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Advances in Bioresearch

ORIGINAL ARTICLE

Chromatographic Fingerprinting and Quality Assessment of Hinguvachadi Choorna and Its Marketed Formulation Using HPTLC

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ABSTRACT
Standardization of classical Ayurvedic polyherbal formulations is essential to ensure quality, safety, and therapeutic consistency. High Performance Thin Layer Chromatography (HPTLC) is widely applied for qualitative and quantitative phytochemical profiling due to its simplicity, reproducibility, and high-throughput capability. The present study aimed to develop and validate an HPTLC fingerprinting method for Hinguvachadi Choorna and its commercially available effervescent tablet formulation, DYS-FIZZ. Chromatographic separation was performed on silica gel 60 F_{254} plates using toluene: ethyl acetate: formic acid (6:3:1 v/v/v) as the mobile phase. Densitometric scanning was carried out at 254 nm and 366 nm. Application volumes ranged from 5–20 µL. Method validation was conducted according to ICH Q2 guidelines, assessing linearity, precision, specificity, and robustness. Fourier Transform Infrared (FT-IR) spectroscopy was employed to identify functional groups present in the formulation. The optimized method produced a well-resolved chromatographic fingerprint with seven prominent phytochemical spots demonstrating good correlation between concentration and peak area. The method showed acceptable linearity, precision, and robustness as per validation criteria. FT-IR analysis confirmed the presence of aromatic compounds, alkaloids, phenolics, and volatile resinous constituents. The developed HPTLC method provides a reliable and reproducible fingerprint profile for quality control of Hinguvachadi Choorna and its effervescent formulation. Further studies involving marker-based standardization and hyphenated analytical techniques are recommended to strengthen quality assurance protocols.
Keywords: *HPTLC fingerprinting, Hinguvachadi Choorna, Polyherbal formulation, Method validation, FT-IR analysis, Quality standardization*

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INTRODUCTION

Polyherbal formulations are an important part of traditional medical systems. However, their intricate phytochemical profiles pose serious analytical challenges in quality control analysis. HPTLC methods have become important in herbal standardization processes due to their ability to resolve multi-component mixtures, generate reproducible fingerprints, and provide visual confirmation of chemical patterns. [1] *Hinguvachadi Choorna* is an Ayurvedic formulation traditionally used for gastrointestinal and respiratory disorders. *Ferula asafoetida, Piper nigrum*, and *Piper longum* are some of its alkaloid-, resinous volatiles-, and phenolic component-containing ingredients, respectively. The well-documented alkaloid piperine from Piper species is generally used as a reference marker in chromatographic standardization. Identification and estimation of piperine and related phytoconstituents in polyherbal preparations through HPTLC have been undertaken in several studies, reflecting the importance of the

technique in the determination of the said class of formulations like Hinguvachadi Choorna. [2] This study now combines HPTLC fingerprinting of a commercial tablet sample, namely DYS-FIZZ, with the FTIR analysis of the corresponding Choorna to construct a comprehensive analytical overview and to propose a method validation framework aligned with ICH Q2 guidelines.

Objectives

To establish an HPTLC fingerprinting and validation approach, supported by FT-IR characterization, for identification of major phytochemical zones and quality control assessment of Hinguvachadi Choorna (DYS-FIZZ).

MATERIAL AND METHODS

Sample materials

Two analytical datasets were used:

- HPTLC report for DYS-FIZZ tablets, containing instrument parameters, chromatograms, densitograms, peak tables, application volumes (5–20 µL), plate information, and wavelength scans at 254 nm and 366 nm.
- FT-IR spectrum of *Hinguvachadi Choorna*, acquired as a single-scan reflective spectrum.

Formulation Details

1. Sample Used: The formulation used in this study was DYS-FIZZ effervescent tablet. The analysis was conducted at a Ministry of AYUSH Approved Ayurvedic Testing Laboratory.
2. Batch Number: Nov. 2025
3. Manufacturer: Parul Institute of Ayurved and Research Pharmacy

Preparation of Extracts

Hinguvachadi Choorna components:

1. Hingu (*Ferula asafoetida*) – alkaloids and resinous volatile compounds
2. Vacha (*Acorus calamus*) – alkaloids and phenolic compounds
3. Haritaki (*Terminalia chebula*) – tannins and phenolic compounds
4. Eshwari (*Aristolochia indica*) – alkaloids and flavonoids
5. Dadima (*Punica granatum*) – phenolics and antioxidants
6. Ajamoda (*Apium graveolens*) – flavonoids and phenolic compounds
7. Dhanyakam (*Coriandrum sativum*) – essential oils and antioxidants
8. Patha (*Cissampelos pareira*) – alkaloids and resins
9. Kustha (*Saussurea lappa*) – essential oils and flavonoids
10. Shati (*Hedychium spicatum*) – essential oils
11. Mundi (*Sphaeranthus indicus*) – essential oils and antioxidants
12. Chithrak (*Plumbago zeylanica*) – bioactive naphthoquinones and related compounds
13. Amlavedasam (*Garcinia pedunculata*) – antioxidants and phenolic compounds
14. Pippali (*Piper longum*) – alkaloids and antioxidants
15. Chavyam (*Piper chaba*) – essential oils and antioxidants

Drug Preparation and Analysis

All the herbal drugs were extracted separately according to the standards of the Indian Pharmacopeia. The salt and alkaline substances were thoroughly washed using demineralized water and dried to obtain a fine powder. The herbal extract and washed salt were mixed together and subjected to dehumidification in a hot air oven.

