

## Research Article

# Development of a New RP-UPLC Method for the Determination of Rabeprazole Sodium in Pharmaceutical Formulation and Application in Dissolution Studies

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## Abstract

**Purpose:** To develop a reverse phase ultra-performance liquid chromatographic (RP-UPLC) method for the estimation of rabeprazole sodium in tablet formulations.

**Methods:** Chromatographic separation was achieved on a Waters Acquity BEH C<sub>18</sub> (50 x 2.1 mm, particle size 1.7 μm) column using an isocratic method with mobile phase composed of acetonitrile and phosphate buffer (pH 7.4) in the ratio of 35:65 (v/v). The flow rate was 0.4 ml/min, temperature of the column was maintained at ambient, injection volume was 5 μL and detection was made at 280 nm. The run time was as short as 2 min. Comparison of system performance with conventional HPLC was made with respect to analysis time, efficiency and sensitivity.

**Results:** The developed method was linear for rabeprazole sodium from 0.03 - 30 μg/ml and the linear regression obtained was > 0.999. Precision, evaluated by intra- and inter-day assays had relative standard deviation (R.S.D) values within 1.5 %. Recovery data were in the range 98.0 - 101.5 % with R.S.D. values < 1.5 %.

**Conclusion:** The method is precise, accurate, linear, robust and fast. The short retention time of 1.49 min allows the analysis of a large number of samples in a short period of time and, therefore, should be cost-effective for routine analysis in the pharmaceutical industry.

**Keywords:** Ultra performance liquid chromatography, Assay, Rabeprazole sodium, Validation

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## INTRODUCTION

Rabeprazole sodium (RAB) is a proton pump inhibitor with actions and uses similar to those of omeprazole. It is given orally as enteric coated rabeprazole tablets and normally taken in the morning. In the treatment of severe ulcerative gastro-oesophageal reflux disease, the usual dose is 20 mg once daily for 4 to 8 weeks; in the USA, a further 8-week course is permitted for healing of erosive oesophagitis [1]. Chemically rabeprazole is 2-[(4)3-methoxypropoxy-3-methyl-2-pyridinyl] sulphanyl 1-H benzimidazole; its structure is shown in Fig 1.

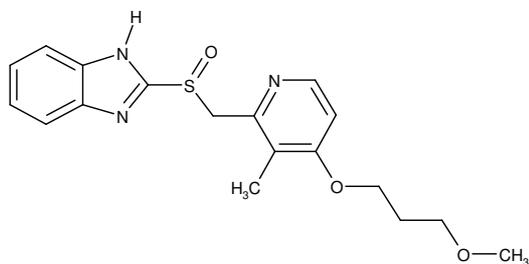


Fig 1: Structure of rabeprazole

Methods that have been reported for the determination of RAB include HPLC [2-5], thin layer chromatography (TLC) [6], UV spectrophotometry [7-9] and voltametry [10]. A method has been reported for the determination of the enantiomer and metabolite of RAB using HPLC [11] while some methods have also been published for the quantitative analysis of RAB in combination with other drugs [12-15].

Ultra performance liquid chromatography (UPLC) is an emerging liquid chromatography technique which enables significant reduction in run time and solvent consumption. To the best of our knowledge, no UPLC method has been reported yet for the determination of RAB in pharmaceutical formulations.

The aim of the present study was to develop a simple UPLC method for the determination

of RAB in tablets and compare the results with those of HPLC.

## EXPERIMENTAL

### Chemicals and reagents

Rabeprazole sodium (RAB) was received as a gift from Metro Labs Ltd, Baddi, India. RAB tablets was purchased from a local pharmacy. (Pepraz<sup>®</sup>, 10 mg, East West Pharma, India). HPLC grade acetonitrile was purchased from Merck India Ltd, Mumbai, India. High purity water was obtained using Millipore Milli-Q water purification system (Billerica, MA, USA). All other reagents were of analytical grade.

