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RESEARCH ARTICLE

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Pioglitazone HCl and Glimepiride in Tablets

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ABSTRACT

The aim of this paper was to develop a simple and validated RP- HPLC method for simultaneous estimation of Pioglitazone HCl (PGZ) and Glimepiride (GLM) in tablet dosage form. Chromatographic separation was achieved on a Kinetics C_{18} column (250 × 4.6 mm, 5µ). The mobile phase comprised of Acetonitrile: 25 mM ammonium acetate buffer (pH 4.6) 60:40%v/v. Isocratic elution mode was used at flow rate of 1 mL/min and all eluents were detected at 230 nm. The validation parameters studied were linearity, accuracy, precision, specificity and robustness. Calibration curves at seven levels for PGZ and GLM were linear in the range of 5-35 µg/mL and 2-14 µg/mL, respectively. Accuracy for both PGZ and GLM were studied in the range of 80-120 % QC standard levels. The method was found to be precise with respect to repeatability and intermediate precision at all QC standard levels. There was no interference from excipient in the analysis of PGZ and GLM. Hence, the proposed method can be used for analysis of routine quality control samples of PGZ and GLM tablets.

KEYWORDS

Pioglitazone HCl, Glimepiride, RP-HPLC, Analytical Method Validation

INTRODUCTION

PGZ is thiazolidinedione class having the hypoglycemic activity and is used for treating the diabetes. Chemically it is, 5-[[4-[2-(5-Ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2, 4-thiazolidinedione[1]. It is an antihypergycemic agent that, in the presence of insulin resistance, increases hepatic and peripheral insulin sensitivity, thereby inhibiting hepatic gluconeogenesis and increase peripheral and fatty acid glucose uptake[2].

Glimepiride, chemically is, 1-[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulphonyl]-3-trans-(4-methylcyclohexyl) urea[3].

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GLM belongs to the class of sulfonyl urea having anti-diabetic activity used in treatment of type 2 diabetes mellitus[4].

Literature survey revealed quantitative analytical methods for simultaneous estimation of PGZ and GLM by UV spectroscopy[5] and RP-HPLC[6-9]

The aim of the present work was to develop a validated RP-HPLC method for the simultaneous analysis of two anti-diabetic drugs, namely PGZ and GLM in tablet formulation.

MATERIAL AND METHODS

Chemicals and Reagents

Pharmaceutical grade PGZ and GLM were kindly supplied as a gift sample from Macleods Pharmaceuticals Ltd, Gujarat. Acetonitrile, methanol and acetic acid used in analysis were of

HPLC-grade and ammonium acetate was of AR-grade, all chemicals and reagents purchased from SD Fine Chemicals, Mumbai, Maharashtra. A freshly prepared double distilled water was used were prepared by Glass Double Distillation Assembly, purchased from Borosil, Mumbai, Maharashtra and further used after filtering through 0.45 μ membrane filter papers purchased from Millipore (India) Pvt. Ltd., Bengaluru, Karnataka. Tablets containing 15 mg of PGZ and 2 mg of GLM were prepared in-house.

Instrumentation and Chromatographic Conditions

JASCO High Pressure Gradient HPLC system equipped with dual PU 2080 plus pumps, multichannel UV detector, UV-2075 and injection loop (20 μL capacity), Rheodyne manual loop injector 7725*i* was used. Data were collected using Borwin Chromatography software (version 1.5). The mobile phase was composed of acetonitrile: 25 mM ammonium acetate buffer (pH 4.6) 60:40 %v/v. Isocratic elution was carried out on a Phenomenex Kinetics C_{18} column (250 × 4.6 mm, 5μ) at a flow rate of 1 mL/min. The wavelength was fixed at 230 nm.

Preparation of Standard Solution

Quantity equivalent to 10 mg of PGZ and GLM were weighed and transferred to separate 10 mL separate volumetric flasks and volume was made up to the mark with methanol. The resulting solutions were of 1000 μg/mL of PGZ and GLM, respectively.

System Suitability Standard

System suitability standard solutions were prepared daily by appropriate diluting standard stock solutions with mobile phase to get 15 μg/mL of PGZ and 2 μg/mL of GLM, respectively. System suitability was determined for six replicates of the mentioned concentrations and evaluated for USP acceptance criteria.

Calibration Curve Standards

Suitable aliquots of the above stock solutions were diluted with the mobile phase to get six different calibration curve standards with concentrations of 2, 4, 6, 8, 10, 12 and 14 μg/mL of PGZ and 5, 10, 15, 20, 25, 30 and 35 μg/mL of GLM, respectively. The calibration curve standards were analyzed in triplicates and the peak areas were plotted against concentrations to get an equation for best fit line. The slope, intercept, and co-efficient of regression were noted.

