Normal Phase Thin Layer Chromatography and Simultaneous Densitometric Determination of Rosiglitazone and Glimepiride in Tablet Dosage Form

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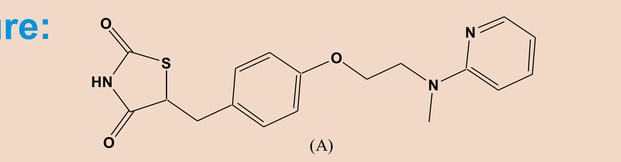
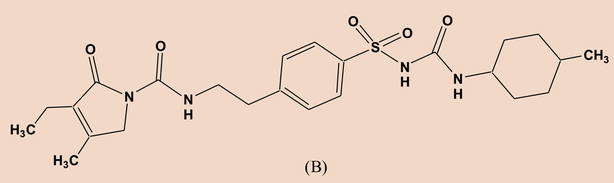
Drug Profile:

Structure:



(A)



(B)

(A) Rosiglitazone (ROS), (B) Glimepiride (GLM)

Category : Antidiabetic

Chemical Name

(A) (RS)-5-[4-(2-[methyl(pyridin-2-yl)amino] ethoxy) benzyl] thiazolidine-2,4-dione
(B) (4-(2-(3-ethyl-4-methyl-2-oxo-2,5-dihydro-1H-pyrrole-1 carboxamido) ethyl)phenylsulfonyl)-3-(4-Methyl cyclohexyl) urea

Empirical Formula : ROS- $C_{18}H_{19}N_3O_3S$
GLM- $C_{24}H_{34}N_4O_5S$

Molecular Weight : ROS- 357.4, GLM- 490.6

Solubility : ROS- Methanol, DMSO, DMF
GLM- Methanol, DMF

Method Reported:

(A) Estimation of ROS by HPLC[1-2] and HPTLC[3-4],
(B) Estimation of GLM by UV[5], HPLC[6], LCMS[7], HPTLC[8]
(C) Simultaneous estimation by UV[9], HPLC[10,11], LCMS[12]

Experimental:

Instrument: CAMAG LINOMAT-IV sample applicator with CAMAG TLC SCANNER III (Densitometer) and winCAT'S 4.0 version software

Reagents and Chemicals :

	Drug/Dosage form/Chemical	Manufacturer
Pure Drug Sample	Rosiglitazone (PIO)	Glenmark Pharmaceuticals Ltd.
	Glimepiride (GLM)	Themis Lab Pvt. Ltd.
Tablet Formulation	*Rosicon-G*	Glenmark Pharmaceuticals Ltd.
Chemicals	Toluene, Methanol, Ethyl acetate and Formic acid	Qualigens
TLC Plate	Pre-coated silica gel G60, F_{254} HPTLC plates	E-Merck

Standard Solution: 250 µg/mL of ROS,
125 µg/mL of GLM in methanol

Selection of mobile phase: Toluene, Methanol, Ethyl acetate and Formic acid solution [7:3:1:0.01 (v/v)]

Selection of Wavelength: After application of spot of standard solution, development and drying of plate, bands were scanned over 200-400 nm wavelength range. Wavelength selected was 245 nm. Typical chromatogram and absorption spectra is shown in Figure 1.

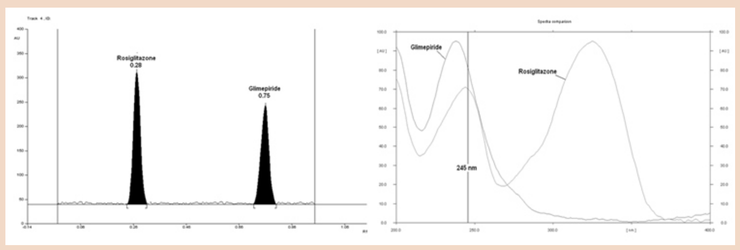


Figure 1: Chromatogram and absorption spectra

Chromatographic conditions:

Stationary Phase : Aluminium precoated TLC plates Silica Gel G60, F254 TLC Plate, size 10 x 10 cm, 200 µm layer thickness
Mode of Application: Band
Band Width : 4 mm
Sample volume : 6 µL
Application rate : 5 sec/µL
Separation Technique: Ascending
Development Chamber: Twin trough glass chamber, 10 x 10 cm.
Saturation Time : 15 min with mobile phase and Spotted plate
Migration Distance : 80 mm
Detection : UV Densitometric scanning
Scanning Mode : Absorbance/ Reflectance
Scanning speed : 20 mm/sec
Slit Dimension : 3 × 0.45 mm
Temperature : 25 ± 5ºC

Preparation of calibration curve: Aliquot portions of working standard solution (1-10 µL) were applied on the TLC plate and densitograms were developed under optimized chromatographic conditions and the calibration curves were obtained. Linearity curves are shown in Figure 2. The curves were found to be linear between concentration range 500-2000 ng/spot for ROS and 250-1000 ng/spot GLM both by height and area. Results are summarized in Table 6.

