chemistry proceedings

MDPI

Proceeding Paper

Standardization and Stability Study of Ayurvedic Formulation—Triphala Churna as Per the ICH Guidelines †

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† Presented at the 27th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-27), 15–30 November 2023; Available online: https://ecsoc-27.sciforum.net/.

Abstract: The research focused on standardizing Ayurvedic formulations, specifically targeting Triphala churna, a complex multi-ingredient traditional medicine. In the context of increasing interest in Ayurveda's potential medical benefits, this study addresses the critical need for comprehensive guidelines as to those in allopathic medicine for formulating, ensuring quality, establishing safety profiles, and assessing efficacy in Ayurvedic preparations. The primary objective is to standardize Triphala churna using High-Performance Thin-Layer Chromatography (HPTLC) and to evaluate the formulation's stability and quality, Forced Degradation studies were performed according to ICH guideline Q1A(R2) and Q2 (R1), focusing on two marker compounds, Gallic acid, and ascorbic acid. There was a comparative analysis between an in-house Triphala churna preparation and a commercially available product to determine the concentration of these marker compounds. The study reveals specific wavelengths for marker compounds and demonstrates a decrease in their concentrations after forced degradation. The significance of standardizing Ayurvedic formulations to ensure their quality, efficacy, and safety. It also highlights the successful development of a validated method to assess the stability profile of Triphala churna. The mobile phase Toluene: Ethyl acetate: Formic acid (6:3:1) was selected for HPTLC due to its efficient separation capabilities. The research indicates that Gallic acid remains stable under acidic and oxidative conditions but not under alkali hydrolysis, in accordance with ICH guidelines. Ascorbic acid's stability under basic and oxidative conditions warrants further investigation. Overall, this study makes a valuable contribution to advancing the standardization of Ayurvedic formulations, with promising results regarding Triphala churna's stability under various stress conditions, including ICH-compliant testing methodologies.

Keywords: stability; marker; efficacy; standardization

Citation: Sinha, A.; Arti; Ghosh, M. Standardization and Stability Study of Ayurvedic Formulation—Triphala Churna as Per the ICH Guidelines. *Chem. Proc.* **2023**, *14*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: 15 November 2023

1. Introduction

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) is an organization that develops and promotes harmonized guidelines for the development, registration, and post-approval of pharmaceutical products worldwide. These guidelines are intended to ensure the safety, efficacy, and quality of pharmaceutical products, and to facilitate the global registration of drugs [1].

The Q1A (R2) guidelines: Stability Testing of New drug substances and products, lay out a framework for the evaluation and testing of new drugs, with the objective to confirm the safety, efficacy, and quality of these materials and products, including the storage temperature, humidity, and duration of the study [2]. The Q2 (R1) ICH guidelines, known as the 'Validation of Analytical Procedures; Text and Methodology' delivers guidance for the validation towards analytical processes used in pharmaceuticals, biotechnology, and other related industries. The main objective of the Q2(R1) guideline is to ensure that

analytical procedures used in the testing of pharmaceutical products are accurate, reliable, and consistent. It is the process of securing that a product meets certain quality and safety standards. Hence standardization of Ayurvedic formulations is important to secure that these formulations are safe and effective [3].

Force degradation study or stress testing is a method used to check the stability of active compounds and products by subjecting them to conditions more severe than accelerated testing [4]. Various parameter affecting force degradation are Acid and Alkali Hydrolysis in which the drug is exposed to a solution of 0.1 N HCl/0.1 N NaOH, to study the hydrolytic destruction of novel drugs in acidic and alkaline conditions respectively [2]. Oxidative degradation in which the drug could potentially produce degradation products by being exposed to 0.1–3% hydrogen peroxide at atmospheric temperature for up to 10% deterioration [2]. Triphala churna is an Ayurvedic herbal formulation that is made by mixing three fruits I.e., Amalaki (*Emblica officinalis*), Bibhitaki (*Terminalia bellirica*), and Haritaki (*Terminalia chebula*) in equal proportions. The three fruits working together synergistically strengthen numerous other systems as well. Triphala is used in Ayurveda to assist for the betterment in the breathing, circulation, urination, reproductive, and neurological issues. Additionally, Triphala has been demonstrated to be a potent antioxidant, shielding cells from the harmful consequences of free radicals. Triphala churna shows pharmacological activities like antioxidant, anti-inflammatory, antidiabetic, antimicrobial, antitumor, and immunomodulatory effects. It has also been found to be beneficial in the management of various diseases, such as diabetes, cancer, cardiovascular diseases, and liver disorders [5].

2. Material and Methods

2.1. Materials

Fresh fruits of Amla, Baheda & Harad and marketed formulation i.e., Divya Triphala churna (labeled to be 100 g in weight and containing Amla, Baheda, Harad in equal amount that is 33.33 g) were purchased from local market, fruits were sun dried and powdered individually.In house Triphala churna was prepared by mixing the three ingredients in the ratio 1:1:1 and stored in air tight container [7]. Pure Gallic acid was purchased from yucca pvt. Ltd. and pure Ascorbic acid was procured from laboratory. TLC plates of 20 cm × 20 cm, aluminum-backed silica gel 60 F254 were purchased.

