

Development of Natural Medicines and Their Application in Chemical Evaluation a Critical Review of Extraction, Analytical Profiling and Quality Standardisation

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ABSTRACT

Natural medicines remain a major source of therapeutic leads and of widely used traditional remedies, but their multi-component nature makes development and quality control more demanding than for single synthetic drugs. This review follows the path from plant selection to a chemically defined, standardised medicine and focuses on the role of chemical evaluation at every step: authentication, extraction, isolation, profiling, quantification and validation. Three analyses support the discussion. First, a chemical-space map of sixteen well-known natural-product drugs, calculated from molecular formulas, shows that four of them (paclitaxel, reserpine, vinblastine and digoxin) exceed the molecular-weight or hydrogen-bond-acceptor limits of the rule of five, which has implications for formulation and analysis. Second, qualitative comparisons rank six extraction techniques and six analytical platforms against practical criteria. Third, a simulated chromatographic-fingerprint example illustrates how a cosine-similarity metric separates a conforming batch (0.9995) from a degraded or adulterated one (0.7815). Requirements for method validation, contaminant control, safety, interactions and regulatory pathways through 2023 are summarised, and priorities for future work—orthogonal analytics, data-driven dereplication and biotechnological supply—are identified.

Index Terms—natural medicines, phytochemical analysis, extraction, HPLC, mass spectrometry, NMR, chromatographic fingerprint, standardisation, method validation.

1.INTRODUCTION

Medicines derived from plants, microorganisms and marine organisms have shaped therapy for centuries. Artemisinin from *Artemisia annua* [1], paclitaxel from the Pacific yew [2], quinine from cinchona bark and morphine from the opium poppy are examples of natural products that became cornerstone drugs, and systematic reviews of new approvals show that natural products and their derivatives continue to supply a large share of small-molecule drugs [3], [4]. Beyond isolated drugs, traditional and complementary medicines are used by large populations; most WHO member states report their use [5].

Developing a natural medicine is difficult because the active material is rarely a single compound. A plant extract contains hundreds of constituents whose levels vary with species, chemotype, growth conditions, harvest time and processing [6], and several constituents may act together or antagonise each other [7]. The medicine has to be defined chemically, not only botanically, if it is to be reproducible, safe and comparable between batches. Chemical evaluation—the identification and quantification of constituents and contaminants with validated analytical methods—is the tool that links the plant to the finished product [8], [9].

Existing reviews usually treat extraction, analytical chemistry or regulation separately [10], [11], [8]. This article joins them along the development pathway and adds three original elements: (i) a chemical-space analysis of sixteen natural-product drugs calculated from their formulas; (ii) transparent, criterion-based comparisons of extraction and analytical techniques; and (iii) a simulated fingerprint example that makes the similarity-based quality criterion concrete. Section II describes the chemical space and the representative compounds, Section III the development pathway, Section IV extraction and isolation, Section V analytical platforms, Section VI standardisation and validation, Section VII safety, formulation and regulation, and Section VIII future directions.

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2.. CHEMICAL SPACE OF NATURAL MEDICINES

Natural-product drugs occupy a different region of chemical space from typical synthetic drugs. They usually contain more oxygen atoms and stereocentres and often more complex ring systems [12], [13]. The rule of five of Lipinski and co-workers predicts poor oral absorption when a compound has more than five hydrogen-bond donors, more than ten acceptors (counted as nitrogen plus oxygen atoms), a molecular weight above 500 or a high lipophilicity [14]. Many successful drugs lie beyond these limits, especially for natural products and their derivatives [15].

