- Short communication

Development and Validation of RP-HPLC Method for Analysis of Rabeprazole Sodium

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(Received: November 11, 2018; Accepted: January 10, 2019; Published (Web): April 01, 2019)

Rabeprazole sodium, a 4-(3-methoxypropoxy)-3-methylpyridinyl derivative of timoprazole, is a proton pump inhibitor that is used for the treatment of acid-peptic diseases, such as duodenal, gastric and esophageal ulceration.[1,2] A simple, decisive and responsive reverse phase liquid chromatography (RP-HPLC) method was developed and validated for assaying rabeprazole sodium. The main purpose of the present study was to establish a relatively simple, single step and inexpensive HPLC method for the determination of rabeprazole sodium in tablet dosage form, since most of the previous methods have been found to be relatively complicated and expensive. This proposed method has been validated as per ICH guidelines.[3]The developed method has been found to be rapid and sensitive which can be used to determine the potency of rabeprazole sodium in bulk and pharmaceutical dosage form.

Rabeprazole sodium standard was obtained from Department of Pharmacy, Dhaka University. The commercial sample of rabeprazole sodium tablet was purchased from various drug stores. All the chemicals used were of HPLC grade.

A reversed phase waters C_{18} (4.6 × 150 mm, 5 µm) column was utilized. For data acquisition and analysis, Empower Pro software was used. Mettler Toledo analytical balance were employed for weighing. The HPLC system consisted of a binary pump (UV visible pump), column heater and UV detector 20 A. For optimum separation, column temperature was maintained at 35°C using column oven and isocratic elution with methanol : water (50 : 50; v/v) at the flow rate of 1.0 ml/min. The detection was carried out at 290nm whereas injection volume was fixed at 20 µL. The peak purity was checked with the UV detector (Table 1)

Table 1. Optimized chromatogram.

Parameter	Values ±SD
Theoretical plates	390±10
USP Tailing Factor (SD)	0.8±0.02
Capacity factor	2.5±0.05
LOD in µg/ml	0.369
LOQ in µg/ml	4.103
Retention time	9.99±0.9

The mobile phase was prepared with water and methanol at a proportion of 50:50 followed by filtration by using 0.45 µ membrane filters. The standard solution was prepared by adding 100ml of water to the accurately weighed 100 mg rabeprazole sodium. The concentration of this solution was then adjusted to 10 µg/ml.

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Dhaka Un iv. J. Pharm. Sci. **18**(1): 127-130, 2019 (June)
DOI: https://doi.org/10.3329/dujps.v18i1.41428

According to ICH guidelines, the HPLC method was optimized in terms of precision, accuracy and linearity for the assay of rabeprazole sodium. For method optimization, different column and mobile phase were tried but acceptable retention time, good resolution and theoretical plates were observed only with methanol and water (Figures 1 and 2).

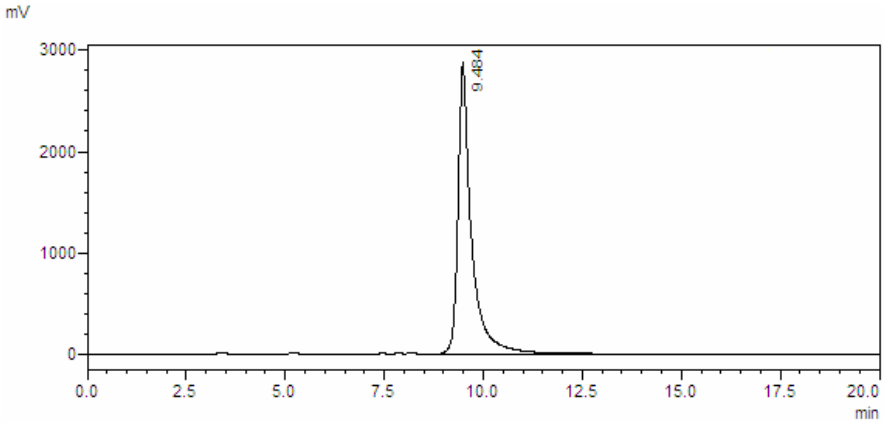

Fig 1. Chromatogram of rabeprazole sodium working standard.

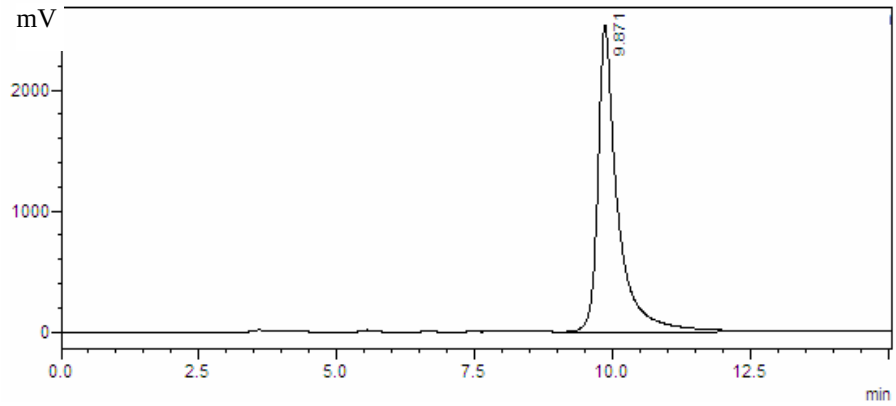

Fig 2. Chromatogram of rabeprazole sodium (sample 3).