### Instrumentation and operating conditions

The HPLC system used for initial chromatographic development was a Waters Alliance separation module with 2487 UV detector. A Waters symmetry C<sub>18</sub> column (150 x 4.6mm, particle size 5µm) was used for separation. The mobile phase consisted of a mixture of phosphate buffer (pH 7.4) and acetonitrile in the ratio 65:35 (v/v). The flow rate was 1.0 ml/min while the injection volume was 20µL and detection was at 280 nm. The column temperature was maintained at ambient.

UPLC analysis was performed on a Waters Acquity UPLC system (Milford, USA) equipped with a binary solvent manager, an auto sampler, and a column manager composed of a column oven and tunable ultra violet (tuv) detector. Chromatographic separation was performed using a Waters Acquity BEH C<sub>18</sub> (50 x 2.1 mm, particle size 1.7 µm) column with the column temperature maintained at ambient while the mobile phase was the same as that used for HPLC. Flow rate was 0.4 ml/min and the detector was set at 280 nm. Data acquisition, handling and instrument control

were performed with the aid of Empower software, version 1.0.

### **Preparation of standards**

A stock solution of 1.0 mg/ml was prepared by dissolving an appropriate amount of RAB in 10mM potassium hydroxide solution. A working solution of 10 µg/ml was prepared from this stock solution by serial dilution.

### **Preparation of test solution from tablets**

Twenty tablets were weighed and powdered. An amount of the powder equivalent to 50 mg of RAB was taken into a 100 mL volumetric flask, and extracted with 10mM potassium hydroxide solution for 10 min. The solution was diluted suitably to 10 µg/ml and filtered through a 0.45 µm Millipore nylon filter paper.

### **Dissolution sample preparation**

Labindia 2000 dissolution apparatus (a six-tablets dissolution unit) (Labindia, Mumbai, India) was used to test the dissolution of the tablets and hence generate test samples for analysis using the developed method. The dissolution test followed the procedure stipulated by United States Pharmacopoeia (USP) using apparatus 2 with paddles. The paddle speed was 100 rpm and the dissolution conditions followed US FDA (United States Food and Drug Administration) recommendation; the dissolution temperature of the media was maintained  $37.0 \pm 0.5$  °C. For delayed release RAB tablets, the US FDA recommends dissolution test in 700 mL of 0.1N HCl for 2 h (samples were collected at intervals for assay of RAB), after which 300 mL of 0.6M Tris-HCl buffer (pH 8.0) was added to the medium and the dissolution test continued for 45 min, during which samples were also withdrawn at intervals for assay of RAB). Auto sampling was used to collect samples (10 ml) at intervals over the test period. The samples were immediately filtered through a 0.45 µm Millipore nylon

filter. The first 2 ml of the sample obtained each time during filtration was discarded in order to clean out the filter paper and thus maintain uniform filtration.

### **Method validation**

#### **System suitability**

Standard solution was used for the system suitability check. System suitability was analysed in terms of USP tailing factor ( $\leq 2.0$ ), theoretical plate counts ( $\geq 30000$ ) and % R.S.D. (relative standard deviation) for five replicate injections (should be  $\leq 2.0$ ).

#### **Precision**

The precision of the method was evaluated by carrying out six independent assays of RAB test (tablet) sample against a qualified reference standard and the % R.S.D. of assay was calculated. The intermediate precision of the method was also evaluated using a different analyst, different columns of the same brand (Waters Acquity, BEH C<sub>18</sub>, 50 x 2.1 mm, particle size 1.7 µm) and instrument (UPLC system, Waters Acquity) in the same laboratory.

#### **Limits of detection (LOD) and quantification (LOQ)**

LOD and LOQ for RAB were determined at signal-to-noise ratios of 3:1 and 10:1, respectively, by injecting series of dilute solution with known concentrations. Precision evaluation was also carried out at the LOQ level by injecting six individual preparations, and calculating the % RSD of the area.

#### **Linearity**

Test solutions were prepared from RAB stock solution at ten concentration levels from LOQ to 300 % of analyte concentration (0.03, 0.5, 1.0, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0 and 30.0 µg/ml). The peak area versus concentration data was treated by least squares linear regression analysis.