The moisture-free powder was then mixed with:

Sodium Bicarbonate (5% weight by weight)

Sodium Saccharin (1.8% weight by weight)

Starch (5% weight by weight)

These components were used to prepare the **Fizz tablet**. The **weight of each tablet** is 1 gm.

Methodology for Preparation of the Effervescent Tablet

Preparation of Herbal Extracts:

Each herbal component was extracted individually as per the specified solvent combinations (alcoholic, hydroalcoholic, and water-based extracts) to ensure the correct profile of active compounds.

Mixing and Drying

The fine powder of herbal extracts was mixed with sodium bicarbonate, sodium saccharin, and starch in specified proportions. The mixture was then subjected to dehumidification in a hot air oven to ensure it was moisture-free.

Formulation of Tablets:
After drying, the powder was mixed with **Sodium Bicarbonate** (5%), **Sodium Saccharin** (1.8%), and **Starch** (5%). The resulting mixture was then compressed into effervescent tablets with the specified weight (1 gm).

Testing for Fizz Tablet Properties:
Once the tablets were prepared, they were tested for their effervescence, stability, and dissolution properties. Tablets that met the required criteria were selected for further analysis.

Batch Details
Batch Number: Nov. 2025
Manufacturer: Parul Institute of Ayurved and Research Pharmacy

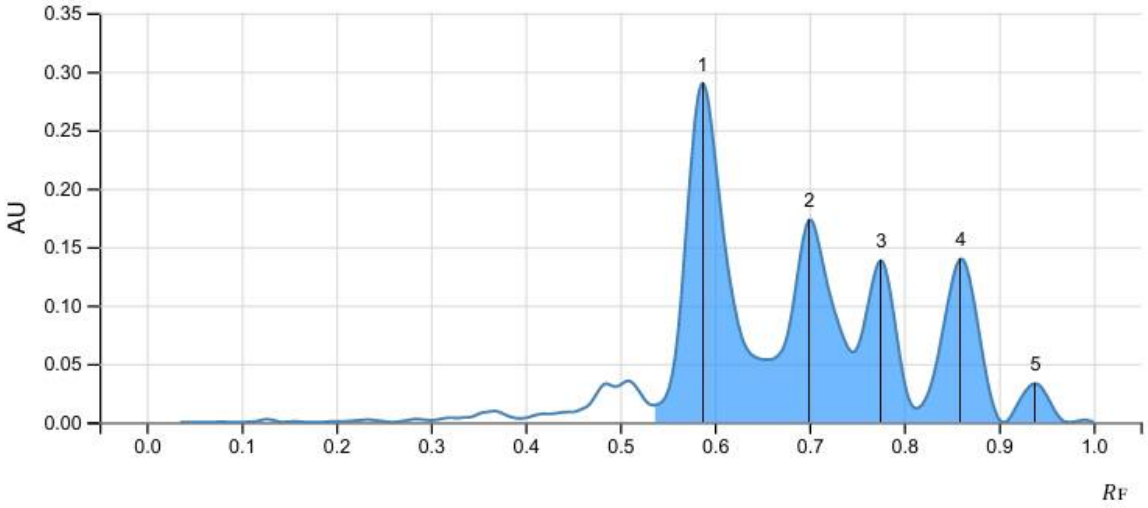
HPTLC instrumentation and chromatographic conditions
All experimental conditions were extracted from the laboratory-generated VisionCATS report:
Stationary phase: Merck HPTLC silica gel 60 F_{254} (100 × 100 mm).
Sample application: Linomat 5; dosage speed 150 nL/s; pre-dosage 0.20 µL; application position Y = 8 mm; track spacing fixed.
Applied volumes: 5, 10, 15 and 20 µL.
Mobile phase: toluene:ethyl acetate:formic acid (6:3:1 v/v/v).
Chamber saturation: 20 minutes with pad.
Development distance: 70 mm; drying time: 5 minutes.
Densitometry: TLC Scanner 4; absorbance mode (254 nm, deuterium lamp) and fluorescence mode (366 nm, mercury lamp with K400 filter).
Scan settings: slit 6 × 0.45 mm; data resolution 100 µm; Savitzky–Golay smoothing; lowest-slope baseline correction.

FT-IR spectroscopy
The FT-IR spectrum of *Hinguvachadi Choorna* was evaluated for characteristic functional groups indicative of the formulation's botanical constituents. Peaks were interpreted qualitatively.

Validation framework
The method was assessed against ICH-aligned criteria for herbal HPTLC methods, including:
- Linearity (peak area vs. application volume)
- Precision (Rf reproducibility and relative area % across tracks)
- Specificity (chromatographic separation and wavelength behavior)
- Accuracy (to be determined through recovery studies)
- Detection limits (to be evaluated upon availability of standards)
- Robustness (mobile phase and development variations)

