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Simultaneous determination of pioglitazone and glimepiride in their pharmaceutical formulations

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ABSTRACT

Two sensitive and precise methods were developed and validated for the simultaneous determination of pioglitazone hydrochloride and glimepiride as the bulk drugs and in their pharmaceutical formulations. Among the techniques adopted were chromatography [coupled TLC-densitometry and HPLC].Method I : Densitometric separation of the drugs was performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of chloroform: toluene: glacial acetic acid: ethanol [4.5:4.5:1:1, v/v/v/v]. Densitometric evaluation of the separated zones was performed at 228 nm and 268 nm. The two drugs were satisfactorily resolved with RF values 0.4and 0.65 for pioglitazone hydrochloride and glimepiride, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity 3-15µg/spot for pioglitazone hydrochloride and 0.1-3 µg/spot for glimepiride, precision (intra-day RSD 1.178% and inter-day RSD 1.152 % for pioglitazone hydrochloride, and intra-day RSD 1.101 % and inter-day RSD 0.999 % for glimepiride), accuracy (99.94 ± 1.30 % for pioglitazone hydrochloride and 100.74 ±1.58 % for glimepiride) and specificity, in accordance with ICH guidelines. Method II: chromatographic separation using a 250 mm x 4.6 mm, i.d. C_{18}Lichrosorb™ 10µm analytical column. The mobile phase consisted of phosphate buffer [pH: 4]: methanol: acetonitrile: triethylamine [40:20:40:0.1, v/v/v/v] The average retention times under the conditions described were 4 minutes for pioglitazone hydrochloride and 7.5 minutes for Glimepiride, accuracy and reliability of the method was assessed by evaluation of linearity 5-175 µg/mL for pioglitazone hydrochloride and 5-30µg/mL for Glimepiride, precision (intra-day RSD 0.295% and inter-day RSD 0.215 % for pioglitazone hydrochloride, and intra-day RSD 0.345 % and inter-day RSD 0.231 % for glimepiride), accuracy (99.80 ± 1.16 % for pioglitazone hydrochloride and 99.47 ±2.07 % for glimepiride) and specificity, in accordance with ICH guidelines.

Keywords: Glimepiride; High-performance liquid chromatography; Pioglitazone; Thin layer Chromatography.

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 [1].

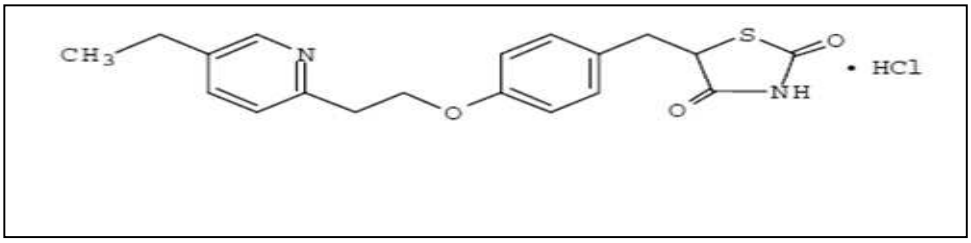

Fig.1. Structural formula of pioglitazone hydrochloride [PGZ]
M.W. [392.90]

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 [2]. The treatment of non-insulin dependent type II diabetes usually starts with diet and exercise, then oral hypoglycemic drugs or insulin may be added [3, 4].

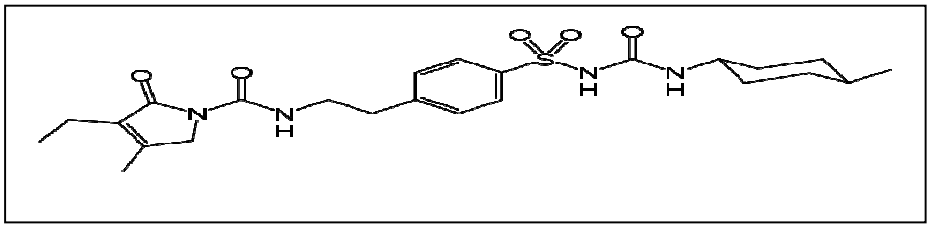

Fig.2. Structural formula of glimepride [GLM]
M.W. [490.617]

The literature survey reveals several analytical methods for quantitative estimation of PGZ and GLM in body fluids and in pharmaceutical formulations. These methods include high-performance liquid chromatography [HPLC] for PGZ [5, 6], for GLM [7, 8] and for both in other combinations [9-24] in addition to thin layer chromatography [25-27] & capillary electrophoresis [28].

