Journal of Chromatography and Separation Techniques

OPEN ACCESS Freely available online

Research Article

Validated Chromatographic Methods for Determination of Pioglitazone and Glimepiride in Pharmaceutical Dosage Form and in Human Plasma

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ABSTRACT

Two simple, sensitive and selective chromatographic methods were described for simultaneous determination of Pioglitazone (PGZ) and Glimepiride (GLM) in their tablet formulation and in human plasma. The first method Thin Layer Chromatography (TLC) was performed on aluminium TLC plates pre coated with silica gel 60 F254 as the stationary phase and a mixture of Toluene: Methanol: Ethyl acetate as the mobile phase by ratio 6:2:4, v/v/v to give compact spots for PGZ at Rf=0.88 and GLM at Rf=0.63 with linearity range and mean percentage recoveries 0.5-5 µg spot-1, 99.00% for PGZ and 0.7-2 µg spot-1, 99.97% for GLM and the chromatogram was scanned at 266 nm. The second method High Performance Liquid Chromatography (HPLC) was developed for determination of the co-administered drug Duloxetine (DUL) with the drugs of interest (PGZ and GLM). Using Xterra C18 column and gradient mobile phase consisting of acetonitrile: water at pH 2.5 adjusted by orthophosphoric acid by ratio (30:70) from 0:10 minutes, then 10:15 minutes by ratio (85:15), respectively with flow rate 1.5 ml/min for the separation of PGZ (tR=1.1), GLM (tR=8.1) and DUL as co-administered drug (tR=2.2). Quantitation was achieved with UV detection at 220 nm. The linearity range was (2:50 µg/ml) for the three drugs and the mean percentage recoveries were found to be 100.26%, 99.53% and 99.89% for PGZ, GLM and DUL, respectively. Both methods were optimized and validated as per ICH guidelines.

Keywords: Pioglitazone; Glimepiride; Duloxetine; TLC-densitometry; HPLC; Human plasma

INTRODUCTION

Pioglitazone Hydrochloride (PGZ) is (RS)-5-(4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl)thiazolidine-2,4-dione (Figure 1). Is a prescription drug of the Thiazolidinedione (TZD) class with hypoglycaemic action [1], used to lower blood glucose levels in the treatment of diabetes mellitus type 2 either alone or in combination with a sulfonylurea, metformin, or insulin [2]. Glimepiride (GLM) is 3-Ethyl-4-methyl-N-[2-(4-{[(trans-4-methylcyclohexyl) carbamoyl] sulfamoyl} phenyl) ethyl]-2-oxo-2, 5-dihydro-1H-pyrrole-1-carboxamide (Figure 2). It is an orally medium-to-long-acting sulfonylurea, which is indicated to treat type 2 diabetes mellitus and its mode of action is to increase insulin production by the pancreas. Sometimes it classified as either the first-generation sulfonylurea [3] or as second-generation [4]. Duloxetine Hydrochloride (DUL) is (S)-N-Methyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propan-1-amine hydrochloride (Figure 3). It is a thiophene derivative and a selective neurotransmitter reuptake inhibitor for serotonin, is mostly prescribed for major depressive disorder, generalized anxiety disorder, fibromyalgia and neuropathic pain. It is the co-administrated with the drugs of interest. Different analytical methods were reported for estimation of the binary mixture including UV spectrophotometric methods, chromatographic methods and mass spectrometry [5-7].

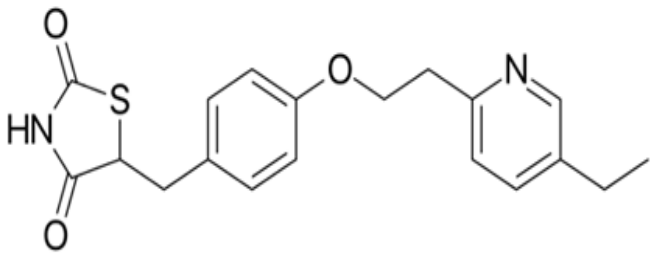

Figure 1: Structural formula of Pioglitazone Hydrochloride (PGZ).

