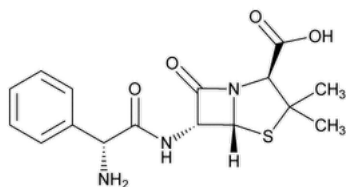


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Ampicillin



$C_{16}H_{19}N_3O_4S$ (anhydrous) 349.41
4-Thia-1-azabicyclo[3.2.0]heptane-2 carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, [2S-[2 α ,5 α ,6 β (S*)]]-;
(2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid CAS RN[®]: 69-53-4;
UNII: 7C782967RD.
Trihydrate 403.46 CAS RN[®]: 7177-48-2; UNII: HXQ6A1N7R6.

DEFINITION
Ampicillin is anhydrous or contains three molecules of water of hydration. It contains NLT 900 µg/mg and NMT 1050 µg/mg of ampicillin ($C_{16}H_{19}N_3O_4S$), calculated on the anhydrous basis.

IDENTIFICATION
• [SPECTROSCOPIC IDENTIFICATION TESTS \(197\)](#), [Infrared Spectroscopy: 197K](#): Except that where the specimen under test is the trihydrate, both it and the [USP Ampicillin Trihydrate RS](#) are undried.

ASSAY
• **PROCEDURE**
Solution A: 6.54 g/L of monobasic potassium phosphate and 0.34 g/L of dibasic potassium phosphate, adjusted with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 5.5 before final dilution
Solution B: Acetonitrile and *Solution A* (2:23)
Solution C: Acetonitrile and *Solution A* (3:7)
Mobile phase: See [Table 1](#).

Table 1

Time (min)	Solution B (%)	Solution C (%)
0	100	0
6	100	0
15	0	100
16	0	100
18	100	0
20	100	0

Solution D: 46.3 g/L of monobasic potassium phosphate and 27.8 g/L of dibasic potassium phosphate, adjusted with 1 N sodium hydroxide or 1 N phosphoric acid to a pH of 6.5 before final dilution
System suitability solution: 0.5 mg/mL of [USP Ampicillin RS](#) and 0.1 mg/mL of [USP Amoxicillin RS](#) in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume.
Standard solution: 0.5 mg/mL of [USP Ampicillin RS](#) in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume. Analyze immediately after

preparation.

Sample solution: 0.5 mg/mL of Ampicillin in acetonitrile, water, and *Solution D* (4:91:5) prepared as follows. Dissolve first in a mixture of acetonitrile, water, and *Solution D* (4:30:5), sonicating if necessary, and dilute with water to volume. Analyze immediately after preparation.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: UV 230 nm

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

Flow rate: 1.5 mL/min

Injection volume: 20 μL

System suitability

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

Suitability requirements

Resolution: NLT 10 between ampicillin and amoxicillin, *System suitability solution*

Tailing factor: NMT 1.4 for ampicillin, *System suitability solution*

Relative standard deviation: NMT 2.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in μg, of ampicillin ($C_{16}H_{19}N_3O_4S$) in each mg of Ampicillin taken:

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

r_U = peak response from the *Sample solution*

r_S = peak response from the *Standard solution*

C_S = concentration of [USP Ampicillin RS](#) in the *Standard solution* (mg/mL)

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

P = potency of [USP Ampicillin RS](#) (μg/mg)

Acceptance criteria: 900–1050 μg/mg on the anhydrous basis

IMPURITIES

• ORGANIC IMPURITIES, PROCEDURE 1

Organic Impurities, Procedure 1 is recommended when the impurity profile includes ampicillin thiazepine.

Solution A, Solution B, Solution C, Mobile phase, Solution D, System suitability solution, Sample solution, and Chromatographic system: Prepare as directed in the Assay.

Standard stock solution: Prepare as directed for the *Standard solution* in the Assay.

Standard solution: 0.005 mg/mL of ampicillin in *Solution D* and water (1:19) from *Standard stock solution*. Transfer an aliquot of the *Standard stock solution* to a suitable volumetric flask, add *Solution D*, using about 5% of the final volume, and dilute with water to volume. Analyze immediately after preparation.

Sensitivity solution: 0.5 μg/mL of ampicillin in *Solution D* and water (1:19) from the *Standard solution*. Transfer an aliquot of the *Standard solution* to a suitable volumetric flask, add *Solution D*, using about 5% of the final volume, and dilute with water to volume.

