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Proceeding Paper

Standardisation and Stability Studies of Ayurvedic Formulation- Trikatu Churna as Per Ich Guidelines †

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Abstract: World is moving back towards the root of medical advancement which is Ayurveda, but the issue faced during Ayurvedic medicine development is that there are no any such guidelines present for formulation and, development, quality assurance, defining the safety profile, and assuring the efficacy of the Ayurvedic formulation as it there for allopathic medicine. This problem led to the unavailability of data regarding particular target-based Efficacy or mechanism of action of particular ingredient in the formulation as mainly Ayurvedic formulations are poly ingredient in nature. This research is designed for performing standardization of Ayurvedic formulation i.e., Trikatu Churna using HPTLC Instrument and carrying out force degradation studies of the Churna extract by using Piperine and Quercetin as marker compound according to ICH guidelines Q1A (R2) and Q2R1). In-house Trikatu churna was compared to the marketed Trikatu churna for analyzing the concentration of piperine and Quercetin both in the churna. The presence of piperine and Quercetin in churna was marketed by comparing the Rf of churna with Marker compounds which was found to be piperine for 0.64 and Quercetin for 0.51 forced degradation data showed the loss in concentration of both pure marker compound and in house Trikatu churna and as well as marketed churna. The use of standardization for Ayurvedic formulation and developed validated method for standardization and estimated the Stability profile of the Trikatu churna. The wavelength is identified for both marker compound and after degradation study there was decrease in concentration of marker Compound and of in house Trikatu churna and as well as marketed churna. The use of Standardization for Ayurvedic formulation discussed and validated method is developed for standardization and the stability profile of the Trikatu churna is estimated.

Keywords: trikatu churna; standardization; HPTLC; piperine; quercetin; ICH guidelines; force degradation; stability profiling; polyherbal formulation

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1. Introduction

Ayurveda, an ancient system of medicine, is gaining renewed attention for its holistic approach to health. However, the development of Ayurvedic formulations faces challenges due to the lack of standardized guidelines for formulation, quality assurance, safety, and efficacy, unlike allopathic medicines. Trikatu Churna, a polyherbal Ayurvedic formulation, requires proper standardization to ensure its effectiveness and stability. This research aims to standardize Trikatu Churna using HPTLC and conduct force degradation studies with piperine and quercetin as marker compounds, following ICH guidelines [1],[10]. The comparison between in-house and marketed formulations will provide insights into their stability and concentration profiles [3].

2. Method

2.1. Preparation of Standard Solutions

Piperine Stock Solution: Weighed 10 mg of standard piperine and transferred it to a 10 mL volumetric flask. Methanol was added to dissolve the powder and the volume was adjusted to 10 mL, resulting in a stock solution with a concentration of 1000 µg/mL.

Dilution of Piperine & Quercetin Stock Solutions: From the 1000 µg/mL stock solution, dilutions ranging from 100 µg/mL to 700 µg/mL were prepared.

2.2. Mobile Phase Optimization

The optimal mobile phase was determined to be a mixture of Toluene: Ethyl acetate: Formic acid: Methanol in the ratio 8.57:8.57:2.28:0.57 after testing several solvent combinations.

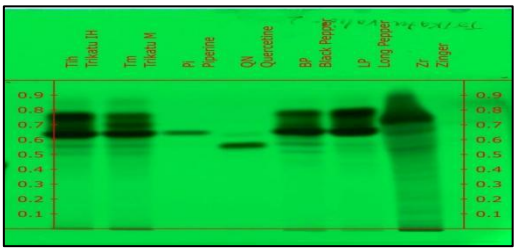


Figure 1. Extraction Procedure (Maceration Method).

Ingredients and Solvent: Weighed 25 g each of black pepper, long pepper, and zingerone, as well as in-house and marketed Trikatu samples. 75 mL of methanol was added to each [11].

