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Development and validation of derivative spectrophotometric method for estimation of pioglitazone HCl and glimepiride in bulk and combine dosage form

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ABSTRACT

Pioglitazone hydrochloride and Glimepiride is antidiabetic drug. A sensitive, precise, accurate and simple first order zero crossing UV spectrophotometric method has been developed for simultaneous Estimation of Pioglitazone hydrochloride and Glimepiride in bilayer tablet dosage form. The quantification was achieved by the first-order derivative spectroscopy method at 225 nm (zero cross point of Glimepiride) for pioglitazone and 248 nm (zero cross point of Pioglitazone) for Glimepiride. Pioglitazone HCl (R^2=0.9912) and Glimepiride (R^2=0.9964) shows Linearity in a concentration range of 5-30µg /ml and 4-20µg/ml respectively. Procedure does not require prior separation of layers of tablet formulation. LOD values for Pioglitazone HCl and Glimepiride are found to be 0.0187µg/mL and 0.132µg/mL, respectively. LOQ values for Pioglitazone HCl and Glimepiride are found to be 0.056µg/mL and 0.40 µg/mL, respectively. The results of analysis have been validated statistically and recovery studies carried out in the range 80-120% to confirm the accuracy of the proposed method. The relative standard deviation was found to be <2.0%. The Proposed method is recommended for routine analysis since it is rapid, simple, accurate and also sensitive and specific.

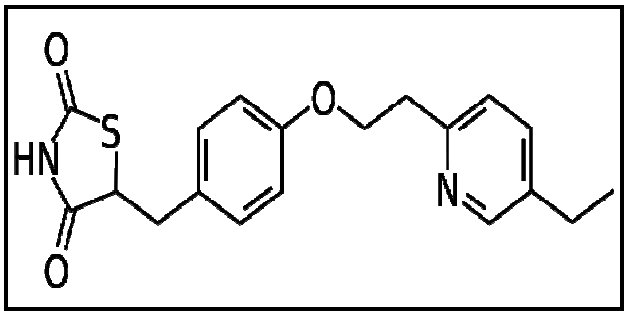
Key words: Pioglitazone hydrochloride, Glimepiride, Derivative spectrophotometry, Validation.

INTRODUCTION

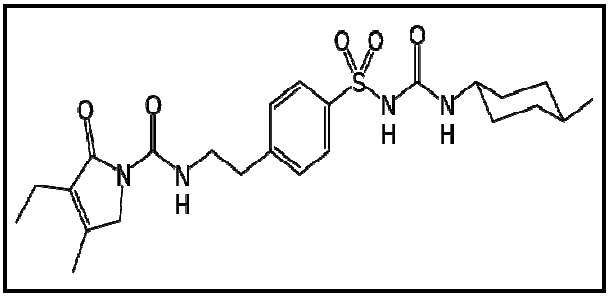
Pioglitazone hydrochloride (PIO) is drug of the class thiazolidinedione (TZD) with hypoglycemic (antihyperglycemic, antidiabetic) action to treat diabetes. Chemically it is (±)-5-[p-[2-(5-Ethyl-2-pyridyl) ethoxy]benzyl]-2,4-thiazolidinedione monohydrochloride. It act by reduces insulin resistance in the liver and peripheral tissues; increases the expense of insulin-dependent glucose; decreases withdrawal of glucose from the liver; reduces quantity of glucose.

Glimepiride (GLM) is an antidiabetic drug belonging to a class of medications known as sulfonylureas. Chemically it is 3-ethyl-N,N-bis(3-ethyl-4-methyl-2-oxo-5H-pyrrol-2-yl)-4- methyl-2-oxo-5H-pyrrole-1-carboxamide. The primary mechanism of action of glimepiride in lowering blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells. Both the drugs are official in I.P. 2010.[1]

The reported methods are time consuming expensive and relatively complicated. The aim of this study was to develop simple, precise, accurate and convenient method for the simultaneous estimation of PIO and GLM in combined dosage form.



Pioglitazone HCl [1]



Glimepiride [1]

MATERIALS AND METHODS

A Jasco V-630 UV- Visible double beam spectrophotometer with 1 cm matched Quartz cells were used for spectral measurement. Schimadzu AX 200 Analytical balance was used for weighing purposes. The Reference Standard of Pioglitazone HCL and Glimepiride was Kindly provided by Cadila pharmaceuticals And Bilayer tablet was formulated in laboratory were utilized for the study. All chemicals and reagent used were of analytical grade.

