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Pharmaceutical Preparation, Physicochemical Standardization and HPTLC Quantification of Withaferin A in Ashwagandha Siddha Ghrita

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Abstract

Background: Ashwagandha (Withania somnifera Dunal) is a premier rejuvenating (Rasayana) herb in Ayurveda, traditionally processed into medicated ghee (Ghrita Kalpana) to extract and deliver both fat-soluble and water-soluble active compounds. While extensively used, there is a lack of standardized manufacturing and analytical protocols to ensure batch-to-batch consistency for this classical formulation.

Aims and Objectives: To establish a reproducible Standard Operating Procedure (SOP) for preparing Ashwagandha Siddha Ghrita, perform its physicochemical standardization across multiple batches, and quantify the bioactive marker Withaferin A using High-Performance Thin-Layer Chromatography (HPTLC).

Materials and Methods: Three batches of Ashwagandha Siddha Ghrita were prepared in a GMP-certified pharmacy following classical Sneha Kalpana principles from Bhaishajya Ratnavali. The formulation utilized Go-ghrita (1 kg) as the lipid base, Ashwagandha Kalka (250 g), Ashwagandha Kwatha (4 L), and Godugdha (4 L). Heating was maintained until traditional endpoint tests (Sneha Siddhi Lakshana) were achieved. Finished products were subjected to organoleptic, physicochemical, and HPTLC analysis (mobile phase: Ethyl acetate:Toluene:Acetic acid, 9:1.1:0.6 v/v; detection at 214 nm).

Results: The manufacturing process yielded a consistent, light yellowish-brown granular ghee with an average yield of 850 g. Physicochemical analysis showed high batch-to-batch reproducibility with the following mean values: specific gravity (1.055 ± 0.0017), saponification value (225 ± 1.00), iodine value (30 ± 1.00), acid value (1.48 ± 0.04), refractive index (1.4646 ± 0.0004), and zero moisture content. HPTLC analysis successfully identified Withaferin A at R_f 0.58, with a quantified concentration of 0.024% w/w in the finished formulation.

Conclusion: The developed SOP yields a highly uniform and stable product. Complete removal of moisture confirms the successful attainment of traditional heating endpoints and suggests long shelf-life. The established physicochemical parameters and Withaferin A marker concentration (0.024% w/w) provide a reliable quality control standard for industrial manufacturing and future research.

Keywords: Ashwagandha, Ghrita Kalpana, HPTLC, Standardization, Withaferin A.

INTRODUCTION

Ashwagandha (Withania somnifera Dunal) is one of the most extensively described medicinal plants in Ayurveda and is categorized under Balya, Brimhana, Rasayana, and Vajikarana drugs. Classical Ayurvedic literature recommends Ashwagandha in conditions associated with Vata predominance, tissue depletion, neuromuscular weakness, debility, emaciation, stress-related disorders, infertility, cognitive decline, and impaired vitality. Its multidimensional therapeutic utility has resulted in increasing contemporary scientific interest toward its adaptogenic, anti-inflammatory, neuroprotective, immunomodulatory, antioxidant, and anabolic properties [1,2].

Modern phytochemical investigations have demonstrated the presence of biologically active constituents such as withanolides, sitoindosides, alkaloids, steroidal lactones, flavonoids, and glycowithanolides in Ashwagandha. Databases such as IMPPAT and phytochemical repositories have identified compounds including withaferin A, withanolide A, withanoside IV, and withanoside V, which contribute significantly to its pharmacological activities including neuroprotection, immunomodulation, anti-stress action, and tissue restorative potential. These phytoconstituents possess varying degrees of lipophilicity, making lipid-based dosage forms especially relevant for optimized extraction and delivery.[3]

Among various Ayurvedic dosage forms, Ghrita Kalpana occupies a unique position due to its capacity to extract both lipid-soluble and water-soluble active principles when prepared according to classical Sneha Kalpana principles. Ghrita is regarded as Samskarasya Anuvartanat, implying its ability to imbibe and potentiate the pharmacodynamic attributes of drugs processed with it. Classical texts also describe Ghrita as Medhya, Balya, Vayasthapana, Agnivardhaka, and Vata-Pitta shamaka, making it particularly suitable for diseases requiring nourishment, rejuvenation, neurocognitive support, and tissue replenishment. Charaka Samhita describes Ghrita as one of the most superior lipid media owing to its ability to carry the properties of associated drugs without losing its intrinsic therapeutic qualities.[4]

