RP-HPLC analysis for the simultaneous estimation of rabeprazole sodium and aceclofenac in a combined dosage form

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ABSTRACT
A rapid, simple and highly sensitive reversed phase high performance liquid chromatographic (RP-HPLC) method has been developed for the quantitative determination of Rabeprazole sodium and Aceclofenac in a combined dosage form. Rabeprazole sodium and Aceclofenac were chromatographed using C-18 column as stationary phase and methanol: acetonitrile: water (60 : 10 : 30 v/v/v) as the mobile phase at a flow rate of 1.0 ml/min at ambient temperature and detected at 280 nm. The retention time (RT) of Rabeprazole sodium and Aceclofenac were found to be 5.611 min and 2.102 minutes, respectively. The linearities of Rabeprazole sodium and Aceclofenac were in the range of 1-10 µg/ml and 3-15 µg/ml, respectively. The limit of detection was found to be 0.091 µg/ml for Rabeprazole sodium and 0.043 µg/ml for Aceclofenac. The proposed method was applied for the determination of Rabeprazole sodium and Aceclofenac in a combined dosage form and result was found satisfactory.

Key Words: Quantitative analysis, validation, capsule, standard addition method, anti-inflammatory, analgesic.

INTRODUCTION
Rabeprazole sodium (figure 1) is chemically sodium salt of 2-[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl] methyl] sulfinyl]-1H-benzimidazole and used as antiulcer agent (Merck index, 2001). It is a proton pump inhibitor, which suppresses gastric acid secretion by specific inhibition of the gastric H+/K+/ATPase enzyme at the secretory surface of the gastric parietal cells (Patel et al., 2007). Aceclofenac (figure 2) is chemically [[2-[(2, 6-Dichlorophenyl) amino] phenyl] acetyl] oxy] acetic acid and has anti-inflammatory and analgesic properties (Indian Pharmacopoeia, 2007). Aceclofenac directly blocks prostaglandin (PGE2) secretion at the site of inflammation by inhibiting interleukin (IL1) and tumour necrosis factor (TNF) in the inflammatory cells (intracellular action) (Garg et al., 2006). Literature review reveals that Spectrophotometric (Kousy et al., 1999; Zawilla et al., 2002; Rahman et al., 2008; El-Gindy et al., 2003; Shah et al., 2008; Misra et al., 2006) HPLC (Singhvi et al., 2007; Park et al., 2008; Ramakrishna et al., 2005; Srinivas et al., 2009; Uno et al., 2005; Singh et al., 2004; Rao et al., 2006; Kulkarni et al., 2006; Jin et al., 2006; Bhinge et al., 2008; Hinz et al., 2006; Musmade et al., 2008) and HPTLC (Joshi et al., 2008) methods have been reported for estimation of individual drugs or in combination with other drugs in formulation. So far no simultaneous method has been reported as this was a new combined dosage form. A single, simple and precise method was to be explored for estimation of Rabeprazole sodium and Aceclofenac in a combined dosage form by RP-HPLC. Therefore, the aim of this work was to develop a RP-HPLC method for the determination of Rabeprazole sodium and Aceclofenac in a combined formulation.

MATERIALS AND METHODS
Rabeprazole sodium and Aceclofenac pure samples were procured as gift samples from Ranbaxy Laboratories Ltd., Gurgaon. Acetonitrile, methanol and water (HPLC grade, Merck chem. Ltd.) were used for mobile phase preparation and as solvents. Altraday® capsules (Ranbaxy Laboratories Ltd., Mumbai), which were claimed to contain 20 mg of...
Rabeprazole sodium IP and 200 mg of Aceclofenac IP, were procured from a local market. An Agilent HPLC1200 instrument equipped with UV-Visible detector, manual injector of 50 µL loop and column Pursuit C-18 (250 mm x 4.6 mm i.d., 5 µm particle size) was used, a weighing balance (Afcoset ER 200A) and a sonicator were used for the study.

Chromatographic conditions
Chromatographic estimations were performed under the following conditions: Pursuit C-18 column (250 mm x 4.6 mm i.d., 5 µm) was used at ambient temperature. The mobile phase comprised methanol: acetonitrile: water (60: 10: 30 v/v/v) was pumped at a flow rate of 1 ml/min. The mobile phase was filtered through Nylon 0.45 µm, 47 mm membrane filter and was degassed before use. The elution was monitored at 280 nm. The injection volume was 10 µl.

Preparation of combined standard solution of rabeprazole sodium and aceclofenac
Accurately weighed 2 mg Rabeprazole sodium and 20 mg of Aceclofenac were transferred to 100 ml volumetric flask. It was dissolved with sufficient methanol and diluted up to mark with methanol to give concentration of 20 µg/ml of Rabeprazole sodium and 200 µg/ml of Aceclofenac. Above solution was further diluted with the same solvent to get the concentration of 1.5 µg/ml of Rabeprazole sodium and 15 µg/ml of Aceclofenac in the mixture.

