DEVELOPMENT OF UV SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF RISEDRONATE SODIUM IN DIFFERENT SOLUTIONS

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Four simple, low-cost, sensitive, accurate, and direct spectrophotometric methods for Risedronate sodium (RIS) estimation have been developed. All methods were based on pyridinyl sensitivity for the UV light at 262 nm. The proposed methods were extensively validated according to the ICH guidelines and proved as following Beer's law over the concentration ranges 6–120, 5–100, 17–170, and 15–150 μg/ml for methods 1, 2, 3, and 4, respectively. All the proposed methods were found to be precise with RSD values less than 2% and accurate with recovery values between 90–110%. These methods can fully fill the needs of QC routine tests plus meet different demands for Research and Development departments.

Keywords: *risedronate sodium, spectrophotometry, validation.*

РАЗРАБОТКА УФ-СПЕКТРОФОТОМЕТРИЧЕСКИХ МЕТОДОВ ОПРЕДЕЛЕНИЯ РИЗЕДРОНАТА НАТРИЯ В РАЗЛИЧНЫХ РАСТВОРАХ

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Разработаны четыре простых, чувствительных и точных спектрофотометрических метода определения ризедроната натрия, основанных на чувствительности пиридинила к УФ-излучению при 262 нм, и проверены в соответствии с принципами ICH. Методы показали соответствие закону Бера в диапазонах концентраций 6–120, 5–100, 17–170 и 15–150 мкг/мл, точность для RSD < 2% и воспроизводимость от 90 до 110%.

Ключевые слова: *ризедронат натрия, спектрофотометрия, валидация.*

Introduction. Risedronate sodium (RIS) is a nitrogen-containing third-generation bisphosphonate drug [1] approved by the FDA in 2000 [2]. It is widely used in the treatment of osteoporosis and Paget's disease. Regarding Risedronate's chemical nature, as well as other bisphosphonates, several analytical challenges are known. Among them there are the following: a) bisphosphonates are strong chelators, which causes interaction with HPLC metallic systems (e.g., injection valves or columns), b) bisphosphonates generally lack chromophore groups in their structures [3, 4], which makes it problematic to detect them by direct UV, c) bisphosphonates are highly ionic, polar with low volatility, making it hard to detect them directly by GC [4–7]. Considering the mentioned difficulties, researchers have put much effort into developing new validated methods to detect RIS in both biological and pharmaceutical samples. The reported techniques used to detect assay RIS in pharmaceutical forms include: ion-pair high-performance liquid chromatographic [4, 6], capillary electrophoresis [7], and spectrophotometric titrations [5, 8–10]. Though both HPLC and capillary electrophoresis are successful approaches, they are time-consuming and require highly sophisticated equipment for routine analysis in Quality Control (QC) laboratories. Even though spectrophotometer devices are widely

available, giving this approach a big preference over others, the previously reported UV titrations seems to have some drawbacks, such as complexity in work [5], long duration [8], need to conduct two measurements to evaluate the results, and indirect detection within a short linearity range [9, 10]. Therefore, it would be of great interest to develop and validate easy, simple, reproducible, accurate, and direct UV methods for RIS detection in four widely used different solutions (water, methanol, 0.1 N NaOH, phosphate buffer saline pH 7.4).

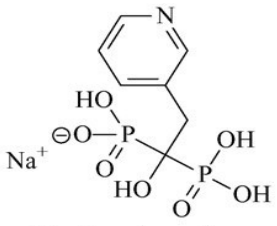
We introduce four direct UV assays for RIS at 262 nm. Meanwhile, we use the RIS specific feature: it has a pyridinyl group that acts as an appreciable chromophore, sensitive enough for direct detection in UV light [4, 8].

Experimental. *Material and methods.* A T80+ UV/VIS spectrophotometer (UK) with 1 cm quartz cells was used for spectrophotometric measurements. Risedronate sodium was kindly donated by PHARMASYR (a Syrian pharmaceutical company in the Damascus countryside, Syria). All the solvents and chemicals used were of analytical reagent grade, and all the solutions were made fresh on a daily basis.

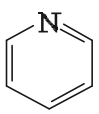
Risedronate stock solutions. Method 1: risedronate sodium stock solution in water (140 μg/ml) was prepared by dissolving 14 mg of RIS in 10 0 mL of distilled water and completed to the mark after 5 min sonicating. Method 2: risedronate sodium stock solution in methanol (100 μg/ml) was prepared by dissolving 10 mg of RIS in 100 ml of methanol and completed to the mark after 5 min sonicating. Method 3: risedronate sodium stock solution in 0.1 N NaOH (180 μg/ml) was prepared by dissolving 18 mg of RIS in 100 ml of 0.1 N NaOH solution and completed to the mark after 5 min sonicating. Method 4: risedronate sodium stock solution in phosphate buffer saline pH 7.4 (160 μg/ml) was prepared by dissolving 16mg of RIS in 100 ml of phosphate buffer saline pH 7.4 and completed to the mark after 5 min sonicating.

