ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A simple electroanlytical method for estimation of norfloxacin and tinidazole individually from pharmaceutical formulation.

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ABSTRACT

In present study, a successful attempt has been made to develop a simple method for the determination of Norfloxacin and Tinidazole individually using Differential Pulse Polarography (DPP) technique. Quantification of Norfloxacin and Tinidazole was done in Britton-Robinson Buffer Norfloxacin pH 6.5 and pH 6.0 respectively using 0.1M KCl as a supporting electrolyte. Both Norfloxacin and Tinidazole exhibit reduction cathodic peak in given respective pH with peak potential (Ep) as -1.30V for Norfloxacin and -0.32V for Tinidazole vs. S.C.E. 0.1N HCl was used as Solvent for the analysis. The parameters were used for 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 determination Norfloxacin and Tinidazole individually in drug formulation.

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

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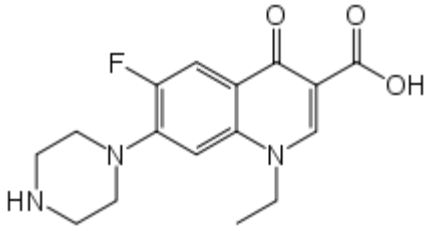
ISSN: 0975-8585

INTRODUCTION

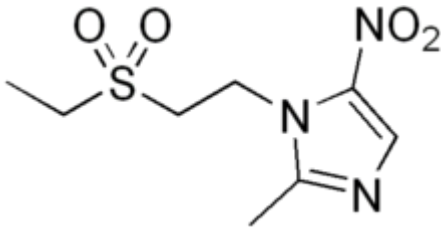
In the topical countries like India, the major problems of health arise due to improper lifestyle, unhealthy environmental conditions, unhygienic and substandard food. Infections caused by the microorganisms like, fungi, protozoa, are most common. Drugs with antifungal and antiprotozoal activity have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



Norfloxacin

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

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. It is highly effective for bacterial and protozoal infections and is available in the tablet form.

ISSN: 0975-8585

Few Chromatographic and spectrophotometric methods have been reported for the simultaneous determination Norfloxacin and Tinidazole. But, Very little attention has been paid to the use of electroanalytical techniques. A literature survey has revealed cyclic voltammetry and D.C polarography methods for the determination of Norfloxacin and Tinidazole individually, The present study gives a simple, rapid, efficient, reliable and economic method for the determination of Norfloxacin and Tinidazole individually in pharmaceutical formulations using Differential Pulse Polarography technique. The proposed method has been validated as per ICH guidelines.

MATERIALS AND METHODS (EXPERIMENTAL)

Instruments:-

Electrochemical workstation- PG STAT 30 with 663 VA Electrode stand (Metrohm)
It is made up of three electrode systems 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.

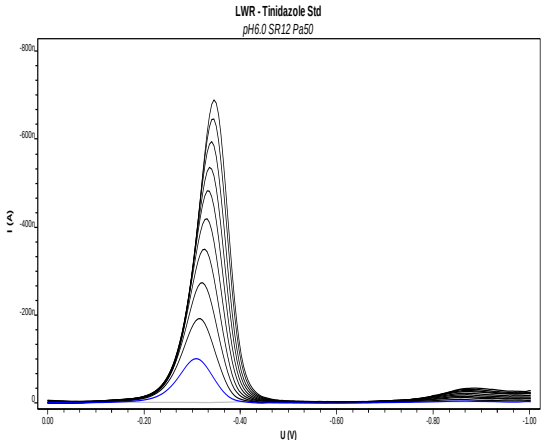
Reagents:-

Standard Norfloxacin and Tinidazole were 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 each 0.04M H_3BO_4 + 0.04M H_3PO_4 + 0.04M CH_3COOH].

