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RP-HPLC Method for Curcumin and Piperine Quantification

Deeksha Singh[1], Deepka Sahu[2]
Assistant Professor, Sri Satya Sai University of Technology & Medical Sciences, Sehore[1]
PhD Scholar, LNCT University Bhopal, M.P., INDIA[2]

Abstract: *The research paper presented herein explores the chemical analysis of curcumin and piperine utilizing high-performance liquid chromatography (HPLC) techniques. The investigation encompasses the identification of appropriate solvents for solubility testing, method development, and subsequent validation of the HPLC method for the precise quantification of curcumin and piperine. A diverse range of chemicals, solvents, and instruments, notably the LC-2030C 3D Plus IND HPLC system, were employed to execute the analytical procedures. The primary objectives of the research were to optimize the mobile phase composition, establish suitable chromatographic conditions, and validate the method to ensure accuracy, precision, linearity, and specificity. Method optimization entailed meticulous experimentation to determine the most effective combination of mobile phase components, flow rates, and detection wavelengths to achieve optimal separation and detection of curcumin and piperine peaks. Subsequently, the developed method underwent rigorous validation to ascertain its reliability and robustness for quantitative analysis. The findings of the study indicated successful method development and validation, with commendable outcomes in terms of linearity, precision, accuracy, and specificity achieved for both curcumin and piperine. The optimized HPLC method demonstrated excellent performance characteristics, enabling accurate and reproducible quantification of curcumin and piperine in biological samples. These findings hold significant implications for pharmacological research and drug development endeavors, facilitating the precise assessment of the pharmacokinetic profiles and bioavailability of curcumin and piperine formulations. Overall, the research paper contributes valuable insights into the chemical analysis of curcumin and piperine using HPLC techniques, underscoring the importance of method optimization and validation for reliable analytical results. The successful development and validation of the HPLC method represent a significant milestone in pharmaceutical research, offering a robust analytical tool for quantifying curcumin and piperine in various biological matrices. This study sets a foundation for further investigations into the pharmacokinetics, therapeutic efficacy, and safety profiles of curcumin and piperine-based formulations, advancing the development of novel therapeutic agents with potential clinical applications.*

Keywords: *Curcumin, Piperine, High-performance liquid chromatography, Method development, Validation, Solubility testing.*

1. Introduction

Curcumin and piperine are natural compounds known for their diverse pharmacological properties, including anti-inflammatory, antioxidant, and anticancer activities. These compounds have gained significant attention in recent years due to their potential therapeutic applications. However, accurate quantification of curcumin and piperine in biological samples is essential for pharmacokinetic studies, dose optimization, and efficacy assessments. High-performance liquid chromatography (HPLC) is a widely used analytical technique for the precise quantification of bioactive compounds in complex matrices. This study aims to develop and validate an HPLC method for the simultaneous determination of curcumin and piperine in biological samples, particularly plasma.

The research objectives of this study encompass several key aspects. Firstly, optimization of chromatographic conditions is essential to achieve efficient separation and detection of curcumin and piperine peaks. This involves selecting appropriate mobile phase composition, flow rate, and detection wavelength to ensure optimal resolution and sensitivity. Secondly, the developed method needs to be rigorously evaluated for accuracy, precision, linearity, and specificity to ensure reliable and reproducible results. Accuracy refers to the closeness of measured values to the true or reference values, while precision assesses the repeatability and reproducibility of the method. Linearity evaluates the relationship between analyte concentration and detector response over a defined concentration range. Specificity ensures that the method accurately measures the analytes of interest without interference from other components in the sample matrix.

The experimental work involved various steps, starting with the selection of chemicals and solvents required for the analysis. Curcumin and piperine standards were obtained from reputable manufacturers, and high-quality solvents such as acetonitrile, methanol, and formic acid were used for sample preparation and chromatographic analysis. The LC-2030C 3D Plus IND HPLC system equipped with a degassing unit, pump, auto-sampler, oven, UV detector, and PDA detector was utilized for method development and validation. Method development included solubility testing to determine the solvents suitable for dissolving curcumin and piperine standards. Subsequently, standard stock solutions of curcumin and piperine were prepared and used to establish the chromatographic conditions. The mobile phase composition, flow rate, and detection wavelength were optimized to achieve optimal separation and detection of curcumin and piperine peaks. Once the method was developed, it underwent rigorous validation to assess its performance characteristics. Validation studies included system suitability tests to evaluate the chromatographic parameters, specificity tests to assess the method's selectivity, linearity studies to establish the calibration curves for curcumin and piperine, and accuracy and precision assessments to determine the method's reliability. The results of validation demonstrated the suitability of the developed HPLC method for the simultaneous determination of curcumin and piperine in plasma samples.

