

**A MODIFIED REVERSE PHASE LIQUID CHROMATOGRAPHIC METHOD
DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF
GLIMEPRIDE AND PIOGLITAZONE IN BULK AND DOSAGE FORMS****Kavuri Dwarathi*, D. Dhachinamoorthi**

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ABSTRACT

A modified reverse phase liquid chromatographic method was developed and validated for the simultaneous estimation of glimepiride and Pioglitazone in bulk and pharmaceutical dosage forms. The developed and validated method was simple, precise, accurate and economical reverse phase liquid chromatography method. Estimation of drugs in this combination was done with a C₁₈ column (Zorbax 100-5 column, 250mm x 4.6mm) passing mobile phase of composition phosphate buffer pH 7: Acetonitrile in ratio 45:55 v/v. The flow rate was 1 ml/min and the column effluents were identified at 230 nm. The retention time of Pioglitazone and Glimepiride were 6.7 min and 8.3 min respectively. The method was found to be linear over a range of 10-50µg/ml for Pioglitazone and 3-15µg/ml for Glimepiride. The validated method proved as reproducible one with a %RSD value of less than 2 and having the selectivity and accuracy within the specified limits. Assay of marketed dosage forms was determined and found to be with 98% and 99% for Pioglitazone and Glimepiride respectively. The method was validated according to the guidelines of International Council for Harmonization (ICH) and was successfully utilized in the simultaneous estimation of the commercial dosage forms. This liquid chromatographic method can be applied for the qualitative and quantitative determination of selected drugs.

KEYWORDS: Pioglitazone, Glimepiride, RP-HPLC, Method development and validation.**INTRODUCTION**

Pioglitazone is chemically designated as *RS*)-5-(4-[2-(5-ethylpyridin-2-yl) ethoxy] benzyl) thiazolidine-2,4-dione is a hypoglycemic drug with a pKa value of 14.64. Its molecular formula is C₁₉H₂₀N₂O₃S and it has a molecular weight of 165.52gm/mole. It is soluble in methanol, slightly soluble in acetone and acetonitrile. It is official drug in Martindale,^[1] Merck index^[2] and Indian Pharmacopeia 2007 and 2014.^[3]

Glimepiride is an oral hypoglycemic drug which is chemically designated as 1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl-3-(trans-4-methyl cyclohexyl) urea. The molecular formula is C₂₄H₃₄N₄O₅S and molecular weight is 490.62 gm/mol. It is a white to off-white crystalline powder. That is soluble in methanol and dimethyl formamide. It is official drug in Martindale,^[1] Merck index,^[2] and Indian Pharmacopeia 2007 and 2014.^[3]

After extensive literature survey it was proved that very few methods were reported for the estimation of Pioglitazone and Glimepiride individually or in combination by RP-HPLC^[4-22] but few methods had been reported for simultaneous estimation of these two

drugs. So we attempted to develop an accurate, rapid, precise, stable, sensitive and economically viable liquid chromatographic method for the simultaneous determination of selected drugs in the present research.

MATERIALS AND METHODS**Equipment used**

The chromatographic separation was performed on Agilent 1120 compact liquid chromatographic system integrated with a variable wavelength programmable UV detector and a Rheodyne injector equipped with 20µl fixed loop. A reverse phase C₁₈ [Zorbax 100-5 column, 250mm x 4.6mm] was used. Elico SL-210 double beam UV visible spectrophotometer and Axis AGN204-PO electronic balances were used for spectrophotometric determinations and weighing purposes respectively.

Reagents and chemicals

Pharmaceutical grade pure Pioglitazone and Glimepiride gift samples were procured from Mylan Laboratories, Hyderabad. Marketed formulation Tablets with dose of 50 mg of Pioglitazone and 15 mg of Glimepiride were procured from local market (Pioglar by Ranbaxy Pharma). HPLC grade Acetonitrile and Water were

procured from Merck specialties private limited, Mumbai.

Chromatographic conditions

Zorbax-C₁₈ column 5 μ m [250mm x 4.6mm] was used for the chromatographic separation at a detection wavelength of 230 nm. Mobile phase with a composition of Phosphate buffer pH 7 and Acetonitrile in a ratio of 45:55 v/v was selected for elution and same mixture was used in the preparation of standard and sample solutions. Flow rate was adjusted to 1 ml/min and the injection volume was 20 μ l.

