Simultaneous Estimation of Metformin and Glimipride by Ultraviolet Spectrophotometry.

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Abstract

The simple, accurate, economical and reproducible spectrophotometric methods for simultaneous estimation of two-component drug mixture of Metformin and Glimipride in combined tablet dosage form have been developed. Developed methods are based on direct estimation of Metformin at 234.0 nm and Glimipride at 239.0 nm. For estimation first developed method involves formation and solving of simultaneous equation. The developed methods obey Beer's law in the concentration ranges 5-25µg/ml. The results of analysis were validated statistically.

Key Words

Simultaneous Spectrophotometric Analysis, Metformin, Glimepiride.

Introduction

Combined tablets of metformin and Glimipride are available containing 500 mg of metformin and 1 mg of glimepiride. Metformin hydrochloride chemically 1, 1- dimethlybiguanide hydrochloride is white crystalline powder, hygroscopic and freely soluble in water because metformin's insulin-sensitizing effect occurs mainly at the liver, combination with thiazolidinediones (TZDs), which mainly sensitize muscle to insulin-mediated glucose uptake, is a rational therapeutic strategy. The aim of this paper was to explore the possibility of using techniques of absorbance ratio or Q-analysis method and derivative spectrophotometry methods for quantifying metformin and pioglitazone simultaneously in their mixture form. Glimipride is a medium-to-long acting sulfonylurea anti-diabetic drug and is chemically 3- ethyl-4-methyl-*N*-(4-[*N*-((1*r*,4*r*)-4methylcyclohexylcarbamoyl)sulfamoyl]-phenethyl)-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide (Figure 2).
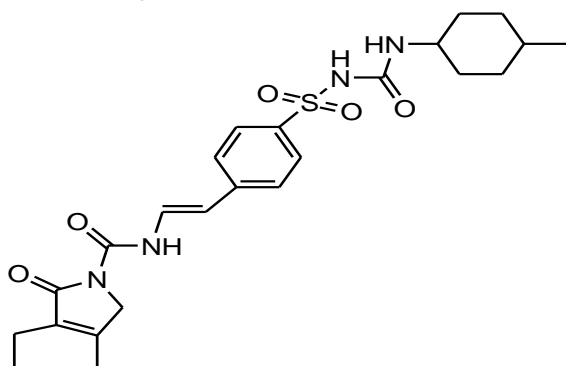
Structure of Glimipride.

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Glimipride acts as a secretagogue. 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. Glimipride is not yet official in any of the pharmacopoeia.
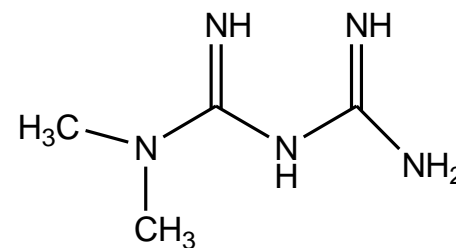
Structure of Metformin.

Materials and Methods

Instrument: A Jasco UV-630 UV/VIS Spectrophotometer was used with 1 cm matches quartz cell.

Solvent used: 0.1N NaOH solution.

Preparation of stock Solution

GLM (10mg) and MET(10mg) were accurately weighed and transferred to two separate 100 ml volumetric flask, dissolved in 0.1N NaOH solvent to obtained stock solution of 100 µg/ml each. The stock solutions of both the drugs were further diluted separately with solvent to obtain 5-25µg/ml solution each and scanned in spectrum mode from 400-200nm. The overlain spectra of both the drug obtained to determine the λmax. GLM has λmax 239nm while MET has λmax 234nm.

Method (Simultaneous Equation Method)

From the stock solution, working standard solution of drugs were prepared by appropriate dilution and

were scanned in the entire U.V. range .Two wavelengths selected for the method are 239 nm and 234 nm that are absorption maxima of GLM and MET respectively in0.1 N NaOH solution A series dilution were prepared of standard solutions GLM and MET 5-25 µg/ml for both. For the estimation of drugs in the commercial formulations, twenty tablets containing 1 mg of GLM and 500 mg of MET were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. Quantity of powder equivalent to 10 mg of MET was transferred to 100ml volumetric flask, dissolved in sufficient quantity of 0.1N NaOH solvent, sonicated and volume was adjust up to mark with solvent to obtain a stock solution of 100 µg/ml of MET and further diluted to get 10 µg/ml This solution was then filtered through Whatmann filter paper # 41. Absorbances of these solutions were measured at appropriate wavelengths, and values were substituted in the respective formula to obtain their respective concentrations. A1 and A2 are absorbances of the sample solution measured at 228 and 234 nm respectively. The absorbances (A1 and A2) of the sample solutions were recorded at 234 and 239nm, respectively and concentration of both components were calculated using above mentioned equations.

$$Cx = \frac{A1\ ay2 - A2\ ay1}{ax1ay2 - ax2\ ay1}$$

$$Cy = \frac{A1\ ax2 - A2\ ax1}{ay1\ ax2 - ay2\ ax1}$$

Validation
The methods were validated with respect to accuracy, linearity, and Limit of detection (LOD) and limit of quantification (LOQ).

Accuracy (Recovery Test)
To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%).

Linearity
The linearity of measurement was evaluated by analyzing different concentration of the standard solution of GLM and MET. For both the method, the Beer-Lambert's concentration range was found to be 5-25 µg/ml.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
The LOD and LOQ of GLM and MET were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines.

