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Research Article

NOVEL POLAROGRAPHIC METHODS FOR DETERMINATION OF PIOGLITAZONE HCL IN PURE FORM AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

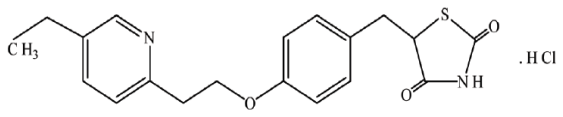
A sensitive methods are described for the determination of pioglitazone HCl(PGZ-HCl) as anti-diabetic drug in its pure form and pharmaceutical formulations. The proposed methods depends on the polarographic activity of PGZ-HCl in Britton-Robinson buffer over the pH range2-12 using direct current (DC) and differential pulse polarography (DPP), and it showed well-defined two cathodic peaks with high selectivity. Its electrochemical behavior at a dropping mercury electrode (DME) and stating mercury drop electrode (SMDE) has been investigated. Polarograms of the drug at DME & SMDE in B–R buffer at pH 6.0 exhibited two 2-electron irreversible cathodic peaks, the first peak (Ep_1) is in the range of potential at -0.05V to -0.10V,while the second peak (Ep_2) is in the potential ranges at -0.975V to -1.10V versus Ag/AgCl. The first and second peaks may be attributed to the reduction of oxy group ($peak_1$) and C=N group ($peak_2$), respectively.

The diffusion current–concentration relationship was found to be rectilinear over the range1.6–224 $\mu g.mL^{-1}$ and 1.6–28 $\mu g.mL^{-1}$ for Ep_1 and over the ranges 1.6–256 $\mu g.mL^{-1}$ and 1.6–32 $\mu g.mL^{-1}$ for Ep_2 using DME & SMDE, respectively, with limit of quantifying PGZ-HCl was 1.6 $\mu g.mL^{-1}$, and relative standard deviation (RSD) ±4.0% & ±4.3% for Ep_1 & Ep_2 using DME and ±3.6% & ±3.8% for Ep_1 & Ep_2 using SMDE. The peaks were characterized as being irreversible, diffusion-controlled although adsorption phenomenon played a limited role in the electrode process. The proposed methods were novel, simple, accurate and successfully applied to the determination PGZ-HCl in pharmaceuticals and the average percentage recovery was in agreement with that obtained by the official USP method.

Keywords: Pioglitazone HCl, Differential Pulse Polarographic Analysis, Direct Current, DME, SMDE, Pharmaceutical Formulations.

INTRODUCTION

Pioglitazone hydrochloride(Schem.1),(±)-5-{4-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl}-2,4thiazolidinedione hydrochloridsalt, is an oral anti-diabetic agent that has been shown to affect abnormal glucose and lipid metabolism associated with insulin resistance by enhancing insulin action on peripheral tissues in animal models [1,2]. It is used in the treatment of type-II diabetes (non-insulin-dependent diabetes mellitus, NIDDM also known as adult on set diabetes) [3,4]. Pioglitazone decreases insulin resistance in the periphery and liver, resulting in increased insulin-dependent glucose disposal and decreased hepatic glucose output.



Schem 1: Chemical structure of pioglitazone HCl.

Several analytical methods have been reported for the determination of pioglitazone HCl in pure form, pharmaceuticals and biological fluids. Most of the reported method sare chromatographic, and no official methods have been reported for the determination of pioglitazone HCl. The reported methods include: high performance liquid chromatography with ultraviolet detection (HPLC UV) [5-15], mass spectroscopy (HPLC/MS) [16], tandem mass spectroscopy (HPLC/MS/MS) [17-19], high performance thin layer chromatography(HPTLC) [20,21], thin layer chromatography(TLC) [22,23], capillary electrophoresis (CE) [24,25] and miceller electro-kinetic chromatography (MEKC)[26].Other reported methods include a potentiometric method[27] and UV spectrophotometric methods [28,29].

Previously reported methods are costly and time consuming to be used in routine laboratories. We therefore, developed a simple and rapid polarographic method with a one-step for determination of pioglitazone HCl in pharmaceutical preparations. Electrochemical methods were proved to be useful for sensitive and selective determination in pharmaceutical compounds. These methods do not require tedious pre-treatment and involve limited pre-separation, and consequently reduce the cost of analysis [30,31]. To date no polarographic procedure has been reported for the assay of pioglitazone HCl.

PGZ freebase and its hydrochloride salt have very low aqueous solubility's, and the hydrochloride salt (PGZ-HCl) is used in the pharmaceutical formulations. Pioglitazone (PGZ) is a non-polar drug and cannot effectively break down the lattice structure of water and hence its aqueous solubility is low. Different techniques are used to enhance the aqueous solubility of drugs including salt formation [32], co-crystal formation [33], addition of co-solvents [34], hydrotropes [35], surface active agents [36] and ionic liquids [37]. Pioglitazone hydrochloride (PGZ-HCl) is used in pharmaceutical formulations, however the aqueous solubility of PGZ-HCl is still low and reports of a number of investigations deal with solubilization of PGZ or PGZ-HCl [38, 39]. The pKa of pioglitazone HCl was estimated and found to equal 5.8 and 6.1, respectively, with a mean pKa value of 5.95.And complement of our researches on some pharmaceuticals [40], including anti-diabetic drugs [41,42],the aim of this study was to develop a rapid, economical, precise and accurate method for the determination of pioglitazone in tablets. The method described is quite suitable for the routine analysis of tablets.

MATERIALS AND METHODS

Instruments and apparatus

Electrochemical Analyzer (797VA Computrace Analyzer-Metrohm/Switzerland) was used. The electrode assembly of a dropping mercury electrode (DME), static mercury drop electrode (SMDE) and hanging mercury drop electrode (HMDE) as a working electrode. Ag/AgCl was the reference electrode and a platinum wire supplied as auxiliary electrode. All measurements were done at room temperature 25±2°C, Nitrogen gas was used for deoxygenation. pH – meter from Radio meter company model Ion Check was used for the studying the pH effects. The measurements were semi-automated and controlled through the programming capacity of the apparatus.

