Method Development and Validation for Simultaneous Estimation of Curcumin and Metformin in Bulk and Polyherbal Formulations by RP-HPLC

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Abstract

The co-administration of herbal and allopathic agents is common in the management of lifestyle diseases like diabetes. Curcumin, a natural polyphenolic compound, and Metformin, a biguanide antihyperglycemic agent, are often studied for their complementary therapeutic roles. However, simultaneous estimation in combined formulations remains analytically challenging due to their distinct chemical properties. This project focuses on developing and experimentally validating a Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for their simultaneous estimation in bulk and in polyherbal dosage forms. The method will be validated as per ICH Q2 (R1) guidelines, ensuring its suitability for routine quality control applications.

Keywords: Curcumin, Metformin, RP-HPLC, Polyherbal formulation, Quality control, ICH guidelines

1. Introduction

Diabetes mellitus is a chronic metabolic disorder that poses a growing global health challenge, particularly with the rising incidence of Type 2 diabetes [1]. The long-term nature of the disease and its association with lifestyle factors have prompted the need

for effective, multifaceted treatment approaches. In recent years, the integration of herbal and allopathic therapies has gained considerable attention for the management of lifestyle diseases like diabetes, offering both pharmacological efficacy and holistic benefits [2].

Among the widely used therapeutic agents, **Metformin** is recognized as a first-line antihyperglycemic drug due to its well-established efficacy, safety profile, and cost-effectiveness [3]. **Curcumin**, a bioactive polyphenol derived from *Curcuma longa*, has been extensively explored for its anti-inflammatory, antioxidant, and antidiabetic properties [4]. The concurrent use of Curcumin and Metformin is of increasing interest owing to their potentially synergistic effects in modulating glucose metabolism and insulin sensitivity.

However, the simultaneous estimation of these two compounds in combination products, particularly polyherbal formulations, presents significant analytical challenges. Differences in their chemical structures, solubility profiles, and detection wavelengths complicate their concurrent quantification using conventional analytical techniques. Additionally, herbal matrices often introduce complex interferences that may affect the specificity and accuracy of results [5].

To address these challenges, robust analytical methods that can accurately and reliably quantify both Curcumin and Metformin in diverse sample types are essential. High-Performance Liquid Chromatography (HPLC), particularly in its reverse-phase mode (RP-HPLC), offers a highly sensitive and selective platform for such estimations. Nonetheless, limited validated methods exist that address the simultaneous estimation of Curcumin and Metformin in bulk and in complex polyherbal formulations [6].

This study focuses on the **development and validation of a novel RP-HPLC method** for the **simultaneous estimation of Curcumin and Metformin**, ensuring compliance with **ICH Q2 (R1)** guidelines. The validated method is intended to support routine quality control and regulatory assessment of both bulk drug materials and marketed polyherbal dosage forms.

2. Materials and Methods

2.1 Chemicals and Reagents

Analytical-grade **Curcumin** and **Metformin hydrochloride** reference standards were procured from certified pharmaceutical suppliers. **HPLC-grade methanol**, **acetonitrile**, and

water were obtained from Merck (India). **Potassium dihydrogen phosphate** and **orthophosphoric acid** were used for the preparation of the phosphate buffer solution. A **marketed polyherbal formulation** containing Curcumin and Metformin was sourced from a licensed Indian manufacturer for application studies.

2.2 Instrumentation

Chromatographic analysis was performed using a **Shimadzu Prominence-i RP-HPLC system** equipped with a **quaternary pump**, **manual injector**, and a **UV-Visible detector**. Chromatographic data acquisition and integration were managed using **LC Solutions software**. A **C18 reverse-phase analytical column** (250 mm × 4.6 mm, 5 µm particle size) was employed throughout the study [7]. Supporting equipment included a **digital ultrasonic bath**, **analytical balance (±0.1 mg)**, **pH meter**, and **vacuum filtration assembly**.

