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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PIOGLITAZONE AND GLIMEPIRIDE IN TABLET DOSAGE FORM BY RP-HPLC

Ravi Sharma*[1], Gagan Sharma [1], Darpan Chopra [1] and Pavan Kumar Jain [2]

Choksi Laboratories Limited [1], Manoramaganj, Indore, Madhya Pradesh, India
Shri Ramnath Singh College of Pharmacy [2], Gormi, Bhind, Madhya Pradesh, India

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Correspondence to Author:

Mr Ravi Sharma

Choksi Laboratories Limited, Manoramaganj, Indore, Madhya Pradesh, India

ABSTRACT

A simple reverse phase liquid Chromatographic method has been developed and subsequently validated for simultaneous determination of Pioglitazone and Glimepiride in combination. The separation was carried out using a mobile phase of phosphate buffer (pH-4.5): Acetonitrile (45:55) v/v and using methanol as diluent. The column used was Inertsil ODS (250 mm x 4.6 mm i.d., 5µm) with flow rate of 1 ml/min using UV detection at 225 nm. The described method was linear over a concentration range of 5-50µg/ml and 5-25 µg/ml for the assay of Pioglitazone and Glimepiride respectively. The retention times of Pioglitazone and Glimepiride were found to be4.6 and 7.7min respectively. Results of analysis were validated statistically and by recovery studies. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Pioglitazone and Glimepiride bulk drug and in its pharmaceutical dosage form.

INTRODUCTION: Pioglitazone (PIO) is a thiazolidinedione antidiabetic agent. Chemically Pioglitazone [1] is [(+) - 5- [[4- [2- (5-ethyl- 2- pyridinyl) ethoxy] phenyl] methyl] - 2, 4-] thiazolidinedione monohydrochloride. It is not official in BP, USP, EP and IP. Literature surveys revealed method of analysis for pioglitazone and glimepiride in single dosage form by HPLC. Glimepiride (GLI) [2] is a sulfonyl urea antidiabetic agent. Chemically glimepiride is 1- [[p- [2- (3- ethyl- 4- methyl- 2- oxo- 3- pyrroline- 1- carboxamido) ethyl] phenyl] sulfonyl] - 3- (trans- 4- methylcyclohexyl) urea. It is official in BP [3], USP [4] but not official in IP till 2007. Pioglitazone and Glimepiride in combined tablet dosage form [5] are available in the market.

This paper presents the method for estimation of Pioglitazone and Glimepiride as two component dosage form was developed on RP-HPLC [6-7]. The statistical treatment of the assay of both the components reveals that these methods are precise, robust, rugged and accurate [8-9]. So far, no method has been reported for estimation of PIO and GLI in combined dosage form has less retention time by HPLC, hence we attempted to develop a simple, accurate, and economical analytical method. This paper describes validated RP-HPLC for simultaneous estimation of PIO and GLI in combination, using phosphate buffer (pH 4.5) and Acetonitrile (45:55) v/v and using methanol as diluent. The column used was Inertsil ODS (250 mm x 4.6 mm i.d., 5µm) with flow rate of 1 ml /min using UV detection [10-11] at 225 nm.

Experimental:

Chemicals, reagents and Instrumental Conditions: Standard bulk drug sample Pioglitazone and Glimepiride were provided by Ranbaxy Laboratories Ltd., Dewas (M.P.), in the month of May, 2010. Tablets of combined dosage form were procured from the local market. All other reagents used were of HPLC grade. Chromatographic separation was performed on a Shimadzu LC-20 AT HPLC (Double pump). Wavelength of detection [10-11] chosen was 225nm. A reverse phase Inertsil ODS (250 mm x 4.6 mm i.d., 5µm) column was used for the analysis. The mobile phase comprised of a mixture of phosphate buffer (pH-4.5) and Acetonitrile (45:55) v/v and using methanol as diluent with a flow rate of 1ml/min. The injection volume was 10 µL.

