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# "ANALYTICAL FINGERPRINTING (HPTLC, HPLC AND GC-MS) FOR QUALITY CONTROL OF CLASSICAL AYURVEDIC FORMULATIONS: A SCOPING REVIEW"

**Tushar Tapadiya**[1]

**AFFILIATIONS:**
1. Research Assistant, IRA Consultancy and Research Organisation, Bhosari MIDC, Pune, Maharashtra, India

**CORRESPONDENCE:**
Tushar Tapadiya
**EMAILID:**
tushartapadiya11@gmail.com

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**ABSTRACT**

**Background:** Classical Ayurvedic formulations are complex mixtures of many ingredients, often transformed by processing. Single-marker tests cannot capture this complexity, and chromatographic fingerprinting has become the main tool for establishing identity, consistency and authenticity. **Objective:** To map how high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS) and related techniques have been applied to classical Ayurvedic formulations, and to identify methodological gaps. **Methods:** A structured narrative review following scoping principles, charting published fingerprinting and marker-quantification studies by dosage form, technique, markers and validation. **Results:** HPTLC is the most widely used technique, applied to *churna*, *vati*, *ghrita* and *kwatha* for both fingerprinting and validated quantification of markers such as gallic acid, piperine and bacosides. HPLC and UPLC-QToF-MS methods have been used for *arishta*, *kwatha* and tablets, and GC-MS has documented the formation and loss of compounds during fermentation. Studies of marketed products show large variation, for example bacoside A from about 4% to 32% w/w across four brands of a *Brahmi* product. Gaps include frequent tentative peak assignment without reference standards, limited method validation, lack of acceptance criteria for fingerprints, and little linkage between chemical profiles and biological activity. **Conclusion:** Fingerprinting is now feasible for most classical formulations. Validated methods, reference standards, defined acceptance criteria and activity-linked markers are needed for fingerprints to become regulatory specifications.

**KEYWORDS:** HPTLC; HPLC; GC-MS; Chemical fingerprint; Marker compounds; Quality control; Polyherbal formulations

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## INTRODUCTION

A classical Ayurvedic formulation may contain ten, twenty or more ingredients, prepared by decoction, fermentation, cooking in fat or trituration with herbal juices. Its therapeutic value is thought to derive from the combination rather than from any single constituent. Quality control based on one marker compound, or on general physicochemical parameters such as ash and extractive values, cannot confirm that all ingredients are present in the right proportions or that the process was carried out correctly[1].

Chromatographic fingerprinting addresses this problem by recording a characteristic pattern of many constituents. A fingerprint can confirm identity, detect adulteration or omission of ingredients, compare batches, and track changes during processing and storage. International guidance on herbal quality control accepts chromatographic fingerprints as a component of identity testing[2], and the Ayurvedic Pharmacopoeia of India includes thin-layer chromatographic identity tests in many monographs[3]. This review maps how fingerprinting techniques have been applied to classical Ayurvedic formulations and what is needed for them to support regulation.

## METHODS OF THE REVIEW

This review follows scoping principles. Studies were identified through PubMed, PubMed Central, DOAJ, Crossref-indexed records and journal archives using the terms HPTLC, HPLC, GC-MS, LC-MS, fingerprint, marker, standardisation and Ayurvedic formulation, together with names of dosage forms. Studies applying chromatographic or spectrometric fingerprinting or marker quantification to classical formulations were charted by dosage form, technique, markers, validation and purpose. Formal screening counts were not recorded.

## TECHNIQUES

**Table 1. Fingerprinting techniques used for Ayurvedic formulations**

| Technique | Strengths | Limitations | Typical uses |
|---|---|---|---|
| HPTLC | Parallel analysis of many samples; visual fingerprints; derivatisation for different compound classes; low cost | Lower resolution than HPLC; quantification requires careful validation | Identity, batch comparison, ingredient tracing, marker quantification |
| HPLC-UV/DAD | Good resolution and quantification; widely available | Needs chromophores; long run times for complex mixtures | Marker assay, fingerprints of decoctions and fermented products |
| UPLC or LC-MS (QToF) | High resolution and structural information; detects many compounds | Cost; data complexity; putative identifications without standards | Comprehensive profiling, authentication |
| GC-MS | Volatile and semi-volatile compounds; library identification | Not suitable for non-volatile or thermolabile compounds without derivatisation | Essential oils, fermentation products, fatty acids in *sneha* |

General characteristics drawn from the reviewed literature and standard texts [1,4].

HPTLC is particularly suited to Ayurvedic formulations because it allows many samples, including each raw ingredient and the finished product, to be run side by side on one plate and visualised under different conditions[4]. LC-MS techniques can detect hundreds of compounds and are increasingly used for comprehensive profiling, while GC-MS is the method of choice for volatile constituents and for fermented and lipid-based products.

