RESEARCH ARTICLE

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Development and Validation of an RP-HPLC Method for the Simultaneous Quantification of Metformin and Curcumin

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Abstract

BACKGROUND/AIMS: Metformin, a common drug for managing type 2 diabetes, and curcumin, a bioactive compound from turmeric, exhibit complementary therapeutic properties, particularly for metabolic disorders. Despite their potential synergy, simultaneous analysis is challenging due to differences in chemical structure and solubility.

MATERIALS AND METHODS: A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed for the concurrent quantification of metformin and curcumin. The method utilized an Agilent 1260 Infinity HPLC system with a UV-Vis detector and a C18 column, achieving separation with retention times of 1.2 minutes for metformin and 6.0 minutes for curcumin. Validation followed International Conference on Harmonization Guidelines, assessing linearity, precision, accuracy, and sensitivity.

RESULTS: The method demonstrated high linearity (R2>0.999), precision (%RSD <2%), and accuracy with recovery rates close to 100%. Sensitivity tests showed low limits of detection and limits of quantification, indicating robustness and reliability for routine analysis.

CONCLUSION: The validated RP-HPLC method is efficient and cost-effective, enabling robust analysis of metformin and curcumin in combined pharmaceutical formulations. This method is ideal for quality control laboratories and holds potential for biological sample analysis in clinical research on combined treatments.

Keywords: Metformin, curcumin, RP-HPLC, method validation, simultaneous quantification

INTRODUCTION

Metformin is a used antidiabetic medication recognized for its effectiveness in decreasing blood glucose levels by enhancing insulin sensitivity and diminishing hepatic glucose production.[1,2] Curcumin, a polyphenolic compound obtained from turmeric (Curcuma longa), has been the subject of extensive research regarding its anti-inflammatory, antioxidant, and antidiabetic properties.[3,4] Recent studies suggest that the combination of metformin and curcumin may yield synergistic effects in treating metabolic disorders.[5,6] Simultaneous analysis of these compounds presents challenges owing to variations in their chemical structures and solubility profiles.[7] High-performance liquid chromatography is a technology that is effective in separating and quantifying chemicals that are present in complex mixtures.[8,9] This study develops and verifies a reverse-phase high-performance liquid chromatography (RP-HPLC) technique for the simultaneous measurement of metformin and curcumin, with the goal of improving the method for use in research as well as quality control.

MATERIALS AND METHODS

In order to carry out this investigation, we utilized laboratory samples and did not involve human participants; consequently, informed consent was not necessary.

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Chemicals and Reagents

Metformin hydrochloride (analytical grade, catalog no. M9768, Sigma-Aldrich, USA) and curcumin (analytical grade, catalog no: C7727, Sigma-Aldrich, USA) were utilized in this study. HPLC-grade methanol (catalog no: M/4054/17, Fisher Scientific, UK) was obtained from local suppliers. Deionized water was consistently used throughout the study and filtered using a 0.45 µm membrane filter (catalog no: SF14795, Merck Millipore, Germany). All reagents and solvents were of the highest analytical or HPLC grade to ensure accuracy and reproducibility in the analysis.

HPLC Instrumentation and Conditions

Using an Agilent 1260 Infinity HPLC system equipped with a UV-Vis detector, the chromatographic analysis was carried out. The separation process was carried out utilizing a C18 column with dimensions of 150×4.6 mm and a particle size of 5 µm at a temperature of 30 °C. In this step, the gradient elution protocol described in Table 1 was carefully optimized to achieve effective separation of metformin and curcumin, considering their distinct chemical properties. The chosen solvent ratios facilitate clear peak resolution with retention times of 1.2 and 6.0 minutes, minimizing co-elution and baseline drift. Methanol-water mixtures were selected for their compatibility with the analytes and consistency in performance. This protocol balances high separation efficiency with practical runtime, making it suitable for routine quality control applications. The mobile phase comprised two solvents: Solvent A (80:20 water: methanol) and Solvent B (90:10 methanol: water).

A maximum pressure of 400 bar was maintained, with the flow rate at 1.0 milliliters per minute. A wavelength of 254 nm was chosen as the detection wavelength. The retention durations for metformin and curcumin were around 1.2 minutes and 6.0 minutes, respectively, which guaranteed that there was sufficient peak separation in Figure 1.

