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International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.3, pp 1580-1588, July-Sept 2011

Method Development and Validation of Rabeprazole in Bulk and Tablet dosage form by RP-HPLC Method

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Abstract: A precise and feasible high-performance liquid chromatographic (HPLC) method for the analysis of the novel antacid drug Rabeprazole in tablet dosage form has been developed. The analysis was carried out on a Symmetry C18 (4.6 x 150mm, 5 µm, Make: XTerra) or equivalent column, using a mixture of phosphate buffer (pH 5.5), methanol (30:70) as the mobile phase using a low pressure gradient mode with flow rate at 0.9ml/min and analysis was performed at wavelength 284 nm using Photo Diode Array (PDA) detector at ambient temperature.. The injection volume was 20µl.The retention time of the drug was 2.657 min. The method produced linear responses in the concentration range of 20 to 60µg/ml of Rabeprazole. The LOD and LOQ values for HPLC method were found to be 2.96and 10.1µg/ml respectively. The method was found to be applicable for determination of the drug in tablets.
Key words: RP-HPLC, PDA, Rabeprazole, Tablet dosage forms.

Introduction

Rabeprazole is a substituted benzimidazole that inhibits gastric acid secretion and primarily used in the treatment of Ulcerative Gastroesophageal Reflux Disease (GERD). It is chemically 2-[[[4-(3-methoxy propoxy)-3-methyl-2-pyridinyl] sulfinyl]-*H*-benzimidazole [1,2] (Figure 1). Rabeprazole is officially used as PDR1[3].Rabeprazole belongs to a class of antisecretary compounds that suppress gastric acid secretion by inhibiting the gastric H+, K+ATPase at the secretary surface of the gastric parietal cell [4,5]. Because this enzyme is regarded as the acid (proton) pump within the parietal cell, rabeprazole has been characterized as a gastric proton pump inhibitor [6,7]. Rabeprazole blocks the final step of gastric secretion[8].In gastric parietal cells, rabeprazole is protonated, accumulates and is transformed to an active sulfonamide2 [9,10].

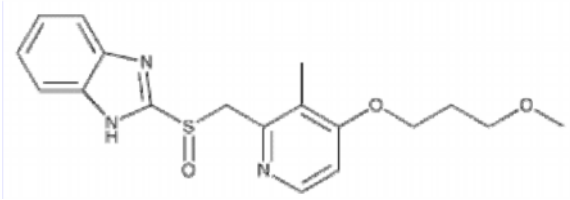


Figure 1. Chemical structure of rabeprazole

Few HPLC methods for quantitative determination [11] of rabeprazole were reported in Literature [12].Majority of these HPLC methods were applied in the determination of rabeprazole [13] and its metabolites using buffer solutions [14] and biological fluids3-8 [15] and are mainly useful for therapeutic monitoring of rabeprazole [16,17] .The present work describes a new, simple and accurate reverse phase liquid chromatographic method for the estimation of Rabeprazole. The developed method was validated to ensure the compliance in accordance with ICH guidelines.

Materials and Methods:

Chemicals and Reagents

1. Methanol (Merck) : HPLC grade
2. Water : Milli-Q grade
3. Potassium di hydrogen phosphate: AR grade
4. Sodium Hydroxide : AR grade

Instrumentation

The HPLC system consisted of Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters 2695 solvent delivery module in a quaternary gradient mode and a Waters2669 PDA detector. Data acquisition was performed by the Empower 2® software operated on a Pentium® IV microprocessor. Analysis was carried out at 284 nm with reversed phase Symmetry C18 (4.6 x 150mm, 5 µm, Make: XTerra) or equivalent column, using a mixture of phosphate buffer (pH 5.5), methanol (30:70) as the mobile phase using a low pressure gradient mode with flow rate at 0.9ml/min .The mobile phase was degassed and filtered through 0.45 µm membrane filter before pumping into HPLC system.

