Analysis of Risedronate According to USP Using the Agilent 1260 Infinity Bio-inert Quaternary LC System

Application Note

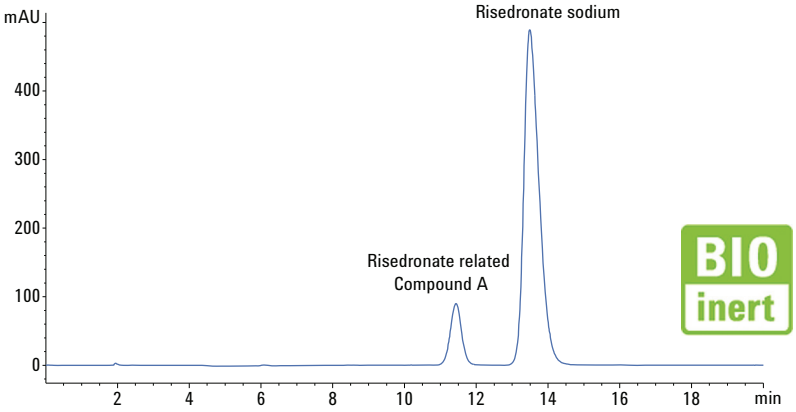
Small Molecule Pharmaceuticals & Generics

Authors

Sonja Schneider
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

This Application Note presents the analyses of USP risedronate sodium substance and of risedronate sodium tablets according to U.S. Pharmacopeia (USP) specifications carried out using the Agilent 1260 Infinity Bio-inert Quaternary LC System. Risedronate sodium is a bisphosphonate used, for example, in osteoporosis therapy. The completely metal-free sample flow path of the 1260 Infinity Bio-inert Quaternary LC System avoids severe peak tailing of phosphate compounds due to the formation of phosphate-Fe(III) complexes. The risedronate sodium assays met or exceeded the USP requirements regarding resolution, tailing factor, and area precision. In addition, high linearity, low limit of detection (LOD), and limit of quantification (LOQ) were found together with high assay precision and accuracy.



Verified for Agilent
1260 Infinity II LC
Bio-inert System

Agilent Technologies

Introduction

Risedronate sodium is a bisphosphonate, which is therapeutically used for the treatment of osteoporosis, Paget's disease of bone, heterotopic ossification, and cancer-induced bone loss[1]. Bisphosphonates are synthetic compounds with a similar structure to pyrophosphates. They are potent antiresorptive drugs with high antifracture activity[2]. The efficacy of the bisphosphonates increased during the development phases from etidronate and clodronate (first generation), over pamidronate, alendronate (second generation), to risedronate as a third generation of bisphosphonate compound, 5,000 times more potent than etidronate[1].

Figure 1 displays the structure of risedronate sodium.

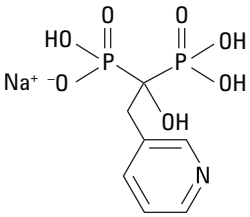


Figure 1. Chemical structure of risedronate sodium.

The presence of phosphate groups can be critical for the analysis of those compounds on a standard stainless steel liquid chromatography system. Severe peak tailing of phosphate compounds is a well described issue in HPLC analysis due to the formation of phosphate-Fe(III) complexes[3,4]. As previously shown for the analysis of adenosine triphosphate (ATP)[5], these complex formations can be avoided by using bio-inert PEEK tubing and modules. The Agilent 1260 Infinity Bio-inert Quaternary LC System has capillaries that are PEEK inside and stainless steel outside, presenting a metal-free sample flow path UHPLC. With this system, a user can analyze a variety of phosphate compounds without the emersion of peak tailing or other unspecific sample retention in the system due to phosphate-iron complexes.

The USP monograph specifies two methods for the analysis of risedronate sodium substance and risedronate sodium tablets using ion chromatography[6]. This Application Note describes the analysis of the USP risedronate sodium substance and of risedronate sodium tablets (Actonel, 5 mg tablets) according to USP specifications. Ethylenediaminetetraacetic acid (EDTA) is supplementally added to the mobile phase to avoid chelation of metal contaminants with risedronate.