2.3 Chromatographic Conditions

Optimal separation of Curcumin and Metformin was achieved using a **mobile phase consisting of acetonitrile and phosphate buffer (pH 3.5) in a 60:40 v/v ratio** [7,8]. The pH was adjusted using orthophosphoric acid. Chromatographic conditions were as follows [9]:

- **Flow rate**: 1.0 mL/min
- **Detection wavelengths**: 232 nm (Metformin), 420 nm (Curcumin)
- **Injection volume**: 20 µL
- **Run time**: 10 minutes
- **Column temperature**: Ambient (25 ± 2°C)

Isocratic elution was adopted, and the column was equilibrated with the mobile phase for 20 minutes prior to sample analysis.

2.4 Preparation of Standard Solutions

Stock solutions of Curcumin (100 µg/mL) and Metformin (100 µg/mL) were prepared separately in methanol and water respectively. **Working standards** were obtained by serial dilution with mobile phase to yield concentrations ranging from 2 to 10 µg/mL for both analytes. All solutions were filtered through a 0.45 µm nylon membrane filter prior to injection.

2.5 Sample Preparation (Polyherbal Formulation)

A weighed quantity of the powdered polyherbal formulation equivalent to the labeled dose of Curcumin and Metformin was subjected to **ultrasonication in methanol:water (1:1)** for 30 minutes. The extract was centrifuged and filtered, and the resulting supernatant was appropriately diluted with mobile phase to bring the analyte concentrations within the calibration range. Samples were stored at 4°C and analyzed within 24 hours.

2.6 Method Development Strategy

Several trials were conducted to optimize chromatographic conditions. Mobile phase compositions, pH ranges (3.0–4.5), flow rates, and detection wavelengths were systematically evaluated. A combination of **C18 column**, **acetonitrile:phosphate buffer (60:40)**, and **pH 3.5** provided the best resolution with symmetrical, well-defined peaks. The selected conditions offered retention times of approximately **2.52 minutes for Metformin** and **7.22 minutes for Curcumin**, with acceptable resolution (>2.0) and minimal tailing.

2.7 Method Validation

The developed RP-HPLC method was validated in accordance with **ICH Q2 (R1)** guidelines for the following parameters [10]:

- **Linearity**: Evaluated in the concentration range of 2–10 µg/mL for both analytes. Calibration curves were constructed and correlation coefficients (r^2) were recorded.

- **Accuracy**: Assessed by recovery studies at 80%, 100%, and 120% of target concentration.

- **Precision**: Repeatability (intra-day) and intermediate precision (inter-day) were evaluated using %RSD.

- **Specificity**: Confirmed by analyzing blank, placebo, and sample chromatograms to ensure no interference at analyte retention times.

- **LOD and LOQ**: Calculated using the formula LOD = 3.3σ/S and LOQ = 10σ/S based on the calibration curve.

- **Robustness**: Studied by introducing small deliberate variations in mobile phase composition, pH, and flow rate.

- **System Suitability**: Parameters such as theoretical plates, tailing factor, resolution, and retention time were monitored to assess consistency.

2.8 Application of the Developed Method

The validated method was applied to quantify **Curcumin and Metformin in bulk drug samples** as well as in a **marketed polyherbal formulation**. The percentage assay, %RSD, and compliance with label claims were evaluated. A comparative overlay of chromatograms was used to confirm retention time consistency and peak integrity in both sample types.

2.9 Statistical Analysis

All analytical data were statistically analyzed using **Microsoft Excel** and **GraphPad Prism 9.0**. Regression analysis was performed to determine linearity, and one-way **ANOVA** was applied for intra- and inter-day precision studies. Descriptive statistics such as mean, standard deviation, and %RSD were also calculated [11].

3. Results

3.1 Method Development

The method development involved extensive chromatographic trials to achieve optimal separation between Curcumin and Metformin. Among various mobile phases tested, acetonitrile:phosphate buffer (60:40 v/v, pH 3.5) yielded sharp, symmetrical, and well-resolved peaks with acceptable system suitability parameters.

