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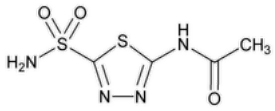
Acetazolamide

Change to read:



$C_4H_6N_4O_3S_2$ ▲222.24▲ (USP 1-May-2024)

Acetamide, *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]-;

N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide CAS RN®: 59-66-5; UNII: O3FX965V0I.

DEFINITION

Acetazolamide contains NLT 98.0% and NMT 102.0% of acetazolamide ($C_4H_6N_4O_3S_2$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*:** ▲197A or▲ (USP 1-May-2024) 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

Change to read:

• **Procedure**

Mobile phase: Dissolve 4.1 g of anhydrous sodium acetate in 950 mL of water. Add 20 mL of methanol and 30 mL of acetonitrile, and mix. Adjust with glacial acetic acid to a pH of 4.0.

Standard solution: 0.1 mg/mL of USP Acetazolamide RS prepared as follows. Transfer an appropriate amount of USP Acetazolamide RS into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the flask volume, and dilute with water to volume.

Sample solution: 0.1 mg/mL of Acetazolamide prepared as follows. Transfer an appropriate amount of Acetazolamide into a suitable volumetric flask, add 0.5 N sodium hydroxide equivalent to 10% of the flask volume, and dilute with water to volume.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 25-cm; ▲5-µm▲ (USP 1-May-2024) packing L1

Flow rate: 2 mL/min

Injection volume: 20 µL

▲Run time: NLT 1.5 times the retention time of acetazolamide▲ (USP 1-May-2024)

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 1.5

Relative standard deviation: NMT 0.73%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Acetazolamide taken:

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

r_U = peak response of acetazolamide from the *Sample solution*

r_S = peak response of acetazolamide from the *Standard solution*

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

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

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

IMPURITIES

• **Residue on Ignition ⟨281⟩:** NMT 0.1%

• **Chloride and Sulfate ⟨221⟩, *Chloride***

Sample solution: Digest 1.5 g with 75 mL of water at about 70° for 5 min. Cool to room temperature, and filter.

Acceptance criteria: A 25-mL portion of the filtrate shows no more chloride than corresponds to 0.10 mL of 0.020 N hydrochloric acid (0.014%).

• **Chloride and Sulfate ⟨221⟩, *Sulfate***

Sample solution: A 25-mL portion of the filtrate prepared in the test for *Chloride and Sulfate, Chloride*

Acceptance criteria: It shows no more sulfate than corresponds to 0.20 mL of 0.020 N sulfuric acid (0.04%).

Change to read:

• **Organic Impurities**

Solution A: 13.61 g/L of monobasic potassium phosphate in water

Mobile phase: Methanol and *Solution A* (10:90)

Standard stock solution: 0.4 mg/mL of USP Acetazolamide RS in *Mobile phase* prepared as follows. Transfer an appropriate amount of USP Acetazolamide RS to a suitable volumetric flask. Add 20% of the flask volume of methanol, and sonicate for 15 min with occasional shaking to dissolve. Dilute with *Mobile phase* to volume.

Standard solution: 0.005 mg/mL of USP Acetazolamide RS from *Standard stock solution* in *Mobile phase*

Sensitivity solution: 0.5 µg/mL ▲▲ (USP 1-May-2024) of USP Acetazolamide RS from *Standard solution* in *Mobile phase*

Sample solution: 1 mg/mL of Acetazolamide in *Mobile phase* prepared as follows. Transfer an appropriate amount of Acetazolamide to a suitable volumetric flask. Add 20% of the flask volume of methanol, and sonicate for 15 min with occasional shaking to dissolve. Dilute with *Mobile phase* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 254 nm

Column: 4.6-mm × 15-cm; 3.5-µm packing L10

Column temperature: 30°

Flow rate: 1.0 mL/min

Injection volume: 30 µL

Run time: NLT 9.5 times the retention time of acetazolamide

System suitability

Samples: *Standard solution* and *Sensitivity solution*

▲[Note—The relative retention times in *Table 1* are provided as information that could aid in peak assignment.]

Table 1▲ (USP 1-May-2024)

Name	Relative Retention Time
▲Acetazolamide related compound D▲ (USP 1-May-2024) [a]	0.45
▲Acetazolamide related compound E▲ (USP 1-May-2024) [b]	0.54
Acetamidothiadiazole[c]	0.82
Acetazolamide	1.0
Mercaptothiadiazole analog[d]	1.6
Chlorothiadiazole analog[e]	1.9
Acetazolamide dimer[f]	6.3

[a] 5-Amino-1,3,4-thiadiazole-2-sulfonamide.

[b] 5-Acetamido-1,3,4-thiadiazole-2-sulfonic acid.

[c] *N*-(1,3,4-Thiadiazol-2-yl)acetamide.

[d] *N*-(5-Mercapto-1,3,4-thiadiazol-2-yl)acetamide.

[e] *N*-(5-Chloro-1,3,4-thiadiazol-2-yl)acetamide.

[f] *N,N'*-{5,5'-[(Hydrosulfonylamino)sulfonyl]bis(1,3,4-thiadiazole-5,2-diyl)}diacetamide.

Suitability requirements

Tailing factor: NMT 2.0, *Standard solution*

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

Signal-to-noise ratio: NLT 14, *Sensitivity solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each impurity in the portion of Acetazolamide taken:

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

r_U = peak area of each impurity from the *Sample solution*

r_S = peak area of acetazolamide from the *Standard solution*

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

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

F = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*.▲The reporting threshold is 0.05%.▲ (USP 1-May-2024)

Table 2

Name	Relative Response Factor	Acceptance Criteria, NMT (%)
▲Acetazolamide related compound D▲ (USP 1-May-2024)	0.48	▲0.15▲ (USP 1-May-2024)
▲Acetazolamide related compound E▲ (USP 1-May-2024)	1.0	▲0.15▲ (USP 1-May-2024)
Acetamidothiadiazole	1.5	▲0.15▲ (USP 1-May-2024)
▲▲ (USP 1-May-2024)	▲▲ (USP 1-May-2024)	▲▲ (USP 1-May-2024)
Mercaptothiadiazole analog	0.46	▲0.15▲ (USP 1-May-2024)
Chlorothiadiazole analog	1.4	▲0.15▲ (USP 1-May-2024)
Acetazolamide dimer	1.0	▲0.15▲ (USP 1-May-2024)
Any unspecified impurity	1.0	▲0.10▲ (USP 1-May-2024)
Total impurities	—	1.0

SPECIFIC TESTS

• **Water Determination ⟨921⟩, *Method I*:** NMT 0.5%

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers. Store at room temperature.

• **USP Reference Standards ⟨11⟩**

USP Acetazolamide RS

Topic/Question	Contact	Expert Committee
ACETAZOLAMIDE	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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