Fig. 1 maps sixteen representative compounds (Table I). Molecular weight and the nitrogen-plus-oxygen count were calculated from the molecular formulas; donors and lipophilicity were not evaluated, so the plot tests two of the four criteria. Twelve compounds fall within the two limits. Four lie outside: paclitaxel ($M = 853.92$, 15 acceptors), vinblastine (810.99, 13), digoxin (780.95, 14) and reserpine (608.69, 11). All four are large, highly oxygenated or nitrogen-rich terpenoid, alkaloid or glycoside structures, and several are known for poor aqueous solubility or a narrow therapeutic window, so formulation and analysis of these compounds are especially demanding. The map shows that chemical evaluation is not assume small-molecule, drug-like behaviour for natural products

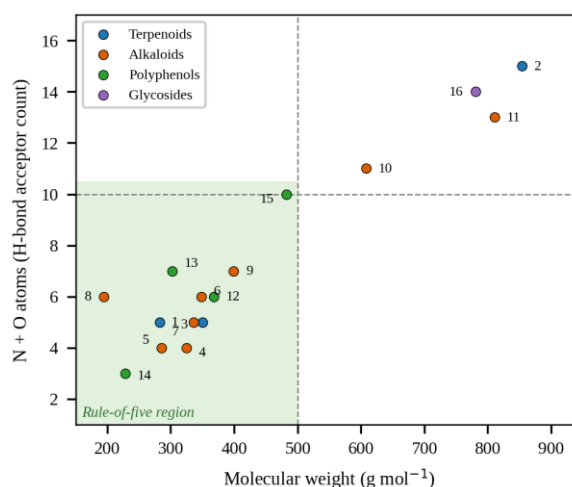


Fig. 1. Chemical-space map of sixteen natural-product drugs: 1 artemisinin, 2 paclitaxel, 3 andrographolide, 4 quinine, 5 morphine, 6 camptothecin, 7 berberine, 8 caffeine, 9 colchicine, 10 reserpine, 11 vinblastine, 12 curcumin, 13 quercetin, 14 resveratrol, 15 silybin, 16 digoxin. Molecular weights and N + O counts were calculated from molecular formulas; the shaded region is the rule-of-five space for these two criteria [14].

TABLE I Representative natural medicines and analytical considerations

Compound (formula, M)	Source	Class and use	Analytical consideration
Artemisinin ($C_{15}H_{22}O_5$, 282.34)	Artemisia annua	Sesquiterpene lactone; antimalarial	Weak chromophore; ELSD, MS or derivatisation
Paclitaxel ($C_{47}H_{51}NO_{14}$, 853.92)	Taxus spp.; semisynthesis	Taxane diterpenoid; anticancer	HPLC-UV near 227 nm; LC-MS
Quinine ($C_{20}H_{24}N_2O_2$, 324.42)	Cinchona bark	Quinoline alkaloid; antimalarial	Fluorescence or UV detection
Morphine ($C_{17}H_{19}NO_3$, 285.34)	Papaver somniferum	Morphinan alkaloid; analgesic	HPLC-UV, LC-MS
Digoxin ($C_{41}H_{64}O_{14}$, 780.95)	Digitalis lanata	Cardiac glycoside; heart failure	Narrow therapeutic index; LC-MS
Camptothecin ($C_{20}H_{16}N_2O_4$, 348.36)	Camptotheca acuminata	Quinoline alkaloid; topoisomerase I scaffold	Lactone-carboxylate equilibrium depends on pH

Compound (formula, M)	Source	Class and use	Analytical consideration
Berberine (C ₂₀ H ₁₈ NO ₄ ⁺ , 336.37)	Berberis, Coptis	Isoquinoline alkaloid; antimicrobial use	UV near 345 nm; cationic, ion-pair or acidic mobile phase
Curcumin (C ₂₁ H ₂₀ O ₆ , 368.38)	Curcuma longa	Diarylheptanoid; anti-inflammatory research	Visible absorbance near 420 nm; unstable, assay-interfering [16]
Silybin (C ₂₅ H ₂₂ O ₁₀ , 482.44)	Silybum marianum	Flavonolignan; hepatoprotective use	UV near 288 nm; diastereomer pair
Andrographolide (C ₂₀ H ₃₀ O ₅ , 350.45)	Andrographis paniculata	Labdane diterpenoid; traditional use	UV near 225 nm; end absorption

3.. FROM PLANT TO STANDARDISED MEDICINE

Fig. 2 summarises the development pathway. Selection draws on ethnobotanical records and the literature, but the choice of material should be tested against ecological and chemical data, because misidentified or poorly documented plant material is a major source of irreproducible results [6]. Authentication combines morphological and microscopic examination with molecular methods; the ITS2 region has been validated as a DNA barcode for medicinal plant species [17], and barcoding of commercial products has detected contamination and substitution [18]. Raw-material quality is controlled by good agricultural and collection practices and by tests described in WHO guidance, including macroscopic and microscopic examination, thin-layer chromatography, ash and extractive values, and limits for contaminants [19], [9].

