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Simultaneous Estimation of Glimepiride and Pioglitazone in Bulk and in Pharmaceutical Formulation by HPTLC Method

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A validated HPTLC method for simultaneous estimation of glimepiride and Pioglitazone in bulk and in tablet formulations is described. Separation was achieved on aluminum sheet of silica gel $60F_{254}$ using toluene : ethyl acetate : methanol (50 : 45 : 5 v/v/v) as mobile phase. Quantification was achieved with UV detection at 230 nm over the concentration range of 200–700 ng/spot and 1500–5250 ng/spot with mean recovery of 98.40 ± 0.675 and 98.75 ± 1.140 for glimepiride and pioglitazone, respectively. R_f values for glimepiride and pioglitazone were found to be 0.49 and 0.61, respectively. The method was validated in terms of accuracy and precision. The proposed method is simple, precise, sensitive and applicable for the simultaneous determination of glimepiride and pioglitazone in bulk powder and in tablets.

Key Words: Glimepiride, Pioglitazone, HPTLC.

INTRODUCTION

Glimepiride (GLIM) is a sulfonyl urea class of antidiabetic drug, chemically 1-({*p*-[2(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl}sulfonyl)-3-(*trans*-4-methylcyclohexyl) urea[1]. Pioglitazone hydrochloride (PIO) is a thiazolidinedione class of antidiabetic drug, chemically (±)-5-{*p*-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl}-2,4-thiazolidinedione hydrochloride[2]. Combination of glimepiride and pioglitazone provides synergistic anti-diabetic effects[3]. This combination is widely used in the treatment of diabetes. A survey of literature revealed that HPLC, HPTLC and gas chromatography method[4–18] have been reported for the estimation of glimepiride and pioglitazone. GLIM is not official in any pharmacopoeia. Only one method[9] has been reported for the simultaneous estimation of both the drugs. However, not a single HPTLC method has been reported for the simultaneous estimation of both the drugs. In the present investigation an attempt has been made to develop simple, accurate, precise and reproducible HPTLC methods for the simultaneous estimation of glimepiride and pioglitazone in bulk powder and in tablet dosage forms.

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EXPERIMENTAL

GLIM and PIO bulk powder were gifted by Sun Pharmaceutical, Baroda, India. All solvents, *viz.*, methanol, toluene and ethylacetate (S.D. Fine Chemicals) were of AR grade. Aluminum sheet of silica gel $60F_{254}$ (layer thickness (0.2 mm) 20 × 20 cm, E. Merck KgaA, Setu Scientific Services, Ahmedabad). A Camag HPTLC with Linomat V auto sprayer and Camag Scanner-III (Anachrom Analytical Services, Bombay), Camag flat bottom and twin trough developing chamber (20 × 20 cm) (Anachrom Analytical Services, Bombay), UV cabinet with dual wavelength UV lamp (Anachrom Analytical Services, Bombay).

Preparation of standard stock solutions

Standard GLIM stock solution (100 μg mL^{-1}): Accurately weighed GLIM (5 mg) was transferred in a 50 mL volumetric flask and dissolved in and diluted to the mark with methanol.

Standard PIO stock solution (750μg mL^{-1}): Accurately weighed PIO (37.5 mg) was transferred in a 50 mL volumetric flask and dissolved in and diluted to the mark with methanol.

Bulk solution

Accurately weighed GLIM (4 mg) and PIO (30 mg) were transferred in a 100 mL volumetric flask and dissolved in and diluted to the mark with methanol. It was further diluted with methanol to get solution having GLIM (10 μg/mL) and PIO (75 μg/mL).

Sample solution

Ten tablets each of two brands were weighed and powdered in a glass mortar and pestle and analyzed as follows: A mass of powder equivalent to one tablet was weighed and transferred to a 50 mL volumetric flask and methanol (40 mL) was added. It was sonicated for 15 min and final volume was made to the mark with methanol. The mixture was then filtered through nylon 0.20 μm-47 mm membrane filter. This solution (2.5 mL) was transferred to a 10 mL volumetric flask and diluted to the mark with methanol.