Experimental

Instrumentation

Agilent 1260 Infinity Bio-inert Quaternary LC System	Model number
Agilent 1260 Infinity Bio-inert Quaternary Pump	G5611A
Agilent 1260 Infinity High performance Bio-inert Autosampler	G5667A
Agilent 1290 Infinity Thermostat for sample cooling	G1330B
Agilent 1290 Infinity Thermostatted Column Compartment with bio-inert solvent heat exchanger	G1316C
Agilent 1260 Infinity DAD VL with bio-inert standard flow cell, 10 mm	G1315D

Acquisition and Evaluation Software

Agilent OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.05 [35]

Chromatographic Conditions

Parameter	Setting
Column	Anion exchange column, 4 × 250 mm, 10 µm, packing L48 + guard column, 4 × 50 mm
Mobile phase	4.8 mM EDTA, pH 9.5
Flow rate	0.8 mL/min
Stop time	20 minutes
Injection volume	20 µL
Sample temperature	4 °C
Column temperature	25 °C
Detection	263 nm/ 4 nm, Ref.: OFF Peak width > 0.05 minutes (1.0 second response time) (5 Hz)

Solvents and Samples

All solvents were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). Ethylenediaminetetraacetic acid (EDTA) was purchased from Fluka (Sigma-Aldrich), St. Louis, USA.

USP Risedronate Sodium hemi-pentahydrate, USP risedronate related Compounds A and C were purchased from LGC Standards, Teddington, UK. Risedronate sodium tablets (Actonel, 5 mg) were purchased from a local pharmacy. Sample and standard solution for the risedronate sodium assay and the risedronate sodium tablets assay were prepared according to USP instructions, as shown in Tables 1 and 2.

Table 1. Risedronate sodium assay, standard and samples.

Standard and sample solutions	
USP risedronate related Compound A stock solution	1 mg/mL risedronate related Compound A in mobile phase
USP risedronate standard solution	1 mg/mL anhydrous risedronate sodium + 0.1 mg/mL risedronate related Compound A
USP sample solution	1 mg/mL anhydrous risedronate sodium

Table 2. Risedronate sodium tablets assay, standard and samples solutions.

Standard and sample solutions	
Standard preparation	0.1 mg/mL anhydrous risedronate sodium
System suitability preparation	0.15 mg/mL anhydrous risedronate sodium + 7.5 µg/mL risedronate related Compound C
Assay preparation	0.1 mg/mL risedronate sodium from risedronate sodium tablets

Results and Discussion

The separation of USP risedronate sodium from the risedronate related Compound A is described in the USP official monographs for the risedronate sodium assay in addition to the calculation of % risedronate sodium substance in the portion of risedronate sodium. Figure 2 displays this separation for the analysis of the system suitability preparation. Table 3 shows the requirements for resolution, tailing factor, and standard deviation together with the experimental results. The percentage of risedronate sodium in the portion of risedronate sodium was calculated using Formula 1. All USP requirements were achieved or exceeded, as shown in Table 3.

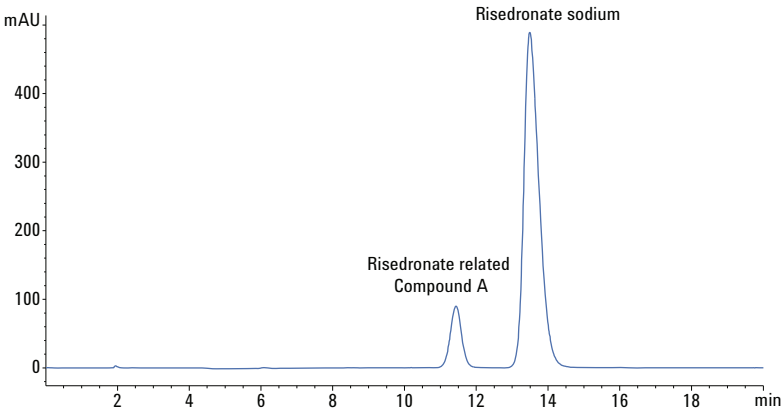


Figure 2. Test for system suitability of the risedronate sodium assay.

Formula 1

$$\%\ Risedronate\ sodium = \frac{r_u}{r_s} \times \frac{C_s}{C_u} \times 100$$

r_u = peak area from sample solution

r_s = peak area from standard solution

C_s = concentration of risedronate sodium in the standard solution (mg/mL)

C_u = concentration of risedronate sodium in the sample solution (mg/mL)

The specified amount of risedronate sodium hemi-pentahydrate in the USP risedronate sodium is 98% to 102%, calculated on the anhydrous basis. With 100.9% risedronate sodium substance, the amount meets the USP specifications.

The analysis of risedronate sodium tablets is also described in the USP monographs. The separation of USP risedronate sodium from the risedronate related Compound C is used for system suitability testing. The amount of risedronate sodium in the risedronate sodium tablets was calculated using Formula 2. Figure 3 displays a risedronate peak in the risedronate sodium tablets preparation (Actonel, 5 mg).

Table 4 shows the requirements and experimental results for system suitability including the amount of risedronate sodium found in the risedronate sodium tablets (Actonel, 5 mg). All USP requirements were achieved or exceeded.

