

Development and Validation of Stability-Indicating High-Performance Thin-Layer Chromatography for estimation of Ranolazine with Green Analytical Assessment

Jitendra Yadav

ju7288096@gmail.com

University Institute of Pharmacy Pt. Ravishankar Shukla University

Harish Bhardwaj

Shri Rawatpura Sarkar University

Shubham Sahu

University Institute of Pharmacy Pt. Ravishankar Shukla University

Rajendra Kumar Jangde

University Institute of Pharmacy Pt. Ravishankar Shukla University

Sanjay J. Daharwal

University Institute of Pharmacy Pt. Ravishankar Shukla University

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“Development and Validation of Stability-Indicating High-Performance Thin-Layer Chromatography for estimation of Ranolazine with Green Analytical Assessment”

Jitendra Yadav^{1*}, Harish Bhardwaj², Shubham Sahu¹, Rajendra Kumar Jangde¹,
Sanjay J. Daharwal¹

¹University Institute of Pharmacy, Pt. Ravishankar Shukla University, Amanaka,
Raipur, CG, 492010

²Faculty of Pharmacy, Shri Rawatpura Sarkar University, Raipur, Chhattisgarh,
492015, India.

Jitendra Yadav: jy7288096@gmail.com

Harish Bhardwaj: harishbhardwaj808@gmail.com

Shubham Sahu: shubhambandhi@gmail.com

Rajendra Kumar Jangde: rjangdepy@gmail.com

Sanjay J. Daharwal: daharwalresearch@rediffmail.com

Corresponding author: jy7288096@gmail.com

Mr. Jitendra Yadav

M.Pharm (Pharmaceutical Analysis)

University Institute of Pharmacy

Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

Abstract

In this work, a new simple, selective, precise, Green assessment based stability indicating high performance thin layer chromatographic method has been established for estimation of ranolazine in pharmaceutical dosage form. Separation was achieved on precoated TLC silica gel 60 F254 aluminium plates (20cm×10cm) using mixture of Toluene: methanol (6:4 v/v). The detection of bands was carried out at 274 nm. The compact bands of RAN were obtained at R_f 0.63±0.04, Linearity (0.998), Range 100-1000 ng/bands, limit of detection (12.1 ng/band) and limit of quantification (36.95 ng/bands) for RAN at 274 nm. Accuracy and precision of this proposed method was evaluated by recovery studies (99.08 % and 99.67% for different tablet formulations). Robustness studies were performed under appropriate condition. Drug was degraded under Acid degradation, Alkali degradation, Neutral degradation, Photolytic degradation, and Thermal degradation. The proposed method was evaluated by greenness assessment methods like Blue applicability grade index (BAGI), Green analytical procedure index (GAPI), Modified green analytical procedure index (MoGAPI), Analytical GREENness metric (AGREE), and Analytical GREENness metric for sample preparation (AGREEprep). We obtain moderate green results: BAGI (67.5), AGREE (0.56) and AGREEprep (0.57). The method was satisfactorily applied for the analysis of different tablet formulations and proved to be specific and accurate for the quality control of these drugs.

Keywords: RAN, LOD, LOQ, BAGI, AGREE, and AGREEprep.

1. Introduction

Ranolazine (RAN) is a piperazine derivative, anti-anginal agent for the treatment of chronic stable angina pectoris [1], with the empirical formula $C_{24}H_{33}N_3O_4$ and a molecular weight of 427.54 g/mol, chemically is 1-piperazine-acetamide, N-(2, 6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy) propyl], show in Fig.-1 [2]. It exhibits antianginal and anti-ischemic properties that are independent of heart rate or blood pressure reductions. It diminishes the late sodium current, which is anticipated to lower sodium influx into ischemic myocardial cells, thereby reducing calcium uptake indirectly through the sodium exchanger [3,4]. It is a second line anti-anginal medication that reduces intracellular calcium excess by blocking late sodium channels, thus improving diastolic tension and alleviating angina symptoms. It affects sodium and potassium ion channel currents during cardiac repolarization, particularly the late inward sodium current. In pathological conditions, ranolazine enhances sodium-calcium exchange, increasing cytosolic calcium and impairing left ventricular relaxation after ischemia and reperfusion [5,6].

Due to its short half-life (7hrs) and inconsistent absorption, RAN, which was once offered as immediate-release capsules, is now accessible in an extended release version. After three days of a 500 mg twice daily dosage schedule, it achieves stable levels; if well tolerated, it may be increased to 1000 mg. It is normally processed by the liver and excreted by the kidneys. Due to their metabolic effects CYP3A4 and CYP2D6 inhibitors should be used with caution. Lactic acid generation is decreased, fatty acid metabolism is inhibited, and glucose oxidation is encouraged by RAN [7–9].

In terms of solubility, Ranolazine is a white to off-white solid that dissolves well in methanol and dichloromethane but is faintly soluble in other solvents and poorly soluble in water. It has a melting point of 122-124°C and pKa of 13.6 indicating that it is strong base [10,11]. Ranolazine is not yet included in any pharmacopoeia and several spectrophotometric UV, AUC D^1 , D^2 and chromatography methods like HPLC [12–14], LC-MS [15] and HPTLC [16] have been developed for its determination, though most of them have focused on its quantification in human plasma. No study has been reported on green evaluation for ranolazine method, stability indicating HPTLC method for ranolazine as per guidelines. The present study is to develop a simple, economical, rapid, precise and accurate HPTLC method for

estimation of RAN in the presence of its degradation products under various stress condition and validation of the methods as per ICH guidelines and Greenness evaluation of method as per green chemistry principles; tools like GAPI, AGREE, AGREEprep, MOGAPI and BAGI [17,18].

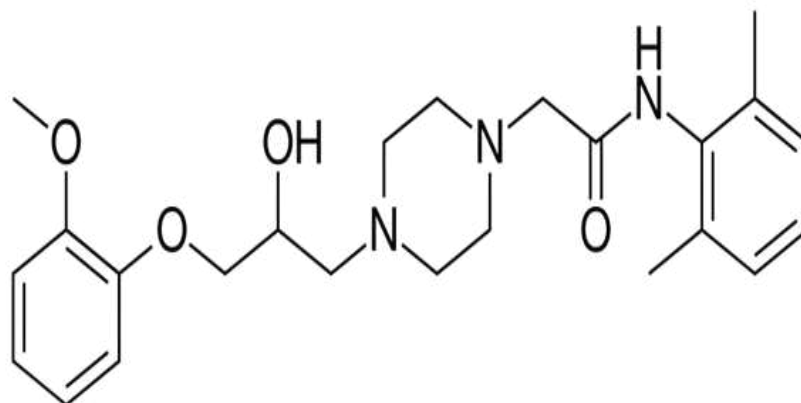


Fig. 1: Structure of Ranolazine

2. Experimental investigation

2.1. Material and method

2.1.1. Material

Ranolazine (RAN) gift sample was kindly provided by Atul bioscience Ltd. Mumbai, Maharashtra, India. AR and HPLC-grade solvents including such as toluene, methanol, ethyl acetate, Chloroform, Ammonia, Iodine, N-hexane, Ethanol, NaOH, HCL and H₂O.

