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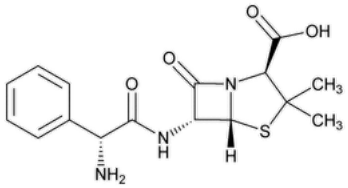
Ampicillin



$C_{16}H_{19}N_3O_4S$ (anhydrous) 349.41

4-Thia-1-azabicyclo[3.2.0]heptane-2 carboxylic acid, [6-(aminophenylacetyl)amino]-3,3-dimethyl-7-oxo-, [2*S*-[2α,5α,6β(*S**)]]-;

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

[c] (4*S*)-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] (4*S*)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

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

[g] (2*S*,5*R*,6*R*)-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] (2*S*,5*R*,6*R*)-6-[(2*R*)-2-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-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] (4*S*,4'*S*)-2,2'-{(1*R*,7*R*,13*R*)-1-Amino-14-[(2*S*,5*R*,6*R*)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-pentaoxo-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 phenylglycine, pivaloyl 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

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine[a]	0.15	1.0
Amoxicillin related compound A (6-aminopenicillanic acid)[b]	0.21	1.0
Ampicilloic acid[c,d]	0.40	1.0
	0.58	
L-Ampicillin[e]	0.65	1.0
Ampicillin	1.0	—
Ampilloic acid[f,g]	1.16	1.0
	1.40	
Ampicillin rearrangement product[h,i]	1.25	1.0
	1.48	
Phenylpyrazinol[j]	1.75	1.0
Pivaloyl phenylglycine[k]	1.87	1.0
Diphenyldiketopiperazine[l]	1.94	1.0
Ampicillin oligomer 2[m]	2.08	1.0
D-Phenylglycylampicillin[n]	2.25	1.0
Pivaloyl aminopenicillanic acid[o]	2.54	1.0
Open ring dimer[p,q]	2.87	1.0
	2.97	
	3.03	
Ampicillin oligomer 1 (dimer)[r]	3.15	1.0
Ampicillinyl-D-phenylglycine[s]	3.86	1.0
Ampicillin oligomer 1 (trimer)[t]	4.19	1.0
Any individual unspecified impurity	—	0.10
Total impurities	—	5.0

[a] (*R*)-2-Amino-2-phenylacetic acid.

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

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

[d] The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.

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

[f] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[g] The system resolves the two isomers of ampilloic acid. The sum of the two isomers is reported.

[h] (4*S*)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

[i] The system resolves the two isomers of ampicillin rearrangement product. The sum of the two isomers is reported.

[j] 3-Phenylpyrazin-2-ol.

[k] (*R*)-2-Phenyl-2-pivalamidoacetic acid.

[l] 3,6-Diphenylpiperazine-2,5-dione.

[m] (4*S*)-2-{1-[(*R*)-2-Amino-2-phenylacetamido]-2-[(1*R*)-2-{carboxy[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[n] (2*S*,5*R*,6*R*)-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.

[o] (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[p] (4*S*)-2-{1-[(*R*)-2-amino-2-phenylacetamido]-2-[(1*R*)-2-{[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[q] The system may resolve the three isomers of open ring dimer. The sum of the three isomers is reported.

[r] (2*S*,5*R*,6*R*)-6-[(2*R*)-2-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-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.

[s] (*R*)-2-((2*S*,5*R*,6*R*)-6-((*R*)-2-Amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetic acid.

[t] (4*S*,4'*S*)-2,2'-{(1*R*,7*R*,13*R*)-1-Amino-14-[(2*S*,5*R*,6*R*)-2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-6-ylamino]-2,5,8,11,14-pentaoxo-1,7,13-triphenyl-3,6,9,12-tetraazatetradecane-4,10-diyl}bis(5,5-dimethylthiazolidine-4-carboxylic acid).

