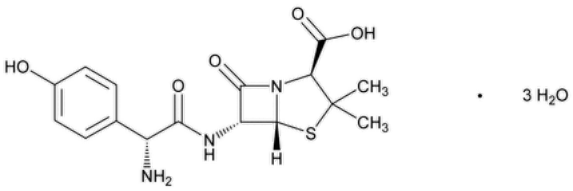
Status: Currently Official on 17-Feb-2025
Official Date: Official as of 01-May-2020
Document Type: USP Monographs
DocId: GUID-BE258FA7-AD04-4D79-B3D5-52E27CDC924B_6_en-US
DOI: https://doi.org/10.31003/USPNF_M4100_06_01
DOI Ref: 0uw2a

Amoxicillin



• $3\ H_2O$

$C_{16}H_{19}N_3O_5S \cdot 3H_2O$ 419.45

4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid,6-[[amino(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-, trihydrate [2*S*-[2α,5α,6β(*S**)]]-; (2*S*,5*R*,6*R*)-6-[(*R*)-(−)-2-Amino-2-(*p*-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate CAS RN®: 61336-70-7; UNII: 804826J2HU.

Anhydrous 365.41 CAS RN®: 26787-78-0; UNII: 9EM05410Q9.

DEFINITION

Amoxicillin contains NLT 900 µg/mg and NMT 1050 µg/mg of amoxicillin ($C_{16}H_{19}N_3O_5S$), calculated on the anhydrous basis.

IDENTIFICATION

Change to read:

• **A.** ▲Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: **197K**▲ (CN 1-May-2020)

• **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**

Diluent: 6.8 g/L of *monobasic potassium phosphate* in *water*. Adjust with a 45% (w/w) solution of *potassium hydroxide* to a pH of 5.0 ± 0.1.

Mobile phase: *Acetonitrile* and *Diluent* (1:24)

Standard solution: 1.2 mg/mL of USP Amoxicillin RS in *Diluent*. [Note—Use this solution within 6 h.]

Sample solution: 1.2 mg/mL of Amoxicillin in *Diluent*. [Note—Use this solution within 6 h.]

Chromatographic system

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

Mode: LC

Detector: UV 230 nm

Column: 4-mm × 25-cm; packing *L1*

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

Sample: *Standard solution*

Suitability requirements

Tailing factor: NMT 2.5

Relative standard deviation: NMT 2.0%

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg/mg, of amoxicillin ($C_{16}H_{19}N_3O_5S$) in the portion of Amoxicillin 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 Amoxicillin RS in the *Standard solution* (mg/mL)

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

P = potency of amoxicillin in USP Amoxicillin RS (µg/mg)

Acceptance criteria: 900–1050 µg/mg of amoxicillin ($C_{16}H_{19}N_3O_5S$) on the anhydrous basis

IMPURITIES

• **Organic Impurities**

Solution A: 2.72 g/L of *monobasic potassium phosphate*. Adjust with *1 N potassium hydroxide* or *20% phosphoric acid* to a pH of 5.0 ± 0.1.

Solution B: *Methanol*

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	97	3
10	97	3
22	75	25
26	97	3

System suitability solution: 12.5 µg/mL each of USP Amoxicillin Related Compound A RS and USP Amoxicillin Related Compound D RS in *Solution A*

Standard solution: 12.5 µg/mL of USP Amoxicillin RS in *Solution A*

Sample solution: 1.25 mg/mL of Amoxicillin in *Solution A*. [Note—Store this solution at 4° and use within 4 h.]

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 10-cm; 5-µm packing *L1*

Temperatures

Autosampler: 4°

Column: 40°

Flow rate: 1.5 mL/min

Injection volume: 10 µL

System suitability

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

[Note—Identify peaks by the relative retention times in *Table 2*.]

Suitability requirements

Resolution: NLT 1.5 between amoxicillin related compound A and the second peak for amoxicillin related compound D, *System suitability solution*

Relative standard deviation: NMT 10%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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

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

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

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

C_S = concentration of USP Amoxicillin RS in the *Standard solution* (µg/mL)

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

F = unit conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*. [Note—The reporting limit is 0.03 times the amoxicillin peak from the *Standard solution*.]

