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VM Wagh
Department of Chemistry, Ramnarain Ruia Autonomous College, Matunga (East), Mumbai, Maharashtra, India

Corresponding Author:
VM Wagh
Department of Chemistry, Ramnarain Ruia Autonomous College, Matunga (East), Mumbai, Maharashtra, India

Simultaneous determination of ofloxacin and tinidazole in combined drug formulation by a simple electroanalytical technique in urine

VM Wagh

Abstract
In present study, a successful attempt has been made to develop a simple method for the simultaneous determination of Ofloxacin and Tinidazole in presence of Urine using Differential Pulse Voltammetry (DPV) technique. Quantification of Ofloxacin and Tinidazole was done in Britton-Robinson Buffer of pH 4.75 using 1M KCl as a supporting electrolyte. Both Ofloxacin and Tinidazole exhibit reduction cathodic peak in given pH with peak potential at -1.21 V for Ofloxacin and -0.25 V for Tinidazole vs SCE. 0.1N CH_3COOH was used as Solvent for the analysis. The parameters used for the method validation are LOD, linearity, assay and recovery. 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 Tinidazole in combined drug formulations in presence of Urine.

Keywords: differential pulse voltammetry (DPV), ofloxacin, tinidazole, britton-robinson buffer, urine

Introduction
Determination of quantity of drugs is very important during various stages of manufacturing of drug and during its clinical trials. Individual determination of several drugs by various electroanalytical methods have been reported [1-4]. Individual determination of drugs in presence of biological matrix such as urine have been reported [5-6]. Simultaneous determination of drugs using conventional methods such as HPLC and spectroscopy have been reported [7-13]. Variation in electrochemical behaviour of ofloxacin and tinidazole simultaneously by differential pulse voltammetry is studied [14]. Little attention is given to the simultaneous determination of drugs using electroanalytical techniques in presence of biological matrix such as Urine. Ofloxacin, $C_{18}H_{20}FN_3O_4$ that is 8-Fluoro-3-methyl-9-(4-methyl-piperazin-1-yl)-6-oxo-2,3-dihydro-6H-1-oxa-3a-aza-phenalene-5-carboxylic acid, (Molecular Weight: 361.37 g/mol), is available in single dose tablet or combined dose tablet. It is used to the treatment of various bacterial infections. Tinidazole, $C_8H_{13}N_3O_4S$ that is 1-(2-ethylsulfonylethyl)-2-methyl-5-nitro-imidazole, (Molecular Weight: 247.27 g/mol) is available in single dose tablet or combined dose tablet. It is used to treat or prevent a variety of bacterial infections.

Objective
The main objective of study is to provide a simple, rapid, efficient, reliable and economic method for the simultaneous determination of Ofloxacin and Tinidazole in combined pharmaceutical formulations in presence of Urine using Differential Pulse Voltammetry 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 Ofloxacin 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]. Further the desired value of pH (4.75) was adjusted with the addition of 1M NaOH.

Analytical method development
Preparation of standard solution
25mg of standard Ofloxacin and 75mg of standard Tinidazole 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 (500µg/ml of Ofloxacin and 1500µg/ml of Tinidazole respectively). Further all the standard solutions containing the mixture of Ofloxacin and Tinidazole were prepared using this stock solution.

Proposed voltammetric method
An aliquot of 20 ml made up of 18 ml Britton-Robinson Buffer adjusted to pH 4.75 by 1M NaOH + 2 ml of 1M KCl +1.0 mL of urine was placed in the dry and clean valtammetric cell. This is treated as a blank solution. 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 optimized operational parameters were as follows: 1] Scan rate- 22.5 $mVs^{-1.}$ 2] Pulse amplitude- 50mV. After recording a voltammogram of blank, aliquots of (0.5ml) each starting from 2.5 ml was added from the standard stock solution and then voltammogram is obtained. Resulted voltammograms were recorded under the optimum experimental conditions. Peak currents were recorded. Calibration curve was prepared by plotting peak current versus concentration of Ofloxacin and Tinidazole.