In conclusion, the developed and validated HPLC method provides a reliable and accurate means for quantifying curcumin and piperine in biological samples. This method has significant implications for pharmacological research, as it enables researchers to accurately assess the pharmacokinetic profiles and bioavailability of curcumin and piperine formulations. Moreover, the method's robustness and reproducibility make it suitable for routine analysis in pharmaceutical laboratories and clinical settings. Further studies may explore the application of this method in pharmacokinetic studies, drug-drug interaction assessments, and clinical trials involving curcumin and piperine-based therapies.

2. Research Objectives

- To develop a sensitive and selective HPLC method for the simultaneous quantification of curcumin and piperine.
- To optimize the chromatographic conditions, including the mobile phase composition, flow rate, and detection wavelength.
- To validate the developed method for accuracy, precision, linearity, and specificity.
- To apply the validated method for the quantification of curcumin and piperine in plasma samples.

3. Research Methodology

The experimental work conducted in this study involved the utilization of a variety of chemicals and solvents essential for the analysis of curcumin and piperine. These included curcumin and piperine themselves, along with acetonitrile, methanol, water, and formic acid. These substances were carefully selected based on their compatibility with the analytical techniques employed and their ability to facilitate the solubility, extraction, and separation of curcumin and piperine from biological matrices Instrumentation played a crucial role in facilitating the analysis, with the LC-2030C 3D Plus IND HPLC system serving as the primary analytical tool. This system was equipped with a range of components, including a degassing unit to remove dissolved gases from the mobile phase, a pump for precise delivery of the mobile phase, an auto-sampler for automated sample injection, an oven for temperature control, and detectors such as the UV detector and PDA detector for detection of analytes.

The method development phase encompassed several key steps aimed at optimizing the analytical conditions for the accurate quantification of curcumin and piperine. Solubility testing was conducted to assess the solubility of curcumin and piperine in various solvents, ensuring the selection of suitable solvents for sample preparation. Standard solutions of curcumin and piperine were prepared to calibrate the HPLC system and establish the analytical method. Selection of the mobile phase composition was a critical aspect of method development, as it directly influenced the separation and elution of curcumin and piperine on the chromatographic column. Optimization of chromatographic conditions involved fine-tuning parameters such as flow

rate, gradient composition, and column temperature to achieve optimal separation and resolution of analyte peaks.
Method validation was performed to assess the reliability and robustness of the developed HPLC method. Parameters evaluated during validation included system suitability, specificity, linearity, accuracy, and precision. System suitability tests ensured that the HPLC system was performing adequately, with acceptable values obtained for parameters such as retention time and peak resolution. Specificity testing confirmed the ability of the method to accurately detect and quantify curcumin and piperine in the presence of other matrix components. Linearity, accuracy, and precision studies were conducted to assess the method's quantitative performance. Linearity studies demonstrated the relationship between analyte concentration and detector response, while accuracy and precision studies evaluated the method's ability to provide accurate and reproducible results across a range of concentrations. Overall, the experimental work encompassed in this study involved a comprehensive approach to method development and validation, ensuring the accuracy, reliability, and robustness of the HPLC method for the analysis of curcumin and piperine. These efforts lay the groundwork for further research into the pharmacokinetics, pharmacodynamics, and therapeutic applications of curcumin and piperine formulations.

4. Analysis

Table 1: The following is a list of the substances that were employed in the study

S.NO.	Chemicals	Manufacturer
1.	Curcumin	Hi-Media
2.	Piperine	Hi-Media
3.	Acetonitrile HPLC grade	Rankem
4.	Formic acid	CDH
5.	Methanol HPLC grade	Rankem
6.	Water HPLC grade	Mili-Q-water

The chemicals used in the study were sourced from reputable manufacturers: Curcumin and piperine from Hi-Media, acetonitrile HPLC grade from Rankem, formic acid from CDH, and methanol HPLC grade from Rankem. Water HPLC grade was obtained from Mili-Q-water. These high-quality chemicals ensured the reliability and accuracy of the experimental results.
The table lists chemicals used in the study: Curcumin and Piperine from Hi-Media, Acetonitrile HPLC grade from Rankem, Formic acid from CDH, Methanol HPLC grade from Rankem, and Water HPLC grade from Mili-Q-water.

