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Azithromycin for Injection

DEFINITION

Azithromycin for Injection is a sterile, dry mixture of azithromycin and a suitable stabilizing agent. It contains NLT 90.0% and NMT 110.0% of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$).

IDENTIFICATION

- **A.** The retention time of the major peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.
- **B.** **Spectroscopic Identification Tests ⟨197⟩, *Infrared Spectroscopy*: 197A**

Standard: 25 mg/mL of USP Azithromycin RS in acetonitrile. Pass the solution through a suitable filter, and remove the solvent by natural evaporation.

Sample: Equivalent to 25 mg/mL of azithromycin from Azithromycin for Injection in acetonitrile. Pass the solution through a suitable filter, and remove the solvent by natural evaporation.

Analysis: Examine the spectra of the *Standard* and the *Sample* in the range between 3800 and 650 cm^{-1}.

Acceptance criteria: The *Sample* exhibits bands at about 900, 995, 1165, 1376, 1456, 1725, and 2936 cm^{-1} similar to the spectrum from the *Standard* similarly obtained.

ASSAY

- **Procedure**

Buffer: 6.7 mg/mL of dibasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (52:48). Adjust with 10 N potassium hydroxide to a pH of 11.0 ± 0.1.

Diluent: Acetonitrile and water (52:48)

System suitability solution: 1 mg/mL each of USP Azaerythromycin A RS and USP Azithromycin RS in a mixture of acetonitrile and water (52:48). Dissolve first in acetonitrile, and then dilute with water to volume.

Standard solution: 1 mg/mL of USP Azithromycin RS in a mixture of acetonitrile and water (52:48). Dissolve first in acetonitrile, and dilute with water to volume.

Sample solution: Nominally equivalent to 1 mg/mL of azithromycin from Azithromycin for Injection in *Diluent* prepared as follows. Reconstitute 3 vials individually as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture with *Diluent*.

Chromatographic system

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

Mode: LC

Detector: UV 215 nm

Columns

Guard: 4.6-mm × 1-cm; 5-µm packing L67

Analytical: 4.6-mm × 15-cm; 5-µm packing L67

Temperatures

Autosampler: 15°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 15 µL

System suitability

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

[Note—The relative retention times for azaerythromycin A and azithromycin are 0.68 and 1.0, respectively.]

Suitability requirements

Resolution: NLT 2.5 between azaerythromycin A and azithromycin, *System suitability solution*

Tailing factor: NLT 0.9 and NMT 1.5, *Standard solution*

Relative standard deviation: NMT 2%, *Standard solution*

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) in the portion of Azithromycin for Injection taken:

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

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* (mg/mL)

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

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

F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

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

IMPURITIES

[Note—The test for *Limit of Azithromycin N-Oxide, Desosaminylazithromycin, and N-Demethylazithromycin* does not quantify aminoazithromycin, formamido analog, methylformamido analog, and 3'-de(dimethylamino)-3'-oxoazithromycin. If these impurities are part of the impurity profile, the *Limit of Aminoazithromycin, Formamido Analog, Methylformamido Analog, and 3'-De(dimethylamino)-3'-oxoazithromycin* test is recommended in addition to the test for *Limit of Azithromycin N-Oxide, Desosaminylazithromycin, and N-Demethylazithromycin*.]

• **Limit of Azithromycin *N*-Oxide, Desosaminylazithromycin, and *N*-Demethylazithromycin**

Buffer: 3.5 g/L of dibasic potassium phosphate

Mobile phase: Acetonitrile and *Buffer* (23:77). Adjust with 5 N potassium hydroxide to a pH of 10.55 ± 0.05.

Standard stock solution: 0.05 mg/mL of USP Azithromycin *N*-oxide RS, 45 µg/mL of USP Desosaminylazithromycin RS, and 160 µg/mL each of USP *N*-Demethylazithromycin RS and USP Azithromycin RS in acetonitrile. Sonicate if necessary to dissolve.

