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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF RABEPRAZOLE AND PANTOPRAZOLE USING RP-HPLC

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ABSTRACT

Introduction: A new RP-HPLC method was developed for simultaneously estimating Rabeprazole and Pantoprazole. Separation was achieved using a Thermosil C18 column (4.0×125mm, 5µ) with a 0.7 ml/min flow rate and a mobile phase ratio of 70:30 (methanol: Sodium acetate buffer pH 3, adjusted with ortho phosphoric acid). Detection occurred at 252nm. The equipment used included the WATERS HPLC Auto Sampler, Separation module 2690, photo diode array detector 996, and Empower software version 2. Retention times were 2.566 mins for Rabeprazole and 3.417 mins for Pantoprazole. Purity percentages were 101.27% for Rabeprazole and 99.97% for Pantoprazole. System suitability parameters (theoretical plates, tailing factor, and resolution) were 4668, 1.3, and 6.0 for Rabeprazole and 6089, 1.2, and 6.0 for Pantoprazole. The method validation, following ICH guidelines, showed linearity in the 5-25µg range for Rabeprazole and 50-250µg for Pantoprazole, both with a correlation coefficient (r^2) of 0.999. Recovery rates were 99.56% and 99.48%, with %RSD for repeatability at 0.86 and 0.82, and for intermediate precision at 0.44 and 0.19, respectively. The method proved precise, robust, and repeatable, with LOD values of 3.17 and 5.68, and LOQ values of 0.0172 and 0.2125, respectively.

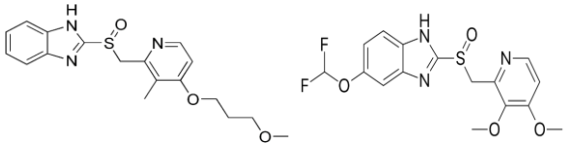
KEYWORDS: Rabeprazole, Pantoprazole, Repeatability, Linearity, Correlation.

INTRODUCTION

Rabeprazole, 2-[[4-(3-methoxypropoxy)-3-methylpyridin -2-yl] methyl sulfinyl]- 1H-benzimidazole [Figure 1] is a proton pump inhibitor that selectively and irreversibly inhibits the gastric H+/K+ ATPase (IC50 = 72 nm). Formulations containing rabeprazole have been used in the treatment of ulcers, pathological hypersecretory conditions, and gastroesophageal reflux disease (GERD). Pantoprazole, 6-(Difluoro methoxy)-2-[(3,4-dimethoxy pyridin-2-yl) methyl sulfinyl]-1H-benzo[d]imidazole [Figure 2] is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H+,K+)-ATPase enzyme system at the secretory surface of the gastric parietal cell. This effect is dose- related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus.[1, 2]

A literature review indicates no existing RP-HPLC method for simultaneous analysis of Rabeprazole and Pantoprazole. Existing methods use spectrophotometry, HPLC, and HPTLC for these drugs individually or with other compounds. Therefore, a new RP-HPLC method is needed for their simultaneous estimation in pharmaceutical forms. This work aims to develop and validate a simple, rapid, accurate, and reproducible RP-HPLC method for this purpose, following ICH guidelines.[3]

RP-HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS ESTIMATION OF RABEPRAZOLE AND PANTOPRAZOLE



Rabeprazole **Pantoprazole**

MATERIALS AND METHODS

Instruments: In this study the present was carried on HPLC (WATERS), which comes with an auto sampler injector with variable UV detector and with a software of Empower-2 and U.V double beam spectrometer.

Reagents & Chemicals: Rabeprazole and Pantoprazole were obtained as gifted samples. Methanol, Acetonitrile is of HPLC grade and MilliQ water was used. For the assay, Ortho phosphoric acid, Potassium hydrogen Phosphate, Dipotassium hydrogen phosphate, 0. 22µ Nylon filter, 0.45µ filter paper were used.

Mobile phase preparation: Prepare a 30% sodium acetate buffer solution in HPLC-grade methanol (70% v/v). Cleavage of siloxane linkages affects silica dissolution and elution properties by controlling ionization. Degas the mixture in an ultrasonic water bath for 5 minutes, then filter through a 0.22 µm filter using vacuum filtration.

Phosphate buffer preparation: Weigh 6.8 grams of sodium acetate and place it in a 1000 ml beaker. Dissolve and dilute it to 1000 ml with HPLC water, then adjust the pH to 3 using orthophosphoric acid. Sonicate and filter the resulting solution.

Diluent's preparation: Mobile phase was used as the diluent.

Preparation of Solutions

Preparation of Pantoprazole Standard Solution

Accurately weigh 10 mg of Pantoprazole working standard and transfer it to a clean, dry 10 ml volumetric flask. Add approximately 2 ml of diluent, sonicate until completely dissolved, and then fill to the mark with the same solvent to create the stock solution. From this stock solution, pipette 1.5 ml into another 10 ml volumetric flask and dilute to the mark with diluent.

Preparation of Rabeprazole Standard Solution

Accurately weigh 10 mg of Rabeprazole working standard and transfer it to a clean, dry 10 ml volumetric flask. Add approximately 2 ml of diluent, sonicate until completely dissolved, and then fill to the mark with the same solvent to create the stock solution. From this stock solution, pipette 3 ml into another 10 ml volumetric flask and dilute to the mark with diluent.

RESULTS AND DISCUSSION

This investigation aimed to develop and validate an RP-HPLC method for the simultaneous estimation of Rabeprazole and Pantoprazole. Literature indicates no existing RP-HPLC methods for this purpose, highlighting the need for development.

Method Development

To select the detection wavelength, Rabeprazole and Pantoprazole were dissolved in the mobile phase at 10 µg/ml concentrations and scanned from 200-400 nm. The isobestic point at 252 nm was identified. After several trials with different columns, flow rates, and mobile phases, an optimized RP-HPLC method was established for the separation and quantification of Rabeprazole and Pantoprazole in API and pharmaceutical forms.[4,9]

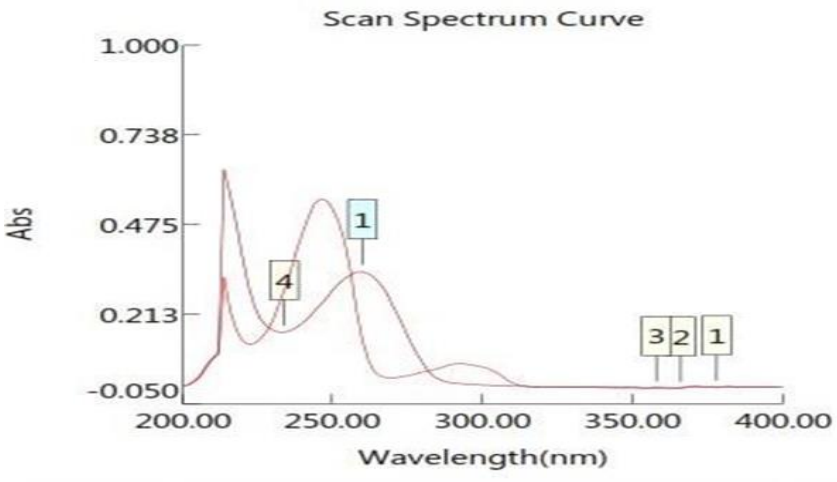


Fig. No. 1: Spectrum showing overlapping spectrum of Rabeprazole and Pantoprazole.

