Journal of Applied Pharmaceutical Science
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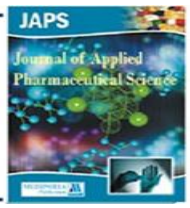

ISSN: 2231-3354
Received on: 06-05-2012
Revised on: 10-05-2012
Accepted on: 18-05-2012
DOI: 10.7324/JAPS.2012.2544

Simultaneous determination of pioglitazone and glimepiride in their pharmaceutical formulations

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ABSTRACT

Four sensitive and precise spectrophotometric methods were developed and validated for the simultaneous determination of pioglitazone hydrochloride (PGZ) and glimepiride (GLM) in their pharmaceutical formulations. Among the methods adopted were direct absorbance, first-derivative (^{1}D), second-derivative (^{2}D) and first-derivative of ratio spectra (^{1}DD). The selectivity of the proposed methods was checked using laboratory prepared mixtures. The proposed methods were successfully applied to the analysis of GLM and PGZ in their mixture and in pharmaceutical dosage forms without interference from other additives.

Keywords: Derivative–ratio; Derivative spectrophotometry; Glimepiride; Pioglitazone.

INTRODUCTION

Pioglitazone hydrochloride (PGZ) is [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2,4-] thiazolidine-dione monohydrochloride (Fig. 1). It is an oral anti-hyperglycemic agent that decreases insulin resistance. It is used in treatment of type-II diabetes mellitus (Hayashi *et al*., 2003). Glimepiride (GLM) is 1-[[*p*-[2-(3-ethyl-4-methyl-2-oxo-3-pyrrolinepyrroline-1-carboxamido) ethyl]-phenyl]-sulfonyl]-3-(*trans*-4-methylcyclohexyl) urea (Fig. 2). It is an oral anti-diabetic drug of sulfonylurea class. It is effective at low doses in patients with non-insulin-dependent diabetes mellitus (Tripathi, 1999). The treatment of non-insulin dependent type II diabetes usually starts with diet and exercise, then oral hypoglycemic drugs or insulin may be added (Muller, 1996 and Draeger, 1995). The literature survey reveals several analytical methods for quantitative estimation of PGZ and GLM in body fluids and in pharmaceutical formulations.

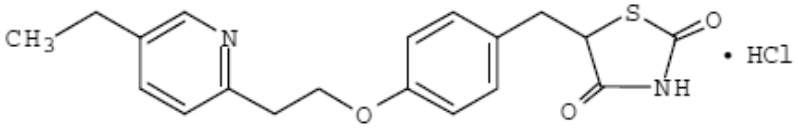

Fig. 1: Structural formula of pioglitazone hydrochloride (PGZ) M.W. (392.90)

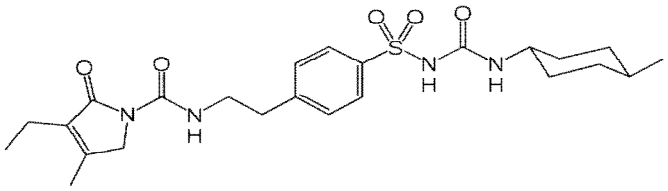

Fig. 2: Structural formula of glimepride (GLM)M.W. (490.617)

These methods include high-performance liquid chromatography (HPLC) for PGZ (Souri *et al.,* 2008 and Sripalakit*et al.,* 2006) for GLM (Khabbaz *et al.,* 2005and Song *et al.,* 2004) and for both in other combinations (Jain *et al.*,2008, Venkatesh *et al.*,2006, Kolte *et al.*,2004, Xue *et al.*,2003, Radhakrishna *et al.*,2002, Yamashita *et al.*,1996, Zhong ,1996 , Zhong, 1989, Kolte *et al.,* 2005, Pistos *et al.,* 2005, Aburuz *et al.,* 2005, Wanjari *et al.,* 2005, Lad *et al.,* 2003, Sahoo *et al.,* 2008, Reddy *et al.,* 2010, Sane *et al.,* 2004) in addition to UV spectrophotometry (Chaturvedi, 2008, Shankar *et al.,* 2005, Goyal , 2007, Chandna *et al.,* 2005, Hegazy *et al.,* 2009), thin layer chromatography (Bhushan *et al.*,2006, Menon *et al.,* 2004 and Gumieniczek *et al.,* 2003) & capillary electrophoresis (Jamali *et al.,* 2004). In modern analytical laboratory, there is always a need for simple, rapid and accurate methods for simultaneous determination of drug combinations that could be used for routine analysis. The present work aimed to develop simple instrumental methods for the quantification of GLM and PGZ in bulk form or in their pharmaceutical formulations.