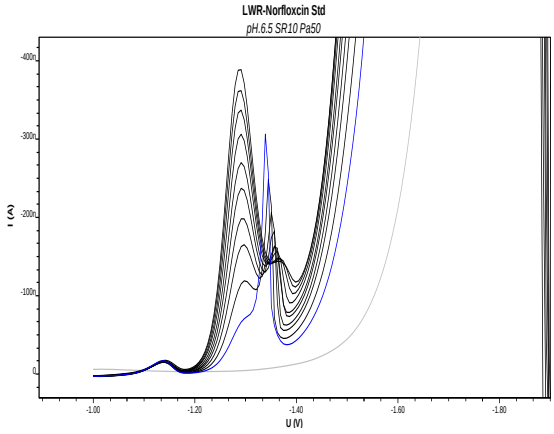
Preparation of standard solutions:-

10mg Norfloxacin standard and 15mg Tinidazole standard was accurately weighed and dissolved in 0.1N HCl individually and made up to a volume of 50 mL in two separate standard flask to give stock solution (200µg mL^{-1} of Norfloxacin and 300µg mL^{-1} of Tinidazole respectively). Further all the standard solutions containing Norfloxacin and Tinidazole were prepared using this stock solution.

ISSN: 0975-8585



Polarograms for Tinidazole standard



Polarograms for Norfloxacin standard

Proposed Polarographic Method:-

An aliquot of 20cm^3 made up of 18 mL Britton-Robinson Buffer adjusted to pH 6.5 for Norfloxacin and pH 6.0 for Tinidazole 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 scans between the potential Norfloxacin 0.0 V to -2.0 V vs. S.C.E was applied. The operational parameters were as follows: For Norfloxacin 1] Scan rate- 10 mV s$^{-1.}$ 2] Pulse amplitude- 50mV and for Tinidazole 1] Scan rate- 12 mV s$^{-1.}$ 2] Pulse amplitude- 50mV. After recording a polarogram of blank, aliquots of (1mL) the required standard Norfloxacin and Tinidazole 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 Norfloxacin and Tinidazole applied. The results were shown in [Table.1]

Table.1: Optimum Conditions and Parameters for the polarographic determination of Norfloxacin and Tinidazole.

Conditions	**Values**	
	Norfloxacin	**Tinidazole**
Solvent	0.1N HCl	0.1N HCl
Optimum pH	Britton-Robinson Buffer of pH 6.5	Britton-Robinson Buffer of pH 6.0
Supporting Electrolyte	0.1M KCl	0.1M KCl
Peak Potential	-1.30V	-0.32V
Conditions	**Norfloxacin**	**Tinidazole**
Scan rate (mVs^{-1})	10 mVs^{-1}	12 mVs^{-1}

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Pulse Amplitude (mV)	50 mV	50 mV

Preparation of sample solution:-

Two commercial brands containing Norfloxacin and Tinidazole were procured. Each brand contained a label claim of 200mg Norfloxacin and 300mg and 500mg of Tinidazole per tablet. Ten tablets of each brand were weighed and powdered for the analysis. The powder equivalent to 10mg of Norfloxacin and 15mg of Tinidazole was accurately weighed, transferred quantitatively to 50 mL volumetric flask; then added 0.1N HCl in it and the mixture was sonicate 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 0.1N HCl. 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 = 5.4383x + 19.4820] and [for Tinidazole: y = 27.6311x + 4.0245]

Analytical Method Validation

System Suitability:-

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by recording polarograms for Norfloxacin (18.18 µg mL^{-1}, 40 µg mL^{-1}, 57.14 µg mL^{-1}) and for Tinidazole (6.84 µg mL^{-1}, 15 µg mL^{-1}, and 21.42 µg mL^{-1}) with five replicates and the mean was used for the whole calculations. The mean % RSD was found to be 0.60 for Norfloxacin and 0.75 for Tinidazole, which was acceptable as it is less than 2%. [Table.2]

Parameters	**Values**	
	Norfloxacin	**Tinidazole**
System suitability(n=5) %RSD	0.60%	0.75%
Linearity range (µg mL^{-1})	9.52 to 66.66 µg mL^{-1}	3.57 to 25 µg mL^{-1}
Slope (m)	5.4383	27.6311
Intercept(c)	19.4820	4.0265
Correlation coefficient (R^2)	0.9996	0.9999
LOD (µg mL^{-1})	3.92 µg mL^{-1}	0.24 µg mL^{-1}
LOQ (µg mL^{-1})	9.52 µg mL^{-1}	0.71 µg mL^{-1}
Intraday precision (n=5)	0.55%	0.65%
Interday precision (n=5)	0.60%	0.45%

Assay	98% to 102%	98% to 102%
Recovery	98% to 102%	98% to 102%

Table.2: Method validation parameter for the determination of Norfloxacin and Tinidazole.

