Simultaneous Identification and Quantification of Three Bioactive Markers in Ayurvedic Polyherbal Formulation Lekhniya Mahakashaya Using HPTLC, with Comprehensive Phytochemical Characterization by HRLC-MS/MS and FTIR

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Title

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ABSTRACT

Lekhniya Mahakashaya (LMK) is a classical polyherbal Ayurvedic formulation described in the Ayurvedic texts for the management of obesity (*Sthaulya*) and metabolic disorders. Traditionally, LMK is indicated for reducing excessive adiposity, correcting impaired metabolism, and restoring metabolic balance through its *Lekhaniya* (scraping) and *Medohara* (anti-obesity) properties. Owing to its therapeutic importance, LMK has gained considerable attention for its potential role in the management of obesity-associated metabolic disturbances. However, despite its widespread traditional use, comprehensive phytochemical characterization and marker-based standardization of the formulation remain limited.

To ensure the quality, consistency, and reproducibility of this important Ayurvedic formulation, the present study employed a comprehensive analytical strategy involving High-Resolution Liquid Chromatography–Tandem Mass Spectrometry Orbitrap (HRLC–MS/MS Orbitrap), High-Performance Thin-Layer Chromatography (HPTLC), and Fourier Transform Infrared (FTIR) spectroscopy. The study focused on the simultaneous identification and quantification of three major bioactive marker compounds-β-asarone, berberine, and curcumin-present in LMK and associated with important medicinal plant ingredients including *Vacha* (*Acorus calamus* Linn.) and other phytoconstituent-rich herbal components of the formulation.

Comprehensive metabolomic profiling using HRLC–MS/MS Orbitrap analysis resulted in the identification of 2,034 metabolites, including 1,712 compounds in positive ionization mode and 322 compounds in negative ionization mode. HPTLC fingerprinting demonstrated 7–8 distinct and reproducible chromatographic peaks within the Rf range of 0.12–0.89, confirming the complex polyherbal nature of LMK. Effective chromatographic separation of β-asarone was achieved using the optimized mobile phase toluene:ethyl acetate (8:3 v/v), yielding a distinct peak at an Rf value of approximately 0.57. Berberine and curcumin were similarly resolved under optimized chromatographic conditions with characteristic Rf values and spectral profiles corresponding to their respective standards.

Quantitative analysis revealed the presence of β-asarone (25.89 µg/100 mg), berberine (0.24% w/w), and curcumin (0.31% w/w) in the methanolic extract of LMK. FTIR spectral analysis

further demonstrated 19 major absorption peaks within the range of 3278–468 cm^{-1}, indicating the presence of hydroxyl, aromatic, aliphatic, and heteroatom-containing functional groups, thereby supporting the chemically diverse phytoconstituent profile of the formulation.

The present study demonstrates that the developed analytical approach is a reliable and reproducible tool for the simultaneous identification and quantification of major bioactive markers in LMK. The integrated HPTLC, HRLC–MS/MS, and FTIR profiling provides a robust framework for phytochemical standardization, quality control, and batch-to-batch consistency assessment of LMK and related Ayurvedic formulations. Furthermore, the identified marker compounds provide a plausible phytochemical basis for the therapeutic relevance of LMK in obesity and metabolic disorders.

Keywords: Analytical method, bioactive compound,β-asarone, berberine, curcumin, HPTLC, FTIR, polyherbal formulations, simultaneous estimation

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Abbreviation	Full form
LMK	Lekhniya Mahakashaya
HRLC–MS/MS	High-Resolution Liquid Chromatography–Tandem Mass Spectrometry
HPTLC	High-Performance Thin-Layer Chromatography
FTIR	Fourier Transform Infrared Spectroscopy
AU	Absorbance Unit
Rf	Retardation Factor
w/w	Weight by Weight
µg	Microgram
mg	Milligram
nm	Nanometer
cm^{-1}	Reciprocal Centimeter
MS	Mass Spectrometry
LC	Liquid Chromatography
Orbitrap	Orbitrap Mass Analyzer
UV	Ultraviolet
TLC	Thin-Layer Chromatography

Background:

Lekhniya Mahakashaya (LMK) is a classical polyherbal Ayurvedic formulation introduced by *Acharya Charaka* in the *Charaka Samhita*. It is distinguished for its scraping or fat-reducing (*Lekhana*), Kapha-alleviating (*Kaphahara*), and lipid-lowering (*Medohara*) effects. Traditionally indicated for conditions such as Obesity (*Sthoulya*), metabolic disorders analogous to type 2 diabetes mellitus (*Prameha*), and dyslipidemia, in the contemporary clinical context, it is increasingly relevant to metabolic syndrome. The formulation comprises

ten medicinal plants, each contributing unique phytoconstituents that synergise to produce therapeutic effects.

Musta (Cyperus rotundus L.) has the highest phenolic content, the most vigorous antioxidant activity (IC50: 448.63 µg/mL for FRAP; 418.74 µg/mL for DPPH), and notable α-glucosidase inhibition (IC50: 383.75 µg/mL). These have been reported to exhibit lipid-lowering, antioxidant, and anti-obesity potentials[1]. *Kushtha (Saussurea lappa C.B. Clarke*) provides costunolide and dehydrocostus lactone, compounds with hepatoprotective, insulin-sensitising, and anti-adipogenic effects[2]. *Haridra (Curcuma longa L.) and* Curcuma longa extract(FCE50) have demonstrated anti-obesity effects in high-fat diet-induced rats by reducing adiposity and modulating lipid metabolism via AMPK activation[3]. *Daruharidra (Berberis aristata D.C.)* contains the isoquinoline alkaloid berberine, which activates AMPK pathways and effects. *Vacha (Acorus calamus L.)* contains β- and α-asarone, as well as acorin, compounds noted for their neuroactive, metabolic-stimulating, and digestive-enhancing properties—Ativisha *(Aconitum heterophyllum Wall*. ex-Royle*)*. Methanol extract of *Aconitum heterophyllum* reduces serum cholesterol and triglycerides in diet-induced obese rats by modulating HMG-CoA reductase and LCAT activity and enhancing faecal fat excretion[4]. *Katurohini (Picrorhiza kurroa Royle ex Benth.)* is rich in picroside I and II as well as apocynin, known for their hepatoprotective, anti-inflammatory, and insulin-sensitizing actions. *Chitraka (Plumbago zeylanica L.)* has shown significant antihyperlipidemic activity in experimental models. Its aqueous root extracts reduce serum cholesterol and triglycerides, inhibit hepatic HMG-CoA reductase, enhance faecal cholesterol excretion, and exhibit antioxidant effects.[5] *Chirabilva (Holoptelea integrifolia (Roxb.)* Holoptelea integrifolia reduces serum lipids and body weight in obese rats by inhibiting hepatic HMG-CoA reductase, enhancing LCAT activity, and increasing faecal lipid excretion. LC-MS/MS identified 3-(7-ethoxy-4-methyl-2-oxo-2H-chromen-3-yl)propanoate (C1) as a potential bioactive compound.[6] *Haimavati (Iris versicolor)* yields flavonoids such as irigenin and iridin, which act as lipid metabolism and free radical scavengers. Despite this rich phytochemical base and use, the formulation's complete chemical fingerprint and mechanistic basis remain underexplored in modern scientific terms (Table 1). The present study addresses this gap through high-resolution chemical profiling of a hydroalcoholic Soxhlet extract of LM using Orbitrap-based HRLC–MS/MS, an alcoholic extract of LM for HPTLC, a LM powder sample for HPTLC, and a LM powder sample for FTIR.

