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SIMULTANEOUS DETERMINATION OF BERBERINE AND CURCUMIN IN A TOPICAL GEL USING A VALIDATED HPLC METHOD

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ABSTRACT

A simple, rapid, and reliable HPLC method was developed and validated for the simultaneous quantification of berberine and curcumin in a laboratory-formulated gel. Chromatographic separation was achieved using an Agilent 1260 Infinity II HPLC system equipped with an EC-C18 column (100 mm × 4.6 mm, 4 µm), and a mobile phase consisting of acetonitrile and 0.1% phosphoric acid (85:15, v/v). The method showed excellent linearity ($R^2 > 0.999$), high sensitivity, and fulfilled all validation criteria in accordance with ICH guidelines. The limits of detection (LOD) and quantification (LOQ) were 0.06/0.20 µg/mL for berberine and 0.004/0.015 µg/mL for curcumin, respectively. Recovery ranged from 100.65% to 101.37% for berberine and from 99.65% to 100.02% for curcumin, with %RSD values below 1.0% for both repeatability and intermediate precision. This validated method is suitable for routine quality control of gel formulations and offers a robust analytical approach for further pharmaceutical development involving berberine and curcumin.

KEYWORDS: Berberine, Curcumin, HPLC, Method validation, Gel formulation, Simultaneous quantification.

1. INTRODUCTION

Curcumin, a polyphenolic compound extracted from *Curcuma longa*, and berberine chloride, an isoquinoline alkaloid found in *Coptis chinensis* and *Phellodendron amurense*, are both bioactive substances with significant pharmacological effects. Curcumin exhibits antioxidant and anti-inflammatory activities primarily through inhibition of NF-κB and COX-2 pathways.[1] while berberine demonstrates antimicrobial, immunomodulatory, and antioxidative properties by suppressing pro-inflammatory cytokines.[2] The combination of these two compounds has been reported to exert synergistic effects in inflammation control, cytoprotection, and tissue regeneration, making them particularly suitable for topical formulations.[3] Although some commercial products combining berberine and curcumin are available on the market, there is currently no official analytical method for their simultaneous quantification in gel formulations, posing challenges for quality control.

A review of the literature reveals that several studies have established methods for the individual quantification of curcumin or berberine in raw materials and finished products using techniques such as UV-Vis spectrophotometry[4,5], high-performance liquid chromatography (HPLC)[6], fluorescence spectroscopy[7], and liquid chromatography–mass spectrometry (LC–MS)[8], among others. While a few studies have reported the simultaneous determination of these compounds in plant extracts[9], their concurrent quantification in pharmaceutical formulations remains limited.

Based on this context, the present study was conducted to develop and validate a robust and reliable HPLC method for the simultaneous determination of berberine and curcumin in a gel formulation prepared in-house. The results are expected to contribute to the standardization and quality control of pharmaceutical products containing both active ingredients.

2. MATERIALS AND METHODS

2.1. Study materials, chemicals, and instruments

Study materials

Test sample: Topical gel containing berberine and curcumin, batch NC01 (manufactured on April 25, 2025), with the following formulation: berberine (30 mg), curcumin (30 mg), PEG (6.84 g), glycerol (4.5 g), Tween 20 (1.5 g), ethanol (9 g), menthol (0.3 g), carbopol 940 (3 g), triethanolamine (1.5 g), and purified water qs to 30 g.

Placebo sample: Same composition as the test sample, excluding berberine and curcumin.

Chemicals: Berberine chloride (CAS: 633-65-8) and curcumin (CAS: 458-37-7) were purchased from Sigma-Aldrich. Methanol, acetonitrile, and 85% phosphoric acid were obtained from Merck (Germany). All solvents were of HPLC grade.

Instruments: Chromatographic analysis was performed using an Agilent 1260 Infinity II HPLC system (USA) equipped with a variable wavelength detector (VWD).