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 given pharmaceutical formulation.

Linearity and Range:-

The linearity for Norfloxacin and Tinidazole were observed individually by addition of standard solution. A good linearity was achieved in the concentration ranges of 9.52 µg mL^{-1} to 66.66 µg mL^{-1} for Norfloxacin and 3.57 µg mL^{-1} to 25 µg mL^{-1} for 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.2]

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 signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. For LOD and LOQ analysis twenty readings for blank recorded then their standard deviation calculated i.e. for LOD= (SD×3+ Mean absorbance of Blank) and for LOQ= (SD×10 + Mean absorbance of Blank.). [Table.2]

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 (9.52 µg mL^{-1} to 66.66 µg mL^{-1} for Norfloxacin and 3.57 µg mL^{-1} to 25 µg mL^{-1} 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 0.55% and 0.60% and for Tinidazole it was 0.65% and 0.45%, respectively. [Table.2]

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Assay:-

The developed Polarographic method was used for determination of Norfloxacin and 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. 40% to140% level. The percentage assay at different levels for Norfloxacin and Tinidazole was found to be from 98.00 % to 102.00 %. The results were shown in [Table.3-4]

Table.3: Assay Results for Norfloxacin

Brand Name	Norflox (Cipla)	Norilet (Dr.Reddy's)
Label claim (mg)	200mg	200mg
Amount found (mg)	198.8 mg	201.2 mg
% RSD (n=5)	0.440	0.607
% Assay	99.4%	100.6 %

Table.4: Assay Results for Tinidazole

Brand Name	Tiniba (Cadila Ltd)	Fasigyn (Pfizer)
Label claim (mg)	300mg	500mg
Amount found (mg)	300.9 mg	499.1 mg
% RSD (n=5)	0.536	0.741
% Assay	100.3%	99.7 %

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.

Accuracy (Recovery):-

The recovery was used to evaluate the accuracy of the method. Accuracy of the method was determined using the standard addition method. A fixed volume of standard Norfloxacin and Tinidazole solution was mixed with different concentrations of pre-analyzed sample solutions and mixtures were analyzed by proposed method. The percent recovery was

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determined at different levels i.e. from 40% to 140% level. The results were shown in [Table.5-6] [1-12]

Table.5: Recovery Results for Norfloxacin

Standard	Level	Conc. of std [µg mL^{-1}]	Conc. of std Found[µg mL^{-1}]	RSD (%) (n = 5)	Recovery (%)
Norfloxacin	0	9.52	9.57	0.58	100.6%
	40 %	17.39	17.35	0.57	99.8%
	80 %	32.0	32.32	0.47	101.01%
	110%	44.44	44.52	0.32	100.2%
	140%	55.17	54.73	0.19	99.22%
				Mean	**100.40%**

Table.6: Recovery Results for Tinidazole

Standard	Level	Conc. of std [µg mL^{-1}]	Conc. Of std Found[µg mL^{-1}]	RSD (%) (n = 5)	Recovery (%)
Tinidazole	0	3.57	3.58	0.71	100.42%
	40 %	6.52	6.50	0.25	99.79%
	80%	12	12.12	0.69	101. %
	110%	16.66	16.61	0.58	99.7%
	140%	20.69	20.58	0.47	99.5%
				Mean	**100.07%**

RESULTS AND DISCUSSION

In the present study quantification of Norfloxacin and Tinidazole have been done from their respective 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 Norfloxacin and Tinidazole in different buffer solutions have been studied i.e. Acetate,

