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Spectrophotometric analysis of a mixture of glyburide and metformin HCl in pharmaceutical preparations

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ABSTRACT

Two simple and rapid spectrophotometric methods were developed for the resolution and analysis of the binary mixture of glyburide (GB) and metformin HCl (MF) in tablets. The first method, zero-crossing first derivative spectrophotometry, depends on measuring the first derivative values at 314.7 nm for GB and 228.6 nm for MF. The second method, ratio first derivative spectrophotometry, depends on measuring the amplitudes of the first derivative of the ratio spectra at 314.7 nm for GB and 238.0 nm for MF. The calibration graphs were linear over the range of 10-125 µg/mL for GB and 2-18 µg/mL for MF. The proposed methods were applied successfully to the assay of these drugs in commercial tablets. The developed methods were able to solve the problem arising from the co-formulation of GB and MF in the ratio of 2.5:500, 5:500 or 2.5:400 (w/w), respectively in addition to the increase of the specific absorbance of MF (the major component) over that of GB (the minor component). The results were statistically compared with those obtained using a reference HPLC method and were found to be in good agreement.

Keywords: Glyburide, Metformin HCl, First derivative spectrophotometry, Ratio derivative spectrophotometry, Pharmaceutical preparations.

INTRODUCTION

Glyburide (GB) is a sulfonylurea hypoglycemic while metformin hydrochloride (MF) is a biguanide hypoglycemic. Both drugs are given by mouth in the treatment of type 2 diabetes mellitus [1]. GB is available in combination with MF in tablets in 2.5:500, 5:500 or 2.5:400 ratios (w/w), respectively. Combination treatment with MF and sulfonylurea is more effective than these drugs alone in improving glycemic control in type 2 diabetes, while also allowing a reduction of the dosage of each drug [2].

In spite of the increasing use of this mixture in the treatment of type 2 diabetes mellitus, few methods have been described for its analysis. A review of the literature revealed that the methods published for the determination of this combination relied mainly on the use of chromatographic methods, such as HPLC [3-6], TLC [7] and liquid chromatography tandem mass spectrometry [8,9]. In addition, Capillary electrophoresis has been used for the determination of this combination together with phenformin [10]. A review of the analytical methods used for bioavailability studies of glyburide/metformin mixture in addition to other oral anti-diabetic drugs was published by Thirumurugu et al. [11]. Although these methods offer a high degree of specificity, the instrumentation limitations preclude their use in routine analysis. To the best of our knowledge, no spectrophotometric methods have been yet described for the determination of both drugs in tablets. Therefore, it was desirable to develop a simple and fast procedure that could be applied in quality control laboratories for the determination of both drugs in the presence of each other.

Derivative spectrophotometry offers greater selectivity than normal spectrophotometry in the simultaneous determination of two or more drugs without previous chemical separation [12-15]. Principles and advantages of this technique have been described by O'Haver and Green [16]. Ratio derivative spectrophotometry is based on the use of the first derivative of the ratio spectra. This method was developed by Salinas et al. [17]. Berzas Nevado et al. extended this method to resolving ternary mixtures [18].

The present paper describes simple and rapid methods for the determination of both GB and MF in synthetic mixtures and in commercial tablets by first derivative spectrophotometry and ratio first derivative spectrophotometry without prior separation of the two drugs.

MATERIALS AND METHODS

Experimental

Apparatus

Spectrophotometric analysis was carried out on a Shimadzu (Kyoto, Japan) UV-1601 PC, UV-Visible double-beam spectrophotometer with matched 1 cm path-length quartz cells.

Suitable settings were: Slit width, 1 nm; scan speed, fast; sampling interval, auto. For first derivative spectra of both drugs: Wavelength range 200-350 nm, $\Delta\lambda$ = 4 nm and scaling factor = 10.

For ratio derivative spectra of GB: Wavelength range 250-328 nm, $\Delta\lambda$ = 8 nm for smoothing of ratio spectra, $\Delta\lambda$ = 4 nm for the first derivative of ratio spectra and scaling factor = 1. For ratio derivative spectra of MF: Wavelength range 212-268 nm, $\Delta\lambda$ = 4 nm for smoothing of ratio spectra, $\Delta\lambda$ = 4 nm for the first derivative of ratio spectra and scaling factor = 1.

Materials and reagents

Pure drug samples were kindly provided by pharmaceutical companies: Glyburide (Pharco Pharmaceuticals, Alexandria, Egypt) and metformin hydrochloride (CID, Cairo, Egypt). Pharmaceutical preparations were purchased from commercial sources. Methanol analytical grade was obtained from Prolabo, France.

Standard solutions

Stock solutions (1 mg/mL) of GB and MF were prepared in methanol. These solutions were stable for at least 7 days when kept in the refrigerator.

Procedure for calibration curves

Working standard solutions of GB and MF were prepared from the previous stock solutions by serial dilutions with methanol to 10-125 µg/mL for GB and 2-18 µg/mL for MF (final concentration).

