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Simultaneous UV Spectrophotometric Method for Estimation of Gliclazide and Metformine Hydrochloride in Tablet Dosage Form

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Abstract: Two simple, accurate, precise, reproducible and economical procedures for simultaneous estimation of Gliclazide(GLZ) and Metformine hydrochloride (MET) in tablet dosage form have been developed. First method based on solving of simultaneous equation using 228 nm (λmax of GLZ) and 234 nm (λmax of MET) as two analytical wavelengths for both drugs in mixture of Water and Methanol (60:40) solvent. Second method based on an equation of area calculation of curve at two wavelength resion (233 to 223nm and 239 to 229 nm).Linearity was observed in the concentration range of 2-24 µg/ml for GLZ and 2-14 µg/ml for MET. The result of analysis have been validated satistically and by recovery study.

Keywords: Gliclazide, Metformine hydrochloride, λmax, Simultaneous estimation method and AUC.

Introduction

Gliclazide(GLZ), a sulphonyl urea derivative is used as an oral hypoglycemic agent. Chemically it is 1-(3-azabicyclo [3.3.0] oct-3-yl)-3-p-methylpheylsulphonylurea. It is official in British Pharmacopoeia 2007[1].

Metformin Hydrochloride (MET) is a biguanide class of antidiabetic drug, chemically is N,N-dimethylimidodicarbonimidic diamide hydro-chloride [2-7] It is an oral anti-diabetic drug from the biguanide class. It is the first-line drug for the treatment of type 2 diabetes, particularly in overweight and obese people and those with normal kidney function,[8,9]and evidence suggests it may be the best choice for people with heart failure[10]- It is also used in the treatment of polycystic ovary syndrome.

The combination of GLZ and MET is used in non insulin dependent diabetes mellitus. Non aqueous method have been reported for analysis of gliclazide [11] in B.P. and metformine hydrochloride [12] in I.P. Several HPLC methods have been developed for gliclazide and metformine hydrochloride in plasma[13]

Literature survey revealed that it is simultaneous Spectrophotometric estimated of gliclazide and metformine hydrochloride in combine dosage forms in NAOH solvent[14] Only. No simultaneous spectrophotometric estimated of gliclazide and metformine hydrochloride in combine dosage forms in mixture of water:methano(60:40) solvent. Simultaneous UV spectrophotometric methods have been reported for estimation of gliclazide and metformine hydrochloride in combine dosage forms. Hence, an attempt has been made to develop new simultaneous UV methods for its estimation in pharmaceutical dosage formulations with good accuracy, simplicity and sensitivity.

Materials and Methods

Instrument:

A Shimadzu UV-1700 UV/VIS Spectro photometer was used with 1 cm matches quartz cell.

Materials:

Gift samples of MET and GLZ were procured from Torrent Pharmaceutical LTD, Indrad, Dist. Mehsana (Gujarat) and Panacea biotech LTD, Baddi, Dist. Solan (H.P.) respectively. Tablets containing both drugs i.e. Metformine Hydrochloride and Gliclazide were purchased from local pharmacy of commercial brand Glizid-M(Panacea biotech)

Solvent used: Mixture of Water:Methanol(60:40)

Preparation of stock Solution:

GLZ (10mg) and MET(10mg) were accurately weighed and transferred to two seperate 100 ml volumetric flask, dissolved in Water:Methanol(60:40) 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 10µg/ml solution each and scanned in spectrum mode from 400-200nm. The overlain spectra of both the drug obtained (Fig No.1) to determine the λmax. GLZ has λmax 228nm while MET has λmax 234nm.

Method I (Simultaneous Equation Method) [15]

From the stock solution, working standared solution of drugs were prepared by appropriate dilution and were scanned in the entire U.V. range .Two wavelengths selected for the method are 228 nm and 234 nm that are absorption maximas of GLZ and MET respectively in Water:Methanol(60:40). A series dilution were prepared of standard solutions GLZ and MET 2-24 µg/ml and 2-14µg/ml respectively. The absorptivity coefficients of GLZ within concentration range of 2-24 µg/ml and MET within concentration range of 2-14 µg/ml were determined at 228 and 234 nm by calibration curve.