The therapeutic rationale of Ashwagandha Siddha Ghrita lies in the synergistic combination of Ashwagandha, Go-ghrita and Godugdha, all of which are traditionally regarded as Brimhana and Rasayana substances and are indicated in conditions associated with tissue depletion, debility and impaired vitality. Such formulations may improve palatability, stability, and incorporation of lipophilic phytoconstituents in chronic degenerative and nutritional disorders. Contemporary pharmaceutical understanding supports the role of lipid matrices in improving solubilization and stability of lipophilic phytoconstituents.

Despite the extensive therapeutic use of Ashwagandha-based formulations, there remains limited pharmaceutico-analytical documentation regarding standardized preparation of Ashwagandha Siddha Ghrita with reproducible manufacturing methodology and analytical characterization. Standardization of classical formulations has become increasingly important in the present era to ensure batch-to-batch consistency, safety, efficacy, and regulatory acceptability. Establishment of a validated Standard Operating Procedure (SOP) based on classical Sneha Siddhi principles is therefore essential for ensuring pharmaceutical reproducibility and quality assurance.

Ayurved Classics provides detailed guidelines regarding Sneha Kalpana, including proportion of Kalka, Sneha, and Drava Dravya, stages of heating, and identification of Sneha Siddhi Lakshana. Scientific validation of these classical pharmaceutical principles through analytical evaluation may contribute toward strengthening the evidence base of Ayurvedic pharmaceutics.

Therefore, the present study was undertaken to develop a standardized SOP for preparation of Ashwagandha Siddha Ghrita based on classical references, followed by preparation of three batches under

controlled GMP conditions, physicochemical standardization of the finished formulation, and HPTLC-based quantification of Withaferin A as a marker compound.

MATERIALS AND METHODS

Study Design

The present pharmaceutico-analytical study was undertaken to develop a standardized Standard Operating Procedure (SOP) for preparation of Ashwagandha Siddha Ghrita based on classical Sneha Kalpana principles described in Bhaishajya Ratnavali.[5] The study included authentication and standardization of raw materials, pharmaceutical preparation of the formulation under GMP conditions, preparation of three batches using identical methodology, and analytical evaluation of the finished product using organoleptic and physicochemical parameters.

Study Site

The pharmaceutical preparation of Ashwagandha Siddha Ghrita was carried out in a GMP-certified Ayurvedic pharmacy. Authentication and analytical standardization of raw materials and finished product were performed at authorized analytical laboratory.

Procurement and Authentication of Raw Materials

Raw materials were procured from an authentic Ayurvedic raw drug supplier. The collected raw drugs were subjected to pharmacognostical and physicochemical evaluation at an authorized analytical laboratory to confirm identity, purity, and quality according to standards prescribed in the Ayurvedic Pharmacopoeia of India (API).

Analytical Standardization of Ashwagandha

The analytical evaluation of Ashwagandha confirmed compliance with API standards with acceptable organoleptic and physicochemical characteristics including moisture content, total ash, acid insoluble ash, and alcohol soluble extractive values. Microscopic examination revealed characteristic identifying features of the root drug.

Analytical Standardization of Go-ghrita & Godugdha

The Go-ghrita and Godugdha used for pharmaceutical processing complied with Ayurvedic Pharmacopoeial standards with acceptable organoleptic and physicochemical parameters.

Pharmaceutical Preparation

Preparation of Ashwagandha Kwatha

One kilogram of coarse Ashwagandha churna was added to sixteen litres of potable water in a stainless steel vessel. The mixture was heated at approximately 80-85°C with intermittent stirring. Heating was continued until the volume was reduced to one-fourth of the initial quantity, yielding approximately four litres of Kwatha. The prepared decoction was filtered through sterile muslin cloth

Preparation of Ashwagandha Siddha Ghrita

The preparation of Ashwagandha Siddha Ghrita was carried out according to the principles of Sneha Kalpana described in Bhaishajya Ratnavali.[5] The formulation was prepared using 1 kg of Go-ghrita as the lipid base, along with 250 g of Ashwagandha Kalka, 4 L of Ashwagandha Kwatha, and 4 L of Godugdha as processing media in accordance with classical Sneha Kalpana methodology.

