RESEARCH PAPER

"QUANTIFICATION OF EUGENOL, CURCUMIN AND CINNAMALDEHYDE FOR HERBAL STANDARDIZATION OF DENTAL PRODUCT BY HPTLC METHOD"

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ABSTRACT

This study presents the development and validation of a simple, rapid, and economical High-Performance Thin-Layer Chromatography (HPTLC) densitometric method for the simultaneous determination of three important bioactive markers—Curcumin, Cinnamaldehyde, and Eugenol—in a herbal tooth powder formulation. Separation was achieved on pre-coated silica gel HPTLC plates using an optimized mobile phase consisting of dichloromethane, ethyl acetate, and formic acid (7.5:2.5:0.1, v/v/v). The optimized chromatographic conditions produced well-defined and distinct bands with (R_f) values of 0.25, 0.50, and 0.86 for Curcumin, Cinnamaldehyde, and Eugenol, respectively. Densitometric analysis was performed at 254 nm.

The analytical method was validated in accordance with ICH Q2(R2) guidelines. Validation results confirmed excellent linearity over the selected concentration ranges, adequate sensitivity, and high specificity without significant interference from the formulation matrix. Accuracy was demonstrated through recovery values ranging from 96.35% to 102.36%, while precision studies showed %RSD values below 2.0%, indicating good repeatability and intermediate precision. The validated method was successfully applied to the analysis of a commercial herbal tooth powder, confirming its suitability for routine quality control, standardization, and quantitative assessment of polyherbal dental formulations in pharmaceutical and herbal industries.

Keywords:HPTLC, , Curcumin, Cinnamaldehyde, Eugenol, Method Validation, Standardization.

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1. INTRODUCTION

Herbal dental products have gained substantial therapeutic prominence due to their natural, multi-targeted clinical benefits, including antimicrobial, anti-inflammatory, and analgesic properties. However, the inherent phytochemical complexity of these botanical formulations poses a significant challenge for ensuring quality, safety, and batch-to-batch uniformity. To overcome the tedious sample clean-up and high solvent consumption often associated with column liquid chromatography, High-Performance Thin-Layer Chromatography (HPTLC) has emerged as an ideal alternative. As a high-throughput planar analytical tool, HPTLC offers distinct advantages for complex matrix analysis, including minimal sample preparation, low solvent waste, and parallel processing, which allows multiple samples and standards to be analysed simultaneously under identical conditions.

Consequently, developing a validated HPTLC method is essential for the reliable quality control and simultaneous densitometric estimation of the formulation's three key bioactive marker compounds: Eugenol (4-allyl-2-methoxyphenol), a clove-derived phenolic local anaesthetic and analgesic used in dental cements; Curcumin [(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione], a turmeric extract celebrated in periodontics for its potent anti-inflammatory and plaque-reducing properties; and Cinnamaldehyde [(2E)-3-phenylprop-2-enal], a cinnamon constituent highly valued for its robust action against cariogenic oral biofilms and its role as a natural flavouring agent. By utilizing automated multi-wavelength scanning, this HPTLC approach ensures precise, cost-effective, and rapid standardization of these structurally diverse markers within complex oral healthcare matrices.

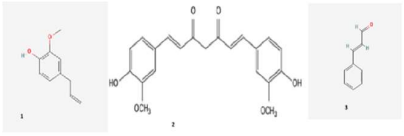

Figure 1 Structure of 1-Eugenol 2-curcumin 3-Cinnamaldehyde

2. MATERIAL AND METHOD

2.1 Chemicals

All API Eugenol, Curcumin and Cinnamaldehyde were obtained from Oxford Lab Fine Chem, Maharashtra, India. Acetonitrileand Methanol was purchased from Thermo fisher scientific India Pvt Ltd."Pre-coated silica gel 60 F_{254} HPTLC plates were procured from Merck.

