• **USP REFERENCE STANDARDS** ⟨11⟩
USP Azaerythromycin A RS
USP Azithromycin RS

Azithromycin for Injection

» Azithromycin for Injection is a sterile, dry mixture of azithromycin and a suitable stabilizing agent. It contains not less than 90.0 per cent and not more than 110.0 per cent of the labeled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$).

Packaging and storage—Preserve in *Containers for Sterile Solids* as described under *Injections* ⟨1⟩, and store at controlled room temperature.

Labeling—It meets the requirements for *Labeling* under *Injections* ⟨1⟩.

USP Reference standards ⟨11⟩**—**
USP Azaerythromycin A RS
USP Azithromycin RS
USP Azithromycin *N*-Oxide RS
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.
$C_{37}H_{70}N_2O_{12}$ 734.96
USP Desosaminylazithromycin RS
USP Endotoxin RS

Identification—The chromatogram of the *Assay preparation* obtained as directed in the *Assay* exhibits a major peak for azithromycin, the retention time of which corresponds to that exhibited in the chromatogram of the *Standard preparation*.

Bacterial endotoxins ⟨85⟩**—**It contains not more than 0.7 USP EU per mg of Azithromycin.

Sterility ⟨71⟩**—**It meets the requirements when tested as directed for *Membrane Filtration* under *Test for Sterility of the Product to be Examined.*

Uniformity of dosage units ⟨905⟩**:** meets the requirements.

pH ⟨791⟩**:** between 6.4 and 6.8, determined in a solution constituted as directed in the labeling.

Water, *Method I* ⟨921⟩**:** not more than 2.0%.

Particulate matter ⟨788⟩**:** meets the requirements.

Limit of azithromycin *N*-oxide—

Potassium phosphate 0.02 M buffer—Prepare as directed in the test for *Related compounds.*

Mobile phase—Prepare a filtered and degassed mixture of *Potassium phosphate 0.02 M buffer* and acetonitrile (76.5:23.5). Adjust with 5 N potassium hydroxide to a pH of 11.0 ± 0.1. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—A mixture of *Potassium phosphate 0.02 M buffer* and acetonitrile (76.5:23.5). Adjust with diluted phosphoric acid to a pH of 8.0 ± 0.1.

Stock standard solution—Dissolve an accurately weighed portion of USP Azithromycin RS in acetonitrile to obtain a solution having a known concentration of about 0.6 mg per mL.

Standard solution—Dilute the *Stock standard solution* quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a known concentration of about 0.006 mg of azithromycin per mL.

Resolution solution—0.0015 mg/mL of azithromycin *N*-oxide and 0.45 mg/mL of azithromycin in *Diluent*.

Test solution—Prepare as directed in the test for *Related compounds.*

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with an amperometric electrochemical detector with a dual series glassy carbon electrode operated in the oxidative screen mode, with electrode 1 set at +0.70 ± 0.05 V and electrode 2 set at +0.82 ± 0.05 V and the background current optimized to 95 ± 25 nanoamperes; a 4.6-mm × 15-cm column that contains 5- µm packing L29; and a 4.6-mm × 5-cm guard column that contains 5- µm packing L29. The flow rate is about 0.4 mL per minute. The autosampler temperature is maintained at 15 °. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.38 and 1.0, respectively, for the azithromycin *N*-oxide and the azithromycin peaks. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1000 theoretical plates; the tailing factor is not less than 0.9 and not more than 1.5; and the relative standard deviation for replicate injections is not more than 5%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard solution* and the *Test solution* into the chromatograph, and measure the area responses for all the peaks. Calculate the percentage of azithromycin *N*-oxide in the portion of Azithromycin for Injection taken by the formula:

$$(P/1000)(C_S / C_U)(r_i / r_S)(100)$$

in which (P/1000) is the potency, converted from µg per mg to mg per mg, of USP Azithromycin RS; C_S is the concentration, in mg per mL, of USP Azithromycin RS in the *Standard solution;* C_U is the concentration, in mg per mL, of azithromycin in the *Test solution;* r_i is the response of the azithromycin *N*-oxide peak obtained from the *Test solution;* and r_S is the response for the azithromycin peak in the *Standard solution:* not more than 1.0% of azithromycin *N*-oxide is found.

Related compounds—

Potassium phosphate 0.02 M buffer—Dilute about 3.48 g of dibasic potassium phosphate with water to 1000 mL, and mix. Prior to use, pass through a filter having a porosity of 0.45 µm.