RESULTS

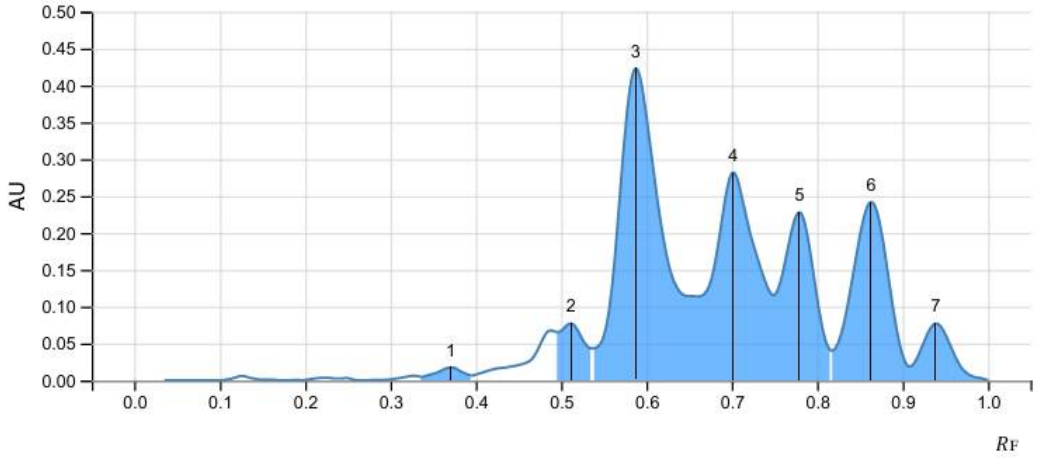
Chromatographic fingerprinting
The HPTLC densitometric chromatogram of Hinguvachadi Choorna revealed five major peaks at R_F 0.587, 0.700, 0.776, 0.860, and 0.939 (Figure 1). Peak 1 was dominant with the highest relative peak area (40.54%), followed by Peaks 2, 4, 3, and 5. The fingerprint pattern obtained in the R_F range of 0.53–0.94 may be used as a reference for the identification and quality control of the formulation.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.535	0.0144	0.587	0.2897	37.46	0.655	0.0535	0.01489	40.54
2	0.658	0.0535	0.700	0.1730	22.38	0.745	0.0601	0.00915	24.91
3	0.745	0.0601	0.776	0.1381	17.85	0.813	0.0118	0.00549	14.95
4	0.813	0.0118	0.860	0.1395	18.04	0.905	0.0000	0.00609	16.57
5	0.905	0.0000	0.939	0.0330	4.27	0.974	0.0000	0.00111	3.03

Fig. 1: HPTLC densitogram of Track 2 with 10.0 µL at 254 nm

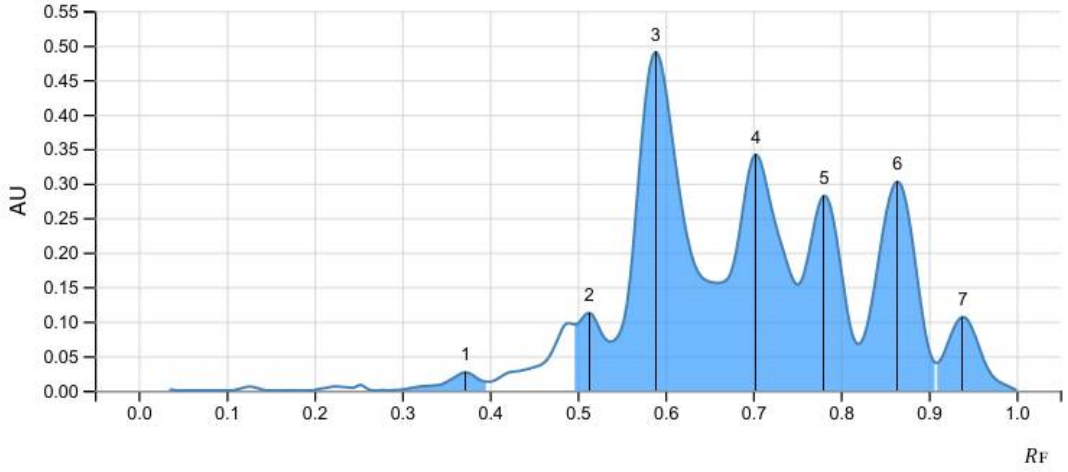
The HPTLC densitometric chromatogram showed seven resolved peaks at R_F 0.371, 0.517, 0.587, 0.702, 0.779, 0.863, and 0.939 (Figure 2). Among these, Peak 3 was dominant with the highest relative area (35.28%), followed by Peak 4 (23.58%) and Peak 6 (17.25%). The chromatographic profile indicated a distinct fingerprint pattern for the formulation.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.335	0.0047	0.371	0.0175	1.30	0.395	0.0065	0.00066	0.96
2	0.495	0.0653	0.511	0.0769	5.72	0.535	0.0431	0.00258	3.74
3	0.537	0.0430	0.587	0.4228	31.43	0.650	0.1142	0.02437	35.28
4	0.661	0.1138	0.702	0.2818	20.95	0.748	0.1154	0.01629	23.58
5	0.748	0.1154	0.779	0.2278	16.93	0.816	0.0404	0.01009	14.60
6	0.818	0.0401	0.863	0.2417	17.97	0.908	0.0185	0.01192	17.25
7	0.908	0.0185	0.939	0.0768	5.71	1.000	0.0002	0.00317	4.59