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. These methods include chromatographic methods; *namely*, TLC densitometry and HPLC.

MATERIALS AND METHODS

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}.

TLC-plates [20 cm x10 cm, 0.25mm] coated with silica gel 60 F_{254} [Merck, Germany] were used.

Camag TLC scanner 3 S/N 130319 with WinCATS software and Camag Linomat 5 auto sampler [Muttenz, Switzerland] with Camag micro syringe [100 µL] were used.

A liquid chromatography consisted of an isocratic pump [Agilent Model G1310A], an ultraviolet variable wavelength detector [Model G1314A, Agilent 1100 series], Rheodyne injector [Model 7725I, Rohnert Park, CA, USA] equipped with 20 µL injector loop, Agilent [USA]. Stationary phase; a 250 mm x 4.6 mm i.d. C_{18} Lichrosorb^{TM} 10 µm analytical column, Alltech [USA] was used. The samples were injected by the aid of a 25 µL Hamilton® analytical syringe.

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 [30], while that of PGZ was found to be 100.47% according to the reference method [29]. Acetonitrile, methanol, potassium dihydrogen orthophosphate and triethylamine were HPLC grade and were supplied by Sigma Aldrich. Glacial acetic acid, ethanol, chloroform, toluene, and ethyl alcohol were spectrophotometric 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 [1 mg mL^{-1}] in methanol were prepared for TLC-densitometric method. For HPLC method, PGZ standard solution [1mg mL^{-1}] and GLM standard solution [0.1mg mL^{-1}] in the mobile phase were prepared. All solutions were freshly prepared on the day of analysis.

Procedures

Chromatographic methods.

TLC-densitometric method.

Aliquots of 0.1-3 µg $spot^{-1}$ of GLM standard solution [1mg mL^{-1}] and of 3-15 µg $spot^{-1}$ of PGZ standard solution [1mg mL^{-1}] were applied in the form of bands on TLC plate. The band length was 4 mm and dosage speed was 150 nL S^{-1}, the bands were applied 12.8 mm apart from each other and 15 mm from the bottom edge of the plate. Linear ascending development was performed in a chromatographic tank previously saturated with chloroform: toluene: glacial acetic

acid: ethanol [4.5:4.5:1:1, v/v/v/v] for 30 minutes at room temperature. The developed plates were air-dried and scanned at 228 nm and 268 nm using deuterium lamp, absorbance mode at 3 mm x 0.45 mm slit dimension and scanning speed of 20 mm S^{-1}.

Calibration curves relating the optical density of each spot to the corresponding concentration of GLM and PGZ were constructed .The regression equations were then computed for the studied drugs and used for determination of unknown samples.

High performance liquid chromatographic method
Linearity
Portions of [0.05-1.75 mL] of PGZ standard solution [1mg mL^{-1}] and [0.5-3 mL] of GLM standard solution [0.1mg mL^{-1}] were transferred to a series of a10mL volumetric flasks. The content of each flask was completed with the mobile phase to volume to get a final concentration of [5-175 µg mL^{-1}] of PGZ and [5-30 µg mL^{-1}] of GLM.

The samples were then chromatographed using the following chromatographic conditions: stationary phase: a 250 mm x 4.6 mm i.d. C_{18} Lichrosorb™ 10µm analytical column , Alltech [USA], mobile phase ; phosphate buffer: methanol: acetonitrile: triethylamine [40:20:40:0.1, v/v/v/v], the final pH-value was adjusted to 4.0 ± 0.2 with *O*-phosphoric acid using a pH-meter. The mobile phase was filtered through 0.45 µm Millipore membrane filter and was degassed for about 30 minutes in an ultrasonic bath prior to use, flow rate 1mL $min.^{-1}$ [isocratically at ambient temperature [~25 ◦C]] with UV detection at 228 nm. Calibration curves relating the peak area ratios of PGZ and GLM to that of standard [75 µg mL^{-1}] and [20 µg mL^{-1}] respectively versus the corresponding concentrations of PGZ and GLM [µg mL^{-1}]. The regression equations were computed and calculations were performed following the external standard technique, concentrations of unknown samples of GLM and PGZ were determined using the obtained regression equations.

