SIMULTANEOUS ESTIMATION OF FAMOTIDINE AND RABEPRAZOLE SODIUM: METHOD DEVELOPMENT AND VALIDATION BY UV-SPECTROPHOTOMETRIC METHOD

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ABSTRACT

Objectives: Study aimed for the simultaneous estimation of Famotidine and Rabeprazole sodium at 263 nm, λ_max of Famotidine and 284 nm, λ_max of Rabeprazole sodium.

Methods: Method development and validation were done by UV spectrophotometric method.

Results: The method developed obeyed Beer-Lambert’s law in the concentration range of 5-30 µg/ml for both famotidine and rabeprazole. The results of the analysis have been successfully validated statistically. The %RSD confirms the precision of the proposed method and Recovery studies show the accuracy of the method.

Conclusion: This method was simple, precise and accurate and was successfully applied to the determination of these drugs in the laboratory mixture.

Keywords: Famotidine, Rabeprazole, Beer-Lambert’s Law, simultaneous estimation.

INTRODUCTION

Famotidine chemically 3-[[2-[(Aminoiminomethyl) amino]-4-thiazolyl]methyl][thio]-N-(aminosulfonyl) propanimidamide is used in the treatment of gastric ulcer, stress ulcer and gastritis [1]. An extensive literature survey revealed titrimetry, HPLC, HPTLC and Spectrophotometric methods for the analysis of Famotidine in bulk and in formulations [2].

Rabeprazole sodium is a potent proton pump inhibitor that suppresses gastric acid secretion. It is used in the treatment of gastroesophageal reflux disorder (GERD) and duodenal ulcer [3]. Chemically Rabeprazole is 2-[[4-(3-methoxypropoxy)-3-methyl(pyridin-2-yl) methane] sulfinyl]-1H-1,3 benzodiazole.

Estimation of Famotidine and Rabeprazole by UV-Visible spectroscopy

Preparation of simulated gastric fluid pH 1.2

Accurately weighed 3.75 g of Potassium chloride was dissolved in distilled water and 3.71 ml of Conc. Hydrochloric acid was added to it. The final volume was made upto 1000 ml with distilled water.

Famotidine and Rabeprazole stock solution

An accurately weighed quantity of Famotidine equivalent to 100 mg was dissolved in 100 ml of simulated gastric fluid in 100 ml volumetric flask to get 1000 μg/ml. Similarly Rabeprazole equivalent to 100 mg was dissolved in 100 ml of simulated gastric fluid in 100 ml volumetric flask to obtain 1000µg/ml.

Study of spectra and selection of wavelength

Aliquot portions of stock solutions of Famotidine and Rabeprazole were diluted appropriately with simulated gastric fluid pH 1.2 to obtain concentration 10 µg/ml of each drug. The solutions were scanned in the range of 400-200 nm in 1 cm cell against blank.

The UV absorbance spectrum of Famotidine and Rabeprazole is shown in fig. 3 and fig. 4. From the spectrum the wavelengths selected for estimation of drugs were 263 nm as λ_max of Famotidine and 284 nm as λ_max of Rabeprazole.

Study of beer-lambert’s law

Aliquots of the working stock solution of Famotidine and Rabeprazole were prepared with simulated gastric fluid of pH 1.2 to get concentrations in the range of 5-30 µg/ml for Famotidine and Rabeprazole. The absorbances of the resulting solutions were measured at their respective λ_max. A calibration curve as concentrations. Absorbance was constructed to study the Beer-Lambert’s Law and regression equation.
Vierodt’s simultaneous equation method

If a sample contains two absorbing drugs each of which absorbs at the $\lambda_{max}$ of the other, it may be possible to determine both drugs by the technique of simultaneous equation method. This method analysis is based on the absorption of drugs at the wavelength maximum of the order. Quantification analysis of Drug1 and Drug2 in a binary mixture was performed with the simultaneous equation.

