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Chemical Standardization of Polyherbal Formulations Using Marker Compounds and Advanced Analytical Techniques

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Abstract

Chemical standardization of polyherbal formulations should be more than just the identification of well-known phytochemicals. Markers must aid in making well-defined decisions regarding the identity of ingredients, quantitative consistency, processing or stability in chemically complex matrices. The 15 purposively selected formulation studies (2009–2026), analyticalmethod research, and authoritative quality guidance are used to examine these decisions in this critical narrative review. Targeted literature consultation was finalized on 17 October 2026; evidence was accessible only via abstracts or indexed publisher summaries is differentiated from accessible full text. The review is a comparison of high-performance thin layer chromatography, liquid chromatography and optical detection, targeted and high-resolution mass spectrometry, quantitative nuclear magnetic resonance and multivariate analysis. In the case of multicomponent, one marker can be used for quantitative analysis, and this is regarded as a calibration strategy instead of an instrumental platform. Examples of the existing applications of multi-marker assays, prescription-ratio investigations, chemical–biological fingerprinting and integrated stability assessment are illustrated by the evidence. But the abundance of the markers, similarity of the fingerprints and apparent chemometric separation are not sufficient for the complete botanical authenticity or product equivalence. Validating reporting internally in a coherent manner, matrix dependent response, the difference between technical replicates and independent batches, and explicit confidence in compound identification are critical issues. A proposed decision-oriented framework is linked to authenticated starting materials, challenged marker panels, routine testing, investigation of atypical profiles and lifecycle monitoring. External validation, interlaboratory transfer, stability relevant measurements and clear reporting of analytical and operational burden are priorities. The framework is a literature-based recommendation that needs to be evaluated in the future; chemical compliance is separate from contaminant control, biological activity and clinical efficacy.

Keywords: Polyherbal formulations; chemical standardization; marker compounds; HPTLC; LC–MS/MS; chromatographic fingerprinting; analytical validation; chemometrics.

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1. Introduction

Polyherbal formulations are made up of several botanicals, and the chemical contributions of each of these should be taken into account when making up the final product. A quality-control procedure should define the material under consideration, how the sample reflects the material of the batch, and which measurable properties are significant for the product. Macroscopic and microscopic identification, physicochemical tests and chemical assays are all concerned with various aspects of this problem. WHO guidance therefore does not consider a marker assay to be a replacement for all other controls; it is a part of a control system for herbal-material identity testing and quality assessment. [1]

The complexity is not only a result of the number of ingredients, but also of the overlapping constituents and effects of preparation. A compound can be found in multiple plants and the concentration of the compound in a finished product can be influenced by the proportions of the ingredients, extraction, processing and the unit of measure used to report the concentration. WHO recommendations for the processing and handling of herbs emphasize documented procedures. When the botanical source or the method of manufacture or the reporting basis is not known, these considerations make it difficult to interpret a chemically precise measurement. [2]

Standardization using markers is already in use, as is combined analytical approaches. Triphala was investigated with the help of a three-marker reversed-phase high-performance liquid chromatography (RP-HPLC) method and untargeted and targeted profiling were linked in formulation with Adhatoda vasica. In addition, HPLC, high performance thin layer chromatography (HPTLC) and high resolution mass-spectrometric characterization with marker-based stability component have been performed on AYUSH SG-5. [3, 4, 5]

The central analytical difference is that between measuring a constituent and making a defensible decision about a formulation. A marker can be quantified without identifying all the ingredients and it can be quantified reproducibly. The fingerprint may look like a reference, but there are significant concentrations that vary. A statistical model can be used to divide samples used in its construction, but not be able to detect a future nonconforming batch. This review will focus on those differences in terms of rationales for markers, sample preparation, complementary analytical platforms, and evidence that would be required for clinical interpretation.

It sets out to compare the information provided by a set of analytical strategies, to look at how published study designs limit the conclusions drawn from them, and to suggest a strategy for using measurements to inform product decisions. The scope covers ayurvedic preparations, unani preparations and some multibotanical products from East Asian countries as methodological comparisons. It does not evaluate clinical effectiveness, give universal acceptance limits, or cover all analytical methods or traditional-medicine systems.

2. Literature search strategy and review scope

This is a critical narrative review and NOT a systematic review or meta-analysis. A web-based literature search and source check were carried out, with a particular focus on 17 October 2026. They pulled PubMed-indexed articles, PubMed Central articles, and primary publisher pages (such as Springer Nature, Wiley, SciELO, and others). Each of the official WHO, ICH, EMA,

FDA and Indian Pharmacopoeial resources have been consulted separately. This workflow didn't include access to subscription databases like Scopus or Web of Science.

Formulation terms were used along with analytical concepts like “polyherbal formulation standardization HPLC HPTLC multi marker”, “polyherbal formulation chemical standardization LC MS 2025 2026 multi marker”, “qNMR Shenmai injection quality control” in searches. Identified papers were checked using exact-title and DOI searches and references and related-article links were followed. There was no minimum year of publication set. The final formulation examples cover the period 2009-2026; some of the methodological papers were kept if they were directly relevant to the older examples. The representative queries and the limitations of the sources of information are recorded in Supplementary File S1.

The comparative evidence table only included formulations with a name and analytical measures that included one or more of the following: marker content, fingerprints, composition changes, or chemical–biological relationships. Full texts were considered if they were in English, or English abstracts. Single plant studies were not directly used as an indicator of performance in a polyherbal matrix. The number of 15 formulation studies were purposively selected to contrast the analytical questions, not a systematic-search yield.

