SPECTROPHOTOMETRIC DETERMINATION OF RISEDRONATE SODIUM IN PHARMACEUTICAL PREPARATIONS BY DERIVATIVE AND CONTINUOUS WAVELET TRANSFORMS

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ABSTRACT

Determination of risedronate sodium (RIS) in its commercial tablet formulations in the presence of the effect of excipients was performed by derivative spectrophotometry and continuous wavelet transform. No preliminary separation step was used for the quantitative analysis by the proposed methods. Firstly direct absorbance measurement (DAM) method was applied to the analysis of RIS in samples, but this method did not give desirable results for the analysis of the commercial tablet samples. For this reason, the signal processing methods, first derivative spectrophotometry (DS), Morlet and Biorthogonal2.8 continuous wavelet transforms (Morlet-CWT and Bior2.8-CWT, respectively) were subjected to the quantitative resolution of the samples containing RIS and tablet excipients without using any separation step. These methods were found to be suitable for the analysis of the related drug. Calibration graphs were obtained by using the relationships between first derivative, CWT signals and concentration. The linearity ranges of DS and CWT methods were found to be 0.8-120 μg/mL, 3.0-100.0 μg/mL respectively and good correlations were observed for the calibration equations. The proposed methods were validated and applied to

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the determination of RIS in pharmaceutical preparations. The experimental results obtained by the proposed methods were statistically compared with each other and with those obtained by spectrophotometric method reported in literature. In the statistical analysis, no significant difference was found between the assay results. It was observed that the DS, Morlet-CWT and Bior2.8-CWT methods were accurate, sensitive, precise, rugged and useful for the quality control of RIS in commercial pharmaceutical samples without the interference of the excipients.

INTRODUCTION

Risedronate sodium used in osteoporosis treatment is a pyridinyl bisphosphonate that inhibits osteoclast-mediated bone resorption and modulates bone metabolism /1/. Commercial tablet preparations containing 5 mg or 35 mg anhydrous RIS in the form of the hemi-pentahydrate with small amounts of monohydrate have been used for oral administration. The chemical name of RIS is [1-hydroxy-2-(3 pyridinyl)ethylidene]bis[phosphonic acid] monosodium salt. The chemical structure of RIS hemi-pentahydrate is shown in Figure 1.

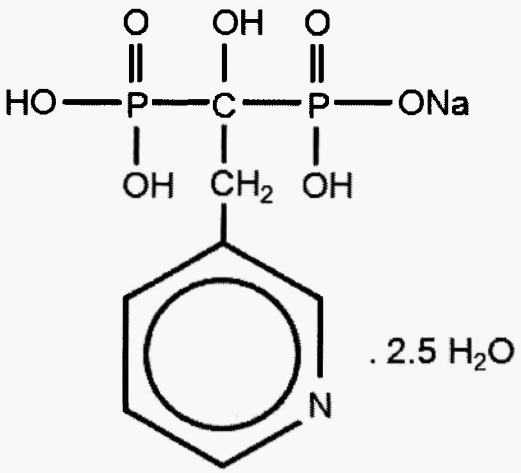

Fig. 1. Chemical structure of Risedronate Sodium

The chemical nature of RIS, as well as other bisphosphonates, causes several analytical difficulties: (a) bisphosphonates are strongly polar and ionic, hindering a simple extraction from a biological fluid into an organic solvent and (b) the capacity for complexation with metal ions and other cations give rise to poor peak shape (severe tailing of the peak), baseline disturbance and irreproducible chromatograms. In addition, bisphosphonates are characterized by low bioavailability, typically with absorption of 1% or less at the therapeutic dose, leading to only low levels (ng mL^{-1}) are present in blood and other biological fluids. Therefore, the development of assays for the quantitation of bisphosphonates in biological fluids presents a formidable challenge to the analyst. The difficulties associated with bioanalysis of bisphosphonates have been well documented /2/.

