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http://www.ijcpa.in

April-June 2016

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article | Volume-3 | Issue-3 | Article ID: 1031

SIMULTANEOUS DETERMINATION OF LEVOFLOXACIN AND ORNIDAZOLE IN COMBINED DRUG FORMULATION BY A SIMPLE ELECTROANALYTICAL TECHNIQUE AND ITSDETERMINATION IN URINE

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Received: 12 May 2016 / Revised: 2 June 2016 / Accepted: 22 June 2016 / Available online : 30 June 2016

ABSTRACT

In present study, a successful attempt has been made to developa simple method for the simultaneous determination of Levofloxacin and ornidazole using Differential Pulse Polarography (DPP) technique. Quantification of levofloxacin and ornidazole was done in Britton-Robinson Buffer of pH 7.0 using1M KCl as a supporting electrolyte. Both levofloxacin and ornidazoleexhibit reductioncathodic peak in given pH with peak potential at -1.30Vfor levofloxacin and -0.45V for ornidazolevs. S.C.E.0.1N acetic acidwas used as Solvent for the analysis. Proposed method was found to be simple, precise, and accurate andcan be successfully applied for routine quality control analysis andsimultaneous determination of levofloxacin and ornidazole in combined drug formulations. The proposed methodhas been validated. The limit of detection for levofloxacin in the standard solution was 0.12 µg/mland in urine was 0.59µg/mlwhile for ornidazole in the standard solution was 0.24µg/mland in urine was 1.16 µg/ml.

Keywords *– Differential Pulse Polarography (DPP),Levofloxacin, Ornidazole, Britton-Robinson Buffer, Pharmaceutical formulations.*

1. 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.

Levofloxacin (LF), $C_{18}H_{20}FN_3O_4$, that is (*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-7*H*-pyrido[*1,2,3-de*]-1,4-benzoxazine-6-carboxylic acid (Molecular weight:- 361.368 g/mol) It is used in the treatment of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections.Levofloxacin is a broad spectrum antibiotic of the fluoroquinolone drug class, and the levo isomer of its predecessor ofloxacin.

Ornidazole (OZ), $C_7H_{10}ClN_3O_3$,that is 1-Chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl)-2-propanol , an anti-parasitic drug is used as an antiprotozoal drug. (Molecular weight: - 219.626g/mol) It is highly effective for bacterial and protozoan infections and is available in the tablet form.

A literature surveys reveals few Chromatographic methods i.e. HPLCHPTLC, Derivative and Extractive spectrophotometric methodsfor the simultaneous determination of Levofloxacin and Ornidazole. Very little attention has been paid to the use of electroanalytical methods.A literature survey has revealed cyclic voltammetry and D.C polarography methods for the determination of Levofloxacin and Ornidazole individually, but its simultaneous determination by using Differential Pulse Voltammetry has not been reported.

Levofloxacin and Ornidazole in combined dosage form is available in the market, has gained great acceptance in diarrhooea, bacterial and protozoal infections. 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.

2. MATERIALS AND METHODS (EXPERIMENTAL)

2.1 Materials

StandardLevofloxacin 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. 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.

2.2 Instrumentation

Electrochemical workstation PG STAT 30 with 663 VA Electrode stand (Metrohm) used in the study was made up of three electrode system namely-

1. Hanging Mercury Drop electrode (HMDE) as the working electrode
2. Saturated calomel electrode as thereference electrode
3. Platinum electrode as thecounter electrode

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

2.3 Analytical method development

2.3.1 Preparation of standard solution

50 mg of standard Levofloxacin and 100 mg of standard Ornidazole was accurately weighed and dissolved in 0.1N Acetic acidand made up to a volume of50 mL in standard flask to give stock solution (1000µg/ml of Levofloxacin and 2000µg/mlof Ornidazole resp).Further all the standard solutions containing the mixture of Levofloxacin and Ornidazole were prepared using this stock solution.

