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UV-visible spectrophotometric method for simultaneous estimation of glibenclamide and metformin

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ABSTRACT

Novel method for simultaneous estimation of Glibenclamide (GLB) and Metformin (MET) was developed using methanol as solvent. Methanol does not interfere in the spectroscopic determination of GLB and MET and shown maximum absorbance at 228 nm and 237 nm respectively. GLB and MET follows Beer-Lambert's law in range of 0.2-1.2 µg/mL and 50-300 µg/mL respectively. LOD and LOQ values of GLB and MET were found to be 0.10 and 0.41 µg/mL and 0.34 and 1.35 µg/mL respectively. The proposed method is can be used for routine analysis since it is rapid, simple, accurate, precise, sensitive and specific.

KEYWORDS

Glibenclamide;
Metformin;
Simultaneous estimation.

INTRODUCTION

Chemically GLB is 5-chloro-N-[2-(4-{[(cyclohexylcarbamoyl)amino] sulfonyl}phenyl)ethyl]-2- methoxybenzamide. is used in the control of mild to moderately severe type II diabetes mellitus (adult, maturity-onset) that does not require insulin, but that can be adequately controlled by diet alone. It stimulates the secretion and enhances the utilization of insulin by appropriate tissues[1].

Chemically MET is 1, 1-Dimethylbiguanide hydrochloride which is used as antidiabetic drug from the biguanide class used in the management of type 2 diabetes. Major action of metformin is increasing glucose transport across the cell membrane in skeletal muscle[2].

Structure of Glibenclamide and Metformin are shown in Figure 1 (a) and (b) respectively.

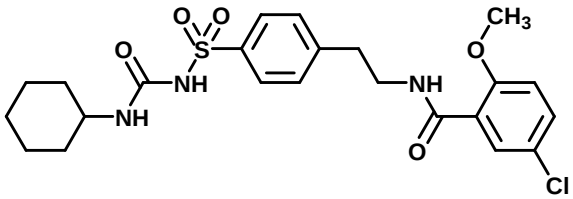

Figure 1: (a) Structure of glibenclamide

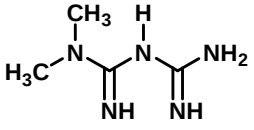

Figure 1: (b) Structure of metformin

Working standard of Glibenclamide and Metformin

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were obtained from Cipla Ltd. and Macleoids Ltd as a gift samples. All chemicals and solvents used for analysis are of AR grade and purchased from Qualigens fine Chemicals, Mumbai,(MS), India.

Instrument used is UV- spectrophotometer Model-UV-1800 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells were used for development analytical method over the range of 200-400 nm.

Marketed formulation *Glucored Forte* tablet containing GLB 5 mg and MET 500 mg was used as sample; purchased from local pharmacy.

EXPERIMENTAL

Preparation of standard stock solutions

An accurately weighed quantity of about 10 mg pure drug of GLB and MET was dissolved in methanol and diluted to 100 mL separately. Further dilutions carried out to get final concentration of 10 µg/mL and 100 µg/mL of GLB and MET respectively.

Selection of analytical wavelengths

Appropriate dilutions were done for each drug from the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. GLB and MET showed absorbance maxima at 228 nm (Figure 2) and at 237 nm (Figure 3) respectively. Figure 4 represents the overlain spectra of both the drugs.

Selection of analytical concentration ranges

From the standard stock solution of GLB and MET, appropriate aliquots were pipetted out into 10 mL volumetric flasks and dilutions were made with methanol to obtain working standard solutions of concentrations 0.2- 1.2 µg/mL and 50-300 µg/mL respectively.

