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PharmTech

International Journal of PharmTech Research
CODEN(USA): IJPRIF ISSN : 0974-4304
Vol.1, No.3, pp 496-499 , July-Sept 2009

DETERMINATION OF PIOGLITAZONE AND GLIMEPIRIDE IN PHARMACEUTICAL FORMULATIONS AND RAT PLASMA BY RP-LC

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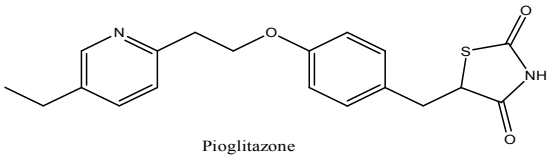
ABSTRACT: A simple and sensitive reverse phase high performance liquid chromatographic method for the determination of Pioglitazone (PIO) and Glimepiride (GLM) was developed on a Shimadzu Class vp series HPLC system with a phenomenex C_{18} column (150x4.6mm, 5µ) using a mobile phase mixture containing methanol and ammonium acetate buffer (pH-3.5) in the ratio of 55: 45. The flow rate was 0.5ml/min and effluents were monitored at 252nm and eluted at 5.63min (PIO) and 7.18min (GLM). Calibration curve was plotted with a range from 25-25000ng/ml for PIO and 10-10000ng/ml for GLM. The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters. The drugs were extracted from rat plasma by simple liquid-liquid extraction using diethyl-ether as extraction solvent. The results were found to be satisfactory and the method can be adapted for the routine quality control of the drugs in formulations and clinical samples.

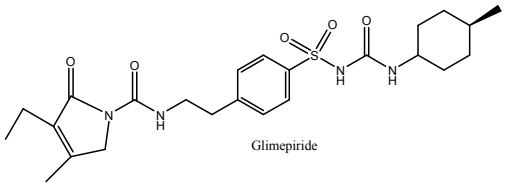
KEY WORDS: pioglitazone, glimepiride, reverse phase HPLC, liquid-liquid extraction, rat plasma, validation

INTRODUCTION:

Pioglitazone (shown in Fig 1a) is (±)-5-[p- [2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2,4-thiazolidinedione where as glimepiride (shown in Fig 1b) is 1-(4-(2-(3-ethyl-4-methyl-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamido)ethyl)phenylsulfonyl)-3-(4-methylcyclohexyl)urea[1] and are used in the treatment of type 2 diabetes. Glimepiride is a sulfonyl urea group oral anti-diabetic drug with prolonged effect and more over it maintains a more physiological regulation of insulin secretion than glibenclamide during physical exercise, suggesting that there may be less risk of hypoglycaemia with glimepiride, where as pioglitazone hydrochloride has been shown to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues (H. lkeda *et al*., 1990; Y. Sugiyama *et al*., 1990). Many patients suffering from type 2 diabetes require treatment with more than one anti-hyperglycemic drug to achieve optimal glycemic control.



Pioglitazone



Glimepiride

The literature reveals that there are some of the methods have been reported for pioglitazone and glimepiride in single dosage forms[2-7] and only few reports were found in combined dosage forms by UV[8] ,HPLC[9-10] and HPTLC[11-12] . The present paper describes a simple, sensitive, validated and economic

method for the determination of glimepiride and pioglitazone.

EXPERIMENTAL:

Materials and Reagents:

Pioglitazone and Glimepiride were obtained from Medley Pharmaceuticals Ltd., Daman and Diu, India. Methanol (HPLC grade, Qualigens, Mumbai), MilliQ water was used through out the analysis. All the other reagents were of AR grade.

Instrumentation:

The HPLC system consisted of a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spinco Win chrome software. The method was developed on a phenomenex C_{18} (150x4.6mm i.d, 5µm). Column maintained at ambient temperature. The mobile consisted of a mixture of methanol and ammonium acetate buffer (pH-3.5) fixed in the ratio of 55: 45 at a flow rate of 0.5ml/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 PIO and GLM were prepared by taking suitable aliquots of drug solution from the standard solutions and the volume was made up to 10ml with mobile phase to get concentrations of 25-25000ng/ml for PIO and 10-10000ng/ml for GLM.

For the analysis of pharmaceutical formulations, ten tablets were weighed and powdered. A quantity equivalent to labeled amount was weighed and transferred into extraction flask, to this suitable amount of methanol was added and the mixture was subjected to vigorous shaking for 30min for complete extraction of drugs, and then centrifuged at 5000rpm for 20min (Remi R8C laboratory centrifuge). Supernatant was collected from each set and diluted with mobile phase and injected to HPLC system for the analysis.

Extraction of drugs from rat plasma

Rat plasma was collected by centrifugation of blood and the drugs were spiked into the plasma and vortex mixed for 3min. The extraction of drugs was done by using diethyl-ether as a solvent. 20µg/ml solution of both PIO and GLM were used for the analysis.

