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A simple electroanalytical method for estimation of Pioglitazone Hydrochloride and Gliclazide individually from pharmaceutical formulation.

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ABSTRACT

A simple, specific, accurate, precise and reproducible method has been developed and validated for the Pioglitazone Hydrochloride and Gliclazide individually using Differential Pulse Polarography (DPP) technique. Quantification of Pioglitazone Hydrochloride and Gliclazide was done in Britton-Robinson Buffer pH 6.0 and pH 2.5 respectively using 1M KCl as a supporting electrolyte. Both Pioglitazone Hydrochloride and Gliclazide exhibit reduction cathodic peak in given respective pH with peak potential (Ep) as -1.62V for Pioglitazone Hydrochloride and -1.180V for Gliclazide vs. S.C.E. .The parameters were used for method validation are linearity; accuracy, precision, LOD and LOQ. Proposed method was successfully applied for routine quality control analysis and determination Pioglitazone Hydrochloride and Gliclazide individually in drug formulation.

KEYWORDS: *Differential Pulse Polarography (DPP), Pioglitazone Hydrochloride (PIO) Gliclazide (GL), Britton-Robinson Buffer,*

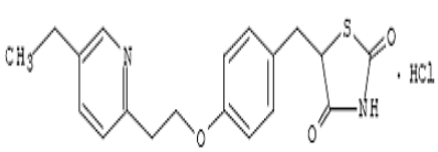
INTRODUCTION

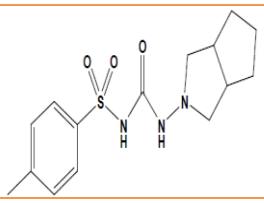
Diabetes develops when the level of blood sugar increases due to insufficient insulin secreted from the pancreas. Blood sugar is then released via urination, leading to “sugary urine”, or diabetes. The disease may give rise to multiple complications, and in severe cases, it can lead to coma. For maintaining constant level of blood sugar in such diabetic patients, long term treatment is needed. Anti-diabetic medications treat diabetes mellitus by lowering glucose levels in the blood.

Pioglitazone Hydrochloride (PIO), belongs to the drug class of thiazolidinedione, is used to decrease insulin resistance. It is an antidiabetic agent to manage a certain type of diabetes like NIDDM (non-insulin-dependent diabetes mellitus, sugar diabetes) called type 2 diabetes. It improves glycemic control while reducing circulating insulin levels. Pioglitazone Hydrochloride decreases plasma glucose concentrations, insulin concentrations, and glycosylated hemoglobin. Additional favorable metabolic effects include decreased hepatic glucose output, lower free fatty acid concentrations, and improved lipid profiles. Thiazolidinediones such as pioglitazone help insulin to work more effectively.

Gliclazide (GL) is a second-generation sulphonylurea oral hypoglycemic agent used in the treatment of non-insulin dependent diabetes mellitus. Gliclazide stimulates insulin secretion by pancreatic beta cells. In the long-term, it reduces hepatic gluconeogenesis, and increases insulin effects by acting at receptor or post-receptor sites. It also inhibits platelet aggregation and increases fibrinolysis .Sometimes insulin that is being produced by the body is not able to help sugar get inside the body’s cells. Sulfonylureas help insulin get into the cells where it can work properly to lower blood sugar.

Structure





Different analytical methods including HPLC, Evaporative Light Scattering Detection and simultaneous spectrophotometric estimation of gliclazide and metformin hydrochloride in combined dosage forms have been reported. The literature survey revealed that for metformin methods on UV absorption , High Performance Liquid Chromatography (HPLC) have been reported.

A literature surveys reveals few Chromatographic methods i.e. HPLC HPTLC, Derivative and Extractive spectrophotometric methods for the simultaneous determination of Pioglitazone Hydrochloride and Gliclazide. Very little attention has been paid to the use of electroanalytical methods.

The present study gives a simple, rapid, efficient, reliable and economic method for the determination of Pioglitazone Hydrochloride and Gliclazide individually in pharmaceutical formulations using Differential Pulse Polarography technique. The proposed method has been validated as per ICH guidelines.

