

A NEW VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS DETERMINATION OF PIOGLITAZONE AND GLIMEPIRIDE IN THE COMBINED TABLET DOSAGE FORM

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ABSTRACT

A new simple, precise, rapid and sensitive isocratic RP-HPLC method with UV detection in wavelength of 230 nm for Pioglitazone and Glimepiride in the combined tablet dosage form has been developed and validated. Chromatography was performed with mobile phase containing a mixture of acetonitrile and phosphate buffer (pH-6) in the ratio of 60:40, (v/v) with flow rate 1.5 ml/min by using C₁₈phenomenx luna as a column. The calibration graph of Pioglitazone and Glimepiride was found to be linear over the range of 240 - 350 µg/ml and 32 - 48 µg/ml with correlation coefficient of 0.997, 0.990 respectively. The retention times of Pioglitazone and Glimepiride were found to be 2.71 and 4.41 respectively. System suitability, specificity, linearity, accuracy robustness, LOD and LOQ parameters were validated for the developed method. The new method developed was successfully applied to estimate the amount of Pioglitazone and Glimepiride in the formulations by easily available low cost materials.

KEYWORDS

Pioglitazone and Glimepiride RP-HPLC, new method development, validation

1.1 INTRODUCTION

Pioglitazone (Figure 1) is chemically [(±) 5 [(4 [2 [5 ethyl 2 pyridinyl) ethoxy] phenyl] methyl] 2,4] thiazolidinedione monohydro-chloride¹. This is a thiazolidine Dione derivative. It is one type of PPAR-α agonist, insulin sensitizer used to reduce insulin resistance. It is not official in IP, BP, EP and USP. Glimepiride (Figure 2) is a sulfonylurea urea derivative chemically [[p [2 (3 ethyl 4 methyl 2 oxo 3 pyrroline 1 oxamide) ethyl]phenyl] sulfonyl] 3 (trans 4 methyl cyclohexyl) urea² and are used in the treatment of type 2 diabetes. Glimepiride is an oral anti-diabetic drug with prolonged effect and more over it maintains a more physiological regulation of insulin secretion, 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. Many patients suffering from type 2 diabetes require treatment with more than one anti-hyperglycemic drug to achieve optimal glycemic control.

The literature reveals that there are some of the methods have been reported for Pioglitazone and Glimepiride in single dosage forms and only few reports were found in combined dosage forms by UV, HPLC^{3,4,5,6}. Hence we have developed a new simple, rapid, accurate and economic analytical method for determination of Pioglitazone and Glimepiride in a combined tablet dosage form using UV detection at 230nm^{7,8}. The developed method was validated as per ICH (Q2A) guidelines^{9,10}.

Figure 1: Chemical structure of Pioglitazone:

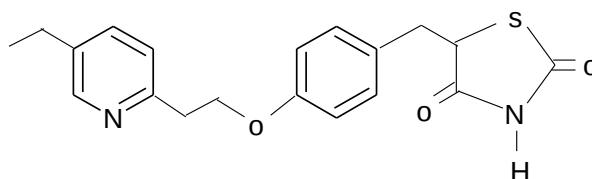
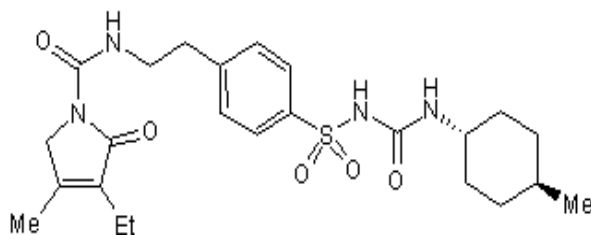


Figure 2: Chemical structure of Glimepiride:



1.2 EXPERIMENTAL:

1.2.1. Materials and Reagents:

Pioglitazone and Glimepiride were procured from Medley Pharmaceuticals Ltd. Tablets of combined dosage form were purchased from the local drug store. Potassium di hydrogen phosphate AR Grade, Acetonitrile, and Methanol HPLC grade from Merck chemicals, Mumbai.

1.2.2 Chromatography conditions:

chromatography separation was performed on a Shimadzu LC-10 at Vp series by using uv-vis SPD 10 AVp detector at the wavelength of 230 nm. A reverse phase HPLC C₁₈ phenomenx luna column (4.6 X 250 mm) was used. The mobile phase consists of acetonitrile and phosphate buffer (pH-6) in the ratio of 60:40, (v/v) with flow rate 1.5 ml/min. injection volume was 20µl.

1.2.3 Solutions:

1.2.3.1 Standard preparation:

Transferred about 165.4 mg of Pioglitazone working standard and 20.3 mg of Glimepiride in to 100 ml volumetric flask, dissolved in

mobile phase by sonication and diluted to volume with mobile phase and mixed. Pipetted out 5 ml of the above solution into 50 ml of volumetric flask, diluted to volume with mobile phase.

