

UPLC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF RISEDRONATE IN FORMULATION

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ABSTRACT

Rapid and sensitive Ultra performance liquid chromatography method was developed and validated for quantification of Risedronate in pharmaceutical formulation. The analysis is resolved by using symmetry C18, (100 × 2.1 mm), 1.7 μ m column; make BEH, in an Isocratic mode, with mobile phase containing Methanol and Water in the ratio of 70:30 v/v was used. The flow rate was 0.8 ml/min and the analyte was monitored at 273 nm by UV detection. The retention time for Risedronate sodium was 2.29 mins. The method was validated for system suitability, linearity (correlation coefficient 0.999), precision, accuracy (recovery studies 98%-102%), specificity, ruggedness, robustness, LOD (2.98 μ g/ml) and LOQ (9.94 μ g/ml). This present method is simple, highly sensitive, precise and accurate and has the potential of being useful for routine quality control.

Keywords: Risedronate; UPLC; Validation

INTRODUCTION

Risedronate [1, 2] is chemically [1-hydroxy-2-(3-pyridinyl) ethylidene] bis [phosphoric acid] monosodium salt, it is having Molecular formula C₇H₁₀NO₇P₂Na. It is soluble in water and in aqueous solutions and insoluble in organic solvents. It is used as Antiosteoporosis [3, 4] agent, used in treating Paget's disease [5] of the bone (osteitis deformans), postmenopausal and glucocorticoid induced osteoporosis.

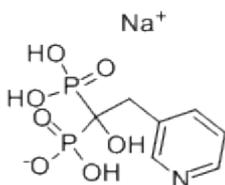


Figure 1: Structure of Risedronate

In the literature survey [6-10], few HPLC methods are reported for estimation of Risedronate in formulation by using an ion pairing agent. So far to our present knowledge, no validated UPLC method is available for the estimation of Risedronate in formulations. UPLC method has many advantages over HPLC.

EXPERIMENTAL

Materials and Methods

Risedronate standard was obtained from Hetero drugs Ltd., Hyderabad, as a gift sample. Risedronate tablets (Risofos 35mg-cipla) were procured from local market. UPLC grade Methanol and water were obtained from Spectrochem Pvt. Ltd., Mumbai. The LC system of Waters Acquity UPLC with UV detector was used for this study and chromatographic separation was achieved on Symmetry - C18 (2.1*100mm, 1.7 μ m, Make: BEH) column as stationary phase with isocratic mode.

Preparation of Mobile phase

UPLC Water 300ml (30%) and 700 ml of Methanol UPLC (70%) were mixed well and degas in ultrasonic water bath for 5 minutes. Filtration carried through 0.45 μ m filter under vacuum filtration.

Standard Preparation

About 10mg of Risedronate Working standard into a 10 ml volumetric flask added about 7 ml of diluent and sonicated 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 filtered through 0.45 μ m filter.

Sample Preparation

The whole 20 tablets had crushed together and finely powdered. Sample powder equivalent to 10 mg of Risedronate was taken into a 10 ml volumetric flask and about 7 ml of diluent was added and sonicated to dissolve and made the volume up to the mark with diluent and filtered. Further pipette 0.7 ml of the above solution into a 10ml volumetric flask and diluted up to the mark with diluent. Mix well and filter through 0.45 μ m filter.

Injected 20 μ l of the standard, sample into the chromatographic system and measured the area for the Risedronate peak and calculated the %purity by using the formulae.

RESULTS AND DISCUSSION

Optimization of the method [11-13]

To develop a rugged and suitable UPLC method for the quantitative determination of Risedronate, the analytical conditions were selected after testing the different Parameters such as diluents, buffer, buffer concentration, organic solvent for mobile phase and mobile phase composition and other chromatographic conditions. Our preliminary trials using different composition of mobile phases consisting of water and methanol, did not give good peak shape.

Method Validation [14, 15]

Linearity

Linearity of method was ascertained by injecting each level (50, 60, 70, 80 & 90 μ g/ml) of standard solution into the chromatographic system and measured the peak area. By plotting a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area), the correlation coefficient was determined. The linearity graph was shown in Figure no.3.