MATERIALS AND METHODS (EXPERIMENTAL)

INTRODUCTION TO WORKSTATION

Electrochemical workstation- PG STAT 30 with 663 VA Electrode stand (Metrohm)

It is made up of three electrode system namely-

1) Hanging Mercury Drop electrode (HMDE) as the working electrode
2) Saturated calomel electrode as the reference electrode
3) Platinum electrode as the counter electrode

The pH measurements were made with Euiptrances model No. 610.

REAGENTS

Standard PIO and GL was obtained from local pharmaceutical company. All the solutions were prepared in double distilled water. All the reagents use were of AR grade. Britton-Robinson buffer solutions-[100ml of 0.04M H_3BO_4 + 0.04M H_3PO_4 + 0.04M CH_3COOH]. Further the desired value of pH (6.0) and (2.5) was adjusted with the addition of 1M NaOH.

ANALYTICAL METHOD DEVELOPMENT

PREPARATION OF STANDARD SOLUTION

Preparation of 500 µg/mL stock solution of the standard PIO

Accurately weighed 0.025 gm of PIO was transferred into a 50 cm^3 volumetric flask and diluted up to the mark with ethanol: water (80:20V/v)

Preparation of 1000 µg/mL stock solution of the standard GL

Accurately weighed 0.050 gm of GL was transferred into a 50 cm^3 volumetric flask and diluted up to the mark with methanol: water (60:40V/v).

Further all the standard solutions containing PIO and GL were prepared using this stock solution.

PROPOSED VOLTAMMETRIC METHOD

An aliquot of 20cm3 made up of 18 mL Britton-Robinson Buffer adjusted to pH 6.0 for PIO and pH 2.5 for GL by 1M NaoH + 2 mL of 1M KCl as a supporting electrolyte was placed in the dry and clean Voltammetric cell. Then it was purged with highly pure nitrogen gas for 180s. A negatively directed DP scans between the potential 0.0 V to -2.0 V vs. S.C.E was applied. The operational parameters were as follows: For PIO 1] Scan rate- 10 mV s-1. 2] Pulse amplitude- 50mV and for GL 1] Scan rate- 10 mV s-1. 2] Pulse amplitude- 50mV. After recording a polarogram of blank, aliquots of

(0.3mL) the required standard PIO (100µg/ml) and (0.5mL) of standard GL (500µg/ml) solutions were added from the standard stock solution. Resulted polarograms were recorded under the optimum experimental conditions. Peak currents were recorded. Calibration curve was prepared by plotting peak current versus concentration of PIO and GL applied. The results were shown in [**Table-1**]

Table.1: Optimum Conditions and Parameters for the polarographic determination of PIO and GL

Conditions	Values	
	Pioglitazone Hydrochloride (PIO)	**Gliclazide (GL)**
solvent	with ethanol: Water (80:20V/v)	with methanol: Water (60:40V/v)
Optimum PH	Britton-Robinson Buffer of pH 6.0	Britton-Robinson Buffer of pH 2.5
Supporting Electrolyte	1M KCl	1M KCl
Peak Potentials	-1.62V	-1.18V
Scan rate (mVs-1)	15 mVs-1	15 mVs-1

PREPARATION OF SAMPLE SOLUTION

Two commercial brands containing of PIO and GL were procured. Each brand contained a label claim of 30mg of PIO and 30mg of GL per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder equivalent to 10mg of PIO and 50mg of GL was accurately weighed, transferred quantitatively to 50 mL two separate volumetric flask; then added ethanol: water (80:20V/v) and methanol: water (60:40V/v) in two separate flasks and the it was vortexed for 10mins, the solution was filtered through Whatman filter paper no 41.and finally volume of the solution was made up to 50 mL with respected solvents. Polarograms for the sample solutions were analyzed by the method described as above. Polarograms were recorded under the optimum experimental conditions. The amount of PIO and GL was calculated from resulting peak current values using already constructed calibration graph. (**Figure-2**).