Standard solution: 0.001 mg/mL of azithromycin *N*-oxide, 0.9 µg/mL of desosaminylazithromycin, and 3.2 µg/mL each of *N*-demethylazithromycin and azithromycin from *Standard stock solution* in *Mobile phase*

Sample solution: Nominally equivalent to 0.3 mg/mL of azithromycin in *Mobile phase* from Azithromycin for Injection

Chromatographic system

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

Mode: LC

Detector: Amperometric electrochemical

Electrodes: Dual series glassy carbon

Mode: Oxidative screen

Electrode 1: +0.70 ± 0.05 V

Electrode 2: +0.82 ± 0.05 V

Column: 4.6-mm × 15-cm; 3-µm packing L49

Autosampler temperature: 5°

Flow rate: 1 mL/min

Injection volume: 50 µL

System suitability

Sample: *Standard solution*

[Note—See *Table 1* for relative retention times.]

Suitability requirements

Tailing factor: NMT 2.0 for azithromycin and NMT 2.6 for *N*-demethylazithromycin

Relative standard deviation: NMT 10.0% for azithromycin *N*-oxide, desosaminylazithromycin, *N*-demethylazithromycin, and azithromycin

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of each specified impurity in the portion of Azithromycin for Injection taken:

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

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

r_S = peak response of each specified impurity from the *Standard solution*

C_S = concentration of the relevant impurity Reference Standard in the *Standard solution* (mg/mL)

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

P = potency of the relevant Reference Standard (mg/mg)

Acceptance criteria: See *Table 1*. The reporting threshold is 0.05%.

Table 1

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azithromycin *N*-oxide[a]	0.17	1.0
Desosaminylazithromycin[b]	0.27	0.3
Erythromycin A oxime[c,d]	0.35	—
N-Demethylazithromycin[e]	0.50	1.0
Azaerythromycin A[c,f]	0.85	—
Azithromycin	1.00	—

[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*)-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] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[d] (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.

[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-[[3,4,6-trideoxy-3-methylamino-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[f] 9-Deoxo-9a-aza-9a-homoerythromycin A.

Change to read:

• **Limit of Aminoazithromycin, Formamido Analog, Methylformamido Analog, and 3'-De(dimethylamino)-3'-oxoazithromycin** (if present)

Buffer: 3.5 g/L of dibasic potassium phosphate in water

Mobile phase: Acetonitrile and *Buffer* (46:54). Adjust with 10 N potassium hydroxide to a pH of 11.0 ± 0.1.

Diluent: Acetonitrile and water (46:54)

Standard stock solution: 0.09 mg/mL of USP Desosaminylazithromycin RS, 0.21 mg/mL of USP *N*-Demethylazithromycin RS, and 0.30 mg/mL of USP Azithromycin RS in acetonitrile

Standard solution: 0.0018 mg/mL of desosaminylazithromycin, 0.0042 mg/mL of *N*-demethylazithromycin, and 0.006 mg/mL of azithromycin in *Diluent*

Sample solution: Nominally equivalent to 0.6 mg/mL of azithromycin from Azithromycin for Injection in *Diluent*. Reconstitute 3 vials individually, as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture with *Diluent*. The *Sample solution* must be injected immediately after preparation.

Blank: Use the *Diluent*.

Chromatographic system

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

Mode: LC

Detector: Amperometric electrochemical

Electrodes: Dual series glassy carbon

Mode: Oxidative screen

Electrode 1: +0.70 ± 0.05 V

Electrode 2: +0.82 ± 0.05 V

Columns

Guard: 4.6-mm × 1-cm; 5-µm packing L67

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

Temperatures

Autosampler: 15°

Column: 40°

Flow rate: 1 mL/min

Injection volume: 25 µL

System suitability

Sample: *Standard solution*

[NOTE—See *Table 2* for relative retention times.]

Suitability requirements

Resolution: NLT 1.5 between desosaminylazithromycin and *N*-demethylazithromycin

Tailing factor: NMT 1.5 for azithromycin

Relative standard deviation: NMT 5% for azithromycin

Analysis

Samples: *Standard solution*, *Sample solution*, and *Blank*

Disregard any peaks corresponding to those obtained from the *Blank*.

