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QUANTIFICATION OF ANTI-DIABETIC COMBINATION DRUGS BY HPTLC METHOD

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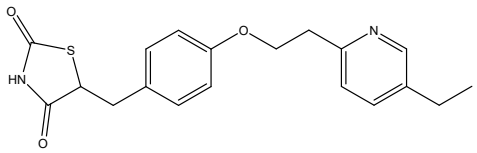
ABSTRACT

Pioglitazone and Glimepride are used for the treatment of type-II diabetes mellitus. Accurate, rapid, simple, precise high performance thin layer chromatographic method (HPTLC) for Pioglitazone and Glimepride has been established and validated according to ICH guideline. Separation was achieved on silica gel 60 F254 plates with Toluene: Ethyl acetate: Diethyl ether (6:3:1v/v/v) used as mobile phase. The Rf values for Pioglitazone and Glimepride were found to be 0.88 and 0.79.Results were linear in range of 6000-12000 ng/mL for Pioglitazone and 800-1600 ng/mL for Glimepride. The repeatability testing for sample and standard solutions were found as %RSD NMT 2.0% which was within the acceptable limits. Percentage recovery for Pioglitazone is 100.06% and for Glimepride is 99.94%. LOD for Pioglitazone and Glimepride was found to be 2.75ng/mL and 1.10ng/mL and LOQ for Pioglitazone and Glimepride was 8.34ng/mL and 3.33 ng/mL. The developed method was validated for linearity, precision, accuracy, robustness, limit of detection, limit of quantification according to ICH guideline.

Keywords: HPTLC, Pioglitazone, Glimepride, LOD, LOQ, PIO, GLI

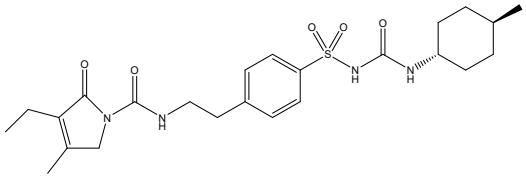
INTRODUCTION

Pioglitazone is a thiazolidinedione derivative chemically it is 5-[[4-[2-(5-ethylpyridin-2-yl) ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione **[1]**. It is one of the PPAR-alpha agonist, insulin sensitizer used to reduce the insulin resistance **[2]**. Glimepride (GLI) is a sulfonylurea derivative chemically-[[p-[2-(3-ethyl-4-methyl-2-oxo-3- pyyroline-1-oxamide) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea and also used in type 2 diabetes **[3]**. Glimepride binds to ATP-sensitive potassium channel receptors. Reducing potassium conductance and causing depolarization of the membrane which stimulates calcium ion influx through voltage-sensitive calcium channels. This increase in intracellular calcium ion concentration induces the secretion of insulin **[4]**.



Pioglitazone

Figure 1: Structure of Pioglitazone



Glimepride

Figure 2: Structure of Glimepride

MATERIALS AND METHODS

Chemicals and reagents

Pioglitazone and Glimepride drugs were obtained as a gift from Arbindo Pharma Pvt Ltd. The reagents Methanol, Acetonitrile, Toluene, Ethyl acetate, Diethyl ether of Thermo scientific India.

Instrumentation

The HPTLC system manufactured by AETRON consists of Linomat – 5 sample applicator, variable wavelength programmable AETRON TLC Scanner – 3, AETRON Twin-trough chambers, Hamilton syringe (100 µL). Chromatographic analysis was performed on aluminum packed silica gel 60 F254 HPTLC plates (Merck, Darmstadt, Germany) using Spraylin software. Quantification is done through visible light, UV-254, 365nm with EOS utility using JUST TLC or AETRON IDS software.

Mobile phase preparation

For better resolution of Pioglitazone and Glimepride- Toluene: Ethyl acetate: Diethyl ether was used as mobile phase in the ratio of 6:3:1 v/v/v.

Preparation of Standard stock solution

Accurately weighed 150mg of Pioglitazone and 20mg of Glimepride in 100ml volumetric flask ,3/4th diluent was added. The solution

was sonicated for 30 minutes to dissolved and made up to volume with diluent. The solution were filtered through 0.45µ Millipore Nylon filter.

