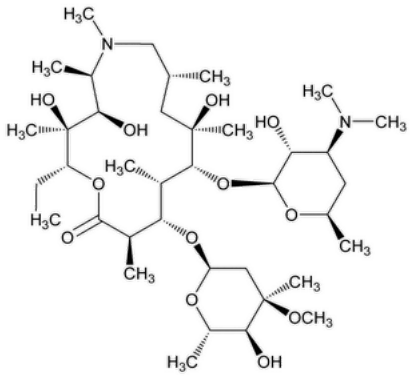
Status: Currently Official on 13-Feb-2025
Official Date: Official as of 01-Dec-2023
Document Type: USP Monographs
DocId: GUID-34C99575-55AF-407A-8C43-79D17394E453_7_en-US
DOI: https://doi.org/10.31003/USPNF_M6740_07_01
DOI Ref: zb3x9

Azithromycin



• $x\ H_2O$

$C_{38}H_{72}N_2O_{12}$ 749.00
$C_{38}H_{72}N_2O_{12} \cdot H_2O$ 767.01
$C_{38}H_{72}N_2O_{12} \cdot 2H_2O$ 785.03

1-Oxa-6-azacyclopentadecan-15-one, 13-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-, [2*R*-(2*R**,3*S**,4*R**,5*R**,8*R**,10*R**,11*R**,12*S**,13*S**,14*R**)];
(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one;
9-Deoxo-9a-aza-9a-methyl-9a-homoerythromycin A

Anhydrous CAS RN®: 83905-01-5.

Monohydrate CAS RN®: 121470-24-4.

Dihydrate CAS RN®: 117772-70-0.

DEFINITION

Azithromycin is anhydrous or contains 1 or 2 molecules of water of hydration. It contains the equivalent of NLT 945 µg/mg and NMT 1030 µg/mg of azithromycin ($C_{38}H_{72}N_2O_{12}$), calculated on the anhydrous basis.

IDENTIFICATION

• **A. Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197K:** If a difference appears in the IR spectra of the analyte and the Standard, dissolve equal portions of the test specimen and the USP Reference Standard in equal volumes of methanol. Evaporate the solutions to dryness on a water bath, and dry at 80° for 30 min under vacuum. Perform the test on the residues.

• **B.** The retention time of the azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

• **Procedure**

Solution A: 10 M potassium hydroxide

Solution B: 6.7 g/L of dibasic potassium phosphate, adjusted with *Solution A* to a pH of 11.0

Solution C: 6.7 g/L of dibasic potassium phosphate, adjusted with phosphoric acid to a pH of 8.0

Mobile phase: Acetonitrile and *Solution B* (60:40)

Diluent: Acetonitrile and *Solution C* (60:40)

System suitability solution: 0.5 mg/mL each of USP Azithromycin RS and USP Azaerythromycin A RS prepared as follows. Dissolve USP Azithromycin RS and USP Azaerythromycin A RS first in acetonitrile, using 5% of the final volume, and then dilute with *Diluent* to volume.

Standard solution: 0.53 mg/mL of USP Azithromycin RS prepared as follows. Dissolve USP Azithromycin RS first in acetonitrile, using 2% of the final volume, and then dilute with *Diluent* to volume.

Sample solution: 0.53 mg/mL of Azithromycin prepared as follows. Dissolve Azithromycin first in acetonitrile, using 2% of the final volume, and then dilute with *Diluent* to volume.

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L67

Column temperature: 40°

Flow rate: 1 mL/min

Injection volume: 10 µL

System suitability

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

[NOTE—The relative retention times for azaerythromycin A and azithromycin are 0.7 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 3.0 between azaerythromycin A and azithromycin, *System suitability solution*

Tailing factor: 0.8–1.5 for azithromycin, *Standard solution*

Relative standard deviation: NMT 1.10% for azithromycin, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the quantity, in µg, of azithromycin ($C_{38}H_{72}N_2O_{12}$) in each milligram of Azithromycin 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 Azithromycin RS in the *Standard solution*

C_U = concentration of Azithromycin in the *Sample solution*

P = potency of USP Azithromycin RS (µg/mg of azithromycin)

Acceptance criteria: 945–1030 µg/mg on the anhydrous basis

IMPURITIES

• **RESIDUE ON IGNITION ⟨281⟩:** NMT 0.3%, moistening the charred residue with 2 mL of nitric acid and 5 drops of sulfuric acid

Change to read:

• **ORGANIC IMPURITIES**

Solution A: 1.8 mg/mL of anhydrous dibasic sodium phosphate in water. Adjust with 1 N sodium hydroxide or 10% phosphoric acid to a pH of 8.9.