Calculate the percentage of each impurity in the portion of Azithromycin for Injection 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 azithromycin from the *Standard solution*

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

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

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

F = conversion factor, 0.001 mg/µg

Acceptance criteria: See *Table 2*.

Table 2

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Erythromycin A iminoether[a,b]	0.20	—
3'-(*N*,*N*-Didemethyl)azithromycin (aminoazithromycin)[c] + 3'-(*N*,*N*-didemethyl)-3'-*N*-formylazithromycin (formamido analog)[d]	0.25	1.0
Azithromycin F[a,e]	0.30	—
Desosaminylazithromycin[f,g]	0.31	—
3'-*N*-Demethyl-3'-*N*-formylazithromycin (methylformamido analog)[h]	0.32	1.0
N-Demethylazithromycin[f,i]	0.35	—
Erythromycin A oxime[a,j]	0.42	—
Azaerythromycin A[a,k]	0.63	—
3'-De(dimethylamino)-3'-oxoazithromycin[l]	0.72	1.0
3'-*N*-Demethyl-3'-*N*-[(4-methylphenyl)sulfonyl]azithromycin[a,m]	0.85	—
Azithromycin	1.00	—

Name	Relative Retention Time	Acceptance Criteria, NMT (%)
Azithromycin B (3-deoxyazithromycin)[a,n]	1.64	—
Any other unspecified impurity	—	0.2
Total impurities[o]	—	3.0

[a] Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only.

[b] (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.

[c] (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.

[d] (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.

[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-hexamethyl-14-hydroxymethyl-11-[[3-dimethylamino-3,4,6-trideoxy-β-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

[f] These impurities are controlled using the *Limit of Azithromycin N-Oxide, Desosaminylazithromycin, and N-Demethylazithromycin* test. They are listed here for information only.

[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*-methyl)formamido-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-(dimeth ylamino)-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] 9-Deoxo-9a-aza-9a-homoerythromycin A.

[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-methylphenylsulfonyl)-*N*-methylamino]-3,4,6-trideoxy-ß-D-*xylo*-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.▲ (ERR 1-Aug-2023)

[n] (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.

[o] Total impurities include desosaminylazithromycin and *N*-demethylazithromycin.

SPECIFIC TESTS

- **Bacterial Endotoxins Test ⟨85⟩:** Meets the requirements
- **Sterility Tests ⟨71⟩:** Meets the requirements
- **Particulate Matter in Injections ⟨788⟩:** Meets the requirements
- **pH ⟨791⟩:** 6.4–6.8, determined in a solution constituted as directed in the labeling
- **Water Determination ⟨921⟩, *Method I*:** NMT 2.0%
- **Other Requirements:** It meets the requirements under *Injections and Implanted Drug Products ⟨1⟩*.

ADDITIONAL REQUIREMENTS

- **Packaging and Storage:** Preserve as described under *Packaging and Storage Requirements ⟨659⟩, Injection Packaging, Packaging for Constitution*. Store at controlled room temperature.
- **Labeling:** It meets the requirements for *Labeling ⟨7⟩, Labels and Labeling for Injectable Products*.

Change to read:

- **USP Reference Standards ⟨11⟩**

USP Azaerythromycin A RS

9-Deoxo-9a-aza-9a-homoerythromycin A.

$C_{37}H_{70}N_2O_{12}$ ▲734.97▲ (ERR 1-Aug-2023)

USP Azithromycin RS

USP Azithromycin *N*-oxide RS

(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.

$C_{38}H_{72}N_2O_{13}$ ▲765.00▲ (ERR 1-Aug-2023)

USP *N*-Demethylazithromycin RS

(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.

$C_{37}H_{70}N_2O_{12}$ ▲734.97▲ (ERR 1-Aug-2023)

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▲ (ERR 1-Aug-2023)

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

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

Chromatographic Database Information: Chromatographic Database

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