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Original Article

White Analytical Chemistry Aligned Validation and Stability Indicating RP-HPTLC Method for the Determination of Dabigatran Etexilate in Pharmaceutical Formulation

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ABSTRACT

Background: Oral direct thrombin inhibitors like dabigatran etexilate are frequently used to prevent and treat thromboembolic diseases. Regular quality control and stability evaluation necessitate an analytical technique that is straightforward, dependable, and ecologically friendly. **Objectives:**To initiate and verify green, stable, RP-HPTLC method to measure dabigatran etexilate quantitatively in pharmaceutical dosage forms complying with International Council for Harmonisation (ICH) validation standards. **Materials and Procedures:** Chromatographic separation was performed under optimal conditions using Reverse Phase-High Performance Thin Layer Chromatography (RP-HPTLC) with 2-Propanol:water: Glacial acetic acid in the ratio 6:4:0.2 as mobile phase. Validation was performed in accordance with all the suitable parameters. Stress studies under various conditions were carried out to ascertain the stability capacities. Green analytical principles were made possible by the use of eco-friendly solvents. **Results:** The method obtained outstanding linearity (R^2 = 0.9994) over 100–800 ng/spot. The LOQ was 69.649 ng/spot, while the LOD was 23.018 ng/spot. Robustness, accuracy, and precision were all within reasonable bounds (%RSD <2%). Dabigatran etexilate in pharmaceutical formulations was satisfactorily quantified, as indicated by the assay results. Forced stress studies demonstrated drug separation from its break down products effectively and validated the approach's stability-indicating character. **Conclusion:** The new RP-HPTLC method is easy to use, quick, accurate, precise, reliable, economical, and eco-friendly. It is appropriate for Dabigatran etexilate pharmaceutical analysis, stability research, and routine quality monitoring.

Keywords: Dabigatran Etexilate; Forced Degradation; Green Analytical Chemistry; RP-HPTLC; Stability-Indicating Method; Validation of Methods.

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INTRODUCTION

A common oral direct thrombin inhibitor used to prevent and treat thromboembolic disorders, dabigatran etexilate.[1] is especially useful for lowering the risk of stroke in people with non-valvular atrial fibrillation. The chemical structure of Dabigatran Etexilate is shown in Figure 1.Dabigatran Etexilate is a recommended treatment option in many clinical settings because it offers similar anticoagulant efficacy to warfarin without requiring regular international normalised ratio (INR) monitoring. However, the most commonly reported side effects are bleeding and gastrointestinal disturbances, which are mainly attributed to the tartaric acid in the capsule formulation, which enhances drug absorption by lowering gastric pH·Ensuring the safety and quality of pharmaceutical goods requires effective impurity monitoring. Several process- and degradation-related impurities of dabigatran have been reported, arising from raw materials, intermediates, synthesis, or storage conditions. According to ICH guidelines.[2], these impurities should be identified and quantified to ensure product quality, efficacy, and patient safety. Only a small number of publications provide trustworthy stability-indicating RP-HPTLC methods for the detection of related chemicals, even though various analytical techniques have been described for the assay determination of dabigatran. A comparison of details in reported analytical methods and present proposed method is provided in Table 1.

RP-HPTLC[3] is a simple, rapid, economical, and sensitive chromatographic technique widely used for pharmaceutical analysis. It is well suited for routine quality control and impurity profiling because of its high sample throughput, low solvent consumption, excellent separation efficiency, and ease of operation. For the examination of Dabigatran Etexilate

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Table 1: Comparison of White analytical chemistry aligned RP HPTLC method with other reported methods

Criterion	Previously reported analytical methods for Dabigatran Etexilate	Novelty of the proposed WAC-aligned RP-HPTLC method
Analytical platform	Reported methods are UV, RP-HPLC, RP-UPLC	Alternative planar chromatographic platform- RP HPTLC
Chromatographic separation	Reported methods are column based chromatographic approaches.	Low resource chromatographic approach of HPTLC separation with densitometric quantification.
Solvent consumption	Relatively high reported for conventional liquid chromatographies reported.	Potentially reduced solvent consumption and waste which is typical for planar chromatography.
Environmental assessment	There is no reported methods so far for environmental assessment .	Explicitly evaluated in present method.
Analytical performance assessment	Accuracy, precision, linearity, specificity etc.	Analytical performance incorporated through red dimension.
Practical/economic evaluation	Rarely integrated into method comparison.	Simplicity, throughput, resource requirements and practical utility considered by blue dimension.
WAC assessment	Not reported for the earlier dabigatran methods identified	Integrated into method evaluation.