2.2. Simultaneous Quantification Procedure for Berberine and Curcumin in the Gel Formulation

Chromatographic conditions

An HPLC method was developed to simultaneously quantify berberine and curcumin using an Agilent Poroshell 120 EC-C18 column (100 × 4.6 mm, 4 µm). The detection was carried out at 425 nm using a UV detector. The mobile phase flow rate was set at 1.0 mL/min, injection volume at 1.0 µL, and column temperature maintained at 40 °C.

The mobile phase consisted of acetonitrile and 0.1% phosphoric acid in water (v/v), with different ratios evaluated to optimize the separation efficiency.

Preparation of standard solutions

Individual stock solutions of berberine/curcumin: Accurately weigh 10 mg of berberine or curcumin reference standard into a 25 mL volumetric flask, add 15 mL of mobile phase, ultrasonicate until completely dissolved, then dilute to volume with mobile phase.

Mixed stock solution of berberine and curcumin: Accurately weigh 10 mg of each standard (berberine and curcumin), place into a 25 mL volumetric flask, add 15 mL of mobile phase, ultrasonicate until dissolved, then dilute to volume.

Working standard solutions: Prepare by diluting the stock solutions with mobile phase to the required concentrations. All solutions were filtered through a 0.22 µm PTFE membrane filter prior to analysis.

Preparation of sample and placebo solutions

Sample solution: Accurately weigh approximately 0.25 g of gel into a 25 mL volumetric flask, add 15 mL of extraction solvent, sonicate (without heating) for 30 minutes, and dilute to volume with extraction solvent. Filter through a 0.22 µm PTFE membrane filter.

Placebo solution: Prepared in the same manner, using 0.25 g of the placebo gel (devoid of active ingredients).

Based on the physicochemical characteristics of berberine, curcumin, and the gel matrix, various extraction solvent systems were investigated to optimize recovery. The content of berberine and curcumin in the samples was determined using an external standard method by comparing peak areas with those of standard solutions at equivalent concentrations.

Quantification and content calculation

Sample and standard solutions were analyzed under the optimized chromatographic conditions. The percentage recovery of each active ingredient was calculated using the following formula.

$$\%\ Recovery = \frac{C_t \times V}{1000 \times m \times 0.1\%} \times 100\%$$

Where: C_t is the concentration of berberine or curcumin in the test solution (µg/mL), determined from the calibration curve; V is the volume of the test solution (mL); m is the mass of gel sample used (mg); 0.1% is the labeled content of each active ingredient in the formulation.

2.3. Method Validation

The analytical method was validated according to ICH guidelines[10], including the following parameters: system suitability, specificity, linearity range, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision.

3. RESULTS AND DISCUSSION

3.1. Optimization of Chromatographic Conditions

The results of evaluating different mobile phase systems to optimize chromatographic separation are presented in Table 1.

Table 1: Effect of Mobile Phase on the Retention, Tailing, and Resolution of Berberine and Curcumin.

Mobile Phase	Berberine		Curcumin		R_s
	t_R (min)	T	t_R (min)	T	
Acetonitrile – 0,1% H_3PO_4 (50:50, v/v)	1.272	1.492	3.932	1.270	20.619
Acetonitrile – 0,1% H_3PO_4 (85:15, v/v)	0.916	1.235	1.296	1.328	4.396
Acetonitrile – 1% H_3PO_4 (85:15, v/v)	0.884	1.286	1.307	1.421	4.779
**Legend: t_R: Retention time; T: Tailing factor; R_s: Resolution*					

All tested mobile phase systems enabled baseline separation of berberine and curcumin peaks. However, the acetonitrile – 0,1% H_3PO_4 (50:50, v/v) system resulted in prolonged retention times and a high tailing factor for berberine (T = 1.492), indicating strong secondary interactions with the stationary phase. Increasing the acetonitrile proportion to 85% significantly reduced retention times and improved peak symmetry. Among these, the mobile phase consisting of acetonitrile – 0.1% H_3PO_4 (85:15, v/v) provided the most symmetrical and stable peaks with a satisfactory resolution (Rs = 4.396). Further increasing the acid concentration to 1% slightly improved Rs (4.779), but also increased peak tailing and may reduce column lifespan. Therefore, the acetonitrile – 0.1% H_3PO_4 (85:15, v/v) system was selected as the optimal mobile phase for simultaneous quantification of berberine and curcumin.

