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Standardization and Stability Studies of Ayurvedic Formulation—Hingvastika Churna as per ICH Guidelines †

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Abstract

Ayurveda has earned global attention, but it lacks the standardized guidelines for formulation, quality assurance, and efficacy assessment limits its scientific validation. This paper focuses on the standardization of Hingvastika Churna using HPTLC, with piperine and ferulic acid as marker compounds, following ICH Q1A (R2) and Q2 (R1) guidelines. In-house and marketed samples were compared, and both markers were successfully identified (R_f: 0.52 for piperine, 0.35 for ferulic acid). Method validation and forced degradation studies revealed significant instability under acidic conditions, with piperine showing 39.68% and ferulic acid 62.21% degradation. Moderate degradation was observed under oxidative and basic stress. These findings highlight the importance of scientific standardization in ensuring the quality and stability of Ayurvedic formulations.

Keywords: Ayurveda; Hingvastika Churna; standardization; HPTLC; piperine; ferulic acid; ICH guidelines; force degradation; stability profiling; polyherbal formulation

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1. Introduction

Ayurveda is once again gaining recognition for its holistic approach to health, and its demand is increasing daily as 80% of developing countries rely on traditional medicine [1]. However, unlike modern allopathic medicines, Ayurvedic formulations still lack clear guidelines for standardization, quality control, safety, and efficacy [2,3]. Hingvastika Churna, composed of *Piper nigrum*, *Piper longum*, *Ferula asafoetida*, and *Zingiber officinale Roscoeis* [4] well known for its numerous therapeutic benefits, such as relieving indigestion, gas, and bloating. With carminative herbs like hing, it expels gas from the digestive tract, alleviating discomfort [5]. Its antiemetic qualities, attributed to dry ginger, make it useful for managing nausea and vomiting [6]. This polyherbal formulation requires proper scientific validation to confirm its effectiveness and stability. This study focuses on standardizing Hingvastika Churna using HPTLC and evaluating its stability through forced degradation studies, with piperine and ferulic acid selected as marker compounds, in line with ICH guidelines [7,8]. Piperine is recognized as the principal bioactive phytoconstituent of *Piper nigrum* (black pepper) [9], responsible for its characteristic pungency [10,11] and diverse pharmacological activities like antibacterial [12], antioxidant [13], and anti-inflammatory [14,15]. Similarly, ferulic acid is the major phenolic compound found in *Ferula asafoetida* (asafoetida), contributing to its antioxidant and therapeutic properties

[16]. By comparing in-house and marketed formulations, the research aims to provide valuable insights into their quality, stability, and concentration profiles.

2. Method

2.1. Standard Solutions Preparation

- In a 100 mL volumetric flask, 50 mL of methanol was added, 100 mg of piperine and ferulic acid reference standards were weighed precisely, and sonicated to dissolve the contents fully. Methanol was then added to get the level up to 100 mL. The standard's final concentration was 1 mg/mL. (1000 ppm)
- **Dilution of Piperine & Ferulic Acid Stock Solutions**: The standard stock solution of 1000 ppm was further diluted to make standard to different concentrations ranging from 100 ppm to 700 ppm.

2.2. Mobile Phase Optimization

After reviewing various mobile phases for ferulic acid and piperine in TLC [17,18], a combination of Toluene, ethyl acetate, formic acid, and methanol (6.5:3.5:1:0.4) was selected to prepare the mobile phase for ferulic acid and piperine.

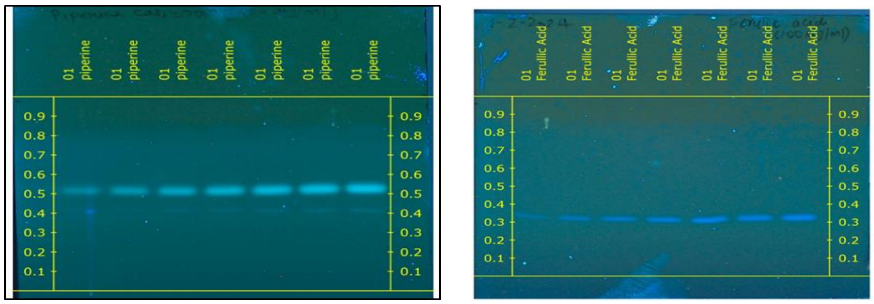

Figure 1. TLC plate showing the separated bands of piperine and Ferulic acid at R_f value of 0.52 and 0.35, respectively by the developed mobile phase.