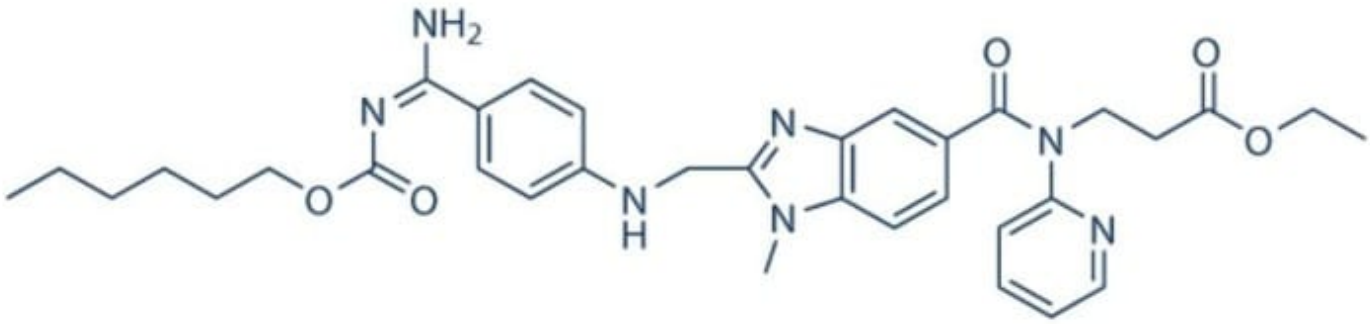


Figure 1: Structure of Dabigatran Etexilate

and its associated impurities, it is crucial to create a validated, stability-indicating RP-HPTLC approach.

As a result of the enhanced focus on Green Analytical Chemistry (GAC).[4]Comprehensive approaches to evaluate the ecological sustainability of analytical procedures have been developed. Among these, the Analytical GREEnness Metric Approach (AGREE).[5], Red Analytical Performance Index (RAPI).[6], and Blue Applicability Grade Index (BAGI).[7] are well known evaluators for several facets of sustainability and analytical performance. Unlike conventional validation parameters that primarily focus on analytical performance, these assessment tools provide a comprehensive evaluation by integrating environmental, practical, and analytical considerations

The combined application of AGREE, RAPI, and BAGI offers a comprehensive evaluation of analytical methods because each tool addresses a distinct aspect of method assessment. BAGI focuses on the practical applicability of analytical methods for routine laboratory use, RAPI integrates analytical performance with environmental sustainability, whereas AGREE mainly assesses method adherence to the Green Analytical Chemistry. The combined use of all three assessment tools offers a more balanced evaluation of analytical methods.

White Analytical Chemistry (WAC) concept integrates analytical efficiency, environmental sustainability, and practical applicability into a unified framework. Therefore, this forms the prime reason to evaluate developed method using White Analytical Chemistry principles through AGREE, RAPI, and BAGI assessment tools.