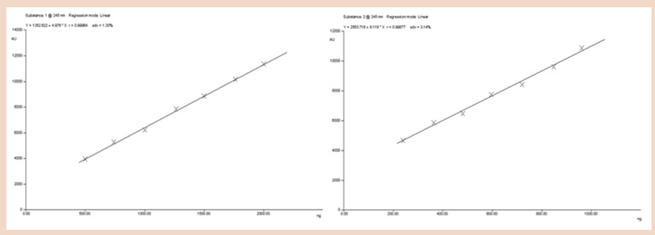


Figure 2: Linearity by area for ROS and GLM

Application of Proposed Method for Estimation in Marketed Formulation:

Twenty tablets were weighed and finely powdered. An accurately weighed tablet powder equivalent to 25.0 mg of ROS was transferred into a 25 ml volumetric flask containing little methanol. The powder was dissolved in 25 ml methanol and the solution was sonicated for 30 min. The solution was cooled to room temperature and diluted up to the mark with methanol and filtered. A 6.25 ml of clear filtrate was transferred to a 25 ml volumetric flask and then volume was made up to the mark with methanol and used as working sample solution. Two bands of working standard and six bands of sample solution of equal volume (6 µL) were applied on TLC plate and the plate was developed and scanned as per optimized chromatographic conditions.

$$\% \text{ Labelled claim} = \frac{Ew \times D \times Avg.Wt.}{Va \times Ws \times Lc} \times 100$$

Ew =Drug estimated in applied volume (µL)
D = Dilution factor
Va = Volume of sample applied
Ws = Weight of sample
Lc = Labelled claim of drug (mg/ml)

Table 1. Results of assay

Component	Label claim (mg)	% of labeled claim* ± SD	% RSD
ROS	2	100.07 ± 0.1435	0.1434
GLM	1	100.03 ± 0.1334	0.1334

*Each value is a mean of five determinations

Validation of proposed method:

Precision:

Precision of estimation of ROS and GLM by proposed methods was ascertained by replicate analysis of homogenous samples of tablet powder.

Table 2. System, method and intermediate precision data

Formulation	Percent labeled claim by area*		System Precision	Method Precision	Intermediate Precision		
					Interday	Intraday	Different Analysts
ROSICON-G	ROS	Mean	99.88	99.80	99.59	99.86	99.39
		SD	0.383	0.413	0.552	0.226	0.136
		% RSD	0.383	0.414	0.554	0.226	0.134
	GLM	Mean	99.71	99.83	99.52	99.77	99.47
		SD	0.331	0.225	0.287	0.062	0.187
		% RSD	0.332	0.226	0.289	0.062	0.188

Each value is a mean of five determinations

Accuracy:

Accuracy of Proposed method was ascertained on the basis of recovery studies were carried out by standard addition method.

Table 3. Results from recovery analysis

ROSICON-G Tablet (Avg. Wt. 172.23 mg)						
Sr. No.	% Spiking Level	Wt. of sample + std. ROS# + std. GLM# (mg)	Amount of standard drug recovered by area (mg)		% Recovery*	
			ROS	GLM	ROS	GLM
1	80	602.82 + 1.0 + 0.5	1.01	0.50	101.20	99.80
2	100	602.05 + 3.0 + 1.5	2.99	1.50	99.70	99.73
3	120	602.67 + 5.0 + 2.5	4.99	2.50	99.78	100.04
				Mean	100.23	99.86
				± SD	0.8439	0.1613
				% RSD	0.8420	0.1615

*Each value is a mean of six determinations, #Added in the form of standard stock solution,

Specificity:

The specificity of the method was ascertained by how accurately and specifically the analyte of interest are estimated in the presence of other components (e.g. impurities, degradation products, etc.) by exposing the sample to different stress conditions such as acidic (0.1 N HCl), alkaline (0.1N NaOH), oxidizing (3% H_2O_2), heat (60°C) and UV radiations for 24 h and then analyzing them by proposed method.

Table 4. Results of specificity study

Sr. No.	Sample	% labeled claim by area	
		ROS	GLM
1.	Normal	99.73	99.13
2.	Acid	42.02	99.75
3.	Alkali	31.72	99.45
4.	Oxide	90.32	99. 03
5.	Heat	99.91	99.43
6.	Sunlight	93.96	98.53

Ruggedness:

It is a degree of reproducibility of test results obtained by the analysis of the same samples under variety of conditions such as different laboratories, different analyst and different instruments and different days. Results are shown in Table 2.

Robustness:

It is the ability of the analytical method to remain unaffected by small but deliberate variation in method parameter and provide its reliability during normal usage.

Table 5. Results of Robustness

Method Parameter		ROS			GLM		
		Mean*	SD	% RSD	Mean*	SD	% RSD
Wavelength	243 nm	100.75	0.7374	0.7319	100.36	0.6049	0.6027
	247 nm	98.69	0.7018	0.7111	98.97	0.4799	0.4849
Temperature	22°C	98.42	0.5230	0.5314	98.56	0.3409	0.3458
	28°C	99.55	0.9378	0.9420	99.86	0.7877	0.7887
Saturation period	8 min	99.08	0.8050	0.8125	99.33	0.5716	0.5755
	12 min	98.60	0.5305	0.5381	98.90	0.4306	0.4354

LOD and LOQ:

Table 6. Analytical Performance Data

Parameters	ROS		GLM	
	By height	By area	By height	By area
Linear dynamic range (ng/band)	500–2000	500–2000	250–1000	250–1000
Slope	0.155	4.976	0.285	8.118
Y-intercept	76.554	1352.622	151.010	2563.718
Correlation coefficient (r)	0.999	0.999	0.998	0.997
LOD (µg/mL)	165.37	130.42	127.11	94.49
LOQ (µg/mL)	501.13	395.22	385.19	286.34

Result and Discussion:

Results of marketed formulation of ROS and GLM were found to be 100.07±0.1435 and 100.03±0.1334 respectively.
The average recovery values are obtained were 100.23±0.8439 and 99.86±0.1613.
The proposed method is simple fast cost effective and therefore can be applied for routine quality control of pharmaceutical preparations.

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