2.1.2. Pharmaceutical matrix

RENOLZIN-500 SR contains 500 mg of RAN, manufactured in India and marketed by KNOLL HEALTHCARE PVT. Ltd and RANOMAS-500 contains 500 mg of RAN, manufactured in India and marketed by XENON PHARMA Pvt. Ltd.

2.1.3. Instrumentation

Chromatographic separation of drug was performed on Merck TLC plate precoated TLC silica gel 60 F254 Aluminium plates (20cm x 10cm with 250 mm thickness of layer) from E. Merck, Germany. The samples were applied onto the plates as band width of 4 mm using Linomat-5, 100µl sample syringe (Hamilton, Switzerland) with applicator (Linomat-5). Linear ascending development was carried out in twin through glass chamber (20cm x 10cm). Densitometric analysis was performed with a

CAMAG TLC scanner operated by VisionCATs software (Version 3.2). Electronic balances (Sartorius), Cyclo mixer (Remi), reflex condenser, Heating Mantel (Remi) were used for this study.

2.1.4. Preparation of Standard Stock Solutions and working solution

A Standard stock solution of RAN was prepared by accurately weighing 10 mg of RAN and transferring in into a 10 ml volumetric flask. The drug was dissolved in methanol and volume was made up to the mark with methanol to obtain a stock solution (1mg/ml). For working solution of RAN, 1 ml of stock solution was transferred into 10 ml volumetric flask and the volume was made up to the mark with methanol to obtain working solution (0.1mg/ml).

2.1.5. Selection of detection wavelength

In order to determine to the absorbance maxima, different drug concentration from standard stock solution were spotted on the plate and developed. Following development, plate was dried and scanned to get in situ spectra in the 200-400 nm range. Additionally a UV spectrophotometer was used to scan the medication in the 200-400nm range.

2.1.6. Mobile Phase optimization

Precoated TLC silica gel 60 F254 Aluminium plates were activated at 60° C for 5 minutes after methanol cleaning. Standard solutions of Ranolazine, along with their deteriorated samples were applied using glass capillaries and allowed to dry. Various mobile phase compositions were tested in glass beaker after saturation. The plates were spotted and analyzed under UV light after the solvent front reached approximately three-fourths of the plate length.

2.1.7. Chromatographic separations

On TLC silica gel 60 F254 Aluminium plates that had already been coated, the chromatographic separation was carried out using a mobile phase made up of Toluene: methanol (6:4 v/v). The plates were developed for 7.30 min in a CAMAG twin trough chamber that had been previously saturated with mobile phase. The plates were air dried and then dried in a hot air oven after being developed to a distance of 70 mm at 274nm; the separated zones were then scanned and measured.

2.1.8. Solution stability

To demonstrate their stability during the assessment procedure the two original and test solutions were inspected for an entire day at room temperature. The original and test solutions were inspected for the study showed that there no significant difference in the peak area of 1000 ng/bands of RAN for either solution. Within the required time span, there were no noticeable degradations.

2.1.9. Analysis of commercial tablet formulation

Accurately weigh and finely powder twenty tablets. Transfer separately a quantity of the powdered material equivalent to 13.8 mg of RENOLZIN-500 SR (equivalent to 10 mg as per its equivalent weight) and 13.5 mg of RANOMAS-500 (equivalent to 10 mg as per its equivalent weight) into 10 ml of volumetric flask and made up to the mark with methanol then filter the solution-A. Take a 1 ml of solution-A was transferred into 10 ml volumetric flask and the volume was made up to the mark with methanol to obtain solution-B at 274 nm.

2.2. Validation of quantitative experiment

The method was confirmed to be in accordance with ICH guideline Q2 (R2) for the evaluation of numerous parameters, such as specificity, linearity and range, precision, Accuracy, robustness, LOD and LOQ [19,20].

2.2.1. Specificity

The analysis of standard and tablet solutions confirmed the selectivity and specificity of the technique for RAN. The chromatograms showed no interference from tablet excipient and the peak purity analysis indicated that the observed peak was due to RAN alone.

2.2.2. Linearity and range

Aliquots of 1 to 10 μ l of a 0.1 mg/ml RAN standard solution were applied to give a concentration range of 100-1000 ng/band. The chromatograms were recorded and scanned at 274 nm wavelength. The linearity was evaluated by constructing a calibration curve of peak area versus ranolazine concentration and calculating the regression equation and correlation coefficient (r^2)

2.2.3. Precision

A solution comprising 300ng/bands and 500ng/bands of RAN was spotted on TLC plate and subject to six analyses without the plate being moved in order to ensure measurement repeatability. Intraday precision was determined by analyzing three concentrations (400, 600 and 800 ng/bands) thrice in one day, while interday precision involved the same concentration analyzed over three consecutive days. To evaluate the measurement variability, the percentage RSD of the peak regions was computed.

2.2.4. Accuracy

To evaluate accuracy, recovery studies were carried out using the conventional approach. A pre analyzed sample solution containing 300 ng/bands was mixed standard RAN at 50% (150 ng/bands), 100% (300 ng/bands), and 150% (450 ng/bands) of the initial concentration. The method accuracy was demonstrated by the acceptable recoveries, which fell between 98% and 102%.

2.2.5. Robustness

The influence of slight modification to the chromatographic setting was examined in order to access the methods robustness. The modifications were the change of toluene: methanol mobile phase from 6:4 to 5.9:4.1 and 6.1:3.9, the change of detection wavelength from 274 nm to 272 nm and 276 nm and the change of chamber saturation time from 20 min to 18 min and 22 min. Effect of RAN on peak area and Rf value at 300 ng/bands was studied and %RSD was found low thus confirming reliability of method for varying conditions.

2.2.6. Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ values represent the lowest concentration of RAN that can be detected and quantified with sufficient accuracy and precision. The LOD and LOQ were calculated based on the standard deviation (σ) of the response and the slop (s) of the calibration curve according to the ICH guidelines. The following formula can be used to calculate both. $LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$.

2.3. Forced degradation study

Forced degradation of ranolazine substance was performed under acid condition, basic condition, neutral condition, thermal condition and photolytic condition. After the degradation, 1000 ng RAN per band was applied [21,22].