For the estimation of drugs in the commercial formulations, ten tablets containing 80 mg of GLZ and 500 mg of MET were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, a standard addition method was used. An accurately weighed 18.4 mg of pure GLZ was added to finely powdered sample to bring the concentration of GLZ in linearity range. With this addition, the ratio of MET to GLZ was brought to 1:2. Quantity of powder equivalent to 20 mg of GLZ and 10 mg of MET was transferred to 100 ml volumetric flask , dissolved in sufficient quantity of Water:methanol(60:40) solvent, sonicated and volume was adjust up to mark with solvent to obtain a stock solution of 200 µg/ml of GLZ and 100 µg/ml of MET. This solution was then filtered through Whatmann filter paper # 41. Further dilutions were made from this stock solution to get required concentration. Absorbances of these solutions were measured at appropriate wavelengths, and values were substituted in the respective formula to obtain their respective concentrations. Results of tablet analysis are shown in Table No.2 The analysis procedure was repeated six times (n=6).

A set of two simultaneous equation as developed using these absorptivity coefficients as:

$A_1=(52C_1+95C_2)\times10^{-3}$ 1

$A_2=(43.2C_1+105C_2)\times10^{-3}$.....2

Where C1 and C2 are concentrations of GLZ and MET respectively in µg/ml in sample solution. 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 228 and 234nm, respectively and concentration of both components were calculated using above mentioned equations (1 and 2)

Method II (Area Under Curve Method)

For the selection of analytical wavelength solution of GLZ and MET (10 µg/ml each) were prepared separately by appropriate dilution of stock solution and scanned spectrum mode from 400 to 200nm . From the overlain spectra of both drugs (Fig No.1) , area under the curve in the range of 233-223nm (for GLZ) and 239-229nm (for MET) were selected for the analysis. The calibration curve for GLZ and MET were prepared in the concentration range of 2-24 µg/ml and 2-14 µg/ml at their respective AUC range. The calibration curve were plotted against concentration v/s area.

For the estimation of drugs in the commercial formulations, ten tablets containing 80 mg of GLZ and 500 mg of MET were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, a standard addition method was used. An accurately weighed 18.4 mg of pure GLZ was added to finely powdered sample to bring the concentration of GLZ in linearity range. The stock solution were obtained 200 µg/ml GLZ and 100 µg/ml MET. This solution was then filtered through Whatman filter paper # 41. Further dilutions were made from this stock solution to get required concentration. Area of these solutions were measured at appropriate wavelengths, and values were substituted in the respective formula to obtain their respective concentrations. Results of tablet analysis are shown in Table No.2 The analysis procedure was repeated six times (n=6).

A set of two equation as developed using these absorptivity coefficients as:

$$\int_{223}^{233} Ad\lambda_1=k_1C_1+k_2C_2\ldots\ldots\ldots\ldots\ldots\ldots\ldots3$$

$$\int_{229}^{239} Ad\lambda_2=k_3C_1+k_4C_2\ldots\ldots\ldots\ldots\ldots\ldots\ldots4$$

Where area of curve between 233-223nm and between 239-229nm are represented by $\int Ad\lambda_1$ and $\int Ad\lambda_2$ respectively,C1 and C2 are concentration of GLZ and MET respectively in µg/ml k1, k2 ,k3,and k4 are constants. Finally equation would be

$$\int_{223}^{233} Ad\lambda_1=.50C_1+.779C_2\ldots\ldots\ldots\ldots\ldots5$$

$\int_{229}^{239} Ad\lambda_2 = .333C_1 + 1.01C_2$ 6

Sample solution were scanned and area was calculated within indicated wave length ranges. The concentration of both components were calculated using above mentioned equation (5 and 6)

Validation:[16,17]

The methods were validated with respect to accuracy, linearity, Sensitivity 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%). Percent recovery for GLZ and MET, by both the methods, was found in the range of 98.94-100.25(Table No.2)

Linearity:

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of GLZ and MET.For both the method ,the Beer-Lambert's concentration range was found to be 2-24 µg/ml and 2-14 µg/ml for GLZ and MET respectively

Sensitivity

High Molar absorptivity and low Sandell's sensitivity for the respective method reveals that all these methods are highly sensitive. (Table no.1)

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ of GLZ and MET were determined by using standard deviation of the response and slope approach as defined in International Conference on Harmonization (ICH) guidelines.The LOD and LOQ was found to be as in Table no.1.