EXPERIMENTAL

Instruments

A double beam UV-visible spectrophotometer (Shimadzu, Japan) model UV-1601 PC, with 1 cm quartz cells, connected to an IBM-compatible computer was used. The software was UV-PC personal spectroscopy software version 3.7. The spectral band width was 2 nm with wavelength-scanning speed of 2800 nm min^{1}.

Materials and reagents

Reference GLM and PGZ standards pure samples were kindly supplied by Takeda pharmaceuticals America, Inc. The purity of GLM was found to be 99.80% according to the official method (USP, 2011) while that of PGZ was found to be 100.47% according to the reference method (Hegazy *et al.,* 2009). Methanol was spectroscopic grade. Pharmaceutical dosage form (Duetact® 2mg and 4mg) tablets were kindly supplied by Takeda pharmaceuticals America, Inc. All calculations and samples preparation for reference material and pharmaceutical formulation were done regarding the salt forms.

Standard solutions

Stock standard solutions of PGZ and GLM (0.2 mg mL^{-1}) in methanol were prepared for the proposed spectrophotometric methods. All solutions were freshly prepared on the day of analysis.

Procedures

Direct spectrophotometric method

Spectral characteristics of PGZ and GLM

Two aliquots (0.2 mL) of GLM and (3 mL) of PGZ were separately transferred to a series of 10 mL volumetric flasks. Each flask was completed to volume with methanol to obtain final concentration of 4 µg mL^{-1} of GLM and 60 µg mL^{-1} of PGZ the spectrum of each solution was scanned and recorded separately.

Linearity

Portions equivalent to (0.5-4.5 mL) of PGZ standard stock solution each (0.2 mg mL^{-1}) were separately transferred to a series of 10 mL volumetric flasks. Each flask was completed to the volume with methanol to reach the concentration range of 10-90 µg mL^{-1}. The absorbance of the peaks of PGZ was measured at 268nm. Calibration graph was constructed by plotting the absorbance versus concentrations. The regression equation was then computed for PGZ at the specified wavelength and used for its determination of unknown samples.

First-derivative (^1D) method

Linearity

Standard serial concentrations in the range of 10-90 µg mL^{-1} of PGZ were prepared as under section 2.4.1.2. The amplitudes of the first derivative peaks of PGZ were measured at 279.4 nm with $\Delta\lambda$= 4 nm and scaling factor = 10. Calibration graph was constructed by plotting peak amplitude versus concentrations. The regression equation was then computed for PGZ at the specified wavelength and used for determination of its unknown samples.

Second-derivative (^2D) method.

Linearity

Standard serial concentrations in the range of 10-90 µg mL^{-1} of PGZ were prepared as described under section 2.4.1.2. The amplitudes of the second-derivative peaks of PGZ were measured at 242.3 nm, 274 nm and 287 nm with $\Delta\lambda$ = 8 nm and scaling factor =100.

Calibration graphs were constructed by plotting the peak amplitudes versus concentrations. The regression equations were then computed for PGZ at the specified wavelengths and used for determination of unknown samples of it.

First-derivative of ratio spectra (^1DD) method.

Linearity

Standard serial concentrations in the range of 2-18 µg mL^{-1} for GLM were prepared as under section 2.4.1.2. and accurately 3 mL of PGZ standard solution (0.2 mg mL^{-1}) was transferred to a 10-mL volumetric flask and volume completed with methanol to get final concentration of 60 µg mL^{-1} of PGZ to be used as a divisor.

The spectra of the prepared standard solutions were scanned (200-400 nm) and stored into the PC. The stored spectra of GLM were divided (the amplitude of each wavelength) by the spectrum of 60 µg mL^{-1} of PGZ. The first-derivative of the ratio spectra (^{1}DD) with $\Delta\lambda$ =4 nm and scaling factor of 10 was obtained. The amplitude of the first-derivative peaks of GLM were measured at 237.2 nm and 248 nm. Calibration graphs were constructed relating the peak amplitudes of (^{1}DD) to the corresponding concentrations. The regression equations were then computed for GLM at the two specified wavelengths and used for determination of unknown samples of it.

Analysis of laboratory prepared mixtures

Laboratory prepared mixtures containing different ratios of GLM and PGZ were analyzed using the suggested methods, aliquots of GLM and PGZ were mixed to prepare different mixtures and the procedures were followed as mentioned under each method, the concentrations from the corresponding regression equations were calculated.

Assay of pharmaceutical formulations (Duetact® 2 mg, 4 mg tablets)

Twenty tablets were weighed from each dosage form and the average weight was calculated, tablets were crushed to furnish a homogenous powder and certain amount of powdered tablets were dissolved by the aid of an ultrasonic bath for 2 hours and filtered. The solutions were diluted to the same concentration of the appropriate working solutions then the procedures were followed as described under each method.