1. for the determination by first derivative spectrophotometry. The first derivative spectra were recorded against methanol and the first derivative values were measured at 314.7 nm for GB and 228.6 nm for MF.
2. for the determination by ratio derivative spectrophotometry. The first derivative of the ratio spectra (the spectra of GB divided by the spectrum of a 18 µg/mL MF solution and the spectra of MF divided by the spectrum of a 1 µg/mL GB solution) were recorded. The amplitudes at 314.7 nm for GB and 238.0 nm for MF were measured.

Procedure for analysis of tablets

Twenty tablets were weighed and then powdered. Two accurately weighed amounts of the powder, one contains 20 mg GB and the other contains 20 mg MF, were transferred into two separate 100 mL volumetric flasks, and diluted to the mark with methanol. Flasks were sonicated for 30 min, filtered and then analyzed as described under *Procedure for calibration curves*. The concentration of each drug was determined using either the calibration curve or the corresponding regression equation.

RESULTS AND DISCUSSION

GB and MF are co-formulated in tablets in the ratio of 2.5:500, 5:500 or 2.5:400 (w/w), respectively. Moreover the specific absorbance of MF (the major component) is higher than the specific absorbance of GB (the minor component) [19]. This rendered analysis of such mixture by conventional spectrophotometry challenging. Therefore, we resorted to first derivative and ratio first derivative spectrophotometry in an attempt to analyze the mixture of the two drugs in their tablets.

Fig. 1A shows the absorption spectra of GB and MF in methanol which overlap sufficiently to demonstrate the resolving power of the proposed methods. As it may be seen, GB could be directly determined in presence of MF by measuring the absorbance at 300 nm where MF does not absorb. However, in the presence of high concentration of MF, as that present in tablets, it was impossible to determine GB by direct spectrophotometry since MF shows a linear background absorption in the wavelength region of 290-320 nm. Moreover, MF could not be directly determined in the presence of GB by conventional UV spectrophotometry due to the marked overlap of their spectra. These problems have been solved satisfactorily by the proposed methods.

'Zero-crossing' first derivative spectrophotometry

Fig. 1B shows the first derivative spectra of both drugs where sharp bands of large amplitudes of GB and MF were produced which could offer more specific determination of these drugs. The zero-crossing method is the most common procedure for conducting analytical calibration so GB was determined by measurement of its first derivative amplitude at the zero-crossing of MF at 314.7 nm ($1D_{314.7}$)[a] while MF was determined by measurement of its first derivative amplitude at the zero-crossing of GB at 228.6 nm ($^1D_{228.6}$).

