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SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF PIOGLITAZONE AND GLIMEPIRIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING ABSORBANCE RATIO METHOD

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ABSTRACT

Glimepiride and Pioglitazone in combination are available as tablet dosage forms in the ratio of 2: 15. A simple, reproducible and efficient spectrophotometric method has been developed for the simultaneous estimation of Glimepiride and Pioglitazone in bulk and tablet dosage forms. The sampling wavelengths selected are 227 nm and 235 nm, Absorption Ratio Method, the sampling wavelengths selected are 251 nm (iso-absorptivity wavelength) and 235 nm.

KEYWORDS: Pioglitazone, Glimepiride, UV-Visible Spectrophotometric, Validation.

INTRODUCTION

Diabetes Mellitus commonly referred to as diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period.[1,2] Anti-diabetics such as sulfonylurea and thiazolidinedione derivatives are commonly prescribed hypoglycaemic drugs for non-insulin dependent type II diabetes mellitus. Pioglitazone hydrochloride, (±)-5-{4-[2-(5-ethyl-2- pyridyl) ethyl]benzyl}-2,4-thiazolidinedione hydrochloride salt is a member of thiazolidinedione class, which exerts its glucose-lowering effect by binding to peroxisome

proliferator-activated receptors gamma. (PPARγ), thus increasing the receptor sensitivity to insulin. Glimepiride, 1-H-pyroll-1-carboxamide-3-ethyl-2,5-dihydro-4-methyl-N-[2-[4-[[[[(4-methylsiklohexyl) amino] carbonyl] amino] sulfonyl] [phenyl] ethyl]-2-oxo-trans, is a member of sulfonylurea drugs, which can increase the secretion of insulin by functioning islet β - cells. In the past few decades, several generations of sulfonylurea drugs have been developed for common use such as glimepiride.[3,4]

This generation of hypoglycemic drugs is much more potent hence are effective at much lower dosages. Several analytical methods have been reported for the determination of pioglitazone and Glimepiride in bulk and pharmaceutical dosage form. Even though various methods were reported in the literature for estimation of glimepiride and pioglitazone individually or in combination with other drugs no method had been reported for simultaneous estimation of these two drugs using Multiwavelength spectroscopy method in bulk drug and pharmaceutical dosage forms.[5,6] Aim and objective of the present work was to develop & validate UV-Spectrophotometric methods for the simultaneous estimation of Pioglitazone and Glimepiride in combined dosage form by simultaneous equation method.

MATERIALS AND METHODS

Table no. 1: List of Instruments/equipments.

Sr. No.	Instrument / Equipment	Make	Model
1.	UV spectrometry	Shimadzu Corporation	UV-1800 240V
2.	Weighing Balance	Shimadzu Corporation	BL-22OH (Electronic balance)

Table no. 2: Apparatus and Glass wares.

Sr. No.	Glass wares	Make
1.	Volumetric flasks (25 ml)	Borosil, India
2.	Beaker	Borosil, India
3.	Measuring Cylinder (250 ml, 1000 ml, 2000 ml)	Borosil, India

Table no. 3: List of Drugs.

Sr. No.	Name of drug	Supplied By	Quantity
1.	Pioglitazone	Lupine Pharmaceuticals	2.0 gm
2.	Glimepiride	Lupine Pharmaceuticals	2.0 gm

Marketed Formulation Available

Brand Name: PIOGLAR-G

Manufactured by: RANBAXY

Labeled claim: Pioglitazone – 15 mg

Glimepiride – 2 mg

Reagents and Chemicals

All reagents and chemicals used were of AR analytical grade.

- Methanol

MATERIALS AND METHODS

Pioglitazone and glimepiride were obtained as gift samples from Lupin Pharmaceuticals Pune.

1. Simultaneous spectrophotometric determination of Pioglitazone and Glimepiride by Q-Analysis or Absorption Ratio Method.

Preparation of standard stock solution[4,5]

The standard stock solutions of Pioglitazone and Glimepiride were prepared by dissolving separately 10 mg of drug each in 100 ml methanol. Aliquots of working stock solutions of Pioglitazone and Glimepiride were diluted with methanol solution.

Selection of sampling wavelength for analysis[5,6,7]

Appropriate dilutions were made with methanol to give concentration of 10µg/ml. Further the solution was scanned in UV range from 200-400 nm and the spectrum was recorded. From the spectrum, wavelengths chosen were 251 nm (isobestic point) for pioglitazone and Glimepiride respectively. The selected two wavelengths were utilized for the measurement of absorbance of each drug and further analysis was done.