Chemicals and reagents

- Pioglitazone HCl was kindly provided by Dr. Reddy's/ India
- Analytical reagent grade of methanol (BDH) was used.

- Britton–Robinson buffer pH 6.0 [43].
- Standard stock solution 400 µg.mL^{-1} (1.018×10^{-3}M) of pioglitazone HCl was prepared in methanol.
- Pharmaceutical preparations were purchased from the local market.

The solution was found to be stable for at least 2 weeks when stored in the refrigerator. The pH was adjusted with a NaOH solution (2M). All solutions and reagents were prepared with double-distilled deionised water and analytical grade chemicals. Ultrapure mercury from Metrohm Company was used throughout the experiments.

Procedure

25 mL of working standard of PGZ-HCl was transferred to the cell. The solution was well mixed by automatic mixer and deoxygenated with nitrogen gas for 5 min. The polarograms of PGZ-HCl were recorded by scanning the potential toward the negative direction applying the direct current polarography (DC),differential pulse polarography positive polarity (DPPPP) and differential pulse polarography negative polarity (DPPNP), in the potential range from +200mV to -1500mV. All data were obtained at room temperature.The number of experiments (n=5) according to this value, the statistical calculations were done.

Sample preparation

Twenty tablets of Pioglit (15mg, 30mg and 45mg PGZ-HCl) and Pioglit met (15mg PGZ-HCl with 500mg or 850mg metformin HCl) BPI, Aleppo-Syria, Actaoze Asia (15mg, 30mg and 45mg PGZ-HCl), Asia, Aleppo-Syria and Defast (15mg and 30mg PGZ-HCl) Unipharma, Damascus-Syria, were weighed and ground to a fine powder. A quantity equivalent to 1 × 10^{-3} M of PGZ-HCl was accurately weighed, dissolved in 40 mL of methanol, transferred to a 50 mL volumetric flask and diluted to the mark with methanol. The content of the flask was sonicated for about 15 min then solution was filtered to separate the insoluble excipients. Aliquots of the drug solution were introduced into the electrolytic cell and the general procedure was carried out.

RESULTS AND DISCUSSION

Effect of supporting electrolyte

The effect of supporting electrolyte was examined using different supporting electrolytes including:Britton–Robinson, acetic acid–sodium acetate, sodium citrate–citric acid and sodium tartrate–tartaric acid buffers (each 0.1M) was studied by DPP.B-R buffer was selected for further work because it gave the highest peak current and the best peak shape of the PGZ-HCl, it was added methanol with electrolyte in 32%(v/v). The addition of methanol, to the electrolyte solution, decreases the effect of adsorption on the electrode process. However, relatively better defined peak shapes were recorded with the B–R buffer electrolyte containing 32% (v/v) methanol as a solubilizer for PGZ-HCl; it also decreased the adsorption interferences.

Effect of pH

The electrochemical behavior of PGZ-HCl has been investigated, in 0.1M B-R buffer containing 32% (v/v) methanol, at different pH from 2.0 to 12.0 on the peak current (Ip) and peak potential (Ep) were examined. The polarograms of the DPP for 7.87 µg.mL^{-1} and 15.72µg.mL^{-1} PGZ-HCl in B-R at different pH values using DME shown in Fig.1 & 2. Two reduction peaks were observed, the first peak (Ep_1) is in the range of potential at -0.008V to -0.040V, while the second peak (Ep_2) is in the potential ranges at -0.975V to -1.150V, Fig.2, a. It was found that the cathodic peak$_{1\&2}$ current at DME were increased at pH values between 2.0–4.0,with constant Ip_1 & Ip_2 at pH values between 4.0 - 7.0,thenalmostdecrease at pH values between 7.0-9.0 and then increased after pH >9.0, (fig.1 & 2.a). Therefore, the proper value of pH was 6 for both two peaks.

The Ep_1 was shifted to more negative direction when the solution pH was increased from 2.0 to 6.0 and then constant between 6.0 to12.0, Fig.1,b, it is observed that the reduction potential of PGZ-HCl shifted to more negative values with pH increase, indicating the presence of chemical reaction with participation of protons[44]. Also, PGZ-HCl produced a well-defined cathodic Ep_2over the pH range of 2.0–12.0 in B-R buffer. Reduction of PGZ-HCl at the DME was found to be pH-dependent as the Ep values were shifted to more negative values upon increasing the pH, indicating the irreversible nature of the reduction process. A plot of Ep *vs.* pH gave two straight lines with one break at pH 6 assigning a theoretical pKa value of PGZ-HCl a mean pKa value of 5.95. Fig.2.b. The relation between Ep and the pH of the solution is represented by the following equations:

$Ep_{2,1}$ = -0.9487-0.0103pH (R^2=0.9994)over the pH range 2 -5.

$Ep_{2,2}$ = -1.0373-0.0103pH (R^2=0.9998) over the pH range 6 -12.

The $Ep_2$of PGZ-HCl was shifted linearly towards more negative potential values with increasing the pH between 2 to 5 and 6 to 12 by 0.0103 V/pH. The slope of the line at two cases was -0.9487 and

-1.0373V per pH unit, with mainly slope of -0.993 V/pH, shows that the reduction mechanism of PGZ-HCl involves the same number of electrons and protons.

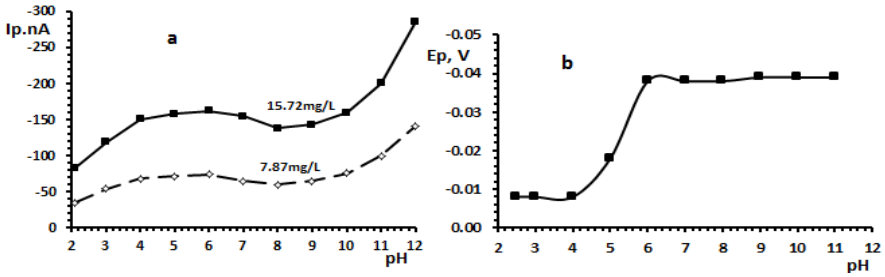


Fig. 1: The effect of pH values using DME of PGZ-HCl for Ip_1 in B-R buffer 0.1M containing 32% (v/v) methanol: a- Ip (for two concentration, 7.87&15.72 µg.mL^{-1}), b- Ep(for one concentration 15.72µg.mL^{-1}).