Fig. 2: Track 2 with 10.0 µL @254 nm

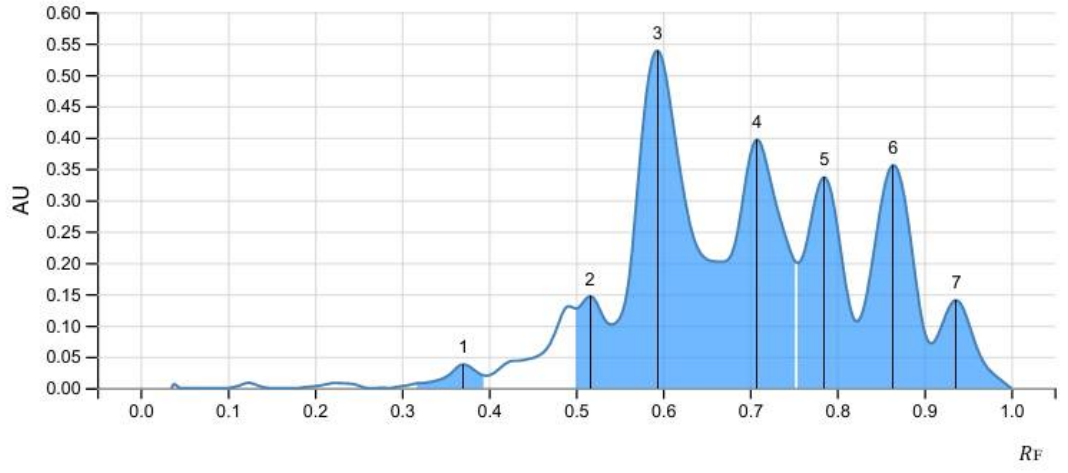
The densitometric chromatogram revealed seven major peaks at R_F 0.373, 0.513, 0.589, 0.703, 0.781, 0.865, and 0.939. Peak 3 showed the maximum relative area (34.51%), while Peaks 4, 6, and 5 contributed 23.14%, 17.10%, and 14.58%, respectively (Figure 3). The peak pattern remained well resolved and reproducible.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.285	0.0000	0.373	0.0263	1.58	0.397	0.0129	0.00122	1.32
2	0.497	0.0957	0.513	0.1124	6.76	0.537	0.0708	0.00386	4.20
3	0.537	0.0708	0.589	0.4902	29.50	0.658	0.1560	0.03172	34.51
4	0.660	0.1560	0.703	0.3417	20.57	0.750	0.1539	0.02127	23.14
5	0.750	0.1539	0.781	0.2821	16.98	0.819	0.0674	0.01341	14.58
6	0.819	0.0674	0.865	0.3024	18.20	0.908	0.0400	0.01572	17.10
7	0.910	0.0399	0.939	0.1065	6.41	1.000	0.0002	0.00472	5.14

Fig. 3: Track 3 with 15.0 µL @254 nm

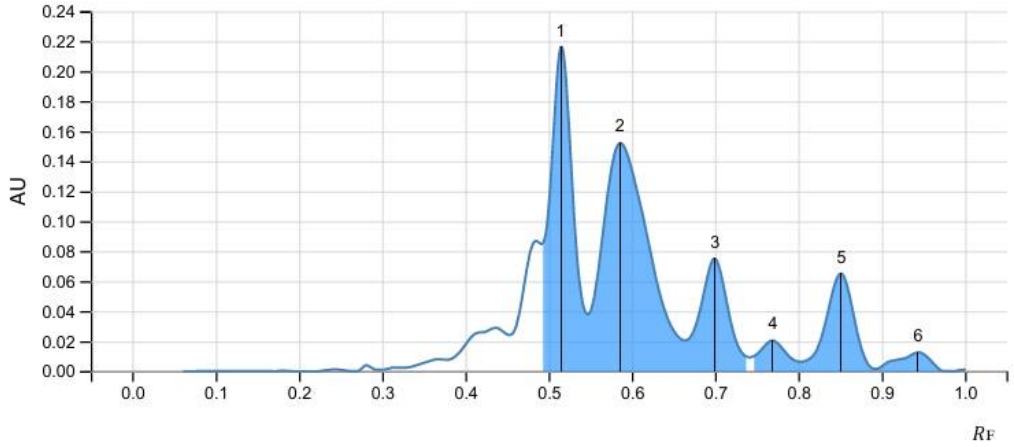
The chromatogram exhibited seven well-resolved peaks at R_F 0.371, 0.516, 0.594, 0.708, 0.785, 0.865, and 0.937. The most prominent component was Peak 3 with a relative area of 34.25%, followed by Peak 4 (22.07%) and Peak 6 (16.98%) (Figure 4). The fingerprint pattern confirmed consistent chromatographic separation of the constituents.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.315	0.0069	0.371	0.0378	1.94	0.397	0.0199	0.00171	1.52
2	0.500	0.1271	0.516	0.1464	7.50	0.540	0.1016	0.00519	4.62
3	0.540	0.1016	0.594	0.5387	27.60	0.666	0.2017	0.03845	34.25
4	0.668	0.2016	0.708	0.3966	20.32	0.753	0.2004	0.02477	22.07
5	0.755	0.1997	0.785	0.3363	17.23	0.823	0.1065	0.01642	14.63
6	0.823	0.1065	0.865	0.3557	18.23	0.908	0.0714	0.01906	16.98
7	0.908	0.0714	0.937	0.1401	7.18	1.000	0.0002	0.00666	5.94