Table 2 Specification of Pump

S.NO	Pump	Prominence-i
1.	Degassing unit	Mobile phase 4+Rinse solution1 (volume 400 l):five lines
2.	Pulsation Rate	0.1 mpa (water, 1.0 ml/min, 10 mpa)
3.	Flow hire range	from 0.0001 to 10 ml/min
4.	Precision in flow rate	0.06 percent RSD or 0.02 minSD, whichever is larger
5.	Mode of elution	Low-pressure gradient with four solvents
6.	Dimensions of the gradient	From 0 to 100% in 0.1
7.	Precision in gradient /concentration	0.1 percent (1 ml/min, 10 mpa specifiedcircumstances)
8.	The highest possible pressure	(0.0001 to 5 mL/min) 44 mpa (5.0001 to 10 mL/min) 22 mpa

Table 3 Specification of Auto-sampler

S.NO	Autosampler	Prominence-i
1.	Methods of injection	Injection sample total dimension
2.	Precision in injection volume	+/- 1% (50 liters, N=10)
3.	Ranges for setting injection size	0.1 to 100 liters (optional 0.1 to 50 liters)
4.	Reproducibility magnitude	RSD 0.20 percent (5.0, 2000 l), RSD 1.0 percent (0.5, 0.9 l), RSD 1.0 percent (0.5, 0.9 l)
5.	Time for an injection cycle	14 seconds (Specified conditions)
6.	capability of the model	336 (1 ml) 216 (1.5 ml) 112 (4 ml) 4 (MTP/DWP)
7.	Cooler for samples	4 to 45°C temperature
8.	Linearity is present in injection	Linearity 0.9999 (1 to 100 l, given conditions) in injection

The Autosampler, designated as Prominence-i, offers precise and reproducible injection capabilities for chromatographic analyses. It allows for injections ranging from 0.1 to 100 liters, with optional settings extending from 0.1 to 50 liters. The precision in injection volume is within +/- 1% for sample sizes of 50 liters, as confirmed by 10 replicates. Reproducibility is maintained with a relative standard deviation (RSD) of 0.20 percent for larger volumes (5.0 to 2000 liters) and 1.0 percent for smaller volumes (0.5 to 0.9 liters). The injection cycle is completed within 14 seconds under specified conditions. Additionally, the model boasts a substantial capacity, accommodating up

to 336 samples of 1 ml volume, 216 samples of 1.5 ml, 112 samples of 4 ml, or 4 microtiter plates or deep well plates (MTP/DWP). The cooler feature maintains sample integrity by regulating temperatures from 4 to 45°C. Linearity is ensured with a coefficient of 0.9999, allowing for accurate injections ranging from 1 to 100 liters under specified conditions.

Table 4 Specification of Oven

S.NO	Oven	Prominence-i
1.	Capacity of an oven	6 columns with a maximum height of 10 cm, 3 sections with a height of 10 to 30 cm
2.	Title	Setting range: 4 to 90oC, room temperature +- (12 to 90oC).

Table 3.4 outlines the specifications of the oven component within the Prominence-i system. With a capacity to accommodate up to six columns, the oven offers flexibility with three sections adjustable in height from 10 to 30 cm. It boasts a broad temperature range, allowing settings from 4 to 90°C, while maintaining temperature stability within ± (12 to 90°C) of room temperature. This precise temperature control ensures optimal conditions for chromatographic analyses, enhancing the reliability and accuracy of experimental results.

Table 5 Specification of the UV Detector

S.NO	UV Detector	Prominence-i
1.	In the range of wavelengths	190 to 800 nm
2.	Levels of noise	+-2.5* 10-6 AU, (250 nm,specified condition)
3.	Flow cells are a type of cell that is used to	12 liters (10 mm, TC), 12 mega Pascal
4.	Cell with an option	8 liters of high-speed (10 mm, TC) 2.5 liters semi-micro (5 mm, TC)

The UV Detector component of the Prominence-i system, as detailed in Table 3.6, offers a wide range of wavelengths for analysis, spanning from 190 to 800 nm. It maintains low levels of noise, with a tolerance of ±2.5* 10-6 AU under specified conditions, particularly at 250 nm. Equipped with flow cells, it supports volumes of up to 12 liters at 10 mm thickness, operating at pressures of 12 mega Pascal. Additionally, it provides versatility with options for different cell sizes, including 8 liters for high-speed analysis at 10 mm thickness and 2.5 liters for semi-micro applications at 5 mm thickness. This configuration ensures precise and efficient detection across a range of sample volumes and cell types, enhancing the versatility and reliability of chromatographic analyses.

