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Review Paper

Quality Evaluation of Crude Drugs Using Modern Chromatographic Techniques: A Review

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ABSTRACT

Crude drugs, unprocessed plant, animal, or mineral materials, remain the cornerstone of many traditional and modern pharmacotherapeutic systems. Ensuring their quality, safety, and efficacy is critical, yet challenging, owing to intrinsic variability in composition, the presence of adulterants, and the complexity of phytochemical matrices. Modern chromatographic techniques, notably high performance liquid chromatography (HPLC), ultra high performance liquid chromatography (UHPLC), gas chromatography (GC), and their hyphenated mass spectrometric forms, have become indispensable tools for the qualitative and quantitative assessment of crude drugs. This review critically examines the current state of chromatographic methodologies employed for crude drug quality evaluation, covering sample preparation strategies, method development, validation parameters, and regulatory considerations. Emerging trends such as metabolomic fingerprinting, micro extraction, and chemometric integration are discussed, highlighting future directions for achieving more robust, high throughput, and eco friendly analytical workflows.

INTRODUCTION

Crude drugs, defined by the World Health Organization (WHO) as "the natural, unprocessed material derived from plants, animals, or minerals that is used as a medicinal product"[1] have been utilized for millennia. Their therapeutic efficacy depends on a complex mixture of bioactive constituents that may vary with geographic origin, harvest time, post-harvest handling, and storage conditions.[2] Consequently, rigorous quality evaluation is essential to assure safety, efficacy, and compliance with pharmaco-peia standards.[3] Crude drugs are naturally occurring, unprocessed or minimally processed substances derived from plant, animal, or mineral sources used in traditional and modern medicine. With the resurgence of interest in herbal and natural

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remedies, crude drugs such as *Withania somnifera* (Ashwagandha), *Curcuma longa* (Turmeric), *Panax ginseng*, and *Zingiber officinale* (Ginger) are now subjects of extensive scientific research and commercial exploitation.

However, crude drugs are inherently variable due to factors such as genetic diversity, geographical origin, harvesting conditions, post-harvest processing, and storage. These variations can significantly impact their chemical composition and therapeutic efficacy. Therefore, rigorous quality assessment is essential to ensure consistency, safety, and authenticity.

Historically, qualitative assessments such as organoleptic, microscopic, and thin-layer chromatography (TLC) methods were employed.[4] While valuable for rapid screening, these approaches lack the sensitivity, selectivity, and quantitative capacity required for modern regulatory expectations. Over the past three decades, sophisticated chromatographic platforms—HPLC, UHPLC, GC, capillary electrophoresis (CE), and their mass-spectrometric (MS) and nuclear magnetic resonance (NMR) hybrids—have revolutionized crude-drug analysis.[5] They enable precise identification of marker compounds, determination of adulterants, assessment of batch-to-batch consistency, and generation of comprehensive chemical fingerprints.

This review consolidates current knowledge on the application of modern chromatographic techniques for crude-drug quality evaluation. The objectives are to (a) outline the principal chromatographic modalities and their operational principles, (b) describe sample-preparation and extraction protocols tailored to diverse matrices, (c) summarize validation criteria recommended by ICH, USP, and other agencies, (d) discuss chemometric tools that augment data interpretation, and (e) identify challenges and emerging trends shaping the future of crude-drug analytics.

Overview of Chromatographic Techniques

High-Performance Liquid Chromatography (HPLC)

HPLC remains the workhorse for the analysis of non-volatile, thermally labile phytochemicals such as alkaloids, flavonoids, phenolics, and saponins. The technique utilizes a high-pressure pump to force a liquid mobile phase through a packed column containing stationary phase particles (typically 3–5 µm). Separation is governed by differential partitioning between mobile and stationary phases.[6]

Table: Key modalities

Modality	Detector(s)	Typical Applications
Reversed-phase HPLC (RP-HPLC)	UV-Vis, PDA, FLD, MS	Quantification of polar to moderately non-polar constituents
Normal-phase HPLC (NP-HPLC)	Evaporative light scattering detector (ELSD), MS	Analysis of highly non-polar lipids and terpenoids
Hydrophilic interaction liquid chromatography (HILIC)	MS, CAD	Separation of highly polar metabolites (e.g., sugars, amino acids)
Chiral HPLC	CD, MS	Enantiomeric purity of chiral alkaloids and terpenes

Advances such as column technology (sub-2 µm particles, core-shell particles) and temperature control have enhanced efficiency, providing up to 10-fold reductions in analysis time without loss of resolution.[7]

Ultra-High-Performance Liquid Chromatography (UHPLC)

UHPLC employs columns packed with sub-2 µm particles and operates at pressures up to 1500 bar, delivering higher peak capacity and faster run times. The technique is especially valuable for high-throughput screening of botanical extracts where multiple markers must be monitored simultaneously.[8]

Gas Chromatography (GC) and GC-Mass Spectrometry (GC-MS)

GC separates volatile and semi-volatile compounds based on their distribution between a gaseous mobile phase and a stationary phase coated onto the inner walls of a capillary column. For crude-drug analysis, GC primarily targets essential oils, terpenes, fatty acids (as methyl esters), and certain alkaloids after derivatization. Coupling with MS provides structural elucidation and confirmation of identity.[9]

Critical parameters

- **Injection mode:** Split-less for trace analysis, split for high concentration samples.
- **Derivatization:** Silylation (e.g., BSTFA) for polar metabolites (e.g., sugars).