RESULTS AND DISCUSSION:

Optimization of the method

A reversed-phase column procedure was proposed as a suitable method for the simultaneous determination of pioglitazone and glimepiride in combined dosage form and rat plasma. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of methanol and ammonium acetate buffer (pH-3.5) in the ratio of 55: 45 was used fixed at flow rate of 0.5ml/min was used for the elution of the drugs.

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 PIO and GLM were 5.63 and 7.18 min, respectively.

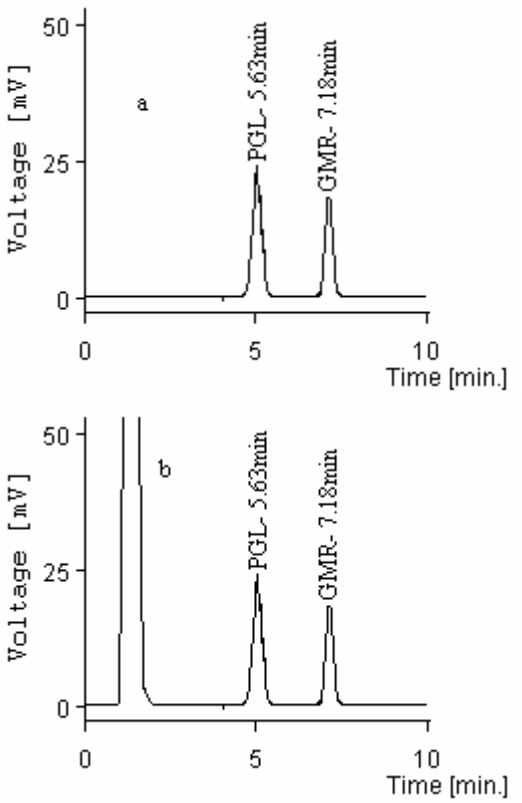


Fig 1-A typical chromatogram showing the peaks of pioglitazone (5.63min) and glimepiride (7.18min) (a) in pure forms, (b) rat plasma

Validation of the method

The linearity of the method was tested from 25-25000ng/ml for PIO and 10-10000ng/ml for GLM. Linearity solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in ng/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 0.9998 and 0.9986 for PIO and GLM respectively (sensitive than previous reports [9, 10]. 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 PIO and GLM ranged from 98-102% and 97-103%, 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 quantitation (LOQ) for PIO and GLM were found to be 10ng/ml, 23ng/ml and 4ng/ml, 10g/ml, respectively

indicates the sensitivity of the method. To determine the robustness of the developed method experimental conditions were purposely altered and RSD of the peak areas of PIO and GLM were found not greater than 2.0 illustrate the robustness of the method (Table 3).

APPLICATION OF THE METHOD TO PHARMACEUTICAL FORMULATIONS:

The method is sensitive and specific for the quantitative determination of PIO and GLM and also subjected to validation for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Each sample was analyzed in triplicate after extracting the drug as mentioned above in experimental section. The amount of rimonabant was found to be within the range of 96%-104%. None of the tablet excipients were found to interfere with the analyte peak and the results were shown in Table 4.

CONCLUSION:

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of pioglitazone and glimepiride from pure and pharmaceutical formulations and rat plasma. The mobile phase is simple to prepare and the run time was less than 10min which consumes only 5ml of mobile phase shows that the method was economical. The sample recoveries in all formulations were in good agreement with their respective label claims and samples extracted from plasma suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of pioglitazone and glimepiride in combined dosage forms and can also be used for dissolution or similar studies along with clinical samples.

Table-1 Recovery of PIO and GLM

Sample ID	Concentration of drug (µg/ml)				% Recovery	
	Pure Drug		Formulation			
	PIO	GLM	PIO	GLM	PIO	GLM
50%	30	4	60	8	99.12	98.88
100%	60	8	60	8	98.73	100.07
150%	90	12	60	8	99.29	98.73

n=3

Tabel-2 Precision data for PIO and GLM

Nominal concentrations (µg/ml)		Mean±S.D, %RSD	
PIO	GLM	PIO	GLM
15	2	14.67± 0.28, 1.90	1.96± 0.021, 1.07
30	4	28.76± 0.65, 2.26	3.93± 0.045, 1.15
45	6	44.36± 0.91, 2.05	5.86± 0.113, 1.93

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

%R.S.D= (S.D/mean) x100

Table-3 Robustness data (n=3)

Condition	PIO[a]	GLM[a]
Standard condition	0.86	0.79
Methanol 53%	1.09	1.11
Methanol 57%	1.21	1.29
Flow rate 0.4ml/min	1.39	1.31
Flow rate 0.6ml/min	1.28	1.38
pH 3.3	1.71	1.67
pH 3.7	1.49	1.81

Each value obtained from 15µg/ml and 2µg/ml of PIO and GLM respectively

[a]%RSD value

Table-4 Results of the determination of pioglitazone and glimepiride in Tablets (n=6)

Brand	Amount taken (mg)		Amount found (mg)		%RSD	Assay (%)	
	PIO	GLM	PIO	GLM		PIO	GLM
Glimy	15	2	14.79	1.92	1.87	98.6	96.0
G Tase G	15	2	14.81	1.94	2.03	98.7	97.0

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