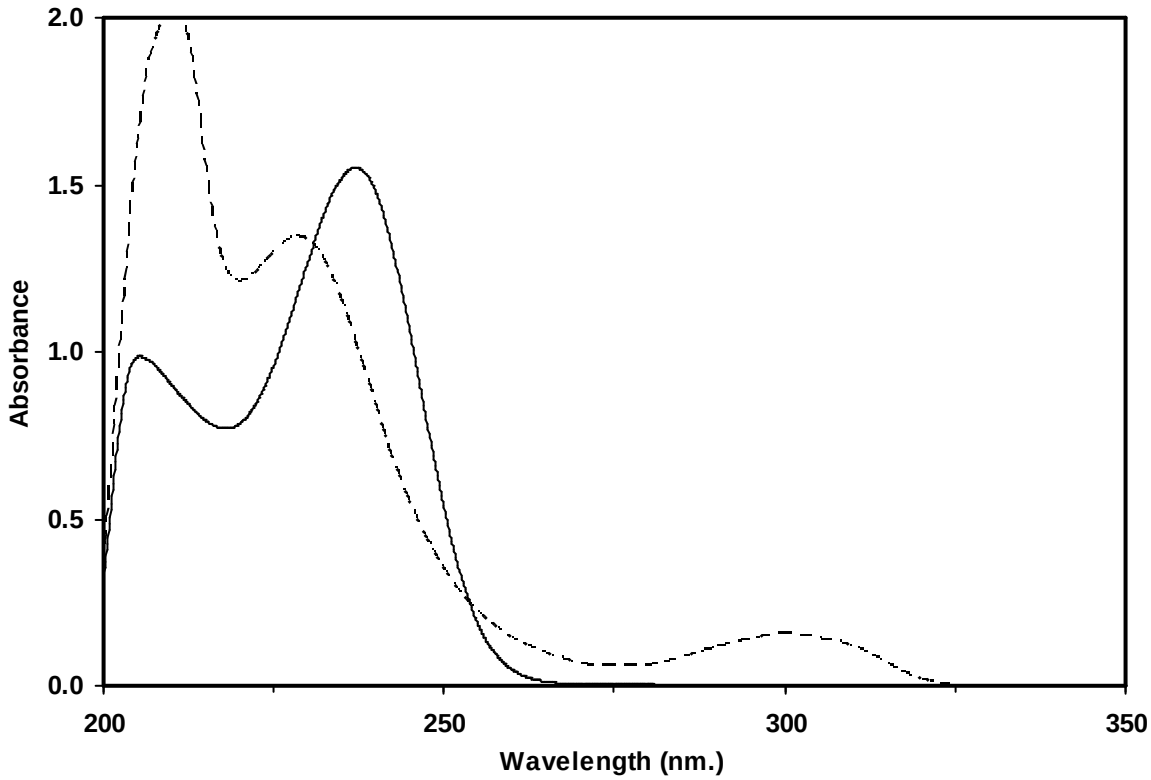

Fig. 1A. Zero-order spectra of 25 µg/mL GB (---) and 18 µg/mL MF (—) in methanol

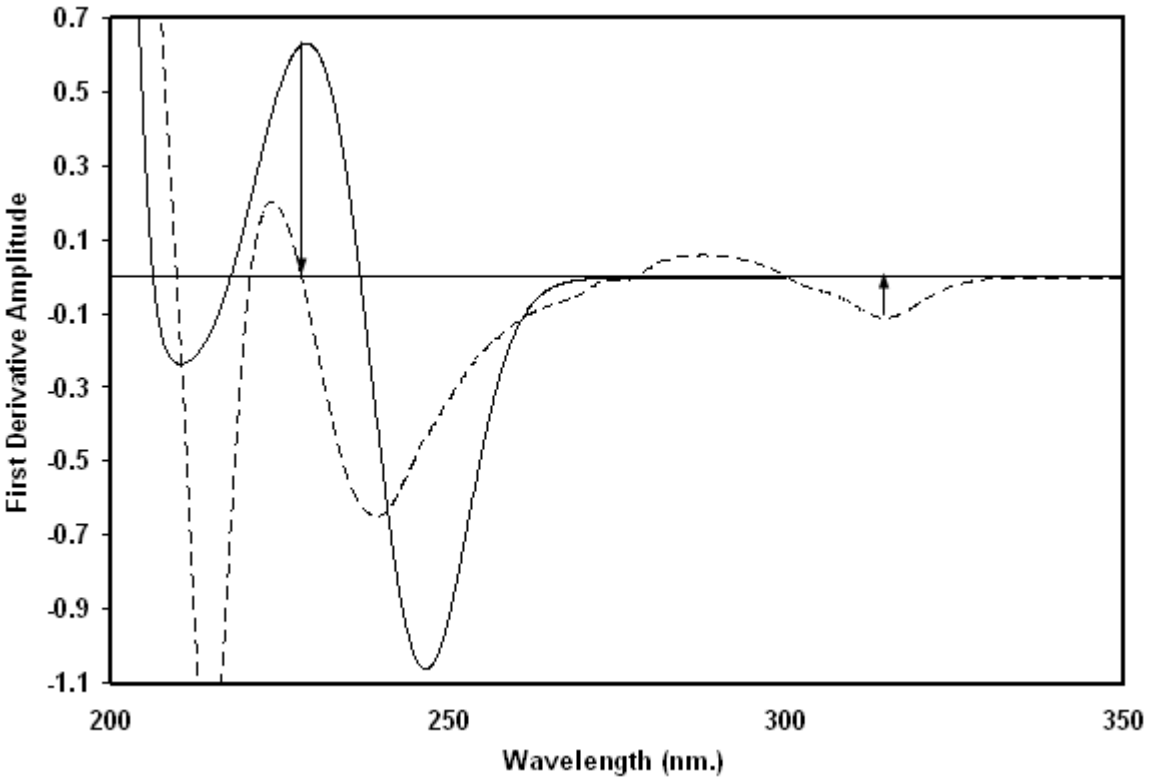

Fig. 1B. First derivative spectra of 25 µg/mL GB (---) and 18 µg/mL MF (—) in methanol.

Ratio first derivative spectrophotometry

Fig. 2A shows the ratio spectra of different concentrations of GB standards (spectra divided by the spectrum of a solution containing 18 µg/mL of MF) while Fig. 2B shows their first

derivatives. As it can be seen, the amplitude at 314.7 nm ($^1DD_{314.7}$)[a] in the ratio derivative spectra corresponds to GB present in the solution, so it can be used for its quantitative determination.

Likewise, Fig. 3A and Fig. 3B show the ratio spectra of different concentrations of MF standards (spectra divided by the spectrum of 1 µg/mL GB solution) as well as the corresponding first derivative spectra, on the basis of which MF can be quantified by measuring the amplitude at 238.0 nm ($^1DD_{238.0}$).

The influence of Δλ for obtaining the first derivative of the ratio spectra was tested to obtain the optimum wavelength interval; Δλ = 4 nm was considered as suitable for both drugs. For selecting the standard solution as divisor, different concentrations were tested and different calibration curves were obtained. The best results in terms of signal– to– noise ratio, sensitivity and repeatability were obtained by using the spectra of 18 µg/mL MF and 1 µg/mL GB solutions as divisors in the determination of GB and MF, respectively.