Preparation of sample solution
Ten tablets of TZ and OF combination were weighed and powdered for the analysis. The amount equivalent to average weight of a tablet i.e. 1.096 g (Ofloxacin - 200mg Tinidazole-600mg), was accurately weighed and transferred quantitatively to 500 ml beaker; then about 300 ml of 0.1N acetic acid was added in it and the mixture was sonicated for 10 mins with intermittent shaking. The volume was made up to the 400 ml by adding 100 ml of 0.1N acetic acid. The solution was then filtered through Whatman filter paper no. 41 to remove the excipients present in the tablet. Voltammograms for the sample solutions were analyzed by the method described as above. Voltammograms were recorded under the optimum experimental conditions. The amount of Ofloxacin and Tinidazole was calculated from resulting peak current values using already constructed calibration graph.

(For Ofloxacin: y = 8.5969x + 79.8077) and (for Tinidazole: y = 11.1512x + 240.4160)

Analytical method validation
Limit of detection
The limit of detection (LOD) in absence of Urine and in presence of urine for OF and TZ were determined by comparing peak current of blank and sample in the ratio 3:1 [Figure 1-4]. The replicates for blank solution were recorded 20 times and the mean current value at the reduction potential of Ofloxacin (i.e. at -1.21 V) and Tinidazole (i.e. at -0.35 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. LOD in absence of Urine and in presence of urine for OF and TZ obtained are as follows.

Table: Type of LOD

Drug	LOD (µg/ml)	
	In the absence of urine	In urine
TZ	0.93	1.76
OF	11.11	16.66

Linearity and Range
The linearity for Ofloxacin and Tinidazole were observed simultaneously by addition of standard solution. A good linearity was achieved in the concentration ranges of 53.2 µg/ml to 88.2 µg/ml µg/ml for Ofloxacin (Plot: 1) and 159.6 µg/ml to 264.7 µg/ml for Tinidazole (Plot: 2). The calibration curves were constructed with peak current (ip) against concentration (c) Figure [5]. The slope, Intercept, regression equation and correlation coefficient for the Ofloxacin and Tinidazole was obtained is given in Table [1].

Assay
The developed Polarographic method was used for simultaneous determination of Ofloxacin and Tinidazole from a drug formulation in presence of Urine. The sample solutions were analyzed by the developed method described above. Voltammograms were recorded under the optimum experimental conditions. Resulting peak currents of Ofloxacin and Tinidazole were measured and the amount of Ofloxacin and Tinidazole calculated using already constructed calibration graph. Assay studies were carried out at three different levels lower, middle and higher levels. 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 pre-analyzed sample of Ofloxacin and Tinidazole solution was mixed with different concentrations of standard solution and mixtures were analyzed in presence of Urine by proposed method. The percentage recovery was determined at different levels i.e. from lower, middle and higher levels. The results of percentage recovery analysis for Ofloxacin and Tinidazole are shown in Table [3].

Result and Discussion
In the present study simultaneous quantification of Ofloxacin and Tinidazole have been done from the drug formulation using Differential Pulse Voltammetry technique in presence of urine. Simultaneous quantification has many advantages over individual quantification such as

1. Saving of time
2. Saving of various recourses such as chemicals, energy, analysis time etc.

The developed method was validated and the results are shown in [1] to [3]

The percentage assay and percentage recovery at three different levels for both Ofloxacin and Tinidazole was found to be well within the acceptable limit of 98.00 % to 102.00 %. The results were shown in Table [2] and Table [3].

Conclusion

Limit of detection for both Ofloxacin and Tinidazole in presence of urine was found to be greater than the limit of detection in absence of urine. The Differential Votammetric technique can be used for detection and determination of Ofloxacin and Tinidazole in presence of urine.

Tables and Figures

Table 1: Method validation parameters for determination of and TZ

Parameters	Values	
	Tinidazole	**Ofloxacin**
Linearity range (µg/ml)	159.6 µg/ml to 264.7 µg/ml	53.2 µg/ml to 88.2 µg/ml
Slope (m) [a)]	11.1512	8.5969
Intercept(c) [a)]	240.4160	79.8077
Correlation coefficient (R^2)	1.0000	0.9997
LOD (µg/ml) in absence of Urine	0.93	11.11
LOQ (µg/ml) in presence Urine	1.76	16.66
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 TZ and OF in presence of urine:-

Drug	Level	Labeled claim (mg/tablet)	Drug found in mg	% Assay
TZ	Lower	600	590.49	98.42
	Middle	600	596.58	99.43
	Higher	600	597.86	99.64
	Average % Assay			99.16
	Standard Deviation (SD)			0.66
	% RSD			0.66
OF	Lower	200	202.93	101.47
	Middle	200	203.84	101.92
	Higher	200	204.56	102.28
	Average % Assay			101.89
	Standard Deviation (SD)			0.41
	% RSD			0.40