Bioassay-guided fractionation links activity to fractions and finally to compounds [20]. Its risks are well known: frequent “actives” such as polyphenols, quinones and aggregators may react with assay components rather than act on the intended target, and a substantial proportion of reported natural-product hits may be false [21], [22]. Early dereplication, which compares chromatographic and spectral data with databases to recognise known compounds, saves effort and prevents rediscovery [23]. For products intended for use as multi-component extracts, the chemical definition may remain at the level of a characterised extract, whereas isolation to a single compound follows the small-molecule route [7].

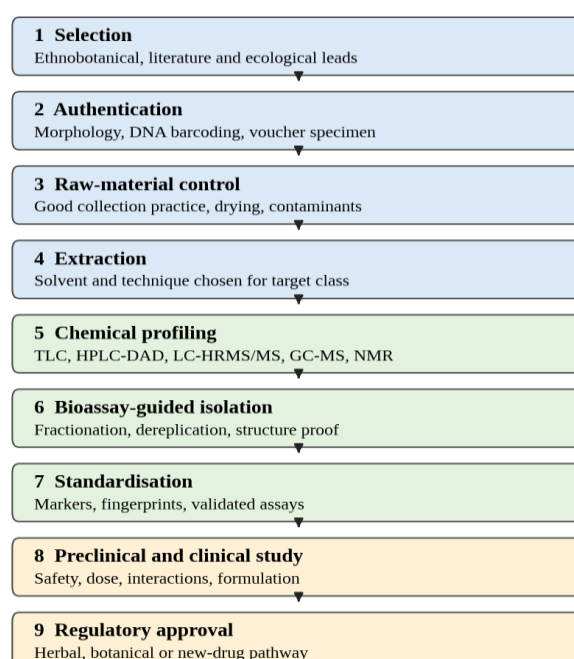


Fig. 2. Development pathway of a natural medicine with chemical evaluation at each stage. Blue: raw material and extract; green: analysis and standardisation; amber: clinical and regulatory stages.

4. EXTRACTION AND ISOLATION

The extraction technique must give an acceptable yield of the target class while preserving its integrity [24], [10]. Maceration and percolation are simple, scalable and gentle but slow and solvent-intensive; Soxhlet extraction is exhaustive but exposes constituents to prolonged heat. Ultrasound-assisted extraction uses cavitation to disrupt cell walls and shortens the extraction time [25]. Microwave-assisted extraction heats the solvent and plant moisture rapidly, with benefits in speed and solvent consumption but a risk to thermolabile compounds [26]. Supercritical CO₂ gives solvent-free extracts of lipophilic compounds and tuned by pressure, temperature and polar modifiers such as ethanol [27]. Pressurised liquid extraction uses elevated temperature and pressure to speed up extraction with small solvent volumes [24].

Fig. 3 scores six techniques on five criteria. The scores are the author's qualitative judgments derived from the cited reviews and are not measured values. Supercritical CO₂ has the best combined solvent and selectivity profile, ultrasound and pressurised liquid extraction offer speed at moderate scale, and conventional maceration remains the most scalable and gentlest although the least efficient. Emerging natural deep eutectic solvents are attractive for green extraction of polar compounds [28], and guidelines for green extraction emphasise renewable solvents, reduced energy and safe products [29]. Whichever technique is chosen, the extraction solvent determines which constituents are analysed later, so it must be reported as part of the product definition.



Fig. 3. Qualitative scoring (1 = low, 3 = high favourability) of six extraction techniques on five criteria. Scores are the author's judgment based on the cited literature and are not experimental data.

Isolation of pure compounds from fractions uses open-column, flash and preparative liquid chromatography. High-speed counter-current chromatography is a support-free liquid-liquid method that avoids irreversible adsorption of sensitive compounds on solid supports and is well suited to polar and labile natural products [30].

