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International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.3, pp 1213-1219, July-Sept 2011

A Simple ElectroAnlytical Method for Simultaneous Determination of Norfloxacin and Tinidazole from Combined Pharmaceutical Drug Formulation

P.V. Rege[1]*, P.A. Sathe[1], V.S. Salvi[2]

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

Corres. author: pvr_2008@yahoo.co.in / pralhad1806@gmail.com

Abstract: In present study, a successful attempt has been made to develop a simple method for the simultaneous determination of norfloxacin and tinidazole using Differential Pulse Polarography (DPP) technique. Quantification of norfloxacin and tinidazole was done in Britton-Robinson Buffer of pH 6.5 using 1M KCl as a supporting electrolye. Both norfloxacin and tinidazole exhibit reduction cathodic peak in given pH with peak potential at -1.32V for norfloxacin and -0.42V for tinidazole vs. S.C.E. 0.1N HCl was used as Solvent for the analysis. The parameters used for the method validation are linearity; accuracy, precision, robustness, ruggudness, 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 tinidazole in combined drug formulations.

Keywords: Differential Pulse Polarography (DPP), Norfloxacin, Tinidazole, Britton-Robinson Buffer, Pharmaceutical formulations.

Abbreviations used: NF- Norfloxacin, TZ- Tinidazole.

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 synthetic chemotherapeutic agent occasionally used to treat common as well as complicated urinary tract infections It is sold under various brand names with the most common being Noroxin. In form of ophthalmic solutions it is known as Chibroxin. Norfloxacin is a second generation synthetic fluoroquinolone (quinolone) developed by Kyorin Seiyaku K.K. (Kyorin).

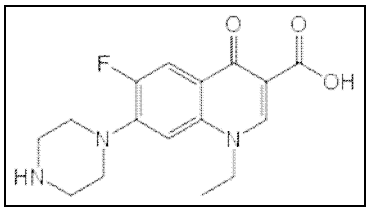
Tinidazole, $C_8H_{13}N_3O_4S$ that is 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole derivative, an anti-parasitic drug is used as an antiprotozoal drug. (Molecular weight: - 247.273 g/mol) It is highly effective for bacterial and protozoan infections and is available in the tablet form.

A literature surveys reveals few Chromatographic methods i.e. HPLC[1], HPTLC[2], Derivative and Extractive spectrophotometric methods for the simultaneous determination of Norfloxacin and Tinidazole[3]. Very little attention has been paid to the use of electroanalytical methods [4-10]. A literature survey has revealed cyclic voltammetry[4] and D.C polarography[5] methods for the determination of norfloxacin and tinidazole individually, even adsorptive stripping[6] and differential pulse stripping Voltammetry[7] methods have been reported but its simultaneous determination by using Differential Pulse Polarography has not been reported.

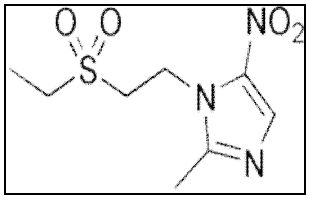
Norfloxacin and tinidazole in combined dosage form is available in the market, has gained great acceptance in diarrhooea, bacterial and protozoal infections. In many cases, drugs with two active ingredients are prescribed to the patients to have an added advantage. Many of these antibacterial drugs are found in combination with antifungal and antiprotozoal drugs which are highly effective against fungal and protozoal infections.

The present study gives a simple, rapid, efficient, reliable and economic method for the simultaneous determination of norfloxacin and tinidazole in combined pharmaceutical formulations using Differential Pulse Polarography technique [8-10]. The proposed method has been validated as per ICH guidelines [11-12].

Structure:-



Norfloxacin



Tinidazole

Materials and Methods (Experimental)

Instruments

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 tinidazole 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].

Preparation of standard solutions

20mg of standard Norfloxacin and 30mg of standard tinidazole 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 (400µg/ml of norfloxacin and 600µg/ml of tinidazole resp). Further all the standard solutions containing the mixture of norfloxacin and tinidazole 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 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 -2.0 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, initial aliquots of (1.0ml) followed by (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 tinidazole applied.

INTRODUCTION TO WORKSTATION

AUTOLAB PGSTAT 30 WITH 663 VA STAND (METROHM)

Three- Electrode system consist of
3 M KCl Electrode:-Reference electrode.
Hanging Mercury Drop Electrode:-Working electrode.
Platinum Electrode:- Counter Electrode.

