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RESEARCH ARTICLE

A Validated HPLC Method for the Determination of Rabeprazole in Bulk and Pharmaceutical Dosage form.

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ABSTRACT

A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of Rabeprazole in bulk and tablets dosage forms. The separation was achieved on C18 analytical column (250 mm × 4.6 mm i.d., 5.0 µm) using acetonitrile and phosphate buffer (pH 7) in the ratio 60:40 v/v as mobile phase and at a flow rate of 1.0 mL/min. Detection was carried out using a UV detector at 282nm. The total chromatographic analysis time per sample was about 10.0min with Rabeprazole eluting at retention time of about 13min. The method was validated for accuracy, precision, specificity, linearity and sensitivity. Validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible. The standard curve was linear over the concentration range of 25-150µg/mL with R^2 close to one (1.0002). The limit of detection (LOD) and limit of Quantitation (LOQ) obtained for Rabeprazole were 0.02µg/mL and 0.05µg/mL, respectively. The developed and validated method was successfully applied for the quantitative analysis of Aptizole® Tablets. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of Rabeprazole in tablet dosage form.

KEY WORDS: Analytical method development, Reversed phase HPLC, ICH guidelines, Tablet dosage forms, Accuracy and precision

1. INTRODUCTION:

Rabeprazole (Sodium) is a proton pump inhibitor (PPI) belonging to anti-secretory and gastric mucosal protecting group of drug. According to IUPAC, the Rabeprazoel (sodium) is (*RS*)-2-([4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfinyl)-1*H*-benzo[*d*]imidazole.

Rabeprazole (Sodium) produces its pharmacological action by reducing the concentration of gastric acid by hindering enzyme action in gastric parietal cells, thus putting off movement of hydrogen ion into gastric lumen **(1).**

Upon oral administration, Rabeprazole has an absolute bioavailability of approximately 52% and Maximum plasma concentrations are achieved after 3-4 h. It undergoes a complete and non-enzymatic metabolism. The metabolites are eliminated via renal route with an elimination half-life of about 1 h **(2,3)**.

There are various methods in the literature for the qualitative and quantitative analysis of the Rabeprazole in the bulk and the pharmaceutical dosage forms. The method was developed and validated under the light of International Conference on Harmonization (ICH) guidelines **(4, 5)**. And for the statistical evaluation of results, standards guidelines were followed **(6, 7).** Hence, our aim was to establish an easy and convenient high pressure liquid chromatography (HPLC) technique, which not only useful for researcher but also for the analysts working in the pharmaceutical quality control labs.

2. MATERIALS & METHODS:

2.1 APPARATUS & CHROMATIC CONDITIONS:

An isocratic elution HPLC system of Shimadzu with LC20AD pump and SPD-20A UV-visible detector was used working via Lab-Solution software. The separation was carried on column (*thermolab)* with C_{18} packaging and 250 x 4.6mm dimensions (5µm internal diameter). The analysis of elution was completed at 282nm on 40°C temperature (Achieved by Shimadzu Column Oven). The run time was set at 10 minutes for this analysis at flow rate of 1.4 ml/minute.

2.2 CHEMICALS & REAGENTS:

The working standards of Rabeprazole (99.97% purity) was received from Global Pharmaceuticals Pvt. Ltd, Islamabad, as gift sample. The Aptizole tablets (Global Pharmaceuticals, Islamabad) claiming film coated tablet containing 40mg Rabeprazole were purchased from the local pharmaceutical market. The acetonitrile, potassium dihydrogen phosphate and disodium hydrogen phosphate used in the research were of HPLC grade. All the chemicals purchased from the local franchise of Sigma Aldrich.

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2.3 PREPARATION OF MOBILE PHASE:

The mobile phase was prepared by mixing phosphate buffer and acetonitrile in 60:40 (v/v) ratios. The phosphate buffer was prepared by dissolving 13.6gm of potassium dihydrogen phosphate in 1000ml of distilled water and adjusting the pH of the buffer to 7 with the help of 3.6% disodium hydrogen phosphate. The final mobile phase was then filtered by passing through 0.5µm membrane filter and degassed before use.

2.4 PREPARATION OF STANDARD SOLUTION:

Standard solution was prepared by dissolving Rabeprazole (Sodium) equivalent to 100mg of Rabeprazole in mobile phase (final concentration, 1 mg/mL). Then, 1ml of the above solution was diluted to 100ml using the same solvent (final concentration, 10µg/mL). The solution should be stayed for 2 hours in dark. This solution was filtered through 0.2µm membrane filter and 20 µL of this solution was injected for HPLC analysis. Unknown assay samples were quantified based on the AUC of the above standard.

2.5 TABLETS SAMPLE PREPARATION:

For the assay of Rabeprazole, 20 tablets were weighed; their contents were crushed into fine powder and mixed thoroughly. An amount of tablet powder equivalent to 100mg of Rabeprazole was accurately weighed and transferred in a 100mL volumetric flask and dissolved in mobile phase (final concentration, 1 mg/mL). Then, 1ml of the above solution was diluted to 100ml using the same solvent (final concentration, 10µg/mL). The solution should be stayed for 2 hours in dark. This solution was filtered through 0.2µm membrane filter and 20 µL of this solution was injected for HPLC analysis.

3. RESULTS & DISCUSSION:

3.1 SYSTEM SUITABILITY:

Before performing the main analysis, the system suitability was evaluated **(8)**. For this purpose, various parameters were calculated as per their standard procedure e.g. retention time (for Rabeprazole), theoretical plates number of the column (for column efficiency), tailing factor, relative standard deviation of peak area and retention time. The table 1 shows the result for these parameters. The column efficiency was much better for analysis i.e. ≥2000. The tailing factor was also within range i.e. ≥1.2. Moreover, the calculated relative standard deviation for the retention time and peak area (mean of 6 replicates) also within acceptance criteria. Depending on all these information, it reflects that the proposed method will be suitable for routine analysis.