Where it is intended for use in preparing veterinary products:

Table 5

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine[a]	0.15	2.0
Amoxicillin related compound A (6-aminopenicillanic acid)[b]	0.21	2.0
N-Formyl ampicilloic acid[c]	0.26	1.0
Ampicilloic acid[d,e]	0.40	2.0
	0.58	
L-Ampicillin[f]	0.65	2.0
Ampicillin	1.0	—
Ampilloic acid[g,h]	1.16	2.0
	1.40	
Ampicillin rearrangement product[i,j]	1.25	2.0
	1.48	
Phenylpyrazinol[k]	1.75	2.0
Pivaloyl phenylglycine[l]	1.87	2.0
Diphenyldiketopiperazine[m]	1.94	2.0

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Ampicillin oligomer 2[n]	2.08	2.0
D-Phenylglycylampicillin[o]	2.25	2.0
Pivaloyl aminopenicillanic acid[p]	2.54	2.0
Open ring dimer[q,r]	2.87	0.50
	2.97	0.50
	3.03	0.50
Ampicillin oligomer 1 (dimer)[s]	3.15	4.5
Ampicillinyl-D-phenylglycine[t]	3.86	2.0
Ampicillin oligomer 1 (trimer)[u]	4.19	2.0
Any individual unspecified impurity	—	0.5
Total impurities	—	5.0

[a] (*R*)-2-Amino-2-phenylacetic acid.

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

[c] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido](carboxy)methyl}-3-formyl-5,5-dimethylthiazolidine-4-carboxylic acid.

[d] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[e] The system resolves the two isomers of ampicilloic acid. The sum of the two isomers is reported.

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

[g] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[h] The system resolves the two isomers of ampilloic acid. The sum of the two isomers is reported.

[i] (4*S*)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

[j] The system resolves the two isomers of ampicillin rearrangement product. The sum of the two isomers is reported.

[k] 3-Phenylpyrazin-2-ol.

[l] (*R*)-2-Phenyl-2-pivalamidoacetic acid.

[m] 3,6-Diphenylpiperazine-2,5-dione.

[n] (4*S*)-2-{1-[(*R*)-2-Amino-2-phenylacetamido]-2-[(1*R*)-2-{carboxy[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[o] (2*S*,5*R*,6*R*)-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.

[p] (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid.

[q] (4*S*)-2-{1-[(*R*)-2-amino-2-phenylacetamido]-2-[(1*R*)-2-{[(4*S*)-4-carboxy-5,5-dimethylthiazolidin-2-yl]methylamino}-2-oxo-1-phenylethylamino]-2-oxoethyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[r] The system may resolve the three isomers of open ring dimer.

[s] (2*S*,5*R*,6*R*)-6-[(2*R*)-2-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-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.

[t] (*R*)-2-((2*S*,5*R*,6*R*)-6-((*R*)-2-Amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetic acid.

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

• **Organic Impurities, Procedure 4**

Organic Impurities, Procedure 4 is recommended when the impurity profile includes ampilloyl aminopenicillanic acid and penicillanyl ampicillinamide.

Solution A: 3.4 g/L of dibasic sodium phosphate dodecahydrate and 1.4 g/L of monobasic potassium phosphate adjusted with phosphoric acid to a pH of 5.5

Solution B: Acetonitrile

Mobile phase: See *Table 6*.

Table 6

Time (min)	Solution A (%)	Solution B (%)
0	99	1
1.5	95	5
6.5	90	10
7.5	89	11
13.5	84	16
16.5	75	25
18	60	40
25	99	1

Standard solution: 30 µg/mL of USP Amoxicillin Related Compound A RS, 30 µg/mL of D-phenylglycine, and 25 µg/mL of USP Ampicillin RS in *Solution A*

Sample solution: 2.5 mg/mL of Ampicillin in *Solution A*. Store the *Sample solution* in the refrigerator, and use within 9 h.

Chromatographic system

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

Mode: LC

Detector: UV 220 nm

Column: 4.0-mm × 15-cm; 3-µm packing L1

Column temperature: 40°

Flow rate: 1.3 mL/min

Injection volume: 5 µL

Autosampler temperature: 4°

System suitability

Sample: *Standard solution*

Suitability requirements

Resolution: NLT 1.5 between D-phenylglycine and amoxicillin related compound A

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 7*. Disregard any peak with an area less than 0.03 times the area of the ampicillin peak in the *System suitability solution*.