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Amoxicillin related compound I[a] (D-hydroxyphenylglycine)	0.32	1.0
Amoxicillin related compound D[b,c] (amoxicillin open ring)	0.53	1.0
	0.68	1.0
Amoxicillin related compound A[d] (6-aminopenicillanic acid)	0.78	0.5
Amoxicillin related compound B[e,f] (L-amoxicillin)	0.87	—
Amoxicillin	1.0	—
Amoxicillin related compound G[g] (D-hydroxyphenyl glycylamoxicillin)	2.9	1.0
Amoxicillin related compound E[h,i] (amoxicillin penilloic derivative)	4.5	1.0
Amoxicillin related compound M[j] [*N*-(penicillan-6-yl) open ring amoxicillinamide]	6.0	1.0
Amoxicillin related compound F[e,k] (phenylpyrazinediol)	6.3	—
Amoxicillin related compound C[l] (amoxicillin rearrangement product)	6.4	1.0
Amoxicillin related compound E[h,i] (amoxicillin penilloic derivative)	6.7	1.0
Amoxicillin related compound J[m] (amoxicillin open ring dimer)	8.8	1.0
Amoxicillin related compound L[n] [*N*-(penicillan-6-yl)amoxicillinamide]	9.0	1.0
Any unspecified individual impurity	—	1.0
Total impurities	—	5.0

[a] (*R*)-2-Amino-2-(4-hydroxyphenyl)acetic acid.

[b] The chromatographic system resolves two penicilloic acids from each other.

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

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

[e] These compounds are listed for information only and are not to be reported.

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

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

[h] The chromatographic system resolves two penilloic acids from each other.

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

[j] (2*S*,5*R*,6*R*)-6-(2-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-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.

[k] 3-(4-Hydroxyphenyl)pyrazin-2-ol.

[l] (4*S*)-2-[5-(4-Hydroxyphenyl)-3,6-dioxopiperazin-2-yl]-5,5-dimethylthiazolidine-4-carboxylic acid.

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

[n] (2*S*,5*R*,6*R*)-6-{(2*S*,5*R*,6*R*)-6-[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido]-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.

SPECIFIC TESTS

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

• **Dimethylaniline ⟨223⟩:** Meets the requirements

• **pH ⟨791⟩**

Sample solution: 2 mg/mL

Acceptance criteria: 3.5–6.0

• **Water Determination ⟨921⟩, *Method I*:** 11.5%–14.5%

• **Sterility Tests ⟨71⟩, *Test for Sterility of the Product to Be Examined, Direct Inoculation of the Culture Medium*:** Where the label states that Amoxicillin is sterile, it meets the requirements, except to use Fluid Thioglycollate Medium containing polysorbate 80 solution (5 mg/mL) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, to use Soybean-Casein Digest Medium containing polysorbate 80 solution (5 mg/mL) and an amount of sterile penicillinase sufficient to inactivate the amoxicillin in each tube, and to shake the tubes once daily.

• **Bacterial Endotoxins Test ⟨85⟩:** Where the label states that Amoxicillin is sterile or Amoxicillin must be subjected to further processing during the preparation of injectable dosage forms, it contains NMT 0.25 USP Endotoxin Units/mg of amoxicillin.

ADDITIONAL REQUIREMENTS

• **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

• **Labeling:** Where it is intended for use in preparing injectable dosage forms, the label states that it is intended for veterinary use only and that it is sterile or must be subjected to further processing during the preparation of injectable dosage forms. Label all other Amoxicillin to indicate that it is to be used in the manufacture of nonparenteral drugs only.

• **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;
6-Aminopenicillanic acid.

$C_8H_{12}N_2O_3S$ 216.26

USP Amoxicillin Related Compound D RS

(4*S*)-2-{[(*R*)-2-Amino-2-(4-hydroxyphenyl)acetamido](carboxy)methyl}-5,5-dimethylthiazolidine-4-carboxylic acid;
Amoxicillin open ring.

$C_{16}H_{21}N_3O_6S$ 383.42

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

Topic/Question	Contact	Expert Committee
AMOXICILLIN	Documentary Standards Support	SM12020 Small Molecules 1
REFERENCE STANDARD SUPPORT	RS Technical Services RSTECH@usp.org	SM12020 Small Molecules 1

Chromatographic Database Information: Chromatographic Database

Most Recently Appeared In:
Pharmacopeial Forum: Volume No. PF 43(3)

Current DocID: GUID-BE258FA7-AD04-4D79-B3D5-52E27CDC924B_6_en-US

DOI: https://doi.org/10.31003/USPNF_M4100_06_01

DOI ref: 0uw2a