2.3.2 Proposed voltammetric method

An aliquot of 20cm^3made up of 18 mL Britton-Robinson Buffer adjusted to pH 7.0 by 1M NaoH+ 2 mL of 0.1M KCl as a supporting electrolyte was placed in the dry and clean valtammetric 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 -1.6 V Vs. S.C.E was applied.The operational parameters were as follows:1] Scan rate-20 $mVs^{-1.}$ 2] Pulse amplitude- 50mV. After recording a voltammogram of blank, aliquotsof (0.5ml) each of the required standard solutionswasadded from the standardstock solution.Resulted voltammograms were recorded under the optimum experimental

conditions. Peak currents were recorded. Calibration curve was prepared by plotting peak current versus concentration ofLevofloxacin and Ornidazole applied.

2.3.3 Preparation of sample solution

Commercial brand named L-Cin OZ (Lupin Ltd.) containing Levofloxacin andOrnidazole in combination was procured. This brand contained a label claim of 250 mg of Levofloxacin and 500mg of Ornidazole per tablet.Ten tablets of each brand were weighed and powdered for theanalysis. The powder equivalent to50mg of Levofloxacin and 100mg of Ornidazole was accurately weighed, transferred quantitatively to 50 mL volumetric flask; then added 0.1N Acetic acidin it and the mixture wasvortexed for 10mins, the solution was filtered through Whatman filter paper no. 41 and finally volume of the solution was made up to 50mL with distilledwater.Voltammograms for the sample solutions were analyzed by the method described as above. Voltammograms were recorded under the optimum experimental conditions. The amount of Levofloxacin and Ornidazole was calculated from resulting peak current values using already constructed calibration graph.

(ForLevofloxacin: y = 16.4582x + 133.0684) and

(For Ornidazole: y = 3.3720x + 187.3081)

2.4 Analytical method validation

2.4.1 System suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recordingvoltammogramforLevofloxacin (47 µg/ml,

111 µg/ml, 166.6 µg/ml) and for Ornidazole (95 µg/ml, 222 µg/ml, 333 µg/ml with five replicates and the mean was used for the whole calculations. The % RSDwas found to be 0.88forLevofloxacin and 0.62 for Ornidazole,which was acceptable as it is less than 2%.

2.4.2 Specificity

The specificity of method was confirmed by observing the voltammograms of both the combined standard solution and the drug sample solutions. Thevoltammograms 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 voltammogram. This gives the validity of method for the determination of both drugs from combined pharmaceutical formulation.

2.4.3 Linearity and range

The linearity for Levofloxacinand Ornidazole wereobserved simultaneously byaddition of standard solution. A good linearity was achieved in the concentration ranges of 25-200 µg/ml for Levofloxacin and 0.84 – 500µg/ml forOrnidazole. The calibration curves were constructedwith concentration (C) against peak current (Ip). The slope, Intercept, regression equation and correlation coefficient for the Ornidazole was obtained is given in **(Table 1).**

2.4.4 Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantification (LOQ) for LF and OZ 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 Levofloxacin (i.e. at -1.30 V) and Ornidazole (i.e. at -0.45 V) 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 ofLevofloxacinwere0.12µg/mland 25.0µg/ml.AndOrnidazolewas found to be 0.24µg/mland 0.84µg/mlrespectively.The LOD in Urine sample for Levofloxacin was 0.59 µg/ml and for Ornidazole it was 1.16µg/ml**. (Table 4)**

2.4.5 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 thevoltammograms of standard solutions ofLevofloxacin and Ornidazole i.e. whole concentration ranges (25-200 µg/ml for Levofloxacin and 0.84 – 500µg/ml for Ornidazole) both at intra-day (three 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 Levofloxacinfound to be 0.10% and 1.4% and for Ornidazoleit was 0.08% and 1.6%, respectively.