2.2 Instrumentation

HPTLC analysis was performed using a CAMAG HPTLC system (Muttenz, Switzerland). Samples and standards were formulated and applied as bands using a CAMAG Linomat 4 automated applicator equipped with a 100 µL Hamilton syringe. Chromatographic separation was carried out on pre-coated silica gel 60 F_{254} HPTLC plates procured from Merck. Following plate development in a CAMAG twin-trough glass chamber, post-chromatographic evaluation and photodocumentation were executed using a Reprostar 3 system equipped with a digital camera. Quantitative densitometric scanning was performed utilizing a CAMAG TLC Scanner III operated in the absorbance mode. System command, data acquisition, and multi-wavelength processing were controlled via WinCATS software.

2.3 Chromatographic and scanning conditions

Chromatographic separation was performed in an ascending mode at room temperature using pre-coated Silica Gel G60 F_{254}aluminum sheets (10 * 10cm, layer thickness 0.2mm, Merck) as the stationary phase. The optimized mobile phase consisted of dichloromethane: ethyl acetate: formic acid (7.5:2.5: 0.1, v/v/v). Plates were developed to a migration distance of 80 mmin a twin-trough chamber following a 30-minute chamber saturation period, and subsequently derivatized using anisaldehyde sulfuric acid as the spraying agent. Samples were applied as 6 mm wide bands with an inter-band spacing of 6mm, a track distance of 12mm, and a spraying rate of 159 nL/sec. Densitometric scanning was executed at a speed of 20 mm/secusing a CAMAG TLC Scanner IV equipped with a deuterium lamp operated in absorption/fluorescence measurement mode at a detection wavelength of 254 nm (monochromatic bandwidth20nm). The slit dimensions were maintained at 10.00 *0.45 mm

2.4 Preparation of Stock Solution

Eugenol (EUG) Standard Stock Solution

An accurately weighed quantity of 100 mg of Eugenol reference standard was transferred into a 100 mL volumetric flask, dissolved in acetonitrile (ACN), and the volume was made up to the mark with the same solvent to obtain a master stock solution containing 1000 µg/mL of Eugenol.

Cinnamaldehyde (CIN) Standard Stock Solution

An accurately weighed quantity of 40 mg of Cinnamaldehyde reference standard was transferred into a 100 mL volumetric flask, dissolved in ACN, and diluted to volume with the same solvent to obtain a master stock solution containing 400 µg/mL of Cinnamaldehyde.

From this solution, 1.0 mL was accurately transferred into a 10 mL volumetric flask and diluted to volume with ACN to obtain a working standard stock solution containing 40 µg/mL of Cinnamaldehyde.

Curcumin (CUR) Standard Stock Solution:

An accurately weighed quantity of 20 mg of Curcumin reference standard was transferred into a 100 mL volumetric flask, dissolved in ACN, and diluted to volume with the same solvent to obtain a master stock solution containing 200 µg/mL of Curcumin.

From this solution, 1.0 mL was accurately transferred into a 10 mL volumetric flask and diluted to volume with ACN to obtain a working standard stock solution containing 20 µg/mL of Curcumin.

Preparation of standard stock solution of mixture

A combined standard mixture stock solution was prepared by accurately weighing 100 mg of Eugenol, 4 mg of Cinnamaldehyde, and 2 mg of Curcumin into a 100 mL volumetric flask. The contents were dissolved in a sufficient quantity of ACN, and the volume was made up to the mark with the same solvent. The resulting combined standard mixture stock solution contained: Eugenol: 1000 µg/MlCinnamaldehyde: 40 µg/mLCurcumin: 20 µg/mL.This combined standard mixture stock solution was used for the preparation of calibration standards and method validation studies.

2.5 Validation

The method was validated as per ICH Q2 (R2) guideline for parameter like linearity, precision, LOD, LOQ, accuracy, robustness and system suitability Parameters.

2.5.1 System Suitability Parameters

System suitability parameters were evaluated by analysing a mixed standard solution containing Curcumin (40 ng/band), Cinnamaldehyde (80 ng/band), and Eugenol (2000 ng/band) in triplicate (n = 3). Chromatographic performance was assessed by determining the retardation factor (Rf) and its percentage relative standard deviation (%RSD). Peak purity of each analyte was evaluated by comparing the UV absorption spectra recorded at three different positions of the chromatographic peak, namely the start (s), apex (m), and end (e). The spectral correlation coefficients, r(s,m) and r(m,e), were calculated to confirm peak homogeneity and purity.

