

Original Research Article

## U.V Visible Spectrophotometric Determination, Validation and In vitro Interaction of Rabeprazole with Chlorazepate

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**Abstract:** In the present work a simple, rapid, accurate and precise method has been developed and validated for the determination of Rabeprazole in bulk using UV/visible spectroscopy. Rabeprazole is used for the treatment of GERD and duodenal ulcer. In this development methanol is used as a solvent and solution was scanned at 200 to 400nm range, rabeprazole showed maximum absorbance at 284nm. Linearity was observed in the concentration range of 12-18ug/ml. Percent recovery was found from 99.86% - 100.14%, %RSD for repeatability was calculated 0.628% and for precision inter and intraday was found from 0.488% - 0.77% representing the accuracy, repeatability and precision of the method. Validation of the method was carried out for its linearity, accuracy, precision, specificity according to ICH guidelines. On the basis of results obtained it is concluded that the proposed method can be used for the routine analysis of rabeprazole. After method validation interaction studies were performed with chlorazepate dipotassium, both were taken in 1:1 ratio and readings were observed on UV/Visible spectrophotometer for 3 hours at buffers 4, 7.4 and 9. Results support to the fact that rabeprazole interact with chlorazepate dipotassium.

**Keywords:** rabeprazole, spectrophotometric determination, validation, interaction, chlorazepate

### INTRODUCTION

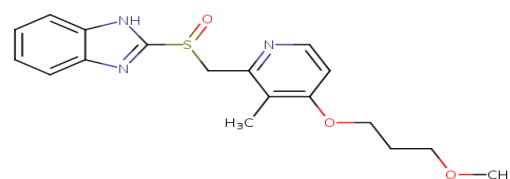
Rabeprazole is chemically 2-({[4-(3-methoxypropoxy)-3-methyl-2-pyridyl] methyl} sulfinyl)-1H-benzimidazole sodium [1-4]. It belongs to second generation of proton pump inhibitors i.e. esomeprazole and rabeprazole. These are prodrugs derived from the timoprazole a pyridylmethylsulfinyl benzimidazole [4, 5, and 6]. As proton pump is the last step of acid secretion, it is most preferable site for inhibition of acid secretion. Its mechanism of action is inhibition of H<sup>+</sup>/K<sup>+</sup> ATP ase which is located in the gastric parietal cells. Its indication includes GERD2 and duodenal ulcers. Rabeprazole showed more potent inhibitory action and less drug interaction as compared to other PPIs [7-9].

Literature survey reveals that several methods have been developed for rabeprazole including capillary electrophoresis [1], LC-MS [3], RP-HPLC [4-7, 9], derivative Spectrophotometric methods for determination of rabeprazole alone as well as in combination [11-13]. All above developed methods require high instrumentation, high technology like diode array detectors, mass detectors employing electron spray ionization, complex reactions in spectrophotometry along with use of expensive reagents. It is also found that most of the methods are

for determination of rabeprazole in combine dosage forms and for plasma determinations.

Therefore we worked on simple reagents and simpler technique which allow us simple, quick, rapid, cost effective but accurate and precise determination of rabeprazole.

Moreover literature survey reveals that there is metabolic interaction of proton pump inhibitors with other drugs. PPIs interact with P450 both as competitive inhibitors and inducers. Decrease in clearance is observed in diazepam, carbamazepin and phenytoin [14]. It is also suggested that PPIs interact with benzodiazepine so care should be given [15]. Rabeprazole is new member so we perform in-vitro interaction studies between rabeprazole and dipotassium chlorazepate as we expect to behave like its other member.



Structure of Rabeprazole

## METHODOLOGY

### Material and reagents

Analytical grade methanol was used as a solvent procured from Merck ltd. All the glassware was of A class.

### Equipment

Uv/ Vis 1800 shimadzu ( Japan ) having quartz cells of 1cm and slit width of 2cm was used for analysis.

### Preparation of solutions

75mg of standard rabeprazole was dissolved in 100ml methanol. Then dilution was made by taking 2ml from stock in 100ml methanol to get 15ppm concentration. Sample aliquots were prepared from stock solution to get concentration of 12 to 18ppm.

### Selection of wavelength

The solution of rabeprazole was scanned in the UV range of 200-400nm against blank separately. Rabeprazole showed lambda max at 284nm.

## FOR INTERACTION STUDIES

### Preparation of simulating full stomach (buffer pH 4.0)

3.725 g of potassium chloride were dissolved in deionized water in one liter for the preparation of chloride buffer of pH 4. pH was adjusted with 0.1 N HCl.