General procedure. Method 1: into 10 mL volumetric flasks, aliquot volumes from the aqueous RIS stock solutions corresponding to 6–120 μg/mL were transferred and completed to the volume with distilled water. The absorbance at 262 nm was measured against distilled water as a blank. A calibration curve relating the absorbance versus drug concentrations in μg/mL was constructed; consequently, the regression equation was derived. Method 2: into 10 mL volumetric flasks, aliquot volumes from the RIS stock solution corresponding to 5–100 μg/mL were transferred and completed to the volume with methanol. The absorbance at 262 nm was measured against methanol as a blank. A calibration curve relating the absorbance versus drug concentrations in μg/mL was constructed; consequently, the regression equation was derived. Method 3: into 10 mL volumetric flasks, aliquot volumes from the RIS stock solution corresponding to 17–170 μg/mL were transferred and completed to the volume with 0.1 N NaOH. The absorbance at 262 nm was measured against 0.1 N NaOH solution as a blank. A calibration curve relating the absorbance versus drug concentrations in μg/mL was constructed; consequently, the regression equation was derived. Method 4: into 10 mL volumetric flasks, aliquot volumes from the RIS stock solution corresponding to 15–150 μg/mL were transferred and completed to the volume with phosphate buffer. The absorbance at 262 nm was measured against phosphate buffer saline pH 7.4 as a blank. A calibration curve relating the absorbance versus drug concentrations in μg/mL was constructed; consequently, the regression equation was derived.

Results and discussion. Risedronate sodium is one of the bisphosphonate drugs characterized by the nitrogen atom in a heterocycle named pyridinyl



Risedronate sodium



Pyridine

Therefore, RIS four stock solutions were scanned in the UV range (200–400 nm) showing λ_{max} at 262 nm in all solutions (Fig. 1).

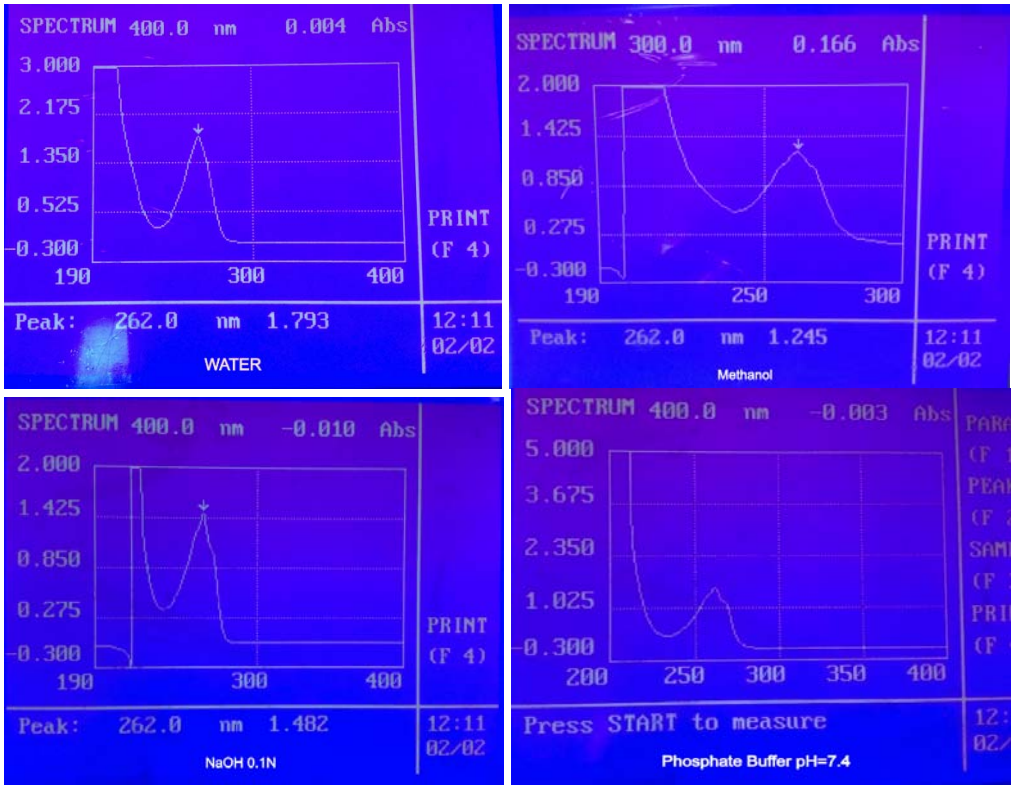


Fig. 1. UV spectrum of Risedronate sodium at 262 nm in four solutions.