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Received: 24-Oct-2022, Manuscript No. JCGST-22-19811; **Editor assigned:** 26-Oct-2022, PreQC No. JCGST-22-19811 (PQ); **Reviewed:** 10-Nov-2022, QC No. JCGST-22-19811; **Revised:** 17-Nov-2022, Manuscript No. JCGST-22-19811 (R); **Published:** 25-Nov-2022, DOI:10.35248/2157-7064.22.S1.004.

Citation: Riad SM, Farid M, Rahman MHA, Atef D (2022) Validated Chromatographic Methods for Determination of Pioglitazone and Glimepiride in Pharmaceutical Dosage Form and in Human Plasma, J Chromatogr Sep Tech. S1:004

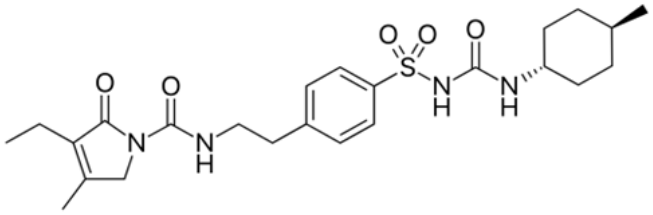

Figure 2: Structural formula of Glimepiride (GLM).

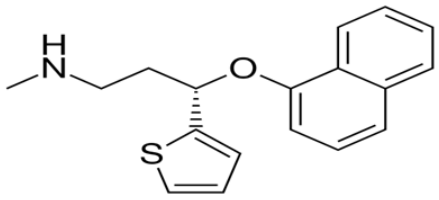

Figure 3: Structural formula of Duloxetine Hydrochloride (DUL).

MATERIALS AND METHODS

Apparatus

The HPLC system: Waters LC 2695 instrument coupled to Waters 996 PDA detector was used. A Xterra, C18 column, 100* 4.6 mm (length* ID), 5 um particle size was used.

The TLC-Densitometric system: Camag TLC scanner 3 S/N 130319 operated with win CATS software, Linomat 5 autosampler (Switzerland), Camag micro syringe (100 µL) and TLC aluminum sheet (20 × 20 cm) precoated with silica gel 60 F254. (Merck KgaA, Darmstad, Germany) were used.

Chemicals and reagents

Pioglitazone (PGZ) and Glimepiride (GLM) were kindly supplied by Hikma pharmaceuticals company; 6th October, Giza, Egypt. Their purity was found to be 100.47% for PGZ according to the reference method and 99.80% for GLM according to the official method (USP, 2011).

Duloxetine (DUL) was supplied by EVA Pharma company; Giza, Egypt. Its purity was found 100.5 ± 0.43 by official methods.

Glimepiride plus tablet labelled to contain 4 mg of GLM, 30 mg of PGZ was manufactured by Andalous (batch number 158120)

Methanol, acetonitrile of HPLC grade, orthophosphoric acid, toluene, ethyl acetate were obtained from Merck® (Darmstadt, Germany).

Standard stock solutions

For HPLC; GLM, PGZ and DUL were prepared by dissolving 0.01 gm in 100 ml methanol to obtain concentration 100 µg/ml.

For TLC; PGZ was prepared by dissolving 0.05 gm in 50 ml methanol to obtain concentration 1 mg/ml and GLM was prepared by dissolving 0.025 gm in 50 ml methanol to obtain concentration 0.5 mg/ml.

Laboratory prepared mixture

For HPLC; different aliquots of the drugs were accurately transferred from their stock solutions and diluted with acetonitrile: water (1:1).

For TLC; different aliquots of the drugs were accurately transferred from their stock solutions and mixed with methanol to prepare solutions of different ratios.