The specified amount of risedronate sodium hemi-pentahydrate in the risedronate sodium tablets is 90% to 110%, calculated on the anhydrous basis. With 102.4% risedronate sodium substance, the amount meets the USP specifications.

Table 3. USP requirements and experimental results for the risedronate sodium assay.

Risedronate sodium assay	USP requirements	Experimental results
Resolution	≥ 2.3 between risedronate and risedronate related compound A	3.02
Tailing factor	≤ 1.6 for risedronate peak	1.39
Relative standard deviation RT	≤ 1% for risedronate peak from three replicate injections	0.15%
% Risedronate sodium	98.0%–102.0%	100.9%

Table 4. USP requirements and experimental results for the risedronate sodium tablets assay.

Risedronate sodium tablets assay	USP requirements	Experimental results
Resolution	≥ 2.5 between risedronate and risedronate related Compound C	3.11
Relative standard deviation area	≤ 2% for risedronate peak from three replicate injections	0.17%
% Risedronate sodium	90.0%–110.0%	102.4%

Formula 2

$$\% \textit{ Risedronate sodium} = \frac{r_u}{r_s} \times \frac{C_s}{C_u} \times 100$$

r_u = peak area from assay preparation

r_s = peak area from standard solution

C_s = concentration of risedronate sodium in the standard preparation (mg/mL)

C_u = concentration of risedronate sodium in the assay preparation (mg/mL)

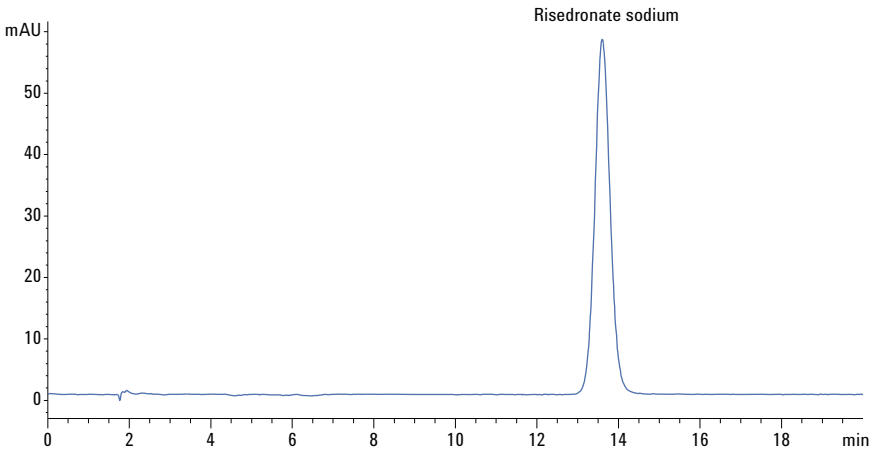


Figure 3. Analysis of risedronate sodium tablets.

Linearity was determined using a dilution series from 2 mg/mL down to 16 µg/mL risedronate sodium in mobile phase. The response factors were within the ± 5% range for all analyzed concentration levels, see Figure 4. Together with a coefficient of determination (R^2) of 1, excellent linearity is represented.

LOD and LOQ were defined as the signal-to-noise ratio of 3:1, respectively 10:1. Table 5 shows the results of the evaluation. In addition to a very high linearity, low LOD and LOQs of 0.254 µg/mL and 0.849 µg/mL, respectively, were achieved.

Assay precision and accuracy was determined by the threefold preparation of risedronate sodium substance at amounts of 0.08, 0.1, 0.12 and 0.8, 1, 1.2 mg/mL, representing the amounts used for the risedronate sodium and risedronate sodium tablets assay ± 20%. The percentage of risedronate sodium in the assays was calculated using Formulas 1 and 2. The assay precision for the concentration at approximately 0.1 mg/mL, ranged form 0.7% to 3.1%, and the accuracy was between 92.5% and 98.5% risedronate. The assay precision for the concentration at approximately 1 mg/mL, ranged from 0.6% to 1.9%, and the accuracy was between 93.9% and 99.3% risedronate. Table 6 displays the detailed results for assay precision, accuracy, and % of risedronate sodium. The percentage of risedronate in all concentrations was between 94.3% and 101.4%.