The HRLC–MS/MS Orbitrap dataset was analysed using multiple spectral databases for the identification of both polar and non-polar phytoconstituents in positive and negative electrospray ionisation modes (ESI+ and ESI−). A total of 1,712 metabolites were identified in positive ionisation mode and 322 metabolites in negative ionisation mode through mzCloud-assisted spectral matching and confidence-based annotation algorithms. Phenolic compounds, including gallic acid and related acidic phytoconstituents, were predominantly detected in the negative ionisation mode, whereas alkaloids, curcuminoids, and other lipid-soluble constituents such as berberine and curcumin were more prominent in the positive ionisation mode.

The negative-ion mode primarily revealed polar and acidic metabolites, including phenolic acids and flavonoids **(Table 2),** characterized by hydroxyl and carboxyl functional groups associated with antioxidant potential and high polarity. In contrast, the positive-ion mode highlighted relatively non-polar to moderately basic compounds such as alkaloids, curcuminoids, and terpenoid derivatives **(Table 3).** These phytoconstituents, possessing aromatic, methoxy, and nitrogen-containing functional groups, are reported to participate in metabolic regulation through mechanisms including AMP-activated protein kinase (AMPK) activation, insulin sensitization, and modulation of inflammatory signalling pathways. Such phytochemical attributes correlate well with contemporary therapeutic approaches targeting obesity and metabolic syndrome.

The HPTLC fingerprint profile of *Lekhniya Mahakashaya* (LMK) was generated using methanolic extracts (2–10 µL) developed in the solvent system toluene:ethyl acetate:acetic acid (8:2:0.1 v/v/v). Chromatographic scanning at 254 nm, 366 nm, and after derivatization at 540 nm revealed 7–8-well-resolved and reproducible peaks within the Rf range of 0.12-0.89, demonstrating the polyherbal complexity and consistency of the formulation across different applied concentrations (Figure 2).

The present HPTLC analysis also enabled the identification and quantification of β-asarone in LMK, a classical Ayurvedic formulation indicated for the management of obesity (*Sthaulya*). Chromatographic separation using silica gel 60 F_{254} plates and a mobile phase consisting of toluene:ethyl acetate (8:3 v/v) produced a well-resolved and compact peak corresponding to β-asarone at an Rf value of approximately 0.57. The close agreement between the Rf values and spectral characteristics of the sample and reference standard confirmed the specificity of the method and established the presence of β-asarone in LMK, attributable to its constituent drug

Vacha (*Acorus calamus* Linn.). Quantitative estimation revealed β-asarone content of 25.89 µg per 100 mg of LMK extract, indicating a measurable and reproducible level of this bioactive constituent within the formulation. The detection of β-asarone serves as a significant phytochemical marker for *Vacha* and contributes toward establishing a reliable fingerprint profile for LMK, thereby supporting quality assurance and batch-to-batch consistency.

Berberine, a pharmacologically important isoquinoline alkaloid, was identified and quantified in LMK using HPTLC. Standard berberine solution (100 µg/mL) was applied in varying concentrations (1–4 µL), while LMK test samples were applied in the range of 2–5 µL. Chromatographic separation was achieved using an optimized mobile phase consisting of n-propanol: formic acid: water (9:0.1:0.9 v/v/v). The chromatograms exhibited distinct and compact bands with an Rf value of approximately 0.31. The spectral overlay and corresponding absorbance intensity of the sample closely matched that of the reference standard, confirming the presence of berberine in LMK. Quantitative analysis revealed a berberine content of 0.24% w/w in the methanolic extract of LMK (**Figures 3 and 4**).

Curcumin quantification was performed using the mobile phase chloroform: ethyl acetate: formic acid (7.5:6:0.5 v/v/v), with densitometric scanning carried out at 420 nm. Curcumin reference standard (100 µg/mL) and LMK extracts (2-6 µL) produced distinct yellow fluorescent bands with Rf values ranging from 0.45 to 0.54. Calibration plots demonstrated satisfactory linearity within the selected concentration range. Quantitative estimation showed the presence of curcumin at 0.31% w/w in the methanolic extract of LMK (**Figures 5 and 6**).

The identified bioactive markers-β-asarone, berberine, and curcumin-have recognized pharmacological relevance in obesity and metabolic disorders through their reported effects on lipid metabolism, inflammatory signalling pathways, oxidative stress, and metabolic regulation. Their presence in LMK provides a plausible phytochemical basis for the formulation's classical therapeutic indications. However, considering the polyherbal nature of LMK, its pharmacological effects are likely mediated through synergistic interactions among multiple phytoconstituents. Further studies integrating multi-marker analysis with advanced metabolomic and pharmacological investigations may provide deeper insight into the mechanistic basis of LMK.(**Figures 7 and 8**)

The FTIR dataset for LMK comprises 19 absorption peaks, each corresponding to specific bond vibrations and functional groups. The wavenumbers from approximately 3278 to 468 cm^{-1}, indicating the presence of a broad spectrum of organic functional groups in the sample like The

identified signals encompass hydroxyl groups (O–H), aliphatic chains (C–H), unsaturated bonds (C=C in various configurations, and C=C=C), nitro groups (N–O), sulfur and nitrogen containing groups (thiocyanates, isothiocyanates, sulfonates), multiple C–O containing functionalities (esters, ethers, and alcohols), as well as halogens (fluoro-, chloro-, and iodo-compounds)[7]. This diversity of functional groups reflects the complex chemical nature of the sample, indicating that LMK is a multi-component herbal formulation containing a broad spectrum of phytochemicals. Such a rich assortment of chemical functionalities suggests that a wide range of molecular species (from polar phenolic compounds to nonpolar terpenic or alkane-like substances, including organosulfur and organohalogen compounds) are present. The FTIR peaks profile is placed below in **Figure 8.**

Table. 1: Formulation Composition of *Lekhaniya Mahakashaya*:

S.NO	INGREDIENT	LATIN NAME	PART USED	QUANTITY
1	*Musta* API	*Cyperus rotundus L.*	Rz.	1 part
2	*Kushtha* API	*Saussurea lappa C.B. Clarke*	Rt. Bk.	1 part
3	*Haridra* API	*Curcuma longa* L.	Rz.	1 part
4	*Daruharidra* API	*Berberis aristata* D.C.	St.	1 part

5	*Vaca* API	*Acorus calamus* L.	Rz.	1 part
6	*Ativisha* API	*Aconitum heterophyllum* Wall. ex-Royle)	Rt.	1 part
7	*Katurohini (Katuki* API)	*Picrorhiza kurroa*	Rz.	1 part
8	*Citraka* API	*Plumbago zeylanica* L.	Rt.	1 part
9	*Chirabilva* API	*Holoptelea integrifolia*	St. Bk.	1 part
10	*Hemavati*	*Iris versicolor*	Rt.	1 part

Materials and methods

Raw material sourcing and Authentication

All the botanicals prescribed in the classical LM formulation were procured in February 2025 from the Khari Baoli herbal market in Old Delhi. Each herb was authenticated at the Raw Drug Repository (RRDR) laboratory of All India Institute of Ayurveda (AIIA), and voucher specimens were assigned accession numbers (RRDR/AIIA/175-183) for future reference. The authenticated samples were deposited in the institute's herbarium, and aliquots were stored in airtight containers for subsequent analysis.

