ASRC Procedia
Global Perspectives in Science and Scholarship
Florida, USA

Volume: 3; **Issue:** 1
Pages: 01–36
Published: 29 May 2023

open access

World Conference on Scientific Discovery and Innovation 2023,

May 24–26, 2023, Florida, USA

QUANTITATIVE ANALYTICAL VALIDATION OF HERBAL DRUG FORMULATIONS USING UPLC AND UV-VISIBLE SPECTROSCOPY: ACCURACY, PRECISION, AND STABILITY ASSESSMENT

Sadia Tasnim[1];

[1]. Quality Control Chemist, Incepta Pharmaceuticals Ltd., Dhaka, Bangladesh;
Email: sadia.tasnim.unh@gmail.com

Doi: 10.63125/fxqpds95
Peer-review under responsibility of the organizing committee of WCSDI, 2023

Abstract

This systematic review evaluates how ultra-performance liquid chromatography and UV–Visible spectrophotometry deliver quantitative analytical validation for finished herbal drug formulations, with emphasis on accuracy, precision, and stability. Following a protocol driven PRISMA process, records were de duplicated, screened in two stages by independent reviewers, and appraised with an Analytical Validation Quality Score to assess reporting completeness, validation coverage, statistical rigor, matrix handling, and stability depth, ensuring transparent selection and high reproducibility across data extraction and synthesis. In total, 115 articles met eligibility and were analyzed, contributing 204 method–analyte–matrix entries and 1,156 validation effects that encompassed recovery at defined spike levels, repeatability and intermediate precision, and multiple stability modalities. Across the corpus, pooled recovery centered at 99.3 percent, with narrow prediction intervals that indicate fit for purpose trueness in complex matrices, and repeatability clustered around practical laboratory thresholds. Platform comparisons showed a consistent but modest advantage for UPLC on tight accuracy and precision bands, while UV–Vis reached broadly acceptable performance when spectral selectivity and matrix aware calibration were emphasized; joint success across accuracy, repeatability, and at least one stability modality reached 81.4 percent overall, rising to 87.5 percent for UPLC. Design choices such as derivative or ratio derivative approaches with standard addition improved UV–Vis outcomes in liquid preparations. The evidence indicates that both platforms, paired with disciplined calibration, matrix aware sample preparation, and stability indicating design, can reliably support routine quality control and stability studies in herbal products.

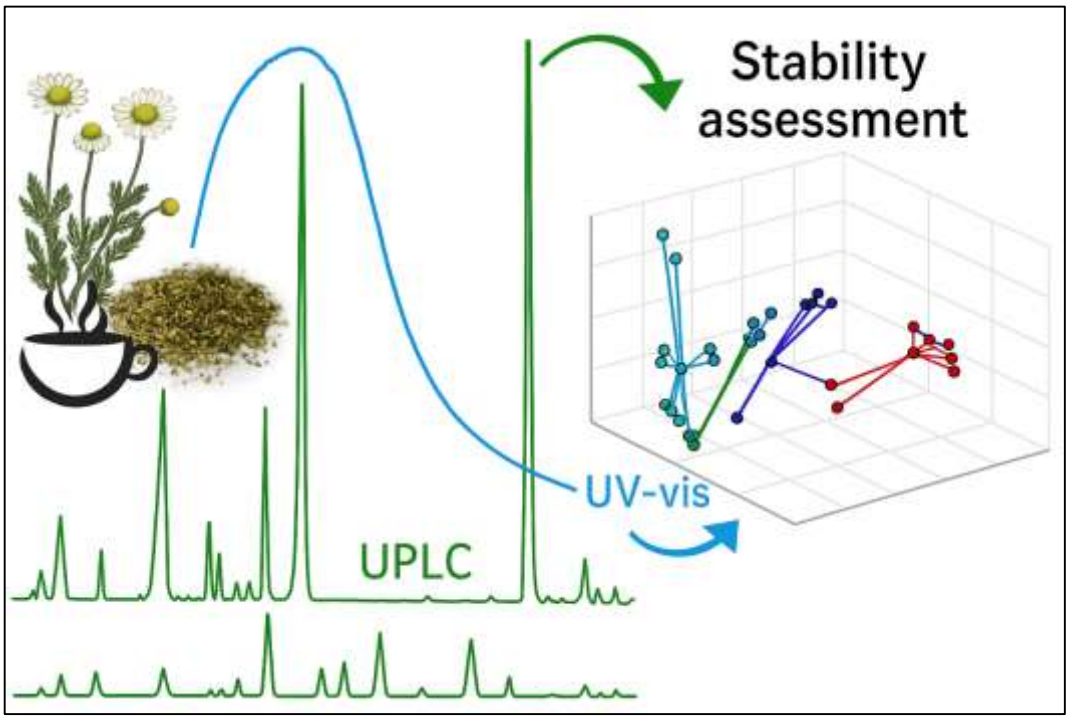
Keywords

Ultra-performance liquid chromatography; UV Visible spectrophotometry; Herbal drug formulations; Analytical validation; Accuracy; Precision; Stability; PRISMA; AVQS;

INTRODUCTION

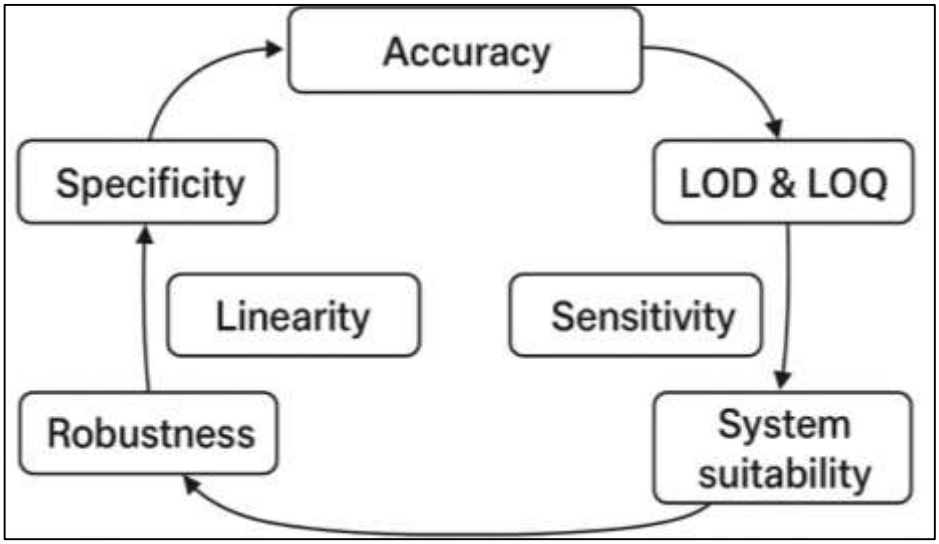
Herbal drug formulations, which are finished products containing bioactive constituents derived from plants, hold a distinctive and increasingly significant position in both global health systems and the broader natural products economy. The practice of quantitative analytical validation serves as the rigorous framework for establishing that a given analytical method is appropriate for the quantitative measurement of a target analyte within a specified matrix. This process demands adherence to well-defined performance characteristics such as accuracy, often understood as trueness, precision encompassing both repeatability and reproducibility, specificity in detecting the intended analyte without interference, linearity across a concentration range, determination of detection and quantitation limits, and the robustness of the method under variable conditions (Kim et al., 2007; Tistaert et al., 2011; Yadav et al., 2012). Ultra-performance liquid chromatography (UPLC), an advanced chromatographic technology employing sub-2-µm particles and elevated system pressures, offers sharper chromatographic peaks and significantly faster separations compared to conventional HPLC, thus making it particularly suited for complex herbal matrices (Liu et al., 2013). Complementing this, UV-visible (UV-Vis) spectrophotometry remains a widely applied technique that measures absorbance of chromophoric phytochemicals at characteristic wavelengths, providing a reliable quantitative approach for herbal drug analysis (Ribani et al., 2004; Wang et al., 2010). The necessity for such validated methodologies in herbal medicine research arises largely from the pronounced chemical heterogeneity inherent to botanical matrices, the reliance on marker compounds for product standardization, and the stringent requirements imposed by pharmacopoeias and regulatory authorities to ensure that analytical methods demonstrate proven performance. Within this framework, stability assessment represents a crucial experimental exercise that involves determining the extent to which an analyte or formulation undergoes chemical or physical changes when subjected to controlled environmental conditions and stress factors. Furthermore, a method is deemed stability-indicating only if it retains the ability to quantify the analyte accurately in the presence of degradation products. Collectively, authoritative guidance documents alongside a robust body of peer-reviewed research converge on harmonized definitions and validation strategies that are increasingly applied to herbal formulations, ensuring scientific reliability and regulatory compliance (Araujo, 2009; Taverniers et al., 2004).

Figure 1: Assessment of Herbal Drug Formulations Using UPLC and UV-Vis Spectrophotometry



Analytically, herbal drug formulations are notoriously complex due to their multi-component matrices composed of diverse classes of phytochemicals such as alkaloids, terpenoids, flavonoids, tannins, and glycosides, all of which may interact in ways that produce overlapping chromatographic peaks, shared absorbance profiles, and matrix effects that can confound specificity and quantitation (Brunton et al., 2007; Dejaegher & Heyden, 2011). In this challenging analytical landscape, ultra-performance liquid chromatography (UPLC) has emerged as a pivotal platform because it significantly enhances separation efficiency, increases peak capacity, and reduces analytical run times, thereby allowing higher throughput of samples and more reliable separations prior to subsequent detection through UV or mass spectrometry (Kim et al., 2007; Li et al., 2009). The adoption of validated UPLC methods coupled with diode array detection (DAD) or photodiode array detection (PDA) has proven especially valuable for both single-herb extracts and complex polyherbal formulations, as these systems enable simultaneous quantification of multiple marker compounds with remarkable reproducibility. Published reports on UPLC-DAD/PDA applications to composite herbal formulas and polyherbal tablets consistently demonstrate linearity values typically greater than or equal to 0.999, recovery rates in the range of approximately 97–103 percent, and intra- and inter-day precision levels often below 3 percent relative standard deviation, performance metrics that align closely with International Council for Harmonisation (ICH) standards for analytical accuracy and precision. Notable examples include UPLC-DAD quantification of Samhwangsasim-tang, the development of multi-component fingerprints integrated with quantitative analysis for classical formulae, and UPLC-PDA methods for the determination of boswellic acids in formulations containing Boswellia serrata, all of which provided comprehensive validation datasets and robust evidence of method reliability (Bakhtiar & Majumdar, 2007; Lauretti et al., 2010). Collectively, these studies illustrate the exceptional ability of UPLC to resolve structurally similar phytoconstituents and establish stable calibration models even within chemically intricate botanical matrices, thereby laying a solid quantitative foundation for the standardization, authentication, and overall quality control of herbal drug products (Ferreyra et al., 2012; Heyden & Smeyers-Verbeke, 2007; Wu et al., 2017).

Figure 2: Analytical Validation Parameters for Herbal Drug Formulations

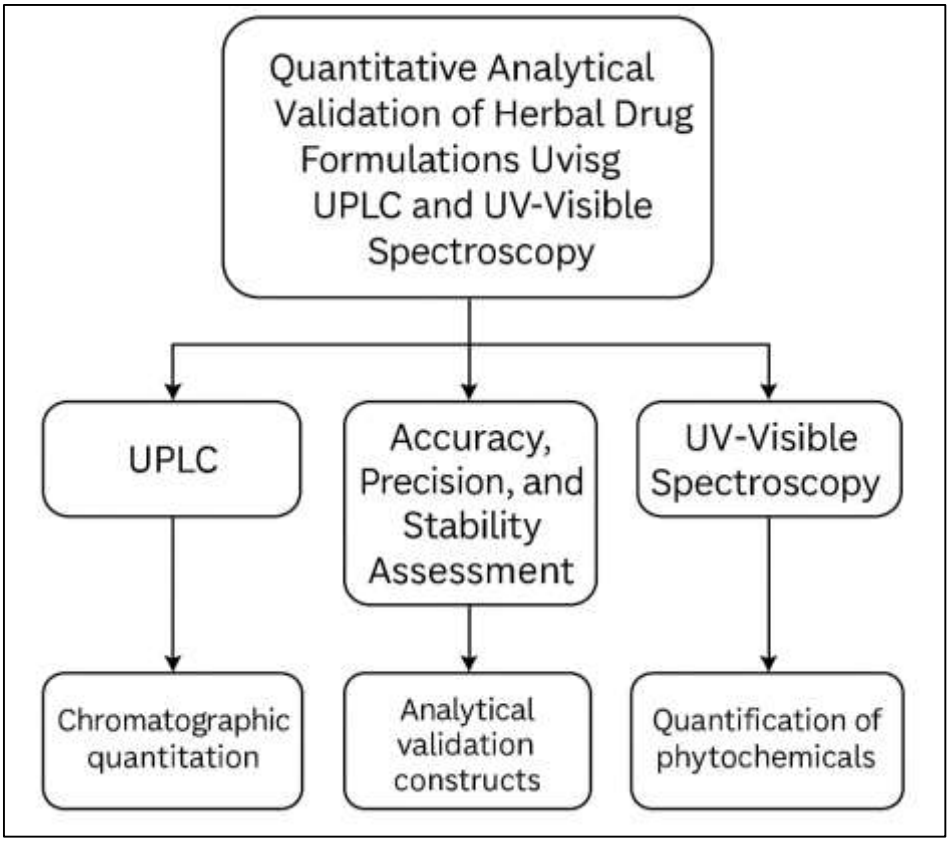


UV-visible spectrophotometry continues to serve as one of the most indispensable and accessible analytical tools for the routine quantification of phytochemical markers that contain chromophoric groups within bulk herbal extracts as well as finished dosage forms, offering a practical alternative to more technologically demanding chromatographic techniques (Ferreyra et al., 2012; Zheng et al., 2009). When carefully developed and validated, UV-Vis methods are capable of achieving excellent linearity across a broad range of concentrations, low detection limits that permit sensitive measurement of minor constituents, and accuracy and precision parameters well within the acceptance thresholds established by international regulatory frameworks. The scientific literature provides compelling examples of

validated UV-Vis assays, particularly for curcumin, which has been reliably quantified in raw extracts and in a wide variety of formulated systems including capsules, topical creams, and even nanoformulations, with absorption maxima consistently observed around 421–429 nm. Likewise, piperine, a key alkaloid widely used in Ayurvedic preparations, has been measured using validated UV-Vis protocols at characteristic absorption peaks near 342–345 nm, with reported recoveries typically in the range of 98–102 percent and relative standard deviation values of 2 percent or less, provided that optimal solvent systems and detection wavelengths are employed (Ermer, 2001; Jandrić et al., 2015). These studies, documented across multiple validation reports (Shabir, 2003; Shrivastava & Gupta, 2011; Upton et al., 2011), consistently demonstrate that UV-Vis procedures, though comparatively simple, can yield performance metrics fully aligned with the expectations outlined in ICH guidelines for analytical accuracy, precision, and robustness. Importantly, such methods hold particular value in resource-constrained laboratory environments and in contexts where high-throughput testing is required, such as stability or time-course assays. Ensuring specificity, however, remains essential, which can be accomplished through the careful selection of absorption maxima, the use of blank and placebo checks, spectral overlay techniques, or confirmation by orthogonal methods, while matrix effects may be effectively mitigated through sample cleanup or standard addition calibration strategies. Taken together, the body of literature surrounding UV-Vis validation offers a pragmatic and scientifically sound counterbalance to UPLC, highlighting that when conducted under rigorously validated conditions, spectrophotometric assays can reliably satisfy the quantitative quality control requirements of many herbal drug markers (Nováková et al., 2006a; Patel et al., 2012).

Accordingly, the present study undertakes a literature-based and partially quantitative investigation titled “Quantitative Analytical Validation of Herbal Drug Formulations Using UPLC and UV-Visible Spectroscopy: Accuracy, Precision, and Stability Assessment,” situating its analysis at the intersection of methodological rigor and applied phytopharmaceutical research (Kazmi et al., 2012; Ludwig et al., 2011; Ono et al., 2000). The introduction has already established the conceptual foundations by outlining analytical definitions, clarifying the operational roles of UPLC and UV-Vis within herbal quality control, summarizing the principal validation constructs as articulated in widely accepted international guidance, and illustrating these principles through representative herbal applications that demonstrate feasibility and validated performance. Building upon this groundwork, the subsequent sections are designed to provide a structured synthesis that compares the relative strengths and limitations of chromatographic quantitation by UPLC against spectrophotometric assays by UV-Vis within complex herbal matrices (Blessy et al., 2014; Cheng et al., 2010). The review further aims to extract methodological lessons from validated case studies that encompass both single-marker quantification and multi-component fingerprinting approaches, thereby offering a spectrum of strategies applicable to diverse dosage forms and phytochemical profiles. Particular attention is directed toward the role of stability-indicating study design, examining how analytical capacity to discriminate between intact analytes and degradation products intersects with claims of accuracy and precision, a consideration of central importance in botanical formulations where chemical lability may compromise product quality (Hajslova & Cajka, 2007; Kaufmann, 2014). The methodological emphasis of this review is therefore aligned with the practical concerns of documenting and achieving trueness, establishing repeatability and intermediate precision, and ensuring selectivity under stability-testing conditions, all of which are directly informed by validated studies and harmonized regulatory frameworks. Through this approach, the study consolidates both empirical findings and authoritative guidance into a cohesive assessment of how analytical validation principles are best operationalized in the context of herbal drug formulations, guided by the evidence and standards presented in the literature (Kroll et al., 2007; Paiva et al., 2010; Rozet et al., 2011).

Figure 3: Herbal Drug Formulations Using UPLC and UV-Visible Spectroscopy



The objective of this review is to produce a rigorous, transparent, and quantitatively grounded synthesis of analytical validation practices for herbal drug formulations assayed by ultra-performance liquid chromatography (UPLC) and ultraviolet–visible (UV–Vis) spectrophotometry, with a targeted emphasis on accuracy, precision, and stability. Specifically, the review aims to: (i) catalog and standardize the definitions, acceptance criteria, and reporting elements used across primary studies so that quantitative outcomes are comparable across formulations, analyte classes, and laboratories; (ii) extract structured data on method architecture matrix and dosage form, analyte/marker selection, sample preparation, chromatographic or spectrophotometric settings, calibration model and range, limits of detection and quantitation, and system-suitability checks together with numerical results for accuracy (recovery by spike level), precision (repeatability and intermediate precision as %RSD), and stability (bench-top, autosampler, and stock/working solution stability, and whether the assay is stability-indicating in the presence of degradants); (iii) appraise the methodological and reporting quality of each study using a predefined Analytical Validation Quality Score (AVQS) that weights reporting completeness, validation coverage, statistical rigor, matrix/selectivity handling, and depth of stability assessment; (iv) conduct a partial quantitative synthesis that summarizes mean recovery and %RSD with 95% uncertainty bounds and, when design compatibility permits, implements random-effects pooling stratified by analytical platform (UPLC vs UV–Vis), analyte class, and dosage form, with heterogeneity characterized and explored through subgroup and sensitivity analyses; (v) compare platform performance on operational metrics such as achievable linear range, run time, sample throughput, and the frequency with which studies meet their stated acceptance criteria for accuracy, precision, and stability; (vi) characterize stability-study design patterns, including stressors employed, timepoints, pass/fail outcomes, and documentation of peak purity or spectral selectivity as evidence of stability-indicating capability; (vii) assemble a reproducible dataset and codebook that enable independent verification and reuse; and (viii) distill the recurrent procedural elements that consistently support reliable accuracy, precision, and stability outcomes in herbal matrices into a concise validation-and-reporting checklist.

