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Development and Validation of analytical method for Simultaneous Estimation of Glibenclamide and Metformin HCl in Bulk and Tablets using UV – visible spectroscopy

Patil Sudarshan S.*, Bonde C. G.
School of Pharmacy and Technology Management, SVKM's NMIMS University, Shirpur, Dist. Dhulia
***Corres.author : sud_pharma@yahoo.co.in**
Mobile No. 09881429057

ABSTRACT: Simple spectrophotometric method has been developed for simultaneous estimation of Glibenclamide and Metformin HCl in combined dosage form. The method employed simultaneous equation method for analysis using methanol as a solvent. The two wavelengths 229.5 nm and 237 nm were selected for estimation of Glibenclamide and Metformin HCl respectively. Linearity was observed in the concentration range of 3-15 µg/ml and 2-10 µg/ml for Glibenclamide and Metformin HCl respectively. The recovery studies ascertained the accuracy of the proposed method and the results were validated as per ICH guidelines. The method can be employed for estimation of pharmaceutical formulations with no interference from any other excipients and diluents.
Keywords*:* Glibenclamide, Metformin HCl, Simultaneous equation

INTRODUCTION

Glibenclamide is 1-[4-[2-(chloro-2-methoxy benzamido) ethyl]-benzenesulphonyl]-3cyclohexylurea,5-chloro-N-[2-[4[[[(cyclohexyl(amino)carbonyl]-amino]sulphonyl] phenyl]
ethyl]-2-methoxy benzamide or 1-[[p-[-2-(5-chloro-o-anisamido)ethyl]phenyl]-sulphonyl-3-cyclohexylurea,a sulphonyl urea derivative is a second generation oral hypoglycemic agent which is more potent than those of first group[1] and is used to assist 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 is drug of choice for initiating treatment in noninsulin-dependent diabetes when diet and weight control fails. It stimulates the secretion and enhances the utilization of insulin by appropriate tissues[2]. Metformin chemically N, N-dimethylimidodicarbonimidic diamide hydrochloride is used as antidiabitic drug from the biguanide class used in the management of type 2 diabetes. Major action of metformin lay in increasing glucose transport across the cell membrane in skeletal muscle[3]. The chemical structure of Glibenclamide and Metformin HCL are shown shown in fig. 1.

Several assay techniques have been described for quantitative determination of glibenclamide in biological fluids; these include procedures based on high performance liquid chromatography (HPLC) [4-12], fluorometry[13], radioimmunoassay[14-16] and gas chromatography[17]. A few reports deal with the analysis of the drug in these dosage forms; such procedures include: micellar electrokinetic capillary chromatography [18], RP-HPLC[19], fluorometry[20], TLC-UV spectrophotometry[21], derivative spectrophotometry[22], UV spectrophotometry [23] and colorimetry[24].

Few UV Spectrophotometric methods [25,26], HPLC [27, 28,29, 30] and ion-pair HPLC[31] method have been reported for the estimation of MET.

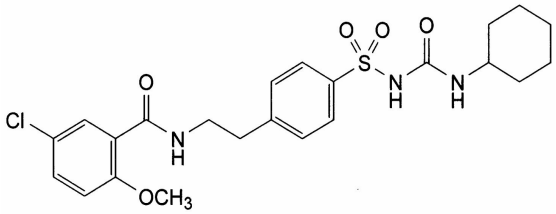
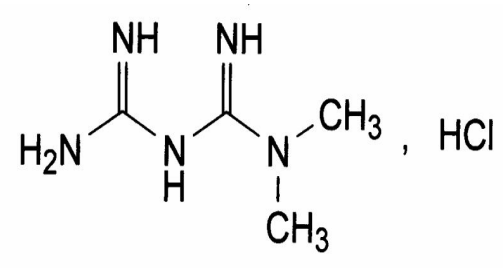


Fig.1(a) : Chemical structure of Glibenclamide



(b)

Fig.1(b) : Chemical structure of Glibenclamide (a) and Metformin HCl

EXPERIMENTAL

Instrument

Absorption spectral measurements were carried out with a Perkin Elmer Lambda 25 model UV – Visible spectrophotometer.