The formulation, measurement strategy, named markers or signal coverage, sampling unit, validation information and conclusions based on the available record were considered during extraction. Full-text and abstract/summary access are distinguished in Table 3. No formal risk-of-bias score or numerical SANRA score was given, and narrative synthesis was done to highlight the scientific reasoning and transparent presentation of evidence, as emphasized in the domains by SANRA. [6]

3. Principles of marker selection

3.1. Distinguish analytical purpose from biological attribution

EMA differentiates analytical markers (analytical control) from active markers which are believed to be active in the therapeutic effect. The difference is in the evidence for the role of a marker – analytical convenience is not evidence of therapeutic responsibility. On the other hand, the biologically relevant constituent can also be helpful analytically, as long as it is appropriate. WHO guidance acknowledges that characteristic patterns or more than one marker substance may be required when one is not. [7, 8]

Four uses are distinguished for this review: ingredient-associated identification, quantitative consistency, processing or stability monitoring and control of relevant undesirable constituents. These are practical categories and not a substitute for regulatory terminology. A marker can be used for more than one purpose, however, evidence for one use should not be equated to evidence for another. Especially, if a stable release-assay marker is used, it may not be appropriate for detecting degradation in the mixture at other sites.

Table 1. Marker purposes and the evidence needed to support them.

Purpose	Useful evidence	Interpretive boundary
Ingredient-associated identity	Authenticated ingredient and formulation profiles; testing against plausible substitutes and omitted-ingredient preparations.	Occurrence alone is insufficient when the compound is shared by several ingredients.

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Quantitative consistency	Traceable reference material, selective assay, defined reporting basis, and an appropriate product-specific range.	Consistent concentration does not establish complete formulation identity.
Processing or stability monitoring	Documented changes during relevant processing, storage, or controlled degradation challenges.	Loss of one marker does not describe every chemical or physical change.
Undesirable-constituent control	Risk-based identification of the target, selective low-level measurement, and applicable justified limits.	A positive marker assay cannot substitute for separate contaminant or residue tests.
Activity-related interpretation	Reproducible association with an appropriate biological endpoint and independent confirmation.	Association is not proof of causal contribution, synergy, or clinical equivalence.

The categories are the review's practical synthesis; analytical/active marker terminology follows EMA and marker-selection principles follow WHO. [7, 8]

3.2. Botanical discrimination and panel coverage

Shared markers show how important it is to have a panel reason. Gallic acid, chebulagic acid and chebulinic acid strategy reported for Triphala are relevant to tannin chemistry and the constituent distributions are similar, so that only certain identities can be made on the basis of one of the three. A complementary approach is ingredient-level profiling: Rahman et al. analyzed 14 accessions of A. vasica and then looked at four markers for the quinazoline alkaloids in the plant in the formulations. This approach supports the evidence of the chosen ingredient, but not necessarily of all the other botanicals in the formulation. [3, 4]

A helpful development exercise is to list each candidate and the contribution it is intended to make to the botanical or processing aspects of the development and then look for overlaps. Choosing a number of chemically similar constituents can increase the confidence in one fraction without the other ingredient being represented in some. The goal is not just to specify one marker for each quality attribute of an herb, but to provide a documented rationale for the use of each of the constituents or complementary tests that support each important quality attribute.

Also, marker panels should be challenged with realistic changes in composition. This is not a completely novel approach, as Hu et al. explored Sishen formula in 16 prescription ratios, with complementary mass-spectrometric strategies and an 18-marker targeted assay. The additional opportunity is to see if a proposed routine panel can identify blinded omissions, substitutions or altered processing of independent material, not just different ratios. [9]

3.3. Analytical suitability and reference materials

The selection of a reference material should take into account the reference-material identity, the assigned content, stability and availability. There is no automatic relationship between area purity and mass fraction assigned for the calibration. The calculation basis (including any water or solvent contribution, and chemical form) should be recorded. Similarly, if the marker is abundant, it is only helpful if it still has a high degree of selectivity and measureability in the final matrix. [7, 8]

The proposed selection logic is summarized in figure 1. It is vital that it gives feedback – if they do not see an appropriate composition challenge in the image, then they should ask the panel to

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reconsider or add an orthogonal test. The more markers that are added without testing this discrimination, the larger the analytical load without benefit to the quality decision.

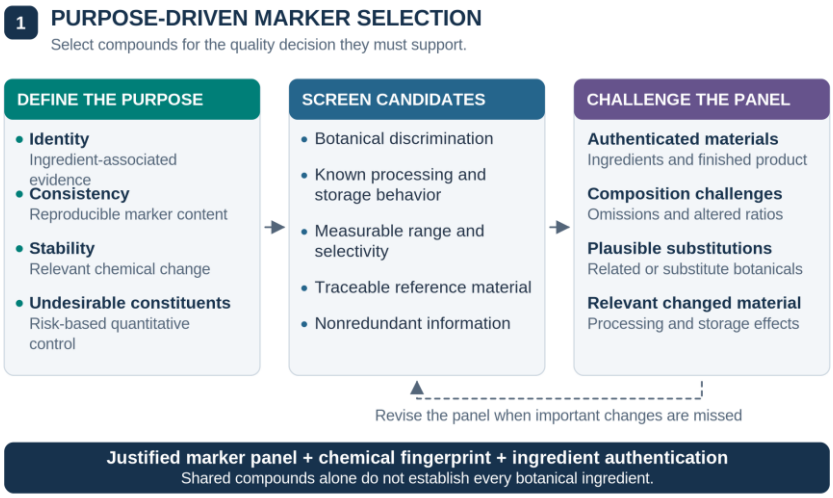


Figure 1. Purpose-driven selection and challenge of marker panels. The conceptual workflow links the intended decision to candidate screening, authenticated materials, and realistic composition challenges. It is an original synthesis, not a validated universal selection algorithm. Regulatory marker terminology and published ratio-challenge evidence inform the framework. [7, 8, 9]

4. Sampling, extraction, and the reporting basis

The analytical result represents the material prepared for the analysis. WHO quality-control and processing guidance facilitates documented sampling, identification, handling and preparation. The particle size or segregation in a polyherbal powder may impact the amount used in the extraction process and the phase separation or sediment in a liquid preparation alters the significance of sampling the supernatant only. Therefore, the preparation procedure should include a description of homogenization, the fraction of the sample being taken, the amount of mass or volume of sample, and any moisture correction. [1, 2]

The result should clearly indicate the product that was received, the dry product, the amount of extract solids or the administered unit for quantitative comparison. These denominators are used to solve different questions. Methodologically, it should not be said that a product is chemically inferior or superior if the concentration of the marker differs between the two products, unless there is a difference in the excipient content and/or concentration. Both product-basis and a justified secondary normalization may be helpful and the conversion should be demonstrated and not assumed.