Proportions and sample preparation. As classical texts provide no explicit ratios for these herbs, each ingredient was weighed in equal proportions by mass. The dried plant materials (tubers, roots, rhizomes, and bark as applicable) were separately cleaned, air-dried, and milled into coarse powder using a stainless-steel grinder. Equal quantities of each powdered drug were combined to yield a homogeneous coarse mixture.

Batch preparation: The coarse powder was prepared in-house following the Good Manufacturing Practices

SAMPLE PREPARATION FOR HR-LCMS:

The hydroalcoholic extract of LM was prepared using the Soxhlet extraction method. A 20 g sample of the coarse powdered formulation was packed into a filter paper thimble and placed in the extractor chamber. A hydroalcoholic solvent in a 70:30 ratio of ethanol to distilled water was prepared, and 200 mL of this solvent was added to the round-bottom flask. The Soxhlet apparatus was assembled and heated gently at 60–70°C. The solvent evaporated, condensed, and percolated through the sample, extracting the phytoconstituents in repeated cycles over 6–8 hours. The process continued until the siphon tube appeared colourless, indicating exhaustive extraction. The obtained extract was dried in a hot air oven at 40–45°C. The dried extract was stored in an airtight amber-colored glass container for HR-LCMS analysis.

Preparation of Standard Solutions

Standard stock solutions of β-asarone, berberine, and curcumin were prepared separately by accurately weighing the reference standards and dissolving them in methanol. The stock solutions were further diluted to obtain suitable working standard concentrations for chromatographic analysis and quantitative estimation.

Preparation of LMK Sample Solution

Accurately weighed 2 g of *Lekhniya Mahakashaya* (LMK) powder was extracted with 10 mL of methanol and sonicated for 15 min at 40 °C. The extract was filtered through Whatman filter paper followed by a 0.22 µm PTFE syringe filter. The resulting filtrate was used for HPTLC fingerprinting and marker analysis.

Chromatographic Conditions for HPTLC Analysis

HPTLC analysis was performed using a CAMAG HPTLC system equipped with a Linomat-V sample applicator, TLC Scanner-IV, TLC Visualizer, and VisionCATS software. Precoated silica gel 60 F_{254} HPTLC plates (20 cm × 10 cm) were used as the stationary phase.

For β-asarone estimation, the optimized mobile phase consisted of toluene:ethyl acetate (8:3 v/v). Standard and sample solutions were applied as bands using a 100 µL Hamilton syringe

with the Linomat-V applicator. Plate development was carried out in a CAMAG twin-trough chamber pre-saturated with the mobile phase for 20 min at room temperature (25 ± 2 °C).

After development, the plates were air-dried and visualized under UV light at 254 nm and 366 nm, followed by derivatization and scanning at 540 nm wherever required. Densitometric scanning was performed at marker-specific wavelengths. Berberine was scanned at 420 nm, curcumin at 350 nm, and β-asarone at 254 nm. Quantitative estimation and spectral documentation were carried out using VisionCATS software.

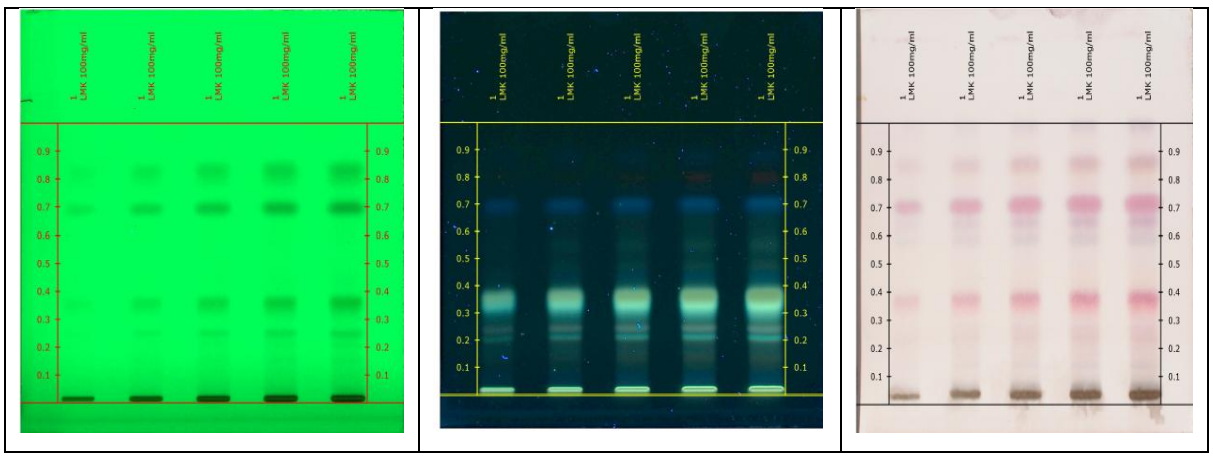
Identification and Quantification of Bioactive Markers in LMK

The identification of β-asarone, berberine, and curcumin in LMK was carried out by comparing the retention factor (Rf) values and spectral overlays of the sample peaks with those of corresponding reference standards. Well-resolved compact peaks corresponding to β-asarone, berberine, and curcumin were observed in the chromatographic profiles.

β-asarone was identified at an Rf value of approximately 0.57, confirming the presence of *Vacha* (*Acorus calamus* Linn.) in the formulation. Berberine and curcumin were also successfully identified through characteristic chromatographic behavior and spectral matching with their respective standards.

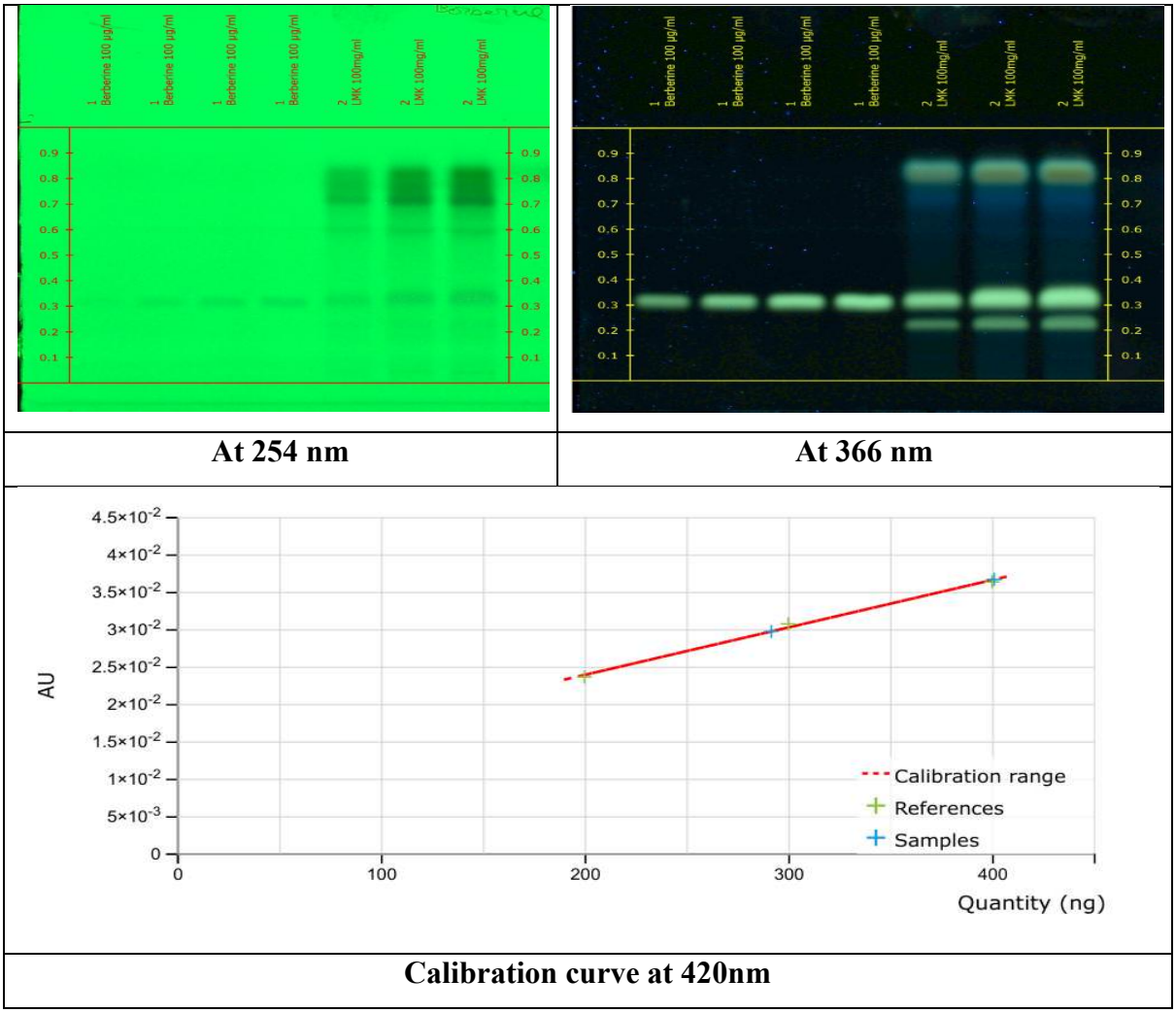
Quantitative estimation revealed the presence of β-asarone (25.89 µg/100 mg of extract), berberine (0.24% w/w), and curcumin (0.31% w/w) in LMK. Calibration curves constructed using different concentrations of standard solutions demonstrated satisfactory linearity for all three markers. The developed HPTLC method enabled simultaneous identification and quantification of the selected bioactive markers within the complex polyherbal matrix of LMK.

