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SIMULTANEOUS QUANTIFICATION OF OFLOXACIN AND ORNIDAZOLE IN COMBINED DRUG FORMULATION BY A SIMPLE ELECTRO-ANLYTICAL TECHNIQUE

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Keywords:

linear sweep Voltammetry (LSV), Ofloxacin, Ornidazole, Britton-Robinson Buffer, Pharmaceutical formulations

Abbreviations:

OF- Ofloxacin;
OZ- Ornidazole;
Linear sweep Voltammetry- LSV

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ABSTRACT

In present study, a successful attempt has been made to develop a simple method for the simultaneous determination of ofloxacin and ornidazole using linear sweep Voltammetry (LSV) technique. Quantification of ofloxacin and ornidazole was done in Britton-Robinson Buffer of pH 5.5 using 0.1M KCl as a supporting electrolyte. Both ofloxacin and ornidazole exhibit reduction cathodic peak in given pH with peak potential at -1.30 V for ofloxacin and -0.42V for ornidazole vs. S.C.E. 0.1N HCl was used as Solvent for the analysis. The method was validated in the terms of its linearity, accuracy, precision, robustness, ruggedness, LOD and LOQ. Proposed method was found to be simple, precise, and accurate and can be successfully applied for routine quality control analysis and simultaneous determination of ofloxacin and ornidazole in combined drug formulations.

INTRODUCTION: In the topical countries like India, the major problems of health arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. Infections caused by the microorganisms like, fungi, protozoa, are most common. Drugs with antifungal and antiprotozoal activity have been used in the treatment of the same.

In many cases, drugs with two active ingredients are prescribed to the patients to have an added advantage. Many of these antibacterial drugs are found in combination with antifungal and antiprotozoal drugs which are highly effective against fungal and protozoal infections.

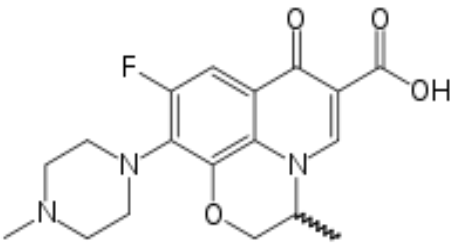
Ornidazole, $C_7H_{10}ClN_3O_3$ that is 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole, is used as an antiprotozoal drug. (Molecular weight: - 219.625 g/mol)

Ofloxacin $C_{18}H_{20}FN_3O_4$ that is (*RS*)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)- 10- oxo- 4- oxa- 1- azatricyclo [7.3.1.0 $^{5,\ 13}$] trideca- 5(13), 6, 8, 11-tetraene-11-carboxylic acid is used as a antibacterial drugs (Molecular weight: - 361.368 g/mol).

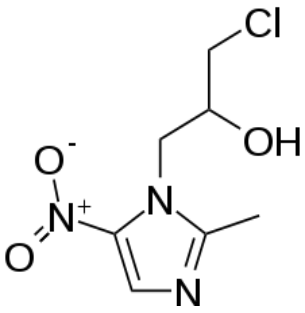
It is highly effective for bacterial and protozoan infections and is available in the tablet form. Few Chromatographic such as HPLC [1], HPTLC [2], and Derivative and Extractive spectrophotometric methods spectrophotometric methods [3-5] have been reported for the simultaneous determination of ofloxacin and ornidazole. But, Very little attention has been paid to the use of electroanalytical techniques [6-8]. A literature survey has revealed cyclic voltammetry and D.C polarography methods for the determination of ornidazole and ofloxacin individually, but its simultaneous determination by using linear sweep Voltammetry has not been reported.

Objective: The main objective of study is to provide a simple, rapid, efficient, reliable and economic method for the simultaneous determination of ofloxacin and ornidazole in combined pharmaceutical drug formulations using Linear Sweep Voltammetry technique. The proposed developed method to be subsequently validated as per ICH guidelines [9-10].

Structure:



OFLOXACIN



ORNIDAZOLE

MATERIALS AND METHODS:

Instruments:

Introduction to Electrochemical 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 ofloxacin and ornidazole was obtained from local pharmaceutical company. All the solutions were prepared in double distilled water. All the reagents use were of AR grade.

Preparation of standard solutions: 10mg of standard Ofloxacin and 25mg of standard ornidazole was accurately weighed and dissolved in 0.1N HCl and made up to a volume of 50 mL in standard flask to give stock solution (200 µg/mL of ofloxacin and 500 µg/mL of ornidazole respectively) Further all the standard solutions containing the mixture of ofloxacin and ornidazole were prepared using this stock solution.

Proposed Polarographic Method: An aliquot of 20cm^3 made up of 18 mL Britton-Robinson Buffer adjusted to pH 5.5 by 1M NaOH + 2 mL of 0.1M 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 scan between the potential of 0.0 V to -2.0 V vs. S.C.E was applied. The operational parameters were as follows: 1] Scan rate: 15 mV $s^{-1.}$ 2] Pulse amplitude: 50mV (**Table 2**). After recording a polarogram of blank, aliquots of (0.5mL) the required standard ornidazole 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 OF and OZ in µg/mL (ppm).