Selection of analytical concentration range[5,6,7,8]

From working standard solution of pioglitazone 0.02, 0.04, 0.06, 0.08 and 0.10 ml were pipette out and each was diluted to 10 ml to get the concentrations 0.2, 0.4, 0.6, 0.8 and 1.0 µg/ml. Similarly, from working standard solution of Glimepiride 1.50, 30, 4.50, 60, and 7.50 ml were pipette out and each was diluted to 10 ml to get the concentrations…in µg/ml. The absorbance of each of this solution was measured at selected wavelengths and plotted against concentration. The concentration range over which the drug obeyed Beer's law was chosen. The range was found to be 2-30 µg/ml for Glimepiride for (r^2 = 0.993) and 2-10 µg/ml for Pioglitazone (r^2 = 0.995).

Determination of Absorptivity at analytical wavelengths[5-8]

For each drug appropriate aliquots were pipetted out from standard stock solution and a series of dilutions of different concentration were made for pioglitazone the concentration range taken was 1.50 µg/ml to 7.50µg/ml and similarly the concentration range for Glimipiride was 0.20 µg/ml to 1.00 µg/ml. The absorbances of said concentrations for both the drugs were noted at selected analytical wavelengths. These absorbances were then divided by concentration in gm/lit to get absorptivities. Where, Q_{Pio} and Q_{Glim} are Q values, a_{PIO} and a_{GLIM} are absorptivities at isobestic point for pioglitazone and glimipiride respectively. These values were found to be Q_{GLIM}(1.2070), a_{GLIM} (2.1517), Q_{PIO}(1.2075), a_{PIO} (2.1517).

$$\mathbf{Q_{Glim}} = \frac{\text{Absorbance of Glimepiride at 235nm}}{\text{Absorbance of Glimipiride at 251nm.}}$$

$$\mathbf{Q_{Pio}} = \frac{\text{Absorbance of Pioglitazone at 235 nm}}{\text{Absorbance of Pioglitazone at 251 nm.}}$$

$$\mathbf{a_{Pio}} = \frac{\text{Absorbance of Pioglitazone at 251nm}}{\text{Concentration of Pioglitazone}}$$

$$\mathbf{a_{Glim}} = \frac{\text{Absorbance of Glimipiride at 251 nm}}{\text{Concentration of Glimipiride}}$$

Where, Q_{Pio} and Q_{Glim} are Q values, a_{PIO} and a_{GLIM} are absorptivities at isobestic point for pioglitazone and glimipiride respectively. These values were found to be

Q_{GLIM} = 1.2070 a_{GLIM} = 2.1517

Q_{PIO} = 1.2075 a_{PIO} = 2.1517

$$Qp = \frac{Absorbance\ Glimepiride}{Absorbance\ of\ pioglitazone}$$

$$CGlim = \frac{Qp - QPio}{QGlim - QPio}\ x\ \frac{A}{a\ Glim}$$

$$CPio = \frac{QG - QGlim}{QPio - QGlim}\ x\ \frac{A}{aPio}$$

Analysis of powder mixture[10-12]

By using working standard solutions of Pioglitazone and Glimepiride, further dilutions were made to get Pioglitazone and Glimepiride in concentration of 10µg/ml. The absorbance of this mixture was measured at 235 nm and 251 nm by using formula.

Procedure for analysis of tablet formulation[10-13]

Twenty tablets each containing 15 mg of Pioglitazone and 2 mg of Glimepiride were weighed and powdered. Powder equivalent to 50 mg of Pioglitazone and Glimepiride was weighed accurately and transferred to 50 ml volumetric flask. The solution was filtered through filter paper and first few ml were rejected. 10 ml of this filtrate was further diluted to 100 ml with methanol. From this solution, further dilutions were made using methanol to get the final concentration of 10 µg/ml of Pioglitazone and 10 µg/ml of Glimepiride. The solution was scanned in the range of 200-400 nm against blank. Absorbances were recorded at wavelengths 235 nm and 251 nm.

Procedure for Recovery Studies[9-13]

Recovery studies were carried out by applying the method to drug sample present in tablet dosage form to which known amount of Pio and Glim corresponding to 80, 100, 120% of Pioglitazone and 80, 100, 120% of Glimepiride was added (standard addition method In 80% recovery study amount of standard added was 1.20 mg of pioglitazone. In 100% recovery study the amount of standard added was 1.50 mg of pioglitazone. In 120% recovery study the amount of pioglitazone standard added was 1.80 mg. In 80% recovery study the amount of glimipiride standard added was 0.16 mg. In 100% recovery study the amount of standard added was 0.20 mg of glimipiride In 120% recovery study the amount of glimipiride standard added was 0.24 mg. Further dilutions were made from this stock solution to get required concentration.

