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HPLC-BASED STANDARDIZATION OF PANCHAKOLA DRUGS: A SYSTEMATIC REVIEW

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ABSTRACT

Panchakola is a classical Ayurvedic formulation comprising five pungent drugs—Pippali, Pippalimula, Chavya, Chitraka, and Shunthi—and is widely used for **Deepana, Pachana, and Amapachana**. Despite its extensive therapeutic applications, the quality and efficacy of Panchakola drugs are influenced by variations in raw material source, geographical origin, processing methods, and phytochemical composition. High-Performance Liquid Chromatography (HPLC) has emerged as a robust and reliable analytical technique for the qualitative and quantitative standardization of Ayurvedic drugs. The present systematic review compiles and compares HPLC-based studies on individual Panchakola constituents, summarizes the commonly employed phytochemical markers, evaluates analytical performance and reproducibility, and proposes indicative standard ranges based on published literature. The review highlights the significance of HPLC in quality control, authentication, adulteration detection, and chemical fingerprinting of Panchakola drugs while identifying current research gaps and future perspectives for pharmacopeial standardization.

KEYWORDS: Panchakola, High-Performance Liquid Chromatography (HPLC), Piperine, Plumbagin, 6-Gingerol, Standardization, Ayurveda.

1. INTRODUCTION

Ayurvedic medicine primarily emphasizes the use of polyherbal formulations, which are considered superior due to their synergistic therapeutic action, safety, and holistic approach toward disease management. Among such classical combinations, **Panchakola** is an important formulation frequently described in Ayurvedic literature.

It is composed of five pungent drugs, namely **Pippali (*Piper longum* Linn. fruit)**, **Pippalimula (*Piper longum* Linn. root)**, **Chavya (*Piper retrofractum* Vahl)**, **Chitraka (*Plumbago zeylanica* Linn.)**, and **Shunthi (*Zingiber officinale* Roscoe)**. These drugs are well known for their **Deepana, Pachana, and Agnivardhana** properties and are extensively used in the management of digestive disorders, metabolic diseases, and as preparatory agents in Panchakarma therapies.

Although Panchakola drugs are widely used and classically accepted, variations in their **quality and therapeutic efficacy** are frequently observed. Such variations are mainly attributed to differences in geographical source, cultivation and harvesting conditions, post-harvest processing, and phytochemical composition of the raw materials. These factors significantly influence the concentration of bioactive

constituents, which may ultimately affect safety, consistency, and clinical efficacy. Therefore, scientific evaluation and analytical standardization of Panchakola drugs have become essential to ensure reproducibility and quality assurance in Ayurvedic pharmaceutics.

In recent years, modern analytical techniques have played a pivotal role in the quality assessment of herbal medicines. Among various tools, chromatographic techniques are particularly valuable for the separation, identification, and quantification of phytoconstituents present in complex herbal matrices. **High-Performance Liquid Chromatography (HPLC)** has gained wide acceptance due to its high sensitivity, accuracy, reproducibility, and suitability for multi-component analysis.

Several studies have reported validated RP-HPLC methods for the quantitative estimation of **piperine**, the principal alkaloid present in the fruits and roots of *Piper longum*, establishing it as a reliable chemical marker for standardization purposes.[1] Similarly, HPLC analysis of *Piper retrofractum* (Chavya) has confirmed piperine as the major bioactive constituent and has demonstrated that HPLC provides more accurate results than UV spectrophotometry by minimizing interference from structurally related compounds.[2]

Plumbago zeylanica (Chitraka), categorized as *Upavisha* in Ayurveda, contains **plumbagin**, a bioactive naphthoquinone compound associated with both therapeutic and toxic effects. RP-HPLC and HPTLC studies have established plumbagin as the principal biomarker of *Plumbago zeylanica* roots.[3] Furthermore, HPLC-based evaluation of classical *Shodhana* procedures has shown a significant reduction in plumbagin content following *Nimajjana* in lime water, thereby providing scientific validation for traditional detoxification methods.[4]