Preparation of sample solution: Two commercial brands containing of ofloxacin and ornidazole in combination were procured. Each brand contained a label claim of 200 mg of ofloxacin and 500 mg of

ornidazole per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder (45mg) equivalent to 10mg of ofloxacin and 25mg of ornidazole was accurately weighed, transferred quantitatively to 50 mL volumetric flask; then added 0.1N HCl in it and the mixture was vortexed for 10mins, the solution was filtered through Whatmann filter paper no. 41 and finally volume of the solution was made up to 50 mL with distilled water. Polarograms for the sample solutions were analyzed by the method described as above. Polarograms were recorded under the optimum experimental conditions. The amount of ofloxacin and ornidazole was calculated from resulting peak current values using already constructed calibration graph.

(For ofloxacin: y= 16.6541x + 18.7124) (**Figure 2**) and (for ornidazole: y = 6.8830x + 1.6322) (**Figure 2**)

Analytical Method Validation [9-10]:

System Suitability: System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recording polarogram for ofloxacin (22.22 µg/mL) and for ornidazole (55.55 µg/mL) with five replicates and the mean was used for the whole calculations. The % RSD was found to be 0.69 for ofloxacin and 0.46 for ornidazole, which was acceptable as it is less than 2% (**Table 1**).

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 ofloxacin and ornidazole were observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of 4.87µg/mL to 36.73µg/mL for ofloxacin and 12.19µg/mL to 91.83µg/mL for ornidazole. The calibration curves were constructed with concentration (C) against peak current (Ip). The slope, Intercept, regression equation and correlation coefficient for the ornidazole was obtained is given in (**Table 1**) **(Figure 1 and 2)**

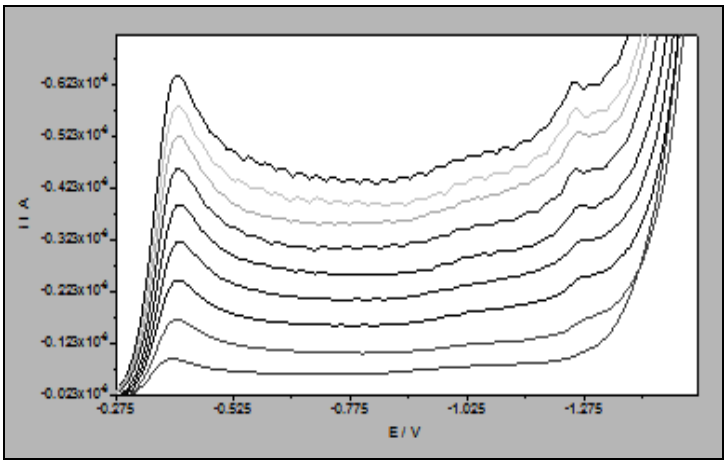
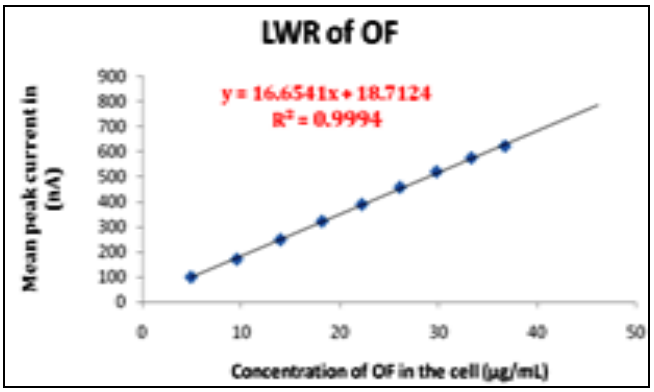


FIG. 1: POLAROGRAM FOR OFLOXACIN AND ORNIDAZOLE STANDARD



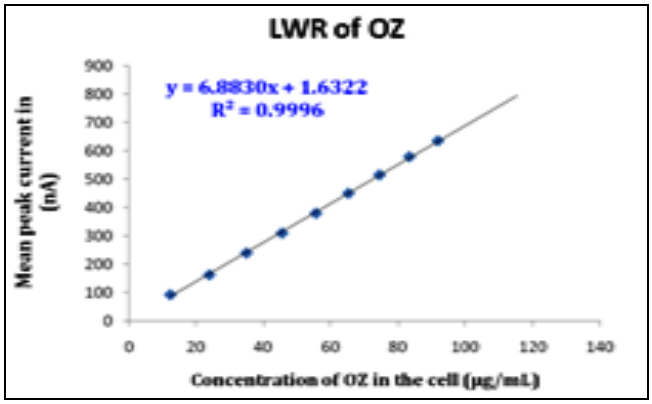


FIG. 2: LINEARITY GRAPH FOR; 1). OFLOXACIN STANDARD AND 2). ORNIDAZOLE STANDARD

Limit of Detection and Limits of Quantitation: The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. The LOD and LOQ of ofloxacin were 1.98µg/mL and 4.87µg/mL. And ornidazole was found to be 0.98µg/mL and 2.48µg/mL respectively (**Table 1**).

