International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
www.ijprd.com

SIMULTANEOUS DETERMINATION OF NORFLOXACIN AND METRONIDAZOLE IN COMBINED DRUG FORMULATION BY A SIMPLE ELECTROANALYTICAL TECHNIQUE

V. M. Wagh[1*],
P.A. Sathe [1], P.V. Rege [1], V.S. Salvi[1]

[1]Department Of Chemistry, Ramnarain Ruia College, Matunga (East), Mumbai-19.

ABSTRACT
In present study, a successful attempt has been made to develop a simple method for the simultaneous determination of Norfloxacin and Metronidazole using Differential Pulse Polarography (DPP) technique. Quantification of Norfloxacin and Metronidazole was done in Britton-Robinson Buffer of pH 6.5 using 1M KCl as a supporting electrolyte. Both Norfloxacin and Metronidazole exhibit reduction cathodic peak in given pH with peak potential at -1.40V for Norfloxacin and -0.48V for Metronidazole vs. S.C.E. 0.1N CH_3COOH was used as Solvent for the analysis. The parameters used for the method validation are 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 Norfloxacin and Metronidazole in combined drug formulations.

KEYWORDS : *Differential Pulse Polarography (DPP), Norfloxacin, Metronidazole, Britton-Robinson Buffer, Pharmaceutical formulations.*

Correspondence to Author

V. M. WAGH

Department Of Chemistry, Ramnarain Ruia College, Matunga (East), Mumbai-19.

Email: vwagh123@gmail.com

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.

Norfloxacin$C_{16}H_{18}FN_3O_3$ that is (1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid) (Molecular weight: 319.331 g/mol)] is used in the treatment of bacterial infection. Norfloxacin is a second generation synthetic fluoroquinolone (quinolone) developed by Kyorin Seiyaku K.K. (Kyorin).

Metronidazole, $C_6H_9N_3O_3$ that is 2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethanol, is an antibiotic, amebicide, and antiprotozoal (Molecular weight: 171.15 g/mol g/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. HPLC HPTLC, Derivative and Extractive spectrophotometric methods for the simultaneous determination of Norfloxacin and Metronidazole. 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 Norfloxacin, but its simultaneous determination with Metronidazole by using electroanalytical technique has not been reported.

Norfloxacin and Metronidazole 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.

OBJECTIVE

The main objective of study is to provide a simple, rapid, efficient, reliable and economic method for the simultaneous determination of Norfloxacin and Metronidazole in combined pharmaceutical formulations using Differential Pulse Polarography technique.

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 Norfloxacin and Metronidazole 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

50mg of standard Norfloxacin and 50mg of standard Metronidazole was accurately weighed and dissolved in 0.1N CH_3COOH and made up to a volume of 50 mL in standard flask to give stock solution (1000µg/ml of Norfloxacin and 1000µg/ml of Metronidazole respectively). Further all the standard solutions containing the mixture of Norfloxacin and Metronidazole were prepared using this stock solution.

PROPOSED VOLTAMMETRIC METHOD

An aliquot of 20cm^3 made up of 18 mL Britton-Robinson Buffer adjusted to pH 6.5 by 1M NaOH + 2 mL of 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 -1.50 V vs. S.C.E was applied. The operational parameters were as follows: 1] Scan rate- 15 $mVs^{-1.}$ 2] Pulse amplitude- 50mV. After recording a polarogram of blank, aliquots of (0.5ml) each of the required standard solutions was

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 Norfloxacin and Metronidazole applied.

PREPARATION OF SAMPLE SOLUTION

One commercial brand containing of Norfloxacin and Metronidazole in combination were procured. This brand contained a label claim of 400mg of Norfloxacin and 500mg of Metronidazole per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder (1.1425 g) equivalent to 400mg of Norfloxacin and 500mg of Metronidazole was accurately weighed, transferred quantitatively to 500 mL volumetric flask; then added 0.1N CH_3COOH 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 500 mL with 0.1N CH_3COOH. Polarograms for the sample solutions were analyzed by the method described as above. Polarograms were recorded under the optimum experimental conditions. The amount of Norfloxacin and Metronidazole was calculated from resulting peak current values using already constructed calibration graph.