The results of the two proposed methods showed no significant differences with those obtained by the reference method [3] as regards to accuracy and precision.

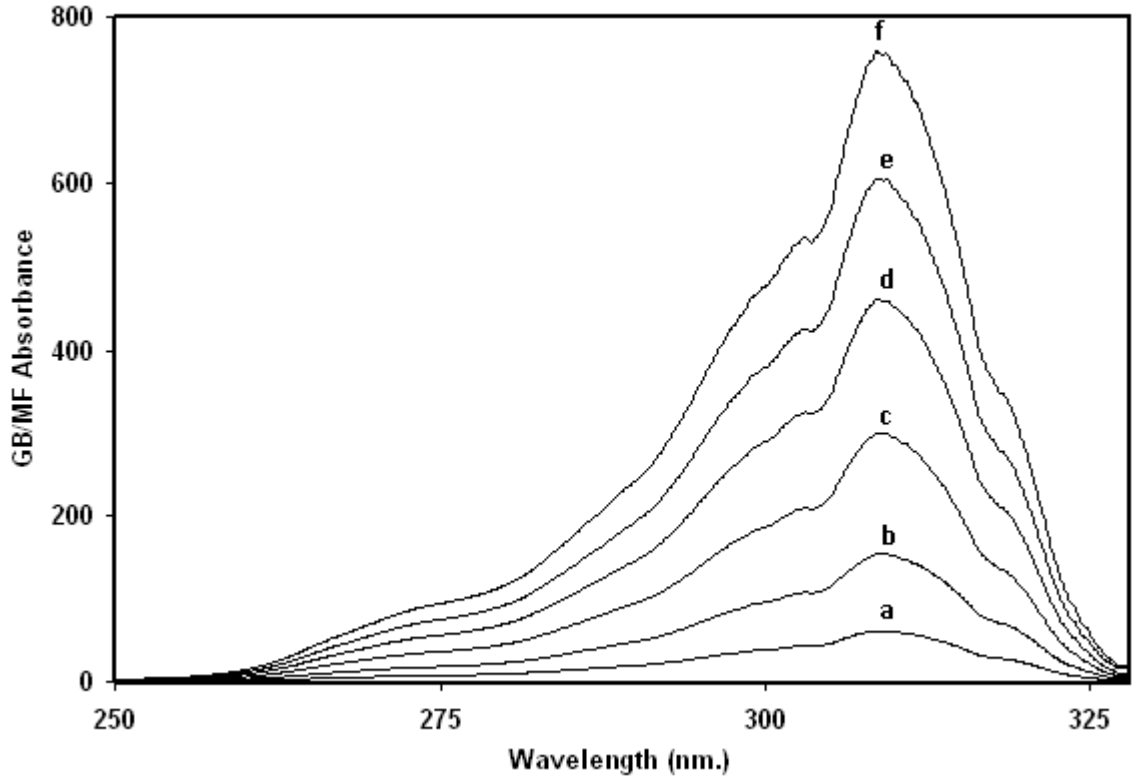

Fig. 2A. Ratio spectra of GB of (a) 10 µg/mL, (b) 25 µg/mL, (c) 50 µg/mL, (d) 75 µg/mL, (e) 100 µg/mL, and (f) 125 µg/mL; when 18 µg/mL MF is used as divisor.