## APPLICATIONS BY DOSAGE FORM

**Table 2. Representative fingerprinting and marker studies of Ayurvedic formulations**

| Formulation | Dosage form | Technique | Purpose and findings |
|---|---|---|---|
| *Mahasudarshan churna* | *Churna* | HPTLC | Validated quantification of major biomarkers |
| *Sulaharan Yoga* | Tablet | TLC and HPTLC | Detection of gallic acid, gingerols, piperine, brucine, strychnine and umbelliferone in in-house and |

| | | | |
|---|---|---|---|
| | | | marketed products |
| *Brahmi ghrita* | *Ghrita* | HPTLC | Fingerprint with 9, 6 and 8 spots under different detection conditions; tentative bacoside assignment |
| *Brahmi* capsules, four brands | Capsule | UV assay of bacoside A | Bacoside A 4.3–32% w/w; significant batch differences |
| *Nayopayam kwatha* | *Kwatha* | Multiple techniques | Standardisation protocol |
| *Pathyashadangam kwatha* | *Kwatha* | HPLC and HPTLC | Marker-based standardisation |
| *Cirivilvadi kashaya* | *Kwatha* | Phytochemical indicators | Differences between fresh and commercial products |
| *Abhayarishta* | *Arishta* | HPLC-DAD | Chemical changes during fermentation; standardisation |
| *Balarishta*, *Chandanasava* | *Arishta*, *asava* | GC-MS | Compounds retained, lost and formed during fermentation |
| *Divya-Swasari-Vati* | Tablet | UPLC/QToF-MS and HPLC-DAD | Rapid comprehensive quality evaluation |
| Polyherbal formulation and ingredients | Mixed | Spectrophotometry and HPTLC | Comparative profiling of formulation against ingredient plants |

**1. Powders and tablets**

For *churna* and tablets, fingerprinting is used to confirm the presence of each ingredient and to quantify key markers. A validated HPTLC method quantified major biomarkers in *Mahasudarshan churna*[5], and TLC and HPTLC detected markers of several ingredients in *Sulaharan Yoga* tablets[6]. Combined UPLC/QToF-MS and HPLC-DAD methods have been developed for rapid quality evaluation of a tablet formulation[14].

**2. Decoctions**

For *kwatha*, fingerprinting helps to standardise a preparation that is often made fresh or in different ways. Protocols for *Nayopayam kwatha* and *Pathyashadangam kwatha* used HPTLC and HPLC[9,10], and differences in phytochemical indicators between fresh and commercial *Cirivilvadi kashaya* were detected[11].

**3. Fermented products**

Fermentation changes the chemical profile, so fingerprints of *arishta* and *asava* must be based on the finished product rather than the raw herbs. HPLC tracked chemical changes during fermentation of *Abhayarishta*[12], and GC-MS showed that in *Chandanasava* 30 compounds disappeared and 32 new ones formed during fermentation[13]. GC-MS has also been used to confirm the absence of methanol in fermented products[16].

**4. Lipid-based products**

For *ghrita* and *taila*, the lipid matrix complicates analysis. HPTLC fingerprints of *Brahmi ghrita* have been reported, together with fat constants such as acid, saponification and iodine values[7].

**5. Mineral-containing products**

Chromatographic fingerprints address only the organic fraction. For *bhasma* and *rasa* preparations, they must be complemented by X-ray diffraction, elemental analysis and other techniques that characterise the inorganic fraction[17,18].

## EVIDENCE OF VARIABILITY

Fingerprinting and marker assays have repeatedly shown that products sold under the same name differ. In four marketed *Brahmi* capsule products, bacoside A ranged from about 4% to 32% w/w, with significant differences between batches of the same brand, and several products failed pharmacopoeial limits for ash and moisture[8]. Differences between fresh and commercial decoctions have also been detected[11]. Such variability is precisely what fingerprint specifications are intended to control.

## METHODOLOGICAL GAPS

- **Tentative assignments.** Peaks or bands are often assigned to compounds on the basis of retention factor or retention time alone, without co-chromatography with reference standards; for

example, a single spot was assigned to bacosides in a *ghrita* fingerprint[7].

- **Validation.** Many studies do not report specificity, linearity, precision, accuracy, limits of detection and quantification, or robustness.
- **Acceptance criteria.** Fingerprints are often presented descriptively, without defined criteria for how similar a batch must be to a reference.
- **Reference materials.** Authenticated reference samples of raw materials and reference batches of formulations are scarce.
- **Link to activity.** Markers are usually chosen for availability rather than biological relevance, so a compliant fingerprint does not guarantee activity.

## RECOMMENDATIONS

- Develop validated, transferable methods for priority formulations, combining HPTLC for routine identity with HPLC or LC-MS for quantification.
- Establish reference standards and reference batches, prepared according to classical procedures from authenticated raw materials.
- Define acceptance criteria, such as the presence of specified bands or peaks and similarity indices relative to a reference fingerprint.
- Include fingerprints of each raw ingredient alongside the finished product to confirm the presence of all ingredients.
- Select markers linked to biological activity wherever possible, and use chemometric methods to relate fingerprints to activity.

## CONCLUSION

Chromatographic fingerprinting, led by HPTLC and increasingly supported by HPLC, LC-MS and GC-MS, is now applied to all major classes of Ayurvedic formulations and has revealed substantial variability among marketed products. To function as a regulatory tool, fingerprinting needs validated methods, reference standards, defined acceptance criteria and markers that reflect biological activity. Achieving these would bring the quality control of classical formulations closer to the standards expected for modern herbal medicines.

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