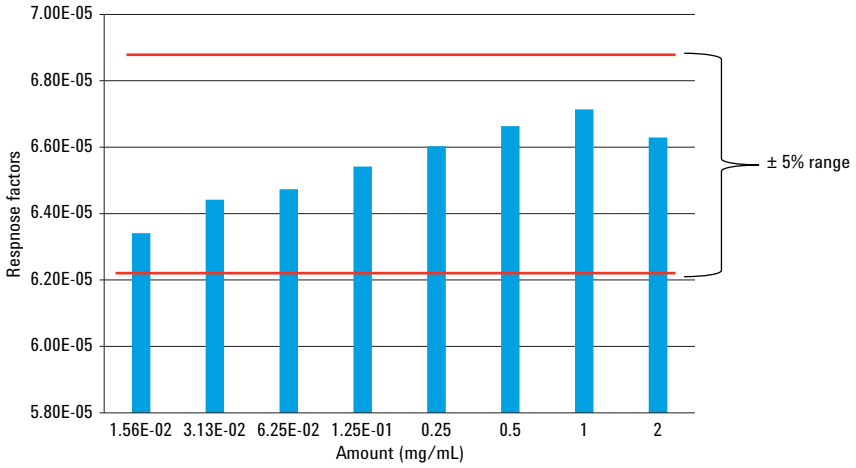


Figure 4. Linearity for risedronate sodium concentration from 2 mg/mL–16 µg/mL.

Table 5. Linearity, LOD and LOQ of the risedronate assays.

Range	R^2	LOD (µg/mL)	LOQ (µg/mL)
0.016 mg/mL–2 mg/mL	1	0.254	0.849

Table 6. Precision and accuracy for USP risedronate assays.

Concentration (mg/mL)	Peak area (AU)	% Risedronate	Calculated risedronate amount (mg/mL)	Assay precision (%RSD)	Accuracy (%)
0.08	1187	100.5	0.08	3.1	98.5
0.08	1121	94.9	0.07		93.0
0.08	1178	99.7	0.08		97.8
0.1	1418	96.0	0.09	1.6	94.2
0.1	1438	97.4	0.10		95.4
0.1	1393	94.3	0.09		92.5
0.12	1691	95.4	0.11	0.7	93.6
0.12	1680	94.8	0.11		93.0
0.12	1704	96.2	0.11		94.3
0.8	11,953	101.4	0.79	1.9	99.3
0.8	11,525	97.8	0.77		95.7
0.8	11,848	100.5	0.79		98.4
1	14,329	97.2	0.95	1.3	95.2
1	14,505	98.4	0.96		96.4
1	14,138	95.9	0.94		93.9
1.2	17,274	97.7	1.15	0.6	95.7
1.2	17,067	96.5	1.13		94.5
1.2	17,234	97.5	1.15		95.4

Conclusion

This Application Notes shows the analysis of the USP Risedronate sodium substance and of Risedronate Sodium tablets (Actonel, 5 mg tablets) according to USP specifications using the Agilent 1260 Infinity Bio-inert Quaternary LC System. System suitability for both assays met or exceeded the USP requirements. The percentage of risedronate sodium in both the drug substance and the risedronate sodium tablets was 100.9% and 102.4%, respectively, which was found to be within the USP requirements range. The assays showed high linearity with $R^2 = 1$ with low LODs and LOQs and the assay precision and accuracy was in the ± 10 % range.

The 1260 Infinity Bio-inert Quaternary LC System is ideally suited for the analysis of phosphorylated compounds such as risedronate sodium to ensure optimal analysis conditions such as peak shape, linearity, high precision, and accuracy.

References

1. J. DeRuiter and R. Clark, Bisphosphonates: Calcium Antiresorptive Agents, Endocrine Module, **2002**.
2. H.M. Jeong *et al.*, "Risedronate increases osteoblastic differentiation and function through connexin 43", *Biochemical and Biophysical Research Communications*, **2013**.
3. Shi *et al.*, "Novel direct detection method for quantitative determination of intracellular nucleoside triphosphates using weak anion exchange liquid chromatography/ tandem mass spectrometry", *Rapid Communications in Mass Spectrometry*, 16: 1092-1099, **2002**.
4. Nagaoka *et al.*, "Adduct Formation at C-8 of Guanine on in vitro Reaction of the Ultimate Form of 2-Amino-l-methyl-6-phenylimidazo[4,5-b]pyridine with 2-Deoxyguanosine and Its Phosphate Esters", *Japanese Journal of Cancer Research*, 83: 1025-1029, **1992**.
5. Analysis of phosphate compounds with the Agilent 1260 Infinity Bio-inert Quaternary LC System, Agilent Application Note, Publication Number 5991-0025EN, **2012**.
6. U.S. Pharmacopeia Monographs 35 for Risedronate: 4568- 4571, **2012**.

www.agilent.com/chem

Published in the USA, May 1, 2016
5991-2404EN

Agilent Technologies