Azithromycin for Oral Suspension

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In accordance with the Rules and Procedures of the Council of Experts, the Small Molecules 1 Expert Committee has revised the Azithromycin for Oral Suspension monograph. The purpose of this revision is to widen the Organic Impurities acceptance criterion for N-demethylazithromycin from NMT 0.50% to NMT 0.7% to accommodate FDA-approved drug products with different limits.

The Azithromycin for Oral Suspension Revision Bulletin supersedes the currently official monograph.

Should you have any questions, please contact Claire Chisolm, Senior Scientist II (301-230-3215 or cnc@usp.org).
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.

• 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 Oral Suspension 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

  [Note—Solutions containing azithromycin are stable up to 12 h at 10°.]

  **Solution A**: Dissolve 8.7 g of dipotassium hydrogen phosphate anhydrous in 1000 mL of water and adjust with potassium hydroxide or dilute phosphoric acid to a pH of 8.2.

  **Solution B**: Acetonitrile

  **Mobile phase**: Solution A and Solution B (30:70)

  **Diluent**: Acetonitrile, methanol, and water (40:40:20)

  **Standard solution**: 0.6 mg/mL of USP Azithromycin RS in Diluent. Sonicate in cool water to dissolve as needed.

  **Sample solution**: Nominally 0.6 mg/mL of azithromycin in Diluent prepared as follows. Transfer an accurately measured portion of the constituted suspension to a suitable volumetric flask. Add Diluent equal to 50% of the volume of the flask, and sonicate for 20 min with shaking in cool water. Dilute with Diluent to volume. Pass a portion of this solution through a suitable filter of 0.45-µm pore size.

  **Chromatographic system**
  (See *Chromatography* (621), System Suitability.)

  **Mode**: LC

  **Detector**: UV 210 nm

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

  **Temperatures**

  **Autosampler**: 10°
Column: 30°  
Flow rate: 2 mL/min  
Injection volume: 50 µL  
Run time: NLT 2 times the retention time of azithromycin

System suitability
Sample: Standard solution  
Suitability requirements  
Tailing factor: NMT 2.0  
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution  
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:

\[ \text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times P \times F \times 100 \]

- $r_U$ = peak response of azithromycin 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: 90.0%–110.0%

PERFORMANCE TESTS

- **Deliverable Volume** (698): Meets the requirements  
- **Uniformity of Dosage Units** (905).  
  For single-unit containers: Meets the requirements  
- **Dissolution** (711).  
  [Note—Solutions containing azithromycin are stable up to 12 h at 10°.]  
  Medium: Sodium phosphate buffer, pH 6.0 (14.2 g/L of sodium phosphate, dibasic, anhydrous in water, adjusted with dilute hydrochloric acid to pH 6.0); 900 mL  
  Apparatus 2: 50 rpm  
  Time: 30 min  
  Solution A: Dissolve 8.7 g of dipotassium hydrogen phosphate anhydrous in 1000 mL of water and adjust with potassium hydroxide or dilute phosphoric acid to a pH of 8.2.  
  Solution B: Acetonitrile  
  Mobile phase: Solution A and Solution B (35:65)

Standard stock solution: 0.55 mg/mL of USP Azithromycin RS prepared as follows. Transfer an accurately weighed amount of USP Azithromycin RS to a suitable volumetric flask. Add acetonitrile to fill 5% of the volume of the flask and sonicate in cool water for 5 min to dissolve completely. Dilute with Medium to volume.

Standard solution  
For Azithromycin for Oral Suspension labeled to contain 100 mg/5 mL: 0.11 mg/mL of USP Azithromycin RS in Medium from Standard stock solution
For Azithromycin for Oral Suspension labeled to contain 200 mg/5 mL: 0.22 mg/mL of USP Azithromycin RS in Medium from Standard stock solution

Sample solution: Pass a portion of the solution under test through a suitable filter.

Chromatographic system
(See Chromatography (621), System Suitability.)