Filtration: After 7 days of maceration, the mixtures were filtered using Whatman filter paper to obtain liquid extracts [4].

Concentration: The liquid extracts were concentrated using a rotary evaporator, followed by further evaporation in a water bath to remove any remaining solvent, leaving a solid mass.

3. Chromatographic Procedure

Sample Application: Diluted piperine and quercetin samples (100 µg/mL to 700 µg/mL) were applied to a TLC plate using a Linomat 5 applicator (5 mm band width).

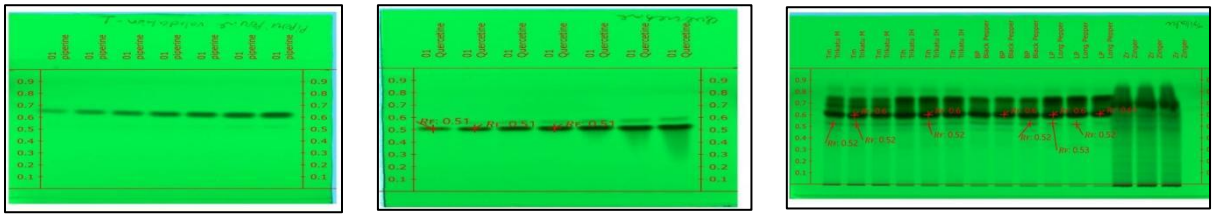
Plate Development: The TLC plate was developed in a dual trough chamber with the optimized mobile phase. The plate was allowed to saturate at room temperature for 20 min. The run distance was set to 70 mm.

Post-Development: The developed plate was removed, air-dried, and visualized using a TLC Visualizer.

Scanning: The plate was scanned using a Camag TLC Scanner 4, focusing on the UV-visible range (200–800 nm). Piperine was identified at a wavelength of 342 nm, following ICH Q2 (R1) guidelines.

4. Visualization and Analysis

Photographs of the plates were captured, and the TLC plate was scanned to confirm the presence and concentration of piperine and quercetin in both in-house and marketed Trikatu samples.



HPTLC plate of (**A**). Piperine (**B**). Qurecetine (**C**). individual ingridients

Figure 2. Development and Validation of Method Using Hpltc.

Table 1. Validation parameters.

Sr. No	Parameter	Values Obtained for Piperine	Values Obtained for Qurecetine
1	LOD	31.57785803	31.26391
2	LOQ	95.69047887	94.73911

Sr. No.	Parameters	Piperine	Qurecetine
1	Absorption Maxima	342	375
2	Regression Equation	y = 0.072x + 1.00	y = 0.02x + 0.1
3	Correlation Coefficient	0.9988	0.993
4	Specificity	specific	specific
5	Intra-day precision (%RSD)	0.237%	0.38%
6	Inter-day precision (%RSD)	0.18%	0.0873%
7	Repeatability (%RSD	0.401%	0.258%
8	Linearity range	100–700µg/mL	100–700µg/mL
9	Robustness (%RSD)	0.228%	0.100

