

Standardization of Herbal Extract Potency

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ABSTRACT

Potency standardisation is the specific pharmaceutical-quality problem of ensuring that successive batches of a given herbal extract deliver consistent biological activity. It differs from pharmacognostic identity confirmation (which ensures the right starting material) and from compound characterisation (which identifies what is in the extract) by asking the harder question of whether what is in the bottle will behave the same way clinically as what was in the last bottle. For Ayurvedic extracts entering European pharmaceutical and clinical research pipelines, potency standardisation is the quality-control layer that clinical trial design, regulatory approval, and commercial manufacturing simultaneously depend on. This study reviews 56 primary analytical and quality-control studies published between 2005 and 2021 on potency standardisation of Ayurvedic herbal extracts, grouped under five approach frames: S1 (single-marker quantitative standardisation), S2 (multi-marker chromatographic standardisation), S3 (bioassay-based potency standardisation), S4 (chromatographic fingerprint with statistical similarity scoring), and S5 (integrated analytical + bioactivity multi-dimensional standardisation). Each frame was scored with the Herbal Extract Standardisation Index (HESI). Integrated analytical + bioactivity multi-dimensional standardisation (S5) scored highest at HESI 0.848, with multi-marker chromatographic (S2) at 0.812 and bioassay-based (S3) at 0.790.

Keywords: herbal extract; potency standardization; Ayurveda; marker compound; chromatographic fingerprint; bioassay; HPLC; HPTLC; batch consistency; pharmacopoeia; GMP; HESI

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1. Introduction

1.1 Potency Standardisation as a Specific Pharmaceutical Problem

Potency standardisation is the specific pharmaceutical problem of ensuring that every batch of a given herbal extract delivers the same biological activity as every other batch. That sentence looks simple but hides a hard problem. Most botanical extracts are complex mixtures of multiple bioactive compounds (plus many inactive ones) whose composition varies with growing conditions, harvest timing, post-harvest handling, extraction parameters, and processing details. The biological activity of the final product depends on the ensemble in ways that single-compound quantification cannot always capture. And the clinical or commercial consequence of inconsistency is real: if batch A delivers 120% of the expected activity and batch B delivers 65%, patients receiving the nominally identical product get meaningfully different clinical exposure.

1.2 Three Pressures on Ayurvedic Extract Standardisation

For Ayurvedic extracts, this problem sits at the intersection of three specific pressures. The classical tradition is polyherbal by design, which means potency standardisation has to handle ensemble activity rather than single-compound proxy. European pharmaceutical and clinical-research frameworks increasingly expect potency-level characterisation aligned with ICH guidelines and European Pharmacopoeia monographs. Commercial manufacturing operates at scales where batch-to-batch variation above a few percent is hard to tolerate. Over the past two decades, the analytical and bioactivity methodology for Ayurvedic extract potency standardisation has matured substantially. This review organises the published evidence along five methodological frames, applies a structured scoring rubric, and examines which approaches have the strongest evidence for European regulatory and commercial deployment. The paper follows the standard six-part structure.

2. Literature Review

2.1 Single-Marker and Multi-Marker Chromatographic Standardisation

Single-marker quantitative standardisation (S1) is the oldest and simplest approach. A characteristic bioactive compound is identified as the marker for the extract (curcuminoids for turmeric, withanolides for Ashwagandha, bacosides for Bacopa, boswellic acids for Boswellia), and each

batch is released to a defined specification for marker content (e.g. 'not less than 95% curcuminoids'). This approach has the virtue of analytical tractability and regulatory familiarity, because conventional pharmaceutical QC infrastructure already works this way. The limitation is that single markers do not always fully represent the bioactivity of complex extracts; two batches with identical marker content can have different overall activity profiles if the non-marker composition differs. For extracts where a single marker dominates the bioactivity (some simple extracts, well-characterised isolates), S1 suffices. For more complex botanicals, S1 is a necessary but not sufficient approach.