Procedure

One kilogram of Go-ghrita was taken in a stainless steel vessel and melted under mild heat. To the melted Ghrita, 250 g of Ashwagandha Kalka and four litres of freshly prepared Ashwagandha Kwatha and

Godugdha (cow's milk) were added. The mixture was subjected to controlled heating with continuous stirring throughout the procedure to prevent settling and charring of Kalka at the base of the vessel. Heating was continued until attainment of classical Sneha Siddhi Lakshana. The final product was filtered while warm through sterile muslin cloth and allowed to cool. The prepared Ghrita was then stored in airtight amber-coloured containers.

Assessment of Sneha Siddhi Lakshana

Completion of Sneha Siddhi was assessed using classical parameters including:

- Absence of crackling sound
- Formation of wick (Varti) from Kalka
- Soft and non-sticky nature of Kalka
- Appearance of clear Ghrita
- Development of characteristic odour and colour

Batch Preparation

A total of three batches of Ashwagandha Siddha Ghrita were prepared using identical raw material proportions and pharmaceutical procedures under standardized GMP conditions.

SOP for Preparation of Ashwagandha Siddha Ghrita

1. Procurement of authentic raw materials
2. Authentication and analytical standardization of raw drugs
3. Preparation of Ashwagandha Kwatha using 1:16 water ratio reduced to one-fourth
4. Filtration of prepared Kwatha
5. Melting of Go-ghrita under mild heat
6. Addition of Ashwagandha Kalka, Kwatha and Godugdha
7. Controlled heating with continuous stirring
8. Observation of Sneha Siddhi Lakshana
9. Filtration of finished Ghrita
10. Cooling and storage in airtight containers
11. Analytical evaluation of finished product

Fig 1: a) Raw Ashwagandha b) Preparation of Ghrita c) Final product Ashwwagandha Ghrita

Analytical Evaluation of Finished Product

The finished Ashwagandha Siddha Ghrita was subjected to organoleptic and physicochemical analysis at an authorized analytical laboratory.

HPTLC Analysis

High Performance Thin Layer Chromatography (HPTLC) analysis of the finished Ashwagandha Siddha Ghrita was carried out at authorized analytical laboratory (Vasu Research Centre, Vadodara, Gujarat). Withaferin A was selected as the marker compound for qualitative and quantitative evaluation. Chromatographic separation was performed on silica gel 60 F254 HPTLC plates using Ethyl acetate:Toluene:Acetic acid (9:1.1:0.6 v/v) as the mobile phase. Detection and quantification were carried out at 214 nm and the chromatographic profile of the test sample was compared with the standard Withaferin A reference

Statistical Presentation

Analytical observations obtained from the three prepared batches were compiled and expressed as mean ± standard deviation to assess batch reproducibility and consistency of the pharmaceutical process.

RESULTS

Analytical Evaluation of Raw Ashwagandha

The raw Ashwagandha used for preparation of Ashwagandha Siddha Ghrita complied with standards prescribed in the Ayurvedic Pharmacopoeia of India. The sample was observed as a dry buff-coloured fine powder with characteristic odour and bitter acrid taste. Physicochemical evaluation revealed moisture content of 4.1%, total ash value of 4.34%, acid insoluble ash of 0.49%, and alcohol soluble extractive value of 18.72%, all of which were within the prescribed pharmacopoeial limits. Microscopic examination further confirmed the authenticity of the drug by demonstrating characteristic starch grains, tracheids, and parenchymatous cells.

Analytical Evaluation of Go-ghrita

The Go-ghrita used for pharmaceutical processing complied with Ayurvedic Pharmacopoeial standards and exhibited acceptable organoleptic and physicochemical characteristics. It was light yellow in colour with characteristic pleasant odour and taste. Analytical evaluation revealed specific gravity of 1.0199, Reichert Meissel value of 27, moisture content of 0.37%, saponification value of 219, iodine value of 33, and unsaponifiable matter of 1.14%, all of which were within the prescribed limits, confirming the suitability of the lipid base for preparation of Ashwagandha Siddha Ghrita.

