Research Article

Simultaneous Estimation of Metformin and Pioglitazone in Combined Dosage Form

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ABSTRACT

The quantitative estimation is the method to determine how much of each constituent is in the sample. Estimation of a given drug or medicine in the dosage forms needs the quantitative analysis of that drug or medicinal in it. The first quantitative analyses were gravimetric, made possible by the invention of a precise balance. It was soon found that carefully calibrated glassware made considerable saving of time through the volumetric measurement of gravimetrically standardized solutions. The current objective of this research work is to establish a rapid, accurate and inexpensive method for the estimation of metformin and piogliatzone in combined dosage form.

Keywords: metformin, pioglitazone, simultaneous, accuracy.

INTRODUCTION

The quantitative estimation is the method to determine how much of each constituent is in the sample. Estimation of a given drug or medicine in the dosage forms needs the quantitative analysis of that drug or medicinal in it. The first quantitative analyses were gravimetric, made possible by the invention of a precise balance. It was soon found that carefully calibrated glassware made considerable saving of time through the volumetric measurement of gravimetrically standardized solutions. Although in recent years, spectrophotometric methods are extensively used, but it would be wrong to conclude that instrumental methods have totally replaced chemical methods. In fact, chemical steps are often an integral part of an instrumental method. The sampling, dissolution, change in oxidation state, removal of excess reagent, pH adjustment, addition of complexing agent, precipitation, concentration and the removal of interferences are the various chemical steps which are part of an instrumental method. In recent years HPLC (High Performance Liquid Chromatography) is extensively used, because HPLC is not limited by sample volatility or thermal stability. HPLC is able to separate macromolecules and ionic species, labile natural products, polymeric material and a wide variety of other high molecular weight poly-functional group because of the relatively high pressure necessary to perform this type of chromatography; a more elaborate experimental setup is required.[1,2]

Simultaneous equations method

If a sample contains two absorbing drugs (X and Y) each of which absorbs at the max of the other , it may be possible to determine both drugs by the technique of simultaneous equations (Vierordt's method) provided that certain criteria apply.

The information required is:

(a) The absorptivities of X at 1 and 2, a_{x1} and a_{x2} respectively

(b) The absorptivities of Y at 1 and 2, a_{x1} and a_{x2} respectively

(c) The absorbance of the diluted sample at 1 and 2, A_1 and A_2 respectively.

Let C_x and C_y be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that at 1 and 2 the absorbance of

the mixture is the sum of the individual absorbance of X and Y.

At 1 $A1 = a_{x1}bc_x + a_{y1}bc_y$ (1)
At 2 $A2 = a_{x2}bc_x + a_{y2}bc_y$ (2)

For measurements in 1 cm cells, b = 1.
Rearranging eq. (2).

$$C_y = A_2 – a_{x2}c_x / a_{y2}$$

Substituting for Cy in eq. (1) and rearranging gives

$C_x = A_2\, ay_1 – A_1\, a_{y2} /\ a_{x2}ay_1 – ax_1\, a_{y2}$ (3)
$Cy = A_1\, ax_2 – A_2\, ax_1\ / ax_2\, ay_1 – ax_1\, ay_2$ (4)

Modified equations containing a symbol (b) for path-length can be used for application in situations where A1 and A2 are measured in cells other than 1 cm path-length.
Criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested [2,3] that place limits on the relative concentrations of the components of the mixture. The criteria are that the ratios

$$\frac{A_2/A_1}{a_{x2}/a_{x1}} \quad \& \quad \frac{a_{y2}/a_{y1}}{A_2/A_1}$$

Should lie outside the range 0.1-02 for the precise determination of Y and X respectively.

Pioglitazone hydrochloride (PIO) is chemically [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl] -2, 4-]thiazolidinediones monohydro chloride. It is a potent agonist for peroxisome proliferators activated receptor-gamma (PPARγ), activation of which modulates the transcription of a number of insulin responsive genes involved in the control of glucose and lipid metabolism.

Metformin hydrochloride (MET) is chemically (N, N dimethyl imidodicarbonimidic diamide hydrochloride) is a member of the biguanides of oral antihyperglycemic improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization.[4-6]

EXPERIMENTAL

Materials: Shimadzu UV-1700 spectrophotometer with spectral band width of 1.8nm, wavelength accuracy of ± 2nm & matched quartz cell of 10mm optical path length was used for all spectral & absorbance measurement. All chemicals used were of analytical reagent grade and double distilled water was used to prepare the solvent medium. Pharmaceutical grade MET and PIO procured from Concept pharmaceuticals, Roorkee, India were used as received.

