



# Standardizing Traditional Polyherbal Decoctions by High-Performance Thin-Layer Chromatography Fingerprinting

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## Article Info

Volume 1, Issue 1, 2026

[doi: 10.62715/ijep.2026.v1.i1.pp14-19](https://doi.org/10.62715/ijep.2026.v1.i1.pp14-19)

Received: 23 December 2025

Accepted: 13 February 2026

Published: 06 April 2026

## Abstract

Decoctions of several herbs boiled together are the commonest dosage form in Ayurveda, traditional Chinese medicine and many folk traditions, and they are among the hardest herbal preparations to standardize. The product is an aqueous mixture of hundreds of compounds from several species, altered by heat, with no single constituent that represents the whole. Chromatographic fingerprinting treats the entire pattern as the quality attribute, and high-performance thin-layer chromatography (HPTLC) is well suited to it: plates are run in parallel, results are images that can be compared by eye, and the technique is recognised in pharmacopoeias. This article proposes a stepwise procedure for developing an HPTLC fingerprint for a traditional polyherbal decoction. It covers the definition of a reference decoction, sample preparation from an aqueous matrix, the use of single-herb and omission decoctions to assign zones to ingredients, standardized chromatographic conditions with a system suitability test, validation, and the setting of acceptance criteria from batch-to-batch variation. The procedure is assembled from pharmacopoeial chapters, guidelines and the published literature. It is a proposal and has not been validated on a particular formulation here. Its limits, especially the gap between chemical consistency and therapeutic equivalence, are discussed.

**Keywords:** HPTLC fingerprinting, Polyherbal decoction, Herbal quality control, Method validation, Traditional medicine, Chemometrics

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## 1. Introduction

A decoction is made by boiling coarsely cut plant material in water and straining it. Ayurvedic texts call the form *kwatha* or *kashaya*, Chinese medicine calls it *tang*, and household practice everywhere knows it as a strong tea. Most classical decoctions combine several herbs, on the reasoning that the ingredients reinforce or moderate one another. Pharmacology gives that reasoning some support: interactions between constituents of plant mixtures, including effects on solubility and absorption, are documented (Williamson, 2001; Wagner and Ulrich-Merzenich, 2009).

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The same complexity defeats conventional quality control. A single-marker assay confirms that one compound from one herb is present in the right amount and says nothing about the other ingredients. Regulators accept that for herbal products the whole preparation is the active substance ([European Medicines Agency, 2011](#)), and the World Health Organization's quality control methods include chromatographic identity tests for that reason ([World Health Organization, 2011](#)). Fingerprinting extends the idea: the complete chromatographic pattern is compared with that of a reference, so that missing, substituted or degraded ingredients show up as changes in the pattern ([Liang et al., 2004](#); [Xie et al., 2006](#)).

This article sets out a procedure for building such a fingerprint for a polyherbal decoction by HPTLC. It is written for laboratories in small manufacturing units, teaching hospitals and pharmacopoeial programmes that already hold the basic equipment. The procedure is proposed and is not accompanied by an application to a specific formula.

## 2. Why HPTLC Suits Decoctions

Thin-layer chromatography has been the standard identity test for herbal drugs since the atlases of Stahl and of Wagner and Bladt established characteristic patterns for hundreds of drugs ([Wagner and Bladt, 1996](#)). HPTLC is the instrumental form of the technique: plates with fine, uniform silica particles, automated band-wise application, controlled development and electronic documentation ([Reich and Schibli, 2007](#)). The European Pharmacopoeia and the United States Pharmacopeia now carry general chapters that fix the parameters of the method for botanical identification ([Council of Europe, 2019](#)).

Three properties matter for decoctions. Many samples run side by side on one plate under identical conditions, so that a batch, the reference and the single herbs can be compared directly. The plate can be viewed under short-wave and long-wave ultraviolet light and again after derivatization, which gives several fingerprints from one run and reveals compound classes, such as terpenoids and sugars, that absorb poorly. The stationary phase is used once, so crude aqueous extracts rich in tannins and polysaccharides cannot foul a column. Against these stand lower resolving power than column chromatography and sensitivity to humidity and operator practice, which the standardized method is designed to contain. Comparative reviews of fingerprinting techniques treat the planar and column methods as complementary ([Tistaert et al., 2011](#)).