The extraction procedure should be assessed in a way that is relevant to its own purpose. An extensive laboratory extraction can be used to aid in the compositional evaluation but cannot measure the quantity delivered to a patient when administered. Development should consider how recovery and/or instability is affected by solvent composition, extraction time, temperature, filtration, dilution, and holding time. To determine if the original sample was exhaustively extracted, one could compare successive extractions or make suitable recovery experiments.

The study unit should also be clear and concise. Repeat injections estimate instrumental variability, separate preparations from a homogenized sample add preparation variability, and independently manufactured batches allow for information about manufacturing variation. These levels cannot be switched. Tables and statistical analysis should maintain the order of manufacturer, lot, preparation of samples and injection to avoid giving an impression of wide batch consistency that would be misleading as to the precision.

5. Complementary analytical techniques

5.1. HPTLC: parallel profiling and selected-marker measurement

HPTLC allows for the analysis of ingredient extracts, reference samples and formulation samples to be compared and analyzed simultaneously. Ladva et al. used marker based HPTLC for SJT-DI-02 using marmelosin, gallic acid, lupeol and asarones as markers. Pillai and Pandita have studied the analyte specific chromatographic condition of Pillai Pandita (Draksharishta) with respect to the study of Gallic acid, Catechin and Resveratrol. Boini et al. then reported simultaneous measurement of five markers applied to three separate batches of Jwarahara Kwatha Choornam (JKC). These are various implementations – several compounds measured does not necessarily mean a single common separation procedure. [10, 11, 12]

The most important information for transfer are application volume, loading range, layer type, development conditions, derivatization (if applicable), detection wavelength and the timings of image acquisition. The review suggests that the plausibility of matrix components should be assessed for a band that is assigned to a marker to see if it is resolved. Matching retention factors or colours can be used to help identify the identity of a molecule, but should not be used as an absolute molecular identification. The criteria for the visual fingerprint and densitometric assay should be specified separately.

5.2. HPLC/UHPLC with optical detection

When selected constituents are capable of being separated and detected at a suitable wavelength, HPLC and ultra-high-performance liquid chromatography (UHPLC) with ultraviolet or diode-array detection can be useful. Seven marker RP-HPLC analysis of JKC were reported by Misro et al. which includes andrographolide, piperine, picroside-I, picroside-II, α-cyperone, 6-gingerol, and 6-shogaol. This increases the range of the measured panel when compared with the 5 marker HPTLC investigation done for the same named formulation. It does not imply that HPLC is always superior, as the required marker coverage and workload, and the decision to be made, vary. [12, 13]

This should be compared in practice in terms of resolution of critical pairs, extraction compatibility, calibration behaviour, total turnaround time and sample information retained. A shorter instrument run can be compensated for by more difficult clean up or repeat analysis. Data from the diode-array spectrometer can be used to explore a suspect peak, but a peak that is apparently "pure" does not necessarily exclude the presence of all co-eluting peaks. The best routine procedure is the one that has been shown to perform for the specific matrix and use.

5.3. Targeted LC–MS/MS: selective measurement with matrix control

Targeted liquid chromatography–tandem mass spectrometry (LC–MS/MS) is a combination of chromatographic behavior and selected mass-spectrometric transitions. Chandra et al. have

quantified 28 compounds in 20 batches of Mentat tablets using UHPLC–MS/MS by switching the polarity and multivariate analysis. This is a representative example of broader targeted coverage as part of a complex formulation, but is not necessarily representative of the whole phytochemical composition. [14]

Special attention is needed for matrix effects. In quantitative LC–MS/MS, Matuszewski et al. separated the ionization-related from the extraction recovery. The implication for the polyherbal analysis is that a good spike-recovery result is not enough to prove freedom from ion-suppression or enhancement. In relation to observed source of bias, pre-extraction and post-extraction comparisons, appropriate internal standards, dilution or matrix-matched should be considered. A calibration procedure which works well for one dosage form/excipient combination may not work the same way in the new dosage form/excipient combination. [15]

The Unani formulation Majoon-e-Nisyan is a good example of a non-Ayurvedic formula. Five marker readouts, such as a combined α- and β-boswellic acid measure, β-asarone, luteolin, 6-gingerol, and piperine were measured by Thakkar et al. They reported the recovery of 87.5–94.1%, with low intra- and interday relative standard deviations. These measure various properties, and a small variability doesn't eliminate recovery bias. Acceptability should be based on a criterion of intended use, rather than on a recovery interval that is universal and applied once the results are found. [16]

5.4. High-resolution profiling and identification confidence

High resolution LC–MS can provide information on features not included in a targeted panel and can be used to select candidates for confirmation. This is demonstrated in the untargeted-to-targeted sequence that is employed by Rahman et al. The Sishen study also explored the compositional analysis at various prescription ratios by multidimensional separation and ion-mobility/high-resolution measurements. This information is helpful if it resolves an uncertainty which a more limited routine assay cannot resolve. [4, 9]

Feature detection, tentative annotation and confirmed compound identification should still be separate. The Metabolomics Standards Initiative puts an emphasis on the reporting of the degree of confidence in the identification as well as the supporting evidence for the assignments. The accuracy of the mass or the library match does not equal the confirmation by an authentic standard and independent matching properties. Special care should be used for isomeric constituents and features that are not resolved. Therefore, peaks or features should be reported as such in a review table and not as metabolites that are "silently" identified. [17]