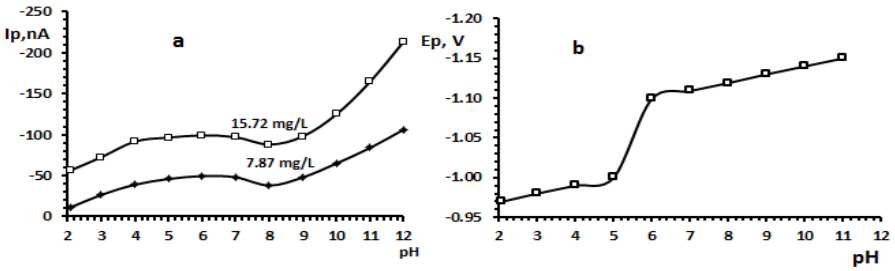


Fig. 2: The effect of pH values using DME of PGZ-HCl for Ip_2 in B-R buffer 0.1M containing 32% (v/v) methanol: a- Ip (for two concentration,7.87&15.72 µg.mL^{-1}), b- Ep(for one concentration 15.72 µg.mL^{-1}).

Study of methods and its conditions

The DC-polarograms of PGZ-HCl at pH 6, using DME, exhibited two irreversible reduction waves (Fig.3,a). The half-wave potentials of the reduction waves shifted to more negative values upon the increase of pH. This behaviour indicated the involvement of protons in the electrode reaction and that the proton-transfer reaction precedes the electrode process proper [45,46].

The polarographic methods of PGZ-HClwere investigated at pH 6 using DC, DPPPP and DPPNP in presence of 0.1M B-R buffer as electrolyte with 32% (v/v) methanol. DPPPP was found to give the greatest sensitivity. The polarographic methods of PGZ-HCl using DME & SMDE were given two well-defined reduction peaks, the first peak (Ep_1) is in the range of potential at -0.05 to -0.10V,while the second peak (Ep_2) is in the potential ranges at -0.975 to -1.150 V, Fig.3.

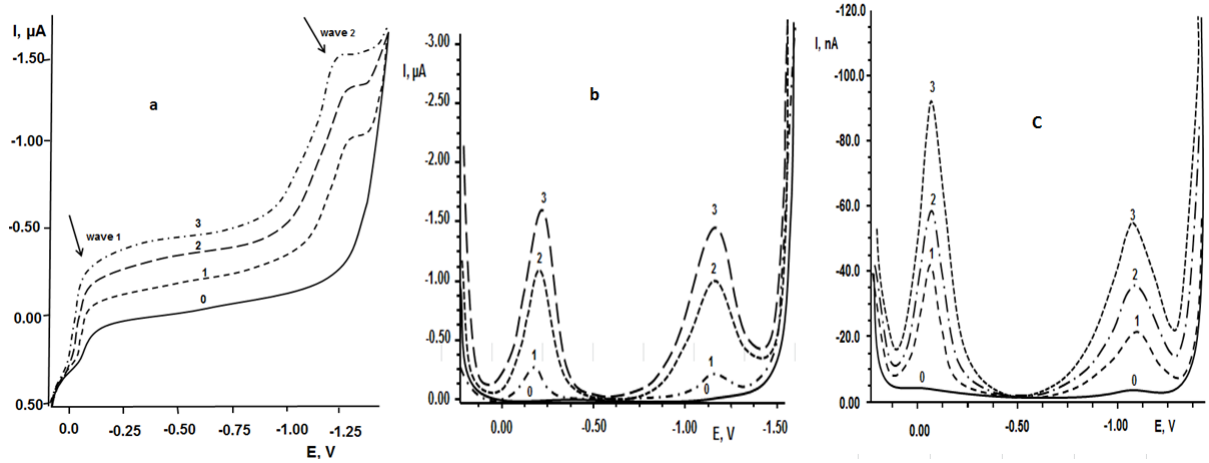


Fig. 3: The Polarographic Curves for the determination of PGZ-HCl in B-R buffer 0.1M containing 32% (v/v) methanol at pH 6.0, (a) DC using DME (0-elec, 1- 8, 2- 12.8 & 3- 16µg.mL^{-1}), (b) DPPPP using DME (0-elec, 1- 16, 2- 96 & 3- 144µg.mL^{-1}), (c) DPPPP using SMDE (0-elec, 1- 9.6, 2- 16 & 3- 24µg.mL^{-1}).

Effect of pulse amplitude

The effect of pulse amplitude on polarograms of DPP using DME for the determination of PGZ-HCl at pH 6 was studied over the range of 10–100 mV. The peak current Ip_1 & Ip_2 increases proportionally with increasing of pulse amplitude positive polarity (DPPPP). Therefore the value of pulse amplitude 60 mV for DPPPP was chosen as optimum value, (Fig.4, a.)

Effect of pulse time

The effect of pulse time on polarograms was studied over the range of 0.01–0.10sec, Fig.4,b. As the follows: Ip_1&Ip_2 decreases with increasing pulse time from 0.01sec to 0.03sec and over that it was constant, so that the proper value of pulse time for Ip_1 & Ip_2 were 0.04sec. Ep has become increasingly positive value with increasing pulse time.