RESULTS AND DISCUSSION

Direct spectrophotometric method

PGZ can be determined directly at 268 nm without any interference from GLM (zero absorbance) till concentration 10 µg mL^{-1} of GLM (Fig. 3). A linear relationship was obtained in the range of 10-90 µg mL^{-1} for PGZ. The corresponding regression equation was computed and found to be:

$A = 0.019\ C - 0.061$ (r=0.9994), at 268 nm

Where, A is the absorbance of PGZ at 268 nm, C is the concentration of PGZ (µg mL^{-1}) and r is the correlation coefficient. The precision of the proposed method was confirmed and the mean percentage recoveries were found to be 101.52 at 268 nm.

First-derivative method (^{1}D) method

Derivative spectrophotometry is a powerful tool in quantification of mixtures of drugs. A simple, rapid and selective spectrophotometric technique was proposed and applied for the determination of PGZ, either in raw material or in pharmaceutical formulations containing GLM .This was done by applying the first-derivative (^{1}D) ultraviolet spectrophotometry. The method could solve the problem of spectral bands overlapping between PGZ and GLM without sample pretreatment or separation steps of the two analyzed drugs. The absorption spectra of PGZ and GLM showed overlapping, little interference and error probability affected the use of direct spectrophotometry for determination of PGZ in the presence of GLM, when the first derivative spectra (Fig. 4) were examined, it was found that PGZ can be determined at 279.4 nm, where GLM has no contributions. This allows accurate determination of PGZ in presence of GLM till the concentration of 12 µg mL^{-1} of GLM but at higher levels interference increases.

A linear relationship was obtained in the range of 10-90 µg mL^{-1} for PGZ. The corresponding regression equation was computed and found to be:

$^1D = 0.013\ C - 0.041$ (r=0.9994), at 279.4 nm

Where ^{1} D is the peak amplitude of the first-derivative curve ($\Delta A/\Delta\lambda$) at 279.4 nm, C is the concentration of PGZ (µg mL^{-1}) and r is the correlation coefficient. The precision of the proposed method was confirmed by the analysis of different samples in triplicates. The mean percentage recoveries were found to be 99.91at 279.4 nm.

Second-derivative (^{2}D) method

The second -derivative (^{2}D) ultraviolet spectrophotometry was applied for the determination of PGZ, either in raw material or in pharmaceutical formulations.

The absorption spectra of PGZ and GLM showed overlapping , little interference and error probability affect the use of direct spectrophotometry and first-derivative method (^{1}D)for determination of PGZ in the presence of GLM ,especially at higher levels of GLM .When the second derivative spectra (Fig. 5) were examined, it was found that PGZ could be determined at 242.3nm, 274nm and 287nm, where GLM has no contribution (zero crossing) at 242.3nm, the clear zero crossing of GLM allowed accurate determination of PGZ in presence of any level of GLM. A linear relationship was obtained in the range of 10-90 µg mL^{-1} for PGZ. The corresponding regression equations were computed and found to be:

$^2D = 0.0179\ C + 0.1289$ (r=0.9985), at 242.3 nm

$^2D = 0.0148\ C - 0.0176$ (r=0.9995), at 274 nm

$^2D = 0.0135\ C - 0.1208$ (r=0.9994), at 287 nm

Where ^{2}D is the peak amplitude of the second-derivative curve ($\Delta A/\Delta\lambda$) at the corresponding wavelengths, C is the concentration of PGZ (µg mL^{-1}) and r is the correlation coefficient.

The mean percentage recoveries were found to be 101.4 at 242.3 nm, 99.98 at 274nm and 100.24 at 287nm.

Derivative ratio spectrophotometric method

Derivative ratio spectrophotometric method was used to determine GLM in presence of PGZ. The zero-order of the derivative ratio spectra of GLM and the first-order of the derivative ratio spectra were presented in figure 6 & figure 7, respectively. The concentration of the devisor was studied, it was found that upon dividing by 60 µg mL^{-1} of PGZ product led to the best results in terms of sensitivity, repeatability and signal to noise ratio. Linear calibration graphs were obtained for GLM in concentration range of 2-18 µg mL^{-1} by recording the peak amplitude at 237.2 and 248.4 nm using 60µg mL^{-1} of PGZ as a devisor. The regression equations were computed and found to be:

(^{1}DD) = 0.0296 C + 0.002 (r=0.9995), at 237.2 nm
(^{1}DD) = 0.0436 C + 0.0093 (r=0.9998), at 248.4 nm

Where ^{1}DD is the peak amplitude of the first-derivative curve for (GLM/PGZ), C is the concentration of GLM (µg mL^{-1}) and r is the correlation coefficient. The precision of the proposed method was checked by the analysis of different concentrations of authentic samples in triplicates. The mean percentage recoveries were found to be 100.176 at 237.2 nm and 100.55 at 248.4 nm.