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 were proceeded 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 and proceeded as described under each method.

RESULTS AND DISCUSSION

Chromatographic methods
TLC-densitometry
A TLC-densitometric method could be used for the simultaneous determination of PGZ and GLM without prior separation. Different solvent systems were tried for the separation of PGZ

and GLM. Satisfactory results were obtained by using a mobile phase composed of chloroform: toluene: ethanol: glacial acetic acid [4.5:4.5:1:1, v/v/v/v] where R_f = 0.4 and 0.65 for PGZ and GLM, respectively. The separation allowed the determination of PGZ and GLM with no interference [Fig. 3]. The linearity was confirmed by plotting the measured peak area versus the corresponding concentrations at 228 nm over a range of 0.1-3 µg spot^{-1} for GLM and at 268 over a range of 3-15µg spot^{-1} for PGZ, where a linear response was obtained, regression equations were found to be:

A = 0.139 C + 0.803 [r=0.9989] for PGZ
A= 0.2208 C + 0.5162 [r=0.9986] for GLM

Where A is the integrated area under the peak x10^{-4} for PGZ and GLM, C is the concentration of PGZ and GLM in µg spot^{-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 recovery was found to be 99.94 for PGZ and 100.74 for GLM.

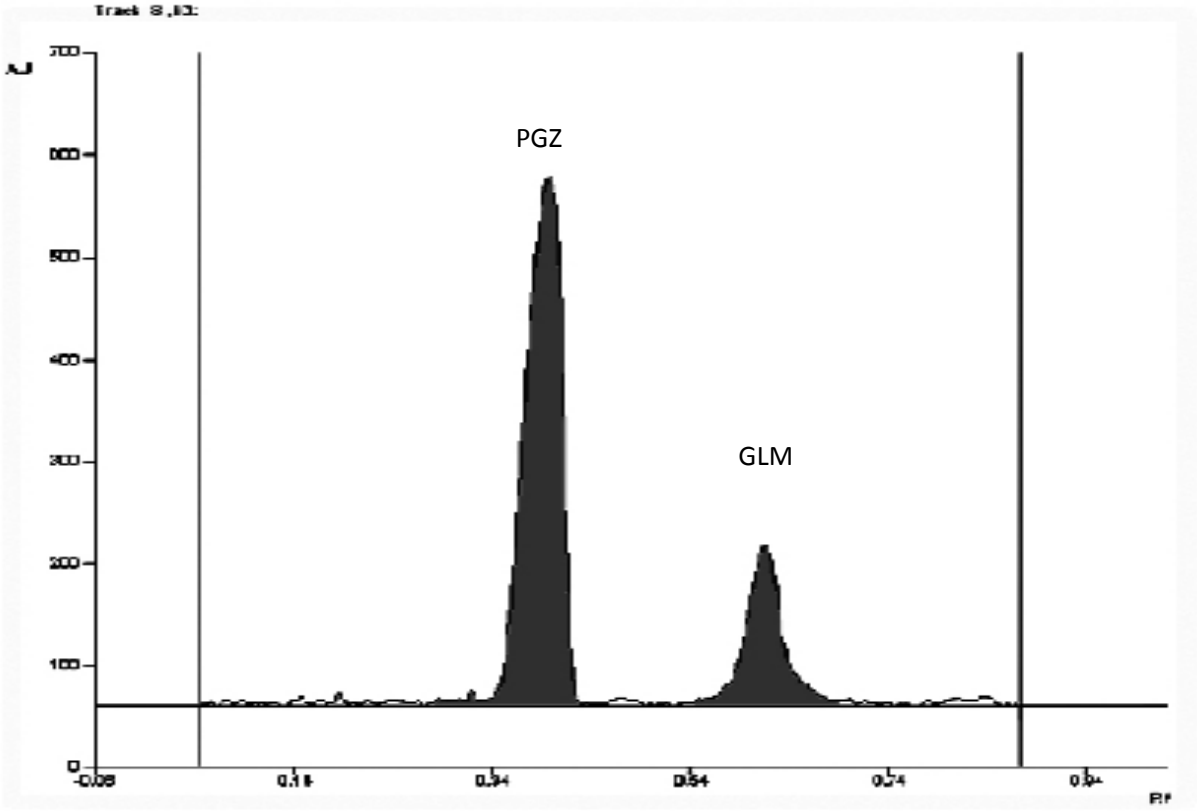

Fig.3. TLC-densitometric resolution of PGZ and GLM

High performance liquid chromatography method