Preparation of working standard solutions, and sample solution: A standard solution of PIO (sol-1) was prepared, by taking 150 mg of PIO was transferred to 50ml volumetric flask and 25ml of diluent was added and sonicated to dissolve, then was made up to the mark with the diluent. A standard solution of GLI (sol-2) was prepared, by taking 20 mg of GLI was transferred to 100ml volumetric flask and 50ml of diluent was added and sonicated to dissolved, then was made up to the mark with the diluent. A standard mixed solution of PIO and GLI (100 ml) was prepared, by taking 5ml of each solution, accurately weighed, in separate 100-ml volumetric flasks. They were dissolved in mobile phase and then the volume was made up to the mark to get PIO-150 µg/ml, GLI-20µg/mL. For test solution 20 tablets were taken and weighted, powdered and triturated well. A quantity of tablet powder equivalent to average weight was mixed with methanol (50ml) and the volume made up to 100ml to get final test solution of 150:20 µg/ml. For each drug, appropriate aliquots were pipetted and were filtered through a 0.45µm Teflon filter.

Method validation:

Linearity: The developed method has been validated as per ICH guidelines [8-9]. Every 20 µL of the working standard solution of PIO in the mass concentration range of 5 to 50 µg/mL, and that for GLI in the mass concentration range of 5 to 25µg/mL were injected into the chromatographic system. The chromatograms were developed and the peak area was determined for each

concentration of the drug solution. Calibration curves of PIO and GLI were obtained by plotting the peak area ratio versus the applied concentrations of PIO and GLI. The linear regression coefficients were found to be 0.9854 and 0.9926for PIO and GLI respectively.

Precision: Repeatability of the method was checked by injecting replicate injections of the solution 150µg/mL and 20µg/mL of PIO and GLI respectively and the% RSD was found to be 1.01% and 1.31%. For showing method precision six preparation of sample is prepared at 100% of nominal concentration was prepared by same analyst and injected in duplicate. The average area thus obtained is used to calculate the assay of all six preparations. After calculation of assay %RSD of all six assays is calculated and found to be 1.01% and 1.31% for pioglitazone and glimepiride respectively.

Accuracy: Accuracy of the method was tested by carrying out recovery studies at different spiked levels. The estimation was carried out as described earlier. At each level, three determinations were performed and results obtained. The amounts recovered and the values of percent recovery were calculated, results are shown in **Table 1** and **Table 2**.

TABLE 1: RECOVERY STUDIES OF PIO (n=6)

Std 157.6 mg in to	50ml → 5 ml in to → 100 ml			
Potency	99.6%w/w			
Test X mg in to	50ml → 5 ml in to → 100 ml			
Std Area	1845869	1845670	Avg. 1845766	Factor 0.9072
Test	Area	Amount Added	Amount Recovered	% Recovery
50%	998591	77.05	77.03	100.0
50%	987899	76.21	76.14	100.1
50%	985323	76.01	75.82	100.3
100%	1892136	145.97	147.84	98.7
100%	1890544	145.84	146.74	99.4
100%	1889536	145.77	144.87	100.6
150%	2897581	223.53	225.96	98.9
150%	2896537	223.45	224.84	99.4
150%	2890859	223.01	222.96	100.0
Mean				99.71
SD				0.6344
%RSD				1.0

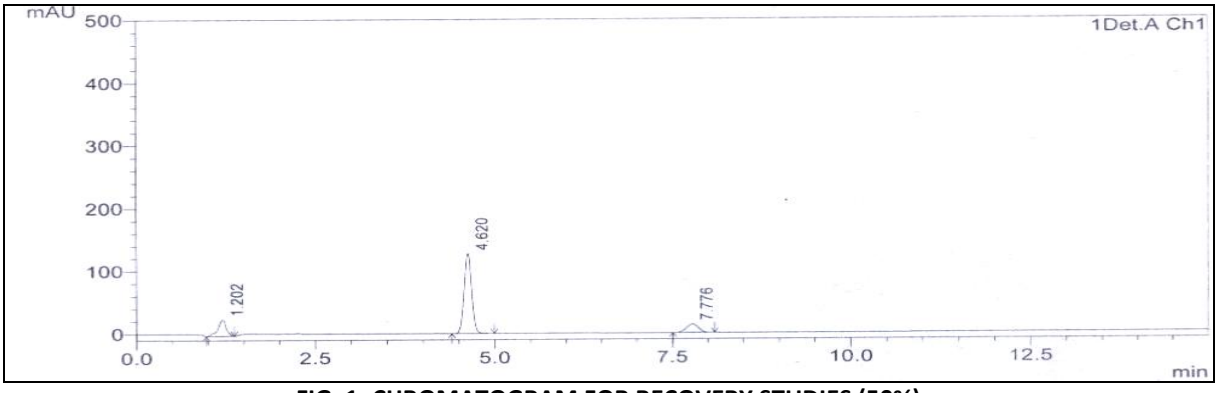

FIG. 1: CHROMATOGRAM FOR RECOVERY STUDIES (50%)