It has been observed that rabeprazole with buffer having pH 6.8 gave standard chromatogram.[4] In this method, the HPLC grade water utilized, had a pH of 6.8 ± 0.05. For this reason, only (50 : 50 v/v) methanol and water were used. This particular method not only produced sharp peak but also provided the easy mobile phase preparation with a flow rate of 1ml/min and an acceptable retention time (t_R) of 9.91 ± 0.9 min, theoretical plates and good resolution of drug. Well defined symmetrical peak was obtained upon measuring the response of eluent under the optimized conditions after thorough experimental trials. Two columns were used for performance investigation, including waters C_{18} (4.6 × 150 mm, 5 micron) and waters C_{18} (4.6 × 250 mm, 5 micron). The UV detector response of rabeprazole sodium was studied and the best wavelength was found to be 290 nm which showed highest sensitivity.

For the construction of calibration curves, seven calibration standard solutions were prepared over the concentration range. Linearity was determined for rabeprazole sodium in the range of 10-200 µg/ml. The correlation coefficient (r^2) value was 0.99 (n=6) and the regression equation for the calibration curve was found to be y =450919x-1478376. System suitability test was performed every time before formulation analysis (Table 2).

Formulation was analyzed as described in experimental section. Assay values were (100 ± 8) % for both the formulations. The precision of repeatability was studied by replicate (n=6) analysis of tablet solutions. It was also studied in terms of intra-day changes in peak area of drug solution on the same day and on three different days over a period of one week (Table 3). Accuracy and precision study of the method was calculated by recovery studies at three level by standard addition method (Table 4).

Table 2. System suitability parameters.

SI. No.	Name	Retention time	Area	Theoretical plates	Peak height
1	Rabeprazole sodium	9.99	87845818	400	1234567
2	Rabeprazole sodium	9.99	87569875	398	1438763
3	Rabeprazole sodium	10.01	86547985	390	2034561
4	Rabeprazole sodium	9.95	88741259	410	1430781
5	Rabeprazole sodium	9.89	85214798	392	1351380
6	Rabeprazole sodium	9.92	84369871	396	1256576
AVG			86714.93433		
SD			1666938.82		
% RSD			1.9		

Table 3. Results of potency determination.

SI. No.	Name	Retention time	Area	Injection	Potency
1	SD	9.50	87845818	2	99.00 %
2	SD	9.48	87581561	2	95.77 %
3	Sample 1	10.10	84898578	6	99.10 %
4	Sample 2	9.70	82285678	6	94.12 %
5	Sample 3	9.87	87345613	6	100.25 %
6	Sample 4	10.20	82245618	6	94.38 %
7	Sample 5	9.90	69938034	6	80.00 %
8	Sample 6	10.14	62162964	6	70.56 %
9	Sample 7	10.00	57719657	6	66.41 %
10	Sample 8	9.92	46229825	6	52.90 %

Table 4. Result of precision study.

Precision	Estimated amount in percentage		
	Injection of each sample (n=3)	Area	Assay
Inter-day	1	87845818	99.00%
	2	82285678	94.11%
	3	84785484	86.15%
Intra-day	1	87845818	99.54%
	2	87654157	98.54%
	3	87952479	99.54%

Table 5. Result of robustness study.

Parameter	Level	System suitability parameters (n = 3)			
		Retention time in minute	Theoretical plates	Capacity factor	Assay (%)
Flow rate in ml/min	0.5	15.87	390±10	2.5±0.05	96%-100%
	0.8	12.98			
	1.0	09.99			
% of organic solvent	40	12.05	390±10	2.5±0.05	96%-100%
	50	09.99			
	60	03.25			
pH of mobile phase	4.0	N/A	390±10	2.5±0.05	96%-100%
	6.8	09.99			
	9.2	10.20			
Columns	250 x 4.6	09.99	390±10	2.5±0.05	96%-100%
Wavelength in nm	290	09.99	390±10	2.5±0.05	96%-100%
	300	N/A			
Column temp.	35	09.99	390±10	2.5±0.05	96%-100%

Rabeprazole sodium is more soluble than rabeprazole. Solubility profile is more important for dissolution and method validation. According to ICH guidelines, buffer medium in a mobile phase must be compatible with HPLC and compound. As for rabeprazole analysis, pH optimization was a key factor in proposed method because rabeprazole is rapidly degraded in acidic medium.[4] Besides, % of organic solvent is also changed for the validation method.

Robustness was examined by observing the change of small variations in different parameters such as flow rate (±0.5 ml), mobile phase composition (±20%), pH of inorganic solvent (±5.5), temperature (±5°C) and wavelength at 300 nm and 290 nm (Table 5).

In conclusion, the proposed method makes use of fewer amount of solvents and changed a set of conditions that required a short time. So the method developed here can therefore be used for routine analysis of rabeprazole sodium in bulk and its tablet dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and thus can be conveniently adopted for quality control analysis.

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