2.2. Instruments

A Hamilton microliter syringe (Linomat syringe 659.0014, Hamilton-Bonaduz Schweiz, Camag, Switzerland), pre-coated silica gel aluminum Plate 60 F254, (20 cm × 10 cm, 10 cm × 10 cm, 100 m thickness; E. Merck, Darmstadt, Germany), Linomat 5 sample applicator (Camag, Switzerland), twin trough chamber (20 cm × 10 cm, 10 cm × 10 cm Camag, Switzerland), TLC Visualizer 2 (Camag, Switzerland) for photo documentation, and a TLC scanner 4 (Camag, Switzerland) operated by the vision CATS software (version 2.31, Camag, Switzerland) were used while performing the study [7].

2.3. Methodology

The method utilized to develop a validated HPTLC method and perform the stability study for analysis of the Triphala churna and its constituents by.

2.3.1. Extraction Process

Collecting the individual ingredients of Triphala churna and carrying out extraction of the powder of individual ingredients, in-house and marketed Triphala churna [6].

Extraction of Ascorbic acid and Gallic acid from Amla fruit using Methanol as a Solvent: 20 g of Amla powder was macerated in 100 mL of methanol for 72 h. Filtered from whattman filter paper, concentrated and stored.

- Extraction of Gallic acid from Baheda and Harad fruit using Methanol as a Solvent: 20 g of Baheda and Harad powder were taken separately and macerated in 100 mL of methanol for 72 h. Filtered from whattman filter paper, concentrated and stored.
- Extraction of Gallic acid from the in-house mixture of equal amount of Amla, Baheda, & Harad powder as well as Marketed Triphala (Divya churna 100 g) using Methanol as a solvent:20 g of mixed powder and Marketed churna were taken separately and macerated in 100 mL of methanol for 72 h. Filtered from whattman filter paper, concentrated and stored.

2.3.2. Standardization of Triphala Churna and Its Constituents by HPTLC

Developing a validated HPTLC method for analysis of the Triphala churna and its constituents [8].

- **Chromatography Procedure:**

Preparation of standard: 10 mg Ascorbic acid (marker compound) was dissolved in 10 mL of methanol and 10 mg of Gallic acid (marker compound) was dissolved in 10 mL of methanol simultaneously.

Sample: Extract of Amla powder, extract of Baheda powder, extract of Harad powder, extract of in-housed prepared Triphala churna and extract of marketed Triphala churna using Mobile phase: Toluene: Ethyl acetate: Formic acid (6:3:1) with Saturation time of 1 h [6].

- **Wavelength identification and Selection:** HPTLC Plate was developed by spotting pure Gallic acid and Ascorbic acid under the selected mobile system and scanned in densitometer in UV-visible range **(200–800 nm)**, identification is done to confirm the identity of the product [3]. A single peak was obtained at **271 nm** at **Rf 0.45** for Gallic acid and at **265 nm** at **Rf 0.23** for Ascorbic acid [3].
- **High Performance Thin Layer Chromatographic (HPTLC)** study of Gallic acid marker, Ascorbic acid marker and Extracts of Amla, Harad and Baheda, In house Triphala and Marketed Triphala churna [8].

2.3.3. Forced Degradation Studies

Conducting stability studies for the extract of individual ingredients, in house preparation and marketed churna of the Churna [4].

- **Degradation Studies by HPTLC:** Samples taken: Gallic acid marker (G), Ascorbic acid marker (A), Amla Extract (Am), Harad extract (H), Inhouse Triphala churna (Ti) Extract, Marketed churna (Tm) Extract [4].
- **Acidic Hydrolysis:** (Samples were stressed with 0.1 M HCl and simultaneously control was also performed. Readings were taken at interval of one days, three days and five days.
- **Oxidative degradation:** Samples were stressed with 3% H_2O_2, simultaneously control was also performed. Readingswere obtained at interval of one day, three days and five days.
- **Basic hydrolysis:** Samples were stressed with 0.1 M NaOH. Readings were obtained at interval of One day, three days and five days.

3. Result and Discussions

- HPTLC Study The plate was developed by spotting pure **Gallic acid** under the selected mobile system Toluene: Ethyl acetate: Formic acid (6:3:1) and scanned in densitometer in UV-visible range (200–800 nm) by using Camage TLC scanner 4. Identification is done to confirm the identity of the product (ICH Q2 R1). A single peak was obtained at 271 nm.

- The plate was developed by spotting pure Ascorbic acid under the selected mobile system and scanned in densitometer in UV-visible range (200–800 nm) by using Camage TLC scanner 4.A single clear peak was obtained at 265 nm.
- HPTLC of Extract of Gallic acid marker, Ascorbic acid marker and Extracts of Amla, Harad and Baheda, Inhouse Triphala and Marketed Triphala churna.
- Also, the Rf value for in–House Triphala preparation and Marketed Triphala formulations was also found to be 0.47 for both the samples.