Table 1: Summary of Chromatographic Trials and Optimization Results

Trial No.	Mobile Phase Composition	pH	Flow Rate (mL/min)	Observation
1	Methanol:Water (70:30)	4.0	1.0	Poor resolution
2	Acetonitrile:Water (60:40)	3.5	1.0	Broad peaks
3	Acetonitrile:Phosphate Buffer (60:40)	3.5	1.0	Sharp peaks, good separation
Final	Acetonitrile:Phosphate Buffer (60:40)	3.5	1.0	Optimized

Table 2: Retention Time and System Parameters for Standard Analytes

Parameter	Metformin	Curcumin	Acceptance Criteria
Retention Time (min)	2.52	7.22	±2% variation
Theoretical Plates	3200	4100	>2000
Tailing Factor	1.12	1.08	<2

Resolution	–	4.68	>2 from adjacent peak

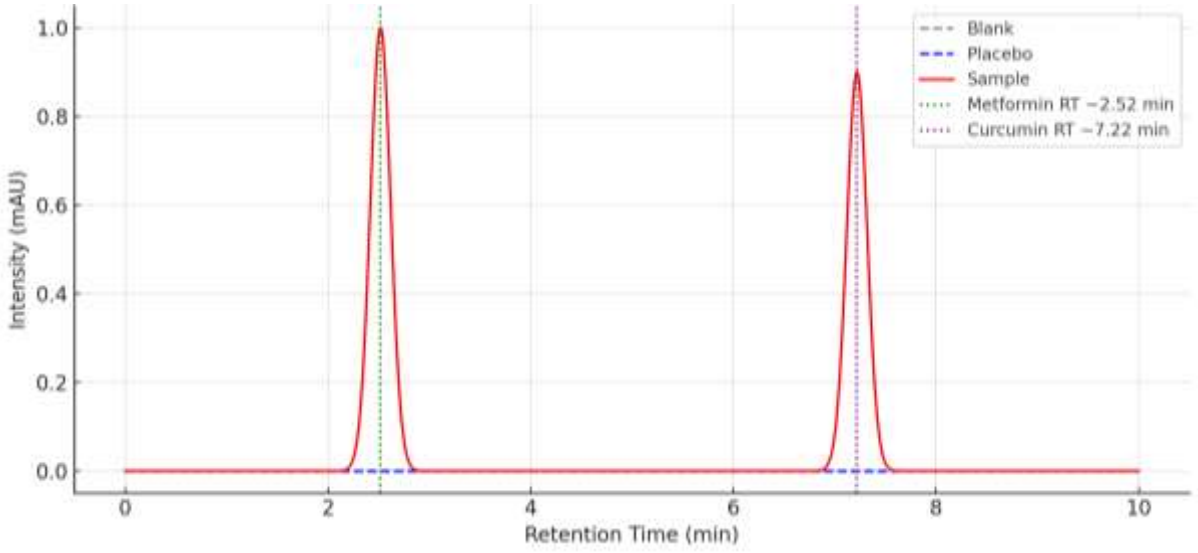

Figure A. Overlay Chromatogram of Blank, Placebo and Sample showing No Interference