In the case of *Zingiber officinale* (Shunthi), **6-gingerol** has been identified as the major pharmacologically active constituent. Several validated HPLC methods have been developed for its quantitative estimation, demonstrating good linearity, precision, and accuracy, and highlighting the importance of standardized analytical protocols for quality control of ginger-based drugs.[5,6]

Although individual Panchakola drugs have been extensively studied using HPLC, a **systematic and consolidated evaluation** of chromatographic conditions, marker compounds, and analytical trends across all Panchakola constituents is still limited. Hence, the present review aims to compile and critically assess reported HPLC methodologies employed for the phytochemical evaluation and standardization of Panchakola drugs, with special emphasis on marker compounds and their relevance to quality control in Ayurvedic pharmaceutics.

2. Principle and Working of HPLC

Principle and Working of High-Performance Liquid Chromatography (HPLC)

A. Basic Principle

High-Performance Liquid Chromatography (HPLC) operates on the principle of differential partitioning of components between a stationary phase and a mobile phase. In this technique, a liquid mobile phase is propelled at high pressure through a column packed with a stationary phase. The constituents of a sample mixture interact with the stationary phase to varying extents depending on their polarity, molecular size, and chemical affinity.

Due to these differential interactions, each component migrates through the column at a distinct rate and elutes at a characteristic retention time. This phenomenon enables efficient separation, identification, and quantification of individual compounds. In the context of Ayurvedic formulations, which comprise complex phytochemical mixtures, this principle allows effective resolution of bioactive markers such as piperine, plumbagin, gingerols, and shogaols, thereby establishing HPLC as a highly dependable analytical technique.[7]

B. Instrumentation and Working Mechanism

Major Components of an HPLC System

1. Solvent Reservoir

Stores the mobile phase, which may consist of methanol, acetonitrile, water, or their appropriate mixtures.

2. Pump

Delivers the mobile phase at a constant and controlled high pressure to maintain a uniform flow through the chromatographic column.

3. Injector / Autosampler

Introduces an accurately measured volume of the sample into the mobile phase stream.

4. Chromatographic Column

Contains the stationary phase, most commonly C18-bonded silica. Actual separation of constituents occurs within this column based on their interaction with the stationary phase.

5. Detector

Detects the eluted compounds and converts them into measurable electrical signals. UV–Visible detectors are widely used for herbal and Ayurvedic drug analysis.

6. Data Acquisition System

Processes detector signals and generates chromatograms, where peak area or peak height is directly proportional to the concentration of analytes[7].

C. Working Mechanism

1. The mobile phase is pumped from the solvent reservoir under high pressure.
2. The prepared sample is injected into the flowing mobile phase.
3. The sample mixture enters the chromatographic column.
4. Individual constituents interact differently with the stationary phase.
5. Compounds separate and elute at specific retention times.
6. The detector records each eluted compound as a signal.
7. A chromatogram is generated for qualitative and quantitative interpretation.

Flow Diagram of HPLC Operation

Solvent Reservoir
↓
Pump
↓
Injector
↓
Column
↓
Detector
↓
Data System
↓
Chromatogram

