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Spectrophotometric Determination of Pioglitazone Hydrochloride in Tablets and Urine

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A simple, sensitive and precise visible spectrophotometric method is developed for the determination of pioglitazone hydrochloride in pure form, in tablets and in human urine. The method is based on the reaction of pioglitazone hydrochloride with tetrazolium blue reagent in presence of sodium hydroxide at 65 ºC to form a pink-violet product with maximum absorbance at 518 nm. The optimum reaction conditions and reaction stoichiometry were evaluated. Linear calibration graph from 5-45 µg/mL of pioglitazone hydrochloride was obtained with minimum detectability of 1.4 µg/mL and a correlation coefficient of 0.9999. The relative deviation (n = 10) was 1.6. The method was applied to the determination of the drug in glustin tablets and gave good results. The method was successfully applied to the determination of pioglitazone hydrochloride in spiked human urine.

Key Words: Pioglitazone hydrochloride, Tetrazolium blue, Spectrophotometry, Glustin tablets, Human urine.

INTRODUCTION

Pioglitazone hydrochloride (Fig. 1), (±)-5-{*p*-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl}-2,4-thiazolidinedione hydrochloride is an oral antidiabetic agent used in the treatment of type 2 diabetes mellitus[1]. 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 antihyperglycemic drug to achieve optimal glycemic control.

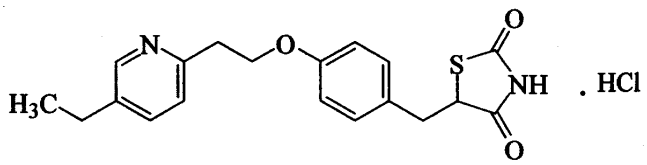


Fig. 1. Structure of pioglitazone hydrochloride

Various methods such as flow-injection chemiluminescence[2], voltammetry[3], HPLC[4-14], micellar electrokinetic chromatography (MEKC)[10], LC[15], LC-MS/MS[16,17], TLC[18-20], HPTLC[21] and capillary electrophoresis (CE)[22] have been used for the determination of pioglitazone hydrochloride in tablets and biological samples either when present alone or in mixtures with other antihyperglycemic drugs in multicomponent dosage forms. Also, potentiometric sensors[23] and ion selective membrane sensors[24] were fabricated for the determination of pioglitazone hydrochloride in some pharmaceutical formulations and plasma. Some spectrophotometric methods are available for the determination of pioglitazone hydrochloride. A colorimetric method based on the formation of an ion associated complex with bromocresol green, bromocresol purple, bromophenol blue and bromothymol blue have been described for the determination of pioglitazone in pure and dosage forms[25]. Other reagents such as diazotized sulphanilic acid in an alkaline medium[26], tropaeolin-OOO, wool fast blue, saffranin-O and methylene blue[27] have been used for the analysis of pioglitazone hydrochloride samples. Other reported spectrophotometric methods include: UV spectrophotometry[26,28,29], first-derivative UV spectrophotometry[5], second-derivative UV spectrophotometry[15,25] and methods using derivative, ratio derivative isosbestic and chemometric-assisted spectrophotometry in binary mixture with metformin and in ternary mixture with metformin and pioglitazone acid degradate[30].

The aim of this study is to develop a simple, economical, precise and accurate spectrophotometric method to determine pioglitazone hydrochloride in pure form as well as in its tablets and in urine.

EXPERIMENTAL

A UV-visible spectrophotometer (Ultrospec 2100 Pro/80-2112-21/Amersham Bioscience) with 1 cm quartz cells have been used throughout this work.

Pure pioglitazone hydrochloride sample was kindly supplied from Saudi Pharmaceutical Industries and Medical Appliances Corporation/Al-Qassim Pharmaceutical Plant (SPIMACO), Saudi Arabia. Glustin tablets, 15 mg pioglitazone hydrochloride per tablet (Takeda Chemical Industries, Ltd., Osaka, Japan) were purchased from the local market.

A stock solution containing 500 µg/mL of pioglitazone hydrochloride was prepared by dissolving 25 mg in 50 mL of ethanol.

All chemicals and solvents used were of spectroscopic grade and distilled water was used throughout this work. Ethanol (99.8 %, BDH-England) was used. Solutions of 0.5 % tetrazolium blue (Riedel-de Haen, Germany) in water and 0.02M NaOH (WINLAB) in ethanol were prepared.

