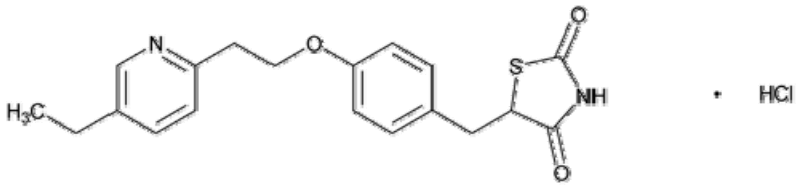
Pioglitazone

USP Method Pioglitazone HCl RS
USP Method Pioglitazone HCl Assay



Original Manufacturer:	Takeda Pharmaceuticals (patent started expire 2011)
Brand Names:	Actos, Glustin , Glizone, Pioz, CND, USV, Zactos
Combination Drugs:	ActoplusMet (Pioglitazone and Metformin) Competact (Pioglitazone and metformin) Duetact (Pioglitazone and Glimepiride)

Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action.

Actos was the tenth-best selling drug in the U.S. in 2008, with sales exceeding $2.4 billion

The first patent for Pioglitazone expired in 2011. However, there is very good reason to believe that other Actos patents (which expire later) will provide protection against generic competition for this medication.

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany

www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Pioglitazone Hydrochloride

USP34 – NF29 S1

USP Columns:

YMC 18 ODS-A Assay and Organic Impurities 4.6 mm x 15 cm, 5 µm

Equivalent Column:

Purospher®STAR RP-18 endcapped (5 µm) 150x4.6 mm	(1.51455.0001)

Recommended Solvents and Reagents:

Acetonitrile	isocratic grade for liquid chromatography LiChrosolv®	(1.14291)
Methanol	for liquid chromatography LiChrosolv®	(1.06018)
Water	Water for chromatography LiChrosolv® or freshly purified water from Milli-Q water purification system	(1.15333)
Benzophenone		(8.01801)
Ammonium Acetate	Use ACS reagent grade.	
Acetic Acid	Acetic acid (glacial) 100%. Use ACS reagent grade.	

USP Standards

Pioglitazone Hydrochloride (50 mg)	USP Product Number:	1539905

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

USP Method for Pioglitazone Assay

Mobile phase
Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)

Standard solution
Prepare a 0.5 mg/mL solution of USP Pioglitazone Hydrochloride RS in methanol, and dilute with Mobile phase to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride.

System suitability stock solution
0.5 mg/mL of USP Pioglitazone Hydrochloride RS and 0.13 mg/mL of benzophenone in methanol

System suitability solution
Dilute System suitability stock solution with Mobile phase to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride and 13 µg/mL of benzophenone.

Sample solution
Prepare a 0.5 mg/mL solution of pioglitazone hydrochloride in methanol, and dilute with Mobile phase to obtain a solution containing 50 µg/mL of pioglitazone hydrochloride.

Chromatographic system (*See Chromatography 621, System Suitability.*)
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 2.5°C
Flow rate: 0.7 mL/min
Injection size: 20 µL
[Note—Adjust the flow rate so that the retention time of the pioglitazone peak is about 7 min.]

System suitability (*Samples: System suitability solution and Standard solution)*
•Approximate relative retention times for pioglitazone and benzophenone are 1.0 and 2.6, respectively.

Suitability requirements
•Tailing factor: NMT 1.5 for pioglitazone and benzophenone, System suitability solution
•Resolution: NLT 15 between pioglitazone and benzophenone, System suitability solution
•Relative standard deviation: NMT 2.0% for six replicate injections, Standard solution

Analysis (Samples: Standard solution and Sample solution)
Calculate the percentage of $C_{19}H_{20}N_2O_3S{\cdot}HCl$ in the portion of Pioglitazone Hydrochloride taken:

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

r_U = peak response from the Sample solution
r_S = peak response from the Standard solution
C_S = concentration of USP Pioglitazone Hydrochloride RS in the Standard solution (µg/mL)
C_U = concentration of Pioglitazone Hydrochloride in the Sample solution (µg/mL)

