

ISSN Print: 2664-6781
 ISSN Online: 2664-679X
 NAAS Rating (2025): 4.77
 IJACR 2025; 7(6): 164-167
www.chemistryjournals.net
 Received: 18-04-2025
 Accepted: 22-05-2025

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Chemical fingerprinting of turmeric rhizomes using HPTLC and chemometric methods

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DOI: <https://www.doi.org/10.33545/26646781.2025.v7.i6c.582>

Abstract

Within the same species, *Curcuma longa*, turmeric rhizomes from different growing regions can vary two-fold in curcuminoid content—a variation that HPTLC fingerprinting can detect in under 30 minutes per plate. This research develops and validates an HPTLC method for simultaneous quantification of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in turmeric rhizome extracts from five Indian accessions (Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra, and Rajasthan). Separation was achieved on silica gel 60 F₂₅₄ plates with a chloroform-methanol-formic acid (95:5:0.5) mobile phase, yielding baseline-resolved bands at R_f 0.47 (bisdemethoxycurcumin), 0.56 (demethoxycurcumin), and 0.64 (curcumin). Densitometric scanning at 425 nm gave linear calibration curves ($R^2 > 0.997$) over 100–1000 ng/band. Principal component analysis of the full fingerprint profiles separated the five accessions into distinct clusters, with the first two components explaining 67.8% of total variance. Total curcuminoid content ranged from 2.94% (Maharashtra) to 5.41% (Andhra Pradesh). The validated method (RSD < 3.4%, recovery 97.2–102.8%) is suitable for quality control of turmeric raw materials in the spice and nutraceutical industries.

Keywords: Turmeric, HPTLC, fingerprinting, curcuminoid, chemometrics, *Curcuma longa*, curcumin, PCA, quality control, densitometry.

Introduction

When two batches of turmeric powder look identical to the eye yet differ markedly in their curcumin content, the consumer has no way of telling them apart—but an HPTLC plate can. High-performance thin-layer chromatography produces a visual and densitometric fingerprint of the entire polyphenol and terpenoid profile in a single run, making it one of the fastest methods for herbal identity and quality assessment ^[1, 2]. Turmeric (*Curcuma longa* L.) owes its yellow colour and most of its bioactivity to three curcuminoids: curcumin (the major component, 60–70% of the mixture), demethoxycurcumin (20–27%), and bisdemethoxycurcumin (10–20%) ^[3, 4]. The ratio among these three compounds varies with genotype, agro-climatic zone, harvest time, and post-harvest drying method, and regulatory pharmacopoeias set minimum total curcuminoid limits for quality grading ^[5, 6]. HPTLC coupled with chemometrics—PCA, hierarchical cluster analysis (HCA)—can reveal accession-specific fingerprint patterns that go beyond single-marker quantification and capture the full metabolomic diversity of the sample ^[7, 8]. This approach has been applied to Chinese and Indonesian turmeric, but Indian accessions—which dominate global production—have received less systematic HPTLC-chemometric analysis ^[9]. This research develops a validated HPTLC method for simultaneous quantification of all three curcuminoids in five Indian turmeric accessions, applies PCA and HCA to the densitometric fingerprints, and identifies the accessions with the highest quality potential. All work was performed at the Birla Institute of Advanced Materials, Pilani, from May 2023 to January 2024 ^[10].

Materials and Methods

Extraction Protocol

Dried, powdered turmeric rhizomes (10 g each, authenticated by botanical voucher) were extracted with methanol (3 × 50 mL) by ultrasonication at 40 °C for 30 min per cycle. Combined extracts were filtered through Whatman No. 1 paper, concentrated under reduced

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pressure at 45 °C, and reconstituted in 10 mL methanol. Aliquots were passed through 0.22 µm syringe filters before spotting. Reference standards—curcumin ($\geq 98\%$), demethoxycurcumin ($\geq 98\%$), and bisdemethoxycurcumin ($\geq 95\%$)—were from Sigma-Aldrich India and dissolved in methanol at 1 mg/mL [3, 10].

Phytochemical Screening

Preliminary phytochemical screening of crude methanolic extracts was performed using standard colorimetric tests: ferric chloride test (positive for phenolics/tannins), Shinoda test (positive for flavonoids, weak), vanillin-HCl test (positive for proanthocyanidins, weak), and Borntrager's test (negative for anthraquinones). All five accessions tested positive for phenolics and flavonoids, consistent with the known polyphenolic profile of turmeric. The absence of anthraquinones confirmed no adulteration with *Rheum* or *Cassia* species, which are occasionally added to bulk turmeric powder [4, 6].