Preparation of solutions:

Preparation of Phosphate buffer:

Weigh 7.0 grams of Potassium di hydrogen phosphate into a 1000ml beaker, dissolve and diluted to 1000ml with HPLC water. Adjusted the pH to 5.5 with Sodium Hydroxide.

Preparation of mobile phase

Mix a mixture of above buffer 300mL (30%) and 700 ml of Methanol HPLC (70%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

Mobile phase as diluent.

Preparation Standard Solution:

Accurately weigh and transfer 10mg of Rabeprazole Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Preparation Sample Solution:

Weigh 5 Rabeprazole Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Rabeprazole into a 10 mL volumetric flask. Add about 7 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

Chromatographic parameters.

Equipment : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector.
Column : Symmetry C18 (4.6 x 150mm, 5 µm, Make: XTerra) or equivalent
Flow rate : 0.9mL per min
Wavelength : 284 nm
Injection volume : 20 µl
Column oven : Ambient
Run time : 5 min

Linearity study

Preparation of stock solution:

Accurately weigh and transfer 10mg of Rabeprazole API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Linearity was performed by taking from stock solution aliquots of 2,3,4,5 and 6ml were taken in 10ml volumetric flasks and diluted upto the mark with mobile phase such that the final concentration of Rabeprazole in the range of 20-60 µg/ml. Volume of 20 µl of each sample was injected in five times for each concentration level and calibration curve was constructed by plotting the peak area versus the drug concentration. The observations and calibration curve is shown in **Table 1, Fig.1.**

Acceptance Criteria:

Correlation coefficient should be not less than 0.999.

Linearity:

TABLES (1) Linearity Results: Table (1) Linearity of Rabeprazole

S.No	Linearity Level	Concentration	Area
1	I	20µg/ml	939926
2	II	30µg/ml	1390971
3	III	40µg/ml	1860230
4	IV	50µg/ml	2285771
5	V	60µg/ml	2779976
Correlation Coefficient			1.000

