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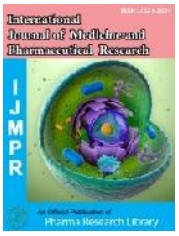

**Research Article** **Open Access**

# Reverse Phase High Performance Liquid Chromatographic Technique for the Determination of Rabeprazole in Pure and It's Dosage Form

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**A B S T R A C T**

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Rabeprazole, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Symmetry C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol and water (80:20 v/v) as the mobile phase at a flow rate of 0.8ml/min, the detection was carried out at 280nm. The retention time of the Rabeprazole was 2.379 ±0.02min respectively. The method produce linear responses in the concentration range of 20-100mg/ml of Rabeprazole. The method precision for the determination of assay was below 2.0%RSD.The method is useful in the quality control of bulk and pharmaceutical formulations.

**Keywords:** Rabeprazole, RP-HPLC, validation.

**A R T I C L E I N F O**

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## 1. Introduction

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

**Introduction to HPLC**

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance.

**1.2.1 Types of HPLC Techniques**

**1.2.1.1 Based on Modes of Chromatography**

These distinctions are based on relative polarities of stationary and mobile phases

**Reverse phase chromatography:**

In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

**Normal phase chromatography:**

In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.

## 2. Materials and Methods

**Materials:** Rabeprazole (Pure), Water and Methanol for HPLC, Acetonitrile for HPLC

**Method Development**

The selected method was used for method development of the drug molecules Rabeprazole as per ICH guidelines. By taking different mobile phases and by changing columns trials have been done and validation process is done for the optimized method.

**Optimized Chromatographic Conditions**:

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.

| | | |
|---|---|---|
| Temperature | : | 45ºC |
| Column | : | Symmetry C18 (4.6 x 150mm, 5µm) |
| Mobile phase | : | Methanol: Water (80:20 v/v) |
| Flow rate | : | 0.8ml/min |
| Wavelength | : | 280nm |
| Injection volume | : | 10µl |
| Run time | : | 6minutes |

## 3. Results and Discussion

**Optimized Chromatogram (Standard)**

| | |
|---|---|
| Mobile phase ratio | : Methanol: water (80:20 v/v) |
| Column | : Symmetry C18 (4.6×150mm) 5µ |
| Column temperature | : 45ºC |
| Wavelength | : 280nm |
| Flow rate | : 0.8ml/min |
| Injection volume | : 10µl |
| Run time | : 6minutes |

**Specificity**

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitative Rabeprazole in drug product.

**Precision:** The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

**Repeatability:** Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

**Accuracy:** Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

**Limit of Detection for Rabeprazole**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

**Result:**

=3.3×53204/38050

=4.6µg/ml

**Quantitation limit**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

**LOQ=10×σ/S**

Where, σ = Standard deviation of the response

S = Slope of the calibration curve

**Result:** =10×53204/38050

=13.9µg/ml

**Robustness:** The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Rabeprazole. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±10%. The standard and samples of Rabeprazole were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

**Table 1:** Optimized Chromatogram (Standard)

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Rabeprazole | 2.379 | 3119086 | 258364 | 1.2 | 5837 |

**Table:2 Optimized Chromatogram (Sample)**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count |
|---|---|---|---|---|---|---|
| 1 | Rabeprazole | 2.380 | 3118812 | 258374 | 1.2 | 5264 |

**Table 3: Results of system suitability for Rabeprazole**

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate Count | US Tailing |
|---|---|---|---|---|---|---|
| 1 | Rabeprazole | 2.317 | 2274631 | 239458 | 5728 | 1.2 |
| 2 | Rabeprazole | 2.302 | 2284721 | 239582 | 5093 | 1.2 |
| 3 | Rabeprazole | 2.323 | 2238127 | 236493 | 5391 | 1.2 |
| 4 | Rabeprazole | 2.343 | 2259349 | 249482 | 6139 | 1.2 |
| 5 | Rabeprazole | 2.321 | 2204850 | 239452 | 5281 | 1.2 |
| **Mean** | | | 2252336 | | | |
| **Std. Dev.** | | | 31827.08 | | | |
| **%RSD** | | | 1.41307 | | | |

**Table 4:** Chromatographic Data for Linearity Study

| Concentration Level (%) | Concentration «g/ml | Average Peak Area |
|---|---|---|
| 33 | 20 | 791554 |
| 66 | 40 | 1647073 |
| 100 | 60 | 2283804 |
| 133 | 80 | 3058339 |
| 166 | 100 | 3839630 |

