

**RESEARCH ARTICLE**

## Development and Validation of RP-HPLC for the Rabepazole sodium in Pharmaceutical formulations and Human Plasma

Prasanna Reddy Battu<sup>1\*</sup> and MS Reddy<sup>2</sup>

<sup>1</sup>Dept. Quality Control and Analytical R and D, Smilax Laboratories Limited, Jeedimetla, Hyderabad-500055, A.P, India.

<sup>2</sup>Department of Entomology and Plant Pathology, Auburn University, USA

\*Corresponding Author E-mail: [drbpkreddy@gmail.com](mailto:drbpkreddy@gmail.com)

**ABSTRACT**

A simple, selective, accurate high Performance Liquid Chromatographic (HPLC) method was developed and validated for the analysis of Rabepazole sodium. Chromatographic separation achieved isocratically on a C<sub>18</sub> column [Use Inertsil C<sub>18</sub>, 5 $\mu$ , 150 mm x 4.6 mm] utilizing a mobile phase of acetonitrile/phosphate buffer (70:30, v/v, pH 7.0) at a flow rate of 0.8 ml/min with UV detection at 228 nm. The retention time of Rabepazole sodium was 2.44 min. The method is accurate (99.15-101.85%), precise (intra-day variation 0.13-1.56% and inter-day variation 0.30-1.60%) and linear within range 0.1-30 $\mu$ g/ml (R<sup>2</sup>=0.999) concentration and was successfully used in monitoring left over drug. The detection limit of Rabepazole sodium at a signal-to-noise ratio of 3 was 1.80ng/ml in human plasma while quantification limit in human serum was 5.60 ng/ml. The proposed method is applicable to routine analysis of Rabepazole sodium in pharmaceutical formulations as well as in human plasma samples.

**KEY WORDS** Rabepazole sodium, RP-HPLC, Validation, Human blood samples, Pharmaceutical dosage forms.

**INTRODUCTION:**

Rabepazole sodium belongs to a class of antisecretory compounds (substituted benzimidazole proton pump inhibitors) that do not exhibit anticholinergic or histamine H<sub>2</sub>-receptor antagonist properties, but suppress gastric acid secretion by inhibiting the gastric H<sup>+</sup>/K<sup>+</sup> ATPase (hydrogen-potassium adenosine triphosphatase) at the secretory surface of the gastric parietal cell<sup>1</sup>. Rabepazole may also be used with antibiotics to get rid of bacteria that are associated with some ulcers. Rabepazole is chemically 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole sodium. It has an empirical formula of C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>NaO<sub>3</sub>S and a molecular weight of 381.43. Soluble in water and methanol. It is not yet any of the pharmacopoeias<sup>2, 3</sup>. Literature survey revealed HPLC<sup>4</sup>, spectrophotometric and spectrofluorometric methods for determination of rabepazole in bulk and dosage forms<sup>5</sup>. It is a centrally and peripherally acting analgesic and antipyretic agent.

**EXPERIMENTAL:****Instrumentation:**

A High Performance Liquid Chromatograph system, with LC solutions data handling system (Shimadzu-LC2010) with an auto sampler was used for the analysis. The data was recorded using LC 2010 solutions software.

The purity determination performed on a stainless steel column 150 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5 $\mu$ m diameter (Inertsil C<sub>18</sub>, 5 $\mu$ , 150 mm x 4.6 mm, make: Shimadzu Ltd, Japan) with the mobile phase containing acetonitrile and phosphate buffer in the ratio of 70:30 (v/v pH 7.0) at ambient temperature. Flow rate was kept at 0.8 ml/min and the elution was monitored at 228 nm.

**Materials and Chemicals:**

Rabepazole sodium working standard, used from Smilax Laboratories Limited. For the estimation of Rabepazole sodium in bulk and commercial formulations of rabepazole sodium brand (Rabezne-20, OBOI laboratories), 20 tablets were obtained from retail pharmacies. Each tablet was labeled contain 20 mg of Rabepazole sodium and had an expiry of not less than 365 days at the time of study. HPLC grade Sodium dihydrogen phosphate (NaH<sub>2</sub>PO<sub>4</sub>). Disodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>), Acetonitrile- procured from Merck, India. High pure water was prepared by using Millipore Milli Q plus purification system.

**Preparation of Mobile Phase:**

**Buffer preparation:** Weighed accurately 0.1gm of sodium dihydrogen phosphate (NaH<sub>2</sub>PO<sub>4</sub>) and 0.5gm of disodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>) into a clean dry 1000ml glass beaker and dissolved in 300ml water.