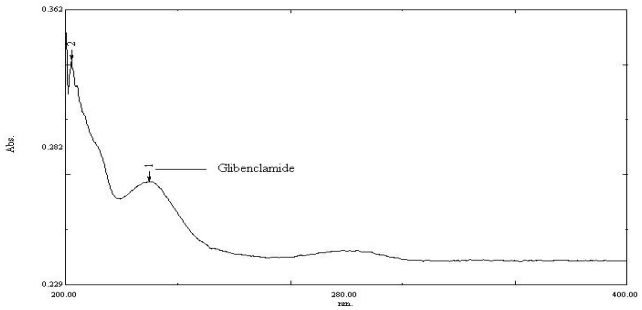

Figure 2 : UV spectrum of GLB

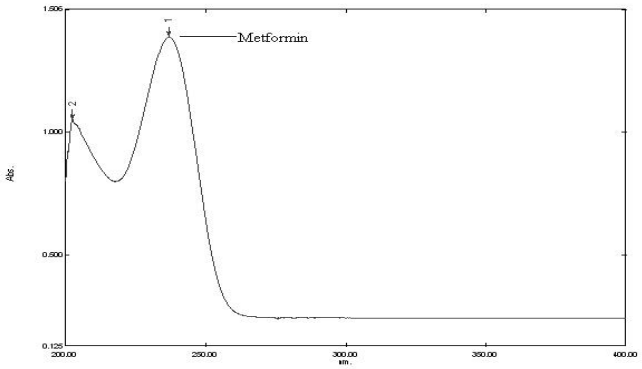

Figure 3 : UV spectrum of MET

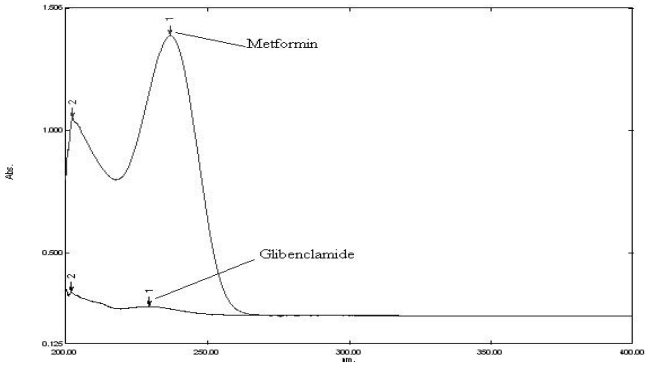

Figure 4 : Overlain spectrum of the GLB and MET

Absorbance for these solutions were measured at 228 nm and 237 nm respectively (TABLE 1) and a calibration curve of absorbance against concentration was plotted (Figure 5, 6).

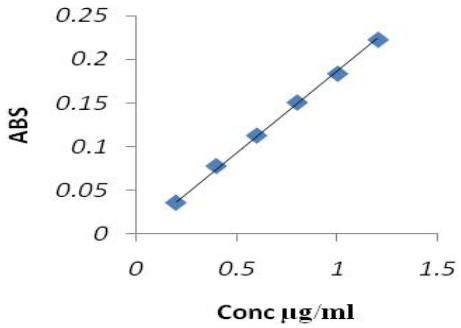

Figure 5 : Calibration curve of GLB

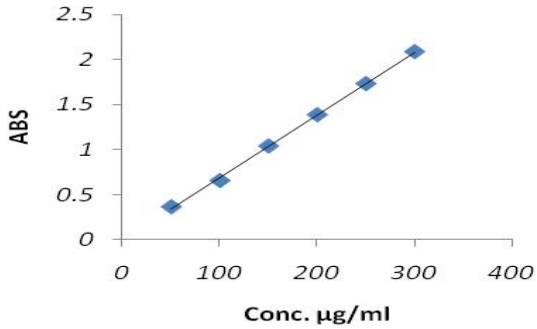

Figure 6 : Calibration curve of MET

Both drugs followed the Beer-Lamberts law in the range of 0.2-1.2 µg/mL and 50-300 µg/mL for GLB and MET respectively. TABLE 2 summaries the optical characteristics of both the drugs.

TABLE 1 : Standard calibration table for GLB and MET

Sr. No.	For Glibenclamide		For Metformin	
	Conc. (µg/mL)	Abs.* at 228 nm	Conc. (µg/mL)	Abs.* at 237 nm
1.	0.2	0.036	50	0.363
2.	0.4	0.078	100	0.654
3.	0.6	0.113	150	1.039
4.	0.8	0.151	200	1.386
5.	1	0.184	250	1.732
6.	1.2	0.223	300	2.088

