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A simple electroanalytical method for simultaneous quantification of ofloxacin and ornidazole from combined drug formulation

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ABSTRACT

In present study, an attempt has been made to develop a simple method for the simultaneous determination of ofloxacin and ornidazole using Differential Pulse Polarography (DPP) technique. Quantification of ofloxacin and ornidazole was done in Britton-Robinson Buffer of pH 5.5 using 1M KCl as a supporting electrolyte. Both ofloxacin and ornidazole exhibit reduction cathodic peak in given pH with peak potential at -1.25 V for ofloxacin and 0.35V 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.

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KEYWORDS

Differential pulse polarography (DPP);
Ofloxacin;
Ornidazole;
Britton-Robinson buffer;
Pharmaceutical 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 and spectrophotometric methods have been reported for the simultaneous determination of ofloxacin and ornidazole. But, Very little attention has been paid to the use of electroanalytical techniques. A

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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 Differential Pulse Polarography has not been reported.

The present study gives a simple, rapid, efficient, reliable and economic method for the simultaneous determination of ofloxacin and ornidazole in pharmaceutical formulations using Differential Pulse Polarography technique. The proposed method has been validated as per ICH guidelines^{9,10}.

EXPERIMENTAL

Materials and methods

Instruments

Electrochemical workstation- PG STAT 30 with 663 VA Electrode stands (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 Euiptances 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.

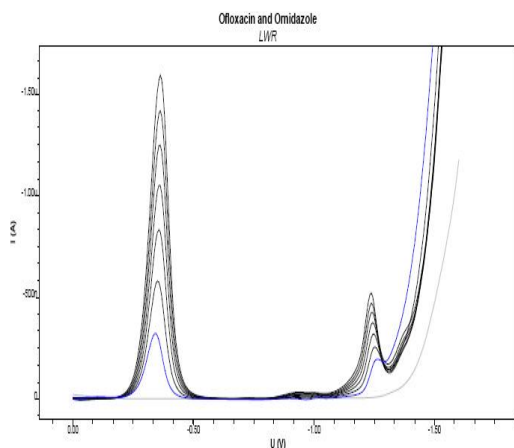


Figure 1 : Polarogram for ofloxacin and ornidazole standard

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 ($200\mu\text{g mL}^{-1}$ of ofloxacin and $500\mu\text{g mL}^{-1}$ of ornidazole resp). 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 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. 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 ornidazole applied.

Preparation of sample solution

Two commercial brands containing of ofloxacin and ornidazole in combination were procured. Each brand contained a label claim of 200mg of ofloxacin and 500mg of ornidazole per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder 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 Whatman 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: $y = 13.2418x + 134.0892$) and (for

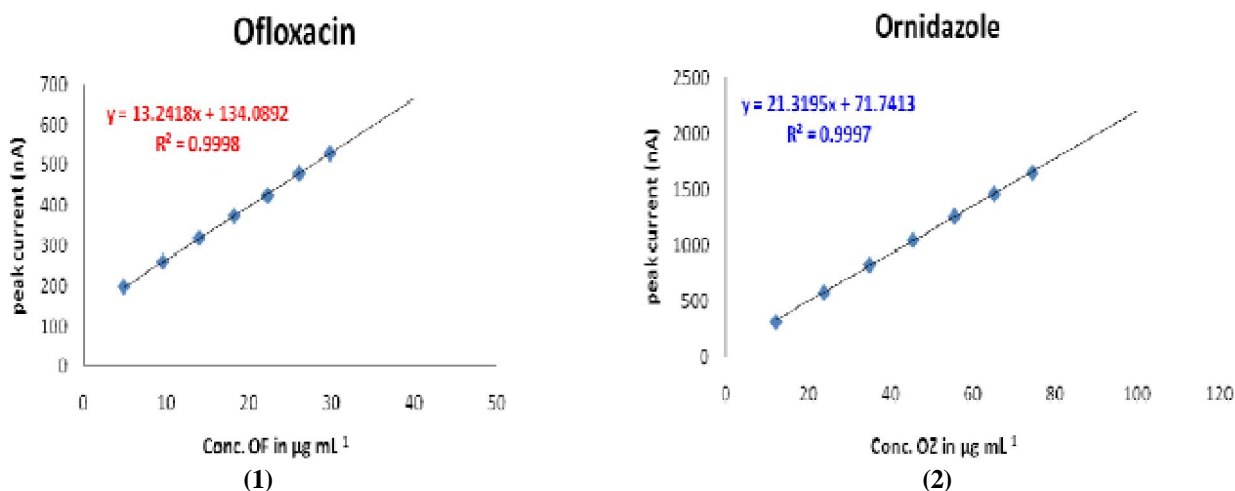


Figure 2 : Linearity graph for (1) Ofloxacin standard ans (2) ornidazole standard

