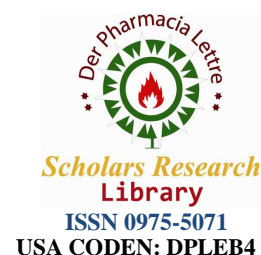




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# Estimation of pioglitazone and glimipride in its pharmaceutical dosage form by spectrophotometric methods

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## ABSTRACT

Two simple, accurate, precise and reproducible spectrophotometric methods for the simultaneous estimation of Pioglitazone (PIO) and Glimipride (GLIM) have been developed. First method used was simultaneous equation method, in this two wavelengths (216 and 225 nm) were selected for the measurement of absorbance. Second method, absorption ratio method in which measurements are made based on the absorptivity at the isobestic point (228 nm) and absorption maxima of PIO (216 nm). The absorption maximum wavelengths of PIO, GLIM were observed at 216 and 225 nm respectively and the isobestic point at 228 nm. Linearity ranges from 5-25 µg/ml for both drugs. The proposed methods are recommended for routine analysis of PIO and GLIM as it is rapid, precise, accurate and reproducible. These methods were validated according to the ICH guidelines.

**Keywords:** Pioglitazone, Glimipride, Simultaneous Equation Method, Absorption Ratio Method.

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## INTRODUCTION

Absorption spectroscopy is one of the most useful and widely used tools available to the analyte for quantitative analysis. The relation between the concentration of analyte and the amount of light absorbed is the basis of most analytical applications of molecular spectroscopy. This method of analysis is gaining importance as it is simple, rapid, precise, highly accurate and less time consuming. Simultaneous estimation of a drug formulation is used to quantify the components of the formulation. In this communication we are dealing with Pioglitazone and Glimipride as a combined dosage form.

Pioglitazone is a prescription drug of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action. It is chemically (RS)-5-(4-[2-(5-ethylpyridin-2-

yl)ethoxy]benzyl)thiazolidine-2,4-dione (Figure 1). Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ) and to a lesser extent PPAR- $\alpha$ . [1-2] It modulates the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in the muscle, adipose tissue, and the liver. It is used for the treatment of diabetes mellitus type 2 (previously known as non-insulin-dependent diabetes mellitus, NIDDM) in monotherapy and in combination with a sulfonylurea, metformin, or insulin.[3]

Glimepiride is a medium-to-long acting sulfonylurea anti-diabetic drug and is chemically 3-ethyl-4-methyl-*N*-(4-[*N*-((1*r*,4*r*)-4-methylcyclohexylcarbamoyl)sulfamoyl]phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide (Figure 2). glimepiride acts as a secretagogue.[4] It lowers blood sugar by stimulating the release of insulin by pancreatic beta cells and by inducing increased activity of intracellular insulin receptors. It is used for the treatment of diabetes mellitus type 2.