### Preparation of simulating blood pH (buffer pH 7.4)

Phosphate buffer of pH 7.4 was prepared by dissolving 0.6 gm of potassium dihydrogen o-phosphate, 6.4 g of disodium hydrogen o-phosphate and 5.85 g of sodium chloride in sufficient deionized water to produce 1000 mL and the pH was adjusted.

### Preparation of simulating intestinal pH (buffer 9.0)

Ammonia buffer of pH 9 was prepared by dissolving 4.98g of ammonium chloride in 1000 mL of deionized water. 10% ammonia was used to adjust the pH of solution.

### Procedure for interaction

Amount equivalent to 20 mg each of rabeprazole and dipotassium chlorazepate were separately transferred to 100 mL volumetric flasks and volumes were made up to the mark with concomitant mixing of drugs into simulated gastric juice. The concentration of each solution was  $200 \mu\text{g mL}^{-1}$ . Equal volumes of rabeprazole: dipotassium chlorazepate (1:1) were taken into a reaction flask to produce the final concentration of each drug to  $100 \mu\text{g mL}^{-1}$  and kept at  $37^\circ\text{C}$  in water bath with constant stirring. 2 mL of this mixture was withdrawn to record its absorbance at zero time. Further the samples were withdrawn periodically at the interval of 30 minutes for 3 hours. The above

procedure was repeated for pH 7.4 and 9. Its results are mentioned in the form of table 1D showing percentages of both drugs in combination in three mentioned buffers. At pH4, % availability of rabeprazole increases right from time zero because absorbance increases when drugs were in combination than alone. This trend was observed in pH 7.4 and pH 9 as well. Furthermore when rabeprazole was taken in pH 7.4 after some time colour of the solution begins to changes and it darkens with the passage of time. All the data support to the fact that rabeprazole has interaction with chlorazepate dipotassium.

### Validation parameters

#### Linearity

Linearity was performed to check that the system gives a linear response and obeys and excellent linearity obtained with correlation coefficient value 0.999. The standard curve, slope, y intercept and coefficient of determination were obtained from linear regression analysis as shown in Table 1a.

#### Accuracy

Accuracy was performed by spiking the known amounts of analyte and it was evaluated as the percentage of recovery at 80, 100 and 120%. Results are demonstrated in table 1b, high recovery in the range of 99.86% -100.14% and %RSD 0.358 shows the high degree of accuracy for the determination of analyte.

#### Repeatability

Repeatability was evaluated with nine replicates of the standard solution of 15ug/ml of rabeprazole. %RSD was calculated 0.628 which is very promising within range.

#### Precision

Intraday precision was performed by two different analysts on the same day while intraday was performed by two different analysts other day; results are summarized in table 1c indicates mean of three absorbance performed by each analyst, their SD and %RSD.

#### Specificity

The specificity of the method was documented by preparing Placebo and sample. Both scanned at the wavelength of active i.e. rabeprazole, no hindrance was observed in placebo and in sample.

#### Range

Samples were prepared for the range 80 to 120% and results were very promising.

#### Ruggedness

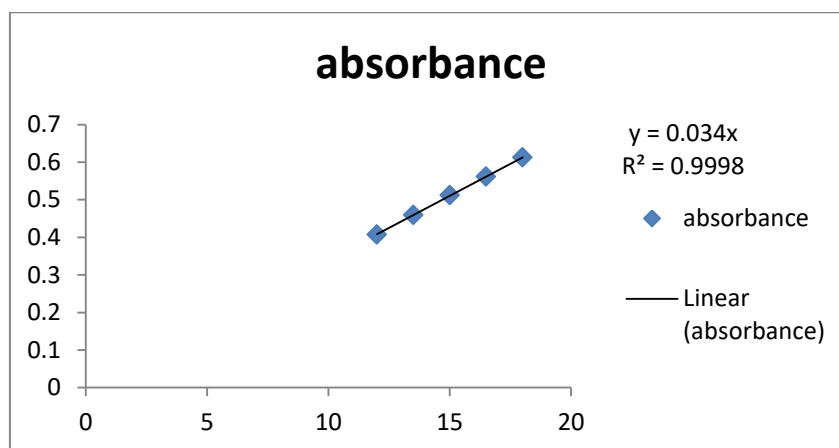
Excellent results obtained when two analyst perform the same method in two different days employing similar conditions as shown in **table 1c**.%

RSD was calculated for analyst 1 and 2 on day 1 was found 0.602% , 0.488% and on second day found to be 0.703% and 0.77% respectively.

