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Acetazolamide Extended-Release Capsules

To view the Notice from the Expert Committee that posted in conjunction with this accelerated revision, please click www.uspnf.com/rb-acetazolamide-erc-20240628.

DEFINITION
Acetazolamide Extended-Release Capsules contain NLT 90.0% and NMT 110.0% of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$).

IDENTIFICATION
- **A.** The UV spectrum of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **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**

Buffer: 1.38 g/L of sodium phosphate monobasic, prepared as follows. Transfer a suitable quantity of sodium phosphate monobasic to an appropriate volumetric flask. Dissolve in 95% of the flask volume of water. Adjust with 2 M phosphoric acid to a pH of 3.0 and dilute with water to volume.

Mobile phase: Acetonitrile and *Buffer* (10:90)

Diluent: Methanol and *Buffer* (90:10)

Standard stock solution: 0.4 mg/mL of USP Acetazolamide RS in *Diluent*. Sonicate to dissolve.

Standard solution: 0.02 mg/mL of USP Acetazolamide RS in *Mobile Phase*, from the *Standard stock solution*

Sample stock solution: Nominally 0.4 mg/mL of acetazolamide, prepared as follows. Transfer a portion of the contents from NLT 10 Capsules to a suitable volumetric flask. Add about 60% of the flask volume of *Diluent*. Sonicate for NLT 30 min with intermittent shaking and maintain at room temperature. Dilute with *Diluent* to volume. Pass the solution through a suitable filter.

Sample solution: Nominally 0.02 mg/mL of acetazolamide in *Mobile phase*, from the *Sample stock solution*

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

Mode: LC

Detector: UV 265 nm. For *Identification A*, use a diode array detector in the range of 200–400 nm.

Column: 4.6-mm × 15-cm; 5-µm packing L1

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 1.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) in the portion of Capsules 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 = nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

Change to read:

• **Dissolution** ⟨711⟩

Test 1

Medium: Acetate buffer, pH 4.5 with 2.2% polysorbate 20,[1] prepared as follows. Dissolve 3.0 g of ▲sodium acetate▲ (RB 1-Jul-2024) in 900 mL of water. Add 22 mL of polysorbate 20, and adjust with glacial acetic acid to a pH of 4.5. Dilute with water to 1000 mL; 900 mL.

Apparatus 2: 75 rpm

Times: 0.5, 2, 5, and 12 h

Standard stock solution: 0.28 mg/mL of USP Acetazolamide RS prepared as follows. Transfer USP Acetazolamide RS to a suitable volumetric flask. Add 10% of the flask volume of methanol, and sonicate to dissolve. Dilute with *Medium* to volume.

Standard solution: 0.017 mg/mL of USP Acetazolamide RS in *Medium*, from the *Standard stock solution*

Sample solution: Pass a portion of the solution under test through a suitable filter. Dilute the filtrate with *Medium*, if necessary. Replace the amount of solution withdrawn at each time point with the same volume of *Medium*.

Blank: *Medium*

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 265 nm

Cell: 1.0 cm

System suitability

Sample: *Standard solution*

Suitability requirements

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of acetazolamide ($C_4H_6N_4O_3S_2$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (A_U/A_S) \times C_S \times D$$

A_U = absorbance of the *Sample solution*

A_S = absorbance of the *Standard solution*

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

D = dilution factor of the *Sample solution*, if necessary

Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{[(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of acetazolamide in the portion of the sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 1*.

Table 1

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	0.5	NMT 25

Time Point (*i*)	Time (h)	Amount Dissolved (%)
2	2	40–65
3	5	70–90
4	12	NLT 85

The percentages of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: Acetate buffer, pH 4.5 with 2.2% polysorbate 20, prepared as follows. Weigh and transfer 2.99 g of sodium acetate into a 1000-mL volumetric flask. Add 14.0 mL of 2 N acetic acid and dilute with water to volume. The measured pH should be 4.5. Transfer the resulted solution to a suitable container and add about 22.0 mL of polysorbate 20. Shake vigorously and further sonicate for about 10–15 min; 900 mL.

Apparatus 2: 75 rpm

Times: 1, 2, and 7 h

Standard stock solution: 0.22 mg/mL of USP Acetazolamide RS prepared as follows. Transfer a suitable amount of USP Acetazolamide RS to a suitable volumetric flask. Add 5% of the flask volume of methanol, and sonicate to dissolve if necessary. Dilute with *Medium* to volume.

Standard solution: 0.022 mg/mL of USP Acetazolamide RS from the *Standard stock solution* in *Medium*. [NOTE—The *Standard solution* may be stable for 22 h at room temperature.]

Sample solution: At the specified time points, withdraw a suitable volume of the solution under test. Pass through a suitable filter of 0.45-µm pore size, discarding an appropriate volume of filtrate so that a consistent result can be obtained. Dilute 1.0 mL of the filtrate with *Medium* to 25 mL. Replace the amount of solution withdrawn at each time point with the same volume of *Medium*. [NOTE—The *Sample solution* may be stable for 22 h at room temperature.]