Selection of common solvent:
Main criteria for media selection was solubility and stability, i.e. PIO and GLM should be soluble as well as stable for sufficient time in selected media. Both the drugs show solubility in 0.1N HCl, it is economical and hence selected for analysis.

Preparation of standard drug solution:
Standard stock solutions containing Pioglitazone (PIO) and Glimepiride (GLM) were prepared individually by dissolving 10 mg of PIO and 10 mg of GLM separately in 50 ml of 0.1 N HCl, It was then sonicated for 10 minutes and the final volume of both the solutions were made up to 100 ml with same solvent to get stock solutions containing 100µg/ mL each of PIO and GLM in two different 100 ml volumetric flasks.

Determination of absorption maxima:
Solutions of PIO and GLM (10 µg/ml each) were prepared separately by appropriate dilution of standard stock solutions and scanned over the range of 400 to 200 nm. The absorption spectra thus obtained were derivatised from first order. First order derivative spectra were selected for analysis of both the drugs. From the overlain spectra (Figure 1) wavelengths selected for quantitation were 248.0 nm (zero cross point of PIO HCl) for GLM and 225.0 nm (zero cross point of GLIM) for PIO HCl.

Derivative Spectroscopy:
Method:
The aliquots were prepared from stock solution of PIO and GLM to obtain the concentration 5-30 µg/ml of PIO and 4-20 µg/ml of GLM. All the solutions were scanned from 400-200nm and the resultant spectra are derivatise. First order spectra for various concentration of PIO were obtain at 225nm the detecting wavelength of PIO and no GLM

interferences were observed as D 1=0. So any change in GLIM concentration has no effect on quantitative determination of PIO HCl. (figure 2) Similarly, at 248nm the detecting wavelength of GLM, first order derivative spectra for varying concentrations of GLM were obtained for its determination and no PIO interferences were observed as D1= 0. So any change in PIO concentration has no effect on quantitative determination of PIO HCl. (figure 3)

Application of proposed method to Tablet formulation

Twenty tablets of PIO+GLM (each tablet containing 30 mg of PIO and 1 mg of GLM present in tablet) were weighed and finely powdered. Accurately weighed powder samples equivalent to 30 mg of PIO and 1 mg GLM were transferred to a 100 ml volumetric flask, to this accurately weighed 9 mg of GLM was added. About 50 ml of solvent 0.1 N HCl was added to the flask and placed in an ultrasonic bath at room temperature for 15 min. The solution was cooled and made up to volume and then filtered through 0.45 µm whatman filter paper. Resulting solution was equivalent to 300 µg/ml PIO and 100 µg/ml GLM. Accurately measured 1ml of filtrate transferred to 10 ml volumetric flask and diluted up to the mark. The resultant solution was scanned and the spectra derivetised to first order. The absorbance's was recorded at 225nm and 248nm. The content of PIO and GLM were calculated and % labeled claim was determined and results shown in **Table 1**

Table 1: Analysis of Tablet formulation

Drug	Label claim (mg/tab)	% Drug found	±SD
PIO	30	99.77	1.05
GLM	1	98.87	0.52

(n=6)

Table 2: Recovery study of PIO and GLM

Drug	Level of addition (%)	Amount added (µg/ml)	Amount recovered (µg/ml)	%Recovery ± SD
PIO	80	8	7.97	99.65 ± 0.78
	100	10	10.17	101.77 ± 1.67
	120	12	11.88	99.02 ± 1.56
GLM	80	8	7.93	99.20 ± 0.90
	100	10	9.90	99.04 ± 1.25
	120	12	21.78	98.26 ± 1.21

Values expressed mean±SD (n=3)

