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_{37}H_{70}N_{2}O_{12}, 734.96). 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:

$$\frac{P/1000(C_i / C_0)(r_i / r_0)(100)}{1}$$

in which (P/1000) is the potency, converted from μg per mg to mg per mg, of USP Azithromycin RS; $C_i$ is the concentration, in mg per mL, of USP Azithromycin RS in the Standard solution; $C_0$ 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 $r_0$ 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-Demethyiazithromycin 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-demethyiazithromycin, 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-demethyiazithromycin, 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 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 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 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 azithromycin per mL.

Test 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 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.
tion; ri

tion; ri

concentration, in mg per mL, of azithromycin in the

tion; ri

Desosaminylazithromycin 0.31 0.3 (in which

CU

Injection taken by the formula:

ﬁed impurities meet the limits specified in

mycin peak in the

CU

-N

Demethylazithromycin RS in the

CU

Desosaminylazithromycin RS

in mg per mL, of USP Azithromycin RS in the

in mg per mL of azithromycin in the Test solu-
tion; ri is the response of the desosaminylazithromycin peak ob-
tained from the Test solution; and rri is the response for the desosam-

CU

in mg per mL, of USP N-demethylazithromycin RS in the

Standard solution; CU is the concentration, in mg per mL of azithromycin in the Test solu-
tion; rri is the response for the desosaminylazithromycin peak in the

Calculated the percentage of desosaminylazithromycin in the portion of Azithromycin for Injection taken by the formula:

(P)(Ci / CS)(ri / rri)(100)

in which P is the potency, in mg per mg, of USP Desosaminylazithromycin RS; Ci is the concentration, in mg per mL of USP Desosaminylazithromycin RS in the Standard solution; CS is the concentration, in mg per mL of USP Azithromycin RS in the Standard solution; and ri is the response for the desosaminylazithromycin peak obtained from the Test solution.

Calculate the percentage of N-demethylazithromycin in the portion of Azithromycin for Injection taken by the formula:

(P)(Ci / CS)(ri / rri)(100)

in which P is the potency, in mg per mg, of USP N-Demethylazithromycin RS; Ci is the concentration, in mg per mL, of USP N-Demethylazithromycin RS in the Standard solution; CS is the concentration, in mg per mL, of azithromycin in the Standard solution; and ri 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)(Ci / CS)(ri / rri)(100)

in which (P/1000) is the potency, converted from µg per mg to mg per mg, of USP Azithromycin RS; Ci is the concentration, in mg per mL, of USP Azithromycin RS in the Standard solution; CS is the concentration, in mg per mL, of azithromycin in the Standard solution; and ri 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

<table>
<thead>
<tr>
<th>Peak Identification</th>
<th>Relative Retention Time</th>
<th>Limit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3′-(N,N-Dimethyl)azithromycin (aminoaizithromycin) + 3′-(N,N-dimethyl)-N-formylazithromycin</td>
<td>0.25</td>
<td>1.0</td>
</tr>
<tr>
<td>Desosaminylazithromycin</td>
<td>0.31</td>
<td>0.3</td>
</tr>
<tr>
<td>3′-N-Demethyl-3′-N-formylazithromycin</td>
<td>0.32</td>
<td>1.0</td>
</tr>
<tr>
<td>N-Demethylazithromycin</td>
<td>0.35</td>
<td>1.0</td>
</tr>
<tr>
<td>3′-De(dimethylamino)-3′-oxoazithromycin</td>
<td>0.72</td>
<td>1.0</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>1.00</td>
<td>—</td>
</tr>
</tbody>
</table>

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 Azithromycin 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 azathromycin 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 °C. The autosampler temperature is maintained at 15 °C. 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 azathromycin A and azithromycin peaks; and the resolution, R, between the peaks due to azathromycin 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%. Proceedure—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 (C38H37N3O12) in the portion of Azithromycin for Injection taken by the formula:

(P/1000)(Ci / CS)(ri / rri)(100)

in which (P/1000) is the potency, converted from µg per mg to mg per mg, of USP Azithromycin RS; Ci is the concentration, in mg per mL, of USP Azithromycin RS in the Standard preparation; CS is the nominal concentration, in mg per mL, of azithromycin in the Assay preparation; and ri and rri are the peak area responses for azithromycin obtained from the Assay preparation and the Standard preparation, respectively.

Table 1 (Continued)
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_{2}O_{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 to dissolve. Diluent: Sample solution 1:
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 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_{2}O_{12}) in the portion of Azithromycin for Oral Suspension taken:
Result = (r_{U}/r_{S}) × (C_{S}/C_{U}) × P × F × 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)
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_{2}O_{12}) in the portion of Azithromycin for Oral Suspension taken:
Result = (r_{U}/r_{S}) × (C_{S}/C_{U}) × P × F × 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)
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