5.5. QAMS and qNMR: alternative quantitative strategies

Quantitative analysis of multicomponents by a single marker (QAMS) is a calibration strategy, but not an instrument. It calculates a few constituents based on relative response relationships to a reference marker. Xu et al. integrated QAMS, fingerprinting and chemometrics for 28 batches of Qizhi Xiangfu Pills, and 7 quantitative constituents were quantified. The method can decrease the use of multiple standards once the relative response and identification procedure has been determined. It is dependent upon the suitability of those relationships under the analytical conditions used. [18]

A different measurement principle is quantitative nuclear magnetic resonance (qNMR) which is not calibration-free or sample effect free. Malz and Jancke discussed the requirements for the

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quantitative validity of the experiment, which include appropriate acquisition, signal integration and reference procedures in qNMR validation. Overlapping signals may restrict the measurement of certain constituents or groups in a complex mixture with confidence. [19]

Importantly, direct evidence in the form of multi-components is available. Li et al. adopted an NMR method to determine the quantitative amounts of total ginsenosides, polysorbate 80 and 20 primary metabolites in six brands of Shenmai injection. This expands the scope of compositional accounting beyond selected secondary metabolites, but no more than an equivalent number of individual therapeutic markers: an excipient and a grouped ginsenoside measurement are included. Nor does chemical accounting consider the different needs of an injectable dosage form. [20]

Table 2. Analytical roles and the questions that should govern platform selection.

Strategy	Principal contribution	Important constraint	Appropriate decision focus
HPTLC	Parallel visual fingerprints and selected-marker densitometry.	Band overlap; development, detection, and image timing require control.	Is the characteristic profile present, and are validated marker amounts consistent?
HPLC/UHPLC–DAD	Resolved targeted assays with optical and retention information.	Chromophore dependence and co-elution; representative extraction remains essential.	Can routine marker concentrations and selected fingerprint features be measured reliably?
Targeted LC–MS/MS	Selective measurement of a predefined analyte panel.	Ion suppression/enhancement, shared transitions, and standard availability.	Does selective quantification resolve a question inaccessible to the routine assay?
LC–HRMS profiling	Broader feature discovery and support for structural annotation.	Identification confidence and data-processing choices; not all features are confirmed compounds.	What changed, and which features merit targeted confirmation?
QAMS calibration	Multiple concentrations estimated through relative responses to a reference.	Response-factor and peak-identification transfer must be demonstrated.	Can standard consumption be reduced without unacceptable quantitative bias?
qNMR	Orthogonal quantification of suitable signals or constituent groups.	Signal overlap, acquisition conditions, and reference assignment.	Can an independent measurement or broader compositional balance resolve uncertainty?

Qualitative synthesis of the selected applications and methodological studies; not a universal sensitivity, cost, or accuracy ranking. [10, 13, 14, 15, 17, 18, 19, 20]

Figure 2 summarizes complementary rather than competing roles. The selection starts from the quality question and matrix, not from a presumed hierarchy in which the most sophisticated instrument must always produce the most useful result.

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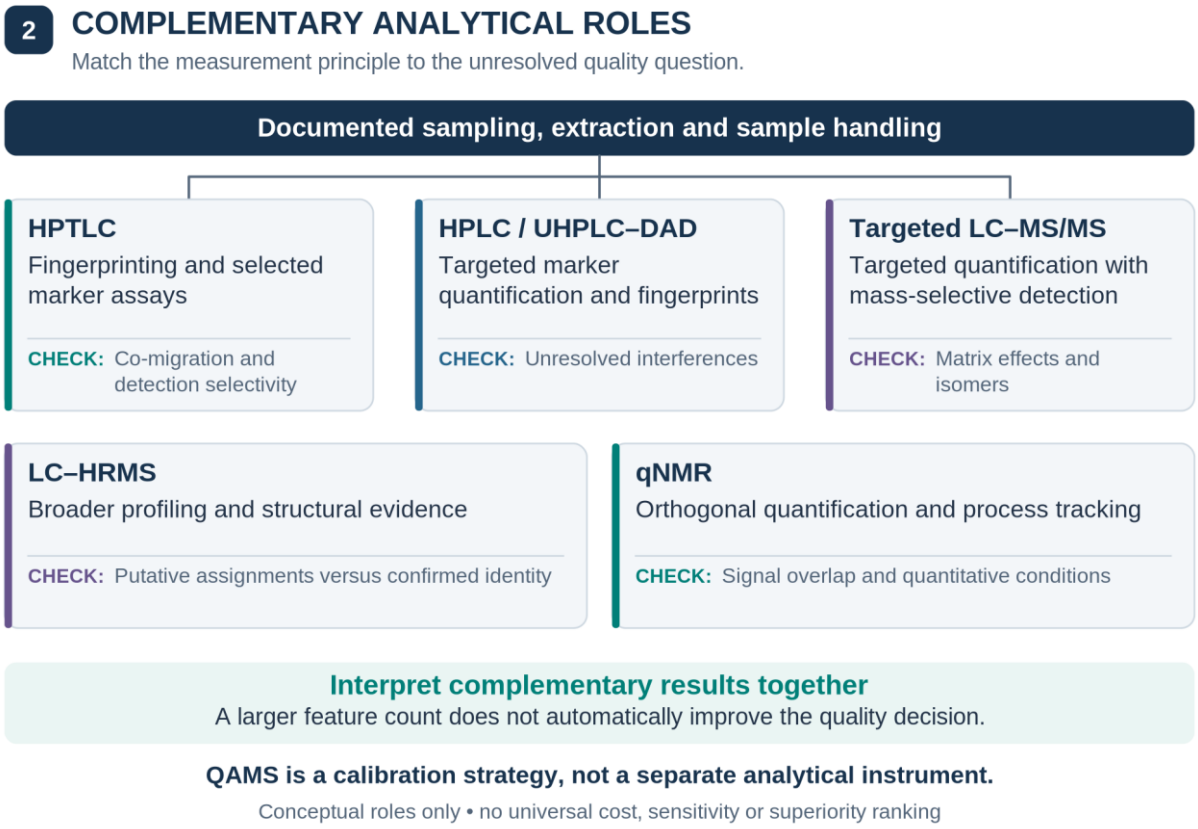


Figure 2. Complementary analytical roles in a shared sample-preparation workflow. Each platform addresses a different information need and retains characteristic limitations. QAMS is distinguished from an instrumental technique. This conceptual figure contains no experimental chromatograms, spectra, or comparative performance data. [13, 14, 15, 17, 18, 19, 20]

6. Comparative evaluation of formulation studies

The selected evidence is organized in Table 3 according to the formulation and sampling unit, and not according to the sophistication of the instrument. Examples include powders, tablets, fermented preparations, pills, a formulation of honey and compound herbal injections. They have different matrices and intended uses; the examples of injectable preparations are not intended to be extrapolated to the safety or regulatory requirements of oral preparations.