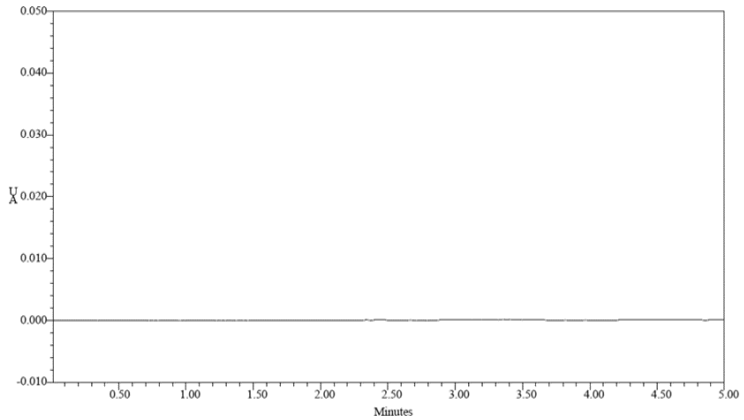


Fig. No. 2: Chromatogram showing blank preparation (mobile phase).

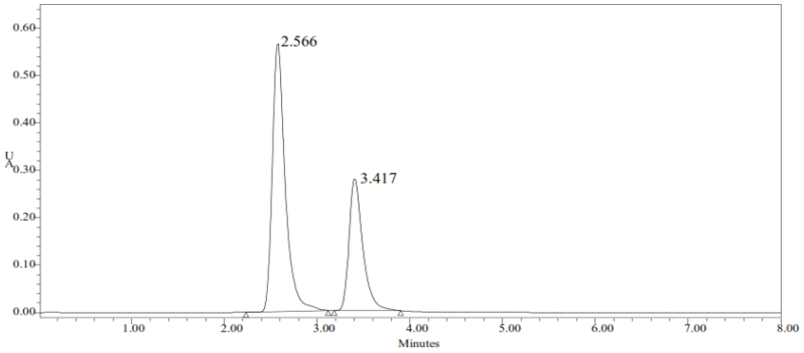


Fig. No. 3: Chromatogram showing Simultaneous estimation of sample preparation.

Method Validation

Specificity: System suitability for specificity was tested by injecting a blank to check for impurity interference at the analytical peak's retention time. The test for Rabeprazole and Pantoprazole showed no impurity interference.

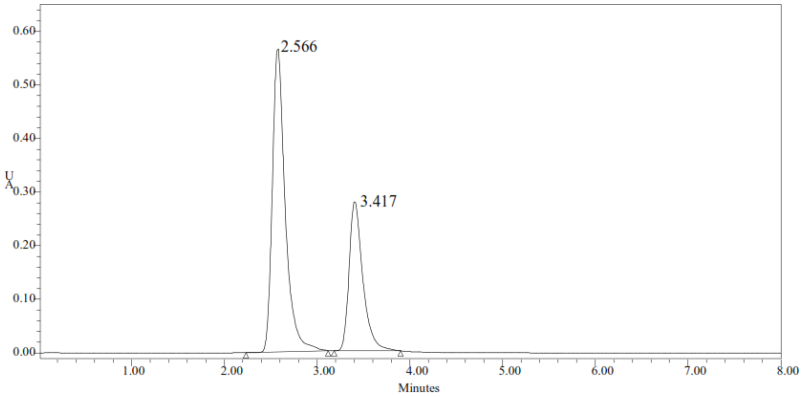


Fig. No. 4: Chromatogram showing standard injection.

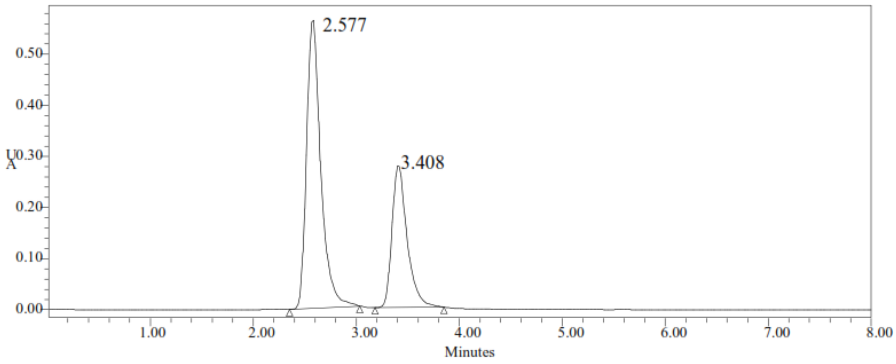


Fig. No.5: Chromatogram showing sample injection.

SYSTEM SUITABILITY

Table No. 1: Results for system suitability of Rabeprazole.

Injection	RT(min)	Peak area	TP	TF
1	2.432	124452	1564.32	1.24
2	2.531	124857	1534.54	1.21
3	2.746	121804	1523.37	1.31
4	2.754	128383	1523.74	1.24
5	2.799	123154	1560.38	1.31
6	2.782	125344	1535.87	1.25
Mean		126635	-	-
SD		521.0		-
%RSD		0.5	-	-

Table No.2: Results for system suitability of Pantoprazole.

Injection	RT(min)	Peak area	TP	TF
1	5.233	434308	4415.31	1.16
2	4.012	436747	4332.43	1.16
3	4.512	436752	4322.54	1.16
4	4.522	498950	4314.17	1.16
5	4.055	458626	4321.21	1.16
6	4.053	445285	4387.14	1.17
Mean		44531.2	-	-
SD		1237.3	-	-
%RSD		0.2	-	-

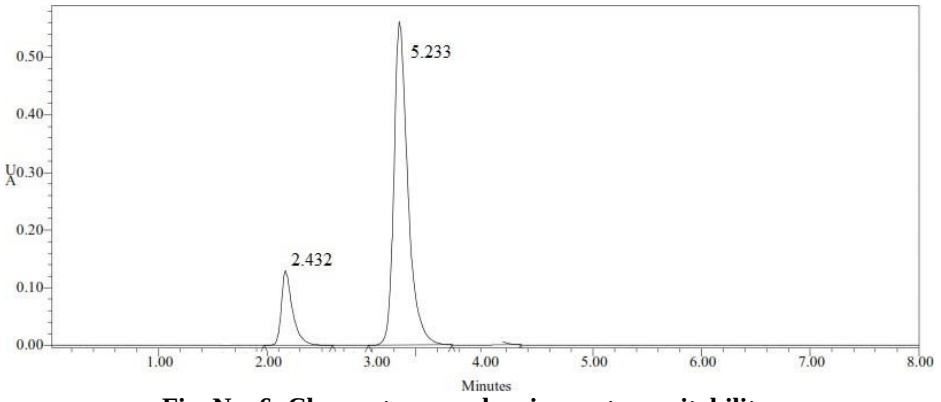


Fig. No. 6: Chromatogram showing system suitability.