Mode: LC
Detector: UV 210 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Autosampler: 10°
Column: 50°
Flow rate: 2 mL/min
Injection volume: 100 µL
Run time: NLT 2 times the retention time of azithromycin

System suitability
Sample: Standard solution
Suitability requirements
Tailing factor: NMT 2.0
Relative standard deviation: NMT 2.0%

Analysis
Samples: Standard solution and Sample solution
Calculate the percentage (Q) of the labeled amount of azithromycin (C_{38}H_{72}N_{2}O_{12}) dissolved:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{L} \right) \times D \times \left( \frac{d}{W} \right) \times V \times 100
\]

\(r_U\) = peak response of azithromycin 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)
\(L\) = label claim of Azithromycin for Oral Suspension (mg/5 mL)
\(D\) = dilution factor, necessary only if the Sample solution requires dilution (mL/mL)
\(d\) = density of the Sample solution (g/mL)
\(W\) = weight of Azithromycin for Oral Suspension taken (g)
\(V\) = volume of Medium, 900 mL

Tolerances: NLT 75% (Q) of the labeled amount of azithromycin (C_{38}H_{72}N_{2}O_{12}) is dissolved.

IMPURITIES
Change to read:

● Organic Impurities
Solution A: Dissolve 1.8 g of disodium hydrogen phosphate dihydrate in 1000 mL of water and adjust with dilute phosphoric acid to a pH of 8.9.
Solution B: Acetonitrile and methanol (75:25)
Mobile phase: See Table 1.

Table 1
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>25</td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>30</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>80</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>81</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>90</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

**Buffer:** Dissolve 1.73 g of ammonium dihydrogen phosphate in 1000 mL of water and adjust with ammonia solution to a pH of 10.0 ± 0.05.

**Diluent:** Buffer, methanol, and acetonitrile (35:35:30)

**System suitability solution:** 0.015 mg/mL of USP Azithromycin Related Compound F RS and 0.025 mg/mL of USP Desosaminy lazithromycin RS in Diluent

**Standard solution:** 0.04 mg/mL of USP Azithromycin RS in Diluent. Sonicate in cool water to dissolve as needed.

**Sample solution:** Nominally 4.0 mg/mL solution of azithromycin in Diluent prepared as follows. Transfer a portion of the constituted suspension, equivalent to about 400.0 mg of azithromycin, to a 100-mL volumetric flask. Add 70 mL of Diluent and sonicate in cool water for about 15 min. Dilute with Diluent to volume. Pass a portion of this solution through a suitable filter of 0.45-µm pore size.

**Chromatographic system**
(See Chromatography (621), System Suitability.)

- **Mode:** LC
- **Detector:** UV 210 nm
- **Column:** 4.6-mm × 25-cm; 5-µm packing L1
- **Temperatures**
  - Autosampler: 10°
  - Column: 60°
- **Flow rate:** 0.9 mL/min
- **Injection volume:** 100 µL
- **Run time:** NLT 2 times the retention time of azithromycin

**System suitability**

- **Samples:** System suitability solution and Standard solution
- **Suitability requirements**
  - **Resolution:** NLT 1.5 between desosaminy lazithromycin and azithromycin related compound F, System suitability solution
  - **Relative standard deviation:** NMT 5.0%, Standard solution

**Analysis**

- **Samples:** Standard solution and Sample solution
Calculate the percentage of each impurity in the portion of Azithromycin for Oral Suspension taken:

\[
\text{Result} = \left( \frac{r_U}{r_S} \right) \times \left( \frac{C_S}{C_U} \right) \times P \times F_1 \times \left( \frac{1}{F_2} \right) \times 100
\]

- \( r_U \) = 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)
- \( F_1 \) = conversion factor, 0.001 mg/µg
- \( F_2 \) = relative response factor (see Table 2)

Acceptance criteria: See Table 2. Disregard any peaks at relative retention times before 0.29 and after 1.31.

