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Research Article

SELECTIVE AND VALIDATED SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF ROSIGLITAZONE AND PIOGLITAZONE WITH 2, 4-DNP

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ABSTRACT:

New, simple and sensitive spectrophotometric methods for the determination of rosiglitazone and pioglitazone have been developed. The method is based on the oxidation of 2, 4-dinitrophenyl hydrazine (2, 4- DNP) and coupling of the oxidized product with rosiglitazone and pioglitazone to give intensely colored chromogen. Rosiglitazone and pioglitazone showed maximum absorbance at 421 nm and 480 nm with linearity was observed in the concentration range of 15.0-55µg/ml and 30.0-110.0µg/ml respectively. The relative standard deviations of 0.00903 % for rosiglitazone and 0.65 % for pioglitazone were obtained. The recoveries of rosiglitazone and pioglitazone tablets are in the range 97.846±0.52, 99.49±0.96 respectively. The proposed method is simple, rapid, precise and convenient for the assay of rosiglitazone and pioglitazone in commercial tablet preparations.

Keywords: Rosiglitazone, Pioglitazone, Oxidation, 2, 4- DNP, Spectrophotometry, Pharmaceutical formulation

INTRODUCTION:

Rosiglitazone maleate, (Figure 1), chemically [(±)-5-[4-[2-[N-methyl-N(2-pyridyl) amino] ethoxy] benzyl]-2,4-dione thiazolidine] maleate, it's a potent new oral anti hyperglycaemic agent that reduces insulin resistance in patients with type 2 diabetes by binding to peroxisome proliferator-activated receptors gamma [1-3]. Several methods have been reported for the determination of rosiglitazone in pharmaceutical preparations such as UV-spectrophotometry [4, 5], high performance thin layer chromatography [6-8], high performance liquid chromatography [9, 10] and visible spectrophotometric methods available for determination of rosiglitazone in tablets [11].

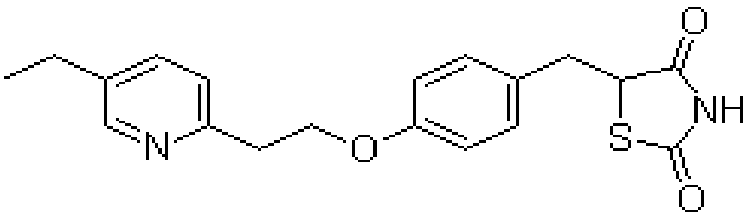


Figure 1: Chemical Structure of Pioglitazone.

Pioglitazone (Figure 2), chemically (*RS*)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2, 4-dione is an oral ant diabetic agent that has been shown to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues in animal models [12, 13]. Various method cited in literature for its determinations involve high performance liquid chromatography (HPLCH) [14, 15], PLC/MS [16], TLC [17], HPTLC [18], capillary electrophoresis [19], chemiluminescence [20], membrane selective electrode [21] potentiometry [22] and one spectrophotometric method was developed [23]

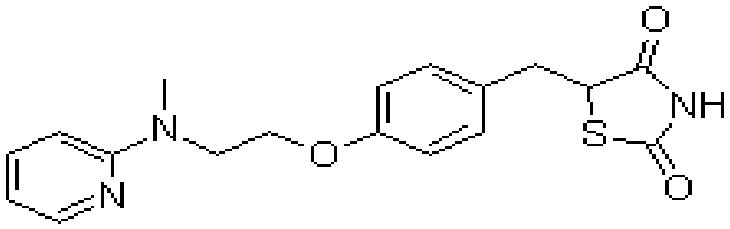


Figure 2: Chemical Structure of Rosiglitazone.

2, 4-dinitrophenyl hydrazine (2, 4- DNP) has been used as a chromogenic reagent for the spectrophotometric determination of many drugs [24, 25]. However, the reaction between 2, 4- DNP with rosiglitazone and pioglitazone has not been investigated so far. The present study describes the evaluation of 2, 4- DNP as a chromogenic reagent in the development of simple and rapid spectrophotometric method for the determination of rosiglitazone and pioglitazone in its pharmaceutical dosage forms.