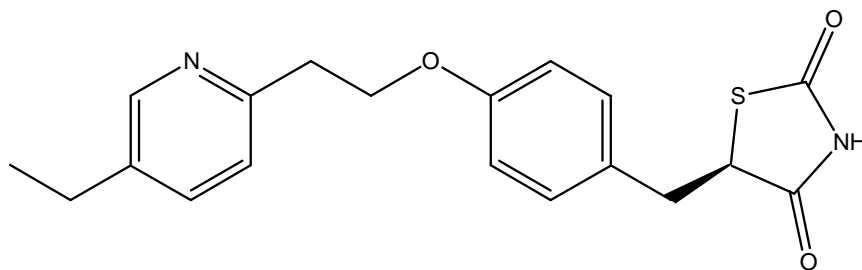


Figure 1: Chemical structure of pioglitazone

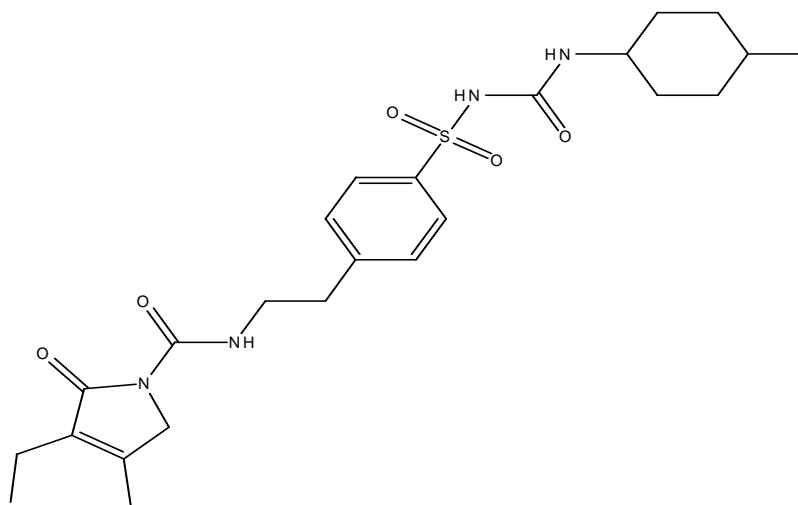


Figure 2: Chemical structure of glimepiride

Drug is available in tablet dosage form as PIO -30 mg and GLIM- 2 mg (Duetact) in the market. Literature survey revealed that PIO has been estimated with other drugs using UV[5], HPLC[6-12]. GLIM has been determined along with other drugs by UV [13], HPLC [9,11-12,14-15]. Since, no spectrophotometric method has been reported yet for simultaneous estimation of PIO and GLIM, so the present study is focussed on a successful attempt to estimate PIO and GLIM using more economical UV spectrophotometric method.

## MATERIALS AND METHODS

### Instrument

A double-beam Shimadzu UV-Visible spectrophotometer, 1800, with a pair of 1cm matched quartz cells were used to measure the absorbance of the solutions.

### Materials

Vinca life sciences, Baddi gifted the standard sample of PIO and GLIM and their marketed combination (Duetact) were purchased from the market.

**Solvent:** 0.1 N NaOH was used as the solvent.

### Stock solution

Stock solution of PIO (1000 $\mu$ g/ml) and GLIM (1000 $\mu$ g/ml) were prepared separately in 0.1 N NaOH for analysis.

### Absorption maxima

Stock solution was diluted to 10 $\mu$ g/ml of PIO and 10 $\mu$ g/ml of GLIM respectively and then was scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption of both the drugs. PIO showed maximum absorption at 216 nm and GLIM at 225 nm (Figure 3).

