

BRIEFING

Zoledronic Acid. A new USP Pending Monograph is being proposed based on validated methods of analysis. The HPLC procedures in the test for *Assay* and for *Limit of Phosphate and Phosphite* are based on analyses performed with Mitsubishi Kasei MCI Gel SCA04 Polymer Anion brand of L31 column. The typical retention times for the zoledronic acid peak is 8–9 min. The HPLC procedure in the test for *Limit of Imidazole and Zoledronic Related Compound A* is based on analysis performed with Inertsil Phenyl brand of L11 column. The typical retention time for zoledronic acid is 6 min.

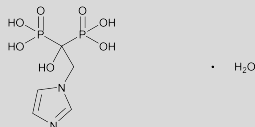
Description and Solubility: White crystalline powder. Very soluble in 0.1 N sodium hydroxide solution; sparingly soluble in water and in 0.1 N hydrochloric acid; practically insoluble in organic solvents.

(SM3: L. Santos, E. Gonikberg.) RTS—C85761

Add the following:

►Zoledronic Acid

Draft 1



$C_5H_{10}N_2O_7P_2 \cdot H_2O$ 290.10
Phosphonic acid, [1-hydroxy-2-(1H-imidazol-1-yl)ethylidene]bis-, monohydrate;
(1-Hydroxy-2-imidazol-1-ylethylidene)diphosphonic acid, monohydrate [165800-06-6].
Anhydrous 272.09

DEFINITION

Zoledronic Acid contains NLT 98.0% and NMT 102.0% of $C_5H_{10}N_2O_7P_2$, calculated on the anhydrous basis.

IDENTIFICATION

- **A. INFRARED ABSORPTION** (197K)
- **B.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **PROCEDURE**

Mobile phase: Add 0.47 mL of formic acid to 2.5 L of water, and adjust with 2 N sodium hydroxide to a pH of 3.5.

Standard solution: 1 mg/mL of USP Zoledronic Acid RS in *Mobile phase*

Sample solution: 1 mg/mL of Zoledronic Acid in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 15-cm; 5-μm packing L31

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 20 μL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Column efficiency: NLT 1000 theoretical plates

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of $C_5H_{10}N_2O_7P_2$ in the portion of Zoledronic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of Zoledronic Acid from the *Sample solution*

r_S = peak response of zoledronic acid from the *Standard solution*

C_S = concentration of USP Zoledronic Acid RS in the *Standard solution* (mg/mL)

C_U = concentration of Zoledronic Acid in the *Sample solution* (mg/mL)

Acceptance criteria: 98.0%–102.0% on the anhydrous basis

IMPURITIES

- **HEAVY METALS, Method III (231):** NMT 10 ppm

• **LIMIT OF IMIDAZOLE AND ZOLEDRONIC RELATED COMPOUND A**

Buffer: Mix 2 mL of triethylamine and 800 mL of water in a 1-L volumetric flask. Adjust with phosphoric acid to a pH of 3.0, and dilute with water to volume.

Mobile phase: Methanol and *Buffer* (1:20)

System suitability solution: 3 μg/mL of USP Imidazole RS, 3 μg/mL of USP Zoledronic Related Compound A RS, and 2 mg/mL USP Zoledronic Acid RS in *Mobile phase*

Standard solution: 3 μg/mL of USP Imidazole RS and 3 μg/mL of USP Zoledronic Related Compound A RS in *Mobile phase*

Sample solution: 2 mg/mL of Zoledronic Acid in *Mobile phase*

Chromatographic system

(See *Chromatography* (621), *System Suitability*.)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 25-cm; 5-μm packing L11

Flow rate: 0.8 mL/min

Injection size: 10 μL

Run time: At least 7 times the retention time of zoledronic acid

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 2.0 between imidazole and zoledronic related compound A peaks and NLT 2.0 between zoledronic related compound A and zoledronic acid peaks, *System suitability solution*

Tailing factor: NMT 2.0 for zoledronic acid peak, *System suitability solution*

Relative standard deviation: NMT 5.0% for each of imidazole and zoledronic related compound A peaks, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of imidazole or zoledronic related compound A in the portion of Zoledronic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of imidazole or zoledronic related compound A from the *Sample solution*

r_S = peak response of imidazole or zoledronic related compound A from the *Standard solution*