5. ANALYTICAL PLATFORMS FOR CHEMICAL EVALUATION

A. Planar and Liquid Chromatography

High-performance thin-layer chromatography (HPTLC) provides inexpensive, parallel fingerprints of many samples on one plate and is a standard identity test in pharmacopoeial monographs and in WHO guidance [9], [8]. High-performance liquid chromatography with diode-array detection (HPLC-DAD) is the workhorse for quantification of marker compounds, because it combines good precision and wide availability with spectral confirmation of peak identity [8]. Its limitations are evident in Table I: compounds with weak chromophores, such as artemisinin, require evaporative light-scattering, mass spectrometric or derivatisation-based detection.

B. Mass Spectrometry and Metabolomics

Gas chromatography-mass spectrometry is the method of choice for volatile constituents, whereas liquid chromatography coupled to high-resolution tandem mass spectrometry (LC-HRMS/MS) provides sensitive profiling of non-volatile compounds in a single run, with molecular formulas and fragmentation data for annotation [11]. Spectral libraries and community platforms such as molecular networking group structurally

related compounds and speed up dereplication [31]. Matrix effects such as ion suppression mean that quantification by LC-MS requires internal standards and matrix-matched calibration.

C. Nuclear Magnetic Resonance

NMR spectroscopy is unrivalled for unambiguous structure determination and is inherently quantitative, since signal intensity is proportional to the number of nuclei. Quantitative ^1H NMR determine the purity of reference compounds and the content of constituents without compound-specific calibration standards [32], [33], and NMR-based metabolomics offers reproducible profiling of plant extracts [34]. Its drawbacks are low sensitivity and the capital cost of the instrument [11].

D. Spectroscopic Fingerprints and Colorimetric Assays

Infrared and near-infrared spectroscopy provide rapid, non-destructive fingerprints that, with multivariate methods such as principal component analysis [35], identify species or detect adulteration, although they need reference data and are less specific than chromatography. Colorimetric assays remain popular for total phenolic and total flavonoid content. The Folin–Ciocalteu method for total phenolics measures the reducing capacity of a sample [36], and several antioxidant assays respond to any reducing compound, so results indicate class-level capacity and should not be interpreted as proof of a specific constituent or of biological benefit [37], [38].

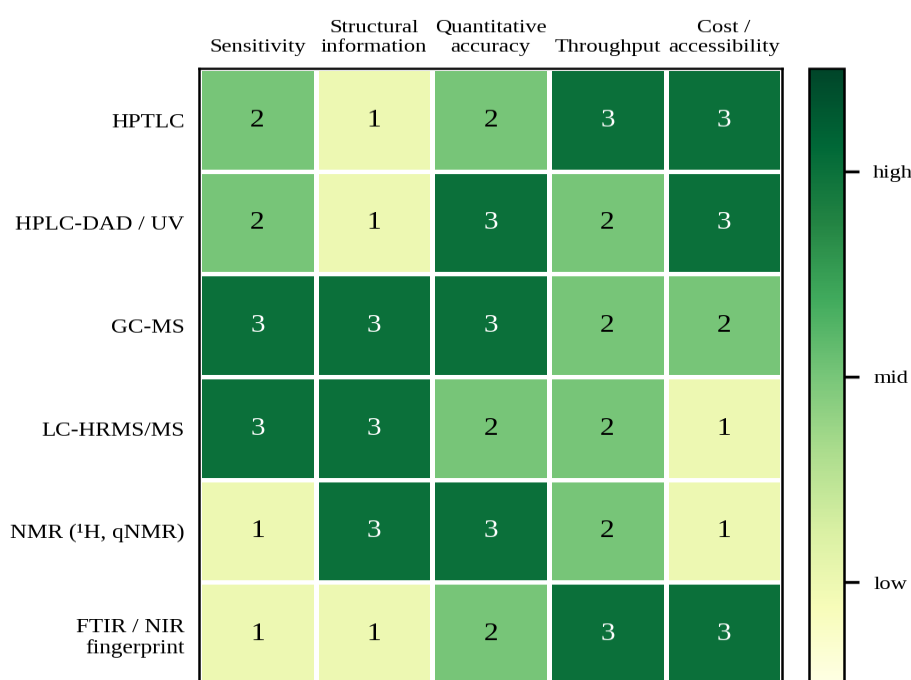


Fig. 4. Qualitative comparison (1 = low, 3 = high favourability) of six analytical platforms. Scores are the author's judgment from the cited literature. GC-MS applies only to volatile or derivatised compounds.