Table No:1 Optical Characteristics and Validation data of Gliclazide and Metformine Hydrochloride.

Parameters	Gliclazide		Metformine	
	Method I	Method II	Method I	Method II
Working λ (in water:methanol 60:40)	228	223-233	234	229-239
Beer Lamberts Law range	2-24 µg/ml	2-24 µg/ml	2-14 µg/ml	2-14 µg/ml
Molar absorptivity (lit/mole/cm)	16817.372	162352.32	17423.224	168601.16
Sandell's sensitivity(mcg/sq cm/0.001)	.019063	.00199	.023753	.000992
LOD	.5542	.33	.3982	.2940
LOQ	1.6794	1	1.73971	.8910
Slope	.052(at228), .0432(at 234)	.502(at223-233) .333(at229-239)	.1052(at234), .095(at228)	1.018(at229-239) .779(at223-233)
Regression coefficient(r^2)	.9991(at 228) .9991(at 234)	.998(223-233) .996(229-239)	.9991(at 234) .9994(at228)	.999(229-239) .996(223-233)

Results and Discussion

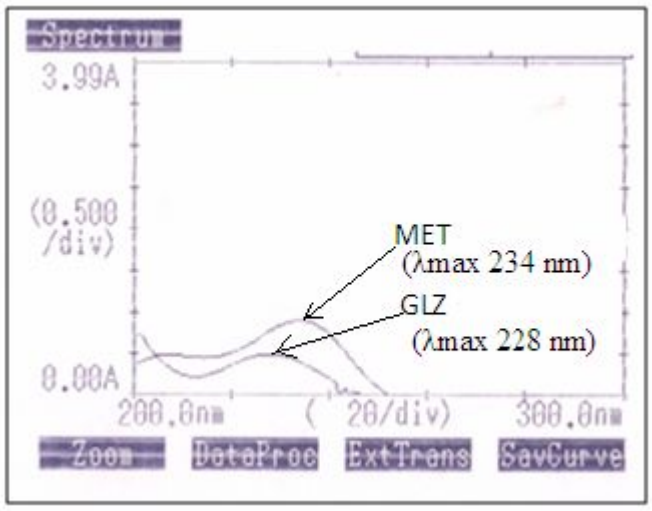
The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of GLI and MET. In simultaneous equation method, wavelengths selected for analysis were 228 nm for GLI and 234 nm for MET. In area under curve method, the area under curve in the range of 233-223 nm (for GLI) and 239-229 nm (for MET) were selected for the analysis. In both the methods linearity were observed in the concentration range of 2-24 µg/ml and 2-14 µg/ml for GLI and MET respectively. In method I, concentration of the individual drug present in the tablet sample solution was determined by solving the simultaneous equation at 228 nm and 234 nm using the respective absorptivity value. In method II, concentration of tablet sample solution were determine by solveing two equation at the range of 233-223nm and 239-229nm using respective absorptivity value. Percent label claim for GLI and MET in tablet analysis, by both the methods, was found in the range of 98.52% to 100.43 %. S.D.and R.S.D. for six determinations of tablet sample, by both the methods, was found to be less than ± 2.0 indicating the precision of both the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for GLI and MET, by both the methods, was found in the range of 98.94% to 100.25 %., The results of validation parameters shown in table no. 1 are satisfactory, indicates the accuracy of proposed methods for estimation of GLZ and MET. These methods can be employed for routine analysis of these two drugs in combined tablet dosage form. The results obtained for tablets and recovery study is summarized in table no. 2.

Table No:2 Result of analysis of tablet Formulation:

Methods	Drugs	Label claim	Amount Found	% label claim	S.D.*	R.S.D.*	%Recovery*
I	GLI	80	80.10	100.43	0.5454	0.6809	99.12
	MET	500	492.6	98.52	0.7811	0.1585	98.94
II	GLI	80	79.32	99.15	0.7467	0.9413	100.25
	MET	500	494.15	98.83	0.9775	0.1978	99.56

* indicates mean of six determinations.

Fig.No:1 Overlain spectra of Gliclazide and Metformine Hydrochloride in Water : Methanol (60:40) solvent.



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