

Analytical Standardization of Haritaki (*Terminalia chebula* Retz.) Using Modern Chromatographic Techniques a Comprehensive Review

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Abstract—Haritaki (*Terminalia chebula* Retz.), belonging to the family Combretaceae, is one of the most important medicinal fruits described in Ayurveda and is extensively used either alone or as an ingredient of classical formulations such as Triphala and Abhayarishta. Its pharmacological and therapeutic relevance is largely associated with a complex phytochemical profile dominated by hydrolysable tannins, phenolic acids and related polyphenolic constituents. However, substantial variation in geographical origin, maturity, genotype, processing, extraction procedure and storage conditions can influence the qualitative and quantitative composition of Haritaki preparations. Consequently, reliable analytical standardization is essential for ensuring identity, purity, consistency, reproducibility and quality of raw material and finished herbal products. Modern chromatographic techniques have substantially improved the characterization and quality assessment of *T. chebula*. High-performance thin-layer chromatography (HPTLC) is useful for fingerprinting and routine marker estimation, whereas high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC/UHPLC) permit sensitive and selective simultaneous determination of characteristic compounds. Liquid chromatography coupled with mass spectrometry (LC-MS/MS and UHPLC-MS) further enables comprehensive metabolite profiling and structural characterization. Reported chromatographic investigations have identified gallic acid, chebulic acid, chebulagic acid, chebulinic acid, corilagin, ellagic acid, punicalagin, chebulanin and other hydrolysable tannins as important analytical constituents. A validated HPLC-PDA method has demonstrated simultaneous determination of gallic acid, corilagin, chebulagic acid, ellagic acid and chebulinic acid in *Terminalia* species, while more recent UPLC-PDA work has achieved rapid separation of major polyphenols in *T. chebula* fruit within approximately 10 minutes. LC-MS/MS methods have expanded the analytical panel to multiple phenolics and tannins simultaneously. This review critically discusses the phytochemical basis, marker selection,

chromatographic methodologies, method-validation requirements, fingerprinting approaches, advantages and limitations of modern chromatographic techniques, and future prospects for establishing an integrated analytical standardization framework for Haritaki.

Index Terms—Haritaki; *Terminalia chebula*; chromatographic standardization; HPTLC; HPLC; UPLC; LC-MS/MS; chebulagic acid; chebulinic acid; gallic acid; hydrolysable tannins.

I. INTRODUCTION

Medicinal plants constitute an important component of traditional healthcare systems and continue to contribute to contemporary herbal pharmaceutical development. Unlike synthetic drugs, medicinal plants contain complex mixtures of primary and secondary metabolites, and their chemical composition can vary according to species, variety, geographical origin, environmental conditions, maturity, harvesting season, processing and storage. Therefore, quality control of herbal drugs requires approaches capable of assessing both individual marker compounds and the overall chemical profile.¹

Haritaki, botanically identified as *Terminalia chebula* Retz., is an important medicinal plant of the family Combretaceae. The mature fruit is traditionally used in Ayurveda under the name Haritaki and is regarded as a versatile medicinal drug. It is also extensively used in other traditional systems, including Tibetan, Siddha, Unani and East Asian systems. Contemporary phytochemical investigations have demonstrated that the fruit contains a diverse group of tannins, phenolic acids, flavonoids, triterpenoids and other constituents. A recent comprehensive review reported approximately 149 identified compounds from *T.*

chebula, including tannins, phenolic acids, lignans, triterpenes, flavonoids and volatile constituents. 2

Among these constituents, hydrolysable tannins are particularly important from an analytical-standardization perspective. Gallic acid, chebulic acid, chebulagic acid, chebulinic acid, corilagin and ellagic acid are frequently investigated as characteristic compounds. Earlier chromatographic investigations demonstrated that numerous tannin-related constituents can be separated and quantified simultaneously. A reverse-phase HPLC study reported determination of 14 constituents in *T. chebula* fruit, including gallic acid, chebulic acid, punicalagin, corilagin, chebulanin, ellagic acid, chebulagic acid and chebulinic acid. 3

The increasing commercialization of *T. chebula*-based extracts and Ayurvedic formulations makes reliable analytical standardization particularly important. Conventional organoleptic and microscopic examination remains useful for authentication, but these methods alone cannot adequately establish chemical consistency. Chromatographic techniques therefore provide a critical bridge between classical pharmacognostic identification and modern quality-control requirements.