2.3. Extraction of Ingredients

- Weighed accurately 25 g of *Piper longum, Piper nigrum* and Asafoetida and in-house Hingvastika churna, and marketed Hingvastika churna. They were immersed in 250 mL of methanol for 7 days using cold maceration for the extraction of piperine and ferulic acid, with intermittent shaking every few hours.
- After maceration, individual ingredients and churna were filtered by Whatman filter paper 1 to get the desired by biomarkers.
- A rotary evaporator was used to dry the filtrates, and any leftover solvent was then evaporated in a water bath to leave a solid mass.

2.4. Chromatographic Conditions

- **Sample preparation**: Standard stock solutions of piperine and ferulic acid (1000 ppm) were serially diluted with methanol to obtain working standard solutions of concentration ranging from 100 to 700 ppm.
- Using a solvent system of Toluene, ethyl acetate, formic acid, and methanol (6.5:3.5:1:0.4), silica gel 60 F254 pre-coated plates (20 × 10 cm) were employed. Thin layer chromatography was developed using ascending mode with a 5 mm applied bandwidth on the plate. TLC Plate was developed for up to 70 mm.
- Photographs of plate were taken at 254 nm and 366 nm for visualization.

3. Validation of the Developed Plate

- The developed TLC plate was then validated for piperine and ferulic acid in accordance with ICH Q2 (R1).
- Both analytes exhibited high specificity, confirming that the method can accurately distinguish each compound without interference from excipients or other components, with Rf values for piperine and ferulic acid at 0.52 and 0.35, respectively.
- The straight-line equations were found to be y = 0.14x − 0.0037 for piperine and y = 0.21x + 0.0149 for ferulic acid, with correlation coefficients (R^2) of 0.9921 and 0.9992, respectively, indicating excellent linearity within the concentration range of 100–700 ppm.
- Both analytes passed precision and repeatability parameters with %RSD of 0.13% and 0.33% respectively.
- The robustness of the method, assessed by minor variations in analytical conditions like change in saturation time and mobile phase ratio, showed %RSD values of 0.38% for piperine and 0.61% for feulic acid, confirming method reliability.
- Limit of detection for ferulic acid and piperine were found to be 6.85 ppm and 8.06 ppm, respectively, and limit of quantification was found to be 20.78 ppm and 24.42 ppm for ferulic acid and piperine, respectively.

4. Mass Spectra of Piperine and Black Pepper Extract

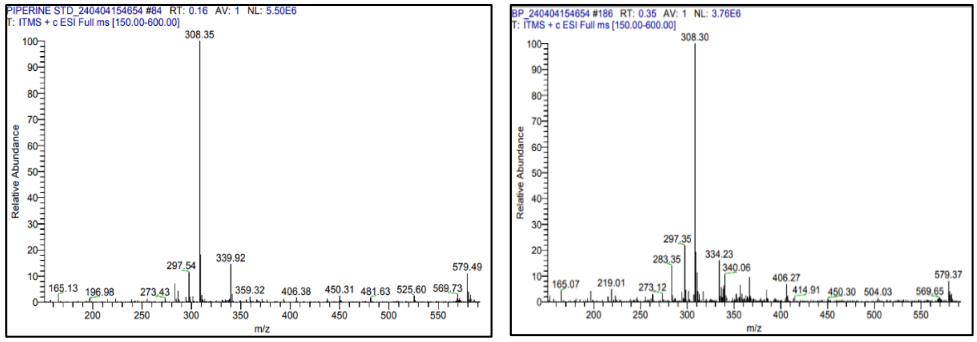

Figure 2. Standard solution of piperine and hing extract was analyzed by HPTLC-MS, both presented similar profiles, both of which had a molecular ion peak at m/z 308 [$M^- + Na^+$], authenticating the presence of piperine in the black pepper extract.

5. Mass Spectra of Ferulic Acid and Hing Extract

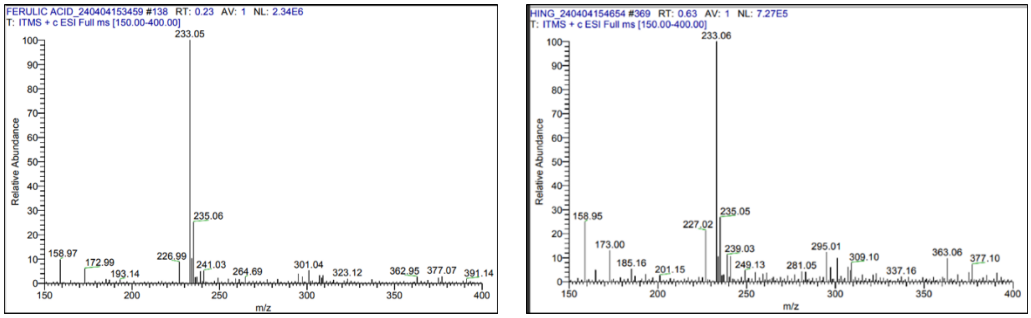

Figure 3. Standard solution of ferulic acid and hing extract was analyzed by HPTLC-MS, both presented similar profiles both of which had a molecular ion peak of 233 [$M^- + K^+$]. Authenticating the presence ferulic acid in Hing extract.