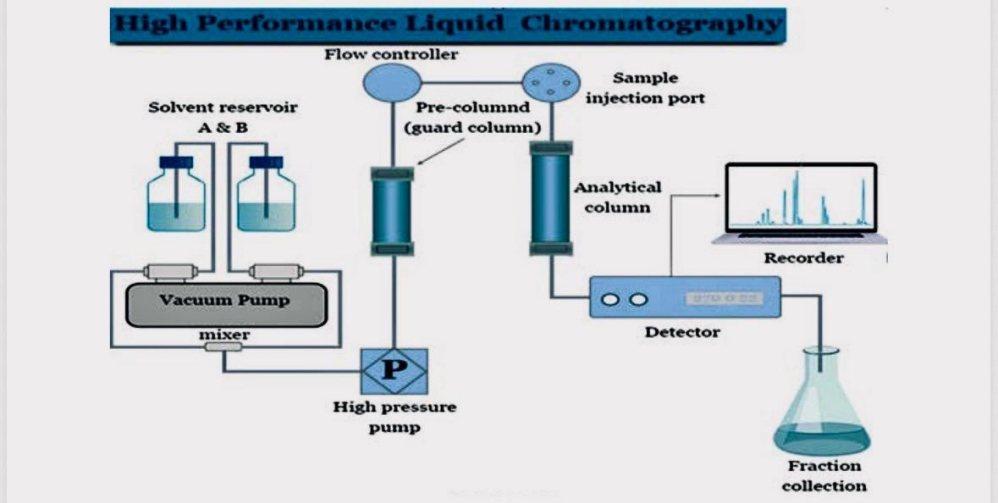

Figure 1: High performance liquid chromatography.[7]

4. Suitability of HPLC for Ayurvedic Samples

HPLC is highly appropriate for the analysis and standardization of Ayurvedic formulations as it:

- Enables accurate identification and quantification of marker compounds
- Ensures batch-to-batch uniformity
- Detects adulteration and substitution
- Supports pharmacopoeial and regulatory compliance
- Integrates traditional Ayurvedic concepts with modern analytical science

Therefore, HPLC is regarded as a gold-standard analytical technique for the quality control and standardization of various Ayurvedic dosage forms such as **churna, kwatha, avaleha, vati, ghrita**, and complex polyherbal formulations like **Panchakola.**[7]

5. Literature Review – HPLC-Based Standardization of Panchakola Drugs

Ayurvedic polyherbal formulations require robust analytical tools for quality control due to inherent variability in raw materials, geographical sources, and processing methods. Among modern analytical techniques, **High Performance Liquid Chromatography (HPLC)** has emerged as the most reliable and widely accepted method for the qualitative and quantitative evaluation of bioactive markers in Ayurvedic drugs. Panchakola, comprising five pungent drugs—Pippali, Pippalimula, Chavya, Chitraka, and Shunthi—has been extensively studied using HPLC to establish marker-based standardization. The available literature for each constituent drug is reviewed below.

A. Pippali (Piper longum Linn.)
Piperine is recognized as the principal bioactive marker of Pippali and is commonly employed for its HPLC-based standardization.

Santosh et al. developed a validated RP-HPLC method for the estimation of piperine in *Piper longum* fruit and root. The method demonstrated excellent linearity in the range of **0.005–0.1%** with a correlation coefficient (**r = 0.998**). The piperine content in Pippali fruit was reported as **0.879% w/w**, confirming piperine as a reliable quantitative marker.[1]

Kurangi and Jalalpure further reported a stability-indicating RP-HPLC method for piperine with a broader calibration range of **0.5–20 µg/mL** and an excellent regression coefficient (**R^2 > 0.999**). The method showed high sensitivity with LOD and LOQ values of **0.015 µg/mL** and **0.044 µg/mL**, respectively, supporting its suitability for formulation analysis.[8]

Uday C. Basak quantified piperine in *Piper longum* spikes using HPLC and observed significant variation depending on extraction technique and geographical source. Piperine content in Extraction–1 (methanol and ethanol extracts) was found to range from **1.93% to 5.29% (dry weight basis)** in female spikes. Piperine content in Extraction–2 (ethyl acetate:water and methanol:water extracts) ranged from **4.24% to 5.72% (dry weight basis)** in female spikes. indicating natural variability and the need for defining acceptable standard ranges.[9]

B. Pippalimula (Piper longum Linn., Root)
HPLC studies on Pippalimula also rely on piperine as the principal marker, though reported concentrations are lower than those in fruit.

Santosh et al. developed a validated RP-HPLC method for the estimation of piperine in *Piper longum* fruit and root.

The method demonstrated excellent linearity in the range of 0.005–0.1% with a correlation coefficient (r = 0.998). The piperine content in *Piper longum* root was reported as **0.31% w/w**, confirming piperine as a reliable quantitative marker for root standardization.[1]

Manisha Mohapatra and Uday Chand Basak conducted an HPLC-based comparative study on extraction efficiency from *Piper longum* roots. The piperine content varied between **0.156% and 0.49% (dry weight basis)** depending on the extraction method, highlighting processing-related variability.[10]

Santosh et al. also reported a recovery value of 99.4%, indicating high precision and accuracy of the RP-HPLC method for piperine estimation and supporting its application in quality control of Piper longum root samples.[1]

C. Chavya (Piper retrofractum Vahl)
Chavya, botanically identified as *Piper retrofractum*, is chemically characterized by piperine and related alkaloids. HPLC is routinely applied for its quality assessment.