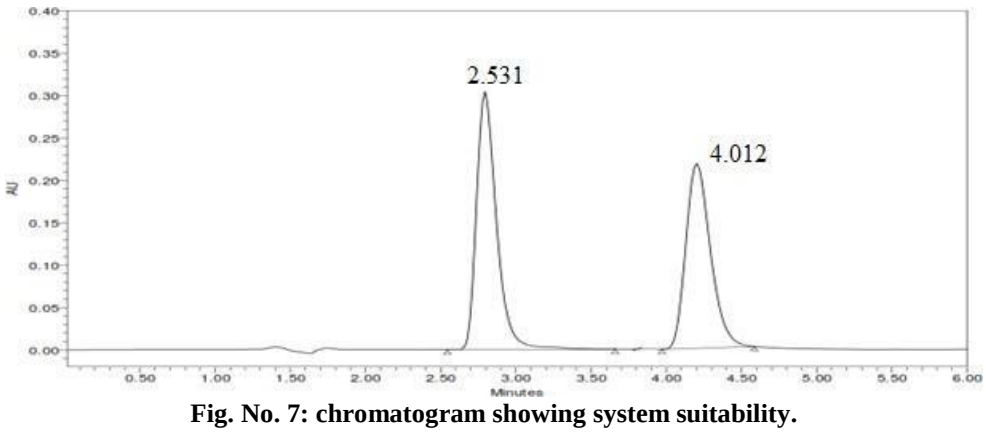


Fig. No. 7: chromatogram showing system suitability.

Linearity: The linearity study for Rabeprazole (5-25 µg) and Pantoprazole (50-250 µg) showed a correlation coefficient of 0.999 for both, indicating excellent linearity. Each concentration level was injected into the chromatographic system, and the area was used to calculate the correlation coefficient. The chromatograms are shown in below figures and results are tabulated in below. Calibration graph for Rabeprazole and Pantoprazole are shown below.

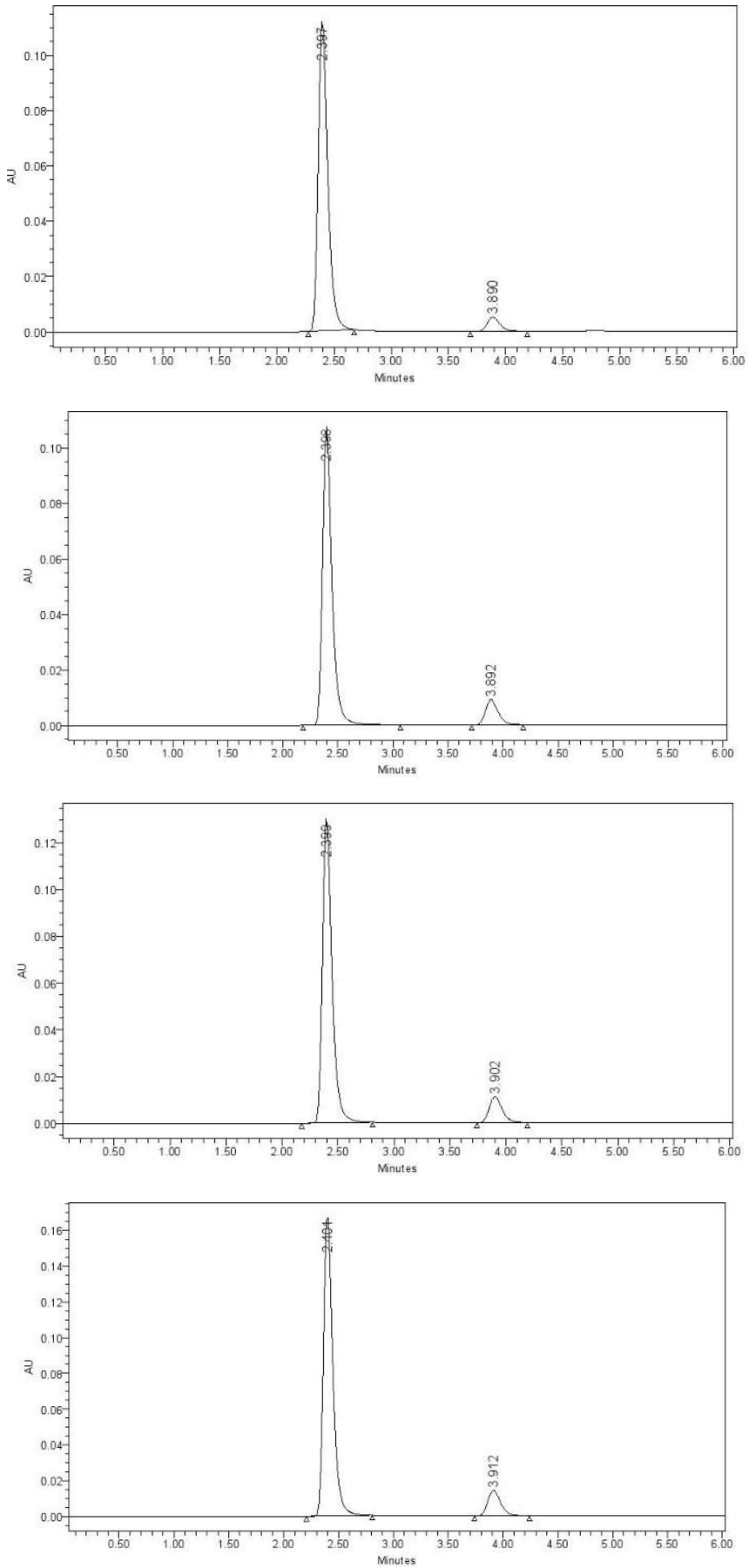


Fig. No. 8: Chromatograms showing linearity level-1 to level 5 (5ppm-25 ppm of Rabeprazole and 50ppm - 250ppm of Pantoprazole) injections.

Table No. 3: Linearity Results of Rabeprazole.

S. No	Linearity Level	Concentration	Area
1	I	5 ppm	471543
2	II	10 ppm	656277
3	III	15 ppm	794999
4	IV	20 ppm	946124
5	V	25 ppm	1002139
Correlation Coefficient			0.999

Rabeprazole r2 = 0.999.

Table No. 4: Linearity Results of Pantoprazole.

S. No	Linearity Level	Concentration	Area
1	I	5 ppm	471543
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3	III	15 ppm	794999
4	IV	20 ppm	946124
5	V	25 ppm	1002139
Correlation Coefficient			0.999

Pantoprazoler2 = 0.999.