Table 3. Selected primary formulation studies: analytical coverage, sample design, and interpretive limits.

Study / formulation	Analytical coverage	Sampling unit / access	Critical interpretation
Pawar et al., 2009 Triphala [3]	RP-HPLC: gallic, chebulagic, and chebulinic acids; blend comparison.	Formulation and composition variants; P.	Shared markers support composition assessment but do not individually identify all ingredients.
Ladva et al., 2014 SJT-DI-02 [10]	HPTLC: marmelosin, gallic acid, lupeol, and asarones.	Named laboratory formulation; F.	Chemical-class coverage and botanical discrimination are different attributes.
Chandra et al., 2015 Mentat tablets [14]	28 compounds by UHPLC–MS/MS; multivariate analysis.	20 batches; A.	A comparatively broad targeted panel remains a selected chemical window.

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Pillai and Pandita, 2016 Draksharishta [11]	HPTLC assays of gallic acid, catechin, and resveratrol.	3 in-house batches and 2 marketed samples; F.	Reported calibration ranges and quantitation limits require reconciliation before transfer.
Rahman et al., 2019 *A. vasica*-containing products [4]	Untargeted profiling followed by four isolated quinazoline alkaloid markers.	14 botanical accessions plus formulations; A.	Ingredient-focused evidence cannot be extended automatically to every component.
Li et al., 2022 Shenmai injection [20]	qNMR: total ginsenosides, polysorbate 80, and 20 primary metabolites.	Products from 6 manufacturers; A.	Manufacturer count is not batch count; grouped analytes and excipients must be distinguished.
Long et al., 2023 Triphala [21]	HPLC fingerprint: 19 common peaks; similarity and multivariate analysis.	20 batches from 4 geographical areas; A.	Similarity above 0.9 coexisted with sample clustering.
Yang et al., 2023 Shenmai injection [22]	HPLC fingerprinting, 28 characteristic peaks, and antioxidant-linked analysis.	30 batches from 3 manufacturers; F.	Chemical–bioassay association is not clinical equivalence or established causality.
Thakkar et al., 2023 Majoon-e-Nisyan [16]	Five LC–MS/MS marker readouts, including combined boswellic acids.	Unani honey-based formulation; batch number unavailable in abstract; A.	Combined-isomer measurements and recovery bias require explicit interpretation.
Misro et al., 2024 JKC [13]	Seven-marker RP-HPLC, including 6-gingerol and 6-shogaol.	Formulation method-development study; F.	Coverage is broader than the selected five-marker HPTLC panel, not proof of universal superiority.
Gandhi et al., 2024 AYUSH SG-5 [5]	Eight-marker HPLC; HPTLC stability component; LC–QTOF-MS/MS.	Formulation and marker-based stability investigation; P.	Integrated analysis and stability-oriented work are already established contributions.
Xu et al., 2024 Qizhi Xiangfu Pills [18]	23 common fingerprint peaks; seven constituents measured by QAMS.	28 batches; A.	No significant difference from external-standard results is not a formal equivalence test.
Hu et al., 2024 Sishen formula [9]	Multidimensional/high-resolution profiling plus 18-marker targeted quantification.	16 designed prescription ratios; F.	Replicate analytical samples must not be counted as independent manufacturing batches.
Boini et al., 2025 JKC [12]	Simultaneous five-marker HPTLC assay.	3 independent formulation batches; P.	Supports application to the tested batches, not population-wide batch consistency.
Seo, 2026 Maziren-Wan [23]	30-marker LC–MS/MS profiling of different preparations.	Reference extract and 2 commercial granule preparations; A.	Preparation-dependent differences are not automatically defects or validated classification.

6.1. Coverage, specificity, and comparison across matrices

The studies demonstrate the inadequacy of the "number of markers" comparison variable. The Mentat investigation consists of 28 targeted measurements in 20 batches while the Shenmai NMR study contains an excipient and a collection of constituents. These panels are for addressing various questions. Likewise, there are two types of JKC methods based on the different principle of coverage and detection. A valid head-to-head comparison would be possible if matched samples were available, if there was a common intended use question, and if there were criteria defined for bias, precision, and discrimination. [12, 13, 14, 20]

Another difficulty in market comparison is that there are differences in formulations. A reference Maziren-Wan extract was compared with two commercial granule preparations with a 30-marker panel. The study demonstrates chemical differences that are preparation dependent,

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but is not a large manufacturing-batch survey of three preparations. Concentration comparisons may be affected by excipients, extraction and presentation; the analytical difference between products does not necessarily indicate a poor manufacturing process or suggest that the products are not clinically equivalent. [23]

6.2. Similarity and validation reporting require interpretation

Long et al. found 19 common peaks in 20 batches of Triphala and found the similarity in the fingerprints to be more than 0.9 even after dividing into two clusters. This is not an inverse relationship: the features that are most important for a global resemblance measure don't necessarily have to be the features that best differentiate groups. The observation confirms the need to use suitable absolute marker measurements in combination with meaningful fingerprint similarity criteria, instead of using a single similarity coefficient for all cases as a pass/fail criterion. [21]

In the Draksharishta paper a concrete reporting problem comes up. The other two analytes have similar range–limit discrepancies in their Table 2, which provides a calibration range of 300–700 ng for gallic-acid and a quantitation limit of 900 ng. These values are published, and need to be clarified prior to transfer of the procedure. [11]