Preparation of Sample solution

Accurately weighed 20 tablets and average weight of the tablets was calculated.10 tablets were weighed and transferred into 100 ml volumetric flask with 70ml of diluent. The solution is sonicated for 30 minutes with intermediate shaking and made up to volume with diluent. Further solution is filtered through 0.45µ Millipore Nylon filter. Further, 6ml filtered solution is transferred into 10ml volumetric flask, diluted with diluents up to the volume.

Optimization of HPTLC method

The HPLTC method was optimized on 10cm×10cm aluminium TLC plate coated with silica gel 60 F_{254}. The plates were pre washed with methanol and activated at 60^0 C for 5 min. The samples were spotted in form of bands with 8mm width by using AETRON sample applicator equipped with 100 µL Hamilton syringe. The application rate was 20µL/sec. Ascending mode of development on the plate was performed at 25 ± 2^0c using Toluene: Ethyl acetate: Diethyl ether (6:3:1v/v) as mobile phase in a twin through glass chamber(AETRON,10cm×10cm) which is previously saturated with 10ml mobile phase for 30 minutes. The mobile phase was allowed to run up to 8cm. The plates were dried in air, after development. Documentation was performed at 365 nm with an AETRON TLC scanner equipped with Aetron IDS software, using a deuterium light source **(Table 1)**.

Method Validation

The following parameters were validated according to ICH Q2 (R1) guidelines include linearity, precision, accuracy, robustness, limit of detection, limit of quantification.

System suitability

System suitability can be defined as a process of checking the system, before or during the analysis of unknowns, to ensure system performance. Accurately weighed 150mg of Pioglitazone and 20mg of Glimepride in 100ml volumetric flask $3/4^{th}$ diluent was added. The solution was sonicated for 30 minutes to dissolved and made up to volume with diluent. Further, 6ml filtered solution is transferred in to 10ml volumetric flask, diluted with diluents up to the volume. The solution was filtered through 0.45µ Millipore Nylon filter.30 µL of standard solutions of drug were injected in triplicate into chromatographic system**.** %RSD was calculated for Pioglitazone and Glimepride **(Table 2, Figure 3)**.

Table 1: Optimized parameters for HPTLC method

Stationary phase	**10cm×10cm silica gel 60 F_{254} Aluminium sheets**
Mobile phase	**Toluene: Ethyl acetate: Diethyl ether (6:3:1v/v)**
Dosage speed	**20µL/sec**
Band length	**8mm**
Sample volume	**30µL**
Detection wavelength	**365nm**

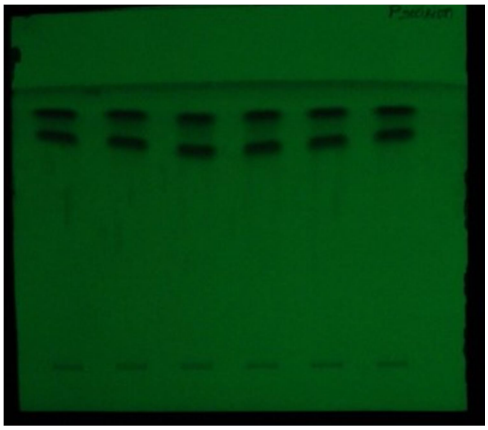


Figure 3: Chromatogram for system suitability

Table 2: System suitability data:

S. No.	Peak area		Rf values	
	PIO	GLI	PIO	GLI
1	1065	983	0.85	0.78
2	1069	972	0.87	0.79
3	1075	966	0.88	0.78
4	1068	985	0.87	0.76
5	1069	965	0.88	0.75
6	1090	953	0.86	0.79
Mean	1072.667	972.33	-	-
SD	0.85	9.54	-	-
%RSD	0.60	0.98	-	-

Acceptance criteria: The %RSD should be NMT 2.0%

Linearity

Linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of the analyte of the sample. The linearity was studied by using Toluene: Ethyl acetate: Diethyl ether (6:3:1v/v) as mobile phase. Pipette out 4ml, 5ml, 6ml, 7ml, 8 ml from standard stock solution and made up with diluents up to the volume. The development was carried out to obtain the calibration curves by plotting areas against corresponding concentrations **(Table 3, Figure 4, 5)**.

Accuracy

The accuracy of the method was determined by performing recovery studies at 3 different concentrations (50%, 100%, 150% levels) by adding known amount of Pioglitazone and Glimepride. At each level 3 determinations

were done. Percentage recovery were studied for both the drugs **(Table 4, 5)**.