High Performance Liquid Chromotography (HPLC) assays for RIS in biological fluids using detection based on the native UV absorbance /3/, fluorescence /4/, and electrochemical /5/ properties of the analytes have been reported in literature. Assays for bisphosphonates in biological matrices employing precolumn chemical derivatization /6,7/, postcolumn phosphomolybdate complex formation /8/, and indirect fluorescence detection schemes /9/ have also been reported. RIS has been determined in human urine using Gas Chromotography – Mass Spectrophotometry (GC-MS) following acylation and silylation to form a volatile derivative /10/. More recently, RIS in human urine based on enzyme linked immunosorbent assay (ELISA) has been reported /11/.

There are two studies in the literature for the analysis of RIS from pharmaceutical formulations by HPLC /12,13/. Bisphosphonates are strong chelators; they readily interact with metals in HPLC systems (e.g. in injection valves or HPLC columns), giving rise to poor peak shape and irreproducible chromatograms. There is an approach to reduce the adsorptive character of bisphosphonates by adding tailing suppressors in the mobile phase /14,15/. Another way to overcome these interactions with the chromatographic set-up is to use a completely non-metallic system /16,17/. Irrespective of these preventive measures, the selection of the column packing material remains critical, since the use of a metal-free system prevents only the chelation of bisphosphonates with metal ions but not the specific interaction of bisphosphonates with the column packing material. Because of the chelation feature of RIS, the HPLC system costs too much money and time for analysis of this substance in pharmaceutical preparations.

In the study of Taha and Youssef /18/ two spectrophotometric methods were developed for determination of RIS in bulk powder and pharmaceutical formulations. The first method is based on the measurements of difference in absorbance of RIS in 10^{-2} M HCl and in 10^{-2} M NaOH at 262 nm. The second method is based on the oxidation of RIS with ceric (IV) sulphate and subsequent measurement of the excess unreacted cerium (IV) sulphate at 320 nm. Both methods include time-consuming sample preparation steps, and use of chemicals which can cause contamination and recovery problems. Parameters of validation were not evaluated.

In recent years, wavelet transform (WT) is a powerful signal processing tool for data reduction, de-nosing, baseline correction and resolution of the overlapping signals /19-21/. In the spectral studies, the continuous wavelets transform (CWT) methods and their applications in analytical chemistry have significantly amplified the potential power of various spectrophotometric techniques. CWT methods in combination of zero-crossing and ratio treatment have been used for the quantitative resolution of two-component and three-component mixtures /22-26/.

In the previous studies on the RIS analysis, HPLC and spectrophotometric methods require many additional chemical and graphical procedures as mentioned above. For these reasons, it is clear that the RIS analysis in samples requires new, simple, fast, cheap and very easy applicable analytical methods.

In this study, signal processing methods, DS, Morlet-CWT and Bior2.8-CWT were developed and applied to the determination of the RIS drug in tablet dosage forms in the presence of excipient effect. These proposed analytical methods do not need any separation step and they eliminated the interference effect of excipients due to the mathematical algorithms of the derivative and wavelet transforms. The validation of the developed methods was performed by analyzing intra-day and inter-day samples and by using the standard addition technique, and other validation parameters. After that DS, Morlet-CWT and Bior2.8-CWT approaches were applied to the real samples containing RIS drugs and a good coincidence was observed for the experimental results, which were statistically compared with each other as well as literature methods.

EXPERIMENTAL

Apparatus

The spectrophotometric measurements were carried out by using an Agilent 8453 model UV-Vıs spectrophotometer with a diode array detector (DAD) (190 – 1100 nm). UV spectra of reference and sample solutions were recorded in 1 cm quartz cells.

Reagents and solutions

RIS standard was kindly supplied by Fleminginfo Lab. (India). It was tested for purity by controlling its melting point, UV and IR spectra. No impurities were found. Stock standard solution (1000µg/mL) was prepared in deionized water. Deionized water was made in the laboratory using the Milli Q system. The stock solution was kept at +4 ^{0}C. Standard solutions were prepared daily by diluting the stock solution with water.

Sample solutions

Pharmaceutical preparations of RIS was obtained from local pharmacies. Ten tablets (5 mg per tablet) were weighed and powdered. Powder equivalent to one average tablet was accurately weighed and dissolved in 100 mL deionized water with ultrasonication for 15 min. After centrifugation for 20 min, at 4000 rpm, 2.5 mL of supernatant was diluted to 5 mL with water, and processed by the proposed analytical methods. Deionized water was used as blank.