Fig. 4: Track 4 with 20.0 µL @254 nm

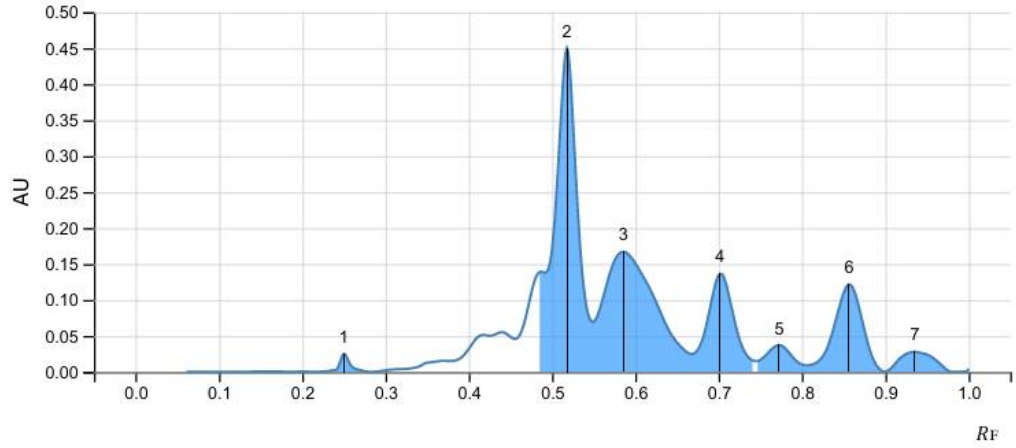
Mid-polarity regions (Rf 0.54–0.71) contributed the greatest proportion of total peak area. Fluorescence scanning at 366 nm revealed additional selectivity toward aromatic and alkaloid constituents.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.492	0.0848	0.515	0.2160	39.91	0.547	0.0379	0.00692	29.14
2	0.547	0.0379	0.585	0.1521	28.10	0.663	0.0205	0.00987	41.55
3	0.665	0.0205	0.700	0.0750	13.86	0.740	0.0092	0.00302	12.73
4	0.742	0.0092	0.769	0.0205	3.78	0.802	0.0063	0.00081	3.41
5	0.802	0.0063	0.852	0.0650	12.01	0.892	0.0015	0.00255	10.72
6	0.892	0.0015	0.944	0.0127	2.34	0.976	0.0000	0.00058	2.46

Fig. 5: Track 1 with 5.0 µL @366 nm

The chromatogram exhibited seven well-resolved peaks at R_F 0.250, 0.518, 0.585, 0.702, 0.773, 0.856, and 0.935. The most prominent component was Peak 2 with a relative area of 36.82%, followed by Peak 3 (30.53%) and Peak 4 (12.89%). Minor constituents were represented by Peaks 6 (12.03%), 5 (3.54%), 7 (3.30%), and 1 (0.90%) (Figure 6). The fingerprint pattern demonstrated clear chromatographic separation of the constituents.

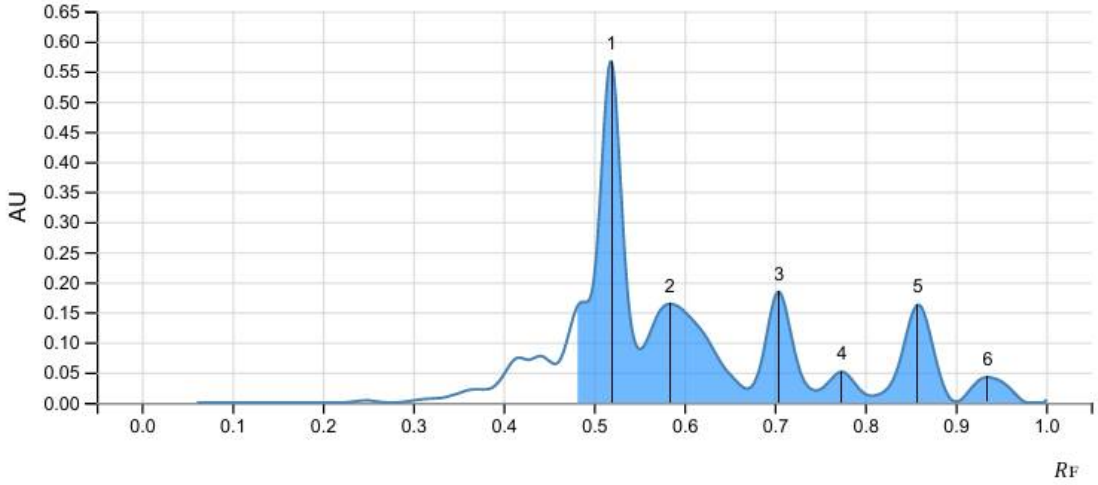


Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.219	0.0000	0.250	0.0249	2.59	0.282	0.0000	0.00036	0.90
2	0.484	0.1377	0.518	0.4497	46.65	0.548	0.0694	0.01467	36.82
3	0.548	0.0694	0.585	0.1668	17.31	0.666	0.0248	0.01216	30.53
4	0.668	0.0246	0.702	0.1363	14.14	0.744	0.0149	0.00513	12.89
5	0.745	0.0148	0.773	0.0370	3.84	0.805	0.0090	0.00141	3.54
6	0.805	0.0090	0.856	0.1213	12.59	0.898	0.0003	0.00479	12.03
7	0.900	0.0003	0.935	0.0278	2.88	0.979	0.0000	0.00131	3.30

Fig. 6: Track 2 with 10.0 µL @366 nm

The chromatogram displayed six distinct peaks at R_F 0.519, 0.584, 0.705, 0.774, 0.858, and 0.935. Among them, Peak 1 was the dominant component with a relative area of 39.72%, followed by Peak 2 (25.70%) and Peak 3 (13.60%). Other constituents included Peaks 5 (13.12%), 4 (4.01%), and 6