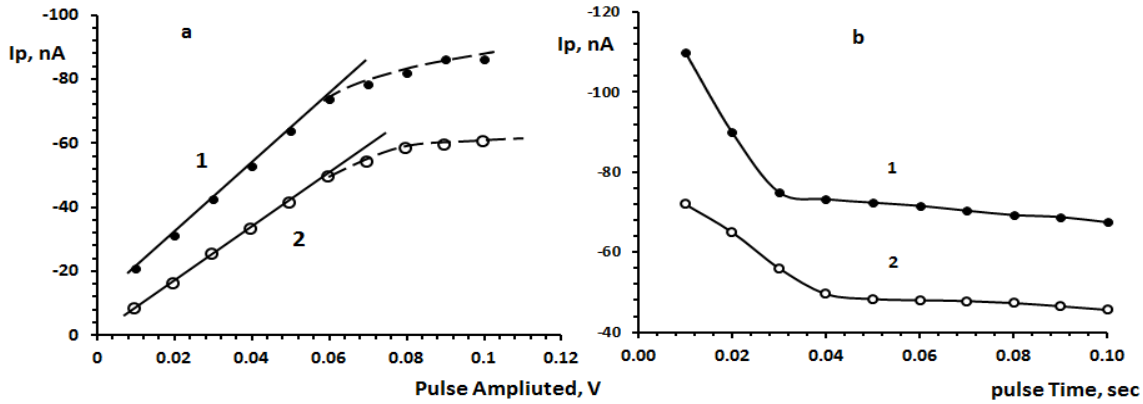


Fig. 4: The effect of pulse amplitude and pulse time of PGZ- HCl at 7.87µg.mL^{-1}for Ip1&Ip2in B-R buffer 0.1M containing 32% (v/v) methanol at pH 6.0: (a) Ip_1&Ip_2*vs.* pulse amplitude and (b)Ip_1&Ip_2*vs.* pulse time using DME.

Effect of electrode sort

DPP polarograms were studied for standard solutions of PGZ-HCl in the potential range from +0.2V to -1.5V in 0.1 M B-R buffer containing 32% (v/v) methanol at pH 6.0 by using DME and SMDE electrodes. Well-defined electrochemical reduction peaks for PGZ-HCl were noticed at Ep_1 and Ep_2. It was found that, the diffusion factor using DME was greater than their value using SMDE as been: K_{DME}=3.22$K_{SMDE}$for Ip_1 and K_{DME}=4.32K_{SMDE} for Ip_2

The electrochemical behaviour of PGZ- HCl

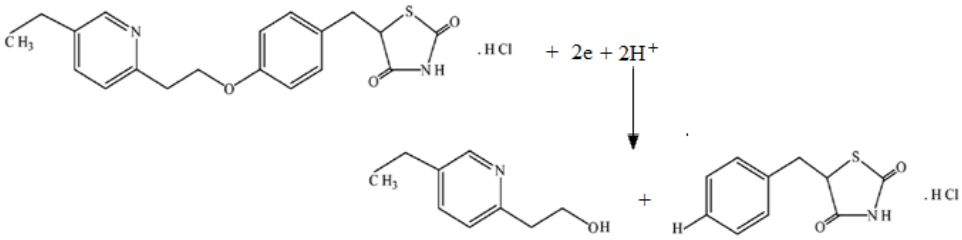
The chemical structure of PGZ-HCl is characterized by the presence of oxy and C=N groups which are susceptible for the reduction into another groups at DME & SMDE as shown in (scheme 1).

The differential pulse polarographic behavior was investigated for PGZ-HCl compound in 0.1 M B-R buffer containing 32% (v/v) methanol at pH 6.0 using DME & SMDE were given two well-defined reduction peaks, as

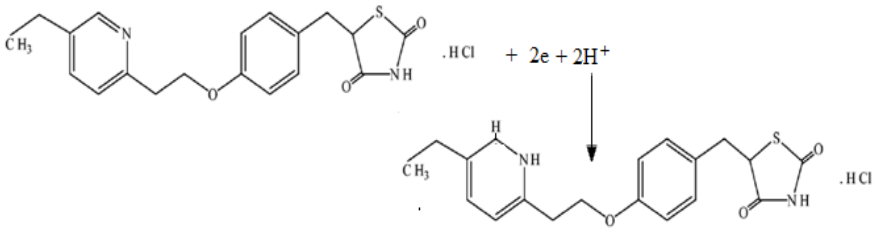
shown in (Fig.3,c), The first peak (Ep_1) in the range of potential at -0.05V to -0.10V was observed. The first peak may be attributed to the reduction of oxy group ($peak_1$) . A proposed mechanism, for the electrochemical reduction of this electro-active group, was studied and depending on the relationship between $W_{1/2}$ and number of electrons, it was calculated and confirmed that the number of electron of this reduction operating were two electron. This mechanism suggests that the electrochemical reaction is an irreversible process. Such quantitation depends not only on the corresponding peak potentials but also on the width of the peak. The width of the peak (at half-height) is related to the electron stoichiometry[47]:

$$W_{1/2} = \frac{3.52RT}{nF}$$

The number of electrons transferred during the reduction process was accomplished through comparing the $peak_1$ height of PGZ-HCl with that obtained from an equimolar solution of an earlier studied compound having similar functional group and of nearly identical value of the diffusion-coefficient, namely, danazol[48].The electrode reaction is suggested to proceed as follows:



The second peak (Ep_2) is in thepotentialranges at -0.975 to -1.150 V, (Fig.3,c)a polarographic $peak_2$ was observed as a well-defined 2-electron irreversible cathodic $peak_2$ which may be attributed to reduction of the C=N double bonds of the pyridyl ring of the target molecule.A proposed mechanism, for the electrochemical reduction of this electro-active group, was studied and depending on the relationship between $W_{1/2}$ and number of electrons[47], it was calculated and confirmed that the number of electrons of this reduction operating were two electrons.The number of electrons transferred during the reduction process was accomplished through comparing the $peak_2$ height of PGZ-HCl with that obtained from an equimolar solution of an earlier studied compound having similar functional group and of nearly identical value of the diffusion-coefficient, namely,glipizide[49]. The electrode reaction is suggested to proceed as follows:



Calibration curves

Calibration curves for the determination of PGZ- HCl compound in 0.1M B-R buffer containing 32% (v/v) methanol at pH 6.0 by DPP using DME and SMDE electrodes were studied. The first peak Ip_1 were proportional to the concentration of PGZ- HCl over the ranges, 1.6–224µg.mL^{-1} and 1.6–28µg.mL^{-1} using DME & SMDE, respectively, fig. 5. The limit of quantifying PGZ-HCl was 1.6 µg.mL^{-1}with the relative standard deviation (RSD) of ±4.0% using DME and ±3.6% using SMDE.The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.211µg.mL^{-1} and 0.195µg.mL^{-1}(LOD),0.640µg.mL^{-1} and 0.590µg.mL^{-1}(LOQ) using DME & SMDE respectively. The second peak Ip_2 were proportional to the concentration of PGZ-HCl over the ranges, 1.6–256 µg.mL^{-1} and 1.6–32 µg.mL^{-1} using DME and SMDE respectively, Fig.6. The limit of quantifying PGZ-HCl was 1.6µg.mL^{-1}, with RSD of ±4.3% using DME and ±3.8% using SMDE. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.224µg.mL^{-1} and 0.221µg.mL^{-1}(LOD),0.680µg.mL^{-1} and 0.640µg.mL^{-1}(LOQ)using DME&SMDE, respectively, tables 1-3.