Analysis of laboratory mixture by proposed method

In order to see the feasibility of the proposed method for simultaneous estimation Famotidine and Rabeprazole in pharmaceutical formulations, the method was first tried for the estimation of the drugs in standard laboratory mixture.

Accurately weighed quantities of Famotidine and Rabeprazole sodium 100 mg taken in 100 ml volumetric flask and dissolved in simulated gastric fluid of pH 1.2 by vigorous shaking. The suitable dilutions were made to get a final concentration of about 10 $\mu$g/ml Famotidine and 10 $\mu$g/ml rabeprazole. The absorbances of the resulting solutions were measured at 263 nm and 284 nm in 1 cm cell against a blank. Amount of each drug was determined using simultaneous equation as following:

\[ C_1 = \frac{A_1a_1y_2 - A_2a_2y_1}{ax_1y_2 - ax_2y_1} \quad \ldots \ldots \ldots \quad (Equ. 1) \]

\[ C_2 = \frac{A_2ax_1 - A_1ax_2}{ax_1y_2 - ax_2y_1} \quad \ldots \ldots \ldots \quad (Equ. 2) \]

Where,

$A_1$ and $A_2$ are absorbance of Sample solution of Drug1 and Drug2 respectively.

$ax_1$ = Absorptivity of Drug1 at A1 nm

$ax_2$ = Absorptivity of Drug 1 at A2 nm

$ay_1$ = Absorptivity of Drug 2 at A1 nm

$ay_2$ = Absorptivity of Drug 2 at A2 nm

Validation of proposed method

Validation of an analytical method is the process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application. The UV method was validated in terms of accuracy, precision, LOD, LOQ, linearity and sensitivity.

Linearity

It was determined by measuring the absorbance of various concentrations of both drugs Famotidine and Rabeprazole at their own respective $\lambda_{max}$ and at the $\lambda_{max}$ of other drug. The response was plotted against concentration of the analyte. Linearity of the calibration curve was demonstrated by applying the least square regression analysis to the plot obtained.

Accuracy

To determine accuracy of the proposed method, three different levels of drug concentrations were prepared from independent stock solution and analyzed. Accuracy was assessed as the mean percentage Bias.

Precision

Intra-day and inter-day variation was taken to determine intermediate precision of the proposed method. Different levels of drug concentrations in triplicates were prepared three different times in a day and studied for intra-day variation. %Relative standard deviation (%RSD) were calculated which should be less than 2%.

Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD was determined using the relation $3.3 \sigma/s$ where ‘$\sigma$’ is the standard deviation of the response and ‘$s$’ is the slope of the calibration curve.

The standard deviation of the response can be obtained either by measuring the standard deviation of the blank response or by calculating the residual standard deviation of the regression line or by calculating the standard deviation of the y-intercept of the regression line, i.e. the standard error of the estimate. Similarly, LOQ was determined using the relation $10 \sigma/s$.

RESULTS

An attempt has been made to develop a simple, sensitive, reproducible, fast and economic analytical method for the simultaneous estimation of Famotidine and Rabeprazole in bulk form using Simultaneous equation method. This method uses the absorbance at two selected wavelengths. One is the $\lambda_{max}$ of Famotidine and other being the $\lambda_{max}$ of Rabeprazole. From the stock solution, working standard solutions of Famotidine and Rabeprazole were prepared by appropriate dilution and were scanned in the entire UV range to determine the $\lambda_{max}$ of both the drugs.

Famotidine have $\lambda_{max}$ at 263 nm and Rabeprazole at $\lambda_{max}$ 284 nm. A series of standard solutions were prepared for both Famotidine and Rabeprazole and absorbances of solutions were recorded at 263 nm and 284 nm to plot a calibration curve of absorbance versus concentration. Regression equation and Absorptivity values of Famotidine and Rabeprazole were determined at selected wavelengths are presented in table 1.