2.4.6 Assay

The developed Voltammetric method was used for determination of Levofloxacin and Ornidazole from acommercial brand of formulations. The sample working solutions were analyzed by the developed method described above. Voltammograms were recorded under the optimum experimental conditions. Resulting peak currents of Levofloxacin andOrnidazolewere measured and the amount of Levofloxacin and Ornidazole calculated using already constructed calibration graph. Assay studies were carried out at three different levels i.e. lower, middle and higher 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).**

2.4.7 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.

2.4.8 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 wasmixed withdifferent concentrations of preanalyzed sample solutions andmixtures were analyzed by proposed method. The percent recovery was determined at different levels i.e.from 50% to 200% level. The results of recovery analysis for Ornidazole are shown in **(Table 3)**

3. RESULTS AND DISSCUSSION

In the present study quantification of Levofloxacin and Ornidazole have been done from the formulations using Differential Pulse Polarography technique. The developed method was validated as per the ICH guidelines (Table1-3). But before the method development and subsequent validation, optimization of the conditions for the analyte was done i.e. pH, supporting electrolyteand also the parameters i.e. 1] scan rate 2] Pulse amplitude has been studied. During optimization of the conditions,the Voltammetric response of Levofloxacin andOrnidazole 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 thewhole 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 7.0 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. The1M 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. As the concentration

of OZ increases the slight negative shift in potential was observed whereas the increase in the concentration of LF tends a positive shift in the potential.

The Differential Pulsevoltammograms ofLevofloxacin andOrnidazolewere recorded at various scan rates. A scan rate of 20 mVs^{-1} was chosen as a best for theanalysis. The height of peak increase gradually with concentration of Levofloxacin and Ornidazole 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 stearateor talc powder.

Table 1: Method validation parameters for determination of LF and OZ

Parameters	Values	
	Levofloxacin	**Ornidazole**
System suitability (n=3) %RSD	0.88%	0.62%
Linearity range (µg/ml)	25-200 µg/ml	0.84 – 500 µg/ml
Slope (m)[a)]	16.4582	3.3720
Intercept(c) [a)]	133.0684	187.3081
Correlation coefficient (R^2)	0.9999	0.9990
LOD (µg/ml)	0.12 µg mL^{-1}	0.24µg mL^{-1}
LOQ (µg/ml)	25.0 µg mL^{-1}	0.84 µg mL^{-1}
Intraday precision (n=3)	0.08%	0.10%
Interday precision (n=3)	1.4 %	1.6 %
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: Results of assay studies for LF and OZ

Brand Name	**L-Cin OZ (Lupin) Ltd**	
	Levofloxacin	**Ornidazole**
Labeled claim (mg)	250 mg	500mg
Drug found in mg	253.02 mg	499.1 mg
% RSD (n=5)	0.440	0.941
% Assay	99.4%	99.8 %

Table 3: Results of recovery studies for LF and OZ

Standard	**Level**	**Conc. Of std [µg/ml]**	**Conc. of std Found [µg/ml]**	**RSD (%) (n = 5)**	**Recovery (%)**
Ornidazole	50 %	46.51	45.75	0.57	98.37%
	150%	90.91	92.21	0.47	101.43%
	250%	173.91	169.27	0.32	98.09%
				Mean	**99.30%**
Levofloxacin	50 %	23.26	22.93	0.25	98.48%
	150%	45.45	45.40	0.69	99.88%
	250%	86.96	87.06	0.58	100.11%
				Mean	**99.49%**

Table 4: Comparison of detection limit in standard solution and Urine

PARAMETERS	OZ	LF
Limit of Detection (Solvent)	0.24 µg/ml	0.12 µg/ml
Limit of Detection (Urine)	1.16 µg/ml	0.59 µg/ml