Solution B: Acetonitrile and methanol (3:1)

Solution C: 1.73 mg/mL of monobasic ammonium phosphate. Adjust with ammonia TS to a pH of 10.0 ± 0.05.

Solution D: Methanol, acetonitrile, and *Solution C* (7:6:7)

Mobile phase: See *Table 1*.

Table 1

Time (min)	Solution A (%)	Solution B (%)
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

System suitability solution: 0.0165 mg/mL of USP Azithromycin Related Compound F RS and 0.027 mg/mL of USP Desosaminylazithromycin RS in *Solution D*

Standard solution: 86 µg/mL of USP Azithromycin RS in *Solution D*

Sample solution: 8.6 mg/mL of Azithromycin in *Solution D*

Chromatographic system

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

Mode: LC

Detector: UV 210 nm

Column: 4.6-mm × 25-cm; 5-µm packing L1

Column temperature: 60°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

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

Suitability requirements

Peak-to-valley ratio: NLT 1.4, *System suitability solution* Calculate the peak-to-valley ratio:

$$\text{Result} = H_P/H_V$$

H_P = height above the baseline of the desosaminylazithromycin peak

H_V = height above the baseline of the lowest point of the curve separating the desosaminylazithromycin and azithromycin related compound F peaks

Tailing factor: 0.8–1.5, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

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

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times P \times F_1 \times (100/F_2)$$

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

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

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

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

P = potency of USP Azithromycin RS (µg/mg of azithromycin)

F_1 = conversion factor, 0.001 mg/µg

F_2 = relative response factor (see *Table 2*)

Acceptance criteria: See *Table 2*. Disregard peaks eluting before azithromycin *N*-oxide and after 3-deoxyazithromycin (azithromycin B). Disregard peaks with a response less than 0.1 times the response of the azithromycin peak in the *Standard solution* (0.1%).

Table 2

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
Azithromycin *N*-oxide[a]	0.29	0.43	0.5
3'-(*N*,*N*-Didemethyl)-3'-*N*-formylazithromycin[b,c]	0.37	1.7	0.5
Erythromycin A iminoether[d]	0.42	1.0	0.5
3'-(*N*,*N*-Didemethyl)azithromycin(aminoazithromycin)[e]	0.43	1.0	0.5
Azithromycin related compound F[c,f]	0.51	3.8	0.5
Desosaminylazithromycin[g]	0.54	1.0	0.3
3'-*N*-{[4-(Acetylamino)phenyl]sulfonyl}-	0.55	12	0.15

Name	Relative Retention Time	Relative Response Factor	Acceptance Criteria, NMT (%)
3',3'-didemethylazithromycin[h]			
N-Demethylazithromycin[i]	0.61	1.0	0.7
Erythromycin A oxime[j]	0.64	1.0	0.5
Azithromycin C (3"-*O*-demethylazithromycin)[k]	0.73	1.0	0.5
3'-De(dimethylamino)-3'-oxoazithromycin[l]	0.76	1.5	0.5
▲3'-*N*-{[4-(Acetylamino)phenyl]sulfonyl}-3'-demethylazithromycin▲ (ERR 1-Dec-2023) [m]	0.79	10	0.5
Azaerythromycin A[n]	0.83	1.0	0.5
Azithromycin impurity P[o]	0.92	1.0	0.2
Azithromycin	1.0	—	—
2-Desethyl-2-propylazithromycin[p]	1.23	1.0	0.5
3'-*N*-Demethyl-3'-*N*-[(4-methylphenyl)sulfonyl]azithromycin[q]	1.26	5	0.5
3-Deoxyazithromycin (azithromycin B)[r]	1.31	1.0	1.0
Any individual, unidentified impurity	—	1.0	0.2
Total impurities	—	—	3.0

[a] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylazinoyl)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[b] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[c] The system may resolve two rotamers. The limit is for the sum of the two rotamers.

[d] (3*R*,4*R*,5*S*,6*R*,9*R*,10*S*,11*S*,12*R*,13*S*,15*R*,*Z*)-12-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-6-ethyl-4,5-dihydroxy-10-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-3,5,9,11,13,15-hexamethyl-7,16-dioxa-2-azabicyclo[11.2.1]hexadec-1-en-8-one.