Method: Simultaneous equations method was used i.e. if a sample contains two absorbing drugs (X and Y) each of which absorbs at the max of the other , it may be possible to determine both drugs by the technique of simultaneous equations (Vierordt's method) provided that certain criteria apply.
Two equations are constructed based upon the fact that at 1 and 2 the absorbance of the mixture is the sum of the individual absorbance of X and Y.

At 1 $A1 = a_{x1}bc_x + a_{y1}bc_y$ (1)
At 2 $A2 = a_{x2}bc_x + a_{y2}bc_y$ (2)

For measurements in 1 cm cells, b = 1.

Solvent System

The solvent system was prepared by dissolving the 0.1 N HCl & Dimethyl Formamide in the ratio of 9:1 in a volumetric flask.

Preparation of stock Solution

Pure Drug

Accurately weighted 100mg pure drug of MET & PIO were dissolved in the solvent system in a two different 100ml of volumetric flasks. The solution was kept on the sonicator for 15min and then volume was makeup to the 100ml.

Marketed formulation

The average weight of 10 tablets was taken and it was found 0.999mg which contain claim of 500mg MET & 15mg PIO. Accurately weighted 1gm of drug was taken and in a 100 ml of volumetric flask and dissolved by adding a solvent system. The solution was sonicated for 15 min after that the volume was make up to the 100ml by adding the solvent system. The solution was kept overnight because the formulation contains the sustained release METFORMIN. After that the solution was sonicated for 15min and filtered. Now the solution was diluted by serial dilution method to achieve the conc. of MET 50µg/ml & PIO 1.5 µg/ml.

Determination of λmax

From the stock solutions, a working standard was prepared. The absorption spectrum for Pioglitazone was recorded using the concentration of 6µg/ml and it was found to show two absorption maxima at 269nm. For Metformin hydrochloride, the absorption spectrum was recorded using 8µg/ml solution and the maximum absorption was found to be 237nm.(table no. 1)[6]

Preparation of Standard Curve

The Calibration curves were prepared for PIO and MET in the concentration range of 2-16 µg/ml and 2-16 µg/ml at selected wave Lengths by diluting aliquot portions of stock solution of each drug. The plot of Beer's law limit is shown in fig no. 1,2.

Simultaneous Estimation

First prepare the dilutions of 2 to 20µg/ml from stock solution of MET & PIO After that take its absorbance at both λmax of PIO & MET respectively. Absorptivity value of both drugs was found by dividing its absorbance value with its concentration as shown in table. The marketed formulation of drug was also subjected at both wavelength of 237nm & 269nm to find out the absorbance.[6,7]

RESULTS AND DISCUSSIONS

Amounts of Metformin and Pioglitazone were determined by solving the simultaneous equations.

Two simultaneous equations were formed using absorptivity coefficient values.

$A1 = 0.004 \times C1 + 0.008\ C2$ ---------- (1)

$A2 = 0.002 \times C1 + 0.031 C2$ --------- (2)

The concentrations of Metformin and Pioglitazone were calculated using following two equations.

$C_1 = A_2\ a_{y1} - A_1\ a_{y2} /\ a_{x2} a_{y1} - a_{x1}\ a_{y2}$ --- (3)

$C_2 = A_1\ a_{x2} - A_2\ a_{x1} / a_{x2}\ a_{y1} - a_{x1}\ a_{y2}$ --- (4)

$C_1 = 145.6 \times 8 - 211.4 \times 31 / 108$ --- (2)

$C_2 = 211.4 \times 2 - 145.6 \times 4 / 108$

Where C1 and C2 are concentration of Metformin and Pioglitazone respectively in gm/liter in then sample solution, A1 and A2 are the absorbance of the mixture at 237 nm and 269 nm respectively. C1(metformin) was calculated to be 498.89mg while amount of pioglitazone was found out to be 14.77 mg. the accuracy of the method was found out and reported in table no. 2, 3,4.

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Metformin and Pioglitazone from pure and in pharmaceutical dosage forms. The solvent system is simple to prepare and economical. The sample recoveries in all

formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Metformin & Pioglitazone in combined dosage forms and can also be used for dissolution or similar studies.