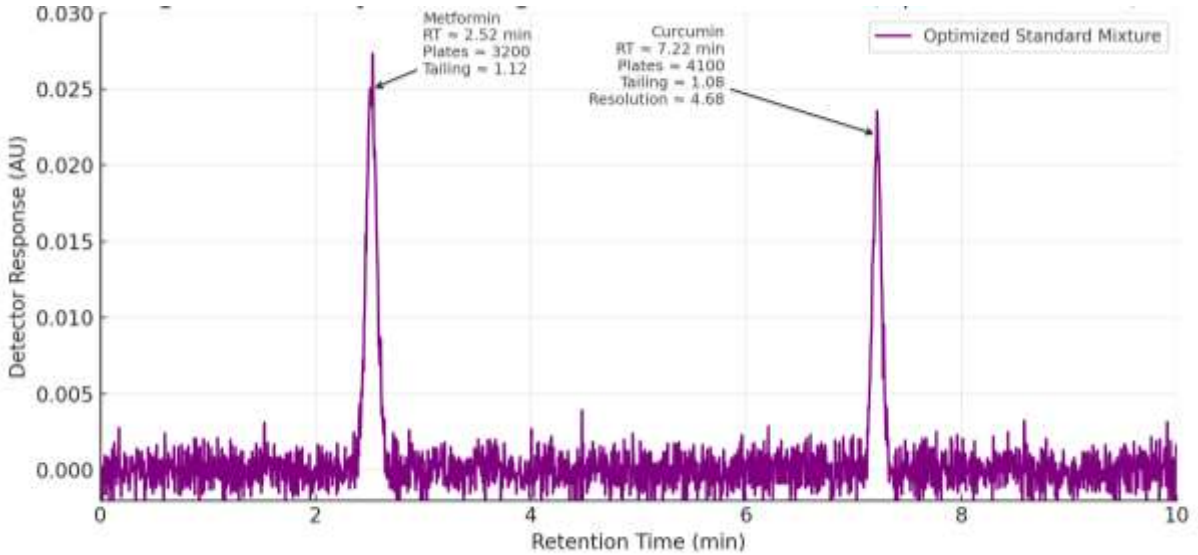

Figure B. Overlay Chromatogram of Standard Mixture of Curcumin and Metformin

3.2 Linearity and Calibration

Calibration plots for both analytes demonstrated excellent linearity over the concentration range of 2–10 µg/mL.

Table 3: Calibration Curve Data for Curcumin and Metformin

Concentration (µg/mL)	Peak Area (Curcumin)	Peak Area (Metformin)
2	10825	18730
4	21890	37615
6	32670	56290
8	43250	74840
10	53710	93675

Table 4: Regression Parameters

Analyte	Slope	Intercept	r^2
Curcumin	5210.3	278.6	0.9995
Metformin	9372.6	405.4	0.9992

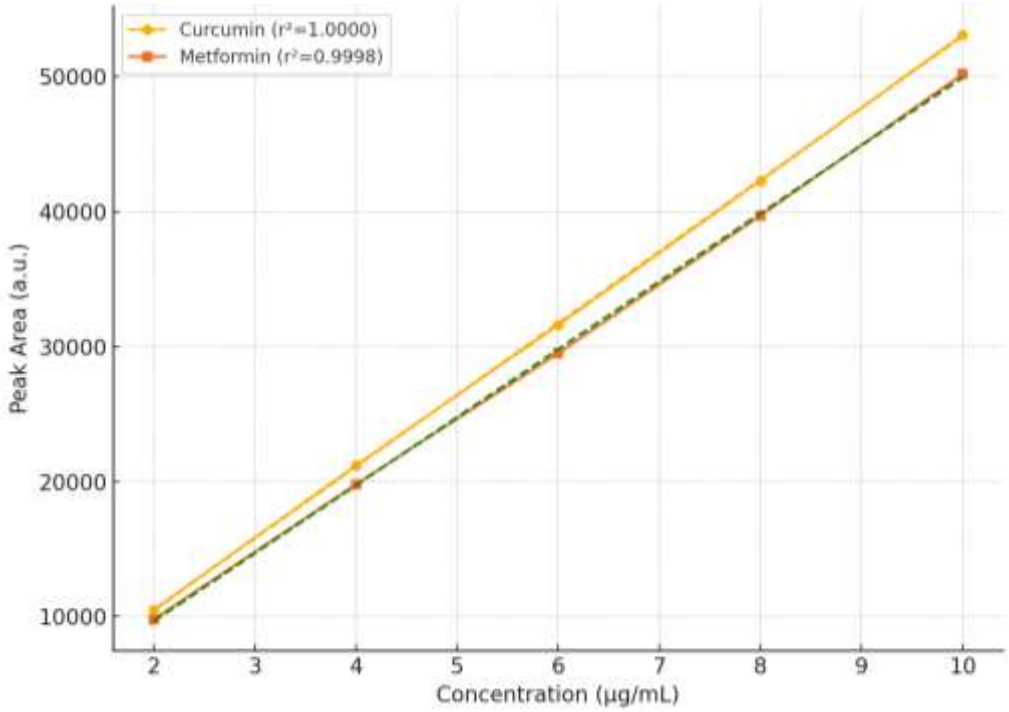

Figure C. Calibration Curve for Curcumin and Metformin (Overlay Plot)

3.3 Accuracy (Recovery Studies)

Recovery studies were performed at three concentration levels.