II. BOTANICAL AND PHARMACOGNOSTIC PROFILE 4

2.1 Botanical classification

Taxonomic category	Classification
Kingdom	Plantae
Division	Angiosperms
Class	Magnoliopsida
Order	Myrtales
Family	Combretaceae
Genus	Terminalia
Species	<i>Terminalia chebula</i> Retz.

The medicinally important material is primarily the mature fruit. The fruit is generally ovoid to obovoid, yellowish-green to brownish or blackish depending upon maturity and processing, with longitudinal ridges and a characteristic astringent taste.

2.2 Importance of botanical authentication 5

Authentication is the first stage of standardization. Misidentification, substitution with related *Terminalia*

species or use of inappropriate varieties can produce considerable variation in chemical composition. Chemical profiling of commercial *Fructus Chebulae* has demonstrated that different *Terminalia chebula* varieties may differ in their concentrations of tannin-related constituents.

Consequently, chromatographic standardization should ideally be performed after proper botanical authentication rather than being used as a substitute for taxonomic identification.

III. AYURVEDIC PERSPECTIVE OF HARITAKI 6

Haritaki occupies an important position in Ayurvedic materia medica and is traditionally described as having broad therapeutic applications. Classical Ayurvedic literature attributes multiple properties and therapeutic actions to Haritaki, particularly in disorders involving Vata, digestion, bowel function and systemic metabolic balance.

Its importance extends beyond single-drug use because it is a principal component of Triphala, which combines Haritaki, Bibhitaki and Amalaki. It is also incorporated into numerous polyherbal preparations. From an analytical perspective, however, traditional classification and modern chemical standardization address different aspects of quality. Ayurvedic identity, pharmacognostic characteristics and therapeutic attributes should therefore be integrated with chemical fingerprinting and quantitative marker analysis.

IV. PHYTOCHEMICAL PROFILE OF TERMINALIA CHEBULA 7

The phytochemical complexity of *T. chebula* is a major reason why a single-marker approach may be insufficient for comprehensive standardization.

4.1 Major phytochemical groups

The principal groups reported include:

- Hydrolysable tannins
- Phenolic acids
- Ellagitannins
- Gallotannins
- Flavonoids
- Triterpenoids

- Phenolic glycosides
- Other secondary metabolites

The fruit is particularly rich in tannin-related compounds. Reviews of *T. chebula* consistently identify tannins and phenolic compounds as major chemical classes.

4.2 Important analytical constituents 8

Constituent	Chemical group	Analytical significance
Gallic acid	Phenolic acid	Common quantitative marker
Chebolic acid	Phenolic/tannin-related compound	Characteristic constituent
Chebulagic acid	Hydrolysable tannin	Important characteristic marker
Chebulinic acid	Hydrolysable tannin	Important characteristic marker
Corilagin	Ellagitannin	Useful fingerprint/quantitative marker
Ellagic acid	Polyphenolic compound	Quality-control marker
Punicalagin	Hydrolysable tannin	Useful for comprehensive profiling
Chebulanin	Hydrolysable tannin	Useful in advanced profiling
Pentagalloyl glucose	Gallotannin	Chemotaxonomic/analytical relevance

A 2004 RP-HPLC investigation demonstrated the feasibility of simultaneously determining 14 tannin-related constituents from *T. chebula* fruit.

V. NEED FOR ANALYTICAL STANDARDIZATION

Standardization of Haritaki should address several quality attributes:

1. Botanical identity
2. Macroscopic and microscopic identity
3. Physicochemical characteristics
4. Extractive values
5. Moisture content
6. Ash values
7. Microbial quality

8. Contaminant assessment
9. Chromatographic fingerprint
10. Quantification of selected marker compounds
11. Batch-to-batch consistency
12. Stability of the chemical profile

Chromatography is particularly valuable because it can simultaneously provide qualitative and quantitative information.

A standardized extract should ideally demonstrate not only the presence of one marker but also an appropriate chromatographic pattern representing the characteristic phytochemical profile of the drug.

VI. HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY 9

6.1 Principle

HPTLC is an advanced form of planar chromatography that allows simultaneous analysis of multiple samples on a single plate. Following chromatographic development, separated constituents can be visualized under suitable wavelengths or after derivatization.

