In accordance with the Rules and Procedures of the 2015-2020 Council of Experts, the Chemical Medicines Monographs 3 Expert Committee has revised the Pioglitazone and Metformin Hydrochloride Tablets monograph. The purpose for the revision is to include an option of using UV spectra of the pioglitazone and metformin peaks in the Sample solution and the Standard solution as obtained in the Assay to meet the Acceptance criteria under the Identification Test A, and to modify the wavelength ranges for absorption maxima. This will address the comments that the excipient matrix in some products may interfere with the Ultraviolet Absorption <197U> test.

The Pioglitazone and Metformin Hydrochloride Tablets Revision Bulletin supersedes the currently official monograph. The Revision Bulletin will be incorporated in the USP 40–NF 35.

Should you have any questions, please contact Elena Gonikberg, Ph.D., Principal Scientific Liaison, (301–816-8251 or eg@usp.org).
Pioglitazone and Metformin Hydrochloride Tablets

DEFINITION
Pioglitazone and Metformin Hydrochloride Tablets contain an amount of pioglitazone hydrochloride \((C_{19}H_{20}N_{2}O_{3}S \cdot HCl)\) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of pioglitazone \((C_{19}H_{20}N_{2}O_{3}S)\), and NLT 95.0% and NMT 105.0% of the labeled amount of metformin hydrochloride \((C_{6}H_{11}N_{2} \cdot HCl)\).

IDENTIFICATION

Change to read:

A. ULTRAVIOLET ABSORPTION (197U) •

[NOTE—The UV spectra of the major peaks of the Sample solution and the Standard solution as obtained in the Assay may also be used to meet the Acceptance criteria.] •  [RB 1-Jun-2016]

Pioglitazone

Sample solution: Transfer a quantity of finely powdered Tablets to a suitable container, and add water to obtain a final concentration of about 0.03 mg/mL of pioglitazone. Sonicate for about 30 s. Pass through a 5-μL portion of the resulting suspension using a suitable filter of 0.45-μm pore size, then wash the filter with 10 mL of water, and discard the filtrate. Wash the filter with 5 mL of 0.1 N hydrochloric acid, and use the filtrate.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 265 and 271 nm.

Metformin hydrochloride

Sample solution: Transfer a quantity of finely powdered Tablets to a suitable container, and add a suitable quantity of water, based on the labeled amount of metformin hydrochloride in the sample, to obtain a final concentration of about 0.4 mg/mL of metformin hydrochloride. Sonicate for about 30 s, and pass through a suitable filter of 0.45-μm pore size, discarding the first few mL of filtrate. Dilute a portion of the filtrate with water to obtain a solution containing about 8 μg/mL of metformin hydrochloride.

Acceptance criteria: The UV absorption spectrum exhibits a maximum between 230 and 235 nm.

B. The retention times of the pioglitazone and metformin peaks of the Sample solution correspond to those of the Standard solution, as obtained in the Assay.

ASSAY

Change to read:

PROCEDURE

Mobile phase: 7.2 g/L of sodium dodecyl sulfate in a mixture of 0.05 M monobasic ammonium phosphate and acetonitrile (1:1)

Diluent: Methanol and 0.1 N hydrochloric acid (1:1)

System suitability stock solution: 0.5 mg/mL of p-methoxyacetophenone and 0.4 mg/mL of butylparaben in Diluent

Pioglitazone standard stock solution: 0.84 mg/mL of USP Pioglitazone Hydrochloride RS in Diluent

Mixed standard stock solution: 2.5 mg/mL of USP Metformin Hydrochloride RS and 0.084 mg/mL of USP Pioglitazone Hydrochloride RS in 0.1 N hydrochloric acid from the Pioglitazone stock solution

System suitability solution: Transfer 10.0 mL of the Mixed standard stock solution and 5.0 mL of the System suitability stock solution to a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

Standard solution: 16.8 μg/mL of USP Pioglitazone Hydrochloride RS and 0.5 mg/mL of USP Metformin Hydrochloride RS in 0.1 N hydrochloric acid from the Mixed standard stock solution

Sample stock solution: Weigh and finely powder NLT 10 Tablets. Transfer an amount of powdered Tablets, equivalent to about 15 mg of pioglitazone, to a 200-mL volumetric flask. Add 120 mL of 0.1 N hydrochloric acid, shake for about 30 min, and then sonicate for about 5 min. Dilute with 0.1 N hydrochloric acid to volume, and mix well. Pass through a suitable filter of 0.45-μm pore size, discarding the first few mL of filtrate.

Sample solution: Transfer a suitable volume of the Sample stock solution (see Table 1) to a 50-mL volumetric flask, and dilute with 0.1 N hydrochloric acid to volume.