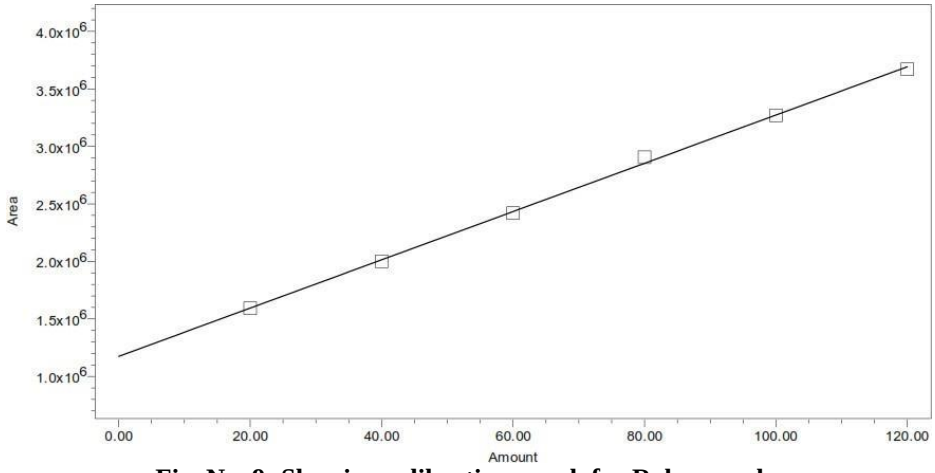


Fig. No. 9: Showing calibration graph for Rabeprazole.

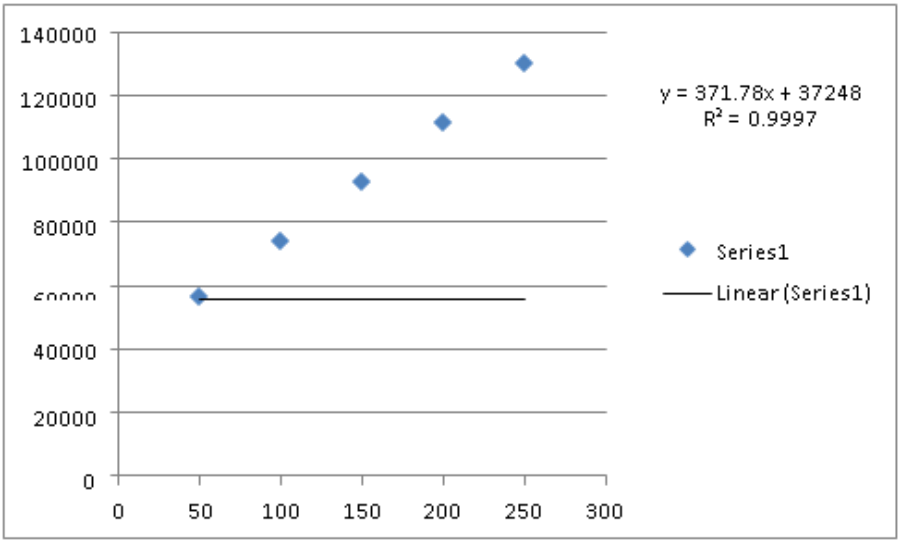


Fig. No. 10: Showing calibration graph for Pantoprazole.

Accuracy: The accuracy study for Rabeprazole and Pantoprazole at 50%, 100%, and 150% levels was conducted in triplicate. The % recovery was 99.56% for Rabeprazole and 99.47% for Pantoprazole, meeting the criteria of NLT 98% and NMT 102%.

Table No. 5: Details of Accuracy at 50%.

Inj	Name	RT	Area	Height
1	Rabeprazole	2.563	1380019	154378
2	Rabeprazole	2.561	1375579	157824
3	Rabeprazole	2.559	1418031	153550
4	Pantoprazole	3.431	480884	42541
5	Pantoprazole	3.467	480319	41325
6	Pantoprazole	3.431	480227	27655
Mean			839330.4	
SD			502815.3	
%RSD			0.535	

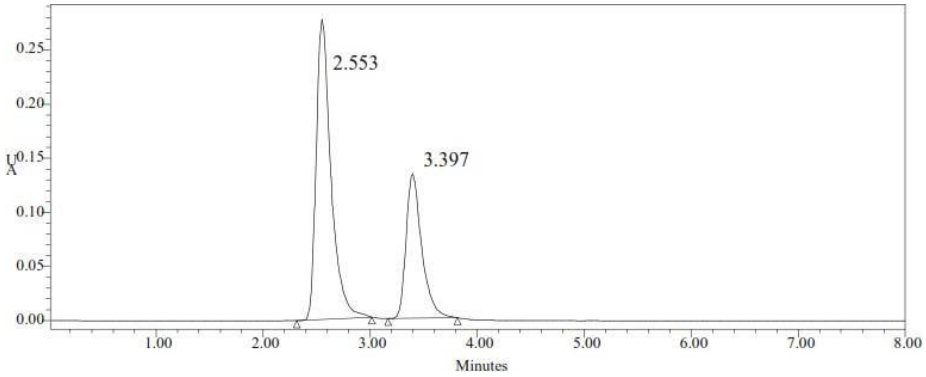
Table No. 6: Details of Accuracy at 50%.

Inj	Name	RT	Area	Height
1	Rabeprazole	2.553	7.3483	85023
2	Rabeprazole	2.554	7.6284	85462
3	Rabeprazole	2.564	7.3004	84162
4	Pantoprazole	3.397	342518	27155
5	Pantoprazole	3.413	328965	21606
6	Pantoprazole	3.519	368858	21832
Mean			841512.5	
SD			2538864.4	
%RSD			0.438	

Table No. 7: Details of Accuracy at 150%.

Inj	Name	RT	Area	Height
1	Rabeprazole	2.574	2208291	241385
2	Rabeprazole	2.573	2188199	253406
3	Rabeprazole	2.644	2733155	258276
4	Pantoprazole	3.436	7321448	597236
5	Pantoprazole	3.439	7342578	516428
6	Pantoprazole	3.537	7452861	548321
Mean			1482671.3	
SD			748319.25	
%RSD			0.572	

Accuracy – 100%
Accuracy – 150%.