Table 3: Results of recovery studies for TZ and OF in presence of Urine:-

Standard	Level	Conc. Of std added [µg/ml]	Conc. of std Found [µg/ml]	Recovery (%)
TZ	Lower	31.91	32.42	101.58
	Middle	91.84	92.36	100.57
	Higher	147.06	147.47	100.28
	Average % Recovery			100.81
	Standard Deviation (SD)			0.69
	% RSD			0.68
OF	Lower	10.64	10.52	98.87
	Middle	30.61	30.91	100.96
	Higher	49.02	50.52	103.06
	Average % Recovery			100.96
	Standard Deviation (SD)			2.09
	% RSD			2.07

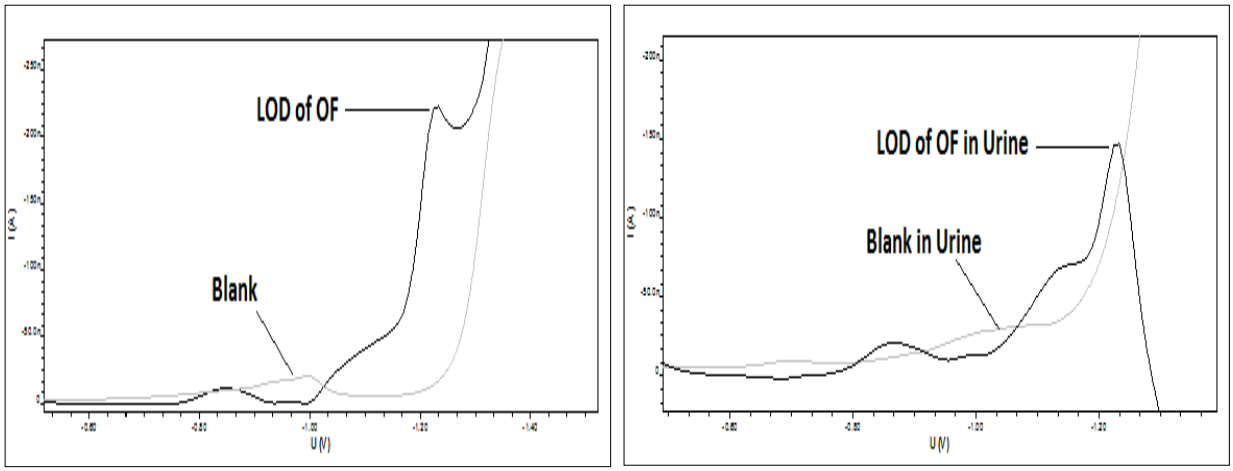


Fig 1, 2: Voltammograms showing the limit of detection for of in the absence of urine and in urine respectively.