2.5.2 Linearity

Linearity of the developed HPTLC method was evaluated by applying mixed standard solutions containing Curcumin (20–100 ng/band), Cinnamaldehyde (40–200 ng/band), and Eugenol (1000–5000 ng/band). Aliquots of 2.0–10.0 µL of the mixed standard solution (CUR: 10 µg/mL, CIN: 20 µg/mL, and EUG: 500 µg/mL) were applied as bands using a Hamilton syringe under a nitrogen stream. Calibration curves were constructed by plotting peak area against the corresponding concentration of each analyte, and linear regression analysis was performed.

2.5.3 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

2.5.4 Robustness

Following parameters were altered one by one for determination of robustness of the method and their effect was observed by standard preparation.

1. Mobile phase composition (± 0.8 mL), in optimized ratio.
2. Saturation time (±5 min), optimized saturation time 30min.
3. TLC Plates 10*10 cm & 20*20 cm.determinations for each alteration were carried out and %RSD was measured

2.5.5 LOD and LOQ

The LOD is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels and LOQ of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy.

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

2.5.6 Accuracy

Accuracy of the developed HPTLC method was evaluated by the standard addition technique. Pre-analysed blank matrix was spiked with known amounts of Curcumin (CUR), Cinnamaldehyde (CIN), and Eugenol (EUG) at three concentration levels corresponding to 50%, 100%, and 150% of the target concentration. The target concentration was 40 ng/spot for CUR, 80 ng/spot for CIN, and 2000 ng/spot for EUG. Accordingly, the spiked concentrations were 20, 40, and 60 ng/spot for CUR; 40, 80, and 120 ng/spot for CIN; and 1000, 2000, and 3000 ng/spot for EUG. Each level was analysed in triplicate, and the percentage recovery was calculated from the peak areas obtained to assess the accuracy of the method.

2.5.7 Standardization Process

For the standardization of the tooth powder formulation, an accurately weighed quantity of 1.0 g of the sample was transferred into a 100 mL stoppered conical flask and extracted by sonicating with 20 mL of HPLC-grade Acetonitrile for 25 minutes to ensure efficient recovery of the target active markers. The resulting mixture was filtered through a Whatman No. 41 filter paper, and the remaining residue on the filter paper was rinsed with an additional 5 mL of acetonitrile to capture any residual components. The collected filtrates were combined, transferred quantitatively into a 25 mL volumetric flask, and diluted to volume with acetonitrile to prepare the final tooth powder stock solution. From this stock solution, a volume of 4.0 µL was applied as a band using a Hamilton syringe under a continuous stream of nitrogen gas onto the stationary phase for subsequent chromatographic development and densitometric evaluation.

3. RESULTS AND DISCUSSION

3.1 Selection of Wavelength

An ideal wavelength is the one that gives good response of the drugs that are to be detected. For selection of wavelength, mixture of standard solution of Curcumin, Cinnamaldehyde, and Eugenol was scanned over the range of 800-200 nm wavelengths. From Mixture of all three drugs, Curcumin, Cinnamaldehyde, and Eugenol showed maximum absorbance at 254 nm in UV-visible mode.

3.2 Selection of Diluent Medium

The diluent medium is depending on common solubility of Curcumin, Cinnamaldehyde, and Eugenol. The standard sample of Curcumin (0.1 mg), Cinnamaldehyde (0.2mg), and Eugenol (5.0 mg) was taken in 1 mL volumetric flask of different solvents and rotated in test tube rotator. Acetonitrile was used as a diluent.

3.3 Selection of Stationary Phase

The resolution of Curcumin, Cinnamaldehyde, and Eugenol was achieved on TLC plates precoated with silica gel G60 F254 on an aluminium support (E. Merck). The size of the silica gel particle was 2 µm and thickness of sorbent layer was 0.2 mm. The plates were supplied in 20 * 20 cm size, which was cut in to appropriate sizes for method development (10 × 10).

