Stability indicating method development and validation of assay method for the estimation of rizatriptan benzoate in tablet

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Abstract A simple, sensitive, precise and specific high performance liquid chromatography method was developed and validated for the determination of rizatriptan in rizatriptan benzoate tablet. The separation was carried out by using a mobile phase consisting of acetonitrile: pH 3.4 phosphate buffer in ratio of 20:80. The column used was Zorbax SB CN 250 mm × 4.6 mm, 5 μm with a flow rate of 1 ml/min using UV detection at 225 nm. The retention time of rizatriptan and benzoic acid was found to be 4.751 and 8.348 min respectively. A forced degradation study of rizatriptan benzoate in its tablet form was conducted under the condition of hydrolysis, oxidation, thermal and photolysis. Rizatriptan was found to be stable in basic buffer while in acidic buffer was found to be degraded (water bath at 60°C for 15 min). The detector response of rizatriptan is directly proportional to concentration ranging from 30% to 160% of test concentration i.e. 15.032 to 80.172 mcg/ml. Results of analysis were validated statistically and by recovery studies (mean recovery = 99.44). The result of the study showed that the proposed method is simple, rapid, precise and accurate, which is useful for the routine determination of rizatriptan in pharmaceutical dosage forms.

1. Introduction

Rizatriptan benzoate is N,N-dimethyl-2-[5-(1H-1,2,4-triazol-1-ylmethyl)-1H-indol-3-yl]ethanamine. It is an anti migraine drug, which selectively activates 5-HT1B/1D receptors. Physical properties are white to off white crystalline powder, soluble in water, melting point 178–180°C, and stable under ordinary condition. So far, no method has been reported for the estimation of rizatriptan in rizatriptan benzoate, hence I attempted to develop a simple, accurate economical and analytical method. A study of forced degradation of rizatriptan was also reported.
This paper describes validated HPLC for the estimation of rizatriptan using a mobile phase consisting of acetonitrile: 
\[ \text{pH 3.4 phosphate buffer in ratio of 80:20.} \]
The column used was CN 250 mm \( \times \) 4.6 mm, 5 \( \mu \) with a flow rate of 1 ml/min using UV detection at 225 nm.

2. Materials and methods

2.1. Equipment

HPLC equipped with a pump, injector and PDA detector Waters 2695, 2996, HPLC equipped with a pump, injector and UV detector, Waters 2695, 2487, HPLC equipped with a pump, injector and UV detector, Agilent 1200 series, Balance-Sartorius, Mettler Toledo, Photo stability chamber-Newtronic, Oven-Skan.

2.2. Materials

Rizatriptan benzoate standard (Alkem), tablets were procured from a local market. Potassium dihydrogen phosphate (Merck), Acetonitrile (Merck) Orthophosphoric acid (Merck), Milli Q water, Hydrogen (Merck) Hydrochloric acid (Merck), Sodium hydroxide (Merck), and Column Zorbax SB CN 250 \( \times \) 4.6 mm, 5 \( \mu \).

Preparation of 0.01 M potassium dihydrogen phosphate buffer pH 3.4:

Dissolve 2.7218 g of potassium dihydrogen orthophosphate into 2000 ml water, mix and adjust the pH at 3.4 with orthophosphoric acid solution (Mix 10 ml orthophosphoric acid (88%) into 100 ml water), filter through a 0.45 \( \mu \) nylon filter, mix and degas.

Preparation of mobile phase.

Mix the above buffer and acetonitrile in the ratio of 80:20, and degas.

Use suitable high performance liquid chromatography equipped with the following.

Column: Zorbax SB CN 250 \( \times \) 4.6 mm, 5 \( \mu \).
Flow rate: 1.0 ml/min

Figure 3  Linearity plot for rizatriptan.

**Table 1**  Linearity.

<table>
<thead>
<tr>
<th>Spike level in %</th>
<th>Concentration of rizatriptan in mcg/ml</th>
<th>Peak areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>15.032</td>
<td>1281515</td>
</tr>
<tr>
<td>50</td>
<td>25.054</td>
<td>2105110</td>
</tr>
<tr>
<td>80</td>
<td>40.086</td>
<td>3354257</td>
</tr>
<tr>
<td>100</td>
<td>50.108</td>
<td>4181675</td>
</tr>
<tr>
<td>120</td>
<td>60.129</td>
<td>5032026</td>
</tr>
<tr>
<td>140</td>
<td>70.151</td>
<td>5839565</td>
</tr>
<tr>
<td>160</td>
<td>80.172</td>
<td>6653218</td>
</tr>
<tr>
<td>Slope</td>
<td>82660</td>
<td></td>
</tr>
<tr>
<td>y-intercept</td>
<td>40355</td>
<td></td>
</tr>
<tr>
<td>r-value</td>
<td>0.99998</td>
<td></td>
</tr>
<tr>
<td>RSS</td>
<td>699915125</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1  Rizatriptan benzoate.

Figure 2  HPLC Chromatogram of standard.
**Table 2** Accuracy.