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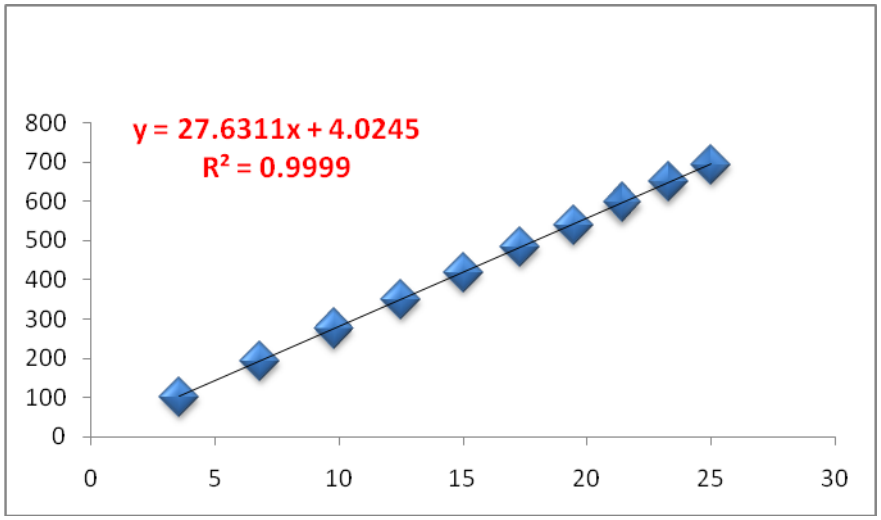
Phosphate and Britton-Robinson Buffer. Britton-Robinson buffer was prepared by mixing 0.04M Boric acid, 0.04M Phosphoric acid and 0.04M Glacial acetic acid. Further pH was adjusted with 1M NaOH. In the Britton-Robinson Buffer the whole pH range i.e. pH 2.0 to pH 10.0 has been studied.

As the pH was shifted from acidic to basic there is change in peak potential was observed. 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, finally Britton-Robinson Buffer of pH 6.5 and pH 6.0 was chosen as the best for Norfloxacin and Tinidazole respectively. As the concentration of Tinidazole increases the slight negative shift in potential was observed whereas the increase in the concentration Norfloxacin tends a positive shift in the potential.

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 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 $10mVs^{-1}$ uniform peak shape and peak height was small as compared to that of higher scan rate than $15mVs^{-1}$, so a scan rate of $10mVs^{-1}$ and $12mVs^{-1}$ was chosen as a best for the analysis of norfloxacin and tinidazole respectively. 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.

Linearity graph for Tinidazole standard



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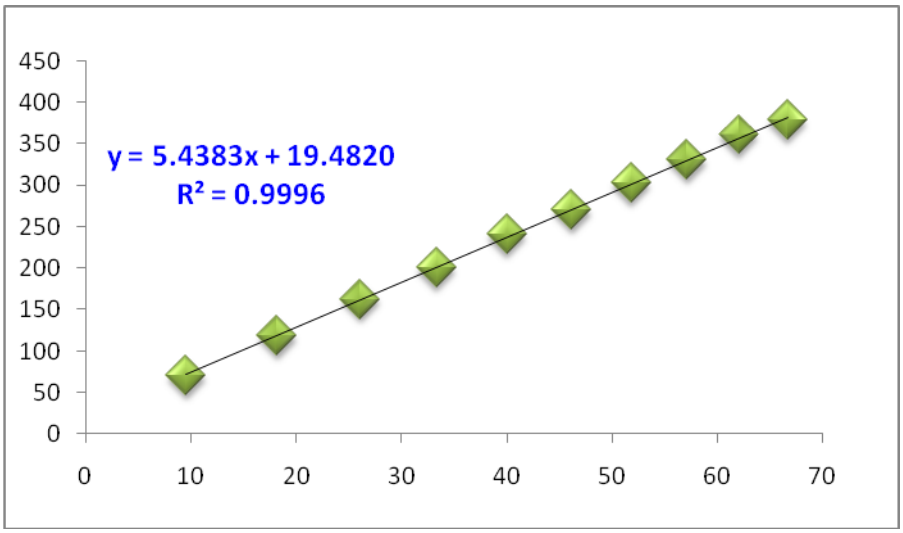
Linearity graph for Norfloxacin standard



CONCLUSION

Application to analysis of pharmaceutical formulation:-

A new polarographic method has been developed and subsequently validated for the quantifications of Norfloxacin and Tinidazole in the formulation individually. The advantages of this method for analytical Purposes lie in its rapid determination 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 spectrophotometric methods. Therefore presented method can be recommended for routine quality control analysis of Norfloxacin and Tinidazole in the pharmaceutical 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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