Result and Discussion

The method discussed in the present work provides a convenient and accurate way for simultaneous analysis of MET and GLM. In simultaneous equation method, wavelengths selected for analysis were 234.0 nm (λmax of Metformin) and 239.0 nm (λmax of Glimipride). In this method linearity for detector response was observed in the concentration range of 5-25 µg/ml for both MET and GLM. Absorptivity coefficient were Calculated for both the drugs at selected wavelengths and substituted in equations for determining concentration of MET and GLM in tablet sample solution. Percent label Claim for MET and GLM in tablet analysis. Low values of LOD and LOQ indicated good sensitivity of proposed method. Accuracy of proposed methods was ascertained by recovery studies. The percent recovery for MET and GLM, by this method, was found 99.16 and 99.21. The proposed method could be employed for routine quality control of Metformin and Glimipride in combined dose tablet formulation.

References

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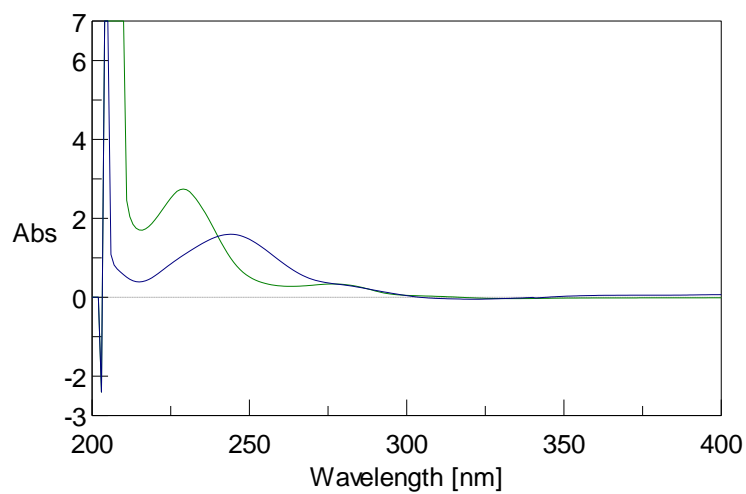6. Analytical Method Validation; Michael E. Swartz, Ira S. Krull, Marcel Dekker, Inc.I[st] edition, 2009; 56-65.

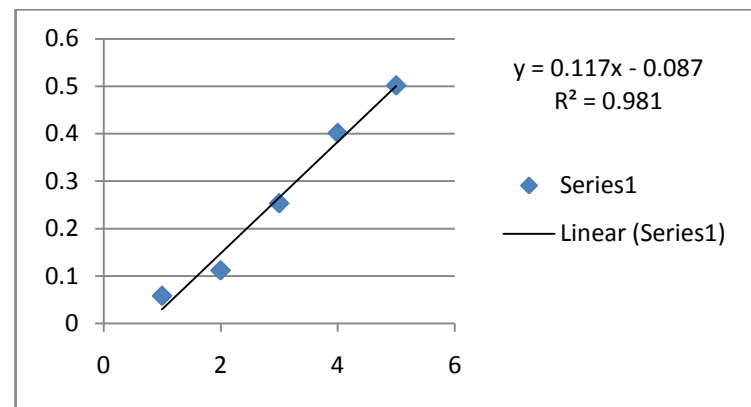**Fig. 1:** Overlain Spectra of Metformin (MET) and Glimipride (GLM).

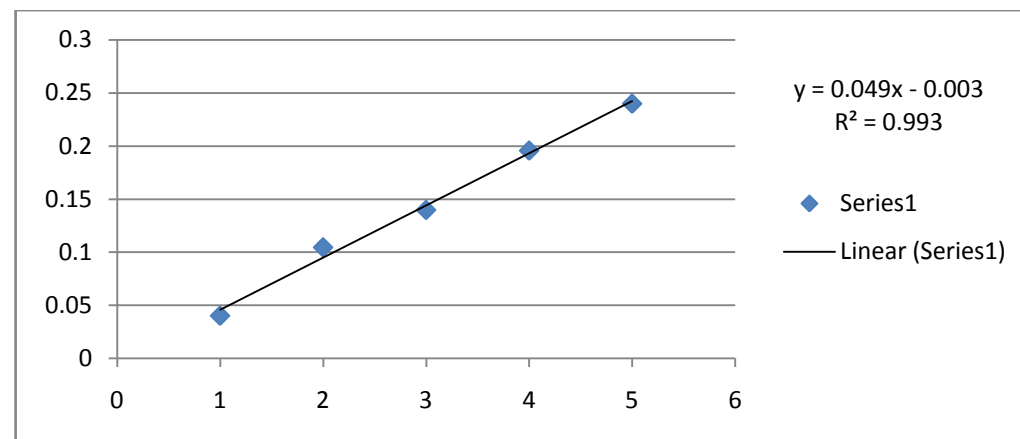**Fig 2:** Calibration curve of Metformin at 234 nm.

Fig 3: Calibration curve of Glimipride at 239 nm.

Table 1: Validation Parameters.

Parameters	234nm.		239nm.	
	MET	**GLM**	**MET**	**GLM**
Beers law limit(µg/ml)	5-25	5-25	5-25	5-25
Regression equation	y=0.023x-0.087	y=0.044x-0.015	Y=0.096x-0.070	y=0.009x-0.001
Slope(m)	0.023	0.044	0.096	0.009
Intercept(c)	0.087	0.015	0.070	0.001
S.D.	0.1876	0.07	0.15	0.0911
LOD	26.91	5.24	5.15	33.40
LOQ	81.56	15.90	15.62	101.22
Correlation coefficient (r2)	0.981	0.990	0.980	0.993

Table 2: Accuracy by percentage recovery method.

Sr. No.	Tablet Formulation (µg/ml)	Level of addition (%)	% estimated (mean ± SD)	
			Metformin	**Glimipride**
1.	5	80	99.58 ± 0.56	99.36 ± 0.97
2.	5	100	99.02 ± 0.36	98.99 ± 1.01
3.	5	120	98.90 ± 0.42	99.28 ± 1.04

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