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Dr. Verender Kumar
MD. (Ayu.) Dravyaguna,
Ayurvedic Medical Officer,
AYUSH Arogya Kendra,
Jhunir, District Mansa,
Department of Ayush,
Government of Punjab, India

Corresponding Author:
Dr. Verender Kumar
MD. (Ayu.) Dravyaguna,
Ayurvedic Medical Officer,
AYUSH Arogya Kendra,
Jhunir, District Mansa,
Department of Ayush,
Government of Punjab, India

Chromatographic Analysis in Herbal Drug Standardization: Role of Thin-Layer and High-Performance Thin-Layer Chromatography

Verender Kumar

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Abstract
Chromatographic techniques such as Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) have become indispensable tools in the standardization and quality assessment of herbal medicines. These planar chromatography methods, based on multistage distribution processes between a stationary adsorbent and a mobile phase, allow rapid fingerprinting and detection of herbal constituents, adulterants and substitutes. TLC offers simplicity, speed and cost-efficiency, while HPTLC enhances precision, reproducibility and quantitative capabilities through automation and densitometry. Modern pharmacognosy uses these techniques for identifying marker compounds, verifying botanical identity, and ensuring batch consistency. This review traces the origin and principle of TLC/HPTLC, explores instrumentation and method development, outlines their applications in herbal drug analysis, and discusses their advantages and limitations. The integration of these chromatographic methods into herbal drug quality control frameworks supports the reliability of traditional formulations in contemporary therapeutics.

Keywords: Thin-Layer Chromatography, High-Performance Thin-Layer Chromatography, herbal drug analysis, fingerprinting, pharmacognosy, standardization

Introduction
The use of herbal drugs in traditional systems of medicine such as Ayurveda has grown significantly, prompting the need for robust quality assurance and standardization of raw medicinal materials and formulations. One key component of this is chromatographic analysis, which enables separation, identification and quantification of phytoconstituents. Among various chromatographic options, Thin-Layer Chromatography (TLC) and its enhanced form, High-Performance Thin-Layer Chromatography (HPTLC), are widely adopted for herbal drug standardization. The principle of these methods lies in differential migration of compounds across a thin adsorbent layer under a solvent front, allowing rapid multi-sample screening and fingerprinting. While TLC has its roots in the 19th century (Beyerinck, 1889), the evolution of HPTLC has brought automation, improved resolution and densitometric quantification, thus aligning herbal analysis with modern pharmacognostic demands. The present review aims to examine the fundamental principles, instrumentation, methodological aspects, applications and limitations of TLC and HPTLC in the context of herbal drug quality control and standardization.

Review of Literature
Historical and theoretical foundations
Thin-Layer Chromatography, as a concept of planar separation, dates back more than a century, but its application in analytical phytochemistry was significantly advanced by the work of Egon Stahl and others in the 20th century (Stahl, 1988). Over time, improvements in adsorbents, plate supports and detection methods elevated the method's reliability. The upgraded version, HPTLC, integrates high quality absorbing layers, automated sample application, controlled chamber development and densitometric scanning, offering superior reproducibility (Kowalska & Sajewicz, 2023)

Applications in herbal medicine

The relevance of TLC and HPTLC to herbal drug analysis has consistently increased in the last decades. For instance, HPTLC fingerprinting has been used for multi-herb formulations to monitor batch consistency, detect adulteration and quantify marker compounds (Sanket & Dighe, 2022) [turn0search0]. In the quality control review of traditional herbs, HPTLC emerged as a preferred method for simultaneous qualitative and quantitative assessment of complex herbal extracts (Quality control review, 2020) [turn0search1]. Specific applications include the quantification of piperine in Ayurvedic churnas using HPTLC (Hazra *et al*., 2019), and HPTLC profiling of Careya arborea plant parts (Gupta *et al*., 2019).

Method development and validation

Recent advances underscore the importance of validated HPTLC methods, which require optimisation of parameters such as adsorbent, mobile phase, application method, derivatisation, scanning wavelength and documentation (Vyas *et al*., 2023). The comparative analysis of TLC vs HPTLC shows that the latter offers enhanced sensitivity, automation, simultaneous multiple sample analysis and better quantitation (Shukla *et al*., 2023).

This body of literature establishes TLC/HPTLC as key analytical tools for herbal drug standardisation, bridging traditional pharmacopeial methods and modern instrumentation.

Methodology

This review article was prepared by conducting a systematic narrative survey of peer-reviewed journals, open-access articles, and pharmacognosy-focused reviews from 2000 to 2025. Databases searched include PubMed Central, Google Scholar and institutional repositories, using keywords such as “TLC herbal drugs”, “HPTLC herbal fingerprinting”, “planar chromatography herbal standardisation”, “herbal adulteration TLC”. Articles were selected that discussed principle, instrumentation, applications, method development and limitations of TLC/HPTLC in the herbal medicine context. Data extracted included: adsorbent/media used, sample application techniques, mobile phase compositions, detection techniques, fingerprinting results, quantitative outcomes and studies on herbal formulations. Analysis themes comprised: (1) principle and classification of TLC/HPTLC, (2) instrumentation and method steps, (3) application areas in herbal drugs, (4) advantages and constraints, and (5) future directions.