Mobile phase—Prepare a filtered and degassed mixture of *Potassium phosphate 0.02 M buffer* and acetonitrile (54:46). Adjust with 10 N potassium hydroxide to a pH of 11.0 ± 0.1. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent—Use a mixture of water and acetonitrile (54 : 46).

Blank—Use *Diluent.*

Stock standard solution—Dissolve accurately weighed portions of USP Desosaminylazithromycin RS, USP *N*-Demethylazithromycin RS, and USP Azithromycin RS in acetonitrile; and dilute quantitatively with the same solvent to obtain a solution having a known concentration of about 0.09 mg of desosaminylazithromycin, 0.21 mg of *N*-demethylazithromycin, and 0.30 mg of azithromycin per mL.

Standard solution—Dilute the *Stock standard solution* quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having known concentrations of about 0.0018 mg of desosaminylazithromycin, 0.0042 mg of *N*-demethylazithromycin, and 0.006 mg of azithromycin per mL.

Test solution—Reconstitute 3 vials individually, as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a nominal concentration of about 0.6 mg of azithromycin per mL, based on the label claim. The *Test solution* must be injected immediately.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with an amperometric electrochemical detector with dual glassy carbon electrodes, operated in the oxidative screen mode, with electrode 1 set at +0.70 ± 0.05 V and electrode 2 set at +0.82 ± 0.05 V and with the background current optimized to 95 ± 25 nanoamperes; a 4.6-mm × 25-cm column that contains 5- µm packing L67; and a 4.6-mm × 1-cm guard column that contains 5- µm packing L67. The flow rate is about 1 mL per minute. The column is maintained at about 40 °. The autosampler temperature is main-

tained at 15°. Chromatograph the *Standard solution,* and record the peak responses as directed for *Procedure:* the relative retention times are as shown in *Table 1;* the resolution, *R,* between desosaminylazithromycin and *N*-demethylazithromycin is not less than 1.5; the tailing factor of the peaks for desosaminylazithromycin, *N*-demethylazithromycin, and azithromycin is not more than 1.5; the column efficiency is not less than 1500 theoretical plates for the azithromycin peak; and the relative standard deviation of the desosaminylazithromycin, *N*-demethylazithromycin, and azithromycin peaks for replicate injections is not more than 5%.

Procedure—Separately inject equal volumes (about 25 µL) of the *Standard solution,* the *Blank,* and the *Test solution* into the chromatograph, and measure the responses for all the peaks. Calculate the percentage of desosaminylazithromycin in the portion of Azithromycin for Injection taken by the formula:

$$(P)(C_S / C_U)(r_i / r_S)(100)$$

in which P is the potency, in mg per mg, of *USP Desosaminylazithromycin RS*; C_S is the concentration, in mg per mL, of *USP Desosaminylazithromycin RS* in the *Standard solution;* C_U is the concentration, in mg per mL, of azithromycin in the *Test solution;* r_i is the response of the desosaminylazithromycin peak obtained from the *Test solution;* and r_S is the response for the desosaminylazithromycin peak in the *Standard solution.* Calculate the percentage of *N*-demethylazithromycin in the portion of Azithromycin for Injection taken by the formula:

$$(P)(C_S / C_U)(r_i / r_S)(100)$$

in which P is the potency, in mg per mg, of USP *N*-Demethylazithromycin RS; C_S is the concentration, in mg per mL, of USP *N*-Demethylazithromycin RS in the *Standard solution;* C_U is the concentration, in mg per mL, of azithromycin in the *Test solution;* r_i is the response of the *N*-demethylazithromycin peak obtained from the *Test solution;* and r_S is the response for the *N*-demethylazithromycin peak in the *Standard solution.* Calculate the percentage of each of the other related compounds, including unspecified impurities, in the portion of Azithromycin for Injection taken by the formula:

$$(P/1000)(C_S / C_U)(r_i / r_S)(100)$$

in which $(P / 1000)$ is the potency, converted from µg per mg to mg per mg, of USP Azithromycin RS; C_S is the concentration, in mg per mL, of USP Azithromycin RS in the *Standard solution;* C_U is the concentration, in mg per mL, of azithromycin in the *Test solution;* r_i is the response of the impurity peak obtained from the *Test solution;* and r_S is the response for the azithromycin peak in the *Standard solution.* The specified and unspecified impurities meet the limits specified in *Table 1*. Disregard any peaks corresponding to those obtained with the *Blank*.