Chemicals

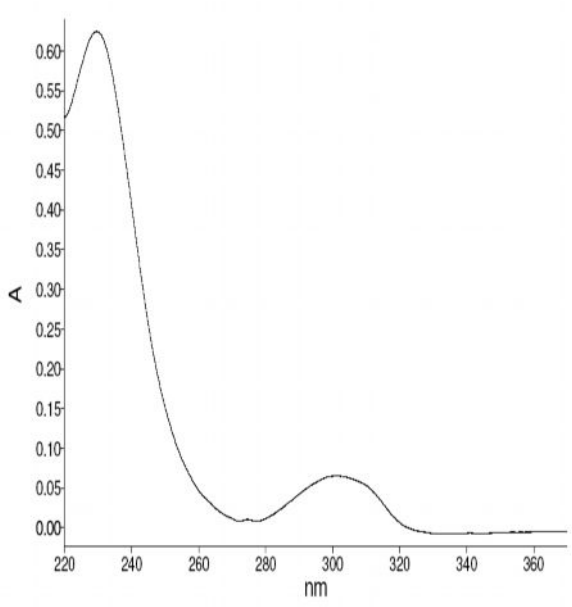
Glibenclamide (GLB) and Metformin HCl (MET) were supplied by Wockhardt research centre, India as gift sample and used as such. Methanol used was spectro grade from Qualigen fine chemicals Ltd, India. Water used was generated by double distillation.

Preparation of stock solution

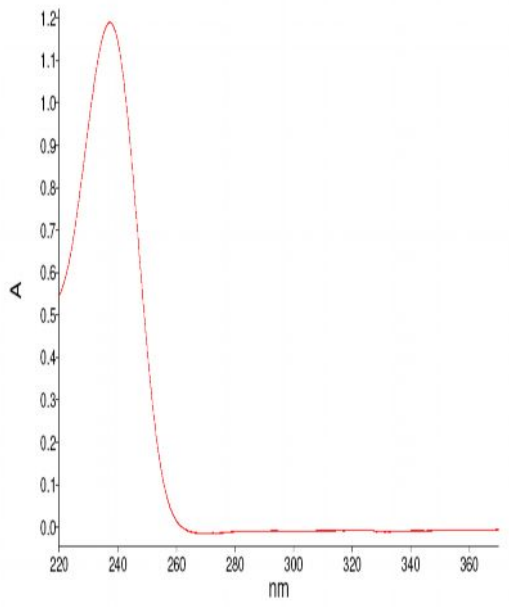
GLB and MET (Metformin HCl equivalent to 10 mg of metformin 10 mg) were accurately weighed and transferred to two separate 100 ml volumetric flasks. Each drug was dissolved in 50 ml of methanol, shaken manually for 10 min and volume was made up to the mark with the same solvent to obtain final concentration 100 µg/ml each.

Selection of λ max

An appropriate aliquot portions of 1, 2, 3, 4 and 5 ml of GLB from standard stock solutions of GLB was transferred to separate 10 ml volumetric, dissolved in methanol and volume was made up to the mark to obtain concentrations 10, 20, 30, 40 and 50 µg/ml of GLB. The same procedure followed to obtain concentrations 10, 20, 30, 40 and 50µg/ml of MET. Drug solutions were scanned separately between 200 nm to 400 nm. The spectrum of both drugs was recorded; **Fig 1-2** and two wavelengths 229.5 nm (λ max of GLB) and 237.0 nm (λ max of MET) were selected for further study.