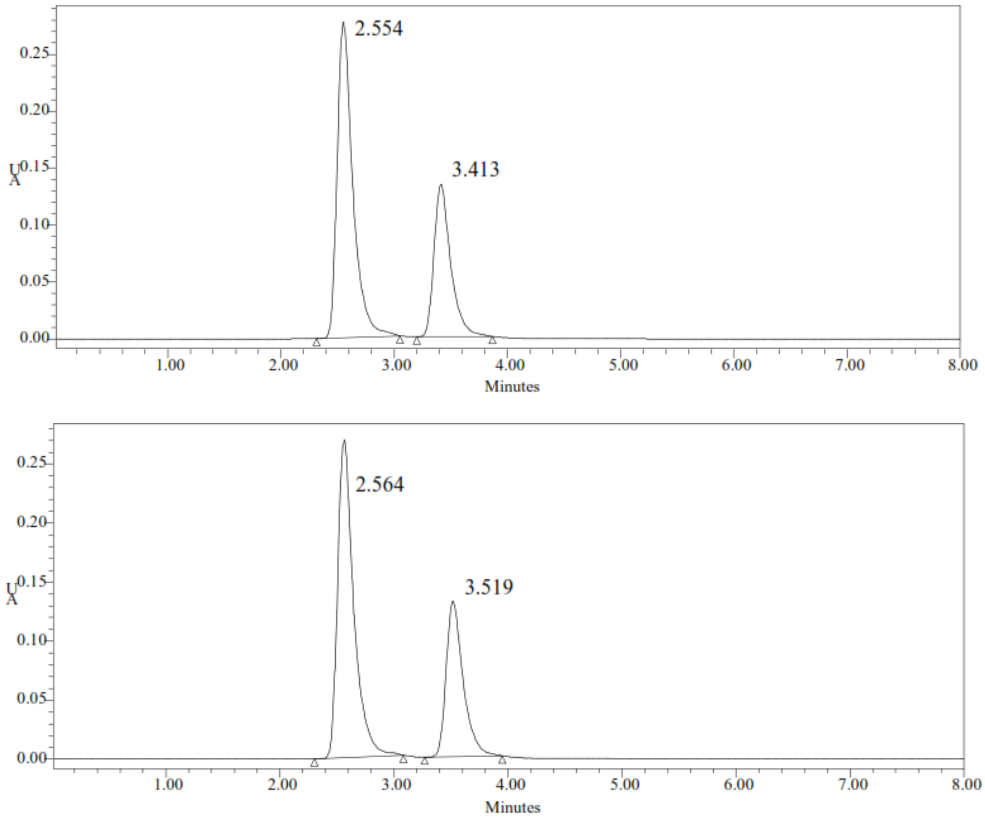
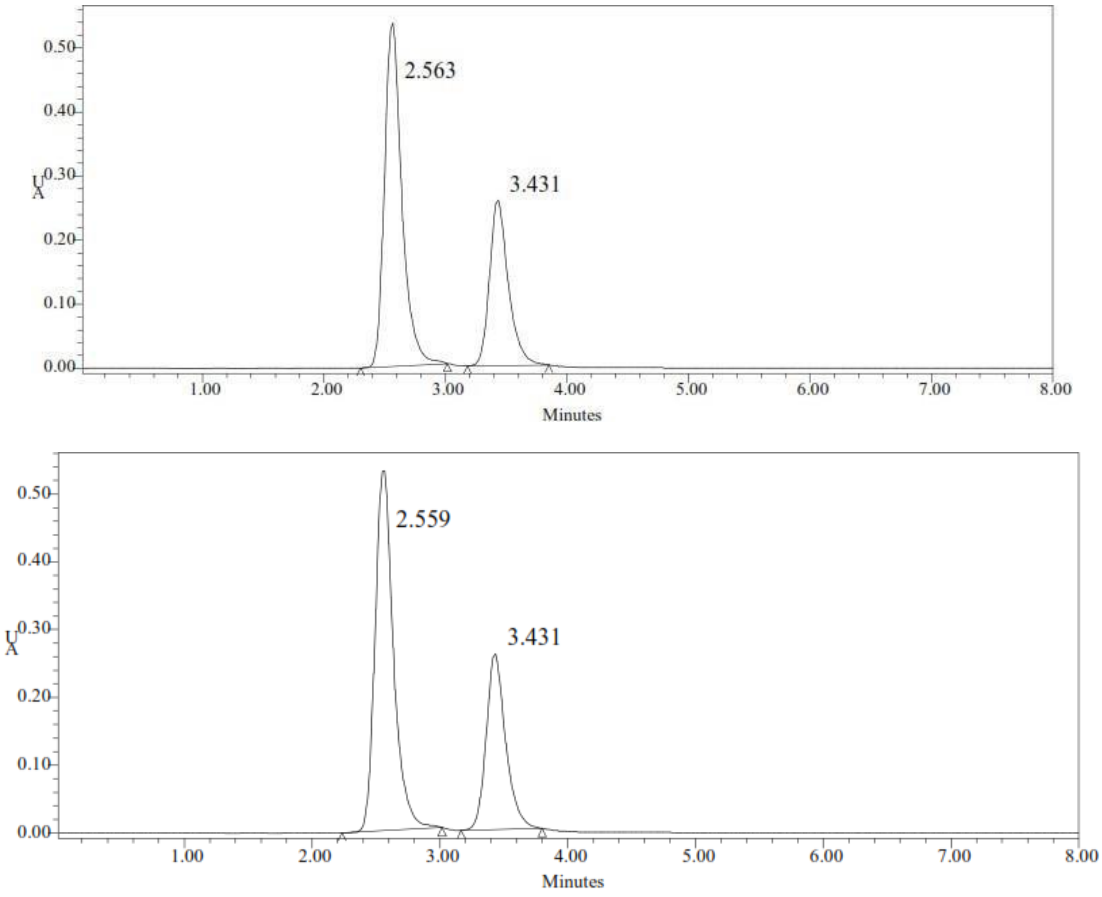


Fig. No. 11: Chromatograms showing accuracy-50% injection-1, 2, 3.



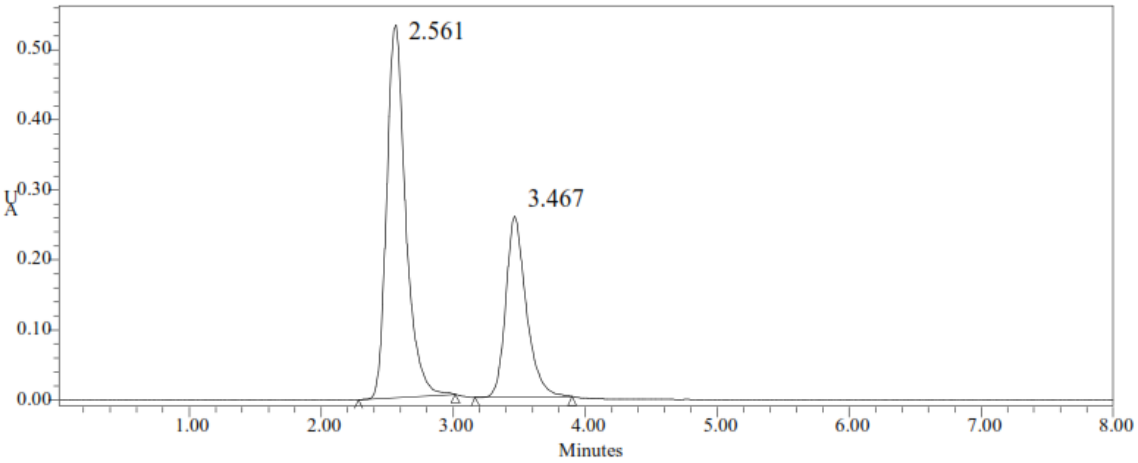


Fig. No. 12: Chromatogram showing accuracy -100%injection-1, 2, 3.