3.4 Optimization of Mobile Phase

Chromatographic separation was performed on silica gel 60 F_{254}precoated TLC plates using a mobile phase consisting of dichloromethane, ethyl acetate, and formic acid (7.5:2.5:0.1, v/v/v). The optimized mobile phase provided sharp, symmetrical, and well-resolved bands for all analytes by minimizing ionization effects. Under the optimized conditions, curcumin, cinnamaldehyde, and eugenol were satisfactorily separated with distinct Rfvalues of 0.25, 0.50, and 0.86 respectively.

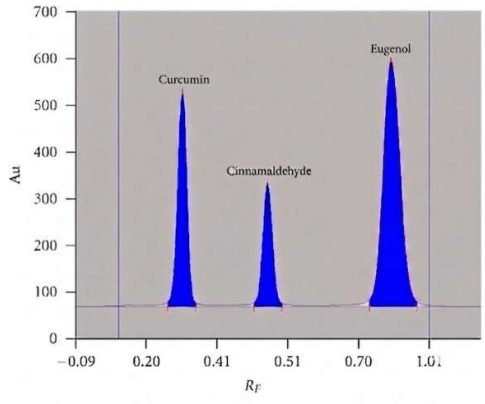

Figure 2Densitogram of CUR, CIN and EUG.

Photograph of Developed HPTLC plate showing resolved spots of Curcumin, Cinnamaldehyde, and Eugenol.

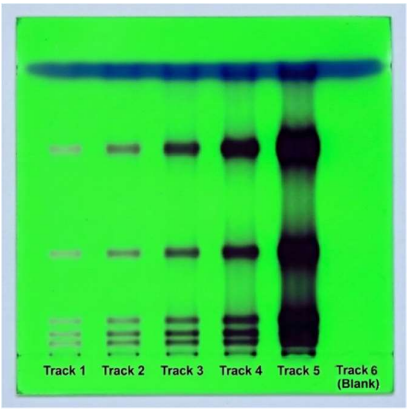

Figure 3 Photograph of developed HPTLC plate

3.5 VALIDATION

3.5.1 System Suitability Parameters Result

Table 1 System Suitability Parameters Result

Drug	Rf Value (Mean Area ± S.D)	%RSD	Peak Purity
Curcumin	0.25 ± 0.002	0.9	0.994
Cinnamaldehyde	0.50 ± 0.002	0.41	0.996
Eugenol	0.86 ± 0.003	0.41	0.997

System suitability studies showed well-resolved and reproducible chromatographic peaks for curcumin, cinnamaldehyde, and eugenol with Rf values of 0.25 ± 0.002, 0.50 ± 0.002, and 0.86 ± 0.003, respectively. The low %RSD values (<1%) and high peak purity values (>0.99) confirmed the precision, specificity, and reliability of the developed HPTLC method.

3.5.2 Linearity

Linearity of the developed method was evaluated by constructing calibration curves over concentration ranges of 20–100 ng/spot for curcumin, 40–200 ng/spot for cinnamaldehyde, and 1000–5000 ng/spot for eugenol. A good linear relationship between peak area and concentration was observed for all analytes, with correlation coefficients (R^2) of 0.9986, 0.9963, and 0.9980 for curcumin, cinnamaldehyde, and eugenol, respectively, indicating excellent linearity within the studied ranges.

Table 2 Concentration and Area for linearity

Sr. no	EUG		CIN		CUR	
	Conce	Ar	Conce	Ar	Conce	Ar

	ntration	ea	ntration	ea	ntration	ea
1	1000	1031.25	40	746.12	20	1042.15
2	2000	1964.8	80	1458.3	40	1955.6
3	3000	2779.1	120	2182.44	60	2785.44
4	4000	3695.5	160	2735.1	80	3712.2
5	5000	4538.9	200	3312.85	100	4522.11
Regression Equation	y = 0.9x + 84.81		y = 16.611x + 78.04		y = 44.92x + 89.78	
R^2	0.998		0.9963		0.9986	

