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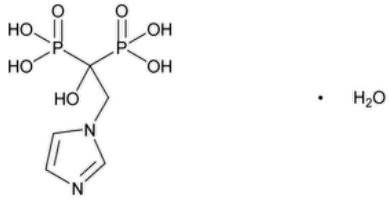
Zoledronic Acid



$C_5H_{10}N_2O_7P_2 \cdot H_2O$ 290.10

Phosphonic acid, [1-hydroxy-2-(1*H*-imidazol-1-yl)ethylidene]bis-, monohydrate;

(1-Hydroxy-2-imidazol-1-ylethylidene)diphosphonic acid, monohydrate CAS RN®: 165800-06-6.

Anhydrous CAS RN®: 118072-93-8. 272.09

DEFINITION

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

IDENTIFICATION

- **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** 197K or 197A
- **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**

Perchloric acid solution: Carefully add 10 mL of perchloric acid to 1 L of water, then add 2 mL of phosphoric acid, and mix.

Buffer: 0.037 g/L of edetate disodium and 10.8 g/L of octanesulfonic acid sodium salt in *Perchloric acid solution*

Mobile phase: Acetonitrile and *Buffer* (4:96)

System suitability solution: 2 µg/mL each of USP Imidazole RS, USP Zoledronic Acid Related Compound A RS, and sodium nitrate; 5 µg/mL of USP Zoledronic Acid Related Compound B RS; and 1.2 mg/mL of USP Zoledronic Acid RS in *Mobile phase*

Standard solution: 1 mg/mL of USP Zoledronic Acid RS in *Mobile phase*. Sonicate with occasional shaking for about 15 min to achieve complete dissolution.

Sample solution: 1 mg/mL of Zoledronic Acid in *Mobile phase*. Sonicate with occasional shaking for about 15 min to achieve complete dissolution.

Chromatographic system

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

[Note—Plastic HPLC vials are recommended.]

Preconditioning of the instrument: Rinse the instrument without a column with a mixture of acetic acid and water (25:75) at 5 mL/min for 20 min. Then, rinse with water at 5 mL/min for about 2 h.

Preconditioning of the column: Rinse with *Mobile phase* at 0.6 mL/min for about 1 h. During the rinsing, inject the *Sample solution* 10–15 times, applying a run time of about 3 min for each injection.

Mode: LC

Detector: UV 215 nm

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

Flow rate: 0.6 mL/min

Injection volume: 5 µL

Run time: NLT 3 times the retention time of zoledronic acid

System suitability

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

Suitability requirements

Resolution: NLT 1.0 between the nitrate and zoledronic acid related compound B peaks, *System suitability solution*

Tailing factor: NMT 1.4 for the zoledronic acid peak, *System suitability solution*

Relative standard deviation: NMT 0.73%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of zoledronic acid ($C_5H_{10}N_2O_7P_2$) 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

Change to read:

• **Organic Impurities**

Perchloric acid solution, Buffer, Mobile phase, and **System suitability solution:** Prepare as directed in the *Assay*.

Standard stock solution: 0.4 mg/mL of USP Zoledronic Acid RS in *Mobile phase*. Sonicate with occasional shaking for about 15 min to achieve complete dissolution.

Sensitivity solution: 1 µg/mL of USP Zoledronic Acid RS in *Mobile phase* from *Standard stock solution*

Standard solution: 0.002 mg/mL of USP Zoledronic Acid RS in *Mobile phase* from *Standard stock solution*

Sample solution: 2 mg/mL of Zoledronic Acid in *Mobile phase*. Sonicate with occasional shaking for about 30 min to achieve complete dissolution.

Chromatographic system: Proceed as directed in the *Assay*, except for *Injection volume* and *Run time*.