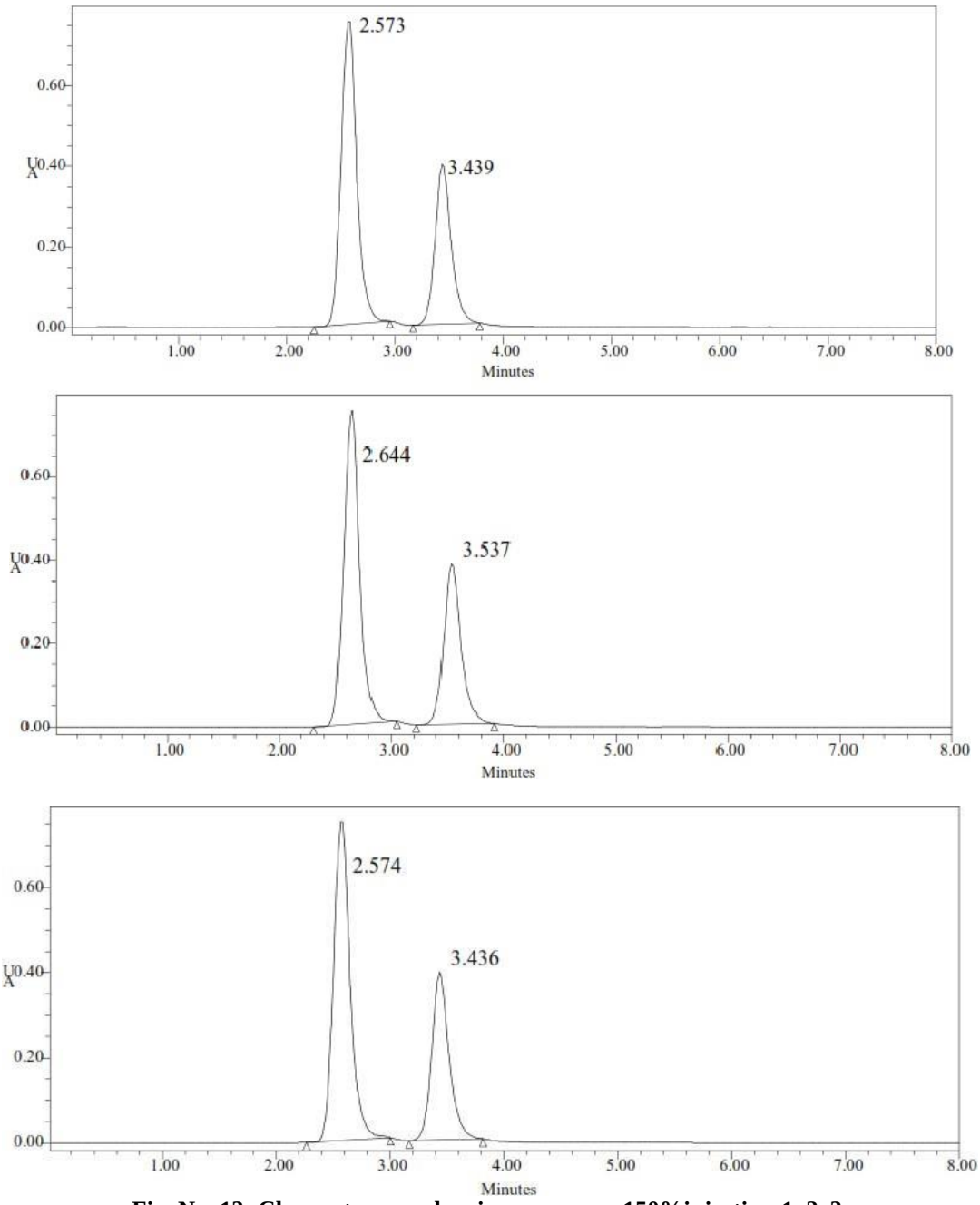


Fig. No. 13: Chromatogram showing accuracy -150%injection-1, 2, 3.

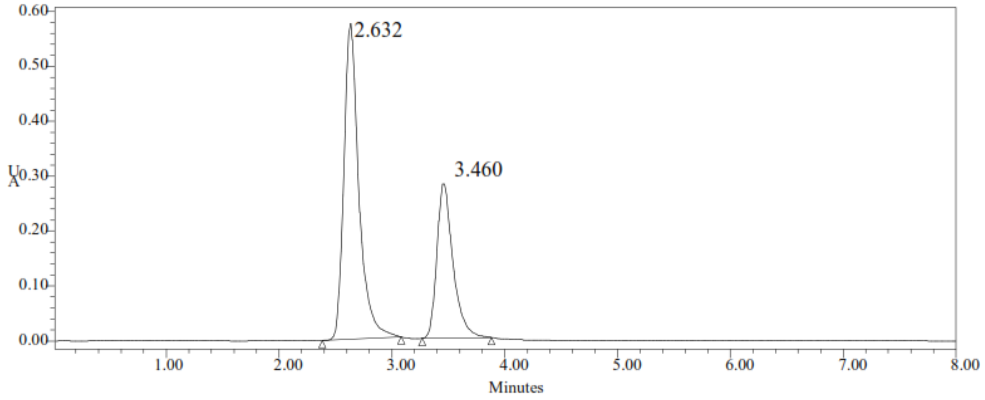
Table No. 8: Showing accuracy results for Rabeprazole.

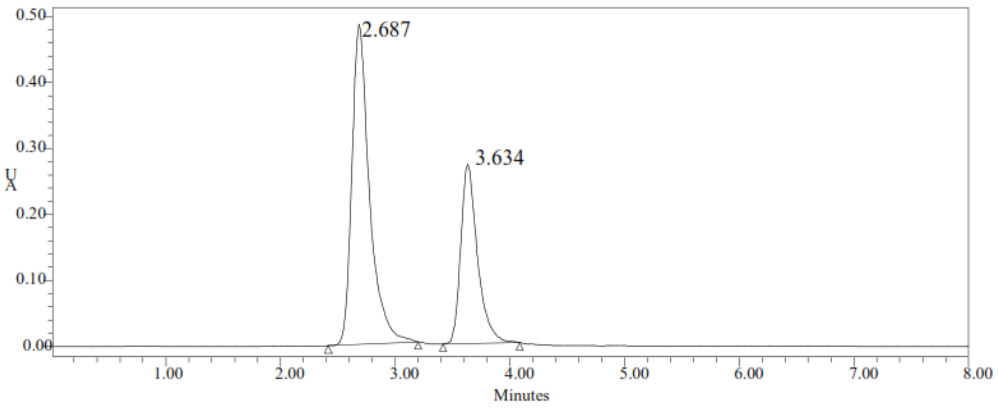
%Conc. (at specific level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50	2630409	5	4.96	99.91%	99.56%
100	5277055	10	9.98	99.18%	
150	7514836	15	15.02	99.60%	

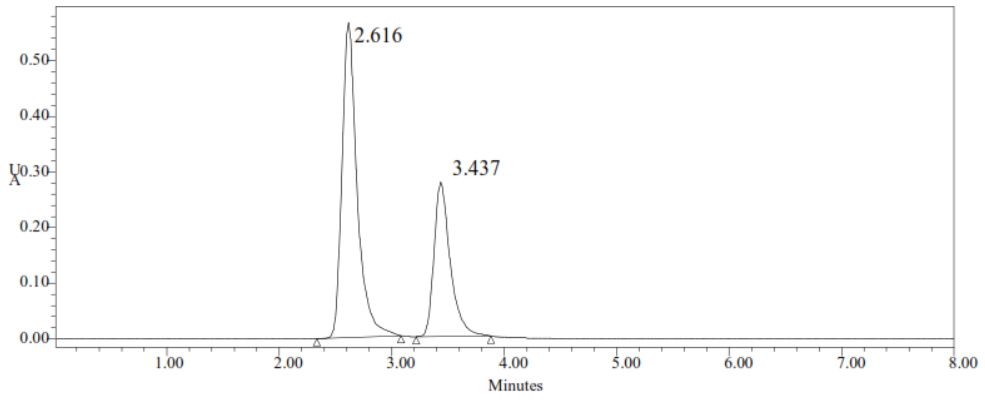
Table No. 9: Showing accuracy results for Pantoprazole.