General procedure: Aliquots (0.1-0.9 mL) of standard pioglitazone hydrochloride solution (500 µg/mL) were transferred into a series of 20 mL test tubes, 0.8 mL of 0.02M NaOH solution was added to each tube followed by 0.6 mL of tetrazolium blue solution (0.5 %). The tubes were heated at 65 ºC for 1 h, then cooled to room temperature and their constituents transferred quantitatively to a series of 10 mL volumetric flasks. The volume was completed with ethanol and absorbance of the pink-violet product was measured at 518 nm against a reagent blank similarly prepared. The calibration curve was plotted by relating the absorbance against drug concentration in µg/mL.

Procedure for tablets: Ten glustin tablets were finely ground and mixed. An amount of the fine powder equivalent to 25 mg pioglitazone hydrochloride was weighed and dissolved in ethanol, then filtered into a 50 mL volumetric flask and completed to volume with ethanol. The obtained solution labeled to contain 500 µg/mL pioglitazone hydrochloride was analyzed by the proposed method as detailed above.

Procedure for spiked urine: 25 mg of pure pioglitazone hydrochloride were weighed accurately and transferred into a 50 mL volumetric flask. 1.0 mL of human urine was added and the volume was completed with ethanol to prepare alcoholic solution claimed to contain 500 µg/mL pioglitazone hydrochloride to be analyzed as described above.

RESULTS AND DISCUSSION

Pioglitazone hydrochloride was found to react with tetrazolium blue in alcoholic alkaline medium to yield a pink-violet coloured product with λ_{max} 518 nm, Fig. 2.

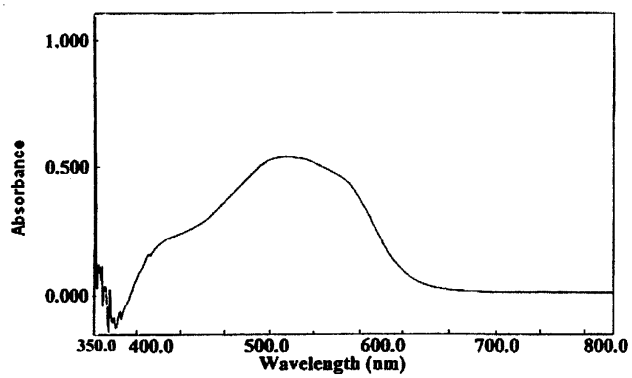


Fig. 2. Absorption spectrum of pioglitazone hydrochloride (20 µg/mL)/ tetrazolium blue product in presence of ethanolic NaOH

The absorption properties of the coloured product, as well as the influence of different parameters on colour development, were studied to determine optimal conditions for the assay.

Effect of tetrazolium blue concentration: Tetrazolium blue was used as a colour-producing reagent. To study the effect of concentration of the reagent on the colour of the product, various volumes of 0.5 % of the reagent in the range 0.1-0.8 mL were mixed with 35 µg/mL of pioglitazone hydrochloride in NaOH medium and heated at 65 ºC for 1 h. It was found that the absorbance gradually increases with increasing the reagent concentration up to 0.6 mL which gave maximum colour intensity after which the absorbance gave constant values (Fig. 3).

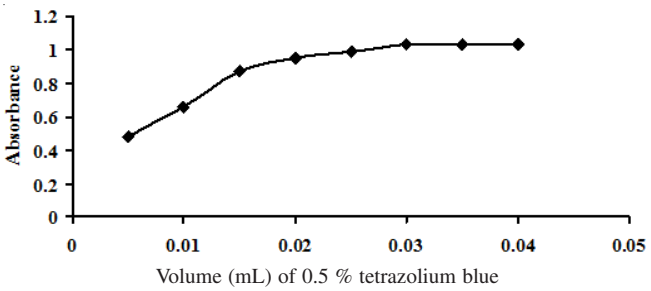


Fig. 3. Effect of concentration of tetrazolium blue on the absorbance of pioglitazone hydrochloride (35 µg/mL)/tetrazolium blue product at 518 nm in presence of ethanolic NaOH

Effect of sodium hydroxide concentration: The effect of addition of different volumes of 0.02M NaOH in the range 0.1-1.2 mL was studied. The absorbance was found to increase with increasing volume of NaOH. Maximum colour intensity was obtained upon using 0.8 mL of 0.02M NaOH as shown in Fig. 4.

