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Development and validation of RP-HPLC/UV methods for the estimation of Risedronate sodium in pure and pharmaceutical dosage form

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Abstract: Recent study was conducted to develop and validate analytical methods for estimation of Risedronate sodium in pure and pharmaceutical dosage form using UV Spectroscopy and RP- HPLC method. The first method (Method A) based on the UV Spectroscopy using 0.1M HCl as diluent λ_{max} was found at 261 nm. Linearity existed perceived in the concentration between 10-50 µg/mL (r^2 = 0.999) for the method. The method was validated pertaining to linearity, precision and accuracy studies, LOD and LOQ consistent with ICH guidelines. The second method (Method B), based on determination of Risedronate sodium tablet dosage form by RP-HPLC method. Chromatographic separation was carried out on a C_{18} (150x4.6 mm x 5 µ) SS Column using Methanol:Ammonium formate (85:15) as the mobile phase at a flow rate of 1.0 mL/min. The chromatographic analysis was carried out in the reflectance and absorbance mode at 254 nm and retention time of the drug was found to be 1.11 mL/min for standard and tablet. Linear responses of the drug were in the concentration range of 200-1000 µg/mL. The accuracy of the method was assessed by standard dilution method and found to be 98-102% . Results of the analysis were validated statistically using prism software. The method established was found to be simple, precise, linear, accurate and sensitive. The developed method can be used for routine quality control analysis of Risedronate sodium in pure and pharmaceutical dosage form.

Keywords: Risedronate sodium; method development; validation; RP-HPLC; UV-Spectrophotometry; ICH guidelines

1 Introduction

Risedronate sodium, chemically known as [1-hydroxy-2-(3-pyridinyl)ethylidene] bis[phosphonic acid] monosodium salt (**Figure 1**).[1] It is a fine white, odorless, crystalline powder; soluble in water, aqueous solutions, and essentially, insoluble in organic solvents.[1-2] It has affinity for hydroxyl apatite crystals in bone and acts as an antiresorptive agent. At the cellular level, inhibits osteoclasts. It reduces bone turnover & preservation of bone mineralization. It causes potent antiosteoclast activity and bone dependently increases bone mass and biomechanical skeletal strength. In post-menopausal woman decreases in bone turnover were observed in 1 month and reached maximum in 3-6 months.[3]

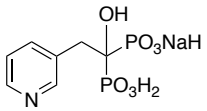

Figure 1. Structure of Risedronate Sodium

The literature survey revealed that the methods like HPLC,[4-5] LC/MS[6] and spectrophotometric methods Risedronate metal-complexes[7-8] were reported. The objective of the present work is to develop and validate UV Spectroscopy and RP-HPLC methods for estimation of Risedronate sodium as per ICH guidelines.[9]

2 Result and Discussion

2.1 UV Spectroscopy method

Absorbance spectra of Risedronate sodium shown in the (**Figure 2**) the wavelength of 261 nm was chosen for determination of Risedronate sodium using 0.1N HCl as a diluent respectively. The Assay percentage obtained by UV-method was found to be 100.59%. Various mixture of composition of Risedronate sodium was prepared at different concentration which obeys the Beer's and Lambert's law from 10-50 µg/mL with co-relation coefficient of 0.999. Accuracy should be nine determinations over a minimum of three concentrations levels covering specific ranges that is 80%, 100%, 120%. The percentage recovery was found to be 98.50%-101.1% respectively. Repeatedly applying the analytical

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method to multiple samplings (at least 6) of homogeneous samples was performed on the same day, same system and percentage recovery was done. The relative standard deviation for the assay of six sample preparations was found to be 0.5371. Mean of percentage recovery is 100.99%. Ruggedness study was carried out within-laboratory variations at different days, different analysts and different equipment. The diluent used is 0.1M HCl. Percentage RSD of the assay is 0.5870 and mean of percentage recovery is 100.3%.

