

# Evaluation of the USP Risedronate Sodium Assay

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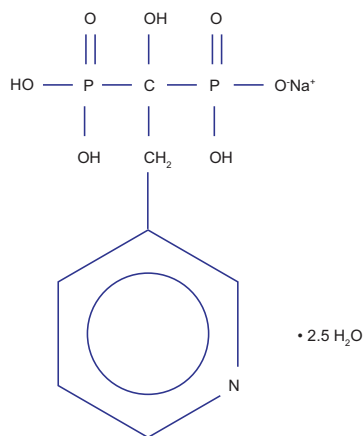


## Abstract

Risedronate (Figure 1) is a pyridinyl bisphosphonate and is one of the most popular first-line drugs available for the prevention and treatment of osteoporosis, a debilitating disease estimated by the National Osteoporosis Foundation to affect 61 million Americans age 50 and older by 2020. Risedronate is available as a tablet for oral administration that contains the equivalent of 5, 30, 35, or 75 mg of anhydrous risedronate sodium in the hemi-pentahydrate form with small amounts of the monohydrate. In October 2010, a second-generation risedronate in a delayed-release formulation was approved by the U.S. Food and Drug Administration (FDA).

The U.S. Pharmacopeia (USP) monograph describes an ion chromatography (IC) method to assay risedronate in the drug substance and product (risedronate, 35 mg tablets). The method specifies a USP L48 column with an edetate disodium (EDTA) eluent at pH  $9.5 \pm 0.1$  followed by UV detection at 263 nm. The Thermo Scientific Dionex IonPac™ AS7 column is a USP L48 column and is used in this method. The Dionex IonPac AS7 column is a high-capacity, high-efficiency, hydrophobic, anion-exchange column specifically developed for the analysis of a wide range of polyvalent anions, including polyphosphonates, hexavalent chromium, cyanide, and iodide. This study evaluates and validates the USP monograph method for risedronate analysis and identifies the critical points of the method for successful chromatography.

FIGURE 1. Risedronate sodium hemi-pentahydrate chemical structure.



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## Equipment

A Thermo Scientific Dionex ICS-3000 system was used for this study. For individual components of the system and preparation of solutions, standards and samples refer to Dionex Application Note 289.<sup>1</sup>

## Method Conditions

Column:	Dionex IonPac AS7 analytical, 4 × 250 mm (P/N 035393) Dionex IonPac AG7 guard, 4 × 50 mm (P/N 035394)
Mobile Phase:	4.8 mM EDTA, pH 9.5 ± 0.1
Injection Volume:	20 µL
Flow Rate:	0.8 mL/min
Column Temperature:	25 °C
Detection:	UV absorbance, 263 nm
System Backpressure:	~1430 psi
Total Run Time:	20 min

## Results and Discussion

### Separation and Detection

The presence of two polar phosphonate groups makes the retention of risedronate on commonly used RP columns (e.g., the Thermo Scientific Acclaim™ 120 C18 column) difficult. In addition, the metal chelation property of risedronate can cause poor peak shape and analyte recovery in systems that are not metal-free. Chelating agents, such as EDTA, are added to the mobile phase to prevent metal contaminants from chelating with risedronate. In addition, ion-pairing reagents typically are added to the mobile phase to enable retention and separation on a RP column. However, ion-pairing RP methods typically are not as robust as ion-exchange methods.

In this method, separation of risedronate from its related substances is achieved using a Dionex IonPac AS7 column with 4.8 mM EDTA mobile phase adjusted to pH 9.5. At pH 9.5 ± 0.1, risedronate is readily ionized into a polyvalent anion and EDTA is predominantly trivalent. Separation is likely achieved by the trivalent EDTA anion eluting the polyvalent risedronate anion. Risedronate and its related compounds elute within 15 min and the detection is by UV absorbance at 263 nm. The experimental results for the assay amount, resolution between risedronate and its related compounds, tailing factor, and peak area RSD either meet or exceed the USP specifications and are presented in Table 1.

Figure 2, trace A, shows the separation of risedronate from related compound C, and trace B shows the separation of risedronate and its related compound A using the conditions described in the monograph. The retention times of related compound C, related compound A, and risedronate were approximately 9.4, 10.2, and 11.9 min, respectively. Figure 3 shows a risedronate peak in the drug product (Actonel®, 35 mg tablet) preparation.