EXPERIMENTAL

Apparatus

A Shimadzu UV-visible spectrophotometer model 1800 with 1 cm matched quartz cell was used for the absorbance measurements. Systonics electronic balance was used for weighing the samples.

Chemicals and Reagents

All employed chemicals were of analytical grade and high-purified water was used throughout the study. Rosiglitazone and pioglitazone pure samples were obtained as a gift samples from Dr. Reddy's Laboratories, Hyderabad, India.

2, 4-dinitrophenyl hydrazine (2, 4- DNP) 0.08 %(w/v)

0.08 g of 2, 4- DNP was accurately weighed transferred into a 100 ml calibrated flask, dissolved in 10ml distilled water, and make up the volume up to the mark with distilled water to obtain a solution of 0.08% (w/v). The solution was freshly prepared and protected from light during the use.

10 N Sodium hydroxide solution

40 g of sodium hydroxide is accurately weighed and transferred into a 100.0ml volumetric flask and made up to the mark with distilled water.

Potassium iodate 4% (w/v)

4 g of potassium iodate is accurately weighed and transferred into a 100.0 ml volumetric flask and made up to the mark with distilled water.

Standard solutions

Rosiglitazone and pioglitazone stock solutions (1000 µg/ml) were prepared separately by dissolving in distilled water. Working solutions of the drug were prepared by dilution of the stock solution. The tablet forms of rosiglitazone and pioglitazone which are used in the determination was Reglit®(2 mg) (Dr. Reddy's Laboratories, Hyderabad, India) and PIOZ 15 (15 mg) (Dr. Reddy's Laboratories, Hyderabad, India) respectively.

Selection of Analytical Wavelengths for Rosiglitazone and Pioglitazone

A 1.5 ml quantity of 0.08% 2, 4- DNP solution, 1.5 ml 0f 4 % potassium iodate and 1ml of 10N sodium hydroxide were added into two test tubes and 3.5 ml of rosiglitazone and 3.0 ml pioglitazone stock solutions were added. The immediate colored complex was formed. The solutions were made up to 10ml with water. The absorption spectrums of the complex were determined against blank solution and the wavelengths of maximum absorption ($\lambda_{max)}$ of the products of the reactions were noted.

Effect of Reagent Concentration

The effect of varying the concentration of 2,4-DNP was carried out using reagent concentrations of 0.01, 0.02, 0.03, 0.04 ...0.08% in 10N NaOH and 4 % potassium iodate. After mixing 1.5 ml of each reagent concentration with the drug solutions of Rosiglitazone and pioglitazone and made up to 10 ml with water, the absorbance readings of the complex formed were made at 421 nm and 480 nm on the UV-visible spectrophotometer.

Optimization Studies

Effect of 2, 4- DNP Concentration

The studying of 2, 4 -DNP concentrations revealed that the reaction was dependent on 2, 4 -DNP reagent. The absorbance of the reaction solution increased as the 2,4- DNP concentration increased, and the highest absorption intensity was attained at 2,4- DNP concentration of 0.08 % (w/v). Higher 2, 4- DNP concentrations up to 1.5 % had no effect on the absorption values. Further experiments were carried out using 0.08 %.

Preparation of calibration curve

Standard solutions of rosiglitazone and pioglitazone in water, having final concentrations in the range of 15.0-55 µg/ml and 30.0-110.0 µg/ml, were transferred into a series of 10 ml volumetric flasks, to these solutions 1.5 ml of 0.08% 2, 4- DNP, 1.5 ml of 4% potassium iodate and 1 ml of 10N sodium hydroxide was added. The mixture was then gently shaken until the appearance of colour chromogen. The contents were diluted up to 10 ml with distilled water. The absorbance of each solution was measured at 421 nm and 480 nm respectively against the reagent blank prepared in the same manner, without the analyte and the absorption spectra and calibration curve are represented in the (Figure 3 and 4) respectively.