2.3.1. Acid condition

For the acid investigation, 10 mg of the medication was weighed accurately and transferred into 10 ml volumetric flask. Methanolic HCL (1N) was added and made up to the mark. The solution was refluxed at 75° C for 2 hours. After this it was neutralized with NaOH (1N). An appropriate dilution was made from this solution and 1000 ng/bands were applied.

2.3.2. Basic condition

For the Basic investigation, 10 mg of the medication was precisely weighed and diluted in 1N methanolic NaOH in 10 ml volumetric flask. After refluxing the mixer for 2 hours at 75°C, 1 N HCL was used to neutralize it. This solution was diluted appropriately, using 1000 ng/bands.

2.3.3. Neutral condition

For the Neutral investigation, 10 mg of the medication was precisely weighed and diluted in methanol: water (80:20). After refluxing the solution for 1 hour at 75°C. The solution was diluted appropriately, using 1000 ng/bands.

2.3.4. Photolytic condition

For the Photolytic investigation, 10 mg of the drug was accurately weighed and kept hot air oven for 3 days at 80° C. then, it was transferred into 10 ml volumetric flask and volume was made up to mark with methanol. After this solution was diluted appropriately, using 1000 ng/bands.

2.3.5. Thermal condition

For the thermal condition, 10 mg of the drug was accurately weighed and kept hot air oven for 3 days at 80° C. then, it was transferred into 10 ml volumetric flask and volume was made up to mark with methanol. After this an appropriate dilution was made and 1000 ng/band was applied.

2.4. Greenness evaluations

2.4.1. Blue applicability grade index

Blue applicability grade index is new metric to assess the applicability of analytical methods to existing green metrics. It evaluates ten attributes including analysis type and automation, generating a pictogram and score, > 60 is practical, shown with five methods. The assessments are provided via an open source desktop and web application [23].

2.4.2. Green Analytical Procedure Index

GAPI is an advanced visual tool designed to assess the environmental impact of spectrophotometric procedures from sampling to final analysis. It evaluates process greenness using a pictogram and color coding across different phases. The environmental impact is measured at each stage with a unique five pentagram symbol, featuring fifteen parameters represented on a color scale: red for high, yellow for medium and green for low impact [24].

2.4.3. Modified Green Analytical Procedure Index

MoGAPI overcomes the limitation of the original GAPI instrument which did not provide an overall numerical score for direct comparison of the methods. The software application evaluates the safety and environmental impact of analytical procedures, applying five pentagrams with fifteen subdivisions for waste management and sample collection, and integrating quantitative measures with visual indicators. Each subchapter earns credits according to how green it is and the overall score is calculated by dividing the credits earned by the maximum credits that could be earned, excluding steps that don't apply [25].

2.4.4. Analytical GREEnness metric

AGREE was founded in 2020 with the goal of improving eco friendly analytical technique based on the 12 principles of green analytical chemistry. It produces a simpler result by using a measure for input and custom weighting of these principles. The overall score is shown by a clock-like graphic, where each principle is graded from 0 to 1. Greenness is represented by color and width, ranging from dark green to red [26].

2.4.5. Analytical GREENness metric for sample preparation

AGREEp is a statistic that assesses how sample preparation techniques affect the environment in accordance with green sample preparation (GSP) principles. It provides energy sustainability grading system from 0 to 1 by analyzing energy use, waste output, and solvent safety using open-source software. To assist researchers in comparing cutting edge techniques with conventional protocols for more environmentally friendly lab operations the tool includes a color coded pictogram [27].

3. Results and discussion

3.1. Selection of detection wavelength

The 200-400 nm range was used to scan spectra of RAN (1000 ng/bands). Since 274 nm was determined to be wavelength, it was chosen as the detecting wavelength.

3.2. Optimization of HPTLC experiments

Ranolazine was effectively separated and quantified by methodically optimizing the HPTLC technique. To produce compact, symmetrical, and well resolved peaks, various chromatographic parameters were examined, including stationary phase, mobile phase composition, chamber saturation time, migration distance and detection wavelength. Precoated silica gel 60 F254 aluminum plates were chosen as the stationary phase because to their superior repeatability and separation efficiency. During method development, a number of solvent solutions containing varying amounts of ethanol, ethyl acetate, N- hexane, chloroform, methanol, and toluene were assessed sharp peaks for ranolazine were produced by the toluene: methanol (6:4) mixture among the investigated the solvent systems. A saturation time of 20 min enhanced resolution and reproducibility with a fixed migration distance of 70 mm ensuring satisfactory drug separation and developing time 7.30 min. Scanning at 274nm showed peak area of ranolazine show in Fig.-2. Under the optimized chromatographic conditions, well resolved peaks were obtained with R_f values of approximately 0.63 ± 0.04 as show in Fig.-3. Developed HPTLC plate visualized under UV light RAN at 274 nm showing in Fig.-4. The optimized HPTLC technique achieved excellent peak, resolution and were validated according to ICH Q2 (R1) guideline, making the method suitable for estimation in pharmaceutical dosage form RAN.