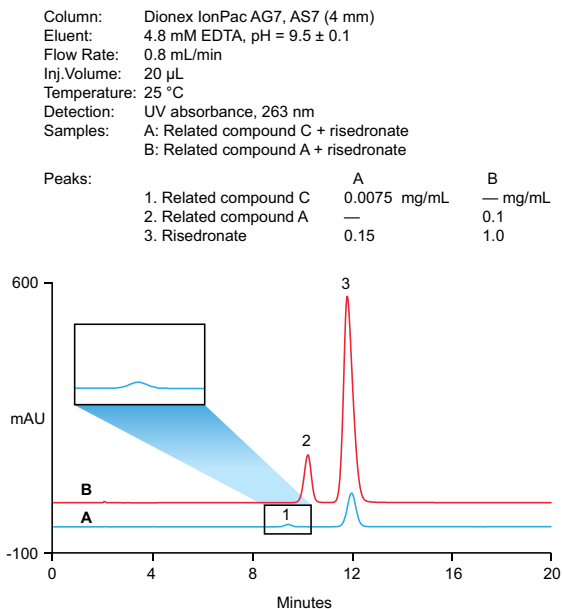
Table 1. Specifications				
Drug Substance				
	Tailing Factor	Resolution <sup>a</sup>	% RSD <sup>c</sup>	% Risedronate
USP Spec.	≤ 1.5	≥ 2.3	≤ 1.0	98.0%–102.0%
Experimental	1.3	2.5	0.32	99.6%
Drug Product				
	Resolution <sup>b</sup>	% RSD <sup>c</sup>	% Risedronate	
USP Spec.	≥ 2.5	≤ 1.0	90.0%–110.0%	
Experimental	4.4	0.19	98.3%	

<sup>a</sup>Related compound A and risedronate

<sup>b</sup>Related compound C and risedronate

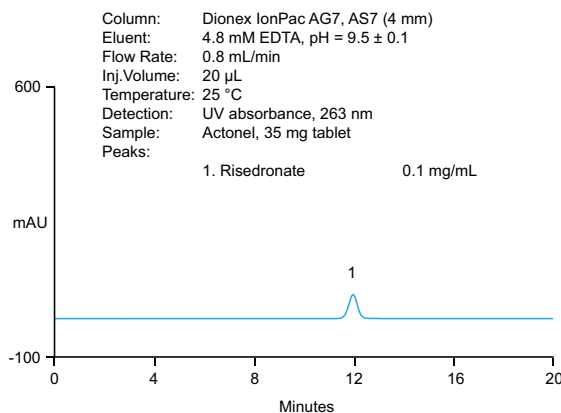
<sup>c</sup>n = 3

**FIGURE 2. Overlay of chromatograms of risedronate standard with related compound C in trace A and related compound A in trace B.**



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**FIGURE 3. Chromatogram of Actonel, 35 mg tablet preparation showing risedronate.**



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### Method Performance

The linearity, limit of detection (LOD), and limit of quantification (LOQ) were evaluated to determine the suitability of the method for this analysis. To determine linearity, the system was calibrated using duplicate injections of five concentrations. Table 2 shows the linearity, LOD, and LOQ for risedronate determined in the drug substance and drug product using UV detection.

Table 2. Linearity, LOD, LOQ for Risedronate					
	Analyte	Range (mg/mL)	Coefficient of Determination (r <sup>2</sup> )	LOD (µg/mL)	LOQ (µg/mL)
<b>Substance</b>	Risedronate	0.5–1.5	1.00	0.08	0.3
<b>Product</b>	Risedronate	0.05–0.15	0.9999	0.08	0.3

The average percent assay of risedronate in the drug substance prepared at 0.75, 1.00, and 1.25 mg/mL anhydrous risedronate is 99.5%, 99.6%, and 99.1%, respectively. Average percent assay of risedronate in the drug product (tablets) prepared at 0.075, 0.100, and 0.125 mg/mL anhydrous risedronate is 97.1%, 98.3 %, and 97.7%, respectively. The USP monograph specifies % assay for risedronate in the drug substance to be 100.0 ± 2.0% and in the drug product to be 100.0 ± 10.0%. All the values obtained in this validation successfully meet the specifications. For both substance and product analysis, the method shows a peak area precision of <1.0% at the test concentration (100%) and <2.0% for concentrations at 75% and 125% of the test method concentration. The accuracy and precision values are reported in Tables 3 and 4.