Application to pharmaceutical preparations

The proposed method has been successfully applied for the analysis of PGZ-HCl in its commercial tablets. Pharmaceutical preparation determined using Differential Pulse Polarography with positive amplitude at DME and SMDE in 0.1M B-R buffer containing 32% (v/v) methanol at pH 6.0. There were no interferences within containing of met form in HCl in some tablets. The results of quantitative analysis for PGZ-HCl were calculated by calibration curves method, Table 4&5.

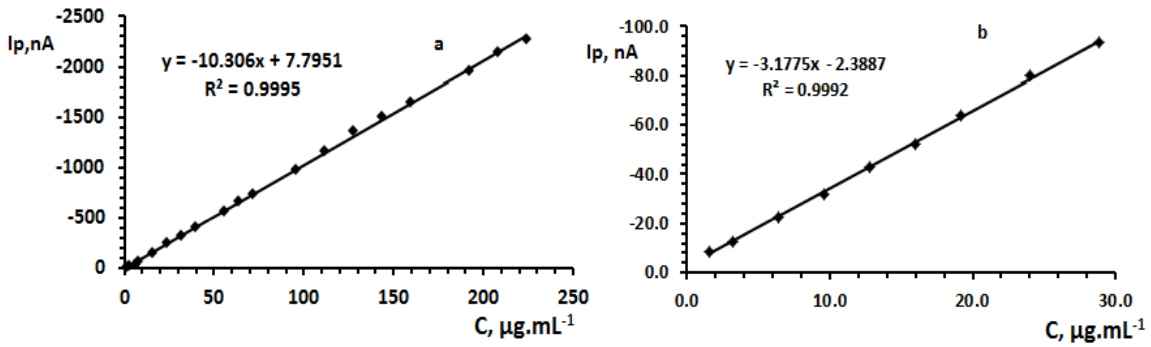


Fig. 5: Calibration curves for the determination of PGZ-HCl of Ip_1using DPPPP (a) DME and (b)SMDEinB-R buffer 0.1M containing 32% (v/v) methanol at pH 6.0·

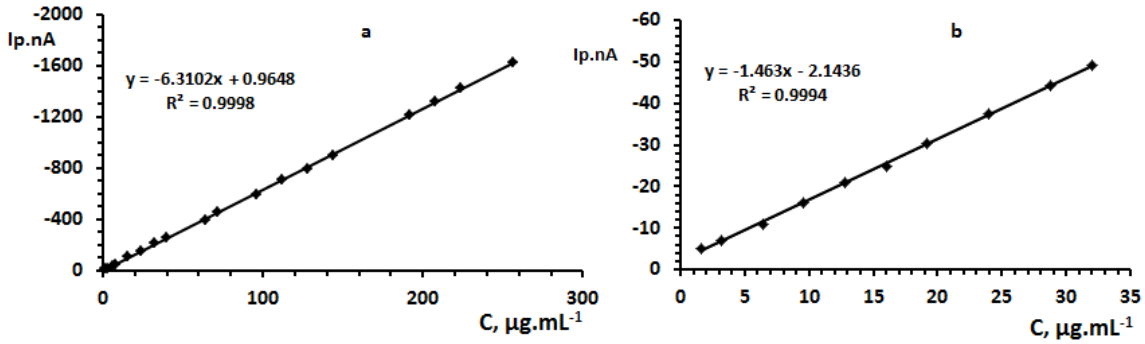


Fig.6: Calibration curves for the determination of PGZ-HCl of Ip_2 using DPPPP (a)DMEand (b)SMDE in B-R buffer 0.1M containing 32% (v/v) methanol at pH 6.0.

Table 1: The optimum parameters established for determination of PGZ-HCl using differential pulse polarography with positive amplitude in B-R buffer 0.1M containing 32% (v/v) methanol at pH 6.0.

Parameters	Operating modes	
Electrode	DME & SMDE	
Supporting electrolytes	B-R buffer 0.1M containing 32% (v/v) methanol	
pH	6.0	
Solvent for PGZ-HCl	methanol	
Pulse amplitude	60mV	
Purge gas	Pure N_2	
Purge time	300 sec	
Initial potential	0.2V	
Final potential	-1.5V	
Scan rate	10 mV/s	
Reduction peaks	Peak1	Peak2
Peak Potential	Ep_1: -0.05 to -0.10 V	Ep_2 :-0.975 to -1.150 V
Linearity	1.6 - 224µg.mL^{-1}, DME	1.6 - 256µg.mL^{-1}, DME
range of concentration	1.6 - 28µg.mL^{-1},SMDE	1.6 - 32µg.mL^{-1}, SMDE
RSD%		
at DME	4.0%	4.3%
SMDE	3.6%	3.8%
Regression equation		
at DME	* y = -10.306x+7.7951; (R^2=0.9995)	* y = -6.3102x+0.9648; (R^2=0.9998)
SMDE	* y = -3.1775x-2.3887; (R^2=0.9992)	* y = -1.463x-2.1436; (R^2=0.9994)
LOD (3.3SD)		
at DME	0.211	0.224
SMDE	0.195	0.221
LOQ (10SD)		
atDME	0.640	0.680
SMDE	0.590	0.640

* y= nA, x= concentration ofPGZ-HCl,µg.mL^{-1}).

Table 2: Evaluation of accuracy and precision of the proposed method DPPPP for determination of PGZ-HCl(Ip_1) on DME and SMDE.

