RESEARCH PAPER

Stability Indicating Spectrophotometric Method Of Pioglitazone Hcl And Glimepiride In Bulk And Pharmaceutical Dosage Form

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Abstract

Simple, rapid, precise and accurate spectrophotometric methods have been developed for determination of Glimepiride (GLIM) and Pioglitazone (PIO) by simultaneous equation method and stability study method in combined dosage form. The simultaneous equation method is based on measurement of absorbance at 217nm and 228 nm as two wavelengths selected for quantification of GLIM and PIO. Pioglitazone and Glimepiride were subjected to stress degradation under different conditions recommended by ICH. The sample so generated was used for degradation studies using the developed method. The proposed methods were validated and can be used for analysis of combined dosage tablet formulation containing GLIM and PIO.

Keywords: Pioglitazone HCl; Glimepiride; Simultaneous equation method; Stability indicating; Spectrophotometric method; ICH guidelines.

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1. Introduction

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.Fig.1 [1]

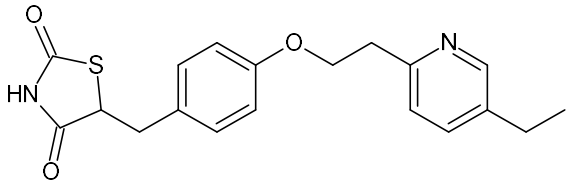

Fig.1-Chemical structure of Pioglitazone

Pioglitazone selectively stimulates the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) and to a lesser extent PPAR-α. [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. [1]

Glimepiride is a medium-to-long acting sulfonylurea anti-diabetic drug and is chemically 3-ethyl-4-methyl-N-(4-[N-((1r, 4r)-4-methylcyclohexylcarbamoyl) sulfamoyl] phenethyl)-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamide .fig.2[1]

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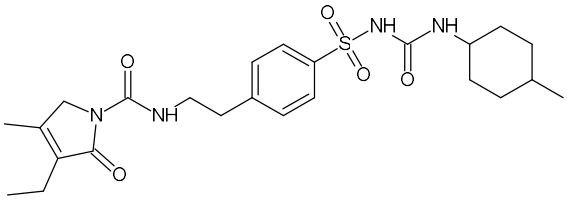

Fig.2-Chemical structure of Glimepiride

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. [1]

Drug is available in tablet dosage form as PIO -15 mg and GLIM- 2 mg (PIOZ*-G) in the market.

Literature survey revealed that Pioglitazone has been estimated with other drugs using HPLC[2-9] and Glimepiride has been determined along with other drugs by UV[10], and HPLC[7-9,11-12].. Since, no spectrophotometric method has been reported yet for stability study estimation of PIO and GLIM in 0.1 N NaOH, so the present study is focused on a successful attempt to estimate PIO and GLIM using more economical UV spectrophotometric method.

MATERIALS AND METHODS

Instrumentation

An UV-visible double beam spectrophotometer (JASCO V-630) with Matched quartz cells corresponding to 1 cm path length. AUX – 220 single pan electronic balance was used for weighing the materials.

Reagent and chemicals

Pioglitazone (PIO) and Glimepiride (GLIM) were procured from Cassel Research Lab, Chennai as a gift sample. Sodium hydroxide (Analytical grade) was procured from SD Fine Chemicals, Mumbai. All the chemicals used were of analytical grade and were used without further purification. Distilled water was used as a solvent throughout the study**.**

Preparation of Stock Solution

Pioglitazone

Pioglitazone is soluble in 0.1 N NaoH. Standard stock solution of Pioglitazone was prepared by dissolving 10 mg Pioglitazone in 100 ml of 0.1N sodium Hydroxide to produce a concentration of 100 µg/ml. which is the standard stock solution**.**

Glimepiride

Glimepiride is soluble in 0.1 N NaoH. Standard stock solution of Glimepiride was prepared by dissolving 10 mg of Glimepiride in 100 ml of 0.1N sodium Hydroxide to produce a concentration of 100 µg/ml. which is the standard stock solution.