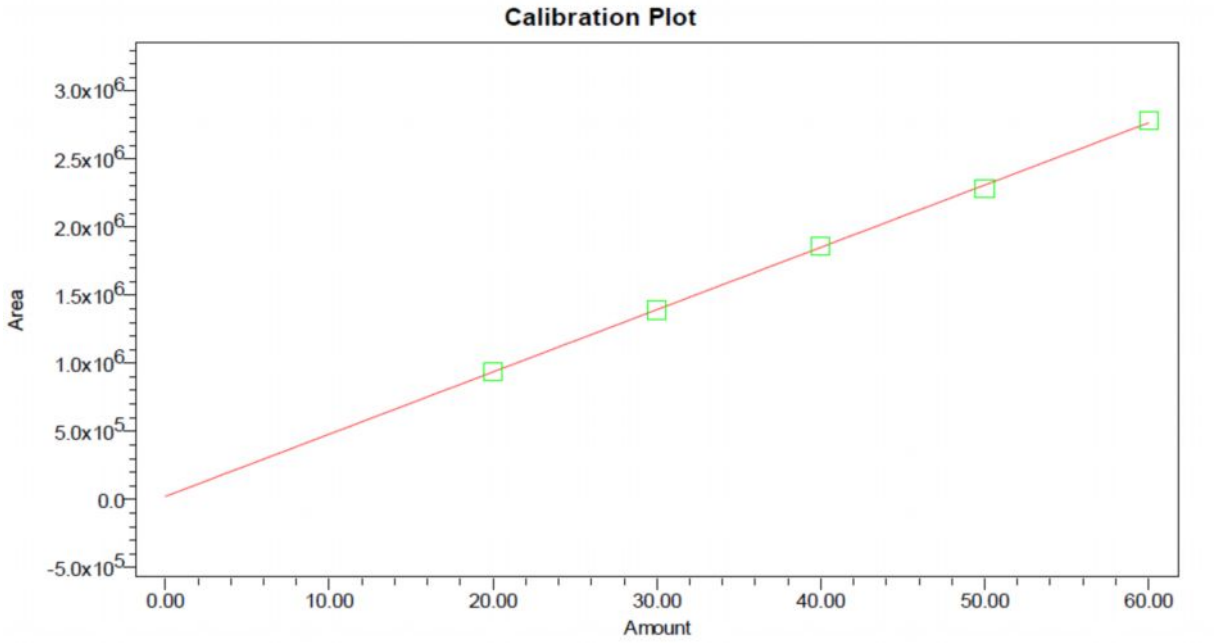


Figure 1:- Linearity Plot

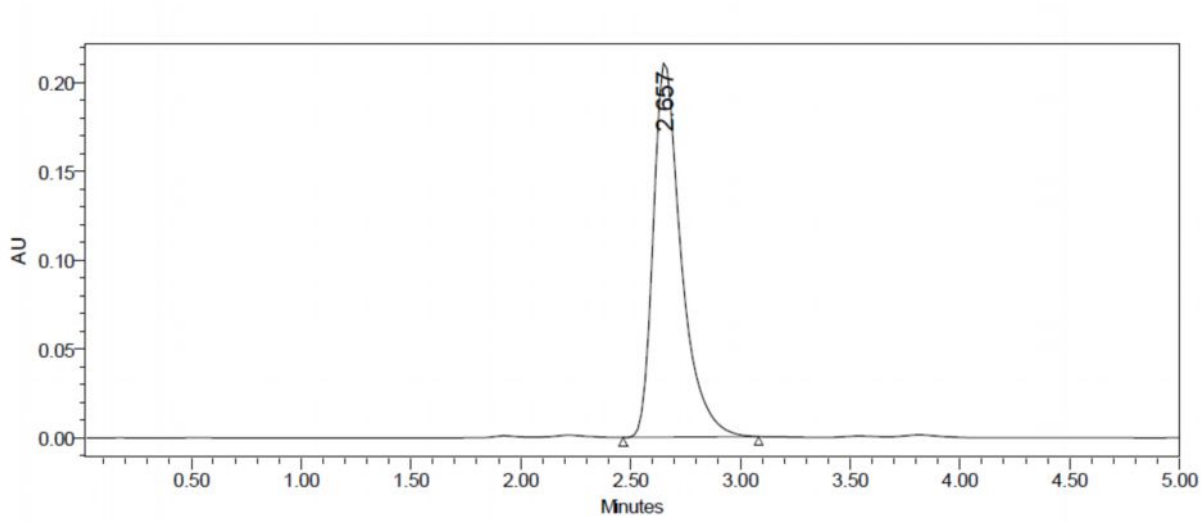


Figure 2: chromatogram of Rabeprazole

Assay:

Assay was performed by accurately weighed amount of powder equivalent to 10.00mg of Rabeprazole was quantitatively transferred to 10 ml of calibrated flask with the aid of diluent. The volume was made up to mark, sonicate for 10min. From the stock solution aliquot of 0.1ml was taken in 10ml volumetric flasks and diluted upto the mark with mobile phase and filtered, such that the final concentration of Rabeprazole was 10µg/ml. The chromatogram was shown in figure-3. Table-2.

Calculation:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. Wt}}{\text{Label Claim}} \times 100$$

Where:

AT = Peak Area of Rabeprazole obtained with test preparation.

AS = Peak Area of Rabeprazole obtained with standard preparation.

WS = Weight of working standard taken in mg.

WT = Weight of sample taken in mg.

DS = Dilution of Standard solution.

DT = Dilution of sample solution.

P = Percentage purity of working standard .

System Suitability:

Tailing factor for the peak due to Rabeprazole in Standard solution should not be more than 2.0.

Theoretical plates for the Rabeprazole peak in Standard solution should not less than 2000.Table-5.

