Development and Validation of RP-HPLC Method for the Assay of Risedronate Sodium

Pragati Ranjan Satpathy1,*, A. Srinivasa Rao2, P. Ravikumar1, JVC. Sharma1 and V. Mohan Goud1

1 Jagnapally B.R. Pharmacy College, Yenkapally (V), Moinabad (M), R.R. District, Hyderabad-500075, Andhra Pradesh, India
2 Bhaskar Pharmacy College, Yenkapally (V), Moinabad (M), R.R. District, Hyderabad-500075, Andhra Pradesh, India

* Corresponding author: Pragati Ranjan Satpathy; e-mail: spragatiranjan@ymail.com

ABSTRACT
A method based on RP-HPLC with indirect UV detection was developed for the determination of phosphates and phosphites as impurities in Risedronate sodium. RP separation of the phosphates and phosphites was achieved by adding Tetra butyl ammonium hydroxide as an ion-pairing agent in the mobile phase. Potassium hydrogen phthalate was added to the mobile phase as an ionic chromophore in order to obtain high background absorption of the mobile phase. Separation was performed on a C18 column using a mixture of pH 8.2 buffer, acetonitrile and methanol as the mobile phase along with indirect UV detection at 262 nm. The retention time was found to be Rt = 2.292. The validation of this method included determination of its specificity, accuracy, precision, linearity, LOD, LOQ, and robustness. The LOD was 0.86 g/mL for phosphates and 0.76 g/mL for phosphites. The LOQ was 2.60 g/mL for phosphates and 2.29 g/mL for phosphites. The developed method is suitable for quantitative determination of phosphates and phosphites as impurities in QC of Risedronate sodium.

Keywords: Risedronate, Phosphites, Ionic Chromophore, LOD and LOQ

INTRODUCTION
Risedronate [1-hydroxy-1-phosphono-2-(pyridin-3-yl)ethyl] phosphonic acid [figure-1] is a bisphosphonate used to strengthen bone, treat or prevent osteoporosis, and treat Paget's disease of bone. It is also called as Risedronic acid used for the acute treatment of Osteoporosis and for the treatment postmenopausal and glucocorticoid-induced osteoporosis.

![Figure 1. Image of Risedronate](http://ijps.aizeonpublishers.net/content/2014/1/ijps438-441.pdf)

The action of risedronate on bone tissue is based partly on its affinity for hydroxyapatite, which is part of the mineral matrix of bone. Risedronate also targets farnesy1 pyrophosphate (FPP) synthase. Include abdominal pain, anxiety, back pain, belching, bladder irritation, bone disorders and pain, bronchitis, bursitis, cataracts, chest pain, colitis, constipation, depression, diarrhoea, difficulty breathing, dizziness, dry eyes, eye infection, flu-like symptoms, gas, headache, high blood pressure, infection, insomnia, itching, joint disorders and pain, leg cramps, muscle pain, muscle weakness, nausea, neck pain, nerve pain, pain, pneumonia, rash, ringing in ears, sinus problems, sore throat, stomach bleeding, stuffy or runny nose, swelling, tendon problems, tumor, ulcers, urinary tract infection, vertigo, vision problems, and weakness.

MATERIAL AND METHODS
Chemicals and reagents
The Risedronate has been collected from Suven Life Pharmaceuticals Ltd Hyderabad. All used reagents were HPLC grade as; "Methanol, acetonitrile, Tetra butyl ammonium hydroxide, Bio phosphoricacid, Potassium hydrogen phthalate," purchased from Rankem India. All other chemicals were of
analytical reagent grade unless specified. All the glass were washed with detergent, rinsed thoroughly with distilled water, and dried prior to use.

**Chromatographic (HPLC) conditions**
Chromatographic separation was performed on a Waters HPLC with alliance with Auto sampler, Empower 2.0 software, Symmetry C18 (4.6 x 150mm, 5mm, Make: Thermosil), and UV detection of 262 nm at ambient temperature. The injection volume was 10µl with a flow rate of 0.5 ml/min per minute and a run time of 10 minutes.

**Mobile phase and solutions**
Mixed a mixture of buffer, Acetonitrile and methanol in the ratio of 450: 480:70. and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

**Standard Solution Preparation**
Accurately weigh and transfer 10mg of Risedronate Working standard into a 10 ml volumetric flask add about 7 ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

**Sample Solution Preparation**
Weigh 5 Residronate Tablets and calculate the average weight. Accurately weigh and transfer the sample equivalent to 10 mg of Add about 7 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with diluent. Mix well and filter through 0.45µm filter. Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

**System suitability:** Tailing factor for the peak due to Risedronate in Standard solution should not be more than 2.0 Theoretical plates for the Risedronate peak in Standard solution should not less than 2000.

**Validation parameters**

**Accuracy**
Preparation of standard solution: Accurately weigh and transfer 10 mg of Risedronate Working standard into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

**Preparation Sample solutions**
For preparation of 50% solution: Accurately weigh and transfer 6.54 mg of risedronate API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

**For preparation of 100% solution**
Accurately weigh and transfer 10.0mg of Risedronate API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

**For preparation of 150% solution**
Accurately weigh and transfer 14 mg of Risedronate API sample into a 10 mL volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.7 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Mix well and filter through 0.45µm filter.

**Procedure**
Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Risedronate and calculate the individual recovery and mean recovery values. The % Recovery for each level should be between 98.0 to 102.0%.