Mixture (Marketed tablet)

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accuracy GLIM weighed quantity of powder equivalent to 15 mg and 2 mg of Pioglitazone and Glimepiride respectively and transferred into 100 ml volumetric flask containing 50 ml Sodium Hydroxide, sonicate for 15 min., volume was adjusted to mark with same solvent up to 100 ml to produce a concentration 150 µg/ml of Pioglitazone and 20 µg/ml of Glimepiride respectively . Then filter with Whatmann filter paper No.41. And these filtrate used for further studies.

Determination of λmax-

Standard stock solution containing 100 µg/ml of Pioglitazone (PIO) and Glimepiride (GLIM) was prepared in 0.1N sodium Hydroxide. Different aliquots were taken from the stock, diluted to 10ml mark with same solvent to obtain series of concentrations. The solution were scanned on spectrophotometer in the UV range 200-400 nm Pioglitazone (PIO) and Glimepiride (GLIM) showed absorbance maxima at 217nm and 228nm. The optical characteristic and linear regression data is summarized in table 1 and fig.3&4.

TABLE 1: SUMMARY OF OPTICAL CHARACTERISTIC AND REGRESSION STUDIES OF PIOGLITAZONE AND GLIMEPIRIDE

Parameter	**UV-Spectrophotometric**	
	Pioglitazone	Glimepiride
λ max (nm)	217	228
Beer's Law limits (µg/ml)	5-20 (µg/ml)	5-25 (µg/ml)
Regression equation (*Y)	0.040x	0.036x
Slope (m)	0.040	0.036

Intercept (c)	0.001	0.004
Standard deviation	0.2978	0.2433
Correlation co-efficient (r)	0.999	0.997

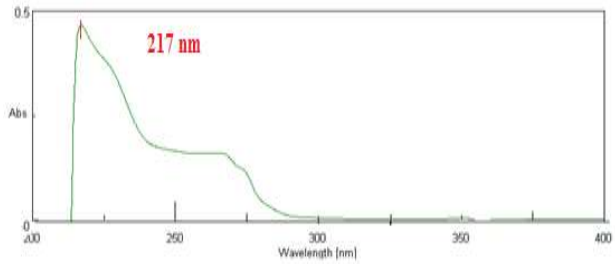

Fig.3- λmax of Pioglitazone shows at 217 nm

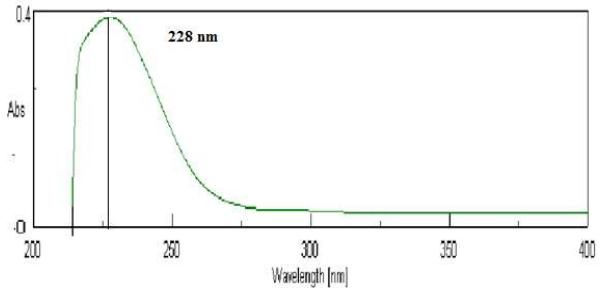

Fig.4- λmax of Glimepiride shows at 228 nm

METHOD DEVELOPMENT

Simultaneous Equation Method [13]

Two wavelengths selected for the 217 nm and 228 nm absorption maxima of GLIM and PIO respectively. From stock solution of Pioglitazone transferred 2 ml of solution in each three 10 ml volumetric flask and dilute up to 10 ml with 0.1 N NaOH.The concentration of solution become 20 µg/ml. From stock solution Glimepiride transferred 2.5 ml in each three 10 ml of volumetric flask and dilute up to 10 ml with 0.1N NaOH.The concentration of solution become 25 µg/ml.From stock solution of mixture transferred 1 ml each three 10 ml volumetric flask and dilute up to 10 ml with 0.1 N NaOH.The concentration of solution become 10 µg/ml.This solution was measured at selected wavelength and absorptivity was determined as a mean of three independent determinations. Concentration of the drug in the samples was obtained using the following equation:

