. K., Nurfadhila, L., & Utami, M. R. (2023). Analysis and validation of metformin in human plasma using the HPLC method. Journal of Pharmaceutical and Sciences, 6(2), 741–749.

Nopi, N. S., & Isnaeni, N. (2023). Validasi Metode Analisis Penetapan Kadar Bahan Baku Lapatinib Ditosylate secara KCKT-DAD. *Eruditio: Indonesia Journal of Food and Drug Safety*, *4*(1), 33-42.

Oliveira, M. A. L. et al. (2018). Simultaneous determination of antituberculosis drugs in FDC formulations: A review. Analytical Methods, 10, 1103–1116.

Patala, R., & Awilia, N. (2023). Analisis Kadar Parasetamol Generik Dibandingkan Bermerek Dagang. *JIFI (Jurnal Ilmiah Farmasi Imelda)*, *7*(1), 7-12.

Poole, C. (2009). *Handbook of Methods and Instrumentation in Separation Science: Volume 1 (Vol. 1).* Academic Press.

Pradana, D. A., Kristin, E., Nugroho, A. K., Nugrahaningsih, D. A. A., Mustofa, M., & Wibowo, A. (2023). Bioanalytical method Validation of metformin hydrochloride in human plasma by HPLC-UV for preliminary Population-Based Pharmacokinetic Modeling study. *Indonesian Journal of Chemistry*, *23*(4), 937-947.

Prajapati, S. K., Jain, A., & Bajpai, M. (2024). Development and validation of the RP-HPLC method for quantification of tavaborole. *Analytical Methods*, *16*(30), 5280-5287.

Priyadi, S., Darmadji, P., Santoso, U., & Hastuti, P. (2015). Distribusi plumbum, cadmium pada biji kedelai, dan deprotonasi gugus fungsional karboksil asam sitrat dalam khelasi. *Agritech*, *34*(4), 407-414.

Putri, A. K., & Yuwono, M. (2023). Analisis Kualitatif dan Kuantitatif P-Aminophenol dalam Sediaan Sirup Parasetamol dengan Metode HPLC. *Journal of Islamic Pharmacy*, *8*(1), 27-33.

Rohayati, A., Hasanah, A. N., Saptarini, N. M., & Aryanti, A. D. (2015). Optimasi kondisi pemisahan glibenklamid kombinasi metformin dalam plasma darah menggunakan KCKT. *Indonesian Journal of Pharmaceutical Science and Technology*, *2*(3), 96.

Sangeetha, M., & Mahalakshmi, A. S. (2025). A Review on Analytical Method Development and Validation for the Simultaneous Estimation of Levothyroxine and Liothyronine in Combined Pharmaceutical Dosage Form by RP-HPLC. International Journal of Research in Pharmacy and Allied Science, 4(9), 54-60.

Sarılmışer, H. K., Özçelik, N. O., Arslan, B. Ö., Gökalp, M., Ustaoğlu, A., Dude, U. K., & Knezevic, Z. (2017). Development and validation of a simple HPLC assay method for ibuprofen, pseudoephedrine hydrochloride and chlorpheniramine maleate in syrup form. *J. Chem. Metrol*, *11*(2), 61.

Setyaningrum, L. (2022). Validasi metode analisis penetapan kadar parasetamol dan kafein dalam sediaan tablet secara simultan menggunakan RP-HPLC. *Jurnal Katalisator, 7*(2), 323–335.

Suhendi, A., & Ramly, E. A. (2021). Validasi Metode Analisis Kapsul Rifampisin Dengan HPLC-PDA. *Farmasains*, *8*(1), 31-36.Umar, S., Saafrida, S., & Lucida, H. (2021). Validasi Metoda Analisis Penetapan Kadar Ketoprofen pada Tablet Salut Enterik secara Kromatografi Cair Kinerja Tinggi dan Spektrofotometri UV. *Jurnal Sains Farmasi & Klinis*, *8*(2), 200-207.

Umar, S., Saafrida, & Lucida, H. (2021). Validasi metoda analisis penetapan kadar ketoprofen pada tablet salut enterik secara kromatografi cair kinerja tinggi dan spektrofotometri UV. Jurnal Sains Farmasi & Klinis, 8(2), 200–207.

Woottisin, N., Kongkiatpaiboon, S., Sukprasert, S., & Sathirakul, K. (2020). Development and validation of stability indicating HPLC method for determination of caffeic acid, vitexin and rosmarinic acid in Thunbergia laurifolia leaf extract. *Pharmacognosy Journal*, *12*(3).

Yashin, Y. I., & Yashin, A. Y. (2020). Contribution of Russian Experts to the Development of High-Performance Liquid Chromatography. *Journal of Analytical Chemistry*, 75(10), 1252-1263.