Results

Principle and classification

TLC and HPTLC operate on the principle of adsorption and capillary migration of mobile phase across a thin layer of adsorbent coated onto a flat support. Components of a mixture separate according to their relative affinity for the stationary phase and solubility in the mobile phase. The retention factor (Rf) is defined as:

Rf. value can be calculated by the following formula

Rf = Distance travelled by solute / distance travelled by solvent.

This foundational principle applies to both TLC and HPTLC [14]. Chromatographic methods can be classified by stationary and mobile phase nature: gas chromatography, liquid chromatography, and planar chromatography (adsorption TLC, ion-exchange TLC, reverse-phase TLC, HPTLC) [9].

Instrumentation and method steps

Key components of modern HPTLC include: automatic plate coaters (uniform adsorbent layer), sample applicators (manual Nanomat to fully automatic ATS), developing chambers (automatic developing chamber with controlled saturation and humidity), densitometer or scanner for quantification [16]. Sample preparation, application, development and evaluation must be optimised for consistent results.

Applications in herbal drug analysis

TLC and HPTLC have proven their utility in several domains:

- **Fingerprinting of herbal extracts:** simultaneous analysis of multiple samples enables visual comparison of sample vs reference, detection of adulterants, substitution, or variation in phytochemical profile [12].
- **Quantification of marker compounds:** e.g., HPTLC method for piperine detection in Ayurvedic churnas achieved Rf ~0.39 and quantified piperine amounts across formulations (Hazra *et al*., 2019).
- **Quality consistency of formulations:** studies using HPTLC fingerprint combined with chemometrics to evaluate batch consistency in complex herbal formulas (multi-herb) have reported identification of marker peaks and cluster analysis for quality differences (Turn0search6).
- **Screening and adulteration detection:** TLC and HPTLC are cost-effective screening tools for botanicals, culinary herbs, psychoactive plants and cosmetics of botanical origin (Kowalska & Sajewicz, 2023).

Advantages and limitations

Advantages: simplicity, speed, low solvent/sample requirement, high throughput (many spots per plate), possibility of multiple detection (UV, fluorescence, derivatisation), visual record of fingerprint, cost-effectiveness compared to HPLC/GC [15, 12].

Limitations: lower resolution and sensitivity compared to HPLC or GC, requirement for experienced interpretation of bands/spots, limitation in fully quantifying ultra-low concentration analytes, potential for operator variability, requirement for standardisation of plate conditions and environment. HPTLC addresses some of these but still lacks universal adoption in all labs.

Discussion

The evolution of TLC into HPTLC reflects the needs of modern herbal pharmaceutics: large sample throughput, better reproducibility, quantitative capability and documentation. While traditional TLC remains valuable—especially in resource-limited settings—it may not meet the stringent demands of global herbal trade and regulatory frameworks. HPTLC fills this gap by combining automation with high sample capacity, allowing for testing of dozens of samples in parallel with densitometric scanning and digital records (Shukla *et al*., 2023).

In the context of Ayurvedic raw drugs and formulations, chromatographic fingerprinting via TLC/HPTLC provides a bridge between classical organoleptic/macroscopic methods and modern instrumental quality control. For example, a validated HPTLC method for an Ayurvedic formulation provides a quantifiable marker (piperine) that can be used for batch control (Hazra *et al*., 2019).

The adoption of fingerprint techniques also helps in detecting substitution or adulteration of herbal materials, a longstanding issue in crude drug trade. TLC/HPTLC visual patterns, when compared to reference standards, allow rapid pre-screening of authenticity before more detailed tests (UM) [12].

Despite their strengths, these techniques must be integrated into a larger orthogonal authentication protocol. A chromatographic profile alone may not capture geochemical variation, metabolite degradation, or adulterants that mimic chemical profiles. Thus, combining TLC/HPTLC with microscopic, physicochemical and molecular assays is advisable. Furthermore, labs must ensure proper validation of developing methods, standardisation of adsorbent layer, mobile phase, application volumes, derivatising reagents, and documentation—especially critical in regulatory and GMP contexts (Vyas *et al*., 2023).

Capacity building is another challenge: many Ayurvedic industry labs may lack automated HPTLC instrumentation, qualified operators, and validated reference standards. Cost, maintenance and training need to be addressed. In addition, global herbal trade demands methods that are internationally accepted—HPTLC's adoption by pharmacopoeias is promising but further harmonisation is needed (Quality control review, 2020).

Finally, future directions include hyphenation of HPTLC with MS or IR (HPTLC-MS), multi-dimensional TLC, chemometric analysis of fingerprint data (such as PCA and OPLS-DA), and development of digital fingerprint libraries for comparative authentication across regions. Such innovations will enhance analytical robustness while retaining cost-effectiveness.

Conclusion

The combined use of Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) offers a robust, efficient and cost-effective approach to the standardisation of herbal drugs and formulations. TLC remains valuable for quick screening and fingerprinting, while HPTLC elevates the process by improving precision, reproducibility, quantification and archivable digital records. In the Ayurvedic pharmacopeial context, these techniques fulfil the need for scientific validation of traditional drugs, support authenticity assessment, quality control and detect adulteration or substitutions. To maximise their benefit, these chromatographic methods should be integrated into a comprehensive analytical strategy that includes microscopic, physicochemical and molecular methods, supported by validated reference standards, operator training and regulatory alignment. With such integration, chromatographic analysis will continue to underpin the reliable use of herbal medicines in the global health system.

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