System suitability

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

Suitability requirements

Signal-to-noise ratio: NLT 3, *Sensitivity solution*

Resolution: NLT 10 between ampicillin and amoxicillin, *System suitability solution*

Tailing factor: NMT 1.4 for ampicillin, *System suitability solution*

Relative standard deviation: NMT 10.0%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

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

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

C_S = concentration of [USP Ampicillin RS](#) in the *Standard solution* (mg/mL)

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

P = potency of [USP Ampicillin RS](#) (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See [Table 2](#).

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine ^a	0.27	0.5
Amoxicillin related compound A (6-aminopenicillanic acid) ^b	0.31	0.5
Ampicilloic acid ^c	0.45	1.0
Ampicillin thiazepine analog ^d	0.65	0.3
Ampicillin	1.0	—
Ampicillin rearrangement product (isomer 1) ^e	1.8	0.4
Ampicillin rearrangement product (isomer 2) ^e	2.0	0.3
Ampicillin oligomer 2 ^f	2.2	0.6
D-Phenylglycylampicillin ^g	2.5	0.8
Ampicillin oligomer 1 (dimer) ^h	2.6	1.0
Ampicillin oligomer 1 (trimer) ⁱ	2.9	0.4
Any individual unspecified impurity	—	0.25
Total impurities	—	3.0

^a (R)-2-Amino-2-phenylacetic acid.

^b (2S,5R,6R)-6-Amino-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^c (4S)-2-[(R)-2-Amino-2-phenylacetamido](carboxy)methyl-5,5-dimethylthiazolidine-4-carboxylic acid.

^d (S)-6-[(R)-2-Amino-2-phenylacetamido]-2,2-dimethyl-7-oxo-2,3,4,7-tetrahydro-1,4-thiazepine-3-carboxylic acid.

^e (4S)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

^f (4S)-2-[1-[(R)-2-Amino-2-phenylacetamido]-2-[(1R)-2-(carboxyl(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl)methylamino]-2-oxo-1-phenylethylamino]-2-oxoethyl)-5,5-dimethylthiazolidine-4-carboxylic acid.

^g (2S,5R,6R)-6-[(R)-2-[(R)-2-Amino-2-phenylacetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

^h (2S,5R,6R)-6-[(2R)-2-[2-[(R)-2-Amino-2-phenylacetamido]-2-[(4S)-4-carboxy-5,5-dimethylthiazolidin-2-yl]acetamido]-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

ⁱ (4S,4'S)-2,2'-[(1R,7R,13R)-1-Amino-14-[(2S,5R,6R)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-penta-oxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl]bis(5,5-dimethylthiazolidine-4-carboxylic acid).

• **ORGANIC IMPURITIES, PROCEDURE 2, DIMETHYLANILINE (223):** Meets the requirements

Organic Impurities, Procedure 2 is recommended when dimethylaniline is used during the production of Ampicillin.

• **ORGANIC IMPURITIES, PROCEDURE 3**

Organic Impurities, Procedure 3 is recommended when the impurity profile includes phenylpyrazinol, pivaloyl D-phenylglycine, pivaloyl 6-aminopenicillanic acid, diphenyldiketopiperazine, and open ring dimer.

Solution A: 4 g/L of monobasic sodium phosphate dihydrate adjusted with 1 N sodium hydroxide to a pH of 5.0

Solution B: Acetonitrile

Mobile phase: See [Table 3](#).

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	98	2
20	90	10
40	85	15
50	80	20
55	75	25
60	75	25
62	98	2
70	98	2

Diluent: Acetonitrile and *Solution A* (2:98)

System suitability solution: 1.5 mg/mL of [USP Ampicillin System Suitability Mixture RS](#) in *Diluent*

Standard solution: 15 µg/mL of [USP Ampicillin RS](#) in *Diluent*

Sample solution: 1.5 mg/mL of Ampicillin in *Diluent*. Store the sample in the refrigerator, and discard after 60 min.

Chromatographic system

(See [Chromatography \(621\), System Suitability](#).)

Mode: LC

Detector: UV 220 nm

Column: 4.6-mm × 15-cm; 5-µm packing L7

Column temperature: 40°

Flow rate: 1.5 mL/min

Injection volume: 20 µL

Autosampler temperature: 4°

System suitability

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

Suitability requirements

Resolution: NLT 1.5 between the pivaloyl phenylglycine and diphenyldiketopiperazine peaks, *System suitability solution*

Tailing factor: NMT 2.0, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

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

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

C_S = concentration of [USP Ampicillin RS](#) in the *Standard solution* (mg/mL)

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

P = potency of ampicillin in [USP Ampicillin RS](#) (µg/mg)

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See [Table 4](#) and [Table 5](#). The limits in [Table 5](#) are to be used only where Ampicillin is intended for use in preparing veterinary products.

Table 4