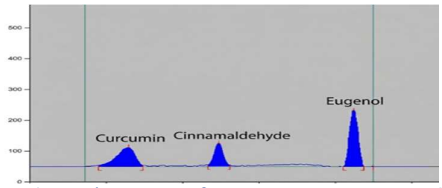

Figure 4Densitogram of CUR+CIN+EUG at 254 nm (20+ 40+ 1000 ng/spot)

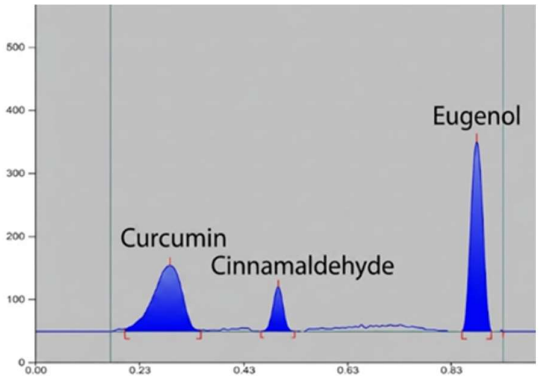

Figure 5Densitogram of CUR+CIN+EUG at 254 nm (40+ 80+ 2000 ng/spot)

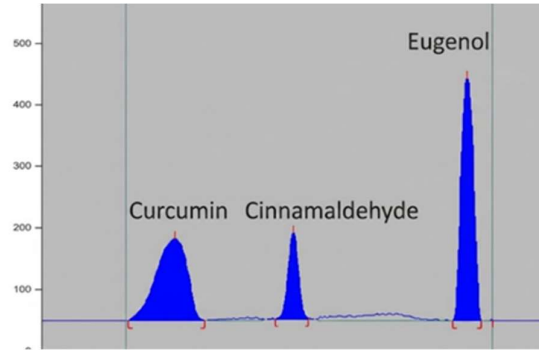

Figure 6Densitogram of CUR+CIN+EUG at 254 nm (60+ 120+ 3000 ng/spot)

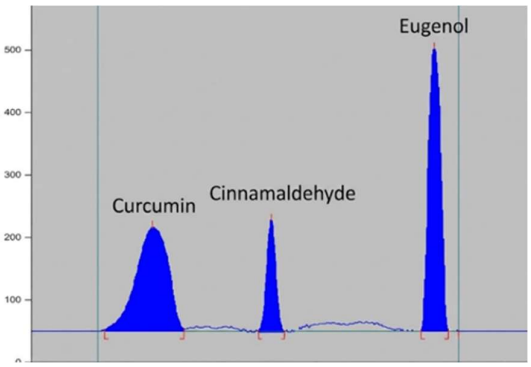

Figure 7Densitogram of CUR+CIN+EUG at 254 nm (80+ 160+ 4000 ng/spot)

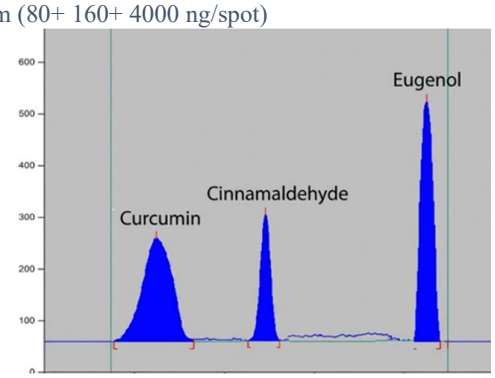

Figure 8Densitogram of CUR+CIN+EUG at 254 nm (100+ 200+ 5000 ng/spot)

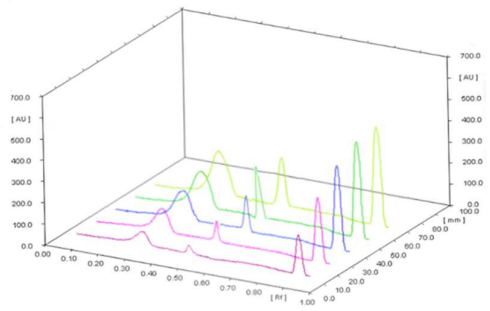

Figure 9Overlain 3D graph of CUR+CIN+EUG

3.5.3 Precision

3.5.3.1 Repeatability

Table 3 Result Table for Repeatability

Sr No.	CUR (60 ng/spot)	**CIN** (120 ng/spot)	EUG (3000 ng/spot)
1.	2776.9	2181.9	3263.2
2.	2770.2	2182.3	3261.5
3.	2775.3	2180.1	3265.2
4.	2778.4	2179.6	3264.9
5.	2771.2	2175.9	3255.9
6.	2769.4	2170.4	3260.4
Mean	2773.5	2178.3	3261.8
SD	3.78	4.51	3.46
%RSD	0.13	0.20	0.10