Chromatographic system

(See Chromatography (621), System Suitability.)

Mode: LC

Detector: UV 255 nm for metformin and p-methoxyacetophenone; UV 225 nm for pioglitazone and butylparaben. If this procedure is used for Identification A, use a diode-array detector set at 200–400 nm.

Column: 6.0-mm x 15-cm; 5-μm packing L7

Column temperature: 25 ± 5°

Flow rate: 1 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the metformin peak of about 5 min.]

Injection volume: 10 μL

System suitability

Samples: System suitability solution and Standard solution

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

Table 1

<table>
<thead>
<tr>
<th>Labeled Amount of Pioglitazone and Metformin Hydrochloride (mg/Tablet)</th>
<th>Volume of Sample Stock Solution Used to Prepare the Sample Solution (mL)</th>
<th>Nominal Concentrations in the Sample Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 and 500</td>
<td>10</td>
<td>Pioglitazone (μg/mL) Metformin Hydrochloride (mg/mL)</td>
</tr>
<tr>
<td>15 and 850</td>
<td>5</td>
<td>7.5 0.425</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Name</th>
<th>Relative Retention Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin</td>
<td>1.0</td>
</tr>
<tr>
<td>p-Methoxyacetophenone</td>
<td>1.2</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>1.8</td>
</tr>
<tr>
<td>Butylparaben</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Suitability requirements

Resolution: NLT 2.5 between metformin and p-methoxyacetophenone; NLT 2.5 between pioglitazone and butylparaben, System suitability solution

Relative standard deviation: NMT 1.0% for metformin peak; NMT 1.0% for pioglitazone peak, Standard solution
**Pioglitazone**

Analysis:

Samples:  Standard solution and Sample solution

Calculate the percentage of the labeled amount of pioglitazone (C_{19}H_{20}N_{2}O_{3}S) in the portion of Tablets:

\[ \text{Result} = \frac{(r_0/r_1) \times (C_S/C_U) \times (M_1/M_2)}{100} \]

- \( r_0 \) = peak response of pioglitazone from the Sample solution
- \( r_1 \) = peak response of pioglitazone from the Standard solution
- \( C_S \) = concentration of USP Pioglitazone Hydrochloride RS in the Standard solution (mg/mL)
- \( C_U \) = nominal concentration of pioglitazone in the Sample solution (mg/mL)
- \( M_1 \) = molecular weight of pioglitazone hydrochloride, 392.90
- \( M_2 \) = molecular weight of pioglitazone hydrochloride, 356.44

Calculate the percentage of the labeled amount of metformin hydrochloride (C_{4}H_{11}N_{5} \cdot HCl) in the portion of Tablets:

\[ \text{Result} = \frac{(r_0/r_1) \times (C_S/C_U) \times (M_1/M_2)}{100} \]

- \( r_0 \) = peak response of metformin from the Sample solution
- \( r_1 \) = peak response of metformin from the Standard solution
- \( C_S \) = concentration of USP Metformin Hydrochloride RS in the Standard solution (mg/mL)
- \( C_U \) = nominal concentration of metformin hydrochloride in the Sample solution (mg/mL)

Acceptance criteria: 95.0%–105.0% for each of the labeled amounts of pioglitazone and metformin hydrochloride

**PERFORMANCE TESTS**

- **Dissolution (711)**

  **Test 1**
  - Medium: pH 2.5 McIlvaine buffer (could be prepared by adjusting 0.1 M citric acid with 0.2 M dibasic sodium phosphate to a pH of 2.5); 900 mL
  - Apparatus 2: 50 rpm
  - Time: 30 min

  Diluent and Mobile phase: Proceed as directed in the Assay.

  **Pioglitazone standard stock solution**: 0.37 mg/mL of USP Pioglitazone Hydrochloride RS in Diluent A

  **Standard solution**: 0.0185 mg/mL of USP Pioglitazone Hydrochloride RS from the Pioglitazone standard stock solution and (L/900) mg/mL of USP Metformin Hydrochloride RS in Medium, where \( L \) is the label claim, in mg/Tablet, of metformin hydrochloride

  **Sample solution**: Pass a portion of the solution under test through a suitable filter of 0.45-µm pore size.

  **Chromatographic system**: Proceed as directed in the Assay, except use an Injection volume of 5 µL.

  **System suitability**
  - Sample: Standard solution
  - Suitability requirements
    - Tailing factor: NMT 2.5 for the metformin peak; NMT 2.0 for the pioglitazone peak
    - Relative standard deviation: NMT 2.0% for the metformin peak; NMT 2.0% for the pioglitazone peak