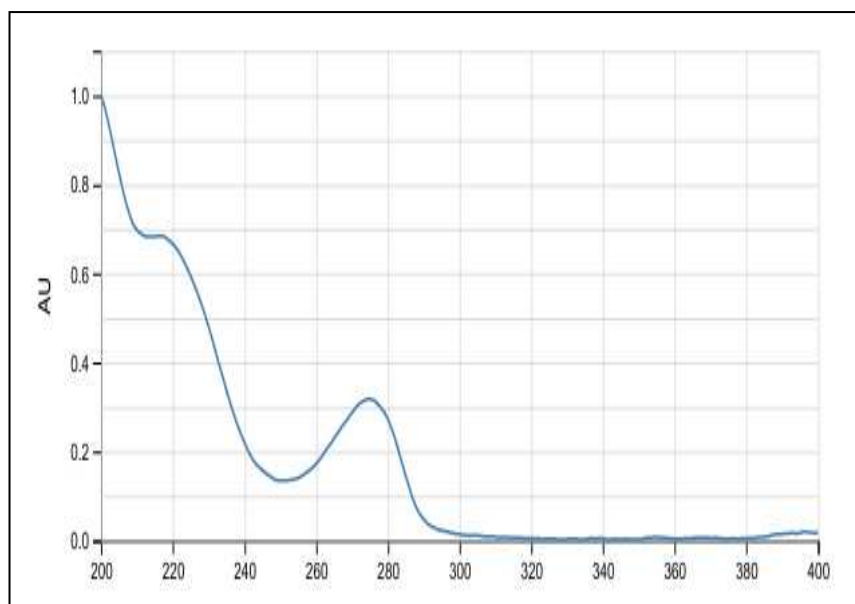


Fig. 2: UV scans of RAN. at 274 nm

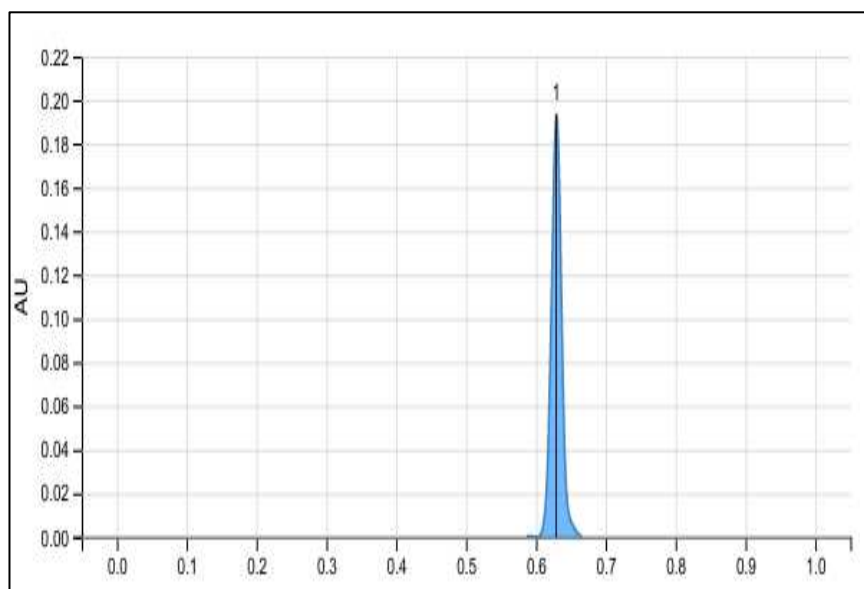


Fig. 3: Densitogram of RAN. (R_f -0.63) at 274 nm

3.3. Method validation

3.3.1. Specificity

The Investigated validated the specificity of the approach by confirming that excipients in the tablet formulation had no effect on the separation of ranolazine. The uniformity of peaks, which is crucial for identifying impurities and guaranteeing reliable analytical data in quantitative studies, was shown in Fig.-7 by peak purity spectra analysis of material.

3.3.2. Linearity and Range

Aliquots of 1, 2, 3,4,5,6,7,8,9 and 10 µl of the working standard solution (0.1 mg/ml) of ranolazine were applied as bands to obtain a concentration range of 100-1000 ng/band. The regression analysis yielded a correlation coefficient (r^2) of 0.9996, indicating excellent linearity between peak area and concentration and regression equation of $y=2.942 \times 10^{-6}x+4.29 \times 10^{-4}$. The calibration curve is depicted in Fig.-5 and the detailed linearity results are summarized in Table 1, UV spectrum show in Fig.-6 and 3D densitogram of RAN at 274 nm show in Fig.-7 and Comparative validation parameter show in table 6.

Table 1: Analytical determination of RAN: HPTLC experiments at 274 nm (n=3)

S.N.	Conc. (ng)	Peak area			Peak area means $\pm$ S.D	% RSD
		(n-1)	(n-2)	(n-3)		
1	100	0.00077	0.00075	0.00074	0.000753 ± 0.0000105	1.39
2	200	0.00100	0.00102	0.00098	0.00100 ± 0.00002	2.0
3	300	0.00129	0.00129	0.00127	0.001283 ± 0.0000115	0.90
4	400	0.00165	0.00162	0.00164	0.001637 ± 0.0000153	0.93
5	500	0.00189	0.00192	0.00191	0.001907 ± 0.0000153	0.80
6	600	0.00219	0.00220	0.00217	0.002187 ± 0.0000153	0.70
7	700	0.00250	0.00251	0.00246	0.002490 ± 0.0000265	1.06
8	800	0.00282	0.00282	0.00280	0.002813 ± 0.0000115	0.41
9	900	0.00309	0.00308	0.00308	0.003083 ± 0.0000058	0.19
10	1000	0.00333	0.00331	0.00334	0.003327 ± 0.0000153	0.46