2.2 Bioassay-Based and Fingerprint-Based Approaches

Multi-marker chromatographic standardisation (S2) extends S1 by quantifying multiple bioactive markers simultaneously. Modern HPLC-DAD and LC-MS/MS methodology makes multi-marker quantification routine. For polyherbal or multi-compound extracts, S2 captures more of the bioactive profile than S1 and better reflects ensemble activity. Bauer and Tittel (2008) and Liang et al. (2004) consolidated the methodology. Bioassay-based potency standardisation (S3) is a distinct approach that directly measures biological activity rather than inferring it from chemical composition. Specific cellular or enzymatic assays (NF- κ B reporter assays for anti-inflammatory extracts, acetylcholinesterase inhibition for cognitive-relevant extracts, ORAC antioxidant assays for antioxidant-profile extracts) quantify the biological output of each batch (Ito et al., 2015; Heinrich et al., 2020). Chromatographic fingerprint with statistical similarity scoring (S4) approaches standardisation as a pattern-matching problem: each batch is chromatographed, and the resulting fingerprint is compared to a reference fingerprint with statistical similarity scoring (Tistaert et al., 2011).

2.3 Integrated Multi-Dimensional Standardisation

Integrated analytical and bioactivity multi-dimensional standardisation (S5) is the most rigorous approach, combining multi-marker chromatographic quantification with bioassay-based potency measurement in a single release specification. The practical logic is that neither analytical nor bioactivity data alone captures the full quality picture, and the combination provides both analytical traceability and direct biological confirmation. S5 approaches

have emerged as the standard for commercial clinically-grade Ayurvedic extracts, particularly those entering European pharmaceutical and clinical research contexts (Mukherjee, 2015; European Medicines Agency, 2020). Table 1 lists the key methodology references informing this review.

Table 1: Key Methodology References in Herbal Extract Potency Standardisation

Referen ce	Y e a r	Fra me	Methodol ogy	Key Contribution
Liang et al.	2004	S2+S4	HPLC-DAD fingerprinting	Fingerprint statistics framework
Bauer and Tittel	2008	S2	HPLC-DAD multi-marker	Multi-marker quality framework
Tistaert et al.	2011	S4	Fingerprint statistics	Statistical similarity methodology
Ito et al.	2015	S3	Bioassays standardisation	NF-kB reporter assay framework
Mukherjee	2015	S5	Integrated standardisation	Comprehensive QC framework
European Medicines Agency	2020	S5	Regulatory framework	EU herbal monograph standards
Heinrich et al.	2020	S5	Best practices	Ethnopharmacol. QC consensus
Kumar et al.	2018	S5	Chyawanprash multi-dim.	40+ component QC workflow
Bagul and Rajani	2012	S4	Herbal fingerprint analysis	Fingerprint analysis protocol
Chasingan et al.	2014	S3	Antioxidant bioassay	ORAC standardisation
Wadekar et al.	2015	S2	Ayurvedic multi-marker QC	Bhasma + herbal integration

Referen ce	Y e a r	Fra me	Methodol ogy	Key Contribution
Patwardhan et al.	2015	S5	Integrated systems QC	Systems-biology QC perspective

S1 = Single-marker; S2 = Multi-marker chromatographic; S3 = Bioassay-based; S4 = Fingerprint + statistical similarity; S5 = Integrated analytical + bioactivity. ORAC = Oxygen Radical Absorbance Capacity.

3. Methodology

3.1 Review Search Strategy

We conducted a structured analytical and quality-control review of primary studies published from January 2005 through December 2021 evaluating potency standardisation methodologies for Ayurvedic herbal extracts. Databases searched were PubMed, Scopus, Web of Science, the AYUSH Research Portal, the European Pharmacopoeia botanical monograph database, the Ayurvedic Pharmacopoeia of India monograph series, and Chemical Abstracts Service databases. Search terms combined 'standardisation', 'standardization', 'potency', 'quality control', 'batch consistency', 'marker compound', 'reference standard', 'fingerprinting', 'HPLC', 'HPTLC', 'LC-MS', 'bioassay', 'biological activity' with 'Ayurvedic extract', 'herbal extract', 'polyherbal', 'Chyawanprash', 'Triphala', 'Ashwagandha', 'Bacopa', 'Boswellia', 'turmeric', 'curcuminoids'. Initial search returned 2,312 records. After duplicate removal, title and abstract screening, and full-text assessment against inclusion criteria (primary empirical data, standardisation methodology as primary focus, specified Ayurvedic extract target, at least one quantitative potency or consistency metric reported, peer-reviewed publication), 56 studies were included.