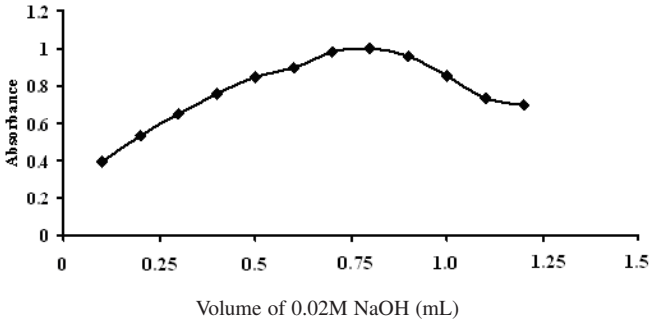


Fig. 4. Effect of concentration of ethanolic NaOH on the absorbance of pioglitazone hydrochloride (35 µg/mL)/tetrazolium blue product at 518 nm

Effect of temperature: The reaction of pioglitazone hydrochloride and tetrazolium blue is slow at room temperature. It was found that heating using water bath greatly enhances the reaction and the absorbance increases with increasing the reaction temperature. 60-70 ºC gave maximum colour intensity. 65 ºC was chosen as the best temperature for the reaction where higher temperatures cause a loss of the solvent (Fig. 5).

Effect of heating time: As mentioned earlier, the reaction needs heating at 65 ºC. Time of the reaction is very important parameter to ensure complete reaction. Therefore, the effect

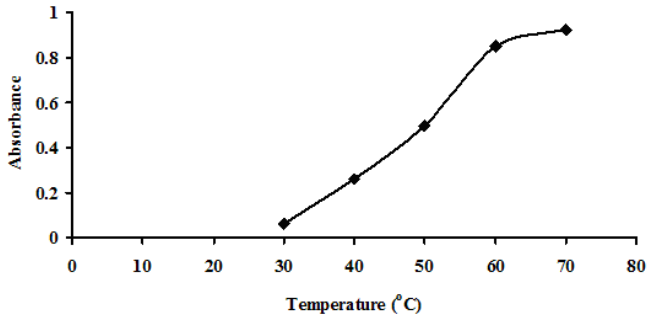


Fig. 5. Effect of temperature on the reaction of pioglitazone hydrochloride (35 µg/mL) and tetrazolium blue at 518 nm in presence of ethanolic NaOH

of time was studied by measuring the absorbance at different time intervals from 10-80 min. It was found that development of the colour of the product was completed after 1 h in the water bath at 65 ºC and remained stable for at least 24 h (Fig. 6).

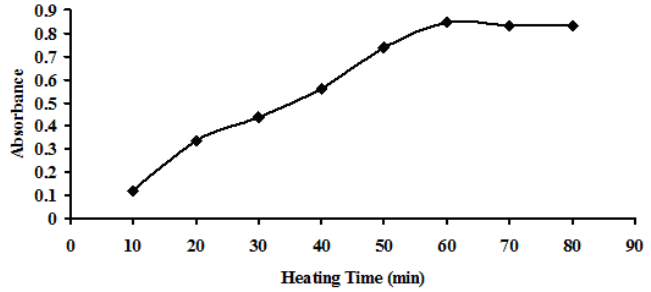


Fig. 6. Effect of heating time at 65 ºC on the reaction product of pioglitazone hydrochloride (35 µg/mL) and tetrazolium blue in presence of ethanolic NaOH at 518 nm

Application of continuous variation method[31] indicated a ratio of 4:1 drug-reagent (Fig. 7). The reaction pathway can be represented as shown in **Scheme-I**.