Electrode	$C_{PGZ-HCl}$ taken, µg.mL^{-1}	$C_{PGZ-HCl}$ found, $\bar{X}$, µg.mL^{-1}	SD, µg.mL^{-1}	Analytical standard error, $\frac{SD}{\sqrt{n}}$, µg.mL^{-1}	Confidence limits $\bar{X} \pm \frac{SD}{\sqrt{n}}$ t, µg.mL^{-1}	RSD %
DME	1.60	1.591	0.064	0.029	1.59 ± 0.079	4.0
	3.20	3.16	0.120	0.054	3.16 ± 0.150	3.8
	6.40	6.14	0.215	0.096	6.14 ± 0.267	3.5
	8.00	7.94	0.262	0.117	7.94 ± 0.325	3.3
	16.0	15.89	0.477	0.213	15.8 ± 0.592	3.0
	24.0	24.42	0.659	0.295	24.42 ± 0.819	2.7
	32.0	32.29	0.807	0.361	32.29 ± 1.003	2.5
	40.0	39.67	0.912	0.408	39.67 ± 1.133	2.3
	56.0	55.72	1.114	0.498	55.72 ± 1.383	2.0
	64.0	64.85	1.167	0.522	64.85 ± 1.449	1.8
	72.0	72.00	1.152	0.515	72.00 ± 1.430	1.6
	96.0	95.85	1.438	0.643	95.85 ± 1.785	1.5
	112.0	113.23	1.472	0.658	113.23 ± 1.827	1.3
	160.0	159.79	1.917	0.858	159.79 ± 2.381	1.2
	192.0	191.16	2.103	0.940	191.16 ± 2.610	1.1
	208.0	208.88	3.133	1.401	208.88 ± 3.890	1.5
	224.0	222.38	4.003	1.790	222.38 ± 4.970	1.8

SMDE	1.60	1.641	0.059	0.026	1.641 ± 0.073	3.6
	3.20	3.23	0.103	0.046	3.230 ± 0.128	3.2
	6.40	6.40	0.179	0.080	6.400 ± 0.222	2.8
	8.00	7.94	0.199	0.088	7.940 ± 0.246	2.5
	12.8	12.87	0.296	0.132	12.870 ± 0.367	2.3
	16.0	15.88	0.318	0.142	15.880 ± 0.394	2.0
	19.2	19.28	0.328	0.147	19.280 ± 0.407	1.7
	24.0	24.32	0.340	0.152	24.320 ± 0.423	1.4
	28.8	28.72	0.345	0.154	28.720 ± 0.428	1.2

* n=5, t=2.776

Table 3: Evaluation of accuracy and precision of the proposed methodDPPPP for determination of PGZ-HCl(Ip_2) on DME and SMDE.

Electrode	$C_{PGZ-HCl}$ taken, µg.mL^{-1}	$C_{PGZ-HCl}$ found, $\overline{X}$, µg.mL^{-1}	SD, µg.mL^{-1}	Analytical standard error, $\frac{SD}{\sqrt{n}}$, µg.mL^{-1}	Confidence limits $\overline{X} \pm \frac{SD}{\sqrt{n}}$ t, µg.mL^{-1}	RSD %
DME	1.60	1.584	0.068	0.030	1.591 ± 0.085	4.3
	3.20	3.140	0.126	0.056	3.140 ± 0.156	4.0
	6.40	6.320	0.234	0.106	6.320 ± 0.290	3.7
	8.00	7.960	0.279	0.125	7.960 ± 0.346	3.5
	16.0	16.21	0.519	0.232	16.21 ± 0.644	3.2
	24.0	24.15	0.725	0.324	24.15 ± 0.900	3.0
	32.0	32.22	0.902	0.403	32.22 ± 1.120	2.8
	40.0	40.72	1.059	0.474	40.72 ± 1.315	2.6
	56.0	55.88	1.285	0.575	55.88 ± 1.596	2.3
	64.0	63.74	1.339	0.599	63.74 ± 1.662	2.1
	72.0	71.95	1.511	0.611	71.95 ± 1.696	1.9
	96.0	95.64	1.626	0.727	95.64 ± 2.019	1.7
	112.0	112.74	1.691	0.756	112.74 ± 2.099	1.5
	144.0	143.78	1.869	0.836	143.78 ± 2.321	1.3
	192.0	191.91	2.294	0.944	191.91 ± 2.621	1.1
	208.0	208.07	2.289	1.024	208.07 ± 2.841	1.1
	224.0	225.19	2.927	1.309	225.19 ± 3.634	1.3
	256.0	256.72	4.108	1.837	256.72 ± 5.100	1.6
SMDE	1.60	1.680	0.064	0.029	1.680 ± 0.079	3.8
	3.20	3.320	0.116	0.052	3.320 ± 0.114	3.5
	6.40	6.370	0.204	0.091	6.370 ± 0.253	3.2
	8.00	8.000	0.240	0.107	8.00 ± 0.297	3.0
	12.80	12.890	0.335	0.150	12.89 ± 0.416	2.6
	16.00	15.940	0.383	0.171	15.94 ± 0.475	2.4
	19.20	19.320	0.406	0.182	19.32 ± 0.504	2.1
	24.00	24.000	0.432	0.193	24.00 ± 0.536	1.8
	28.80	28.820	0.461	0.206	28.82 ± 0.572	1.6
	32.00	32.030	0.448	0.200	32.03 ± 0.556	1.4

* n=5, t=2.776

Table 4: Determination of PGZ-HCl in some pharmaceutical formulations by DPPPP methods using DME and SMDE at pH 6.0 using calibration curves method.