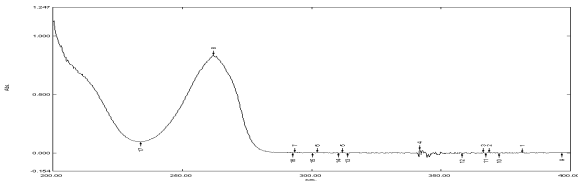

Figure 2. UV Spectrum of Risedronate sodium

2. 2 HPLC Method

Various chromatographic separations were carried out, best separation was achieved in Methanol: Ammonium formate buffer (85:15, pH was adjusted to 4.5 with dilute formic acid (**Figure** 4#**3**) and UV detection was carried out at 254 nm (**Table** 5#**1**). The retention time of Risedronate Sodium was 1.11 mL/min. The assay percentage was found to be 98-100.01%. The linearity of response was determined by preparing standard solution containing 200, 400, 600, 800 & 1000 µg/mL solution of Risedronate sodium and injecting 20 µL of the sample in triplicate into the HPLC system. The correlation coefficient was found to be 0.9993. Accuracy was assessed by standard dilution method. Added known quantities of the pre-analyzed to the drug product at 80%, 100% and 120% in triplicate and analyzed by HPLC-grade method. The percentage recovery of Risedronate sodium was found to be 98.93%-101.64%, the system suitability of this method the responses generated by the standard should exhibit a reasonable relative standard deviation. The standard solution of Risedronate sodium was indicating satisfactory, Percentage RSD of the peak area is 0.3510 and theoretical plates are 2657, tailing factor is 1.47 respectively (**Table** 8#**2**). Repeatability applying the analytical method to multiple samplings (at least 6) of a homogeneous sample at 100% of test concentration. The Percentage RSD for peak area is 0.3532, percentage recovery is 0.3531 respectively (**Table** 9#**3**).

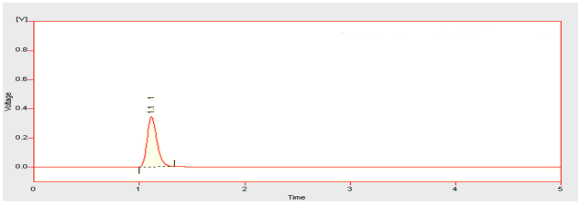

Figure 3. Optimized Chromatogram

Table 1. Chromatographic condition

S.No	Parameters	Description
1.	Column	C_{18} :150X4.6 mm, 5 µ, SS Column
2.	Mobile Phase	Methanol: Buffer (85:15)
3.	Diluent	Mobile Phase
4.	Flow rate	1.0 mL/min
5.	Detection	UV-254 nm
6.	Temperature	25°C
7.	Injection volume	20 µL
8.	Run time	10 min

Table 2. System Suitability for Risedronate sodium by HPLC method

S.No	Retention Time	Peak area	Theoretical plates	Tailing factor
1	1.12	2213.854	2622	1.41
2	1.11	2207.143	2544	1.41
3	1.11	2204.353	2588	1.37
4	1.10	2190.975	2660	1.37
5	1.10	2199.654	2657	1.47
6	1.10	2194.295	2589	1.37
MEAN	1.10666667	2201.712		
SD	0.00745356	7.729049		
RSD	0.67351445	0.351047		

Table 3. Repeatability of Risedronate sodium by HPLC method

S No	AUC	Percentage
1	2257.918	100.41%
2	2268.43	100.87%
3	2282.497	101.50%
4	2278.809	101.34%
5	2275.94	101.21%
6	2270.045	100.95%
Mean	2272.27	101.05
S.D	8.02589	0.0035
R.S.D	0.35320	0.3531

3 Conclusion

The rapid RP-HPLC and UV spectroscopy method developed for quantitative analysis of Risedronate sodium in pharmaceutical dosage forms. Method repored is precise, accurate, linear, robust, and specific. This newly developed RP-HPLC and UV-spectroscopic method for Risedronate sodium assay determination was found to be capable of giving good resolution; this method was completely showing satisfactory data for all the parameters tested. The methods reported provides excellent performance in terms of sensitivity, speed and relatively low solvent consumption when compared to most of the earlier reported chromatographic methods. The method developed were also validated according to International Conference on Harmonization (ICH) guidelines and is suitable routine analysis of Risedronate in pure drug and formulations.

4 Experimental

Materials & methods: Risedronate sodium pure drug, Osteodronate 35mg tablets were supplied by Hambran Pharmaceuticals, Punjab, India. Hydrochloric acid (Analytical grade) was obtained from Loba Chemical Ltd. Mumbai, India. Acetonitrile and methanol (HPLC Grade) were obtained from SD Finechem Ltd, India. Ammonium formate and Formic acid (AR Grade) were procured from Merck, India. Double beam spectrophotometer, Shimadzu model 1700, data acquisition was made with Lambda 25 software. The HPLC instrument used here was SPD- 20A Shimadzu, Japan.