**Table 5:** Results of repeatability for Rabeprazole

| S. No | Peak name | Retention time | Area(µV*sec) | Height (µV) | USP Plate Count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Rabeprazole | 2.356 | 2259464 | 245362 | 5938 | 1.2 |
| 2 | Rabeprazole | 2.356 | 2275915 | 248293 | 5827 | 1.2 |
| 3 | Rabeprazole | 2.357 | 2282117 | 240795 | 5032 | 1.2 |
| 4 | Rabeprazole | 2.358 | 2278675 | 230139 | 5978 | 1.2 |
| 5 | Rabeprazole | 2.359 | 2282448 | 249605 | 6183 | 1.2 |
| **Mean** | | | 2275724 | | | |
| **Std.dev** | | | 9476.485 | | | |
| **%RSD** | | | 0.416416 | | | |

**Table 6:** Results of Intermediate precision for Rabeprazole

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Rabeprazole | 2.380 | 2236184 | 202188 | 5472 | 1.2 |
| 2 | Rabeprazole | 2.383 | 2238020 | 201837 | 6193 | 1.2 |
| 3 | Rabeprazole | 2.385 | 2239352 | 201273 | 5980 | 1.2 |
| 4 | Rabeprazole | 2.385 | 2242466 | 203923 | 7163 | 1.2 |
| 5 | Rabeprazole | 2.389 | 2244692 | 202938 | 6182 | 1.2 |
| 6 | Rabeprazole | 2.389 | 2247654 | 201982 | 7684 | 1.2 |
| **Mean** | | | 2241395 | | | |
| **Std.Dev.** | | | 4333.851 | | | |
| **%RSD** | | | 0.193355 | | | |

**Table 7:** Results of Intermediate precision Day 2 for Rabeprazole

| S.No | Peak Name | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Rabeprazole | 2.380 | 2236184 | 217363 | 5928 | 1.2 |
| 2 | Rabeprazole | 2.383 | 2238020 | 218467 | 6183 | 1.2 |
| 3 | Rabeprazole | 2.385 | 2239352 | 218346 | 5927 | 1.2 |
| 4 | Rabeprazole | 2.385 | 2242466 | 221736 | 5163 | 1.2 |
| 5 | Rabeprazole | 2.389 | 2244692 | 228361 | 4827 | 1.2 |
| 6 | Rabeprazole | 2.346 | 2263431 | 217553 | 5019 | 1.2 |
| **Mean** | | | 2244024 | | | |
| **Std.Dev.** | | | 9988.458 | | | |
| **%RSD** | | | 0.445114 | | | |

**Table 8:** The accuracy results for Rabeprazole

| %Concentration (at specification Level) | Area | Amount Added (ppm) | Amount Found (ppm) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 1172485 | 30 | 29.9 | 99.7 | 100.0% |
| 100% | 2314753 | 60 | 59.9 | 99.8 | |
| 150% | 3480210 | 90 | 90.5 | 100.6 | |

**Table 9:** Results for Robustness

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 3119086 | 2.379 | 5837 | 1.2 |
| Less Flow rate of 0.7 mL/min | 2640811 | 2.763 | 5361 | 1.2 |
| More Flow rate of 0.9 mL/min | 2640354 | 2.234 | 5231 | 1.2 |
| Less organic phase | 2640758 | 2.765 | 4503 | 1.5 |
| More organic phase | 2640125 | 2.236 | 4491 | 1.5 |