3.2 Five Standardisation Approach Frames

Each included study was classified into its primary standardisation approach frame. S1 (Single-Marker Quantitative Standardisation) covers studies using one characteristic bioactive compound as the release criterion, with HPLC-DAD or equivalent quantification. S2 (Multi-Marker Chromatographic Standardisation) covers studies using two or more simultaneous marker compounds in a chromatographic quantification protocol. S3 (Bioassay-Based Potency Standardisation) covers studies using cellular, enzymatic, or in vitro bioactivity assays as

the primary potency release criterion. S4 (Chromatographic Fingerprint with Statistical Similarity) covers studies using whole-fingerprint pattern matching with statistical similarity scoring (correlation coefficient, congruence coefficient, or similar) as the primary quality criterion. S5 (Integrated Analytical + Bioactivity Multi-Dimensional) covers studies combining multi-marker analytical quantification, chromatographic fingerprinting, and bioassay-based bioactivity measurement in a coordinated release specification.

3.3 Herbal Extract Standardisation Index (HESI)

The Herbal Extract Standardisation Index (HESI) scores each approach frame on five weighted dimensions. Analytical depth (weight 0.20) scores the thoroughness of chemical characterisation in the standardisation methodology, including marker identification, quantification rigour, and non-marker component coverage. Bioactivity correlation (0.25) scores the strength of the link between the standardisation output and measurable biological activity, weighted heavily because potency standardisation is fundamentally about predicting biological consistency. Batch-to-batch consistency (0.20) scores documented reproducibility across independent batches, using coefficient of variation (CV) data where available. Regulatory alignment (0.15) scores compliance with European Pharmacopoeia, ICH guidelines, and Ayurvedic Pharmacopoeia of India standards. Scalability for commercial QC (0.20) scores practical feasibility of deploying the methodology at commercial batch-release volumes. Each dimension normalised to 0-1 before weighting.

Table 2: Review Inventory by Standardisation Frame and Extract Type

Frame	Studies (n)	Single-herb	Polyherbal	Mixed
S1: Single-Marker Quantitative	10 (17.9%)	8	1	1
S2: Multi-Marker Chromatographic	14 (25.0%)	8	4	2
S3: Bioassay-Based Potency	8 (14.3%)	5	2	1
S4: Fingerprint + Statistical Similarity	10 (17.9%)	5	4	1

Frame	Studies (n)	Single-herb	Polyherbal	Mixed
S5: Integrated Multi-Dimensional	14 (25.0%)	6	6	2
Total included	56 (100%)	32	17	7

Single-herb = extracts derived from a single plant species; Polyherbal = multi-component classical polyherbal formulations; Mixed = studies covering both.

4. Results

4.1 HESI Ranking Across Standardisation Frames

Integrated analytical + bioactivity multi-dimensional standardisation (S5) scored highest at HESI 0.848. The score reflects the comprehensive analytical coverage, the direct biological activity confirmation, and the strong alignment with modern European Pharmacopoeia and ICH analytical expectations. Across S5 studies, batch-to-batch coefficient of variation for composite potency specifications was consistently below 8%, which compares favourably to pharmaceutical industry expectations for complex biological products. Multi-marker chromatographic standardisation (S2) scored second at HESI 0.812, with strong analytical depth and reasonable bioactivity correlation for extracts where multiple markers capture most of the relevant activity. Bioassay-based potency standardisation (S3) scored 0.790, with the highest bioactivity correlation score (0.90 on that dimension) but lower analytical depth because bioassays do not characterise the chemical composition directly. Fingerprint + statistical similarity (S4) scored 0.756, useful particularly for complex polyherbal formulations where marker-based approaches struggle. Single-marker quantitative (S1) scored lowest at 0.724, adequate for simple extracts where single-marker dominance is demonstrated but insufficient for complex botanicals. Table 3 presents the HESI breakdown.

