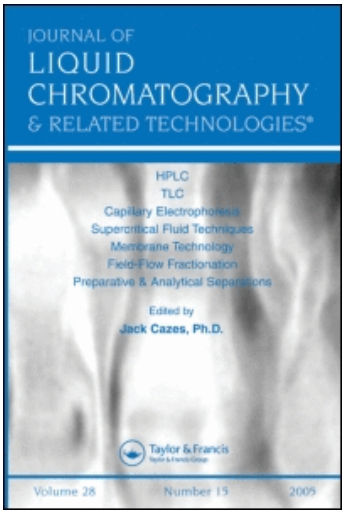

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Stability Indicating Ion-Pair HPLC Method for the Determination of Risedronate in a Commercial Formulation

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ABSTRACT

A simple, rapid, and reproducible analytical procedure has been developed for the assay of risedronate in pharmaceutical dosage forms. The method is based on ion-pair liquid chromatography with UV detection. Separation is performed on an Eclipse XDB C_{18} ($4.6 \times 150\,mm^2$, 3.5 μm particles) column, using 5 mM tetrabutylammonium phosphate as counter-ion in the mobile phase. The proposed method was extensively validated according to ICH guidelines for the assay determination. A linear relationship was found in the concentration range studied from 50 to 150 μg risedronate sodium per 25 μL injection. The method

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precision was below 1.2% relative standard deviation (RSD) ($n = 9$). The mean recovery of risedronate from commercial tablets was found to be in the range of 99.3–100.6%. The limit of detection (LOD) and limit of quantification (LOQ) of risedronate were found to be 30 and 100 ng, respectively. Since the method is stability indicating, it is also well suited for shelf-life studies of risedronate pharmaceutical preparations.

Key Words: Ion-pair HPLC; UV detection; Assay; Validation; Stability indicating; Risedronate; Eclipse XDB C_{18}; Actonel tablets.

INTRODUCTION

Risedronate sodium is a pyridinyl bisphosphonate that inhibits osteoclast mediated bone resorption and modulates bone metabolism. The chemical name of risedronate sodium is [1-hydroxy-2-(3-pyridinyl) ethylidene]*bis* [phosphonic acid] monosodium salt (Fig. 1). Procter & Gamble have formulated risedronate sodium as a prescription drug under the brand name Actonel™, and is widely used in the treatment of osteoporosis and Paget's disease.

A few methods have been published in the literature for the determination of risedronate in biological fluids: for example, by using GC-MS following acylation and silylation to form a volatile derivative,[1] enzyme linked immunosorbent assay (ELISA),[2] and column-switching ion-pair HPLC with UV detection.[3] But all these methods involve complicated sample pretreatment procedures, or require instrumentation typically not available in a routine pharmaceutical quality control laboratory. Moreover, they lack the simplicity, convenience, and speed preferred for an assay of active pharmaceutical

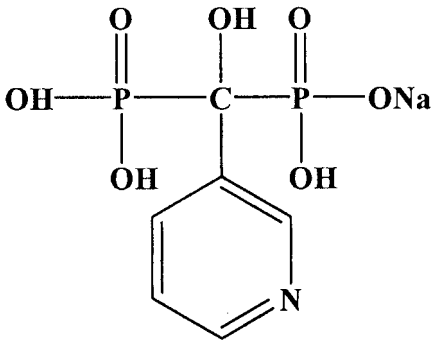

Figure 1. Chemical structure of risedronate sodium.

ingredient (API) in formulation. Therefore, a simple, accurate, and reproducible analytical method is necessary for risedronate in quality control and in shelf-life studies of formulation products. To date, no high performance liquid chromatography (HPLC) method has been reported for the determination of risedronate in pharmaceutical preparations.

The bisphosphonate class of drugs are known to be extremely difficult to analyze using liquid chromatography and, hence, development of their HPLC assay is a tough challenge to the analyst.[4–7] Because they are very strong chelators, they readily interact with metals in HPLC systems (e.g., in injection valve or tubing or HPLC columns), giving rise to poor peak shape and irreproducible chromatography. For this reason, several substances of this class are used as detergents. There is an approach to reduce the adsorptive character of bisphosphonates by adding tailing suppressors in the mobile phase.[3,8] Another way to overcome these interactions with the chromatographic set-up, is to use a completely non-metallic system.[9–11] 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.