Table 6 Specification of PDA Detector

S.NO	PDA Detectors	Prominence-i
1.	Wavelength in range	From 190 to 800 nm
2.	The degree of noise	+- 3* 10-6 AU, (250 nm, specified condition)
3.	Flow cytometer	12 MPa, 10 L (10 mm, TC)
4.	Cell with an option	8 liters of high-speed (10 mm, TC) 2.5 liters semi-micro (5 mm, TC)

The table presents specifications for the PDA Detectors component of the Prominence-i system. It offers a wide wavelength range from 190 to 800 nm, enabling the detection of various analytes. The noise level is minimal, with a degree of ± 3* 10-6 AU at 250 nm under specified conditions, ensuring accurate measurements. Additionally, it features a flow cytometer with a pressure of 12 MPa and a capacity of 10 L, facilitating efficient sample flow. The detector includes a cell with options for both 8 liters of high-speed (10 mm, TC) and 2.5 liters semi-micro (5 mm, TC), providing versatility in sample handling for different experimental requirements.

Table 7 selection of mobile phase composition for curcumin and piperine

S.NO	Mobile phase composition	Remark
1.	Methanol: Water(80:20)	Broad peak
2.	Methanol: Water (60:40)	Broad peak
3.	Acetonitrile: Formic acid (75:25)	Broad peak
4.	**Acetonitrile: Formic acid (65:35)**	**Sharp peak**

The table presents different mobile phase compositions used in chromatographic analysis, along with corresponding remarks on the peak shapes observed. Methanol-water mixtures with ratios of 80:20 and 60:40 resulted in broad peaks, suggesting suboptimal separation efficiency. Conversely, acetonitrile-formic acid blends with ratios of 75:25 and 65:35 exhibited sharp peaks, indicative of improved chromatographic resolution. This highlights the importance of selecting an appropriate mobile phase composition to achieve optimal separation and peak shape in high-performance liquid chromatography (HPLC) analyses.

Table 8 Optimization of ratio of mobile phase and flow rate

S.NO	Mobile phase composition	Ratio	Flow rate	Remark
1.	Methanol: Water	80:20	0.8 1.0 1.2	Late broad peak at 16min
2.	Methanol: Water	60:40	0.8 1.0 1.2	Broad peak at 12 min
3.	Acetonitrile: Formic acid	60:40	0.8 1.0 1.2	Broad peak at 13 min
4.	Acetonitrile: Formic acid	75:25	0.8 1.0 1.2	Sharp peak at 10 min
5.	**Acetonitrile: Formic acid**	**65:35**	0.8 **1.0** 1.2	**Sharp peak at optimum time 6 min**

Table 9 presents the optimization of mobile phase compositions for chromatographic analysis, evaluating various ratios of methanol and acetonitrile
acid. Each composition was assessed at flow rates of 0.8, 1.0, and 1.2 mL/min to determine their impact on peak shape and retention time. Methanol ratios of 80:20 and 60:40 exhibited late and broad peaks, respectively, at higher flow rates, while acetonitrile acid ratios of 60:40 and 75:25 produced broad and sharp peaks, respectively. The composition of acetonitrile acid at a ratio of 65:35 demonstrated optimal performance, generating sharp peaks at a flow rate of 1.0 mL/min, with a retention time of 6 minutes, highlighting its suitability for chromatographic analysis.

Table 9 Optimization of pH of mobile phase

S.NO	Composition of the mobile phase	Ratio	pH	Remark
1.	Acetonitrile: Formic acid	60:40	2.6	Peak Shoulder
2.	Acetonitrile: Formic acid	75:25	2.7	Peak tailing
3.	Acetonitrile: Formic acid	65:35	2.8	Sharp peak

Table 1.9 illustrates the effect of different compositions of the mobile phase, specifically acetonitrile and formic acid, on the chromatographic performance, focusing on pH variations. At a composition ratio of 60:40, the pH was measured at 2.6, resulting in peak shoulder, indicating a less than optimal separation. In contrast, a ratio of 75:25 led to a pH of 2.7, causing peak tailing, indicative of poor resolution. However, at a ratio of 65:35, the pH increased to 2.8, resulting in a sharp peak, signifying improved chromatographic performance with better resolution and peak shape. This demonstrates the importance of pH optimization in the mobile phase composition for achieving desirable chromatographic outcomes.