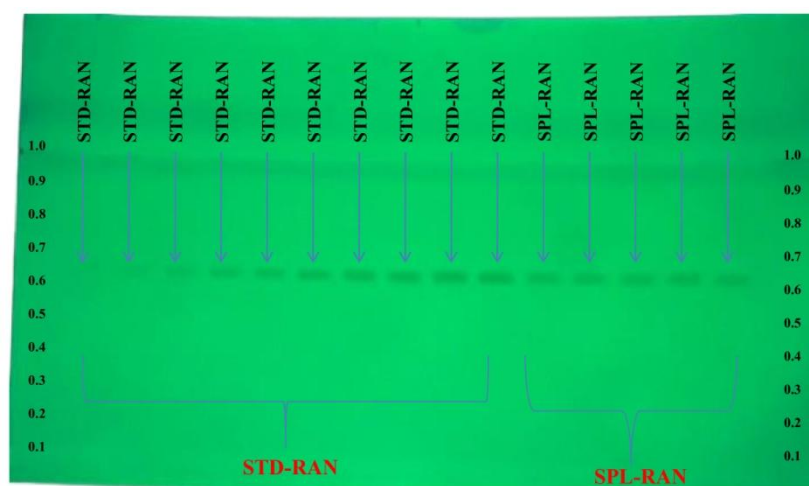


Fig. 4: Developed HPTLC plate visualized under UV light RAN at 274 nm

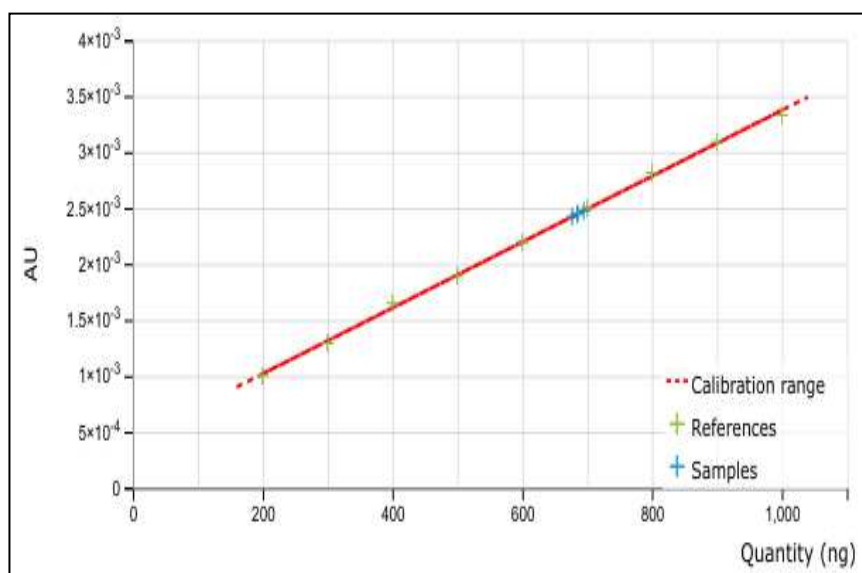


Fig. 5: Linearity image of RAN

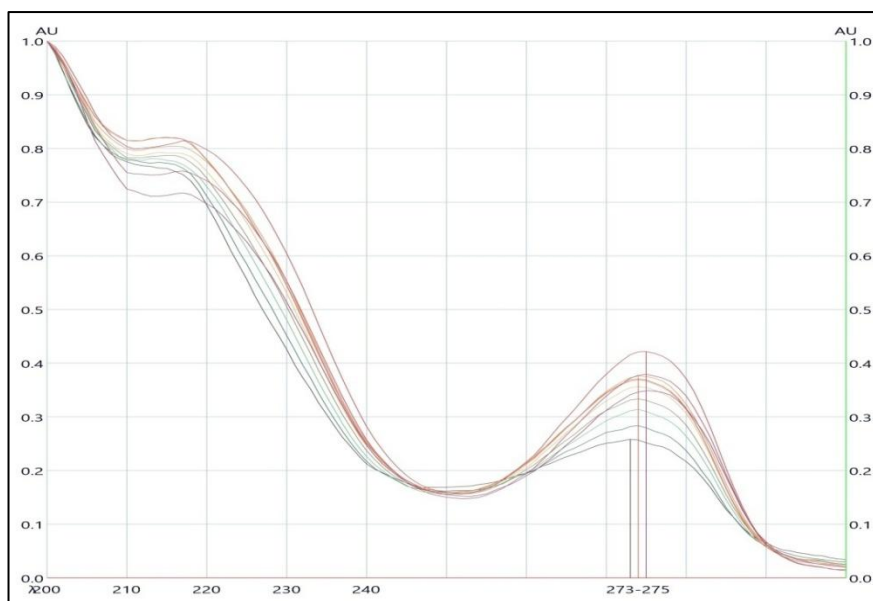


Fig. 6: UV spectrum for RAN

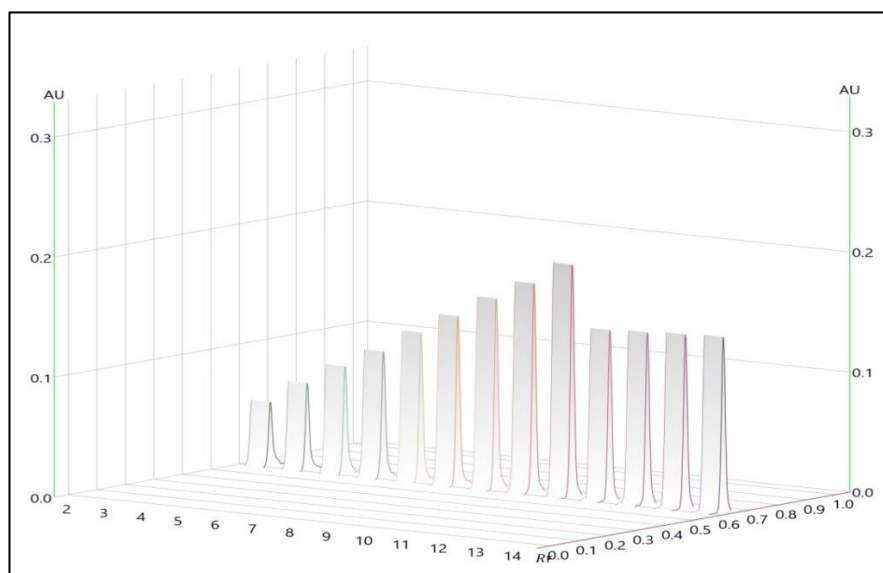


Fig. 7: 3D densitogram of RAN at 274 nm

3.3.3. Precision

Repeatability, intraday, and interday evaluations assessed precision. For repeatability studies, this was found to be 1.32% RSD at 500 ng/band (table 2). For intraday precision studies, which were found to be 0.61%, 0.26% and 1.02% at 400,600 and 800 ng/band and Interday precision studies, the %RSD values were found to be 1.83, 0.70 and 0.41 at 400,600 and 800 ng/band (table 2).

Table 2: Repeatability, Intraday and Interday Precision of RAN at 274 nm

Repeatability								
Conc. (ng)	Peak area						Peak area Mean	% RSD
	1	2	3	4	5	6		
500	0.00192	0.00186	0.00189	0.00189	0.00191	0.00186	0.001888	1.32
Precision								
Conc. (ng)	Intraday precision				Interday precision			
	Peak area mean $\pm$ SD		% RSD		Peak area Mean $\pm$ SD		%RSD	
400	0.001650 $\pm$ 0.0000100		0.61		0.001640 $\pm$ 0.0000300		1.83	
600	0.002197 $\pm$ 0.0000058		0.26		0.002187 $\pm$ 0.0000153		0.70	
800	0.002827 $\pm$ 0.0000289		1.02		0.002813 $\pm$ 0.0000115		0.41	

3.3.4. Accuracy

Accuracy evaluation of the HPTLC method for ranolazine showed excellent recovery rate at 0.62 % RSD for (RENOLZIN-500 SR) and 0.52 %RSD for (RANOMAS-500) at three concentration levels with 2%RSD. The minimal variation between nominal and recovered concentrations confirms the method's reliability and feasibility for routine Ranolazine estimation show in table 3.

Table 3: Pre-analyzed Concentration and Recovery study of RAN (n=3)

Pre-analyzed Concentration of RAN (n=3)							
Formulation		Conc.(ng)	Mean	Pre– analyzed Conc. ± SD	%RSD	Mean Conc. Obtained (%) ± SD	
RENOLZIN-500 SR		300	0.001293	293.78±1.962	0.66	97.92±0.65	
RANOMAS-500		300	0.001313	300.5± 1.962	0.65	99.52 ± 1.23	
Recovery study of RAN (n=3)							
Formulation	Level (%)	Std. Conc. spiked	Total Conc.	Mean Conc. Found	Mean Spiked Conc. Recovered	Mean % Recovery	Overall Mean % Recovery ±%RSD
RENOLZIN-500 SR	50	150	450	443.34	149.563	99.70	98.99±0.62
	100	300	600	589.50	295.723	98.57	
	150	450	750	737.93	444.15	98.70	
RANOMAS-	50	150	450	450.14	149.643	99.76	

500	100	300	600	597.43	296.936	98.97	99.56±0.52
	150	450	750	750.39	449.89	99.97	

3.3.5. Robustness

Robustness method was demonstrated through controlled modification in operational variable such mobile phase, chamber saturation and wavelength. Precision data complied with validation norm, showing %RSD value from 0.44% to 1.60%, significantly under the 2% acceptance threshold show in table 4.