Accuracy

Preparation of 40 µg/ml solution:

Further pipette 0.4 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter. It is taken as 100% target solution.

Accuracy and Recovery:

It was done by recovery study. Sample solutions were prepared by spiking at about 50 %, 100% and 150 % of specification limit to Placebo and analyzed by the proposed HPLC method. Results are shown in **Table-3.**

Assay: Table (2) Assay of Rabeprazole

S.no	Rabeprazole			
	Standard Rt	Sample Rt	Standard area	Sample area
01	2.657	2.671	1868062	1803214
02	2.654	2.673	1900478	1811537
MEAN			1884270	1807375
STDEV			22921.8	5884.8
%RSD			1.22	0.33

Accuracy: Table (3) Accuracy of Rabeprazole

%Concentration(at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	951730	5.0	5.03	100.7%	99.8%
100%	1881869	10.0	9.95	99.5%	
150%	2815614	15.0	14.8	99.3%	

Precision: Table (4) Precision of Rabeprazole

SI.No	Injection number (80 mcg/ml)	Retention Time of Rabeprazole	Area of Rabeprazole
1	Injection-1	2.669	1724358
2	Injection-2	2.668	1777933
3	Injection-3	2.655	1767353
4	Injection-4	2.666	1729271
5	Injection-5	2.662	1782024
	AVRG		1749729
	STDEV		27398.33
	%RSD		1.5

Table (5) :-System Suitability of Rabeprazole by RP-HPLC

Drug name	USP tailing	USP theoretical plates
Rabeprazole	1.6	2183.4
Acceptance criteria	In between 0.5 to 2.0	Above 2000

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.

System precision:

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions. Standard solution of (40µg/ml) were prepared as per test method and injected for 5times. Results are shown in **Table -4.**

Acceptance Criteria:

The % RSD for the area of five standard injections results should not be more than 2%.

Limit of Detection:

Preparation of 0.7% solution At Specification level (0.28µg/ml solution):

Pipette 1mL of 10µg/ml from stock solution into a 10 ml of volumetric flask and dilute up to the mark with diluent. Further pipette 0.7mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.**figure8**

Acceptance Criteria:

- S/N Ratio value shall be 3 for LOD solution.

Limit of Quantification:

Preparation of 0.23% solution At Specification level (0.092µg/ml solution):

Pipette 1mL of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.Further pipette 0.23mL of above diluted solution into a 10 ml of volumetric flask and dilute up to the mark with diluent.**figure-9**

Acceptance Criteria:

- S/N Ratio value shall be 10 for LOQ solution.

Robustness

Table (6) Robustness of Rabeprazole

Proposed variations		**USP Plate Count**	**SP Tailing**
Variation in mobile phase composition	**10% less**	2854.3	1.4
	***Actual**	2253.6	1.5
	10% more	2111.0	1.4
Variation in flow rate	**0.6ml/min**	2168.0	1.4
	0.8ml/min	2253.6	1.5
	1.0ml/min	2074.3	1.4

Ruggedness: **Table (7): Ruggedness of Rabeprazole**

	Retention Time of Rabeprazole	Area of Rabeprazole
Standard(80mcg)	2.669	1890004
Analyst(1)(80mcg)	2.672	1885751
Analyst(2)(80mcg)	2.672	1892673
Analyst(3)(80mcg)	2.667	1861622
Analyst(4)(80mcg)	2.668	1871563
AVRG		1880323
STDEV		13249.5
%RSD		0..70