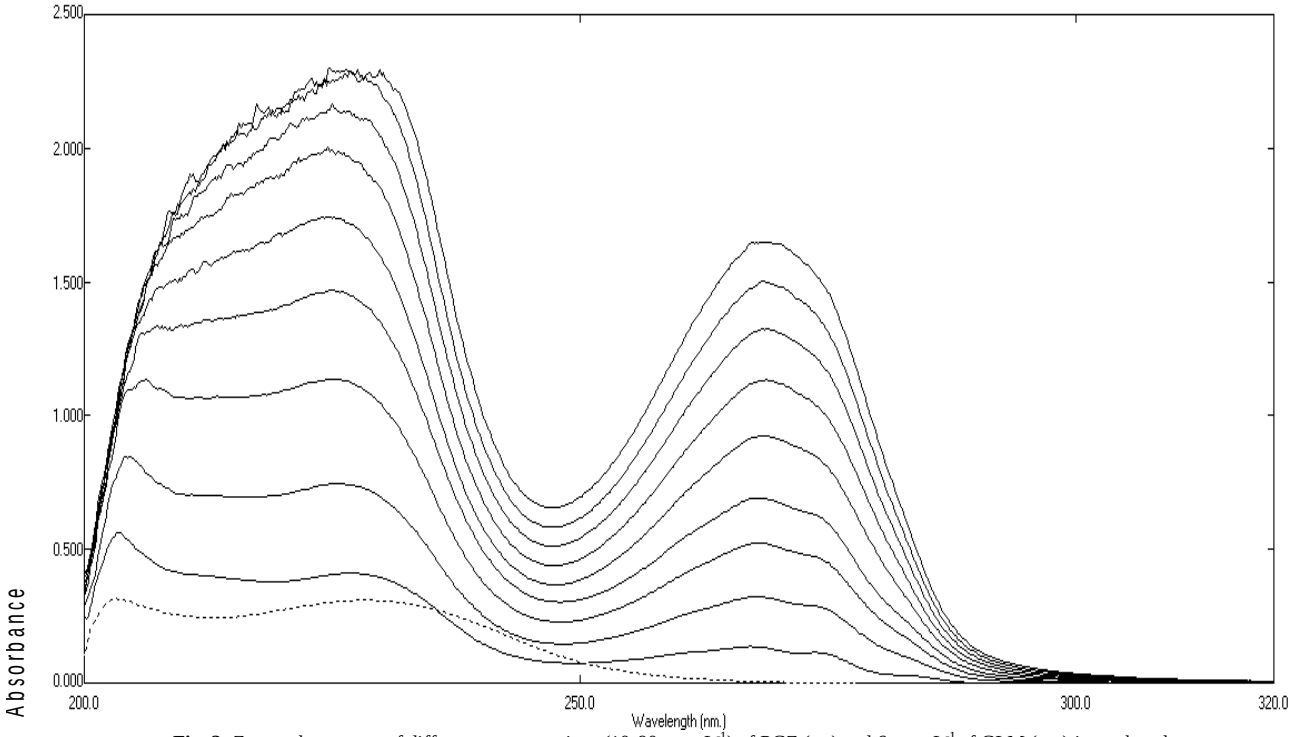

Fig. 3: Zero-order spectra of different concentrations (10-90 µg mL^{-1}) of PGZ (–) and 6 µg mL^{-1} of GLM (….) in methanol.