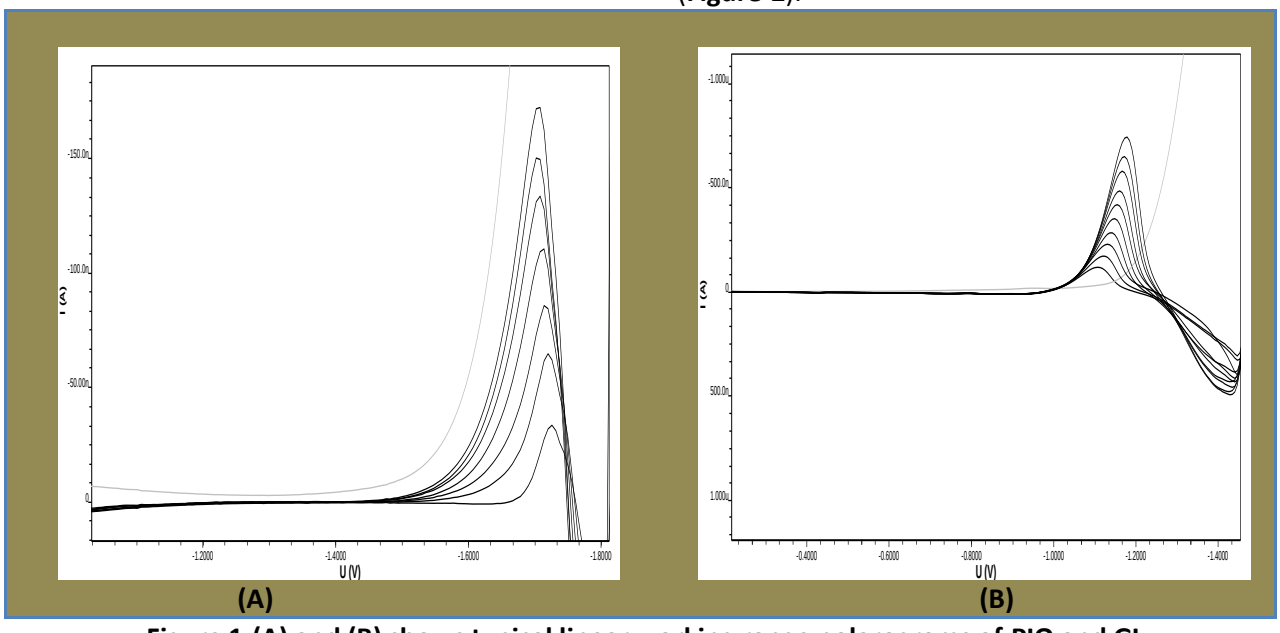
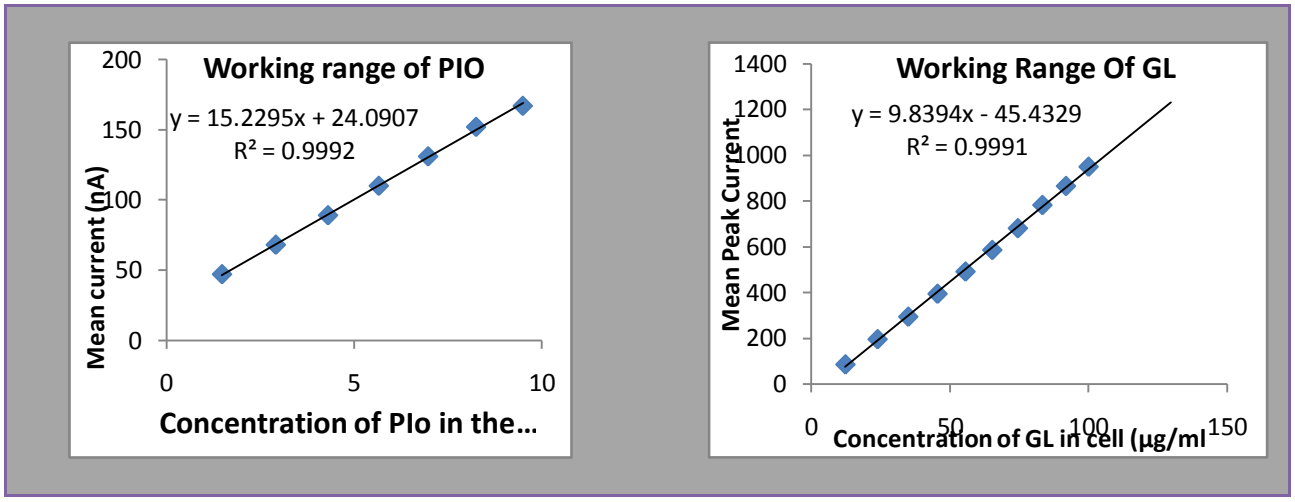