(1)



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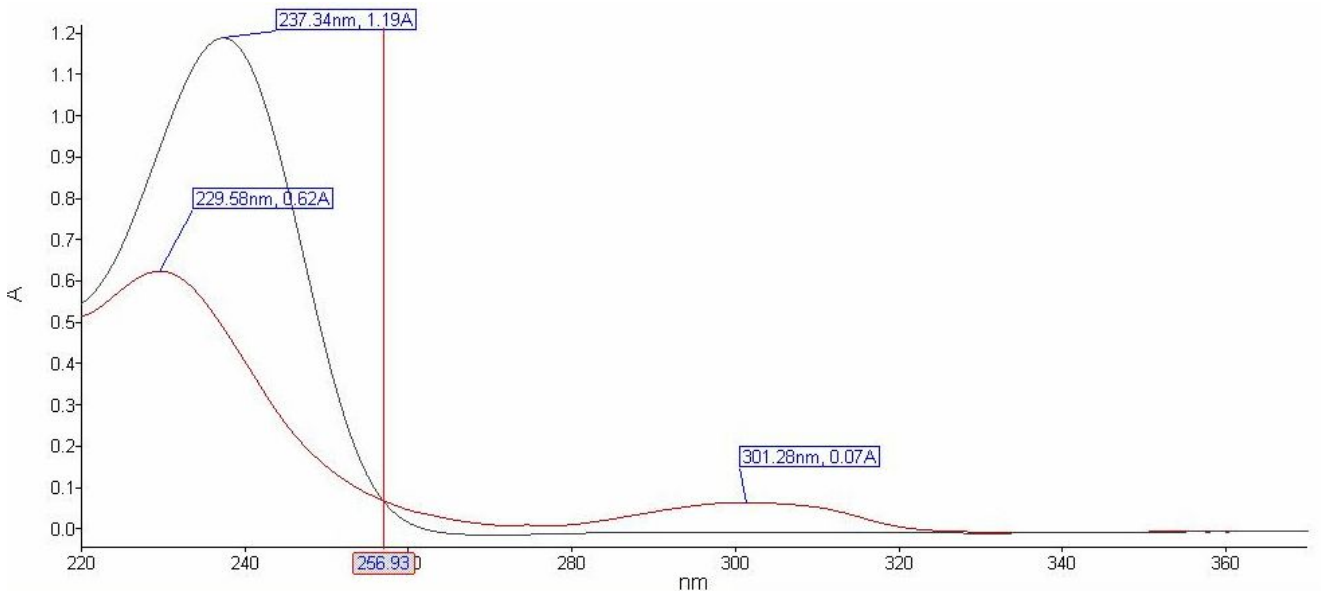

(3)

Fig.2: UV Spectra of GLB (1) ,MET (2) and overlain spectra (3) of GLB and MET

Method: Simultaneous Equations

Different aliquots were taken from the stock solutions and diluted with the same solvent to prepare a series of concentrations. The absorbances of these solutions were measured at 229.5 nm and 237 nm for GLB and M ET, respectively and calibration curves were plotted at selected wavelengths; the optical characteristics and linearity data is shown in table 1. The E (1%, 1cm) of each drug at both wavelengths was determined; results are presented in table 2. The overlain spectra of BF and HCTZ are shown in fig. 2.

Two simultaneous equation (in two variables C_1 and C_2) were framed by using E (1%, 1cm)

$A_1 = (72.13)\, C_1 + (91.85)\, C_2$ (I)

$A_2 = (55.33)\, C_1 + (119.55)\, C_2$ (II)

Where, C1 and C2 are the concentrations of GLB and

MET measured in g /100 ml, in the sample solutions. A_1 and A_2 are the absorbances of the sample solutions, at selected wavelength i.e. 229.5 nm and 237 nm, respectively. By applying the Cramer's rule (Beckett and Stenlake, 2005) to equations I and II, the concentrations C_{GLB} and C_{MET} can be determined as follows:

$C_{GLB} = A_2(55.33) - A1(119.55)/ - 3541.08$ (III)

and

$C_{MET} = A_1(91.85) - A_2(72.13) / - 3600.1$ (IV)