In general, most of the bisphosphonates lack chromophore groups that make it difficult to use UV detection for their analyses. But, risedronate sodium having a pyridinyl group is sufficiently sensitive for direct UV detection. Moreover, risedronate sodium is readily ionizable in solution and, hence, it can be analyzed by a liquid chromatographic technique using reversed-phase conditions with mobile phase containing ion-pair reagents. Therefore, ion-pair reversed-phase liquid chromatography using a non-metallic system with UV detection, is feasible for risedronate determination.

The current study describes the development and validation of a simple, rapid, and accurate ion-pair HPLC method for the determination of risedronate in Actonel™ tablets. Since the method is stability indicating, it is also well suited for shelf-life studies of risedronate pharmaceutical preparations.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Dionex 40 gradient pump, LC 20 Chromatography Enclosure, AD 20 absorbance detector, and a Rheodyne injection valve equipped with a 25-μL sample loop. HPLC was controlled and monitored by Dionex PeakNet ver. 5.01 software. The chromatographic separation was performed using a Zorbax Eclipse XDB C_{18} ($150 \times 4.6\,mm^2$

ID and 3.5 μm particles) column, obtained from Agilent Technologies. An Accumet Meter (Model 07256) from Fisher Scientific was used for pH measurements.

Chemicals and Reagents

Risedronate sodium, Actonel™ and placebo (contains only Actonel™ excipients) tablets were obtained from Procter & Gamble Pharmaceuticals (Norwich, NY). Ion-pair reagent, tetrabutylammonium phosphate was obtained as a 1.0 M solution in water from Aldrich Chemicals (Milwaukee, WI). Acetonitrile 99.93+% HPLC grade and methyl alcohol 99.93+% HPLC grade were also purchased from Aldrich Chemicals (Milwaukee, WI). Dibasic sodium phosphate 7-hydrate crystals and disodium ethylenediamine tetraacetate dihydrate were obtained from J. T. Baker (Phillipsburg, NJ). All standards and stock solutions were made using Nanopure water with resistivity of 18 mΩ or greater.

Chromatographic Conditions

Chromatographic analysis was carried out at ambient temperature (~22°C). The mobile phase used for the separation was a mixture of acetonitrile and 0.01 M sodium phosphate buffer (pH 7.5) in the ratio 10 : 90 (v/v), containing 5 mM tetrabutyl ammonium phosphate (ion pair reagent) and 1 mM EDTA. The sodium phosphate buffer was prepared by dissolving 2.68 g of dibasic sodium phosphate 7-hydrate in 1000 mL of water and then adjusting the pH to 7.5 with dilute phosphoric acid. The mobile phase was filtered under vacuum through 0.45-μm nylon filters (Whatman International, Maidstone, England). The mobile phase flow rate was 1.0 mL/min. The detection was accomplished using UV detection at 262 nm.

Solutions

A stock solution of risedronate was prepared by dissolving 25 mg of the compound in mobile phase. Appropriate dilutions were made in mobile phase to final concentrations of 0.05, 0.075, 0.1, 0.125, 0.15 mg/mL for the calibration curve. The mobile phase was injected as the blank. The average weights of an Actonel™ 5 and 50 mg tablets were established to be 248.6 and 247.3 mg, respectively. Risedronate sample solutions (0.06, 0.09, and 0.12 mg/mL) were prepared by dissolving appropriate amounts of crushed

and ground Actonel^{TM} tablets, respectively, in 25 mL of mobile phase. Equivalent weights of placebo powder (as used to prepare risedronate solutions from Actonel^{TM} tablets solutions) were dissolved in mobile phase to prepare placebo solutions. All solutions were prepared in triplicates.

Degraded Samples for Specificity Study

For acid and base degradations, risedronate sodium was refluxed with 1 N HCl or 1 N NaOH at 70 $\pm$ 1°C for 16 hr, and then neutralized by adjusting pH to 7.0 with either 2 N NaOH or 2 N HCl. The solutions were further diluted to required concentration (0.08 mg/mL) with mobile phase. For aqueous degradation, risedronate sodium was refluxed with water at 70 $\pm$ 1°C for 16 hr, and then diluted to required concentration with mobile phase. For photo degradation, a solution of risedronate sodium [0.08 mg/mL, prepared in water–acetonitrile (90 : 10)] was exposed to ultraviolet light (254 nm). A solid sample of risedronate sodium, kept at 70°C for 24 hr, was used for thermal degradation studies.

