A RP-HPLC METHOD FOR DETERMINATION OF DICLOFENAC WITH RABEPRAZOLE IN SOLID DOSAGE FORM

Arunadevi S. Birajdar*, Subramania Meyyanathan, Bhojraj Suresh.

Department of Pharmaceutical Analysis, J.S.S. College of Pharmacy, Ootacamund, Tamilnadu-643 001, India.

ABSTRACT
To develop and validate simple, reproducible, stability indicating reverse phase high performance liquid chromatography for the simultaneous determination of Rebeprazole and Diclofenac in pharmaceutical solid dosage form. The method was based on HPLC separation of both drugs in reverse phase mode using Phenomenox C18 column with Waters HPLC system by using mobile phase composition of acetonitrile and 50mM ammonium acetate buffer (pH 3.6) (60:40 v/v) at flow rate 1 ml/min. Detection wavelength used at 254 nm. Loratidine was used as internal standard. Linearity was obtained in the concentration range of 1.0-3.2 µg/ml for rabeprazole and 6.0-16.0 µg/ml diclofenac. The mean values of the correlation coefficient, slope and intercept for rabeprazole and diclofenac were 0.998, 0.0186, +0.010 and 0.999, 0.0193, -0.0098 respectively. The method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) for rabeprazole 30, 95 ng/ml and for diclofenac 125, 375 ng/ml respectively. Statistical analysis showed that the method is reproducible and selective for the estimation of rabeprazole and diclofenac in pharmaceutical formulations. This method can be applicable for bioequivalence studies. Keywords: Rebeprazole; Diclofenac; Loratidine(IS); RP-HPLC.

INTRODUCTION
Rabeprazole chemically 2-([4-(3-Methoxypropoxy)-3-methyl-2 pyridyl]methyl)sulfinyl)-1H-benzimidazole sodium. It belongs to a class of proton-pump inhibitors. It suppresses gastric acid secretion by specifically inhibiting the H+/K+-ATPase enzyme system at the secretory surface of the gastric parietal cell Clinically, rabeprazole is used to heal, relieve symptoms and prevent a relapse of acid-peptic diseases, such as duodenal, gastric and esophageal ulceration [1]. Diclofenac sodium, monosodium (2-[(2, 6-dichloroanilino) phenyl acetate, is a potent analgesic, non-
steroidal anti-inflammatory drug [NSAID]. It is used in inflammatory and painful diseases of rheumatic and non-rheumatic origin \[^1\]. Literature survey revealed that various reports on stability in aqueous media \[^2\], analytical methods such as HPLC \[^3\], UV \[^4\], enantiomeric determination \[^5\], estimation of photo degradation products by UV and HPLC \[^6,7\] have been reported for individual estimation of rabeprazole from its formulations.

Literature survey revealed that limited reports on analytical techniques for estimation of individual drug diclofenac from formulations like potentiometric and fluorimetric determination \[^8\]. There were no methods so far reported for simultaneous determination of both drugs for simultaneous estimation by RP- HPLC which was simple and reproducible.

It is essential to develop simple, precise, accurate HPLC methods for simultaneous determination of both drugs in solid dosage from. Therefore, in this study we developed reproducible methods which can be used in laboratory. HPLC method can be applied in future for estimation same drugs from biological samples. The validation of this method carried out as per ICH guidelines \[^9,10\].

![Figure 1](image-url)
Chemical structure of Rebeprazole, Diclofenac and Loratidine (IS).

MATERIAL AND METHODS

Materials

Pharmaceutical grade Rabeprazole and Diclofenac were kindly supplied as a gift sample by Shreechem Pharmaceuticals Pvt ltd. New Mumbai, India. Loratidine (IS) procured from Apex drugs and intermediates Medka (A.P.) All chemicals and solvents of HPLC grade and were purchased from Qualigens fine Chemicals, Mumbai, India. Water HPLC grade was obtained from a Milli-QRO water purification system.

HPLC method

LC system used consisted of pump model (Waters 1515 isocratic solvent delivery system) with universal loop injector (Rheodyne 7725 i) of injection capacity 20 μL. Detector consisted Waters 2487 duel wavelength absorbance detector. The column used was C18 (25 cm X 4.6 mm i.d. 5 μm particle size) phenomnex, USA, at ambient temperature. Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously.

The optimal composition of mobile phase was composition of acetonitrile and 50mM ammonium acetate buffer (pH 3.6) (60:40 v/v). The flow rate was set to 1 ml/min and UV detection was carried out at 254 nm due to overlay point of each component spectrum observed. Loratidine was used as an internal standard. Stock solution was prepared by dissolving 10 mg of rabeprazole and diclofenac in 100 ml volumetric flask separately with mixture of acetonitrile and water (1:1). Stock solution of loratidine (internal standard) was prepared 10 mg in separate 100 ml volumetric flask with same mixture. All solutions were stored at + 20 °C, these solutions were shown to be stable during the period of study. From the above stock solutions, dilutions were made for working standard the concentration range of rebeprazole 1.0–3.2 μg/ml and of diclofenac 6.0–16.0 μg/ml respectively, and each concentration solution contains 20 μg/ml of loratidine as an internal standard. A volume of 20 μl of each working standard was injected into column. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area ratios of analyte to internal standard vs. the corresponding drug concentration.