<table>
<thead>
<tr>
<th>Level no./spike level in %</th>
<th>Actual Amount of rizatriptan added in mg</th>
<th>Amount of Rizatriptan found in mg</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>4.94</td>
<td>4.92</td>
<td>99.66</td>
</tr>
<tr>
<td>100</td>
<td>10.00</td>
<td>9.95</td>
<td>99.49</td>
</tr>
<tr>
<td>150</td>
<td>14.99</td>
<td>14.81</td>
<td>98.79</td>
</tr>
<tr>
<td>Over all % RSD</td>
<td></td>
<td></td>
<td>0.28</td>
</tr>
</tbody>
</table>

Wavelength: 225 nm  
Injection volume: 10 μl  
Column oven temperature: 25 °C  
Sample compartment temp: 25 °C  
Run time: 12 min  
Diluent: mobile phase  
Preparation of standard Solution:  
Weigh and transfer accurately about 73 mg (equivalent to 50 mg of rizatriptan) of rizatriptan benzoate working standard into a 100 ml volumetric flask. Dissolve and dilute to the required volume with diluent. Further dilute 5 ml of the above standard solution to 50 ml with diluent.  
Preparation of sample solution for 10 mg:  
Weigh and transfer five tablets into a 250 ml volumetric flask. Add 180 ml of diluent shake well and sonicate for 15 min with intermittent shaking and dilute to the required volume with diluent. Filter the required amount of solution through a 0.45 μ PVDF/nylon filter.  
Further dilute 5 ml of the above standard solution to 20 ml with diluent.  
Procedure:  
Separately inject 10 μl of blank, standard solution (five replicate injections) and sample solution into the chromatographic system. Record the chromatograms and measure the peak area count for rizatriptan peak.  
The retention time of rizatriptan peak is about 5 min.  
Disregard the peak area count of benzoic acid at the retention time of about 9.2 min (RRT about 1.9).  
Evaluation of system suitability:  
From standard solution:  
(1) The % RSD for the peak areas of rizatriptan from five replicate injections should not be more than 2.0.  
(2) The tailing factor for rizatriptan should be not more than 2.0.  

3. Validation method

3.1. Linearity

The linearity of rizatriptan was performed using the standard solution in the range of 15.032–80.172 mcg/ml (about 30–160% of test concentration). A graph was plotted with concentration (in mcg/ml) on x-axis and peak areas of rizatriptan on y-axis. Slope, y-intercept, correlation coefficient (r-value) and residual sum of squares (RSS) were determined Fig. 3. The results are tabulated in Table 1:(See Figs. 1, 2 and 4).  

3.2. Accuracy

A known amount of rizatriptan benzoate API working standard was spiked to 50%, 100% and 150% in 10 mg tablets uniformity of dosage units test concentration. The amount of rizatriptan was quantified as per the test method. The %
recovery was calculated from the amount found and the actual amount added. The results are tabulated in Table 2.

### 3.3. Robustness

Robustness of the method was verified by deliberately varying the following instrumental conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>% Assay</th>
<th>% Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated sample</td>
<td>98.11</td>
<td>–</td>
</tr>
<tr>
<td>Acid-treated sample</td>
<td>93.99</td>
<td>4.20</td>
</tr>
<tr>
<td>Base-treated sample</td>
<td>95.35</td>
<td>2.81</td>
</tr>
<tr>
<td>Peroxide-treated sample</td>
<td>95.21</td>
<td>2.96</td>
</tr>
<tr>
<td>Heat-treated sample</td>
<td>99.53</td>
<td>#</td>
</tr>
<tr>
<td>UV–visible treated sample</td>
<td>96.41</td>
<td>1.73</td>
</tr>
</tbody>
</table>

By changing the flow rate by ±10%, by changing the temperature by ±5 °C, by changing the wavelength by ±2 nm, by changing the organic content by ±2% (absolute), and by changing the pH of buffer in mobile phase by ±0.1 units. The results are tabulated in Table 3.

### 4. Forced degradation

Forced degradation study was carried out by treating the sample under the following conditions. (Table 4 and Figs. 5–9)

(a) Degradation by hydrochloric acid (acid treated sample)

The sample was treated with 5 ml of 1 N hydrochloric acid and kept on a water bath at 60 °C for 20 min. The treated sample solution was analyzed as per the test method.

(b) Degradation by sodium hydroxide (base treated sample)

![Figure 5](image5.png)

**Figure 5** HPLC chromatogram of acid treated sample.

![Figure 6](image6.png)

**Figure 6** HPLC chromatogram of base treated sample.
The sample was treated with 5 ml of 1 N sodium hydroxide and kept on a water bath at 60 °C for 15 min. The treated sample solution was analyzed as per the test method.

(c) Degradation by hydrogen peroxide (peroxide treated sample)

The sample was treated with 5 ml of 50% hydrogen peroxide solution and kept on water bath at 60 °C for 5 min. The treated sample solution was analyzed as per the test method.

Table 5  Summary of system suitability.

<table>
<thead>
<tr>
<th>Name of experiment</th>
<th>Tailing factor</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>System precision,</td>
<td>1.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Method precision</td>
<td>1.3</td>
<td>0.27</td>
</tr>
<tr>
<td>Ruggedness</td>
<td>1.08</td>
<td>0.10</td>
</tr>
<tr>
<td>Filter paper selection study</td>
<td>1.15</td>
<td>0.03</td>
</tr>
<tr>
<td>Specificity</td>
<td>1.3</td>
<td>0.18</td>
</tr>
</tbody>
</table>
5. Results and discussion

In order to develop an effective method for the analysis of the drugs in pharmaceutical formulations, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection of wavelength, ideal mobile phase and their proportions, optimum pH and concentration of standard solution were studied. The method was developed with Column Zorbax SB CN 250 × 4.6 mm, 5 μ using flow rate: 1.0 ml/min, wavelength 225 nm at room temperature. The linearity of rizatriptan was performed using the standard solution in the range of 15.032 mcg/ml to 80.172 mcg/ml (about 30–160% of test concentration).

6. Conclusion

The HPLC method for the assay of rizatriptan in rizatriptan benzoate tablet was found to be simple, precise, accurate, rapid and validated. The mobile phase is simple to prepare and economical. The sample recoveries in formulation were in good agreement with their label claim. Hence it can be easily and conveniently adopted for a routine analysis of rizatriptan in tablet.

Acknowledgement

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Further reading


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