Fig. 4 shows that no platform dominates. HPTLC and spectroscopic fingerprints offer throughput and low cost but little structural information; HPLC-DAD is a robust quantitative tool; LC-HRMS/MS and NMR give the structural depth needed for identification. In practice, orthogonal combinations are most informative: LC-HRMS/MS to annotate, NMR to confirm and quantify selected markers, and HPLC-DAD for routine release testing [11], [33].

6. STANDARDISATION, FINGERPRINTING AND METHOD VALIDATION

Standardisation fixes the chemical definition of the product through marker compounds, chromatographic fingerprints and limits for contaminants, so that batches are consistent in composition [8], [9]. Markers may be active constituents, characteristic compounds, or analytical markers with no proven activity. A quantitative method for a marker must be validated according to ICH Q2(R1) [39], which was updated in 2023 as Q2(R2) to cover modern analytical techniques [40]. Table II lists the principal parameters and commonly used acceptance criteria; limits vary with the matrix and with the regulatory framework, and herbal matrices usually receive wider criteria than pure drug substances.

TABLE IIPrincipal method-validation parameters for marker quantification

Parameter	What is demonstrated	Commonly applied criterion
Specificity	Marker peak is resolved from neighbouring constituents and matrix	Resolution ≥ 1.5 ; peak-purity check with DAD or MS
Linearity	Response is proportional to concentration	≥ 5 levels; coefficient of determination ≥ 0.99
Accuracy	Closeness to the true value (spike recovery)	Recovery about 90–110 % in botanical matrices
Precision	Repeatability and intermediate precision	Relative standard deviation ≤ 2 –5 %
LOD / LOQ	Lowest detectable and quantifiable amount	LOD = $3.3\sigma/S$; LOQ = $10\sigma/S$
Robustness	Resistance to small changes in method settings	Results within criteria on varying pH, temperature, flow rate

The detection and quantification limits of a calibration with slope S and residual standard deviation σ are estimated as [39]

$LOD = 3.3 \sigma / S, \quad LOQ = 10 \sigma / S \quad (1)$

Chromatographic fingerprints evaluate the whole profile instead of single markers. A common measure of agreement between a sample fingerprint y and a reference fingerprint x , each a vector of detector responses at n time points, is the cosine similarity [8]:

$\rho = \sum x_i y_i / [(\sum x_i^2)^{1/2} (\sum y_i^2)^{1/2}] \quad (2)$

Fig. 5 illustrates the use of the measure on simulated chromatograms of seven peaks. Batch B differs from the reference by random variation of $\pm 5\%$ in peak heights and has a similarity of 0.9995, whereas batch C, in which two peaks are reduced by 50–65 % and an additional peak appears, as would occur in degradation or adulteration, has a similarity of 0.7815. The chromatograms and values are synthetic and serve only to demonstrate the metric; acceptance thresholds for real products must be set from validated batch data. Similarity does not replace marker assays, because a fingerprint dominated by a few large peaks remain similar even if a minor active constituent changes, and adulteration sometimes be detected only with DNA or targeted chemical tests [18].