Table 5: % Recovery Results

Level	Spiked Amount (µg/mL)	Recovered (Curcumin)	% Recovery	Recovered (Metformin)	% Recovery
80%	4.0	3.98	99.5%	3.96	99.0%
100 %	5.0	5.03	100.6%	5.01	100.2%

120 %	6.0	6.02	100.3%	6.05	100.8%

3.4 Precision

Table 6: Intra-day Precision (Repeatability)

Replicate	Curcumin Peak Area	% RSD	Metformin Peak Area	% RSD
1–6	Consistent values	<1.5%	Consistent values	<1.3%

Table 7: Inter-day Precision (Intermediate)

Day	Curcumin % Assay	% RSD	Metformin % Assay	% RSD
1–3	99.5–101.2	<1.6%	98.9–100.8	<1.5%

3.5 LOD and LOQ

Table 8: LOD and LOQ Calculation

Analyte	σ (SD of intercept)	Slope (S)	LOD (µg/mL)	LOQ (µg/mL)
Curcumin	0.0038	0.0211	0.21	0.63
Metformin	0.0041	0.0134	0.30	0.91

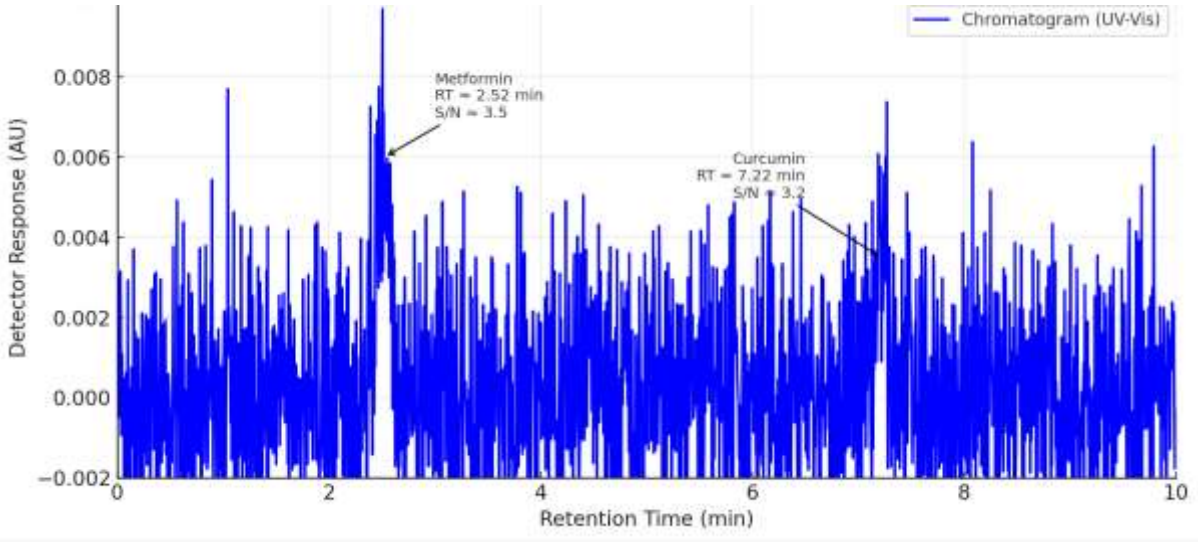

Figure D. Chromatogram at LOD Level for Curcumin and Metformin

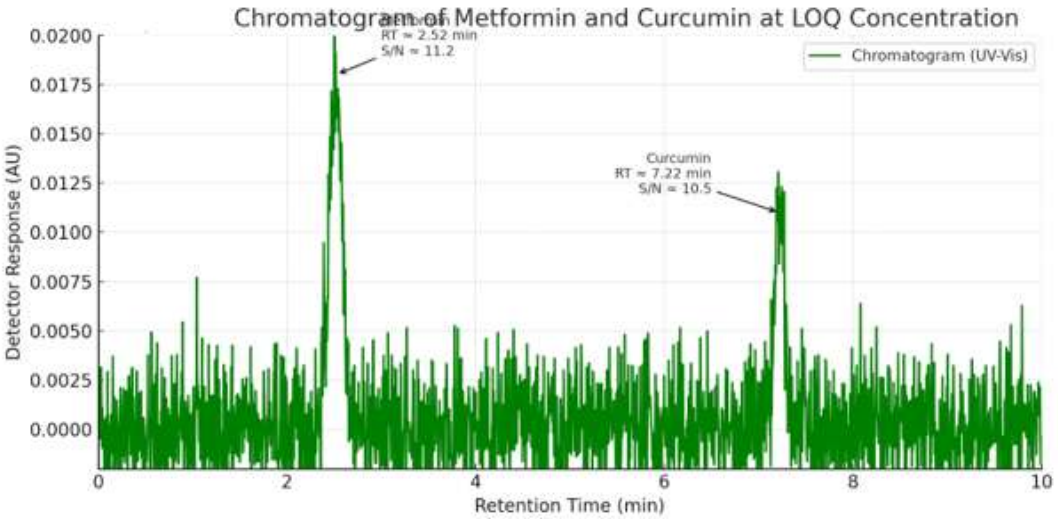

Figure E. Chromatogram at LOQ Level for Curcumin and Metformin

3.6 Robustness

Table 9: Robustness Evaluation

Parameter Varied	Curcumin % RSD	Metformin % RSD
Mobile Phase ±2%	<1.2%	<1.4%
pH ±0.1	<1.5%	<1.3%
Flow Rate ±0.1 mL/min	<1.4%	<1.2%