Degradation Studies by HPTLC:

Degradation studies of Treated Gallic acid: -

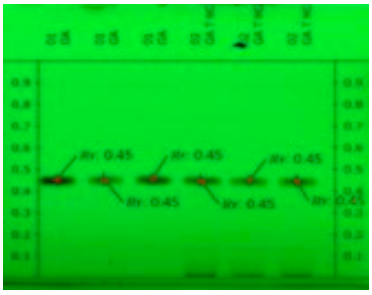

Figure 1. Acidic Treated.

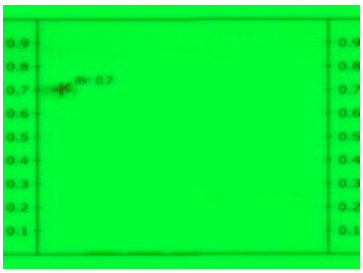

Figure 2. Basic Treated.

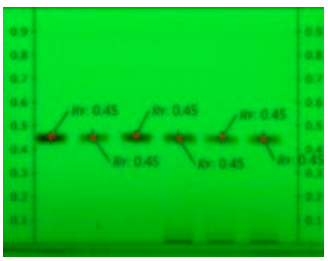

Figure 3. Oxidative Treated.

Degradation studies of Treated Ascorbic acid:

Figure 4. Basic Treated.

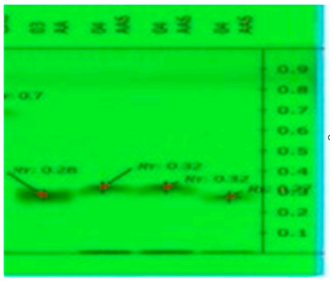

Figure 5. Acidic Treated.

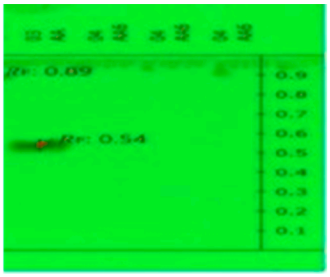

Figure 6. Oxidative Treated.

Comparative Degradation studies of treated in house Triphala Churna (Ti) extract and Treated Marketed Triphala Churna (Tm).

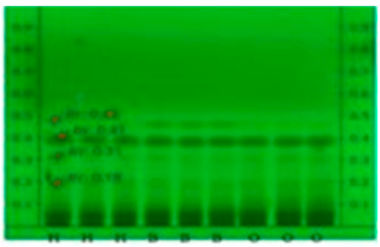

Figure 7. In-House Triphala Extract treated in short UV Range.

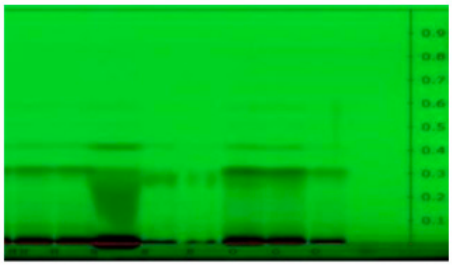

Figure 8. Marketed Triphala Extract treated in short UV Range.

Table 1. Percentage Loss in concentration under Acid, Oxidative & Alkali stressed condition.

Sample	% LOSS OF CONCENTRATION								
	Acidic Stressed			Oxidative Stressed			Alkali Stressed		
	1st day	3rd day	5th day	1st day	3rd day	5th day	1st day	3rd day	5th day
Pure Gallic acid	2.3	2.7	3.8	3.8	4.5	5.2	100	100	100
Pure Ascorbic acid	15	17	19	100	100	100	100	100	100
Amla extract	1.3	3.3	4.3	8.8	11	12	11	11	12
Baheda extract	8.4	14	14	11	11	15	21	22	29
Harad extract	10	15	16	12	15	16	25	26	29
Triphala in-house	3.1	6.8	8.8	0.6	1	5.9	27	28	29
Triphala marketed	8.2	13	17	10	12	14	10	12	14

4. Conclusions

The force degradation studies were performed (according to ICH Q1A (R2)) for three individual plant extract and also for in house preparation of Triphala and marketed formulationof Triphala. The above study shows that there was slight change in concentration of Gallic acid when kept in stressed condition. Pure Gallic showed stability under acidic hydrolysis and oxidative degradation as there was very less percentage loss of concentration in limit. But for alkali hydrolysis Gallic acid was unstable and Gallic acid was not detectable even within 30 min of time frame. In case of Ascorbic acid, it was difficult to come on explanation of stability in basic and oxidative condition as it needs further detection of the compound formed after subjecting it to stress condition because the compounds formed after stressed condition are not visible in UV range. Extract of plants and in house preparation Triphala Churna shows greater concentrationof Gallic acid and shows greater stability when stressed under various conditions as per ICH guidelines than marketed formulation Triphala Churna.

Hence, it may be concluded that in house preparation of Triphala is more stable and superior inquality than marketed formulation of Triphala Churna.

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