Preparation of sample solution

Two commercial brands containing of norfloxacin and tinidazole in combination were procured. Each brand contained a label claim of 400mg of norfloxacin and 600mg of tinidazole per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder (59.5 mg) equivalent to 20mg of norfloxacin and 30mg of tinidazole 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 norfloxacin and tinidazole was calculated from resulting peak current values using already constructed calibration graph.

(For Norfloxacin: y = 4.7200x + 9.9763) and (for Tinidazole: y = 13.6384x + 38.9043)

Analytical Method Validation [11-12]

System Suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recording polarogram for norfloxacin (27.90 µg/ml, 44.44 µg/ml, 59.57 µg/ml) and for tinidazole (41.86 µg/ml, 66.66 µg/ml, 89.36 µg/ml) with five replicates and the mean was used for the whole calculations. The % RSD was found to be 0.40 for norfloxacin and 0.36 for tinidazole, 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 tinidazole were observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of 19.04 µg/ml 66.66 µg/ml for norfloxacin and 28.57 µg/ml to 100µg/ml tinidazole. 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 1).

Table 1: Method validation parameter for the determination of Norfloxacin and Tinidazole.

Parameters	**Values**	
	Norfloxacin	**Tinidazole**
System suitability (n=5) %RSD	0.40%	0.36%
Linearity range (µg/ml)	19.04 to 66.66 µg/ml	28.57 to 100 µg/ml
Slope (m) [a)]	4.7200	13.6384
Intercept(c) [a)]	9.9763	38.9043
Correlation coefficient (R^2)	0.9998	0.9996
LOD (µg/ml)	5.9 µg mL^{-1}	0.3 µg mL^{-1}
LOQ (µg/ml)	19.04 µg mL^{-1}	1.1 µg 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

Limit of Detection and Limits of Quantitation

The limit of detection (LOD) and the limit of quantification (LOQ) for NF and TZ 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 -1.32 V) and Tinidazole (i.e. at -0.42 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 5.9 µg/ml and 19.04 µg/ml. And tinidazole was found to be 0.3 µg/ml and 1.1 µg/ml 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 norfloxacin and tinidazole i.e. whole concentration ranges (19.04 µg/ml to 66.66 µg/ml for norfloxacin and 28.57 µg/ml to 100 µg/ml for tinidazole) 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 norfloxacin found to be 0.75% and 0.60% and for tinidazole it was 0.65% and 0.45%, respectively.

Assay

The developed Polarographic method was used for determination of tinidazole 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 norfloxacin and tinidazole were measured and the amount of norfloxacin and tinidazole calculated using already constructed calibration graph. Assay studies were carried out at three different levels i.e. 60%, to 140% level. The percentage assay at three different levels for tinidazole 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.

Table 2: Result of Assay studies of Norfloxacin and Tinidazole

Brand name	Nor-TZ (Omega Biotech)		Norflox-TZ (Cipla)	
	Norfloxacin	**Tinidazole**	**Norfloxacin**	**Tinidazole**
Labeled claim (mg)	400mg	600mg	400mg	600mg
Drug found in mg	398.8 mg	599.1 mg	401.2 mg	600.9 mg
% RSD (n=5)	0.440	0.941	0.607	0.836
% Assay	99.7%	99.85 %	100.3 %	100.15%

Table 3. Results of Recovery Experiment

Standard	Level	Conc. Of std [µg/ml]	Conc. of std Found [µg/ml]	RSD (%) (n = 5)	Recovery (%)
Norfloxacin	0	19.04	19.15	0.58	100.6%
	60 %	26.26	26.21	0.57	99.8%
	100 %	42.55	43.0	0.47	101.01%
	120%	50.0	50.10	0.32	100.2%
				Mean	**100.40%**
Tinidazole	0	28.57	28.62	0.71	100.2%
	60 %	40.0	39.76	0.25	99.4%
	100 %	63.82	64.45	0.69	100.98%
	120%	76.59	76.36	0.58	99.7%
				Mean	**100.07%**

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 tinidazole 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 60% to 120% level. The results of recovery analysis for tinidazole are shown in (Table 3).