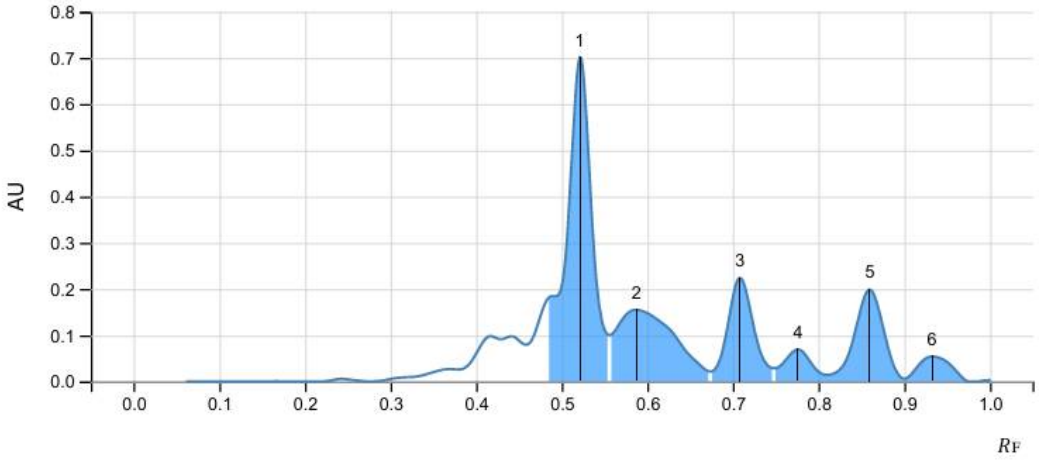
(3.85%) (Figure 7). The chromatographic fingerprint indicated a well-resolved distribution of phytochemical constituents.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.482	0.1610	0.519	0.5665	48.44	0.550	0.0891	0.01945	39.72
2	0.552	0.0890	0.584	0.1638	14.01	0.668	0.0234	0.01259	25.70
3	0.669	0.0230	0.705	0.1838	15.71	0.745	0.0200	0.00666	13.60
4	0.745	0.0200	0.774	0.0512	4.37	0.806	0.0112	0.00196	4.01
5	0.806	0.0112	0.858	0.1622	13.87	0.900	0.0016	0.00643	13.12
6	0.900	0.0016	0.935	0.0421	3.60	0.979	0.0000	0.00188	3.85

Fig. 7: Track 3 with 15.0 µL @366 nm

The chromatogram revealed six well-defined peaks at R_F 0.521, 0.587, 0.708, 0.776, 0.860, and 0.932. The major constituent was Peak 1 with a relative area of 42.04%, followed by Peak 2 (21.58%) and Peak 3 (13.87%). Additional components were observed as Peaks 5 (13.68%), 4 (4.61%), and 6 (4.23%) (Figure 8). The chromatographic fingerprint confirmed consistent separation of the detected compounds.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.484	0.1793	0.521	0.7008	49.99	0.555	0.1006	0.02396	42.04
2	0.556	0.1004	0.587	0.1550	11.06	0.673	0.0216	0.01230	21.58
3	0.674	0.0215	0.708	0.2240	15.98	0.747	0.0287	0.00790	13.87
4	0.748	0.0285	0.776	0.0692	4.93	0.810	0.0141	0.00263	4.61
5	0.810	0.0141	0.860	0.1986	14.17	0.900	0.0061	0.00780	13.68
6	0.900	0.0061	0.932	0.0541	3.86	0.976	0.0000	0.00241	4.23

Fig. 8: Track 4 with 20.0 µL @366 nm

Method performance

Linearity: Peak area increased proportionally with sample load, indicating acceptable linearity for quantitative work.

Precision: Rf variation across tracks remained within a narrow window (±0.01–0.03), confirming chromatographic repeatability.

Specificity: Dual-wavelength scans enhanced discrimination between partially overlapping zones. Some mid-Rf clusters may contain co-eluting phytoconstituents, necessitating confirmation via reference standards.

Overall suitability: The method demonstrates strong potential as a fingerprinting approach and is amenable to full validation.

FT-IR interpretation

The *Hinguvachadi Choorna* spectrum showed:

- Broad O–H stretching (3200–3600 cm^{-1}), consistent with phenolics and alcohols.
- Aliphatic C–H stretches (2850–2950 cm^{-1}).
- Aromatic C=C and conjugated carbonyl bands (1600–1700 cm^{-1}).
- Strong absorption in the 1000–1300 cm^{-1} region, attributable to C–O stretching in glycosides, ethers and resin constituents.

These signals corroborate the presence of Piper-derived alkaloids, asafoetida volatiles and other polyphenolic components detected chromatographically.