$$Cx = \frac{A1\ ay2 - A2\ ay1}{Ax1 ay2 - ax_2\ ay_1} \quad \text{.....eq. 1}$$

$$Cy = \frac{A1\ ax2 - A2\ ax1}{ay1\ ax2 - ay2\ ax1} \quad \text{.....eq. 2}$$

Where,

A1 and A2 are absorbance of mixture at λ1 and λ2 respectively,

ax1 and ax2 are the absorptivities of PIO at λ1 and λ2 respectively and

ay1, ay2 are the absorptivities of GLIM at λ1 and λ2 respectively.

Cx and Cy are the concentrations of PIO and GLIM in µg/ml respectively

METHOD VALIDATION [14]

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for different parameters like Linearity, Accuracy, Precision, Ruggedness, Limit Of Detection (LOD) and Limit Of Quantification (LOQ) as per the ICH (International Conference on Harmonization).[9,12,13]

Linearity

Various aliquots were prepared from the stock solution (100µg/ml) ranging from 1- 10µg/ml. The samples were scanned in UV-VIS Spectrophotometer using Sodium hydroxide as blank. It was found that the selected drug shows linearity between the 1-10 µg/ml. The optical

characteristic and linear regression data is summarized in table 1, calibration curve shown in fig 5&6.

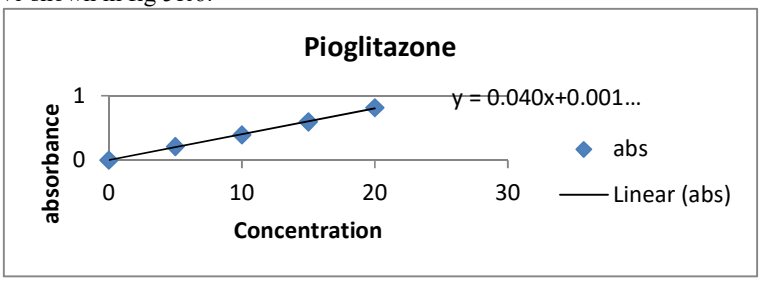

Fig.5-Callibration curve for Pioglitazone in 0.1 N NaOH

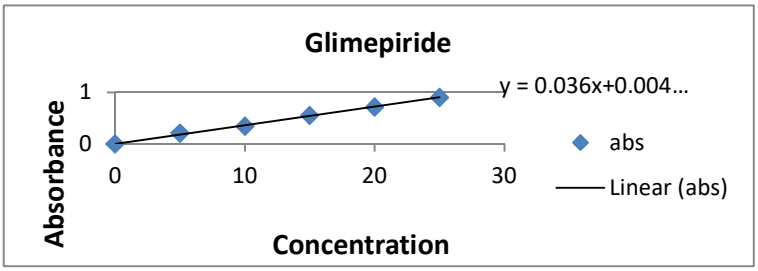

Fig.6- Calibration curve for Glimepiride in 0.1N NaOH

Precision

Precision of the method was demonstrated by intraday and interday variation studies. In intraday variation study, 9 different solutions of same concentration that is 10µg/ml were prepared and analyzed three times in a day i.e. morning, afternoon and evening and evening and the absorbance were noted. The result was indicated by % RSD. In the interday variation study, solutions of same concentration 10µg/ml were prepared and analyzed three times for three consecutive days and the absorbances were noted. The result was indicated by % RSD in table 2.

TABLE 2: RESULT OF PRECISION OF PIOGLITAZONE AND GLIMEPIRIDE

Level of % recovery	% recovery of dugs		Standard Deviation		%R.S.D	
	Pioglitazone	Glimepiride	Pioglitazone	Glimepiride	Pioglitazone	Glimepiride
80	97.05	97.42	0.005	0.02	0.57	0.0205
100	98.03	98.10	0.03	0.05	0.030	0.050
120	98.98	99.31	0.01	0.005	0.011	0.0050

Accuracy:

The accuracy of an analytical procedure express the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found.