**RESULTS**

**Table-1a: Linearity of the Developed Method**

Conc. Ug/ml	Absorbance nm
12	0.4075
13.5	0.4594
15	0.5122
16.5	0.5612
18	0.6122



**Fig-1: Linearity of the Developed Method**

**Table-1b: Accuracy and Recovery of the Developed Method**

s.no	Level	Amount recovered in mg	% recovery
1	80%	8.01 n=3	100.14
2	100%	9.9 n=3	99.856
3	120%	12.03 n=3	100.07
%RSD=	Within limit		
0.358			

**Table-1c: Precision of the Developed Method**

Intraday	Day 1		Intraday day 2		
	Analyst 1	Analyst 2	Analyst 1	Analyst 2	
Mean absorbance ( n=3)	0.507	0.5127	0.512	0.518	
S.D	0.0031	0.0025	0.0036	0.004	Results within limits
%RSD	0.602	0.488	0.703	0.77	

**Table-1d: Interaction of Rabepazole with Chlorazepate Dipotassium**

TIME (min)	% RAB	% CHLORAZEPATE	% RAB	% CHLORAZEPATE	% RAB	% CHLORAZEPATE
	pH4		pH 7.4		PH 9	
0	139.24	118.34	130.27	118.41	126.2	123.38
30	140.94	121.76	110.63	122.73	126.21	123.26
60	144.1	121.32	111.33	122.62	127.91	124.84
90	147.65	124.22	108.27	127.3	122.92	127.27
120	146.9	122.28	104.22	123.88	125.07	123.99
150	147.39	123.34	107.77	124.18	128.66	122.66
180	146.86	123.86	109.44	124.42	127.87	123.23

## DISCUSSION

Rabeprazole is a member of proton pump inhibitors belonging to second generation of the class. It is antisecretory in action as it inhibits H/K ATPase. It is widely used for the treatment of stomach related disorders. It is very effective in the treatment of GERD and ulcers.

Since its use is much alone and in combination dosage forms, necessarily a method is require for its quantification, determination and estimation in bulk as well as in dosage form. Literature survey reveals that methods are available including capillary electrophoresis method, LC- MS, RP- HPLC and derivative spectrophotometric techniques for determination of rabeprazole. These methods acquire expensive instrumentation like HPLC, mass detectors employing electron spray ionization, diode array detectors, complex reactions, expensive reagents and are time consuming as well. It is also found that most of the methods were for combined dosage form analysis and in plasma.

However we have worked on simple and inexpensive reagents to develop such a method that could be easily employed for the routine analysis of rabeprazole. For that purpose a single solvent was selected in which we can prepare all our desired solutions without involving complex reagents and avoiding complex reactions. We selected methanol as our main reagent. All the stocks solution as well as dilutions were prepared using methanol as a solvent. When solution of concentration of 15ug/ml was scanned in the UV range of 200- 400nm it showed maximum absorbance at 284 nm without any hindrance of diluents.

The proposed method was then validated according to ICH guidelines for linearity, accuracy, recovery, precision, repeatability, specificity and ruggedness. Results show great linearity having coefficient regression of 0.9998, percent recovery in the range 99.86%-100.14% and great specificity. Intra and interday precision is also obtained and ruggedness was also established. %RSD was also found from 0.488% to 0.77% for interday, intraday and ruggedness. %RSD for repeatability was calculated and found 0.628% which is very promising.

From above results we concluded that our proposed method is simple, quick, involve cost effective and simple reagents, easy technique and also has been validated according to ICH guidelines for the above discussed parameters therefore it can easily be employed for the routine analysis of rabeprazole.

Our literature survey suggested that PPIs interact with benzodiazepines therefore we performed

in-vitro interaction studies between rabeprazole and chlorazepate dipotassium. Our result showed that rabeprazole has strongly interacted with chlorazepate dipotassium and interaction is pH dependent. The selected drugs are more interactive at pH 4 and 7.4 than at pH 9. Furthermore development of colour took place at pH 7.4 when both drugs were in combination. This opens a new path to investigate further as there is a great indication of some complex formation. It is recommended that dose should be carefully adjusted and monitored incase when both drugs given in combination.

## CONCLUSION

From above results we concluded that our proposed method is simple, quick, involve cost effective and simple reagents, easy technique and also has been validated according to ICH guidelines for the above discussed parameters therefore it can easily be employed for the routine analysis of rabeprazole. Interaction studies indicated that rabeprazole is interactive with chlorazepate dipotassium at pH 7.4.

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