Analytical evaluation of Godugdha

The Godugdha used for pharmaceutical processing complied with Ayurvedic Pharmacopoeial standards and exhibited acceptable organoleptic and physicochemical characteristics. Analytical evaluation revealed specific gravity of 1.028, lactometer reading of 33, fat content of 5.0%, pH of 6.6, total solid content of 13.45%, and ash value of 0.35%, indicating suitability of the milk for use as a Drava dravya during Sneha Kalpana.

Pharmaceutical Observations During Sneha Paka

Three batches of Ashwagandha Siddha Ghrita were prepared under standardized GMP conditions using identical pharmaceutical procedures. Progressive reduction in aqueous content and characteristic changes in colour, odour, and consistency were observed. During Sneha Paka, progressive frothing, reduction of aqueous content, gradual development of yellowish-brown colour, characteristic aroma, thickening of Kalka, disappearance of crackling sound, and successful Varti formation were observed, indicating attainment of classical Sneha Siddhi Lakshana.

The average yield obtained from the three batches was approximately 850 g.

Organoleptic Characteristics of Final Product

The finished Ashwagandha Siddha Ghrita was obtained as a granular semisolid preparation with light yellowish-brown colour, characteristic odour, and pleasant taste. These organoleptic characteristics remained consistent across all three batches.

Physicochemical Evaluation of Ashwagandha Siddha Ghrita

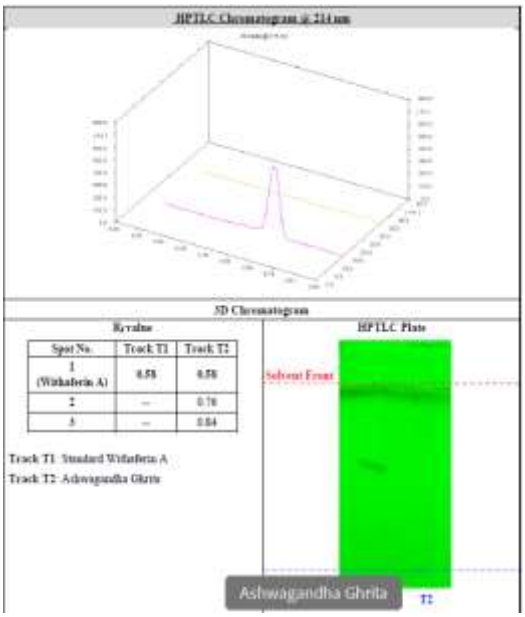
The finished formulation was subjected to physicochemical analysis and the observations obtained from three independently prepared batches were compiled as mean ± standard deviation.

Table 2: Batch-wise Physicochemical Evaluation of Ashwagandha Siddha Ghrita

Parameter	Batch I	Batch II	Batch III	Mean ± SD
Specific gravity	1.053	1.056	1.056	1.055 ± 0.0017
Saponification value	224	226	225	225 ± 1.00
Unsaponifiable matter (%)	1.08	1.11	1.12	1.10 ± 0.02
Iodine value	29	31	30	30 ± 1.00
Reichert Meissel value	28	29	30	29 ± 1.00
Moisture content	Nil	Nil	Nil	Nil
pH	6.2	6.3	6.4	6.3 ± 0.10
Refractive index	1.4642	1.4648	1.4649	1.4646 ± 0.0004
Acid value (mg KOH/g)	1.44	1.49	1.51	1.48 ± 0.04

HPTLC analysis of Ashwagandha Ghrita

HPTLC analysis of the finished Ashwagandha Siddha Ghrita demonstrated the presence of Withaferin A, a characteristic marker constituent of Ashwagandha. The chromatographic profile of the test sample showed a peak corresponding to the reference standard at an Rf value of 0.58. Quantitative estimation revealed a Withaferin A content of 0.024% w/w in the finished formulation, confirming retention of the marker phytoconstituent following Sneha Kalpana processing.