Received on 05.02.2009

Modified on 13.02.2009

Accepted on 22.02.2009

© AJRC All right reserved

Asian J. Research Chem. 2(1): Jan.-March, 2009; Page 49-51

**Mobile phase:** Mix 700ml of acetonitrile to the buffer, the mobile phase was sonicated for 15 min and then it was filtered through 0.45µm membrane filter paper.

**Stock solutions:**

Rabeprazole sodium was powdered finely. A quantity equivalent to 25 mg was transferred into a 25 ml volumetric flask and dissolved in 10 ml of methanol. The volume was then diluted with the mobile phase and filtered through Whatman No. 1 filter paper. One milliliter of the resulting solution was then diluted to 10 ml with mobile phase. From this 1,2and 3 ml samples were taken and their volume was made up to 10 ml each. A chromatogram of these solutions was obtained by injecting 20 µl of each sample into the chromatographic system.

**Method Validation:**

The method was validated for the parameters like specificity, range and linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. In addition, system suitability parameters were also calculated. To demonstrate specificity in the presence of excipients used in formulation, rabeprazole sodium was spiked (at approximately 25 µg/ml) in drug product, chromatogram was observed and compared with that of raw material. To evaluate the linearity, the LOD and LOQ of the method in reference drug and in serum, different serial dilutions (0.0970, 0.190, 0.80, 1.50, 3.10, 6.20, 12.50 and 25 µg/ml) were prepared from the standard stock solutions in 25 ml volumetric flasks and volume made up with diluent which is mixture of 70:30 Acetonitrile and methanol. The samples were injected (10 µl) and signals from the samples were recorded at 2.02 minute which were compared with those of blank. LOD and LOQ values were calculated as signal-to-noise ratio of 3:1 and 10:1 respectively. To determine accuracy of the method, working standard of rabeprazole sodium was prepared in triplicate at three concentration levels (10, 20 and 25 µg/ml) and analyzed. Repeatability of the method was checked by analyzing six replicate samples of rabeprazole sodium (at the 100% concentration level) and calculating relative standard deviation (%RSD). To determine intermediate precision, standard solutions of rabeprazole sodium at eight concentration levels were analyzed three times within the same day (intra-day variation) and three other days (inter-day variation).

**Table 1: Accuracy/recovery of Rabeprazole sodium**

Parameters	Conc (µg/ml)	% Recovery	% RSD
Assay (Spiking method)	10	96.02	1.70
	20	101.54	4.80
	25	95.15	4.60
Assay	6.20	99.18	0.5
	12.40	100	1.5
	25	99.99	0.3
Assay (in serum)	12.40	100	0.5
	6.20	100	1.2
	3.10	100	0.7

**Assay in Formulations:**

In case of marketed formulations, five accurately weighed tablets were crushed to a fine powder and an amount equivalent to 10 mg of rabeprazole sodium was added into different 100 ml volumetric flasks and volume was made up with acetonitrile and methanol mixture. The samples were filtered through a 0.45-µm-membrane filter; different serial dilutions (3.10, 6.20, 12.40, 25µg/ml) were made from this solution in 25 ml volumetric flask and were injected for HPLC analysis.

**Assay in Serum:**

One volume of plasma was de-proteinated by nine volume of acetonitrile and filtered through 0.45 µm Millipore filter paper that was used to make serial dilution of Rabeprazole (0.0970 µg/ml to 25 µg/ml). Three replicates of each dilution were injected to HPLC system and linearity was evaluated. Repeatability of the method was checked by analyzing six replicate samples of rabeprazole sodium (at the 100% concentration level) and calculating relative standard deviation (%RSD).

**RESULTS AND DISCUSSION:**

For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [ICH 1996] and [USP 2002] have recommended the accomplishment of accuracy tests, precision, specificity, linearity of the method