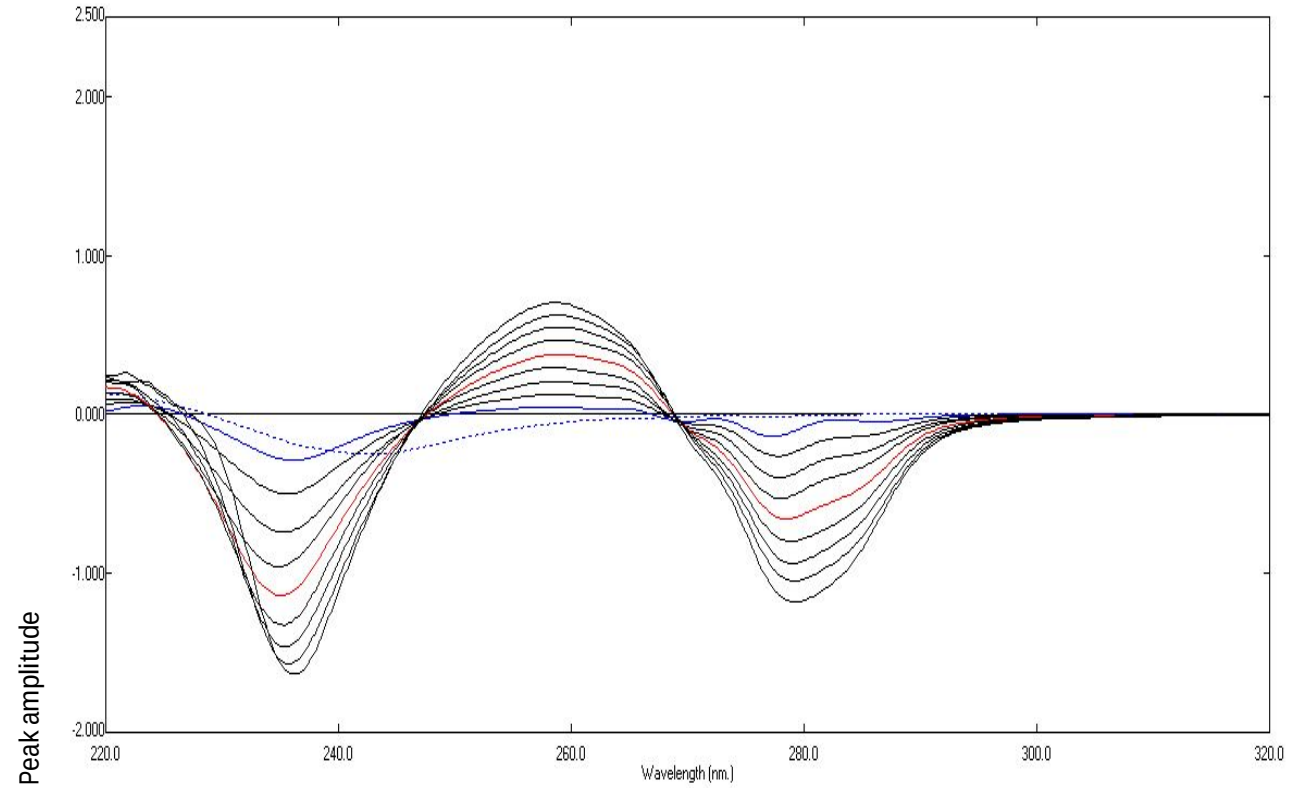

Fig. 4: First-derivative spectra for different concentrations (10-90 µg mL^{-1}) of PGZ(–)and 10 µg mL^{-1} of GLM (….) in methanol.