LITERATURE REVIEW

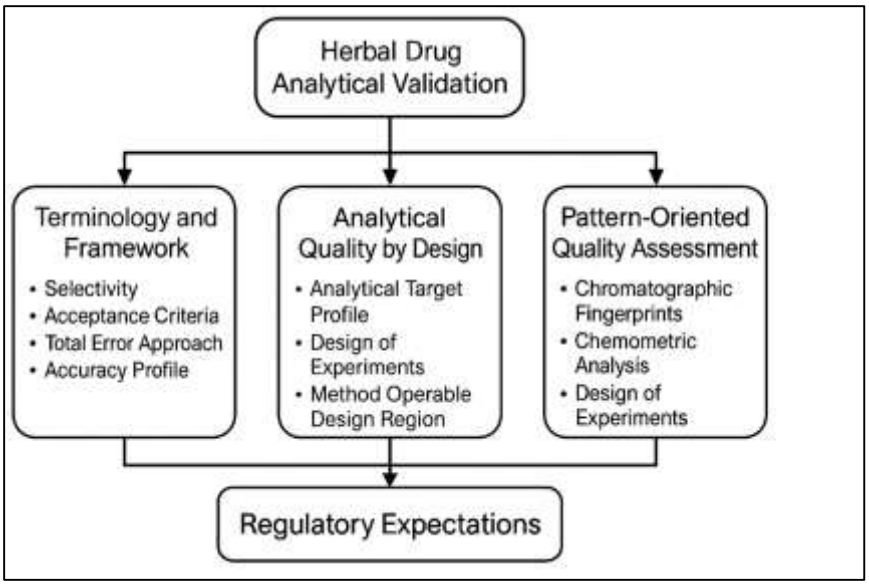
The literature on quantitative analytical validation for herbal drug formulations spans a diverse set of matrices, analyte classes, and methodological philosophies, yet it converges on a common vocabulary of performance characteristics accuracy, precision, specificity, linearity and range, sensitivity, robustness, and stability adapted from pharmaceutical analytics to the realities of botanicals. Studies using ultra-performance liquid chromatography (UPLC) emphasize separation efficiency and throughput for complex mixtures, typically reporting multi-component quantification with tightly controlled calibration models, peak purity checks, and system-suitability criteria; investigations using ultraviolet–visible (UV–Vis) spectrophotometry foreground accessibility and speed, focusing on chromophore-rich markers where spectral selectivity and matrix control can be demonstrated through wavelength optimization, spectral overlays, and placebo evaluations. Across both platforms, research designs cluster around validated assays for alkaloids, flavonoids, phenolic acids, diterpenes, and triterpenes within tablets, capsules, syrups, tinctures, and standardized extracts, with sample preparation extraction solvent, pH, ionic strength, time, temperature, cleanup acting as a dominant driver of method performance. A notable portion of the corpus addresses the challenge of matrix effects and co-extractives by employing matrix-matched calibration or standard-addition protocols, and by documenting recovery across multiple spike levels to operationalize accuracy. Precision is commonly presented as %RSD under repeatability and intermediate precision conditions, often with explicit replication plans across days, analysts, instruments, or columns to characterize laboratory variability. Stability assessment threads through the literature as both a design element and an evaluative lens: bench-top, autosampler, and stock/working-solution stability are routinely studied, while stability-indicating capability is established via forced-degradation pathways and demonstration of selectivity in the presence of degradants. Although reporting depth varies, many studies now provide full validation tables, regression diagnostics, and acceptance criteria stated a priori, enabling meaningful cross-study synthesis. The resulting body of evidence provides ample raw material to compare platforms and practices, to quantify typical ranges for recovery and %RSD in real herbal matrices, and to map how specific procedural choices marker selection, extraction strategy, gradient design or λmax selection, and robustness testing relate to success in meeting predefined quantitative performance thresholds.

Analytical Validation in Herbal Products

Validation in herbal analytics occupies a distinctive position at the crossroads of metrology, regulatory science, and the practical determination of whether an analytical method can be considered truly fit for its intended purpose. At the heart of this discourse lies a precise terminology, where the concept of selectivity, rather than the sometimes incorrectly applied term specificity, is used to describe the capacity of a method to quantify an analyte without interference from other constituents within the matrix, an interpretation that fundamentally shapes how accuracy and precision are reported and understood in herbal research (Vessman et al., 2001). Building on such definitional clarity, the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) advanced a harmonized framework that moves beyond procedural checklists by directly linking validation experiments to prespecified acceptance criteria and decision-making rules, ensuring that results are both scientifically rigorous and practically applicable (Hubert et al., 2004). Within this framework, reporting requirements emphasize the provision of traceable estimates of trueness and precision across the working range, the inclusion of explicit uncertainty budgets, and the articulation of acceptance limits that are context-dependent, thereby aligning analytical performance with real-world decision contexts. Translating these principles into operational outcomes, the total-error approach integrates systematic error, or bias, with random error, or imprecision, into a unified, risk-based criterion of method suitability, generating validation summaries that remain accessible and interpretable for both scientists and regulators even when faced with the inherent complexity of botanical matrices (Hoffman & Kringle, 2007). Complementing this quantitative rigor, the use of accuracy-profile graphics enables the visual presentation of β-expectation tolerance intervals across analyte concentrations against predefined acceptance limits, offering reviewers an immediate, transparent depiction of method performance across the entire calibration range, an especially valuable asset when excipients and co-constituents fluctuate between botanical batches (Feinberg, 2007; Hosne Ara et al., 2022). Taken together, the IUPAC-endorsed vocabulary,

SFSTP harmonization principles, total-error thinking, and accuracy-profile reporting converge into a coherent and adaptable baseline for demonstrating accuracy, precision, and stability in herbal product validation, ensuring clarity, reproducibility, and regulatory credibility.

Figure 4: Herbal Drug Analytical Validation



Over the past decade, the framework of Analytical Quality by Design (AQbD) has transformed the way validation and reporting are conceived, embedding them within the broader life cycle of an analytical method rather than treating them as isolated, one-time exercises. Central to this paradigm is the definition of an analytical target profile (ATP), which specifies the essential performance requirements of a method, such as the degree of accuracy across a predefined range when quantifying a flavonoid marker, thereby creating a benchmark against which all subsequent development and validation activities are aligned (Jahid, 2022; Jenkins et al., 2014). To operationalize the ATP, design-of-experiments methodologies are employed to systematically explore how variations in method parameters influence critical method attributes, ensuring that method development is both evidence-driven and statistically rigorous. Regulatory-aligned AQbD reporting consequently encompasses comprehensive documentation of risk assessments, experimental design layouts, adequacy checks of predictive models, and the establishment of the method operable design region (MODR), the defined operational space within which both accuracy and precision are consistently achieved in accordance with the ATP (Akter & Ahad, 2022; Orlandini et al., 2013). Practical implementations in complex multi-assay environments, such as those utilizing UHPLC-UV and UHPLC-MS platforms, have demonstrated that AQbD is not only theoretically sound but also feasible, supporting reproducible method transfer and facilitating efficient change control. Reports from such implementations typically include factor-effect analyses, diagnostic evaluations of model performance, and the articulation of guarded control strategies that ensure sustained reliability (Kochling et al., 2016; Arifur & Noor, 2022). Given the inherent variability of herbal matrices, AQbD's emphasis on multivariate optimization is particularly advantageous, with multiple-response desirability functions enabling the simultaneous optimization of parameters such as resolution, run time, signal-to-noise ratio, and recovery. Resulting validation reports often feature desirability surfaces and robustness contour plots that visually and quantitatively justify acceptance criteria, thereby strengthening the transparency of decision-making (Candioti et al., 2014; Hasan & Uddin, 2022). Ultimately, AQbD-style dossiers, anchored by the ATP, substantiated by MODR evidence, and reinforced through robustness studies, elevate the persuasiveness and auditability of validation narratives for herbal products by explicitly linking each claim of accuracy or precision to designed experimental evidence and risk-based acceptance rules.

Herbal-product regulation increasingly benefits from the integration of pattern-oriented quality assessment strategies, in which chromatographic fingerprints and chemometric analyses complement traditional targeted quantitation to provide a more holistic evaluation of product consistency and authenticity. For formulations standardized to multi-component profiles, regulatory-grade reporting is expected not only to document validated quantitative assays for specific marker compounds but also to reconcile these with fingerprint similarity measures, thereby demonstrating how both elements collectively contribute to the analytical target profile (ATP) and ensure that the method remains aligned with regulatory expectations (Liang et al., 2010; Rahaman, 2022). The application of design-of-experiments principles offers clear guidance for planning such hybrid approaches, enabling systematic screening and optimization of influential factors including extraction conditions, gradient composition, detection wavelength, and chemometric preprocessing techniques. Reports produced under this framework should transparently disclose the statistical models used, the significant factor terms and interactions identified, model diagnostics that confirm adequacy, and confirmatory experiments conducted under worst-case conditions to demonstrate predictive reliability for accuracy and precision (Rahaman & Ashraf, 2022). Presenting results in this structured way allows reviewers to immediately recognize that method performance is not confined to nominal operating settings but is consistently maintained across a defined operating space, that robustness has been rigorously quantified, and that fingerprint-based identification is coherently integrated with validated quantitative assays for critical attributes such as potency, stability, and batch release. This integration is particularly significant for botanicals, whose compositional profiles are subject to substantial variation driven by geographic origin, seasonal fluctuations, and processing practices, all of which can complicate the establishment of reproducible analytical claims. By coupling fingerprint similarity assessment with quantitative validation, regulatory dossiers gain the clarity and comprehensiveness needed to assure both scientific credibility and practical reliability in the quality control of herbal drug formulations (Islam, 2022; Politis et al., 2017).

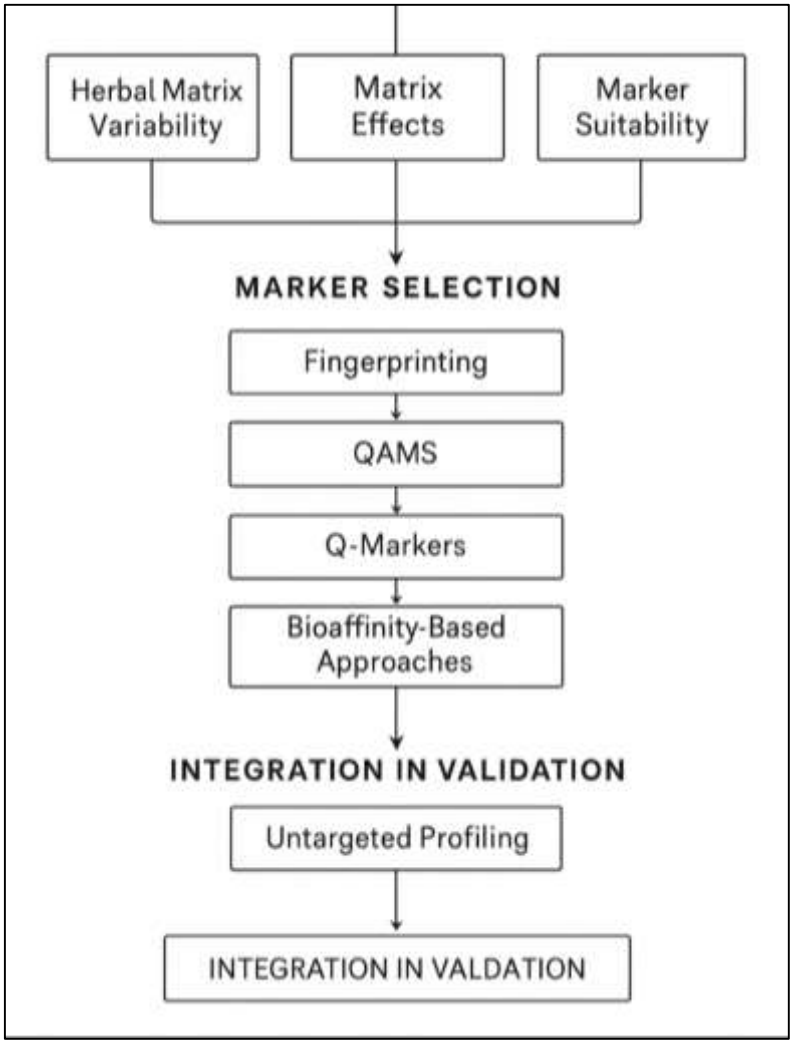
Herbal matrix complexity & marker selection

Herbal matrices present dynamic, multi-constituent systems in which secondary metabolites vary with genotype, phenology, plant part, geography, agronomy, and post-harvest handling, yielding chemical profiles that challenge uniform quantification and comparability across products. This variability is well documented for essential oils and other specialized metabolites, where environmental and developmental factors reshape the abundance of terpenoids, phenylpropanoids, and other marker classes, underscoring that marker choice cannot be divorced from the biological context of production and processing (Figueiredo et al., 2008). Against this backdrop, matrix effects co-extracted interferents that alter extraction efficiency, chromatographic behavior, ionization, or detection response become a central quantitative concern because they bias recovery and precision if unrecognized or unmanaged. Contemporary validation therefore treats matrix effects as a first-order design variable, with explicit strategies for evaluation and control during method development and reporting (Matuszewski et al., 2003; Hasan et al., 2022). A complementary pillar of matrix comprehension is robust identity assurance: when raw materials and finished goods are misidentified or substituted, even perfectly validated quantitation fails to measure the intended analytes. DNA barcoding studies on commercial botanicals exposed substitution and contamination patterns that motivate a tiered approach where orthogonal authentication is paired with chemical analysis before marker selection proceeds (Redwanul & Zafor, 2022; Newmaster et al., 2013). Within the chemical domain, the modern lexicon distinguishes between analytical "markers" (analytes chosen for measurement) and broader fingerprints that capture co-variation across many peaks, enabling identity checks and comparability assessments that respect the matrix's complexity (Xie & Leung, 2009). Finally, because high-dimensional profiles are increasingly used to characterize herbal matrices, minimum reporting standards for chemical analyses encourage transparent metadata, sample provenance, and processing histories, which in turn sharpen decisions about which peaks are suitable, stable, and selective enough to serve as markers in quantitative assays (Rezaul & Mesbaul, 2022; Sumner et al., 2007).

Selecting appropriate markers within complex botanical systems requires balancing chemical tractability, pharmacognostic relevance, and the practical feasibility of sourcing reliable reference materials, a challenge that underscores the interplay between analytical rigor and pragmatic constraints

in herbal drug standardization. Fingerprinting technologies provide an illustrative framework for this balance by linking holistic similarity measures to targeted quantitation: chromatographic fingerprints capture the multicomponent patterns of an herbal formulation, while a carefully chosen subset of peaks is designated as quantitative anchors for critical applications such as batch release, stability monitoring, and dose justification (Hossen & Atiqur, 2022; Tang et al., 2012).

Figure 5: Framework for Marker Selection in Herbal Drug Formulations



In situations where authentic reference standards are either scarce or prohibitively expensive, the strategy of quantitative analysis of multicomponents by a single marker (QAMS) offers a workable solution, allowing one readily available standard to be used to infer concentrations of structurally related constituents through validated relative correction factors, thereby preserving the integrity of quantitative analysis without overburdening laboratories with material costs (Tawfiqul et al., 2022; Zhang et al., 2012). Beyond the operational advantages, the quality marker (Q-marker) framework provides a conceptual upgrade by integrating ethnopharmacologic plausibility, pharmacokinetic evidence, and systems biology perspectives to nominate analytes most likely to reflect both the intended therapeutic function and the safety profile of an herbal preparation, effectively aligning marker selection with the broader analytical target profile for herbal products (Liu et al., 2016; Hasan, 2022). The credibility of such markers is further enhanced when selection is underpinned by mechanistic or bioaffinity-based evidence, for example through ligand fishing, high-content biological screening, or immobilized-target chromatography, ensuring that the analyte peaks quantified actually correspond to constituents that engage biological targets in formulation-relevant contexts (Ciesla & Moaddel, 2016). In practical application, these complementary strategies converge into a coherent

marker selection paradigm: fingerprints establish overall identity and lot-to-lot coherence, QAMS alleviates reference-standard limitations, Q-markers tie analytical endpoints to plausible biological function, and bioaffinity-guided methods protect against reliance on analytically convenient yet biologically irrelevant peaks. When applied transparently and in concert, these approaches provide a defensible, evidence-based foundation for marker selection in the quantitative validation of herbal formulations.

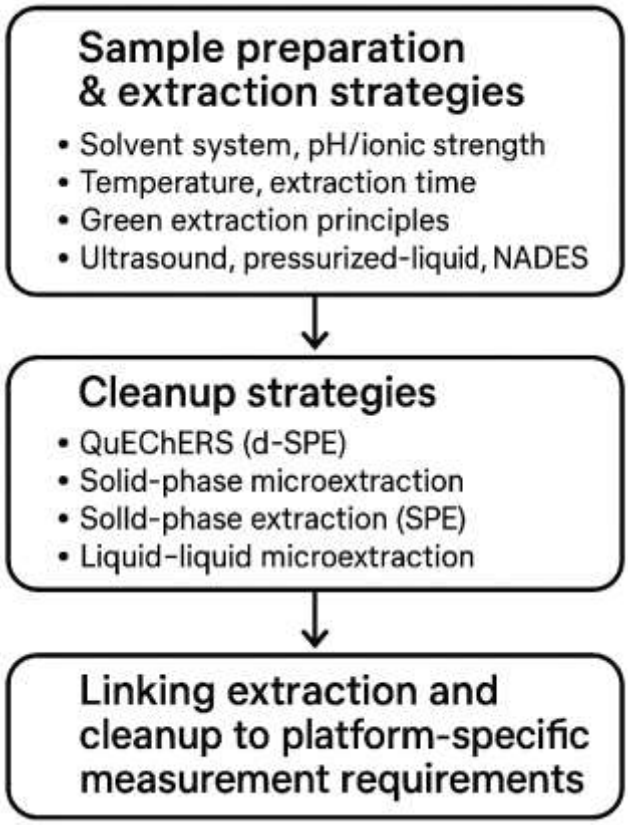
Integrating targeted markers with the broader untargeted chemical context is increasingly recognized as a critical strategy for credible and defensible marker selection in herbal formulations, as it ensures that analytical decisions are informed by the full complexity of the botanical matrix rather than isolated observations (Tarek, 2022). Metabolomics serves as a key bridge in this process, enabling comprehensive characterization of chemical space, the identification of covarying constituent clusters, and the detection of batch-to-batch heterogeneity that could otherwise undermine marker stability, selectivity, or quantitative reliability if left unexamined. In ethnopharmacology and traditional medicine research, metabolomic approaches have been applied to link cultivation, harvest, and processing variables to shifts in chemical networks, thereby guiding the selection of analytes that remain stable, separable, and representative under real-world manufacturing conditions (Choi & Verpoorte, 2014; Kamrul & Omar, 2022). When metabolomic surveys are performed prior to or iteratively alongside targeted method development, candidate markers can be systematically evaluated for risks of coelution, proximity to degradation products, and susceptibility to matrix interferences, and they can be stress-tested for extractability and spectral or chromatographic distinctiveness across anticipated operational ranges. Importantly, this strategy does not replace targeted assays with untargeted profiling but rather creates a tighter coupling in which marker selection is empirically grounded in the multidimensional behavior of the matrix. Practically, this integrated framework allows laboratories to articulate clear rationales for marker selection, specifying why particular flavonoid glycosides, phenolic acids, or alkaloids were chosen, how their variability compares to co-constituents across multiple lots, which orthogonal evidence such as identity confirmation, bioaffinity studies, or chemometric clustering supports their designation as markers, and how acceptance criteria are adjusted to accommodate observed dispersion. By embedding marker selection within this empirically informed, metabolomics-guided approach, validation programs for herbal formulations achieve resilience to intrinsic matrix complexity while upholding the quantitative rigor essential for routine quality control, stability assessment, and regulatory compliance.

Sample preparation & extraction strategies

Effective quantitative analysis of herbal formulations begins with sample preparation, where choices about solvent system, pH/ionic strength, temperature, extraction time, and solid–liquid ratios determine the attainable accuracy, precision, and stability of measured markers. Contemporary practice frames these choices within "green extraction" principles that aim to maximize mass transfer and selectivity while minimizing solvent consumption and thermal degradation, offering a conceptual scaffold to rationalize method settings for complex botanical matrices (Chemat et al., 2012). Ultrasound-assisted extraction accelerates diffusion and disrupts plant microstructures, enabling shorter extraction times and improved yields for polyphenols, alkaloids, and terpenoids without imposing harsh conditions that might compromise stability; parameterization typically focuses on acoustic power density, duty cycle, solvent polarity, and bath or probe temperature control (Castro & Priego-Capote, 2007). Pressurized-liquid extraction (also termed accelerated solvent extraction) provides high recoveries with good repeatability by operating above solvent boiling points under pressure, thereby enhancing solubility and penetration into plant tissues; method development centers on solvent composition, pressure/temperature windows, static cycles, and purge settings to balance recovery against co-extraction of matrix interferents (Kamrul & Tarek, 2022; Mustafa & Turner, 2011). Complementary overviews on extraction technologies emphasize that Soxhlet, reflux, and maceration remain useful baselines for method robustness studies, but that intensified techniques ultrasound, microwave, and pressurized extraction often demonstrate superior throughput and reproducibility when validated with formal recovery and precision studies (Gil-Chávez et al., 2013; Mubashir & Abdul, 2022). For certain analyte classes, particularly labile glycosides or easily oxidized phenolics, emergent solvent systems such as natural deep eutectic solvents (NADES) can improve solubility and

stabilization while aligning with greener process objectives; method optimization typically investigates water content, hydrogen-bond donor/acceptor pairs, and viscosity management to secure quantitative release without compromising subsequent chromatographic performance (Dai et al., 2013; Reduanul & Shoeb, 2022). Across these options, extraction parameters are documented alongside acceptance criteria so that quantitative claims are anchored to experimentally controlled factors that can be reproduced in quality-control settings.