1.2.3.2 Test preparation:

For estimating the tablet dosage form, 20 tablets were powdered, accurately weighed 1346.7mg of ground tablet powder (equivalent to 150mg of Pioglitazone and 20.3 mg of Glimepiride) transferred it into a 100 ml of volumetric flask, to this 100ml of mobile phase added, shaken the flask on a rotator shaker for 30 min with intermediate shaking and sonicated for 15 min. Centrifuge the portion of above solution at 4000 rpm for 5 min. pipette out 5 ml of above clear solution and transfer it to 50 ml volumetric flask and make up the volume with mobile phase.

1.3 SYSTEM SUITABILITY STUDIES:

The resolution, number of theoretical plates and peak asymmetry were calculated for the working standard solutions and is as shown in **Table 1**.

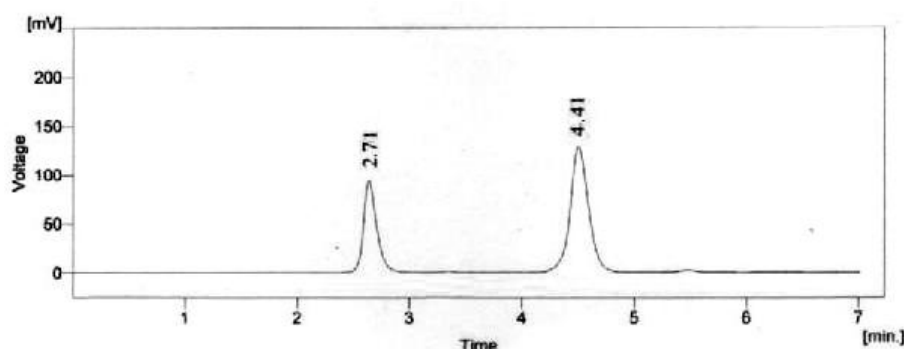
Table 1: system suitability parameters

Parameter	Pioglitazone	Glimepiride
Resolution	7.395	
Tailing Factor	1.122	1.273
Number of theoretical plate	3888	2577

The results obtained showed the suitability of system for the analysis of these drugs in

combination. Figure 3 illustrates the typical chromatogram of standard solution.

Figure 3: Typical chromatogram of Pioglitazone and Glimepiride



1.4 Method Validation:

As per ICH guidelines, the method validation parameters checked were specificity, precision, accuracy, linearity, robustness, limit of detection and limit of quantification.

1.4.1. SPECIFICITY

In order to check the specificity of the method, a blank solution and then a drug solution of 20 μ l was injected. Then the chromatograms of Pioglitazone and Glimepiride were recorded. The study demonstrated that there was no interference and also no change in retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

1.4.2. ACCURACY

The accuracy of the method was determined by recovery experiments. The recovery

studies were carried out six times and the % recovery and %RSD were calculated. From the results obtained, recoveries of standard drugs were found to accurate.

1.4.3. PRECISION

Precision of the method was checked by injecting replicate injections of Pioglitazone and Glimepiride and the % RSD was calculated. For this six samples were prepared at 100% of nominal concentration by same analyst and injected. The average area thus obtained is used to calculate the assays of all six preparations. Then the % RSD of all six assays was found to be 1.35% and 1.31% for Pioglitazone and Glimepiride respectively. Results for accuracy and precision are shown in Table 2.

TABLE 2: RECOVERY STUDIES

Name of the Drug	Spiking level (%)	Amount added (µg/ml)	Amount recovered (µg/ml) n=3	% recovery	Average % recovery	% RSD
Pioglitazone	80	8	8.104	101.3	99.9	1.35
	100	10	9.98	99.8		
	120	12	11.832	98.6		
Glimepiride	80	0.8	0.812	101.5	100.3	1.31
	100	1	1.005	100.5		
	120	1.2	1.186	98.9		

1.4.4. Linearity:

The linearity of the method was determined at 5 concentration levels ranging from 240 to 361.83 µg/ml for Pioglitazone and 32 to 50 µg/ml for Glimepiride. The chromatograms were developed by injecting 20 µl from that the peak area was determined for each concentration of the drug solution. Calibration

curve was constructed by plotting response factor against concentration of drugs. The linear regression coefficients of Pioglitazone and Glimepiride were found to be 0.997 and 0.990 respectively. Figure 4 and 5 shows the linearity curves for Pioglitazone and Glimepiride respectively.

