Simultaneous Estimation of Gliclazide and Metformin Hydrochloride in Combined Dosage Forms

H. K. JAIN AND R.K. AGRAWAL*
Department of Pharmaceutical Sciences, Dr. Hari Singh Gour Vishwavidyalaya
Sagar–470003, MP.

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Three simple, rapid, accurate, economical and reproducible procedures for simultaneous estimation of gliclazide and metformin hydrochloride in two component tablet formulation have been developed. First method is based on an equation of area calculation of curve at two wavelength regions (228.6 to 224 nm and 235 to 231 nm). Second method employs simultaneous equation at two wavelengths corresponding to 226.3 and 233.2 nm. Third method employs derivative spectroscopy at zero crossing points (226 and 232.9 nm) in first order derivative spectra. Both the drugs obey Beer's law in the concentration ranges employed for these methods. The results of analysis have been validated statistically and by recovery studies.

The combination of gliclazide and metformin hydrochloride is used in non-insulin dependent diabetes mellitus. Non aqueous methods have been reported for analysis of gliclazide[1] in B.P. and metformin hydrochloride[2] in I.P. Several HPLC methods for determination of gliclazide and metformin hydrochloride in plasma[3-5] also have been reported. No method is reported so far for simultaneous estimation of gliclazide and metformin hydrochloride in combined dosage forms. Present work illustrates three simple, rapid, accurate, economical and reproducible procedures for simultaneous estimation of gliclazide and metformin hydrochloride in two component tablet formulation.

A Shimadzu UV 1601 (Japan) recording spectrophotometer with 10 mm matched quartz cells was employed for this work. Sodium hydroxide AR in double distilled water (0.01 N NaOH) was used for preparation of solutions. Standard stock solutions of gliclazide and metformin hydrochloride (100 µg/ml) were prepared in 0.01 N NaOH. Two different batches (A and B) were separately used for preparation of tablet sample solutions. Twenty tablets were weighed and ground to a fine powder. An accurately weighed powder sample equivalent to 75 mg of metformin hydrochloride was dissolved in about 150 ml of 0.01 N NaOH by intermittent shaking. The solution was filtered through a Whatman Filter Paper No. 41 into a 250 ml volumetric flask. The residue after filtration was washed with 50 ml of 0.01 N NaOH and the volume was made up to the mark with same. This solution was diluted ten fold. Three different methods were used for the analysis.

In the method I, area claculation[6] involves calculation of integrated value of absorbance with respect to wavelength in indicated region of wavelengths. Area calculation processing item calculates the area bounded by the curve and horizontal axis. Here horizontal axis represents baseline. Area calculation ($\int_{\lambda 2} A\, d\lambda$) = $\alpha + \beta$ where α is the area of portion bounded by curve data and a straight line connecting the start and end points of data. And β is the area of portion bounded by a straight line connecting the start and end points on the curve data and horizontal axis. $\lambda 1$ and $\lambda 2$ are wavelengths representing start and end points of curve region.

This method involves area calculation in regions of 228.6 to 224 nm and 235 to 231 nm. These regions were selected on the basis of repeated observation that plot area calculation of pure single drug vs concentration (2 to 20 µg/ml of gliclazide and 4 to 32 µg/ml of metformin hydrochloride) showed linearity.

*For correspondence

Mixed standards having the composition of 4 µg/ml gliclazide + 36 µg/ml metformin hydrochloride and 8 µg/ml gliclazide + 32 µg/ml metformin hydrochloride were prepared and scanned in the spectrum mode and applying data processing mode, area under curve was calculated between wavelength range 228.6 to 224 nm. The obtained values were substituted in equation (1) and K_1 and K_2 were calculated. Similarly the values of K_3 and K_4 were calculated from equation (2) by submitting the area of curve between wavelength range 235 to 231 nm. The higher and lower ranges of chosen mixed standards were selected such that they lie on either side on ratio of two components in formulation.

$$\int_{224.0}^{228.6} d\lambda = K_1 C_1 + K_2 C_2 \quad \text{.....(1)}$$

$$\int_{231}^{235} A\, d\lambda = K_3 C_1 + K_4 C_2 \quad \text{.....(2)}$$

Where area of curve between 228.6 to 224 nm and between 235 to 231 nm are represented by $\int_{224.0}^{228.6} d\lambda$ and $\int_{231}^{235} A\, d\lambda$ respectively; C_1 and C_2 are concentrations of gliclazide and metformin hydrochloride respectively in µg/ml, K_1, K_2, K_3, and K_4 are constants. Finally equation would be

$$\int_{224.0}^{228.6} A\, d\lambda = 0.1915\ C_1 + 0.3077\ C_2 \quad \text{.....(3)}$$

$$\int_{231}^{235} A\, d\lambda = 0.14856\ C_1 + 0.3051\ C_2 \quad \text{.....(4)}$$

Sample solutions were scanned and area was calculated within indicated wavelength ranges. Concentration of both components were calculated using above mentioned equations (3 and 4).