Procedures

Construction of HPLC calibration curve: Aliquots of PGZ, GLM and co-administered drug (DUL) were accurately transferred from their stock standard solutions (100 µg/ml) equivalent to 0.5-50 µg into separate series of 10 mL volumetric flasks; the volumes were completed to the mark with acetonitrile: water (1:1). A 20-µL aliquot of each solution was injected into column Xterra, C18, 100*4.6 mm (length*ID), 5 um particle size using gradient mobile phase consisting of acetonitrile: Water pH at 2.5 adjusted by orthophosphoric acid (30:70) from 0:10 minutes then 10:15 minutes turn to 85% acetonitrile and 15% water by flow rate 1.5 ml/min and detection at 220 nm. The calibration curves relating the peak areas × 10-3 of PGZ, GLM and DUL versus their concentration were plotted and the corresponding regression equation were calculated.

Assay of laboratory prepared mixture: The peak areas or the peak area ratios to external standard of the laboratory-prepared mixture were scanned and processed as described for the calibration for each of the proposed TLC or HPLC methods, respectively. The concentration of each drug in each mixture was calculated using the specified regression equations.

Application of pharmaceutical formulations: Ten glimepiride plus tablets (each tablet labelled to contain 4 mg of GLM, 30 mg of PGZ) were accurately weighed and finely powdered. An amount of the powdered tablet equivalent to one tablet was accurately weighed and transferred into a beaker, extract with (three times 10 mL) methanol and sonicated for 60 min. The solution was filtered into a 50-mL volumetric flask and completed to volume with methanol. Four different solutions were prepared from the previous prepared tablet solution having the concentrations of PGZ and GLM (18.75, 2.5), (22.5, 3), (37.5, 5), and (33.75, 4.5) µg/ml. For TLC, 10 µL was applied onto TLC plates, whereas for HPLC analysis, the last solution was further diluted by transferring 0.05-5 mL aliquots of it to 10-mL volumetric flasks and the volumes were completed with the acetonitrile: water (1:1). Also this was made for co-administered drug (DUL).The general procedures described above for each method were followed to determine the concentration of pioglitazone and glimepiride in the prepared dosage form solutions.

Spiked plasma samples: Aliquots of human plasma (5 mL) was spiked with concentrations 0.5, 1, 5, 10, 20, 30, 40, 50 µg/mL of stock solution (100 µg/ml) of the three drugs were transferred into centrifugation tubes. Di ethyl ether (5 ml) was added to each tube. After vortex mixing for 5 min., the mixtures were centrifuged at 3500 rpm for 15 min, at room temperature. The supernatant was evaporated using nitrogen gas in a heating block set at 45°C. Dried samples were reconstituted with the mobile phase and aliquots of 20 µL were injected and eluted with the mobile phase under the reported chromatographic conditions. A blank experiment was carried out simultaneously. The peak area ratio to external standard was plotted versus the concentration of the drugs in µg mL-1 to get the calibration graph. The regression equation was calculated.

RESULTS AND DISCUSSION

The aim of work was to develop two simple chromatographic methods for the simultaneous determination of PGZ and GLM in

dosage form and determination of PGZ, GLM and co-administered drug (DUL) in plasma without previous separation for HPLC.

HPLC

Good chromatographic separation of PGZ, GLM and co-administered drug (DUL) in plasma was achieved by using a Utilized Xterra C18 column and mobile phase consisting of acetonitrile: water at pH 2.5 adjusted by orthophosphoric acid (30:70) from 0:10 minutes then 10:15 minutes 85% acetonitrile and 15% water by flow rate 1.5 ml/min (Figure 4). Several trials were done to reach the optimum chromatographic separation, and the suggested chromatographic system allows complete baseline separation in reasonable time [8,9].

TLC

In this work, a TLC densitometric method was used for the determination of PGZ and GLM was achieved using mixture of toluene: methanol: ethyl acetate as the mobile phase by ratio (6:2:4, v/v/v). Densitometric scanning was performed at 266 nm as show in Figure 5.