A simple isocratic high-performance liquid chromatography method was developed for the determination of PGZ and GLM in pure form and in pharmaceutical formulations using a 250 mm x 4.6 mm, i.d. C_{18}Lichrosorb™ 10µm analytical column. The mobile phase consisted of phosphate buffer [pH: 4]: methanol: acetonitrile: triethylamine [40:20:40:0.1, v/v/v/v] and the final

pH was adjusted to 4.0 ± 0.2 using *O*-phosphoric acid. The mobile phase was chosen after several trials to reach the optimum stationary /mobile –phase matching. System suitability parameters were tested by calculating the capacity factor, tailing factor, the sensitivity factor and resolution. The average retention times under the conditions described were 4 minutes for PGZ and 7.5 minutes for GLM. One sample could be chromatographed in 10 minutes. The chromatographic system in this work allowed complete baseline separation of PGZ from GLM [Fig. 4]. Calibration graphs were obtained by plotting the peak area ratios of drug to that of external standard versus concentrations of PGZ and GLM, Linearity ranges were found to be 5-175 µg mL^{-1} for PGZ and 5-30µg mL^{-1} for GLM using the following regression equations:

A = 0.0129 C + 0.0318 [r=0.9994] for PGZ
A = 0.0513 C - 0.0063 [r=0.9979] for GLM

Where A is the peak area ratio, C is the concentration of PGZ and GLM [µg mL^{-1}] and r is the correlation coefficient. The mean percentage recoveries were found to be for 101.42% and101.4% for PGZ and 100.75% and 100.92% for GLM for the dosage form 2, 30 mg and 4, 30 mg respectively.