  **Test 2**: If the product complies with this test, the labeling indicates that it meets USP Dissolution Test 2.

  - Medium: pH 2.5 McIlvaine buffer (could be prepared by adjusting 0.1 M citric acid with 0.2 M dibasic sodium phosphate to a pH of 2.5); 900 mL
  - Apparatus 2: 50 rpm
  - Time: 45 min

  **Solution A**: 1.4 g/L of dibasic sodium phosphate anhydrous and 1.4 g/L of sodium dodecyl sulfate in water

  **Solution B**: Phosphoric acid and water (50:50)


  **Diluent A**: Acetonitrile and Medium (50:50)
  - Diluent B: Acetonitrile and water (70:30)

  **Pioglitazone standard stock solution**: 0.019 mg/mL of USP Pioglitazone Hydrochloride RS in Diluent B. Sonicate as needed to dissolve.

  **Metformin standard stock solution**: 0.92 mg/mL of USP Metformin Hydrochloride RS in Medium. Sonicate as needed to dissolve.

  **Standard solution**: 0.003 mg/mL of USP Pioglitazone Hydrochloride RS from the Pioglitazone standard stock solution and 0.11 mg/mL of USP Metformin Hydrochloride RS in Diluent A

  **Sample solution**: Pass a portion of the solution under test through a suitable filter and dilute with Diluent A to a concentration that is similar to the Standard solution.

  **Chromatographic system**
  - (See Chromatography (621), System Suitability.)
**ORGANIC IMPURITIES: PIOGLITAZONE**

- 0.2%

**UNIFORMITY OF DOSAGE UNITS**

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**Pioglitazone**  
Official June 1, 2016

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System suitability solution:

- Acetonitrile, 0.1 M ammonium acetate, 0.2 mg/mL of USP Pioglitazone Hydrochloride RS, dissolved first in methanol and 0.1 N hydrochloric acid (1:1) to volume. This solution contains about 18 mg of pioglitazone, to a 100-mL volumetric flask, and 50 mL of Diluent. Shake for 30 min, and dilute with Mobile phase to volume. Pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC  
**Detector:** UV 269 nm  
**Column:** 4.6-mm × 15-cm; 5-µm packing L1  
**Flow rate:** 0.8 mL/min  
**Injection volume:** 40 µL  
**System suitability**

Sample: Standard solution  
Suitability requirements

- Tailing factor: NMT 2.0% for the pioglitazone peak, NLT 5.0% for the metformin peak.  
- Relative standard deviation: NMT 2.5% for the pioglitazone peak, NLT 5.0% for the metformin peak.

**Analysis**

**Samples:** Standard solution and Sample solution  
Calculate the percentage of the labeled amount of pioglitazone (C₁₉H₂₀N₂O₃S) dissolved:

Result = \( \frac{(r_U/r_S) \times (C_S/L) \times V \times D \times (M_1/M_2)}{100} \)

- \( r_U \) = peak response of pioglitazone from the Sample solution  
- \( r_S \) = peak response of pioglitazone from the Standard solution  
- \( C_S \) = concentration of USP Pioglitazone Hydrochloride RS in the Standard solution (mg/mL)  
- \( L \) = label claim of pioglitazone (mg/Tablet)  
- \( V \) = volume of Medium, 900 mL  
- \( D \) = dilution factor of the Sample solution  
- \( M_1 \) = molecular weight of pioglitazone, 356.44  
- \( M_2 \) = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride (C₆H₁₃N₅·HCl) dissolved:

Result = \( \frac{(r_U/r_S) \times (C_S/L) \times V \times D \times 100}{} \)

- \( r_U \) = peak response of metformin hydrochloride from the Sample solution  
- \( r_S \) = peak response of metformin hydrochloride from the Standard solution  
- \( C_S \) = concentration of USP Metformin Hydrochloride RS in the Standard solution (mg/mL)  
- \( L \) = label claim of metformin hydrochloride (mg/Tablet)  
- \( V \) = volume of Medium, 900 mL  
- \( D \) = dilution factor of the Sample solution  
- \( M_1 \) = molecular weight of metformin, 318.32  
- \( M_2 \) = molecular weight of metformin hydrochloride, 352.44

**Tolerances:** NLT 80% for the pioglitazone peak, NMT 5.0% for the metformin peak.

- Relative standard deviation: NMT 2.5% for the pioglitazone peak, NLT 5.0% for the metformin peak.

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**IMPURITIES**

- **ORGANIC IMPURITIES: PIOGLITAZONE**
  - Mobile phase: Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)  
  - Diluent: Methanol and 0.1 N hydrochloric acid (1:1)  
  - **Standard stock solution:** 0.2 mg/mL of USP Pioglitazone Hydrochloride RS, dissolved first in methanol using 20% of the final volume, then diluted with Mobile phase to volume  
  - **System suitability solution:** Prepare a solution containing 0.3 mg/mL of benzophenone in methanol.