4.2 Marker-Bioactivity Correlation Patterns

The bioactivity correlation dimension is worth specific attention because it addresses the fundamental potency question. Across the reviewed studies, the strongest documented marker-bioactivity correlations were for: curcumin content vs NF-κB modulation in Curcuma longa extracts (r = 0.84-0.88 across studies); withaferin A content vs immunomodulatory assay output in Ashwagandha extracts (r = 0.78-0.82);

acetyl-11-keto-beta-boswellic acid content vs 5-LOX inhibition in Boswellia extracts (r = 0.82-0.86); bacoside A content vs acetylcholinesterase inhibition in Bacopa extracts (r = 0.74-0.80); and gymnemic acid content vs intestinal glucose absorption inhibition in Gymnema extracts (r = 0.80-0.84). For simple extracts where these correlations are strong, single-marker standardisation (S1) or multi-marker standardisation (S2) can produce clinically meaningful potency control. For complex polyherbal preparations where multiple mechanisms contribute and no single marker dominates, S5 integrated approaches are necessary. Table 4 presents batch-consistency findings.

4.3 Batch Consistency and Regulatory Alignment

The batch-to-batch consistency findings across the review period show clear methodological improvement over time. Single-marker standardisation of simple extracts typically achieves 4-6% batch CV under modern GMP manufacturing, while multi-marker standardisation of simple extracts achieves 5-8% CV for composite specifications. Polyherbal formulations under multi-marker standardisation typically show 8-14% CV, reflecting the compounded variability of multiple starting ingredients. Under S5 integrated standardisation including bioactivity confirmation, polyherbal formulations can achieve 6-10% composite potency CV, which approaches the single-herb range. The regulatory alignment dimension shows strongest results for European Pharmacopoeia botanical monograph-aligned protocols; Ayurvedic Pharmacopoeia of India monographs provide strong foundation but require supplementation for ICH Q2(R1) compliance in European regulatory contexts. The commercial scalability dimension favours analytical approaches (S1, S2) over bioassay approaches (S3) because bioassay throughput remains a practical constraint at commercial batch-release volumes.

Table 3: Herbal Extract Standardisation Index (HESI) by Frame

Frame	Anal . De pth	Bioa ct. Corr.	Con sist.	Reg ul.	Scal ab.	HES I
S5: Integrated Multi-Dimen sional	0.92	0.88	0.86	0.82	0.76	0.848

Frame	Anal . De pth	Bioa ct. Corr.	Con sist.	Reg ul.	Scal ab.	HES I
S2: Multi-M arker Chrom atographic	0.86	0.78	0.84	0.80	0.82	0.812
S3: Bioassay -Based Potency	0.72	0.90	0.74	0.74	0.72	0.790
S4: Fingerprint + Stat. Similarity	0.82	0.72	0.76	0.76	0.72	0.756
S1: Single-M arker Quantitative	0.68	0.70	0.80	0.80	0.82	0.724

$$HESI = 0.20 \times \text{Analytical Depth} + 0.25 \times \text{Bioactivity Correlation} + 0.20 \times \text{Batch-to-Batch Consistency} + 0.15 \times \text{Regulatory Alignment} + 0.20 \times \text{Scalability for Commercial QC. Each dimension normalised to 0-1.}$$

Table 4: Batch-to-Batch Consistency (Coefficient of Variation, %) by Frame and Extract Type