Method II is based on simultaneous equations. The absorptivity coefficients of gliclazide within concentration ranges of 1 to 8 µg/ml and metformin hydrochloride 6 to 36 µg/ml were determined at 226.3 and 233.2 nm. The overlain spectra of gliclazide and metformin hydrochloride with markers at the indicated wavelengths used for analysis are represented in fig.1 A set of two simultaneous equations was developed using these absorptivity coefficients as :

$$A_1 = (40.68\ C_1 + 64.85\ C_2) \times 10^{-3} \quad \text{..... (5)}$$

$$A_2 = (37.81\ C_1 + 76.39\ C_2) \times 10^{-3} \quad \text{.....(6)}$$

Where, C_1 and C_2 are concentrations of gliclazide and metformin hydrochloride respectively, in µg/ml in the sample solution. A_1 and A_2 are absorbances of the sample solution measured at 226.3 and 233.2 nm, respectively. The absorptivities reported are a mean of five independent determinations of pure single drug in given concentration range.

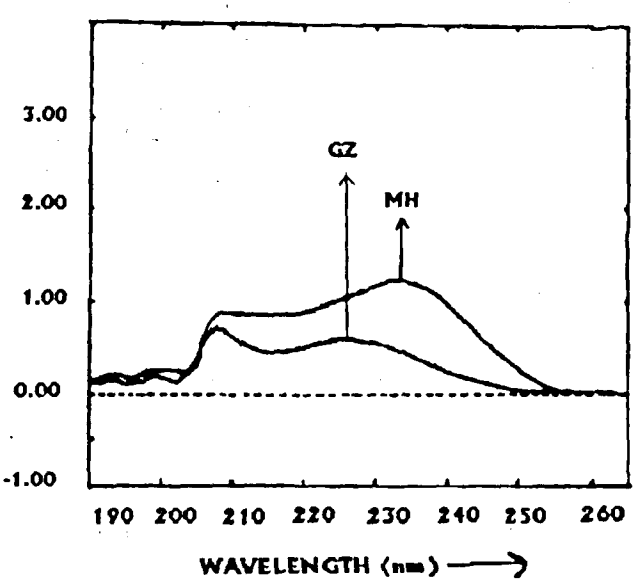

Fig. 1: Overlain spectra of gliclazide (GZ) and metformin hydrochloride (MH).

The absorbance (A_1 and A_2) of the sample solutions were recorded at 226.3 and 233.3 nm, respectively and the concentration of both components were calculated using above mentioned equations (5 and 6).

Method III employs derivative spetrophotometry. First derivative spectra revealed that gliclazide and metformin hydrochloride showed zero absorbance at 226 and 232.9 nm respectively. As at the zero crossing point on the first derivative spectrum of one drug, the other drug showed substantial absorbance. Hence these two wavelength could be effectively employed for undertaking the estimation of gliclazide and metformin hydrochloride, without any interference from the other drug in their combined formulation. Standard stock solutions were mixed in different proportions i.e. 1:15, 1:6.25, 1:3, 1:1 and 2:1 concentration ratio of gliclazide and metformin hydrochloride, respectively and found that calibration curve showed linearity in concentration range of 2 to 10 µg/ml of gliclazide and 4 to 36 µg/ml of metformin hydrochloride. Absorbances of the sample solutions were recorded at 226 and 332.9 nm from first derivative spectra and amount of drug present in sample solution was calculated from the calibration curve.

The results of analysis, obtained in triplicate with two different batches of tablet formulations, were validated

TABLE 1: STATISTICAL ANALYSIS OF RESULTS.

Method	Batch	Tablet Component	Label Claim (mg/tab)	% of Label Claim estimated ± S.D.	Coeff. Of variation	Standard error
I	A	GZ	80	101.53±0.6244	0.616	0.360
		MH	500	99.09±0.5993	0.605	0.346
	B	GZ	80	101.51±0.5214	0.514	0.301
		MH	500	99.36±0.5593	0.561	0.322
II	A	GZ	80	102.51±0.8268	0.807	0.477
		MH	500	98.1±0.7013	0.715	0.405
	B	GZ	80	102.81±0.7512	0.731	0.434
		MH	500	98.15±0.6421	0.654	0.371
III	A	GZ	80	99.18±0.8011	0.808	0.463
		MH	500	99.82±0.3725	0.373	0.215
	B	GZ	80	98.61±0.3015	0.306	0.174
		MH	500	100.32±0.3917	0.391	0.226

Each value is an average of three estimations along with standard deviation. GZ stands for gliclazide and MH for metformin hydrochloride. A and B are two different batches of tablets.

statistically and recovery studies were carried out by adding known amounts of drugs to pre-analysed sample solutions. Statistical analysis was done using established formulae[7]. The values of standard deviation and coefficient of variation were significantly low (Table 1) and recovery was close to 100 % (Table 2) indicating the reproducibility of the methods.

First and third methods are limited to a spectrophotometer having processing functions like area calculation and derivative spectroscopy in data processing mode. Third method does not require complex calculation while first and second method require equation based calculation. It is concluded that the proposed methods can be successfully employed for routine simultaneous

TABLE 2: RECOVERY STUDIES.

Conc. Of added drug 2.0 µg/ml GZ and 12.5 µg/ml of MH		% Recovery					
		Method I		Method II		Method III	
GZ (µg/ml)	MH (µg/ml)	GZ	MH	GZ	MH	GZ	MH
1.6	10.0	100.74	99.97	99.12	98.93	99.77	99.96
2.0	12.5	100.45	100.58	100.45	100.23	100.54	99.65
2.4	15.0	99.94	100.73	100.78	99.56	99.83	100.43

GZ stands for gliclazide and MH for metformin hydrochloride.

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