Preparation and analysis of tablet formulations

Contents of twenty '**Daonil'** Tablets (containing 5 mg of GLB and 500 mg of MET) were weighed and ground to fine powder. For the analysis of drugs, a standard addition method was used. An accurately weighed 250 mg of pure GLB was added to finely powdered samples to bring the concentration of GLB in linearity range. With this addition, the ratio of GLB to MET in samples was brought to 1:2. A quantity of sample equivalent to 250 mg of GLB and 500 mg of MET was transferred into 100 ml volumetric flask containing 40 ml of methanol, sonicated for 10 min, the volume was made upto the mark and filtered through Whatmann filter paper (No. 41). An appropriate volume 0.1 ml of this solution was transferred to 100 ml volumetric flaks, dissolved and volume was adjusted to mark. The absorbances of the solutions were measured at 229.5 nm and 237.0 nm against blank. The concentrations of two drugs in sample were determined by using equations **III** and **IV.** The results are reported in the Table 3.

Validation of Method (ICH guidelines, 2005)

The method was validated with reference to accuracy, precision, and ruggedness.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of BF and HCTZ to preanalysed tablet solutions. The resulting solutions were then re-analysed by proposed methods; the results are shown in table 4.

Precision

Precision of the methods was studied as intra-day, interday and repeatability. Intra-day study was performed by analyzing, the three different concentration of drug for

three times in the same day. Inter-day precision was performed by analyzing three different concentration of the drug for three days in a week. Repeatability was performed by analyzing same concentration of drugs for six times. The results are shown in table 5.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by different analysts using similar operational and environmental conditions. The results are shown in table 5.

Table 1: Optical characteristics and linearity data

Parameters	GLB	MET
Absorption maximum (nm)	229.5	237
Beer's law limit (µg/mL)	3-15	2-10
Correlation coefficient	0.9999	1
Regression equation Y = m X + C	Y= 0.071X	Y= 0.118X + 0.0003
Intercept (C)	0	0.0003
Slope (m)	0.071	0.118

Table 2: E (1%, 1cm) for GLB and MET

* E(1%,1cm) at 229.5 nm ± SD		* E(1%,1cm) at 237 nm ± SD	
GLB	MET	GLB	MET
ax_1= 72.13 ± 0.57	ay_1=91.85 ± 0.31	ax_2=55.33 ± 0.70	ay_2= 119.55 ± 0.71

*mean of ten estimations

Table 3: Analysis of tablet formulation

Brand (DAONIL)		*% Amount found ± SD
GLB 250mg + MET 500mg	GLB	99.81 ± 0.30
	MET	99.97 ± 0.19

*mean of five estimations

Table 4: Results from Recovery Studies

Pre-analysed sample solution [µg/ml]	**Excess drug added [µg/ml ,n = 3]**	**Amount recovered [µg/ml]**	**% Recovery**	**% R.S.D.**
GLB 5	0	4.99	99.75	0.11
	4	3.99	99.75	0.66
	5	5.01	100.2	0.86
	6	6.03	100.5	0.17
MET 10	0	9.98	99.8	0.27
	8	7.97	99.6	0.70
	10	10.05	100.5	0.65
	12	11.98	99.83	0.59

Table 5: Results from precision and ruggedness

Parameters	**GLB**	**MET**
Precision (%RSD)		
Intra-day (n = 3)	0.23 – 0.92	0.79 – 1.120
Inter-day (n = 3)	0.17 – 1.81	1.12 – 1.73
Repeatability (n=6)	0.61	0.35
Ruggedness (%RSD)		
Analyst 1 (n = 3)	0.12	0.61
Analyst 2 (n = 3)	0.16	0.70

RESULTS AND DISCUSSION

Two wavelengths 229.5 nm (λmax for GLB) and 237 nm (λmax for MET) were selected for analysis of the drugs in methanol. Linearity was observed in the range 3 - 15 µg/ml (r^2=0.9999) for GLB and 2-10 µg/ml (r^2 =1) for MET. The amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. Both the methods were found to be precise as indicated by the repeatability, inter-day, intra-day analysis, showing %RSD less than 2. The results did not show any statistical difference between operators suggesting that methods developed were rugged. The results of precision and ruggedness are shown in table 5. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical formulations containing both these drugs.

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