Injection volume: 10 µL

Run time: NLT 5 times the retention time of zoledronic acid

System suitability

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

Suitability requirements

Resolution: NLT 1.0 between the nitrate and zoledronic acid related compound B peaks, *System suitability solution*

Tailing factor: NMT 1.4 for the zoledronic acid peak, *Standard solution*

Relative standard deviation: NMT 5.0%, *Standard solution*

Signal-to-noise ratio: NLT 10, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of any individual impurity in the portion of Zoledronic Acid taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (1/F) \times 100$$

r_U = peak response of ▲any individual impurity▲ (ERR 1-Mar-2022) 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)

F = relative response factor (see *Table 1*)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria NMT (%)
Sodium nitrate[a]	0.6	—	—
Zoledronic acid related compound B	0.7	0.53	0.5
Zoledronic acid	1.0	—	—
Zoledronic acid related compound A	3.1	3.0	0.15
Imidazole	4.4	4.4	0.15
Any unspecified impurity	—	1.0	0.10
Total impurities	—	—	0.8

[a] For peak identification only.

• **Limit of Phosphate and Phosphite (if present)**

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.

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

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

System suitability solution: 13 µg/mL of phosphoric acid from *Phosphoric acid stock solution*, 10 µg/mL of phosphorous acid from *Phosphorus acid stock solution*, and 1 mg/mL of USP Zoledronic Acid RS in *Mobile phase*

Standard solution: 0.013 mg/mL of phosphoric acid from *Phosphoric acid stock solution* and 10 µg/mL of phosphorous acid from *Phosphorus acid stock solution*

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

Chromatographic system

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

Mode: LC

Detector: Refractive index

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

Temperatures

Column: 35°

Detector: 35°

Flow rate: 1 mL/min

Injection volume: 100 µL

Run time: NLT 4 times the retention time of zoledronic acid

System suitability

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

Suitability requirements

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

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

Analysis

Samples: *Mobile phase*, *Standard solution*, and *Sample solution*. [Note—Disregard any peak observed in the blank.]

Calculate the percentage of phosphate (determined as phosphoric acid) and phosphite (determined as phosphorus 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 acid or phosphorus acid in the *Standard solution* (mg/mL)

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

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria NMT (%)
Zoledronic acid	1.0	—
Phosphate[a]	1.4	0.50
Phosphite[b]	2.0	0.50

[a] Determined as phosphoric acid.

[b] Determined as phosphorus acid.

SPECIFIC TESTS

• **Bacterial Endotoxins Test ⟨85⟩:** Where the label states that Zoledronic Acid must be subjected to further processing during the preparation of injectable dosage forms, the levels of bacterial endotoxins are such that the requirement under the relevant dosage form monograph(s) in which Zoledronic Acid is used can be met.

• **Microbial Enumeration Tests ⟨61⟩** and **Tests for Specified Microorganisms ⟨62⟩:** The total aerobic microbial count does not exceed 10^2 cfu/g, and the total combined molds and yeasts count does not exceed 100 cfu/g.

• **Water Determination ⟨921⟩, *Method I, Method Ia*:** For the monohydrate form, 5.0%–7.5%. For the anhydrous form, NMT 0.5%. [Note—Hydranyl solvent and Hydranal titrant 5 are suitable.]

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in well-closed containers. Store at room temperature.

• **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms to ensure acceptable levels of bacterial endotoxins, it is so labeled.

• **USP Reference Standards ⟨11⟩**

USP Imidazole RS

1*H*-Imidazole.

$C_3H_4N_2$ 68.08

USP Zoledronic Acid RS

USP Zoledronic Acid Related Compound A RS

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

$C_5H_6N_2O_2$ 126.12

USP Zoledronic Acid Related Compound B RS

1-Hydroxy-2-[1-(2-hydroxy-2,2-diphosphonoethyl)-1*H*-imidazol-3-ium-3-yl]-1-phosphonoethylphosphonate.

$C_7H_{16}N_2O_{14}P_4$ 476.10

Auxiliary Information - Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
ZOLEDRONIC ACID	Documentary Standards Support	SM32020 Small Molecules 3
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM32020 Small Molecules 3

Chromatographic Database Information: Chromatographic Database

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