TABLE 2: RECOVERY STUDIES OF GLI (n=6)

Std 20.4 mg in to	50ml ⟶ 5 ml in to ⟶ 100 ml			
Potency	98.96%w/w			
Test X mg in to	50ml ⟶ 5 ml in to ⟶ 100 ml			
Std Area	1845869	1845670	Avg. 1845766	Factor 0.9072
Test	Area	Amount Added	Amount Recovered	% Recovery
50%	159229	10.48	10.49	99.9
50%	155331	10.22	10.18	100.4
50%	157533	10.37	10.31	100.6
100%	310290	20.42	20.38	100.2
100%	309983	20.4	20.35	100.2
100%	310838	20.46	20.42	100.2
150%	467538	30.77	30.59	100.6
150%	460583	30.31	30.11	100.7
150%	463251	30.49	30.55	99.8
Mean				100.28
SD				0.3046
%RSD				0.30

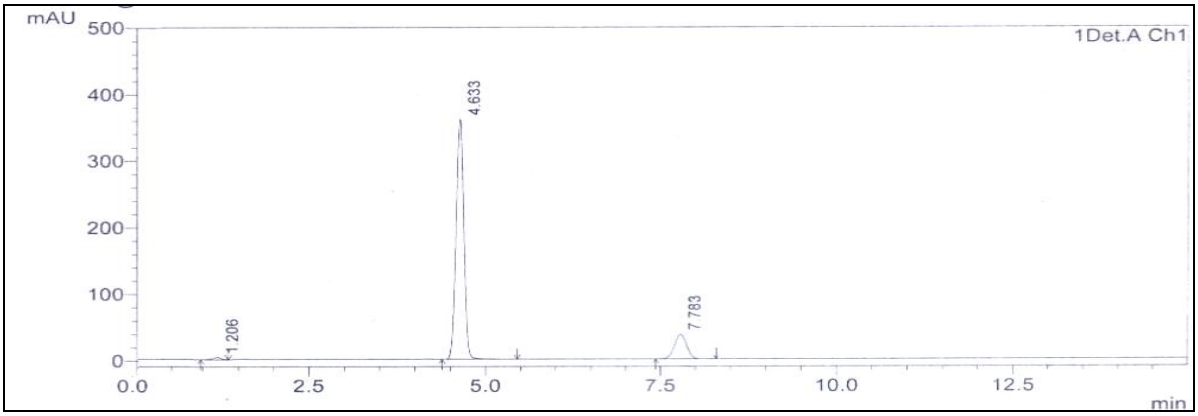

FIG. 2: CHROMATOGRAM FOR RECOVERY STUDIES (100%)

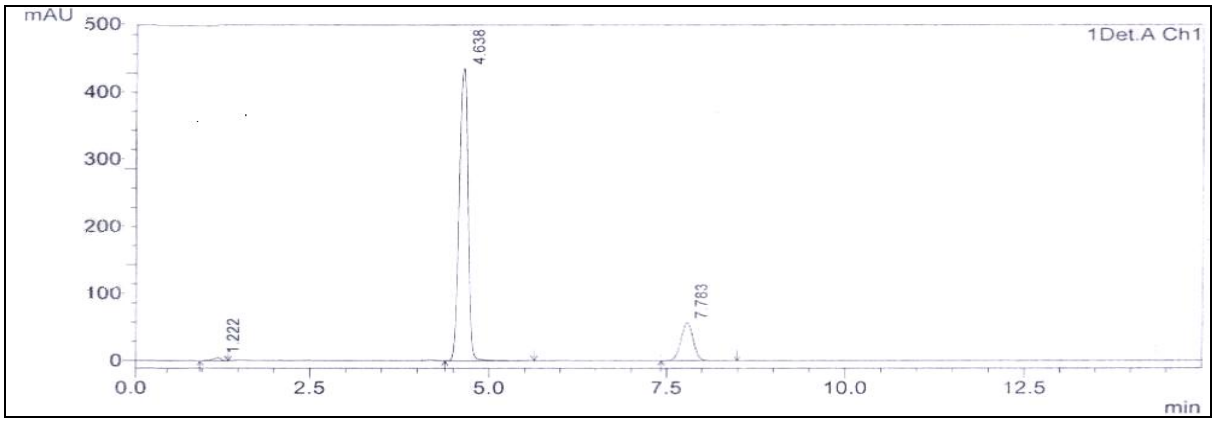

FIG. 3: CHROMATOGRAM FOR RECOVERY STUDIES (150%)