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 265 nm

Cell: 0.5 cm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of acetazolamide ($C_4H_6N_4O_3S_2$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (A_U/A_S) \times C_S \times D$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from the *Standard solution*

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

D = dilution factor of the *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of acetazolamide in the portion of the sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 2*.

Table 2

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	22–42
2	2	40–60
3	7	NLT 80

The percentages of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Test 3: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 3*.

Medium: Acetate buffer, pH 4.5 with 2.2% polysorbate 20, prepared as follows. Dissolve 3 g of sodium acetate in 1000 mL of water. Adjust with acetic acid to a pH of 4.5. Add 22 g of polysorbate 20 and stir to dissolve; 900 mL.

Apparatus 2: 75 rpm

Times: 1, 2, 5, and 12 h

Standard stock solution: 0.55 mg/mL of USP Acetazolamide RS in methanol. Sonicate to dissolve, if necessary.

Standard solution: 0.011 mg/mL of USP Acetazolamide RS from the *Standard stock solution* in *Medium*

Sample solution: At the specified time points, withdraw a suitable volume of the solution under test. Pass through a suitable filter of 0.45-µm pore size, discarding an appropriate volume of filtrate so that a consistent result can be obtained. Dilute 2.0 mL of the filtrate with *Medium* to 100 mL. Replace the amount of solution withdrawn at each time point with the same volume of *Medium*.

Instrumental conditions

(See *Ultraviolet-Visible Spectroscopy* ⟨857⟩.)

Mode: UV

Analytical wavelength: 265 nm

Blank: *Medium*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of acetazolamide ($C_4H_6N_4O_3S_2$) in the sample withdrawn from the vessel at each time point (*i*):

$$\text{Result} = (A_U/A_S) \times C_S \times D$$

A_U = absorbance from the *Sample solution*

A_S = absorbance from the *Standard solution*

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

D = dilution factor of the *Sample solution*

Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of acetazolamide in the portion of the sample withdrawn at time point *i* (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

Tolerances: See *Table 3*.

Table 3

Time Point (*i*)	Time (h)	Amount Dissolved (%)
1	1	20-45
2	2	35–60
3	5	65-90
4	12	NLT 85

The percentages of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

▲**Test 4:** If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 4*.

Medium: Acetate buffer, pH 4.5 with 2.2% (w/v) polysorbate 20 (Dissolve 2.99 g of sodium acetate in 800 mL of water. Adjust with glacial acetic acid to a pH of 4.5. Add 22 g of polysorbate 20 and dilute with water to 1000 mL.); 900 mL

Apparatus 2: 75 rpm with sinker[2]

Times: 1, 2, 5, and 12 h

Buffer: Dissolve 4.11 g of sodium acetate, anhydrous in 1000 mL of water. Adjust with glacial acetic acid to a pH of 4.0.

Mobile phase: Methanol and *Buffer* (25:75)

Standard solution: (*L*/900) mg/mL of USP Acetazolamide RS, where *L* is the label claim in mg/Caspule, prepared as follows. Transfer a quantity of USP Acetazolamide RS to an appropriate volumetric flask and dissolve in 5% of the flask volume of methanol. Sonicate to dissolve, if necessary. Allow to cool and add 60%–70% of the flask volume of *Medium*. Sonicate to dissolve, if necessary. Dilute with *Medium* to volume.

Sample solution: At the specified time points, withdraw a suitable volume of the solution under test. Pass through a suitable filter of 0.45-µm pore size, discarding an appropriate volume of filtrate so that a consistent result can be obtained. Replace the amount of solution withdrawn at each time point with the same volume of *Medium*.

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

Column: 4.6-mm × 15-cm; 5-µm packing L1

Flow rate: 1 mL/min

Injection volume: 5 µL

Run time: NLT 1.5 times the retention time of acetazolamide

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.0

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the concentration (C_i) of acetazolamide $C_4H_6N_4O_3S_2$ in the sample withdrawn from the vessel at each time point (*i*):

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

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)

Calculate the percentage of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at each time point (*i*):

$$\text{Result}_1 = C_1 \times V \times (1/L) \times 100$$

$$\text{Result}_2 = [(C_2 \times V) + (C_1 \times V_S)] \times (1/L) \times 100$$

$$\text{Result}_3 = \{(C_3 \times V) + [(C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

$$\text{Result}_4 = \{(C_4 \times V) + [(C_3 + C_2 + C_1) \times V_S]\} \times (1/L) \times 100$$

C_i = concentration of acetazolamide in the portion of the sample withdrawn at time point i (mg/mL)

V = volume of *Medium*, 900 mL

L = label claim (mg/Capsule)

V_S = volume of the *Sample solution* withdrawn at each time point and replaced with *Medium* (mL)

The percentages of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.

Tolerances: See *Table 4*.

