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A SIMPLE ELECTROANLYTICAL METHOD FOR ESTIMATION OF ROSIGLITAZONE MALEATE AND METFORMIN HYDROCHLORIDE INDIVIDUALLY FROM PHARMACEUTICAL FORMULATION.

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ABSTRACT

A simple, specific, accurate, precise and reproducible method has been developed and validated for the Rosiglitazone Maleate and Metformin Hydrochloride individually using Differential Pulse Polarography (DPP) technique. Quantification of Rosiglitazone Maleate and Metformin hydrochloride was done in Britton-Robinson Buffer pH 5.0 and pH 4.0 respectively using 1M KCl as a supporting electrolyte. Both Roziglitazone Maleate and Metformin Hydrochloride exhibit reduction cathodic peak in given respective pH with peak potential (Ep) as -1.20V for Rosiglitazone Maleate and -1.44V for Metformin Hydrochloride vs. S.C.E. 0.1N HCl was used as Solvent for the analysis. 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 Rosiglitazone Maleate and Metformin Hydrochloride individually in drug formulation.

Key words: Differential Pulse Polarography (DPP), Rosiglitazone Maleate (RM), Metformin hydrochlorid (MET), Britton-Robinson Buffer

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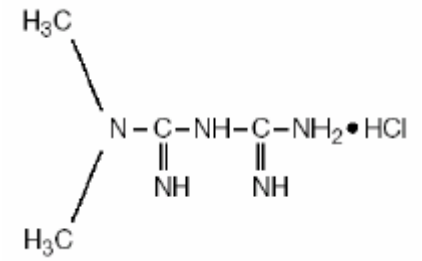
INTRODUCTION

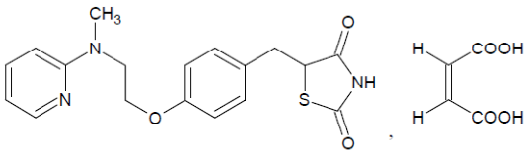
Diabetes develops when the level of blood sugar increases due to insufficient insulin secreted from the pancreas. Blood sugar is then released via urination, leading to “sugary urine”, or diabetes. The disease may give rise to multiple complications, and in severe cases, it can lead to coma. For maintaining 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.

Biguanides: This group of anti diabetic drugs is used in the treatment of diabetes due to its ability to reduce hepatic glucose content; for example Metformin. **Metformin** is N, N-dimethylimidodicarbonimidic diamide hydrochloride. It is an oral anti-diabetic drug from the Biguanides class. It is the first-line drug for the treatment of type II diabetes, particularly in

overweight and obese people and those with normal kidney function.



Thiazolidinediones: This class of anti diabetic drugs are also known as 'Glitazones'. e.g. Roziglitazone. **Rosiglitazone maleate** is an oral anti diabetic drug and chemically, it is (±)-5-{p-[2- (methyl-2-pyridylamino) ethoxy] benzyl}-2, 4-thiazolidinedione maleate. Rosiglitazone maleate improves glycemic control while reducing circulating insulin levels. Pharmacological studies in animal models indicate that rosiglitazone improves sensitivity to insulin in muscle and adipose tissue and inhibits hepatic gluconeogenesis.



Different analytical methods including HPLC, Evaporative Light Scattering Detection[12] 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 Rosiglitazone Maleate and Metformin Hydrochloride. Very little attention has been paid to the use of electroanalytical methods.

OBJECTIVE

The present study gives a simple, rapid, efficient, reliable and economic method for the determination of Rosiglitazone Maleate and Metformin Hydrochloride 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 RZ and MT 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 (6.5) was adjusted with the addition of 1M NaOH.

ANALYTICAL METHOD DEVELOPMENT

PREPARATION OF STANDARD SOLUTION

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

Accurately weighed 0.050 gm of RM was transferred into a 50 cm^3 volumetric flask and diluted up to the mark with methanolic HCl (60:40) .

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

Accurately weighed 0.050 gm of MET was transferred into a 50 cm^3 volumetric flask and diluted up to the mark with Distilled water.

Further all the standard solutions containing RM and MET were prepared using this stock solution.