TABLE 2 : Optical characteristics and other parameters

Parameters	GLB	MET
Working wavelength (nm)	228	237
Linearity range (µg/mL)	0.2-1.2	50-300
Limit of detection (µg/mL)	0.10	0.41
Limit of quantitation (µg/mL)	0.34	1.35
Y= mx + c		
Slope	0.186	0.006
Intercept	0.115	0.012
Regression Coefficient	0.999	0.999

Procedure for analysis of mixture

The method was tested by analyzing a solution containing known concentration of both drugs. The mixed standards were prepared using standard stock solution in the ratio of 1:100 containing 0.8, 1.0 and 1.2 µg/mL of GLB and 80, 100 and 120 µg/mL of MET respectively. The scanning of mixed standard solutions was carried out in the range of 400 nm to 200 nm in spectrum mode (TABLE 3). The absorbance of mixed standard solutions was measured at 228 nm and 237 nm. The concentrations of GLB and MET present in mixed standards were calculated using simultaneous equation. (TABLE 4)

Procedure for analysis of tablet formulation

Twenty tablets were weighed accurately; the average weight was determined and then triturated to a fine powder. A quantity equivalent to 5 mg of GLB and 500 mg of MET was weighed and transferred to a 100 mL volumetric flask containing 70 mL methanol the contents were sonicated for 20 min and volume was made up to 100 mL with methanol and filtered through Whatman filter paper no. 41 to give the stock solution containing 50 µg/mL of GLB and 5000 µg/mL of MET. Various dilutions of the tablet stock solutions were scanned and the absorbances of these solutions were measured at 228 nm and 237 nm respectively and the concentrations of the two drugs in the sample solutions were calculated using simultaneous equations. The analysis procedure was repeated six times. The results of marketed tablet formulation are given in TABLE 5.

TABLE 3 : Absorbance of mixed standards containing GLB and MET

Sr. No.	Mixed Standards		Abs. at 228 nm	Abs. at 237 nm
	Conc. of GLB (µg/mL)	Conc. of MET (µg/mL)		
1.	0.8	80	0.152	0.580
2.	1.0	100	0.183	0.653
3.	1.2	120	0.225	0.784

TABLE 4 : Results of mixture containing GLB and MET

Sr. No.	Amount Present* (µg/mL)		Amount Found* (µg/mL)		% Amount Found*	
	GLB	MET	GLB	MET	GLB	MET
1	0.8	80	0.79	79.97	98.75	98.96
2	1.0	100	1.00	99.84	100.0	99.84
3	1.2	120	1.21	119.93	100.83	99.94

***each value is a mean of six observations**

TABLE 5: Results of marketed tablet* formulation

Sr. No.	Label Claim (mg/tab)		Amount Found (mg/tab)		% of Label Claim	
	GLB	MET	GLB	MET	GLB	MET
1	5	500	4.96	498.72	99.20	99.74
2	5	500	4.95	499.44	99.00	99.88
3	5	500	4.98	500.36	99.60	100.07
4	5	500	4.99	498.96	99.80	99.79
5	5	500	4.97	499.67	99.40	99.93
6	5	500	5.02	498.88	100.40	99.77
				Mean	99.56	99.86
				SD	0.4966	0.1238
				% RSD	0.4987	0.1239

***tablet formulation:Glucored Forte (Sun Pharma, Sikkim)**

Method validation

Linearity and range

The linearity for GLB and MET were determined at six concentration levels, ranging from 0.2-1.2 µg/mL

and 50-300 µg/mL respectively using working standards.

Accuracy (Recovery study)

Recovery studies were carried out at three levels i.e. 80, 100 and 120 % of the label claim of the Tablet formulation as per ICH guidelines.

To perform recovery studies at 80 % of the test concentration, sample containing 5 mg of GLB and 500 mg of MET was weighed and transferred to a 100 mL volumetric flask. To it, 4 mg of standard GLB and 400 mg of standard MET was added, the mixture was mixed thoroughly. Added 70 mL methanol the contents were sonicated for 20 min with methanol to dissolve the active ingredients and the volume was made up to 100 mL with methanol and filtered through Whatman filter paper no. 41.