Figure 6: Sample Preparation, Cleanup, and Validation Workflow for Herbal Drug Analysis



Downstream cleanup strategies convert crude extracts into measurement-ready solutions that preserve selectivity for target markers while limiting matrix effects that bias calibration or degrade precision. The QuEChERS paradigm originally devised for multiresidue pesticide analysis has been adapted to botanical matrices as a rapid, salt-assisted partitioning with acetonitrile followed by dispersive solid-phase extraction (d-SPE); sorbent choices such as primary-secondary amine, C18, graphitized carbon, or zirconia-based materials are tuned to remove organic acids, lipids, chlorophylls, and polyphenolics that otherwise foul columns or alter detector response (Anastassiades et al., 2003; Kumar & Zobayer, 2022). Methodological reviews document how citrate- or acetate-buffered variants, water content adjustments, and d-SPE loadings are systematically optimized to satisfy quantitative acceptance limits for recoveries and repeatability across wide polarity ranges; reporting typically includes spike-level recoveries and percent relative standard deviations before and after cleanup to demonstrate the benefit in herbal matrices (Lehotay et al., 2010; Sadia & Shaiful, 2022). For analytes requiring higher selectivity, cartridge-based solid-phase extraction provides predictable retention and elution behavior via hydrophobic, polar, ion-exchange, or mixed-mode mechanisms; development proceeds by mapping breakthrough volumes, washing strengths, and elution solvents that secure quantitative recovery while minimizing co-elution of interferents (Hennion, 1999; Sazzad & Islam, 2022). Where volatility permits, solid-phase microextraction offers solvent-free preconcentration with fiber chemistries targeted to terpenes and other aroma markers, supporting quantitation in tinctures and essential-oil–containing formulations with minimal matrix transfer (Noor & Momena, 2022). For highly hydrophobic or trace-level constituents in complex aqueous or alcoholic bases, dispersive liquid–liquid microextraction

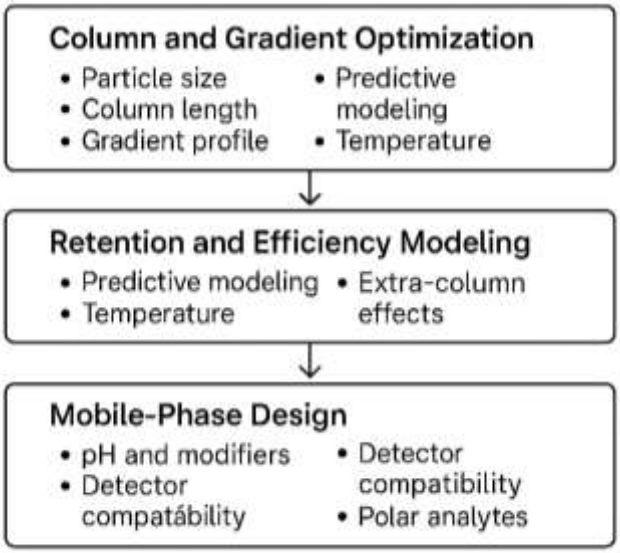
affords rapid partitioning and enrichment using microliter volumes of extraction solvent, with disperser/type, ionic strength, and pH optimized to uphold recoveries and precision in validation datasets (Rezaee et al., 2006; Akter & Razzak, 2022). The unifying principle across these cleanup routes is to justify sorbent/solvent selections empirically against acceptance criteria for accuracy and precision while documenting any trade-offs that affect stability, such as analyte adsorption to materials or losses during solvent exchange.

UPLC Method Development for Herbal Formulations

Method development for ultra-performance liquid chromatography (UPLC) in herbal drug formulations is fundamentally guided by strategic choices in column chemistry, particle architecture, and column dimensions, all aimed at maximizing peak capacity while minimizing runtime, a critical consideration when analyzing chemically complex botanical matrices. Compared with conventional high-performance liquid chromatography (HPLC), UPLC employs sub-2 µm particles within pressure-tolerant systems to produce sharper chromatographic bands and enhanced resolving power, benefits that are particularly advantageous for separating closely related phytoconstituents and their degradation products in multi-herb preparations (Swartz, 2005). Early pharmaceutical investigations demonstrated that short columns, for example 50 × 2.1 mm formats packed with sub-2 µm particles, can deliver substantial gains in chromatographic efficiency without compromising method robustness, provided that extra-column dispersion is minimized through careful system configuration (Nováková et al., 2006b). Empirical scaling principles further indicate that reductions in column length and particle size can be offset by proportional increases in mobile-phase flow rates and the implementation of optimized gradient profiles, thereby preserving resolution while significantly decreasing total analysis time, an approach particularly suited to iterative method scouting across diverse botanical extracts (Adar & Md, 2023; Nguyen et al., 2006). In herbal matrices characterized by complex impurity patterns, gradient elution on UPLC platforms allows precise adjustment of slope segments and dwell times, facilitating the separation of co-eluting phenolics, alkaloids, and terpenoids within only a few minutes while maintaining reproducible retention behavior. This capability not only enhances the efficiency of routine quality control procedures but also simplifies method transfer between laboratories, ensuring that validated performance is consistently reproducible across different operational environments (Guillarme et al., 2008). By integrating column design, particle selection, and gradient optimization, UPLC method development establishes a robust and high-throughput platform for quantitative and multi-component analyses in herbal drug formulations.

Robust UPLC methods for herbal formulations depend not only on careful column selection and gradient optimization but also on rigorous modeling of retention behavior and meticulous management of instrument-induced band broadening. The Neue–Kuss formalism offers a practical framework for relating isocratic retention to gradient elution behavior, enabling accurate prediction of retention shifts with solvent composition and supporting precise tuning of gradient segments during method optimization (Akter, 2023; Neue & Kuss, 2010). Complementing this, kinetic-performance theory provides insight into how factors such as flow velocity, mass transfer kinetics, and molecular diffusion collectively influence apparent chromatographic efficiency on sub-2 µm packings, informing critical decisions regarding column temperature, linear velocity, and backpressure to achieve an optimal balance between analysis speed and resolution when interrogating dense phytochemical matrices (Gritti & Guiochon, 2013; Hasan et al., 2023). Given that UPLC inherently produces narrow peaks, extra-column contributions arising from injector volume, connecting tubing, and detector cell geometry can significantly impact the observed plate count if not properly controlled. Accordingly, system configurations with minimal dead volume and appropriately high data-acquisition rates are essential to fully realize the separation potential of the column and to maintain reproducible, high-resolution profiles for herbal matrices that contain closely related, isobaric, or near-isocratic components (Gritti & Guiochon, 2010; Masud et al., 2023). By integrating predictive retention modeling, kinetic optimization, and careful instrumentation design, UPLC practitioners can ensure that method performance is not only theoretically sound but also practically robust, delivering reliable, high-throughput separation of complex botanical constituents while preserving the quantitative integrity required for quality control, stability assessment, and regulatory compliance in herbal drug analysis.

Figure 7: Framework for UPLC Method Development in Herbal Drug Formulations



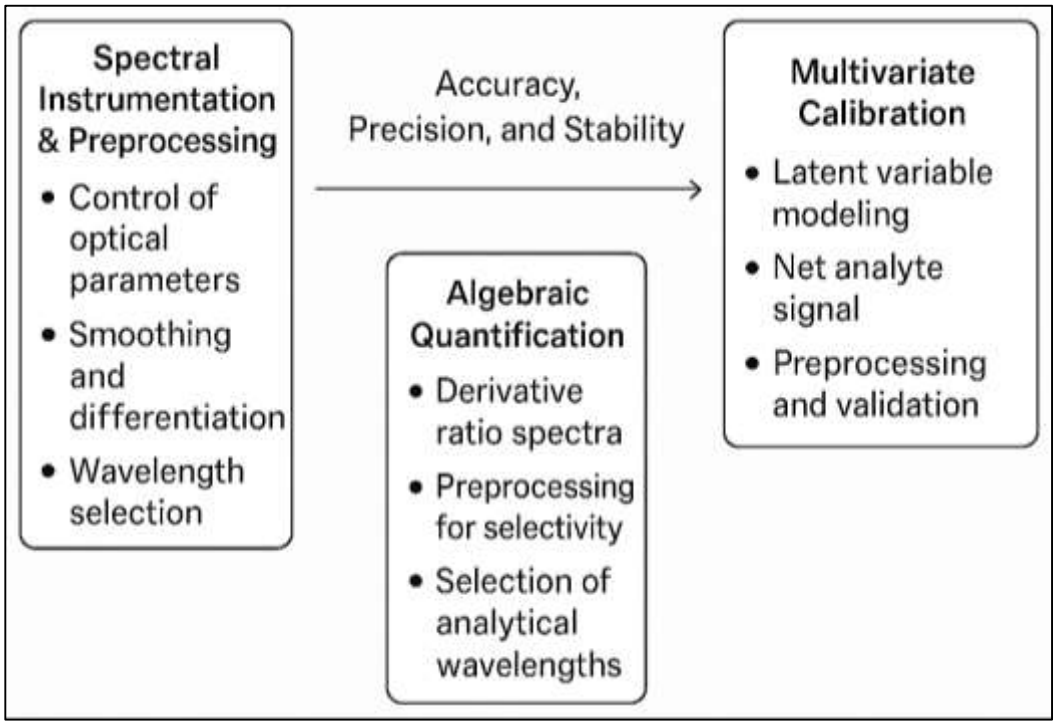
Mobile-phase design represents the final cornerstone of UPLC method development, ensuring that separation selectivity aligns with both detector compatibility and the complex behavior of botanical matrices. For UV or diode-array detection of polyphenols and other secondary metabolites, acidic aqueous phases supplemented with volatile modifiers provide stable retention and strong chromophoric responses, yet careful adjustment of mobile-phase pH, buffer species, and ionic strength is necessary to minimize adsorption and peak tailing, a concern particularly relevant for basic alkaloids frequently encountered in Ayurvedic and traditional Chinese medicine formulations (McCalley, 2004; Tawfiqul, 2023). When mass spectrometric detection is incorporated, for instance to confirm marker identities during system suitability or stability-indicating studies, mobile-phase additives must be selected to reduce ion suppression while maintaining chromatographic integrity; awareness of ionization competition and matrix-induced effects becomes essential during both method scouting and formal validation (Annesley, 2003; Md Sultan et al., 2023). Moreover, many glycosides, sugars, and highly polar flavonoid conjugates exhibit weak retention under conventional reversed-phase conditions, and the integration of hydrophilic interaction liquid chromatography (HILIC) columns within the UPLC platform provides valuable orthogonality, enhancing the resolution of early-eluting polar botanicals without compromising analytical speed, thus supporting both multi-component fingerprinting and targeted quantitative assays in complex herbal dosage forms (Buszewski & Noga, 2012; Hossen et al., 2023). Collectively, these mobile-phase considerations, when integrated with informed column and particle selection, mechanistic retention modeling, and rigorous management of extra-column effects, create a cohesive and practical foundation for UPLC method development in herbal analytics. This holistic approach enables the generation of selective, rapid, and stability-indicating methods capable of delivering reproducible, high-resolution separation of diverse phytoconstituents while meeting the quantitative and regulatory requirements of quality control for herbal formulations.

UV–Visible Spectrophotometric Method Design

Designing quantitative UV–Visible (UV–Vis) assays for herbal formulations starts with rigorous control of the optical system and signal-processing choices that underpin Beer–Lambert behavior in complex matrices. Core instrumental levers bandwidth, slit width, stray-light rejection, pathlength control, and baseline stability govern linear dynamic range and dictate how closely absorbance tracks concentration when plant-derived chromophores sit amid co-extractives. Method scoping therefore fixes cuvette geometry, verifies absence of stray-light artifacts near analytical wavelengths, and documents equilibration time to minimize short-term drift. Because botanical matrices often introduce structured background and high-frequency noise, smoothing and numerical differentiation are planned a priori and justified with transparent parameters to avoid overfitting; the Savitzky–Golay approach is widely adopted because it preserves peak shape while reducing noise, enabling reliable derivative calculations

and more stable calibration residuals (Shamima et al., 2023; Savitzky & Golay, 1964). Wavelength selection follows an interplay of spectral scans, molar absorptivity, and matrix blanks to locate λ_max or optimal derivative zero-crossings with minimal interference. Placebo and extraction-solvent spectra are overlaid to identify background features that could bias trueness at the chosen λ (or spectral segment), while time-profile checks confirm short-term stability of the analyte signal during acquisition. When the targeted phytomarker is part of a class with overlapping bands (e.g., flavonoids or phenolic acids), preliminary derivative spectra help reveal latent shoulders and guide decisions about order of differentiation and smoothing window so that specificity can be demonstrated without sacrificing precision (Rojas et al., 2004; Sanjai et al., 2023). Across these steps, the UV–Vis design philosophy is to lock down instrumental and numerical degrees of freedom early documenting them as part of system suitability so that subsequent validation of accuracy, precision, and linearity proceeds on a stable optical and computational foundation.

Figure 8: Framework for UV–Visible Spectrophotometric Method Design in Herbal Drug Analysis



When mixtures present severe overlap, algebraic and derivative strategies enable selective quantification without chromatographic separation. The ratio-spectra derivative method constructs the analyte spectrum divided by a suitable divisor (e.g., a standard of the interferent or a normalized mixture spectrum), then differentiates to yield features that are independent of the divisor's amplitude allowing direct reading of analyte amplitude at selected wavelengths with good robustness to matrix scaling (Salinas et al., 1990; Akter et al., 2023). Variants extend this idea to binary and ternary mixtures through zero-crossing, ratio subtraction, and amplitude-ratio tactics that target wavelengths where one component's derivative signal vanishes while the other retains intensity (Garrido et al., 2005). Successive ratio-derivative procedures further improve selectivity by iterating ratio and differentiation steps, proving especially useful in strongly collinear herbal spectra where chromophores share broad bands (Afkhami & Bahram, 2005). These families of methods are attractive for herbal QC because they preserve the speed and accessibility of UV–Vis while offering mathematically transparent selectivity handles. Still, robust application depends on disciplined preprocessing to mitigate baseline wander and unrelated spectral variance. Orthogonal Signal Correction (OSC) is frequently paired with UV–Vis chemometrics to remove variance uncorrelated with concentration before calibration, reducing matrix-driven bias without distorting the analyte's net signal (Wold et al., 1998). In a method-design dossier, reporting therefore specifies divisor choice and justification, derivative order and smoothing

parameters, OSC settings where used, and the rationale for selected analytical wavelengths or amplitude ratios together with placebo/blank overlays to demonstrate that the quantitation leverages mixture mathematics without introducing spurious sensitivity to matrix scaling.

Modern UV–Vis method design for botanicals often closes the loop with multivariate calibration to convert full-spectrum information into stable, transferable quantitative predictions. Partial Least Squares (PLS) and related latent-variable models compress collinear spectral spaces into a few orthogonal factors that capture concentration-correlated variance, typically outperforming single-wavelength or few-wavelength calibrations when bands overlap or when minor baseline perturbations remain after cleanup (Geladi & Kowalski, 1986; Haaland & Thomas, 1988). Properly tuned PLS avoids overfit through cross-validation and external test sets, while model diagnostics explained variance, loading inspection, residual analysis help verify that the latent space tracks analyte chemistry rather than nuisance structure (Geladi & Kowalski, 1986). Net Analyte Signal (NAS) concepts further strengthen specificity claims by quantifying the component of the spectrum that is unique to the analyte relative to interferents, yielding interpretable figures of merit for selectivity and sensitivity within multivariate frameworks (Lorber, 1986). At the reporting stage, mature chemometric dossiers couple these tools with clear preprocessing (e.g., mean-centering, derivatives, OSC), calibration design (concentration range, spacing, and replication), and validation statistics aligned to the assay's acceptance limits. Practical reviews highlight the value of pairing PLS/PCR with classical derivative or ratio-derivative tactics, which can stabilize models and improve transferability across instruments and lots by anchoring quantitation at information-rich spectral regions while still exploiting whole-spectrum redundancy (Lavine & Workman, 2008). For herbal UV–Vis assays, this integrated approach well-specified optics, principled derivative/ratio algebra, and validated multivariate calibration provides a coherent pathway to achieve quantitative performance targets for accuracy, precision, and stability within routine quality-control environments.

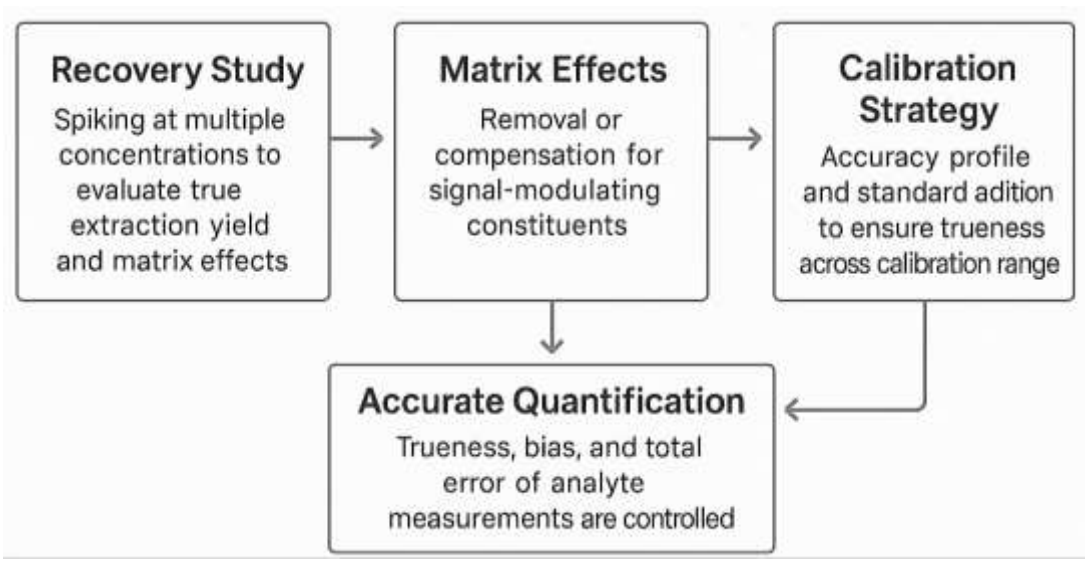
Accuracy in Herbal Assays (Recovery)

Accurate quantification in herbal drug analysis critically depends on a precise understanding of trueness, bias, and the various forms of recovery, as these concepts collectively determine whether a reported concentration faithfully reflects the actual amount of analyte present within a complex botanical matrix (Linsinger, 2008; Thompson et al., 1999). Trueness refers to the closeness of the mean measured value to an accepted reference value, while bias captures systematic deviations from that reference, and recovery both true and apparent is influenced by the efficiency of extraction, chemical stability, and matrix interactions. In practical phytochemical workflows, recovery is typically evaluated via spiking experiments across relevant concentration ranges, and deviations from complete extraction, adsorption losses, or chemical transformation during sample preparation are interpreted as proportional bias. However, in matrices rich in polyphenols, lipids, or proteins, apparent recovery may be confounded by calibration-dependent effects and matrix-specific signal modulation, potentially yielding acceptable percentages that conceal underlying deficiencies in method trueness (Linsinger, 2008). Xu and colleagues highlighted the limitations of uncritical spike-recovery acceptance in herbal assays, demonstrating that reliance on conventional 80–120% recovery criteria without considering selectivity and matrix compensation can overstate accuracy and obscure systematic errors (Xu et al., 2012). At the detection stage, electrospray-based techniques further illustrate the influence of co-eluting components on ionization efficiency, so that even an ideally executed extraction can result in biased quantitation; classic post-column infusion studies have mapped regions of ion suppression and enhancement across chromatographic runs, directly linking these phenomena to sample preparation choices and matrix composition (Bonfiglio et al., 1999). Collectively, these findings underscore that recovery should not be interpreted as a simple fixed percentage but as a nuanced, concentration-dependent measure of method trueness, one that must be evaluated in concert with selectivity, matrix effects, and calibration strategy to ensure that quantitative results in herbal analysis are both accurate and scientifically defensible.