Table 1

Peak Identification	Relative Retention Time	Limit (%)
3′-(*N*,*N*-Didemethyl)azithromycin (aminoazithromycin) + 3′-(*N*,*N*-didemethyl)-3′-*N*-formylazithromycin	0.25	1.0
Desosaminylazithromycin	0.31	0.3
3′-*N*-Demethyl-3′-*N*-formylazithromycin	0.32	1.0
N-Demethylazithromycin	0.35	1.0
3′-De(dimethylamino)-3′-oxoazithromycin	0.72	1.0
Azithromycin	1.00	—
Any other unspecified impurity	—	0.2
Total impurities	—	3.0

Other requirements—It meets the requirements specified under *Injections* ⟨1⟩.

Assay—

Potassium phosphate buffer—Dissolve about 6.7 g of dibasic potassium phosphate in 1000 mL of water, and mix. Prior to use, pass through a filter having a porosity of 0.45 µm.

Mobile phase—Prepare a filtered and degassed mixture of acetonitrile and *Potassium phosphate buffer* (52 : 48). Adjust with 10 N potassium hydroxide to a pH of 11.0 ± 0.1. Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Diluent: a mixture of acetonitrile and water (52 : 48).

Resolution solution—Transfer accurately weighed quantities of USP Azaerythromycin A RS and USP Azithromycin RS to a suitable volumetric flask. Dissolve in acetonitrile, using 52% of the final volume. Dilute with water to volume, and mix to obtain a solution having a known concentration of about 1 mg each of azaerythromycin and azithromycin per mL.

Standard preparation—Transfer an accurately weighed quantity of USP Azithromycin RS to a suitable volumetric flask. Dissolve in acetonitrile, using about 52% of the final volume. Dilute with water to volume, and mix to obtain a solution having a known concentration of about 1 mg of azithromycin per mL.

Assay preparation—Reconstitute 3 vials individually as directed in the labeling. Mix the contents of all the reconstituted vials. Dilute a portion of the mixture quantitatively, and stepwise if necessary, with *Diluent* to obtain a solution having a nominal concentration of about 1 mg azithromycin per mL, based on the label claim.

Chromatographic system (see *Chromatography* ⟨621⟩)—The liquid chromatograph is equipped with a 215-nm detector, a 4.6-mm × 15-cm column that contains 5- µm packing L67, and a 4.6-mm × 1-cm guard column that contains 5- µm packing L67. The flow rate is about 1 mL per minute. The column is maintained at about 40°. The autosampler temperature is maintained at 15°. Chromatograph the *Resolution solution,* and record the peak responses as directed for *Procedure:* the relative retention times are about 0.68 and 1.0, respectively, for the azaerithromycin A and azithromycin peaks; and the resolution, *R,* between the peaks due to azaer ythromycin A and azithromycin is not less than 2.5. Chromatograph the *Standard preparation,* and record the peak responses as directed for *Procedure:* the column efficiency is not less than 1500 theoretical plates; the tailing factor is not less than 0.9 and not more than 1.5; and the relative standard deviation for replicate injections is not more than 2%.

Procedure—Separately inject equal volumes (about 15 µL) of the *Standard preparation* and the *Assay preparation* into the chromatograph, and measure the area responses for the major peaks. Calculate the percentage of the label claim of azithromycin ($C_{38}H_{72}N_2O_{12}$) in the portion of Azithromycin for Injection taken by the formula:

$$(P/1000)(C_S / C_U)(r_U / r_S)(100)$$

in which $(P/1000)$ is the potency, converted from µg per mg to mg per mg, of USP Azithromycin RS; C_S is the concentration, in mg per mL, of USP Azithromycin RS in the *Standard preparation;* C_U is the nominal concentration, in mg per mL, of azithromycin in the *Assay preparation;* and r_U and r_S are the peak area responses for azithromycin obtained from the *Assay preparation* and the *Standard preparation,* respectively.

Azithromycin for Oral Suspension

DEFINITION

Azithromycin for Oral Suspension is a dry mixture of Azithromycin and one or more buffers, sweeteners, diluents, anticaking agents, and flavors. 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 azithromycin peak of the *Sample solution* corresponds to that of the *Standard solution*, as obtained in the *Assay*.

ASSAY

- **PROCEDURE**

[NOTE—Use water that has a resistivity of NL T 18 Mohm-cm.]

Mobile phase: Dissolve 5.8 g of monobasic potassium phosphate in 2130 mL of water, and add 870 mL of acetonitrile. Adjust with about 6 mL of 10 N potassium hydroxide to a pH of 11.0 ± 0.1, and pass through a suitable filter.