Table 3. Accuracy and Precision for the Determination of Risedronate in the Drug Substance					
Drug Substance Preparation	Concentration (mg/mL)	Average Peak Area Response (mAU*min)	% RSD	% Risedronate	Average % Assay
75%-1	0.75	172.1	1.0	98.6	99.5 ± 0.8
75%-2	0.75	175.6		99.9	
75%-3	0.75	174.8		99.9	
100%-1	1.00	233.7	0.3	99.9	99.6 ± 0.5
100%-2	1.00	233.3		99.9	
100%-3	1.01	234.7		99.0	
125%-1	1.25	289.5	1.0	99.6	99.1 ± 0.9
125%-2	1.25	284.5		98.0	
125%-3	1.25	289.6		99.6	
<b>1.01 mg/mL anhydrous USP risedronate standard response 235.9 mAU*min</b>				<b>Average</b>	99.4
				<b>Std. Dev.</b>	0.3

Table 4. Accuracy and Precision for the Determination of Risedronate in the Drug Product (Tablet)					
Drug Product Preparation	Concentration (mg/mL)	Average Peak Area Response (mAU*min)	% RSD	% Risedronate	Average % Assay
75%-1	0.075	17.5	2.0	98.6	97.1 ± 1.5
75%-2	0.075	17.3		97.2	
75%-3	0.075	16.8		95.6	
100%-1	0.100	23.1	0.6	98.0	98.3 ± 0.6
100%-2	0.100	23.1		98.0	
100%-3	0.100	23.3		99.0	
125%-1	0.125	29.0	1.8	98.8	97.7 ± 1.8
125%-2	0.125	29.0		98.8	
125%-3	0.125	28.1		95.6	
<b>0.101 mg/mL anhydrous USP risedronate standard response 23.8 mAU*min</b>				<b>Average</b>	97.7
				<b>Std. Dev.</b>	0.6

Although not required in the USP monograph, this method was evaluated for its ability to pass USP specifications with slight changes in the parameters that can be expected during routine analysis. Column temperature was varied by  $\pm 2$  °C from the control temperature of 25 °C and the pH was varied within the USP specified range of  $9.5 \pm 0.1$  units. In addition, two Dionex IonPac AS7 columns from the same manufactured lot were compared for this analysis. The results from this study are presented in Table 5. All the USP specifications were still met despite the variations in the test method parameters.

Table 5. Robustness for the Analysis of the Drug Substance								
Parameter	Resolution <sup>a</sup> (spec $\geq 2.3$ )	Difference (%)	Resolution <sup>b</sup> (spec $\geq 2.5$ )	Difference (%)	Tailing Factor <sup>c</sup> (spec $\leq 1.5$ )	Difference (%)	Risedronate Peak Area (mAU*min)	Difference (%)
<b>Column Temperature</b>								
23 °C	3.0	+20	4.5	+2	1.18	-8	233.1	-1
25 °C	2.5	—	4.4	—	1.28	—	236.5	—
27 °C	2.9	+16	4.6	+5	1.25	-2	232.7	-2
<b>Mobile Phase pH</b>								
9.38	3.4	+36	4.7	+7	1.15	-10	231.5	-2
9.52	2.5	—	4.4	—	1.28	—	236.5	—
9.63	2.6	+4	4.4	0	1.28	0	231.0	-2
<b>Column Condition</b>								
Column 1 After ~ 550 Injections	2.5	—	4.4	—	1.28	—	236.5	—
Column 2	3.3	+32	5.1	+16	1.45	+13	236.7	+0.1

<sup>a</sup>Resolution between related compound A and risedronate

<sup>b</sup>Resolution between related compound C and risedronate

<sup>c</sup>Tailing factor of risedronate peak

## Conclusions

- The USP monograph for risedronate sodium was used to assay the API in the drug substance and drug product (Risedronate 35 mg tablets).
- The method separated risedronate in 15 min and it was well resolved from related compounds A and C.
- All the experimental results exceeded the USP monograph specifications.
- The results from this study demonstrated a simple, rugged, and accurate method to assay risedronate sodium in the drug substance and product.
- It was observed that using polypropylene bottles for mobile phase preparation and using consistent amounts of sodium hydroxide for mobile phase pH adjustment is critical for successful chromatography.

## References

1. Thermo Fisher Scientific, Inc. Dionex Application Note 289: *Evaluation of the USP Risedronate Sodium Assay*, LPN 2926. Sunnyvale, CA, 2011.

[www.dionex.com/](http://www.dionex.com/)



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