According to the study done by Qizhi Xiangfu, there is no significant difference between the results of QAMS and external standard. That gives support to a method-comparison observation, but it does not prove that there are no differences, it merely fails to detect one. Future transfer studies should provide the magnitude of bias that is important and estimate agreement over the range of interest, using different instruments, columns and laboratories as appropriate to the intended use. [18]

6.3. Existing biological and stability investigations refine the gaps

In 30 batches from three manufacturers, Yang et al. correlated the chromatographic information of Shenmai with the antioxidant activity. The question that remains is whether an association continues in separate chunks and is applicable to a specific product function. Similarly, the AYUSH SG-5 study implies that integrated marker-based stability work is considered a valid evidence. The difference is in the strength and transferability of the quality decision that is achieved, not in the absence of combined techniques. [5, 22]

7. Analytical validation and regulatory considerations

7.1. Validate the intended measurement, not a checklist

ICH Q2(R2) deals with the demonstration of analytical performance and Q14 covers analytical procedure development and considerations for the analytical procedure lifecycle. They have principles that lead to an evaluation that is fit for purpose with a clear relationship between the attribute being measured and the required performance. It is not to be seen as a compulsory document for all herbal assays, but is instead allowed for in Q14 to express that purpose. The requirements that apply will depend upon the product category, jurisdiction and the intended use of regulation. [24, 25]

Selectivity, accuracy, precision, calibration behavior and an appropriate reportable range must be compatible for a quantitative assay. High correlation coefficient is not sufficient to guarantee acceptable residual behavior and performance near a decision limit. Detection and quantitation

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limits are important for low level targets, but for an abundant marker it is key to have a performance at the routine assay concentration. The robustness is typically explored in the development process and should be incorporated in the operating controls. [24, 25]

The following table (Table 4) suggests the questions that should be used to appraise polyherbal studies based on the above. It intentionally refrains from having any fixed universal numerical thresholds. A recovery or precision criterion should be based on the matrix, the target concentration and the consequence of the decision, not just because that is the number that has been reported in a similar paper.

Table 4. Proposed validation and reporting checks for complex formulation matrices.

Question	Evidence to examine	Common interpretive error
Is the intended constituent measured selectively?	Ingredient and finished-matrix interference; reference matching; critical-pair separation or other identity evidence.	Assuming matching retention time or a mass transition proves complete selectivity.
Is extraction distinguished from detector response?	Independent preparations, suitable spike placement, filter tests, and relevant matrix-effect assessment.	Treating post-extraction recovery as proof of extraction completeness.
Is the quantitative range coherent?	Calibration levels, residuals, quantitation limits where relevant, and actual sample concentrations.	Accepting a high correlation coefficient despite an incompatible working range.
What source of variability is estimated?	Replicate level, days, analysts, equipment, and independent batches specified separately.	Calling repeated injections batch-to-batch reproducibility.
Does performance persist during routine use?	Solution stability, carryover, justified robustness challenges, and method-transfer evidence.	Equating initial validation with indefinite suitability.
How does a result trigger a decision?	Predefined product-specific limits, reference profile qualification, and rules for uncertain or atypical results.	Deriving a universal acceptance threshold from a convenience sample.

7.2. Specifications and jurisdiction-specific application

A validated procedure is a different deliverable to a product specification. EMA guidance specifies in terms of tests, analytical procedures and acceptance criteria. A method may be used to reliably measure an attribute without any knowledge of the concentration that a formulation should have. Limits must therefore be based on reference-batch selection, manufacturing consistency, relevant stability behaviour and the applicable monograph or authorization. The average of marketed samples may not be a good target if they are not qualified. [26]

In the case of Indian Ayurvedic products, the official pharmacopoeial system maintained by the Pharmacopoeia Commission for Indian Medicine & Homoeopathy should be referred to, which incorporates the monograph of the ingredient or formulation and any updates to the monograph. This is not to equate WHO recommendations, ICH scientific principles and an Indian monograph. The review suggests a clear mapping of every application to the corresponding official standard, the analytical purpose, and evidence of any additional procedures. Does not establish a common legal requirement for all traditional products. [27]

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FDA botanical-drug guidance covers contexts in which drugs are developed and acknowledges that biological assays can be useful if there is inadequate chemical characterization to support consistency. This advice should not be offered as a guideline for all herbal remedies and traditional medicines. In isolation, WHO contaminant guidance reiterates the importance of dealing with residues and contaminants; a conforming marker result does not imply the absence of other unrelated hazardous substances or microbial contamination. [28, 29]

8. Chemometrics and multivariate quality assessment

Exploratory separation is not a validated predictor of authenticity or acceptability, but may be able to reveal patterns among measured samples by using principal component analysis (PCA) and clustering. Special attention should be paid to supervised models. Westerhuis et al. showed the potential for overfitting in partial least squares discriminant analysis and stressed proper cross-validation and permutation analysis. These concerns come to mind whenever the number of features is large compared to the number of truly independent formulation samples. [30]

This review suggests the unit of independence be defined prior to developing a model. Extracts or injections from the same batch must not be used in the training and test sets as if they are from different batches. If not, the model might be improved by having near-duplicate chemistry in the test set. Randomly splitting technical replicates is a less relevant challenge than holding out complete suppliers, production periods or laboratories in cases where new suppliers or laboratories are involved in the intended use.

The analytical procedure includes also the preprocessing. The result can change due to baseline correction, peak alignment, normalization, scaling, nondetects and feature selection. It is proposed to report on each operation and to limit model fitting and feature selection to the training process. A classifier designed for routine decisions should also include its applicability domain, what should be done with samples that are not in its domain of recognition and what should be done with samples that are not in the domain of confidence of the classifier, instead of being forced into an existing class.