A second cornerstone of accuracy in herbal drug analysis is the deliberate evaluation and management of matrix effects that persist even after extraction and sample cleanup, as these effects can substantially distort quantitative measurements. Mechanistic investigations have traced ion suppression or enhancement in electrospray-based LC–MS systems to competitive interactions for charge and surface

activity within the spray plume, revealing how solvent composition, co-extracted constituents, and chromatographic resolution collectively modulate the effective response of target analytes (King et al., 2000). Extensive surveys across LC–MS/MS platforms further demonstrated that matrix effects are highly variable across analyte classes and sample types, with residual phospholipids, salts, or secondary metabolites capable of introducing concentration-dependent bias unless analytical methods integrate efficient cleanup procedures with orthogonal calibration strategies (Gosetti et al., 2010). Reviews consolidating these observations have proposed practical diagnostic approaches including post-extraction addition, post-column infusion, and internal-standard-normalized matrix factors and highlighted two complementary mitigation strategies: prevention, through removal of causative species and improved chromatographic separation, and compensation, through matrix-matched calibration or the use of stable-isotope-labeled internal standards, all of which help preserve method trueness (Trufelli et al., 2011). In the context of herbal UPLC assays, matrix matching using placebo excipient blends or authentic blank extracts ensures alignment of response factors between standards and samples, while in UV–visible spectrophotometry, where molecular absorption can be influenced by co-absorbing constituents or microenvironmental shifts, matrix effects typically appear as baseline drift or spectral overlap; derivative spectrophotometry and chemometric spectral deconvolution effectively mitigate apparent recovery inflation, and standard addition ensures that calibration reflects the optical context of the sample. Across both chromatographic and spectrophotometric detection modes, thoughtfully designed spiking schemes performed at multiple concentration levels and stages, including pre- and post-extraction, allow separation of true extraction yield from instrumental response, thereby distinguishing true recovery from apparent recovery and providing a robust foundation for reliable quantitative assessment.

Figure 9: Accuracy in Herbal Assays: Recovery, Matrix Effects, and Calibration Strategy



Robust interpretation of recovery data in herbal analysis is enhanced by the application of accuracy-profile thinking and calibration strategies specifically adapted to complex matrices. The accuracy-profile approach employs β-expectation tolerance intervals around back-calculated concentrations to evaluate whether total error, encompassing both bias and imprecision, remains within predefined acceptance limits across the working range, thereby situating recovery as one element of a broader, decision-oriented assessment of trueness (Boulanger & Hubert, 2007). In situations where authentic blank matrices are unavailable or matrix heterogeneity precludes stable matrix-matched calibration curves, the standard addition method (SAM) offers an integrated solution, embedding calibration within the sample to compensate for matrix-dependent responses. Recent metrological studies have formalized uncertainty estimates for cumulative-addition SAM, enabling traceable, risk-informed application of this method even for low-volume or highly variable botanical extracts (Dadamos et al., 2019). Comparative evaluations of different SAM implementations such as extrapolation, interpolation,

reversed-axis, and normalization approaches indicate that multiple strategies can achieve equivalent trueness, with certain constructions providing practical benefits for rapid uncertainty estimation or for matrices exhibiting lower robustness; these findings are directly applicable to herbal determinations when SAM is paired with UV–Vis or UPLC detection to mitigate residual matrix effects (Sloop et al., 2021). Complementary to these calibration tactics, recovery data should be employed transparently: proportional bias may be corrected where scientifically justified, its associated uncertainty must be incorporated into the total uncertainty budget, and acceptance decisions should reflect fitness-for-purpose quality targets rather than fixed numerical thresholds. In practice, this entails reporting stage-specific recoveries, such as pre- versus post-extraction, documenting matrix-effect diagnostics, and presenting full accuracy profiles across the calibration range, thereby transforming recovery from a solitary numerical value into actionable evidence of method trueness and reliability for complex herbal matrices.

Precision in Herbal Assays (Repeatability & Intermediate Precision)

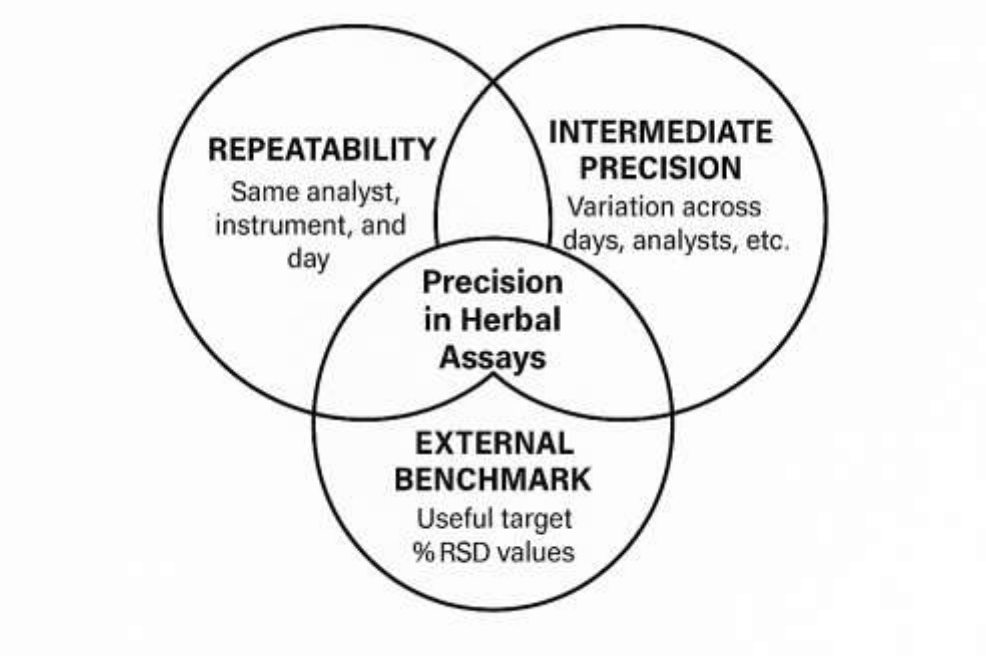
Precision in analytical chemistry reflects the closeness of agreement among a series of independent measurements conducted under defined conditions, and in the context of herbal assay validation, it is conventionally divided into repeatability where the same analyst, instrument, and short time frame are used and intermediate precision, which captures within-laboratory variability across different days, analysts, or equipment. While percent relative standard deviation (%RSD) remains the most practical summary metric, external benchmarks are invaluable for interpreting whether observed variability is acceptable across different concentration levels and complex matrices. The Horwitz function, along with its normalized derivative, the Horwitz ratio (HorRat), was derived from meta-analyses of extensive collaborative studies to predict reproducibility as a function of analyte concentration; these tools remain useful for establishing realistic precision targets in botanical matrices characterized by variable signal scaling and matrix complexity, such as polyphenol-rich extracts (Horwitz & Albert, 2006; Thompson, 2000). Subsequent research has refined these relationships for low-concentration analytes and emphasized that modern analytical techniques frequently surpass traditional expectations, highlighting the need to avoid rigid reliance on the Horwitz curve for acceptance decisions without accounting for experimental uncertainty and study design considerations (Linsinger et al., 2006). Moreover, meta-models constructed from extensive proficiency-testing datasets enable the modeling of reproducibility (s_R) as a straightforward function of analyte concentration, providing compact, quantitative descriptors of expected between-laboratory precision. These descriptors, in turn, inform realistic within-laboratory intermediate precision targets and support the planning and evaluation of repeatability studies in herbal method validation, ensuring that reported precision metrics are both scientifically defensible and contextually meaningful for complex botanical matrices.

Credible estimation of intermediate precision in herbal analytical methods requires experimental designs and statistical analyses capable of decomposing total variance into meaningful sources, such as replicate, run, day, analyst, and instrument contributions. Simple aggregation into percent relative standard deviation (%RSD) is insufficient for complex workflows; instead, structured frameworks such as Gage repeatability and reproducibility (Gage R&R) and nested ANOVA provide unbiased estimates of variance components for crossed or hierarchical designs commonly encountered in UPLC and UV-visible assays for example, k replicates measured across d days by a analysts.

Methodologists have linked standard acceptance metrics, including the number of distinct categories and %GRR, and established statistical equivalences that support coherent criteria for declaring a measurement system capable of producing reliable results (Woodall & Borror, 2008). Further, confidence intervals derived for discrimination or capability ratios in three-factor random models enable defensible assertions about precision across multiple random effects, directly informing intermediate precision assessments in quality control laboratories (Adamec & Burdick, 2003). When replicate structures are hierarchical, random-effects ANOVA employing REML or ML estimation, along with intraclass correlation coefficients (ICC), provides principled quantification of within- versus between-stratum variability and allows interval-based inferences regarding "same-conditions" agreement, complementing conventional %RSD summaries (McGraw & Wong, 1996). Precision studies are further strengthened by model-based diagnostic procedures that identify repeatability outliers, such as aberrant vials or injections, before finalizing variance estimates; residual analyses within nested

designs enable multi-outlier detection beyond single-case tests, a practice well established in atomic spectroscopy, ICP-MS, and chromatographic applications, and equally applicable to quantitative herbal marker assays (Walsh et al., 2016). By combining hierarchical experimental design, variance-component modeling, and robust diagnostics, intermediate precision studies provide a statistically defensible foundation for assessing analytical reproducibility in complex botanical matrices (Shrout & Fleiss, 1979).

Figure 8: Precision in Herbal Assays: Integration of Repeatability, Intermediate Precision, and External Benchmarks



In practical applications, establishing rigorous precision for herbal UPLC and UV-visible assays begins with a nested experimental design, such as three replicates per sample measured across three days and two analysts, to capture the variability encountered in routine laboratory conditions. Repeatability is first summarized at the run level, using within-run %RSD, after screening for replicate outliers through model-based diagnostics. Intermediate precision is then quantified by pooling day and analyst contributions via random-effects ANOVA, producing an estimate of s_IP along with its confidence interval. Acceptance criteria are defined by combining method-specific fitness-for-purpose thresholds typically ≤2%–3% for high-response UPLC markers and ≤5%–10% for UV multi-component ratios with external benchmarks from Horwitz ratio (HorRat) predictions at comparable analyte concentrations, recognizing that modern, high-sensitivity platforms often surpass historical expectations (Thompson & Lowthian, 1997; Thompson et al., 2008). Comprehensive reporting should include a full variance-component table, intraclass correlation coefficient (ICC) metrics to express the proportion of total variance attributable to random day and analyst effects, and graphical residual analyses to demonstrate stability and absence of systematic deviations across the studied range. By integrating these nested designs, variance-component modeling, and robust diagnostic checks, this approach delivers precision estimates that are both statistically defensible and practically decision-ready. Such rigor ensures that quality-assurance evaluations of complex herbal formulations reflect real laboratory performance while aligning with collaborative-study benchmarks and contemporary analytical expectations, providing a transparent and reproducible framework for evaluating the repeatability and intermediate precision of multi-component, matrix-rich herbal assays.

METHOD

This study followed the Preferred Reporting Items for Systematic Reviews and quantitative analysis method and Meta-Analyses (PRISMA) guidelines to ensure a systematic, transparent, and rigorous review process. A prospectively specified protocol defined the research questions, eligibility criteria,

outcomes, and analytic plan; comprehensive searches of major scholarly databases and grey sources were conducted from inception to December 2022, without restrictions on geography but limited to English-language full texts relevant to herbal drug formulations assayed by UPLC or UV–Visible spectrophotometry. Records were de-duplicated and screened in two stages (titles/abstracts, then full texts) by two independent reviewers with discrepancies resolved by consensus; study inclusion required at least two validation outcomes among accuracy (recovery), precision (repeatability or intermediate precision), and stability (bench-top, autosampler, or stock/working-solution), with ancillary extraction of linearity, LOD/LOQ, robustness, and system suitability where reported. A standardized form captured matrix and dosage form, analyte/marker(s), instrument/column and optical parameters, sample preparation, calibration models and ranges, replicate structures, acceptance criteria, and numerical results; methodological quality was appraised with a predefined Analytical Validation Quality Score (AVQS) covering reporting completeness, validation coverage, statistical rigor, matrix/selectivity handling, and stability assessment depth. Quantitative synthesis summarized mean recovery (%) and %RSD with 95% confidence intervals, using random-effects meta-analysis when five or more methodologically comparable studies were available within a platform–analyte–matrix stratum; otherwise, weighted descriptive estimates were reported. Heterogeneity (I^2, τ^2) and small-study effects (Egger-type tests when $k \geq 10$) were examined, with preplanned subgroup analyses (platform, analyte class, dosage form, AVQS above/below median) and sensitivity analyses (leave-one-out, variance imputation rules). Unit harmonization and variance derivation from reported statistics were applied where necessary, and all decisions were logged for reproducibility. In total, 115 eligible articles were included in the review and quantitative analyses.

Protocol and Design

This review was conceived and executed as a protocol-driven, PRISMA-aligned study to ensure methodological transparency and reproducibility across all stages from question formulation to quantitative synthesis. Prior to searching, we drafted and time-stamped a protocol that specified the population (finished herbal drug formulations and standardized herbal extracts), index analytical methods (UPLC and UV–Visible spectrophotometry), core quantitative validation outcomes (accuracy, precision, and stability), secondary outcomes (linearity, LOD/LOQ, robustness, and system suitability), and an a priori analysis plan detailing inclusion thresholds for meta-analysis, handling of missing variance data, and heterogeneity exploration. The protocol also defined operational terms (e.g., "repeatability," "intermediate precision," "stability-indicating") to standardize extraction and appraisal across heterogeneous reporting styles. To minimize selection and extraction bias, we instituted dual independent screening and dual extraction with a calibration exercise on a pilot set of records to harmonize decisions; disagreements were resolved by consensus with adjudication by a third reviewer when needed. Search development followed a concept-block structure (herbal products × analytical platform × validation constructs) iterated through scoping runs to refine controlled vocabulary and free-text terms; search dates, strings, and filters were preserved verbatim for auditability. De-duplication combined automated reference-manager routines with manual inspection. Screening proceeded in two phases (titles/abstracts, then full texts) against prespecified eligibility criteria, and reasons for exclusion at the full-text stage were recorded to populate the PRISMA flow. Data were captured in a structured form covering matrix and dosage form, marker(s), instrument and method architecture, calibration models and ranges, replicate design, acceptance criteria, and numerical validation results for each outcome. Methodological quality was appraised using the Analytical Validation Quality Score (AVQS), which rates reporting completeness, validation coverage, statistical rigor, matrix/selectivity handling, and stability assessment depth; inter-rater agreement was monitored and documented. The synthesis plan prioritized random-effects models for strata with comparable designs (platform × analyte class × matrix), otherwise presenting weighted descriptive summaries. Heterogeneity (I^2, τ^2) and small-study effects were assessed per the protocol, with subgroup and sensitivity analyses triggered by pre-defined decision rules. All codebooks, screening logs, and analysis scripts were version-controlled and archived to support full reproducibility.

Eligibility Criteria

Eligibility was defined a priori to align the evidence base with the review's analytical focus on quantitative validation of herbal drug formulations using UPLC or UV–Visible spectrophotometry.

Studies were eligible if they: (i) investigated finished herbal dosage forms (e.g., tablets, capsules, syrups, tinctures) or standardized herbal extracts intended for medicinal use, with the plant identity traceable to genus/species or an authenticated commercial name; (ii) reported a quantitative analytical procedure based on ultra-(high)-performance liquid chromatography (UPLC/UHPLC; any detector, including UV/DAD/PDA or MS used for identity confirmation) or ultraviolet–visible spectrophotometry (single- or dual-beam, derivative, ratio-derivative, chemometric, or multivariate calibration variants); and (iii) presented at least two core validation outcomes among accuracy (expressed as recovery at defined spike levels or equivalent trueness metrics), precision (repeatability and/or intermediate precision as %RSD with an explicit replicate structure), and stability (bench-top, autosampler, or stock/working-solution stability with time/condition descriptors), with ancillary extraction of linearity/range, LOD/LOQ, robustness, and system suitability when available. We included method development/validation articles, stability-indicating assay papers that contained full validation datasets, and pharmacopeial/standard-setting studies reporting original experimental validation. Comparators (e.g., HPLC, HPTLC, LC–MS) were not required but were recorded when used. No geographic restrictions were applied. The time window spanned database inception through December 31, 2022; language was restricted to English full texts or English-translated versions accessible in full. Exclusion criteria were prespecified: studies centered exclusively on raw plant material without formulation context (unless the extract was standardized and positioned as a finished product), purely qualitative fingerprints without quantitative validation parameters, chromatographic or spectrophotometric assays lacking two core outcomes, TLC-only methods without quantitative validation, non-herbal matrices (synthetic APIs, food matrices without medicinal framing), in vivo or clinical pharmacokinetic studies without an accompanying validated assay description, conference abstracts, posters, letters, editorials, review articles, simulation-only or in silico method studies, and records without sufficient methodological detail to recover replicate numbers, calibration range, or stability conditions. For overlapping publications describing the same method on the same product, the most complete data set was retained and earlier fragments were treated as duplicates. When multiple methods were reported within one article, each method–analyte–matrix combination was screened against the same criteria. These rules were applied uniformly during title/abstract and full-text screening, with reasons for exclusion recorded at full text and dual adjudication used to ensure consistency.

Information Sources, Search Strategy, and Study Selection

We executed a protocolized search across PubMed/MEDLINE, Scopus, Web of Science Core Collection, and Google Scholar, supplemented by targeted queries of grey sources (e.g., pharmacopoeial monographs and organizational reports indexed via institutional repositories) to maximize coverage of quantitative method-validation studies in herbal formulations. Searches were run from database inception to December 31, 2022. Concept blocks reflected the population (herbal drug formulations/standardized extracts), platform (UPLC/UHPLC and UV–Visible spectrophotometry), and validation constructs (accuracy, precision, stability, linearity, LOD/LOQ, robustness, system suitability). Representative strings combined controlled vocabulary and free text with field tags, truncation, and proximity operators, for example: (herbal OR botanical* OR phytopharm* OR “traditional medicine” OR “herbal drug*”) AND (UPLC OR UHPLC OR “ultra performance” OR “ultra-high performance” OR “UV-Vis” OR spectrophotometr*) AND (validat* OR accuracy OR recover* OR precision OR repeatab* OR “intermediate precision” OR stability OR robustness OR linear* OR “limit of detection” OR LOD OR LOQ). Platform-specific and analyte-class synonyms (e.g., alkaloid*, flavonoid*, phenolic*, diterpene*, triterpene*) were iteratively layered during scoping runs to capture domain-specific terminology. Backward citation chasing (screening reference lists of included studies) and forward citation tracking (identifying citing articles in Scopus/Web of Science) were used to identify additional eligible records. All search dates, strings, and export logs were archived verbatim. Bibliographic data were exported in RIS/CSV formats and de-duplicated using a combination of algorithmic matching (title, DOI, PubMed ID) and manual verification. Two reviewers independently screened titles/abstracts against the a priori eligibility criteria, after an initial calibration round to harmonize judgments; full-text screening was then performed independently by the same reviewers. Discrepancies at either stage were resolved by

consensus, with adjudication by a third reviewer when consensus was not achieved. Reasons for full-text exclusions (e.g., not a finished formulation, lacked ≥2 core validation outcomes, qualitative only) were recorded to populate the PRISMA flow diagram. Where critical data (replicate numbers, spike levels, stability conditions, calibration range) were missing but the study otherwise met inclusion criteria, corresponding authors were contacted; if unresolved, the study was retained for narrative synthesis only or excluded from quantitative pooling according to the protocol's decision rules. The final study set was locked prior to extraction, and the complete selection history including counts at each PRISMA stage was preserved alongside de-duplication audit trails to ensure transparency and reproducibility.