PROPOSED VOLTAMMETRIC METHOD

An aliquot of 20cm3 made up of 18 mL Britton-Robinson Buffer adjusted to pH 5.0 for RM and pH 4.0 for MET 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 Rosiglitazone Maleate 1] Scan rate- 10 mV s-1. 2] Pulse amplitude- 50mV and for Metformin Hydrochloride 1] Scan rate- 10 mV s-1. 2] Pulse amplitude- 50mV. After recording a polarogram of blank, aliquots of (1mL) the required standard Rosiglitazone Maleate and (1mL) Metformin Hydrochloride 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 Rosiglitazone Maleate and Metformin Hydrochloride applied. The results were shown in [Table.1]

Table.1: Optimum Conditions and Parameters for the polarographic determination of RM and MET

Conditions	**Values**	
	Rosiglitazone Maleate (RM)	**Metformin Hydrochloride (MET)**
solvent	with methanolic HCl (60:40)	0.1N HCl
Optimum PH	Britton-Robinson Buffer of pH 5.0	Britton-Robinson Buffer of pH 4.0
Supporting Electrolyte	1M KCl	1M KCl
Peak Potentials	-1.20V	-1.44V
Conditions	Rosiglitazone Maleate	Metformin Hydrochloride
Scan rate (mVs-1)	15 mVs-1	15 mVs-1

PREPARATION OF SAMPLE SOLUTION

Two commercial brands containing of RM and MET were procured. Each brand contained a label claim of 2mg of RM and 500mg of MET per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder equivalent to 10mg of RM and 20mg of MET was accurately weighed, transferred quantitatively to 50 mL two separate volumetric flask; then added Methanolic Hcl and Distilled water 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 RM and MET was calculated from resulting peak current values using already constructed calibration graph.

(For RM: y=1.4074x+ 57.0723) and (for MET: y = 17.3216x + 114.2329)

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 RM (52.17 µg/ml, 92.3 µg/ml, 124.28 µg/ml) and for MET (7.69 µg/ml, 11.65 µg/ml, 17.22 µg/ml with five replicates and the mean was used for the whole calculations. The % RSD was found to be 1.36 for RM and 0.85 for MET, 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 RM and MET were observed by addition of standard solution. A good linearity was achieved in the concentration ranges of 19.04 µg/ml 114.28 µg/ml for RM and 1.98 µg/ml to 18.18 µg/ml MET. The calibration curves were constructed with concentration (C) against peak current (Ip). The slope, Intercept, regression equation and correlation coefficient for the tinidazole was obtained is given in (Table.

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The limit of detection (LOD) and the limit of quantification (LOQ) for RM and MET 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 RM (i.e. at -1.20 V) and MET (i.e. at -1.44V) was calculated. The concentration at which the peak current was found three times 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 RM were 11.04 µg/ml and 18.88 µg/ml. And MET was found to be 0.99 µg/ml and 1.81 µg/ml respectively.

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 (19.04 µg/ml to 114.28 µg/ml for RM and 1.98 µg/ml to 18.18 µg/ml for MET) 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 RM found to be 1.01% and 0.93% and for MET it was 0.53% and 0.76%, respectively.

ASSAY

The developed Polarographic method was used for determination of Rosiglitazone Maleate and Metformin Hydrochloride 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 Rosiglitazone Maleate and Metformin Hydrochloride were measured and the amount of Rosiglitazone Maleate and Metformin Hydrochloride calculated using already constructed calibration graph. Assay studies were carried out at three different levels. The percentage assay at three different levels for Rosiglitazone Maleate and Metformin Hydrochloride was found to be from 98.00 % to 102.00 %. The results were shown in (Table 2).

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

Parameters	Values	
	RM	**MET**
System suitability (n=5) %RSD	1.36%	0.85%
Linearity range (µg/ml)	19.04 to 114.28 µg/ml	1.98 to 18.18 µg/ml
Slope (m) [a)]	1.4704	17.3216
Intercept(c) [a)]	57.0723	114.2329
Correlation coefficient (R^2)	0.9996	0.9996
LOD (µg/ml)	11.04 µg mL^{-1}	0.99 µg mL^{-1}
LOQ (µg/ml)	18.88 µg mL^{-1}	1.81 µg mL^{-1}
Intraday precision (n=5)	1.08%	0.77%
Interday precision (n=5)	0.93%	0.53%
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.

ROBUSTNESS

The robustness of the method was examined by the consistency of peak height and peak shape with the deliberately small changes in the experimental parameter. It is a measure of its capacity to retain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the proposed method, the following variations were made in the analytical method-

1] Scan rate by **±** 0.5 mVs^{-1}. 2] Pulse amplitude **±** 1.0 mV

These parameters were deliberately changed one at a time and the effect of these changes on the assay studies was carried out. The proposed method was found to be robust.