Table 4: Robustness Study of RAN. (300 ng/band)

Parameter	Condition	Mean peak area ± SD	%RSD	Mean R _f
Mobile phase composition (±0.1)	5.9:4.1	0.001293 ±0.000021	1.59	0.67
	6.0:4.0	0.001283 ± 0.0000115	0.90	0.63
	6.1:3.9	0.001303±0.000021	1.60	0.626
Chamber saturation time (±2)	18	0.001287±0.000057	0.44	0.65
	20	0.001283 ± 0.0000115	0.90	0.63
	22	0.00129±0.000057	0.77	0.618
Detection	272	0.001287±0.000057	0.44	0.63
Wavelength (nm) (±2)	274	0.001283 ± 0.0000115	0.90	0.63
	276	0.001303±0.000021	1.60	0.629

3.3.6. Limit of detection and limit of quantification

LOD and LOQ were found to be 12.1ng/band and 36.95ng/band respectively for Ranolazine. The proposed method can detect and quantify small amount of drugs precisely. So, it was concluded that the proposed method is very sensitive in nature.

3.4. Degradation Study

3.4.1. Acid degradation

The densitogram showed peak of RAN at R_f of 0.63 (1) along with one degradation peak at R_f of 0.93 (2) indicating 33.33 % degradation (Fig.-8 and Table 5).

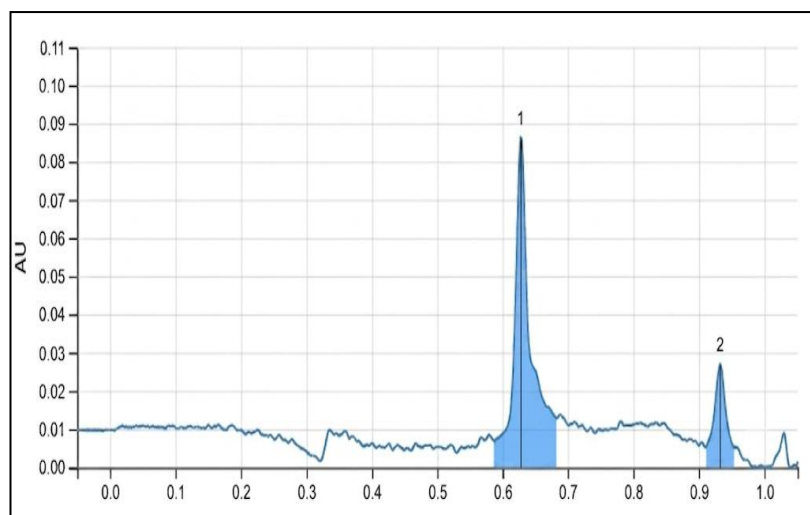


Fig. 8: Densitogram of acid-induced degradation of RAN with one degradant

3.4.2. Alkali degradation

The densitogram showed peak of RAN at R_f of 0.627 along with one peak of degradation product at R_f 0.93 resulting in 20.42% degradation (Fig.- 9 and Table 5).

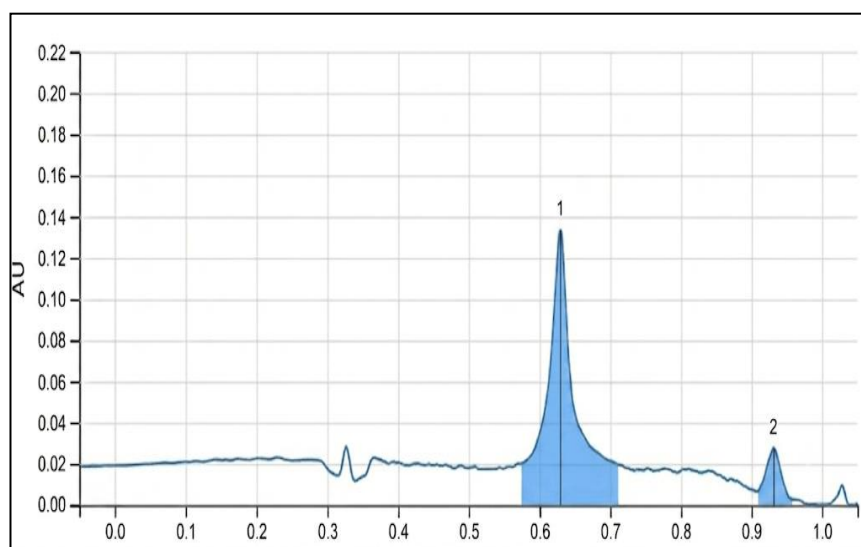


Fig. 9: Densitogram of base-induced degradation of RAN with one degradant

3.4.3. Neutral degradation

The densitogram showed peak of RAN at R_f of 0.66 along with one degradation peak at R_f 0.96 (2) resulting in 54.95% degradation (Fig.-10 and Table 5).

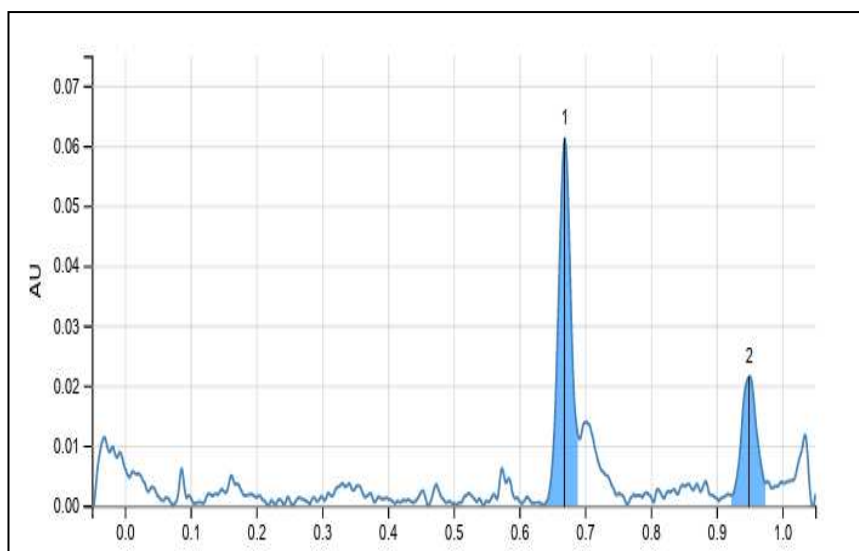


Fig. 10: Densitogram of neutral degradation of RAN with one degradant

3.4.4. Photolytic degradation

The densitogram showed peak of RAN at R_f of 0.63 along with one degradation peak at R_f 0.92 and 1.0 (2) resulting in 30.51% and 5.4 % degradation (Fig.-11 and Table 5).