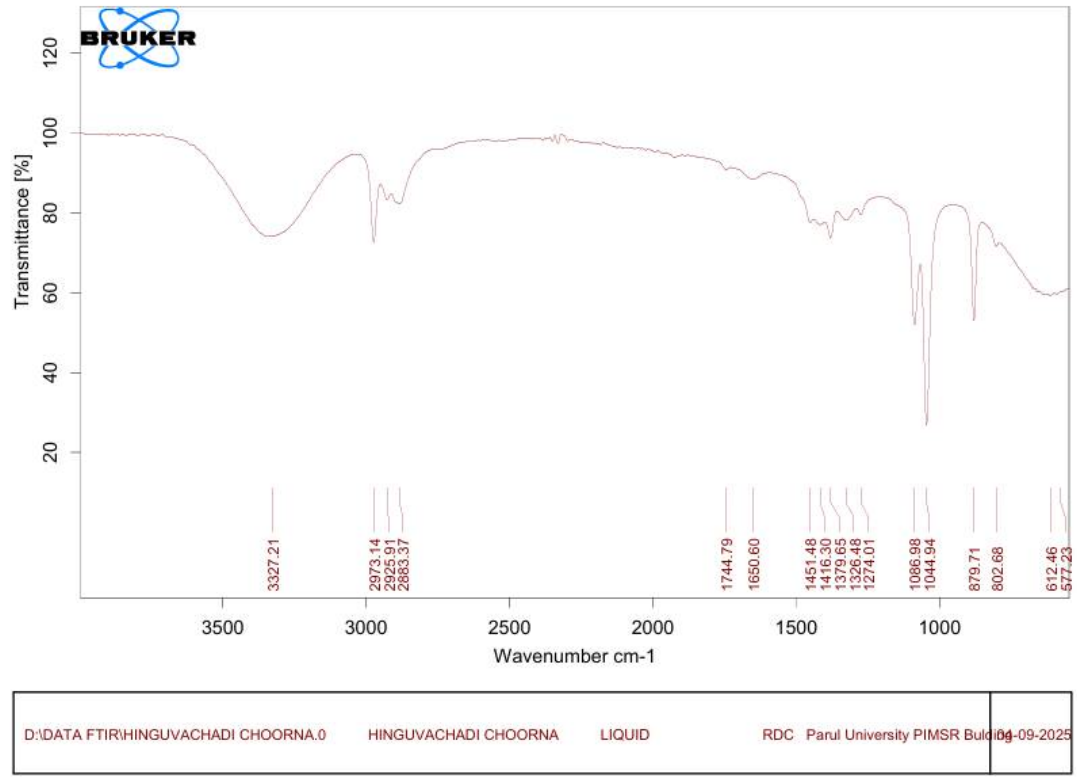


Figure 9: *Fourier Transform Infrared (FT-IR) spectrum* of Hinguvachadi Choorna showing characteristic absorption bands corresponding to major functional groups present in the formulation.

DISCUSSION

The chromatographic fingerprints obtained in the present study represent a consistent and reproducible profile for DYS-FIZZ tablet formulation derived from Hinguvachadi Choorna. The number, position, and relative abundance of peaks provide a basis for batch-to-batch comparison.

These are not included here, but the following should be added to the method to make it fully regulatorily suitable:

• Co-chromatography with authentic piperine and other expected markers.
• Quantitative calibration curves to determine linearity ranges and sensitivity parameters.
• Recovery studies to establish accuracy.

• Hyphenated coupling (HPTLC–MS or LC–MS) for confirmation in the structural characteristics of ambiguous peaks.

Its integration means that FT-IR data provides an orthogonal analytical dimension that enhances general confidence in herbal identity and quality assessment..

In the HPTLC fingerprint, there is evidence of a prominent mid-Rf spot (approximately 0.54-0.71) which is likely to include alkaloid components of Piper species (piperine, piperamides); established digestive, anti-inflammatory, and bioavailability-boosters, as well as secondary mid- to high-Rf spots likely to include asafoetida (Ferula) volatile/resinous components (ferulic acid, sulfides, terpenoids), and other formulation phenolics. These tentative assignments must, of course. [3]

HPTLC Report

- The TLC was performed on silica gel 60 F_{254} plates using mobile phase toluene: ethyl acetate: formic acid (6:3:1). It was scanned at 254 nm (absorbance) and 366 nm (flu
- Over a range of applied sample volumes (5 → 20 µL) there were 6-7 major peaks found in a chromatogram. Examples of Rf/area-% for 20 µL loadings from Track 4 are:

Peak A ≈ Rf 0.315 – 0.371

Major Cluster: Rf = 0.50-0.71 (Group several peaks together; take the largest share, e.g. roughly 0.540-0.666, 0.668-0

Mid-high Rf bands: Rf 0.75-0.91 (Two or three peaks with ~30–40% area)

Probable chemical assignments

1) Major mid-Rf cluster (≈0.50–0.71) - likely: piperine and related piperamides

Evidence:

- Formulations of Hinguvachadi often include Piper longum/ Piper nigrum (Pippali/Maricha); its chief alkaloid is piperine, while in most HPTLC techniques piperine is spotted in the mid-Rf zones on the silica. [4]
- Published HPTLC methods using toluene/ethyl acetate mobile phase systems (and very similar modifications thereof) feature compact spots in mid-Rf region referencing piperine, and is an established standard in Ayurvedic churna QC as an HPTLC piperine marker. [4]In this run the mid-Rf region contributes the largest combined area (≈27–49% for the dominant single mid-Rf peak and additional adjacent peaks), consistent with a major alkaloidal component.