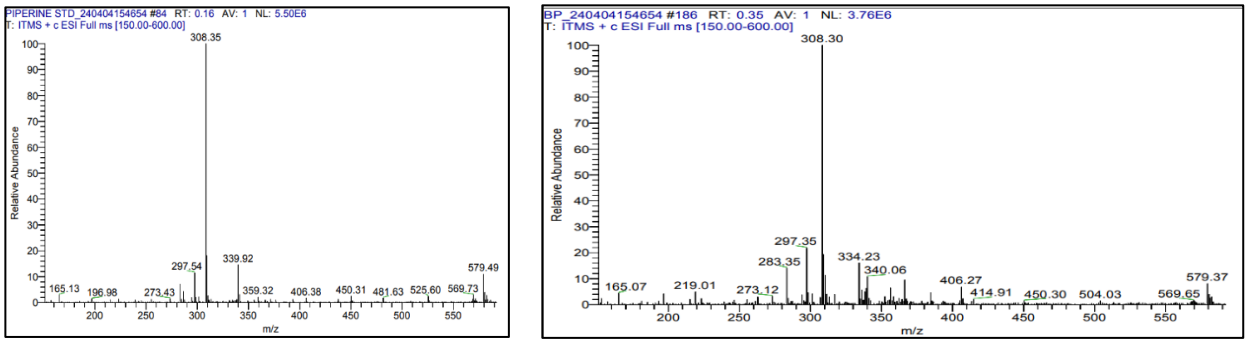


Figure 3. Piperine and black pepper extract.

Mass spectra of standard Piperine and black pepper extract was taken, and we have found that spectra of both Piperine and black pepper extract is same having molecular ion peak of 308[M− + Na+]. This finding confirms the presence of Piperine in black pepper.

5. Degradation Studies by HPTLC

Acidic Degradation

In the degradation studies of Piperine, Quercetine or Trikatu, this includes black pepper, long pepper and ginger. Acidic hydrolysis was conducted by treating the compound with 0.1 M HCl. Readings were taken at one-day intervals [6].

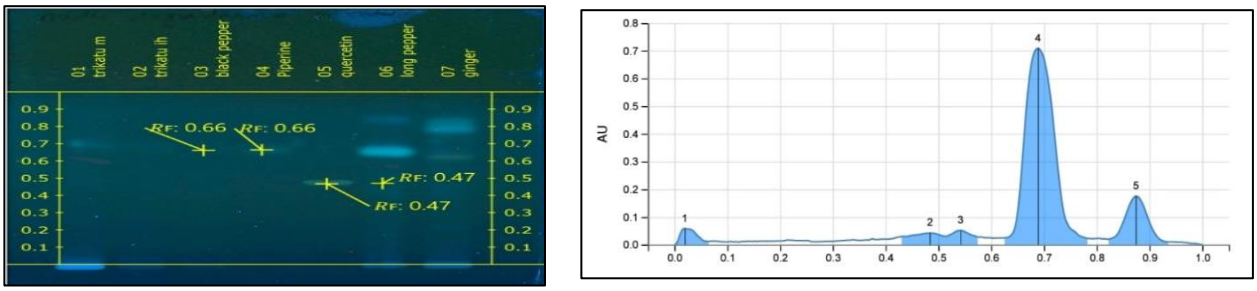


Figure 4. HPTLC Plate of acidic stressed Bp, LP, TM, T IN, Pi, Q with Densitogram.

Quercetin % degradation	
Acidic	
Trikatu (M)	41.67
Trikatu (IH)	38.41
Long Pepper	64.37
Quercetin	35.5
Ginger	84.25

Piperine % degradation	
Acidic	
Trikatu (M)	41.67
Trikatu (IH)	38.41
Black Pepper	64.37
Piperine	35.5

6. Basic Degradation

In the degradation studies of Piperine, Quercetin or Trikatu, this includes black pepper, long pepper and ginger. Basic hydrolysis was conducted by treating the compound with 0.1 M NaOH. Readings were taken at one-day intervals [6].