3.2. Evaluation of Sample Extraction Solvent

The extraction efficiency of berberine and curcumin from the gel matrix was influenced by the nature and composition of the solvent system. When methanol was used as the extraction solvent, only curcumin was effectively recovered (99.89%), whereas berberine was not detected in the chromatogram, indicating that methanol is unsuitable for simultaneous extraction of both analytes.

In contrast, solvent systems composed of acetonitrile and 0.1% phosphoric acid at ratios of 85:15, 90:10, and 95:5 (v/v) yielded high recovery rates for both compounds, ranging from 96.67% to 102.32%. Among them, the 95:5 (v/v) system provided the most efficient extraction, with recovery values of 101.40% for berberine and 99.97% for curcumin. Therefore, this solvent composition was selected for use in subsequent analyses.

3.3. Method Validation Results

System Suitability

System suitability was evaluated by injecting a mixed standard solution of berberine and curcumin (12 µg/mL) six times consecutively. The chromatographic parameters obtained are summarized in Table 2.

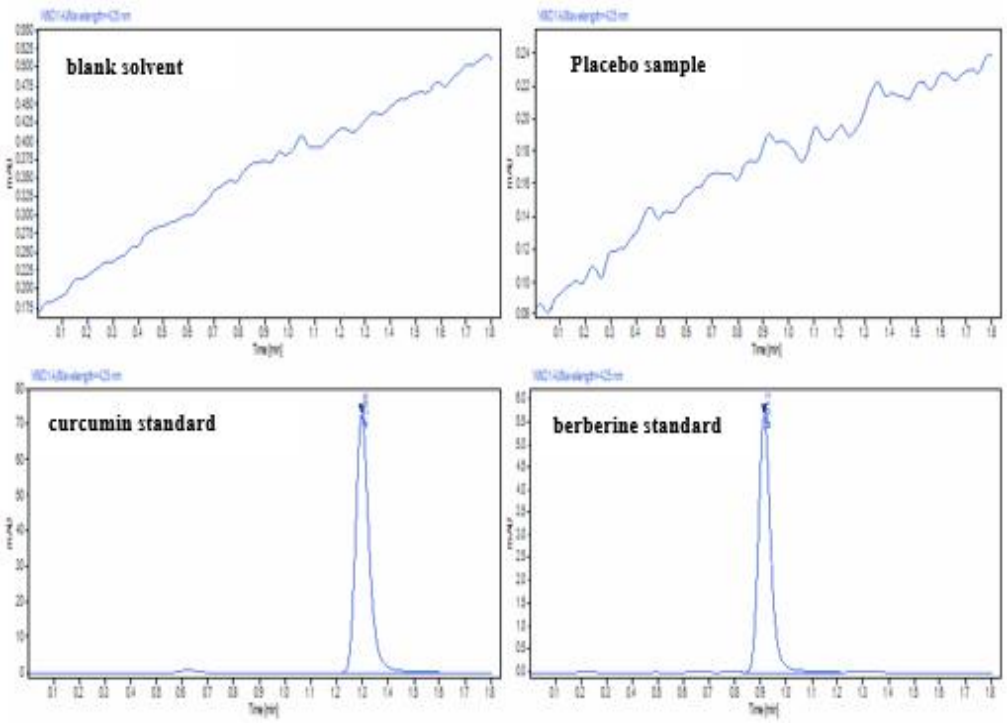
Table 2: System suitability parameters of berberine and curcumin (n = 6).