MATERIALS AND METHODS

Chromatographic analysis was conducted by HPTLC from CAMAG.[8]with Linomat 5 as sample dispenser, TLC Scanner 4, developing chamber with twin-troughs, UV chamber

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Table 2: Linearity of dabigatran Etexilate

Concentration	Peak areas
100	2468
200	2891
400	3720
600	4572
800	5508

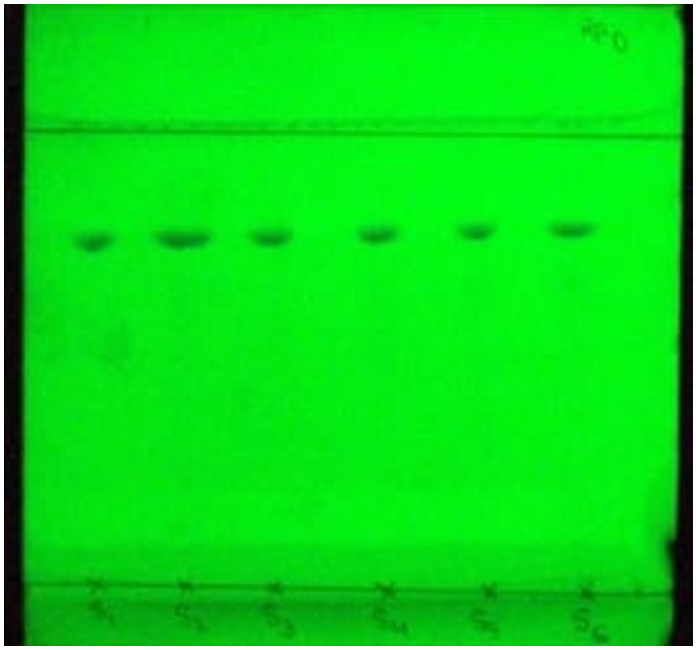


Figure 2: Method development of Dabigatran Etexilate

for visualisation, and winCATS software (Version 1.4.3). Aluminium-backed silica gel 60 F254plates of HPTLC (10 × 10 cm, 250 µm thick; Merck from Germany) made up the stationary phase. A Hamilton 100 µL syringe was sample applier. The forced deterioration investigations were conducted using a hot-air oven and a temperature-controlled water bath.

Chemicals and reagents

The reference standard for dabigatran etexilate mesylate (>99.0% purity) was purchased from Micro Labs in Bengaluru, India. We purchased commercial dabigatran etexilate capsules (Pradaxa®) from a nearby drugstore. We bought hydrogen peroxide, hydrochloric acid, sodium hydroxide, glacial acetic acid, and spectroscopic-grade solvents from Loba Chemicals Pvt. Ltd., Mumbai, India. A Merck Milli-Q Direct-Q3 water purification system was used to obtain high-purity deionised water.[9]

Chromatographic Development

Before being used, HPTLC plates were activated at 60 °C for 15 minutes and prewashed with methanol. Using a CAMAG Linomat 5 applicator, samples were applied as 4 mm bands 15 mm from the plate's lower edge. In a pre-saturated twin-trough chamber, plate development was carried out for 30 minutes to a migration distance of 70 mm. Following drying, a CAMAG TLC Scanner 4 was used to perform densitometric scanning at 254 nm, and winCATS software.[10] was used to record the chromatograms. The optimised chromatographic conditions established during method development are illustrated in Figure 2.

Preparation of Standard and Sample Solutions

A stock solution of the drug was made by dissolving ten milligrams of dabigatran in ten millilitres of methanol (1 mg/mL). The working standard solution (1000 ng/µL) was made by diluting 500 µL of the stock solution to 10 mL with methanol. The capsule sample solution was prepared similarly after the appropriate sample preparation and dilution.

Forced Degradation Studies

In compliance with the guidelines for stability-indicating analytical methods, forced degradation studies.[11] were conducted under acidic (0.1 M HCl), alkaline (0.1 M NaOH), oxidative (3% $H2O_2$), thermal (100 °C), and photolytic (direct sunlight, 6 h/day for 7 days) stress conditions to assess the developed method's stability-indicating capability.

Method validation

Linearity

The working standard solution was diluted with ethanol in the appropriate aliquots to achieve concentrations of 100–800 ng/spot. After applying one microlitre of each solution to RP-HPTLC plates that had already been coated, the plates were developed, dried, and densitometrically scanned at 254 nm[12]Plotting peak area versus concentration allowed for the construction of calibration curves. Table 2 presents the linearity data, while Figures 3 and 4 display the related densitogram and calibration curve

Limit of Detection (LOD)

The concentration of analyte that is lowest yet detectable but not quantifiable is known as the limit of detection (LOD).[13] The following formula was used to compute it.