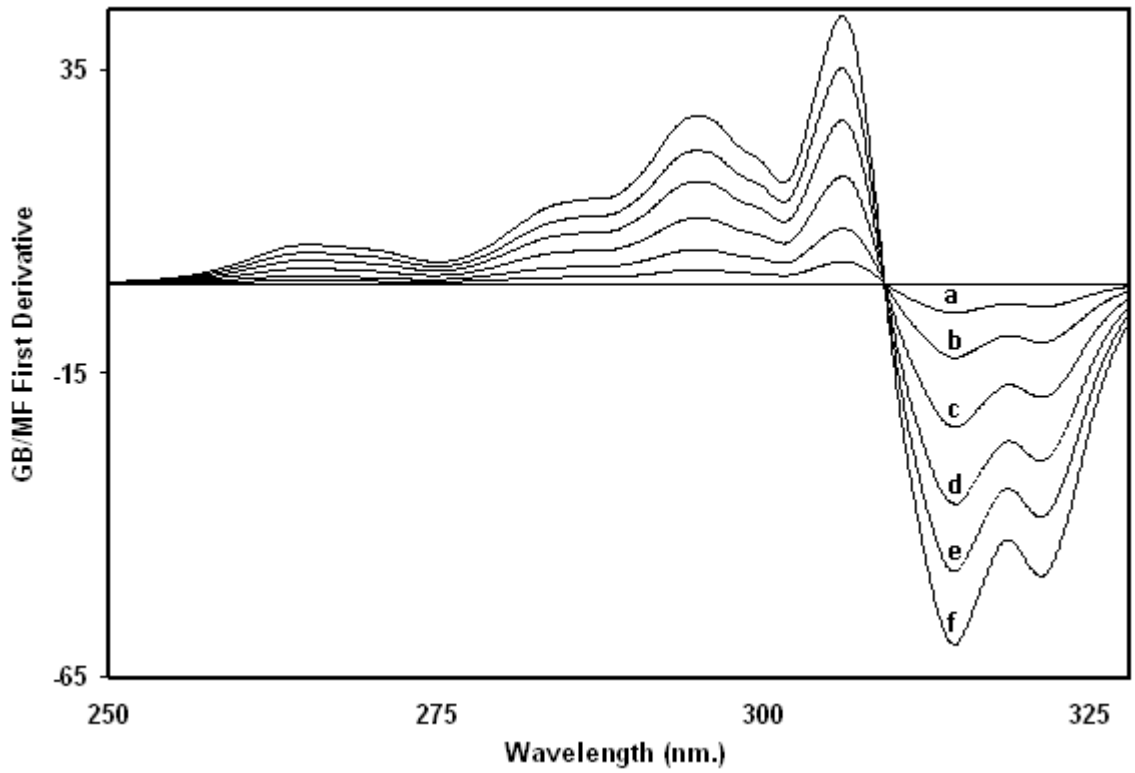

Fig. 2B. First derivative of the ratio spectra of GB of (a) 10 µg/mL, (b) 25 µg/mL, (c) 50 µg/mL, (d) 75 µg/mL, (e) 100 µg/mL, and (f) 125 µg/mL; when 18 µg/mL MF is used as divisor.

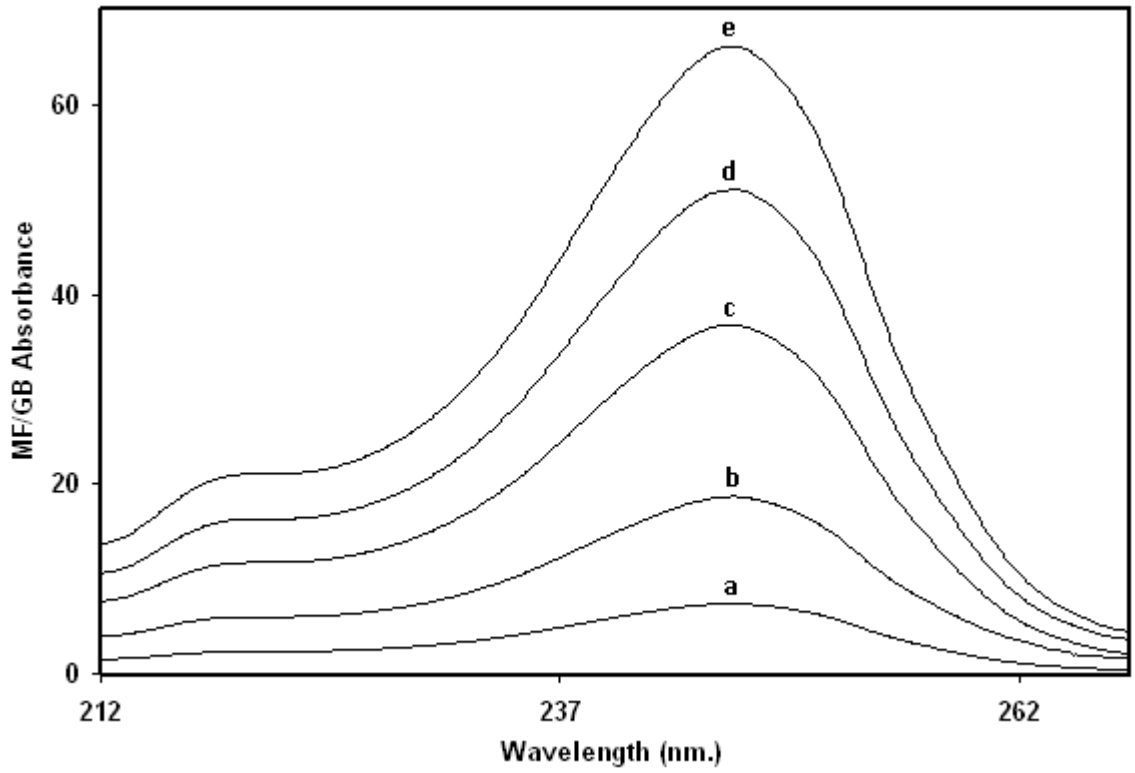

Fig. 3A. Ratio spectra of MF of (a) 2 µg/mL, (b) 5 µg/mL, (c) 10 µg/mL, (d) 14 µg/mL, and (e) 18 µg/mL; when 1 µg/mL GB is used as divisor.