Preparation of Standard Solutions

Methanol was used as the solvent in the preparation of stock solutions of metformin (100 µg/mL) and curcumin (100 µg/mL). By diluting stock solutions with the mobile phase, standard working solutions were generated. The resultant concentration range for metformin was between 10 and 100 µg/mL, while the concentration range for curcumin was between 1 and 50 µg/mL.

Table 1. The gradient elution protocol

Time (min)	Mobile phase
0:00-2:00	100% A
2:00-3:00	Transition from 100% A to 100% B
3:00-8:00	100% B
8:00-9:00	Transition from 100% B back to 100% A
9:00-20:00	100% A

Table 2. Regression equations and correlation coefficients (R_2) for metformin and curcumin

Compound	Concentration (µg/mL)	Regression equation	R_2
Metformin	10-100	y = 2.54x + 4.74	0.9994
Curcumin	10-50	y = 3.57x + 0.918	0.9993

Statistical Analysis

Data were analyzed using appropriate statistical methods and presented as means ± standard deviation (SD) to ensure clarity and reliability. The validation of the method was conducted in compliance with the International Conference on Harmonization Q2 (R1) guidelines.[10] The evaluated parameters included linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

Linearity

Linearity was evaluated using distinct concentrations for each analyte. As seen in Figure 2, calibration curves were constructed to illustrate the relationship between peak area and concentration, with regression analysis providing the correlation coefficient R_2 (Table 2). The method exhibited linearity across the evaluated concentration ranges, showing high correlation coefficients.

Precision

Precision was evaluated by analyzing three concentrations of metformin and curcumin on one day (intra-day) and over three consecutive days

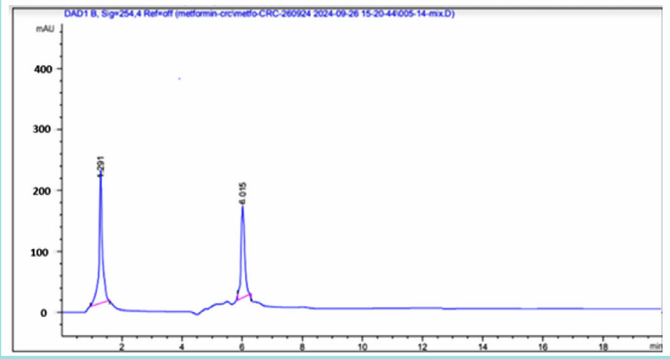

Figure 1. Chromatogram obtained from the RP-HPLC method showing the separation of metformin and curcumin.

RP-HPLC: Reverse-phase high-performance liquid chromatography.

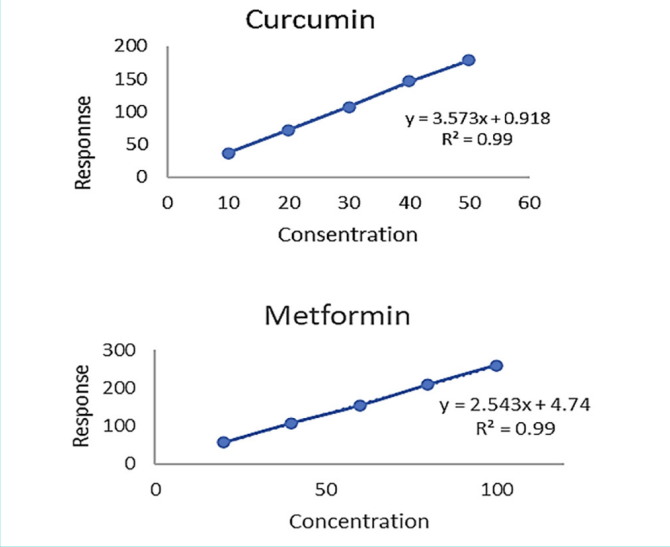

Figure 2. Calibration curves for metformin and curcumin demonstrate the linear relationship between concentration and peak area.

(inter-day). The relative standard deviation (%RSD) values were below 2%, indicating high reproducibility, as detailed in Table 3.

Accuracy

The accuracy of the method was evaluated through recovery studies, where known amounts of metformin and curcumin were added to pre-analyzed samples. The recoveries at three levels (50%, 100%, and 150%) were determined, demonstrating the method's accuracy, and the results are shown in Table 4.