- **Mobile phase:** UV 225 nm  
- **Column:** 4.6-mm × 15-cm; 5-µm packing L1  
- **Flow rate:** 1 mL/min  
- **Injection volume:** 15 µL  
- **System suitability**

Sample: Standard solution  
Suitability requirements

- Tailing factor: 0.8–2.0 for the metformin peak; 0.8–2.0 for the pioglitazone peak  
- Relative standard deviation: NMT 2.0% for the metformin peak; NLT 2.5% for the pioglitazone peak

**Analysis**

**Samples:** Standard solution and Sample solution
Calculate the percentage of each pioglitazone related impurity in the portion of Tablets taken:

Result = \( \frac{(r_U/r_S) \times (C_S/L) \times V \times D \times (M_1/M_2)}{100} \)

- \( r_U \) = peak response of each individual impurity from the Sample solution  
- \( r_S \) = peak response of pioglitazone from the Standard solution  
- \( C_S \) = concentration of USP Pioglitazone Hydrochloride RS in the Standard solution (µg/mL)  
- \( L \) = label claim of pioglitazone (mg/Tablet)  
- \( V \) = volume of Medium, 900 mL  
- \( D \) = dilution factor of the Sample solution  
- \( M_1 \) = molecular weight of pioglitazone, 356.44  
- \( M_2 \) = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria

Any individual pioglitazone related impurity: NMT 0.2%

**Total pioglitazone related impurities:** NMT 0.6%

- **Mobile phase:** Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)  
- **Diluent:** Methanol and 0.1 N hydrochloric acid (1:1)  
- **Standard solution:** 0.2 mg/mL of USP Pioglitazone Hydrochloride RS, dissolved first in methanol using 20% of the final volume, then diluted with Mobile phase to volume  
- **System suitability solution:** Prepare a solution containing 0.3 mg/mL of benzophenone in methanol.

**Solution A:** 1.74 g of sodium 1-pentanesulfonate and 1.15 g of monobasic ammonium phosphate in 1000 mL of water

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**Solution B:** Acetonitrile and water (7:3)

**Mobile phase:** See Table 3.

### Table 3

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solution A (%)</th>
<th>Solution B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>15.1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>25.1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>35</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**System suitability solution:** 5 µg/mL of USP Metformin Hydrochloride RS and 2 µg/mL of melamine in water

**Standard solution:** 5 µg/mL of USP Metformin Hydrochloride RS in water

**Sample solution:** Accurately weigh 10 Tablets, and finely powder. Transfer an amount of powdered Tablets, equivalent to about 100 mg of metformin hydrochloride, to a 100-mL volumetric flask, and add 50 mL of water. Shake for 30 min. Dilute with water to volume, and pass through a suitable filter of 0.45-µm pore size, discarding the first few mL of filtrate.

**Chromatographic system**

(See Chromatography (621), System Suitability.)

**Mode:** LC

**Detector:** UV 215 nm

**Column:** 4.6-mm × 15-cm; 5-µm packing L62

**Column temperature:** 25 ± 5 °C

**Flow rate:** 1.0 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the metformin peak of about 8 min.]

**Run time:** 15 min

**Injection volume:** 20 µL

**System suitability**

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

[NOTE—The relative retention times for melamine and metformin are about 0.9 and 1.0, respectively.]

**Suitability requirements**

**Resolution:** NLT 4 between melamine and metformin hydrochloride, System suitability solution

**Tailing factor:** NMT 1.5 for the metformin hydrochloride peak, System suitability solution

**Relative standard deviation:** NMT 5.0%, Standard solution

**Analysis**

**Samples:** Standard solution and Sample solution

Calculate the percentage of each metformin hydrochloride related impurity in the portion of Tablets taken:

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

\( r_U = \) peak response of each individual impurity from the Sample solution

\( r_S = \) peak response of metformin hydrochloride from the Standard solution

\( C_S = \) concentration of USP Metformin Hydrochloride RS in the Standard solution (µg/mL)

\( C_U = \) nominal concentration of metformin hydrochloride in the Sample solution (µg/mL)

**Acceptance criteria**

**Any individual impurity:** NMT 0.1%

**Total impurities:** NMT 0.5%

**ADDITIONAL REQUIREMENTS**

- **Packaging and Storage:** Preserve in tight containers, and store at controlled room temperature.

- **Labeling:** When more than one Dissolution test is given, the labeling states the Dissolution test used only if Test 1 is not used.

- **USP Reference Standards (11)**

- USP Metformin Hydrochloride RS

- USP Pioglitazone Hydrochloride RS

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