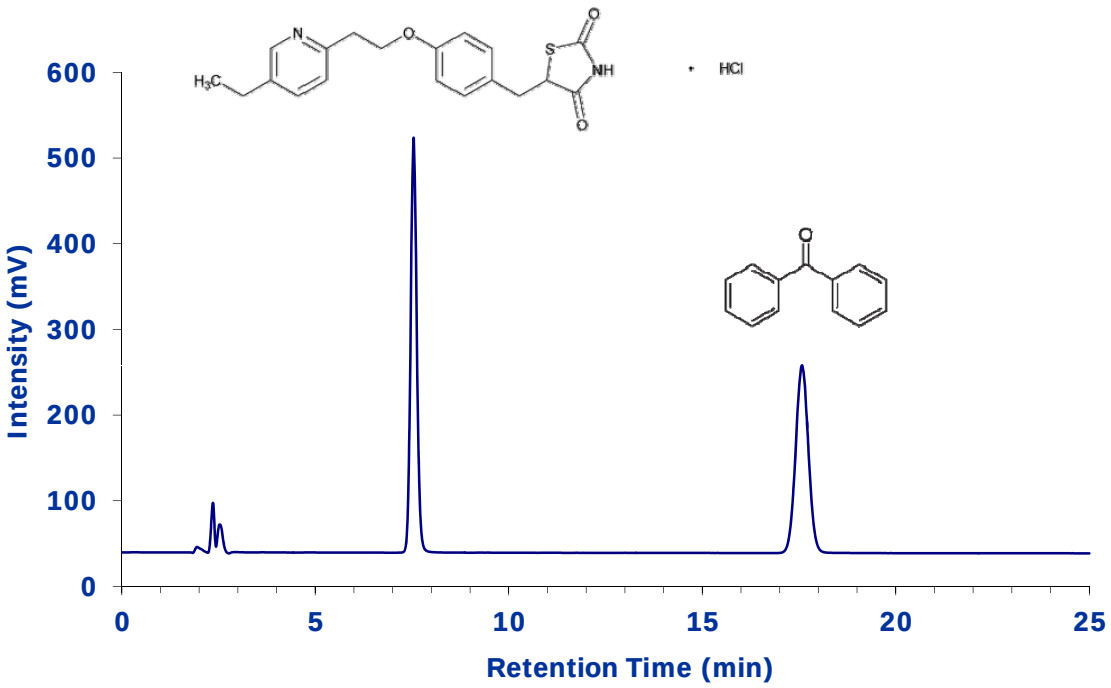
Acceptance criteria:
98.0%–102.0% on the dried basis

USP Method for Pioglitazone HCl Assay

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	20 µL	
Detection:	VWR-Hitachi LaChrom Elite DAD@269 nm	
Cell:	13 µL	
Flow Rate:	0.7 mL/min	
Mobile Phase (v/v):	Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	50 µg/mL of Pioglitazone HCl and 13 µg/mL of benzophenone (SST solution)	
Pressure Drop:	67 Bar (972 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Pioglitazone HCl	7.6	1.0	0.0	1.09
2	Benzophenone (SST)	17.4	2.3	22.0	1.03

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Analysis protocol for Pioglitazone HCl Assay

USP Method Repeatability

No.	Compound	Response (Arbitrary Area Units)	Relative Standard Deviation (%)	N
1	Pioglitazone HCl	5261746	0.2	6

Replicate injections of standard solution (n=6) were analyzed to determine the USP method repeatability. Sample contained 50 ppm of Pioglitazone HCl in mobile phase.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

No.	Compound	LOD (ppm)	LOQ (ppm)	Curve Equation	Regression Coefficient (R^2)
1	Pioglitazone HCl	0.23	0.71	y = 107244x + 30717	1.000

For Pioglitazone HCl, injections were carried out of at seven different concentrations (25-75 ppm) to determine the linearity of the method.

USP Method for Pioglitazone HCl RS

Mobile phase and System suitability stock solution (*Proceed as directed in the Assay.*)

System suitability solution:
Dilute the System suitability stock solution with Mobile phase to obtain a solution containing 25 µg/mL of Pioglitazone hydrochloride and 6.5 µg/mL of Benzophenone.

Sample solution:
0.2 mg/mL of Pioglitazone hydrochloride dissolved in 20% of the final volume with methanol, then diluted with Mobile phase to final volume

Standard solution
1 µg/mL of Pioglitazone HCl prepared by diluting Sample solution with Mobile phase

Chromatographic system (*See Chromatography 621, System Suitability.*)
Detector: UV 269 nm
Column: 4.6-mm × 15-cm; 5-µm packing L1
Column temperature: 25 ± 2.5
Flow rate: 0.7 mL/min
Injection size: 40 µL
Run time: At least four times the retention time of Pioglitazone
[Note—Adjust the flow rate so that the retention time of the Pioglitazone peak is about 7 min.]

System suitability (*Samples: System suitability solution and Standard solution*)
Suitability requirements
•Tailing factor: NMT 1.5 for Pioglitazone and Benzophenone, System suitability solution
•Resolution: NLT 15 between Pioglitazone and Benzophenone, System suitability solution
•Relative standard deviation: NMT 3.0%, Standard solution

Analysis (*Samples: Standard solution and Sample solution*)
Calculate the percentage of each impurity in the portion of Pioglitazone Hydrochloride taken:

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

r_U = = peak response of each individual impurity from the Sample solution
r_S = = peak response of Pioglitazone from the Standard solution
D = = dilution factor used to prepare the Standard solution, 0.005

USP Method for Pioglitazone HCl RS

Acceptance criteria

Individual impurities: See Impurity Table 1.
Total impurities: NMT 0.5%

Table 1.