The major advantages of HPTLC include:

- Low solvent consumption
- Multiple-sample analysis
- Relatively low cost
- Visual fingerprint generation
- Suitable densitometric quantification
- Good applicability to routine herbal-drug quality control

6.2 Application in *T. chebula*

HPTLC has been applied to the estimation of gallic acid and ellagic acid in *T. chebula* extracts. One study evaluating extracts from different geographical locations used HPTLC to quantify these two markers and demonstrated measurable differences between extracts.

HPTLC has also been used for standardization of polyherbal preparations containing *Terminalia* species. A validated HP-TLC protocol quantified gallic acid and quercetin and demonstrated satisfactory analytical performance under the study conditions.

6.3 Role in routine quality control

HPTLC is especially useful when the objective is:

- Raw-drug authentication
- Detection of substitution
- Comparative fingerprinting
- Routine batch testing
- Screening of commercial formulations

However, because *T. chebula* contains numerous structurally related tannins, HPTLC alone may not provide the same quantitative resolution as HPLC or LC-MS/MS.

VII. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY 10

HPLC is currently one of the most important analytical techniques for *T. chebula* standardization.

7.1 RP-HPLC

Reverse-phase HPLC, particularly C18 stationary phases, is widely applicable to polar and moderately polar phenolic compounds.

An early analytical study developed RP-HPLC for determination of 14 constituents in *T. chebula* fruit, demonstrating the potential of HPLC for comprehensive tannin profiling.

7.2 HPLC-PDA

The combination of HPLC with photodiode-array detection provides chromatographic separation together with UV-visible spectral information.

Dhanani et al. developed a validated HPLC-PDA method for simultaneous determination of:

- Gallic acid
- Corilagin
- Chebulagic acid
- Ellagic acid
- Chebulinic acid

The method used an RP-18 column and gradient elution with acetonitrile and dilute trifluoroacetic acid-water. Reported calibration curves showed good linearity, with correlation coefficients above 0.992, while the reported detection and quantification limits demonstrated sensitivity in the low µg/mL range.

This type of multi-marker method is particularly valuable because it moves standardization beyond the traditional single-marker concept.

VIII. UPLC/UHPLC TECHNIQUES

Ultra-performance liquid chromatography provides improved chromatographic efficiency through smaller particle-size stationary phases and higher operating pressures.

A recent UPLC-PDA study developed a rapid method for simultaneous determination of:

- Gallic acid
- Corilagin
- Chebulagic acid
- Ellagic acid
- Chebulinic acid

in *T. chebula* fruit. Separation was achieved using a BEH Shield RP-C18 column within approximately 10 minutes, and the method was validated according to ICH Q2(R1) principles.

Advantages of UPLC

- Shorter analysis time
- Higher resolution
- Lower solvent consumption
- Improved peak capacity
- Greater suitability for high-throughput quality control

For industrial standardization, rapid UPLC methods may offer considerable advantages over longer conventional HPLC methods.

IX. LC-MS AND LC-MS/MS 11

Mass spectrometry substantially expands the analytical capacity of chromatographic techniques by providing molecular-mass information and structural clues.

UHPLC-MS studies have identified multiple constituents in *T. chebula*, including:

- Gallic acid
- Punicalagin
- Geraniin
- Chebulic acid
- Chebulagic acid
- Chebulinic acid

High-resolution mass spectrometry can provide molecular formula information and assist in confirmation of compounds that have similar chromatographic behavior.

9.1 UPLC-MS/MS quantitative analysis

UPLC-MS/MS has been used to simultaneously quantify a broad panel of *T. chebula* constituents. One study quantified nine compounds, including chebulic acid, gallic acid, protocatechuic acid, corilagin, chebulagic acid, chebulinic acid, ellagic acid, ethyl gallate and pentagalloyl glucose.

This approach is particularly useful for:

- Multi-marker standardization
- Pharmacokinetic investigations
- Metabolite profiling
- Extract characterization
- Stability studies
- Authentication of complex preparations

X. CHROMATOGRAPHIC FINGERPRINTING

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A major limitation of conventional single-marker standardization is that one compound may not adequately represent the entire phytochemical profile of a medicinal plant.

For Haritaki, chromatographic fingerprinting can provide a pattern of characteristic peaks corresponding to multiple constituents.