Comparison Method

Stock standard solution of RIS (1000µg/mL) was prepared in deionized water. Different aliquots of standard solution equivalent to 0.15-1.5 mg were transferred into two series of 10 ml volumetric flasks. The first series was completed with 0.01 M hydrochloric acid and the second series with 0.01 M sodium hydroxide. The absorbance difference was measured at 262 nm against the drug in sodium hydroxide as a blank.

RESULTS AND DISCUSSION

In the study, various solvents for the analysis of RIS were tried such as water, methanol, acetonitrile alone or in combinations of different proportions and water was found to be appropriate to reach high absorbance values. Firstly direct absorbance measurement (DAM) method was applied to the determination of RIS in tablet and placebo samples. In the case of DAM, satisfactory results were not obtained due to the interference of excipients (crospovidone, ferric oxide yellow, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polyethylene glycol, silicon dioxide and titanium dioxide). The RIS and excipients show the absorbance at the same wavelength, 262 nm (see Figure 2a). It was observed that ferric oxide yellow and titanium dioxide caused the interference (Figure 3). To overcome this problem, DS, Morlet-CWT and Bior2.8-CWT were applied. The details of the application of the proposed signal processing methods are explained below.

Method application

In the DAM method, the calibration graph was obtained by measuring the original absorbance values at 262 nm. Linear regression analysis and statistical results provided by the DAM are presented in Table 1. The assay results obtained by application of this method to the tablet and placebo samples were not found suitable for the analysis of RIS (see Table 3 and Table 4).

In order to bypass the drawbacks of the DAM method, derivative spectrophotometry, Morlet-CWT and Bior2.8-CWT approaches were applied to the quantitative analysis of RIS in tablets without any interference of excipients.

Derivative spectrophotometry is a useful technique that can be used to resolve overlapping of spectral peaks or existing interferences. The main advantage of the derivative spectrophotometry is assaying single drugs in the presence of tablet excipients and in tablet dosage forms – that is without prior extraction of the drug /27/. As seen in Figure 2b, in the first derivative UV spectra, excipients gave no signal at 258 nm which standard and sample solutions at the same concentration absorbed identically. Therefore 258 nm was selected as marking wavelength (Figure 2b).