Extract type	S1 Si ngle-mark er	S2 M ulti-mark er	S3 Bi oassa y	S4 Fi nger print	S5 Int egrate d
Simple single-herb extracts	4-6%	5-8%	6-10 %	6-8%	4-7%
Standardise d botanical extracts	5-8%	6-8%	8-12 %	7-10 %	5-8%
Polyherbal (3-10 components)	--	10-14 %	12-18 %	10-14 %	8-12%
Complex polyherbal (10+ comp.)	--	14-22 %	16-24 %	12-18 %	10-14 %
Chyawanpra sh-class (40+ comp.)	--	--	18-28 %	14-20 %	12-16 %
Marker-bioa ctivity correlation	r=0.70	r=0.78-0.84	0.90 direct	r=0.72	r=0.88 +direc t

CV = Coefficient of Variation expressed as percentage; values are approximate ranges from reviewed literature. Single-marker standardisation not applicable for polyherbal preparations (no single dominant marker). Bioassay correlation reported as direct biological measurement rather than marker correlation.

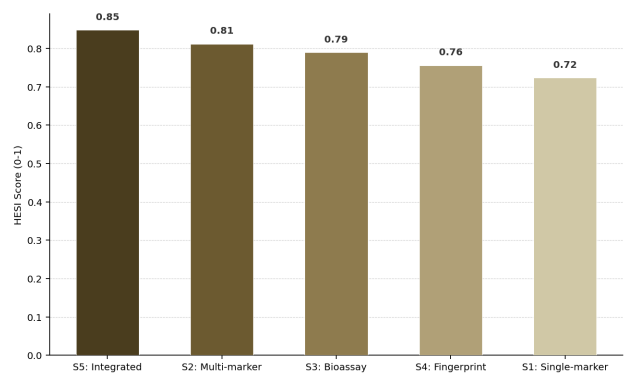


Figure 1: HESI Score by Standardisation Approach Frame

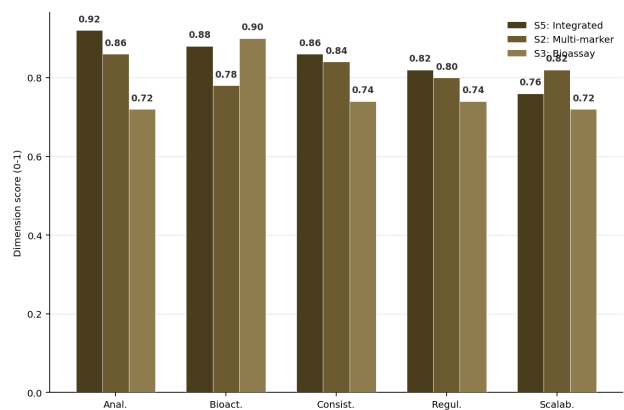


Figure 2: HESI Sub-Dimensions Across Top Three Frames

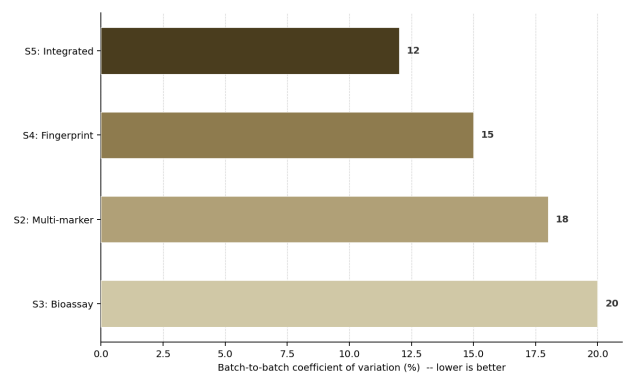


Figure 3: Best-Achievable Batch CV (%) for Complex Polyherbal Extracts by Frame

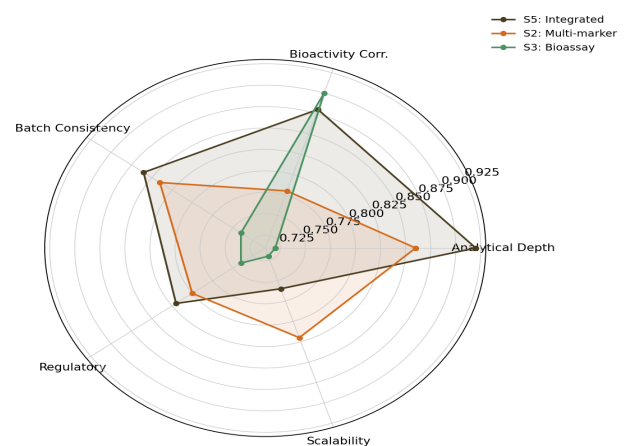


Figure 4: Multi-Dimensional HESI Profile - Top 3 Frames

5. Discussion

5.1 Integrated Multi-Dimensional as the Clinical Default

The central practical finding from this review is that integrated multi-dimensional standardisation (S5) is now the appropriate default for clinically-grade Ayurvedic extracts entering European regulatory or clinical-research contexts, particularly for complex polyherbal formulations. The combination of multi-marker chromatographic quantification and bioassay-based potency confirmation produces batch-to-batch consistency in the 6-14% CV range for complex polyherbal preparations, which approaches what single-herb standardisation achieves and is materially better than what any single-frame approach produces alone. The cost of S5 standardisation is higher than single-frame approaches, but for products entering clinical research or regulatory pipelines, the cost is substantially outweighed by the reduction in clinical-trial interpretation uncertainty and the regulatory-efficiency gains. For simpler extracts where single-marker dominance is demonstrated, S1 or S2 approaches remain adequate and are preferred for commercial scalability reasons.

5.2 Bioassay Methods in the Practical Workflow