Method validation

Calibration curve (linearity): Analysis was performed on 20 × 10 cm HPTLC silica gel $60F_{254}$ aluminum plate. Calibration curves were plotted over a concentration range 200–750 and 1500–5250 ng/spot for GLIM and PIO, respectively. Standard zones were applied to the layer as bands by means of a Camag Linomat V automatic spotter equipped with a 100 μL syringe and operated with the settings: bend length 6 mm, distance between bands 8 mm, distance from the plate side edge 10 mm and distance from the bottom of the plate 10 mm. For the calibration curves accurately measured standard stock solution of GLIM (2, 3, 4, 5, 6, 7 μL) and standard stock solution of PIO (2, 3, 4, 5, 6, 7 μL) were applied to the plate. The plate was developed in developing chamber previously

saturated with the mobile phase for 30 min. After development the plate was dried in air and standard zones were quantified by linear scanning at 230 nm by Camag TLC scanner-III with a deuterium source. The calibration curves were constructed by plotting peak areas *vs.* concentrations with the help of Win-CATS software. Each reading was average of three determinations.

Accuracy (recovery): Accuracy is determined in terms of per cent recovery. Sample solutions (2.5, 5.0, 7.5, 2.5, 5.0, 7.5 μL) were applied to the plate. The first three spots were over-spotted with standard stock solution of GLIM (3 μL) while the remaining three with standard stock solution of PIO (3 μL).

Precision

Method precision (repeatability): Method precision experiment was performed by preparing the standard solution of GLIM and PIO six times and analyzing as per the proposed method. Percentage relative standard deviation should not be more than 2%.

Intermediate precision (reproducibility): It is expresses within laboratory variations as on different days of analysis or equipment within the laboratory.

RESULTS AND DISCUSSION

Several mobile phases were tried to accomplish good separation of GLIM and PIO. Using the mobile phase toluene : ethylacetate : methanol (50 : 45 : 5 v/v/v) and 20 × 10 cm HPTLC silica gel $60F_{254}$ aluminum plate better separation was attained where R_f values were to be 0.49 for GLIM and 0.61 for PIO. A wavelength of 230 nm was used for the quantification of the drugs.

Validation of the proposed methods

Linearity: Linear correlation was obtained between peak areas and concentrations of GLIM and PIO in the concentration ranges 200–700 and 1500–5250 ng/spot, respectively. The linearity of the calibration graphs was validated by the high value of correlation coefficients of the regression (Table-1).

TABLE-1
OPTICAL AND REGRESSION CHARACTERISTICS FOR ANALYSIS OF GLIM AND PIO BY HPTLC METHOD

Parameters	GLIM	PIO
Concentration range	200–700 ng/spot	1500–5250 ng/spot
LOD	23.15 ng/spot	186.62 ng/spot
LOQ	70.15 ng/spot	565.51 ng/spot
Regression equation (Y)	Y = 5.585x + 447.53	Y = 2.472x + 2374
Correlation coefficient (r)	0.9984	0.9988

Accuracy: The per cent recoveries obtained were 98.53–99.23 and 98.39–98.90 for GLIM and PIO, respectively by HPTLC method (Table-2). The low value of SD indicates that both the methods are accurate.

TABLE-2
DATA OF RECOVERY STUDY FOR GLIM AND PIO BY HPTLC METHOD

Content	Amount taken (ng/spot)	Amount added (ng/spot)	% Recovery ± SD (n = 3)
GLIM	300	100	98.53 ± 1.24
	300	200	99.23 ± 1.12
	300	300	98.87 ± 0.69
PIO	2250	750	98.39 ± 0.54
	2250	1500	98.86 ± 1.06
	2250	2250	98.90 ± 1.00

Precision

Method precision: Relative standard deviation of all the parameters is less than 2% (0.67–1.90, Table 3), which indicates that the proposed method is repeatable.