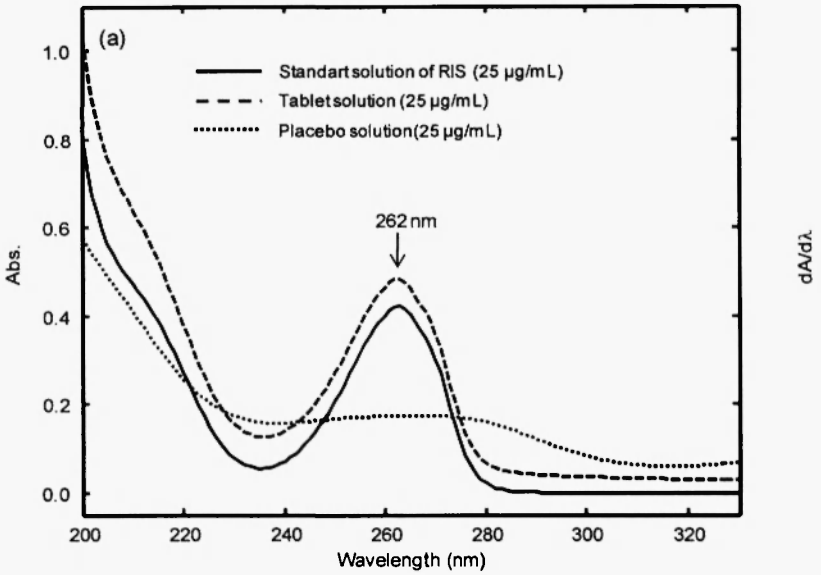

Fig. 2: a) Original absorption spectra

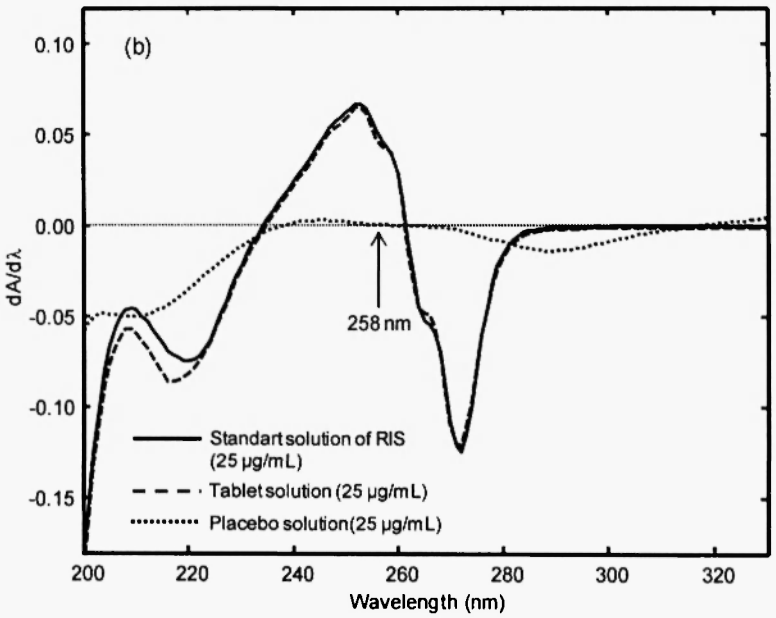

Fig. 2: b) first derivative spectra,

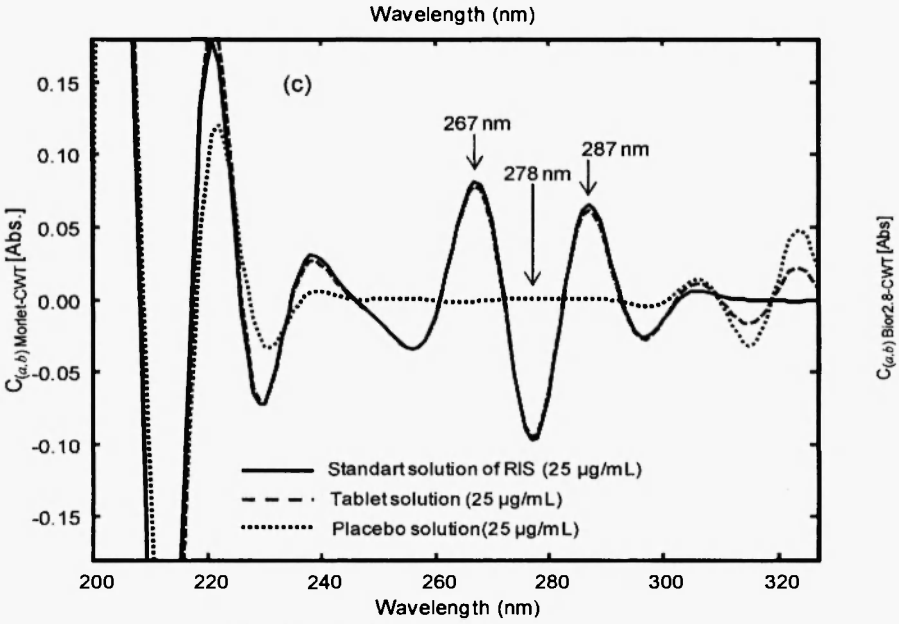

Fig. 2: c) Morlet-CWT spectra

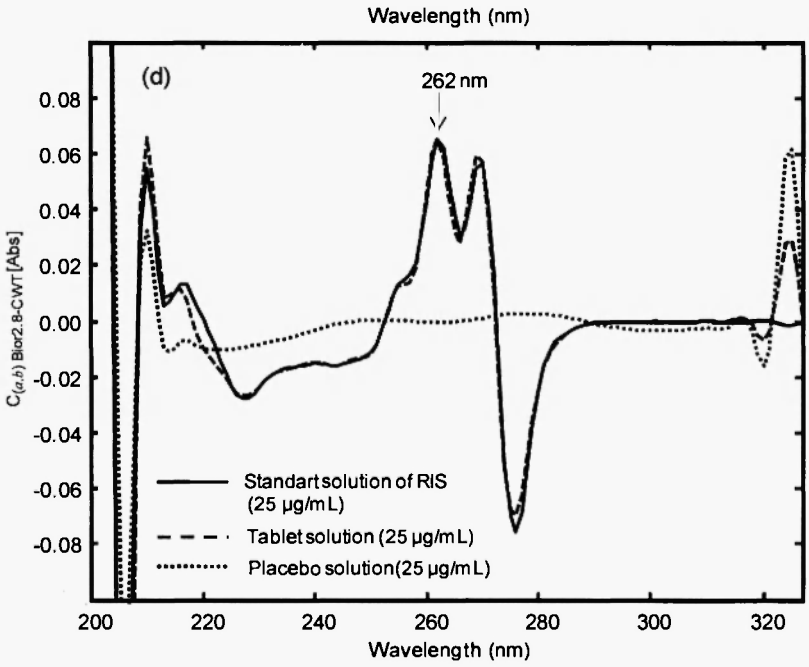

Fig. 2: d) Bior2.8-CWT spectra of RIS, tablet and placebo solutions

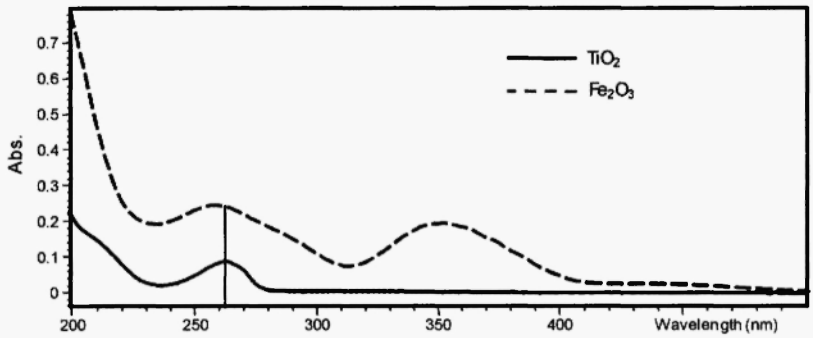

Fig. 3: Absorption spectra of Fe_2O_3 and TiO_2

In the derivative method, calibration equation was obtained by using the concentration and first derivative signals at 258 nm. The linear regression analysis and its results are shown in Table 1. This method gives the suitable results for the RIS analysis in samples.

In application of the continuous wavelet transforms to the resolution of the overlapping spectra of RIS and tablet excipients, various wavelet families were tested to obtain the best recovery results of the determination of RIS in tablets. Bior2.8-CWT (scale parameter, a=8) and Morlet-CWT (a=14) were found to be optimal signal processing methods.

By using Mortlet-CWT, calibration graphs for RIS were obtained by measuring the CWT amplitudes at 267, 278 and 287 nm corresponding to zero crossing points for excipients (Figure 2c). The statistical results