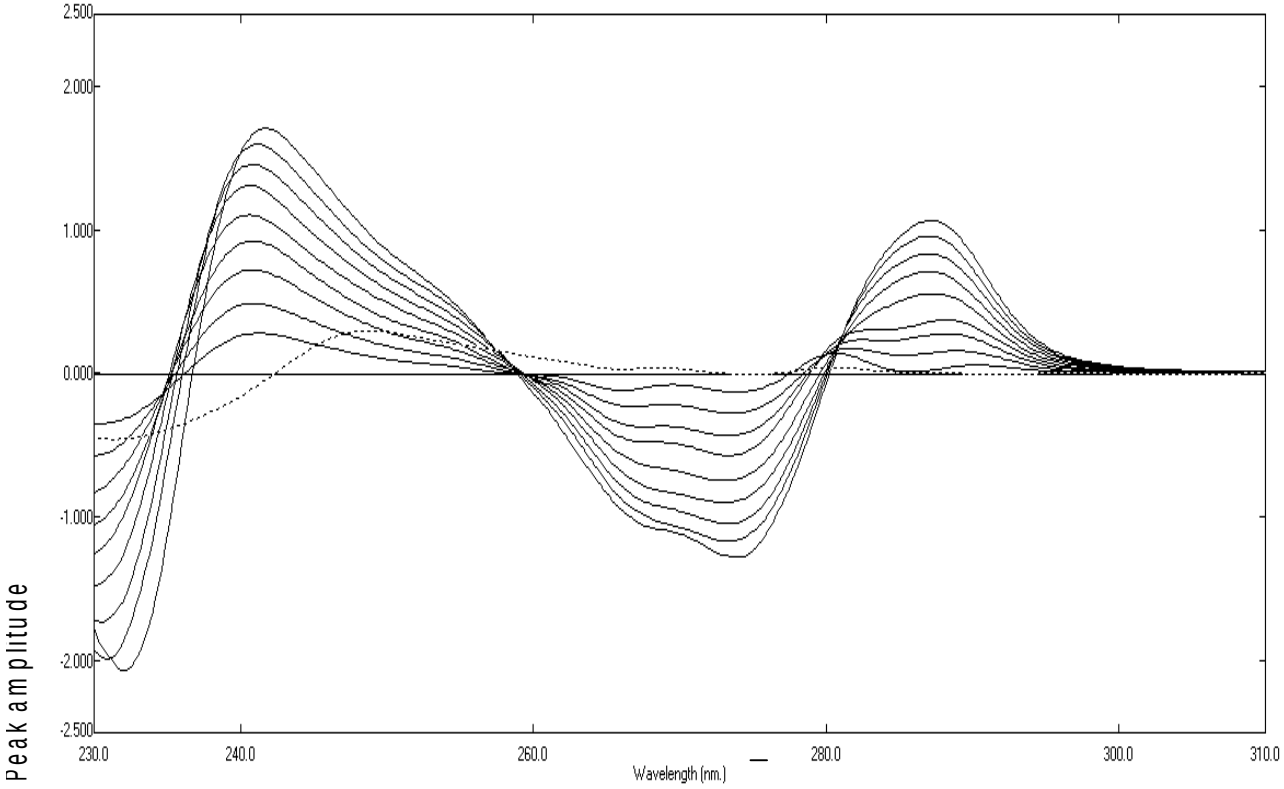

Fig. 5: Second derivative spectra for different concentrations of PGZ (10-90 µg mL^{-1}) ( )and 18µg mL^{-1} (…..) of GLM in methanol.

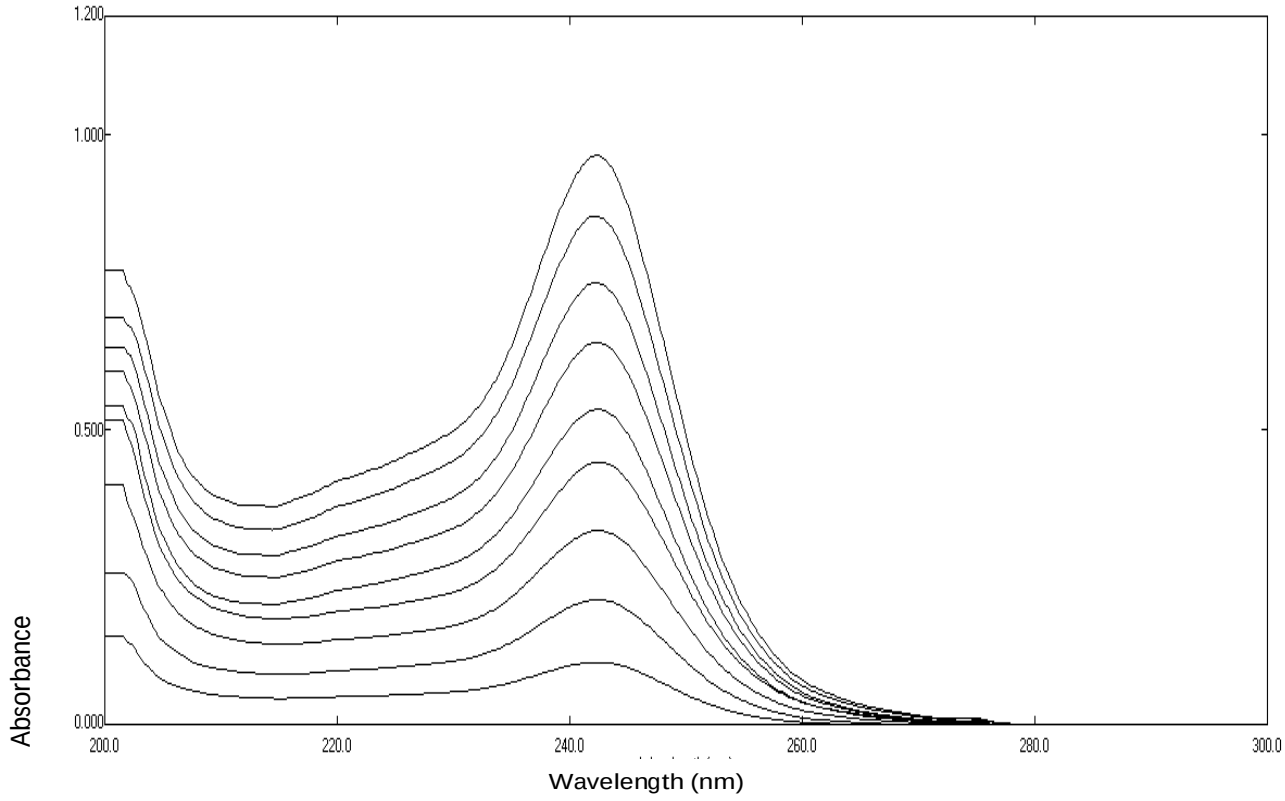

Fig. 6: The ratio spectra of GLM (2-18µg mL^{-1}) using 60 µg mL^{-1} of PGZ as divisor.