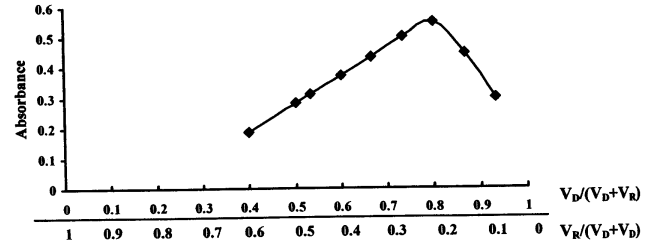


Fig. 7. Stoichiometric reaction of (1×10^{-3} M) pioglitazone hydrochloride with (1×10^{-3} M) tetrazolium blue by the continuous variation method. V_D = volume of drug and V_R = volume of reagent

Determination of pioglitazone hydrochloride: Under the described experimental conditions, standard calibration curve for pioglitazone hydrochloride by the proposed method was constructed by plotting the absorbance *versus* concentration. Conformity to Beer's law was evident over the concentration ranges of 5-45 µg/mL as shown in Fig. 8 and Table-1. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated and cited in Table-1. Spectral data and statistical evaluation of the experimental data gave the values given in Table-1.

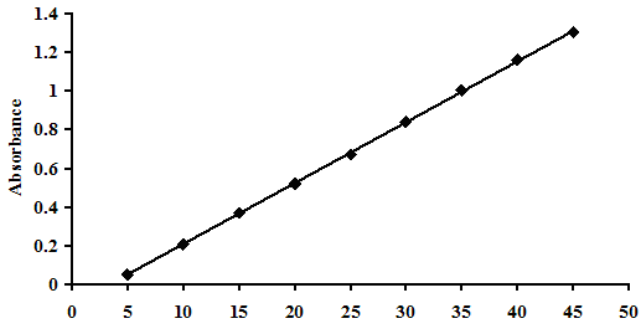


Fig. 8. Calibration curve of pioglitazone hydrochloride/tetrazolium blue product at 518 nm

TABLE-1
SPECTROPHOTOMETRIC DETERMINATION OF PIOGLITAZONE HYDROCHLORIDE WITH TETRAZOLIUM BLUE

Parameter	Proposed tetrazolium blue method
λ_{max} (nm)	518
Concentration range (µg/mL)	5-45
Regression equation parameters:	
Intercept (a)	-0.106
Slope (b)	0.0315
Correlation coefficient (r)	0.9999
LOD (µg/mL)	1.42
LOQ (µg/mL)	4.72
RSD % (n = 10)	1.6

The precision of the proposed method was tested by percentage of standard deviation (% RSD) of ten multiple analyses of solutions containing 20 µg/mL pioglitazone hydrochloride.

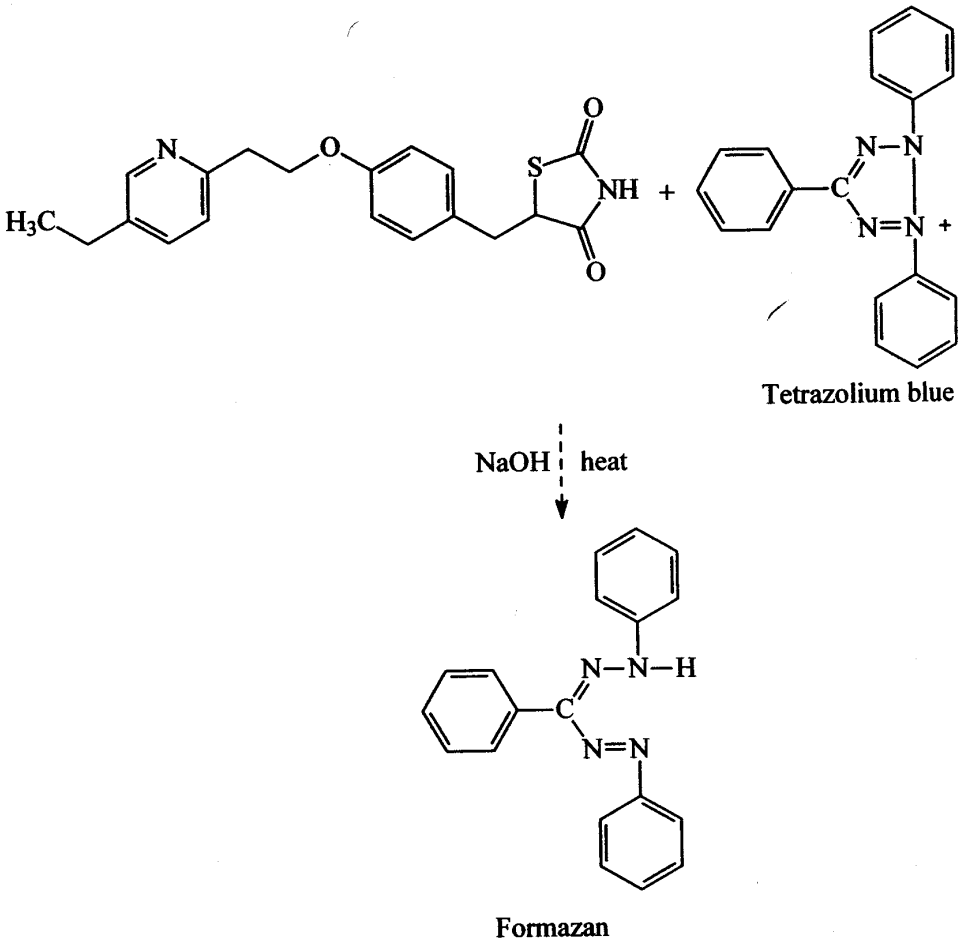
The validity of the method could be proved by analyzing authentic samples of the drug. The results obtained in Table-2 were in good agreement with those given by the comparison spectrophotometric method[29].