Similarly to perform recovery studies at 100 % of the test concentration, tablet powder containing 5 mg of GLB and 500 mg of MET was weighed. To it, 5 mg of standard GLB and 500mg of standard MET was added and at 120 % level, 6 mg of standard GLB and 600 mg of standard MET was added to the tablet powder equivalent to 5 mg of GLB and 500 mg of MET. Added 70 mL methanol the contents were sonicated for 20 min with methanol to dissolve the active ingredients and the volume was made up to 100 mL with methanol and filtered through Whatman filter paper no. 41.

From the stock solutions prepared at each level suitable aliquots were pipetted out and diluted to 10 mL with methanol and were analysed as per the procedure for tablet formulations. The results of the recovery studies were also validated statistically. The results of recovery studies are given in TABLE 6.

TABLE 6 : Results of recovery studies

Level of (%) Recovery	Amount Present (mg/tab)		Amount of standard added (mg)		Total amount Recovered* (mg)		% Recovery*	
	GLB	MET	GLB	MET	GLB	MET	GLB	MET
80	5	500	4	400	8.94	899.24	99.40	99.91
100	5	500	5	500	9.92	998.75	99.20	99.87
120	5	500	6	600	10.92	1097.90	99.27	99.80
						Mean	99.29	99.86
						SD	0.1014	0.0556
						% RSD	0.1021	0.0556

***each value is the mean of three observations**

Precision

Precision of the method was verified by using stock solutions in the ratio of 1:100 containing 1 µg/mL GLB and 100 µg/mL of MET. System repeatability was done by repeating the assay three times of six replicate dilutions of the same concentration after every two hours on the same day for intraday precision. Interday precision was carried out by performing the assay of six sample sets after 24 hours and 48 hours. The results of intermediate precision are given in TABLE 7.

TABLE 7 : Results of intermediate precision

Formulation	Parameter	Intra-day precision*	Inter-day precision*
GLB	Mean	99.56	99.42
	SD	0.1541	0.8964
	% RSD	0.154	0.901
MET	Mean	99.93	99.40
	SD	0.2483	1.0252
	% RSD	0.2487	1.0313

***each value is a mean of six observations**

Limit of detection and limit of quantitation

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in TABLE 2.

LOD = 3.3 (σ / S)

Where, S = slope of calibration curve, σ = standard deviation of the response. The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in TABLE 2.

LOQ = 10 (σ / S)

Where, S = slope of calibration curve, σ = standard deviation of the response.

RESULTS AND DISCUSSION

In the present work, new simultaneous estimation method was developed for the simultaneous spectroscopic estimation of GLB and MET in commercially available tablet dosage form.

The concentrations in the range of 0.2-1.2 µg/mL of GLB and 50-300 µg/mL of MET mixed working

standard and two set of wavelengths gave optimum accuracy, precision, time, economy, and sensitivity for this method. The proposed procedure was successfully applied to the determination of GLB and MET in the commercially available tablets dosage form, and the results are shown below

CONCLUSION

The novel method for simultaneous estimation of GLB and MET was developed using alcoholic solubilization technique. GLB and MET were poorly water soluble drugs therefore methanol was used as a solvent as it is completely soluble in it. methanol did not interfere in the spectroscopic determination of GLB and MET having maximum absorbance at 228 nm and 237 nm respectively. GLB and MET follows Beer-Lambert's law in range of 0.2-1.2 µg/mL and 50-300 µg/mL shows GLB and MET can be estimated in methanol. Commercial formulation containing GLB and MET were analyzed proposed method. Mean assay values in Glucored Forte Plus were found to be 99.56±0.4966 and 99.86 ±0.1238 respectively. The accuracy of method was determined by recovery studies. Pure GLB and MET were added to the preanalyzed tablet powder at three different levels viz. 80, 100, 120% of labeled claims as per the ICH guidelines. Three replicate analyses were carried out at each level. The mean recovery was found to be 99.29±0.1014 % and 99.86±0.0556 % in Glucored Forte samples respectively indicating that the method has required accuracy and there was no interference from the common excipients present in tablets. The RSD value below 2% indicated that the method has required precision. LOD and LOQ values at 228 and 237 were found to be 0.10 and 0.41 µg/mL and 0.34 and 1.35 µg/mL respectively.

Thus, the developed method was simple, accurate and precise and can be used for routine analysis of GLB and MET in pharmaceutical preparation.

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