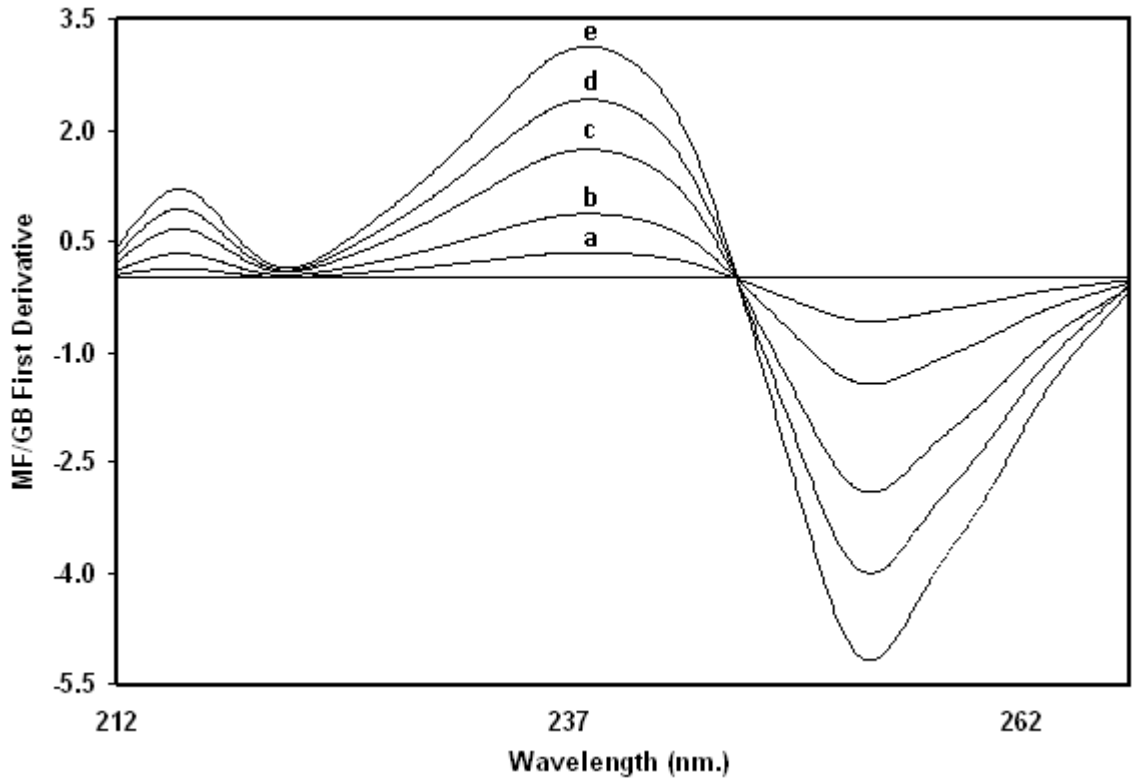

Fig. 3B. First derivative of the ratio spectra of MF of (a) 2 µg/mL, (b) 5 µg/mL, (c) 10 µg/mL, (d) 14 µg/mL, and (e) 18 µg/mL; when 1 µg/mL GB is used as divisor.

VALIDATION

Linearity and range:

The calibration graphs for the determination of GB and MF by the proposed methods were constructed by plotting the concentration versus the derivative amplitude. The graphs were found to be rectilinear over the concentration ranges cited in Table 1.

Statistical analysis [20] of the data gave high values of correlation coefficients (*r*) of the regression equations, small values of the standard deviations of residuals ($S_{y/x}$), of intercept (S_a), and of slope (S_b), and small values of percentage relative standard deviation and percentage relative error (Table 1).

These data proved the linearity of the calibration graphs and the conformity of the measurements of the proposed methods to Beer's law.

Accuracy and precision:

To prove the accuracy of the proposed methods, the results of the assay of GB and MF both in pure forms and in formulations were compared with those of the reference method [3]. Moreover, several synthetic mixtures of GB and MF in different ratios were also assayed.

Statistical analysis of the results obtained by the proposed and reference methods using Student's *t*-test and variance ratio *F*-test showed no significant difference between them regarding accuracy and precision (Tables 2-4). The results obtained for both compounds were precise, as indicated by the small values of the relative standard deviation.

Intraday and interday precisions were assessed using three concentrations and three replicates of each concentration. The relative standard deviations were found to be very small indicating reasonable repeatability and intermediate precision of the two proposed methods (Table 5).

Specificity:

The specificity of the methods was investigated by observing any interference encountered from common tablet excipients. It was shown that these compounds did not interfere with the proposed methods (Table 4).

Limit of detection (LOD) and limit of quantitation (LOQ):

LOD and LOQ were determined according to the United States Pharmacopoeia guidelines [21]. LOD was determined by establishing the minimum level at which the analyte can reliably be detected (signal-to-noise ratio is 3:1) while LOQ was determined by establishing the lowest concentration of analyte that can be determined with acceptable precision and accuracy (signal-to-noise ratio is 10:1) (Table 1).