%Conc. (at specific level)	Average Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean Recovery
50%	1366666	0.5	0.99	99.53%	99.47%
100%	2777487	1.0	1.05	99.38%	
150%	4151234	1.5	1.495	99.52%	

Precision: The precision study, conducted with five injections of Rabeprazole and Pantoprazole, resulted in %RSD values of 0.82 and 0.86, respectively, meeting the NMT 2% criterion.[10.15]







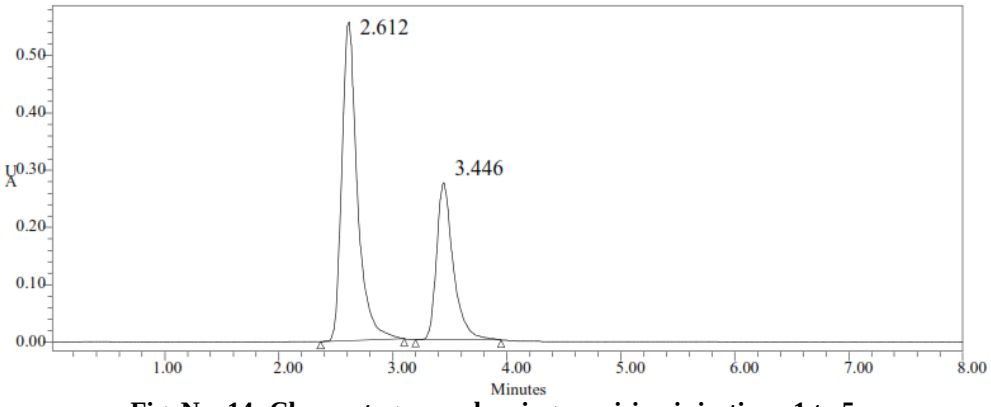


Fig. No. 14: Chromatograms showing precision injections-1 to 5.

Table No. 10: Showing% RSD results for Pantoprazole.

Peak Name: Rabeprazole				
S. No	**Name**	**RT**	**Area**	**Height**
1	Rabeprazole	3.616	2742453	238643.4
2	Rabeprazole	3.634	276250	271543.5
3	Rabeprazole	3.460	2797670	281711.6
4	Rabeprazole	3.446	2793578	274499.8
5	Rabeprazole	3.437	2778483	276713.0
Mean			2774987	
SD.			22806.9	
% RSD			0.82	

Table No. 11: Showing% RSD results for Rabeprazole.

Peak Name: Pantoprazole				
S. No	**Name**	**RT**	**Area**	**Height**
1	Pantoprazole	2.755	5223559	541538.3
2	Pantoprazole	2.687	5208511	485548.5
3	Pantoprazole	2.632	5323569	574440.4
4	Pantoprazole	2.612	5259147	557413.5
5	Pantoprazole	2.616	5273463	565020.1
Mean			5257650	
SD			45206.4	
% RSD			0.86	

Robustness: The robustness study tested flow rate variations from 0.4 ml/min to 0.6 ml/min and mobile phase ratio variations for Rabeprazole and Pantoprazole. The method proved robust at ±5% mobile phase changes but sensitive to flow rate changes, being robust only at lower flow rates. The results indicate that flow rate variations significantly affect the method. The method is robust at ±0.2 ml/min flow rate changes, but only under lower flow conditions.

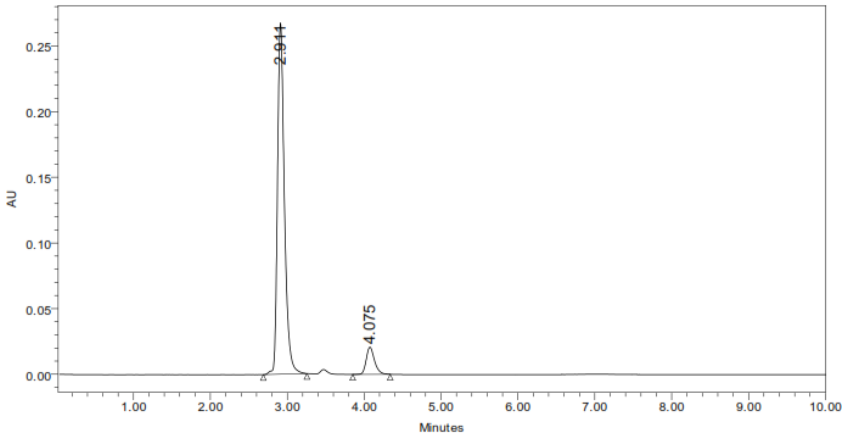


Fig. No. 15: Chromatogram showing less flow rate 0.8ml/min.

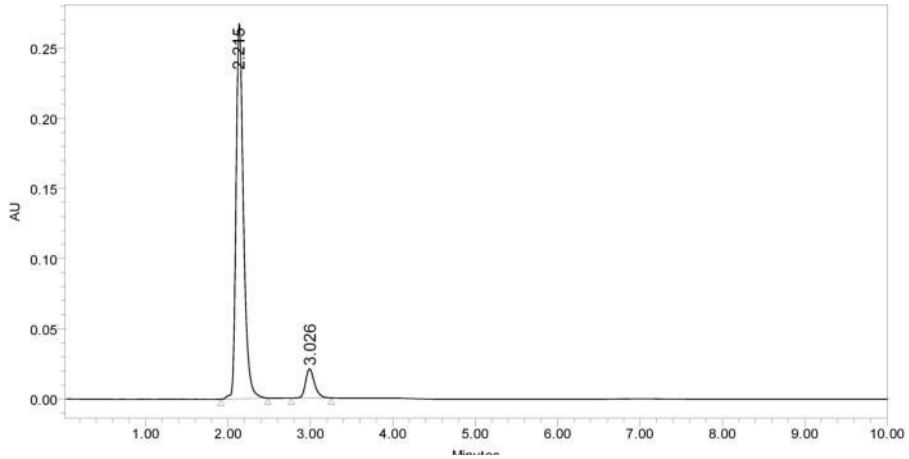


Fig. No. 16: Chromatogram showing more flow rate 1.2 ml/min.

Table No. 12: Details of Robustness Less and More Flow Rate.