Kinetic Studies of Oxidative Degradation

For oxidative degradation, 50 mg of risedronate sodium was refluxed with 100 mL of 10% H_2O_2 at 70 $\pm$ 1°C (water bath). After different time intervals, (0, 30, 60, 120, 180, 240, 300, and 360 min), 1.0 mL aliquots of the solution were transferred into 10-mL volumetric flasks and then diluted to the mark with the mobile phase.

RESULTS AND DISCUSSION

Method Development

Risedronate sodium contains several hydroxide groups and an ionizable pyridine ring, which prompted us to use reverse phase ion-pair HPLC for the development of an assay method in formulation samples. A non-metallic chromatographic system was used for the analysis to minimize potential adsorption sites (e.g., metal surfaces) within the HPLC system. The detector wavelength was set at 262 nm based on the UV data obtained for risedronate sodium in water. Different mobile phase compositions consisting of sodium phosphate buffer (pH 7.5) and acetonitrile with 5 mM tetrabutylammonium phosphate as ion-pair reagent, were used on Dionex RP 300 C_{18},

$4.6 \times 150\,mm^2$ stainless steel columns in order to optimize the separation of risedronate. Among the several mobile phases tested (8–17% acetonitrile), 10% acetonitrile was found to give good retention (~8 min) for risedronate, but a broad peak shape with a small plate count was observed and was, therefore, subjected to further optimization. Although the HPLC tubing and injector were all non-metallic, the column was metallic. Hence, some interaction with risedronate is possible, which may lead to poor peak shape. Addition of 1 mM EDTA to the above mobile phase improved the peak shape but decreased the retention (~4.2 min) of risedronate. Even the manipulation of column length failed to give acceptable chromatography. Interestingly, excellent peak shapes with good retention were achieved with the same eluent on Zorbax Eclipse XDB C_{18} 150 mm stainless steel columns. Therefore, the above chromatography conditions at a flow rate of 1.0 mL/min were finally adopted for the assay determination of risedronate in commercial formulation.

System Suitability Test

System suitability was determined by making six replicate injections of freshly prepared risedronate sodium at 0.08 mg/mL in diluting solution. The relative standard deviation (RSD) of the capacity factors and the peak areas were found to be less than 2%. The number of theoretical plates observed was more than 10,000, and the tailing factor was found to be less than 1.3. All these parameters were calculated on the basis of risedronate peaks.

Method Validation

The proposed method has been extensively validated for assay determination of risedronate according to ICH guidelines[12] using the following parameters.

Specificity

To demonstrate specificity in the presence of excipients used in the formulation, risedronate sodium was spiked in the placebo (at approximately 0.08 mg/mL level) and analyzed for the assay, and the results obtained were compared with the results of pure sample. Reproducibility was observed in both cases (RSD $< 2\%$), suggesting that there is no interference from any excipients present in the tablets. The results of this analysis are illustrated in Fig. 2.

