SIMULTANEOUS DETERMINATION OF ROSIGLITAZONE AND GLICLAZIDE IN PHARMACEUTICAL DOSAGE FORMS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A simple reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of rosiglitazone (RGL) and gliclazide (GLC) in pure and pharmaceutical dosage forms. A phenomenex Gemini reverse phase C₁₈ column (150x4.6mm i.d., 5μ) was used with a mobile phase containing a mixture of acetonitrile and water (pH 3 adjusted with ortho phosphoric acid) in the ratio of 70: 30. The flow rate was 0.6mL/min. and effluents were monitored at 250nm and eluted at 2.41min. (RGL) and 5.22min. (GLC). Calibration curve was plotted with a range from 0.025-2.5μg/mL for RGL and 0.08 to 8μg/mL for GLC. The assay was validated for the parameters like accuracy (>97.87% recovery), precision (intra-day and inter-day with %RSD < 2), robustness and system suitability parameters. Hence the method was found to suitable for the routine quality control of the drugs in pure and pharmaceutical dosage forms.

KeyWords: Rosiglitazone, Gliclazide, RP-HPLC, validation, Pharmaceutical dosage forms

INTRODUCTION

Rosiglitazone [(±)-5-[4-[2-[N-methyl-N(2-pyridyl)amino]ethoxy]benzyl]-2,4-dione thiazolidine] and gliclazide 1-(hexahydrocyclopenta[c]pyrrol-2(1H)-yl)-3-tosylurea (Fig. 1a & 1b) a combination of drugs belong to thiazolidine dione and sulfonyl urea groups of anti-diabetic drugs which is one of the most successful combination used in the treatment of type 2 diabetes. Rosiglitazone is highly bound to plasma proteins (99.8%) and is primarily eliminated via metabolism in the liver by "cytochrome P450 isoenzyme 2C8 exert the glucose-lowering effect by binding to peroxisome proliferator-activated receptors gamma (PPARγ)" thus increasing the receptor sensitivity to insulin¹⁻³ where as gliclazide act by increasing the secretion of insulin by the functioning β-cells of the pancreas⁴.

Fig. 1: structure of (a) rosiglitazone and (b) gliclazide.

The literature reveals few reported methods for rosiglitazone and gliclazide individually on HPLC, HPTLC and LC-MS⁵⁻⁸ and a method on HPTLC⁹ in combination dosage forms. As the combination of rosiglitazone and gliclazide is one of the successful combination therapies of diabetes mellitus type II (thiazolidinedione and sulfonyl ureas). Commercially this combination is available as tablet dosage forms with gliclazide 80mg and rosiglitazone 1 and 2mg dose. Hence it is necessary to develop a method to determine the combination in pure and formulations. The present paper describes a simple, sensitive, validated and economic method for the simultaneous determination of rosiglitazone and gliclazide.

EXPERIMENTAL

MATERIALS AND REAGENTS:

Rosiglitazone (99.5%) and Gliclazide (99%) were obtained from Orchid Chemicals and Pharmaceuticals Ltd, Chennai, India. Acetonitrile and Methanol (HPLC grade, Rankem, New Delhi, India), water (Milli Q). Other reagents were of AR grade. The formulations were purchase from the local pharmacy.

CHROMATOGRAPHIC CONDITIONS:

The HPLC system consisted of Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spincotech software version 1.7. Analysis was carried out at 250nm using a phenomenex Gemini C₁₈ reverse phase column (150x4.6mm i.d., 5μm) at room temperature i.e., 25±2 °C. The mobile phase consisted of Acetonitrile: water (pH 3 adjusted with ortho phosphoric acid) in the ratio of (70: 30, v/v) and that was set at a flow rate of 0.6mL/min.

PREPARATION OF STOCK AND SAMPLE SOLUTIONS:

The standard stock solutions were prepared with methanol to give the final concentration of 1000μg/mL. The working standard solutions of RGL and GLC were prepared by taking suitable aliquots of drug solution from the standard solutions and the volume was made up to 10 mL with mobile phase to get concentrations of 0.025-2.5μg/mL for RGL and 0.08 to 8μg/mL for GLC.

For the analysis of pharmaceutical dosage forms, ten tablets were weighed and powdered. A quantity equivalent to one tablet containing 2 mg of rosiglitazone and 80 mg of gliclazide was transferred into extraction flask, to this suitable amount of methanol was added and the mixture was subjected to vigorous shaking for 30 min. to completely extract drugs, and then centrifuged at 5000rpm for 20min (Remi R8C laboratory centrifuge). Supernatant was collected from each set and diluted with mobile phase to get a final concentration with in linearity range and injected to HPLC system for the analysis.