		Less flow rate					More flow rate				
S.No	**Peak Name**	**RT**	**Area**	**Height**	**USP Plate**	**USP Tailing**	**RT**	**Area**	**Height**	**USP Plate**	**USP Tailing**
1	Rabeprazole	2.911	1580760	151203	2178.1	1.7	2.215	1240203	154007	2089.9	1.6
2	Pantoprazole	4.075	419208	36608	3463.2	1.6	3.026	302311	34781	2415.7	1.6

Detection limit: The LOD for Rabeprazole and Pantoprazole, calculated using the standard deviation of the response (SD) and the slope of the calibration curve (S), was 3.17 and 0.0172, respectively.

Table No. 13: Showing results for LOD.

Drug Name	Standard Deviation	Slope(s)	LOD (µg)
Rabeprazole	373625.50	581075863	3.17
Pantoprazole	5772.40	476579210	0.0172

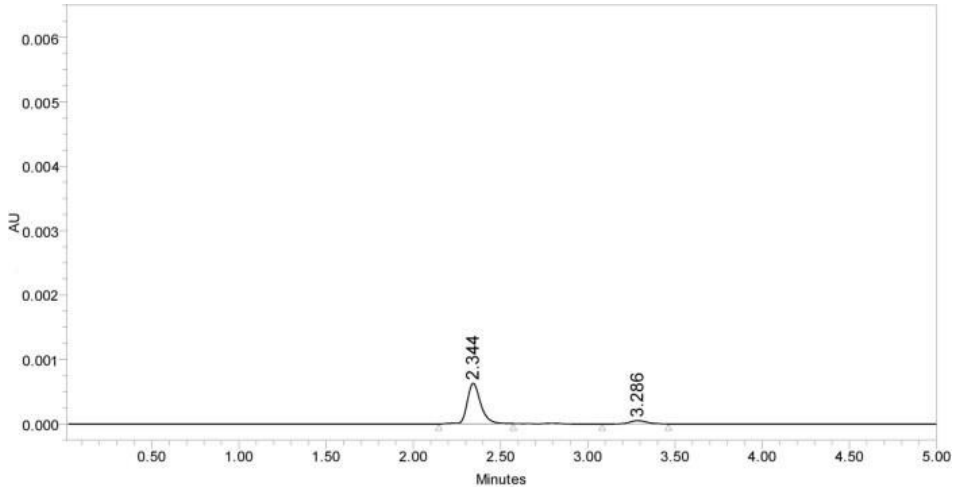


Fig. No. 17: Results of LOD.

Quantitation limit: The LOQ for Rabeprazole and Pantoprazole, calculated using the standard deviation of the response (SD) and the slope of the calibration curve (S), was 5.80 and 0.212, respectively.

Table No. 14: Showing results for LOQ.

Drug Name	Standard Deviation	Slope(s)	LOD (µg)
Rabeprazole	372727.80	574265980	5.80
Pantoprazole	5761.30	478828490	0.212

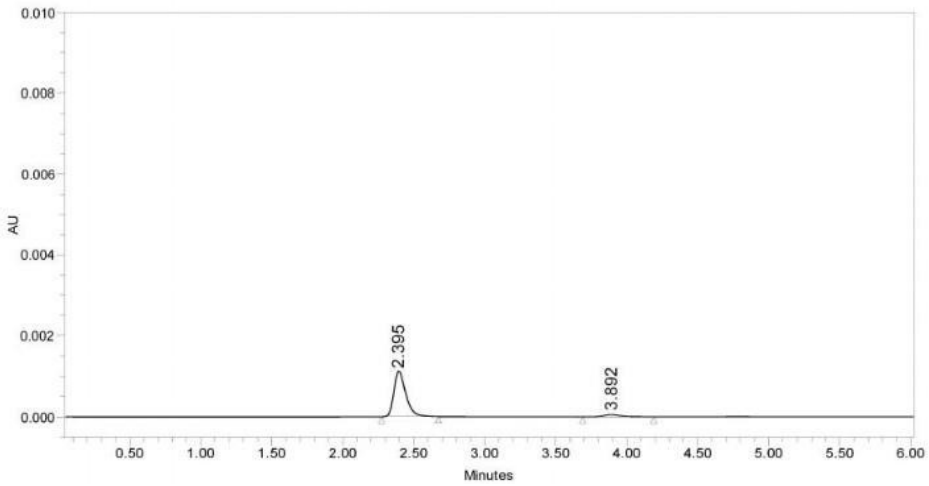


Fig. No. 18: Results of LOQ.

DISCUSSION

In this study, a new RP-HPLC method was successfully established for the simultaneous estimation of Rabeprazole and Pantoprazole. The chromatographic separation was achieved using a Thermosil C18 column (4.0×125mm, 5µ) with a flow rate set at 0.7 ml/min. The mobile phase comprised a mixture of methanol and sodium acetate buffer in a 70:30 ratio (v/v), with the pH adjusted to 3 using orthophosphoric acid. Detection was performed at a wavelength of 252 nm.

The instrumentation utilized for this analysis included the WATERS HPLC system equipped with an auto sampler, Separation Module 2690, a photodiode array detector 996, and Empower software version 2. Under these optimized conditions, the retention times for Rabeprazole and Pantoprazole were found to be 2.566 minutes and 3.417 minutes, respectively. The purity of Rabeprazole was determined to be 101.27%, while Pantoprazole exhibited a purity of 99.97%.

To ensure the reliability of the method, various system suitability parameters were evaluated. For Rabeprazole, the theoretical plate count was 4668, with a tailing factor of 1.3. For Pantoprazole, the theoretical plate count was 6089, with a tailing factor of 1.2. The resolution between the two peaks was calculated to be 6.0, indicating good separation.

The method validation was conducted in accordance with ICH guidelines (ICH Q2 (R1)). The linearity of the method was tested over concentration ranges of 5-25 µg/ml for Rabeprazole and 50-250 µg/ml for Pantoprazole. Both drugs showed excellent linearity with a correlation coefficient (r^2) of 0.999. The accuracy of the method was confirmed by recovery studies, which yielded recovery rates of 99.56% for Rabeprazole and 99.48% for Pantoprazole.

Precision was evaluated through repeatability and intermediate precision studies. The %RSD for repeatability was found to be 0.86 for Rabeprazole and 0.82 for Pantoprazole. The %RSD for intermediate precision was 0.44 for Rabeprazole and 0.19 for Pantoprazole, demonstrating that the method is precise, robust, and repeatable.

The limit of detection (LOD) for Rabeprazole was determined to be 3.17 µg/ml, and for Pantoprazole, it was 5.68 µg/ml. The limit of quantitation (LOQ) was found to be 0.0172 µg/ml for Rabeprazole and 0.2125 µg/ml for Pantoprazole.[16,18]

CONCLUSION

In conclusion, the developed RP-HPLC method proved to be effective for the routine analysis of Rabeprazole and Pantoprazole in both active pharmaceutical ingredients (API) and pharmaceutical dosage forms. The method demonstrated high accuracy, precision, and robustness, making it suitable for quality control and routine analysis purposes.

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