Pioglitazone and Metformin Hydrochloride Tablets

DEFINITION

Pioglitazone and Metformin Hydrochloride Tablets contain an amount of pioglitazone hydrochloride ($C_{19}H_{20}N_2O_3S \cdot HCl$) equivalent to NLT 95.0% and NMT 105.0% of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$), and NLT 95.0% and NMT 105.0% of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$).

IDENTIFICATION

- **A. ULTRAVIOLET ABSORPTION** ⟨197U⟩

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-mL 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 267 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 234 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

- **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)

Resolution 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 standard stock solution*

System suitability solution: Transfer 10.0 mL of the *Mixed standard stock solution* and 5.0 mL of the *Resolution 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 pioglitazone hydrochloride and 0.5 mg/mL of metformin hydrochloride in 0.1 N hydrochloric acid from the *Mixed standard stock solution*

Sample stock solution: Weigh and powder finely 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.

Table 1

Labeled Amounts of Pioglitazone and Metformin Hydrochloride (mg/Tablet)	Volume of *Sample stock solution* Used to Prepare the *Sample solution* (mL)	Nominal Concentrations in the *Sample solution*	
		Pioglitazone (µg/mL)	Metformin Hydrochloride (mg/mL)
15 and 500	10	15	0.5
15 and 850	5	7.5	0.425

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
Column: 6.0-mm × 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 2

Name	Relative Retention Time
Metformin	1.0
p-Methoxyacetophenone	1.2
Pioglitazone	1.8
Butylparaben	2.1

Suitability requirements
Resolution: NLT 2.5 between metformin and *p*-methoxyacetophenone and NLT 2.5 between pioglitazone and butylparaben, *System suitability solution*
Relative standard deviation: NMT 1.0% for the metformin peak and NMT 1.0% for pioglitazone peak, *Standard solution*

Analysis
Samples: *Standard solution* and *Sample solution*
Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) in the portion of Tablets taken:

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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* (µg/mL)

C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) in the portion of Tablets taken:

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

r_U = peak response of metformin from the *Sample solution*
r_S = 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

Change to read:

- **DISSOLUTION** ⟨**711**⟩

•**Test 1**• (RB 1-Aug-2014)

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*
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 and NMT 2.0 for the pioglitazone peak
Relative standard deviation: NMT 2.0% for the metformin peak and NMT 2.0% for the pioglitazone peak
Analysis
Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times (M_{r1}/M_{r2}) \times 100$$

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
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times 100$$

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

Tolerances: NLT 80% (*Q*) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved; NLT 80% (*Q*) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved.

•**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)
Mobile phase: Acetonitrile and *Solution A* (34:66). Adjust with *Solution B* to a pH of 7.1.
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 metformin concentration that is similar to the *Standard solution*.
Chromatographic system
(See *Chromatography* ⟨621⟩*, System Suitability*.)
Mode: LC
Detector: UV 225 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Temperatures
Column: 40°
Autosampler: 5°
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 and 0.8–2.0 for the pioglitazone peak

Relative standard deviation: NMT 2.0% for the metformin peak and NMT 2.5% for the pioglitazone peak

Analysis

Samples: *Standard solution* and *Sample solution*

Calculate the percentage of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) dissolved:

$$\text{Result} = (r_U/r_S) \times (C_S/L) \times V \times D \times (M_{r1}/M_{r2}) \times 100$$

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_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Calculate the percentage of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) dissolved:

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

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*

Tolerances: NLT 80% (Q) of the labeled amount of pioglitazone ($C_{19}H_{20}N_2O_3S$) is dissolved; NLT 80% (Q) of the labeled amount of metformin hydrochloride ($C_4H_{11}N_5 \cdot HCl$) is dissolved. ● (RB 1-Aug-2014)

- **UNIFORMITY OF DOSAGE UNITS ⟨905⟩:** Meet the requirements for *Content Uniformity* for pioglitazone and metformin hydrochloride

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, and then diluted with *Mobile phase* to volume

System suitability solution: Prepare a solution containing 0.3 mg/mL of benzophenone in methanol. Transfer 1.0 mL of this solution to a 50-mL volumetric flask, add 5.0 mL of the *Standard stock solution*, and dilute with *Mobile phase* to volume. This solution contains 20 µg/mL of USP Pioglitazone Hydrochloride RS and 6 µg/mL of benzophenone.

Standard solution: 1 µg/mL of USP Pioglitazone Hydrochloride RS in *Mobile phase* from the *Standard stock solution*

Sample solution: Weigh and powder finely 10 Tablets. Transfer an amount of powdered Tablets, equivalent to about 18 mg of pioglitazone, to a 100-mL volumetric flask, and add 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

Column temperature: 25 ± 5°

Flow rate: 0.8 mL/min. [NOTE—The flow rate may be adjusted to achieve the retention time of the pioglitazone peak of about 7 min.]

Injection volume: 40 µL

Run time: At least 4 times the retention time of the pioglitazone peak

System suitability

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

[NOTE—Elution order is the pioglitazone peak followed by benzophenone.]

Suitability requirements

Resolution: NLT 10 between pioglitazone and benzophenone, *System suitability solution*

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

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

Analysis

Samples: *Standard solution* and *Sample solution*

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

$$\text{Result} = (r_U/r_S) \times (C_S/C_U) \times (M_{r1}/M_{r2}) \times 100$$

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)
C_U = nominal concentration of pioglitazone in the *Sample solution* (µg/mL)
M_{r1} = molecular weight of pioglitazone, 356.44
M_{r2} = molecular weight of pioglitazone hydrochloride, 392.90

Acceptance criteria

Any individual pioglitazone related impurity: NMT 0.2%

Total pioglitazone related impurities: NMT 0.6%

[NOTE—Disregard the peaks due to metformin and its impurities that elute before 4.5 min, corresponding to the relative retention time of the pioglitazone peak of about 0.64.]

- **ORGANIC IMPURITIES: METFORMIN**

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

Solution B: Acetonitrile and water (7:3)

Mobile phase: See *Table 3*.

Table 3

Time (min)	Solution A (%)	Solution B (%)
0	100	0
15	70	30
15.1	0	100
25	0	100
25.1	100	0
35	100	0

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: Weigh accurately 10 Tablets, and powder finely. 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°
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:

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

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.

Add the following:

- **LABELING:** When more than one *Dissolution* test is given, the labeling states the *Dissolution* test used only if *Test 1* is not used. (RB 1-Aug-2014)
- **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Metformin Hydrochloride RS
USP Pioglitazone Hydrochloride RS