Commercial name	Contents, mg/tablet	$\overline{X}$,mg/tablet	$\overline{X}$ ± SD, mg	RSD%	Recovery %
Pioglit ,BPI	15	14.85	14.85 ± 0.075	2.2	99.00
(Aleppo – Syria)	30	30.08	30.08 ± 0.040	1.6	100.27
	45	44.97	44.97 ± 0.085	1.3	99.93
Pioglit met,BPI	15/500mg metformin	15.12	15.12 ± 0.060	2.3	101.47
(Aleppo – Syria)	15/850mg metformin	14.94	14.94 ± 0.025	2.0	99.60
Actaoze Asia,	15	15.22	15.22 ± 0.075	2.4	101.47
Asia(Aleppo – Syria)	30	31.01	31.01 ± 0.040	1.5	103.37
	45	44.57	44.57 ± 0.085	1.1	99.04
DEFAST ,Unipharma	15	15.60	15.60 ± 0.075	2.0	104.00
(Damascus – Syria)	30	30.29	30.29 ± 0.040	1.7	100.97

CONCLUSION

In the proposed method, differential pulse polarographic analysis of PGZ-HCl in pure and pharmaceutical formulations in0.1M B-R buffer containing 32% (v/v) methanol at pH 6.0 has been investigated using DME & SMDE. Two reduction peaks were observed. The first peak (Ep_1) may be attributed to the reduction of oxy group and the second peak (Ep_2) may be attributed to reduction of the C=N double

bonds of the pyridyl ring. The Ip_1were proportional to the concentration over the ranges 1.6 –224μg.mL^{-1} and 1.6–28 μg.mL^{-1} using DME and SMDE respectively, and the Ip_2 were proportional to the concentration of PGZ-HCl over the ranges 1.6–256 μg.mL^{-1} and 1.6–32 μg.mL^{-1}using DME & SMDE, respectively. Applying DME and SMDE over mentioned methods in this context were successfully carried out for the first time. The relative standard deviation (RSD%) did not exceed of ±4.0% and ±3.6% using DME and SMDE, respectively. The proposed method was successfully applied to the analysis of PGZ-HCl in pure and pharmaceutical formulations with average recovery of 99.0% to 104.0%.The results obtained agree well with the contents stated on the labels.

REFERENCES

1. Sohda T, Mizuno K, Momose Y, Ikeda H, Fujita T & Meguro K. Novel thiazolidinedione derivatives as potent hypoglycemic and hypolipidemic agents, J. Med. Chem., 1992; 35, 2617-2626.
2. Kobayashi M,Iwanishi M,EgawaKatsuya,Shigeta Y, Pioglitazone increases insulin sensitivity by activating insulin receptor kinase, Diabetes, 1992; 41: 476-483, 11.
3. Waugh J, Keating GM, Plosker GL, Easthope S, Robinson DM, Pioglitazone: a review of its use in type 2 diabetes mellitus, Drugs, 2006; 66(1):85-109.
4. Chilcott J, Tappenden P, Jones ML, Wight JP, A systematic review of the clinical effectiveness of pioglitazone in the treatment of type 2 diabetes mellitus, Clin. Ther. 2001; 23:1792–1823.
5. Giaginis C, Theocharis S, Tsantili-Kakoulidou A., Investigation of the lipophilic behaviour of some thiazolidinediones Relationships with PPAR-gamma activity, J Chromatogr B AnalytTechnol Biomed Life Sci, 2007; 857:181-187.
6. Sripalakit P, Neamhom P, and Saraphanchotiwitthaya A, High-performance liquid chromatographic method for the determination of pioglitazone in human plasma using ultraviolet detection and its application to a pharmacokinetic study, J Chromatogr B AnalytTechnol Biomed Life Sci , A2006; 843:164–169.
7. Chompootaweep S, Thaworn N, The Pharmacokinetics of Pioglitazone in Thai Healthy Subjects, J Med Assoc Thai., 2006; 89: 2116-2122, 15.
8. Venkatesh P, Harisudhan T, Choudhury H, Mullangi R, and Srinivas NR, Simultaneous estimation of six anti-diabetic drugs-glibenclamide, gliclazide, glipizide, pioglitazone, repaglinide and rosiglitazone: development of a novel HPLC method for use in the analysis of pharmaceutical formulations and its application to human plasma assay, Biomed Chromatogr., 2006 ; 20 :1043-1048.
9. Wanjari DB, Gaikwad NJ, Developed Stability indicating RP-HPLC method for determination of pioglitazone from tablets, Indian journal of pharmaceutical sciences, 2005; Volume:67, Issue:2, Page:256-258.
10. Sane RT, Menon SN, Inamdar S, Mote M, Gundi G, Simultaneous determination of Pioglitazone and Glimepiride by high-performance liquid chromatography, Chromatographia, 2004; 59: 451-453.
11. Kolte BL, Raut BB, Deo AA., Bagool MA, Shinde DB,Simultaneous determination of metformin in combination with rosiglitazone by reversed-phase liquid chromatography, J.Chromatogr.Sci., 2004; 42: 27-31.
12. ZhongWZ,Laking DB, Determination of pioglitazone in dog serum using solid-phase extraction and high-performance liquid chromatography with ultraviolet (229 nm) detection, J. Chromatogr. Biomed. Appl., 1989; 490:377-385.
13. Zhong WZ, Williams MG, Simultaneous quantitation of pioglitazone and its metabolites in human serum by liquid chromatography and solid phase extraction, J. Pharm. Biomed. Anal. 1996; 14:465-473.
14. Yamashita K, Murakami H, Okuda T, and Motohashi M, High-performance liquid chromatographic determination of pioglitazone and its metabolites in human serum and urine, J. Chromatogr. B., 1996; 677:141-146.
15. Dai JD, Jin DJ, Liu YL, Yaowu. Fenxi, Zazhi. 2001; 21:36-39.
16. Xue YH, Turner KC, Meeker JB, Pursley J, Arnold M, Unger S, Quantitative determination of pioglitazone in human serum by direct-injection high-performance liquid chromatography mass spectrometry and its application to a bioequivalence study, J. Chromatogr. B., 2003; 795:215-226.
17. Wang M, and. Miksa IR, Multi-component plasma quantitation of anti-hyperglycemic pharmaceutical compounds using liquid chromatography-tandem mass spectrometry, J. Chromatogr. B., 2007; 856:318-327.
18. Lin ZJ, Ji W, Desai-Krieger D, Shum L, Simultaneous determination of pioglitazone and its two active metabolites in human plasma by LC-MS/MS, J. Pharm. Biomed. Anal., 2003; 33:101-108.
19. Ho ENM, Yiu KCH, Wan TSM, Stewartand BD, Watkins KL,Detection of anti-diabetics in equine plasma and urine by liquid chromatography-tandem mass spectrometry, J. Chromatogr. B., 2004; 811:65-73.
20. Gumieniczek A, HopkalaH,Berecka A, Specific modes of detection – merits of planar chromatography,J. Liq. Chromatogr. Relat. Technol., 2004; 27:2057-2070.
21. Menon R. T. S., Inamdar S., Mote M. and Menezes A., Simultaneous determination of pioglitazone and glimepiride by high-performance liquid chromatography, J. Planar. Chromatogr. Mod. TLC, 2004; 17:154-156.
22. Anna G, Hanna H, Anna B, Dorota K, Normal and reversed-phase thin-layer chromatography of seven oral anti-diabetic agents, J. Planar. Chromatogr. Mod. TLC, 2003; vol. 16, no. 4, pp. 271-275.
23. Ravi B, Deepak G,Aishwarya J, TLC supplemented by UV spectro photometry compared with HPLC for separation and determination of some anti-diabetic drugs in pharmaceutical preparations, JPC- J. of Planar chromatography modern TLC, 2007;19:288-296.
24. Jamali B, Theill GC, Sorensen LL., Generic highly selective and robust capillary electrophoresis method for separation of a racemic mixture of glitazone compounds, J. Chromatogr.A., 2004; 1049:183-187.
25. Jamali B, Bjornsdottir I, Nordfang O, Hansen SH, Investigation of racemization of the enantiomers of glitazone drug compounds at different pH using chiral HPLC and chiral CE., J. Pharm. Biomed. Anal. 2008; 46:82-87.
26. Radhakrishna T, Sreenivas D, Reddy GO, Determination of pioglitazone hydrochloride in bulk and pharmaceutical formulations by HPLC and MEKC methods, J. Pharm. Biomed. Anal., 2002; 29:593-607.
27. Mostafa GAE, Al-Majed A, Characteristics of new composite- and classical potentiometric sensors for the determination of pioglitazone in some pharmaceutical formulations, J. Pharm. Biomed. Anal., 2008; Vol. 48, No. 1, pages 57-61, in press.
28. GowriSankar D, Rajendra KJM, Reddy MVVN, UV Spectrophotometric Methods for the Determination of Anti-diabetic Drugs, Asian J. Chem., 2004; 16(1) :537-539.
29. GowriSankar D, Rajendra Kumar JM, Reddy MVVN, Extractive Spectrophotometric Determination of Pioglitazone Hydrochloride Using Both Acidic and Basic Dyes, Asian J. Chem., 2004; 16 (1):245-251.
30. Sayin F,Kir S, Analysis of diflunisal by electrochemical methods, J. Pharm. Biomed. Anal., 2001; 25, 153-163.
31. OzkanSA,Uslu B,Zuman P, Electrochemical reduction and oxidation of the antibiotic cefepim on a carbon electrode, Anal.Chim.Acta, 2002; 457, 265-274.
32. Serajuddin ATM, Salt formation to improve drug solubility, Adv Drug Del Rev, 2007; 59: 603 616.
33. Babu NJ, and Nangia A, Solubility advantage of amorphous drugs and pharmaceutical cocrystals, Crys Grow Des, 2011; 11: 2662-2679.
34. Li A, and Yalkowsky SH., Predicting cosolvency. 1. Solubility ratio and solute logKow, IndEngChem Res, 1998; 37: 4470-4475.
35. Kim JY, Kim S, Papp M, Park K, and Pinal R, Hydrotropic solubilization of poorly water-soluble drugs, J Pharm Sci, 2010; 99: 3953-3965.
36. Anton N, and Vandamme TF, Nano-emulsions and micro-emulsions: Clarification of the critical differences, Pharm Res, 2011; 28: 978-985.