Compound	Relative Retention Time (RRT)	Limit (%)
Hydroxypioglitazone[a]	0.7	0.2
Pioglitazone	1.0	0.2
Didehydropioglitazone[b]	1.4	0.3
N-Alkylpioglitazone[c]	3.0	0.5
Any other individual impurity	-	0.2

a = (±)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}-5-hydroxythiazolidine-2,4-dione.

B =(Z)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzylidene}thiazolidine-2,4-dione.

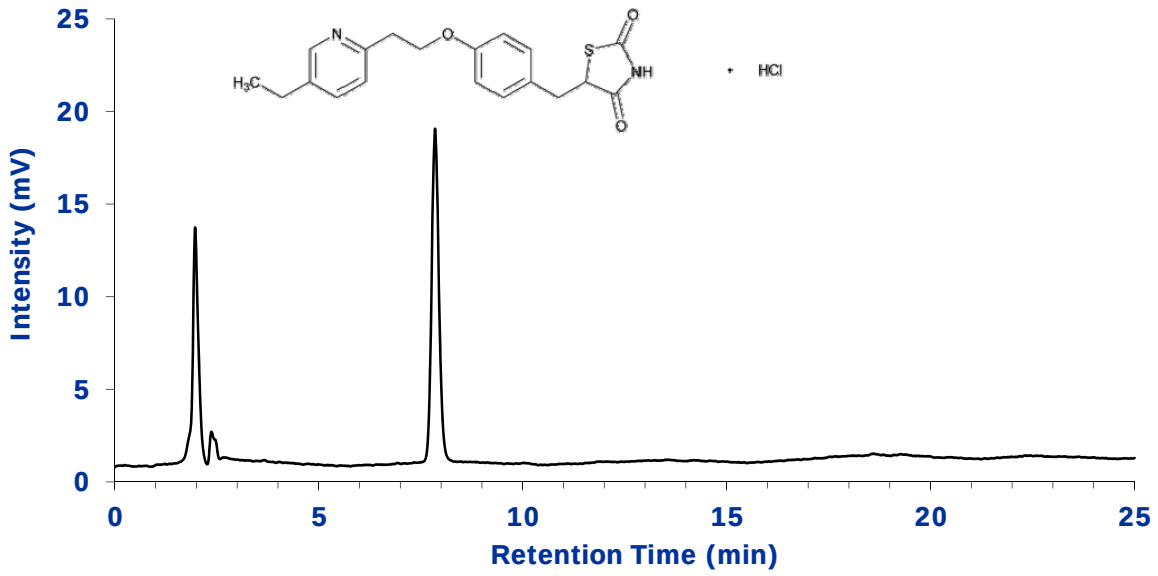
c = (±)-5-{4-[2-(5-Ethylpyridin-2-yl)ethoxy]benzyl}-3-[2-(5-ethylpyridin-2-yl)ethyl]thiazolidine-2,4-dione.

USP Method for Pioglitazone HCl RS

Purospher®STAR RP-18endcapped

Chromatographic Conditions

Column:	Purospher®STAR RP-18endcapped (5 µm) 150x4.6 mm	1.51455.0001
Injection:	40 µL	
Detection:	VWR-Hitachi LaChrom Elite DAD@269 nm	
Cell:	13 µL	
Flow Rate:	0.7 mL/min	
Mobile Phase (v/v):	Acetonitrile, 0.1 M ammonium acetate, and glacial acetic acid (25:25:1)	
Temperature:	Ambient	
Diluent	Mobile phase	
Sample:	1 µg/mL of Pioglitazone HCl	
Pressure Drop:	67 Bar (972 psi)	



Chromatographic Data

No.	Compound	Time (min)	Relative Retention Time (RRT)	Resolution	Asymmetry (T_{USP})
1	Pioglitazone HCl	7.6	1.0	0.0	1.09

Merck Millipore is a division of Merck KGaA, Darmstadt, Germany
www.merck-chemicals.com/chromatography
chromatography@merckgroup.com

Analysis protocol for Pioglitazone HCl RS

USP Method Repeatability

No	Compound	Response (Arbitrary Area Units)	Relative Standard Deviation (%)	N
1	Pioglitazone HCl	221512	0.5	6

Replicate injections of standard solution (n=6) were analyzed to determine the USP method repeatability. Sample contained 1 µg/mL of Pioglitazone HCl