The accuracy of the method was determined by preparing solutions of different concentrations that is 80%, 100% and 120% in which the amount of marketed formulation was kept constant (10mg) and the amount of pure drug was also kept

constant 10mg for 80%, 100% and 120% respectively. The solutions were prepared and the accuracy was indicated by % recovery. The recovery values for Pioglitazone and Glimepiride ranged from 97% to 103 % shown in table 3.

Drug	Intraday			Interday		
	% Mean	S.D	%R.S.D	% Mean	S.D	%R.S.D
Pioglitazone	99.68	0.3176	0.31	99.6	0.05	0.050
Glimepiride	99.6	0.4092	0.41	99.65	0.02	0.020

TABLE 3: RESULT OF RECOVERY STUDY (ACCURACY) OF PIOGLITAZONE AND GLIMEPIRIDE

Limit of detection (LOD)

The limit of detection (LOD) was determined based on standard deviation of response of the calibration curve. The standard deviation of absorbance of calibration curve and slope of the calibration curves was used. According to following formula was used to calculate the LOD. The result was shown in table 4.

TABLE 4: RESULT OF LOD AND LOQ OF PIOGLITAZONE AND GLIMEPIRIDE

Parameter	Drug	
	Pioglitazone	Glimepiride
LOD	1.69 mg/ml	1.68 mg/ml
LOQ	5.13 mg/ml	5.11 mg/ml

LOD=3.3 X S.D/S

Where; S.D=standard deviation

S=Slope of calibration curve

Limit of quantification (LOQ)

The limit of quantification (LOQ) was determined based on standard deviation of response of the calibration curve. The standard deviation of absorbance of calibration curve and slope of the calibration curves was used. According to following formula was used to calculate the LOQ. The result was shown in table 4.

LOQ=10 X S.D/S

Where; S.D=standard deviation

S=Slope of calibration curve

Assay of marketed formulation (Tablet)

For analysis of commercial formulation, twenty tablets were weighed, average weight determined and crushed into fine powder. An accurately weighed quantity of powder equivalent to 15 mg and 2 mg of Pioglitazone and Glimepiride respectively and transferred into 100 ml volumetric flask containing 50 ml Sodium Hydroxide, sonicate for 15 min., volume was adjusted to mark with same solvent up to 100 ml to produce a concentration 150 µg/ml of PIO and 20 µg/ml of GLIM. Then filter with Whatmann filter paper No.41. And these filtrate used for further dilutions using 0.1 N NaOH. Calculate amount found (mg/ml) and % of label claim estimated of PIO and GLIM in marketed formulation. Result was shown in table no.5

TABLE 5: ANALYSIS DATA OF MARKETED FORMULATION

Sr.no	Label claim (mg/tablet)		Amount found (mg/ml)		% of label claim estimated	
	Pioglitazone	Glimepiride	Pioglitazone	Glimepiride	Pioglitazone	Glimepiride
1	15	2	14.55	1.99	97%	99.5

DEGRADATION STUDIES [15, 16 & 17]

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Pioglitazone and Glimepiride using the method developed.

Stress degradation by hydrolysis under acidic condition:
To 1ml of stock solution (100µg/ml) of Pioglitazone and Glimepiride (tablet), 5ml of 1N HCL was added in 10ml of volumetric flask and kept for 3hours. After 3hours solution was neutralized and diluted with 0.1N sodium hydroxide up to the mark (10µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis. The result was shown in table no 6, Acidic degradation of Pioglitazone and Glimepiride (tablet) after 3hours shown in fig 7.