Pyridinyl leads to sensitivity to UV light at 262 nm [11]. The proposed methods were validated according to the ICH guidelines [12]. The methods were tested for linearity, precision, and accuracy. Linear regression plots were obtained for all the methods by plotting the values of the absorbance vs. final concentrations. Linear regression analysis of the data gave the following equations with the r value higher than 0.99% in all the methods:

Method 1: $A = 0.0131C + 0.0038$ ($r = 0.9998$),
Method 2: $A = 0.0109C - 0.0067$ ($r = 0.9996$),
Method 3: $A = 0.0082C - 0.0171$ ($r = 0.9997$),
Method 4: $A = 0.0089C + 0.0158$ ($r = 0.998$),

where A is the absorbance at 262 nm, C is the concentration in µg/mL, and r is the correlation coefficient.

Limit of quantitation and limit of detection. The limit of detection (LOD) means the lowest amount of RIS that can be detected at 262 nm, while the limit of quantitation (LOQ) is the lowest amount of RIS that can be quantitatively determined with suitable precision and accuracy.

TABLE 1. Summarizes the Results: Concentration Ranges, Slopes, Intercepts, Equations, Correlation Coefficients, Detection Limit, and Quantitation Limit for all the Methods

Parameter	Method 1	Method 2	Method 3	Method 4
Concentration range, µg/mL	6–120	5–100	17–170	15–150
Slope	0.0131	0.0109	0.0082	0.0089
Intercept	0.0038	–0.0067	– 0.0171	0.0158
Equation	$y = 0.0131x + 0.0038$	$y=0.0109x - 0.0067$	$y = 0.0082x - 0.0171$	$y=0.0089x+0.0158$
Correlation coefficient (r)	0.9998	0.9996	0.9997	0.998
Limit of detection (LOD), µg/ml	0.07	0.499	0.34	2.2
Limit of quantification (LOQ), µg/mL	0.229	1.5	1	6.66

LOQ and LOD were calculated for all the four methods according to the following equations: LOD = = 3.3 S_a/b, LOQ= 10 S_a/b, where S_a is the standard deviation of the intercept of the regression line, and *b* is the slope of the regression line.

Precision was determined in terms of repeatability or intraday precision and intermediate precision (between days). As for intraday precision, according to ICH recommendations, six solutions with the same concentration of RIS were prepared. The mean recovery and RSD values show that all the methods are precise with RSD values 0.66, 1.4, 1.06, and 0.4 for methods 1, 2, 3, and 4, respectively. Moreover, intermediate (interday) precision was determined by preparing three different concentrations (25, 50, 75 μg/ml) and calculating the mean recovery and RSD values for each method, which were less than 2% for all the methods.

Accuracy was determined by preparing three different concentrations with three replicates for each, taking the mean recovery percentage, which demonstrated all the methods as accurate. The results for intraday and interday precision and accuracy are shown in Tables 2 and 3.

TABLE 2. Repeatability/intraday and Interday Precision

Added	Found	Recovery	%Mean recovery	SD	RSD
Method 1					
50	51.52	103.04%	101.81%	0.0068	0.668
50	50.6	101.20%			
50	50.52	101.04%			
50	51.13	102.26%			
50	50.9	101.80%			
50	50.75	101.50%			
Method 2					
50	50	100.00%	101.17%	0.0142	1.4037
50	50.33	100.66%			
50	51.62	103.24%			
50	49.51	99.02%			
50	50.98	101.96%			
50	51.07	102.14%			
Method 3					
50	51.1	102.20%	101.39%	0.01075	1.0611
50	50.98	101.96%			
50	50.74	101.48%			
50	50.98	101.96%			
50	49.52	99.04%			
50	50.86	101.72%			
Method 4					
75	77.2	102.93%	103.09%	0.00427	0.4144
75	77.88	103.84%			
75	77.2	102.93%			
75	77.55	103.40%			
75	77.2	102.93%			
75	76.87	102.49%			

Interday Precision*(n = 3)								
Concentration,	Method 1		Method 2		Method 3		Method 4	
μg/ml	Conc.found	RSD%	Conc.found	RSD%	Conc.found	RSD%	Conc.found	RSD%
25	24.8	0.38	24.34	0.75	25.90	1.34	25.03	0.56
50	50.31	1.17	50.43	0.89	50.21	1.09	50.69	1.2
75	74.18	0.27	76.14	1.14	76.35	0.59	77.28	0.85

*Average of three estimations.

TABLE 3. Accuracy Study for all the Methods

Concentration, μg/ml	Mean concentration found	Recovery, %	Mean recovery ± SD, %
25	25.18	100.72	100.77 ± 0.0073
50	50.85	101.70	
75	74.93	99.90	
25	27.79	111.16	106.89 ± 0.0312
50	51.83	103.66	
75	79.6	106.13	
50	55.21	110.42	106.07 ± 0.035
100	106.14	106.14	
150	152.48	101.65	
25	24.62	98.48	101.84 ± 0.024
50	51.98	103.96	
75	77.31	103.08	

Conclusions. The four validated spectrophotometric methods described in this paper were found to be simple, accurate, and rapid. They are only one-step methods that need no further processes compared with the previously reported ones. Therefore, they can be applied for risedronate routine determination in bulk powders in four inexpensive, common, and easily obtained solutions for QC labs and industrial new inventive trails.

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