System suitability:-

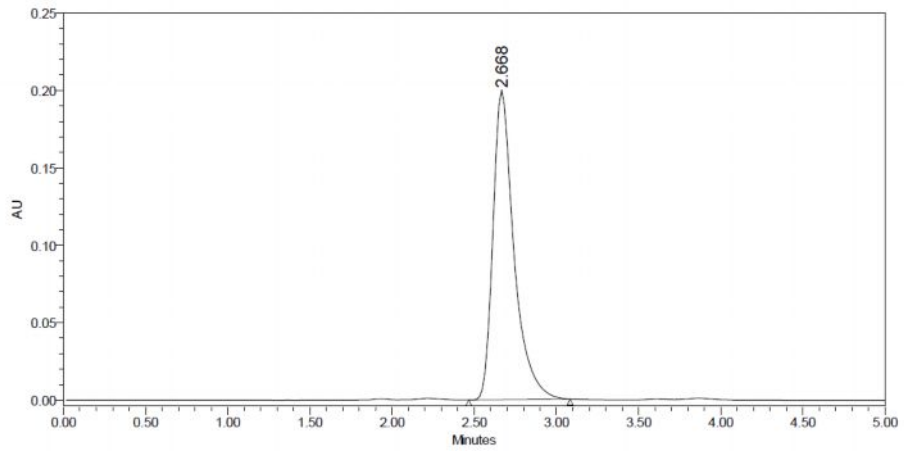


Figure 3: System suitability of Rabeprazole

	Name	Retention Time (min)	Area (µv*sec)	Height (µv)	USP Plate count	USP Tailing
1	Rabeprazole	2.668	1777933	199669	251.0	1.5

Robustness:-

More organic solvent

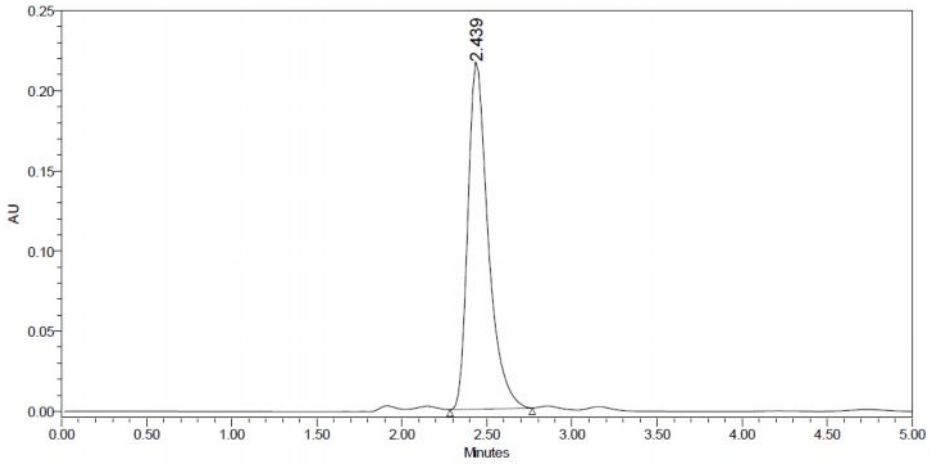


Figure 4: chromatogram indicating robustness (more organic solvent)

Less organic solvent

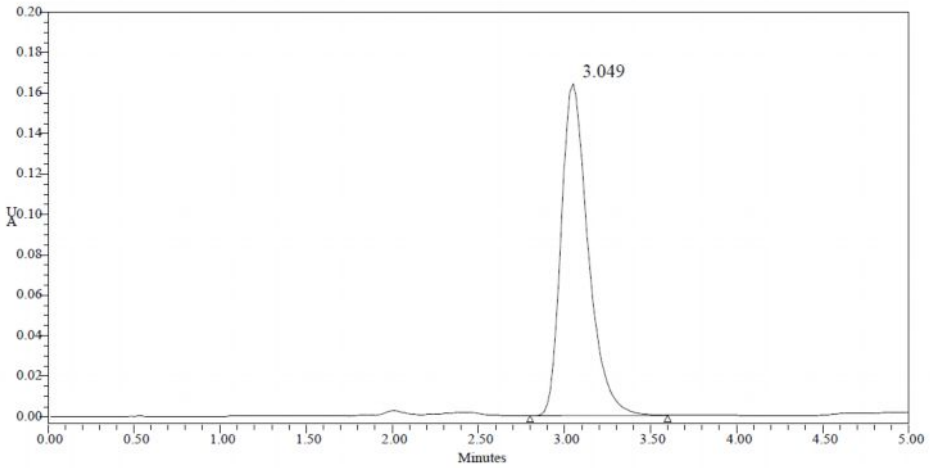


Figure 5: chromatogram indicating robustness (less organic solvent)