obtained by applying the linear regression analysis to the concentration and CWT signals are given in Table 1. In a similar manner, the calibration graph at 262 nm was obtained by using Bior2.8-CWT method (Figure 2d). These signal processing methods were applied to the determination of RIS in tablets without requiring any preliminary separation step.

Validation

The developed methods for the determination of RIS in pharmaceutical preparations were validated with respect to stability, linearity, sensitivity, specificity, precision, accuracy and ruggedness /28,29/.

Table 1

Linear regression analysis and its statistical results obtained by using the proposed methods

Methods	DAM	DS	Morlet-CWT			Bior2.8-CWT
λ(nm)	262	258	267	278	287	262
Linearity Range (μg/mL)	3.0-100.0	0.8 - 120.0	3.0-100.0	3.0-100.0	3.0-100.0	3.0-100.0
m	1.62E-02	5.70×10^{-4}	7.48E-04	-3.76E-03	2.54E-03	2.36E-03
n	8.30E-03	-1.80×10^{-4}	3.16E-03	-1.32E-04	5.12E-04	3.09E-03
r	0.9998	0.9995	0.9998	0.9996	0.9997	0.9996
SE(m)	1.19E-03	0.08×10^{-4}	1.56E-04	4.78E-05	2.77E-05	2.82E-05
SE(n)	1.39E-04	0.03×10^{-4}	3.00E-05	2.48E-04	1.44E-04	1.47E-04
SE(r)	1.29E-02	0.05x10-3	2.78E-03	4.44E-03	2.58E-03	2.62E-03
LOD (μg/mL)	0.58	0.30	0.39	0.53	0.45	0.49
LOQ (μg/mL)	1.95	0.80	1.30	1.75	1.50	1.64