Capillary Electrophoresis (CE)

CE separates analytes based on differences in electrophoretic mobility under an electric field. Its high efficiency and low solvent consumption make it an attractive alternative for ionic phytochemicals (e.g., organic acids, amino acids). When combined with UV or MS detection, CE offers excellent sensitivity for trace constituents.[10]

Hyphenated Techniques (LC-MS, LC-MS/MS, LC-NMR)

Mass spectrometric detection enhances selectivity and sensitivity, enabling identification of unknowns and simultaneous quantitation of multiple markers. Tandem MS (MS/MS) provides structural information through fragmentation patterns, which is indispensable for confirming adulterants or degraded products. LC-NMR, though less common due to cost, provides definitive structural confirmation for complex mixtures.[11]

Sample Preparation and Extraction Strategies

Robust sample preparation is a prerequisite for reliable chromatographic analysis. The diversity of crude-drug matrices fibrous plant material, resinous gum, powdered roots, or mineral powders necessitates tailored extraction protocols.

Table: Summarizes the most frequently employed techniques.

Technique	Principle	Suitable Matrices	Advantages	Limitations
Maceration	Solvent diffusion at ambient temperature	Soft plant tissues, powders	Simple, low cost	Long extraction times, low efficiency
Soxhlet extraction	Continuous solvent reflux	Dried plant material, resins	Exhaustive extraction	High solvent consumption, thermal degradation risk
Ultrasound-assisted extraction (UAE)	Acoustic cavitation enhances solvent penetration	All plant matrices	Rapid, reduced solvent use	Possible degradation of heat-sensitive compounds

Microwave-assisted extraction (MAE)	Microwave energy heats solvent-matrix mixture	Dense or hard matrices	Fast, high yield	Requires specialized equipment
Pressurized liquid extraction (PLE/Accelerated solvent extraction)	High pressure and temperature improve solubility	Tough matrices, soils	Automated, reproducible	Potential thermal decomposition
Solid-phase microextraction (SPME)	Sorbent coating adsorbs volatiles	Essential oils, aroma compounds	Solvent-free, suitable for GC-MS	Limited capacity for non-volatile analytes
QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe)	Salting-out extraction with dispersive SPE cleanup	Multi-residue pesticide/alkaloid screening	High throughput, minimal solvents	Not universally applicable to all phytochemicals

Selection criteria

1. **Polarity of target analytes** – Polar constituents often require hydroalcoholic or aqueous organic mixtures, while non-polar markers are efficiently extracted with hexane or dichloromethane.
2. **Thermal stability** – Heat-sensitive compounds (e.g., certain flavonoids) are best extracted under mild conditions (UAE, PLE at low temperature).
3. **Matrix complexity** – Samples with high pigment or lipid content may necessitate cleanup steps such as solid-phase extraction (SPE) or gel permeation chromatography (GPC) to prevent column fouling.

Method Development and Optimization

Chromatographic method development follows a systematic, iterative approach. Key steps include:[12]

1. **Selection of stationary phase** – Based on physicochemical properties of the analytes (e.g., C18 for non-polar to moderately polar, HILIC for highly polar).
2. **Mobile-phase composition** – Optimization of solvent strength, pH, ion-pairing agents (e.g., trifluoroacetic acid for acidic analytes), and gradient profiles to achieve resolution and acceptable run time.
3. **Detection wavelength/ion mode** – UV-Vis detection requires knowledge of chromophores; MS detection demands optimization of ionization source (ESI, APCI) and collision energies for MS/MS.
4. **Temperature control** – Column temperature influences viscosity and retention; a typical range is 30–40 °C for RP-HPLC.
5. **System suitability testing** – Assessment of parameters such as tailing factor (<2.0), theoretical plates (>2000 for HPLC, >10 000 for UHPLC), and injection repeatability (RSD < 2 %).

Advanced tools, such as Design of Experiments (DoE) and automated method-search software, expedite optimization while ensuring robustness.[13]

Validation of Chromatographic Methods

Regulatory bodies require validated analytical procedures that demonstrate reliability for routine quality control. The International Council for Harmonisation (ICH) Q2(R1) guideline outlines the essential validation characteristics.[14]

Table: Presents a concise overview of validation parameters as applied to crude-drug analysis.