Table 7

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
D-Phenylglycine[a]	0.21	0.5
Amoxicillin related compound A (6-aminopenicillanic acid)[b]	0.32	0.5
Ampicilloic acid[c]	0.46	1.0
	0.57	1.0
Ampicillin thiazepine analog[d,e]	0.72	—
L-Ampicillin[f]	0.84	0.5
Ampilloyl aminopenicillanic acid[g]	0.87	0.5
Ampicillin	1.00	—
Ampilloic acid[h]	1.15	1.0
	1.34	1.0
Ampicillin rearrangement product[i]	1.24	1.0
Pivaloyl phenylglycine[e,j]	1.47	—
Phenylpyrazinol[e,k]	1.84	—
Diphenyldiketopiperazine[e,l]	1.94	—
Pivaloyl aminopenicillanic acid[e,m]	1.95	—
D-Phenylglycylampicillin[n]	2.08	1.0
Ampicillin oligomer 1 (dimer)[o]	2.16	1.0
Penicillanyl ampicillinamide[p]	2.27	1.0
Ampicillinyl-D-phenylglycine[q]	2.64	1.0
Any individual unspecified impurity	—	1.0
Total impurities	—	5.0

[a] (*R*)-2-Amino-2-phenylacetic acid.

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

[c] (4*S*)-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] These impurities are listed for information only. They are not to be reported. They are not to be included in total impurities.

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

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

[h] (4*S*)-2-{[(*R*)-2-Amino-2-phenylacetamido]methyl}-5,5-dimethylthiazolidine-4-carboxylic acid.

[i] (4*S*)-2-(3,6-Dioxo-5-phenylpiperazin-2-yl)-5,5-dimethylthiazolidine-4-carboxylic acid.

[j] (*R*)-2-Phenyl-2-pivalamidoacetic acid.

[k] 3-Phenylpyrazin-2-ol.

[l] 3,6-Diphenylpiperazine-2,5-dione.

[m] (2*S*,5*R*,6*R*)-3,3-Dimethyl-7-oxo-6-pivalamido-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid.

[n] (2*S*,5*R*,6*R*)-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.

[o] (2*S*,5*R*,6*R*)-6-[(2*R*)-2-{2-[(*R*)-2-Amino-2-phenylacetamido]-2-[(4*S*)-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.

[p] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

[q] (*R*)-2-((2*S*,5*R*,6*R*)-6-((*R*)-2-Amino-2-phenylacetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxamido)-2-phenylacetic acid.

SPECIFIC TESTS

• **Sterility Tests ⟨71⟩**

Sample solution: Dissolve 6 g in 800 mL of *Fluid D* containing sufficient sterile penicillinase to inactivate the ampicillin, and swirl the vessel until dissolution is complete before filtering.

Acceptance criteria: Where the label states that Ampicillin is sterile, it meets the requirements when tested as directed for *Test for Sterility of the Product to Be Examined, Membrane Filtration*.

• **Crystallinity ⟨695⟩:** Meets the requirements

• **pH ⟨791⟩**

Sample solution: 10 mg/mL

Acceptance criteria: 3.5–6.0

• **Water Determination, *Method I* ⟨921⟩:** NMT 2.0% where it is labeled as Ampicillin (anhydrous); between 12.0% and 15.0% where it is labeled as Ampicillin (trihydrate)

• **Bacterial Endotoxins Test ⟨85⟩:** Where the label states that Ampicillin is sterile or must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.15 USP Endotoxin Unit/mg of ampicillin.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers.

• **Labeling:** Label to indicate whether it is anhydrous or is the trihydrate. Where the quantity of ampicillin is indicated in the labeling of any preparation containing Ampicillin, this shall be understood to be in terms of anhydrous ampicillin ($C_{16}H_{19}N_3O_4S$). Where it is intended for use in preparing injectable dosage forms, the label states that it is the trihydrate and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms.

If a test for *Organic Impurities* other than *Procedures 1* and *2* is used, then the labeling states with which *Organic Impurities* test the article complies. Where it is intended for use in preparing veterinary products, the label so states.

Change to read:

• **USP Reference Standards ⟨11⟩**

USP Amoxicillin RS

USP Amoxicillin Related Compound A RS

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

▲$C_8H_{12}N_2O_3S$ 216.26▲ (ERR 1-Apr-2021)

USP Ampicillin RS

USP Ampicillin System Suitability Mixture RS

This is a mixture which contains ampicillin, pivaloyl phenylglycine [(*R*)-2-phenyl-2-pivalamidoacetic acid; $C_{13}H_{17}NO_3$; 235.28], diphenyldiketopiperazine (3,6-diphenylpiperazine-2,5-dione; $C_{16}H_{14}N_2O_2$; 266.29), and other related compounds.

USP Ampicillin Trihydrate RS

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

Topic/Question	Contact	Expert Committee
AMPICILLIN	Documentary Standards Support	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

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