Repeatability (instrument precision) of the developed method was assessed by analyzing six replicate measurements (n = 6) at selected concentration levels of 60 ng/spot for curcumin, 120 ng/spot for cinnamaldehyde, and 3000 ng/spot for eugenol. The %RSD values obtained were 0.13%, 0.20%, and 0.10%, respectively. Since all %RSD values were well below the acceptable limit of 2.0%, the method demonstrated excellent precision, repeatability, and reliability for quantitative analysis.

3.5.3.2 Intraday and Interday Precision

The precision of the developed method was verified by determining intraday and interday variations at three different concentration levels for each analyte. For intraday precision, the %RSD values ranged from 0.81% to 1.20% for curcumin, 0.79% to 1.14% for cinnamaldehyde, and 0.75% to 0.96% for eugenol. For interday precision, the %RSD values were found to be between 1.08%–1.43% for curcumin, 1.04%–1.51% for cinnamaldehyde, and 0.84%–1.35% for eugenol. All calculated %RSD values were well within the acceptable regulatory limit of less than 2.0%, confirming the excellent intermediate precision, repeatability, and reproducibility of the proposed method.

3.5.3.3 Robustness

Following parameters were altered one by one for determination of robustness of the method and their effect was observed by standard preparation.

1. Mobile phase composition (± 0.8 mL), in optimized ratio.
2. Saturation time (±5 min), optimized saturation time 30min.
3. TLC Plates 10*10 cm & 20*20 cm.

determinations for each alteration was carried out and %RSD was measured.

Table 4Result table for robustness

Parameter	**Level Change**	**Effect on peak area**					
		CUR (60ng/spot)		**CIN (120ng/spot)**		**EUG(3000ng/spot)**	
		Mean ± SD	**%RSD**	**Mean ± SD**	**%RSD**	**Mean ± SD**	**%RSD**
Mobile Phase: Dichloromethane : Ethyl acetate: formic acid (7.5:2.5:0.1,V/V/V)	7:2:0.5,V/V/V	2739.8 ± 35.90	1.31	2362.3 ± 28.04	1.18	3434.9 ± 56.36	1.64
	7:3:0.1,V/V/V	2730.53 ± 30.00	1.09	2354.03 ± 46.56	1.97	3444.9 ± 38.48	1.11
	8:2.5:0.5,V/V/V	2720.7 ± 26.53	0.97	2340.6 ± 35.81	1.53	3435.9 ± 31.67	0.92
Saturation time	25 min (-5 min)	2840.7 ± 34.84	1.22	2379.4 ± 25.27	1.06	3445.6 ± 39.52	1.14
	35 min (+5 min)	2868.7 ± 41.96	1.46	2417.9 ± 28.28	1.16	3445.7 ± 39.33	1.14
Size of TLC Plate	10*10 cm	2743.8 ± 30.60	1.11	2368.7 ± 33.32	1.40	3450.7 ± 37.89	1.09
	20*20 cm	2923.8 ± 35.49	1.21	2433.6 ± 25.87	1.06	3283.9 ± 28.28	0.86

3.5.4 LOD and LOQ

The sensitivity of the developed HPTLC method was assessed by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ) for each analyte. The LOD values were found to be 0.15 µg/spot for curcumin, 0.22 µg/spot for cinnamaldehyde, and 0.50 µg/spot for eugenol, while the corresponding LOQ values were 0.47 µg/spot, 0.67 µg/spot, and 1.75 µg/spot, respectively. The low LOD and LOQ values obtained indicate the high sensitivity of the developed method and its suitability for the simultaneous quantification of curcumin, cinnamaldehyde, and eugenol, even at low concentration levels.