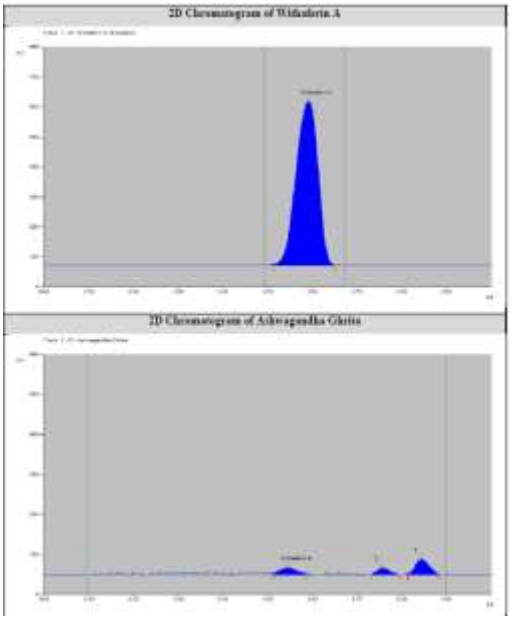


Fig. 2 a) HPTLC Chromatogram @214 nm b) 2D chromatograms of Withaferin A & Ashwagandha Ghrita

DISCUSSION

The present study successfully established a standardized Standard Operating Procedure (SOP) for the preparation of Ashwagandha Siddha Ghrita based on the classical principles of Sneha Kalpana described in Bhaishajya Ratnavali. Preparation of classical Ayurvedic formulations under standardized pharmaceutical conditions is essential to ensure batch-to-batch consistency, reproducibility, quality assurance, and wider scientific acceptability. The low inter-batch variation observed in the present study demonstrates that adherence to a standardized manufacturing process can produce a formulation with consistent physicochemical characteristics, supporting the feasibility of integrating traditional pharmaceutical principles with contemporary quality control measures.

Ashwagandha (Withania somnifera Dunal) is one of the most important Rasayana drugs in Ayurveda and is traditionally indicated in conditions associated with Dhatu Kshaya, neuromuscular weakness, chronic fatigue, stress, infertility, and degenerative disorders. Its therapeutic potential has been extensively validated through modern research, with documented adaptogenic, antioxidant, anti-inflammatory, immunomodulatory, anxiolytic, and neuroprotective activities attributed primarily to withanolides, steroidal alkaloids, and related phytoconstituents [2]. The IMPPAT database further identifies several biologically active constituents, including Withaferin A, Withanolide A, and Withanosides, which contribute to the pharmacological profile of the drug [6].

Selection of Ghrita as the dosage form is pharmaceutically justified because lipid-based formulations facilitate the extraction, incorporation, and stabilization of lipophilic phytoconstituents while also permitting transfer of water-soluble constituents during Sneha Paka. Classical Ayurvedic literature describes Ghrita as Yogavahi, capable of assimilating and potentiating the properties of incorporated drugs, making it an appropriate vehicle for Rasayana formulations. Similarly, inclusion of Godugdha serves both classical and pharmaceutical purposes. Beyond its traditional description as Brimhana, Balya, and Rasayana, milk provides proteins, phospholipids, and lipid components that may enhance dispersion of phytoconstituents within the lipid matrix during processing, thereby contributing to the overall pharmaceutical characteristics of the formulation.

The pharmaceutical process consistently demonstrated classical Sneha Siddhi Lakshana, including disappearance of crackling sound, formation of a soft non-sticky Kalka, successful Varti formation, characteristic aroma, and development of a clear medicated Ghrita. These observations indicate progressive elimination of aqueous content and completion of Sneha Paka, while the average yield of approximately 850 g across all three batches further reflects reproducibility of the standardized manufacturing procedure.

The quality of any medicated formulation depends largely on the quality of its raw materials. Analytical evaluation confirmed that Ashwagandha, Go-ghrita, and Godugdha complied with the standards prescribed in the Ayurvedic Pharmacopoeia of India. The low moisture content, acceptable ash values, and satisfactory alcohol-soluble extractive value of Ashwagandha indicated good-quality raw material with minimal contamination and adequate phytochemical content [7]. Likewise, the analytical profile of Go-ghrita, including Reichert-Meissel value, moisture content, saponification value, and iodine value, confirmed its suitability as a pharmaceutical base for medicated Ghrita preparations [8]. Establishing compliance of all starting materials is particularly important because variability in raw ingredients directly influences the quality, reproducibility, and stability of classical formulations.

The finished Ashwagandha Siddha Ghrita exhibited characteristic organoleptic properties, including a light yellowish-brown colour, pleasant odour, and semisolid granular consistency, which remained consistent across all batches. The colour transition observed during processing is likely attributable to the transfer of herbal pigments and phytoconstituents into the lipid phase, reflecting successful incorporation of plant constituents during Sneha Paka.