Figure 4: linearity curve for Pioglitazone

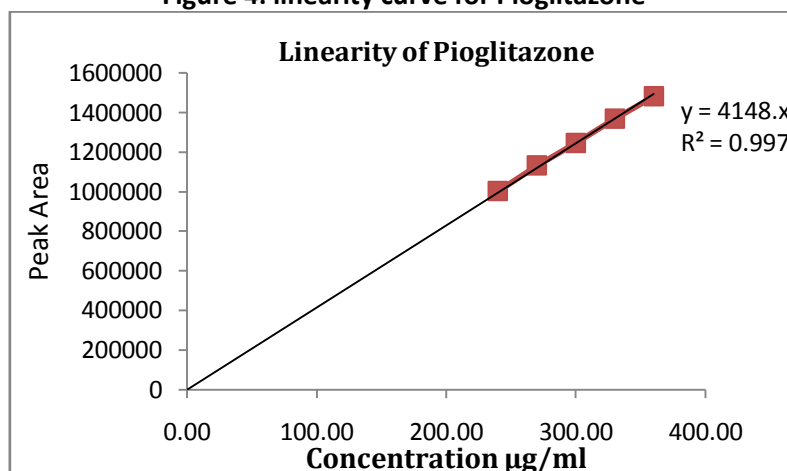
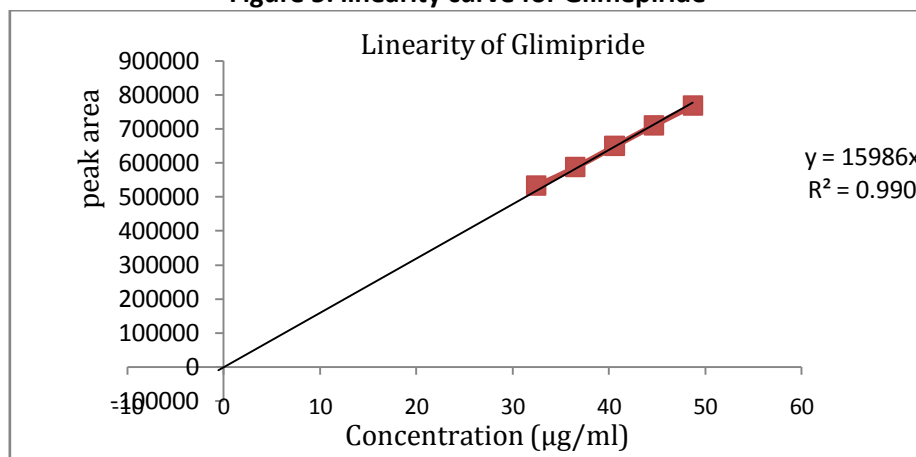


Figure 5: linearity curve for Glimepiride



1.4.5. ROBUSTNESS:

Robustness of assay method was carried out by changing the flow rate of mobile phase and by altering the wavelength and by varying pH and composition of mobile phase. It was noted that there was no significant changes in the chromatograms demonstrates that the developed method is robust.

1.4.6. Quantification Limit:

The LOD (Limit of detection) is the lowest concentration of the analyte that gives a measurable response (signal to noise ratio 1:3). The LOQ (Limit of quantification) is the

smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio 1:10). The LOD and LOQ of the method were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method.

The LOD of Pioglitazone and Glimepiride found to be 0.75 µg/ml and 0.1 µg/ml respectively. The LOQ was 7 µg/ml and 1 µg/ml for Pioglitazone and Glimepiride respectively.

1.5. SUMMARY

The summary of the method validation results were shown in Table 3.

S.NO	PARAMETERS	LIMIT	OBSERVATION
1	System suitability (%RSD of tailing factor)	Suitable	Pio:1.122 Gli:1.273
2.	Specificity	No interferences	Specific for both.
3	Precision:	RSD NMT 2.0%	Pio :1.35; gli:1.31
4	Linearity	Correlation coefficient NLT 0.999	Pio: 0.997; gli:0.990
5	Accuracy	%Recovery range 98-102 %	Pio: 99.9; gli: 100.3
6	Robustness	RSD NMT 2%	Robusted
7	LOD	S:N Ratio should be more than 3:1	Pio : 0.75 µg/ml gli : 0.1 µg/ml
8	LOQ	S:N ratio should be more than 10:1	Pio : 7 µg/ml Gli : 1 µg/ml

1.6. APPLYING THE METHOD FOR MARKETED FORMULATION:

For applying the proposed method to marketed formulation, the six samples were prepared as per the procedure for test preparation and then it was allowed to run

under same chromatographic conditions as per the proposed method. The % label claim was found to be 101.3% and 102% for Pioglitazone and Glimepiride respectively. The results were depicted in **Table 4**.

Table 4: Analysis of formulation

Drugs	Labeled amount(mg)	Amount found(mg)	%Label claim
Pioglitazone	15	15.2	101.3
Glimepiride	2	2.04	102

1.7. RESULTS AND DISCUSSION

The proposed method was found to be simple and sensitive with linearity in the concentration range of 240 to 361.83 µg/ml for Pioglitazone and 32 to 50 µg/ml for Glimepiride. The method was found to be accurate and precise as indicated by results of recovery studies and %RSD not more than 2%. LOD and LOQ for Pioglitazone were found to be 0.75 µg/ml and 7µg/ml respectively and for Glimepiride were 0.1µg/ml and 1 µg/ml respectively. The proposed method was found to be specific as there is no interference from common tablet excipients like lactose, starch etc.

1.8. CONCLUSION

Though the linearity range of this method is slightly more as compared to the reported RP-HPLC method, the newly developed RP-HPLC method leads to better resolution and peak symmetry. Hence the developed RP-HPLC method for the simultaneous determination of Pioglitazone and Glimepiride can be used for routine analysis of both these components in combined dosage form.

1.9. REFERENCES

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