4.1 Method A: UV Spectroscopy

Solvent of selection: The solubility of Risedronate sodium was determined in a variety of solvents as per Indian pharmacopoeia standards. Solubility was carried out in polar and non-polar solvents. From the solubility data 0.1 N HCl was selected as solvent for the analysis of Risedronate sodium.

Determination of absorption maxima: Risedronate sodium, 100 mg was taken in 100 mL standard flask and volume was made up to the mark with 0.1 N HCl to obtain 1000 µg/mL. A 10 mL of the above solution was Pipetted-out into a 100 mL standard flask and diluted to 100 mL

with 0.1M HCl to obtain 100 µg/mL. From the 100 µg/mL stock solution. Final concentration of 10 µg/mL was obtained by diluting 1 mL stock solution to 10 mL with 0.1N HCl. The above solution was scanned over range of 200-400 nm (**Figure 2**).

Analysis of marketed formulation by UV method: Twenty tablets of Risedronate sodium (Osteodronate 35 mg) was accurately weighed and finely powdered. A portion of the powder, 35.6 mg of was transferred into 50 mL volumetric flask and 5 mL of 0.1N HCl was added. The content of the flask was sonicated for 15 min and diluted up to the mark with 0.1N HCl. The solution was then filtered through Whatmann filter paper (No. 42). From the above solution, 5 mL was pipetted-out into a 100 mL volumetric flask and made up to the mark with same 0.1N HCl to obtain 10 µg/mL (**Table 4**).

Table 4. UV analytical Report

Drug Name	Risedronate Sodium
Brand Name	Osteodronate
Label Claim	35 mg
Amount found	0.0349 g
Percentage purity	99.71% (UV)

4.2 Method B: Reverse Phase High Performance Liquid Chromatography

Chromatographic Conditions: *Mobile phase*: This method was performed by mobile phase of Methanol: Ammonium formate buffer (85:15) pH was adjusted to 4.5 with dilute formic acid (**Table 1** and **Figure 4**); *Absorption maxima:* UV- 254 nm**;** *Injection Volume:* 20µL**;** *Column:* C_{18}:150X4.6 mm, 5 µ, SS Column**;** *Flow rate:* 1.0 mL/min

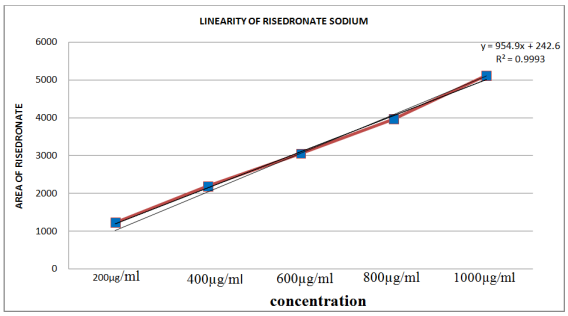

Figure 4. HPLC calibration curve of Risedronate Sodium

Preparation of standard stock solution: Risedronate sodium, 35 mg of working standard was weighed accurately into a 100 mL volumetric flask, dissolved with small quantity of diluent and sonicated. Solution was filtered through 0.45 µ membrane filter. First few mL of the filtrate was discarded. 10 mL of stock solution was pipette out and transferred into a 25 mL volumetric flask and was made up to the volume with mobile phase.

Analysis of marketed formulation by HPLC method: Twenty tablets of Risedronate sodium (Osteodronate35 mg) were accurately weighed, finely powdered and mixed. A portion of the powder 35.6 mg of Risedronate sodium was transferred into a 50 mL volumetric flask and small amount of mobile phase was added. Filtered through 0.45 µ membrane filter paper. A 10 mL of above prepared solution was pipetted-out into a 25 mL volumetric flask and was made up to the volume with mobile phase. Further diluted to obtain 10 µg/mL of the concentration (**Table 4**).

Validation parameters

Linearity: The linearity of an analytical method is ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of proposed methods, various aliquots of standard solution of drug were prepared from stock solution and analyzed. Sample solution of drug with different concentration from 10-50 µg/mL were analyzed by UV Spectrophotometer using 0.1N HCl as blank at 261 nm (**Figure 4**) and HPLC at 254 nm concentration range from 200 -1000 µg/mL (**Figure 5**). Their absorbance and area measured respectively (**Table 5** and **6**).