[e] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-amino-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[f] 3'-*N*-Demethyl-3'-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[g] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[h] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-acetamidophenylsulfonyl)amino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[i] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[j] (3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*S*,12*R*,13*S*,14*R*,*E*)-6-[[3,4,6-Trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-14-ethyl-7,12,13-trihydroxy-4-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-10-(hydroxyimino)-3,5,7,9,11,13-hexamethyloxacyclotetradecan-2-one.

[k] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[l] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3,3-dimethyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-oxo-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[m] ▲(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-acetamidophenylsulfonyl)-*N*-methylamino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.▲ (ERR 1-Dec-2023)

[n] 9-Deoxo-9a-aza-9a-homoerythromycin A; 6-Demethylazithromycin.

[o] Specified unidentified impurity.

[p] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-propyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[q] (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[*N*-(4-methylphenylsulfonyl)-*N*-methylamino]-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[r] (2*R*,3*R*,4*S*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-4,10-dihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

SPECIFIC TESTS

• **Optical Rotation** ⟨781S⟩, *Procedures, Specific Rotation*

Sample solution: 20 mg/mL in dehydrated alcohol

Acceptance criteria: −45° to −49°, at 20°

• **Crystallinity** ⟨695⟩**:** Meets the requirements, except where it is labeled as amorphous, most of the particles do not exhibit birefringence and extinction positions

• **pH** ⟨791⟩

Sample stock solution: 4 mg/mL in methanol

Sample solution: 2 mg/mL obtained by mixing equal volumes of *Sample stock solution* and water

Acceptance criteria: 9.0–11.0

• **Water Determination** ⟨921⟩, *Method I*

Where it is labeled as anhydrous: NMT 2.0%

Where it is labeled as the dihydrate: 4.0%–5.0%

Where it is labeled as the monohydrate: 1.8%–4.0%, except that it may be 4.0%–6.5% when the requirements of the *Loss on Drying* test are met

• **Loss on Drying:** Where it is labeled as Azithromycin monohydrate and has a water content of 4.0%–6.5% (see *Thermal Analysis* ⟨891⟩)

[Note—The quantity taken for this procedure may be adjusted, if necessary, for instrument sensitivity.]

Analysis: Determine the percentage of volatile substances by thermogravimetric analysis in an appropriately calibrated instrument, using about 10 mg of Azithromycin. Heat the specimen at the rate of 10°/min between ambient temperature and 150° in an atmosphere of nitrogen at a constant flow rate of about 35 mL/min. From the thermogram plot the derivatives of the loss on drying (percentage loss/min), and identify the inflection points of the two weight loss steps at about 70° and 130°.

Acceptance criteria: NMT 4.5% between ambient temperature and the inflection point at about 70°, and 1.8%–2.6% between the inflection point at about 70° and the inflection point at about 130°.

ADDITIONAL REQUIREMENTS

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

• **Labeling:** Label it to indicate whether it is anhydrous, the monohydrate, or the dihydrate. The amorphous form is so labeled. Where the quantity of azithromycin is indicated in the labeling of any preparation containing Azithromycin, this shall be understood to be in terms of anhydrous azithromycin ($C_{38}H_{72}N_2O_{12}$).

• **USP Reference Standards** ⟨11⟩

USP Azaerythromycin A RS

9-Deoxo-9a-aza-9a-homoerythromycin A; 6-Demethylazithromycin.

$C_{37}H_{70}N_2O_{12}$ 734.97

USP Azithromycin RS

USP Azithromycin Related Compound F RS

3'-*N*-Demethyl-3'-*N*-formylazithromycin; (2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-13-[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-(*N*-methyl)formamido-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$C_{38}H_{70}N_2O_{13}$ 762.98

USP Desosaminylazithromycin RS

(2*R*,3*S*,4*R*,5*R*,8*R*,10*R*,11*R*,12*S*,13*S*,14*R*)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3,4,6-trideoxy-3-dimethylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

$C_{30}H_{58}N_2O_9$ 590.80

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

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

Chromatographic Database Information: Chromatographic Database

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

Current DocID: GUID-34C99575-55AF-407A-8C43-79D17394E453_7_en-US

DOI: https://doi.org/10.31003/USPNF_M6740_07_01

DOI ref: zb3x9