More flow

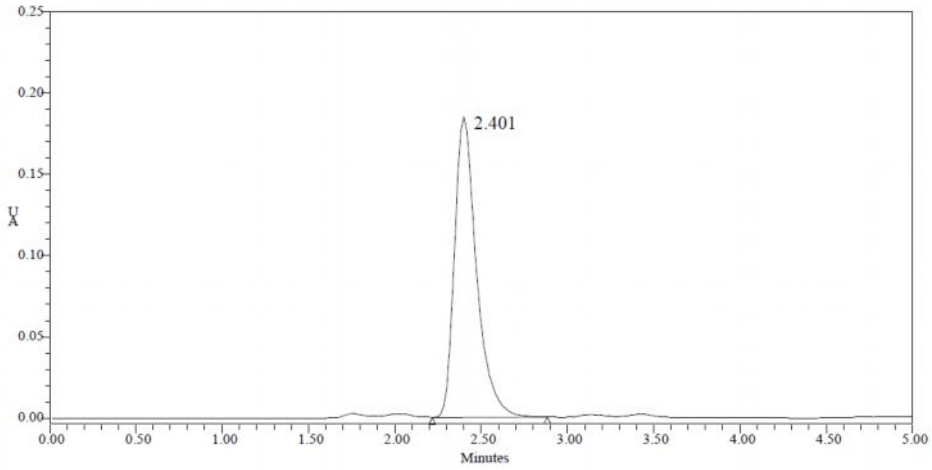


Figure 6: chromatogram indicating robustness (More flow)

Less flow

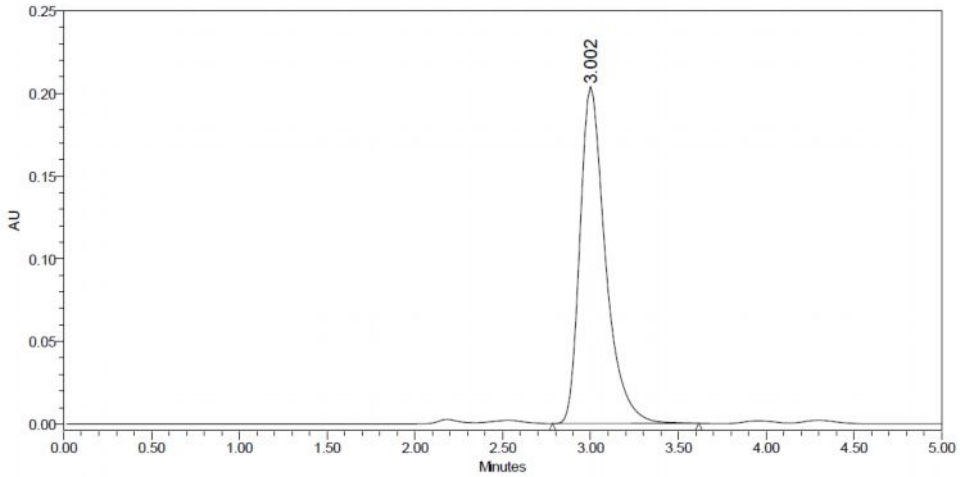
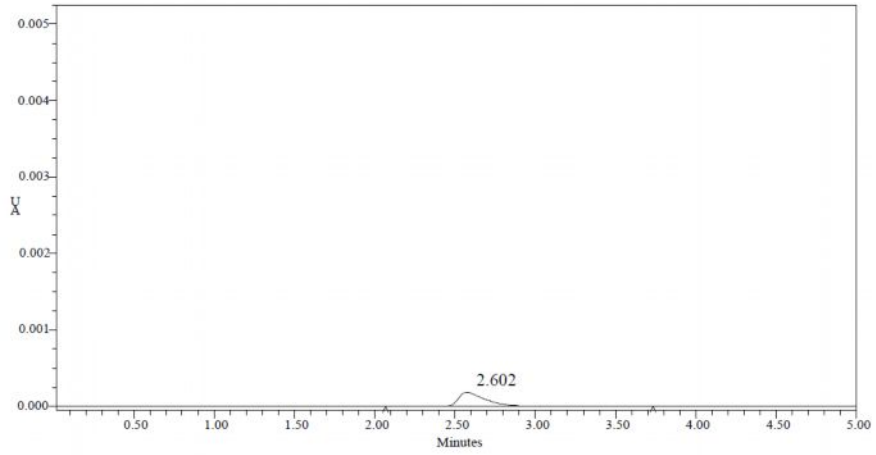