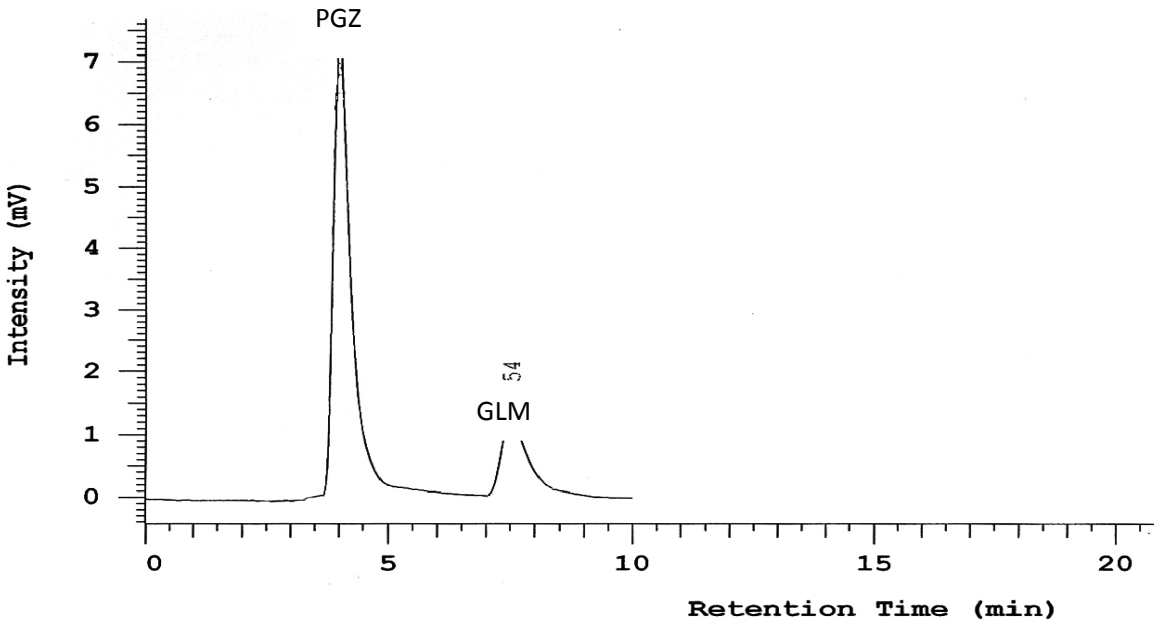

Fig.4 Liquid chromatographic separation of PGZ (4 min.) from GLM (7.5 min.).

The robustness of the HPLC method was investigated by analysis of samples under a variety of experimental conditions such as small changes in the PH [4-4.5], small changes in phosphate buffer / methanol / acetonitrile ratio from [40/20/40 to 30/25/45] in the mobile phase and changing the analytical column using a 250mm x 4.6mm i.d.C_{18} Zorbax™ 10µm analytical column, Agilent [USA]. The effect on retention time and peak parameters was studied. It was found that the method was robust when the column and the mobile phase ratio were varied. During these investigations, the retention times were modified, however the areas and peak symmetry were conserved.

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

Drug determined	TLC-densitometry method	HPLC method
PGZ	101.33 ± 0.94	99.89 ± 1.27
GLM	100.37 ± 0.66	99.89 ± 0.88

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

Preparation		TLC-densitometric method	HPLC method
Duetact® tablets (2,30 mg) Batch No: A16139	PGZ	99.70 ± 1.17	101.42 ± 1.20
	GLM	99.82 ± 0.63	100.75 ± 0.84
Duetact® tablets (4,30mg) Batch No:A16112	PGZ	100.29 ± 1.10	101.40 ± 0.56
	GLM	99.76 ± 0.79	100.92 ± 1.18

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

Parameter	TLC-densitometry method		HPLC method	
	PGZ	GLM	PGZ	GLM
	at 268nm	at228nm	at 228nm	
Range	3-15µg spot^{-1}	0.1-3µg spot^{-1}	5-175µg mL^{-1}	5-30µg mL^{-1}
Slope	0.139	0.2208	0.012	0.051
Intercept	0.803	0.5162	0.031	-0.006
Mean	99.94	100.74	99.80	99.47
S.D.	1.30	1.58	1.16	2.06
Variance	1.69	2.50	1.34	4.28
Coefficient of Variation %	1.31	1.57	1.16	2.08
Correlation coefficient (r)	0.9989	0.9986	0.9999	0.9979
R.S.D.(%)[a]	1.178	0.999	0.215	0.231
R.S.D.(%)[b]	1.152	1.101	0.295	0.345

[a] *the interday (n=6) relative standard deviations of (60µg mL^{-1})of PGZ by HPLC-method and (10µg spot^{-1}) for the TLC-densitometric method, and of (10 µg mL^{-1}) of GLM by the HPLC-method and (1.5µg spot^{-1})for the TLC-densitometric method.*

[b] *the intraday (n=6)relative standard deviations of (60µg mL^{-1}) of PGZ by HPLC-method and (10µg spot^{-1}) for the TLC-densitometric method, and of (10 µg mL^{-1})of GLM by HPLC-method and (1.5µg spot^{-1})for the TLC-densitometric method.*

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	TLC-densitometric method		HPLC-method		Official method for GLM [30]	Reference method for PGZ [29]
	GLM	PGZ	GLM	PGZ		
	at 228nm	at 268nm	at 228 nm			
Mean	100.74	99.942	99.47	99.80	99.80	100.47
S.D.	1.58	1.30	2.07	1.16	1.39	1.34
variance	2.50	1.69	4.28	1.34	1.93	1.80
n	5	5	6	6	6	6
F-test	1.29 (5.19)*	1.07 (6.26)*	2.22 (5.05)*	1.34 (5.05)*		
Student's t-test	1.038 (2.228)*	0.665 (2.262)*	0.324 (2.228)*	0.931 (2.262)*		

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 [31], confirming good accuracy and excellent precision.

CONCLUSION

The suggested methods are found to be simple, 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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