ornidazole: $y = 21.3195x + 71.7413$).

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 ofloxacin ($18.18 \mu\text{g mL}^{-1}$) and for ornidazole ($45.45 \mu\text{g mL}^{-1}$) 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%.

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 $4.87 \mu\text{g mL}^{-1}$ to $29.78 \mu\text{g mL}^{-1}$ for ofloxacin and $12.19 \mu\text{g mL}^{-1}$ to $74.46 \mu\text{g mL}^{-1}$ ornidazole. The calibration curves were constructed with concentration

(C) against peak current (I_p).

The slope, Intercept, regression equation and correlation coefficient for the ornidazole was obtained is given in (TABLE 1).

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 $1.0 \mu\text{g mL}^{-1}$ and $3.92 \mu\text{g mL}^{-1}$. And ornidazole was found to be $0.5 \mu\text{g mL}^{-1}$ and $1.66 \mu\text{g mL}^{-1}$ respectively.

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 ($4.87 \mu\text{g mL}^{-1}$ to $29.78 \mu\text{g mL}^{-1}$ for ofloxacin and $12.19 \mu\text{g mL}^{-1}$ to $74.46 \mu\text{g mL}^{-1}$ 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.

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

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TABLE 1 : Method validation parameter for the determination of ornidazole

Parameters	Values	
	Ofloxacin	Ornidazole
System suitability (n=5) %RSD	0.69%	0.46%
Linearity range ($\mu\text{g mL}^{-1}$)	4.87-29.78 $\mu\text{g mL}^{-1}$	12.19-74.46 $\mu\text{g mL}^{-1}$
Slope (m) ^{a)}	13.2418	21.3195
Intercept(c) ^{a)}	134.0892	71.7413
Correlation coefficient(R ²)	0.9998	0.9997
LOD ($\mu\text{g mL}^{-1}$)	1.0 $\mu\text{g mL}^{-1}$	0.5 $\mu\text{g mL}^{-1}$
LOQ ($\mu\text{g mL}^{-1}$)	3.92 $\mu\text{g mL}^{-1}$	1.66 $\mu\text{g mL}^{-1}$
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 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 experiment

Standard	Level	Conc. of std [$\mu\text{g mL}^{-1}$]	Conc. of std found [$\mu\text{g mL}^{-1}$]	SD	RSD (%) (n = 5)	Recovery (%)
Ofloxacin	0	4.87	4.90	1.16	0.58	100.6%
	50 %	9.30	9.29	1.86	0.57	99.8%
	100 %	17.77	17.95	2.08	0.47	101.01%
	150%	25.53	25.60	1.72	0.32	100.2%
					Mean	100.40%
Ornidazole	0	12.19	12.25	2.40	0.71	100.2%
	50 %	23.25	23.12	2.13	0.25	99.4%
	100 %	44.44	44.88	8.94	0.69	101%
	150%	63.82	63.65	9.83	0.58	99.7%
					Mean	100.07%

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. 50%, 100%, 150% level. The percentage assay at three different levels for ornidazole was found to be from 98.00 % to 102.00 %. The results were shown in (TABLE 2).

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 50% to 150% level. The results of recovery analysis for ornidazole are shown in (TABLE 3).

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 1-3). 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 and (2) Pulse amplitude has been studied. 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. 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 i_p as function of concentration are linear.

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 quantification of ornidazole from a formulation. The advantages of this method for analytical purposes lie in the rapid determination of ornidazole in pharmaceutical formulations, 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. Therefore presented method can be recommended for routine quality control analysis of ornidazole in the pharmaceutical formulations.

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