(For Norfloxacin: y = 4.6082x - 39.9329) and (for Metronidazole: y = 14.0295x + 92.3313)

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 Norfloxacin (47.62 µg/ml, 111.11 µg/ml, 183.67 µg/ml) and for Metronidazole (47.62 µg/ml, 111.11 µg/ml, 183.67 µg/ml) with three replicates and the mean was used for the whole calculations. The % RSD was found to be 0.74% for Norfloxacin and 0.21% for Metronidazole, 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 Norfloxacin and Metronidazole were observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of 23.0 µg/ml to 250.0 µg/ml for Norfloxacin and 1.7 µg/ml to 215.0 µg/ml for Metronidazole. Therefore the linear working range selected for both is 24.0 µg/ml to 200.0 µg/ml. The calibration curves were constructed with peak current (ip) against concentration (c). The slope, Intercept, regression equation and correlation coefficient for the Norfloxacin and Metronidazole was obtained is given in (Table 1).

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The limit of detection (LOD) and the limit of quantification (LOQ) for NF and MZ 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 Norfloxacin (i.e. at

-1.300 V) and Metronidazole (i.e. at -0.464 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 of Norfloxacin were 14.0 µg/ml and 23.0 µg/ml. And Metronidazole was found to be 0.48 µg/ml and 1.67 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 Metronidazole i.e. whole concentration range 24.0 µg/ml to 200.0 µg/ml both at intra-day (three times within 24 hour) and inter-day (three times each. during 3 days intervals) to check the precision. The mean % RSD for intra-day and inter-day precision for Norfloxacin found to be 0.58% and 0.40% and for Metronidazole it was 0.17% and 0.23%, respectively.

ASSAY

The developed Polarographic method was used for simultaneous determination of Norfloxacin and Metronidazole from a drug formulation. The sample solutions were analyzed by the developed method described above. Polarograms were recorded under the optimum experimental conditions. Resulting peak currents of Norfloxacin and Metronidazole were measured and the amount of Norfloxacin and Metronidazole calculated using already constructed calibration graph. Assay studies were carried out at three different levels lower, middle and higher levels. The percentage assay at three different levels for both Norfloxacin and Metronidazole 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 pre-analyzed sample of Norfloxacin and Metronidazole solution was mixed with different concentrations of standard solution and mixtures were analyzed by proposed method. The percentage recovery was determined at different levels i.e. from 50% to 250% level. The results of percentage recovery analysis for Norfloxacin and Metronidazole are shown in (Table 3)

RESULT AND DISSCUSSION

In the present study quantification of Norfloxacin and Metronidazole have been done from the drug formulation using Differential Pulse Polarography technique. The developed method was validated and the results are shown in (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 Norfloxacin and Metronidazole 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 6.5 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. As the concentration of MZ increases no shift in potential was observed whereas the increase in the concentration of NF tends a positive shift in the potential.

The Differential Pulse polarograms of Norfloxacin and Metronidazole 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 Metronidazole 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.

Table 1: METHOD VALIDATION PARAMTERS FOR DETERMINATION OF NF AND MZ

Parameters	**Values**	
	Norfloxacin	**Metronidazole**
System suitability (n=3) %RSD	0.74%	0.21%
Linearity range (µg/ml)	23.0 to 250.0 µg/ml	1.7 to 215.0 µg/ml
Working Range	24.0 µg/ml to 200.0 µg/ml	
Slope (m) [a)]	4.608	14.029
Intercept(c) [a)]	- 39.931	92.331
Correlation coefficient (R^2)	0.9998	0.9990
LOD (µg/ml)	14.0 µg mL^{-1}	0.48 µg mL^{-1}
LOQ (µg/ml)	23.0 µg mL^{-1}	1.67 µg mL^{-1}
Intraday precision (n=3)	0.58%	0.17%
Interday precision (n=3)	0.40%	0.23%
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 current (ip), m is the slope, x is the Concentration and c is the intercept.