Precision

Precision refers to the closeness of two or more measurements to each other or it is the degree to which an instrument or process will repeat the same value **(Table 6)**.

LOD and LOQ:

LOD and LOQ are the lowest concentration of the analyte which can be quantified and LOQ can be equivalent to or much higher than LOD. LOD and LOQ for Pioglitazone and Glimepride were calculated from linearity by using the formula **(Table 7)**:

LOD = (3.3 x SD / S)

LOD = (10 x SD / S)

Where SD- standard deviation

S- Slope of the calibration curve

Robustness

A: Chromatographic changes (Band length)

The robustness of this method was studied by altering various parameters like change in dosage speed, change in band length etc.

B: Chromatographic changes (Speed)

The robustness of this method was studied by altering the dosing speed (20ng/sec and 16 ng/sec). **(Table 8)**.

ASSAY RESULTS

20 tablets were weighed accurately and crushed into powder. From the pooled powder weighed equivalent to 150mg Pioglitazone and 20mg Glimepride into 100ml volumetric flask with diluent. From the above solution pipette out 6ml into 10ml volumetric flask and make up to the mark with diluents **(Table 9)**.

Table 3: Report of linearity

SNO	Pioglitazone		Glimepride	
	Con(ng/mL)	Area	Con(ng/mL)	Area
1	6000	721	800	642
2	7500	912	1000	825
3	9000	1078	1200	952
4	10500	1264	1400	1123
5	12000	1445	1600	1284
Regression equation		Y=1.023x+0.833	Y=8.01x+3.333	
Slope(m)		1.203	8.01	
Correlation coefficient(R^2)		0.9999	0.9999	