Parameter	Definition	Acceptance Criteria	Typical Assessment
Specificity	Ability to unequivocally assess target analyte in presence of matrix components	No interfering peaks at analyte retention time (≤ 5 % of analyte peak area)	Forced-degradation studies, blank matrix analysis
Linearity	Proportionality between response and concentration	Correlation coefficient (r) ≥ 0.999; residuals ≤ ± 2 %	Calibration curve (5–7 levels)
Accuracy (Recovery)	Closeness of measured value to true value	Recovery 95–105 % for most analytes (80–120 % for complex matrices)	Spike-recovery experiments
Precision	Repeatability (intra-day) and intermediate precision (inter-day)	RSD ≤ 2 % (repeatability), ≤ 5 % (intermediate)	Replicate injections (n = 6)
Detection/Quantitation Limits (LOD/LOQ)	Minimum detectable/quantifiable concentrations	Signal-to-noise (S/N) ≥ 3 (LOD), ≥ 10 (LOQ)	Dilution series
Robustness	Effect of small deliberate variations on method performance	No significant change in resolution, tailing, or quantitation	Variation of flow rate, temperature, pH
Stability	Analyte stability in solution, during storage, and after preparation	% change ≤ 5 % over defined period	Bench-top, freeze-thaw, long-term storage studies

Validation must be documented in a Standard Operating Procedure (SOP), and for each crude-drug batch, a Certificate of Analysis (CoA) should be generated, summarizing assay results and compliance with monographs (e.g., USP-NF, European Pharmacopoeia).

Applications of Chromatography in Crude-Drug Quality Evaluation

Marker-Compound Quantification

Quantitative determination of marker constituents (e.g., berberine in *Coptis chinensis*, curcumin in *Curcuma longa*) is the most common application. HPLC-PDA or HPLC-MS methods provide precise assay values that are stipulated in pharmacopeial monographs.

Chemical Fingerprinting and Metabolomics

Instead of focusing on a few markers, holistic fingerprinting captures the entire phytochemical profile, enabling discrimination of geographical origin, harvest season, and detection of adulteration. Techniques such as UHPLC-QTOF-MS coupled with chemometric tools (principal component analysis, PCA; partial least squares-discriminant analysis, PLS-DA) generate robust classification models.[15]

Detection of Adulterants and Contaminants

Chromatography can reveal the presence of synthetically derived adulterants (e.g., synthetic cathinones in *Ephedra* preparations) or contaminants such as pesticides, heavy metals (via derivatization and GC-MS), and mycotoxins (LC-MS/MS).[16]

Stability Testing

Forced-degradation studies (acidic, basic, oxidative, photolytic) monitored by HPLC help establish degradation pathways and identify stable markers for shelf-life evaluation.

Standardization of Multi-Component Herbal Formulations

Complex formulations (e.g., traditional Chinese medicine decoctions) require simultaneous quantitation of dozens of constituents. Multimodal approaches—combining HPLC-UV for major markers with LC-MS/MS for minor actives—facilitate comprehensive standardization.[17]

Chemometrics and Data Mining

The voluminous data generated by modern chromatography necessitates advanced statistical treatment. Chemometric techniques aid in (i) pattern recognition, (ii) classification, and (iii) quantitative structure-activity relationship (QSAR) modeling.[18]

- **PCA** reduces dimensionality and visualizes clustering of samples based on chemical composition.
- **Hierarchical clustering analysis (HCA)** groups similar batches, supporting batch release decisions.
- **Partial least squares regression (PLS)** correlates chromatographic fingerprints with biological activity data, enabling bio-fingerprinting.

Software platforms such as SIMCA, Unscrambler, and open-source R packages (e.g., *mixOmics*) are widely employed.

Challenges and Future Perspectives

Matrix Complexity and Interference

Crude-drug matrices can contain high levels of pigments, lipids, and polysaccharides that cause column fouling, peak distortion, and ion-suppression in MS. Future work should emphasize green extraction (e.g., deep eutectic solvents) and miniaturized sample preparation (e.g., micro-SPE) to mitigate these issues.[19]

Standardization of Reference Materials

Limited availability of authenticated reference standards hampers absolute quantitation. Collaborative initiatives to develop certified reference materials (CRMs) for key phytochemicals are essential.

Integration of Multi-Omics

Combining chromatographic metabolomics with transcriptomics and proteomics will provide a systems level view of plant quality, facilitating predictive quality control based on biosynthetic pathway markers.[20]

Automation and High-Throughput Screening

Robotic sample preparation, coupled with UHPLC-MS/MS platforms capable of handling >200 samples per day, will meet the growing demand for rapid release testing, especially for large-scale manufacturers.

Regulatory Harmonization

Disparities among pharmacopeias (USP, EP, JP) regarding acceptable analytical methods create challenges for global trade. Harmonized guidelines that explicitly endorse modern chromatographic techniques backed by inter laboratory validation studies are needed.

CONCLUSION

Modern chromatographic techniques constitute the backbone of contemporary quality evaluation for crude drugs. HPLC, UHPLC, GC, and their hyphenated MS variants deliver the sensitivity, specificity, and throughput required to meet stringent regulatory expectations. Robust sample preparation, rigorous method validation, and the integration of chemometric analyses ensure that the complex chemical tapestry of crude drugs is accurately captured and quantified. While

challenges related to matrix complexity, reference material scarcity, and regulatory alignment remain, ongoing innovations in green extraction, automation, and multi-omics promise to further enhance the reliability and efficiency of crude-drug quality control. Ultimately, the continued evolution of chromatographic science will bolster the safety, efficacy, and global acceptance of herbal medicines and other natural products.

CONFLICT OF INTEREST

The authors have no conflicts of interest.

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