Figure 1-(A) and (B) shows typical linear working range polarograms of PIO and GL



(A) (B)

Figure 2-(A) and (B) shows calibration curve for working range of PIO and GL

ANALYTICAL METHOD VALIDATION

SYSTEM SUITABILITY

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recording polarogram for PIO (3.92 µg/ml) and for GL (55.55 µg/ml) with five replicates and the mean was used for the whole calculations. The % RSD was found to be 1.85 for PIO and 0.54 for GL, which was acceptable as it is less than 2%.

SPECIFICITY

The specificity of method was confirmed by observing the polarograms of both the combined standard solution and the drug sample solutions. The polarograms obtained from the drugs sample solution were found to be identical to those obtained for standard solution. The addition of the standard solution to the drug sample solution did not change the characteristics of differential pulse polarogram. This gives the validity of method for the determination of both drugs from combined pharmaceutical formulation.

LINEARITY AND RANGE

The linearity for PIO and GL were observed by addition of standard solution. A good linearity was achieved in the concentration ranges of 1.47 µg/ml to 9.50 µg/ml for PIO and 12.19 µg/ml to 100.00 µg/ml GL. (**Figure-1**)The calibration curves were constructed with concentration (C) against peak current (Ip). The slope, Intercept, regression equation and correlation coefficient for the PIO and GL was obtained is given in (**Table-2**)

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The limit of detection (LOD) and the limit of quantification (LOQ) for PIO and GL were determined by signal to noise ratio of 3:1 and 10:1 respectively. The replicates for blank solution were recorded 20 times and the mean current value at the reduction potential of PIO (i.e. at -1.62 V) and GL (i.e. at -1.180V) was calculated. The concentration at which the peak current was found three times that of mean blank current was taken as a limit of detection. And the concentration at which peak current was found to be ten times than the mean blank current was selected as limit of quantification.

The LOD and LOQ of PIO were 1.21 µg/ml and 1.42 µg/ml and GL was found to be 1.47 µg/ml and 2.35 µg/ml respectively. (**Table-2**)

INTRADAY AND INTERDAY PRECISON

The intra-day and inter-day precision was used to study the variability of the method. It was checked by recording the polarograms of standard solutions of norfloxacin and tinidazole i.e. whole concentration ranges 1.47 µg/ml to 9.50 µg/ml for

PIO and 12.19 µg/ml to 100.00 µg/ml GL. Both at intra-day (five times within 24 hour) and inter-day (two times each. during 3 days intervals) to check the precision. The mean % RSD for intra-day and inter-day precision for PIO found to be 0.83% and 1.23% and for GL it was 0.68% and 0.96%, respectively. (**Table-2**)

Table 2: METHOD VALIDATION PARAMTERS FOR DETERMINATION OF RM AND MET

Parameters	Values	
	PIO	**GL**
System suitability (n=5) %RSD	1.85%	0.54%
Linearity range (µg/ml)	1.47 to 9.50 µg/ml	12.19 to 100.00 µg/ml
Slope (m) [a)]	15.2295	9.8394
Intercept(c) [a)]	24.0907	45.4329
Correlation coefficient (R^2)	0.9992	0.9991
LOD (µg/ml)	1.21 µg mL^{-1}	1.47 µg mL^{-1}
LOQ (µg/ml)	1.42 µg mL^{-1}	2.35 µg mL^{-1}
Intraday precision (n=5)	0.83%	0.68%
Interday precision (n=5)	1.23%	0.96%
Assay	98% to 102%	98% to 102%
Recovery	98% to 102%	98% to 102%

a) Of the equation y = mx + c, where y is peak area, m is the slope, x is the Concentration and c is the intercept

ASSAY

The developed Polarographic method was used for determination of PIO and GL from different brands of formulations. The sample working solutions were analyzed by the developed method described above. Polarograms were recorded under the optimum experimental conditions. Resulting peak currents of PIO and GL were measured and the amount of PIO and GL calculated using already constructed calibration graph. Assay studies were carried out at three different levels. The percentage assay at three different levels for PIO and GL was found to be from 98.00 % to 102.00 %. The results were shown in (**Table 3 and 4**).