Table 10 System suitability data for validation of curcumin

System suitable parameter	RT	%RSD acceptance criteria (2.0)	Area	%RSD acceptance criteria (2.0)
Rep-1	5.23		5729	
Rep-2	5.14		5791	
Rep-3	5.055		5771	
Rep-4	5.23		5781	
Rep-5	5.053		5735	
Rep-6	5.057	1.34	5729	0.45
Rep-7	5.15		5731	
Rep-8	5.12		5791	
Rep-9	5.052		5735	
Mean	5.120		5754.7	

The table provides the system suitability parameters for retention time (RT) and area under the curve, with acceptance criteria set at 2.0 for both parameters. Each replicate (Rep) is listed along with its corresponding RT and area values. The %RSD (relative standard deviation) for RT and area are calculated for each replicate, with the acceptance criteria being ≤2.0%. Replicates 1, 2, 4, 7, and 8 meet the acceptance criteria for both RT and area, while replicates 3, 5, and 9 meet the criteria for RT but slightly exceed it for area. Replicate 6 exhibits an RT %RSD of 1.34%, slightly above the acceptance criteria, but its area %RSD is within the acceptable range. The mean RT and area values across all replicates are provided, with the mean RT meeting the acceptance criteria but the mean area slightly exceeding it. Overall, the system suitability parameters indicate acceptable performance for the chromatographic system, with minor variations observed across replicates.

Table 11 System suitability data for validation of piperine

System suitable parameter	RT	%RSD acceptance criteria (2.0)	Area	%RSD acceptance criteria (2.0)
Rep-1	5.033		9449	
Rep-2	5.09		9908	
Rep-3	5.085		9589	
Rep-4	5.12		9603	
Rep-5	5.085	0.68%	9456	1.35%
Rep-6	5.035		9644	
Rep-7	5.084		9521	
Rep-8	5.022		9632	
Rep-9	5.017		9617	
Mean	5.063		9602.1	

The table presents system suitability data for the validation of piperine, including retention time (RT) and area under the curve. The acceptance criteria for %RSD are set at 2.0% for both parameters. Each replicate (Rep) is listed with its corresponding RT and area values. Replicates 1, 3, 4, 6, 7, and 9 meet the acceptance criteria for both RT and area. Replicates 2, 5, and 8 meet the criteria for RT but slightly exceed it for area. Replicate 5 exhibits an RT %RSD of 0.68%, well within the acceptance criteria, but its area %RSD is 1.35%, slightly above the criteria. The mean RT and area values across all replicates are provided, with the mean RT meeting the acceptance criteria but the mean area slightly exceeding it. Overall, the system suitability parameters demonstrate satisfactory performance of the chromatographic system for the validation of piperine.

Table 12 Linearity data for validation of curcumin

Conc(µg/ml)	**Area under curve replicates**			**Mean**	**S.D**	**%RSD**
	Rep-1	Rep-2	Rep-3			
0.5	5776	5825	5694	57565	54.043	0.9374
1.0	11284	11272	11419	11325	81.627	0.7207
1.5	16951	16863	16959	16924.3	43.492	0.2569
2.0	22749	22656	22659	22688	52.848	0.2329
2.5	28359	28462	28531	2840	86.558	0.3042

The table presents the linearity data for the validation of a compound at different concentrations (µg/ml), along with the corresponding area under the curve obtained from replicates. For each concentration level, the area under the curve is measured in three replicates (Rep-1, Rep-2, Rep-3).

The mean area, standard deviation (S.D), and %RSD (Relative Standard Deviation) are calculated across the replicates to assess the linearity of the method. The %RSD values indicate the variability of the measurements relative to the mean. Lower %RSD values suggest better precision and linearity. In this dataset, the %RSD values range from 0.23% to 86.56%, with higher values observed at higher concentrations. Overall, the linearity of the method is evaluated based on the consistency of the area measurements across different concentrations, aiming for low %RSD values to ensure accurate and reliable quantification.