Intra-day and Inter-day precision: 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 ofloxacin and ornidazole i.e. whole concentration ranges (4.87µg/mL to 36.73µg/mL for ofloxacin and 12.19µg/mL to 91.83 µg/mL for ornidazole) 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 ofloxacin found to be 0.75% and 0.60% and for ornidazole it was 0.65% and 0.45%, respectively (**Table 1**).

Assay: The developed Polarographic method was used for determination of ornidazole from two 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 ofloxacin and ornidazole were measured and the amount of ofloxacin and ornidazole calculated using already constructed calibration graph. Assay studies were carried out at three different levels i.e. 40%, to 150% level. The percentage assay at three different levels for ofloxacin and ornidazole was found to be from 98.00 % to 102.00 %. The results were shown in **Table 2.**

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 method of standard addition. A fixed volume of standard ornidazole solution was mixed with different concentrations of preanalyzed sample solutions and mixtures were analyzed by proposed method. The percent recovery was determined at different levels i.e., from 40% to 130% level. The results of recovery analysis for ofloxacin and ornidazole are shown in **Table 4.**

RESULTS AND DISCUSSION: In the present study quantification of ofloxacin and ornidazole have been done from the formulations using Differential Pulse Polarography technique. The developed method was validated as per the ICH guidelines (**Table 1, 2, 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 (**Table 4**). During optimization of the conditions, the polarographic response of ornidazole in different buffer solutions have been studied i.e. Acetate, Phosphate and Britton-Robinson Buffer.

TABLE 1: METHOD VALIDATION PARAMETERS FOR THE DETERMINATION OF OFLOXACIN AND ORNIDAZOLE

Parameters	Values	
	Ofloxacin	Ornidazole
System suitability (n=5) %RSD	0.69%	0.46%
Linearity range (µg/mL)	4.87 to 36.73µg/mL	12.19 to 91.83µg/mL
Slope (m) [a)]	16.6541	6.8830
Intercept(c) [a)]	18.7124	1.6322
Correlation coefficient (R^2)	0.9994	0.9996
LOD (µg/mL)	1.98 µg/mL	0.98 µg/mL
LOQ (µg/mL)	4.87 µg/mL	2.48 µg/mL
Intraday precision (n=5)	0.75%	0.65%
Interday precision (n=5)	0.60%	0.45%
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 c Concentration and c is the intercept

TABLE 2: RESULT OF ASSAY STUDIES OF OFLOXACIN AND ORNIDAZOLE

Brand Name	OFNOF (Aristo)		O2 (Medley)	
	Ofloxacin	Ornidazole	Ofloxacin	Ornidazole
Labeled claim (mg)	200mg	500mg	200mg	500mg
Drug found in mg	198.8 mg	499.1 mg	201.2 mg	500.9 mg
% RSD (n=5)	0.440	0.941	0.607	0.836
% Assay	99.4%	99.8 %	100.6 %	100.18%

TABLE 3: RESULTS OF RECOVERY STUDIES OF OFLOXACIN AND ORNIDAZOLE

Standard	Level	Conc. of std [µg/ml]	Conc. of std found [µg/ml]	RSD (%) (n = 5)	Recovery (%)
Ofloxacin	0	4.87	4.85	0.58	99.6%
	40 %	9.30	9.28	0.57	99.8%
	80%	17.77	17.95	0.47	101.01%
	130%	29.16	29.22	0.32	100.2%
				Mean	**100.40%**
Ornidazole	0	12.19	12.09	0.71	99.2%
	40 %	23.25	23.48	0.25	101%
	80%	44.44	44.66	0.69	100.5%
	130%	72.91	72.69	0.58	99.7%
				Mean	**100.07%**

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 5.5 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 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 ofloxacin and ornidazole 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 ornidazole and the response of peak current $\mathbf{i_p}$ as function of concentration is linear (**Table 4**).

TABLE 4: OPTIMUM CONDITIONS AND PARAMETERS FOR THE POLAROGRAPHIC DETERMINATION OFLOXACIN AND ORNIDAZOLE

Conditions	Values	
	Ofloxacin	Ornidazole
Solvent	0.1N HCl	0.1N HCl
Optimum pH	Britton-Robinson Buffer of pH 5.5	Britton-Robinson Buffer of pH 5.5
Supporting Electrolyte	0.1M KCl	0.1M KCl
Peak Potential	-1.30V	-0.42V
Conditions	**Ofloxacin**	**Ornidazole**
Scan rate (mVs^{-1})	15 mVs^{-1}	15 mVs^{-1}
Pulse Amplitude (mV)	50 mV	50 mV

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

CONCLUSION:

Application to analysis of Pharmaceutical Formulation: A new polarographic method has been developed and subsequently validated for the simultaneous quantification of ofloxacin and ornidazole from a combined drug formulation. The advantages of this method for analytical purposes lie in the rapid determination, its cost effectiveness, easy preparation of the sample, good reproducibility and use of inexpensive instrumentation. In addition to this, proposed method is found to be more simple, economic, accurate and practical than chromatography and spectrophotometry methods. Thus presented method can be recommended for simultaneous determination of ofloxacin and ornidazole in routine quality control analysis in combined drug formulations.

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