	Berberine				Curcumin				
Parameter	**t_R (min)**	**S (mAU.s)**	**T**	**N**	**t_R (min)**	**S (mAU.s)**	**T**	**N**	**R_s**
Mean	0.916	9,099	1,255	2359	1,297	94,102	1,336	3254	4,38
%RSD	0,045	0,574			0,040	0,212			
**Legend: t_R: Retention time; S: Peak area; T: Tailing factor; N: Theoretical plates USP; R_s: Resolution.*									

The system suitability results indicated that the number of theoretical plates for both berberine and curcumin exceeded 2,000. The tailing factors (T) were within the acceptable range (0.8 – 1.5), and the resolution (Rs) between the two peaks was greater than 2.0. The relative standard deviations (%RSD) of both peak areas and retention times were below 2%, demonstrating high repeatability and stable performance of the HPLC system. These parameters fully met the system suitability requirements, ensuring the reliability of the method for simultaneous quantification of berberine and curcumin in the gel formulation.

Specificity

The specificity of the method was evaluated by analyzing the chromatograms of the following solutions: blank (sample diluent), berberine standard solution, curcumin standard solution, mixed standard solution of berberine and curcumin, test sample solution, and placebo sample solution. The resulting chromatograms are shown in Figure 1.



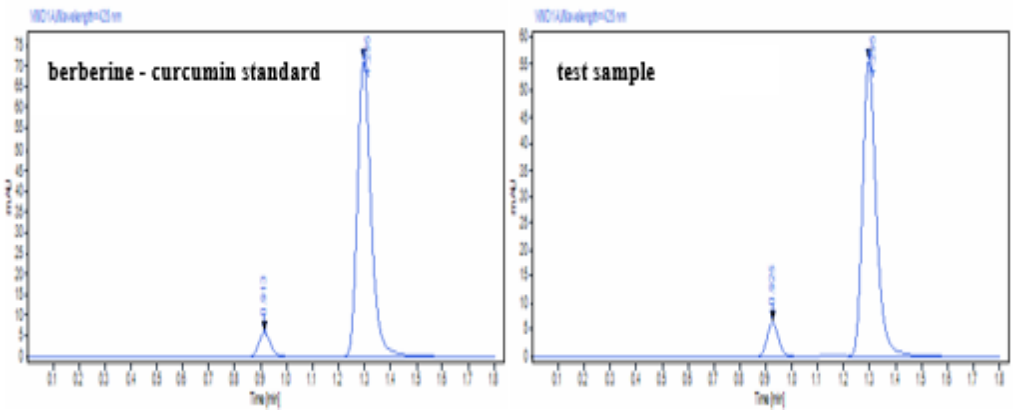


Figure 1: HPLC chromatograms of blank solvent, placebo sample, berberine standard, curcumin standard, berberine–curcumin mixed standard, and test sample solution.

The chromatographic analysis showed that neither the sample diluent nor the placebo solution exhibited any peaks at the retention times of berberine and curcumin, indicating the absence of matrix interference. The average retention times of berberine in the standard and test solutions were 0.918 and 0.916 minutes, respectively; for curcumin, they were 1.297 and 1.296 minutes. The retention time deviations between the test and standard solutions were less than 2.0%, confirming the stability and reliability of the method.

Linearity

The calibration curve was established using five concentrations of a mixed standard solution of berberine and curcumin, ranging from 5 to 15 µg/mL (corresponding to 50 – 150% of the target assay concentration). Each concentration level was injected in triplicate. The chromatographic data obtained were used to construct linear regression equations, as shown in Figure 2.