$LOD = (3.3 \times \sigma)/S$

Limit of Quantitation (LOQ)

The quantitative measurement of lowest analyte concentration with reasonable accuracy andprecisionis limit of quantitation, or LOQ.[14] The following equation was used to compute it.

$(10 \times \sigma)/S = LOQ$

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Table 3: Robustness studies of dabigatran Etexilate

Parameters	Change	Peak area	Average	%RSD
Mobile phase (Propanol: water: Glacial acetic acid)	7:3:0.2	3762	3789.5	1.02
		3817		
	5:5:0.2	2340	2328.5	0.69
		2317		
Sample application volume	10 mm	4193	4166.5	0.89
		4140		
	8 mm	4950	4920.5	0.84
		4891		

%RSD: Percentage Relative Standard Deviation.

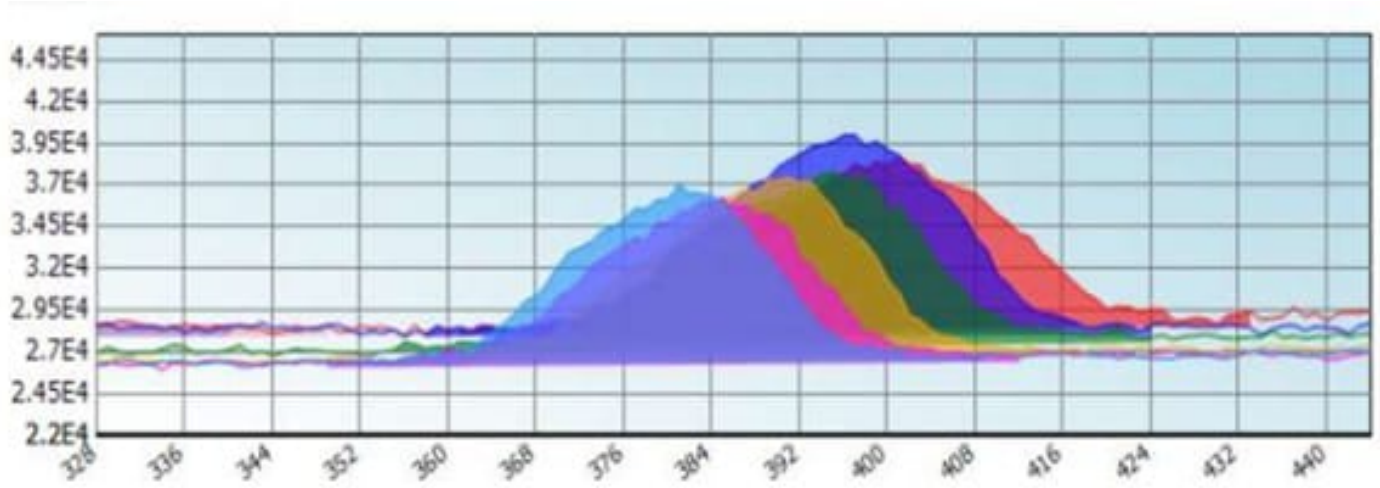


Figure 3: Chromatogram of Dabigatran Etexilate

Precision

The 100% concentration in six replicates on the same day (intra-day precision) and on different days (inter-day precision) was analysed to assess precision.[15] The TLC plate was treated with a 1 µL aliquot, which was then developed, dried, and scanned at 254 nm. The resulting peak regions were utilised to assess precision. Table 4 and Figure 6 display the relevant precision results and densitogram.

Accuracy

The standard addition method was used to evaluate accuracy at concentration levels of 50%, 100%, and 150%.[16] Table 4 and Figure 5 show the accuracy findings and matching densitogram, respectively.

Robustness

Small intentional changes in the mobile phase composition, Sample application volume, and detecting wavelength were considered to study robustness.[17] Table 5 displays the robustness study's findings.