Galih et al. developed and validated an HPLC method for piperine quantification in formulations containing *Piper retrofractum*. The calibration curve was linear over **1–125 µg/mL**, demonstrating good precision and accuracy suitable for phytopharmaceutical analysis.[11]

Ngamdokmai et al. quantified piperine in an anti-cellulite herbal compress formulation containing Java long pepper and reported a piperine concentration of 4.19 mg/g, supporting its use as a marker compound for quality control and standardization.[12]

Cahyono et al. compared UV spectrophotometric and HPLC methods for piperine estimation in *Piper retrofractum* extracts. HPLC analysis revealed piperine contents of **6.8% and 7.3%** in two extract samples, whereas UV spectrophotometry yielded higher values (18.1–19.0%) due to interference from other alkaloids, demonstrating the superior specificity of HPLC for piperine quantification.[2]

D. Chitraka (Plumbago zeylanica Linn.)
Plumbagin is the characteristic naphthoquinone used as the marker compound for Chitraka.

Pallavi et al. employed HPLC for the analytical evaluation of plumbagin in *Plumbago zeylanica* roots after Shodhana by Nimajjana in Sudha Jala. HPLC analysis was carried out at wavelengths of 206 nm and 265 nm, and plumbagin was detected in all four treated root samples. The study reported plumbagin content ranging from 0.29% to 2.50%, confirming the applicability of HPLC for quality assessment and standardization of Chitraka roots.[4]

Unnikrishnan et al. developed and validated an RP-HPLC method for the quantification of plumbagin in *Plumbago zeylanica* roots. The method exhibited linearity in the concentration range of 1–20 µg/mL with a correlation coefficient of 0.9943. HPLC analysis revealed a plumbagin content of 0.1601% in *P. zeylanica* roots, with a retention time of 4.21 min, confirming the suitability of plumbagin as a marker compound for quality control and standardization of Chitraka.[3]

The validated RP-HPLC method demonstrated good linearity, precision, and recovery, supporting the use of plumbagin as a reliable marker compound for the quality control and standardization of Chitraka roots.

E. Shunthi (Zingiber officinale Roscoe, Dry Ginger)
HPLC standardization of Shunthi primarily focuses on **6-gingerol** and **6-shogaol**.

Kajsongkram et al. developed and validated an HPLC method for the simultaneous estimation of 6-gingerol and 6-shogaol in ginger capsules. The method exhibited good linearity over concentration ranges of 20–60 µg/mL for 6-gingerol and 3–7 µg/mL for 6-shogaol, with correlation coefficients of 0.9993 and 0.9994, respectively. The retention times of 6-gingerol and 6-shogaol were 22.712 min and 35.879 min, confirming method specificity and suitability for quality control of ginger-based formulations.[13]

Cho et al. developed an HPLC method for the quantification of 6-gingerol in *Zingiber* species and commercial ginger products. The method exhibited excellent linearity over a concentration range of 0.016–1 mg/mL ($r^2 = 0.9999$) and was successfully applied to the analysis of raw materials as well as commercial food products, demonstrating its suitability for quality control purposes.[14]

Ngamdokmai et al. developed and validated an HPLC-QTOF-MS method for the quantitative determination of active compounds in an anti-cellulite herbal compress containing ginger. HPLC analysis revealed a 6-gingerol content of 0.76 mg/g in the finished formulation, highlighting the applicability of chromatographic techniques for the quality control and standardization of Shunthi-containing herbal preparations.[12]

6. Discussion And Interpretation

The reviewed literature clearly indicates that High-Performance Liquid Chromatography (HPLC) is a highly effective analytical technique for the standardization of Panchakola drugs. Due to its high sensitivity, precision, and reproducibility, HPLC provides a robust scientific basis for quality control of Ayurvedic medicines, which are otherwise prone to variability arising from differences in geographical source, plant part used, harvesting season, and processing methods.