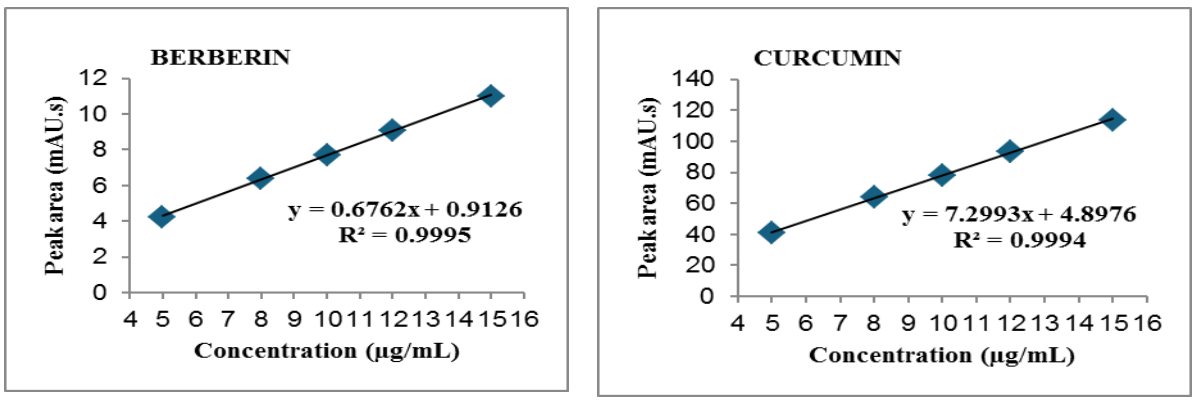


Figure 2: Calibration curves showing the linear relationship between concentration and peak area of berberine and curcumin.

The results demonstrated a clear linear correlation between concentration and peak area for both analytes. The correlation coefficient (R^2) was 0.9995 for berberine (y = 0.6762x + 0.9126) and 0.9994 for curcumin (y = 7.2993x + 4.8976). These R^2 values, which are close to 1.0, confirm that the method exhibits excellent linearity and stability over the tested concentration range.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) were determined based on the signal-to-noise ratio (S/N), corresponding to S/N = 3 for LOD and S/N = 10 for LOQ. The results indicated that the method possesses high sensitivity, with LOD and LOQ values for berberine at 0.06 µg/mL and 0.20 µg/mL, respectively, and for curcumin at 0.004 µg/mL and 0.015 µg/mL, respectively. These findings demonstrate that the method is sufficiently sensitive to quantify both active compounds even at very low concentrations in the gel matrix, meeting the analytical requirements for product quality control.

Accuracy

Accuracy was evaluated using the standard addition method at three concentration levels (70%, 100%, and 130%) by spiking known amounts of berberine and curcumin into the placebo gel matrix. The recovery results for berberine and curcumin are presented in Table 3.

Table 3: Recovery results of berberine and curcumin in placebo gel at three spiking levels (n = 3 per level).

Compound	Spiked concentration level (%)	Added concentration (µg/mL)	Found concentration (µg/mL)	%Recovery	%RSD
Berberine	70	7	7.045	100.65	0.53
	100	10	10.129	101.29	0.35
	130	13	13.178	101.37	0.47
Curcumin	70	7	6.976	99.65	0.27
	100	10	10.002	100.02	0.3
	130	13	12.995	99.97	0.27

The results showed that the recovery of berberine ranged from 100.65% to 101.37%, while that of curcumin ranged from 99.65% to 100.02%, with relative standard deviations (%RSD) below 0.6% for both analytes. These findings demonstrate the high accuracy of the method and its reliability for the quantification of berberine and curcumin in actual gel samples.

Precision

The precision of the method was evaluated using the final gel formulation containing berberine and curcumin (batch NC01), which was prepared according to the developed formulation. Detailed results are presented in Table 4, showing the mean content and relative standard deviation (%RSD) for each analyte over two independent days of analysis (n = 12).

Table 4: Intraday and interday precision of berberine and curcumin determination in gel formulation.

Compound	Intraday		Interday		Mean (n=12)	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Berberine	100,90	0,58	101,19	0,71	101,04	0,68
Curcumin	99,95	0,27	99,92	0,41	99,92	0,41

The results demonstrated good repeatability, with %RSD values of less than 1.0 for both analytes, fully meeting the precision criteria as outlined in ICH guidelines. These findings confirm the reliability of the method for quality control of gel formulations containing berberine and curcumin.

4. CONCLUSION

This study successfully developed and validated an HPLC method for the simultaneous quantification of berberine and curcumin in a gel formulation. The method fully complies with the validation criteria outlined in the ICH guidelines, including specificity, linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). The validation results demonstrated high accuracy, acceptable recovery within pharmaceutical analytical standards, and good reproducibility, confirming the method's reliability for quality control of the gel formulation. This validated method provides a robust analytical tool that contributes to standardizing the quality control process and lays the groundwork for application to other pharmaceutical dosage forms.

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