Analytical Procedure for Commercially Available Formulation

Ten capsules, each containing 110 mg, were weighed and powdered. Ten milligrams of dabigatran were extracted using methanol, sonicated for twenty minutes, and then diluted to ten millilitres. The filtered solution was added to the TLC plate in a 1 µL aliquot, developed, dried, and scanned at 254 nm. To determine the drug content the following formula was used :

$$\%.\ \text{Assay} = (\text{Amount Recovered/Label Claim} \times 100)$$[18]

Forced Degradation Study Procedure

To assess the stability of the devised approach in compliance with ICH Q1A(R2).[19] recommendations, stress studies were conducted under acidic, alkaline, oxidative, hydrolytic, thermal, and photolytic conditions. To identify degradation products,

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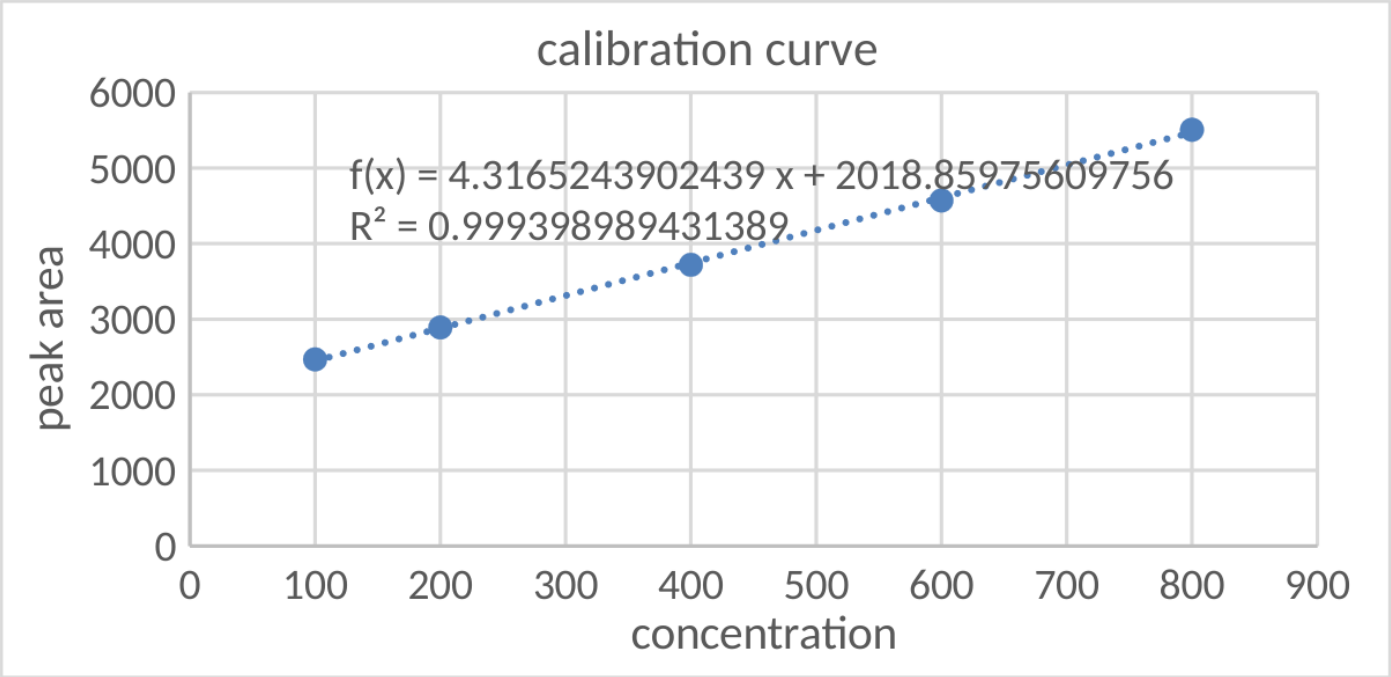