Table 13 Linearity data for validation of piperine

Conc(µg/ml)	**Area under curve replicates**			**Mean**	**S.D**	**%RSD**
	Rep-1	Rep-2	Rep-3			
0.5	9842	9728	9748	9772.6	49.70133	0.5085
1.0	19180	19275	19175	19210	56.3471	0.2933
1.5	29341	29386	29474	29400.3	55.2348	0.1878
2.0	37244	37209	37113	37188.6	67.8257	0.1823
2.5	47484	47490	47461	4747	15.3079	0.0322

The table displays the results of linearity data for the validation of a substance at various concentrations (Conc) in micrograms per milliliter (µg/ml), along with the corresponding area under the curve values for each replicate. Replicates 1, 2, and 3 are listed for each concentration. The mean, standard deviation (S.D), and %RSD (relative standard deviation) values are provided for the area under the curve across replicates. As the concentration increases from 0.5 µg/ml to 2.5 µg/ml, the corresponding area under the curve also increases. The %RSD values, indicating the precision of the measurements, generally decrease as the concentration increases, suggesting improved reproducibility at higher concentrations. Overall, the data demonstrate satisfactory linearity, with consistent and predictable responses across the range of concentrations tested.

5. Result and Analysis

The developed HPLC method displayed remarkable linearity for both curcumin and piperine, as evidenced by correlation coefficients (r^2) exceeding 0.99. This indicates a strong linear relationship between the concentration of analytes and their respective peak areas, confirming the

method's reliability for quantitative analysis. System suitability tests further validated the suitability of the method for accurately quantifying curcumin and piperine in plasma samples, ensuring consistent and reproducible results.

Specificity studies conducted as part of the validation process confirmed the absence of interference from endogenous substances in the chromatographic analysis. This ensures that the observed peaks in the chromatogram correspond solely to curcumin and piperine, without any interference from co-eluting compounds. Such specificity is crucial for accurately identifying and quantifying the target analytes in complex biological matrices. Linearity studies revealed excellent linearity across the concentration range tested, with low %RSD values for both curcumin and piperine. This indicates that the method's response is proportionate to changes in analyte concentration within the specified range, further confirming its suitability for quantitative analysis. The method's ability to accurately measure analyte concentrations over a wide range enhances its applicability in various research and analytical settings.

Accuracy and precision studies provided additional validation of the method's performance. Accuracy assessments demonstrated acceptable recovery of curcumin and piperine from plasma samples, indicating that the method can accurately quantify the analytes in biological matrices. Precision studies, on the other hand, evaluated the method's reproducibility by measuring the variation in results obtained from replicate analyses. The low %RSD values observed in precision studies indicate that the method yields consistent and reproducible results under repeated experimental conditions. In conclusion, the developed HPLC method for the quantification of curcumin and piperine exhibits robust performance characteristics, including satisfactory linearity, specificity, accuracy, and precision. These validation results confirm the method's reliability and suitability for accurately quantifying curcumin and piperine in plasma samples, paving the way for its application in pharmacokinetic studies, bioavailability assessments, and drug development endeavors.

6. Conclusion

In conclusion, the developed HPLC method has proven to be sensitive, selective, and reliable for the simultaneous quantification of curcumin and piperine in plasma samples. The method's satisfactory linearity, accuracy, precision, and specificity meet the stringent requirements for analytical method validation. This validated method holds significant promise for various applications, particularly in pharmacokinetic and pharmacodynamic studies. With its ability to accurately quantify curcumin and piperine levels in biological samples, the validated HPLC method offers valuable insights into their pharmacological effects and therapeutic potential. Researchers can utilize this method to investigate the absorption, distribution, metabolism, and excretion profiles of curcumin and piperine, contributing to a deeper understanding of their mechanisms of action and clinical relevance.

Moreover, the validated method can facilitate the evaluation of the bioavailability and efficacy of curcumin and piperine formulations in preclinical and clinical studies. By precisely measuring drug concentrations in biological matrices, researchers can assess the performance of various formulations, optimize dosage regimens, and enhance therapeutic outcomes. In light of these promising applications, further research may focus on expanding the utility of this method in studying the pharmacokinetics and pharmacodynamics of curcumin and piperine in different disease models and patient populations. Additionally, efforts to refine the method's analytical performance and streamline its application in routine drug analysis could enhance its usability in pharmaceutical research and development. Overall, the validated HPLC method represents a valuable tool for advancing our understanding of curcumin and piperine's therapeutic properties and facilitating the development of novel treatments for various health conditions. Its successful validation underscores its potential to make significant contributions to the fields of pharmacology, drug discovery, and personalized medicine in the years to come.

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