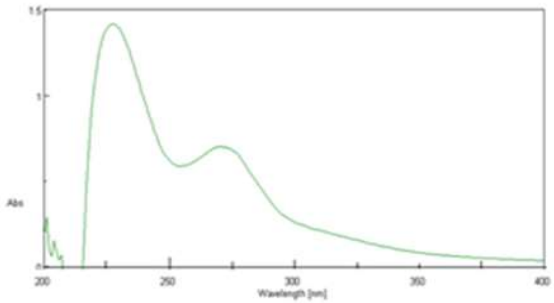

Fig.7-Acidic degradation of marketed formulation (tablet) after 2 hr

Stress degradation by hydrolysis under alkaline condition:
To 1ml of stock solution (100 µg/ml) of Pioglitazone and Glimepiride (tablet), 5ml of 1N NaoH was added in 10ml of volumetric flask and kept for 4hours. After 4hours solution was neutralized and diluted with 0.1N sodium hydroxide up to the mark (10 µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis. The result was shown in table no 6. Alkaline degradation of Pioglitazone and Glimepiride (tablet) after 4hours shown in fig 8.

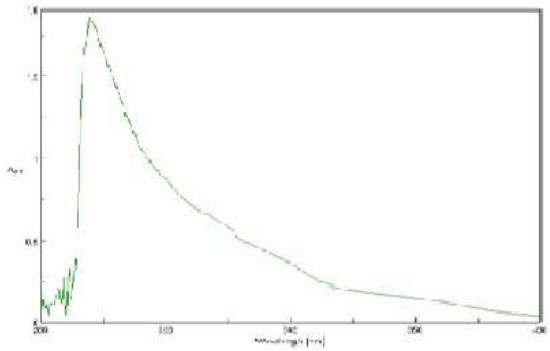

Fig8.-Basic degradation of marketed formulation (tablet) after 2 hr

Oxidative degradation:
To 1ml of stock solution (100µg/ml) of Pioglitazone and Glimepiride (tablet), 5ml of 6% Hydrogen Peroxide was added in 10ml of volumetric flask and kept for 1hours. After 1hours solution was diluted with 0.1N sodium hydroxide up to the mark (10µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis. The result was shown in table no 6. Oxidative degradation of Pioglitazone and Glimepiride (tablet) shown in fig 9.

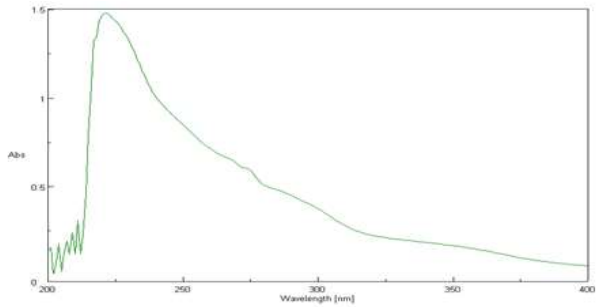

Fig.9-Oxidative degradation of marketed formulation (tablet) after 2 hr

Photolytic degradation:

Photolytic degradation Sample of Pioglitazone and Glimepiride (tablet) was exposed to near ultraviolet lamp in photostablity chamber at 190-320 nm. After that 10mg of sample was dissolved 0.1N NaoH and volume made up to 100 ml. From this solution appropriate dilution (20µg/ml) was made using 0.1N NaoH and the solution was taken in cuvette for the U.V. analysis. The result was shown in table no 6. Photolytic degradation shown in fig 10.