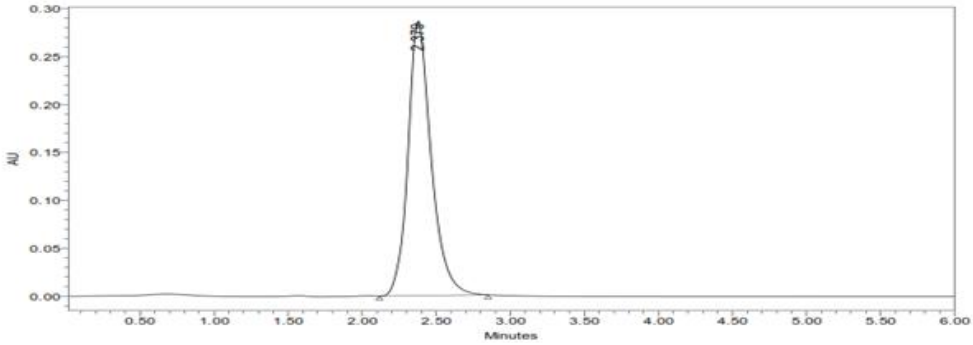


**Figure 1:** Optimized Chromatogram

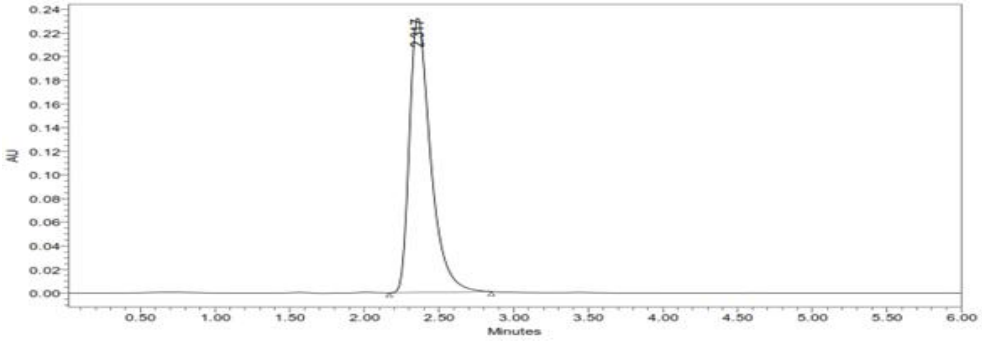


**Figure 2:** Chromatogram showing system sutability

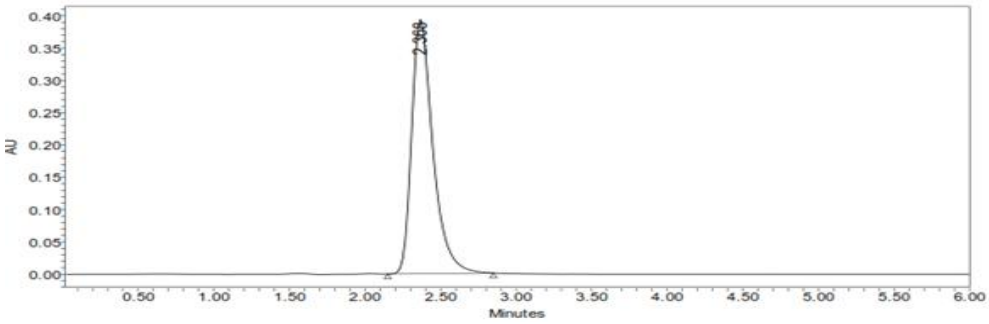


**Figure 3:** Chromatogram showing linearity level

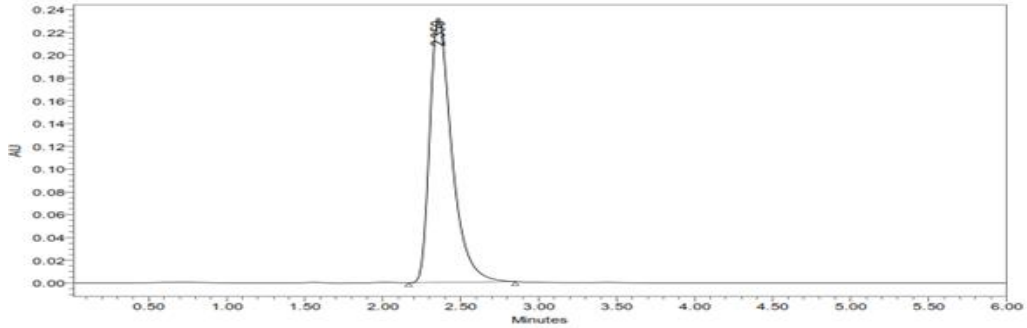


**Figure 4:** Chromatogram showing precision injection

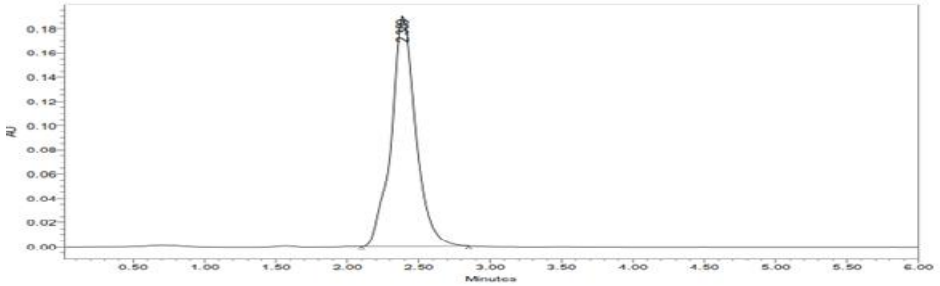


**Figure 5:** Chromatogram showing Day1 intermediate injection -5

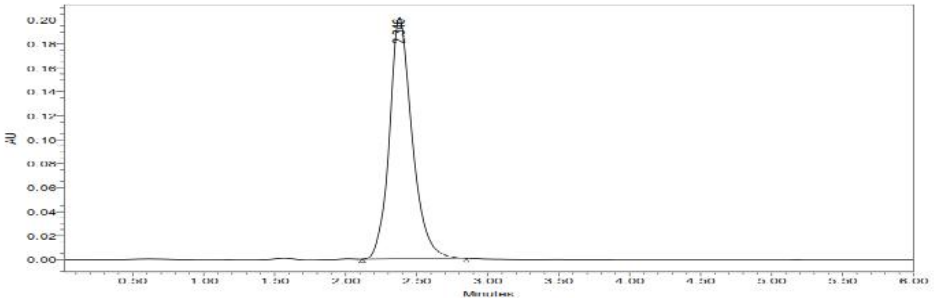


**Figure 6:** Chromatogram showing Day 2 intermediate injection

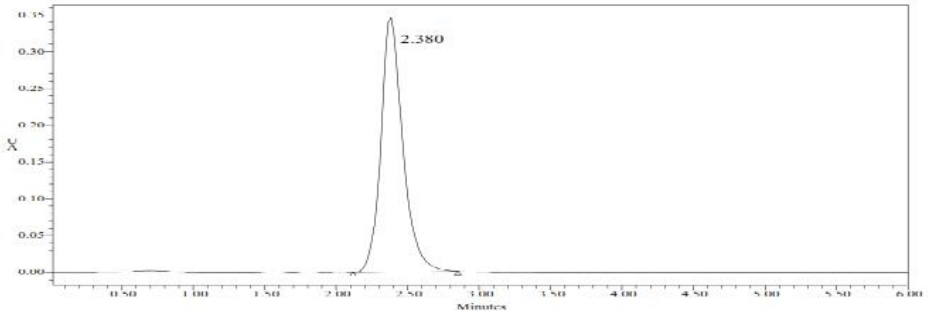


**Figure 7:** Chromatogram showing accuracy-50% injection

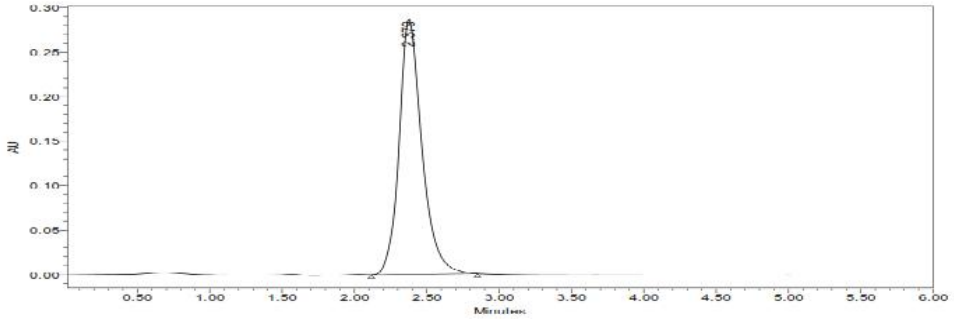