m = Slope of linear regression equation,

n = Intercept of linear regression equation,

r = Correlation coefficient of linear regression equation,

SE(m) = Standard error of slope,

SE(n) = Standard error of intercept,

SE(r) = Standard error of correlation coefficient,

LOD = Limit of detection

LOQ = Limit of quantification

Table 2

Precision and accuracy of the RIS determination by the proposed methods

Method	λ (nm)	Intra-day (n = 6)				Inter-day (n = 6)		
		Added (mg/mL)	Found x ±SE(µg/mL)	Precision RSD %	Accuracy Bias %	Found x ±SE(µg/mL)	Precision RSD %	Accuracy Bias %
DAM	263	5	5.03 ± 0.04	1.94	0.56	4.89 ± 0.03	0.61	-2.10
		50	49.91 ± 0.40	1.96	-0.18	49.35 ± 0.40	0.82	-1.30
		100	96.97 ± 0.41	1.04	-3.03	97.33 ± 0.37	0.38	-2.67
DS	258	5	5.02 ± 0.03	1 34	0 40	4.98 ± 0.02	1.07	-0.40
		50	50.06 ± 0.17	0.82	0.12	50.03 ± 0.04	0.19	0 06
		100	99.72 ± 0.18	0 43	-0.28	99.87 ± 0.27	0.66	-0.13
Morlet-CWT	267	5	4.90 ± 0.03	1.29	-1.92	4.90 ± 0.04	0.58	-2.06
		50	49.12 ± 0.17	0.84	-1.76	49.53 ± 0.21	0.43	-0.94
		100	98.93 ± 0.57	1.40	-1.07	98.46 ± 0.35	0.36	-1.54
	278	5	4.88 ± 0.04	2.11	-2.35	4.93 ± 0.05	1.07	-1.48
		50	48.72 ± 0.19	0.96	-2.56	49.23 ± 0.23	0.46	-1.54
		100	98.71 ± 0.78	1 93	-1.29	98.61 ± 0.35	0 35	-1.39
	287	5	4.90 ± 0.02	0.46	-1.97	4.89 ± 0.02	0 43	-2.11
		50	48.88 ± 0.18	0.36	-2.23	49.42 ± 0 24	0.49	-1.17
		100	99.85 ± 0.40	0.40	-0.15	98.49 ± 0.41	0.41	-1.51
Bior2.8-CWT	262	5	4.87 ± 0.04	2.11	-1.98	4.91 ± 0.04	0 84	-1.87
		50	50 93 ± 0.17	0.83	-0.04	51.08 ± 0.19	0.37	2.15
		100	97.29 ± 0.54	1.54	-1.73	98.37± 0.69	0.70	-1.63

$\bar{x}$: Mean, SE: Standard Error, RSD : Relative standard deviation,

Bias %: [(Found-Added)/Added]x100

Table 3

The recovery results obtained by applying the proposed methods to the analysis of RIS in the placebo medium.

Method		λ(nm)	Added to placebo (µg/mL) 20	40	60
DAM	Found ($\bar{X}$ ± SE)	263	18.5+0.12	37.87±0.35	55.76±0.57
	Recovery (%)		92.48	94.68	93.15
	RSD (%)		1.57	2.29	2.56
DS	Found ($\bar{X}$ ± SE)	258	20.19 ±0 06	40.06 ±0.09	59.93 ± 0.72
	Recovery (%)		100.95	100.15	99.88
	RSD(%)		0.47	0.41	0.49
Morlet-CWT	Found ($\bar{X}$ ± SE)	267	19.83 ±0.27	39.12±0.29	58.66±0.42
		278	19.43±0.09	38.82±0.33	57.80±0 44
		287	19.80±0.12	38.27±013	58.80±0.20
	Recovery (%)	267	99.14	97.81	97.56
		278	97.13	97.04	96.90
		287	98.98	95.68	97.94
	RSD(%)	267	1.35	1.83	1.59
		278	0.45	2.07	1.72
		287	0.62	0.83	0.89
Bior2.8-CWT	Found ($\bar{X}$ ± SE)	262	19.93±0 21	39.30±0.21	58.78±0.48
	Recovery (%)		100.05	97.84	97.84
	RSD(%)		1.97	1.32	2.12

$\bar{X}$ = Mean, SE=Standard Error, RSD= Relative standard deviation

Recovery results are the average of six replicate for each concentration level.