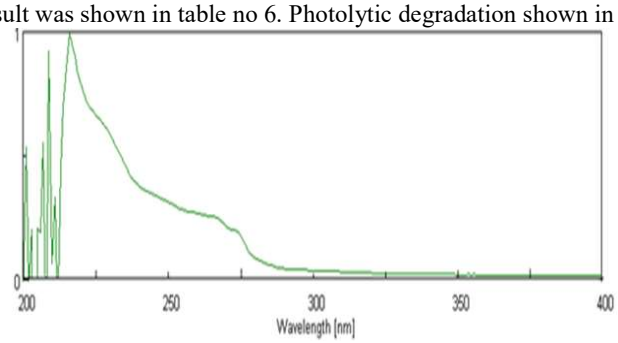

Fig.10-Photolytic degradation of marketed formulation (tablet) after 2 hr

Dry heat induced degradation:

Pioglitazone and Glimepiride(tablet) sample was taken in a Petri plate and exposed to a temperature of 50°c for 48 hours in an oven. Because 40°c and 45°c was not shown any degradation. After 48 hours, 10 mg of the sample was diluted with 0.1N NaoH in order to make the volume up to 100 ml. From this solution, dilutions were carried out to achieve the appropriate concentration (20µg/ml) and the solution was taken in cuvette for the UV-VIS Analysis. The result was shown in table no 6. Dry heat induced degradation of Pioglitazone and Glimepiride (tablet) shown in fig 11.

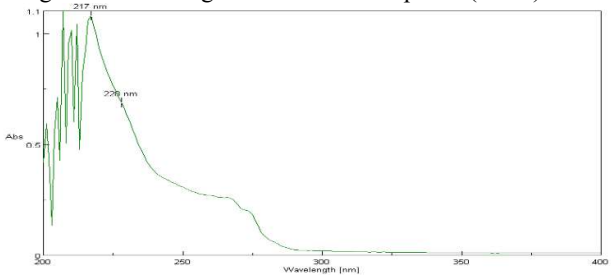

Fig.11—Dry heat induced degradation of marketed formulation (tablet) after 24 hr

RESULT

The proposed methods of analysis for PIO and GLIM in combination were validated as per the recommendations of ICH guidelines for parameter like accuracy, precision, linearity, and range, limit of detection and limit of quantification. The drugs obeys Beer's law in concentration range of 5-20 µg/ml for PIO and 5-25 µg/ml for GLIM with correlation coefficient greater than 0.999. Precision of both the methods were calculated by intraday and inter day variation study and %RSD of observation were found to be less than 2, shown in table 2. Results of recovery studies were within 97-103%, shown in table 3. The stress degradation studies showed that Pioglitazone and Glimepiride (Marketed formulation) i.e. tablet undergoes degradation in acidic, alkaline, oxidative, photolytic conditions whereas it is relatively stable when exposed to dry heat, conditions. Summary of the results of stress degradation studies of Pioglitazone and Glimepiride are shown in the Table 6.

TABLE 6: RESULT OF DEGRADATION STUDY PIOGLITAZONE AND GLIMEPIRIDE

Sr.no	**Stress condition**	**Time**	**% Assay of active substance of drugs**	
			Pioglitazone	**Glimepiride**
1	Acidic degradation	3 hour	83.14%	97.73%
2	Basic degradation	3 hour	99.32%	98.14%
3	Oxidative degradation	3 hour	98.90%	97.96%
4	Photolytic degradation	3 hour	98.77%	98.68%
5	Dry heat induced degradation	24 hour	98.55%	99.32%

DISCUSSION

A simple, accurate, precise, robust and rapid UV visible spectrophotometric method has been developed for simultaneous estimation of PIO and GLIM in pharmaceutical dosage form. The method validation for PIO and GLIM in pharmaceutical dosage form. The present study was concluded to understand the degradation behavior of PIO and GLIM under ICH recommended stress conditions. This study is an example of the development of a stability indicating assay method for PIO and GLIM where forced degradation was carried out under all stress conditions. These drugs showed degradation in all of the studied conditions. However the extent of degradation was different. The method developed for quantitative determination of PIO and GLIM is rapid, precise, accurate and selective. The results of validation tests were found to be satisfactory and therefore, these methods can be applied successfully for routine quality control analysis of PIO and GLIM in bulk and pharmaceutical formulation. The developed method can be conveniently used for the assay determination of in bulk PIO and GLIM drugs and pharmaceutical dosage form.

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