Ruggedness:

To examine the ruggedness of the procedures, the intraday and interday precisions were evaluated as shown in Table 5. The precisions of the proposed methods were fairly high, as indicated by the low values of percentage relative standard deviation (% RSD) for both drugs.

Dosage Forms Analysis

The proposed methods were successfully applied to the assay of GB and MF in their tablets. The results obtained were in good agreement with those obtained with the reference method [3] (Table 4).

Table 1. Analytical performance data of the calibration graphs for the determination of glyburide and metformin HCl by the proposed methods

Parameters	Glyburide		Metformin HCl	
	First derivative method	Ratio derivative method	First derivative method	Ratio derivative method
Linearity range (µg/mL)	10-125	10-125	2-18	2-18
Intercept (*a*)	0.003	0.069	0.007	0.022
Slope (*b*)	0.004	0.481	0.035	0.172
Correlation coefficient (*r*)	0.9999	0.9999	0.9999	0.9999
S.D. of residuals ($S_{y/x}$)	2.102×10^{-3}	2.697×10^{-1}	2.771×10^{-3}	8.224×10^{-3}
S.D. of intercept (S_a)	1.612×10^{-3}	2.068×10^{-1}	2.201×10^{-3}	6.535×10^{-3}
S.D. of slope (S_b)	2.126×10^{-5}	2.729×10^{-3}	2.060×10^{-4}	6.116×10^{-4}
% RSD [a]	0.857	0.612	0.823	0.380
% Error [b]	0.350	0.250	0.336	0.155
LOD (µg/mL)[c]	2.100	1.800	0.250	0.150
LOQ (µg/mL)[d]	7.000	6.000	0.800	0.500
$A^{1\%}$ ($dL.g^{-1}.cm^{-1}$)[e]	40	4810	350	1720
ε ($L.mol^{-1}.cm^{-1}$)[f]	1976	237614	5796	28483

[a] Percentage relative standard deviation for six replicate samples.; [b] Percentage relative error for six replicate samples.; [c] Limit of detection.; [d] Limit of quantitation.; [e] Specific absorbance of the studied mode.
[f] Molar absorptivity of the studied mode.

Table 2. Assay results for the determination of glyburide and metformin HCl in pure forms

Analyte	% Recovery [a]		
	First derivative method	Ratio derivative method	Reference method [3]
Glyburide	100.000	99.670	99.026
	99.000	100.416	100.974
	100.500	99.276	99.675
	101.667	100.221	
	100.250	100.931	
	100.200	99.606	
Mean ± S.D.	100.270 ± 0.860	100.020 ± 0.612	99.892 ± 0.992
t	0.59 (2.36)[b]	0.24 (2.36)	
F	1.331 (5.786)	2.624 (19.30)	
Metformin HCl	98.550	99.700	100.670
	100.000	100.460	99.230
	99.517	99.900	100.253
	100.570	100.060	
	100.814	99.379	
	99.522	100.194	
Mean ± S.D.	99.829 ± 0.822	99.949 ± 0.380	100.081 ± 0.779
t	0.39 (2.36)	0.28 (2.36)	
F	1.23 (19.30)	3.798 (5.786)	

[a] *The average of three determinations.*

[b] *The figures between parentheses are the tabulated values of t and F at P = 0.05.*

Table 3. Assay results for the determination of glyburide and metformin HCl in synthetic mixtures

Analyte	% Recovery [a]		
	First derivative method	Ratio derivative method	Reference method [3]
Glyburide	99.701	99.925	98.906
	99.709	101.192	100.270
	100.445	99.905	99.584
	99.425	101.328	
	100.020	101.571	
	99.270	99.309	
Mean ± S.D.	99.762 ± 0.423	100.538 ± 0.939	99.587 ± 0.682
t	0.48 (2.36)[b]	1.54 (2.36)	
F	2.598 (5.786)	1.89 (19.30)	
Metformin HCl	100.362	100.162	100.765
	100.000	101.348	99.810
	99.708	100.233	100.275
	100.860	100.546	
	98.365	99.407	
	100.005	99.524	
Mean ± S.D.	99.883 ± 0.842	100.203 ± 0.711	100.283 ± 0.478
t	0.75 (2.36)	0.17 (2.36)	
F	3.11 (19.30)	2.22 (19.30)	

[a] *The average of three determinations.*

[b] *The figures between parentheses are the tabulated values of t and F at P = 0.05.*

Table 4. Assay results for the determination of glyburide and metformin HCl in commercial tablets