### Accuracy

The accuracy of the assay method was evaluated in triplicate at six concentration levels (between 10 and 150 %), i.e., 1.0, 3.0, 5.0, 7.5, 10.0, and 15.0 µg/ml. Percent recoveries were calculated.

### Robustness

To determine the robustness of the developed method, experimental conditions were deliberately altered, and system suitability parameters were checked. The flow rate of the mobile phase was 0.4 ml/min. To study the effect of flow rate, flow rate was changed by 0.05 units from 0.35 to 0.45 ml/min, the proportion of acetonitrile in the mobile phase 35 % was changed by ±3.5 %, and UV detection wavelength (280 nm) was changed ± 3nm. To study the effect of pH variation in the mobile phase, pH was altered by ±0.2 units, i.e., to 7.2 and 7.6. Changes in chromatographic parameters, i.e., theoretical plates, tailing factor and % R.S.D. were evaluated for the method.

## RESULTS

### Method validation

Based on International Conference on Harmonization (ICH) guidelines [16], the method is validated with regard to system suitability, linearity, accuracy, precision, LOD, LOQ and robustness as follows.

### System suitability

The system suitability results for the proposed UPLC method are given in Table 1. The results proves that the optimized UPLC method fulfils these requirements within the USP accepted limits indicated in the 'Experimental' section.

### Precision

The % R.S.D. of RAB assay during the method precision and the intermediate

precision was 1.3 and 1.1, respectively, indicating good precision of the method.

**Table 1:** System suitability results

Injection	RT*	Peak area	USP tailing	USP theoretical plates
1	1.492	7019896	1.21	44222
2	1.491	7102321	1.19	45151
3	1.487	7082325	1.20	45132
4	1.493	7095331	1.19	44125
5	1.472	7152345	1.22	45321
<b>Mean</b>	1.487	7090444	1.20	44790
% R.S.D	0.58	0.67	1.08	1.27

\* RT = retention time

### Limits of detection (LOD) and quantification (LOQ)

LOD and LOQ for RAB were 0.007 and 0.03 µg/ml, respectively. The LOQ R.S.D. for the six preparations was 3.2 %, confirming the high precision of the LOQ at 0.03 µg/ml. Since the LOQ and LOD values of RAB are achieved at a very low level, this method can be suitable for cleaning validation in the pharmaceutical industry.

### Linearity

The linearity of the calibration plot for the method was obtained over the calibration ranges tested, i.e., 0.03 - 30 µg/ml, and the correlation coefficient obtained was > 0.999, thus indicating excellent correlation between peak areas and concentrations of the analyte. The regression equation is  $Y_{RAB} = 705304x - 23709$  ( $r^2 = 0.9999$ ).

### Accuracy

Percent recovery of RAB samples ranged from 98.0 to 101.5, and % R.S.D. values were within 1.3 %, showing the good accuracy of the method. The result is shown in Table 2.

## Robustness

In all the deliberately varied chromatographic conditions, the assay results was between 98 and 101 % and no significant changes were obtained in chromatographic parameters. This shows the robustness of the developed method.

**Table 2:** Recovery data

Level (%)	Amount added Concentration (in µg/ml)	% Recovery (n=3)	% R.S.D (n=3)
10	1.0	99.3	0.5
30	3.0	100.9	1.1
50	5.0	98.0	0.8
75	7.5	99.5	1.3
100	10.0	101.5	0.9
150	15.0	99.2	1.2

## Application of the developed method to commercial RAB tablets

When the developed method was used to analyze a commercial brand of RAB tablet formulation, the mean recovery of six replicates was 99.69 % with % R.S.D. of 0.52. The % recovery value indicates non-interference from the excipients present in the dosage form.

Application of the developed method to withdrawn RAB tablet dissolution samples indicates that mean release in acid (pH 1.2) medium was 2.8 % (n = 6)) with % R.S.D of 4.5 and while in alkaline medium (pH 8.0 buffer), the release was 97.2 % (n = 6) with % R.S.D of 3.2.