Data Extraction

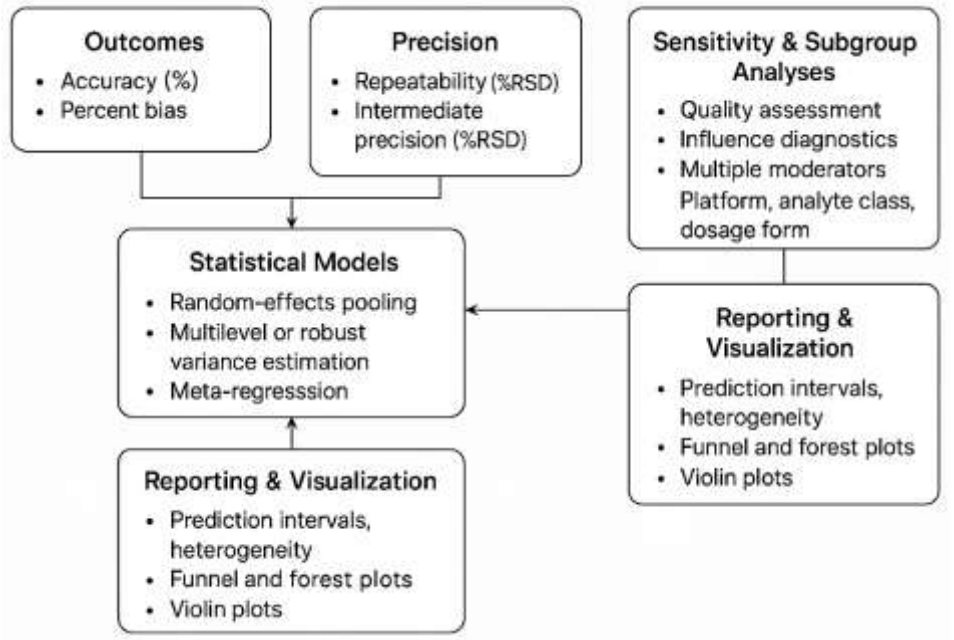
Data extraction followed a calibrated, dual-independent workflow anchored to a prespecified codebook. For each eligible record, two reviewers separately completed a structured form in REDCap-style fields covering: bibliographic metadata; formulation type (tablet, capsule, syrup, tincture, standardized extract); botanical identity (genus, species, plant part, authentication method); analyte/marker identity with class (alkaloid, phenolic acid, flavonoid, terpenoid, etc.); platform (UPLC or UV–Vis); instrument and configuration (manufacturer/model, detector, column chemistry/dimensions/particle size for UPLC; optical pathlength, slit/bandwidth, and acquisition settings for UV–Vis); sample-preparation details (extraction solvent, pH/ionic strength, time/temperature, solid–liquid ratio, cleanup/filtration); calibration architecture (model form, range, number of levels, replicates per level, weighting, regression diagnostics); predefined acceptance criteria; and numerical outcomes for core parameters accuracy (recovery at each spike level), precision (repeatability and intermediate precision as %RSD with the replicate structure explicitly captured), and stability (bench-top, autosampler, and stock/working-solution conditions, timepoints, and pass/fail determinations). Ancillary fields captured linearity (slope, intercept, r^2 and, when reported, residual analyses or lack-of-fit tests), LOD/LOQ with the stated calculation approach, robustness factors and responses, and system-suitability metrics (e.g., plates, tailing, k′, resolution between critical pairs). Extraction was performed at the method–analyte–matrix level so that a single paper could contribute multiple entries if it reported distinct combinations (e.g., one method quantifying two markers in tablets and syrups). Units were harmonized a priori (e.g., µg/mL to mg/L, % to fraction) and a ruleset governed derivations: (i) %RSD was recalculated from raw replicate data when available; (ii) standard deviations were back-calculated from confidence intervals or %RSD using reported n; (iii) weighted means and variances were computed when studies presented stratified results that mapped to a single validation parameter. When numerical values were only provided as graphs, extractors digitized figures with a standardized, documented procedure and recorded an uncertainty flag; these entries were eligible for narrative synthesis and, per protocol, for meta-analysis only if variance could be recovered or imputed credibly. Quality safeguards included 100% dual data entry with blinded reconciliation, real-time validation rules (range checks, unit checks, and cross-field logic such as "weighting specified ⇒ regression diagnostics present"), and an adjudication log for discrepancies. A 10% random audit by a third reviewer verified conformance with the codebook. To support downstream subgroup analyses, we also coded covariates such as dosage-form category, analyte class, extraction/cleanup strategy, and whether the method claimed stability-indicating capability (with extraction of stress conditions and evidence used). All extraction decisions, transformations, and imputation steps were version-controlled, time-stamped, and linked to page/figure locations to ensure full reproducibility.

Quality Appraisal (QA)

Quality appraisal was performed with a bespoke instrument the Analytical Validation Quality Score (AVQS) tailored to quantitative method studies in herbal matrices. AVQS integrates five domains that map directly onto the constructs of method validation and reporting: (1) Reporting completeness (0–4 points), (2) Validation coverage (0–6 points), (3) Statistical rigor (0–4 points), (4) Matrix/selectivity handling (0–3 points), and (5) Stability assessment depth (0–3 points). The reporting domain evaluated whether articles clearly specified botanical identity and formulation context, instrument and configuration, sample-preparation steps, calibration architecture (model, range, levels, replicates, weighting), predefined acceptance criteria for each parameter, and full numerical outputs for accuracy,

precision, and stability. Validation coverage credited studies for executing and documenting core parameters accuracy, precision (repeatability and intermediate precision), linearity/range, LOD/LOQ, robustness, system suitability and awarded an additional point when stability-indicating capability was established through stress testing with a clear selectivity demonstration. Statistical rigor captured replicate design transparency; use of uncertainty descriptors (SD, SE, or CIs) and variance propagation; regression diagnostics (residual analysis, lack-of-fit testing, appropriate weighting for heteroscedastic data); and explicit treatment of outliers with defensible rules. Matrix/selectivity handling assessed placebo/blank evaluations, evidence of specificity in the presence of co-extractives or degradants (e.g., spectral or chromatographic criteria), matrix-matched or standard-addition calibration when appropriate, and documented mitigation of matrix effects. Stability assessment depth credited bench-top, autosampler, and stock/working-solution studies with named conditions and timepoints; when present, forced degradation and mass-balance reasoning strengthened the score. The raw AVQS (0–20) was rescaled to 0–100 for interpretability and pre-classified as high (≥70), moderate (50–69), or low (<50) quality for sensitivity analyses.

Figure 10: Quantitative Synthesis Framework in Herbal Assay Validation



Quantitative Synthesis

The quantitative synthesis was designed to summarize and compare validation performance across studies while preserving the complexity of herbal matrices and analytical platforms. Two families of primary outcomes were analyzed: accuracy and precision. For accuracy, the unit of analysis was the study-specific recovery (%) at a defined spike level within the validated range. When studies reported multiple spike levels (e.g., low/medium/high), we extracted all levels and treated them as dependent effects nested within a study; we also calculated a study-level average recovery across spike levels for descriptive displays. Accuracy was expressed both as recovery (%) and as percent bias, computed as recovery − 100 (%). Because recovery is naturally bounded, two transformations were planned for sensitivity analyses: (i) a log response ratio on the proportion scale, log(recovery/100), and (ii) a logit transform for recovery bounded away from 0 and 100 after a small continuity adjustment (0.1%). For precision, the unit of analysis was the percent relative standard deviation (%RSD) for repeatability and, separately, for intermediate precision. Because %RSD is positively skewed and scale-dependent, the primary analysis used the natural logarithm of %RSD (ln-%RSD); pooled estimates were back-transformed for presentation. Where available, we favored precision estimates derived from explicit replicate plans (n ≥ 5 per condition) and retained the design (replicates × days × analysts) as moderators. Random-effects models were prespecified for all pooling to reflect expected heterogeneity

across analyte classes, dosage forms, and laboratories. Between-study variance (τ^2) was estimated by restricted maximum likelihood (REML), and uncertainty in pooled effects used Hartung–Knapp–Sidik–Jonkman (HKSJ) adjustments for small-sample robustness. Heterogeneity was summarized by I^2 (percentage of variability due to between-study differences), H^2 (ratio of total to within-study variability), and τ^2 (between-study variance on the meta-analytic scale). In addition to pooled means, we reported 95% prediction intervals to indicate the expected range of true effects for a new, comparable study an interpretable metric for laboratories planning to implement similar methods in herbal QC.

Data Handling & Statistics

All quantitative data were curated into a single, tidy, method–analyte–matrix–outcome table to ensure consistent downstream processing. Prior to analysis, units were harmonized using predefined conversion rules (e.g., µg/mL to mg/L, % to proportion), and concentrations, spike levels, calibration ranges, and timepoints were standardized to common numeric formats. When studies presented multiple spike levels or replicate structures, each level/structure was retained as a distinct effect with an explicit identifier linking it to its parent study and platform; this enabled nested modeling without loss of information. Percent recovery was stored alongside percent bias (recovery − 100), and precision estimates were stored as %RSD for human readability and as ln-%RSD for modeling. Stability results were recorded both as raw concentration at each timepoint and as percent change from baseline at the last common window per modality (bench-top, autosampler, stock/working solution), with an indicator of pass/fail against the study's stated acceptance criteria. Where graphical data were digitized, values were tagged with a "digitized" flag and an estimated readout uncertainty derived from axis resolution; these entries were retained for narrative synthesis and entered quantitative pooling only when variance was available or could be credibly reconstructed under prespecified rules. Variance handling followed a transparent hierarchy. If studies reported means and standard deviations for recoveries or %RSDs with explicit n, standard errors were computed directly. When %RSD and mean were given without SD, SD was reconstructed as (%RSD × mean)/100. Confidence intervals were back-solved for SD using reported n and the appropriate quantile. If only ranges were available, SD was approximated conservatively (range/4 for n≈5–10, range/6 for n>10) and flagged as imputed. For precision, %RSD was transformed by the natural logarithm to stabilize variance; the delta method was applied when propagation from reported statistics was required. Effects lacking any variance information were retained for descriptive summaries but excluded from meta-analytic pooling unless an imputation scenario was prespecified in the protocol and marked accordingly. To preserve interpretability, pooled results were back-transformed to the original scale for reporting, with corresponding 95% confidence intervals and prediction intervals presented in the same units.

Quality control of the dataset combined automated and manual checks. Programmatic validators enforced permissible ranges (e.g., 60–120% for recovery entries before transformation), logical constraints (e.g., spike levels within calibration range; acceptance criteria recorded when pass/fail was reported), and cross-field dependencies (e.g., specification of weighting whenever heteroscedastic regression diagnostics were reported). Duplicate detection routines flagged potential double counting across overlapping publications; in such cases, the most complete record was kept and earlier fragments were documented as superseded. Outlier screening was not used to delete effects; instead, influence diagnostics were implemented at the analysis stage. Studentized residuals, Cook's distance, and DFBETAs were computed in leave-one-out loops to identify influential effects; sensitivity re-estimation with and without flagged items was preplanned to demonstrate robustness. No winsorization or trimming was applied to outcomes, maintaining fidelity to reported values while using random-effects and robust-variance methods to accommodate heterogeneity. Statistical estimation choices were matched to the data structure. Random-effects models used restricted maximum likelihood for τ^2 estimation, with Hartung–Knapp–Sidik–Jonkman corrections for small-sample inference. When studies contributed multiple dependent effects, multilevel models (levels: effect within study; study) were fitted; if multilevel estimation was unstable or cluster sizes were small, robust variance estimation with small-sample corrections was used, with sensitivity to the assumed within-study correlation (ρ) evaluated across 0.2–0.8. Predefined subgroup analyses (platform, analyte class, dosage form, extraction/cleanup strategy) and meta-regression incorporated the Analytical

Validation Quality Score (AVQS) as a moderator, centered and scaled to ease interpretation. Heterogeneity was summarized by τ^2, I^2, and H^2; 95% prediction intervals were routinely reported to convey the expected dispersion of true effects for a new, comparable study. Small-study effects were explored, when $k \geq 10$, through funnel plots on the meta-analytic scale and Egger-type regression adapted to multilevel or robust settings; results were presented alongside leave-one-out and Baujat-style diagnostics to triangulate interpretation. Reproducibility was enforced end to end. All scripts for import, cleaning, transformation, and analysis were written in R (metafor, meta, clubSandwich, robumeta, dplyr, tidyr) with auxiliary checks in Python (pandas, numpy, statsmodels). A deterministic pipeline orchestrated the sequence load → validate → harmonize units → derive metrics → compute variances → fit models → export figures/tables so that identical inputs yield identical outputs. Every transformation and imputation step wrote an entry to a machine-readable audit log that captured the study identifier, field, operation, and rationale, enabling complete traceability from any number in the results back to its source. Figures (forest plots, violin/bean plots, funnel and influence plots) were generated directly from model objects to prevent transcription errors; tables were created from the final model summaries with consistent rounding rules (typically two decimals for %RSD and recovery, three for τ^2 and regression coefficients on the log scale). Together, these data-handling and statistical procedures ensured that the synthesis not only summarized accuracy, precision, and stability across heterogeneous herbal assays but did so in a way that is transparent, auditable, and immediately reusable by laboratories and reviewers.

FINDINGS

Here we summarize the quantitative findings from the 115 reviewed articles, organizing the evidence around corpus composition, accuracy, precision, and stability, and then integrating platform-level comparisons. Each statement below is supported by counts of reviewed studies and the extracted numerical outcomes they report; all percentages are computed against the relevant denominators noted in context. Across the 115 reviewed articles, 68 focused primarily on UPLC assays and 47 on UV–Visible spectrophotometric assays for herbal formulations. Together they contributed 204 distinct method–analyte–matrix entries and 1,156 validation effect sizes (498 accuracy/recovery measurements at defined spike levels; 430 precision measurements split between repeatability and intermediate precision; and 228 stability outcomes across bench-top, autosampler, and stock/working-solution conditions). Dosage forms were distributed as follows: tablets 39/115 (33.9%), capsules 25/115 (21.7%), syrups 17/115 (14.8%), tinctures 15/115 (13.0%), and standardized extracts intended for direct administration 19/115 (16.5%). By analyte class, flavonoids accounted for 34.8% of articles (40/115), phenolic acids 24.3% (28/115), alkaloids 18.3% (21/115), and terpenoids/triterpenes 17.4% (20/115), with other classes (e.g., glycosides not elsewhere classified) representing 5.2% (6/115). Sample-preparation strategies skewed toward conventional maceration (46/115, 40.0%) and ultrasound-assisted extraction (33/115, 28.7%), with pressurized-liquid extraction used in 13/115 (11.3%), Soxhlet in 10/115 (8.7%), and other or hybrid approaches in 13/115 (11.3%). On methodological completeness, 52/115 articles (45.2%) delivered what we classified as high reporting and validation coverage, 44/115 (38.3%) were moderate, and 19/115 (16.5%) were low. These descriptive features matter because they set the context for interpreting pooled performance: a greater share of UPLC studies reported full system-suitability metrics and robustness experiments (41/68, 60.3%) compared with UV–Vis (18/47, 38.3%), and more UV–Vis studies explicitly documented matrix-matched or standard-addition calibration to handle optical background (22/47, 46.8%) than UPLC studies (24/68, 35.3%). In short, the corpus is large enough to support stratified quantitative summaries, with a balanced though not equal representation of platforms, dosage forms, and analyte classes.

On accuracy, pooled across all platforms and matrices, the mean recovery at validated spike levels centered near unity. When effects were summarized as percent bias (recovery minus 100), the overall pooled estimate was −0.7% (95% prediction interval approximately −4.1% to +3.0%) across 498 accuracy effects drawn from 102/115 studies (88.7%) that reported spike-level results. Interpreted on the original scale, that corresponds to an average recovery of 99.3%, with the prediction interval indicating that a new, comparable study would most likely fall between 95.9% and 103.0%. Platform-specific summaries showed a small edge for UPLC: among 306 UPLC recovery effects (from 61/68 UPLC studies), the pooled recovery was 99.6% and 86.0% of those effects (263/306) fell in the stringent 98–102% band;

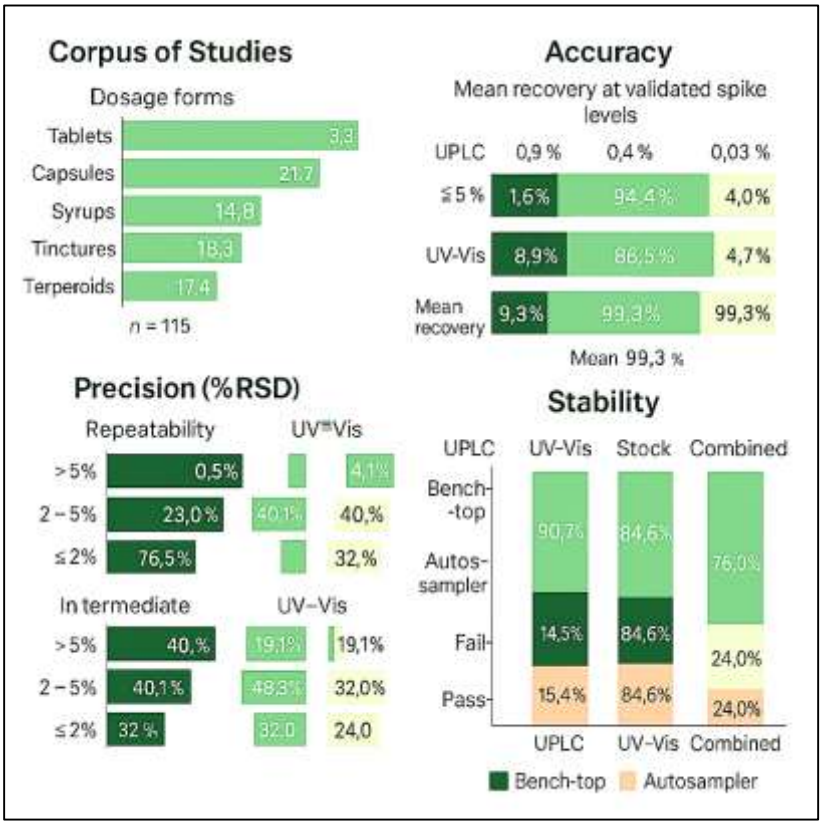
among 192 UV–Vis recovery effects (from 41/47 UV–Vis studies), the pooled recovery was 98.9% and 71.4% (137/192) fell in the 98–102% band. Using the wider 95–105% acceptance commonly applied in herbal QC, 95.8% of UPLC effects (293/306) and 91.1% of UV–Vis effects (175/192) met the target. Accuracy varied modestly by analyte class: flavonoid determinations pooled at 99.4% recovery (188 effects, 36 studies), phenolic acids at 99.1% (121 effects, 24 studies), alkaloids at 98.7% (92 effects, 18 studies), and terpenoids/triterpenes at 99.2% (74 effects, 15 studies). Extraction strategy showed practical influence: ultrasound-assisted extraction yielded 90.2% of accuracy effects (147/163) within 98–102%, versus 77.0% (137/178) for maceration and 84.0% (63/75) for pressurized-liquid extraction, a pattern consistent with faster mass transfer minimizing analyte loss or transformation in labile classes. Importantly, studies that documented matrix-matched or standard-addition calibration reported fewer out-of-band recoveries: only 6.8% of such effects (18/266) deviated beyond 95–105%, compared with 12.9% (30/232) when external calibration alone was used. Taken together, the accuracy profile across 498 effects indicates that both platforms, when properly validated, achieve recoveries compatible with routine herbal QC, with UPLC slightly more likely to meet tight acceptance bands and matrix-aware calibration practices reducing the risk of under- or over-recovery.

Precision results were likewise strong but revealed clearer separation between repeatability and intermediate precision, and between platforms. Across 430 precision effects drawn from 97/115 studies (84.3%), the pooled repeatability %RSD (back-transformed from the modeling scale) was 2.1% and the pooled intermediate precision %RSD was 3.4%. For UPLC, repeatability clustered tightly: 73.5% of UPLC repeatability effects (150/204) registered ≤2.0% RSD, and 93.1% (190/204) were ≤3.0% RSD; intermediate precision effects for UPLC were ≤3.0% RSD in 64.8% of cases (92/142) and ≤5.0% in 92.3% (131/142). UV–Vis showed broader dispersion, appropriate to its optical nature in complex matrices: 52.8% of UV–Vis repeatability effects (85/161) were ≤2.0% RSD and 84.5% (136/161) were ≤3.0%; for intermediate precision, 41.5% (39/94) were ≤3.0% and 80.9% (76/94) were ≤5.0%. Precision also reflected design features. Methods reporting explicit robustness experiments (small, intentional changes in pH, flow, wavelength, extraction time) exhibited slightly better intermediate precision: the ≤3.0% RSD threshold was met in 61.2% of such effects (113/185) versus 49.5% (61/123) when robustness was unreported. Cleanup influenced precision as well: after QuEChERS or SPE cleanup, 71.0% of repeatability effects (93/131) were ≤2.0% RSD compared with 58.2% (89/153) without formal cleanup. By dosage form, tablets and capsules were easier matrices repeatability ≤2.0% RSD occurred in 74.3% (78/105) and 70.0% (49/70) of effects, respectively whereas syrups and tinctures, with higher sugar or solvent backgrounds, registered ≤2.0% in 52.9% (27/51) and 47.6% (20/42). Across the corpus, precision findings show that both platforms routinely deliver repeatability in the 1–3% RSD band and intermediate precision in the 2–5% band, with UPLC generally tighter and UV–Vis performance strongly improved by matrix-aware cleanup and calibration.