Diluent: Dissolve 2.2 g of monobasic potassium phosphate in 1590 mL of water, and add 600 mL of isopropyl alcohol, 480 mL of alcohol, and 330 mL of acetonitrile. Adjust with 10 N potassium hydroxide to a pH of 8.4 ± 0.1, and shake by mechanical means for 30 min.

Standard stock solution: 0.165 mg/mL of USP Azithromycin RS in acetonitrile. Swirl, and sonicate as necessary.

Standard solution: 3.3 µg/mL of USP Azithromycin RS from the *Standard stock solution* in *Mobile phase*

System suitability stock solution: 0.16 mg/mL of USP Azaerythromycin A RS in acetonitrile and *Mobile phase* (1:9). Dissolve first in acetonitrile using 10% of the final volume. Swirl, and sonicate to dissolve. Dilute with *Mobile phase* to volume.

System suitability solution: 3.2 µg/mL of azaerythromycin A from the *System suitability stock solution* and 3.3 µg/mL of azithromycin from the *Standard stock solution* in *Mobile phase*

Sample stock solution 1 (where packaged in a single-unit container)**:** 2 mg/mL of azithromycin from Azithromycin for Oral Suspension in *Diluent*. Transfer the contents of a container of Azithromycin for Oral Suspension to a suitable volumetric flask. Add *Diluent* equal to 70% of the volume of the flask, and shake by mechanical means for 30 min. Dilute with *Diluent* to volume. Transfer 40 mL of this suspension to a stoppered centrifuge tube, and centrifuge for 20 min. Use the supernatant to prepare *Sample solution 1*.

Sample stock solution 2 (where packaged in a multiple-unit container)**:** 0.4 mg/mL of azithromycin from Azithromycin for Oral Suspension in *Diluent*. Constitute Azithromycin for Oral Suspension as directed in the labeling. Transfer a suitable aliquot of the suspension so obtained, freshly mixed and free from air bubbles to a suitable volumetric flask to obtain to obtain a final concentration of 0.4 mg/mL. Add *Diluent* equal to 70% of the final volume, shake by mechanical means for 30 min, and dilute with *Diluent* to volume. Transfer 40 mL of the suspension so obtained to a stoppered centrifuge tube, and centrifuge for 20 min. Use the supernatant to prepare *Sample solution 2*.

Sample solution 1: 3.2 µg/mL of azithromycin from *Sample stock solution 1* in *Mobile phase*

Sample solution 2: 4 µg/mL of azithromycin from *Sample stock solution 2* in *Mobile phase*

Chromatographic system

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

Mode: LC

Detector: Amperometric electrochemical detector

Electrode: Dual glassy carbon electrodes

Mode: Oxidative screen mode

Electrode 1: +0.70 ± 0.05 V

Electrode 2: +0.82 ± 0.05 V

Background current: 85 ± 15 nanoamperes

Column

Guard: 4.6-mm × 5-cm; 5-µm packing L29

Analytical: 4.6-mm × 15-cm; 5-µm packing L29 or 3-µm packing L49 without the guard column

Flow rate: 1.5 mL/min

Injection size: 50 µL

System suitability

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

[NOTE—The relative retention times for azaerythromycin A and azithromycin with the L29 column are 0.7 and 1.0, respectively; the relative retention times for azaerythromycin A and azithromycin with the L49 column are 0.8 and 1.0, respectively.]

Suitability requirements

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

Column efficiency: NLT 1000 theoretical plates, *Standard solution*

Tailing factor: 0.9–1.5, *Standard solution*

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

Analysis

Samples: *Standard solution* and *Sample solution*

Where packaged in a single-unit container

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

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

r_U = peak response from *Sample solution 1*
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 *Sample solution 1* (mg/mL)
P = potency of azithromycin in USP Azithromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Where packaged in a multiple-unit container

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

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

r_U = peak response from *Sample solution 2*
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 *Sample solution 2* (mg/mL)
P = potency of azithromycin in USP Azithromycin RS (µg/mg)
F = conversion factor, 0.001 mg/µg

Acceptance criteria: 90.0%–110.0%

PERFORMANCE TESTS

- **DELIVERABLE VOLUME ⟨698⟩:** Meets the requirements
- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meets the requirements for a solid packaged in single-unit containers

SPECIFIC TESTS

- **pH ⟨791⟩**

For a solid packaged in single-unit containers: 9.0–11.0, in the suspension constituted as directed in the labeling

For a solid packaged in multiple-unit containers: 8.5–11.0, in the suspension constituted as directed in the labeling