Any differences between groups must be explained by a plausible chemical explanation. The Triphala results demonstrate that high overall similarity can be achieved with clustering and the Maziren-Wan comparison illustrates the possibility of formulation presentation along with chemical separation. Both outcomes are not enough to suggest that one cluster is good and the other is poor without having other independent quality criteria. Rather, meaningful endpoints in a model should be associated with authenticated identity, known composition challenges or other attributes that are predetermined. [21, 23]

A useful future report would be able to classify the test samples, and have some indication of uncertainty of correct and incorrect decisions, and performance on likely nonconforming material. It would report prediction error and calibration for independent samples for prediction of concentration or activity. A distinct scores plot is a beginning for investigation, not a conclusion to validation.

9. Proposed decision-oriented standardization framework

This review has synthesized a framework (Figure 3) which has not been validated in a prospective manner. It distinguishes between qualification, routine assessment and

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investigation to ensure advanced characterization is applied when the uncertainty is significant. Not intended to allow release only by chemical testing.

9.1. Qualify materials and establish the analytical question

Qualification should include the botanical identity, proportions of ingredients and/or preparation, relevant processing, dosage form, and sampling plan. The candidate markers and reference fingerprints should be related to the quality attributes they are to be used to evaluate. Development materials should contain representative or realistic materials as well as acceptable reference materials – not just easily available commercial materials. Qualifying a fingerprint for its purpose is more important than just picking it because it is close to the average of the profiles that are available.

9.2. Apply a manageable routine test combination

An appropriate identity/fingerprint procedure may be used in conjunction with validated quantitative assays in the routine assessment. The number of procedures should be based on the information that is needed, not on a need for all the instruments. The control strategy should include the limits to be applied to the samples, features common to the samples to be tested and the conditions under which samples should be investigated. Chemical results should then be taken into account in conjunction with all relevant specifications for the dosage form such as contaminant, microbiological, physical and stability attributes. [26, 29]

9.3. Investigate atypical profiles and monitor continued suitability

The first step in an unexpected result is to investigate the sampling, preparation, reference materials and analytical-system performance. A challenged identity, co-elution, matrix effect or unexpected change can then be investigated by a justified orthogonal method. Repeated testing should not be performed just to find a passing result; the scientific reason for the discrepancy and its impact on the decision of the batch should be discussed. The results may lead to changes in the procedure and/or materials and processing.

As use continues, the review suggests that any changes in the marker measurements and fingerprint characteristics that are relevant should be monitored, and compared to the performance of the method. Any changes in the suppliers, formulation, manufacturing or analytical conditions should be evaluated for their impact on suitability. The robustness of the framework would be demonstrated in the future through decisions made on independent conforming and challenged batches, which could be reproduced, and an explicit mechanism for uncertain results.

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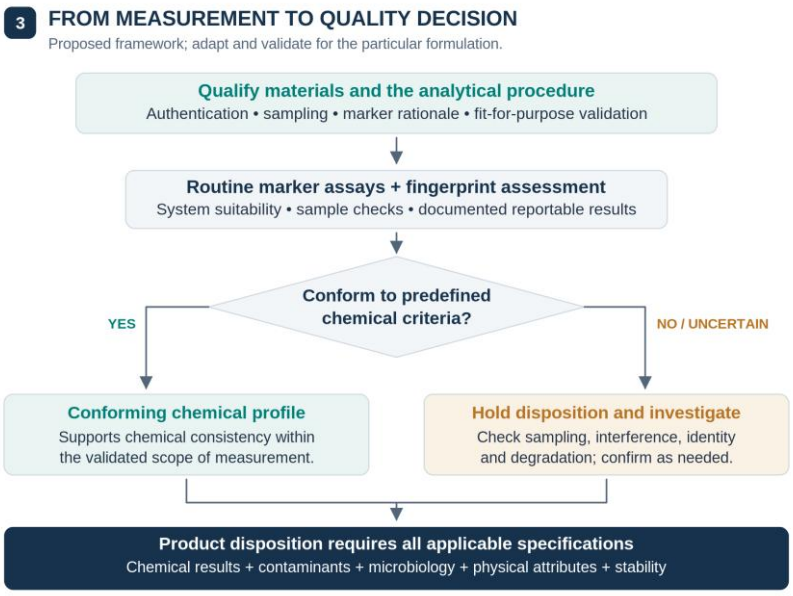


Figure 3. Proposed quality-decision workflow. Chemical assessment is linked to qualified materials, predefined criteria, and investigation of atypical or uncertain results. A conforming chemical profile is only one component of assessment against all applicable specifications. The workflow is conceptual and requires product-specific evaluation. [25, 26, 29]

10. Research gaps and future directions

10.1. From composition sensitivity to validated discrimination

Prescription-ratio studies like the Sishen investigation already show sensitivity to compositional changes that have been designed. The next question is whether a chosen panel can detect realistic errors in independent, blinded samples and be able to separate them from acceptable natural variability. Further research should involve the omission of ingredients, plausible substitutions, ratio changes and processing changes as applicable, with the challenge identity not revealed during analysis. The results should not be just the number of statistically different peaks, but the frequency and consequences of correct, incorrect and uncertain decisions. [9]

10.2. Independent sampling and interlaboratory transfer

The sampling structures chosen are from three JKC batches to wider batch or manufacturer collections but answer different questions. An increased number of injections cannot make up for a small number of independent lots. Future designs should cover across raw-material sources, raw-material production periods and raw-material manufacturing lots; and then hold raw-material source for evaluation. Transfer studies need to be based on common samples and a common reporting basis to separate the differences that are due to the extraction, instrumentation, data processing, or assignment of the reference. [12, 14, 20]

In the case of QAMS and fingerprint model, a design that is particularly informative would be to compare the transferred procedure with an approach that is suitably qualified, with predetermined agreement margins. This would be beyond the point of being statistically insignificant and would actually give the receiving lab a number of how large the differences are. Rather than just whether the mean marker concentrations are near each other, the same

exercise should ask if there is agreement among the different laboratories on their quality classification.