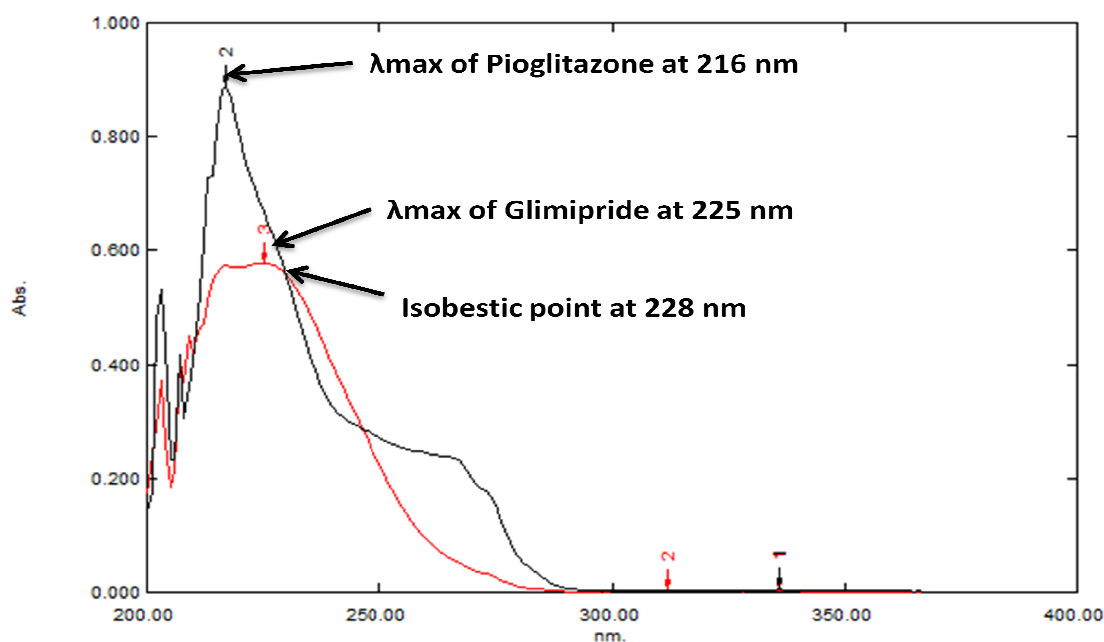


Figure 3: Overlain spectra of PIO and GLIM showing absorption maxima of PIO at 216 nm and GLIM at 225 nm. Isobestic point was found at 228 nm. Graph is plotted between wavelength (nm) on X-axis and absorbance on Y-axis.

### Method I (Simultaneous Equation Method)

Two wavelengths selected for the method are 216 nm ( $\lambda_1$ ) and 225 nm ( $\lambda_2$ ) that are absorption maxima of PIO and GLIM respectively. The stock solution of the samples was further diluted with 0.1N NaOH to get standard solution of concentration 10  $\mu$ g/ml. The absorbance of the

solution was measured at the selected wavelengths and absorptivity was determined as a mean of three independent determinations. Concentration of the drug in the samples was obtained using the following equation:

$$C_x = \frac{A_1 a_{y2} - A_2 a_{y1}}{a_{x1} a_{y2} - a_{x2} a_{y1}} \quad \dots\dots\dots \text{eq. 1}$$

$$C_y = \frac{A_1 a_{x2} - A_2 a_{x1}}{a_{y1} a_{x2} - a_{y2} a_{x1}} \quad \dots\dots\dots \text{eq. 2}$$

Where  $A_1$  and  $A_2$  are absorbance of mixture at  $\lambda_1$  and  $\lambda_2$  respectively,  $a_{x1}$  and  $a_{x2}$  are the absorptivities of PIO at  $\lambda_1$  and  $\lambda_2$  respectively and  $a_{y1}$ ,  $a_{y2}$  are the absorptivities of GLIM at  $\lambda_1$  and  $\lambda_2$  respectively.  $C_x$  and  $C_y$  are the concentrations of PIO and GLIM in g/L respectively.

### Method II (Absorbance Ratio Method)

Two wavelengths were selected, one is 228 nm which is the isobestic point shown in the overlain spectra of PIO and GLIM (Figure 3) and the other is 216 nm which is the absorption maxima of PIO. The absorbance of the sample prepared from stock solution in the same way as in the previous method was measured and the absorbance ratio at the selected wavelengths were calculated. Concentration of the drugs was calculated using the following equation:

$$C_x = \frac{Q_m - Q_2}{Q_1 - Q_2} \times \frac{A}{a_x} \quad \dots\dots\dots \text{eq. 3}$$

$$C_y = \frac{Q_m - Q_1}{Q_2 - Q_1} \times \frac{A}{a_y} \quad \dots\dots\dots \text{eq. 4}$$

Where,  $A$  is the absorbance of sample at isobestic point,  $a_x$  and  $a_y$  are the absorptivities of PIO and GLIM at isobestic point respectively.  $Q_m$ ,  $Q_1$ ,  $Q_2$  are absorbance ratio of mixture, PIO and GLIM respectively at isobestic point to selected wavelength (216 nm).  $C_x$  and  $C_y$  are the concentrations of PIO and GLIM in g/L respectively.

### Method Validation

Method was validated according to the ICH guidelines [16-18] for its accuracy, linearity, precision, limit of detection and limit of quantification.

### Linearity

The linearity of this method was evaluated by linear regression analysis and calculated by least square method and the drug shows linearity in the concentration range of 5-25  $\mu\text{g/ml}$  for both drugs. Standard dilutions were prepared using the required volume from the stock solution and then volume was made up to 10 ml with 0.1 N NaOH to yield the concentrations. Absorbance of the resulting solutions was measured and the calibration curve was plotted between absorbance

and concentration of the drug. Results show excellent correlation between absorbance and analyte concentration (Table 1). Graph for linearity is shown in Figure 4.