A fingerprint-based approach can therefore assess:

Identity → Fingerprint similarity → Marker quantification → Batch consistency

The fingerprint should preferably include characteristic peaks associated with gallic acid, chebulic acid, chebulagic acid, chebulinic acid, corilagin, ellagic acid and other major tannins.

Such a multi-component strategy is more consistent with the chemical complexity of herbal drugs.

XI. COMPARISON OF MODERN CHROMATOGRAPHIC TECHNIQUES

Technique	Major application	Advantages	Limitations
TLC	Preliminary identification	Simple, inexpensive	Limited quantification
HPTLC	Fingerprinting and marker estimation	Multiple samples, economical	Lower resolution than LC-MS

HPLC-UV	Quantification	Robust and widely available	Limited structural information
HPLC-PDA	Multi-marker analysis	Spectral confirmation + quantification	Relatively longer analysis
UPLC-PDA	Rapid multi-marker analysis	Fast, high resolution	Higher instrument cost
LC-MS	Qualitative profiling	Molecular-mass information	Expensive
LC-MS/MS	Targeted quantification	High sensitivity and selectivity	Complex method development
UHPLC-QTOF-MS	Comprehensive metabolomics	Accurate mass and broad profiling	High cost and advanced expertise
GC-MS	Volatile/derivatized compounds	Excellent mass spectral libraries	Less suitable for nonvolatile tannins

XII. MARKER SELECTION FOR HARITAKI

An effective standardization strategy should carefully select analytical markers.

12.1 Gallic acid

Gallic acid is a relatively simple phenolic acid and is frequently used as a marker in herbal products. Its chromatographic determination is comparatively straightforward and can be performed using both HPTLC and HPLC.

12.2 Chebulagic acid

Chebulagic acid is a characteristic hydrolysable tannin of *T. chebula* and is frequently employed as an important standardization marker.

12.3 Chebulinic acid

Chebulinic acid is another major hydrolysable tannin and is often measured alongside chebulagic acid. Commercial standardized extracts have used

combined chebulinic and chebulagic acid content as part of their quality specifications.

12.4 Corilagin

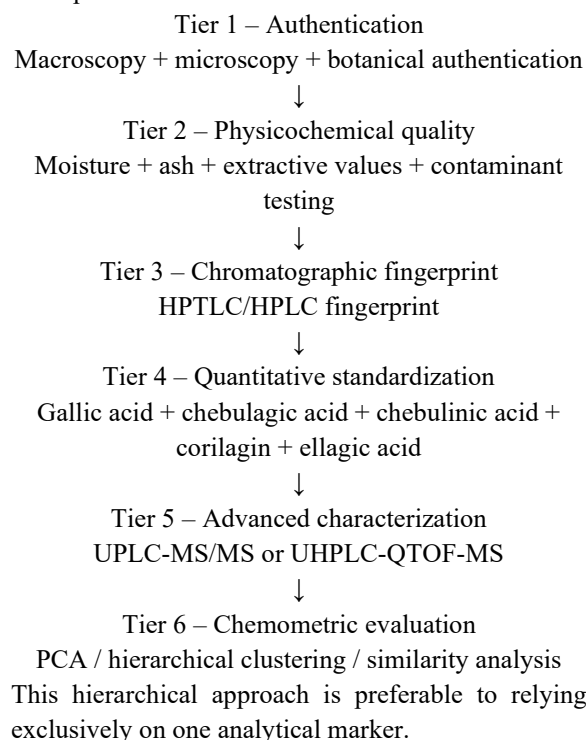
Corilagin provides an additional chemically distinct marker and can strengthen multi-marker standardization.

12.5 Ellagic acid

Ellagic acid is a hydrolysis-related polyphenolic constituent and has been successfully quantified by HPLC and HPTLC. A validated HPLC method has specifically addressed simultaneous determination of gallic acid and ellagic acid in *T. chebula* and other medicinal plants.

XIII. RECOMMENDED MULTI-MARKER STANDARDIZATION MODEL 14

Based on the available chromatographic literature, a practical analytical framework for Haritaki can be conceptualized as follows:



XIV. FACTORS AFFECTING CHROMATOGRAPHIC PROFILE

Several factors may affect the measured concentration of Haritaki constituents.