10.3. Stability-indicating measurements across the finished product

The AYUSH SG-5 example has set the precedence that marker-based stability investigations have been carried out along with other analytical techniques. Future studies should assess if a release panel is able to detect changes that are relevant to the proposed storage period. The retention of the markers, the appearance of new fingerprints, sedimentation or other physical changes, and applicable microbiological properties may necessitate separate measurements. Before making a conclusion on the product level shelf-life, the accelerated kinetic model should be understood in the light of its assumptions and in the context of the real time observations. [5]

10.4. Chemical–biological relationships with defined boundaries

The chemical–antioxidant associations in the Shenmai fingerprint study are examples, and do not imply that they predict clinical benefit. Future investigations should establish the biological endpoint prior to the investigation, use appropriate controls and dose–response testing, and evaluate for analytical interference. Now candidate relationships should be questioned in separate units. A reproducible association can be used to support a quality control rationale without being used as evidence of causality, synergy, therapeutic equivalence, or patient benefit. [22, 28]

10.5. Transparent reporting and practical implementation

More than just a final chromatogram image is needed for re usable analytical evidence. The review suggests that the data on sample identifiers, measurement units, assignment of reference materials, extraction procedures, identification confidence, and processing procedures for deriving fingerprints or statistical models should be reported. If possible, machine-readable signals, results of samples and analysis code should be attached to the manuscript. This takes the lessons learned from chemical reporting to the next level of requirements for formulation comparison. [17]

Analytical performance should be evaluated in conjunction with operational and environmental burdens. The AGREE metric provides a structured approach to analyse analytical greenness, taking into account more than just the volume of solvent used. Running a chromatographic process faster does not necessarily mean that there is less burden on the process for extraction, cleaning up, energy consumption or waste. A beneficial implementation study would be to compare the costs, turnaround time, reagent hazards, waste and trained operator requirements per valid quality decision, and report analytical performance separately. [31]

Figure 4 breaks down these priorities to study designs and measurable outcomes. The priorities are based on the evidence selected and do not necessarily indicate that there is no research related to the priority or represent how often a limitation is found throughout the entire literature.

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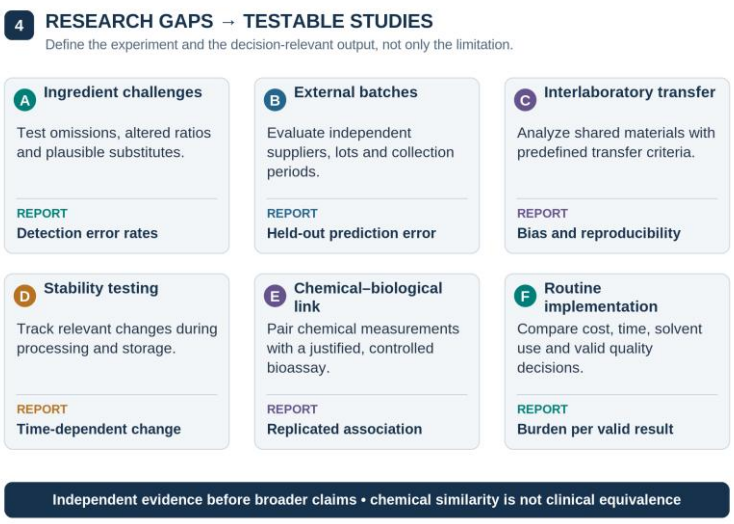


Figure 4. Research priorities linked to testable designs and measurable outcomes. The roadmap distinguishes ingredient discrimination, external model evaluation, interlaboratory transfer, stability, chemical–biological investigation, and implementation. It summarizes proposed future work rather than experimental findings or a quantified prevalence of research deficiencies. [9, 18, 22, 31]

11. Limitations of the review

The purposive search and selection strategy allows for language and accessibility bias, and allows for publication-selection bias. The 15 formulation studies are illustrative, not representative of the field. The detailed appraisal of the validation, stability models and sample selection of several reports was limited as they were only available as abstracts or indexed publisher excerpts. Any information that could not be found was not considered to be a missing procedure.

The review was not carried out independently by reprocessing chromatograms, checking raw calibration data, reproducing chemometric models or conducting experiments. Pooled comparisons of sensitivity and universal acceptance limits are not possible because dosage forms and objectives of analysis are heterogeneous. The suggested workflow and recommendations for studies are thus product-specific and prospective testing is required. The scope is the chemical quality assessment and the selection of a formulation example and discussion of a reported bioassay does not imply endorsement of its therapeutic use.

12. Conclusion

Chemical standardization of polyherbal formulations should be based on establishing links between measurements and attributes of defined quality, rather than presence of one recognized constituent or magnitude of analytical panel. Selected items of evidence illustrate complementary roles of multi-marker assays, fingerprinting, targeted and high-resolution mass spectrometry, QAMS, qNMR and chemical-biological analysis. Their contribution depends on a suitable basis for sampling, rationale for marker selection, reliable identification, coherent validation and a mode of interpretation in line with the study design.

The proposed framework emphasizes three distinctions: Ingredient identity is not synonymous with abundance of one class of markers; analytical similarity does not equal total product

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equivalence; and concordance of chemical profile is not a surrogate for all other quality and safety requirements. Evaluation should be based on performance on independent and specifically challenged material, inter-laboratory transfer, stability-related measurements and pragmatic aspects of implementation. A product-specific battery of routine and investigative tests can then provide reproducible basis for quality decisions, without implying clinically unproven therapeutic conclusions.

Abbreviations

DAD, diode-array detection; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin-layer chromatography; HRMS, high-resolution mass spectrometry; ICH, International Council for Harmonisation; JKC, Jwarahara Kwatha Choornam; LC–MS/MS, liquid chromatography–tandem mass spectrometry; PCA, principal component analysis; QAMS, quantitative analysis of multicomponents by a single marker; qNMR, quantitative nuclear magnetic resonance; QTOF, quadrupole time-of-flight; RP, reversed-phase; SANRA, Scale for the Assessment of Narrative Review Articles; UHPLC, ultra-high-performance liquid chromatography.

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