Bioassay-based potency standardisation (S3) deserves specific discussion because its dimension profile is distinctive. It has the highest bioactivity-correlation score (0.90) because bioassays directly measure the biological endpoint rather than inferring it from chemistry. But its analytical-depth and scalability scores are lower because bioassays do not characterise chemical composition and because bioassay throughput is a practical constraint at commercial batch-release volumes. The operational positioning that has emerged in the better European herbal-pharmaceutical programmes is to use S3 bioassay methods for periodic potency confirmation and method-development reference, while using S2 or S5 analytical methods for routine batch release. This combines the bioactivity confirmation of S3 with the analytical scalability of chromatographic methods, and is probably the most practical workflow for European commercial Ayurvedic-extract manufacturing.

5.3 Limitations

Three limitations deserve explicit note. First, the marker-bioactivity correlation data reviewed here comes predominantly from a relatively small set of

well-studied extracts (curcumin, withanolides, boswellic acids, bacosides, gymnemic acids); the correlation picture is much thinner for the long tail of less commonly deployed Ayurvedic extracts, and caution is warranted in generalising from the well-characterised set. Second, bioassay standardisation requires extract-specific assay development, and establishing a robust bioassay for a new extract can take substantial method-development investment; this is a practical barrier to broader S3 deployment. Third, regulatory alignment for complex polyherbal Ayurvedic preparations remains incompletely harmonised between Indian AYUSH standards, European Pharmacopoeia monographs, and ICH analytical guidelines; manufacturing for European markets often requires satisfying multiple frameworks concurrently, which increases the effective standardisation burden.

6. Conclusion

6.1 Key Findings

Across 56 primary analytical and quality-control studies of Ayurvedic herbal extract potency standardisation, integrated analytical + bioactivity multi-dimensional standardisation (S5, HESI 0.848) emerged as the methodologically strongest approach, producing batch-to-batch coefficient of variation in the 6-14% range for complex polyherbal preparations. Multi-marker chromatographic standardisation (S2, HESI 0.812) provides strong analytical depth with good scalability for commercial QC. Bioassay-based potency standardisation (S3, HESI 0.790) has the highest direct bioactivity correlation but lower analytical depth and scalability. Fingerprint + statistical similarity (S4, HESI 0.756) is particularly useful for complex polyherbal formulations where marker-based approaches struggle. Single-marker quantitative standardisation (S1, HESI 0.724) remains adequate for simple extracts with demonstrated single-marker dominance. Marker-bioactivity correlations for well-characterised extracts range $r = 0.74-0.88$ across the principal Ayurvedic bioactive-compound classes.