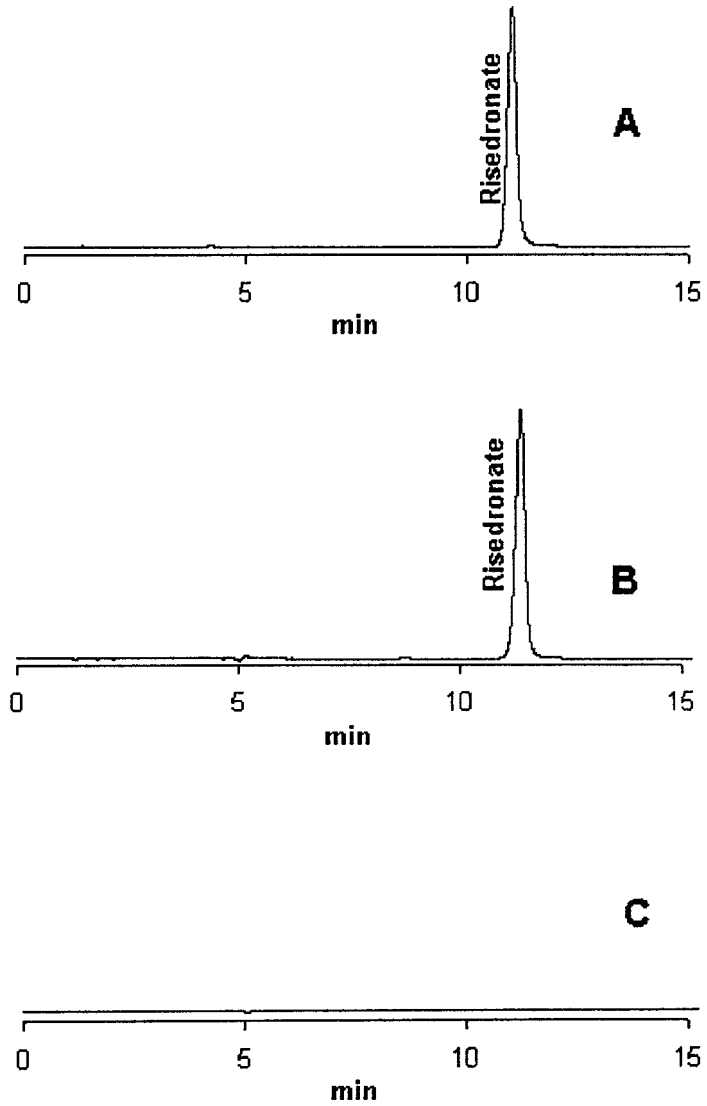

Figure 2. Representative chromatograms: A, risedronate as a bulk; B, risedronate in placebo; C, placebo; eluent: acetonitrile–0.01 M sodium phosphate buffer (pH 7.5) (10 : 90) + 5 mM tetrabutylammonium phosphate buffer + 1 mM EDTA. Column: Zorbax Eclipse XDB-C18, 4.6 × 150 mm^2; UV detection: 262 nm; flow: 1.0 mL/min.

Forced degradation studies were performed to demonstrate the validity of the assay method and provide evidence of its stability indicating property. The samples exposed to acidic, basic, aqueous, oxidative, thermal, and UV stress conditions were analyzed for the assay of risedronate, and the results are presented in Table 1. All the degradation products formed during stress studies were clearly resolved from the risedronate peak. The representative chromatograms of risedronate and its degradation products are shown in Fig. 3. To evaluate the homogeneity of the risedronate peak, all the stress samples were also analyzed at 210 nm. We calculated the response ratio of risedronate using peak areas integrated at both 210 and 262 nm, and found constant value (0.98) in all instances. This suggested that risedronate peak had no detectable impurity peaks embedded, and is free of co-eluting degradation compounds. From these results, it is clear that the proposed assay method is stability indicating and can be used for shelf-life studies of risedronate sodium as bulk and pharmaceutical formulations.

Earlier, it was reported that the decomposition of P–C–P bonds of bisphosphonates under stress conditions led to the releasing of inorganic phosphate and phosphite compounds.[13] Since the phosphate/phosphite does not show any UV sensitivity and it could not be detected as peaks in the stress samples, a corresponding area decrease from risedronate peak was observed (Table 1). Presently we are working on the determination of these inorganic phosphates using electrochemical detection (ED) and the obtained results will be published elsewhere.

The chromatograms obtained from the oxidative degradation revealed that the peak area of risedronate was reduced proportionally with time. Figure 4 shows the plot of log ([risedronate]/10^{-4} mol dm^{-3}) versus time

Table 1. Degradation conditions of risedronate sodium.

Condition	Time (hr)	Assay (%)	RRT[a] of degradation products
1N HCl, ref. at 70°C	16	96.1	0.11, 0.19, 0.37, 0.43
1N NaOH, ref. at 70°C	16	95.5	0.11, 0.19, 0.37, 0.43
Water, ref. at 70°C	16	96.6	0.37, 0.46, 0.76
10 %H_2O_2, ref. at 70°C	6	55.9	0.13, 0.19, 0.23, 0.30, 0.32, 0.37, 0.39, 0.47, 0.66, 0.93, 1.66
Heat dry, 80°C	24	100.2	0.11, 0.19, 0.37, 0.45
Light, UV at 254 nm	24	95.2	0.15, 0.19, 0.21, 0.31, 0.35, 0.37, 0.41, 0.44, 0.47, 0.61, 1.57

Note: ref.: refluxed.

[a]Relative retention time w.r.t risedronate.