Fig. 3: Spectrum of Famotidine
**Fig. 4: Spectrum of Rabeprazole**

**Table 1: Result of analytical method development in SGF pH 1.2**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Famotidine</th>
<th>Rabeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorptivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 263 nm</td>
<td>330.75</td>
<td>366.505</td>
</tr>
<tr>
<td>At 284 nm</td>
<td>200.73</td>
<td>346.55</td>
</tr>
<tr>
<td>Regression equation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.0299</td>
<td>0.0345</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0376</td>
<td>0.0038</td>
</tr>
<tr>
<td>Correlation coefficient ($r^2$)</td>
<td>0.9968</td>
<td>0.9979</td>
</tr>
</tbody>
</table>

The concentration of two drugs in the mixture was calculated by using the equation (1) and (2). The percentage purity of Famotidine and Rabeprazole in laboratory mixture is shown in the table 2.

**Table 2: Determination of Famotidine and Rabeprazole in Laboratory mixture**

<table>
<thead>
<tr>
<th>Mixture content</th>
<th>Label content (µg)</th>
<th>Amount found (µg)</th>
<th>%Amount*</th>
<th>±SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Famotidine</td>
<td>10</td>
<td>9.6</td>
<td>99.60</td>
<td>±0.050</td>
</tr>
<tr>
<td>Rabeprazole</td>
<td>10</td>
<td>9.84</td>
<td>98.40</td>
<td>±0.052</td>
</tr>
</tbody>
</table>

*Mean of six readings

In order to prove the validity and applicability of the proposed method, studies were carried out as per ICH Guidelines and their results are presented in table 3. LOD and LOQ values for Famotidine and Rabeprazole are stated in table 3.

**Table 3: Validation of the proposed method**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Famotidine at 263 nm</th>
<th>Famotidine at 284 nm</th>
<th>Rabeprazole at 263 nm</th>
<th>Rabeprazole at 284 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s Law Limit (µg/ml)</td>
<td>5-30</td>
<td>5-30</td>
<td>5-30</td>
<td>5-30</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>1.927</td>
<td>1.55</td>
<td>0.3535</td>
<td>0.3807</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>5.839</td>
<td>4.72</td>
<td>1.0719</td>
<td>1.1535</td>
</tr>
<tr>
<td>Precision (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interday (%)</td>
<td>0.14-0.29</td>
<td>0.07-0.34</td>
<td>0.25-0.59</td>
<td>0.2-0.71</td>
</tr>
<tr>
<td>Intraday (%)</td>
<td>0.16-0.33</td>
<td>0.23-0.38</td>
<td>0.19-0.21</td>
<td>0.1-0.27</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>0.02-0.15</td>
<td>0.1-0.14</td>
<td>0.07-0.11</td>
<td>0.08-0.19</td>
</tr>
</tbody>
</table>

**DISCUSSION**

UV Spectrophotometric method was successfully developed and validated for the combination of Famotidine and rabeprazole. The calibration curves were found to be linear in the concentration range under study. The Correlation coefficient of these drugs was found to be close to 1.00, indicating good linearity. The % RSD in both intraday and interday precision study was found to be less than 2 which indicates the precision of the method. Satisfactory results were obtained with %RSD value less than 2%; thus confirming the accuracy and precision of the proposed method.

**CONCLUSION**

The proposed method was proved to be simple, accurate, precise, sensitive, rapid, reproducible and economical for the simultaneous estimation of Famotidine and Rabeprazole sodium in combined laboratory mixture. The % RSD values were found to be less than 2% as required by ICH guidelines, which indicates the accuracy of methods; thus this method can be used for routine simultaneous determination of Famotidine and Rabeprazole in bulk drug and can be used to carry out the simultaneous estimation in the dosage form.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTERESTS**

Declared None.

**REFERENCES**

1. Kesur BR, Salunkhe VR, Magdum CS. Development and validation of UV spectrophotometric method for simultaneous