Preparation of Mobile phase

Phosphate buffer pH 7 was prepared by dissolve 0.504 gm of disodium hydrogen phosphate and 0.301gm of Potassium dihydrogen phosphate of HPLC grade water and adjusts the pH to 7.0 with sufficient water was added to produce 100 ml filtered through 0.45 μ membrane filter and sonicated for 15 minutes.

Preparation of Standard solutions

25mg each of Pioglitazone and Glimpiride were accurately weighed and transferred into two 25ml volumetric flasks, dissolved using mobile phase and the volume was made up with the same solvent to obtain primary stock solutions A (Pioglitazone) and B (Glimpiride) of concentration 1000 μ g/ml of each drug. From the primary stock solutions 0.8ml and 0.5 ml were pipette out from A and B respectively, transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 80 μ g/ml and 50 μ g/ml of Pioglitazone and Glimpiride respectively and this solution is (working stock solution A).

Preparation of Sample Solution

Twenty tablets of Pioglitazone and Glimpiride were weighed and crushed. Tablet powder equivalent to 300 mg of Pioglitazone and 40 mg of Glimpiride was weighed accurately and transferred to a 25 ml volumetric flask. The content was dissolved with 10ml of mobile phase and then sonicated for 15min. The volume was made up with the mobile phase and filtered with 0.45 μ m membrane filter and sonicated for 20 min. 0.8 ml of this solution was pipette out and transferred to a 10 ml volumetric flask and the volume was made up with the mobile phase to obtain a concentration of 80 μ g/ml of Pioglitazone and 50 μ g/ml of Glimpiride (working stock solution B).

Optimization of RP-HPLC method

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Pioglitazone and Glimpiride. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Phosphate buffer pH 7 and Acetonitrile (45:55 v/v) using Zorbax-C₁₈ column 5 μ m [250mm x 4.6mm].

Validation of the RP-HPLC method

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

System suitability

System suitability was carried out with five injections of solution of 100% concentration having 50 μ g/ml of Pioglitazone and 15 μ g/ml of Glimpiride in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factors (T) were reported in table 1.

Linearity

For the determination of linearity, appropriate aliquots were pipette out from working stock solution A to a series of 10ml volumetric flasks and volume was made up with the solvent to obtain concentration ranging from 10-50 μ g/ml of Pioglitazone and 3-15 μ g/ml of Glimpiride. Each solution was injected in triplicate. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients. The calibration curves for Pioglitazone and Glimpiride were shown in figure 3 and figure 4 their corresponding linearity parameters were given in table 2.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae $LOD = 3.3 \sigma/s$ and $LOQ = 10 \sigma/s$. The results were given in table 2.

Precision

The repeatability of the method was verified by calculating the %RSD of six replicate injections of 100% concentration (50 μ g/ml of Pioglitazone and 15 μ g/ml of Glimpiride) on the same day and for intermediate precision % RSD was calculated from repeated studies on different days. The results were given in table 3.

Accuracy

To ensure the reliability and accuracy of the method recovery studies were carried out by standard addition method. A known quantity of pure drug was added to pre-analyzed sample and contents were reanalyzed by the proposed method and the percent recovery was reported. The results were given in table 4.

Specificity

Specificity of a method was determined by testing standard substances against potential interferences. The method was found to be specific when the test solution was injected and no interferences were found because of the presence of excipients. The optimized chromatogram of Pioglitazone and Glimpiride without any interference was shown in figure 2.

Robustness

Robustness of the method was verified by altering the chromatographic conditions like wavelength detection, flow rate, etc. and the % RSD should be reported. Small changes in the operational conditions were allowed and the extent to which the method was robust was determined. A deviation of ± 2 nm in the detection wavelength and ± 0.2 ml/min in the flow rate, were tried individually. A solution of 100% test concentration with the specified changes in the operational conditions was injected to the instrument in triplicate. %RSD was reported in the table 5

Ruggedness

Ruggedness of the method was verified by altering method parameters like different instruments, analysts, laboratories, reagents, days etc. The solution containing 50 $\mu\text{g/ml}$ of Pioglitazone and 15 $\mu\text{g/ml}$ of Glimepiride was injected into HPLC three times under different parameters like different analysts. %RSD was reported in the table 6.

Assay of Marketed Formulations

20 μl of sample solution of concentration 50 $\mu\text{g/ml}$ of Pioglitazone and 15 $\mu\text{g/ml}$ of Glimepiride was injected into chromatographic system and the peak responses were measured. The solution was injected three times in to the column. The amount of drug present and percentage purity was calculated by comparing the peak areas of the standards with that of test samples. A typical chromatogram for assay of marketed formulation was shown in figure 5 and the obtained values were reported in the table 7.