**Figure 8:** Chromatogram showing accuracy-100% injection

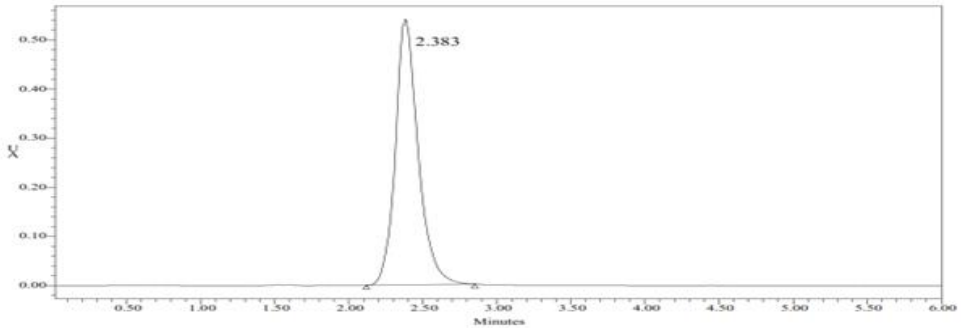


**Figure 9:** Chromatogram showing accuracy-150% injection

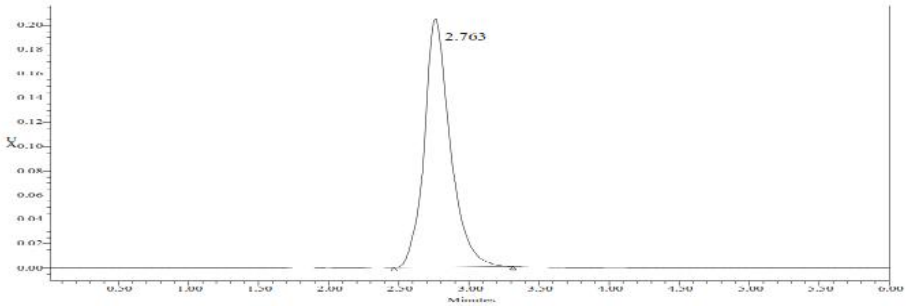


**Figure 10:** chromatogram showing less flow of 0.9ml/min

## 4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Rabeprazole in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Rabeprazole was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: water was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Rabeprazole in bulk drug and in Pharmaceutical dosage forms.

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