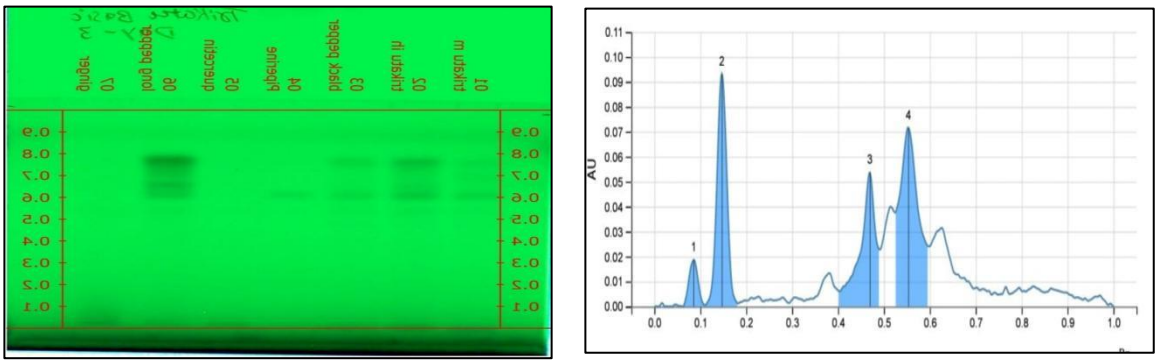


Figure 5. HPTLC Plate of Basic stressed BP, LP, TM, TIN, PI, with Densitogram.

Quercetin % Degradation	
Basic	
Trikatu (M)	46.44
Trikatu (IH)	36.89
Long Pepper	94.23
Quercetin	87.72
Ginger	82.54
Piperine % Degradation	
Basic	
Trikatu (M)	44.41
Trikatu (IH)	35.99
Black Pepper	83.33
Piperine	87.72
Piperine % degradation	
Oxidative	
Trikatu (M)	45
Trikatu (IH)	96.07
Black Pepper	92.86
Piperine	81.37

7. Oxidative Degradation

In the degradation studies of Piperine, Quercetin, or Trikatu (which includes black pepper, long pepper, and ginger), oxidative degradation was conducted by treating the compound with 3% H_2O_2. Readings were taken at one-day intervals.

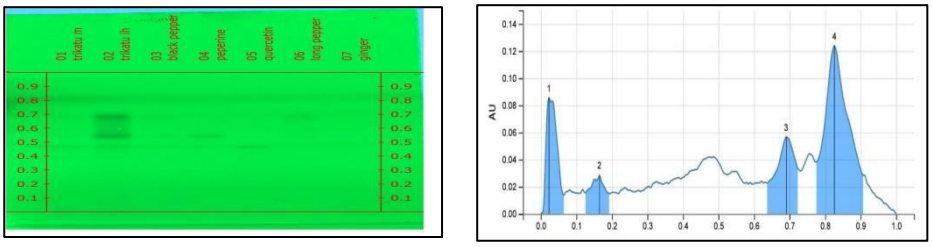


Figure 6. HPTLC Plate of oxidative stressed BP, LP, GN, TM, TIN, PI, Q with Densitogram.

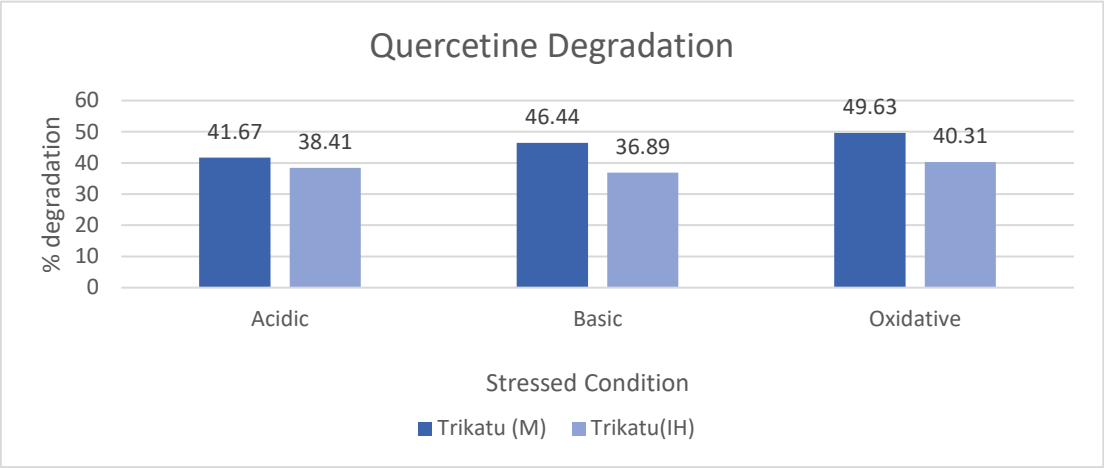
Quercetin % Degradation	
Oxidative	
Trikatu (M)	49.63
Trikatu (IH)	40.31
Long Pepper	59.07
Quercetin	39.12
Ginger	100