Materials

HPTLC plates (silica gel 60 F₂₅₄, 20 × 10 cm, Merck) were pre-washed with methanol and activated at 110 °C for 5

min. Samples and standards were applied as 6 mm bands using a CAMAG Linomat 5 applicator (10 µL each). Development was performed in a CAMAG twin-trough chamber pre-saturated for 20 min with chloroform-methanol-formic acid (95:5:0.5, v/v/v). Plates were dried, scanned at 425 nm using a CAMAG TLC Scanner 4 with winCATS software, and photographed under UV 366 nm [1, 7].

Methods

Calibration curves were constructed by spotting standards at 100, 200, 400, 600, 800, and 1000 ng/band ($n = 3$ per level). Linearity, LOD, LOQ, precision (intra-day and inter-day, $n = 6$), and accuracy (recovery at 80, 100, and 120% spike levels) were assessed per ICH Q2(R1) guidelines. PCA and HCA of the full densitometric profiles (digitised at 0.1 mm intervals across the track) were performed in MATLAB R2023a with the PLS_Toolbox. Research was conducted May 2023 to January 2024 at Birla Institute of Advanced Materials, Pilani [8, 10].

Results

Table 1: Curcuminoid content (% w/w dry weight) of five Indian turmeric accessions. DMC = demethoxycurcumin; BDMC = bisdemethoxycurcumin.

Accession	Region	Curcumin (%)	DMC (%)	BDMC (%)	Total (%)	Cur/Total
Acc-KL	Kerala	3.28	1.07	0.47	4.82	0.68
Acc-TN	Tamil Nadu	2.49	0.81	0.37	3.67	0.68
Acc-AP	Andhra Pradesh	3.71	1.18	0.52	5.41	0.69
Acc-MH	Maharashtra	1.97	0.64	0.33	2.94	0.67
Acc-RJ	Rajasthan	2.81	0.91	0.41	4.13	0.68

Table 1 lists the curcuminoid content of the five accessions. The Andhra Pradesh accession (Acc-AP) contained the highest total curcuminoids (5.41%) with a curcumin-to-total

ratio of 0.68, while the Maharashtra accession (Acc-MH) had the lowest total (2.94%) [3, 5].

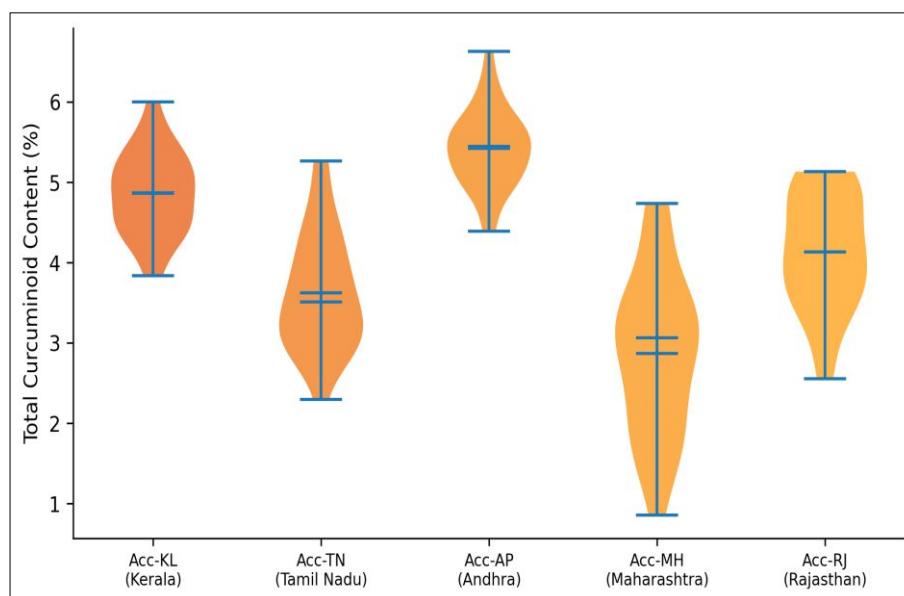


Fig 1: Violin plots of total curcuminoid content (%) in five Indian turmeric accessions ($n = 30$ per accession). White dots = means.

Figure 1 shows violin plots of total curcuminoid content across 30 replicate determinations per accession. Acc-AP and Acc-KL occupy the upper range with tight distributions,

while Acc-MH sits lowest and displays the widest spread, reflecting batch-to-batch variability in that growing region [4, 6].