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, Phosphate buffer pH 7 and Acetonitrile in the ratio 45:55 v/v was selected as mobile phase because of better resolution and symmetric peaks. Pioglitazone and Glimepiride were found to show appreciable absorbance at 230nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Pioglitazone and Glimepiride at different Rts was shown in figure 2.

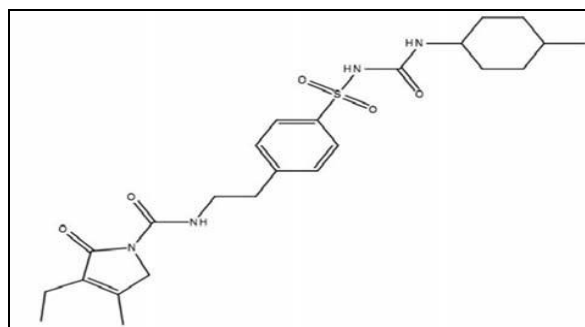
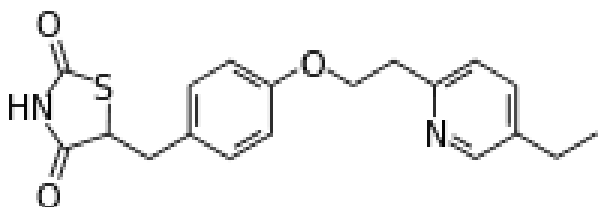


Fig. 1: Chemical Structures of a) Pioglitazone and b) Glimepiride.

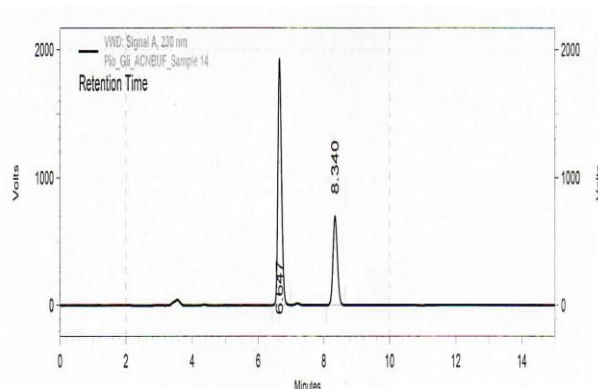


Fig. 2: Optimized chromatogram of Pioglitazone and Glimepiride.

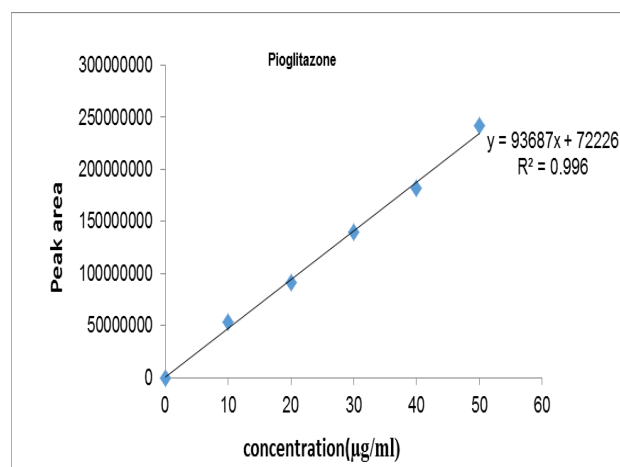


Fig. 3: Calibration plot of Pioglitazone.

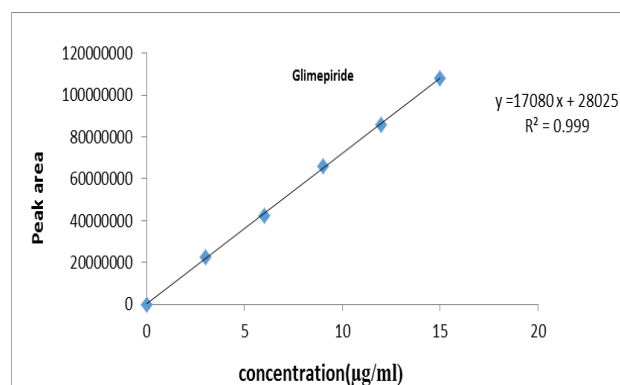


Fig. 4: Calibration plot of Glimepiride.