8. Result and Discussion

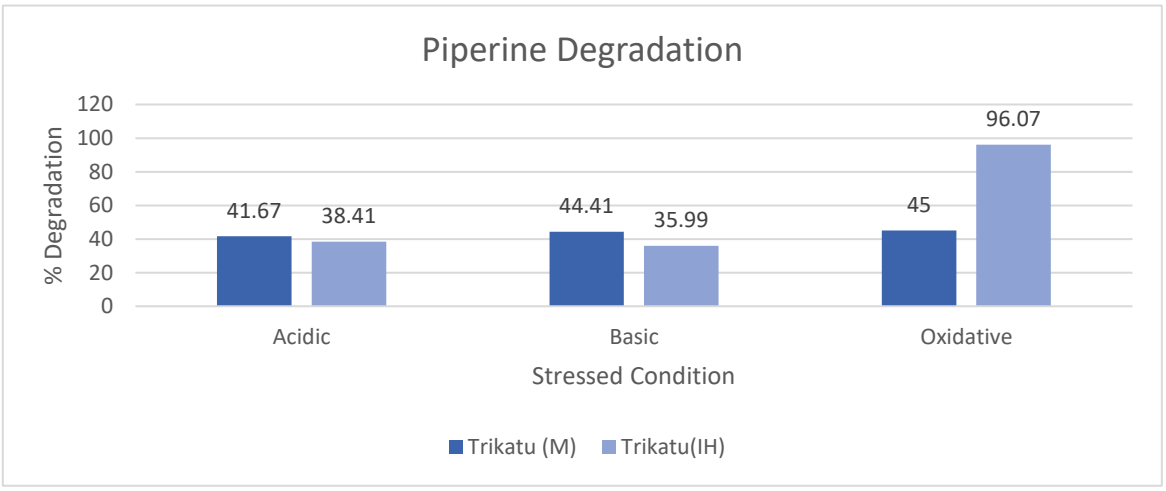
In the forced degradation studies, both quercetin and piperine showed significant degradation under acidic, basic, and oxidative conditions.

For quercetin, the percentage degradation in acidic conditions was 41.67% and 38.41%, while under basic conditions, it was 46.44% and 36.89%. In oxidative conditions, quercetin exhibited 49.63% and 40.03% degradation. These results indicate that quercetin

is most susceptible to oxidative stress, showing the highest degradation in these conditions.



Similarly, piperine degradation was observed to be 41.67% and 38.41% in acidic conditions, and 44.41% and 35.99% in basic conditions. Under oxidative stress, piperine showed 45% and 96.07% degradation, with oxidative stress proving particularly detrimental to piperine stability.



Overall, both compounds displayed higher susceptibility to degradation under oxidative conditions, with piperine being especially unstable. These findings emphasize the need for careful consideration of stability factors during formulation development, particularly for polyherbal formulations like Trikatu Churna, where multiple components may degrade differently under environmental stress.

9. Conclusions

Forced degradation studies offer valuable insights into the stability of Ayurvedic formulations like Trikatu Churna under various conditions. Environmental factors such as temperature, moisture, and pH can affect the therapeutic efficacy and safety of herbal products over time. Stability testing for herbal formulations is challenging due to chemical complexity, but modern techniques like HPTLC and proper guidelines can help generate reliable stability data. This research highlights the need for clear regulatory guidelines for the stability testing of Ayurvedic and herbal medicines, as current regulations are insufficient.

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Data Availability Statement:

Conflicts of Interest:

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