Estimation of PGZ and GLM in Tablets

Tablets containing 15 mg of PGZ and 2 mg of GLM, each, were prepared in-house. Twenty tablets were weighed and finely powdered. A quantity equivalent to 15mg of PGZ and 2mg of GLM was transferred to 100 mL volumetric flask and shaken with 70 mL methanol for 10 min. The excipients were separated by filtration and the volume was made up to the mark with the same solvent. From this solution, suitable aliquot was diluted with mobile phase to get concentrations of 15 μg/ml of PGZ and 2 μg/ml of GLM and subjected to chromatographic analysis under mentioned chromatographic condition. The amount of PGZ and GLM was obtained from the obtained calibration curve line equation.

Validation of the Method

The developed method was validated as per the ICH guidelines[10].

Accuracy and Precision

Accuracy of the method were determined by analyzing the quality control (QC) standard samples at three concentrations level of 80%, 100 % and 120% by standard addition method across the analytical range for PGZ and GLM. The method precision was established by three replicates of three QC levels (80%, 100% and 120%) for the intraday precision and on three successive days for the intermediate precision. The percent recovery of added concentration and % RSD were taken as measures of accuracy and precision, respectively. Also, the results obtained were subjected to one way ANOVA and the between – day mean square compared to the within – day mean square by F – test.

Specificity

To evaluate the specificity of the proposed

method, blank tablets were chromatographed. Absence of peaks in the chromatographic run at the retention time of the PGZ and GLM was taken as indication of specificity.

Robustness

Robustness of the method was studied by applying 2^3 full factorial design. The experimental matrix of 8 experiments was prepared with combination of each factor at each level. The independent variables selected were flow rate of mobile phase, concentration of acetonitrile and pH of the buffer. The dependent variable (Response) studied were number of theoretical plates, tailing and retention time of the last eluted peak.

RESULTS AND DISCUSSION

Various mobile phases were tried, and a mobile phase with composition of Acetonitrile: 25 mM ammonium acetate buffer (pH 4.6) 60: 40 *v/v* was found to resolve PGZ from GLM. The optimum wavelength for detection was 230 nm. The retention time of Pioglitazone and Glimepiride were observed at 4.27 min and 5.20 min respectively (Figure 1).

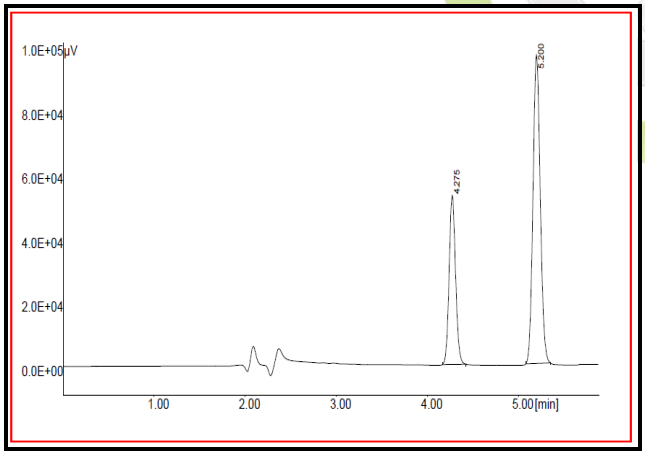

Figure: 1 Representative chromatogram of PGZ (4.27 min) and GLM (5.20 min)

System suitability test verifies that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be conducted. All critical parameters tested met the USP acceptance criteria (Area % RSD < 2, asymmetry < 2 and No. of theoretical plates > 2000).

In calibration studies, it was found that PGZ was linear in the range of 5-35 µg/mL and GLM 2-14 µg/mL. The calibration curves with their respective calibration curve equations and regressions are depicted in Figure 2 and Figure 3, respectively.