Figure 1: Schematic representation of the analytical workflow



At 254 nm	At 366 nm	After derivatization

Figure 2: HPTLC Fingerprint chromatogram of Lekhaniya Mahakashaya Methanolic extract



At 254 nm	At 366 nm

Calibration curve at 420nm

Figure 3: HPTLC chromatogram at 254nm, 366nm and calibration curve of *Berberine estimation*

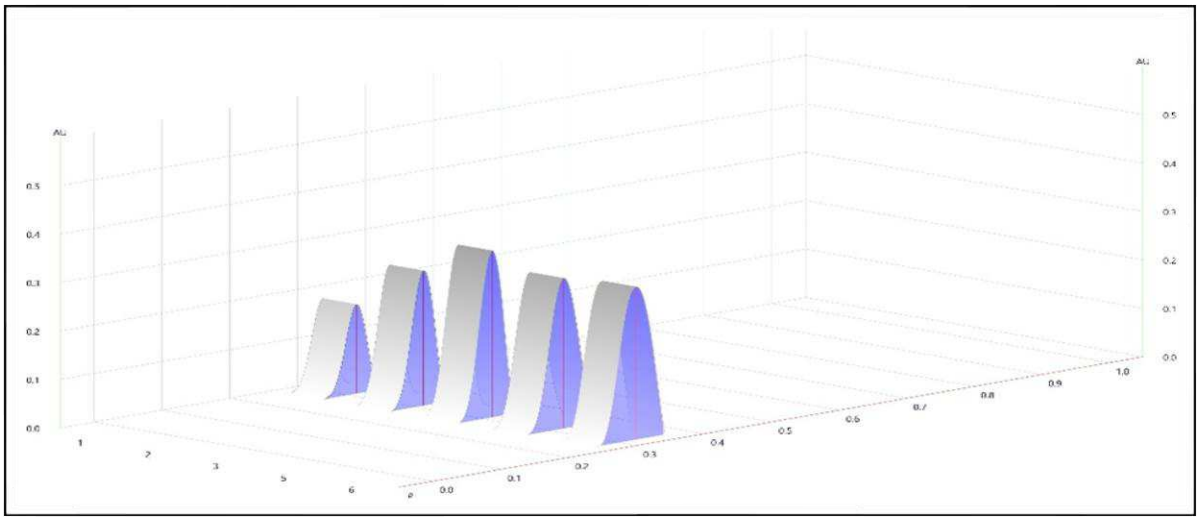


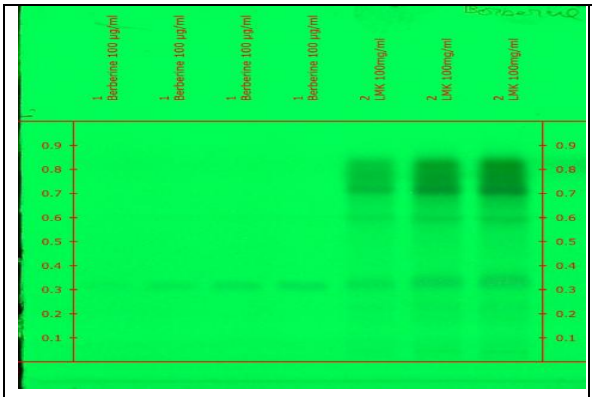
Figure 4: 3D HPTLC densitometric chromatogram of Berberine estimation showing multiple tracks with corresponding Rf values and peak intensities (AU)

Table. 2: Phytochemical Profiling of Hydroalcoholic Extract of *Lekhaniya Mahakashaya:* Negative polarity- HR-LCMS/MS-ORBITRAP

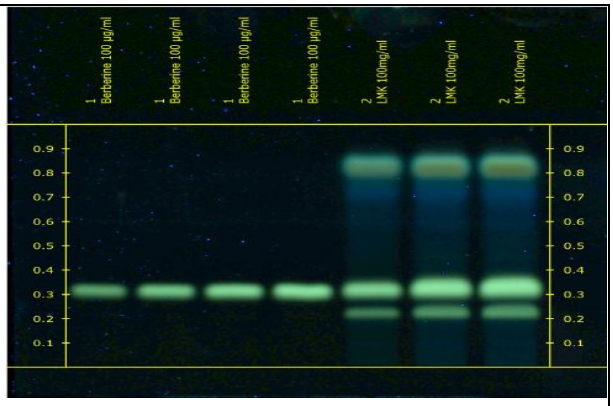
Compound Name	***Molecular Formula***	***MW (Da)***	***RT (min)***	***Area (Max.)***	***# ChemSpider Results***	***mzCloud Best Match (%)***	***Polarity***	***Chemical Class***
Taleranol	$C_{18}H_{26}O_5$	322.18	15.65	3.08×10^7	133	98.4	Negative	Phytoestrogen (lignan derivative)
Curcumin-like compound	$C_{32}H_{38}N_6O_6$	602.28	16.14	1.14×10^7	7	97.1	Negative	Diarylheptanoid analogue