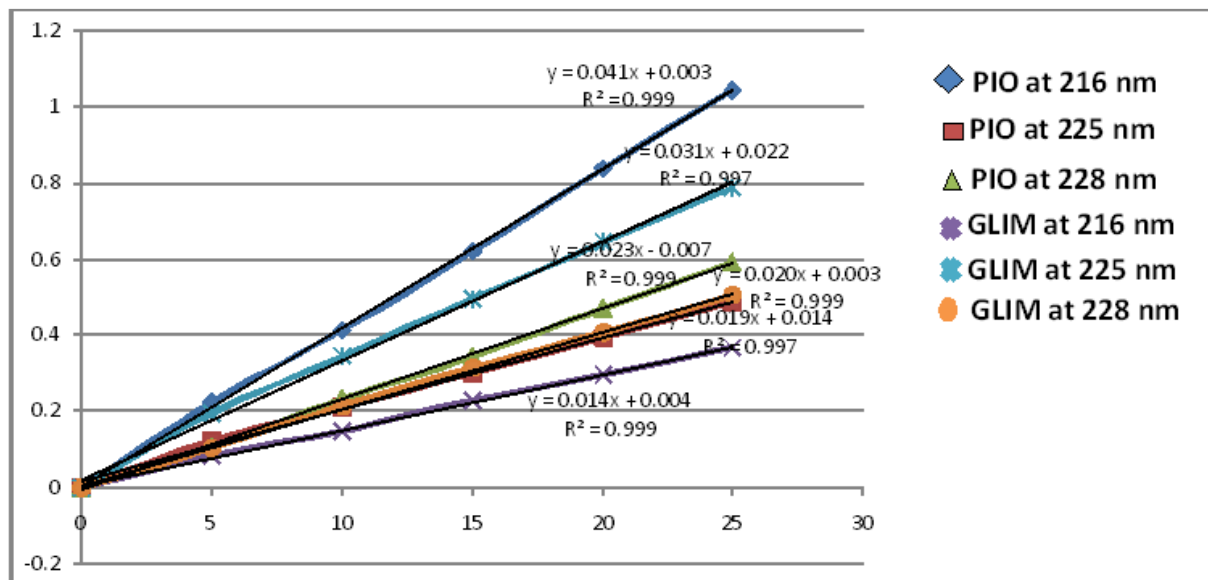


Figure 4: Linearity graphs of PIO and GLIM at 216 ( $\lambda_{max}$  of PIO), 225 ( $\lambda_{max}$  of GLIM) and 228 nm (Isobestic point). From the straight line equation  $y = m x + c$ ,  $m$  (slope),  $c$  (intercept) were observed and the linearity of the calibration curve was determined from correlation coefficient ( $R^2$ ).

Table 1: Optical characteristics

Parameters	216 nm		225 nm		228 nm	
	PIO	GLIM	PIO	GLIM	PIO	GLIM
Beer's law limit ( $\mu\text{g/ml}$ )	5-25	5-25	5-25	5-25	5-25	5-25
Regression equation	$Y = m X + c$					
Slope (m)	0.041	0.014	0.019	0.031	0.023	0.020
Intercept (c)	0.003	0.004	0.014	0.022	-0.007	0.003
Correlation coefficient ( $R^2$ )	0.999	0.997	0.999	0.999	0.997	0.999
Standard deviation SD	0.0005	0.0004	0.0003	0.0003	0.001	0.0005
Limit of detection ( $\mu\text{g/ml}$ )	0.0402	0.0942	0.0521	0.0319	0.143	0.0825
Limit of quantification ( $\mu\text{g/ml}$ )	0.121	0.285	0.157	0.967	0.434	0.25

### Inter-day and Intra-day Precision and Accuracy

Precision and accuracy was studied using solution of concentration 10  $\mu\text{g/ml}$ . Absorbance of the solution was measured for three replicate samples. Intra-day precision studies were run in triplicate in the same day and inter-day on three consecutive days. Precision and accuracy data is shown in Table 2 and 3.