C_S = concentration of USP Imidazole RS or USP Zoledronic Related Compound A RS in the *Standard solution* (mg/mL)

2 / Zoledronic Acid

C_U = concentration of Zoledronic Acid in the *Sample solution* (mg/mL)
Calculate the percentage of any unspecified impurity in the portion of Zoledronic Acid taken:

$$\text{Result} = (r_U/r_T) \times 100$$

r_U = peak response of any unspecified impurity from the *Sample solution*

r_T = sum of all peak responses from the *Sample solution*

Acceptance criteria

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Imidazole ^a	0.5	0.15
Zoledronic related compound A ^b	0.8	0.15
Zoledronic acid	1.0	—
Any unspecified impurity	—	0.10
Total impurities	—	0.50

^a 1*H*-Imidazole.

^b 2-(1*H*-Imidazol-1-yl)acetic acid.

• **LIMIT OF PHOSPHATE AND PHOSPHITE**

Mobile phase: Add 0.47 mL of formic acid to 2.5 L of water, and adjust with 2 N sodium hydroxide to a pH of 3.5.

Impurity stock solution A: 0.65 mg/mL of phosphoric acid in *Mobile phase*

Impurity stock solution B: 0.50 mg/mL of phosphorous acid in *Mobile phase*

System suitability solution: 6.5 µg/mL of phosphoric acid from *Impurity stock solution A*, 5.0 µg/mL of phosphorous acid from *Impurity stock solution B*, and 1 mg/mL of USP Zoledronic Acid RS in *Mobile phase*

Standard solution: 6.5 µg/mL of phosphoric acid from *Impurity stock solution A* and 5.0 µg/mL of phosphorous acid from *Impurity stock solution B* in *Mobile phase*

Sample solution: 1 mg/mL of Zoledronic Acid in *Mobile phase*

Hydrogen ion identification solution: Transfer 1.0 mL of 1 N hydrochloric acid VS to a 100-mL volumetric flask, and dilute with *Mobile phase* to volume.

Chromatographic system

(See *Chromatography* <621>, *System Suitability*.)

Mode: LC

Detector: Refractive index

Column: 4.6-mm × 15-cm; 5-µm packing L31

Column temperature: 35°

Flow rate: 1 mL/min

Injection size: 100 µL

Run time: 4 times the retention time of zoledronic acid

System suitability

Samples: *System suitability solution* and *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between zoledronic acid and phosphate peaks, *System suitability solution*

Relative standard deviation: NMT 10.0% for phosphate and phosphite peaks, *Standard solution*

Analysis

Samples: *Hydrogen ion identification solution*, *Standard solution*, and *Sample solution*

[NOTE—Disregard the peak due to the hydrogen ion, eluting at a relative retention time of about 0.35, and any peak observed in the blank.]

Calculate the percentage of phosphate (determined as phosphoric acid) and phosphite (determined as phosphorous acid) in the portion of Zoledronic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times 100$$

r_U = peak response of phosphate or phosphite from the *Sample solution*

r_S = peak response of phosphate or phosphite from the *Standard solution*

C_S = concentration of phosphoric or phosphorous acid in the *Standard solution* (mg/mL)

C_U = concentration of Zoledronic Acid in the *Sample solution* (mg/mL)

Acceptance criteria

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Zoledronic acid	1.0	—
Phosphate	1.4	0.25 ^a
Phosphite	2.0	0.25 ^b
Total impurities	—	0.50

^a Determined as phosphoric acid.

^b Determined as phosphorous acid.

SPECIFIC TESTS

- **PH** <791>: 2.0–3.0, in a 2.5-mg/mL solution
- **WATER DETERMINATION, Method I** <921>: 5.5%–7.5%. Use a mixture of formamide and acetic acid (1:1) as the solvent.

ADDITIONAL REQUIREMENTS

- **PACKAGING AND STORAGE:** Preserve in well-closed containers. Store at controlled room temperature.

• **USP REFERENCE STANDARDS** <11>

USP Imidazole RS

USP Zoledronic Acid RS

(1-Hydroxy-2-imidazol-1-ylethylidene)diphosphonic acid, monohydrate.

C₅H₁₀N₂O₇P₂ · H₂O 290.10

USP Zoledronic Related Compound A RS

2-(1*H*-Imidazol-1-yl)acetic acid.

C₅H₆N₂O₂ 126.11 ◀(1-Mar-2011)