TABLE-2
DETERMINATION OF PIOGLITAZONE HYDROCHLORIDE IN PURE FORM AND TABLETS BY THE PROPOSED AND REFERENCE METHOS

Preparation	Proposed method	Reference method[29]
Pure pioglitazone HCl		
Found % ± SD	99.9 ± 0.59	100.6 ± 0.53
	n = 9	n= 3
t-Value	0.84 (2.228)	–
F-Value	1.25 (4.46)	–
Glustin tablets* (15 mg/tablet)		
Recovery % ± SD	100.8 ± 1.44	100.1 ± 1.10
t-Value	0.49 (2.776)	
F-Value	1.73 (19.0)	–

*Product of Takeda Chemical Industries, Osaka, Japan. Figures in parentheses are the theoretical *t* and F values at $p = 0.05$ confidence limit.

Statistical analysis[32] of these results using the student's t-test and the variance ratio F-test showed no significant difference between the performances of the methods as regards to accuracy and precision.



Scheme-I: Mechanism of the reaction between pioglitazone hydrochloride and tetrazolium blue in alkaline medium

Good reproducibilities were obtained upon application of the proposed method to different blind experiments of pure samples of pioglitazone hydrochloride; the mean accuracy was 99.9 ± 0.59 (Table-2).

Pharmaceutical applications: In order to evaluate the usefulness of the proposed method, it was applied to the determination of pioglitazone hydrochloride in tablets. The results were listed in Table-2 and agreed well with the comparison method[29]. To avoid the effect of the matrix interference in tablets of pioglitazone hydrochloride, the method of standard additions was constructed to overcome these interferences.

Application to spiked urine: The high sensitivity attained by the proposed method allowed the determination of pioglitazone hydrochloride in human urine. The drug can be directly analyzed in urine without any pretreatment. An extract procedure was not necessary. Results obtained are listed in Table-3, where the recovery of the studied drug is 99.1 from urine.

TABLE-3
DETERMINATION OF PIOGLITAZONE HYDROCHLORIDE IN SPIKED URINE USING TETRAZOLIUM BLUE

Taken (µg/mL)	Found (µg/mL)	Recovery (%)
20.0	19.7	98.5
25.0	25.7	102.8
30.0	28.8	96.0
35.0	34.4	98.3
40.0	40.0	100.0
Mean ± SD	–	99.1 ± 2.51

Conclusion

Pioglitazone hydrochloride reacts with tetrazolium blue in the presence of ethanolic sodium hydroxide to form a pink-violet product. This product which is known as Formazan formed as a reduction product of tetrazolium blue reagent by the drug in alkaline medium[33], which can be used for the spectrophotometric determination of pioglitazone hydrochloride. This product is stable for 24 h. The reaction was found to be temperature dependent, therefore heating at 65 ºC is necessary for the reaction to complete. The method is simple and reasonably sensitive for the determination of microgram amounts of pioglitazone hydrochloride. The proposed method is also easier and cheaper to perform than HPLC separations and do not require expensive reagents. These advantages coupled with acceptable precision make this method suitable for routine quality control. The proposed method was successfully applied to the analysis of pioglitazone hydrochloride in tablets and urine and yield satisfactory results.

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