Table 2: RESULTS OF ASSAY STUDIES FOR NF AND MZ

Brand name	**Nor-Metrogyl (Lekar Pharma)**	
	Norfloxacin	**Metronidazole**
Labeled claim (mg/tablet)	400mg	500mg
Drug found in mg	402.67 mg	496.25 mg
% RSD (n=3)	1.83 %	0.54 %
% Assay	100.67%	99.25 %

Table 3. RESULTS OF RECOVERY STUDIES FOR NF AND MZ

Standard	Level	Conc. Of std [µg/ml]	Conc. of std Found [µg/ml]	RSD (%) (n = 3)	Recovery (%)
Norfloxacin	0	38.09	37.38	0.58	98.13%
	60%	23.25	22.88	0.57	98.41%
	175%	66.67	66.54	0.47	99.81%
	280%	106.38	105.36	0.32	99.04%
				Mean	98.84%
Metronidazole	0	47.62	46.90	0.71	98.48%
	50%	23.25	22.97445	0.25	98.79%
	150%	66.66	66.42247	0.69	99.63%
	250%	106.38	106.6936	0.58	100.29%
				Mean	99.30%

Figure-1. POAROGRAMS OF NF AND MZ

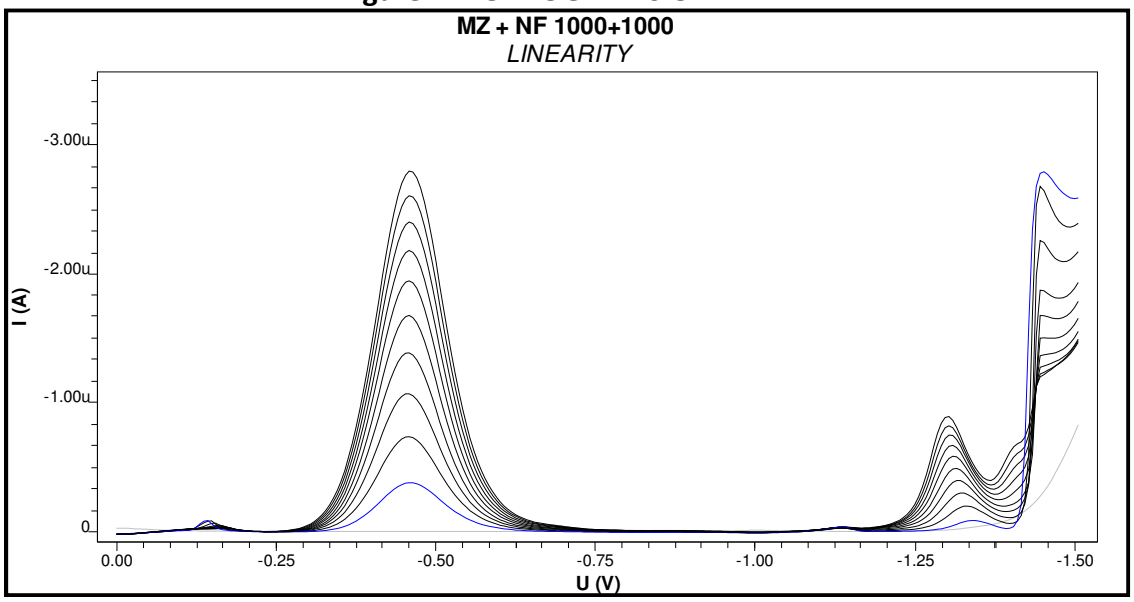
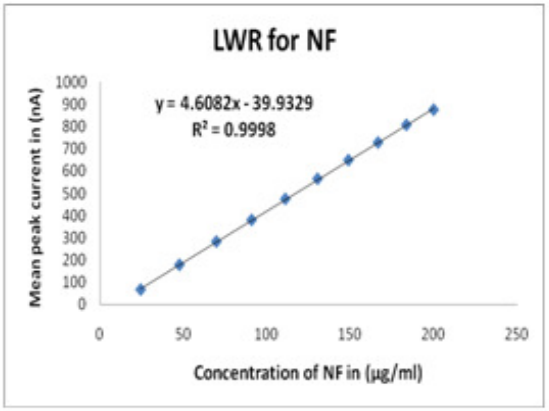
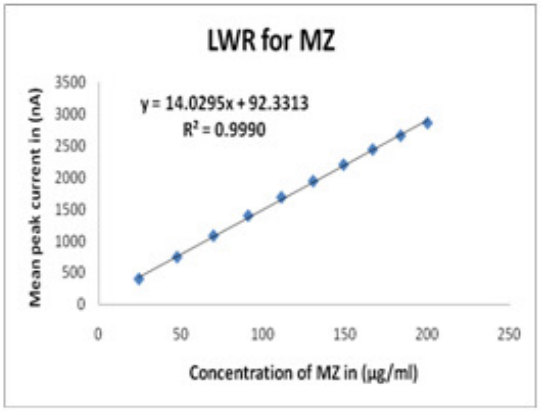