The mixed sample solutions were analyzed to obtain spectra and absorbance value at 251 nm and 235 nm (λ_{max} of Pioglitazone and Glimipiride respectively) were noted. The concentration of Pioglitazone and Glimipiride were calculated from the equation. At each level of the amount of three determinations was performed and results obtained was compared with expected results.

Procedure for precision study[11-15]

Precision of the method was studied as intra-day and inter-day precision. Intra-day precision was determined by analyzing the 1.2, 1.5, 1.8 µg/ml of Pioglitazone and 0.16, 0.20, 0.24 µg/ml of Glimepiride for three times in same day. Inter-day precision was determined by analyzing the same concentration of the solution daily for three days.

1.1 Simultaneous spectrophotometric determination of Pioglitazone and Glimepiride by Q-Analysis or Absorption Ratio Method

Selection of sampling wavelength for analysis[12-16]

From the spectrum, wavelengths chosen were 251 nm (isobestic point) for pioglitazone and Glimepiride respectively. The selected two wavelengths were utilized for the measurement of absorbance of each drug and further analysis was done.

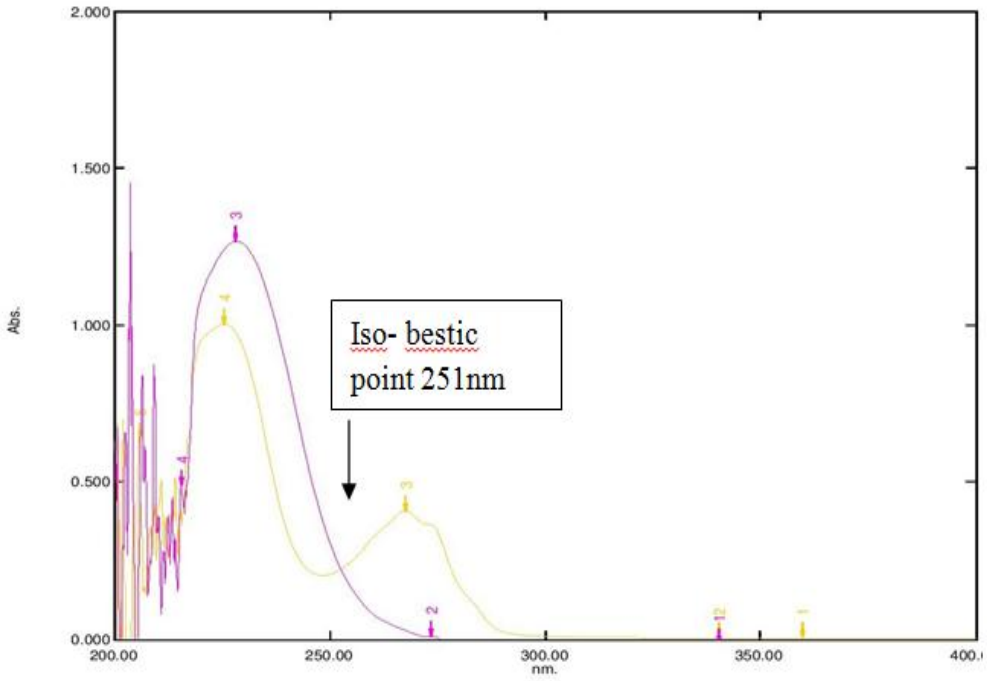

Fig No.1: Overlain spectra of Glimepiride and Pioglitazone.

Determination of Absorptivity at analytical wavelengths[12-16]

The absorbances of said concentrations for both the drugs were noted at selected analytical wavelengths. These absorbances were then divided by concentration in gm/lit to get absorptivities. Where, Q_{Pio} and Q_{Glim} are Q values, a $_{PIO}$ and a $_{GLIM}$ are absorptivities at isobestic point for pioglitazone and glimipiride respectively. These values were found to be Q_{GLIM} (1.2070), a_{GLIM} (2.1517), Q_{PIO}(1.2075), a_{PIO} (2.1517).

Analysis of powder mixture[15-17]

By using working standard solutions of Pioglitazone and Glimepiride, further dilutions were made to get Pioglitazone and Glimepiride in concentration of 10 µg/ml. The absorbance of this mixture was measured at 235 nm and 251 nm by using formula.

Table No. 4: Data of powder mixture Analysis.

Sr. No.	Amount present in (µg/ml)		Amount found in (µg/ml)		Amount found in %	
	Glim	Pio	Glim	Pio	Glim	Pio
1	0.20	1.50	0.18	1.48	90	98.66
2	0.20	1.50	0.19	1.47	95	98
3	0.20	1.50	0.18	1.48	90	98.66
4	0.20	1.50	0.19	1.49	95	99.33
5	0.20	1.50	0.18	1.48	90	98.66

Procedure for analysis of tablet formulation[15-18]

The solution was scanned in the range of 200-400 nm against blank. Absorbances were recorded at wavelengths 235 nm and 251 nm. The concentration of drug was then calculated by using from equation and obtained data is mentioned below.