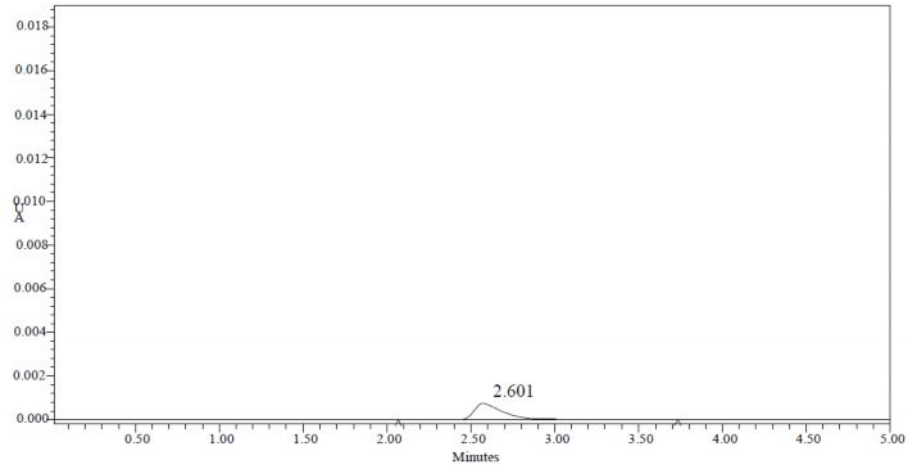
Figure 7: chromatogram indicating robustness (less flow)

Figure 8: Limit of detection of Rabeprazole



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Rabeprazole	2.602	1293	152

Figure 9: Limit of Quantification of Rabeprazole



	Name	Retention Time (min)	Area (µV*sec)	Height (µV)
1	Rabeprazole	2.601	4322	508

Results and discussion

Rabeprazole is also useful in conditions that produce too much stomach acid such as Zollinger-Ellison syndrome. The retention time of Rabeprazole was 2.657 min. The calibration curve was linear over the range 20-60 µg/ml for the determination of Rabeprazole. The linearity of method was statistically confirmed. The correlation coefficients (r^2) for calibration curves were not less than 0.999. The LOD and LOQ values of Rabeprazole were found to be 2.96 and 10.1 respectively. The Precision was expressed as the %RSD of the results. The values obtained for the precision studies presented (Table 4, 5), indicates good repeatability. The analytical recovery at three different concentrations of Rabeprazole was determined and the recovery results are in the range of 98-102% .Therefore proposed validated method was successfully applied to determine Rabeprazole in tablet dosage form.

For the determination of Rabeprazole, the proposed HPLC method was found to be superior due to high percentage recovery which shows that the method was free from interference of excipients used in the formulations. The results of the study indicate that the proposed HPLC method of analysis can be used in quality control department with respect to routine analysis for the assay of the tablets containing Rabeprazole.

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