Precision

The Precision of the method was determined by injecting standard solution of Risedronate for five times and measured the area for all five injections in UPLC chromatographic system. The %RSD for the areas of five replicate injections was found to be within the specified limits.

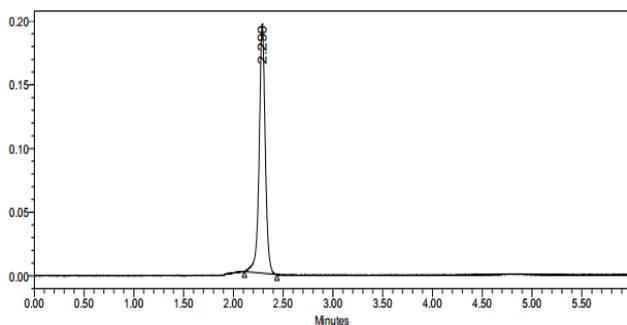


Figure2: Standard chromatogram of Risedronate for Optimized Conditions

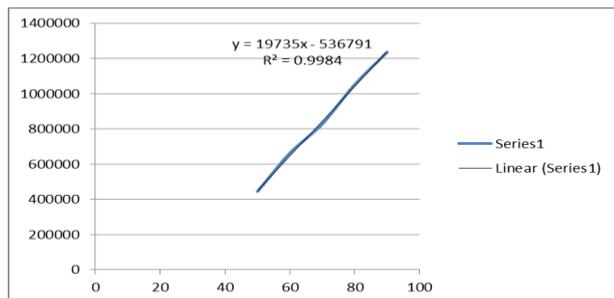


Figure3: Linearity results for standard injections at different levels

Table 3: Accuracy data of Risedronate

Concentration (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%	100.7%
150%	2475069	15.1	15.2	100.9%	

Limit of Detection and Limit of Quantification

The Limit of Detection and Limit of Quantification of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed UPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOD and LOQ of Risedronate were found to be 2.98 and 9.94 respectively.

ROBUSTNESS

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the influence on the method.

On evaluation of the results, it can be concluded that the variation in flow rate and organic composition in the mobile phase do not affect the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10% and organic phase in mobile phase ±10%.

Table 1: Precision results of Risedronate

Risedronate injections	standard	Area
Average		820565.2
Standard Deviation		1075.4
%RSD		0.1

Intermediate Precision/Ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The standard solution was injected for five times on different day and different column and measured the areas for all five injections in UPLC. The %RSD for the area of five replicate injections was determined.

Table2: Results of intermediate precision injections of Risedronate

Risedronate Injection	Area
Average	818984.5
Standard Deviation	492.1
%RSD	0.1

Accuracy

An accuracy study was performed by adding known amounts of Risedronate standard to the placebo preparation. The actual and measured concentrations were compared. Recovery of the method was evaluated at three different concentration levels (corresponding to 50%, 100% and 150% of test preparation concentration). For each concentration level, three sets were prepared and injected in to chromatographic system.

Table4: Result of Robustness for Flow rate changes

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	7698.7	0.9
2	0.8	7412.0	1.0
3	1.0	6945.2	0.9

*Result: Results for actual flow (0.8ml/min) have been considered for Assay standard.

Table 5: Result of Robustness for Change in Organic composition

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6900.7	1.0
2	*Actual	7412.0	1.0
3	10% more	7713.1	0.9

* Result: Results for actual Mobile phase composition Methanol and water (70:30) have been considered for Assay standard.

The UPLC method developed in the present study has been used to quantify Risedronate in the Tablet dosage form. The average drug content of Risedronate was found to be 34.72mg of the labeled amount 35mg mentioned.

CONCLUSION

The developed UPLC method was found to be accurate, linear, precise, rapid and sensitive for the estimation of Risedronate in pharmaceutical formulations. This method has capability to give rapid elution with low retention time and good resolution. The drug recoveries from samples are within the specified limits. Hence the proposed method can be useful for the routine analysis of Risedronate in laboratories.

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