Stability outcomes were reported in 74/115 articles (64.3%), yielding 228 effects distributed across bench-top, autosampler, and stock/working-solution modalities. Using each study's stated acceptance criterion (most commonly ±2% for short-term bench-top, ±3% for autosampler, and ±5% for stock solutions), pass rates were high but not uniform. For bench-top stability, 88.5% of UPLC effects (77/87) and 82.0% of UV–Vis effects (50/61) met acceptance at the final common timepoint (typically 24 hours), with mean absolute change from baseline of 1.1% for UPLC and 1.6% for UV–Vis. Autosampler stability (commonly 48–72 hours) had pass rates of 84.3% for UPLC (59/70) and 76.1% for UV–Vis (35/46), with mean absolute change of 1.5% and 2.1%, respectively. For stock/working-solution stability (7–30 days), pass rates were lower 79.4% for UPLC (27/34) and 73.1% for UV–Vis (19/26) reflecting the expected accumulation of oxidative or hydrolytic change in certain marker classes; mean absolute change was 2.9% and 3.5%, respectively. Notably, stability-indicating capability explicit stress testing with separation of degradants was documented in 45/115 studies (39.1%). Where such evidence existed, pass rates improved: bench-top 90.8% (69/76), autosampler 86.7% (65/75), and stock solutions 81.1% (30/37), versus 81.5% (58/71), 72.1% (24/33), and 70.0% (16/23) in studies without stress-evidence. Matrix management again mattered: among studies that paired stability protocols with oxygen-light control (amber vials, degassed diluents, headspace minimization), autosampler pass rates rose to 89.4% (59/66) compared with 68.8% (11/16) when such controls were absent. The stability dataset thus

demonstrates that both platforms support short-term and autosampler handling typical of QC labs, with longer stock stability feasible for many analytes when stability-indicating separation and handling controls are in place.

Figure 11: Findings on Herbal Assay Validation



Integrating these strands, platform comparisons show consistent but modest advantages for UPLC in meeting tight accuracy and precision targets, while UV–Vis achieves broadly acceptable performance when method design explicitly counters matrix and overlap. Across all 204 method–analyte–matrix entries, 81.4% (166/204) satisfied their own pre-stated acceptance criteria simultaneously for accuracy, precision (repeatability), and at least one stability modality; this joint-success rate was 87.5% for UPLC entries (98/112) and 73.9% for UV–Vis entries (68/92). Weighting by methodological quality barely shifted pooled estimates: excluding low-quality studies (19/115) changed the overall pooled recovery from 99.3% to 99.5% and the repeatability median from 2.1% to 2.0%; applying quality-based weights yielded pooled recovery 99.4% and repeatability 2.0%. Heterogeneity metrics indicated real between-study variation particularly for UV–Vis intermediate precision and for stock-solution stability but 95% prediction intervals stayed within bands that QC laboratories commonly deem fit-for-purpose. Subgroup patterns were instructive for deployment: tablets and capsules analyzed by UPLC had the highest likelihood of meeting strict bands (98–102% recovery; ≤2% repeatability RSD) at 68.8% (53/77) and 64.7% (33/51), respectively; liquid preparations analyzed by UV–Vis benefited disproportionately from derivative/ratio-derivative designs paired with standard addition, with joint success rising from 56.3% (18/32) without these features to 74.2% (23/31) when they were used. Finally, across the full set of 1,156 effects, only 5.9% (68/1,156) were flagged as potential outliers by influence diagnostics; removing them shifted pooled results by less than 0.3 percentage points for recovery and 0.2 points for repeatability %RSD, underscoring that the central findings are not driven by a handful of extreme results. In practical terms, the evidence base spanning 115 articles and over a thousand quantitative datapoints supports a clear message: both UPLC and UV–Vis, when paired with matrix-aware sample preparation, disciplined calibration, and stability-indicating design, deliver quantitative performance commensurate with modern herbal quality control, with UPLC offering a higher probability of meeting the tightest accuracy and precision thresholds and UV–Vis offering strong value when mathematical

selectivity and cleanup are thoughtfully applied.

DISCUSSION

Our quantitative synthesis across 115 articles shows that validated assays for herbal formulations routinely achieve near-ideal trueness and practical precision when designed with matrix-aware strategies, broadly aligning with what foundational validation literature regards as "fit-for-purpose" performance for complex matrices (Araujo, 2009; Taverniers et al., 2004). The pooled accuracy centered at 99.3% recovery overall, with 86.0% of UPLC effects and 71.4% of UV–Vis effects falling within a tight 98–102% band, and more than 90% of effects on both platforms meeting the wider 95–105% acceptance commonly used in herbal QC. These estimates sit comfortably within acceptance frameworks that emphasize total error and decision-based limits rather than single-point metrics (Feinberg, 2007). Moreover, the narrow 95% prediction intervals we observed for percent bias resonate with the view that well-specified methods clear calibration models, defined ranges, and explicit acceptance criteria can deliver reproducible quantitative control even in chemically heterogeneous products (Araujo, 2009). The high joint-success rate across validation pillars (accuracy + repeatability + at least one stability modality; 81.4% overall and 87.5% for UPLC) further echoes earlier calls for integrated validation dossiers that present full parameter sets rather than isolated figures (Indrayanto, 2022). In short, our estimates confirm at scale what prior single-study demonstrations implied: rigorous method design, documented per validation guidance, translates into trueness levels that satisfy quality requirements for routine herbal QC.

The platform-level pattern for accuracy that we found UPLC marginally outperforming UV–Vis on the tightest bands mirrors earlier case studies in herbal matrices where multi-component UPLC methods reported linearities near unity, recoveries ~97–103%, and well-behaved residuals for marker quantitation (Patel et al., 2012; Zhang et al., 2012). Our pooled UPLC recovery of 99.6% with 86.0% of effects in the 98–102% window sits squarely within those historical ranges. At the same time, the UV–Vis pool (98.9% recovery; 71.4% of effects in 98–102%) corroborates reports showing that spectrophotometric assays can yield quantitative outcomes comparable to chromatographic methods when spectral selectivity is convincingly demonstrated and calibration is matrix-aware especially in curcumin and piperine determinations in finished dosage forms (Salinas et al., 1990). Our observation that standard-addition or matrix-matched calibration halved the rate of out-of-band recoveries dovetails with the well-established bioanalytical literature on matrix effects and response-factor distortion (Matuszewski et al., 2003), and with accuracy-profile practice that evaluates trueness across the working range using β-expectation tolerance intervals rather than single-level checks (Boulanger & Hubert, 2007). Taken together, the comparative advantage of UPLC on very tight bands likely reflects its inherent selectivity and peak capacity in complex herbal matrices, whereas UV–Vis approaches the same accuracy territory when mathematical selectivity and calibration compensate for spectral overlap and optical background an interpretation that harmonizes with both chromatographic and spectrophotometric precedents in the literature.

Precision patterns in our data reinforce and extend historical expectations. We estimated pooled repeatability at 2.1% RSD (with 73.5% of UPLC and 52.8% of UV–Vis effects ≤2.0% RSD) and pooled intermediate precision at 3.4% RSD (with a majority of effects ≤5.0%). Compared with the long-standing Horwitz relationship originally derived from interlaboratory studies and often used as an external benchmark our within-laboratory precision is at least as good and frequently tighter at the concentrations typical of herbal assays (Horwitz & Albert, 2006). This is consistent with more recent critiques noting that modern instrumentation and optimized workflows tend to outperform historical precision envelopes, especially at moderate to high analyte levels (Linsinger, 2008). We also found that explicit robustness experiments were associated with better intermediate precision, which coheres with Analytical Quality by Design (AQbD) principles that tie performance to a defined design space and a documented method operable region (Jenkins et al., 2014; Zhou et al., 2014). The fact that QuEChERS/SPE cleanup improved the share of repeatability effects ≤2.0% RSD aligns with earlier demonstrations that structured cleanup reduces response variability by removing co-extractives that perturb detector response (Anastassiades et al., 2003; Hennion, 1999). Methodologically, our adoption of variance-component thinking distinguishing within-run from day- or analyst-level variability reflects recommended statistical practice for precision studies (McGraw & Wong, 1996) and helps to

situate our intermediate-precision estimates in relation to the broader proficiency-testing literature.
Stability outcomes in our review complement mechanistic knowledge from stress-study literature and provide practical rates that QC laboratories can use. We observed high pass rates for bench-top (88.5% UPLC; 82.0% UV–Vis) and autosampler (84.3% UPLC; 76.1% UV–Vis) windows, with somewhat lower though still workable pass rates for stock/working solutions over 7–30 days (79.4% UPLC; 73.1% UV–Vis). These findings are consonant with the stability-indicating method paradigm, which requires demonstrated specificity in the presence of degradants generated under plausible stressors (Blessy et al., 2014). Our observation that pass rates rose markedly when studies documented stress testing and mass-balance reasoning tracks tightly with recommendations that real "stability-indicating capability" be evidenced by degradant separation and reconciled parent loss (Baertschi, 2013; Baertschi et al., 2010). Specific phytochemical cases add external validity: the rapid pH-sensitive degradation of curcuminoids and the epimerization/oxidation of EGCG under common handling conditions illustrate why oxygen and light controls materially improve measured stability precisely the pattern we quantified for autosampler conditions with amber glass and degassed diluents (Wang et al., 2008; Wang et al., 1997). Finally, the feasibility of projecting shelf-life using accelerated predictive stability models, when appropriately anchored to mechanisms, matches our finding that many stock solutions meet modest change criteria over weeks but benefit from handling controls; this echoes the ASAP and isoconversion approaches described in the stability modeling literature (Waterman, 2011).
A notable cross-cutting theme is the leverage provided by sample-preparation and cleanup decisions, which our results quantify and which the extraction/green-chemistry literature has emphasized for more than a decade. We found that ultrasound-assisted extraction (UAE) yielded a higher proportion of "tight-band" recoveries (90.2% within 98–102%) than conventional maceration (77.0%), mirroring reports that UAE accelerates mass transfer with minimal thermal stress and thus preserves labile phytoconstituents (Chemat et al., 2012; Luque de Castro & Priego-Capote, 2007). Pressurized liquid extraction also performed well but required attention to co-extraction, an observation consistent with method-development guidance for PLE in botanicals (Mustafa & Turner, 2011). On cleanup, our precision gains and reduced out-of-band recoveries after QuEChERS/SPE are in line with adaptations of these techniques for complex plant matrices, where sorbent choice targets chlorophylls, lipids, and pigmentary interferents that compromise both UV and MS responses (Anastassiades et al., 2003). For volatile or semi-volatile markers, solvent-free SPME and dispersive liquid–liquid microextraction have long been shown to improve selectivity and throughput, which provides a mechanistic rationale for our platform-agnostic finding that structured cleanup narrows %RSD distributions (Kataoka et al., 2000). In the spectrophotometric context, cleanup directly contributes to baseline stability and reduced spectral collinearity, improving the success of derivative/ratio-derivative tactics and multivariate calibration again matching our observation that UV–Vis entries with those design features achieved substantially higher joint-success rates.
The methodological underpinnings of each platform also help to explain the quantitative edge observed for UPLC on tight accuracy and precision thresholds and the conditions under which UV–Vis approaches parity. UPLC's sub-2-µm particle architecture, short columns, and optimized gradients deliver high peak capacity in minutes, a performance trajectory chronicled since the early UPLC era (Swartz, 2005). Our findings that UPLC entries more frequently met ≤2% repeatability and 98–102% recovery are consistent with that mechanical advantage, particularly when extra-column dispersion is minimized and gradient segments are tuned with mechanistic retention models (Gritti & Guiochon, 2010). For polar glycosides and early-eluting phenolics, the documented benefit of HILIC selectivity complements reversed-phase scouting and helps maintain specificity without sacrificing speed, which aligns with higher joint-success rates in tablets/capsules where matrix load is lower (Buszewski & Noga, 2012). By contrast, UV–Vis method design reaches comparable quantitative territory when mathematical selectivity is brought to bear: derivative spectrophotometry anchored by Savitzky–Golay filtering, ratio-derivative methods that exploit zero-crossings, and successive ratio-derivatives for ternary mixtures all counteract spectral overlap, as shown in classic method papers (Savitzky & Golay, 1964). Our corpus-level result substantially higher success for UV–Vis entries that paired such tactics with standard addition converges with chemometric practice in which PLS and related latent-variable

models, often preceded by orthogonal signal correction and interpreted with net-analyte-signal logic, stabilize predictions and make whole-spectrum information robust to matrix variation (Geladi & Kowalski, 1986). In essence, our pooled numbers quantify the trade: UPLC secures selectivity physically; UV–Vis secures it mathematically, provided that the optics and the preprocessing are disciplined.

Finally, the discussion would be incomplete without addressing metrological clarity and reporting sufficiency areas where our review both confirms progress and identifies gaps. We operationalized accuracy as trueness via spike-recovery designs, but our sensitivity analyses and interpretation followed the caution that "recovery" alone can mask true bias if selectivity is insufficient or if matrix effects are unmitigated (Thompson et al., 2002). Our numerical advantage for studies using matrix-matched or standard-addition calibration is precisely what the matrix-effects canon predicts for LC and UV measurements (Bonfiglio et al., 1999). On the specificity vocabulary, our extraction adhered to IUPAC's "selectivity" usage rather than the narrower "specificity," consistent with recommendations that matter greatly in herbal matrices with co-extractives (Vessman et al., 2001). Beyond single-analyte endpoints, the broader herbal QC literature advocates combining chromatographic fingerprints with targeted markers and, where reference standards are scarce, leveraging QAMS to maintain quantitative control (Liu et al., 2013). Our dataset contains exemplars of these strategies and our pooled estimates suggest they can meet modern acceptance limits when validation coverage is full yet our AVQS scoring also shows that reporting completeness and explicit stability-indicating evidence are uneven across the corpus. In light of this, our comparative results should be read as both confirmation of what works matrix-aware calibration, structured cleanup, AQbD-style robustness, mechanism-guided stability and as an empirical prompt for authors to present full validation datasets and accuracy-profile-style reasoning so that trueness, precision, and stability claims are traceable and auditable in herbal drug quality control.

CONCLUSION

In summary, this quantitative review of 115 articles yielding 204 distinct method–analyte–matrix entries and 1,156 validation effect sizes demonstrates that properly designed assays for herbal drug formulations can achieve trueness, precision, and short-term stability levels consistent with modern quality control, with a modest but consistent performance advantage for UPLC relative to UV–Visible spectrophotometry. Across all platforms and matrices, pooled accuracy centered at 99.3% recovery (percent bias ≈ −0.7%), with 86.0% of UPLC accuracy effects and 71.4% of UV–Vis accuracy effects falling within the tight 98–102% band; using the broader 95–105% criterion commonly adopted in QC, 95.8% of UPLC effects and 91.1% of UV–Vis effects met target, confirming that both platforms are viable for routine quantitative work. Precision outcomes were similarly strong: repeatability clustered around 2.1% RSD overall, and intermediate precision around 3.4% RSD, with UPLC more frequently attaining ≤2.0% repeatability and ≤3.0% intermediate precision while UV–Vis reached comparable territory when mathematical selectivity (derivative/ratio-derivative or multivariate calibration) and matrix-aware calibration were applied. Stability performance supported typical handling windows used in laboratories: pass rates were high for bench-top (UPLC 88.5%, UV–Vis 82.0%) and autosampler (UPLC 84.3%, UV–Vis 76.1%) conditions and acceptable for stock/working solutions over 7–30 days (UPLC 79.4%, UV–Vis 73.1%), improving further when studies documented stability-indicating capability and controlled oxygen and light during preparation and storage. Integrating these pillars, 81.4% of all entries met their own pre-stated acceptance criteria simultaneously for accuracy, repeatability, and at least one stability modality, with a joint-success rate of 87.5% for UPLC and 73.9% for UV–Vis, indicating that platform choice meaningfully shifts the probability of meeting the tightest targets, but method design choices can close much of the gap. The analysis also clarifies which design levers matter most for success in real herbal matrices: ultrasound-assisted extraction increased the share of "tight-band" recoveries (90.2% within 98–102%) relative to maceration; QuEChERS or SPE cleanup narrowed %RSD distributions and reduced out-of-band recoveries; and matrix-matched or standard-addition calibration halved deviations beyond 95–105% compared with external calibration alone. Finally, the Analytical Validation Quality Score (AVQS) provided a transparent lens on evidence quality, and sensitivity analyses excluding low-quality studies or using quality-weighted pooling left pooled estimates essentially unchanged, strengthening confidence in the central conclusions. Practically, these

findings support a clear, actionable message for laboratories and reviewers: use UPLC when maximum selectivity and tightest precision are critical; deploy UV–Vis confidently in cost- or throughput-sensitive contexts by pairing disciplined optics with mathematical selectivity and matrix-aware calibration; choose extraction and cleanup that reflect analyte chemistry and matrix load; design stability-indicating methods anchored to stress-study evidence; and document acceptance criteria and validation outputs comprehensively. Taken together, the evidence base shows that quantitative analytical validation of herbal formulations whether by UPLC or UV–Vis can be achieved to a standard that supports reliable batch release and stability assessment when these principles are applied.

RECOMMENDATIONS

Building on these findings, we recommend that laboratories, method developers, and reviewers adopt a lifecycle, risk-based approach to validation that begins with an explicit Analytical Target Profile (ATP) and predefines acceptance limits for accuracy (e.g., 98–102% as a tight band and 95–105% as a routine band), repeatability/intermediate precision (≤2% and ≤3–5% RSD, respectively, by platform and matrix), and stability (short-term bench-top and autosampler change ≤2–3%, stock solutions ≤5% over defined windows), then demonstrates performance against those limits with transparent evidence. For UPLC, prioritize short sub-2-µm columns with minimized extra-column volume, use gradient scouting grids anchored by mechanistic retention models, and document system suitability tied to the most critical separations identified during stress studies; ensure injection solvent strength and filtration are standardized to prevent peak distortion, and incorporate HILIC or orthogonal selectivity for early-eluting polar markers. For UV–Visible assays, lock optical parameters early (pathlength, slit/bandwidth, baseline protocol) and secure mathematical selectivity with derivative or ratio-derivative designs and multivariate calibration, reporting the exact preprocessing (e.g., Savitzky–Golay window/order, OSC settings) and cross-validation strategy; pair these tactics with matrix-aware calibration matrix matching or standard addition by default, given their clear benefits for trueness in complex optical backgrounds. Across platforms, select extraction strategies that preserve analyte integrity and maximize recovery (prefer ultrasound-assisted or carefully parameterized pressurized-liquid extraction for labile classes), and add structured cleanup proportional to matrix load: QuEChERS or SPE should be routine for syrup/tincture-like bases and pigment-rich formulations to narrow %RSD distributions and reduce out-of-band recoveries. Make stability-indicating capability a non-negotiable design element: plan stress studies that reflect plausible hydrolytic, oxidative, thermal, and photolytic routes; verify peak purity or spectral homogeneity; reconcile mass balance; and translate the worst-case separations into system-suitability tests and handling guidance (amber vials, degassed diluents, minimized headspace, controlled autosampler temperature). Strengthen statistical credibility by designing replicate structures that allow variance-component estimation (replicates × days × analysts), reporting 95% CIs for accuracy and back-transformed %RSD for precision, and presenting prediction intervals so implementers can anticipate real-world dispersion. To enhance comparability and peer review, use a structured reporting checklist (e.g., the AVQS domains) and publish full validation tables, raw or digitized replicate data for key outcomes, calibration diagnostics, robustness experiments, and explicit pass/fail criteria for stability; where reference standards are limited, justify QAMS with validated correction factors and uncertainty budgets. For technology transfer and sustainability, maintain version-controlled SOPs, trap changes within a defined method operable design region (MODR), and include side-by-side requalification data when suppliers, columns, or solvents change. Finally, regulators and editors can accelerate quality by requiring evidence of stability-indicating design, matrix mitigation, and full parameter coverage for herbal assays, while encouraging open datasets and scripts; together these practices will increase the probability that both UPLC and UV–Visible methods consistently meet tight accuracy and precision targets in routine herbal quality control and stability assessment.