Table 1: Absorbance of MET & PIO at both λmax 237 & 269 respectively

	Metformin λ max (nm)				Pioglitazon λ max (nm)			
Conc.	237 (nm)	ax1	269 (nm)	ax2	269 nm	ay2	237 nm	ay1
2	0.009	0.0045	0.004	0.002	0.074	0.037	0.017	0.008
4	0.018	0.0045	0.010	0.002	0.126	0.031	0.02	0.005
6	0.026	0.0043	0.016	0.002	0.192	0.032	0.042	0.007
8	0.036	0.0045	0.022	0.002	0.251	0.031	0.069	0.008
10	0.044	0.0044	0.028	0.002	0.312	0.031	0.089	0.008
12	0.053	0.0044	0.038	0.003	0.372	0.031	0.117	0.009
14	0.062	0.0044	0.046	0.003	0.427	0.03	0.173	0.012
16	0.074	0.0046	0.058	0.003	0.475	0.029	0.191	0.011

Table 2: Optical and regression parameters of MET and PIO in 0.1N HCl and DMF in the ratio of (9:1)

Parameters	MET		PIO	
λmax (nm)	237 nm	269 nm	269 nm	237 nm
Beer's law limit (mg/ml)	2-14	2-10	2-16	2-12
R^2	0.998	0.979	0.988	0.965
Regression equation	y = 0.004x - 0.000	y = 0.003x - 0.003	y = 0.028x + 0.018	y = 0.012x - 0.020
slope (b)	0.004	0.003	0.028	0.012

Table 3: Summary of validation parameters for MET and PIO

S. No.	Parameters	MET	PIO
4.	Accuracy %	99.46-99.78%	98.46-99.95%
5.	Limit of detection, mg mL^{-1}	0.412	0.589
6.	Limit of quantification, mg mL^{-1}	2.5	2.142

Table 4: Summary of estimation of MET & PIO in different brands

S. No.	Brand	Labeled amount (mg)	Amount found [a] (mg)	% of Labeled amount [a]
1.	Pioz-MF	15 (PIO)	14.77 ± 0.029	98.46 ± 0.588
		500 (MET)	498.89 ± 0.172	99.78 ± 0.343

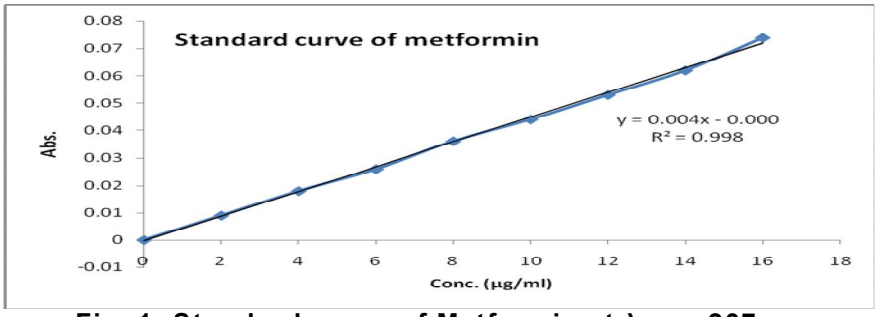

Fig. 1: Standard curve of Metformin at λmax 237nm

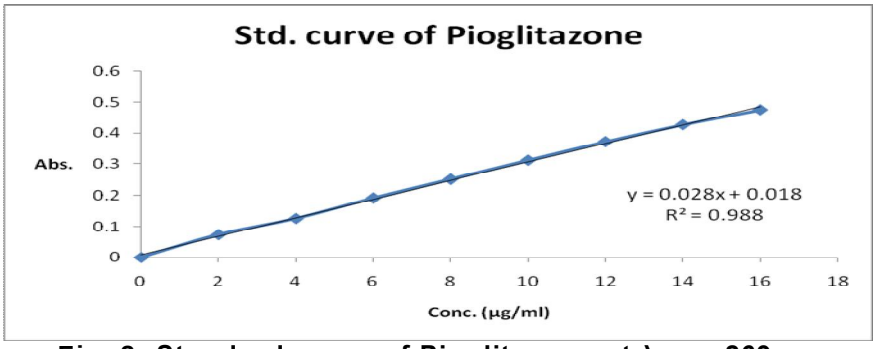

Fig. 2: Standard curve of Pioglitazone at λmax 269nm

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