Table 4

The results obtained by application of the developed analytical methods to two different pharmaceutical dosage forms

	DAM		DS		Morlet-CWT				Bior2.8-CWT		Literature method*	
	263		258		267		278		287		262			
No.	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
1	6.31	44.29	5.19	35.08	5.16	34.8	5.1	36.11	5.13	34.74	5.24	36.69	4.99	35.12
2	6.54	39.54	5.11	35.12	5.17	34.16	5.26	33.42	5.07	34.3	5.27	36.8	4.98	35.22
3	6.06	39.77	5.06	34.93	5.18	34.64	5.12	33.51	5.14	33.31	5.25	36.77	5.03	34.91
4	6.51	45.3	5.13	34.96	5.24	34.49	5.19	36.49	5.23	34.54	5.01	35.08	5.06	34.87
5	6.55	45.12	5.01	35.03	5.25	34.5	5.2	36.52	5.24	35.42	5.03	35.22	4.91	35.03
6	6.17	44.57	5.09	35.11	5.21	34.6	5.15	35.77	5.17	34	5.1	35.73	4.96	35.21
Mean	6.36	43.10	5.10	35.04	5.20	34.53	5.17	35.30	5.16	34.38	5.15	36.05	4.99	35.06
SD	0.21	2.69	0.06	0.08	0.04	0.22	0.06	1.45	0.06	0.71	0.12	0.80	0.05	0.15
RSD (%)	3.27	6.25	1.21	0.23	0.73	0.62	1.14	4.11	1.25	2.07	2.26	2.23	1.06	0.43
SE	0.08	1.10	0.03	0.03	0.02	0.09	0.02	0.59	0.03	0.29	0.05	0.33	0.02	0.06
CL (p=0.05)	0.17	2.15	0.05	0.06	0.03	0.17	0.05	1.16	0.05	0.57	0.09	0.64	0.04	0.12

(I) corresponds to 5 mg Risedronate Sodium® Tablets.

(II) corresponds to 35 mg Risedronate Sodium® Tablets

CL =Confidential limit

Stability

The standard stock solutions of RIS had been stored in two different conditions for 2 months. At the end of this period, UV spectra were taken and no difference was found between the freshly preparad solutions and solutions kept at +4°C and at ambient temperature, indicating that R is highly stable in the above mentioned conditions.

Linearity

In quantitative analysis, the calibration curves were constructed by plotting the dA/dλ values and CWT signals against the concentrations of standard solutions.The regression equations, standard errors of slopes and intercepts, correlation coefficients and linearity ranges are given in Table 1.

Sensitivity (Limit of Detection and Quantification)

The limit of detection (LOD), is the lowest concentration that can be detected and limit of quantification (LOQ), is the lowest concentration of a substance that can be quantified with acceptable precision and accuracy. LOD (k=3,3) and LOQ (k=10) of the methods were established according to the ICH definitions /28/ (C_l = k x S_O/s where C_l is LOD or LOQ, S_O is the standard error of blank determination, s is the slope of standard curve and k is the constant related to the confidence interval).

Specificity

The measured signals at studied wavelengths obtained from tablet solutions were the same as the values obtained from standard solutions containing equivalent concentrations of RIS (Figure 2b, 2c and 2d). The observed results show that developed methods are highly specific.

Standard addition method was also applied to show the specificity of the DS method. No difference was found between the slopes of calibration curve ($5{,}70 \times 10^{-4}$) and standard addition curve ($5{,}67 \times 10^{-4}$) which means there is no interference effect of excipients in this method. So it could be said that the method is specific for the analysis of R in pharmaceutical preparations.