ACCURACY (RECOVERY)

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the standard addition method. A fixed volume of standard PIO and GL solution was mixed with different concentrations of pre-analyzed sample solutions and mixtures were analyzed by proposed method. The percent recovery was determined at different levels. The results were shown in [**Table-5 and 6**]

Table 3: RESULTS OF ASSAY STUDIES FOR PIO

Brand name	Piosys*30 (Systopic lab pvt ltd)
A.P.I	Pioglitazone Hydrochloride
Labeled claim (mg)	30mg
Drug found in mg	29.83
% RSD (n=5)	1.65
% Assay	99.43

Table 4: RESULTS OF ASSAY STUDIES FOR GL

Brand name	Reclide-30 (Dr.Reddy's Lab)
A.P.I	Gliclazide
Labeled claim (mg)	30mg
Drug found in mg	29.90
% RSD (n=5)	0.85
% Assay	99.6

Table 5: RESULTS OF RECOVERY STUDIES FOR PIO

Standard	Level	Conc. Of std [µg/ml]	Conc. of std Found [µg/ml]	Recovery (%)
PIO	50%	0.97	0.98	101
	200%	3.84	3.89	100..3
	400%	7.54	7.52	99.7

Mean	100.6
% RSD	0.84

Table 6: RESULTS OF RECOVERY STUDIES FOR GL

Standard	Level	Conc. Of std [µg/ml]	Conc. of std Found [µg/ml]	Recovery (%)
GL	30%	11.11	11.04	99.36
	90%	31.91	32.18	100.8
	140%	51.02	50.13	98.27

Mean	99.47
% RSD	1.27

RESULT AND DISSCUSSION

In the present study quantification of PIO and GL have been done from the formulations using Differential Pulse Polarography technique. The developed method was validated as per the ICH guidelines. But before the method development and subsequent validation, optimization of the conditions for the analyte was done i.e. pH, supporting electrolyte and also the parameters i.e. 1] scan rate 2] Pulse amplitude has been studied. During optimization of the conditions, the polarographic response of PIO and GL in different

buffer solutions have been studied i.e. Acetate, Phosphate and Britton-Robinson Buffer. Britton-Robinson buffer was prepared by mixing 0.04M Boric acid, 0.04M Phosphoric acid and 0.04M Glacial acetic acid. Further pH was adjusted with 1M NaOH. In the Britton-Robinson Buffer the whole pH range i.e. pH 2.0 to pH 10.0 has been studied.

As the pH was shifted from acidic to basic there is change in peak potential was observed. Finally Britton-Robinson Buffer of pH 6.0 and 2.5 was chosen as the best, due to good separation of both the analytes, more uniform peak shape, less tailing, less broadening of peak, normal base line start and regression analysis. The 1MKCl used as a supporting electrolyte. With 1MKCl more uniform and sharper peaks were observed. Pulse amplitude of 50mV was chosen as optimum as there is loss of resolution at high pulse amplitude.

The Differential Pulse polarograms of PIO and GL were recorded at various scan rates. At higher scan rate than $15mVs^{-1}$ the width of peak increases, its height decrease and peak shape was distorted. At slower scan rate than $15mVs^{-1}$ uniform peak shape and peak height was small as compared to that of higher scan rate than $15mVs^{-1}$, so a scan rate of $15mVs^{-1}$ was chosen as a best for the analysis. The height of peak increase gradually with concentration of norfloxacin and tinidazole and the response of peak current $\mathbf{i_p}$ as function of concentration is linear.

No significant interference was observed from excipients commonly used in the formulation i.e. glucose, sucrose, starch, magnesium stearate or talc powder.

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