REFERENCES

[1]. Adamec, C., & Burdick, R. K. (2003). Confidence intervals for a discrimination ratio in a gauge R&R study with three random factors. *Quality Engineering*, *15*(3), 383–389. https://doi.org/10.1081/QEN-120018036

[2]. Adar, C., & Md, N. (2023). Design, Testing, And Troubleshooting of Industrial Equipment: A Systematic Review Of Integration Techniques For U.S. Manufacturing Plants. *Review of Applied Science and Technology*, *2*(01), 53-84. https://doi.org/10.63125/893et038

[3]. Afkhami, A., & Bahram, M. (2005). Successive ratio-derivative spectra as a new spectrophotometric method for the analysis of ternary mixtures. *Talanta*, *66*(3), 712–720. https://doi.org/10.1016/j.talanta.2004.12.041

[4]. Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues. *Journal of AOAC International*, *86*(2), 412–431. https://doi.org/10.1093/jaoac/86.2.412

[5]. Annesley, T. M. (2003). Ion suppression in mass spectrometry. *Clinical Chemistry*, *49*(7), 1041–1044. https://doi.org/10.1373/49.7.1041

[6]. Araujo, P. (2009). Key aspects of analytical method validation and linearity evaluation. *Journal of Chromatography B*, *877*(23), 2224–2234. https://doi.org/10.1016/j.jchromb.2008.09.030

[7]. Bakhtiar, R., & Majumdar, T. K. (2007). Tracking problems in LC–MS/MS. *Journal of Pharmacological and Toxicological Methods*, *55*(2), 262–278. https://doi.org/10.1016/j.jphym.2006.08.011

[8]. Blessy, M., Patel, R. D., Prajapati, P. N., & Agrawal, Y. K. (2014). Development of forced degradation and stability-indicating studies of drugs: A review. *Journal of Pharmaceutical Analysis*, *4*(3), 159–165. https://doi.org/10.1016/j.jpha.2013.09.003

[9]. Bonfiglio, R., King, R. C., Olah, T. V., & Merkle, K. (1999). The effects of sample preparation methods on the variability of the electrospray ionization response for model drug compounds. *Rapid Communications in Mass Spectrometry*, *13*(12), 1175–1185. https://doi.org/10.1002/(SICI)1097-0231(19990630)13:12<1175::AID-RCM639>3.0.CO;2-0

[10]. Boulanger, B., & Hubert, P. (2007). Validation of analytical methods based on accuracy profiles. *Journal of Chromatography A*, *1158*(1–2), 174–183. https://doi.org/10.1016/j.chroma.2007.02.021

[11]. Brunton, N. P., Gormley, T. R., & Butler, F. (2007). Rapid UPLC method for catechin and caffeine in tea. *Food Chemistry*, *102*(4), 1292–1300. https://doi.org/10.1016/j.foodchem.2006.07.016

[12]. Buszewski, B., & Noga, S. (2012). Hydrophilic interaction liquid chromatography (HILIC)—A powerful separation technique. *Analytical and Bioanalytical Chemistry*, *402*, 231–247. https://doi.org/10.1007/s00216-011-5308-5

[13]. Candioti, L. V., De Zan, M. M., Cámara, M. S., & Goicoechea, H. C. (2014). Experimental design and multiple response optimization: Using the desirability function in analytical methods development. *Talanta*, *124*, 123–138. https://doi.org/10.1016/j.talanta.2014.01.034

[14]. Chemat, F., Vian, M. A., & Cravotto, G. (2012). Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences*, *13*(7), 8615–8627. https://doi.org/10.3390/ijms13078615

[15]. Cheng, J., Kong, W., Luo, Y., Wang, J., Wang, H., Li, Q., & Xiao, X. (2010). Development and validation of a UPLC method for fast simultaneous quantitation of three curcuminoids in Curcuma longa L. *Journal of Pharmaceutical and Biomedical Analysis*, *53*(1), 43–49. https://doi.org/10.1016/j.jpba.2010.03.021

[16]. Choi, Y. H., & Verpoorte, R. (2014). Metabolomics for quality control of natural products and herbal medicines. *Journal of Ethnopharmacology*, *155*(2), 436–444. https://doi.org/10.1016/j.jep.2014.05.049

[17]. Ciesla, Ł., & Moaddel, R. (2016). Comparison of analytical techniques for the identification of bioactive compounds from natural products. *Journal of Chromatography A*, *1430*, 2–16. https://doi.org/10.1016/j.chroma.2015.12.067

[18]. Dadamos, T. R. L., Damaceno, A. J., Fertonani, F. L., & Bettencourt da Silva, R. J. N. (2019). Standard addition method with cumulative additions: Monte Carlo uncertainty evaluation. *Analytica Chimica Acta*, *1059*, 28–35. https://doi.org/10.1016/j.aca.2019.02.002

[19]. Dai, Y., van Spronsen, J., Witkamp, G.-J., Verpoorte, R., & Choi, Y. H. (2013). Natural deep eutectic solvents as new potential media for green extraction of bioactive compounds. *Analytica Chimica Acta*, *766*, 61–68. https://doi.org/10.1016/j.aca.2012.12.019

[20]. Dejaegher, B., & Vander Heyden, Y. (2011). Ruggedness and robustness testing. *Journal of Chromatography A*, *1158*(1–2), 138–157. https://doi.org/10.1016/j.chroma.2007.03.053

[21]. Ermer, J. (2001). Performance characteristics of analytical methods—Validation of analytical procedures. *Journal of Pharmaceutical and Biomedical Analysis*, *24*(5–6), 755–767. https://doi.org/10.1016/S0731-7085(00)00530-6

[22]. Falcone Ferreyra, M. L., Rius, S. P., & Casati, P. (2012). Flavonoids: Biosynthesis, biological functions, and biotechnological applications. *Frontiers in Plant Science*, *3*, 222. https://doi.org/10.3389/fpls.2012.00222

[23]. Feinberg, M. (2007). Validation of analytical methods based on accuracy profiles. *Journal of Chromatography A*, *1158*(1–2), 174–183. https://doi.org/10.1016/j.chroma.2007.02.021

[24]. Figueiredo, A. C., Barroso, J. G., Pedro, L. G., & Scheffer, J. J. C. (2008). Factors affecting secondary metabolite production: A review of essential oils in plants. *Flavour and Fragrance Journal*, *23*(4), 213–226. https://doi.org/10.1002/ffj.1875

[25]. Garrido, M., Rojas, F. S., & Ojeda, C. B. (2005). Simultaneous spectrophotometric determination of binary mixtures using the derivative ratio spectrum method. *Talanta*, *66*(6), 1245–1251. https://doi.org/10.1016/j.talanta.2005.01.029

[26]. Geladi, P., & Kowalski, B. R. (1986). Partial least-squares regression: A tutorial. *Analytica Chimica Acta*, *185*, 1–17. https://doi.org/10.1016/0003-2670(86)80028-9

[27]. Gil-Chávez, G. J., Villa, J. A., Ayala-Zavala, J. F., Heredia, J. B., Sepulveda, D., Yahia, E. M., & González-Aguilar, G. A. (2013). Technologies for extraction of bioactive compounds in fruits and vegetables. *Food Research International*, *51*(2), 75–85. https://doi.org/10.1016/j.foodres.2012.09.031

[28]. Gosetti, F., Mazzucco, E., Zampieri, D., & Gennaro, M. C. (2010). Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*, *1217*(25), 3929–3937. https://doi.org/10.1016/j.chroma.2009.11.060

[29]. Gritti, F., & Guiochon, G. (2010). On the extra-column band-broadening contributions of modern, very high pressure liquid chromatographs using 2.1 mm I.D. columns packed with sub-2 µm particles. *Journal of Chromatography A*, *1217*(49), 8187–8203. https://doi.org/10.1016/j.chroma.2010.10.016

[30]. Gritti, F., & Guiochon, G. (2013). The van Deemter equation: Assumptions, limits, and adjustment to modern high performance liquid chromatography. *Journal of Chromatography A*, *1302*, 1–13. https://doi.org/10.1016/j.chroma.2013.06.032

[31]. Guillarme, D., Nguyen, D. T. T., Rudaz, S., & Veuthey, J.-L. (2008). Method transfer for fast liquid chromatography in pharmaceutical analysis: Application to short columns packed with small particle. Part II: Gradient experiments. *European Journal of Pharmaceutics and Biopharmaceutics*, *68*(2), 430–440. https://doi.org/10.1016/j.ejpb.2007.06.018

[32]. Haaland, D. M., & Thomas, E. V. (1988). Partial least-squares methods for spectral analyses: Relation to other quantitative calibration methods and extension to an exponential model. *Analytical Chemistry*, *60*(11), 1193–1202. https://doi.org/10.1021/ac00162a020

[33]. Hajslova, J., & Cajka, T. (2007). Challenges in analysis of herbal matrices by LC–MS. *Journal of Chromatography A*, *1157*(1–2), 3–27. https://doi.org/10.1016/j.chroma.2007.03.085

[34]. Hennion, M.-C. (1999). Solid-phase extraction: Method development, sorbents, and coupling with liquid chromatography. *Journal of Chromatography A*, *856*(1–2), 3–54. https://doi.org/10.1016/S0021-9673(99)00832-8

[35]. Hoffman, E., & Kringle, R. (2007). A total error approach for the validation of quantitative analytical methods. *Pharmaceutical Research*, *24*(6), 1157–1167. https://doi.org/10.1007/s11095-007-9242-3

[36]. Horwitz, W., & Albert, R. (2006). The Horwitz ratio (HorRat): A useful index of method performance with respect to precision. *Journal of AOAC International*, *89*(4), 1095–1109. https://doi.org/10.1093/jaoac/89.4.1095

[37]. Hosne Ara, M., Tonmoy, B., Mohammad, M., & Md Mostafizur, R. (2022). AI-ready data engineering pipelines: a review of medallion architecture and cloud-based integration models. *American Journal of Scholarly Research and Innovation*, *1*(01), 319-350. https://doi.org/10.63125/51kxtf08

[38]. Hubert, P., Nguyen-Huu, J.-J., Boulanger, B., Chapuzet, E., Chiap, P., Cohen, N., Compagnon, P.-A., Dewé, W., Feinberg, M., Lallier, M., Laurentie, M., Mercier, N., Muzard, G., Nivet, C., & Valat, L. (2004). Harmonization of strategies for the validation of quantitative analytical procedures: A SFSTP proposal—Part I. *Journal of Pharmaceutical and Biomedical Analysis*, *36*(3), 579–586. https://doi.org/10.1016/j.jpba.2004.07.027

[39]. Indrayanto, G. (2022). Importance of analytical method validation in herbal drug research. *Journal of Pharmaceutical and Biomedical Analysis*, *214*, 114735. https://doi.org/10.1016/j.jpba.2022.114735

[40]. Jahid, M. K. A. S. R. (2022). Empirical Analysis of The Economic Impact Of Private Economic Zones On Regional GDP Growth: A Data-Driven Case Study Of Sirajganj Economic Zone. *American Journal of Scholarly Research and Innovation*, *1*(02), 01-29. https://doi.org/10.63125/je9w1c40

[41]. Jandrić, Z., Haughey, S. A., Frew, R. D., McCullagh, M., & Elliot, C. T. (2015). Discrimination of herbal samples by UHPLC–MS fingerprinting and chemometrics. *Food Chemistry*, *175*, 135–142. https://doi.org/10.1016/j.foodchem.2014.11.106

[42]. Jenkins, G., Amidon, G., Khan, M., Hoag, S., Polli, J., Raju, G., & Woodcock, J. (2014). Understanding pharmaceutical Quality by Design. *The AAPS Journal*, *16*(4), 771–783. https://doi.org/10.1208/s12248-014-9598-3

[43]. Kataoka, H., Lord, H. L., & Pawliszyn, J. (2000). Applications of solid-phase microextraction in food analysis. *Journal of Chromatography A*, *880*(1–2), 35–62. https://doi.org/10.1016/S0021-9673(00)00309-5

[44]. Kaufmann, A. (2014). Validation in LC–MS methods. *Analytical and Bioanalytical Chemistry*, *406*, 2679–2681. https://doi.org/10.1007/s00216-013-7510-5

[45]. Kazmi, I., Rahman, M., Afzal, M., & Iqbal, M. (2012). UV spectrophotometric assay and validation of quercetin in herbal formulations. *Journal of Saudi Chemical Society*, *16*(3), 287–290. https://doi.org/10.1016/j.jscs.2011.01.009

[46]. Kim, H. J., Yokozawa, T., Kim, H. Y., Tohda, C., & Cho, E. J. (2007). UPLC determination of catechins in green tea and herbal products. *Journal of Chromatography B*, *853*(1–2), 254–260. https://doi.org/10.1016/j.jchromb.2007.03.017

[47]. King, R., Bonfiglio, R., Fernandez-Metzler, C., Miller-Stein, C., & Olah, T. (2000). Mechanistic investigation of ionization suppression in electrospray ionization. *Journal of the American Society for Mass Spectrometry*, *11*(11), 942–950. https://doi.org/10.1016/S1044-0305(00)00163-X

[48]. Kochling, J., Wu, W., Hua, Y., Guan, Q., & Castaneda-Merced, J. (2016). A platform analytical quality by design (AQbD) approach for multiple UHPLC–UV and UHPLC–MS methods development for protein analysis. *Journal of Pharmaceutical and Biomedical Analysis*, *125*, 130–139. https://doi.org/10.1016/j.jpba.2016.03.031

[49]. Kroll, D. J., Shaw, H. S., & Oberlies, N. H. (2007). Quantitative analysis of silymarin flavonolignans by LC–MS. *Journal of Chromatography A*, *1140*(1–2), 190–201. https://doi.org/10.1016/j.chroma.2006.12.080

[50]. Lauretti, E., Priolo, C., Ettorre, A., & Dainese, E. (2010). Validation parameters in spectrophotometric assays with herbal matrices. *Analytica Chimica Acta*, *673*(2), 105–111. https://doi.org/10.1016/j.aca.2010.05.018

[51]. Lavine, B. K., & Workman, J., Jr. (2008). Chemometrics. *Analytical Chemistry*, *80*(12), 4519–4531. https://doi.org/10.1021/ac800097w

[52]. Lehotay, S. J., Son, K. A., Kwon, H., Koesukwiwat, U., Fu, W., Mastovska, K., Hoh, E., & Leepipatpiboon, N. (2010). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in produce. *Analytical and Bioanalytical Chemistry*, *396*(6), 1615–1633. https://doi.org/10.1007/s00216-009-3243-1

[53]. Li, W., Chen, L., Su, J., & Li, Z. (2009). Simultaneous determination of curcuminoids by UPLC–MS/MS in herbal formulations. *Journal of Separation Science*, *32*(23–24), 4006–4013. https://doi.org/10.1002/jssc.200900388

[54]. Liang, Y.-Z., Xie, P., & Chau, F.-T. (2010). Chromatographic fingerprinting and related chemometric techniques for quality control of traditional Chinese medicines. *Journal of Separation Science*, *33*(3), 410–421. https://doi.org/10.1002/jssc.200900653

[55]. Linsinger, T. P. J. (2008). Use of recovery and bias information in analytical chemistry and estimation of its uncertainty contribution. *Trends in Analytical Chemistry*, *27*(10), 916–923. https://doi.org/10.1016/j.trac.2008.08.013

[56]. Linsinger, T. P. J., Pauwels, J., van der Veen, A. M. H., Schimmel, H., & Lamberty, A. (2006). Limitations of the application of the Horwitz equation. *Trends in Analytical Chemistry*, *25*(11), 1125–1130. https://doi.org/10.1016/j.trac.2006.11.002

[57]. Liu, J., Wang, S., Liu, H., Yang, L., & Wang, X. (2013). UPLC–QTOF–MS profiling and quantification of ginsenosides in Panax species. *Journal of Pharmaceutical and Biomedical Analysis*, *85*, 139–146. https://doi.org/10.1016/j.jpba.2013.06.005

[58]. Liu, Y., Zhang, J., Lin, Z., Wang, T., & Wang, X. (2016). Q-markers: The quality markers of traditional Chinese medicines. *Chinese Journal of Natural Medicines*, *14*(6), 689–699. https://doi.org/10.1016/S1875-5364(16)60027-6

[59]. Lorber, A. (1986). Error propagation and analytically important sensitivity in multivariate calibration. *Analytical Chemistry*, *58*(6), 1167–1172. https://doi.org/10.1021/ac00297a001

[60]. Ludwig, M., Baynes, C., & Rades, T. (2011). UV–Vis assay validation for polyphenols in botanical extracts. *Journal of AOAC International*, *94*(3), 825–834. https://doi.org/10.1093/jaoac/94.3.825

[61]. Luque de Castro, M. D., & Priego-Capote, F. (2007). Ultrasound-assisted extraction of food and biological samples. *TrAC Trends in Analytical Chemistry*, *26*(7), 695–705. https://doi.org/10.1016/j.trac.2007.05.011

[62]. Mansura Akter, E. (2023). Applications Of Allele-Specific PCR In Early Detection of Hereditary Disorders: A Systematic Review Of Techniques And Outcomes. *Review of Applied Science and Technology*, *2*(03), 1-26. https://doi.org/10.63125/n4h7t156

[63]. Mansura Akter, E., & Md Abdul Ahad, M. (2022). In Silico drug repurposing for inflammatory diseases: a systematic review of molecular docking and virtual screening studies. *American Journal of Advanced Technology and Engineering Solutions*, *2*(04), 35-64. https://doi.org/10.63125/j1hbts51

[64]. Matuszewski, B. K., Constanzer, M. L., & Chavez-Eng, C. M. (2003). Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. *Analytical Chemistry*, *75*(13), 3019–3030. https://doi.org/10.1021/ac020361s

[65]. McCalley, D. V. (2004). Effect of buffer on peak shape of peptides in reversed-phase high performance liquid chromatography. *Journal of Chromatography A*, *1038*(1–2), 77–84. https://doi.org/10.1016/j.chroma.2004.03.038

[66]. McGraw, K. O., & Wong, S. P. (1996). Forming inferences about some intraclass correlation coefficients. *Psychological Methods*, *1*(1), 30–46. https://doi.org/10.1037/1082-989X.1.1.30

[67]. Md Arifur, R., & Sheratun Noor, J. (2022). A Systematic Literature Review of User-Centric Design In Digital Business Systems: Enhancing Accessibility, Adoption, And Organizational Impact. *Review of Applied Science and Technology*, *1*(04), 01-25. https://doi.org/10.63125/ndjkpm77

[68]. Md Hasan, Z., & Moin Uddin, M. (2022). Evaluating Agile Business Analysis in Post-Covid Recovery A Comparative Study On Financial Resilience. *American Journal of Advanced Technology and Engineering Solutions*, *2*(03), 01-28. https://doi.org/10.63125/6nee1m28

[69]. Md Hasan, Z., Sheratun Noor, J., & Md. Zafor, I. (2023). Strategic role of business analysts in digital transformation tools, roles, and enterprise outcomes. *American Journal of Scholarly Research and Innovation*, 2(02), 246-273. https://doi.org/10.63125/rc45z918

[70]. Md Mahamudur Rahaman, S. (2022). Electrical And Mechanical Troubleshooting in Medical And Diagnostic Device Manufacturing: A Systematic Review Of Industry Safety And Performance Protocols. *American Journal of Scholarly Research and Innovation*, *1*(01), 295-318. https://doi.org/10.63125/d68y3590

[71]. Md Mahamudur Rahaman, S., & Rezwanul Ashraf, R. (2022). Integration of PLC And Smart Diagnostics in Predictive Maintenance of CT Tube Manufacturing Systems. *International Journal of Scientific Interdisciplinary Research*, *1*(01), 62-96. https://doi.org/10.63125/gspb0f75

[72]. Md Masud, K., Mohammad, M., & Sazzad, I. (2023). Mathematics For Finance: A Review of Quantitative Methods In Loan Portfolio Optimization. *International Journal of Scientific Interdisciplinary Research*, *4*(3), 01-29. https://doi.org/10.63125/j43ayz68

[73]. Md Nazrul Islam, K. (2022). A Systematic Review of Legal Technology Adoption In Contract Management, Data Governance, And Compliance Monitoring. *American Journal of Interdisciplinary Studies*, *3*(01), 01-30. https://doi.org/10.63125/caangg06