37. Mizuuchi H, Jaitely V, Murdan S, and Florence AT, Room temperature ionic liquids and their mixtures: Potential pharmaceutical solvents, Eur J Pharm Sci, 2008;33: 326-331.
38. Seedher N, and Kanojia M, Micellarsolubilization of some poorly soluble drugs: a technical note, AAPS Pharm Sci Tech, 2008; 9: 431-436.
39. Seedher N, and Kanojia M, Co-solvent solubilization of some poorly soluble antidiabetic drugs, Pharm Develop Tech, 2009; 14: 185-192.
40. Mandil H ,Sakur A A,Alulu S, Polarographic Determination of Topiramate in Some Pharmaceuticals, Asian J Chem., 2010; 22 :2129-2135
41. Mandil H ,Sakur A A,Alulu S, Differential Pulse Polarographic Analysis of Glyburide in Pure form and Pharmaceutical Formulations, Asian J Chem., 2012; 24:2980-2984
42. Mandil H ,Sakur A A,Alulu S,Polarographic behavior and quantification of the Anti-diabetic drug Repaglinide in pure form and pharmaceutical formulations, Int J Pharmacy and Pharm Sci, 2013; vol 5 issue 3.
43. Lentner C, Geigy Scientific Tables, 8th ed., Ciba-Geigy, 3 (1984) 58–60.
44. Ocana JA, Barragan FJ, CallejónM,Spectrofluorimetric determination of moxifloxacin in tablets, human urine and serum Analyst,2000;125: 2322.
45. Purvis T, Mattucci ME, Crisp MT, Johnston KP, Williams RO, Rapidly Dissolving Repaglinide Powders Produced by the Ultra-Rapid Freezing Process. AAPS Pharm sci 2007; 8(3):1-9.
46. Zuman P, The Elucidation of Organic Electrode Processes, Academic Press, New York, 1969; 21-24.
47. Joseph W, Analytical Electochemistry,3thd ed., Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
48. Al-Omar M., Al-Majed A, Sultan M, Gadkariem EA, Belal F, Voltammetric study of danazol and its determination incapsules and spiked biological fluids, Journal of Pharmaceutical and Biomedical Analysis 2005;37:199–204.
49. Ghoneim EM, El-Attar MA, HammamE,Khashaba PY, Stripping voltammetric quantification of the anti-diabetic drug glipizide in bulk form and pharmaceutical formulation, Journal of Pharmaceutical and Biomedical Analysis, 2007; 43, 1465–1469.