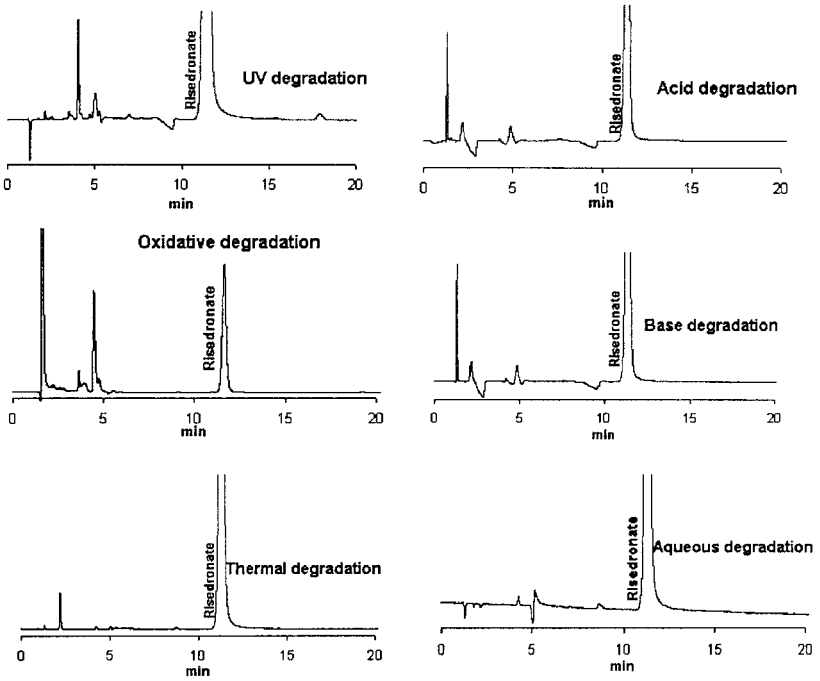

Figure 3. Separation of risedronate from its degraded products; unlabeled peaks are unknown degradation products. Eluent: acetonitrile–0.01 M sodium phosphate buffer (pH 7.5) (10 : 90) + 5 mM tetrabutylammonium phosphate buffer + 1 mM EDTA. Column: Zorbax Eclipse XDB-C18, 4.6 × 150 mm; UV detection: 262 nm; flow: 1.0 mL/min.

for degradation. It indicates a first-order degradation behavior with a rate constant (k) of $2.654 \times 10^{-5}\,\text{sec}^{-1}$ and a correlation coefficient of 0.998. The first-order disappearance rate constant was calculated using the following formula.[14]

$$\log[A] = \log[A]_0 - \frac{kt}{2.303}$$

where $[A]_0$ is the initial concentration of risedronate at $t = 0$ and $[A]_t$ is its concentration at time t. The half-life period ($t_{1/2}$) was calculated according to the formula below,[14] and it was found to be about 435 min.

$$t_{1/2} = \frac{0.693}{k}$$

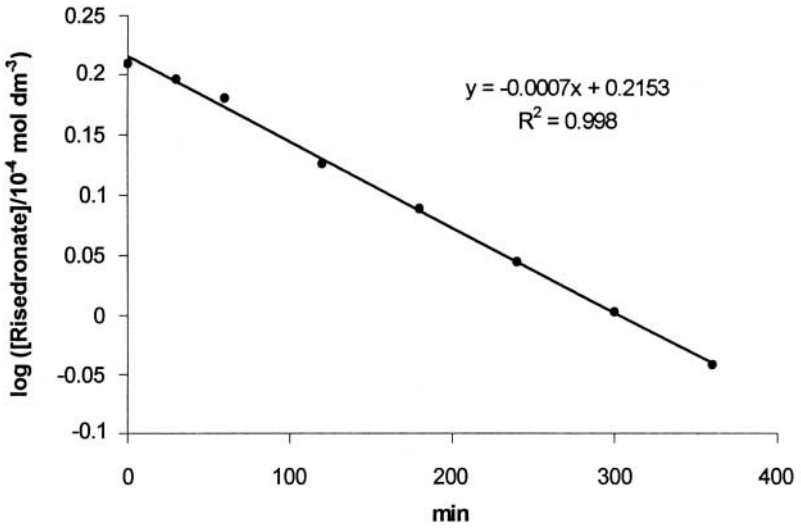

Figure 4. Semilogarithmic plot of risedronate sodium versus time of exposure to 10% H_2O_2 at 70°C for oxidative degradation products.

Precision

The precision of the method for the assay determination of risedronate was studied using the parameters viz. repeatability and intermediate precision. Method repeatability/intermediate precision was assessed by the assay of two, six-sample sets by two different analysts, using different columns and different lots of reagents on different days. The results are summarized in Table 2. The RSD values of repeatability/intermediate precision indicated that the proposed method provides acceptable precision for assay determination.

Linearity

Linearity of the method was checked at five different concentration levels ranging from 50 to 150 μg/mL, prepared from stock solution of working standard. The equation for calibration curve is $y = 2 * 10^7 \times -504.86$ with a correlation coefficient (R^2) equal to 0.999.

Table 2. Repeatability/intermediate precision.