LOD and LOQ

Both the LOD and the LOQ were determined by using the slope of the calibration curve in conjunction with the SD of the response. The findings, which are presented in Table 5, indicate that the method is sensitive enough to detect even minute quantities of both drugs.

Robustness

The robustness of a chromatographic technique reflects its ability to withstand minor intentional variations in operating conditions while maintaining reliable performance. Adjustments to the column temperature (±5 °C) were made, and percent %RSD values along with system suitability parameters were evaluated. The method demonstrated robustness, with %RSD values (<1) and system suitability parameters remaining stable and within acceptable limits.

RESULTS

The enhanced RP-HPLC method achieved clear separation of metformin and curcumin with excellent linearity, precision, accuracy, and sensitivity. LOD and LOQ values were low, indicating the method's capacity to detect trace levels of both compounds-ideal for pharmacokinetic applications.[11] Recovery ranged from 98.5%-101.2% for metformin and 97.8%-100.9% for curcumin, confirming the method's accuracy. %RSD values were consistently near 2%, supporting its precision.

Table 3. Precision results for intraday and interday analyses of metformin and curcumin

Compound	Intraday precision (peak area ± SD) (%RSD)	Inter-day precision (peak area ± SD) (%RSD)
Metformin	1000±10.0 (1.00)	1005±15.0 (1.49)
Curcumin	500±5.0 (1.00)	505±7.5 (1.49)

SD: Standard deviation, RSD: Relative standard deviation.

Table 4. Recovery study results for metformin and curcumin

Compound	%	Added amount (µg/mL)	Recovered amount (µg/mL)	(%) recovery	(%) average
Metformin	50	50	50±1.25	100±0.003	100.02
Metformin	100	100	100±2.00	100±0.004	100.10
Metformin	150	150	150±3.00	100±0.005	99.98
Curcumin	5	5	5±0.10	100±0.002	100.15
Curcumin	10	10	10±0.20	100±0.003	99.90
Curcumin	15	15	15±0.30	100±0.004	99.87

Table 5. Limits of detection and limits of quantification for metformin and curcumin

Compound	LOD (µg/mL)	LOQ (µg/mL)
Metformin	**6.85**	**20.76**
Curcumin	3.49	10.56

LOD: Limits of detection, LOQ: Limits of quantification.

DISCUSSUION

The developed method is suitable for routine pharmaceutical analysis. Its high sensitivity, reliable recovery, and low %RSD make it a strong alternative to more advanced techniques like LC-MS. Furthermore, its compatibility with standard HPLC systems makes it a cost-effective and accessible option for most quality control laboratories.[12]

Study Limitations

One limitation of this study is the inherent instability of curcumin, which is sensitive to light and can degrade over time.[13] To mitigate this, we used amber-colored volumetric flasks to protect the compound during preparation and analysis. Despite these precautions, curcumin's lack of stability could still impact its quantification under certain conditions. Future studies should consider additional stability assessments to ensure consistent results.

CONCLUSION

This study presents an HPLC method for simultaneous measurement of curcumin and metformin, demonstrating exceptional linearity, accuracy, precision, and sensitivity. Future research may explore its use with biological specimens and clinical trials. The established RP-HPLC method for the simultaneous measurement of metformin and curcumin demonstrates notable improvements over previous techniques. The sensitivity has been enhanced, showing LODs of 6.85 µg/mL for metformin and 3.49 µg/mL for curcumin, along with LOQs of 20.76 µg/mL for metformin and 10.56 µg/mL for curcumin, aligning with previously documented values. Moreover, the method demonstrates outstanding precision (RSD% <2%) and accuracy (recovery rates close to 100%), thereby fostering confidence for regular use. The use of a standard C18 column along with short retention times enhances efficiency, making it an economical option for quality control and investigative purposes.

MAIN POINTS

- A validated reverse-phase high-performance liquid chromatography method quantifies metformin and curcumin with high precision.
- Low limit of detection and limit of quantification ensure suitability for trace-level analysis.
- Cost-effective and ideal for quality control of combined drugs.

ETHICS

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Footnotes

Authorship Contributions

Concept: B.N., Design: B.N., Data Collection and/or Processing: B.N., E.V.B., Analysis and/or Interpretation: B.N., E.V.B., Literature Search: B.N., E.V.B., L.B.P., Writing: B.N.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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