6. Degradation Studies

6.1. Acidic Degradation

For acidic degradation, the stock solution of standard references was prepared by dissolving 1 mg of piperine and FA in 1 mL of 0.1 N HCl. Then 0.1 mL was taken from stock solution and volume was made up to 1 mL by methanol.

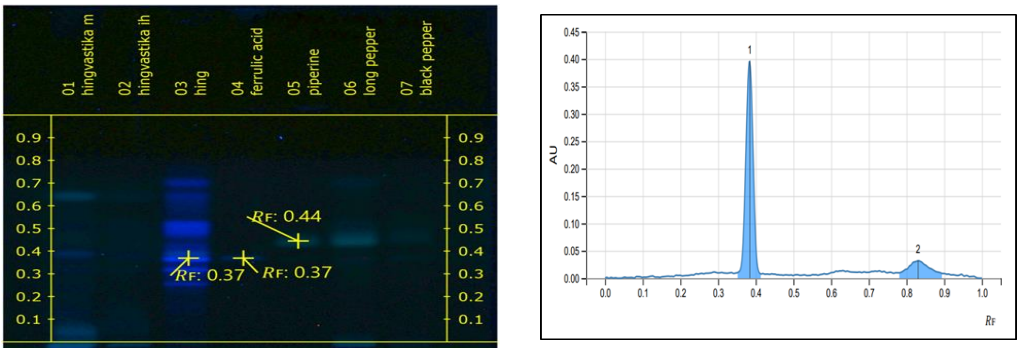

Figure 4. HPTLC plate of acidic stressed HM, HIH, Hing, ferulic acid, piperine and BP.

Sample was prepared by dissolving 10 mg of dried extract in 1 mL of 0.1 N HCl. Further 0.1 mL of this solution was diluted to 1 mL by methanol.

6.2. Basic Degradation

For basic degradation, the stock solution of standard references was prepared by dissolving 1 mg of piperine and FA in 1 mL of 0.1 N NaOH. Then 0.1 mL was taken from stock solution and volume was made up to 1 mL by methanol.

Sample was prepared by dissolving 10 mg of dried extract in 1 mL of 0.1 N NaOH. Further 0.1 mL of this solution was diluted to 1 mL by methanol.

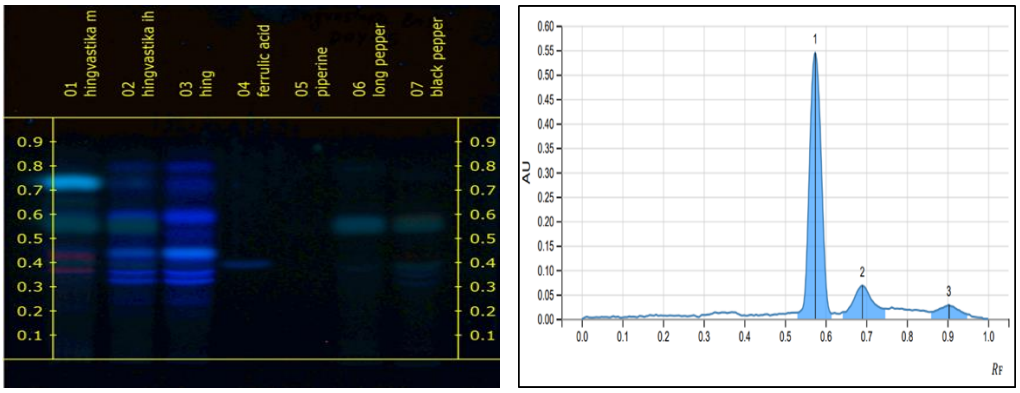

Figure 5. HPTLC plate of basic stressed HM, HIH, Hing, ferulic acid, piperine and BP.

6.3. Oxidative Degradation

For oxidative degradation, the stock solution of standard references was prepared by dissolving 1 mg of piperine and FA in 1 mL of 3% H2O2. Then 0.1 mL was taken from the stock solution, and the volume was made up to 1 mL by methanol.