3.5.5 Accuracy

The accuracy of the developed HPTLC method was evaluated by recovery studies using the standard addition technique at three concentration levels (50%, 100%, and 150%). The mean recovery values ranged from 96.35% to 100.24% for eugenol, 96.35% to 101.25% for cinnamaldehyde, and 97.62% to 102.36% for curcumin. The recoveries obtained were within the acceptable limits recommended by ICH guidelines, demonstrating the accuracy of the method and indicating the absence of interference from formulation excipients during the simultaneous quantification of the three analytes.

Table 5 Result table for Accuracyof EUG

Level of spiking	Amount of std drug (ng/spot)	Amount of std drug recovered (ng/spot)	%Recovery
Un-spike	-	-	-
50%	1500	1482.6	98.84
	1500	1473.3	98.22
	1500	1489.65	99.31
100%	3000	2930.4	97.68
	3000	2890.5	96.35
	3000	3007.2	100.24
150%	4500	4340.25	96.45
	4500	4425.75	98.35
	4500	4471.2	99.36

Table 6 - Result table for Accuracy of CIN

Level of spiking	Amount of std drug (ng/spot)	Amount of std drug recovered (ng/spot)	%Recovery
Un-spike	-	-	-
50%	60	59.088	98.48
	60	58.77	97.95
	60	59.202	98.67
100%	120	119.784	99.82
	120	118.464	98.72
	120	121.5	101.25
150%	180	173.43	96.35
	180	176.004	97.78
	180	177.696	98.72

Table 7Result table for Accuracy of CUR

Level of spiking	Amount of std drug (ng/spot)	Amount of std drug recovered (ng/spot)	%Recovery
Un-spike	-	-	-
50%	30	30.489	101.63
	30	29.358	97.86
	30	29.508	98.36
100%	60	59.874	99.79
	60	58.572	97.62
	60	58.95	98.25
150%	90	91.134	101.26
	90	92.124	102.36
	90	89.856	99.84

3.5.6 Standardization

Table 8 Result table for standardization

Marker Compound	**Chromatogram Area (y)**	**Concentration per injection (ppm) (x)**	**Total Content (mg/100 g)**
Eugenol	87.78	3.30	66.0
Cinnamaldehyde	117.91	2.40	48.0
Curcumin	103.26	0.30	6.0

The developed HPTLC method was successfully applied for the simultaneous quantification and standardization of eugenol, cinnamaldehyde, and curcumin in the formulation. Based on the measured peak areas, the concentrations were determined to be 3.30 ppm for eugenol, 2.40 ppm

for cinnamaldehyde, and 0.30 ppm for curcumin. The corresponding contents in the formulation were calculated as 66.0 mg/100 g, 48.0 mg/100 g, and 6.0 mg/100 g for eugenol, cinnamaldehyde, and curcumin, respectively. These findings demonstrate the suitability, accuracy, and reliability of the developed method for routine quality control and standardization of formulations containing these bioactive marker compounds.

4. CONCLUSION

From this study, it can be concluded that a simple, rapid, accurate, and precise HPTLC method was successfully developed for the simultaneous estimation of curcumin, cinnamaldehyde, and eugenol. The statistical parameters, system suitability, and optimization results confirm the high efficiency, sensitivity, and reproducibility of the method. The proposed method was fully validated in accordance with the ICH Q2(R2) guidelines, with all evaluated parameters—including linearity, accuracy, precision, LOD, and LOQ—falling well within the established acceptance criteria. Consequently, this thin-layer chromatographic densitometric method proves to be highly reliable and well-suited for the routine quality control, standardization, and quantitative analysis of these three active components in pharmaceutical and herbal formulations.