Results and Discussion

In the present study quantification of norfloxacin and tinidazole 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 tinidazole 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 TZ increases the slight negative shift in potential was observed whereas the increase in the concentration of NF tends a positive shift in the potential.

Figure-1. Polarogram for norfloxacin and tinidazole standard

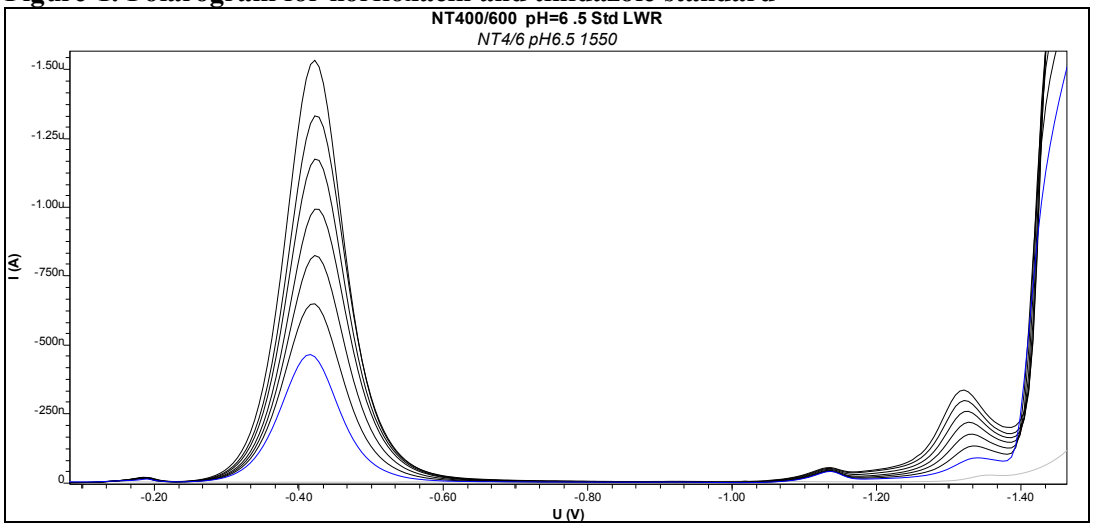
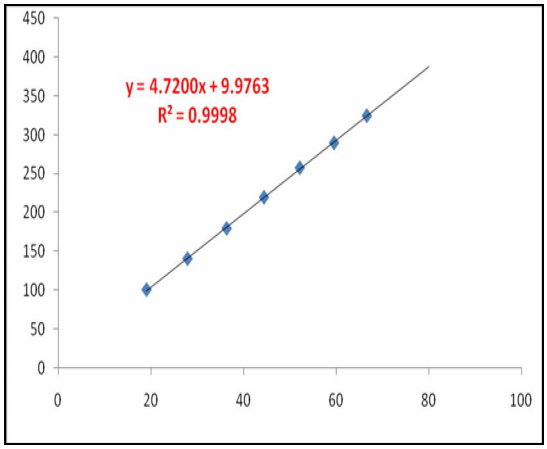
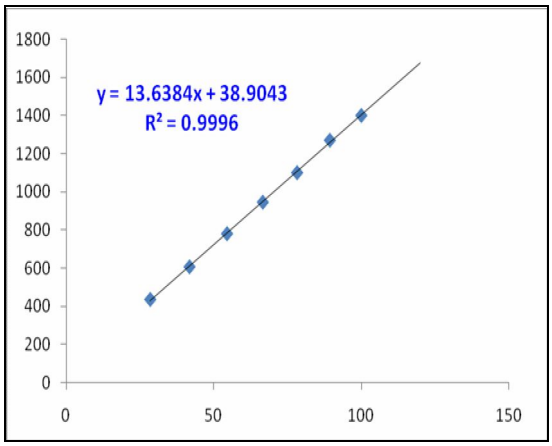


Figure-2. Linearity graphs for----

Norfloxacin standard



Tinidazole standard



The Differential Pulse polarograms of norfloxacin and tinidazole 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.

Conclusion: -

Application to analysis of pharmaceutical formulation-

A new polarographic method has been developed and subsequently validated for the quantification of norfloxacin and tinidazole from a combined drug formulation. The advantages of this method for analytical purposes lie in the rapid determination of both the drugs 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 simultaneous determination of norfloxacin and tinidazole in routine quality control analysis from combined drug formulations.

Acknowledgement:

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

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