Analysis of Pharmaceutical Dosage Forms
To determine the content of rabeprazole and diclofenac simultaneously in capsules (label claim: 20 mg Rabeprazole and 100 mg Diclofenac); twenty capsules granules were weighed; their average weight determined and were finely powdered. The correct amount of powder equivalent to one capsule granule were dissolved in methanol by stirring and sonicated for 30 min. The excipients were separated by filtration. After filtration, an appropriate amount of internal standard was added and diluted up to mark with methanol. Further dilutions are made with mobile phase to get working standard solution containing 2µg/ml of rabeprazole, 10µg/ml of diclofenac and 20µg/ml of loratidine (internal standard). This sample solution injected thrice and recorded chromatogram. The amount of rabeprazole and domperidone were determined. The results are reported in Table 1.

### Table 1: Validation Parameters of Determination of Rabeprazole and Diclofenac

<table>
<thead>
<tr>
<th>Validation parameters</th>
<th>Rabeprazole</th>
<th>Diclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity and range</td>
<td>1 - 3.2</td>
<td>6 - 16</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.998</td>
<td>0.999</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.164</td>
<td>0.120</td>
</tr>
<tr>
<td>LOD (ng/ml)</td>
<td>30</td>
<td>95</td>
</tr>
<tr>
<td>LOQ (ng/ml)</td>
<td>125</td>
<td>375</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>99.78</td>
<td>100.08</td>
</tr>
</tbody>
</table>

### Recovery studies

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method at 80, 100 and 120 % level. From the total amount of drug found, the percentage recovery was calculated. The results are reported in Table 2.
Table 2: Results of Analysis for Formulation and Recovery Studies

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Amount mg/ Capsule</th>
<th>%Recovery ± SD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labeled</td>
<td>Found</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>20.00</td>
<td>20.04</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>100.0</td>
<td>101.24</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

For HPLC method, chromatographic conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried for better separation of drugs with internal standard. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The mobile phase used was mixture of acetonitrile and 50mM ammonium acetate buffer (pH 3.6) in the ratio (60:40 v/v) with 1 ml/min flow rate was quite satisfactory. Loratidine was used as an internal standard, neutralizing the error inherent in sample injection, eliminating random errors. The optimum wavelength fixed for detection was 254 nm at which better detector response for drugs were obtained.

System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. The calibration was linear for rabeprazole at concentration range of 1.0 –3.20 μg/ml, with regression 0.998, intercept + 0.010 and slope 0.0186. The calibration was linear for diclofenac at concentration range of 6.0–16.0 μg/ml, with regression 0.999, intercept -0.0098 and slope 0.0193 respectively. A typical chromatogram and typical 3D chromatogram for rabeprazole, diclofenac and loratidine (internal standard) for sample solution was shown as in (Fig. 2). The average retention time for rabeprazole, diclofenac and loratidine (IS) was found to be 3.214 ± 0.03, 7.070 ± 0.03 and 14.526 ± 0.02 min, respectively.
Sample to sample precision and accuracy were evaluated using, three samples of three different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of one week. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference on the assay, which was tested on an intra – day and inter – day basis. The % R.S.D. values was found to be less than 3% shows that proposed method provides acceptable intra –day and inter – day variation of rabeprazole and diclofenac and conformed precision and accuracy reported in Table 1. The mean recoveries were found in the range of 98.20 – 99.08 %.

**Figure 2**

Typical and 3D chromatogram of sample solution with IS
CONCLUSION

The new HPLC method developed and validated for simultaneous determination of rabeprazole and diclofenac pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

ACKNOWLEDGEMENT

The author’s thank to Shreechem Pharmaceuticals Pvt Ltd. New Mumbai, India. for providing gift samples of Rabeprazole and Diclofenac Cadila Pharmaceuticals Ltd, also to Apex drugs and intermediates Medka (A. P.) for providing a gift sample of Loratadine. The authors are thankful to Mr. Supe (Drug Inspector).The author’s are grateful to “His Holiness Jagadguru Sri Sri Shivarathree Deshikendra Mahaswamigalavaru” of Sri Suttur Mutt, Mysore and AICTE (QIP) cell for providing facilities to carry out this work.

REFERENCES


For Correspondence:
Miss. Arunadevi S. Birajdar
Mobile phone: 09586715521
E-mail: aruna_birajdar@rediffmail.com