**Precision**
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The % RSD should not be more than 2%.

**Linearity**
Working dilutions of Risedronate in the range of 50-90µg/ml was prepared by taking suitable aliquots of working standard solutions of drug in different 10ml volumetric flask and diluting up to the mark with mobile phase. 10µl quantity of each dilutions was injected into the column at a flow rate of 0.8ml/min, the drug in the elute was monitored at 262nm and the corresponding chromatogram were recorded. From these the mean peak areas were calculated and a plot of concentration vs peak areas was constructed and acceptance Criteria: Correlation coefficient should be not less than 0.999.

**Limit of Detection**
Preparation of 0.15% solution At Specification level (0.075µg/ml solution): Pipette 1 ml of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluents. Further pipette 0.15ml of above diluted solution into a 10 ml of volumetric flask and dilutes up to the mark with diluents and the acceptance Criteria: S/N Ratio value shall be 3 for LOD solution.
Limit of Quantification
Preparation of 0.5% solution At Specification level (0.25µg/ml solution): Pipette 1 ml of 10µg/ml solution into a 10 ml of volumetric flask and dilute up to the mark with diluents. Further pipette 0.5ml of above diluted solution into a 10 ml of volumetric flask and diluted up to the mark with diluents and the acceptance Criteria: S/N Ratio value shall be 10 for LOQ solution.

Robustness
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. It was observed from the chromatograms that the results were within the limits. This indicates that the method developed is robust.

RESULT AND DISCUSSION
An Analytical method development by HPLC carried out in this work resulted in sharp peak of Risedronate with negligible fronting and tailing factors. The sharp peak resembles the purity of the sample.

![Figure 2](link) Standard chromatogram of Risedronate showing sharp peak at 2.292min at 262nm.

Chromatogram obtained with a mixture of pH 8.2 buffer, Acetonitrile and methanol in the ratio of 450:480:70

<table>
<thead>
<tr>
<th>%Concentration (at spec level)</th>
<th>Area</th>
<th>Amount Added (µg)</th>
<th>Amount Found (µg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>1082543</td>
<td>6.54</td>
<td>6.64</td>
<td>101.9%</td>
<td>101.0%</td>
</tr>
<tr>
<td>100%</td>
<td>1615409</td>
<td>10.0</td>
<td>9.94</td>
<td>99.4%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>2497572</td>
<td>15.1</td>
<td>15.3</td>
<td>101.8%</td>
<td></td>
</tr>
</tbody>
</table>

Mean Recovery was found to be 101.0%

<table>
<thead>
<tr>
<th>S. No.</th>
<th>RT</th>
<th>Peak area</th>
<th>Average peak area</th>
<th>Standard deviation</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2.286</td>
<td>820056</td>
<td>820565</td>
<td>1075.4</td>
<td>0.1</td>
</tr>
<tr>
<td>2.</td>
<td>2.289</td>
<td>820460</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>2.290</td>
<td>819150</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>2.292</td>
<td>821962</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>2.292</td>
<td>821199</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The %RSD of the drug was found to be 0.1.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Peak name</th>
<th>Concentrate</th>
<th>RT</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Risedronate</td>
<td>50</td>
<td>2.293</td>
<td>609634</td>
</tr>
<tr>
<td>2.</td>
<td>Risedronate</td>
<td>60</td>
<td>2.294</td>
<td>723134</td>
</tr>
<tr>
<td>3.</td>
<td>Risedronate</td>
<td>70</td>
<td>2.296</td>
<td>849765</td>
</tr>
<tr>
<td>4.</td>
<td>Risedronate</td>
<td>80</td>
<td>2.295</td>
<td>993452</td>
</tr>
<tr>
<td>5.</td>
<td>Risedronate</td>
<td>90</td>
<td>2.295</td>
<td>1104531</td>
</tr>
</tbody>
</table>

Average Baseline Noise obtained from Blank : 53µV
Signal Obtained from LOD solution (0.15% of target assay concentration): 158 µV
S/N = 158/53= 2.98
Figure 3. Linear curve for Risedronate sodium by taking peak area vs Concentration.

The regression co-efficient of the drug was found to be 0.999 under the concentration of ranges of 50-70µg/ml.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Peak Name</th>
<th>Rt</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Risedronate</td>
<td>2.288</td>
<td>666</td>
<td>158</td>
</tr>
</tbody>
</table>

Average Baseline Noise obtained from Blank: 53µV
Signal Obtained from LOD solution (0.15% of target assay concentration): 158 µV
S/N = 158/53= 2.98

CONCLUSION

Through the modern analytical study, it can be concluded that more rapid, precise, specific, sensitive, economic, reproducible, isocratic reverse phase HPLC method was developed and validated for quantitative determination of Risedronate. The run time around 2.2 ± 0.1 min allows the analysis of a large number of samples in short period of time. The method was validated successfully using parameters like accuracy, precision, linearity, LOD, LOQ and robustness. This approach will unquestionably build an innovative way out on behalf of maintaining the quality, consistency as well as. These efforts will ensure therapeutic functionality of the drugs. The developed RP-HPLC method presented here is more advantageous as the method was robust with low retention times and sharp peak with reduced fronting and tailing.

REFERENCES

2. Walash M, metwally m, el shaheny r. (2010). University of mansoura, faculty of pharmacy, department of analytical chemistry, 35516, mansoura, egypt.
7. Walash M, metwally m, el shaheny r. (2010). University of mansoura, faculty of pharmacy, department of analytical chemistry, 35516, mansoura, egypt.

© 2014: AIZEON Publishers; All Rights Reserved
This is an Open Access article distributed under the terms of the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.