3.2 ACCURACY:

In order to check the accuracy of the method, solution of Rabeprazole with different concentration (25, 50, 75, 100, 125, and 150%) was prepared and then analyzed over HPLC with the help of developed method. During this step, six samples of each concentration were prepared and their mean was used for further calculations. These calculations (percentage recovery) are shown in the table 2. The results in the given table show that the recovery of Rabeprazole from the prepared samples ranges from 99.78% to 100.23% i.e. within ±1% range. Moreover, the RSD (relative standard deviation) also lies within acceptance range i.e. ≤2.0. These all observations and calculations indicate the method's accuracy due to narrowness of theoretical and actual yields.

3.3 PRECISION:

The precision of the method was checked by inter day and intraday repeatability and reproducibility **(9)**. The repeatability of method was analyzed by replicate analysis (n=6) by injecting the sample solution into the HPLC system. The results are shown in the table 3 which indicates that the proposed method is good with high precision. Moreover, the low RSD values indicate the high degree of correctness of method.

Similarly, for reproducibility was checked by replicate analysis (n=18) of samples over 3 consecutive days. From results (given in table 3), the low calculated RSD reflects that the method has a good inter-day reproducibility.

3.4 LINEARITY:

The linearity of the method was checked by preparing different strengths solution of Rabeprazole from 25% to 150%. Then, a linear regression equation was derived by plotting the graph between the sample dissolved and recovered by the method. From the observation and calculation (given in table 4), it is cleared that the correlation coefficient (R^2) equal to unity and comes under t he acceptance criteria ($R^2 \geq 0.999$). Moreover, the calculated Y-intercept is 0.0193 which is also less than ±2%. Therefore, depending upon calculated values of R^2 and Y-intercept, the developed method should be considered having a high degree of linearity **(10)**.

3.5 LIMIT OF QUANTIFICATION (L.O.Q) AND LIMIT OF DETECTION (L.O.D):

Calibration curves were constructed in a very low concentration region (0.05 to 1.0% of the target concentration) of Rabeprazole (0.10 to 0.20µg/mL) for the calculation of the limit of detection (LOD) and the limit of quantification (LOQ) using Eqs. (1) and (2), respectively.

$$LOD = \frac{3.3\sigma}{S} \quad (1)$$

$$LOQ = \frac{10\sigma}{S} \quad (2)$$

Where σ is the residual standard deviation of the regression line, S is the slope of the standard curve. The LOD and LOQ obtained for Rabeprazole were 0.02µg/mL and 0.05µg/mL, respectively".

3.6 APPLICATION TO PHARMACEUTICAL DOSAGE FORM:

1. The proposed method was also applied to the pharmaceutical dosage (Tablets in this case) form of the Rabeprazole. For this purpose 3 batches were selected and 6 replicates of each batch were analyzed by the HPLC, from the results (Table 5), it was observed that the obtained results are in good agreement with the claimed amount of Rabeprazole by the manufacturer.

Sr. No.	Parameters	Rabeprazole
1	Retention time (min)	8.3
2	Plate number	3416
3	Tailing factor	0.853
4	RSD of peak area (n=6)	0.721
5	RSD of retention time (n=6)	0.84

Table No. 1: System suitability

Sr. No.	Concentration level (%age)					
	25	**50**	**75**	**100**	**125**	**150**
1	100.2	99.95	100.01	100.12	100.17	99.91
2	99.78	99.98	100.09	100.18	100.21	99.82
3	99.91	100.21	100.11	99.97	99.85	99.99
4	100.13	100.14	99.89	100.23	99.95	100.21
5	100.07	100.05	99.95	100.05	99.91	100.14
6	99.84	100.07	99.91	99.79	100.19	99.89
Mean	99.98	100.07	99.99	100.05	100.04	99.99
%RSD	0.17	0.10	0.09	0.16	0.16	0.15

Table No. 2: Accuracy of Method

Sr. No.	Recovery (%age)		
	Day 1	**Day 2**	**Day 3**
1	99.79	100.11	100.10
2	99.95	100.04	100.22
3	100.22	100.09	100.17
4	99.85	100.15	99.89
5	100.09	99.97	99.92
6	99.94	99.82	99.99
Mean	99.97±0.15	100.03±0.12	100.04±0.13
Inter day (n=18)		**100.01±0.13**	

Table No. 3: Inter day and intraday precision of the method:

Sr. No.	Drug Dissolved	Drug Recovered
1	25	24.96
2	50	50.13
3	75	74.90
4	100	100.09
5	125	124.78
6	150	150.17
Correlation Coefficient (R^2)=1.0002		
Y-intercept=0.0193		
Regression Equation: 0.0193+1.0002x		

Table No. 4: Linearity of the Method:

B. No.	Drug Recovered (mg)±SD
1	40.14±0.531
2	40.07±0.780
3	39.93±0.612
Note: n=6; SD=Standard Deviation	

Table No. 5: Assay Results of Rabeprazole Tablets (Aptizole):

4. CONCLUSION:

A simple isocratic RP-HPLC method has been developed for the determination of Rabeprazole in bulk and tablet dosage form, using a UV detector. The method was validated for accuracy, precision, specificity and linearity. The method has a relatively short run time (10min) that allows quantifying a large number of samples in routine and quality control analysis of tablets. In order to reduce cost of analysis and to increase sample throughput during routine analysis, the method is being further optimized, employing statistical experimental design.

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