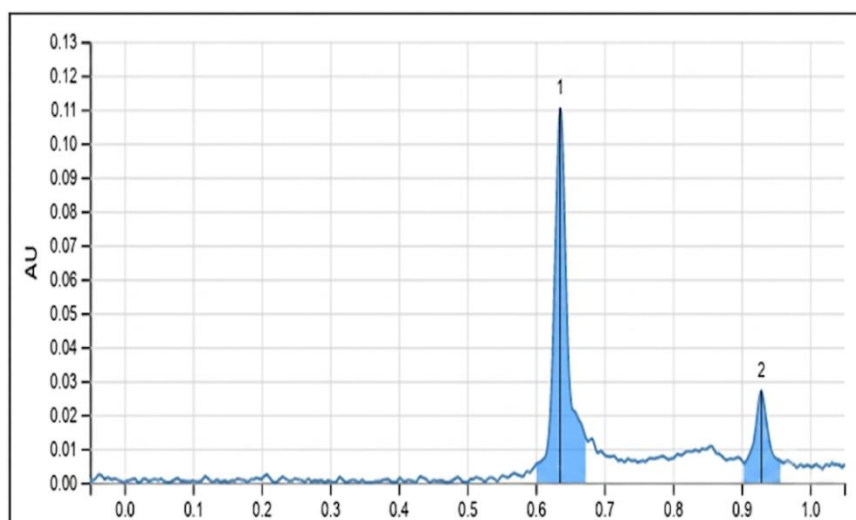


Fig. 11: Densitogram of Photolytic degradation of RAN with one degradant

3.4.5. Thermal degradation

The densitogram showed peak of RAN at R_f of 0.63 along with one degradation peak at R_f 0.926 (2) resulting in 36.93 % degradation (Figure-12 and Table 5).

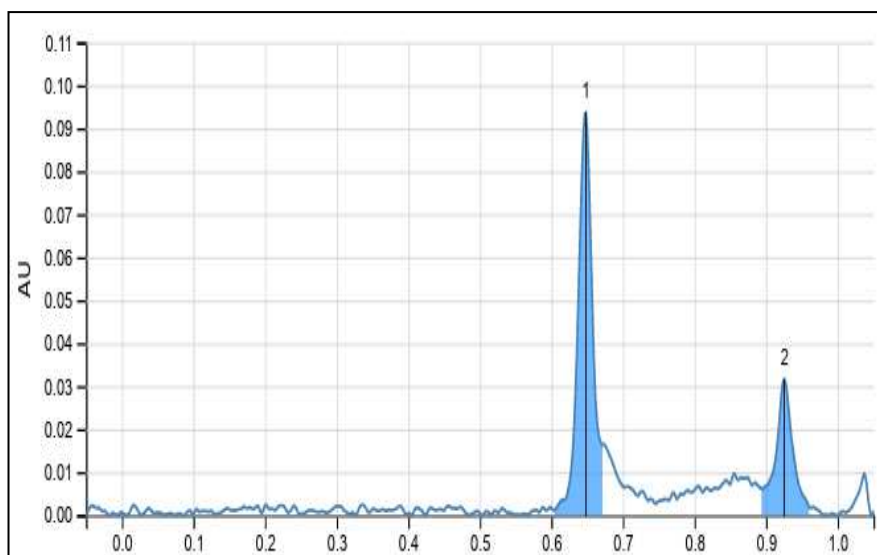


Fig. 12: Densitogram of thermal degradation of RAN with one degradant

Table 5: Summary of forced degradation studies of RAN

Degradation Condition	% Recovery	% Degradation	R _f of RAN. in densitogram	R _f of degradants
Acid degradation	66.67	33.33	0.63	0.93
Alkali degradation	79.58	20.42	0.627	0.932
Neutral degradation	45.05	54.95	0.66	0.96
Photolytic degradation	69.09	30.51	0.638	0.92
Thermal degradation	60.07	39.93	0.63	0.926

Table 6: Comparison of validation parameters of HPTLC method developed and reported in present work by us and other research groups for RAN.

Parameter	HPTLC (Reported in present work)	HPTLC (Reported by Rahul et. Al (28))	HPTLC (Reported by Khedkar A N et. al (29))	HPTLC (Reported in Abburu et. Al (30))
Mobile phase	Toluene: Methanol (7:3)	Methanol: 10 milli molar ammonium acetate	Chloroform: methanol: toluene (5:1:1)	butanol–acetic acid–water (6:2:2 v/v)
Linearity range (ng)	100-1000	1000-6000	500-3500	100-400
Slop	2.942×10^{-6}	1.393	-	8.68

Intercept	4.29×10^{-4}	190.47	-	492.1
Correlation Coefficient	0.999	0.998	0.999	0.99912
Intraday Precision % RSD	0.63	0.07624	7.17	-
Intraday Precision % RSD	0.98	0.07624	4.51	-
Detection wavelength (nm)	274	271	273	270
LOD (ng)	12.1	25.31	250	14.90
LOQ (ng)	36.95	76.711	400	49.67

3.5. Greenness assessment of present investigations

GAC is becoming a major advancement in spectrophotometric research with the goal of satisfying current demands without sacrificing those of future generations. Developing a new analytical methodology that complies with environmentally friendly standards established by sustainable practices is necessary to achieve these objectives. These guidelines place a strong emphasis on analytical chemistry procedures that use cleaner, less hazardous solvents, guarantee effective analysis speed, and produce less waste BAGI (b), GAPI (c), MoGAPI (d), AGREE (e), AGREEprep (f) show in Fig.-13 (18).