Method validation

ICH guidelines for method validation were followed for validation of the suggested methods [8,9].

Linearity and range: Linear relationships were obtained by plotting the drug concentrations against peak areas or peak area ratio for each drug, for both chromatographic methods. The corresponding concentration ranges, calibration equations, LOD and LOQ and other statistical parameters are listed in Table 1.

Accuracy: The accuracy of the investigated methods was validated by analyzing pure samples of PGZ, GLM and DUL. The concentrations were calculated from the calculated regression equation. The results are shown in Table 1.

Precision: Precision was evaluated by calculating intra- and inter day precision by repeating the assay of three different concentrations three times in the same day and assaying the same samples in triplicate on three successive days, using the developed chromatographic methods and calculating RSD%. Results in Table 1 indicate satisfactory precision of the proposed methods.

Application of the method

The suggested methods were successfully applied for determination of glimepiride plus tablet and in plasma. The results shown in Table 2 were satisfactory and with good agreement with the labelled amounts (Table 2). In order to validate the suggested chromatographic methods, an overall system suitability testing was done to determine if the operating systems are performing properly. Good results were obtained and shown in Tables 3 and 4. When results obtained by applying the proposed methods for analysis of the interested drugs compared to those obtained by applying the reported method they showed no significant difference regarding accuracy and precision, and results were given in Table 5 [10-13].

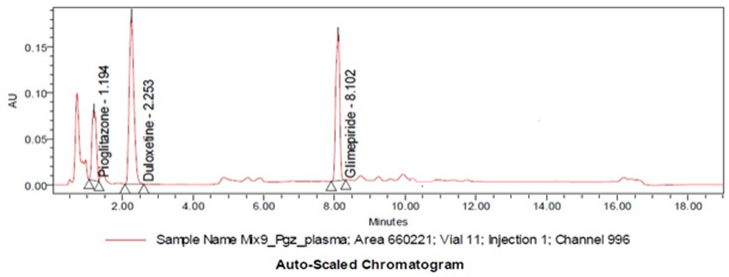

Figure 4: HPLC chromatogram of mixture of PGZ (tR=1.194), GLM (tR=8.102) and co administrated drug (DUL) (tR=2.253) in plasma.

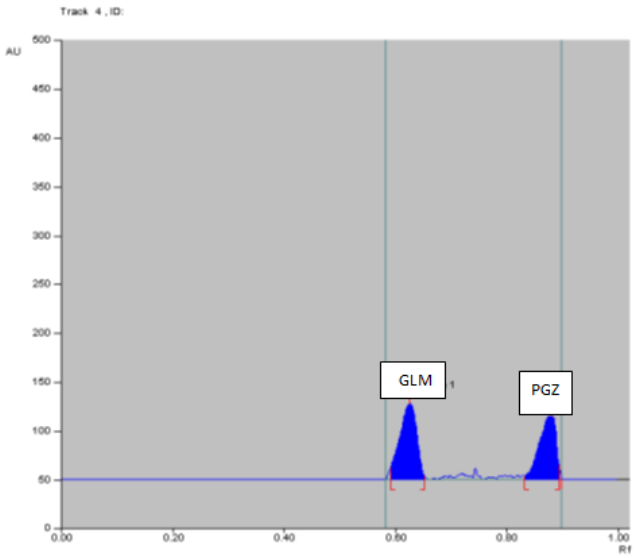

Figure 5: TLC chromatogram of separated peaks of PGZ (Rf = 0.88) and GLM (Rf =0.63).

Table 1: Results of regression and validation parameters of the proposed chromatographic methods for determination of pioglitazone, glimepiride and duloxetine.