Figure 3: Absorption spectra of 2, 4 DNP with rosiglitazone and pioglitazone against the reagent blank.

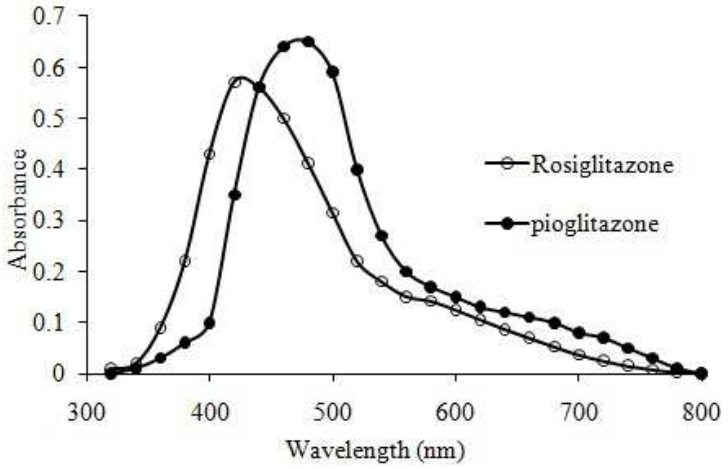
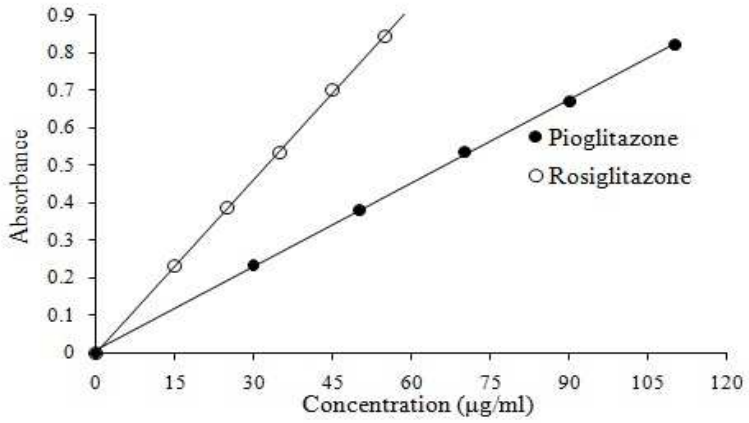


Figure 4: Calibration graphs of rosiglitazone and pioglitazone.



Analysis of commercial pharmaceutical preparations

Tablets

An appropriate amount of rosiglitazone and pioglitazone were dissolved in water so as to prepare 1000 µg/ml solution. An aliquot of this solution was diluted with water to obtain concentrations of 35 µg/ml and 30 µg/ml respectively. To that solution 1.5 ml of 0.08% 2, 4 -DNP, 1.5 ml of 4 % potassium iodate and 1 ml of 10N sodium hydroxide is added. The mixture was then gently shaken until the appearance of colour chromogen. The contents were diluted up to 10 ml with distilled water.

General procedure:

Several standard solutions of rosiglitazone and pioglitazone were taken in individual standard flasks. To each standard flask, 1.5 ml of 0.08% 2, 4- DNP, 1.5 ml of 4 % potassium iodate and 1 ml of 10N sodium hydroxide was added. The mixtures were then shaken until the appearance of color chromogen. The absorbance was measured at λ max at 421 nm and 480 nm for rosiglitazone and pioglitazone respectively against a blank similarly prepared by omitting the drug solution with water. The concentration of rosiglitazone and pioglitazone in each standard flask was obtained by interpolating the corresponding absorbance value from Beer's plot of standard rosiglitazone and pioglitazone solutions.

Quantification

The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated. Regression analyses of the Beer's law plots at their respective λmax values revealed a good correlation. Graphs

of absorbance versus concentration showed zero intercept, and are described by the regression equation, $Y = bX + c$ (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in μg/ml) obtained by the least-squares method. The results are summarized in Table 1.