5. ACKNOWLEDGMENTS

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6. REFERENCES

1. Kulkarni, Kiran, Gorakhnath Jagtap, and Shrikant Magdum. "A comprehensive review on herbal drug standardization." Am. J. PharmTech Res 9 (2019): 97-122.
2. Yadav, N. P., and V. K. Dixit. "Recent approaches in herbal drug standardization." Int J IntegrBiol 2.3 (2008): 195-203.
3. Choudhary, Neeraj, and Bhupinder Singh Sekhon. "An overview of advances in the standardization of herbal drugs." Journal of Pharmaceutical Education and Research 2.2 (2011): 55-70.
4. Gurdeep R. Chatwal,Sham K. Anand 'Instrumental Method of Chemical Analysis ' 5th Edition, Himalya Publication House 2014 pp 2.666-2.587, 2.674-2.700, 2.599-2.616.
5. Francis Rouessac,AnnickRouessac "Chemical Analysis, Modern Instrumentation Methods and Techniques" 2nd Edition, John Wiley & Sons Ltd 1994 pp 31-58,63-89,127-133
6. Foudah AI, Shakeel F, Alqarni MH, Ross SA, Salkini MA, Alam P. Simultaneous estimation of cinnamaldehyde and eugenol in essential oils and traditional and ultrasound-assisted extracts of different species of cinnamon using a sustainable/green HPTLC technique. Molecules. 2021 Apr 3;26(7):2054.https://doi.org/10.3390/molecules 26072054
7. Al Tamim A, Alharbi H, Hawsawi E, Aldosari Z, Alqahtani A. Optimized HPLC-DAD Method for Quantifying Coumarin and Cinnamaldehyde in Local Cinnamon Sample. Applied Food Research. 2025 Sep 10:101336.https://doi.org/10.1016/j.afres.2025. 101336.
8. Shaikh SU, Jain VA. Development and validation of a RP-HPLC method for the simultaneous determination of curcumin, piperine and camphor in an ayurvedic formulation. Int J Pharm Pharm Sci. 2018;10(4):1-0. DOI: http://dx.doi.org/10.22159/ijpps.2018v10i4.25 216
9. Thakur NS, Gupta D, Tiwari AD, Dubey PK. Simultaneous Estimation of Cinnamaldehyde, Cinnamic Acid, and Eugenol in Herbal Formulation by Ultraviolet Spectrophotometry.
10. Thyagaraj VD, Koshy R, Kachroo M, Mayachari AS, Sawant LP, Balasubramanium M. A validated RP-HPLC-UV/DAD method for simultaneous quantitative determination of rosmarinic acid and eugenol in Ocimum sanctum L. Pharmaceutical Methods. 2013 May 1;4(1):1-5.https://doi.org/10.1016/j.phme.2013.08.003.
11. Patole VC, Chaudhari SP. Method Development and Validation for the Simultaneous Estimation of Thymol and Eugenol by Using RP-HPLC in Pure and in Emulgel Formulation. Indian Drugs. 2021 Jul;58(7):59-65.doi. 10.53879/id.58.07.11895
12. Gopaiah KV, Babu GV, Kiran KR, Raju KD, Kishor M, Babu P, Sravani P, Fareena SD, Prasad VD. Exploration And Standardization Of Bioactive Phytopharmaceuticals Derived From Indian Medicinal Plants With Antidiabetic And Anti-Inflammatory Properties: A Comprehensive Review.http://doi.org/10.53555/jab.v11si9.
13. Kadam PV, Yadav KN, Bhingare CL, Patil MJ. Standardization and quantification of curcumin from Curcuma longa extract using UV visible spectroscopy and HPLC. Journal of

Pharmacognosy and Phytochemistry. 2018;7(5):1913-8.
14. Singh A, Avupati VR. Development and validation of UV-spectrophotometric method for the estimation of curcumin in standardised polyherbal formulations. Journal of Young Pharmacists. 2017 Oct 1;9(4). doi. 10.5530/jyp.2017.9.96
15. Peram MR, Jalalpure SS, Joshi SA, Palkar MB, Diwan PV. Single robust RP-HPLC analytical method for quantification of curcuminoids in commercial turmeric products, Ayurvedic medicines, and nanovesicular systems. Journal of Liquid Chromatography & Related Technologies. 2017 Jun 15;40(10):487-98.https://doi.org/10.1080/10826076.2017.1329742

7. LIST OF FIGURES AND TABLES