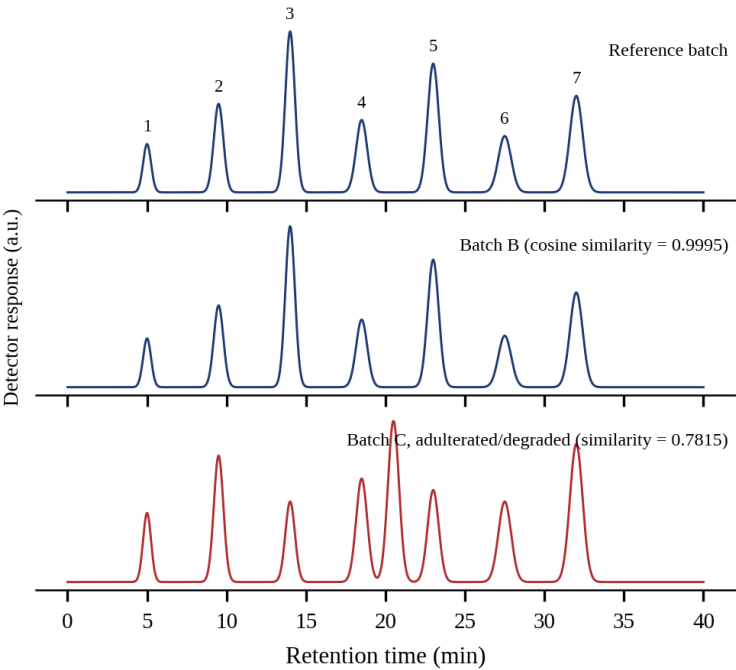


Fig. 5. Simulated HPLC fingerprints (synthetic data for demonstration): reference batch with seven numbered peaks, a conforming batch B and a degraded or adulterated batch C. Cosine similarities relative to the reference were calculated from Eq. (2).

7. SAFETY, FORMULATION AND REGULATION

Natural does not mean safe. Adverse reactions arise from toxic constituents, contaminants such as heavy metals, pesticides and mycotoxins, and adulteration with undeclared synthetic drugs, and under-reporting makes monitoring difficult [41]. Herbal products also interact with prescribed drugs, most often by induction or inhibition of metabolising enzymes and transporters; St John's wort is a well-known example [42]. Chemical evaluation includes limit tests for contaminants [9] and, in clinical studies, characterisation of the exact material administered [6].

Formulation is decisive. Curcumin shows the problem clearly: it has very low systemic bioavailability because of poor absorption and rapid metabolism [43], and it is chemically reactive and prone to interfere with assays [16]. Evaluating such compounds requires stability testing of the dosage form and assays that tolerate reactive compounds. Regulatory pathways differ. In the EU, the directive on traditional herbal medicinal products allows registration on the basis of long-standing use, provided quality and safety are shown [44]. In the United States, botanical drugs may be developed under FDA guidance that accepts a chemically characterised, multi-component product, with emphasis on batch-to-batch consistency and on controls over the botanical raw material [45]. In both systems, validated chemical evaluation is the basis for product identity and consistency.

8. CHALLENGES AND FUTURE DIRECTIONS

Several priorities follow from the analysis. (1) Reproducibility of plant material and reporting of extraction conditions should be improved, as recommended by best-practice guidelines in phytopharmacology [6]. (2) Orthogonal analytical strategies that pair LC-HRMS/MS with NMR and shared spectral databases need wider adoption to speed up identification [11], [31]. (3) Artificial intelligence and machine learning are beginning to support structure annotation, activity prediction and genome mining in natural-product discovery, and these tools need high-quality chemical data to work [46]. (4) Biotechnological supply may relieve dependence on wild harvest; the semi-synthetic production of artemisinin through engineered yeast is the best-known example [47]. (5) Assays that discriminate real activity from assay interference should be built into discovery pipelines [22], [21]. (6) Fingerprint acceptance limits and the relation between chemical profile and clinical effect need to be established with real batch data rather than inferred from similarity values alone [8], [7]. (7) Green extraction, including supercritical fluids and eutectic solvents, should be applied together with validated analysis to ensure that new solvents do not alter the chemical definition of the product [28], [29].

9. CONCLUSION

Chemical evaluation underpins every stage in the development of a natural medicine: it authenticates the material, guides extraction, identifies and quantifies constituents, supports standardisation and provides the evidence needed for safety and regulatory review. The chemical-space analysis shows that natural-product drugs often lie beyond conventional drug-like limits and need tailored analytical and formulation strategies; the technique comparisons show that extraction and analytical platforms are complementary and should be combined; and the fingerprint example demonstrates how similarity metrics help detect departures from a reference profile. Reliable natural medicines will depend on well-characterised starting material, orthogonal and validated analysis, and transparent reporting

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