Prostaglandin K_1 analogue	$C_{12}H_{20}ClN_4OPS$	334.08	6.57	4.79×10^7	41	96.0	Negative	Prostaglandin derivative
Allyl Phenyl Sulfide	$C_9H_{10}S$	150.05	1.34	5.38×10^7	34	94.2	Negative	Organosulfur compound
Phenyl Sulfoxide	$C_{12}H_{10}OS$	202.05	6.34	1.99×10^7	40	90.8	Negative	Sulfoxide
(2E)-4-(Phenylsulfanyl)-2-buten-1-ol	$C_{10}H_{12}OS$	180.06	1.23	2.26×10^8	116	94.8	Negative	Thioether compound
(5E)-5-Benzylidene-6,7-dihydro-1-benzothiophen-4(5H)-one	$C_{15}H_{12}OS$	240.06	8.06	2.39×10^7	44	92.7	Negative	Benzothiophene derivative
Thieno[3,2-e][1]benzofuran	$C_{10}H_6OS$	174.01	1.32	1.06×10^8	3	91.5	Negative	Benzofuran analogue
2,2-Dimethylsuccinic acid	$C_6H_{10}O_4$	146.06	23.6	2.19×10^7	215	93.0	Negative	Dicarboxylic acid
2-Hydroxy-1-(4-methoxyphenyl)propyl hexopyranoside-like compound	$C_{31}H_{46}N_6O_4S$	598.33	10.39	1.27×10^7	3	91.4	Negative	Phenolic glycoside
Octabenzone	$C_{21}H_{26}O_3$	326.19	15.94	4.11×10^7	121	90.2	Negative	Phenolic ketone
2-Methyl-2-propanyl 4-[(3R)-3-{[(benzyloxy)carbonyl]amino}-4-methoxy-4-oxobutyl]-1-piperidinecarboxylate	$C_{23}H_{34}N_2O_6$	434.24	16.91	3.11×10^7	8	89.7	Negative	Piperidine derivative

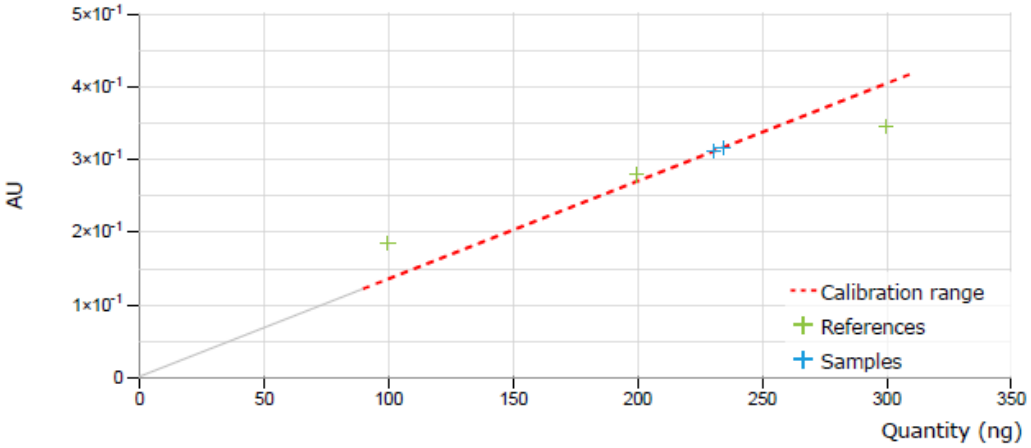
Figure 5: HPTLC chromatogram at 254nm, 366nm and calibration curve of *Curcumin estimation*



At 254 nm



At 366 nm



Calibration curve at 350nm

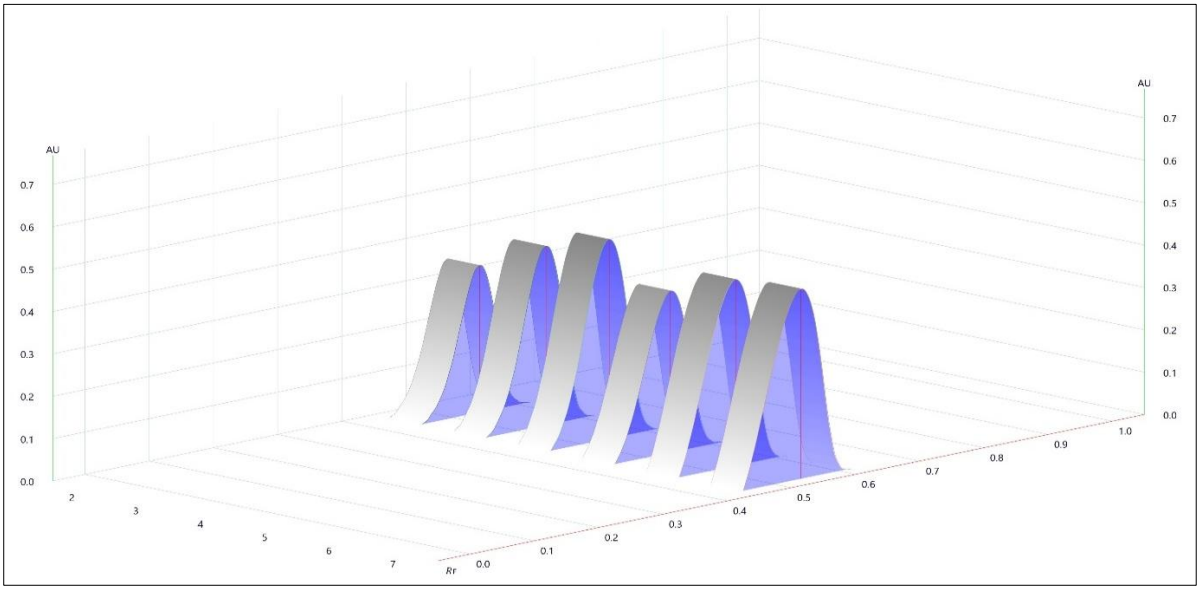


Figure 6: 3D HPTLC densitometric chromatogram of *Curcumin* estimation showing multiple tracks with corresponding Rf values and peak intensities (AU)