Sample	% Recovery [a]					
	Glyburide			Metformin HCl		
	First derivative method	Ratio derivative method	Reference method [3]	First derivative method	Ratio derivative method	Reference method [3]
Glucovance® 500/5 tablets[b]	101.145	101.215	100.971	101.654	101.763	100.992
	100.010	101.191	100.813	101.846	101.960	100.931
	99.855	100.074	99.486	100.560	101.537	101.657
Mean ± S.D.	100.337 ± 0.704	100.827 ± 0.652	100.423 ± 0.816	101.353 ± 0.694	101.753 ± 0.212	101.193 ± 0.403
t	0.139	0.669		0.345	2.132	
F	1.34	1.57		2.97	3.62	
Glucovance® 500/2.5 tablets[c]	99.145	99.814	100.915	100.000	98.125	101.016
	99.810	99.425	98.372	99.328	98.870	99.737
	99.985	100.451	99.013	98.862	99.555	98.401
Mean ± S.D.	99.647 ± 0.443	99.897 ± 0.518	99.433 ± 1.323	99.397 ± 0.572	98.850 ± 0.715	99.718 ± 1.308
t	0.265	0.565		0.390	1.009	
F	8.91	6.52		5.22	3.34	
Glimet® tablets[d]	100.000	100.402	101.435	101.145	98.658	99.050
	98.834	98.458	101.215	100.015	100.295	99.621
	101.446	100.730	102.400	100.600	98.667	101.659
Mean ± S.D.	100.093 ± 1.308	99.863 ± 1.228	101.683 ± 0.630	100.587 ± 0.565	99.207 ± 0.943	100.110 ± 1.372
t	1.896	2.284		0.557	0.940	
F	4.31	3.80		5.89	2.12	

[a] *The average of three determinations.*

[b] *Labeled to contain 500 mg MF and 5 mg GB; manufactured by Merck Santé, France, batch number 4038A.*

[c] *Labeled to contain 500 mg MF and 2.5 mg GB; manufactured by Merck Santé, France, batch number 4037A.*

[d] *Labeled to contain 400 mg MF and 2.5 mg GB; manufactured by Chemipharm Pharmaceuticals Industries, Egypt for Marcyrl Co., batch number 040042.*

N.B. Tabulated t-value at P = 0.05 is 2.78, tabulated F-value at P = 0.05 is 19.00

Table 5. Accuracy and precision data for the determination of glyburide and metformin HCl by the proposed methods

	Parameters	Glyburide Concentration (µg/mL)						Metformin HCl Concentration (µg/mL)					
		First derivative method			Ratio derivative method			First derivative method			Ratio derivative method		
		25	50	100	25	50	100	5	10	14	5	10	14
Intraday	% Recovery	99.856	99.572	99.857	100.960	99.844	99.267	100.540	99.380	100.750	99.300	100.640	99.621
		99.428	99.572	99.143	101.560	100.092	99.298	99.460	99.920	99.657	99.420	100.290	99.664
		99.285	99.858	99.286	101.004	100.100	99.456	100.080	99.190	99.657	100.640	100.990	99.836
	Mean	99.523	99.667	99.429	101.175	100.012	99.340	100.027	99.497	100.021	99.787	100.640	99.707
	± S.D.	0.297	0.165	0.378	0.334	0.146	0.101	0.542	0.379	0.631	0.741	0.350	0.114
	% RSD	0.299	0.166	0.380	0.331	0.146	0.102	0.542	0.381	0.631	0.743	0.348	0.114
	% Error	0.094	0.052	0.120	0.105	0.046	0.032	0.171	0.120	0.200	0.235	0.110	0.036
Interday	% Recovery	99.856	99.572	99.857	100.960	99.844	99.267	100.741	99.380	100.750	99.300	100.640	99.621
		100.000	99.078	100.286	101.324	99.126	100.224	101.082	99.920	99.657	99.420	100.180	99.603
		99.580	99.858	99.429	101.880	99.738	99.844	99.620	99.920	99.551	100.580	101.400	99.000
	Mean	99.812	99.503	99.857	101.388	99.569	99.778	100.481	99.740	99.986	99.767	100.740	99.408
	± S.D.	0.213	0.395	0.429	0.463	0.388	0.482	0.765	0.312	0.664	0.707	0.616	0.353
	% RSD	0.214	0.397	0.429	0.457	0.389	0.483	0.761	0.313	0.664	0.709	0.612	0.356
	% Error	0.068	0.125	0.136	0.145	0.123	0.153	0.241	0.099	0.210	0.224	0.193	0.112

N.B. Each result is the average of three separate determinations

CONCLUSION

The two developed methods can be used for the determination of GB and MF in their binary mixture in pharmaceutical formulations. The zero-crossing derivative spectrophotometry is more rapid and simple than ratio derivative spectrophotometry; however the ratio derivative spectrophotometry has greater sensitivity and accuracy. These proposed methods could be regarded as useful alternative to the reported chromatographic and electrophoretic techniques in the routine quality control of pharmaceutical formulations, allowing qualitative and quantitative information to be simultaneously and rapidly achieved with a relatively inexpensive instrumentation.

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