Table-1: Optical characteristics.

S.No	Parameter	Values	
		Rosiglitazone	**Pioglitazone**
1.	λ_{max}/ nm	421nm	480nm
2.	Beers law limits (μg/ml)	15.0-55	30.0-110.0
3.	Molar absorptivity (1 /mol/cm)	5.59×10^3	2.78×10^3
4.	Correlation coefficient (R)	0.9998	0.9996
5.	Sandell's sensitivity(ng cm^{-2})	0.0649	0.127
6.	Regression equation (y)	y = 0.0154x + 0.0005	y = 0.0074x + 0.007
7.	Slope, *b*	0.0154	0.0074
8.	Intercept, *c*	0.0005	0.007
9.	Relative standard deviation%	0.00903	0.65
10.	Limit of detection (μg/ml)	0.42	0.66
11.	Limit of quantification(μg/ml)	1.28	1.98

Validation of the method

The validity of the method for the assay of rosiglitazone and pioglitazone were examined by determining the precision and accuracy. This was determined by analyzing six replicates of the drug within the Beer's law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. To study the accuracy of the methods, recovery studies were carried out by the standard calibration curve method. For this, known quantities of pure rosiglitazone and pioglitazone were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results are given in Table 2, 3. The average percent recoveries obtained were quantitative indicating good accuracy of the methods.

Table-2: Results of recovery study by standard addition method for Rosiglitazone.

S.no	Standard Rosiglitazone (µg)	Sample Rosiglitazone (µg)	Absorbance at 421nm	Amount of Rosiglitazone from std.graph	Recovery of std (mg)	% Recovery
1	15	10	0.385	26.5	16.5	110.0%
2	25	10	0.534	36.5	26.5	106.0%
3	35	10	0.699	48.0	38.0	108.0%

Table 3: Results of recovery study by standard addition method for Pioglitazone.

S.no	Standard Pioglitazone (µg)	Sample Pioglitazone (µg)	Absorbance at 421nm	Amount of Pioglitazone from std.graph	Recovery of std (mg)	% Recovery
1	30	20	0.390	50	20	100.0%
2	50	20	0.500	68	18	90.0%
3	70	20	0.530	89	19	95.0%

Precision

The precision of the proposed methods was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and finding out the absorbance by the proposed method in all the three drugs. The results are given in Table 4.

Table-4: Evaluation of accuracy and precision.

Drug	S.no	Label Claim (mg)	Amount found* (mg)	% Purity*	Average (%)	S.D	%R.S.D
Rosiglitazone	1	2.0	1.95	97.50	97.846	1.10	1.127
	2		1.92	96.00			
	3		1.96	98.00			
	4		1.96	98.00			
	5		1.98	99.08			
	6		1.97	98.50			
Pioglitazone	1	15.0	14.90	99.33	99.49	0.230	1.05
	2		14.94	99.6			
	3		14.89	99.26			
	4		14.98	99.86			
	5		14.95	99.60			
	6		14.99	99.33			

SD. Standard deviation; RSD.relative standard deviation.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts of bulk samples of rosiglitazone and pioglitazone within the linearity range were taken and added to the pre-analyzed formulation.

Ruggedness: To ascertain the ruggedness of the methods, six replicate determinations at different concentration levels of the drugs were carried out. The intra-day RSD values were less than 1%. The values of between-day RSD for different concentrations of drugs obtained from the determinations and indicate that the proposed method has reasonable ruggedness. The within-day RSD values were less than 1%. The values of inter-day RSD for different concentrations of drugs obtained from the determinations and indicate that the proposed method has reasonable ruggedness.