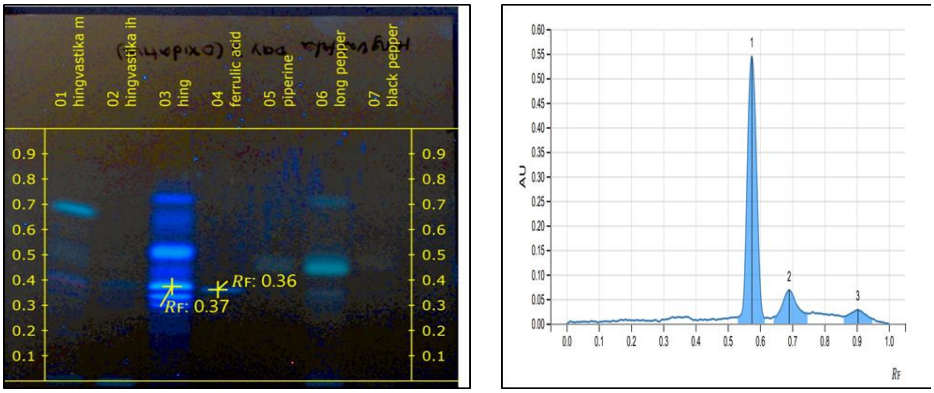

Figure 6. HPTLC plate of oxidative stressed HM, HIH, Hing, ferulic acid, piperine and BP.

Sample was prepared by dissolving 10 mg of dried extract in 1 mL of 3% H_2O_2. Further 0.1 mL of this solution was diluted to 1 mL by methanol.

Table 2. Percent degradation of piperine in acid, basic and oxidative condition.

Piperine % Degradation			
	Acid	**Basic**	**Oxidative**
Hingvastika marketed	41.04	33.91	32.01
Hingvastika In-house	39.68	29.01	30.78
Black Pepper	34.50	35.57	41.03
Long Pepper	41.20	37.36	40.22
Piperine	38.16	41.27	35.18

Table 3. Percent degradation of ferulic acid in acid, basic and oxidative condition.

Piperine % Degradation			
	Acid	**Basic**	**Oxidative**
Hingvastika marketed	41.04	33.91	32.01
Hingvastika In-house	39.68	29.01	30.78
Black Pepper	34.50	35.57	41.03
Long Pepper	41.20	37.36	40.22
Piperine	38.16	41.27	35.18
Ferulic acid % Degradation			
	Acid	**Basic**	**Oxidative**
Hingvastika marketed	76.82	50.11	40.97
Hingvastika In-house	62.21	33.64	36.42
Hing	41.86	36.70	39.64
Ferulic acid	62.95	36.52	39.28

7. Result and Discussion

In the forced degradation investigations, ferulic acid and piperine exhibited substantial degradation under acidic, basic, and oxidative environments.

For piperine, the percentage degradation in acidic conditions was 41.04% and 39.68% for Hingvastika marketed and Hingvastika In-house respectively, while under basic conditions, it was 33.91% and 29.01%. In oxidative conditions, it was 32.01% and 30.78% degradation of piperine in Hingvastika marketed and Hingvastika In-house respectively. These results indicate that piperine is most susceptible to acidic stress, showing the highest degradation in these conditions.

Similarly, for ferulic, the degradation in acidic conditions was 76.82% and 62.21% for Hingvastika marketed and Hingvastika In-house respectively, while under basic conditions, it was 50.11% and 33.64%. In oxidative conditions, it was 40.97% and 36.42% degradation of ferulic acid in Hingvastika marketed and Hingvastika In-house respectively. The above results show that ferulic acid is most vulnerable to acidic stress.

Overall, both substances showed increased vulnerability to acidic degradation, with piperine exhibiting the highest level of instability. Because different components may degrade differently under environmental stress, these findings highlight the importance of carefully considering stability aspects during formulation development, especially for polyherbal formulations like Hingvastika Churna.

8. Conclusions

Studies on forced degradation provide important information about how stable Ayurvedic formulations, such as Hingvastika Churna, are under different circumstances. Over time, environmental elements such as pH, moisture content, and temperature might have an impact on the stability and medicinal effectiveness of herbal products. The chemical complexity of herbal formulations makes stability testing difficult, but with the right guidelines and contemporary methods like HPTLC, trustworthy stability data can be produced. Because existing standards are inadequate, this study emphasizes the necessity for clear regulatory requirements for the stability testing of herbal and Ayurvedic medications.

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