## 3. The Proposed Procedure

The procedure has six steps, summarised in Table 1. Figure 1 shows the material flow from herbs to evaluated fingerprint with the control applied at each stage.

| Table 1: Steps of the proposed fingerprinting procedure and the output of each |                                                                                    |                                                     |
|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------|-----------------------------------------------------|
| Step                                                                           | Action                                                                             | Output                                              |
| 1. Reference decoction                                                         | Prepare the formula from authenticated herbs by the classical method, in replicate | Reference decoctions and written preparation record |
| 2. Test solution                                                               | Convert the aqueous decoction into a solution fit for application                  | Sample preparation method                           |
| 3. Zone assignment                                                             | Run single-herb and omission decoctions beside the full formula                    | Map of zones by source herb                         |
| 4. Chromatography                                                              | Fix plate, mobile phases, development and detection; define suitability test       | Standard operating procedure                        |
| 5. Validation                                                                  | Test specificity, precision, stability and robustness                              | Validation report                                   |
| 6. Acceptance criteria                                                         | Describe the fingerprint and its permitted variation from many batches             | Specification with reference images                 |

### 3.1. Step 1: Define the reference decoction

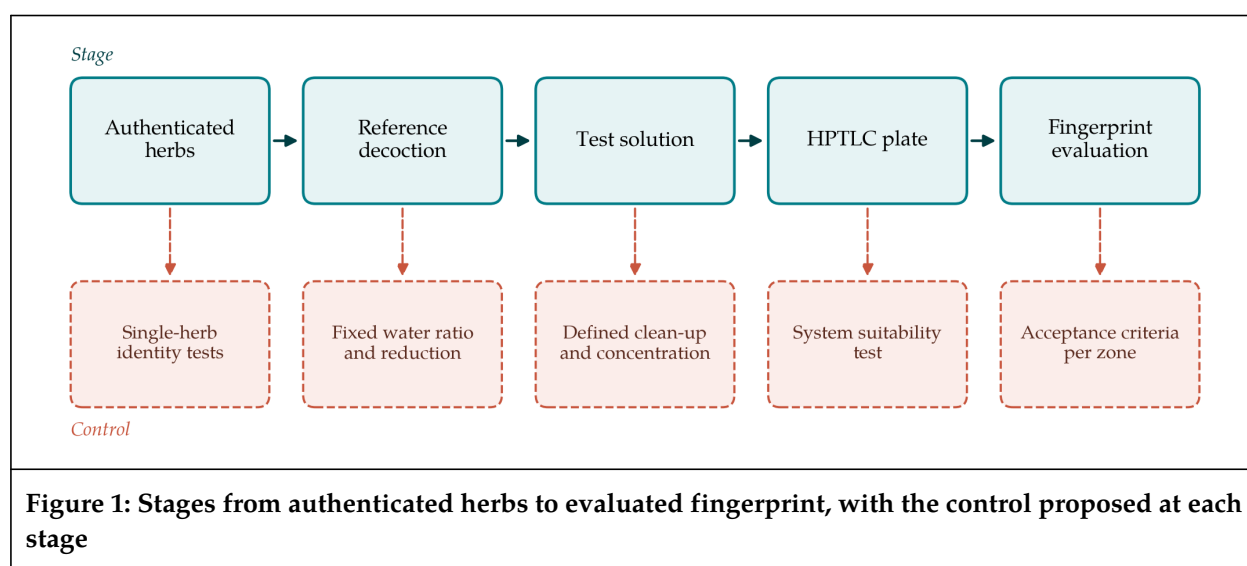
The fingerprint can be no better than its reference. Each ingredient is first authenticated as a single herb, botanically and by its own pharmacopoeial identity test, using material from more than one source. The decoction is then prepared exactly as the governing text or monograph directs. For Ayurvedic formulas the official pharmacopoeia gives the composition and method ([Government of India, 2007](#)); the classical instruction is to boil the coarse powder in a stated multiple of water until a stated fraction remains. Particle size, vessel, heating rate, the endpoint of reduction and the time before straining are written down, because each affects what is extracted. Several independent preparations are made so that the variation of the process itself is known before any commercial batch is judged.

### 3.2. Step 2: Prepare the test solution

A decoction cannot simply be spotted on silica: water spreads the band, and sugars and salts distort the chromatogram. Three routes are in common use. The decoction may be dried under reduced pressure or freeze-dried and the residue taken up in methanol, which leaves most polysaccharides behind. It may be partitioned against ethyl acetate and then *n*-butanol to give fractions of medium and high polarity. Or it may be passed through a reversed-phase solid-phase extraction cartridge. Whichever route is chosen must be applied identically to reference and test samples, with fixed volumes and concentration factor, and stated in the method. Two test solutions of different polarity often serve a complex formula better than one.

### 3.3. Step 3: Assign zones to ingredients

A fingerprint is far more useful when each main zone can be traced to a herb. Decoctions of every single ingredient are prepared under the same conditions and applied next to the full formula. In addition, omission decoctions are made, each lacking one ingredient. A zone that disappears from the corresponding omission track and appears in the single-herb track is assigned to that herb. Where reference substances exist, for example gallic acid and ellagic acid for the *Terminalia* fruits of *Triphala*, they are co-chromatographed to anchor positions. The comparison also shows what boiling together does: zones present in single-herb decoctions may weaken or vanish in the mixture through hydrolysis, precipitation with tannins or loss of volatiles, and new zones may appear.