Figure 4: Calibration curve of Dabigatran Etexilate

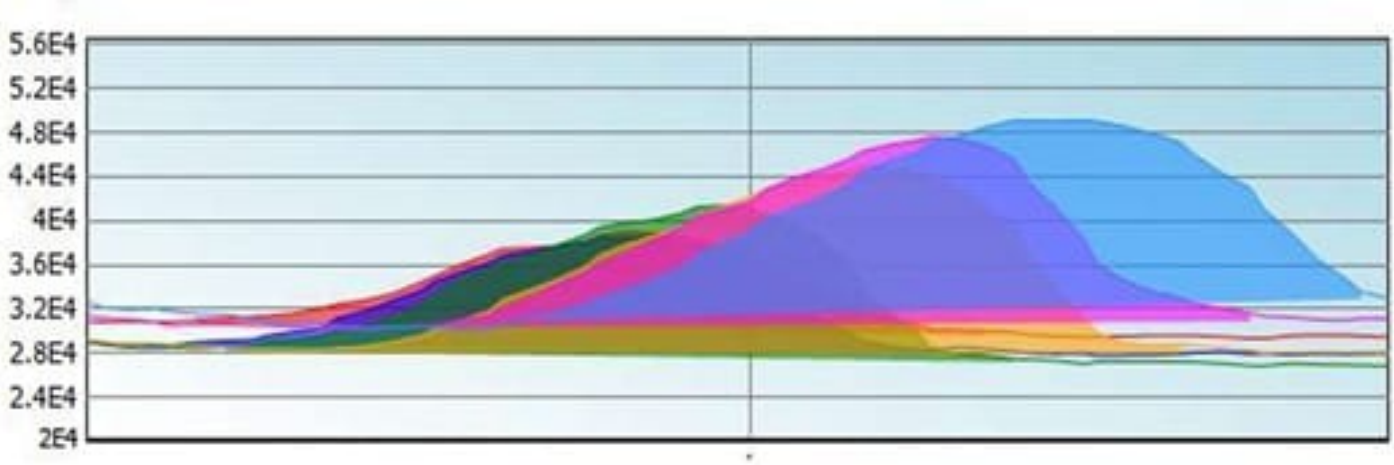


Figure 5: Representative Chromatogram of the Accuracy study

the stressed and unstressed samples were analysed using RP-HPTLC, and the resulting chromatograms and densitograms were documented.

RAPI Tool Evaluation

The Red Analytical Performance Index (RAPI) is a comprehensive assessment tool that evaluates by integrating analytical performance, environmental impact, occupational safety, reagent hazards, waste generation, and energy consumption. The colour-coded scoring system enables comparison of analytical methods based on both sustainability and analytical quality. Higher RAPI scores indicate superior analytical performance and environmental sustainability.[20]

AGREE Tool Evaluation

Based on the 12 principles of Green Analytical Chemistry, the Analytical GREEnness Metric Approach (AGREE).[21] assesses analytical techniques based on factors like sample preparation, reagent toxicity, solvent consumption, energy needs, waste production, occupational safety, and automation. A circular pictogram with a score between 0 and 1 is produced by the software; higher values suggest more adherence to the principles of Green Analytical Chemistry.

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Table 4: Degradation results of DabigatranEtexilate

S.no	Condition		Results (% degradation)	Rf value of dabigatran Etexilate	Rf value of generated impurities
1	Acidic		29.895%	0.761	0.862
2	Basic		90.66%	0.761	0.937
3	Hydrolytic		48.508%	0.761	0.782
4	Peroxide		63.014%	0.761	0.448
5	Thermal (sunlight)	1 h	39.96%	0.761	0.86
		2 h	8.43%		0.78
		4 h	69.17%		0.90
		6 h	27.08%		0.89
6	UV	1 h	43.10%	0.761	0.90
		2 h	14.60%		0.67
		4 h	39.33%		0.87
		6 h	48.52%		0.84

Rf: Retardation factor.