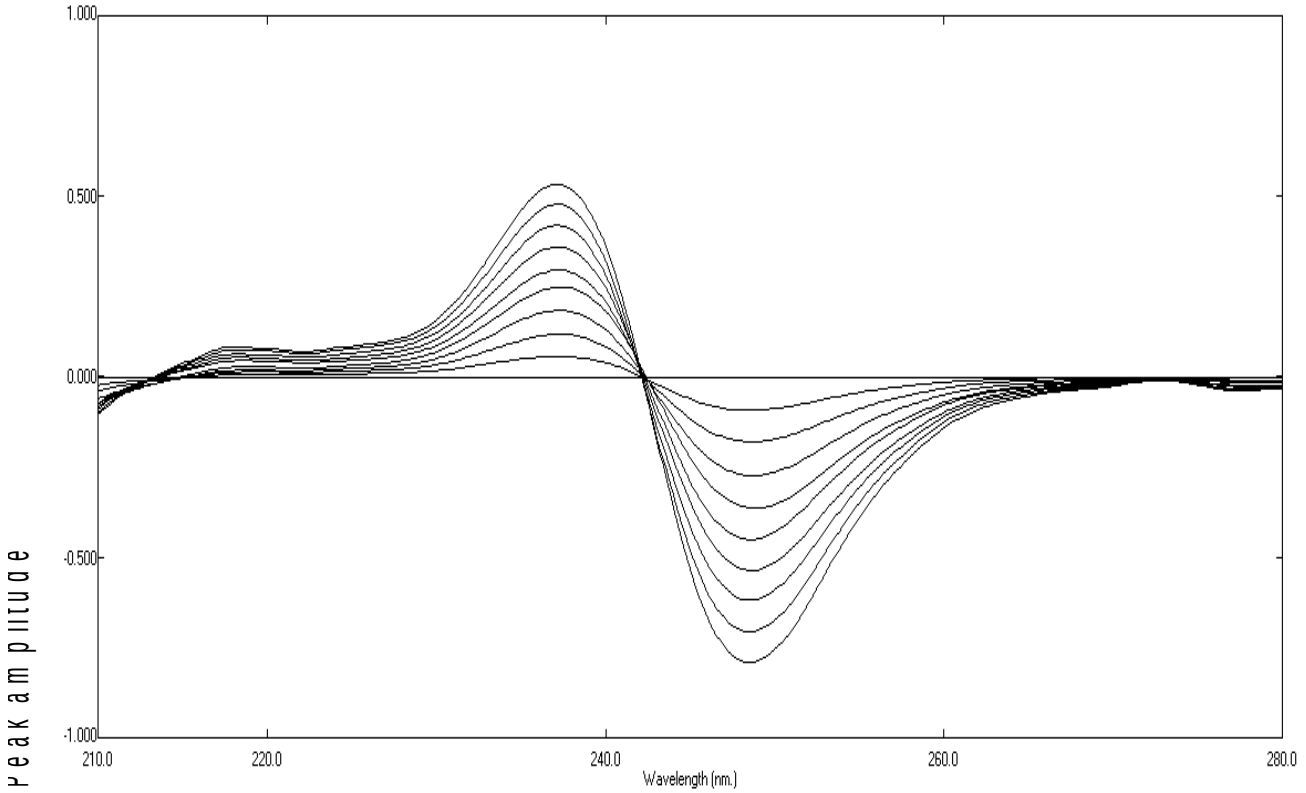

Fig. 7: First order of the ratio spectra of GLM (2-18 µg mL^{-1}) using 60 µg mL^{-1} of PGZ as a divisor.

Table. 1: Determination of PGZ and GLM in laboratory prepared mixtures by the proposed methods.

Drug determined	Direct spectrophotometric method at 268 nm	[1]D-method at 279.4 nm	[2]D-method			[1]DD-method	
			at 242.3 nm	at 274 nm	at 287 nm	at 237.2 nm	at 248.4 nm
PGZ	102.12 ± 0.36	100.78 ± 1.05	101.62 ± 0.87	101.03 ± 1.05	99.68 ± 1.57	---------	---------
GLM	---------	---------	---------	---------	---------	101.39 ± 1.37	100.18 ± 1.13

Table. 2: Determination of PGZ and GLM in Duetact® tablets by the proposed methods.