HPLC-based marker quantification significantly strengthens quality control by enabling accurate estimation of principal bioactive constituents such as piperine in *Pippali*, *Pippalimula*, and *Chavya*; plumbagin in *Chitraka*; and 6-gingerol/6-shogaol in *Shunthi*.

Consistent linearity, recovery, and low detection limits reported across studies demonstrate that HPLC ensures batch-to-batch uniformity and reliable assessment of raw drugs, extracts, and formulations. This analytical precision complements traditional Ayurvedic quality concepts by providing objective chemical validation.

Another important interpretative outcome is the role of HPLC in detecting adulteration and substandard materials.

Compared to UV-spectrophotometric methods, HPLC offers superior specificity by separating marker compounds from structurally related interfering constituents. Variations in retention time, peak shape, or marker concentration effectively indicate substitution, improper processing, or degradation during storage. This is particularly relevant for Piper species and for *Chitraka*, where safety concerns necessitate strict control of plumbagin levels.

HPLC also offers scope for the development of chemical fingerprints, which represent the overall phytochemical profile of a drug. Such fingerprints are especially relevant for polyherbal formulations like Panchakola, where therapeutic efficacy is attributed to synergistic interactions. However, most existing studies remain limited to single-marker estimation, and comprehensive fingerprint-based approaches are still insufficiently explored.

Despite extensive use of HPLC, gaps persist in current research, including lack of standardized sample preparation protocols, limited inter-laboratory validation, and scarcity of formulation-level studies on Panchakola as a combined drug. Therefore, the establishment of harmonized analytical protocols, validated reference standards, and collaborative efforts with pharmacopeial bodies such as API, CCRAS, and the Ministry of AYUSH is essential for strengthening regulatory acceptance and ensuring consistent quality, safety, and global credibility of Panchakola formulations.

7. Research Gaps

Although HPLC has been widely applied for the standardization of individual Panchakola drugs, existing studies are largely limited to single-marker estimation and lack formulation-level evaluation. Variability in sample preparation and chromatographic conditions reduces inter-study comparability, while inter-laboratory validation is scarce. The absence of standardized protocols and pharmacopeial acceptance ranges highlights the need for coordinated, multi-marker HPLC approaches for Panchakola.

8. Need for Standard Protocols

The reviewed studies show wide variation in HPLC conditions, sample preparation, and reported marker ranges for Panchakola drugs, limiting reproducibility and inter-study comparison. Hence, standardized HPLC protocols with validated markers and acceptance limits, developed in collaboration with API, CCRAS, and the Ministry of AYUSH, are essential for uniform quality control and regulatory acceptance.

9. Future Prospects

Future research on Panchakola standardization should focus on the development of multi-marker and HPLC fingerprint-based approaches to better represent the polyherbal nature of the formulation. Standardization of Panchakola as a combined formulation, rather than only individual drugs, is required. Integration of advanced hyphenated techniques such as HPLC-DAD and LC-MS may provide deeper phytochemical insights.

Collaborative efforts with pharmacopeial bodies like API, CCRAS, and the Ministry of AYUSH will be crucial for establishing official standards, validated reference materials, and global acceptance of Panchakola formulations.

10. CONCLUSION

The present review highlights the significance of High-Performance Liquid Chromatography as a reliable and scientifically robust tool for the standardization of Panchakola drugs. HPLC-based marker analysis effectively ensures quality control, detects adulteration, and supports chemical characterization of individual constituents such as piperine, plumbagin, and gingerols.

Although substantial data are available for single drugs, the lack of standardized protocols and formulation-level studies limits broader application. Establishing harmonized HPLC methods, validated reference standards, and collaboration with pharmacopeial bodies is essential to ensure consistent quality, safety, and regulatory acceptance of Panchakola formulations in Ayurvedic pharmaceutics.

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