Table 2: Intra-day precision and accuracy of PIO and GLIM

Drug	Conc. ( $\mu\text{g/ml}$ )	Method 1		Method 2	
		%Accuracy $\pm$ SD	%RSD	%Accuracy $\pm$ SD	%RSD
PIO	10	98.07 $\pm$ 0.266	0.274	99.66 $\pm$ 1.268	1.285
GLIM	10	99.52 $\pm$ 0.302	0.307	100.1 $\pm$ 1.267	1.279

Values expressed mean  $\pm$  SD (n=3)

Table 3: Inter-day accuracy and precision of PIO and GLIM

Drug	Conc. ( $\mu\text{g/ml}$ )	Method 1		Method 2	
		%Accuracy $\pm$ SD	%RSD	%Accuracy $\pm$ SD	%RSD
PIO	10	98.5 $\pm$ 0.251	0.259	100.43 $\pm$ 0.594	0.597
GLIM	10	99 $\pm$ 0.481	0.496	99.73 $\pm$ 0.558	0.565

Values expressed mean  $\pm$  SD (n=3)

### Accuracy (% Recovery)

It is the measure of closeness between the actual value and the analytical value that is calculated by applying the test procedure for a number of times. Recovery was done at three different levels viz. 80%, 100% and 120%, within the beer's limit for both the drugs. The previously analysed sample of concentration 10  $\mu\text{g/ml}$  was spiked with known concentrations of the pure samples and then reanalyzed using the proposed methods. Percentage recovery was calculated using the equations for both the methods. Percentage recovery is given in Table 5.

### Limit of detection and quantification

Limit of detection (LOD) is the minimum concentration of the analyte in the sample which can be analysed by the instrument. Limit of quantification (LOQ) is the minimum concentration of the analyte that can be reliably quantified. The values of LOD and LOQ are given in Table 1.

Table 4: Assay of the tablet mixture

Drug	Label claim (mg/ tab.)	Method 1		Method 2	
		Amount found (mg)	%Drug found $\pm$ SD	Amount found (mg)	%Drug found $\pm$ SD
PIO	10	9.97	99.7 $\pm$ 0.489	9.943	99.43 $\pm$ 0.637
GLIM	0.667	0.662	99.25 $\pm$ 0.543	0.665	99.70 $\pm$ 0.946

Values expressed in mean  $\pm$  SD (n=3)

Table 5: Recovery study of PIO and GLIM

Drug	Amount added ( $\mu\text{g/ml}$ )	Amount recovered ( $\mu\text{g/ml}$ ) Method I	%Recovery $\pm$ SD Method I	Amount recovered ( $\mu\text{g/ml}$ ) Method II	% Recovery $\pm$ SD Method II
PIO	80% (8 $\mu\text{g/ml}$ )	8.02	100.25 $\pm$ 0.735	8.00	100.00 $\pm$ 0.564
	100% (10 $\mu\text{g/ml}$ )	9.95	99.50 $\pm$ 1.268	10.05	100.50 $\pm$ 0.579
	120% (12 $\mu\text{g/ml}$ )	12.01	100.83 $\pm$ 0.510	11.98	99.83 $\pm$ 0.588
GLIM	80% (8 $\mu\text{g/ml}$ )	7.99	99.87 $\pm$ 1.033	8.05	100.62 $\pm$ 0.589
	100% (10 $\mu\text{g/ml}$ )	9.98	99.80 $\pm$ 1.027	9.92	99.20 $\pm$ 1.247
	120% (12 $\mu\text{g/ml}$ )	11.96	99.66 $\pm$ 0.969	12.06	100.50 $\pm$ 0.999

Values expressed mean  $\pm$  SD (n=3)

### Application of the developed method on tablet dosage form

Tablets (Duetact) were weighed, crushed and an accurately weighed sample equivalent to 10mg of PIO which includes 0.667 mg of GLIM and 9.33 mg of pure GLIM was added by standard addition method in order to bring both drugs in 1:1 ratio and the stock solution of this was prepared in 0.1 N NaOH, sonicated for 10 min, was then filtered through Whatman filter paper and then volume was made up to 10 ml with 0.1 N NaOH. This stock solution contains 1000  $\mu\text{g/ml}$  of each drug. Then the appropriate dilution of 10  $\mu\text{g/ml}$  was made using 0.1 N NaOH. All determinations were carried out three times. In Method 1, the absorbance of the prepared

solutions was observed at 216 and 225 nm and then the concentration of both the drugs was calculated using equation 1 and 2. In Method 2, absorbance was measured at 216 and 228 nm for the given dilution and then the concentration for both PIO and GLIM was measured using equation 3 and 4. Results of the assay of tablet dosage form are shown in Table 4.