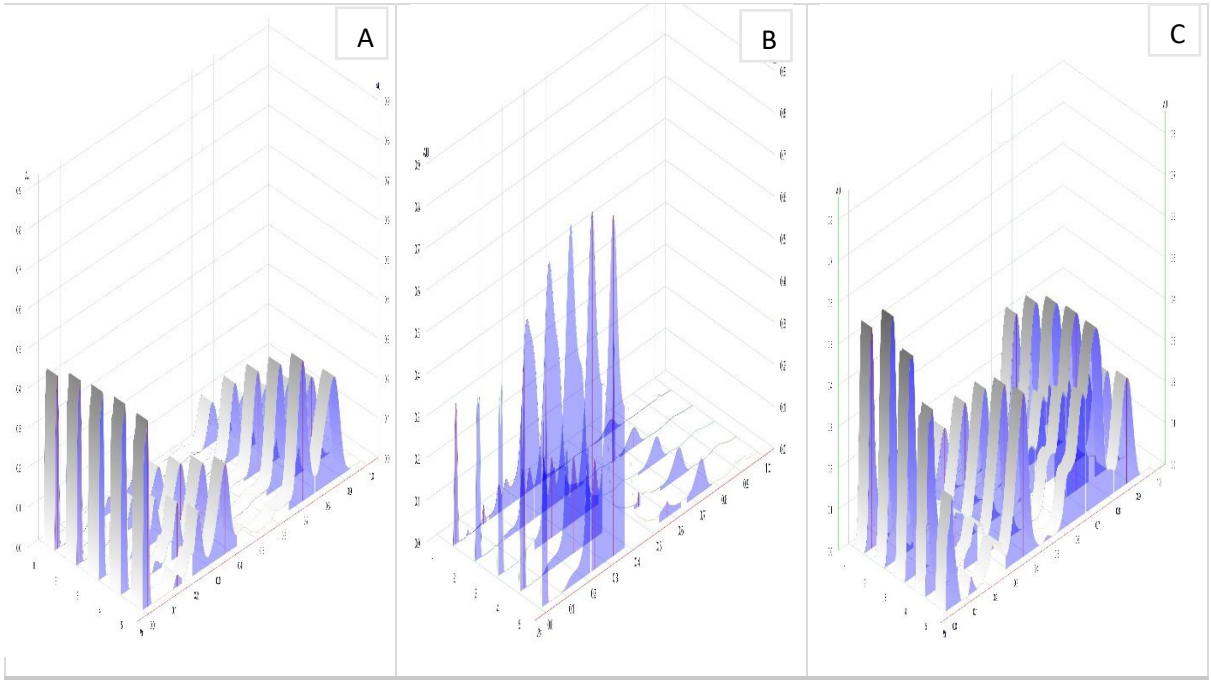


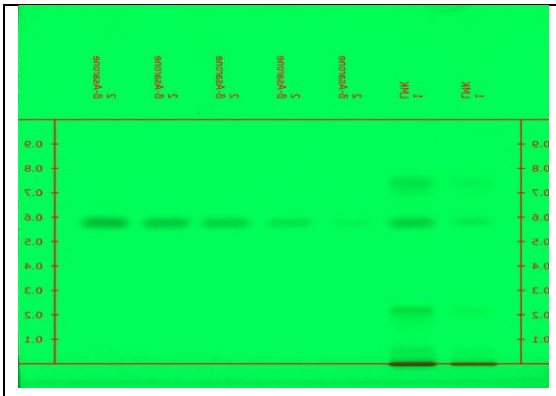
Figure 7: Three-Dimensional HPTLC Fingerprint Profiles of LM at Different Detection Wavelengths(A) 3D HPTLC fingerprint profile at 254 nm, (B) 3D HPTLC fingerprint profile at 366 nm, and (C) 3D HPTLC fingerprint profile at 540 nm.

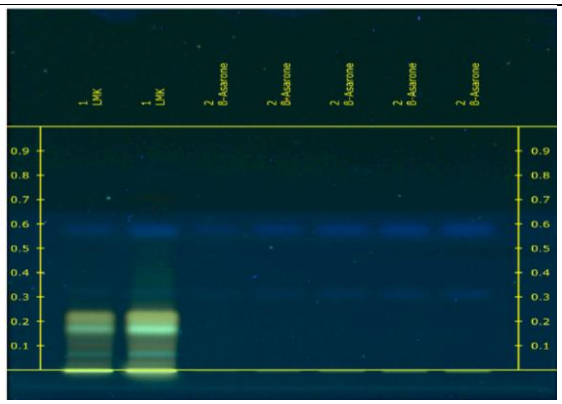
Table 3: Phytochemical Profiling of Hydroalcoholic Extract of *Lekhaniya Mahakshaya:* Positive polarity- HR-LCMS/MS-ORBITRAP

Compound Name	*Molecular Formula*	*MW (Da)*	*RT (min)*	*Area (Max.)*	*# ChemSpider Results*	*mzCloud Best Match (%)*	*Polarity*	*Chemical Class*
Berberine	$C_{20}H_{17}NO_4$	335.12	11.0 – 13.8	2.52×10^{10}	71	97.3	Positive	Isoquinoline alkaloid
Curcumin	$C_{21}H_{20}O_6$	368.13	12.1 – 13.9	2.15×10^{9}	86	98.5	Positive	Diarylheptanoid
Fisetin	$C_{15}H_{10}O_6$	286.05	—	—	—	99.9	Positive	Flavonol

Kaempferol	$C_{15}H_{10}O_6$	286.05	—	—	—	99.9	Positive	Flavonol
Ferulic acid	$C_{10}H_{10}O_4$	194.06	9.33	1.85×10^8	439	99.6	Positive	Phenolic acid
Isoferulic acid	$C_{10}H_{10}O_4$	194.06	10.31	5.39×10^8	439	99.2	Positive	Phenolic acid
Apocynin	$C_9H_{10}O_3$	166.06	8.00–9.75	4.03×10^8	408	98.9	Positive	Phenolic ketone
(+)-ar-Turmerone	$C_{15}H_{20}O$	216.15	13.6–16.3	5.70×10^8	305	89–100	Positive	Sesquiterpenoid
β-Lapachone	$C_{15}H_{14}O_3$	242.09	13.44	1.50×10^8	545	98.2	Positive	Quinone derivative
Linoleoyl Ethanolamide (LEA)	$C_{20}H_{37}NO_2$	323.28	—	—	—	99.3	Positive	Fatty acid amide
Oleoyl Ethanolamide (OEA)	$C_{20}H_{39}NO_2$	325.30	—	—	—	99.1	Positive	Fatty acid amide
Cinnamic acid	$C_9H_8O_2$	148.05	10.39	1.15×10^9	176	—	Positive	Aromatic acid
Betaine	$C_5H_{11}NO_2$	117.08	1.3–24.1	1.09×10^9	219	92–97	Positive	Amino acid derivative
L-/D-Proline & Pipecolinic acid	$C_{5–6}H_{9–11}NO_2$	115–129	1.2–1.6	2.68×10^9	255	99.5–99.9	Positive	Amino acid derivative
9-Oxo-10(E),12(E)-octadecadienoic acid (9-oxo-ODA)	$C_{18}H_{30}O_3$	294.22	16.5–16.6	1.49×10^8	174	95.4–97.5	Positive	Oxo-fatty acid

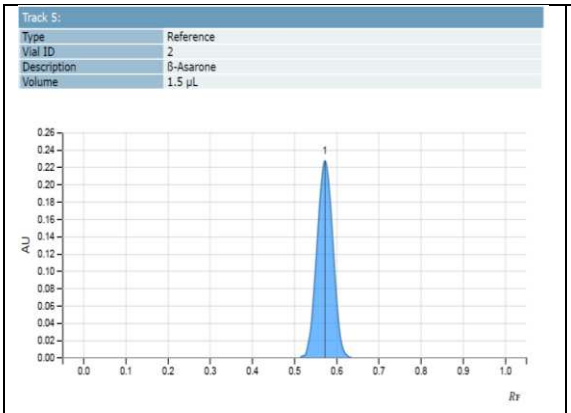
Figure 8: Chromatogram of LMK & β-asarone under different wavelengths