Parameters	TLC		HPLC		
	PGZ	GLM	PGZ	GLM	DUL
Linearity calibration range	1-5 µg/band	0.7-2 µg/band	0.5-50 µg/ml	0.2-24 µg/ml	0.5-50 µg/ml
Slope	0.0011	0.0567	0.0452	0.1455	0.0502
Intercept	0.029	0.0288	0.0294	0.1037	0.0129
LODa	0.336	0.1999	0.17	0.068	0.18
LOQa	1.018	0.605	0.53	0.2	0.55
Wave length	266nm			220nm	
Accuracy Mean %b± SD	99.01 ± 2.68	99.97 ± 0.97	100.26 ± 1.24	99.53 ± 1.85	99.89 ± 1.52
RSD% :Inter day precisionc	1.0007	1.0004	1.004	1.0001	1.002
;Intraday precisionc	1.0015	1.0008	1.008	1.0005	1.005
Correlation coefficient (r)	0.9998	0.9997	0.9999	0.9998	0.9999

Note: a)LOD: Limit of Detection, LOQ: Limit of Quantification; b)Average of three experiments; c)mean value of three samples.

Table 2: Determination of glimepiride plus tablet by the proposed chromatographic methods.e.

Parameters	TLC		HPLC		
Drugs	PGZ	GLM	PGZ	GLM	DUL
Glimepiride plus tablet Mean ± SD	99.55 ± 2.6	100.06 ± 0.97	100.42 ± 1.27	99.99 ± 1.87	100.02 ± 1.54
Spiking in plasma Mean ± SD			100.5 ± 1.33	100.24 ± 1.9	100.16 ± 1.58

Table 3: Statistical analysis of parameters required for system suitability testing of TLC method.

Parameters	PGZ	GLM	Limit
Retention Factor(Rf)	0.88	0.63	-
Resolution (Rs)	3.9		Rs>2
Tailing factor(T)	0.78	1	T<2
Selectivity(α)	1.41		α>1

Table 4: Statistical analysis of parameters required for system suitability for HPLC.

Parameters	PGZ	GLM	DUL	Reference value
Retention time	1.2	8.4	2.3	>1
Resolution	1.4	2.5	4.4	>2
Tailing factor	0.5	0.5	0.5	<2
Capacity factor	1.4	3.6	15.8	>2
Selectivity factor	2.5	4.3		α>1
Column efficiency	3545	3136	7326	N>2000
Height equivalent to theoretical plates	0.01	4.20E-04	0.045	AS HETP increase the column efficiency decrease

Table 5: Statistical comparison of the results obtained by the proposed methods and the reported one.

Parameters	TLC		Reported method		HPLC			Reported method		
	PGZ	GLM	PGZ	GLM	PGZ	GLM	DUL	PGZ	GLM	DUL
Mean	99.72	99.97	99.94	100.74	100.26	99.53	99.9	99.8	99.47	99.8
SD	1.97	0.965	1.69	2.5	1.25	1.85	1.52	1.16	2.07	2.05
N	7	8	4	4	6	6	6	4	4	4
Variance (SD)2	3.88	0.931	2.85	6.25	1.56	3.42	2.31	1.34	4.28	4.2
Student's test	0.378(2.18)*	0.55(2.14)*			0.61(2.23)*	0.057(2.23)*	0.52(2.23)*			
F	2.26 (1.07) *	2.23(1.29) *			1.065(5.05)*	1.38(5.05)*	4.26(5.05)*			

Note: *Statistically in-significant

CONCLUSION

The proposed methods are simple and rapid methods of their analysis especially in quality control laboratories. The suggested chromatographic methods provide simple, accurate, and reproducible for the quantitative analysis of the interested drugs in pharmaceutical formulation and in plasma. The developed TLC method is highly sensitive than the reported method and has the advantages of short run time, large sample capacity, and use of less solvents. The HPLC method gives a good resolution with suitable analysis time and the mobile phase was friendly to environment. It is highly specific to determine PGZ and GLM with co administrated drug (DUL) in plasma.

CONFLICT OF INTEREST

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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