## RESULTS

The methods discussed in the present work provide a convenient way to estimate PIO and GLIM in pharmaceutical dosage form. In simultaneous equation method 216 and 225 nm ( $\lambda_{\max}$  of PIO and GLIM respectively) were selected for analysis and in case of absorption ratio method two wavelengths, 216 and 228 nm ( $\lambda_{\max}$  of PIO and isobestic point respectively) were selected for analysis with the help of the overlain spectrum obtained (Figure 3). Stock solutions were prepared using 0.1 N NaOH as both drugs are soluble in this solvent and both the solutions were found to be stable throughout the experiment. In both the methods linearity for detector response was observed in the concentration range of 5-25  $\mu\text{g/ml}$  for both drugs as shown in Table 1. Regression analysis was made for the slope (m), intercept (c) and correlation coefficient ( $R^2$ ) as shown in Table 1. Higher values of correlation coefficient ( $R^2$ ) indicate good linearity of the calibration curve for both the drugs as is shown in Figure 4.

Sensitivity of the method was determined by calculating limit of detection (LOD) and limit of quantification (LOQ). LOD is the minimum concentration of the analyte in the sample which can be analysed by the instrument. LOQ is the minimum concentration of the analyte that can be reliably quantified. These are calculated according to equation 5 and 6.

$$\text{LOD} = 3.3 (\text{SD/S}) \quad \dots\dots\dots\text{eq. 5}$$

$$\text{LOQ} = 10 (\text{SD/S}) \quad \dots\dots\dots\text{eq. 6}$$

SD is the standard deviation and S is the slope of the calibration curve. Results of LOD and LOQ are given in Table 1.

Precision of the proposed method were determined by inter- and intra-day precision methods. The results are 98.5-99% in case of inter-day precision for simultaneous equation method and from 99.73-100.43% for absorption ratio method. In intra-day precision, results range from 98.07-99.52% for simultaneous equation method and 99.66-100.1% in case of absorption ratio method. %RSD calculated was less than equal to 2 which indicates the accuracy and reproducibility of the method. Results are shown in Table 2 and 3.

Amount of PIO and GLIM in marketed formulation were determined by the proposed method ranges between 99.25-99.7% for simultaneous equation method and 99.43-99.70% for absorption ratio method as shown in Table 4. The proposed method was validated according to the ICH guidelines. Standard deviation (SD) and % relative standard deviation (%RSD) is calculated in Table 2 & 3. Low values of standard deviation show the accuracy, repeatability and reproducibility of both the methods. The accuracy of the method was proved by performing recovery studies on the commercial formulation at 80, 100 and 120% level. Recovery ranges from 99.50-100.83% in simultaneous equation method and from 99.20-100.62% in case of absorption ratio method (Table 5). The results of recovery study indicate that these drugs could

be quantified simultaneously and that there is no interference of the excipients present in the formulation.

## DISCUSSION

Statistical analysis and drug recovery data showed that both methods are simple, rapid, economical, sensitive, precise and accurate and can thereby be easily adopted for routine quality control analysis. Results of this analysis confirm that the proposed method is suitable for the simultaneous determination of these drugs in pharmaceutical formulations with virtually no interference of the additives. Hence, the above method can be successfully applied in simultaneous estimation of PIO and GLIM in marketed formulations.

These results show that the proposed UV spectroscopic method is simple, precise and accurate. They are suitable for the analysis of PIO and GLIM in the bulk and tablet dosage form without the interference of excipients. Statistical data reveals that this method is repeatable and specific for the analysis of these two drugs in combination.

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