### 3.4. Step 4: Fix the chromatographic conditions

The pharmacopoeial chapters prescribe the framework: glass plates coated with silica gel 60 F<sub>254</sub>, samples applied as narrow bands at a fixed distance from the lower edge, development in a saturated twin-trough chamber over a fixed distance after conditioning the plate at controlled relative humidity, and documentation under ultraviolet light at 254 nm and 366 nm and under white light after derivatization

(Council of Europe, 2019). Within that framework the analyst selects mobile phases and reagents. Because a decoction spans a wide range of polarity, two developing systems are usually needed. Derivatization is matched to the chemistry expected: anisaldehyde-sulfuric acid as a general reagent, natural products reagent for flavonoids and phenolic acids, Dragendorff reagent for alkaloids. A system suitability test, in which named reference substances must separate and appear at stated positions, is run on every plate.

### 3.5. Step 5: Validate the method

A qualitative identification method is validated differently from an assay. The general guideline on analytical validation names specificity as the essential characteristic of an identity test (ICH, 2005), and Reich et al. (2008) have adapted the scheme to HPTLC identification of botanicals. Specificity is shown by the ability of the fingerprint to distinguish the formula from the omission decoctions and from formulas made with known adulterants or substitutes. Precision is shown by repeatability on one plate, between plates and between days and analysts, expressed as the variation in position of marker zones. Stability of the analyte in solution and on the plate is checked, and robustness is examined by small deliberate changes in humidity, saturation time and developing distance.

### 3.6. Step 6: Set acceptance criteria

The specification describes, for each detection mode, the zones that must be present, their position, colour and approximate intensity relative to reference substances, and any zone whose presence indicates a fault. It is illustrated with reference images. The criteria are drawn from many batches made with herbs of different origin and harvest, so that natural variation is accepted and abnormal variation is not. Where a visual description proves too coarse, the images can be converted to intensity profiles and compared numerically. Similarity measures and principal component analysis are widely used for chromatographic fingerprints (Wold et al., 1987; Tistaert et al., 2011), and quantitative evaluation of planar chromatograms by scanning densitometry is a mature technique (Spangenberg et al., 2011).

## 4. Fitting the Procedure to Practice

Three practical matters deserve attention. Decoctions are traditionally prepared fresh, and the marketed forms are concentrates, granules or preserved liquids. The fingerprint developed on the freshly made reference therefore becomes the standard against which processed forms are judged, and differences caused by concentration or drying need to be understood and recorded, not hidden by adjusting the criteria. Second, zones that prove discriminating but unidentified can be characterised by eluting them directly from the plate into a mass spectrometer, a coupling that is now routine (Morlock and Schwack, 2010). Third, comprehensive fingerprinting, in which the same plate serves for identity, detection of adulterants and semi-quantitative limits, has been shown for single-herb products (Frommenwiler et al., 2019) and is a reasonable aim for decoctions once the basic method is stable.

Cost and throughput are part of the case for the method. Once the conditions are fixed, a single plate carries the reference decoction, the suitability standards and a dozen or more batch samples, and the images form a permanent record that an inspector can read without specialist software. For a small manufacturer the main investment lies in the application and documentation devices and in training; consumables are modest by comparison with column methods. The discipline lies less in the instrument than in holding every parameter constant from plate to plate.

## 5. Limitations and What Remains to Be Shown

The procedure is a proposal. It has not been carried through on a named formulation in this article, and the effort it requires, particularly the omission decoctions for formulas with ten or more ingredients, has not been measured. For such formulas a reduced design, in which herbs are omitted in botanical or chemical groups, may be necessary, at some cost in interpretability. An interlaboratory exercise on one widely used decoction would show how transferable the fingerprints are in practice.

HPTLC has intrinsic limits. A plate resolves a few dozen zones at best, so minor constituents and co-migrating compounds go unseen, and an ingredient that contributes little extractable matter may leave

no distinct zone at all. Inorganic contaminants, pesticide residues and microbial quality lie outside the method and require their own tests. Most fundamentally, a consistent fingerprint demonstrates chemical consistency and nothing more. Whether two batches with matching patterns are therapeutically equivalent depends on which constituents matter, and for most traditional decoctions that is not known.

## 6. Conclusion

Polyherbal decoctions can be brought under meaningful chemical control without reducing them to a single marker. The route proposed here starts from a carefully defined reference decoction, uses single-herb and omission preparations to tie the pattern to its ingredients, runs the chromatography under pharmacopoeial conditions with a suitability test, and derives acceptance criteria from observed batch variation. Its validation on real formulas, and its transfer between laboratories, are the next tasks.

## Conflict of interest

To be declared by the author(s) before publication.

## Funding

To be declared by the author(s) before publication.

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