Table No.5: Analysis of Tablet Formulation.

Sr. No.	Label Claim (µg/ml)		Amount Found (µg/ml)		% Of Label Claim	
	Glim	Pio	Glim	Pio	Glim	Pio
1.	0.20	1.50	0.19	1.47	95	98
2.	0.20	1.50	0.19	1.48	95	98.66
3.	0.20	1.50	0.18	1.48	90	98.66
4.	0.20	1.50	0.19	1.49	95	99.33
5.	0.20	1.50	0.19	1.48	95	98.66

Table No.5: Statistical analysis of Tablet Formulation.

Component	Mean	Standard Deviation	Co-efficient of Variation	Standard Error
Pioglitazone	94.00	1.236068	0.3787	0.689739
Glimepiride	98.66	0.470234	0.4766	0.308739

Procedure for Recovery Studies[18]

The mixed sample solutions were analyzed to obtain spectra and absorbance value at 251 nm and 235 nm (λ_{max} of Pioglitazone and Glimipiride respectively) were noted. The concentration of Pioglitazone and Glimipiride were calculated from the equation. At each level of the amount of three determinations was performed and results obtained was compared with expected results.

Table No.6: Recovery studies of Pioglitazone and Glimipiride.

Level of % recovery	Preanalysed		Added concentration Ug/ml		Total absorbance ug/ml		Conc.recoverd Ug/ml		Percentage of recovery	
	Pio	Glim	Pio	Glim	Pio 227nm	Glim 235nm	Pio	Glim	Pio	Glim
80	1.50	0.20	1.20	0.16	0.756	0.516	1.15	0.14	95.83	93.75
80	1.50	0.20	1.20	0.16	0.757	0.517	1.17	0.14	97.5	87.5
80	1.50	0.20	1.20	0.16	0.756	0.518	1.18	0.15	98.33	93.75
100	1.50	0.20	1.50	0.20	0.887	0.600	1.43	0.18	95.33	90.00
100	1.50	0.20	1.50	0.20	0.888	0.601	1.44	0.19	96.33	95.00
100	1.50	0.20	1.50	0.20	0.889	0.602	1.43	0.18	95.33	90.00
120	1.50	0.20	1.80	0.24	0.741	0.505	1.72	0.22	95.55	91.66
120	1.50	0.20	1.80	0.24	0.742	0.506	1.76	0.23	96.00	95.83
120	1.50	0.20	1.80	0.24	0.743	0.507	1.78	0.22	95.55	91.66

Table No.7: Statistical analysis of Tablet Formulation.

Level Of percentage recovery	% Mean Recovery		Standard Deaviation		Co –efficient of variation		Standard Error	
	Pio	Glim	Pio	Glim	Pio	Glim	Pio	Glim
80	97.72	91.66	1.273303	1.608439	1.303011	1.936765	0.659042	0.887322
100	95.66	92.22	0.57735	0.626751	0.604175	0.13028	0.448767	0.791232
120	95.7	93.05	0.259808	0.407551	0.271473	0.587373	0.300817	0.719357

Procedure For Precision Study

Table No. 8: Precision studies for Pioglitazone.

Sr. No.	Conc. µg/ml	Measured area (µg/ml) ± S.D, RSD (%)	
		Repeatability (n=2)	Intermediate Precision (n=2)
1	1.20	1.27 ± 0.015275, 1.20	1.27± 0.01219, 0.95
2	1.50	1.42 ± 0.0057, 0.42	1.42 ± 0.0045, 0.32
3	1.80	1.78 ± 0.01, 0.42	1.78 ± 0.01, 0.056

Table No.9: Precision studies for Glimepiride.

Sr. No.	Conc. µg/ml	Measured area (µg/ml) ± S.D, RSD (%)	
		Repeatability (n=2)	Intermediate Precision (n=2)
1	0.16	0.14 ± 0.01311, 9.36	0.14 ± 0.01311, 9.36
2	0.20	0.17 ± 0.01154., 6.79	0.17 ± 0.01142, 6.71
3	0.24	0.24 ± 0.00763, 76.38	0.24 ± 0.0077, 3.01

CONCLUSION

The developed UV method like Absorbance Ratio Method are precise, specific, and accurate. Statistical analysis proves that these methods are suitable for the analysis of Pioglitazone and Glimepiride in bulk and pharmaceutical formulation without any interference from the excipient. These methods have been found to be better than previously reported methods, because of use of less, economical and readily available solvent like methanol.

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