Specificity: The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/mL was injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both PIO and GLI from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

Robustness: To determine the robustness of the method, experimental conditions such as the composition of the mobile phase, pH of the mobile phase, and flow rate of the mobile phase were altered and the chromatographic characteristics were evaluated. No significant change was observed.

Analysis of Formulation: Twenty tablets of PIO and GLI in combination were weighed, their average weight was determined, and finally they were crushed to a fine powder. The tablet powder equivalent to 150mg of PIO and 20mg of GLI was weighed and transferred to a 100mL volumetric flask, first dissolved in 50mL of mobile phase, and then the volume was made up to the mark with the mobile phase. The content was ultrasonicated for 20 min for complete dissolution. The solution was then filtered through a 0.45µm. The selection of the mixed sample solution for analysis was carried out by the optimization of various dilutions of the tablet dosage form, considering the label claim. The results of tablet analysis (n = 6) were found to be 99.2 and 100.9 for PIO and GLI respectively. From the typical chromatogram of PIO and GLI was found that the retention time of PIO was 4.6 min and GLI was 7.7 min. The results analysis is shown in **Table 3**.

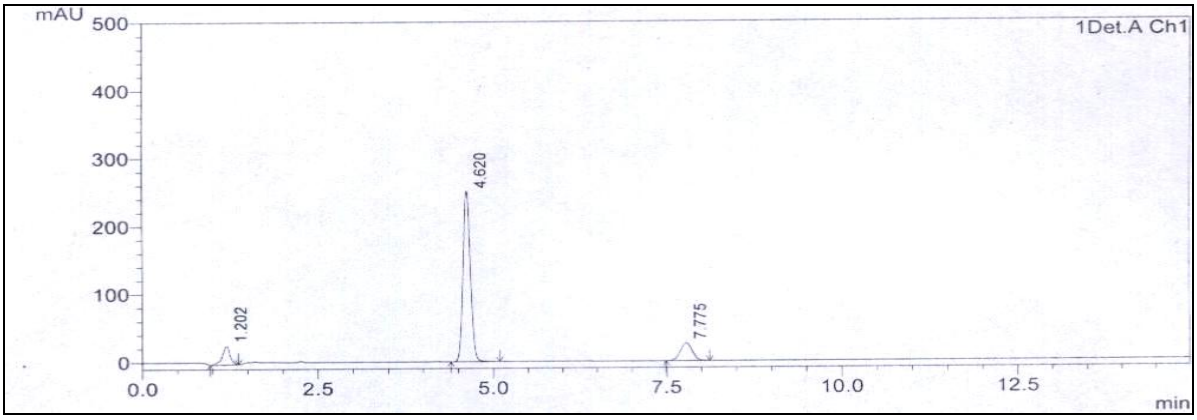

FIG. 4: TYPICAL CHROMATOGRAM OF PIO AND GLI IN SAMPLE SOLUTION

TABLE 3: ANALYSIS OF FORMULATION

Drugs	Labelled Amount (mg)	Amount taken for assay (µg/ml)	*Amount found (mg)	% label claim
Pioglitazone	15	157.6	156.3	99.2
Glimepiride	2	20.4	20.58	100.9

Conclusions: The developed method was validated in terms of accuracy, repeatability, and precision. A good linear relationship was observed for PIO and GLI in the concentration ranges of 5-50µg/mL and 5- 25µg/mL respectively. The correlation coefficient for PIO was found to be 0.9854 and that for GLI was 0.9926. The precision results were good enough to indicate that the proposed method was precise and reproducible. The assay experiment showed that the contents of PIO and GLI estimated in the tablet dosage form were free

from the interference of excipients. This demonstrated that the developed HPLC method was simple, linear, precise, and accurate, and could be conveniently adopted for the routine quality control analysis of PIO and GLI simultaneously, from its pharmaceutical formulations and bulk drug.

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