**System Suitability:**

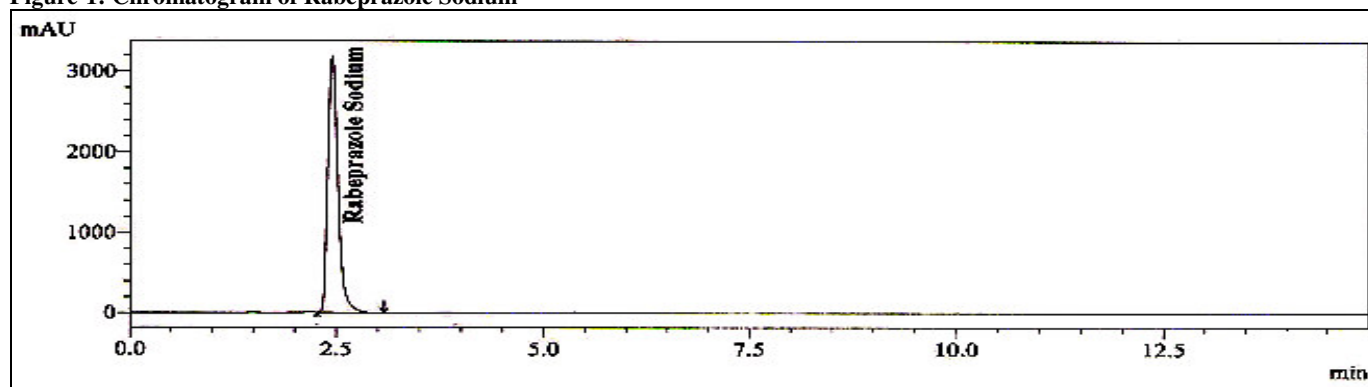
The HPLC system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard. These 10 consecutive injections were used to evaluate the system suitability on each day of method validation.

The system suitability parameters including capacity factor >2, resolution>3 and asymmetric factor<2. All parameters were satisfactory with good specificity for the stability assessment of rabeprazole sodium. Theoretical plates of the column were >3000.

**Accuracy:**

The accuracy of an analytical method is the closeness of test results obtained by that method to true value. In case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product (i.e., "to spike") (USP 2004). In our studies, the later technique was adopted and rabeprazole was spiked in drug product. The result of accuracy given in (Table-1) revealed that the method was found accurate for all above purposes.

Figure-1: Chromatogram of Rabeprazole Sodium

**Precision:**

Precision is the degree of reproducibility or repeatability of the analytical method under normal operating conditions (USP 2004). The method passed the test for repeatability as determined by %RSD of the area of the peaks of six replicate injections at 100% test concentration. The results of intra-and inter-day variation are shown in (Table 2).

**Range and Linearity:**

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range (USP 2004). The linearity of the method was observed with in the expected concentration range demonstrating its suitability for analysis. The correlation coefficient ( $r^2$ ) was found to be 0.999 and value of intercept was less than 25 of the response of 100% of the test concentration in all the cases indicating functional linear relationship between the concentration of analyte and area under the peak

Table 2: Intermediate precision of the method

Concentration ( $\mu\text{g/ml}$ )	Assay in formulation		Serum
	Intra-day variation (%RSD)	Inter-day variation (%RSD)	Intra-day variation (%RSD)
0.0970	0.13	0.90	4.12
0.190	0.35	0.33	0.08
0.80	0.40	1.60	2.70
1.50	0.85	1.08	3.20
3.10	0.26	0.20	1.15
12.5	1.65	0.70	0.40
25	0.25	1.08	3.30

**Limits of Detection and Quantitation:**

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1 (USP 2004, ICH Q2B guidelines, 1996 1997, FDA, Guidance for Industry 2000<sup>6-8</sup>). The lower limit of detection for rabeprazole is 2.40ng/ml in reference material and formulation and 1.70ng/ml serum. Limit of Quantitation (LOQ) is the lowest amount analyte in a sample

that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method (USP 2004). The LOQ values were found to be 8.15ng/ml for raw material, formulations and 5.70ng/ml for serum.

**Specificity:**

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Figure-1). The excipients used in different formulation products did not interfere with the drug peak and thus, the method is specific for Rabeprazole. To further confirm the specificity of the method, UV scans of spiked drug were taken in the range 200-400nm and no significant change was found by comparing the absorbance of pure drug and spiked drug at the analytical wavelength of drug.

**REFERENCES:**

1. Ma JY, Song YH, Sjostrand SE, Rask L, Mardh: cDNA cloning of the beta-subunit of the human gastric H, K-ATPase. *Biochem Biophys Res Commun.* (1991), 180(1): 39-45.
2. Strowing, S.M. and Raskin, P., *Diabetes Care*, (2005), 28, 1562
3. Rosak, C., Petzoldt, R, Wolf, R., Reblin, T. and Dehmel, B., *J. Clin. Pract.*, (2005), 59, 1131.
4. Patel.B.H., B.N. Suhagia, M.M. Patel and J.R. Patel (2007). Determination of pantoprazole, rabeprazole, Esomeprazole, Domperidone and Itopride in pharmaceutical product by reversed phase liquid chromatography using single mobile phase, *journal of chromatographia*, 743-748.
5. Wellington, K, *Drugs*, (2005), 65, 158.
6. FDA: Guidance for Industry, Analytical Procedures and Methods Validation, August 2000
7. ICH Q2B: Validation of Analytical Procedures: Methodology, May (1997).
8. International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use (ICH) Q2B (1996). Validation of Analytical Procedures, Methodology.