14.1 Geographical variation

Environmental conditions and geographical origin may influence secondary metabolite accumulation. Recent UPLC-PDA work examined samples from different regions of India and evaluated geographical variation in major polyphenols.

14.2 Maturity

The concentration and relative distribution of tannins and phenolic compounds may change during fruit maturation.

14.3 Extraction solvent

Water, ethanol, methanol and hydroalcoholic solvents may produce markedly different chemical profiles. Therefore, analytical results should always specify:

- Drug-to-solvent ratio
- Solvent composition
- Extraction temperature
- Extraction duration
- Number of extraction cycles
- Concentration method

14.4 Processing 15

Processing can substantially modify the chemical profile. Importantly, investigations of Ayurvedic fermentation demonstrated that chebulagic and chebulinic acids can undergo hydrolysis, with corresponding changes in chebulic acid, gallic acid and ellagic acid.

This has important implications for standardization of formulations containing Haritaki.

14.5 Storage

Polyphenolic compounds may undergo degradation, hydrolysis or oxidation during storage. Therefore, marker stability should be considered during method development and specification setting.

XV. ANALYTICAL METHOD VALIDATION

Chromatographic methods intended for quality control should be appropriately validated.

Important parameters include:

15.1 Specificity

The method should demonstrate that target compounds can be distinguished from other constituents and matrix components.

15.2 Linearity

Calibration should demonstrate an appropriate relationship between analyte concentration and analytical response.

15.3 Accuracy

Recovery studies should assess closeness of measured values to the accepted/reference value.

15.4 Precision

Both repeatability and intermediate precision should be assessed.

15.5 Limit of detection

LOD establishes the lowest concentration that can be reliably detected.

15.6 Limit of quantification

LOQ represents the lowest concentration that can be quantified with acceptable performance.

15.7 Robustness

The method should remain reliable following deliberate small changes in parameters such as mobile-phase composition, flow rate or column temperature.

15.8 System suitability

Appropriate parameters such as retention time, resolution, theoretical plates and peak symmetry should be monitored.

ICH Q2 provides a recognized framework for analytical-procedure validation, while the updated Q2(R2) guideline expands the framework to modern analytical procedures.

XVI. ROLE OF CHEMOMETRICS IN HARITAKI STANDARDIZATION 16

Modern chromatographic datasets contain considerably more information than individual peak concentrations.

Chemometric tools can extract information from complete chromatographic fingerprints.

Potential approaches include:

- Principal component analysis (PCA)
- Hierarchical cluster analysis
- Partial least-squares analysis
- Similarity analysis

- Multivariate classification
- Fingerprint correlation

These techniques can potentially differentiate:

- Different geographical origins
- Different Terminalia species
- Different varieties
- Adulterated samples
- Processed versus unprocessed material
- Different extraction methods

Thus, future standardization may shift from single-marker estimation toward fingerprint-plus-multivariate authentication.

XVII. CHALLENGES IN CHROMATOGRAPHIC STANDARDIZATION

Despite considerable advances, several problems remain.

17.1 Chemical complexity

Haritaki contains numerous structurally related hydrolysable tannins, making complete separation challenging.

17.2 Lack of universal marker specifications

Different studies use different marker combinations and extraction procedures. Therefore, direct comparison of reported concentrations is often difficult.

17.3 Reference-standard availability

High-purity reference standards for compounds such as chebulagic acid and chebulinic acid can be relatively expensive and may not be routinely available in all laboratories.

17.4 Processing-dependent variation

Raw Haritaki and processed Haritaki cannot necessarily be expected to have identical chromatographic profiles.

17.5 Geographic and botanical variation

Differences between varieties and collection locations can influence marker concentrations.

17.6 Matrix effects

Complex herbal matrices can affect detector response, particularly in LC-MS/MS.

XVIII. FUTURE PERSPECTIVES

The future of Haritaki standardization is likely to involve integration of conventional chromatography with advanced analytical technologies.

18.1 UHPLC-HRMS metabolomics

High-resolution mass spectrometry can characterize hundreds of chromatographic features and potentially provide a much more comprehensive chemical fingerprint.

18.2 Targeted LC-MS/MS

Targeted multiple-reaction-monitoring methods can provide highly sensitive quantification of selected biomarkers.