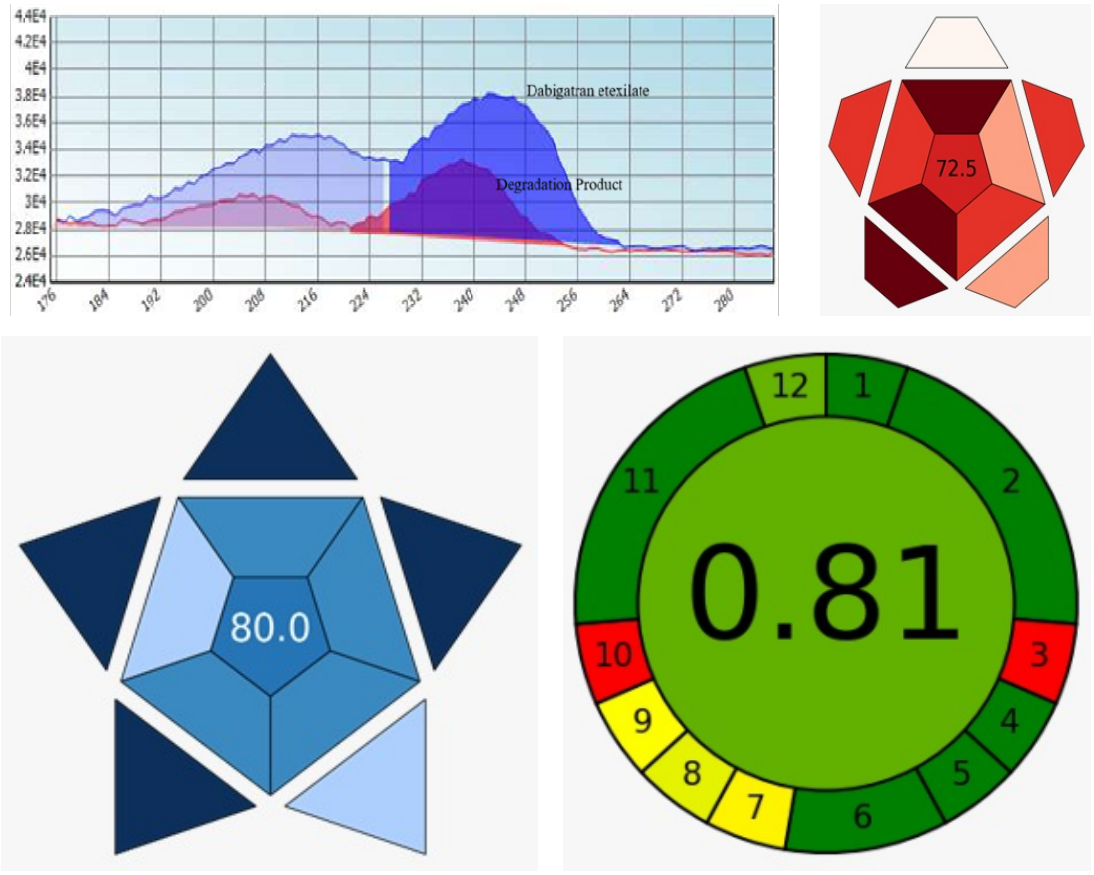


Figure 6: White Analytical Assessment of the Developed RP-HPTLC Method Using RAPI, BAGI, and AGREE Tools.

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BAGI Tool Evaluation

By considering analytical performance, operational simplicity, cost-effectiveness, instrument availability, sample throughput, environmental impact, and overall reliability, the Blue Applicability Grade Index (BAGI) assesses how practically applicable analytical methods are. Higher BAGI ratings are more appropriate for sustainable laboratory applications and routine quality control.[7]

Statistical Analysis

The experiment was conducted in triplicates for every sample or as specified for each validation parameter. To assess the accuracy and resilience of the developed approach, the relative standard deviation (%RSD) was calculated. Utilising linear regression analysis, the calibration curve and correlation coefficient (R^2) were established.