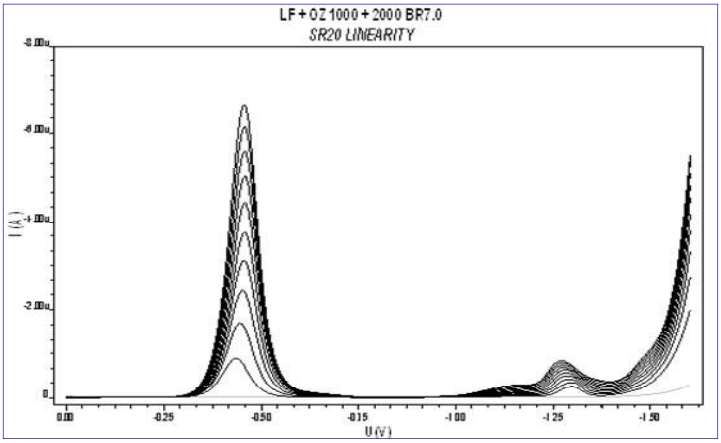
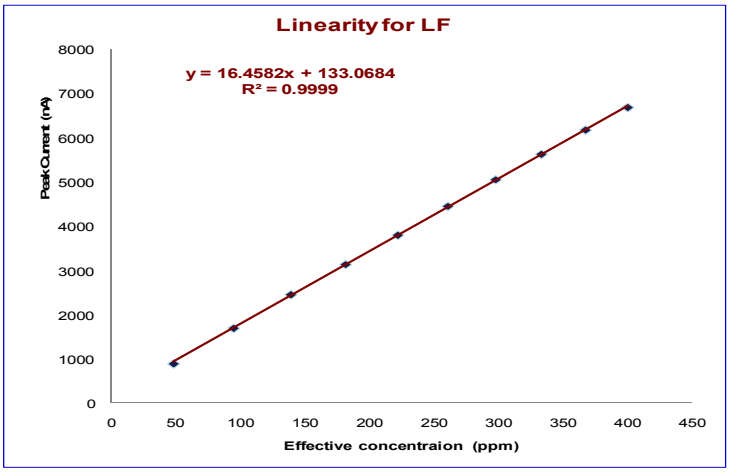
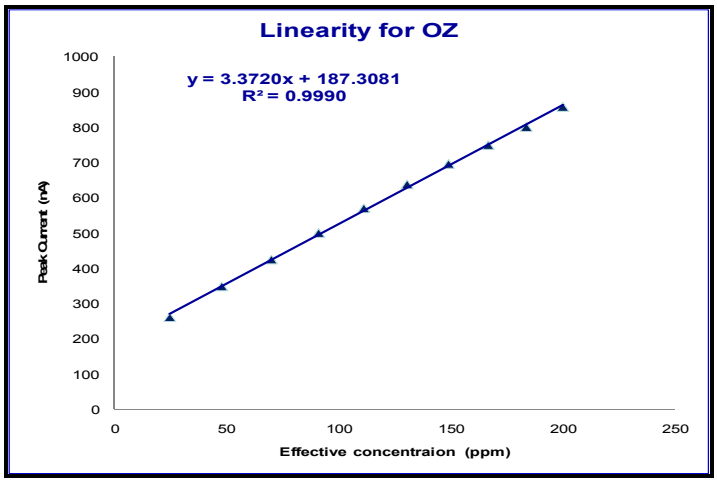


Figure 1 : Voltammogram of LF and OZ



(a)



(b)

Fig. 2 : Calibration curve for standard (a) Levofloxacin (b) Ornidaole

4. CONCLUSION

All the validation parameters were found to be satisfactory hence this method can be used for routine quality control analysis. Detection limit for both levofloxacin and ornidazole in urine sample was found to be more than that in a sample without urine. The advantages of the present method are summarized below:

1. Rapid determination of LF & OZ from the tablet formulation.
2. Easy sample preparation, good reproducibility, cost effective.
3. Can be applied for the drug analysis in any form without any special pretreatment.
4. Simple, Selective, Accurate and Precise.
5. Interference of background matrix can be easily removed.
6. This method can be used in clinical research.

5. ACEKNOWLEDGEMENT

We thank to our Department of Chemistry, St. Xavier's college and Ramnarain Ruia College for providing us all the Necessary instrumentation facilities and their technical assistance.

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