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 Rosiglitazone Maleate and Metformin Hydrochloride 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.5-6] [1-12]

Table 4: RESULTS OF ASSAY STUDIES FOR RM

Brand name	**Glycomet (USV)**
A.P.I	Metformin Hydrochloride
Labeled claim (mg)	500mg
Drug found in mg	492.46
% RSD (n=5)	1.00
% Assay	98.49

Table 5: RESULTS OF RECOVERY STUDIES FOR RM

Standard	Level	Conc. Of std [µg/ml]	Conc. of std Found [µg/ml]	Recovery (%)
RM	50%	17.39	17.68	101.6
	150%	48	48.28	100.5
	250%	74.07	73.89	99.7

Mean	100.6
% RSD	0.76

Table 6: RESULTS OF RECOVERY STUDIES FOR RM

Standard	Level	Conc. Of std [µg/ml]	Conc. of std Found [µg/ml]	Recovery (%)
MET	75%	2.92	2.89	98.9
	175%	6.69	6.61	98.8
	275%	11.21	11.12	99.2

Mean	98.96
% RSD	0.21

Figure- 1. POAROGRAMS OF NF AND TZ

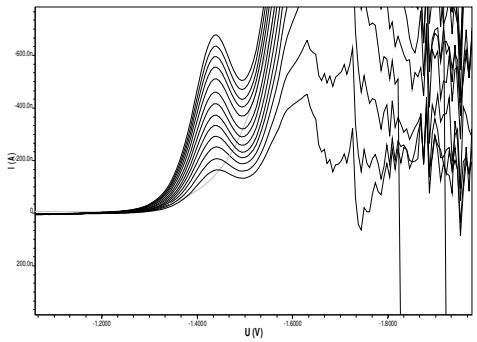


Figure shows typical linear working range of RM

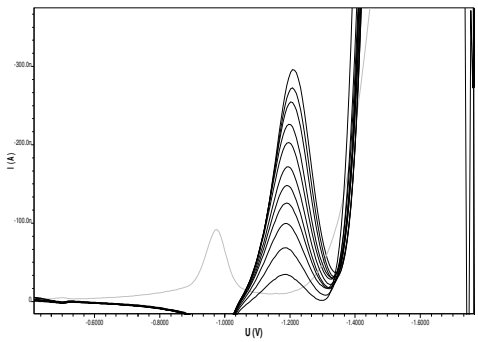
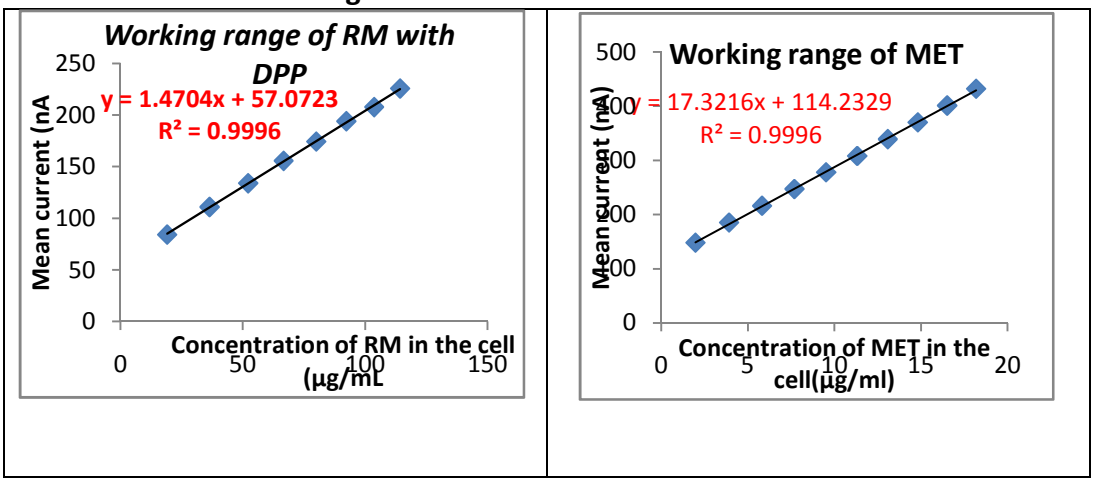


Figure shows typical linear working range of MET

Figure-2. LINEARITY GRAPHES FOR



RESULT AND DISSCUSSION

In the present study quantification of Rosiglitazone Maleate and Metformin Hydrochloride have been done from the formulations using Differential Pulse Polarography technique. The developed method was validated as per the ICH guidelines (Table 1-3). 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 Rosiglitazone Maleate and Metformin Hydrochloride 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.

Table 3: RESULTS OF ASSAY STUDIES FOR RM

Brand name	Windia 2 mg (Glaxo smith)
A.P.I	Rosiglitazone Maleate
Labeled claim (mg)	2mg
Drug found in mg	2.008
% RSD (n=5)	1.74
% Assay	99.75

As the pH was shifted from acidic to basic there is change in peak potential was observed. Finally Britton-Robinson Buffer of pH 5 and 4 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 KCl used as a supporting electrolyte. With KCl 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 Rosiglitazone Maleate and Metformin Hydrochloride 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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