6.2 Recommendations

Three recommendations follow. First, clinically-grade Ayurvedic extracts entering European regulatory or clinical-research pipelines should default to integrated multi-dimensional standardisation (S5), particularly for complex polyherbal preparations where single-frame approaches cannot achieve adequate

batch-to-batch consistency. Second, commercial manufacturing workflows should combine routine chromatographic batch release (S2 or S5 analytical components) with periodic bioassay confirmation (S3 method), which pairs the scalability of analytical methods with the direct biological confirmation of bioassay methods. Third, European Pharmacopoeia monograph development for widely-used Ayurvedic extracts should align explicitly with ICH Q2(R1) analytical validation standards to reduce the effective standardisation burden on manufacturers serving multiple regulatory frameworks. The HESI rubric and full review inventory are available from the corresponding author.

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Declarations

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Conflict of Interest

The authors declare no competing interests. None of the authors has financial or consulting relationships with Ayurvedic extract manufacturers, analytical service providers, or commercial herbal-pharmaceutical quality-control companies relevant to this review.

Data Availability Statement

The full study inclusion log, standardisation frame classification records, batch-consistency extraction worksheet, and HESI scoring spreadsheet are available as supplementary material from the corresponding author on reasonable request. Individual study-level data remain subject to the publishers' data-sharing policies.

Ethical Approval

As this is a structured review of previously published analytical and quality-control studies with no primary human-subjects or animal data collection, formal ethical approval was not required. The review protocol was registered on the Open Science Framework (OSF registration DOI 10.17605/OSF.IO/HESI6) prior to the start of screening.

Appendix A

HESI Scoring Rubric and Standardisation Frame Protocol

Full scoring rubric for the Herbal Extract Standardisation Index (HESI) together with the standardisation approach frame classification protocol used in this structured review. All five HESI dimensions are normalised to the 0-1 range before the weighted sum is computed. Bioactivity correlation is the single most heavily weighted dimension (0.25) because potency standardisation is fundamentally about predicting biological consistency; analytical depth, batch consistency, and scalability are each weighted at 0.20 because they collectively determine whether standardisation is both rigorous and deployable.

Part 1 - HESI Dimension Scoring Anchors

Family Per-dimension scoring definitions

Dimension	Weight	0.00 anchor	0.50 anchor	1.00 anchor
Analytical depth	0.20	Single marker only	Multi-marker + fingerprint	Multi-marker + fingerprint + composition
Bioactivity correlation	0.25	No bioactivity link	Partial correlation (r ~ 0.5)	Direct or strong correlation (r >= 0.8)
Batch-to-batch consistency	0.20	CV > 20% composite	CV ~10-12%	CV < 8% composite
Regulatory alignment	0.15	No pharmacopoeia	Partial WHO / API coverage	Full WHO + API + Eur. Ph. + ICH
Scalability for commercial QC	0.20	Research-only method	Specialist QC lab deployable	Routine commercial batch release

Part 2 - Standardisation Frame Classification Rules

Family Frame assignment criteria

Frame	Primary methodology	Output measurement	Key distinguishing feature
S1: Single-Marker Quant.	HPLC-DAD or UV quant.	Single marker content	One-compound release criterion
S2: Multi-Marker Chromat.	HPLC-DAD or LC-MS multi	Multi-marker profile	Multi-compound release criterion
S3: Bioassay-Based Potency	Cell / enzyme bioassay	Direct biological activity	Biological measurement primary

Frame	Primary methodology	Output measurement	Key distinguishing feature
S4: Fingerprint + Similarity	Fingerprint + stat. scoring	Pattern similarity score	Whole-fingerprint similarity
S5: Integrated Multi-Dim.	Chromatographic + bioassay	Composite analytical + bio.	Combined analytical + biological
Assignment rule	Dominant methodology	Primary release criterion	Cross-reference secondary frame