RESULTS AND DISCUSSION

The chromatographic conditions were optimized by changing the composition of mobile phase where different ratios were experimented to optimize the mobile phase. Finally a mixture of Acetonitrile and water (pH 3 adjusted with ortho phosphoric acid) in the ratio of 70: 30 was used which eluted better resolved peaks (R_s -3.93) of both the drugs which are symmetric with minimum tailing (1.37 and 1.4 for RGL and GLC respectively).

A typical chromatogram obtained by using the aforementioned mobile phase from 20μL of the assay preparation is illustrated in Fig. 2. The retention factors of RGL and GLC were 2.41 and 5.22 min., respectively.

Fig. 2: A typical chromatogram showing the peaks of rosiglitazone and gliclazide in bulk.

Fig. 3: A typical chromatogram showing the peaks of rosiglitazone and gliclazide in pharmaceutical dosage forms.

The linearity of the method was tested from 0.025-2.5 µg/mL for RGL (0.025, 0.05, 0.1, 0.5, 1.0, 1.5 and 2.5 µg/mL) and 0.08 to 8 µg/mL for GLC (0.08, 0.16, 0.32, 1.6, 3.2, 6.4 and 8 µg/mL). Solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/mL. In the simultaneous determination, the calibration graphs were found to be linear for both the analytes in the mentioned concentrations and the correlation coefficients for the regression line were 0.9986 and 0.9991 for RGL and GLC respectively. The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug to the placebo. The recovery was determined at three levels, viz. 50%, 100%, and 150% of the selected concentrations. Three samples were prepared for each recovery level. The recovery values for GLC and RGL ranged from 98.26-101.93% and 97.76-100.34%, respectively (Table 1). The precision (repeatability and intermediate precision) of the method was determined from one lot of combined dosage form. Intra and Inter day studies were performed by taking six replicates of three concentrations. The results are shown in (Table 2). The limit of detection (LOD) and limit of quantification (LOQ) for RGL, GLC was 0.006 µg/mL, 0.008 µg/mL and 0.02 µg/mL, 0.025 µg/mL, respectively was calculated by using signal-to-noise ratio ($S/n=3$ for LOD and $S/n=10$ for LOQ) and by dilute sample solutions. To determine the robustness of the developed method experimental conditions were purposely altered like mobile phase composition (68 to 72%), mobile phase pH (2.9 to 3.1) and flow rate (0.5 to 0.7 mL/min.) and RSD of the peak areas of RGL and GLC were found less than 2.0 illustrate the robustness of the method.

Table 1: Recovery of RGL and GLC (n=3).

Sample ID	Concentration of drug (µg/mL)		% Recovery		%RSD			
	Pure Drug		Formulation					
	GLC	RGL	GLC	RGL	GLC	RGL	GLC	RGL
50%	40	1.0	80	2	99.22	97.87	1.76	2.12
100%	80	2.0	80	2	98.23	100.11	1.89	1.32
150%	120	3.0	80	2	99.49	98.73	2.34	1.90

Table 2: Precision data for RGL and GLC.

	Nominal concentrations (µg/mL)		Mean± S.D	
	GLC	RGL	GLC	RGL
1	40	1.0	39.67± 0.52	0.96± 0.01
2	60	1.5	58.76± 0.95	1.49± 0.01
3	80	2.0	78.35± 1.69	1.86± 0.03

Each mean value is the result of triplicate analysis for three times a day

Table 3: Results of the determination of rosiglitazone and gliclazide in Tablets (n=6).

	labeled	Amount (µg)	Assay	
	amount (mg)	Found± S.D	%RSD	%w/w
GLYROZ-2				
GLC	80	78.76±1.75	2.22	98.45
RGL	2	2.03±0.04	1.97	101.50
ROSINORM-G				
GLC	80	79.32±1.46	1.84	99.15
RGL	2	1.97±0.03	1.52	98.50

APPLICATION OF THE METHOD TO PHARMACEUTICAL DOSAGE FORMS:

The method is sensitive and specific for the quantitative determination of RGL and GLC and also subjected to validation for different parameters, hence applied for the estimation of drug in pharmaceutical dosage forms. Tablets from two different manufacturers were evaluated for the amount of RGL and GLC present in the formulations. Each sample was analyzed in triplicate after extracting the drug as mentioned above in experimental section. The amount of RGL and GLC was found to be within the range of 98%-102%. None of the tablet excipients were found to interfere with the analyte peak (Fig 3) and the results are shown in Table 3.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of rosiglitazone and gliclazide from pure and in pharmaceutical dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of rosiglitazone and gliclazide in combined dosage forms and can also be used for dissolution or similar studies.

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