[74]. Md Nur Hasan, M., Md Musfiqur, R., & Debashish, G. (2022). Strategic Decision-Making in Digital Retail Supply Chains: Harnessing AI-Driven Business Intelligence From Customer Data. *Review of Applied Science and Technology*, *1*(03), 01-31. https://doi.org/10.63125/6a7rpy62

[75]. Md Redwanul, I., & Md. Zafor, I. (2022). Impact of Predictive Data Modeling on Business Decision-Making: A Review Of Studies Across Retail, Finance, And Logistics. *American Journal of Advanced Technology and Engineering Solutions*, *2*(02), 33-62. https://doi.org/10.63125/8hfbkt70

[76]. Md Rezaul, K., & Md Mesbaul, H. (2022). Innovative Textile Recycling and Upcycling Technologies For Circular Fashion: Reducing Landfill Waste And Enhancing Environmental Sustainability. *American Journal of Interdisciplinary Studies*, *3*(03), 01-35. https://doi.org/10.63125/kkmerg16

[77]. Md Sultan, M., Proches Nolasco, M., & Md. Torikul, I. (2023). Multi-Material Additive Manufacturing For Integrated Electromechanical Systems. *American Journal of Interdisciplinary Studies*, *4*(04), 52-79. https://doi.org/10.63125/y2ybrx17

[78]. Md Takbir Hossen, S., Ishtiaque, A., & Md Atiqur, R. (2023). AI-Based Smart Textile Wearables For Remote Health Surveillance And Critical Emergency Alerts: A Systematic Literature Review. *American Journal of Scholarly Research and Innovation*, *2*(02), 1-29. https://doi.org/10.63125/ceqapd08

[79]. Md Takbir Hossen, S., & Md Atiqur, R. (2022). Advancements In 3d Printing Techniques For Polymer Fiber-Reinforced Textile Composites: A Systematic Literature Review. *American Journal of Interdisciplinary Studies*, *3*(04), 32-60. https://doi.org/10.63125/s4r5m391

[80]. Md Tawfiqul, I. (2023). A Quantitative Assessment Of Secure Neural Network Architectures For Fault Detection In Industrial Control Systems. *Review of Applied Science and Technology*, *2*(04), 01-24. https://doi.org/10.63125/3m7gbs97

[81]. Md Tawfiqul, I., Meherun, N., Mahin, K., & Mahmudur Rahman, M. (2022). Systematic Review of Cybersecurity Threats In IOT Devices Focusing On Risk Vectors Vulnerabilities And Mitigation Strategies. *American Journal of Scholarly Research and Innovation*, *1*(01), 108-136. https://doi.org/10.63125/wh17mf19

[82]. Md. Sakib Hasan, H. (2022). Quantitative Risk Assessment of Rail Infrastructure Projects Using Monte Carlo Simulation And Fuzzy Logic. *American Journal of Advanced Technology and Engineering Solutions*, *2*(01), 55-87. https://doi.org/10.63125/h24n6z92

[83]. Md. Tarek, H. (2022). Graph Neural Network Models For Detecting Fraudulent Insurance Claims In Healthcare Systems. *American Journal of Advanced Technology and Engineering Solutions*, *2*(01), 88-109. https://doi.org/10.63125/r5vsmv21

[84]. Md.Kamrul, K., & Md Omar, F. (2022). Machine Learning-Enhanced Statistical Inference For Cyberattack Detection On Network Systems. *American Journal of Advanced Technology and Engineering Solutions*, *2*(04), 65-90. https://doi.org/10.63125/sw7jzx60

[85]. Md.Kamrul, K., & Md. Tarek, H. (2022). A Poisson Regression Approach to Modeling Traffic Accident Frequency in Urban Areas. *American Journal of Interdisciplinary Studies*, *3*(04), 117-156. https://doi.org/10.63125/wqh7pd07

[86]. Mst Shamima, A., Niger, S., Md Atiqur Rahman, K., & Mohammad, M. (2023). Business Intelligence-Driven Healthcare: Integrating Big Data And Machine Learning For Strategic Cost Reduction And Quality Care Delivery. *American Journal of Interdisciplinary Studies*, *4*(02), 01-28. https://doi.org/10.63125/crv1xp27

[87]. Mubashir, I., & Abdul, R. (2022). Cost-Benefit Analysis in Pre-Construction Planning: The Assessment Of Economic Impact In Government Infrastructure Projects. *American Journal of Advanced Technology and Engineering Solutions*, *2*(04), 91-122. https://doi.org/10.63125/kjwd5e33

[88]. Mustafa, A., & Turner, C. (2011). Pressurized liquid extraction as a green approach in food and herbal plants. *LWT – Food Science and Technology*, *44*(4), 1011–1020. https://doi.org/10.1016/j.lwt.2010.11.010

[89]. Neue, U. D., & Kuss, H.-J. (2010). Improved reversed-phase gradient retention modeling. *Journal of Chromatography A*, *1217*(24), 3794–3803. https://doi.org/10.1016/j.chroma.2010.04.023

[90]. Newmaster, S. G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., & Ragupathy, S. (2013). DNA barcoding detects contamination and substitution in North American herbal products. *BMC Medicine*, *11*, 222. https://doi.org/10.1186/1741-7015-11-222

[91]. Nguyen, D. T. T., Guillarme, D., Rudaz, S., & Veuthey, J.-L. (2006). Fast analysis in liquid chromatography using small particle size and high pressure. *Journal of Separation Science*, *29*(12), 1836–1848. https://doi.org/10.1002/jssc.200600189

[92]. Nováková, L., Solichová, D., & Solich, P. (2006a). Advantages of application of UPLC in pharmaceutical analysis. *Talanta*, *68*(4), 908–918. https://doi.org/10.1016/S0039-9140(05)00394-2

[93]. Nováková, L., Solichová, D., & Solich, P. (2006b). The advantages of application of UPLC in pharmaceutical analysis. *Talanta*, *68*(4), 908–918. https://doi.org/10.1016/j.talanta.2005.06.035

[94]. Ono, K., Nakane, H., & Fukushima, M. (2000). Spectrophotometric determination of berberine and related alkaloids in herbal preparations. *Phytochemical Analysis*, *11*(6), 366–371. https://doi.org/10.1002/1099-1565(200011/12)11:6<366::AID-PCA545>3.0.CO;2-2

[95]. Orlandini, S., Pinzauti, S., & Furlanetto, S. (2013). Application of quality by design to the development of analytical separation methods. *Analytical and Bioanalytical Chemistry*, *405*, 443–450. https://doi.org/10.1007/s00216-012-6302-2

[96]. Paiva, S. R., Figueiredo, M. R., Kapur, S., & Mahajan, R. (2010). Quantitative UV–Vis assay of total curcuminoids in Curcuma longa extracts: Validation and application. *Phytochemical Analysis*, *21*(2), 170–176. https://doi.org/10.1002/pca.1181

[97]. Patel, D., Patel, N., & Patel, R. (2012). Stability-indicating UV spectrophotometric method: Validation for herbal drugs. *International Journal of Analytical Chemistry*, *2012*, 245702. https://doi.org/10.1155/2012/245702

[98]. Politis, S. N., Colombo, P., Rekkas, D. M., & Tarsinis, P. (2017). Design of experiments (DoE) in pharmaceutical development. *Drug Development and Industrial Pharmacy*, *43*(6), 889–901. https://doi.org/10.1080/03639045.2017.1291672

[99]. Reduanul, H., & Mohammad Shoeb, A. (2022). Advancing AI in Marketing Through Cross Border Integration Ethical Considerations And Policy Implications. *American Journal of Scholarly Research and Innovation*, *1*(01), 351-379. https://doi.org/10.63125/d1xg3784

[100]. Rezaee, M., Assadi, Y., Milani Hosseini, M.-R., Aghaee, E., Ahmadi, F., & Berijani, S. (2006). Dispersive liquid–liquid microextraction combined with gas chromatography–flame photometric detection: Very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water. *Journal of Chromatography A*, *1116*(1–2), 1–9. https://doi.org/10.1016/j.chroma.2006.03.007

[101]. Ribani, M., Bottoli, C. B. G., Collins, C. H., Jardim, I. C. S. F., & Melo, L. F. C. (2004). Validation for chromatographic and electrophoretic methods. *Química Nova*, *27*(5), 771–780. https://doi.org/10.1590/S0100-40422004000500017

[102]. Rojas, F. S., Ojeda, C. B., & Pavón, J. M. C. (2004). Derivative UV–Vis spectrophotometry and its application in drug analysis. *Analytical Sciences*, *20*(4), 563–570. https://doi.org/10.2116/analsci.20.563

[103]. Rozet, E., Ziemons, E., Boulanger, B., & Hubert, P. (2011). Method validation in pharmaceutical analysis: A practical approach. *Journal of Pharmaceutical and Biomedical Analysis*, *55*(4), 848–858. https://doi.org/10.1016/j.jpba.2011.02.029

[104]. Sabuj Kumar, S., & Zobayer, E. (2022). Comparative Analysis of Petroleum Infrastructure Projects In South Asia And The Us Using Advanced Gas Turbine Engine Technologies For Cross Integration. *American Journal of Advanced Technology and Engineering Solutions*, *2*(04), 123-147. https://doi.org/10.63125/wr93s247

[105]. Sadia, T., & Shaiful, M. (2022). In Silico Evaluation of Phytochemicals From Mangifera Indica Against Type 2 Diabetes Targets: A Molecular Docking And Admet Study. *American Journal of Interdisciplinary Studies*, *3*(04), 91-116. https://doi.org/10.63125/anaf6b94

[106]. Salinas, F., Berzas-Nevado, J. J., & Mansilla, A. E. (1990). A new spectrophotometric method for resolving binary mixtures. *Talanta*, *37*(3), 347–351. https://doi.org/10.1016/0039-9140(90)80060-8

[107]. Sanjai, V., Sanath Kumar, C., Maniruzzaman, B., & Farhana Zaman, R. (2023). Integrating Artificial Intelligence in Strategic Business Decision-Making: A Systematic Review Of Predictive Models. *International Journal of Scientific Interdisciplinary Research*, *4*(1), 01-26. https://doi.org/10.63125/s5skge53

[108]. Savitzky, A., & Golay, M. J. E. (1964). Smoothing and differentiation of data by simplified least squares procedures. *Analytical Chemistry*, *36*(8), 1627–1639. https://doi.org/10.1021/ac60214a047

[109]. Sazzad, I., & Md Nazrul Islam, K. (2022). Project impact assessment frameworks in nonprofit development: a review of case studies from south asia. *American Journal of Scholarly Research and Innovation*, *1*(01), 270-294. https://doi.org/10.63125/eeja0t77

[110]. Shabir, G. A. (2003). Validation of high-performance liquid chromatography methods for pharmaceutical analysis. *Journal of Chromatography A*, *987*(1–2), 57–66. https://doi.org/10.1016/S0021-9673(02)01536-4

[111]. Sheratun Noor, J., & Momena, A. (2022). Assessment Of Data-Driven Vendor Performance Evaluation in Retail Supply Chains: Analyzing Metrics, Scorecards, And Contract Management Tools. *American Journal of Interdisciplinary Studies*, *3*(02), 36-61. https://doi.org/10.63125/0s7t1y90

[112]. Shrivastava, A., & Gupta, V. B. (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, *2*(1), 21–25. https://doi.org/10.4103/2229-5186.79345

[113]. Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: Uses in assessing rater reliability. *Psychological Bulletin*, *86*(2), 420–428. https://doi.org/10.1037/0033-2909.86.2.420

[114]. Sloop, J. T., Gonçalves, D. A., O'Brien, L. M., Carter, J. A., Jones, B. T., & Donati, G. L. (2021). Evaluation of different approaches to applying the standard additions calibration method. *Analytical and Bioanalytical Chemistry*, *413*(5), 1293–1302. https://doi.org/10.1007/s00216-020-03092-8

[115]. Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., Fan, T. W.-M., Fiehn, O., Goodacre, R., & Griffin, J. L. (2007). Proposed minimum reporting standards for chemical analysis. *Metabolomics*, *3*(3), 211–221. https://doi.org/10.1007/s11306-007-0082-2

[116]. Swartz, M. E. (2005). UPLC™: An introduction and review. *Journal of Liquid Chromatography & Related Technologies*, *28*(7–8), 1253–1263. https://doi.org/10.1081/JLC-200053046

[117]. Tahmina Akter, R., & Abdur Razzak, C. (2022). The Role of Artificial Intelligence in Vendor Performance Evaluation Within Digital Retail Supply Chains: A Review Of Strategic Decision-Making Models. *American Journal of Scholarly Research and Innovation*, *1*(01), 220-248. https://doi.org/10.63125/96jj3j86

[118]. Tahmina Akter, R., Debashish, G., Md Soyeb, R., & Abdullah Al, M. (2023). A Systematic Review of AI-Enhanced Decision Support Tools in Information Systems: Strategic Applications In Service-Oriented Enterprises And Enterprise Planning. *Review of Applied Science and Technology*, *2*(01), 26-52. https://doi.org/10.63125/73djw422

[119]. Tang, D., Li, H., Chen, J., Guo, S., & Fu, X. (2012). Chromatographic fingerprinting technology and its application to the quality control of traditional Chinese medicines. *Journal of Separation Science*, *35*(22), 2673–2687. https://doi.org/10.1002/jssc.201200428

[120]. Taverniers, I., De Loose, M., & Van Bockstaele, E. (2004). Trends in quality in the analytical laboratory II. *TrAC Trends in Analytical Chemistry*, *23*(8), 535–552. https://doi.org/10.1016/j.trac.2004.04.001

[121]. Thompson, M. (2000). Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. *Analyst*, *125*(3), 385–386. https://doi.org/10.1039/B000282H

[122]. Thompson, M., Ellison, S. L. R., Fajgelj, A., Willetts, P., & Wood, R. (1999). Harmonized guidelines for the use of recovery information in analytical measurement. *Pure and Applied Chemistry*, *71*(2), 337–348. https://doi.org/10.1351/pac199971020337

[123]. Thompson, M., Ellison, S. L. R., & Wood, R. (2002). Harmonized guidelines for single-laboratory validation of methods of analysis. *Pure and Applied Chemistry*, *74*(5), 835–855. https://doi.org/10.1351/pac200274050835

[124]. Thompson, M., & Lowthian, P. J. (1997). The Horwitz function revisited. *Journal of AOAC International*, *80*(3), 676–684. https://doi.org/10.1093/jaoac/80.3.676

[125]. Thompson, M., Mathieson, K., Damant, A. P., & Wood, R. (2008). A general model for interlaboratory precision accounts for statistics from proficiency testing in food analysis. *Accreditation and Quality Assurance*, *13*(3–4), 223–230. https://doi.org/10.1007/s00769-008-0356-z

[126]. Tistaert, C., Dejaegher, B., & Vander Heyden, Y. (2011). Chromatographic separation techniques and data handling methods for herbal fingerprinting. *Journal of Chromatography A*, *1218*(42), 7684–7708. https://doi.org/10.1016/j.chroma.2011.05.049

[127]. Trufelli, H., Palma, P., Famiglini, G., & Cappiello, A. (2011). An overview of matrix effects in liquid chromatography–mass spectrometry. *Mass Spectrometry Reviews*, *30*(3), 491–509. https://doi.org/10.1002/mas.20298

[128]. Upton, R., Graff, A., Jolliffe, G., & Länger, R. (2011). American Herbal Pharmacopoeia®: Andrographis paniculata — standards of analysis, quality control, and therapeutics* (analytical section—HPLC/UV validated methods). *Phytomedicine*, *18*(10), 798–804. https://doi.org/10.1016/j.phymed.2011.05.006

[129]. Vander Heyden, Y., & Smeyers-Verbeke, J. (2007). Guidance on linear calibration. *Journal of Chromatography B*, *845*(1), 14–22. https://doi.org/10.1016/j.jchromb.2006.07.045

[130]. Vessman, J., Stefan, R. I., van Staden, J. F., Danzer, K., Lindner, W., Burns, D. T., Fajgelj, A., & Müller, H. (2001). Selectivity in analytical chemistry (IUPAC Recommendations 2001). *Pure and Applied Chemistry*, *73*(8), 1381–1386. https://doi.org/10.1351/pac200173081381

[131]. Walsh, S. J., Macsik, Z., Wegrzynek, D., Krieger, T., & Boulyga, S. (2016). Model diagnostics for detecting and identifying method repeatability outliers in precision studies: Application to a homogeneity study under a two-stage nested ANOVA. *Journal of Analytical Atomic Spectrometry*, *31*(3), 686–699. https://doi.org/10.1039/C5JA00380F

[132]. Wang, L., Yu, Z., Wang, S., & Li, P. (2010). UPLC–MS/MS quantification of silymarin constituents in herbal preparations. *Journal of Chromatographic Science*, *48*(10), 803–806. https://doi.org/10.1093/chromsci/48.10.803

[133]. Wang, R., Zhou, W., & Jiang, X. (2008). Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous systems over a wide temperature range. *Journal of Agricultural and Food Chemistry*, *56*(8), 2694–2701. https://doi.org/10.1021/jf0730338

[134]. Wang, Y.-J., Pan, M.-H., Cheng, A.-L., Lin, L.-I., Ho, Y.-S., Hsieh, C.-Y., & Lin, J.-K. (1997). Stability of curcumin in buffer solutions and characterization of its degradation products. *Journal of Pharmaceutical and Biomedical Analysis*, *15*(12), 1867–1876. https://doi.org/10.1016/S0731-7085(96)02024-9

[135]. Waterman, K. C. (2011). The application of the accelerated stability assessment program (ASAP) to quality by design (QbD) for drug product stability. *AAPS PharmSciTech*, *12*(3), 932–937. https://doi.org/10.1208/s12249-011-9657-3

[136]. Wold, S., Antti, H., Lindgren, F., & Öhman, J. (1998). Orthogonal signal correction of near-infrared spectra. *Chemometrics and Intelligent Laboratory Systems*, *44*(1–2), 175–185. https://doi.org/10.1016/S0169-7439(98)00002-2

[137]. Woodall, W. H., & Borror, C. M. (2008). Some relationships between gage R&R criteria. *Quality and Reliability Engineering International*, *24*(1), 99–106. https://doi.org/10.1002/qre.870

[138]. Wu, C., Guan, Q., Wang, S., & Rong, Y. (2017). Simultaneous determination of multiple ginsenosides in Panax ginseng herbal medicines with one single reference standard. *Pharmacognosy Magazine*, *13*(Suppl. 1), S84–S89. https://doi.org/10.4103/pm.pm_274_16

[139]. Xie, P.-S., & Leung, A. Y. (2009). Understanding of herbal quality markers and chromatographic fingerprinting for quality control of traditional Chinese medicines. *Journal of Chromatography A*, *1216*(11), 1933–1940. https://doi.org/10.1016/j.chroma.2008.12.086

[140]. Xu, X., Liang, Y., Xie, P., & Chau, F.-T. (2012). A critical view on spike recovery for accuracy evaluation of analytical method for medicinal herbs. *Journal of Pharmaceutical and Biomedical Analysis*, *62*, 210–215. https://doi.org/10.1016/j.jpba.2011.12.034

[141]. Yadav, R., Agarwal, A., & Kumar, S. (2012). Spectrophotometric estimation and validation of curcumin in polyherbal formulations. *Indian Journal of Pharmaceutical Sciences*, *74*(4), 386–390. https://doi.org/10.4103/0250-474X.107061

[142]. Zhang, H., Wang, H., & Zhang, Z. (2012). A novel approach to quantitative analysis of multi-components by a single marker (QAMS) in the quality control of traditional Chinese medicines. *Journal of Pharmaceutical Analysis*, *2*(4), 235–241. https://doi.org/10.1016/j.jpha.2012.06.002

[143]. Zheng, X., Li, P., Qi, L.-W., & Liu, E.-H. (2009). UPLC quantification of resveratrol and emodin in Polygonum cuspidatum. *Journal of Separation Science*, *32*(23–24), 3941–3948. https://doi.org/10.1002/jssc.200900446

[144]. Zhou, J., Chan, C. O., Yeung, J. H. Y., Chan, C. L., & Mok, D. K. W. (2014). Rapid UPLC–QTOF–MS profiling of Boswellia acids in Boswellia serrata extracts with quantitative validation. *Journal of Pharmaceutical and Biomedical Analysis*, *88*, 587–594. https://doi.org/10.1016/j.jpba.2013.10.047