Figure-2. LINEARITY GRAPHS FOR

NORFLOXACIN STD **METRONIDAZOLE STD**





ACEKNOWLEDGEMENT

We thank to our Department of Chemistry Ramnarain Ruia College for providing us all the Necessary instrumentation facilities and their technical assistance

REFERENCES

1) Lopez fonseca , J M; Gomez Rivera , M.C Garcia Monteagudo , Electrochemistry of ornidazole. J C Dept quim , Fis , Fac farmacia Univ Santiago de compostela 1570 6 Spain) Anal Lett **Jan 1993** 26 (1) *Vol. (55)* 109-124.
2) Lichtig, J.; Andrade, R. F.; Vaz , J. M. Determination of secnidazole in pharmaceutical Table. (Inst. Quim; Univ . Sao Paulo, 05599-070 Sao Paulo, Brazil). Anal Chem. Acta 21 **Oct 1996** , 332 (2-3), *Vol. (58).*161-164
3) ICH, Q2A, Validation of Analytical Procedure: Methodology, In. Proc.Int.Con. Harmonization, Geneva (1994).
4) ICH Q2B, Validation of Analytical Procedure: Methodology, In. Proc. Int. Con. Harmonization, Geneva (1996).
5) Liu,-XJ; Chen,-GN; Xie,-EC; Wang,-SJ; Zhang,-RQ; Ding,-LZ; Ming,-L Yaowu-Fenxi Zazhi. Determination of Norfloxacinby adsorptive voltammetry **1995;** *15(2):* 30-33
6) Jaber,-AMY; Lounici,-Adsorptive differential pulse stripping voltammetry of Norfloxacin and its analytical application. A Analyst (London). **Nov 1994;** *119(11):* 2351-2357
7) Yaber,-AMY; Lounici,-A Anal-Chim-Acta. Polarographic behaviour and their Determination of Norfloxacinin tablets **Jun 1994;** *291(1-2):* 53-64.
8) Pereira F.C,Zanoni M.VB*; Guaratini C.C.I , Fogg A G C boldrinv@unesp.br.D.D Polarographic det'n of CLT after derivitization with procaine Red H-E 3B:-Dept Quim Anal ,Inst Quim. Univ.Estadual Paulista 14800-900 Araraquara SP Brazil J.Pharm biomed Anal 1 **Jan 2002,** *27(1-2*), 201-208.

*****