### Table 2

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
<th>Relative Response Factor</th>
<th>Acceptance Criteria, NMT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin N-oxide(^a)</td>
<td>0.29</td>
<td>0.43</td>
<td>0.50</td>
</tr>
<tr>
<td>3′-((N,N\text{-Didemethyl})-3′-N\text{-formylazithromycin}(^b)</td>
<td>0.37</td>
<td>1.7</td>
<td>0.50</td>
</tr>
<tr>
<td>3′-((N,N\text{-Didemethyl})azithromycin(amaioazithromycin)(^c)</td>
<td>0.43</td>
<td>1.0</td>
<td>0.50</td>
</tr>
<tr>
<td>Azithromycin related compound F(^d)</td>
<td>0.51</td>
<td>3.8</td>
<td>0.50</td>
</tr>
<tr>
<td>Desosaminylazithromycin(^e)</td>
<td>0.54</td>
<td>1.0</td>
<td>0.30</td>
</tr>
<tr>
<td>\textit{N-Demethylazithromycin}(^f)</td>
<td>0.61</td>
<td>1.0</td>
<td>(\text{▲}0.7) (RB 1-(\text{Jun}-2022))</td>
</tr>
<tr>
<td>Azithromycin C (3″-O-demethylazithromycin)(^g,h)</td>
<td>0.73</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3′-De(dimethylamino)-3′-oxoazithromycin(^i)</td>
<td>0.76</td>
<td>1.5</td>
<td>0.50</td>
</tr>
<tr>
<td>Azaerythromycin A(^b,l)</td>
<td>0.83</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Specified unidentified impurity(^b,k)</td>
<td>0.92</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2-Desethyl-2-propylazithromycin(^b,l)</td>
<td>1.23</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3′-N-Demethyl-3′-N-((4-methylphenyl)sulfonyl)azithromycin(^b,m)</td>
<td>1.26</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Name</td>
<td>Relative Retention Time</td>
<td>Relative Response Factor</td>
<td>Acceptance Criteria, NMT (%)</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>-------------------------</td>
<td>--------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>3-Deoxyazithromycin (azithromycin B)(^a)</td>
<td>1.31</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Any individual unspecified degradation product</td>
<td>—</td>
<td>1.0</td>
<td>0.20</td>
</tr>
<tr>
<td>Total degradation products</td>
<td>—</td>
<td>—</td>
<td>3.5</td>
</tr>
</tbody>
</table>

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

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

\(^c\) (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-Dideoxy-3-C-methyl-3-O-methyl-\(\alpha\)-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-\(\beta\)-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

\(^d\) 3'-\((N\text{-Dimethyl})-3'\text{-N-formylazithromycin}; (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-Dideoxy-3-C-methyl-3-O-methyl-\(\alpha\)-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-\(\beta\)-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.


\(^f\) (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-Dideoxy-3-C-methyl-3-O-methyl-\(\alpha\)-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-\(\beta\)-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

\(^g\) Process impurities that are controlled in the drug substance are not to be reported. They are listed here for information only. The unspecified impurities and total impurities limits do not include these impurities.

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

\(^i\) 9-Deoxy-9a-aza-9a-homoerythromycin A.

\(^j\) Specified unidentified impurity.

\(^k\) (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-Dideoxy-3-C-methyl-3-O-methyl-\(\alpha\)-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)-\(\beta\)-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one dihydrate.

\(^m\) (2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-13-[[2,6-Dideoxy-3-C-methyl-3-O-methyl-\(\alpha\)-L-ribo-hexopyranosyl]oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-heptamethyl-11-[[3-[N-(4-methylphenylsulfonfonyl)-N-methylamino]-3,4,6-trideoxy-\(\beta\)-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.

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

**SPECIFIC TESTS**

- **PH** (791)

  **For a solid packaged in single-unit containers**

  **Sample:** The suspension constituted as directed in the labeling

  **Acceptance criteria:** 9.0–11.0
For a solid packaged in multiple-unit containers

Sample: The suspension constituted as directed in the labeling

Acceptance criteria: 8.5–11.0

ADDITIONAL REQUIREMENTS

• Packaging and Storage: Preserve in tight containers.

• USP Reference Standards (11):
  - USP Azithromycin RS
  - USP Azithromycin Related Compound F RS
  - USP Desosaminylazithromycin RS

3’-(N-Demethyl)-3’-N-formylazithromycin;
(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-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-

\[C_{38}H_{70}N_{2}O_{13}\] 762.97

USP Desosaminylazithromycin RS
(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-2-Ethyl-3,4,10,13-tetrahydroxy-3,5,6,8,10,12,14-heptamethyl-

\[C_{30}H_{58}N_{2}O_{9}\] 590.79