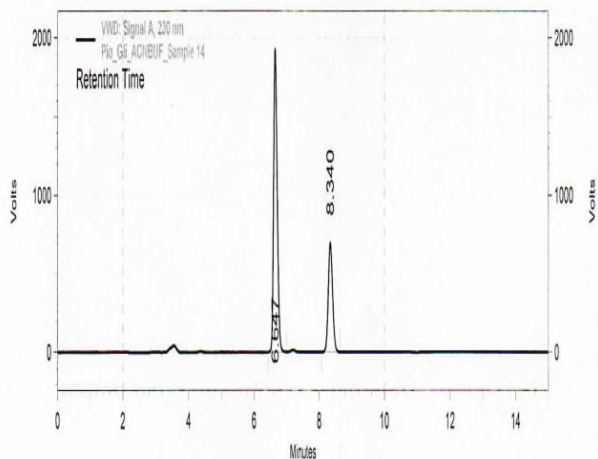


Fig. 5: Assay chromatogram of Pioglitazone and Glimepiride in Tablet formulation.

Table 1: System Suitability Parameters (n=5).

Parameters	Pioglitazone	Glimepiride
Retention time (min)	6.6	8.3
Theoretical plates (N)	8125	9145
Tailing factor (T)	1.2	1.3
Resolution (R _s)	1.657	

*n= No. of determinants

Table 2: Results for Linearity (n=3).

Parameter	Pioglitazone	Glimepiride
Linearity Range (µg/ml)	10-50	3-15
Regression Equation	y = 93687x + 72226	y = 17080x + 28025
Slope (m)	93687	17080
Intercept (c)	72226	28025
Regression Coefficient (r ²)	0.9999	0.9998
Limit of Detection (µg/ml)	0.425	0.52
Limit of Quantitation (µg/ml)	1.40	1.716

*n= No. of determinants

Table 3: Results of Precision (n=6).

Drug	Intraday Precision (%RSD)	Interday Precision (%RSD)
Pioglitazone	1.51	0.478
Glimepiride	1.22	0.85

*n= No. of determinants

Table 4: Results for Accuracy (n=3).

Recovery Level	Pioglitazone				Glimepiride			
	Amount Added (µg/ml)		Amount Found (mg)	%Recovery	Amount Added (µg/ml)		Amount Found (mg)	% Recovery (w/w)
	Std.	Test			Std	Test		
80%	10	30	39	97.5	2	10	11.5	95.8
100%	20	30	48.9	97.8	5	10	14.8	98.6
120%	30	30	59.1	98.5	8	10	17.1	95

*n= No. of determinants

Table 5: Results for Robustness (n=3).

Parameters (n=3)	%RSD	
	Pioglitazone	Glimepiride
Detection wavelength at 228nm	0.018	0.026
Detection wavelength at 232nm	0.03	0.02
Flow rate 0.6ml/min	0.2	0.06
Flow rate 1ml/min	0.032	0.036

*n= No. of determinants

Table 6: Results for Assay (n=3) of Marketed formulation.

Drug	Label claim (mg/tab)	Amount recovered	% Amount found in drug
Pioglitazone	50	49	98%
Glimepiride	15	14.9	99.3%

*n= No. of determinants

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Pioglitazone and Glimepiride at 6.7min and 8.3 min respectively without any interference. The parameters were given in table 1.

Concentration range of 10-50 µg/ml for Pioglitazone and 3-15 µg/ml of Glimepiride were found to be linear with correlation coefficients 0.999 and 0.999 for Pioglitazone and Glimepiride respectively. The results were given in table 2.

The limits of detection for Pioglitazone and Glimepiride were found to be 0.425 µg/ml and 0.52µg/ml respectively and the limit of Quantitation were 1.40µg/ml and 1.716µg/ml respectively. Values were represented in table 2.

The proposed method was found to be precise and reproducible with %RSD of 0.478 and 0.85 for Pioglitazone and Glimepiride respectively. %RSD was reported in table 3.

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 97.5% to 98.5% and 95 to 98.6% for Pioglitazone and Glimepiride respectively. This indicates that the method was accurate. Values obtained were given in table 4. The method was found to be robust after changing the conditions like detection wavelength (\pm 1nm) and flow rate (\pm 0.2 ml). %RSD was calculated for each variation and reported. Values obtained were given in table 5.

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination with a percentage purity of 98% for Pioglitazone and 99% for Glimepiride. The typical chromatogram for assay of marketed formulations was shown in figure.5 and Values obtained were given in table 6.

CONCLUSION

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Pioglitazone and Glimepiride from their formulations. All the validation parameters were found to be within the

limits according to ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged and robust. So the established method can be employed in the routine analysis of the marketed formulations.

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