Acceptance criteria: The correlation coefficient (R2) should be NMT 0.999

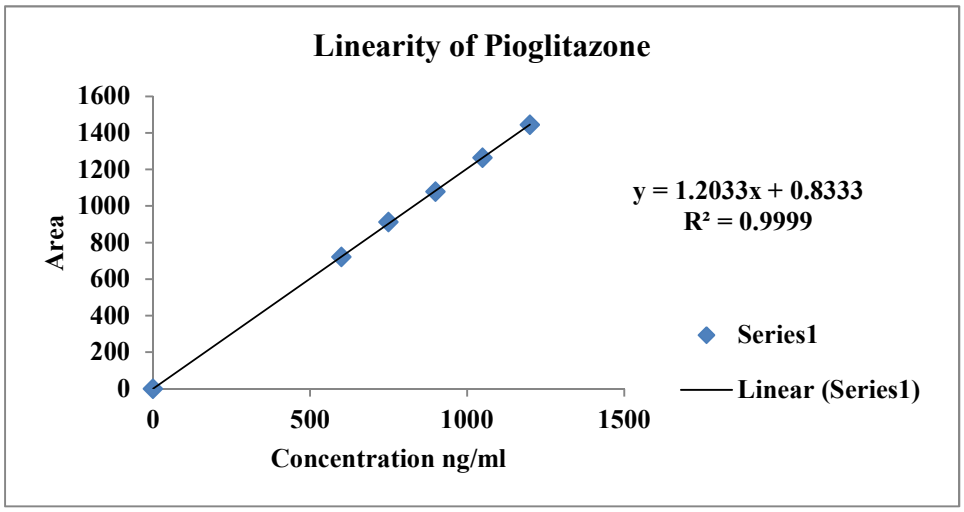


Figure 4: Calibration curve of Pioglitazone

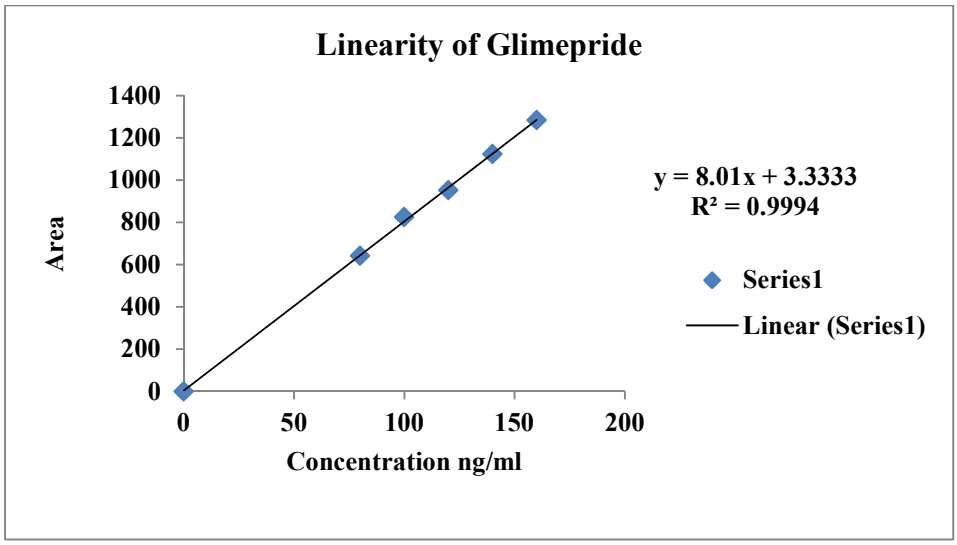


Figure 5: Calibration curve of Glimepride

Table 4: Accuracy data of Pioglitazone

Recovery level	Accuracy of Pioglitazone				Average percentage recovery
	Standard area	Sample peak area	% Recovery	% Mean Recovery	
50	1062	532	100.41	100.066	100.06
	1062	529	99.76		
	1062	531	100.03		
100	1062	1065	100.57	100.016	
	1062	1058	99.82		
	1062	1056	99.66		
150	1062	1594	100.33	100.1	
	1062	1589	100.03		
	1062	1587	99.94		

Table 5: Accuracy data of Glimepride

Recovery level	Accuracy of Glimepride				Average percentage recovery
	Standard area	Sample peak area	% Recovery	% Mean Recovery	
50	955	472	99.06	99.6	99.94
	955	475	99.61		
	955	478	100.13		
100	955	951	99.87	99.99	
	955	958	100.51		
	955	949	99.60		
150	955	1435	100.44	100.25	
	955	1429	100.04		
	955	1432	100.28		

Acceptance criteria: The mean % recovery for each level should not be less than 98.0% and NMT 102%

Table 6: Precision data

Injection No.	Area of Pioglitazone		Area of Glimepride	
	Method precision	System precision	Method precision	System precision
1	1072	1062	973	953
2	1063	1065	972	959
3	1079	1069	987	966
4	1052	1077	979	957
5	1063	1068	985	968
6	1021	1095	979	957
Mean	1058.333	1072.667	999.1667	960
SD	12.04	5.79	20.45	6.08
%RSD	1.12	0.60	1.93	0.62

Acceptance criteria: The %RSD should be NMT 2.0%

Table 7: LOD and LOQ for Pioglitazone and Glimepride

SNO	PIO	GLI
LOD(µg/ml)	2.75	1.10
LOQ(µg/ml)	8.34	3.33

Table 8: Report of Robustness data

Parameter	Area of Pioglitazone	Area of Glimepride
Change in Band length 9mm	1121	970
	1107	965
Change in Band length 7mm	1098	960
	1096	952
Average	1105.5	961.75
Standard deviation	11.38	7.67
%RSD	1.0300	0.798
Change in Dosage speed 16mL/sec	1073	975
	1082	979
Change in Dosage speed 20mL/sec	1092	987
	1095	988
Average	1085.5	982.25
Standard deviation	10.01	6.29
%RSD	0.922	0.640

Acceptance criteria: The %RSD should be NMT 2.0%

Table 9: Results of Assay

Drug	Label claim	% Assay
Pioglitazone	150	99.48
Glimepride	20	99.82

CONCLUSION

Simple, specific and accurate method was developed for simultaneous determination of Pioglitazone and Glimepride by HPTLC method and validated according to ICH Q2 (R1) guideline in terms of linearity, precision, accuracy, robustness, limit of detection, limit of quantification. The linearity was in the range of 6000-12000 ng/ml for Pioglitazone and 800-1600 ng/ml for Glimepride. The best regression values were obtained. The recovery studies were performed for the accuracy of the proposed method and was found to be 100.06% for Pioglitazone and 99.94% for Glimepride. The LOD and LOQ for Pioglitazone was 2.75 µg/ml, 8.34 µg/ml and for Glimepride it was found to be 1.10 µg/ml and 3.33 µg/ml. The Rf values for Pioglitazone and Glimepride were found to be 0.88 and 0.79. Hence the developed method was successfully applied for the estimation of Pioglitazone and Glimepride by HPTLC method.

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