Preparation	Zero-order method at 268nm	[1]D-method at 279.4nm	[1]DD-metod		[2]D-method		
			at 237.2nm	at 248.4nm	at 242.3nm	at 274nm	at 287nm
Duetact® tablets (2, 30 mg) Batch No: A16139							
PGZ	101.34 ± 0.78	101.3 ± 0.94	……..	…….	100.37 ± 1.57	100.23 ± 1.67	99.48 ± 0.45
GLM	----------		100.4±0.66	100.62±0.79	---------	---------	---------
Duetact® tablets (4, 30 mg) Batch No: A16112							
PGZ	99.42 ± 1.57	100.51 ± 0.64	---------	---------	99.95 ± 1.94	100.45 ± 1.37	99.84 ± 1.12
GLM	----------		100.33 ± 1.72	101.13 ± 0.53	---------	---------	---------

Table. 3: Assay parameters and validation sheet for determination of PGZ and GLM.

Parameter	Zero-order method	[1]D-order method	[2]D-method			[1]DD-order method	
	PGZ	PGZ	PGZ			GLM	
	at 268	at 279.4nm	at 242.3nm	at274nm	at 287nm	a237.2nmt	at248.4nm
Range			10-90 µg mL^{-1}			2-18µg mL^{-1}	
Slope	0.019	0.0137	0.0179	0.0148	0.0135	0.0296	0.0436
Intercept	-0.061	-0.0413	0.1289	-0.0176	-0.1208	0.0026	0.0093
Mean	101.52	99.91	101.40	99.98	100.24	100.17	100.55
S.D.	1.16	1.37	0.85	1.56	1.43	0.89	0.68
Variance	1.35	1.88	0.71	2.46	2.04	0.73	0.46
Coefficient of Variation %	1.15	1.37	0.83	1.56	1.42	0.88	0.67
Correlation coefficient (r)	0.9999	0.9994	0.9985	0.9995	0.9994	0.9995	0.9998
R.S.D.(%) [a]	0.995	0.460	0.527	0.501	0.512	0.642	0.631
R.S.D.(%) [b]	0.923	0.535	0.412	0.485	0.535	0.612	0.610

[a] the interday (n=6) relative standard deviations of (60µg mL^{-1})of PGZ by the proposed methods
[b] the intraday (n=6)relative standard deviations of (60µg mL^{-1}) of PGZ by the proposed methods

Table. 4: Statistical comparison for the results obtained by the proposed methods and the official method for analysis of GLM and reference method for analysis of PGZ.

Parameters	Direct spectrophotometric method	^{1}D-method		^{2}D-method		^{1}DD-method		Official method for GLM (USP, 2011) [34]	Reference method for PGZ (Hegazy etal., 2009)
	PGZ					GLM			
	At 268nm	At 279.4nm	At 274nm	At 242.3nm	At 287nm	At 237.2nm	At 248.4nm		
Mean	101.52	99.91	99.98	101.40	100.24	100.18	100.55	99.80	100.47
S.D.	1.16	1.37	1.57	0.84	1.43	0.89	0.67	1.39	1.34
Variance	1.35	1.88	2.46	0.71	2.04	0.73	0.46	1.93	1.80
N	5	5	5	5	5	6	6	6	6
F-test	1.33 (6.26)[a]	1.04 (5.19)[a]	1.37 (5.19)[a]	2.54 (6.26)[a]	1.13 (5.19)[a]	2.64 (5.05)[a]	4.19 (5.05)[a]		
Student's t-test	1.393 (2.262)[a]	0.682 (2.262)[a]	0.552 (2.262)[a]	1.401 (2.262)[a]	0.274 (2.262)[a]	0.564 (2.228)[a]	1.190 (2.228)[a]		

[a]The values in the parenthesis are corresponding theoretical t- and F-values at P = 0.05 (Spiegel,1999).

Statistical analysis

The suggested methods were successfully applied for the determination of PGZ and GLM in their laboratory prepared mixtures with good precision as shown in table 1. The proposed methods were also used for estimating the concentration of both drugs in their pharmaceutical formulations. The results are shown in table 2. Assay parameters and a validation sheet for determination of the studied drugs are shown in table 3. Statistical comparison for the results obtained by the proposed methods and the reference ones for the studied drugs are shown in table 4. The calculated t- and F-values were found to be less than the tabulated ones (Spiegel, 1999) [35], confirming good accuracy and excellent precision.

CONCLUSION

Unlike the mostly recommended HPLC-procedure, the proposed spectrophotometric methods are simple and not expensive. The reagents used in the proposed methods are cheap and available. The procedures applied in each method do not involve any critical reactions or tedious sample preparations. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility of assaying the studied drugs in their mixtures and in their pharmaceutical formulation without interference from the excipients. The suggested methods are found to be accurate and selective with no significant difference of the precision compared with the reference methods of analysis. The proposed methods could be applied successfully, for routine analysis of PGZ and GLM singly, in their mixtures or in their pharmaceutical formulations.

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