Table 3: Optical characteristics data and validation parameters

Parameters	Values for PIO	Values for GLM
Absorption maxima (λ max)	225nm	248nm
Beer's law limit (µg/ml)	5-30	4-20
Regression equation	y = 0.0006x + 0.0009	y = 0.1029x - 0.0198
Correlation coefficient (R2)	R^2 = 0.9912	R^2 = 0.9964
Molar absorptivity	0.807×10^3	0.514×10^3
A(1%,1cm)	82.52 dl $g^{-1}cm^{-1}$	51.47 dl $g^{-1}cm^{-1}$
Accuracy (%Recovery ± SD)	100.14 ± 1.04	98.83 ± 0.5
Precision		
Intraday*(%RSD)	100.16 ± 0.17	99.01 ± 0.96
Interday*(%RSD)	99.97 ± 0.83	99.24 ± 0.83
LOD (µg/ml)	0.0187	0.132
LOQ (µg/ml)	0.056	0.40

Validation:

The method was validated according to ICH Q2B guidelines for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for the analyte.[2][3][5]

Linearity:

To obtain calibration curves for both PIO and GLM, the first derivative spectra of standard drug solutions were recorded at varied concentrations of PIO (5-30 µg/ml) and GLM (4-20 µg/ml) and calibration curves are shown in (figure.-4) and (figure-5) respectively. The spectrum was measured three times for each concentration. The

correlation coefficients of calibration plots for PIO and GLM were 0.9912 and 0.9964 indicating good linearity in both cases that are presented in [Table 3]

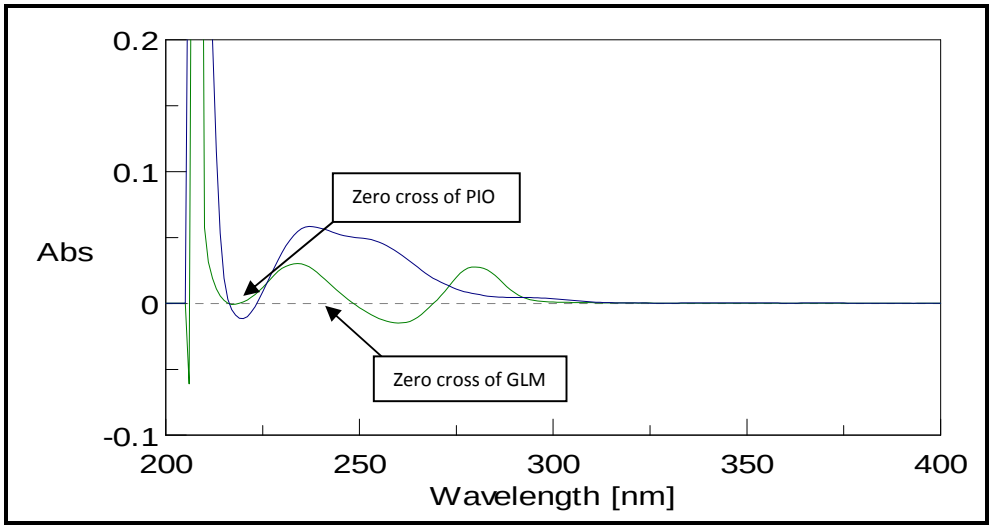


Fig.1: First derivative overlain spectrum of PIO and GlM

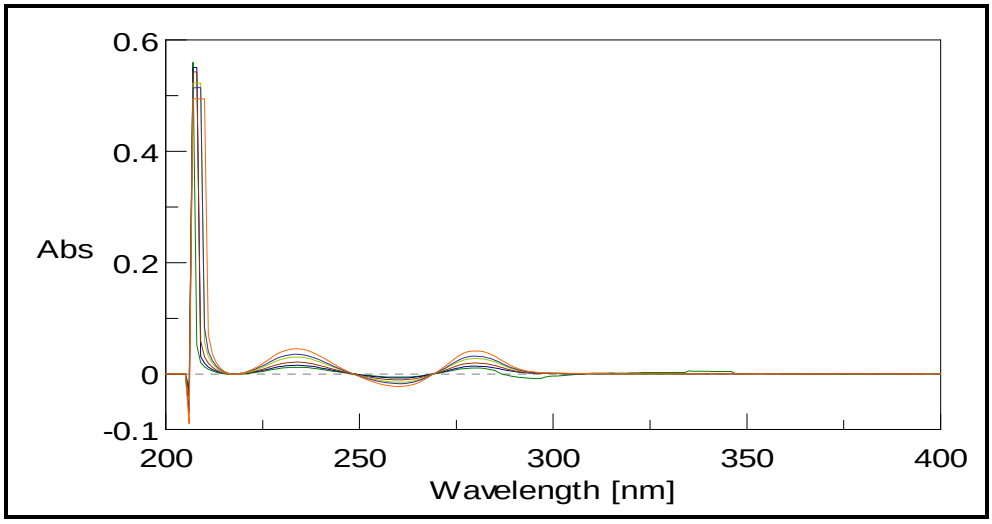


Fig. 2: First order derivative UV spectra of PIO

Accuracy:

To ascertain the accuracy of the proposed methods, recovery studies were carried at three different levels (80%, 100% and 120%). Percent recovery for PIO and GLM, by this method, was found in the range of 99.56-100.14%

Precision

T0 access the repeatability of the present study the Intra-day and inter-day precision of the method was evaluated for mixtures of PIO and GLM at three different independent concentrations by determining their assay. [Table3].

Limit of Detection (LOD) and Limit of Quantization (LOQ):

The LOD and LOQ of PIO and GLM by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3σ/S and 10σ/S respectively, where S is the slope of the calibration curve and σ is the standard deviation of response. The results of the same are shown in Table 3.

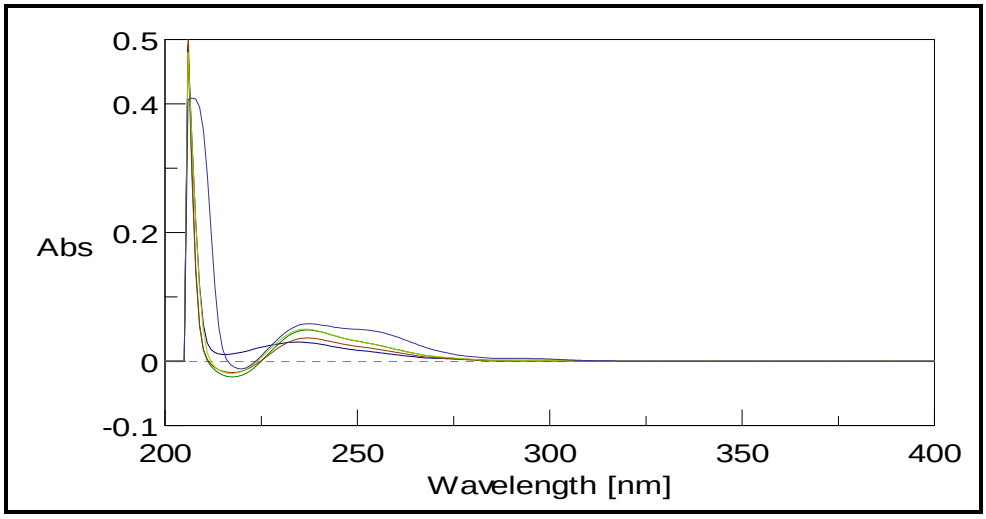


Fig. 3: First order derivative UV spectra of GLM

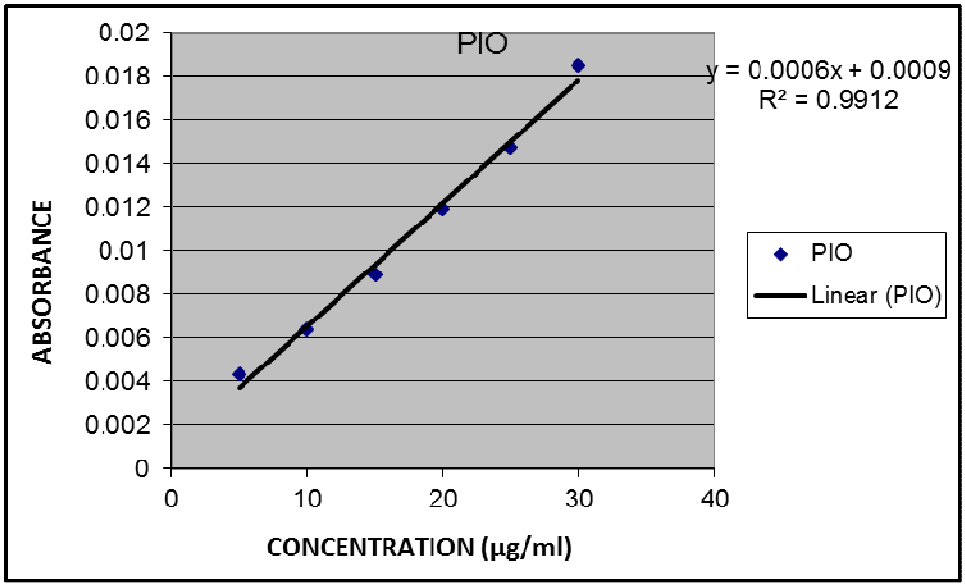


Fig. 4: Calibration curve of PIO

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, precision and recovery studies were performed. The drugs obey beer's law in the concentration range 5-30 µg/ml for PIO and 4-20 µg/ml for GLM for proposed the method with good correlation coefficient. The results of tablet formulation analysis are presented in Table 1. Results of recovery studies are shown in Table 2. The accuracy and reproducibility is evident from the data as results are close to 100 % and low standard deviation. The proposed method is simple, economical, rapid, precise and accurate. Hence these can be used for routine analysis of PIO and GLM in bulk and tablet formulation.