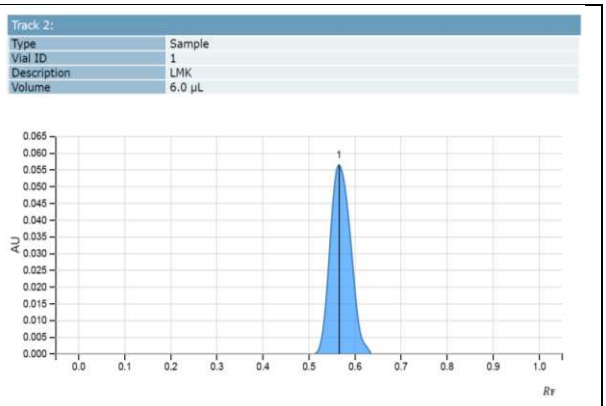




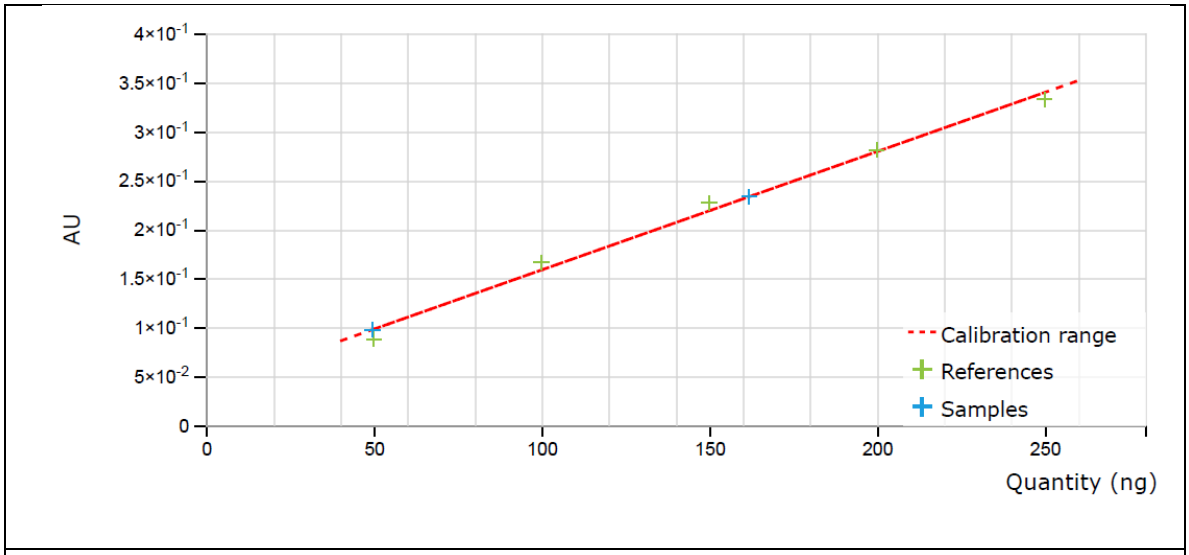
At 254 nm	At 366 nm

Figure 9: Peak densitogram of Curcuminoids NC





β-asarone	LMK



Calibration curve at 254 nm

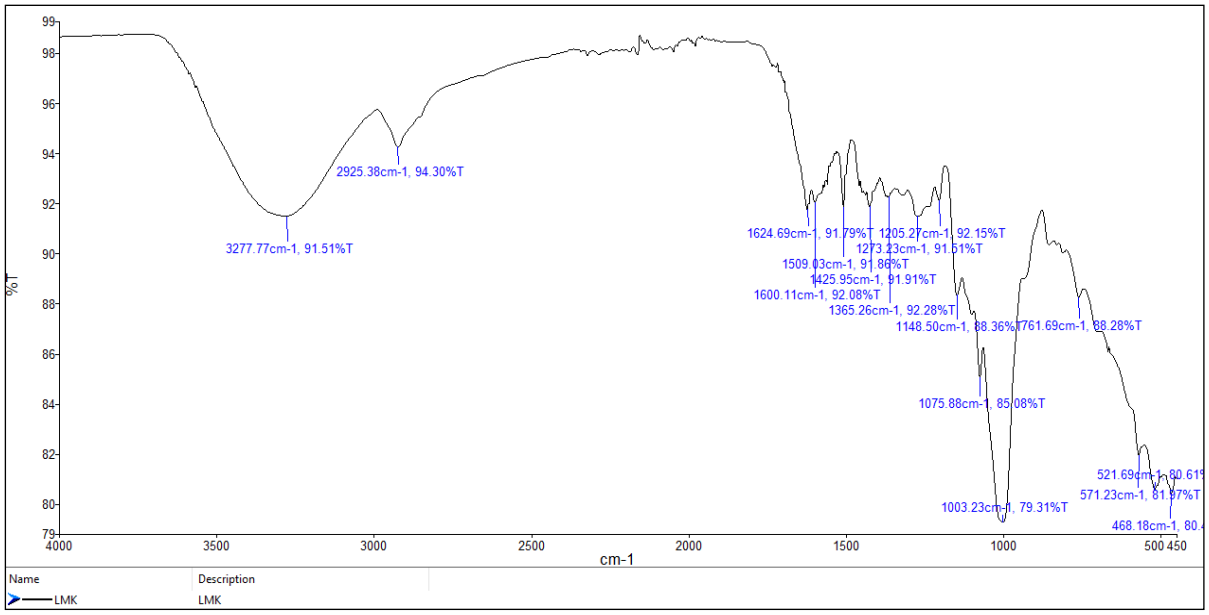


Figure 10. FTIR spectrum of *Lekhniya Mahakashaya* (LMK) showing major absorption peaks

Experimental Design, Materials, and Methods

Sample Collection, Authentication, and Preparation of Extracts Raw materials were procured from the Khari Baoli market, New Delhi, India. Samples were examined for morphological characteristics and classical features according to Ayurveda at the Taxonomy Laboratory, RRDR, Department of Dravyaguna, All India Institute of Ayurveda, New Delhi. (Authentication no.11003/5/08/2025-AIIA/DG/423-432) Only those samples found to be

satisfactory were used for the preparation of LMK. LMK was prepared according to the standards of the Ayurvedic Pharmacopoeia of India[8] under sterile laboratory conditions at the Rasa Shastra and Bhaishajya Kalpana (RSBK) Department laboratory, AIIA, New Delhi.

All ingredients of pharmacopoeia quality were used. The ingredients (**as listed in Table 1**) were washed, dried, powdered, and passed through sieve number 85. The powdered ingredients were transferred to an octagonal blender for proper mixing, then the Powder was stored in an air-tight container for further analysis.

Preparation of LMK Extract

The combined powdered material (20g) was subjected to Soxhlet extraction using a hydroalcoholic mixture (HPLC-grade methanoll and Distilled water in a ratio of 50:50 for 8 hours. The next day, the extract was filtered through vacuum filtration by using a PTFE membrane filter with a 0.45 µm pore size, then concentrated and dried in a hot air oven at 45 °C. For HLCMS/MS-Orbitrap analysis, this extract was used. For HPTLC analysis, the extract was diluted with LC-grade methanol. A 50:50 (v/v) methanol–water mixture was used as the extraction solvent because methanol efficiently extracts both polar and moderately non-polar phytochemicals, while water improves the recovery of highly polar constituents. Three independent extractions of the formulation were prepared under identical conditions (n = 3), and each extract was analysed in triplicate across all analytical platforms. [9].

Phytochemical profiling using HR-LCMS/MS-ORBITRAP

Methods

The HR-LCMS-Orbitarp analysis was performed using a Thermo Scientific Q Exactive Orbitrap instrument equipped with a heated electrospray ionization (HESI) source. Chromatographic separation was achieved using a C18 column (Thermo Hypersil GOLD, 100 × 2.1 mm, 1.9 µm) with a binary solvent system: solvent A (0.1% formic acid in water) and solvent B (acetonitrile with 0.1% formic acid). The gradient started at 5% B, ramped to 95% B over 25 minutes, and returned to initial conditions within 5 minutes, resulting in a total run time of 30 minutes. The flow rate was maintained at 0.3 mL/min. The injection volume was 10 µL. The mass spectrometer operated in full MS scan mode (m/z 1001000) with a resolution of 70,000 (full width at half maximum, FWHM) at m/z 200. The data were acquired under both positive and negative ionisation modes.

Metabolite annotation – Raw LC–MS data were processed in Thermo Xcalibur using automated peak detection and alignment algorithms. A compound was considered positively identified when the measured mass deviated by less than ±5 ppm from the theoretical mass and the MS/MS fragmentation spectrum matched library spectra from the NIST14, METLIN and in-house natural-products databases. Peaks that met the mass accuracy criterion but lacked

high-quality MS/MS matches were classified as “tentatively identified,” recognising that current libraries may not cover all Ayurvedic metabolites.