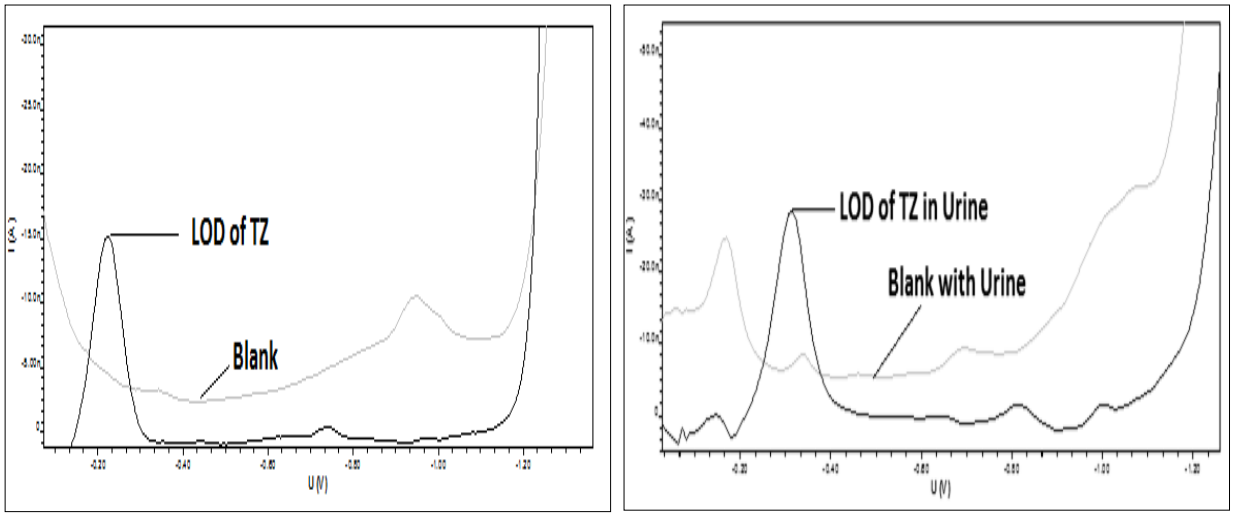


Fig 3, 4: Voltammograms showing the limit of detection for TZ in the absence of urine and in urine respectively.

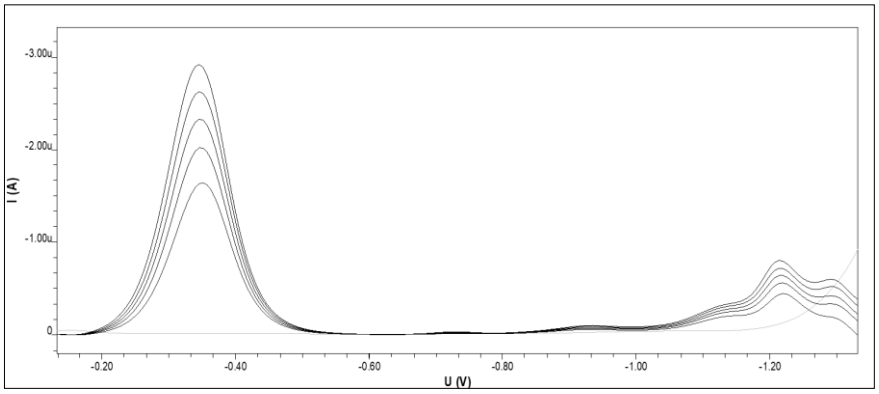
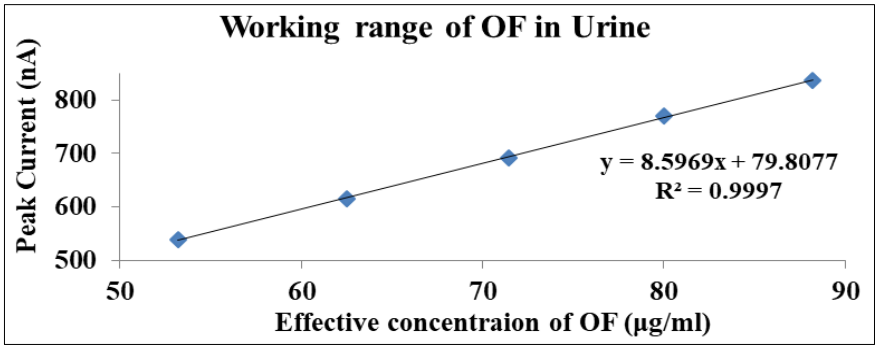
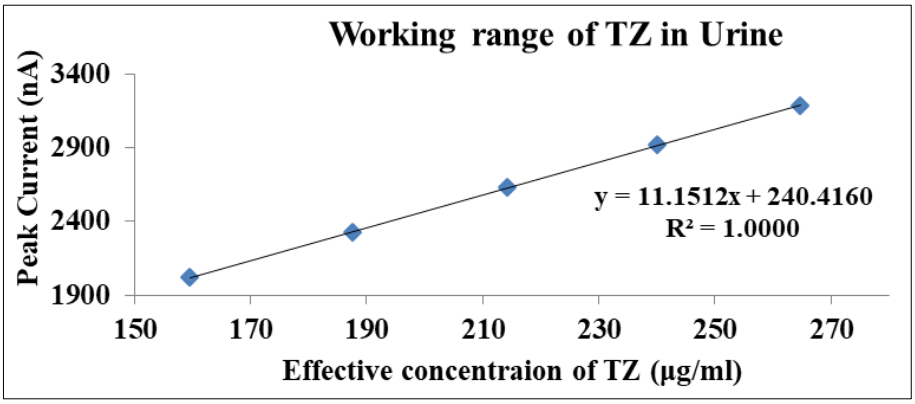


Fig 5: Voltammograms of the working range for TZ and of in urine



Plot 1: The linear working range for OF.



Plot 2: The linear working range for TZ.

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