Physicochemical evaluation further supported the quality and reproducibility of the developed formulation. The slight increase in specific gravity and saponification value following pharmaceutical processing suggests incorporation of herbal constituents into the lipid matrix and minor alterations in fatty acid composition associated with Sneha Paka [4]. Similarly, the marginal reduction in iodine value from raw Go-ghrita to the finished formulation remained within acceptable limits and did not indicate significant oxidative deterioration, while maintenance of the Reichert-Meissel value and unsaponifiable matter demonstrated preservation of characteristic lipid components despite prolonged heating [4].

Among the physicochemical findings, the absence of detectable moisture in the finished formulation is particularly noteworthy. Residual moisture is a major contributor to hydrolytic degradation, microbial proliferation, and reduced shelf stability in lipid-based formulations [9]. Complete removal of moisture therefore not only supports attainment of classical Sneha Siddhi but also indicates improved pharmaceutical stability. Likewise, the low acid value suggests minimal hydrolysis of triglycerides during processing, while the highly consistent refractive index across all three batches reflects uniform composition of the lipid matrix and further substantiates the reproducibility of the standardized SOP [4,10].

HPTLC analysis further strengthened the pharmaceutical standardization of Ashwagandha Siddha Ghrita by confirming the presence of Withaferin A at an Rf value corresponding to the reference standard (Rf 0.58), with a quantified content of 0.024% w/w. Withaferin A is one of the principal bioactive withanolides of Ashwagandha and has been extensively investigated for its anti-inflammatory, antioxidant, immunomodulatory, neuroprotective, adaptogenic, and anticancer properties [2,6]. Detection of this marker compound following completion of Sneha Kalpana indicates that the pharmaceutical process successfully retained an important therapeutically relevant phytoconstituent within the lipid matrix. Beyond confirming formulation authenticity, HPTLC fingerprinting provides a reproducible quality

control tool for batch validation and supports the use of marker-based standardization in classical Ayurvedic formulations.

An important observation in the present study was the remarkably low standard deviation across all physicochemical parameters evaluated in the three independently prepared batches. The consistency observed for specific gravity, refractive index, saponification value, iodine value, Reichert-Meissel value, acid value, and unsaponifiable matter demonstrates the reproducibility of the developed SOP under standardized GMP conditions. Collectively, these findings indicate that the classical principles of Sneha Kalpana, when executed under controlled pharmaceutical conditions and complemented by modern analytical evaluation, can consistently yield a medicated Ghrita of uniform quality suitable for quality assurance and future pharmaceutical standardization.

The present investigation was primarily designed as a pharmaceutico-analytical standardization study and therefore focused on establishing a reproducible manufacturing process and analytical quality profile of Ashwagandha Siddha Ghrita. Although HPTLC-based quantification of Withaferin A provided an important marker for quality control, comprehensive phytochemical characterization using advanced analytical techniques such as LC-MS/MS, GC-MS, or metabolomic profiling was beyond the scope of the present work. Similarly, accelerated stability studies, microbial quality assessment, and evaluation of additional bioactive marker compounds were not undertaken. Incorporation of these parameters in future investigations would provide a more comprehensive pharmaceutical characterization of the formulation and strengthen its evidence base for regulatory standardization.

Further research should also explore detailed chromatographic fingerprinting, long-term stability, and correlation of phytochemical profiles with biological activity. Experimental pharmacological studies and well-designed clinical trials evaluating Ashwagandha Siddha Ghrita in stress-related disorders, neurodegenerative conditions, and other indications requiring Brimhana and Rasayana therapy would further establish its therapeutic utility. Integrating robust pharmaceutical standardization with pharmacological and clinical evidence will facilitate the wider scientific acceptance of this classical formulation and contribute toward the development of evidence-based quality standards for Ayurvedic medicated Ghrita preparations.

CONCLUSION

The present study successfully established a standardized SOP for preparation of Ashwagandha Siddha Ghrita according to classical Sneha Kalpana principles described in Bhaishajya Ratnavali. The formulation demonstrated reproducible physicochemical characteristics across three batches and was further standardized through HPTLC quantification of Withaferin A (0.024% w/w), providing a chromatographic marker for future quality control and batch validation.

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