Pharmacology / mechanism:

Bioavailability enhancer: Piperine, an inhibitor of intestinal/hepatic metabolism of drugs (CYPs, UDP glucuronosyl transferases), affects membrane dynamics, resulting in improved absorption of co-administered compounds. [5]

Anti-inflammatory and antispasmodic actions: Anti-inflammatory and antispasmodic properties: Piperine modulates the synthesis of pro-inflammatory mediators such as TNF α, IL 6, COX 2, and iNOS and has antispasmodic, analgesic, and prokinetic activities. [3]

2) Mid-high Rf bands (≈0.75–0.91) - likely: volatile oils / terpenoids and non-polar resin fractions (Ferula / ginger / other volatile constituents)

Evidence:

Asafoetida (Ferula spp.) resinous fraction including coumarins (sesquiterpenes), sulfides, terpenoids; phenolic esters: ferulic esters. Such compounds were expected to move towards the higher Rf range in non-polar solvent systems, or spot in the middle to upper Rf range in solvent systems of higher polarity.[6] There are several peaks in the current run whose amplitudes occupy the 0.75-0.91 window, thus representing a large area associated with less polar fractions/ volatiles and terpenoids.

Pharmacology / mechanism:

Ferula/asafoetida constituents (e.g., ferulic acid esters, monoterpenes, sulfur compounds) possess antispasmodic and carminative activity (GI smooth muscle relaxation), exert an antimicrobial action against GI pathogens, and exhibit antioxidant activity mechanisms consistent with the alleviation of bloating and flatulence. [7,8]

3) Low-Rf zone (~0.28–0.37; smaller area) likely: more polar phenolics / glycosides

Evidence:

small spot around Rf ~ 0.31-0.37: this is observed only on some samples, and would represent localized areas on silica probes, possibly related to terminalia/punica or other compounds used in preparation. O-H, and C-O FT-IR peaks consistent with presence of phenolics or glycosides.

Pharmacology / mechanism:

Phenolics / tannins contribute antioxidant, astringent, and antimicrobial properties supporting intestinal mucosal protection and modulation of gut flora.

Mechanistic overview

Immediate digestive / carminative effects

Asafoetida and other spicy oil sulfides and irritants act as a salian agent and stimulate smooth muscle relaxation and gastric secretion. This is possibly due to smooth muscle relaxation and mild prokinetic properties.[7,10]

Anti-spasmodic and analgesic activity

Piperine and terpenoids display regulation of inflammatory mediators and nociceptive pathways (e.g., COX-2 and prostaglandins), which may exert antispasmodic action for abdominal. [3]

Antimicrobial modulation of gut flora

Volatile sulfur compounds and phenolics can inhibit gas-producing bacteria or reduce dysbiosis; this is believed to help alleviate gas in herbal medicine.[9]

Bioenhancement and synergistic actions

Piperine functions by increasing the intestinal absorption of co-ingredients (and co-administered substances), by inhibiting enzyme activity thereby increasing permeability, thereby enhancing the effect of other actives, due to its pharmacokinetic properties.[5]

Antioxidant / mucosal protection

Ferulic acid and phenolics scavenge free radicals and protect mucosa, helping recovery from irritative GI conditions. [7]

Role of FT-IR

FT-IR spectra feature broad O-H vibrations, C=O/aromatic and C-O bands indicative of phenolics, esters, and resin compounds. This is an orthogonal verification of the presence of phenolic/resinous and aromatic materials as indicated by the HPTLC analysis.

Degree of confidence & required confirmatory work

Confidence level: *Moderate for class assignments* (i.e., "this region contains piperine-type alkaloids" and "these other bands look like resin/volatile terpenoids/phenolics"); *low for specific molecule identification* (cannot assign a peak uniquely to piperine, ferulic acid, etc., from Rf + FT-IR alone).

Minimum confirmatory experiments I recommend (in order):

1. **Co-chromatography with authentic standards** (piperine, piperlongumine if relevant, ferulic acid, representative terpenoids). Run standards under the exact same HPTLC conditions and compare Rf and densitogram shape.
2. **Derivatization**: Dragendorff's reagent (alkaloids), anisaldehyde-H_2SO_4 (terpenoids/sterols), or vanillin can help class-visualize zones.
3. **HPTLC–MS (or scraping + LC-MS)** of individual spots to obtain molecular mass and fragmentation for unambiguous ID. [10]

Quantitative calibration curves for any selected marker(s) to report content (µg/spot or mg/g) and perform ICH-style validation (linearity, precision, accuracy, LOD/LOQ). VisionCATS data already show linear area vs. load behavior a good start.

Safety & interactions

As piperine is a strong inhibitor of drug-metabolizing enzymes, its final formulation (or high piperine content) may lead to increased exposure of co-administered drugs (such as therapeutic index drugs CYP3A4 and/or UGTs). This interaction description should be flagged in product labeling, and interaction testing may be required if the product is likely to be used extensively with drugs. [5]

Recommendations

1. Addition of reference standards (e.g., piperine, ferulic acid derivatives, resin markers) for compound-specific identification.
2. Utilize derivatization agents-aerialdehyde-sulfuric acid and Dragendorff-to increase the resolution power of chemotypes.
3. Perform robustness testing by varying mobile phase composition, chamber saturation time, and detection wavelengths.
4. Formulate and validate one standard operating procedure (SOP) for regular quality control of *Hinguvachadi Choorna* and its tablet forms.

CONCLUSION

The HPTLC method developed for *Hinguvachadi Choorna* and its commercial tablet dosage form provides reproducible chromatographic fingerprints with clearly defined phytochemical zones. The technique is suitable for routine quality assessment and can be advanced to a fully validated assay following the inclusion of reference standards and expanded validation experiments. FT-IR analysis provides evidence for multi-component chemical nature in the formulation, thus complementing the profiling analysis. This

combined analysis strategy provides a strong base for the standardization of the polyherbal formulation of Ayurvedic drugs.

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