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

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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.

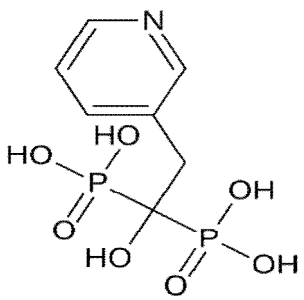


Figure 1. Image of Risedronate

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 farnesyl 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 a10 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 uptothe 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 ofRisedronate 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.

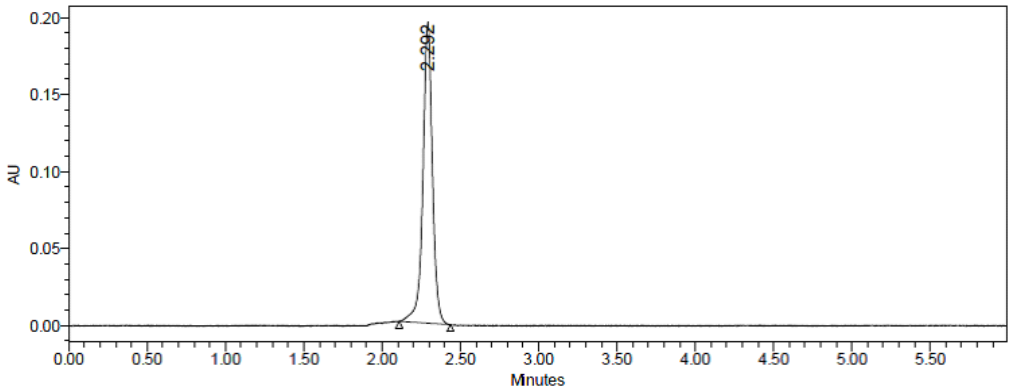


Figure 2. Standard chromatogram of Risedronate showing sharp peak at 2.292min at 262nm.

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

Table 1. Accuracy

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1082543	6.54	6.64	101.9%	
100%	1615409	10.0	9.94	99.4%	101.0%
150%	2497572	15.1	15.3	101.8%	

Mean Recovery was found to be 101.0%

Table 2. Precision

S. No.	RT	Peak area	Average peak area	Standard deviation	% RSD
1.	2.286	820056			
2.	2.289	820460			
3.	2.290	819150	820565	1075.4	0.1
4.	2.292	821962			
5.	2.292	821199			

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

Table 3. Linearity

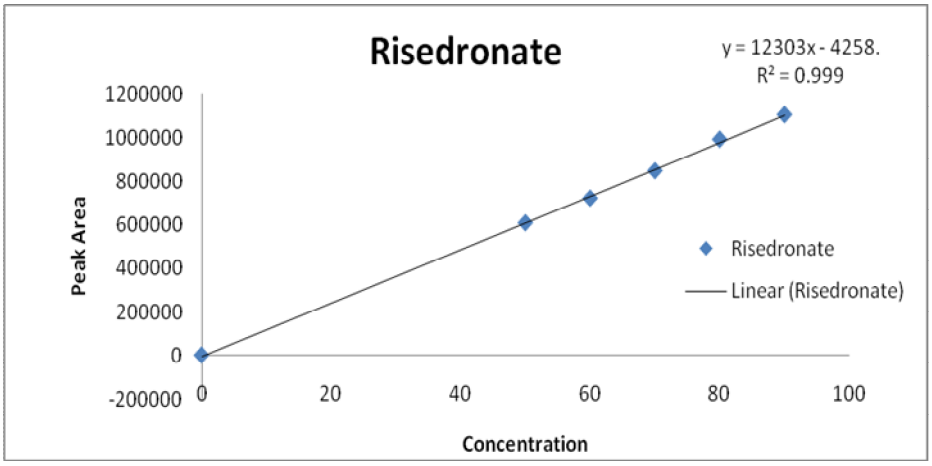
S. No.	Peak name	Concentrate	RT	Area
1.	Risedronate	50	2.293	609634
2.	Risedronate	60	2.294	723134
3.	Risedronate	70	2.296	849765
4.	Risedronate	80	2.295	993452
5.	Risedronate	90	2.295	1104531

Table 4. Limit of Detection

S.No	Peak Name	Rt	Area	Height
1	Risedronate	2.288	666	158

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 4. Limit of Detection

S.No	Peak Name	Rt	Area	Height
1	Risedronate	2.288	666	158

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

Table 5. Limit of Quantification

S.No	Peak Name	Rt	Area	Height
1	Risedronate	2.288	2223	527

Average Baseline Noise obtained from Blank: 53µV
Signal Obtained from LOD solution (0.5% of target assay concentration): 527 µV
S/N = 527/53 = 9.94

Table 6. Robustness

S.No.	Peak name	Condition	RT	Area	Height	USP Plate count	USP Tailing
1.	Risedronate	Increased organic phase	2.263	876461	205066	7713.1	0.9
2.	Risedronate	Decreased organic phase	2.360	898556	195712	6900.7	1.0
3.	Risedronate	Increased flow rate	2.029	761061	192508	6945	0.9
4.	Risedronate	Decreased flow rate	2.269	1002098	205115	7698	0.9

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

1. www.drugbank.ca/drug/DB00884
2. Sethi P.D.(1997). Quantitative analysis of drugs in pharmaceu tical formulations, 3rd edn., cbs publishers and distributors, new delhi. 51.z
3. Ich, q2a. (1994). Text on validation of analytical procedures, international conference on harmonization, geneva, october, 1-7.
4. Ich, q2b. (1996). Vvalidation ofanalytical procedures: methodology, international conference on harmonization, geneva,november, 1-12.
5. Nagi Reddy.N, Venkateshwar Rao.J. (2003). Bisphosphonate mechanism of action, curr rheumatol rep. 5(1):65-74.
6. Reszka AA, Rodan Ga. (2004). Department of bone biology and osteoporosis research, merck research laboratories, west point, pa 19486, usa.
7. Walash M, metwally m, eid m, el-shaheny r.(2010). University of mansoura, faculty of pharmacy, department of analytical chemistry, 35516, mansoura, egypt.
8. Kyriakides D, Panderi I.(2007). Development and validation of a reversed-phase ion-pair high-performance liquid chromatographic method for the determination of risedronate in pharmaceutical preparations. 584(1):153-9.
9. Demetra Kyriakides, Irene Panderi. (2007. feb). Development and validation of a reversed-phase ion-pair high-performance liquid chromatographic method for the determination of risedronate in pharmaceutical preparations. Volume 584, Issue 1, Pages 153–159.
10. Nagi reddy.n, venkateshwar rao.j. (2013). Uplc method development and validation for the estimation of risedronate in formulation,ajppcs, vol 6 suppl 5 (oct-dec).

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