Ethics Statement

This study did not require ethical approval because it solely used commercially available pharmaceutical formulations for analytical evaluation and did not use biological materials, animals, or human subjects.

RESULTS AND DISCUSSION

Linearity

The new RP-HPTLC method exposed good linearity over the investigated (100–800 ng/spot) range with a correlation value (R^2) of 0.9994, proving relationship between concentration and peak area as very much intact (Table 2).

Precision

The developed RP-HPTLC technique demonstrated good repeatability and moderate precision with %RSD values of 1.433 and 1.608 for intra-day and inter-day respectively both below the allowable limit of 2% (Table 3).

Accuracy

The assessment of accuracy of the devised method was led by using the standard addition method at concentrations of 50%, 100%, and 150%. The percentage recoveries varied from 98.17% to 100.08%, indicating that the technique satisfies the ICH Q2(R2) suggested acceptance requirements.

Robustness

With %RSD values ranging from 0.69% to 1.02% after slight intentional changes in chromatographic conditions, the new approach was confirmed to be robust. All the obtained values were below 2% exposing robustness and dependability of the method (Table 5).[16]

Assay

Commercial dabigatran capsules were successfully assayed using the suggested RP-HPTLC method, producing an assay value of 100.82% that is within the acceptable pharmacopeial limit of 98–102%. The method's usefulness for regular pharmaceutical quality control is confirmed by these results (Table 6).

RAPI Tool Evaluation

The devised RP-HPTLC method achieved 72.5 as score from RAPI (Figure 12), indicating good analytical performance with favourable environmental sustainability, supporting its suitability for routine pharmaceutical analysis.

BAGI Tool Evaluation

With a BAGI score of 80.0 (Figure 12), the developed method showed good operational efficiency, analytical applicability, and suitability for regular quality monitoring of pharmaceutical formulations containing dabigatran etexilate.

AGREE Tool Evaluation

The suggested RP-HPTLC method's environmental sustainability was confirmed by an AGREE score of 0.81(Figure 12), which demonstrated outstanding concurrence with the standards of Green Analytical Methodology.

Forced degradation studies

The new RP-HPTLC approach successfully separated dabigatran etexilate from its break down products under acidic, alkaline, hydrolytic, peroxide, thermal, and UV conditions, according to forced degradation studies, validating the method's capacity to indicate stability. Table 7 displays the % degradation and matching Rf values found under each stress situation. Figures 7–11 display representative chromatograms produced under the different stress settings.

CONCLUSION

For dabigatran etexilate's quantitative determination in pharmaceutical formulations, a quick, reproducible, precise, robust, stability-indicating, and eco-friendly RP-HPTLC approach was successfully initiated and validated. According to ICH Q2 (R2) validation rules, the method exposed outstanding linearity, precision, accuracy, robustness, sensitivity, and assay performance. Additionally, the suggested method's environmental sustainability and practical application were validated by greenness assessment utilising RAPI, BAGI, and AGREE. Thus, routine quality control, stability investigations, and sustainable pharmaceutical analysis can all benefit from the suggested approach.

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CONFLICT OF INTEREST

No conflict of interest was declared by authors.

AUTHOR CONTRIBUTIONS

K.E. Pravallika planned and supervised the study. **D. Mounika** performed the experimental work, and manuscript drafting. **Ravi Parimi** contributed to data collection, data analysis and data interpretation. All the authors reviewed and gave their consent for the final manuscript.

ABBREVIATIONS

Reverse-Phase High-Performance Thin-Layer Chromatography (RP-HPTLC); White Analytical Chemistry (WAC); Analytical GREEnness Metric Approach (AGREE); Red Analytical Performance Index (RAPI); Blue Applicability Grade Index (BAGI); International Council for Harmonisation (ICH); Limit of detection (LOD), Limit of Quantitation (LOQ);% RSD (Percentage relative standard deviation) International Normalised Ratio (INR), International Council for Harmonisation. Stability Testing of New Drug Substances and Products (ICH Q1(R2)); International Council for Harmonisation. Validation of Analytical Procedures. (ICH Q2(R2).

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