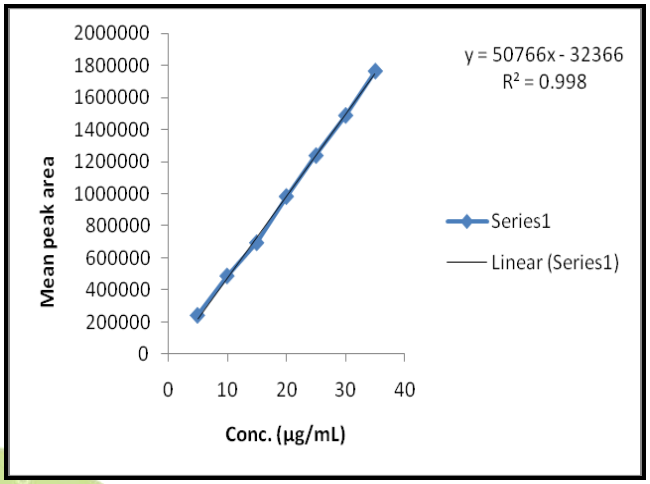

Figure 2: Calibration curve of PGZ

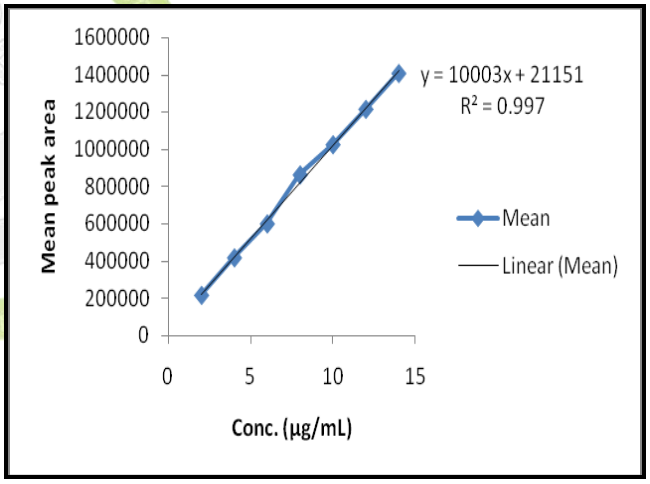

Figure 3: Calibration curve of GLM

When tablets prepared on lab scale were analyzed using the developed method, the results obtained were in good agreement with nominal amount of the drug. The drug content was found to be 99.82 ± 1.94 of the added amount.

The results obtained for accuracy and precision are summarized in Table 1 and Table 2, for PGZ and GLM, respectively. Mean values of concentration found were close to the concentration added and low values of % RSD indicates the acceptable accuracy and precision of the method.

Table 1: Accuracy and Precision studies of PGZ

Concentration added (µg/mL)	Concentration Found (µg/mL)			Within Mean Square	Between Mean Square	F- value
	Day 1	Day 2	Day 3			
(15 + 12) 27 µg/mL (80%)	27.2	26.92	26.9	0.0282	0.00271	0.88
	26.7	27.1	26.7			
	29.9	27	26.9			
Mean	26.99	27.006	26.83			
% RSD	0.934	0.3339	0.4303			
(15 + 15) 30 µg/mL (100 %)	30.3	30.4	30	0.0233	0.2144	0.91
	30.1	30.2	29.8			
	30	30.5	29.7			
Mean	30.133	30.36	29.833			
% RSD	0.506	0.5030	0.5120			
(15 + 18) 33 µg/mL (120%)	33.1	33	32.9	0.0588	0.04777	0.81
	32.9	32.9	33			
	32.6	33.4	33.3			
Mean	32.86	33.1	33.06			
% RSD	0.7657	0.7993	0.6295			

Table 2: Accuracy and Precision studies of GLM

Concentration added (µg/mL)	Concentration Found (µg/mL)			Within Mean Square	Between Mean Square	F- value
	Day 1	Day 2	Day 3			
(2 + 1.6) 3.6 µg/mL (80%)	3.6	3.61	3.54	0.00037	0.00057	1.53
	3.58	3.58	3.57			
	3.56	3.57	3.57			
Mean	3.58	3.5866	3.56			
% RSD	0.5586	0.5803	0.4865			
(2 + 2) 4 µg/mL (100 %)	3.98	3.99	4.01	0.00106	0.00034	0.32
	4	4.04	4.05			
	3.98	3.98	3.96			
Mean	3.9866	4.0033	4.0066			
% RSD	0.2896	0.8029	1.125			
(2 + 2.4) 4.4 µg/mL (120%)	4.39	4.41	4.4	0.00142	0.00201	1.41
	4.37	4.37	4.38			
	4.31	4.33	4.44			
Mean	4.3566	4.37	4.4066			
% RSD	0.9556	0.91533	0.6932			

Table 3: 2^3 factorial design experimental matrix for robustness test

Run No.	Flow rate (mL)	ACN conc. (%)	Buffer pH(mM)	Theoretical Plates	Asymmetry	R.T. of last eluted peak (i.e. GLM) (min)
1	0.8	50	3.6	29458	1.0	14.9
2	1.2	50	3.6	24730	1.03	10.2
3	0.8	70	3.6	19719	1.15	4.9
4	1.2	70	3.6	14494	1.10	3.2
5	0.8	50	5.6	25706	1.06	8.02
6	1.2	50	5.6	20932	1.05	5.3
7	0.8	70	5.6	24121	1.03	9.31
8	1.2	70	5.6	22143	1.04	7.12

Also, when the results of intra-day and inter-day were subjected to one-way ANOVA and F values were calculated at each QC level, the F values were found to be less than the tabulated F values. This indicated that there was no significant difference between intra-and inter-day variability, suggesting good intermediate precision.

When blanked tablets were analyzed as per the mentioned chromatographic conditions, no peak was obtained at the retention times of PGZ and GLM.

During robustness studies, it was observed that there was no significant effect on number of theoretical plates, asymmetry and on retention times of GLM (last eluted peak) by small but deliberate changes in flow rate, acetonitrile concentration and change of buffer pH The experimental matrix of 8 experiments for 2^3 factorial design is depicted in Table 3.

CONCLUSION

A new, simple and accurate method has been developed for simultaneous estimation of PLZ and GLM in tablets. All parameters of validation are within the acceptable range. The developed method is specific as there was no any interfering peak at the retention time of drugs.

Hence the developed method was accurate and can be used for routine analysis of Pioglitazone and Glimepiride in tablet dosage form.

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