Precision

The different concentrations of RIS (5, 50, or 100 μg/ml) in the linear range were analyzed in six independent series in the same day (intra-day precision) and six consecutive days (inter-day precision); within each series every sample was analyzed three times. The RSD % values varied from 0.36 to 2.11 for intra-day precision and 0.19 to 1.07 for inter-day precision (Table 2). These RSD% values were low enough to show the high precision of the method.

Accuracy and Recovery

The accuracy of a method is expressed as the closeness of agreement between the value found and the value that is accepted as a reference value. It is determined by calculating the percent difference (bias %) between the measured mean concentrations and the corresponding nominal concentrations. Table 2 shows the results obtained for intra-day and inter-day accuracy.

The accuracies of the proposed methods were also tested by recovery experiments. The recovery of RIS was calculated by comparing the added and found concentrations in placebo (C_{found} / C_{added} x 100) and expressed as mean recoveries and RSD in each case. The obtained results are shown in Table 3, from which it is clear that the recovery results are satisfactory.

Ruggedness

Ruggedness test for the developed method was performed by the application of the method to the standard solution of RIS (25 μg/mL) by a different analyst. Obtained results were compared statistically (Wilcoxon's Paired Test). The results for the R (25 μg/mL) from different analysts are statistically acceptable ($p= 0.752$, $p>0.05$). Therefore it could be said that the method is rugged.

Analysis of Tablets

DAM, DS, Morlet-CWT and Bior2.8-CWT methods were applied to the RIS drug in two different dosage forms of the tablet preparations (I and II). The assay results obtained from the proposed methods were indicated in Table 4.

The absorbance difference method /18/ mentioned in literature was used as a comparison method to evaluate the validity of the developed methods.

To compare the results obtained by applying the DS, Morlet-CWT and Bior2.8-CWT and literature methods to two different dosage forms of commercial tablets, one way ANOVA-test was applied to the experimental results of all the methods. In this test, no significant difference was reported between the results of the applied methods. The ANOVA-test results for two dosage forms, 5 mg (I) and 35 mg (II) per tablet are summarized in Table 5.

Table 5

The ANOVA results in the application of the DS, Morlet-CWT and Bior2.8-CWT for the (I) and (II) tablets

(I)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F-table*
Between Groups	0.0046	5	0.0009	0.08	1.00	2.62
Within Groups	0.2926	24	0.0122			
Total	0.2971	29				
(II)						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F-table*
Between Groups	2.6495	5	0.5299	0.52	0.76	2.62
Within Groups	24.5896	24	1.0246			
Total	27.2391	29				

SS = Sum of squares, *df* – degree of freedom, *MS* = Mean of squares, F = Calculated F-value, P-value = Confidence level (p= 0.05)

CONCLUSION

In spite of the spectral interference of excipients, the DS, Morlet-CWT and Bior2.8-CWT methods provide the reliable results for the determination of the RIS in tablet samples. In contrast to the above proposed methods, the use of the DAM method did not give desirable results for the analysis of the related drug in samples. In this study, the proposed methods were validated by evaluating the validation parameters such as linearity, sensitivity, specificity, precision, accuracy and ruggudness.

The validated methods were successfully applied to pharmaceutical preparations including 5 mg and 35 mg RIS. The results were compared to

spectrophotometric methods reported in the literature and no significiant difference was found statistically.

RIS is a strong chelator; it readily interacts with metals in HPLC systems (e.g. in injection valves or HPLC columns), giving rise to poor peak shape and irreproducible chromatograms. To overcome these problems, non-metallic systems were used or ion pairs were constituted before RIS was given to the system. In addition, the total analysis period was too long and expensive in HPLC methods in literature.

Spectrophotometric methods in literature which are less sensitive than the proposed method (linearity ranges were 15-150 μg/mL and 2-24 μg/mL respectively) need time consuming chemical reaction procedures. They may also cause contamination and recovery problems. For routine analysis a simple, rapid and inexpensive method should be preferred.

As compared to HPLC and indirect spectrophotometric methods mentioned in literature, the developed methods are more convenient for routine analysis of RIS in bulk powder and pharmaceuticals. It could therefore be concluded that the proposed methods provide alternative approaches for quality control of RIS in pharmaceutical formulations.

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