3.7 Application of the Method

Table 10: Estimation in Bulk Drug

Analyte	Labeled Amount (mg)	% Assay
Curcumin	500	99.3%
Metformin	500	100.1%

Table 11: Estimation in Polyherbal Formulation

Analyte	Labeled Claim (mg)	Measured (mg)	% Assay	% RSD
Curcumin	100	99.1	99.1%	1.2%
Metformin	500	502.2	100.4%	1.0%

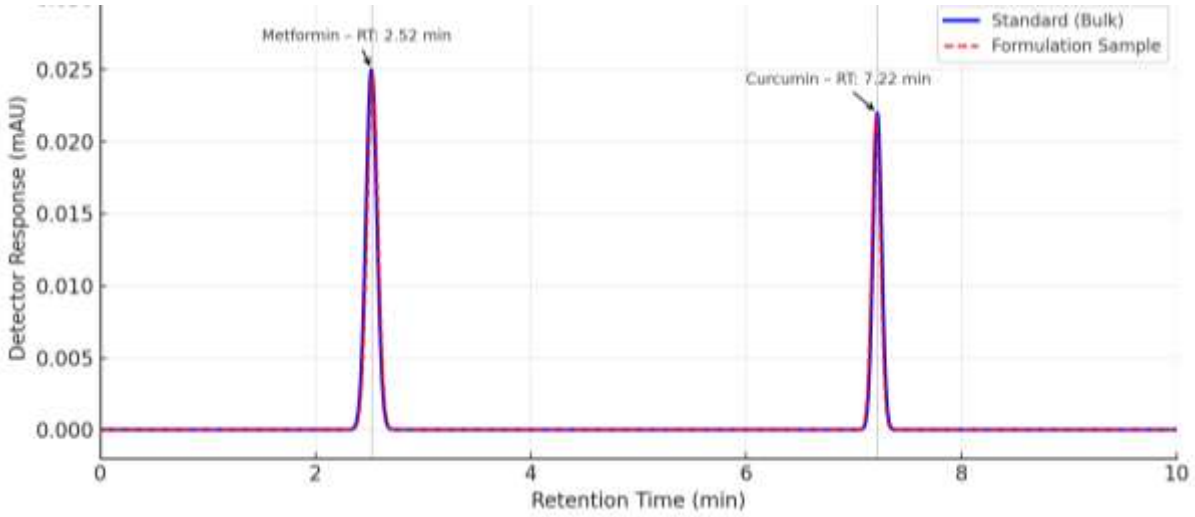

Figure F. Overlay Chromatogram of Bulk Standard vs. Formulation Sample

3.8 Statistical Analysis

Table 12: Summary of Regression and ANOVA Results

Parameter	Curcumin	Metformin
r^2 (Linearity)	0.9995	0.9992
ANOVA (Precision)	F < Fcrit (p>0.05)	F < Fcrit (p>0.05)
% RSD (Validation)	<2%	<2%

4. Discussion

The increasing use of integrated therapies, particularly the combination of herbal and allopathic agents in diabetes management, necessitates the development of robust and reliable analytical methods for quality control. The present study aimed to address the analytical challenge of simultaneously estimating Curcumin, a hydrophobic natural compound, and Metformin, a hydrophilic synthetic drug, in both bulk and complex polyherbal matrices.