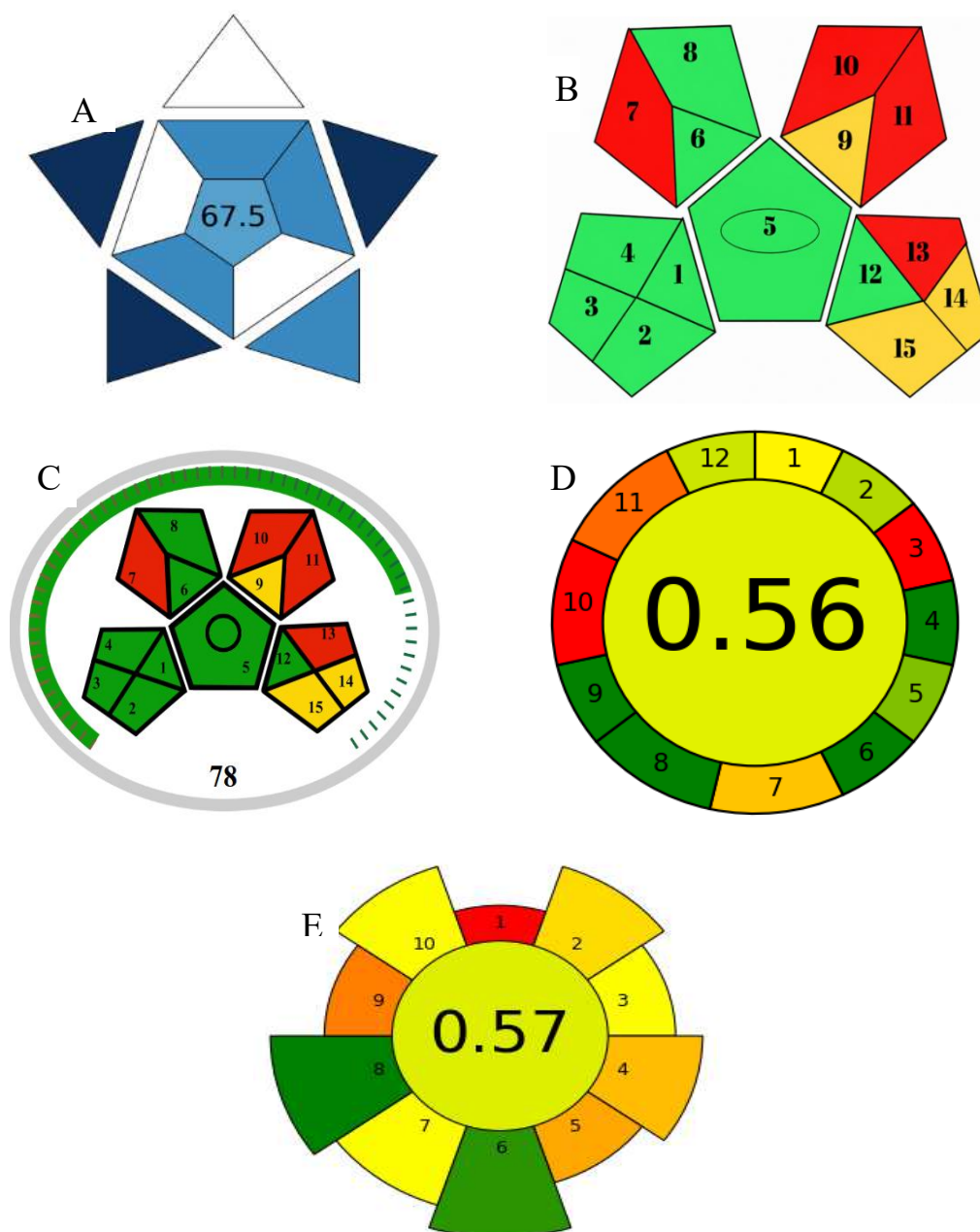


Fig. 13: BAGI (A), GAPI (B), MoGAPI (C), AGREE (D), AGREEprep (E)
Structure

3.5.1. Blue Applicability Grade Index

In the assessed HPTLC method, the BAGI score indicated a moderate to good environmental acceptability. Advantages include low sample and solvent usage, simplicity, low energy requirements, minimal waste, and high efficiency, contributing to its sustainability. However, the use of toxic organic solvents like toluene and methanol slightly reduced the greenness score. Overall, the method is deemed partially green with an acceptable environmental impact according to BAGI evaluation.

3.5.2. Green Analytical Procedure Index

The Green Analytical Procedure Index (GAPI) assesses the environmental impact of analytical methods across their workflow. It features a pictogram with 15 segments color-coded to indicate impact levels green for low, yellow for moderate, and red for high. The analyzed method, though limited in steps, uses hazardous organic solvents such as methanol and toluene, resulting in a moderate impact. While sample handling may fall into yellow to red zones due to solvent usage, instrumentation and lower waste generation contribute to green zones. Overall, the method is considered partially green due to hazardous solvent use despite its fewer process steps.

3.5.3. Modified GAPI

The environmental friendliness of the developed HPTLC method was evaluated by the application of the Modified Green Analytical Procedure Index (MoGAPI) and was found to have a low overall environmental impact, mainly in the green and yellow zones. Some areas were marked red due to harmful organic solvents such as methanol and toluene. However, the method is beneficial because of the low volume of sample, less use of solvents, less waste, and easy sample preparation, which supports its green assessment. Thus, the HPTLC method is considered as an environmentally acceptable and partly green method with a good sustainability profile

3.5.4. AGREE

The AGREE analytical calculator, which considered all 12 GAC principles, was used to assess the HPTLC approach that was created. The GREENness measure score is 0.56 overall.

3.5.5. AGREEprep

The AGREEprep metric evaluated the environmental impact of a HPTLC method for ranolazine, yielding a high eco-friendliness score. The procedure involves simple dilution, leading to low sample volume and minimal solvent use, which reduces chemical waste. While methanol is used, its limited application minimizes the environmental impact. This method is efficient, cost-effective, and aligns with green chemistry principles, as indicated by the AGREEprep evaluation.

4. Conclusion

A quick, accurate and cost effective high performance thin layer chromatography approach dose for the estimation of ranolazine in their tablet dosage form was

successfully developed and validated in this work. From the above study; we can conclude that RAN undergoes degradations. All degradation products formed are well resolved from the drug response. This method was validated according to ICH guidelines, demonstrating satisfactory results in aspects such as linearity, accuracy, precision, specificity, robustness, LOD, and LOQ. The method is suitable for the quantitative determination of Ranolazine in bulk and tablet forms, utilizing readily available solvents with minimal sample preparation. No interference from tablet excipients was noted, confirming specificity. The developed method was evaluated from the perspective of green analytical chemistry using established greenness assessment tools. The method satisfied, to obtained an AGREE score of 0.56, AGREEprep score of 0.57, GAPI, BAGI and MoGAPI indicating moderate greenness for environmental acceptability method.

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Declaration of competing interest

The authors state that none of the work described in this study was influenced by any known competing financial interests or personal relationships.

Data availability

The research described in the article did not use any data.

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