Both the assay and dissolution test analyses were performed within 45 min thus indicating rapidity of analysis using UPLC method.

## DISCUSSION

### Method development and optimization

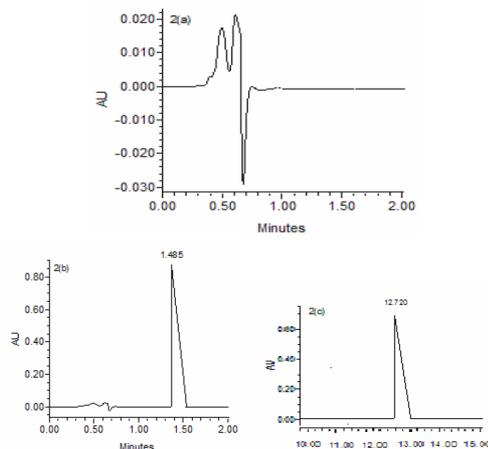
The main aim of the developed method was to achieve separation and quantification of RAB using an isocratic mobile phase with

UPLC system. Developing a UPLC method was to reduce the run time of the method and solvent consumption for routine analysis such as assay, dissolution and content uniformity during quality assurance. Detection of RAB was adequate at 280 nm. The initial trial was conducted using HPLC and chromatographic separation was obtained on a Waters symmetry C<sub>18</sub> column (150 x 4.6mm, particle size 5µm). RAB is an acid labile compound and to avoid any degradation, a mobile phase with basic pH was selected. The mobile phase was optimized in the ratio of 10mM potassium dihydrogen phosphate buffer (pH 7.4, adjusted with potassium hydroxide solution) to acetonitrile of 65: 35 (v/v) with a flow rate of 1.0 ml/min and injection volume 20 µl.

While developing the UPLC method, basic chromatographic conditions such as the column, solvents and UV detection employed in the HPLC method were taken into account. In selecting the UPLC column, its stability at the higher pH was taken into consideration to preserve the long life of the column. For RAB, the recommended Food and Drug Administration (FDA) medium for dissolution solution studies is pH 8.0; the optimized mobile phase pH used in the present study is also on the basic side. Most commercial C<sub>18</sub> columns are not stable at high pH on the longer run, thus shortening their life span. Waters Acquity BEH C<sub>18</sub> (50 x 2.1mm, particle size 1.7 µm) column was found to be more suitable and stable at this pH. The peak was sharp and acceptable and the injection volume was reduced from 20 to 5 µl. The flow rate also is scaled down from 1.0 to 0.4 ml/min. When these operating condition were applied to the developed method, a satisfactory peak was achieved for RAB, which eluted at around 1.49 min giving a total run time of 2 min.

A comparison of HPLC and UPLC (Fig 2) with regard to elution time, sensitivity and other chromatographic parameters when the standard solution was injected, is shown in Table 3. The results indicate that the elution

time of RAB was reduced by about 9-fold compared to that for HPLC. Theoretical plates obtained for UPLC are about 8-fold higher than for HPLC which proves the higher separation efficiency of the UPLC than that of the HPLC method.



**Fig 2:** UPLC chromatograms of blank (a) and standard (b); also, HPLC chromatogram of standard (c)

**Table 3:** Comparative operating parameters of HPLC and developed UPLC methods

System	Flow rate (ml/min)	Injection volume (µL)	USP tailing	USP plate count	Elution time (min)
UPLC	0.4	5	1.2	44790	1.49
HPLC	1.0	20	1.5	4700	12.72

## CONCLUSION

The new, isocratic RP-UPLC method proved to be simple, linear, precise, accurate, robust, rugged and rapid. The developed method was capable of giving faster elution, maintaining good separation more than that achieved with conventional HPLC. The short retention time of 1.49 min allows the analysis of a large number of samples in a short

period of time and is therefore more cost-effective for routine analysis in the pharmaceutical industries. The developed method was successfully applied to the dissolution study of RAB tablet. It is suitable for rapid and accurate quality control of RAB in tablet formulations.

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