Preparation of Calibration Curve
Standard and sample solutions were injected in a column with 50 µl micro-syringe. The chromatogram was run for appropriate minutes with mobile phase, methanol: acetonitrile: water (60 : 10 : 30 v/v/v) which was previously degassed, detection was carried out at wavelength 280 nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc was recorded using Chemstation software. Calibration curves were constructed by plotting peak areas versus concentrations of Rabeprazole sodium and Aceclofenac and the regression equations were calculated. The calibration curves were plotted over a concentration range 1-10 µg/ml and 3-15 µg/ml for Rabeprazole sodium and Aceclofenac respectively.

Analysis of Pharmaceutical Formulation:
Twenty capsules were weighed and the average weight of capsule was determined. Contents were finely powdered by mortar and pestle. The powder equivalent to about 20 mg of Aceclofenac and 2 mg of Rabeprazole sodium was taken in a 100 ml volumetric flask, dissolved and diluted up to mark with methanol. It was then ultrasonicated for 10 minutes. The solution was filtered through Watman filter paper no.42 and first few drops of filtrate were discarded. 0.75 ml of the filtrate was diluted to 10 ml with methanol to get the concentration of 1.5 µg/ml of Rabeprazole sodium and 15 µg/ml of Aceclofenac. 10 µl of this solution was injected into the instrument and chromatographed. The amount of Rabeprazole sodium and Aceclofenac present in the sample solutions were determined by fitting area values of peaks corresponding to Rabeprazole sodium and Aceclofenac to the equations of the line representing the calibration curve of Rabeprazole sodium and Aceclofenac. All determinations were performed in triplicate.

RESULTS AND DISCUSSION
Rabeprazole sodium and Aceclofenac are soluble in methanol; therefore methanol was selected as the common solvent. The formulation was dissolved in methanol with sonication for 10 minutes to ensure complete release of drug from the formulation.
The mixture of methanol: acetonitrile: water (60 : 10 : 30 v/v/v) could resolve Rabeprazole sodium and Aceclofenac with a better peak shape. The combination of this mobile phase offered optimum separation (5.611 min for Rabeprazole sodium and 2.102 min for Aceclofenac) and resolution (Figure 3). The linearity of Rabeprazole sodium and Aceclofenac were in the range of 1-10 µg/ml and 3-15 µg/ml, respectively, with correlation coefficients of 0.9998 for Rabeprazole sodium and 0.9997 for Aceclofenac. The average linear regression equation was represented as y = 35.918x + 2.6676 for Rabeprazole sodium and y = 19.993x + 0.395 for Aceclofenac, where x is the concentration of drug and y is the peak area. The limit of detection was found to be 0.091 µg/ml for Rabeprazole sodium and 0.043 µg/ml for Aceclofenac. The limit of quantification was found to be 0.305 µg/ml for Rabeprazole sodium and 0.142 µg/ml for Aceclofenac. The intraday precision (RSD) was determined for standard Rabeprazole sodium and Aceclofenac three times on the same day and interday precision was calculated for standard Rabeprazole sodium and Aceclofenac, three times over a period of one week. The intraday and interday coefficients of variation for both drugs were found to be in the range of 0.27-0.83% and 0.54-0.81%, respectively. These values indicate that the method is precise. The accuracy of the method was evaluated by calculating the recovery of Rabeprazole sodium and Aceclofenac by a standard addition method at 3 levels of the calibration curve (n = 3). The percentage recovery was found to be 100.7-101.5% for Rabeprazole sodium and 100.1-100.8% for Aceclofenac, ensuring that the method is accurate. Various validation parameters for the proposed RP-HPLC method for determining the Rabeprazole sodium and Aceclofenac contents in their combined marketed formulation and are summarized in Table 1. Potency determination of marketed capsule formulation by proposed method furnished results of 101.08±0.36% and 100.72±0.40% for Rabeprazole sodium and Aceclofenac, respectively. Analysis result indicates that the proposed RP-HPLC method is simple, rapid, precise and accurate for the simultaneous estimation of Rabeprazole sodium and Aceclofenac in its combined formulation.

Table 1. Summary of the validation parameters of the proposed RP-HPLC method.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rabeprazole sodium</th>
<th>Aceclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range (µg/ml)</td>
<td>1-10</td>
<td>3-15</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9998</td>
<td>0.9997</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.091</td>
<td>0.043</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>0.305</td>
<td>0.142</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>100.7-101.5</td>
<td>100.1-100.8</td>
</tr>
<tr>
<td>Precision (%) RSD</td>
<td>Intraday (n = 3)</td>
<td>0.54-0.69</td>
</tr>
<tr>
<td></td>
<td>Interday (n = 3)</td>
<td>0.54-0.81</td>
</tr>
</tbody>
</table>

LOD = Limit of detection, LOQ = Limit of quantification, RSD = Relative standard deviation, n = number of determination.

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REFERENCES