18.3 Chromatographic fingerprint databases

A large reference database containing authenticated Haritaki samples could establish chemical ranges according to:

- Geographic origin
- Variety
- Maturity
- Processing
- Storage period

18.4 Chemometric authentication

Combining chromatographic fingerprints with PCA and supervised machine-learning models may improve detection of adulteration and substitution.

18.5 Quality-by-design approach

Analytical method development can increasingly incorporate risk assessment and systematic optimization rather than relying exclusively on empirical trial-and-error.

XIX. PROPOSED STANDARDIZATION
PROTOCOL FOR HARITAKI

A practical laboratory protocol can be structured as follows:

Step 1: Authentication

Botanical identification and voucher specimen documentation.

Step 2: Sample preparation

Controlled drying, pulverization and storage.

Step 3: Extraction

A predefined extraction solvent and drug-to-solvent ratio.

Step 4: HPTLC screening

Generate a characteristic fingerprint and quantify selected accessible markers.

Step 5: HPLC/UPLC analysis

Quantify a multi-marker panel comprising:

Gallic acid + chebulagic acid + chebulinic acid + corilagin + ellagic acid

Step 6: LC-MS/MS confirmation

Use targeted LC-MS/MS where higher sensitivity and molecular confirmation are required.

Step 7: Fingerprint evaluation

Compare chromatograms against an authenticated reference batch.

Step 8: Validation

Evaluate specificity, linearity, accuracy, precision, LOD, LOQ and robustness.

Step 9: Stability study

Monitor marker concentrations and fingerprint changes during storage.

Step 10: Specification setting

Establish scientifically justified acceptance criteria based on multiple authenticated batches.

XX. DISCUSSION 17

The available literature demonstrates that chromatographic analysis has progressed from simple determination of individual phenolic compounds to comprehensive multi-marker characterization of *T. chebula*. Early RP-HPLC studies established the feasibility of simultaneously separating numerous tannin-related compounds. Subsequent validated HPLC-PDA methods focused on a smaller but analytically useful panel of gallic acid, corilagin, chebulagic acid, ellagic acid and chebulinic acid.

The emergence of UPLC has improved analytical throughput. A recent method achieved separation of five major polyphenols in approximately 10 minutes and applied ICH-based validation, illustrating the transition toward rapid routine quality-control procedures.

Mass-spectrometric techniques provide an additional level of chemical confirmation. UHPLC-MS and UPLC-MS/MS investigations have demonstrated that the chemical profile of Haritaki extends well beyond the commonly measured markers.

An important implication is that no single analytical technique should necessarily be regarded as sufficient for complete standardization. HPTLC is highly useful for routine fingerprinting, HPLC/UPLC is appropriate for quantitative marker analysis, and LC-MS/MS provides advanced qualitative and quantitative characterization.

Furthermore, processing must be considered. Evidence from Ayurvedic formulations indicates that fermentation can modify the concentrations and chemical forms of important T. chebula tannins. Therefore, the chromatographic specification of raw Haritaki should not automatically be applied to every Haritaki-containing formulation.

The most rational approach is consequently a multi-level standardization model combining botanical authentication, physicochemical evaluation, chromatographic fingerprinting and multi-marker quantification.

XXI. CONCLUSION

Haritaki (*Terminalia chebula* Retz.) is a chemically complex medicinal plant characterized predominantly by hydrolysable tannins and phenolic compounds. Modern chromatographic techniques have significantly improved the ability to authenticate, characterize and standardize this important Ayurvedic drug. HPTLC provides a practical and economical approach for fingerprinting and routine marker estimation. HPLC-PDA offers robust multi-marker quantification, while UPLC provides faster and more efficient separation. LC-MS/MS and high-resolution UHPLC-MS extend analytical capabilities to comprehensive metabolite profiling and highly sensitive targeted quantification. Among the currently investigated compounds, gallic acid, chebulagic acid, chebulinic acid, corilagin and ellagic acid represent a useful multi-marker panel for analytical characterization. However, marker concentrations can vary according to botanical material, geographical origin, maturity, extraction procedure, processing and storage. Therefore, future standardization of Haritaki should move from a single-marker model toward an integrated chromatographic fingerprint–multi-marker–mass spectrometric approach, supported by validated analytical procedures and chemometric analysis. Such a framework could improve raw-material authentication, batch-to-batch consistency,

detection of adulteration and quality control of Haritaki-based Ayurvedic formulations.

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