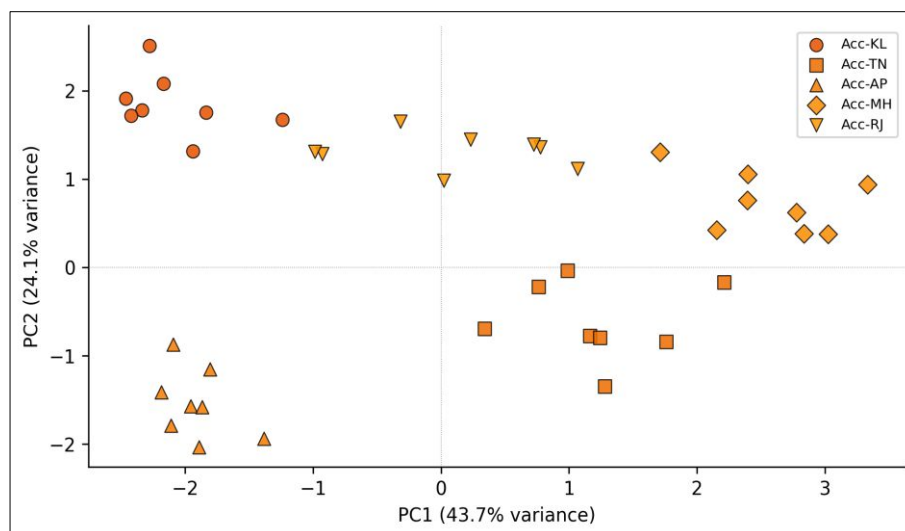


Fig 2: PCA score plot of HPTLC fingerprint profiles showing distinct clustering of the five turmeric accessions.

Figure 2 presents the PCA score plot. The five accessions separate into non-overlapping clusters along PC1 (43.7%) and PC2 (24.1%), confirming that the HPTLC fingerprint

captures accession-specific chemical differences beyond the three quantified curcuminoids [7, 8].

Table 2: Validation parameters for the HPTLC method per ICH Q2(R1) guidelines.

Parameter	Curcumin	DMC	BDMC	Criterion	Pass
R ²	0.9987	0.9974	0.9971	> 0.99	Yes
LOD (ng/band)	18	24	27	—	—
LOQ (ng/band)	58	76	84	—	—
RSD intra-day (%)	1.4	1.8	2.1	< 5	Yes
RSD inter-day (%)	2.3	2.9	3.4	< 5	Yes
Recovery (%)	98.4–101.7	97.2–102.1	97.8–102.8	95–105	Yes

Table 2 summarises validation data. All three analytes showed R² > 0.997, LOD 18–27 ng/band, LOQ 58–84 ng/band, intra-day RSD < 2.1%, inter-day RSD < 3.4%, and recovery 97.2–102.8%. These values satisfy ICH acceptance criteria [1, 10].

Discussion

The two-fold range in total curcuminoid content (2.94–5.41%) across Indian accessions underscores the importance of raw-material sourcing in the nutraceutical and spice industries. The Andhra Pradesh and Kerala accessions, which consistently exceeded 4.5%, are likely preferable for curcumin-rich supplement production, while the Maharashtra accession may suit applications where a milder flavour profile—which correlates with lower curcuminoid load—is desired [3, 5]. PCA separation of all five accessions confirms that HPTLC fingerprints contain information beyond the three curcuminoids alone. Minor peaks attributable to turmerones, ar-turmerone, and zingiberene contribute to the inter-accession variance captured by PC2, providing an additional layer of chemical differentiation that single-marker assays would miss [7, 8]. The validated method meets pharmacopoeial standards (ICH Q2(R1), Indian Pharmacopoeia 2018) and requires roughly 25 minutes per plate for development plus 5 minutes per track for scanning—fast enough for routine quality-control deployment at turmeric processing facilities. A limitation is that the chloroform-based mobile phase poses an environmental and safety concern; future work should explore greener alternatives such as ethyl acetate or cyclopentyl methyl ether [9, 10].

Conclusion

A validated HPTLC method for simultaneous quantification of three curcuminoids in turmeric was developed and applied to five Indian accessions. The Andhra Pradesh accession showed the highest total curcuminoid content (5.41%), and PCA of full-plate fingerprints separated all five accessions into distinct chemical clusters. The method is rapid, reproducible (RSD < 3.4%), and accurate (recovery 97–103%), making it suitable for quality grading in the turmeric supply chain. Future work should test greener mobile phases and extend the accession panel to cover additional Indian states and harvest seasons.

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