TABLE-3
METHOD PRECISION DATA OF HPTLC METHOD FOR ANALYSIS OF GLIMPERIDE AND PIOGLITAZONE

PIO (3000 ng/spot)/ GLIM (400 ng/spot)	R_f		Area	
	PIO	GLIM	PIO	GLIM
1	0.61	0.51	10030.420	2752.28
2	0.61	0.50	9738.750	2859.27
3	0.61	0.49	9974.900	2822.30
4	0.61	0.49	9914.920	2857.57
5	0.61	0.49	10050.560	2784.94
6	0.61	0.49	9856.230	2888.51
Mean	0.6083	0.495	9927.630	2830.47
SD	0.004	0.008	117.340	53.82
%CV	0.67	1.69	1.182	1.90

Intermediate precision: The low values %CV of inter-day (0.68–1.67) and intra-day (0.67–1.75) precision reveals that the proposed method is robust (Table-4).

Analysis of bulk powder and tablets

The proposed validated methods were successfully applied to determine GLIM and PIO in bulk powder as well as in tablets. The percentage recoveries for GLIM and PIO obtained in bulk powder were 99.90 ± 0.201, 99.88 ± 0.254, respectively while the percentage recoveries for GLIM and PIO obtained in tablets were 98.61 ± 0.575, 99.00 ± 1.159, respectively (Table-5). No interference of the excipients with the peaks of interest appeared; hence the proposed method was applicable for the quantitative determination of GLIM and PIO in tablets.

TABLE-4
INTERMEDIATE PRECISION DATA FOR ANALYSIS OF GLIM AND PIO BY HPTLC METHOD

Concentration		Intraday				Interday			
		GLIM		PIO		GLIM		PIO	
GLIM ng/spot	PIO ng/spot	Mean ± SD n = 3	CV (%)	Mean ± SD n = 3	CV (%)	Mean ± SD n = 3	CV (%)	Mean ± SD n = 3	CV (%)
200	1500	1503±20.66	1.37	5908±39.38	0.67	1493±21.22	1.42	5885±40.15	0.68
300	2250	2175±38.00	1.75	8050±117.67	1.46	2160±36.00	1.67	7988±120.25	1.50
400	3000	2715±39.73	1.46	9921±159.44	1.61	2685±40.12	1.50	9858±160.25	1.62
600	4500	3796±33.02	0.87	13540±202.74	1.50	3756±33.56	0.89	12987±210.02	1.61
700	5250	4335±48.01	1.11	15251±180.00	1.18	4325±47.95	1.10	14976±182.30	1.21

TABLE-5
APPLICATION OF PROPOSED METHOD TO THE DETERMINATION OF BULK POWDER AND TABLETS [BRAND-I AND BRAND-II (GLIM 2 mg AND PIO 15 mg PER TABLET]

Content	Sample	% Amount found ± SD n = 3
GLIM	Bulk powder	99.90 ± 0.201
	Brand-I	98.61 ± 0.575
	Brand-II	98.18 ± 0.880
PIO	Bulk powder	99.88 ± 0.254
	Brand-I	98.50 ± 1.132
	Brand-II	99.00 ± 1.159

Comparison with the reported methods

Statistical comparison of the results obtained by proposed method with the results obtained by reported method[9] shows good agreement and indicates no significant difference (Table-6). It also shows that calculated t-values are less than theoretical ones, confirming accuracy and precision at 95% confidence level.

TABLE-6
COMPARISON BETWEEN RESULTS OBTAINED BY THE PROPOSED METHOD AND THE REPORTED METHOD

Parameters	Proposed method		Reported HPLC method[9]	
	GLIM	PIO	GLIM	PIO
Concentration range	200–700 ng/spot	1500–5250 ng/spot	200–1000 ng mL^{-1}	1000–7500 ng mL^{-1}
%Recovery ± SD	98.61 ± 0.57	99.00 ± 1.15	99.15 ± 1.858	99.50 ± 1.215
n	5	5	5	5
Variance	0.324	1.322	3.45	1.476
t-Value (2.31)*	0.621	0.668	—	—

*Figures in parentheses represent corresponding t-tabulated values at p = 0.05

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