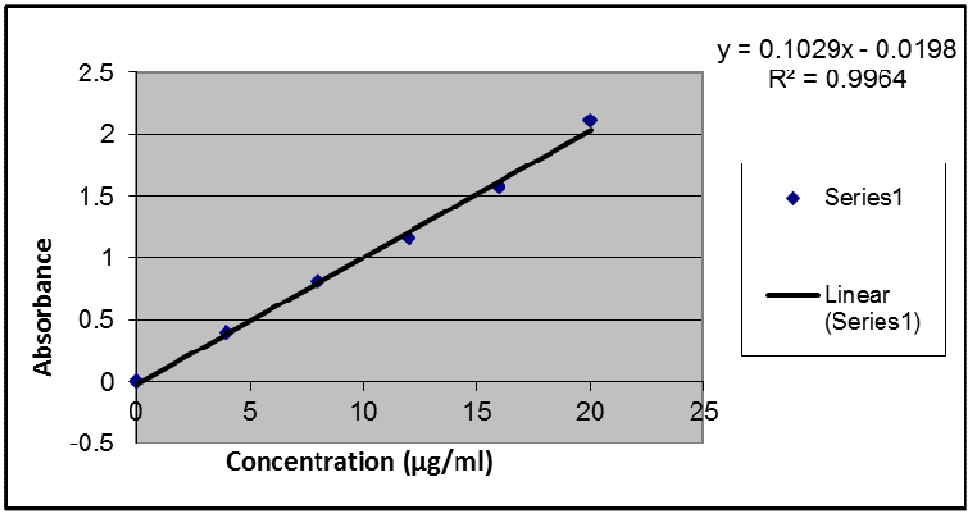


Fig. 5: Calibration curve of GLM
**(n=6)*

CONCLUSION

The proposed method is simple and accurate for estimation of PIO and GLM. The most striking feature of method should be its simplicity, economy and rapidity, which is always preferred by an analyst. The described methods give accurate and precise results for determination of PIO and GLM mixture hence can be used for routine analysis.

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REFERENCES

[1] Indian pharmacopoeia Vol. II and III, New Delhi, The controller of Publications.Govt of India, **2010**.p1418,1916.
[2] Validation of Analytical Procedures: Methodology (in Q2(R1))
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf
[3] ICH Q2A, Text on validation of analytical procedures, International Conference on Harmonization tripartite guidelines, adapted 27 Oct **1994**
[4] Patil PM, Development and Validation of UV derivative spectrophotometric method for determination of Glimepiride, Metformin, and Pioglitazone, in bulk and marketed formulation,JPSI (3), May-June **2012**.
[5] International Conference on Harmonization (ICH), Q2B**,** Validation of Analytical Procedures: Methodology, http://www.fda.gov/downloads/Regulator%20yInformation/Guidances/UCM128049.pdf
[6] SV Patel, GF Patel, SG Pipaliya., , *Inventi Impact: Pharm Ana & Qual Assur* Vol. 2012, Issue 2(**2012**)
[7] Madhuri D.Game, *Journal Pharmacy Research* **2011**,*4(11),4301-4302*
[8] Stenlake J. B. and Backett A. H.; Practical pharmaceutical Chemistry; C. B. S. Publishers And Distributors; New Delhi; 4th Ed., Part II, **1997**, 296-306.