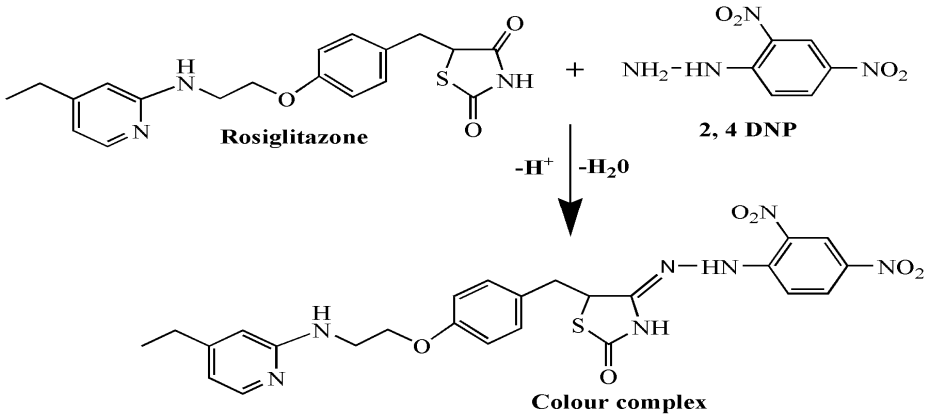
Results and discussion:

Spectral characteristic

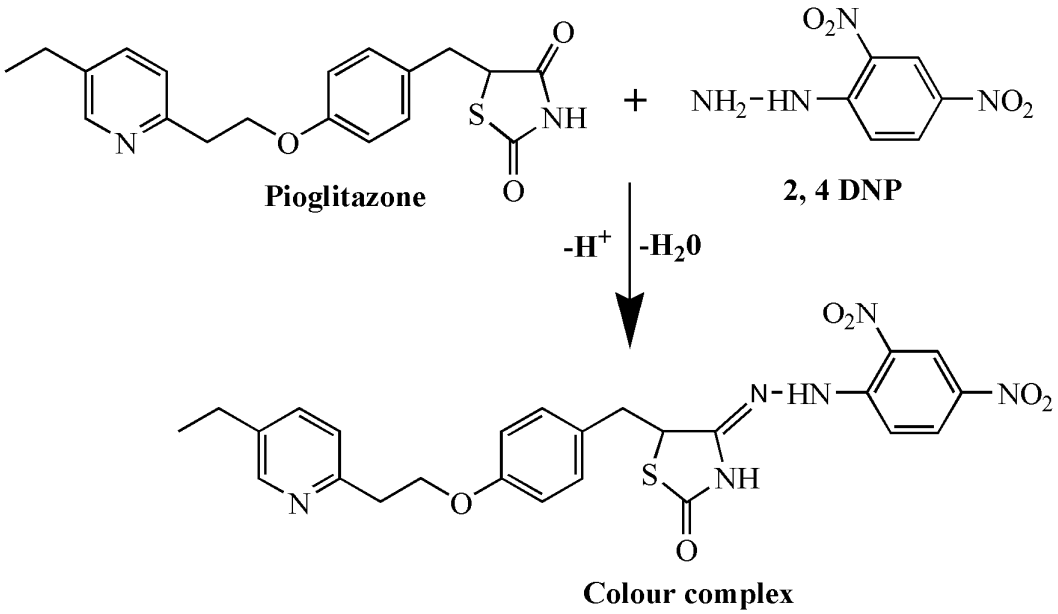
The absorption spectra of the reaction product of oxidized 2,4- DNP with drugs show maximum absorption (λmax) at 421 nm and 480 nm for of rosiglitazone and pioglitazone respectively. The blank solution had negligible absorbance at the λmax in which the drugs were analysed. The thus formed color was stable for more than two hours.

Reaction sequence and stoichiometric relationship: The 2, 4- DNP is oxidized by potassium iodate to give diazonium cation that reacts with drugs by electrophilic substitution at the phenolic ring to give deep colored chromogens. The proposed reaction sequence for rosiglitazone and pioglitazone are shown in Scheme 1 & 2.

Scheme 1: Mechanism of reaction of Rosiglitazone with 2,4 DNP and formation colored chromogen



Scheme 2: Mechanism of reaction of pioglitazone with 2, 4 DNP and formation colored chromogen



CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of rosiglitazone and pioglitazone in pure form and in pharmaceutical preparations.

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