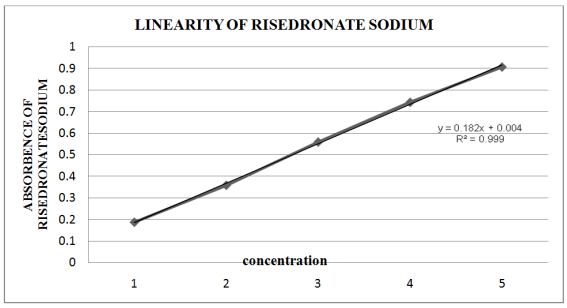

Figure 5. UV Calibration curve of Risedronate sodium

Table 5. Linearity data for UV spectroscopic method

Linearity level	Conc (µg/mL)	Absorbance (n=3)
1	10	0.188
2	20	0.359
3	30	0.559
4	40	0.743
5	50	0.906
Slope		0.182
Intercept		0.004
Correlation Coefficient (r^2)		0.999

Table 6. Linearity data for HPLC spectroscopic method

Linearity level	Conc (µg/mL)	AUC
1	200	1226.248
2	400	2186.702
3	600	3049.747
4	800	3961.439
5	1000	5113.639
Slope		954.9
Intercept		242.6
Correlation Coefficient (r^2)		0.9993

Precision: Precision studies were carried out to establish the repeatability and intermediate precision of the proposed methods using six replicates of same concentrated sample solution.

Repeatability: Repeatedly applying the analytical method to the multiple homogeneous samples (6) at 100% of test concentration in UV Spectroscopy and HPLC method. The results of absorbance and area measured (**Table 3** and **7**).

Table 7. Repeatability of Risedronate sodium

S. No	Absorbance(n=6)	% Recovery
1	0.919	100.102
2	0.923	100.538
3	0.929	101.191
4	0.934	101.736
5	0.931	101.409
6	0.927	100.973
Mean	0.92716	100.992
SD	0.0049	0.54250
RSD	0.7365	0.53717

Intermediate precision: Intermediate precision study was carried out by repeating the complete experiment with different analysts, on different days, different equipment in same laboratory for UV spectroscopy and HPLC methods. The results were presented as % RSD in **Table 8**.

Table 8. Ruggedness data for UV Spectroscopic method

S.NO	Absorbance(n=6)	% Assay
1	0.909	100.00
2	0.914	100.55
3	0.918	100.98
4	0.907	99.78
5	0.905	99.56
6	0.919	101.33
Mean	0.912	100.33
SD	0.005354	0.5893
RSD	0.8976	0.587075

Accuracy: Accuracy determination at 80%, 100% and 120% level were prepared using buffer: Methanol (850:150 v/v) as solvent. At each level, triplicate determinations were performed for HPLC method (**Table 9**). For UV Spectroscopy, three samples of each level were prepared and total nine determinations were done as per ICH guidelines using 0.1N HCl as blank. The results were compared with standard and statistically analyzed. The percentage recovery was presented in **Table 10**.

Table 9. Accuracy data for HPLC method

Conc (%)	Amt added (mg)	Recovered		RSD NMT 2%
		Amt (µg/mL)	Percent	
80	191.4	28.5	100.50	
	191.7	28.7	101.20	0.3443
	191.4	27.9	100.45	
100	191.9	34.3	99.70	
	191.4	35.3	101.64	0.8012
	191.5	35.6	100.1	
120	191.4	41.2	98.93	
	191.7	42.6	99.49	0.2278
	191.4	42.8	99.0	
	Mean		100.01	
	SD		0.010074	0.4688
	RSD		1.0073	

Table 10. Accuracy data for UV method

Conc (%)	Amt added (mg)	Recovered		RSD NMT *2%*
		Amt (µg/mL)	%	
80%	191.4	28.5	99.60	
	191.7	28.7	100.8	0.3380
	191.4	27.9	100.07	
100%	191.9	34.3	99.01	
	191.4	35.3	98.88	0.4311
	191.5	35.6	98.50	
120%	191.4	41.2	101.1	
	191.7	42.6	100.6	0.7605
	191.4	42.8	99.7	
Mean			99.6066	
SD			0.87719	0.5098
RSD			0.87581	

System Suitability: System-suitability tests are the integral part of method development and are used to assure the adequate performance of the chromatographic system. Choice of retention time (Rt), tailing factor (T), theoretical plates (N) and capacity factor (k) was the major task while developing the method and was evaluated for six replicate injections of the drug at a concentration of 10 μg/mL. The results were presented in **Table 2** are within the acceptable limits.

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