Reproducibility and quality control -A pooled quality-control (QC) sample was prepared by combining aliquots of all extracts and injected every ten injections to monitor system stability. The relative standard deviations of retention times and peak areas for ten representative metabolites in the QC sample were all below 5 %. In addition, each of the three biological extracts was injected three times; the resulting extracted-ion chromatograms showed excellent overlap, demonstrating the high reproducibility of the HRLC-MS/MS method.

Chromatographic Conditions:

The chromatographic separation was performed under tightly controlled thermal and flow parameters to ensure optimal peak resolution and reproducibility. The analytical column was maintained at **40 °C** in forced-air mode, with an equilibration time of **1 minute**. The column compartment operated with a **temperature delta of 0.5 °C** and a **fan speed of 5**, providing stable thermal distribution throughout the analysis.

The autosampler temperature was held at **4 °C** to preserve sample stability. Needle penetration was set at a puncture offset of **0 µm**, and both-side needle wash was employed with a **wash duration of 2 seconds** and **wash speed of 10 µL/s**. Sample aspiration and dispensing were performed at **5 µL/s** to prevent bubble formation and maintain injection precision.

The chromatographic method employed four mobile phases: **Solvent A (0.1% formic acid in water)**, **Solvent B (methanol)**, **Solvent C (acetonitrile)**, and **Solvent D (organic gradient phase)**. The flow rate was maintained at **0.3 mL/min** with a pressure operational window of **0–1034 bar** and a ramp limit of **6.0 mL/min²**. A gradient elution program was used, starting with **5% D** from **0–2 minutes** for initial equilibration, followed by a linear rise from **5–95% D** between **2–15 minutes**. A strong wash at **95% D** was maintained from **15–20 minutes**, after which the system returned to **5% D** for **21–30 minutes** to ensure proper column re-equilibration. The injection volume was set according to the instrument’s method configuration.

Mass Spectrometry Conditions:

Mass spectrometric detection was performed using a heated electrospray ionisation (HESI) source in positive mode. Data acquisition employed a **Full MS scan** followed by **data-dependent MS/MS (dd-MS²)** in a Top-5 mode to allow efficient profiling and structural elucidation of analytes.

Full MS Parameters

The instrument operated at a **resolution of 70,000 FWHM at m/z 200**, with a scan range of **85–1100 m/z**. An **AGC target of 1 × 10^6** and a **maximum injection time (IT) of 100 ms** ensured adequate ion accumulation and sensitivity.

MS/MS Parameters

For fragmentation, the dd-MS^2 scans were acquired at a **resolution of 17,500 FWHM at m/z 200** with a scan range of **200–2000 m/z**. The AGC target was set to **1 × 10^5**, with a **maximum IT of 100 ms**. Precursor ions were isolated with a **1.5 m/z window**, and fragmentation was performed using a **stepped normalized collision energy (NCE) of 30 eV**. A **dynamic exclusion of 10 seconds** prevented repeated fragmentation of the same precursor and improved coverage. The method allowed selection of **Top 5 precursor ions per cycle**, and all data were acquired in **profile mode** for high-resolution analysis.

Instrument Control Parameters:
Instrument control utilized a pre-calibrated tune file, **Cal_positive_29082024.mstune**, optimized for positive-ion mode acquisition. Divert Valve A was active throughout the run, operating between **positions 1 and 2**, whereas Divert Valve B remained unused. The syringe system used a **250 µL Hamilton syringe** with an inner diameter of **2.303 mm**, delivering solvent at **3.0 µL/min**.

Lock masses were enabled to ensure continuous mass calibration and maintain sub-ppm mass accuracy. Contact closure functions were not employed, and **in-source CID was set to 0 eV** to avoid unintended fragmentation before MS/MS acquisition.

Data Processing and Compound Identification:
Raw LC–MS data files were processed using **Thermo Xcalibur (Thermo Fisher Scientific, USA)**. Automated peak detection and alignment were performed according to preset signal-processing algorithms. Compound annotation was carried out using standard spectral libraries and curated reference databases.

A compound was considered **positively identified** when two criteria were fulfilled:

1. **Mass accuracy within ±5 ppm of theoretical mass**, and
2. **Fragmentation spectra that matched library reference patterns**, confirming structural identity.

Phytochemical profiling using HPTLC

Methods

HPTLC plate runs were performed according to the following experimental procedure. A clean plate was placed in the TLC visualizer for background documentation prior to each run. Methanol blanks, standard working solutions, or case samples were then applied to the plate as bands (20 × 10 cm) using a CAMAG TLC Linomat 5. The plate was then transferred to the CAMAG TTC development chamber to develop with a selected mobile phase. During development, the plate was first dried for 30 s. A glass chamber underwent a 20-minute humidity-control and tank-saturation step before the plate was lowered into the chamber. The migration distance was set at 70 mm. Once the migration distance was reached, the plate was removed from the mobile phase and dried for five minutes. The plate was then transferred to the CAMAG TLC visualizer for documentation under white light at 254nm and 366 nm. A background image was taken at the beginning of the run, which was automatically subtracted by the software. The plate was transferred to TLC scanner 4 for an intensity scan of the samples after development. The plate was then sprayed with anisaldehyde sulphuric acid reagent for derivatising purposes using the CAMAG Derivatizer. After derivatisation, the plate was transferred to the visualizer for the 3rd set of pictures, which were the final images reported in this article.

Phytochemical profiling using FTIR

Methods

Fourier-Transform Infrared (FTIR) spectroscopy was employed to characterise the functional groups present in the LMK sample. A finely ground powder of LMK (less than 1 gram) was loaded into the sample holder of a PerkinElmer UATR. The sample was then scanned across a mid-infrared (mid-IR) range of 4000 cm^{-1} to 400 cm^{-1} using a Deuterated Triglycine Sulfate (DTGS) detector for optimal signal acquisition[11].

Data acquisition and Reporting

The dataset includes:

- Raw chromatograms, peak intensities, and fragmentation spectra of HRLCMS/MS-Orbitrap data.
- Supplementary data of HRLCMS/MS-Orbitrap files detailing identified compounds.

- Supplementary data of HPTLC fingerprint file, Berberine estimation and curcumin estimation detailing.
- FTIR Functional group data.

Data Availability

Singh, Narayan; Upadhyay,Anjali; Chakraborty, Debajyoti; Patil, Girish; Chaudhari, Swapnil; Jagtap, Chandrasekhar; Yadav, Pramod; R, GALIB; Prajapati, Pradeep Kumar (2026), "Multi-Analytical Dataset on Lekhniya Mahakashaya: HRLC-MS/MS Orbitrap Profiling, HPTLC Fingerprinting with Marker Estimation, and FTIR Spectroscopy", Mendeley Data, V2, doi: 10.17632/7snr6ghw7x.2

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Author Contributions

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review & editing, Analytical validation, Visualization, Methodology, Formal analysis. Pramod Yadav: Validation, Supervision, Methodology, Conceptualization, Writing-review & editing, Statistical analysis. Galib R: Visualization, Validation, Writing-review & editing, Supervision, Methodology, Conceptualization. Pradeep Kumar Prajapati: Supervision, Visualization, Validation, Methodology, Conceptualization, Writing- review & editing, Formal Analysis.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval and consent to participate

Not applicable.

Consent of publication

Not applicable.

Additional information: Supplementary Information

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