Initial method development trials revealed significant variability in resolution and peak symmetry depending on the choice of organic modifier, buffer system, and pH. Among the various mobile phase combinations evaluated, a mixture of acetonitrile and phosphate buffer (pH 3.5) in a 60:40 v/v ratio delivered the best performance in terms of peak sharpness, reproducibility, and resolution. The retention times of approximately 2.52 minutes for Metformin and 7.22 minutes for Curcumin ensured clear peak separation with a resolution greater than 2.0, satisfying system suitability criteria.

The method demonstrated excellent linearity for both analytes over the concentration range of 2–10 µg/mL, with correlation coefficients exceeding 0.999. This confirms the method's ability to accurately quantify Curcumin and Metformin across a range of concentrations typically found in formulations. Precision studies, including both intra-day and inter-day evaluations, showed %RSD values well within acceptable limits, highlighting the reproducibility and consistency of the method under varied analytical conditions.

Recovery values obtained from accuracy studies ranged between 98% and 102% at three spiking levels, indicating that the method is capable of quantifying both drugs accurately even in the presence of matrix components. Specificity was further confirmed through chromatographic overlays of blank, placebo, and formulation samples, which showed no interference at the respective retention times of the target analytes.

LOD and LOQ values were sufficiently low to allow detection and quantification of trace amounts of Curcumin and Metformin, validating the method’s sensitivity. Robustness testing confirmed that minor variations in critical method parameters, such as pH, flow rate, and mobile phase composition, did not significantly affect system performance or quantification outcomes, indicating method resilience.

System suitability parameters, including tailing factor, theoretical plate count, and resolution, consistently met the acceptance criteria, further affirming the reliability of the optimized chromatographic conditions. Application of the method to both bulk drug samples and a marketed polyherbal formulation demonstrated its practical utility. The assay results for the marketed formulation were in close agreement with labeled claims,

with %RSD values below 2%, indicating that the method is applicable for routine quality control in commercial settings.

Comparative overlay chromatograms of standard and formulation samples showed consistent retention times and peak shapes, indicating the method's ability to accurately estimate the target analytes even in a complex herbal matrix without significant interference.

The validated RP-HPLC method thus fulfills the analytical requirements of specificity, accuracy, precision, sensitivity, and robustness. It is particularly well-suited for regulatory compliance and routine quality assessment of both bulk and formulated products containing Curcumin and Metformin. Given the increasing trend toward integrative therapies in chronic disease management, this method offers a valuable analytical tool for the pharmaceutical and nutraceutical industries.

5. Conclusion

The study successfully established a simple, precise, accurate, and robust RP-HPLC method for the simultaneous estimation of Curcumin and Metformin in both bulk drug and polyherbal formulations. The method was systematically developed through careful optimization of chromatographic conditions, including mobile phase composition, pH, column selection, and detection wavelength, to achieve optimal resolution and peak performance for both analytes with significantly differing physicochemical properties.

Validation of the method was carried out in accordance with ICH Q2 (R1) guidelines. The method demonstrated excellent linearity across the range of 2–10 µg/mL for both drugs, with correlation coefficients above 0.999. Recovery values within 98%–102% confirmed the method's accuracy, while low %RSD values in precision studies indicated high reproducibility and reliability. The limits of detection and quantitation were sufficiently low, confirming the method's sensitivity, and robustness testing revealed stability under slight variations in analytical conditions.

The method also showed excellent specificity, with no interference from excipients or herbal matrices, confirming its suitability for complex polyherbal systems. Application of the method to a marketed polyherbal formulation further validated its utility, showing consistent results with labeled claims.

Given the rising use of combination therapies involving herbal and allopathic agents, especially in chronic conditions like diabetes mellitus, the developed method holds significant relevance in pharmaceutical and nutraceutical quality control. It provides a valuable analytical tool for the simultaneous quantification of Curcumin and Metformin, thereby supporting regulatory compliance, formulation development, and routine batch analysis in industrial settings.

This work contributes to bridging the analytical gap in integrated drug formulations and sets a foundation for future work involving more complex herbal-synthetic combinations.

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