Sample	Analyst 1 (mg per tablet)	Analyst 2 (mg per tablet)
Repeatability		
1	5.11	4.96
2	4.99	4.92
3	5.05	5.06
4	5.09	4.98
5	5.00	5.01
6	4.98	5.05
Mean ($n = 6$)	5.04	4.99
%RSD	1.09	1.08
Intermediate precision		
Mean ($n = 12$)	5.02	
%RSD	1.11	

Accuracy/Recovery

Accuracy of the assay method was checked by spiking placebo samples of Actonel tablets with three different concentration levels, i.e., 60, 90, and 120 μg of standard risedronate sodium, and the mixtures were analyzed by the proposed method ($n = 3$). The mean recovery data (at 95% confidence limits) obtained for each level, as well as its percentage recoveries, are tabulated in Table 3.

Table 3. Accuracy in the assay determination of risedronate.

Sample	Taken (μg/mL)	Recovery ($n = 3$) (μg/mL) [mean $\pm$ $t(s/\sqrt{n})$][a]	Percentage recovery
Placebo (for actonel 5 mg)	59.5	59.7 $\pm$ 1.41	100.3
	91.7	92.3 $\pm$ 0.99	100.6
	120.6	119.8 $\pm$ 1.14	99.3
Placebo (for actonel 50 mg)	60.2	59.8 $\pm$ 0.75	99.4
	92.1	92.2 $\pm$ 1.03	100.1
	121.4	121.2 $\pm$ 0.86	99.8

[a]s, S.D.; $t = 4.30$ (at 95% confidence limit and $n - 1$ degrees of freedom).

Limit of Detection and Limit of Quantification

To determine the limit of detection (LOD) and limit of quantification (LOQ), serial dilutions of risedronate sodium were made from the standard stock solution (50 μg/mL). The samples were injected (25 μL) and the measured signals from the samples were compared with those of blank samples. LOD and LOQ values were identified as signal-to-noise ratio of 3 : 1 and 10 : 1, respectively. The LOD and LOQ of risedronate were found to be 30 and 100 ng, respectively.

Robustness

To determine the robustness of the method, experimental conditions such as flow rate, acetonitrile composition, buffer concentration, buffer pH, EDTA concentration, and tetrabutylammonium phosphate concentration were purposely altered, and their effect on chromatographic characteristics such as efficiency, retention time, and tailing factor of risedronate peak were evaluated. The obtained results are presented in Table 4. There are no significant effects observed with the above study, except some changes noticed with buffer and EDTA concentrations. Decrease in buffer or EDTA concentrations reduced the efficiency but increased retention of the risedronate peak was observed.

Stability

The stability of the risedronate in diluting solutions was determined for the samples stored in a refrigerator and at room temperature. The samples were checked after 3 successive days of storage and the data were compared with freshly prepared samples. In each case, the RSD values of the assay were found to be below 2.0%. This indicates that the risedronate sodium is stable in the solution for at least 3 days.

CONCLUSIONS

An ion-pair HPLC method has been developed and subsequently validated for the assay of risedronate in formulation samples. The proposed method was found to be simple, selective, precise, and stability indicating. The method was successfully used for the assay determination of risedronate from Actonel tablets. The results obtained confirmed that the method is highly

Table 4. Robustness studies for the assay method.

	Efficiency (N)[a]	Tailing factor (T)[a]	Retention time (min)[a]
Flow rate			
0.9	12,356	1.21	12.20
1.0	13,302	1.18	11.02
1.1	13,669	1.08	10.00
Acetonitrile composition (%)			
8	12,023	1.22	12.13
10	13,302	1.18	11.02
12	13,586	1.15	9.80
Phosphate buffer concentration (M)			
0.005	11,828	1.31	11.90
0.010	13,302	1.18	11.02
0.025	13,586	1.15	8.50
Phosphate buffer pH			
7.3	12,567	1.25	10.91
7.5	13,302	1.18	11.02
7.7	12,678	1.23	11.10
EDTA concentration (mM)			
0.5	9,278	1.18	13.38
1	13,302	1.22	11.02
1.5	14,232	1.15	8.28
Tetrabutylammonium phosphate concentration (mM)			
3	12,390	1.21	9.92
5	13,302	1.18	11.02
7	12,109	1.24	13.24

[a]Tabulated data is mean of duplicate injections.

suitable for its intended use. The method can be used for stability studies of risedronate sodium as bulk and pharmaceutical dosage forms in the quality control laboratories.

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