Table 4

Time Point (*i*)	Time (h)	Amount Dissolved %
1	1	15–35
2	2	30–50
3	5	55–75
4	12	NLT 80

The percentages of the labeled amount of acetazolamide ($C_4H_6N_4O_3S_2$) dissolved at the times specified conform to *Dissolution* ⟨711⟩, *Acceptance Table 2*.▲ (RB 1-Jul-2024)

• **Uniformity of Dosage Units** ⟨905⟩**:** Meet the requirements

IMPURITIES

Change to read:

• **Organic Impurities**

Buffer: 6.8 g/L of potassium phosphate, monobasic in water

Mobile phase: Acetonitrile and *Buffer* (8:92)

System suitability stock solution: 0.1 mg/mL each of USP Acetazolamide Related Compound D RS and USP Acetazolamide Related Compound E RS in methanol

System suitability solution: 0.002 mg/mL each of USP Acetazolamide Related Compound D RS and USP Acetazolamide Related Compound E RS in *Mobile phase*, from the *System suitability stock solution*

Sensitivity solution: 0.5 µg/mL of USP Acetazolamide RS in *Mobile phase*

Standard stock solution: 0.1 mg/mL each of USP Acetazolamide RS and USP Acetazolamide Related Compound D RS in methanol

Standard solution: 0.002 mg/mL each of USP Acetazolamide RS and USP Acetazolamide Related Compound D RS in *Mobile phase*, from the *Standard stock solution*

Sample solution: Nominally 1.0 mg/mL of acetazolamide, prepared as follows. Transfer a portion of the contents from NLT 10 Capsules to a suitable volumetric flask. Add 10% of the flask volume of methanol, and sonicate to disperse. Add about 70% of the flask volume of *Mobile phase*, and sonicate for NLT 30 min with intermittent shaking while using cold water to maintain the temperature of the ultrasonic bath between 20° and 25°. Dilute with *Mobile phase* to volume. Pass a portion of the solution through a suitable filter.

Chromatographic system

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

Mode: LC

Detector: UV 265 nm

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

Column temperature: 30°

Flow rate: 1.2 mL/min

Injection volume: 10 µL

Run time: NLT 4 times the retention time of acetazolamide

System suitability

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

[Note—The relative retention times in ▲*Table 5*▲ (RB 1-Jul-2024) are provided as information that could aid in peak assignment.]

▲**Table 5**▲ (RB 1-Jul-2024)

Name	Relative Retention Time
Acetazolamide related compound E (free acid)	0.31
Acetazolamide related compound D	0.35
Aminothiadiazole mercaptan[a]	0.46
Acetamidothiadiazole[b]	0.58
Acetazolamide	1.00
Mercaptothiadiazole analog[c]	1.44
Chlorothiadiazole analog[d]	2.54
Acetazolamide dimer[e]	2.86

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

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

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

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

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

Suitability requirements

Resolution: NLT 2.0 between acetazolamide related compound D and acetazolamide related compound E, *System suitability solution*

Relative standard deviation: NMT 5.0% for acetazolamide and acetazolamide related compound D, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of acetazolamide related compound D in the portion of Capsules taken:

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

r_U = peak area of acetazolamide related compound D from the *Sample solution*

r_S = peak area of acetazolamide related compound D from the *Standard solution*

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

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

Calculate the percentage of acetazolamide related compound E (free acid), acetamidothiadiazole, and any unspecified degradation product in the portion of Capsules taken:

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

r_U = peak area of acetazolamide related compound E (free acid), acetamidothiadiazole, and any unspecified degradation product 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 = nominal concentration of acetazolamide in the *Sample solution* (mg/mL)

F = relative response factor (see ▲*Table 6*▲ (RB 1-Jul-2024))

Acceptance criteria: See ▲*Table 6*.▲ (RB 1-Jul-2024) The reporting threshold is 0.05%.

▲Table 6▲ (RB 1-Jul-2024)

Name	Relative Response Factor	Acceptance Criteria, NMT (%)
Acetazolamide related compound E (free acid)	0.49	0.2
Acetazolamide related compound D	—	0.2
Acetamidothiadiazole	0.46	0.2
Any unspecified degradation product	1.0	0.2
Total degradation products	—	1.5

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.
- **Labeling:** The labeling states the *Dissolution* test used only if *Test 1* is not used.
- **USP Reference Standards** ⟨11⟩

USP Acetazolamide RS

USP Acetazolamide Related Compound D RS

5-Amino-1,3,4,-thiadiazole-2-sulfonamide.

$C_2H_4N_4O_2S_2$ 180.20

USP Acetazolamide Related Compound E RS

Potassium 5-acetamido-1,3,4-thiadiazole-2-sulfonate.

$C_4H_4KN_3O_4S_2$ 261.31

[1] Tween 20 by Croda International PLC.

[2] A suitable sinker is available as catalog No. 0104A00116 from https://www.electrolabgroup.com.

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

Topic/Question	Contact	Expert Committee
ACETAZOLAMIDE EXTENDED-RELEASE CAPSULES	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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