Simultaneous Voltammetric Determination of Nitazoxanide and Ofloxacin in Pharmaceutical Formulation

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A simple, sensitive and highly selective electrochemical method was developed for the simultaneous determination of nitazoxanide and ofloxacin in aqueous media (Britton-Robinson buffer, pH-8.36) on a hanging mercury drop electrode (HMDE) using differential pulse polarography (DPP). Using DPP a separation of about 936 mV between the peak oxidation potentials of nitazoxanide and ofloxacin present in binary mixtures was obtained. The quantification limits for the simultaneous determination of nitazoxanide and ofloxacin were 0.083 μg/ml and 0.208 μg/ml, respectively. The proposed method was successfully applied for the simultaneous determination of nitazoxanide and ofloxacin in bulk drug and pharmaceutical tablet formulation.

Key words: Method development, nitazoxanide, ofloxacin, voltammetry

Nitazoxanide is chemically N-(5-nitro-2-thiazoyal) salicylamide acetate)[1-4]. It is used as an antiprotazoal, antihelmenthic, in giardiasis and cryptosporidiosis, in immune-compromised patients, including those with HIV infection[2-4]. It is not official in any pharmacopoeia. Analytical methods reported for quantitative determination of nitazoxanide are spectrophotometric method[5], RP-HPLC method[6,7] and stability indicating RP-LC method[8].

Ofloxacin is chemically (±)-9-flouro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7-H-pyridal(1,2,3de-)-1,4-benzoxazine-6-carboxylicacid and belongs to fluoroquinolone group of antimicrobial agents[2,9]. It is used in chlamydia or chlamydophila infection including nongonococcal urethritis. Ofloxacin is official in BP[10], USP[11], and EP[12]. The assay procedures mentioned in these pharmacopoeias are nonaqueous titration. Reported methods for quantitative determination of ofloxacin in pharmaceutical formulation or biological fluids are differential pulse polarography[13], square wave voltammetry[14], capillary electrophoresis[15], cyclic voltammetry, linear sweep voltammetry and electrochemical impendence spectroscopy[16], spectrophotometry[17] and HPLC[18-20]. Capillary electrophoresis[21], LC with fluorescence detection[22] and fluorimetry[23] are also reported for determination of ofloxacin enantiomers. Stability studies of ofloxacin are also reported[24]. Reported methods for simultaneous determination of nitazoxanide and ofloxacin are simultaneous equation method[25,26] and RP-HPLC method[27,28]. LOQ data is not available for simultaneous equation method but the RP-HPLC method reported LOQ as 0.43 μg/ml and 1.8 μg/ml for nitazoxanide and ofloxacin, respectively. Hence attempts were made to develop more sensitive and selective method for simultaneous determination of nitazoxanide and ofloxacin in bulk drug and pharmaceutical formulation.

All the reagents used were of AR grade. Pure drug samples of ofloxacin and nitazoxanide were kindly gifted by Alembic Pharmaceutical Limited (Vadodara, Gujrat, India) India. The gift samples were used as standards without further purification. Formulation Zenflox NT was purchased from local market.

All the electrochemical experiments were conducted in a three electrode single compartment glass cell. An Ag/AgCl (3.0 mol/L KCl) electrode was used as reference electrode and auxiliary electrode was a Pt electrode. The working electrode was a hanging mercury drop. The polarographic measurements were carried out using Metrohm 757 VA Computrace system (5.757.0010) attached to VA Computrace software 1.0 (Metrohm Ltd., Switzerland).

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Ofloxacin stock solution (1 mg/ml) was prepared by dissolving 50 mg of ofloxacin in methanol. Ofloxacin working standard solution (100 µg/ml) was prepared by diluting 5 ml of ofloxacin stock solution upto 50 ml. Nitazoxanide stock solution (1mg/ml) was prepared by dissolving 50 mg of nitazoxanide in 50 ml of methanol. Nitazoxanide working standard solution (100 µg/ml) was prepared by diluting 5 ml of nitazoxanide stock solution upto 50 ml. Binary mixture solutions containing ofloxacin and nitazoxanide in a ratio same as that in commercial formulation available were prepared by mixing and diluting suitable aliquots of ofloxacin working standard solution and nitazoxanide working standard solution with methanol.

Twenty tablets were accurately weighed and powdered in a mortar. An amount equivalent to one tablet (containing 200 mg of ofloxacin and 500 mg nitazoxanide) was taken in 50 ml volumetric flask and dissolved in 50 ml methanol by sonicating it for five minutes. Then the solution was filtered through Whatman filter paper No. 40 to the 100 ml volumetric flask and volume was made up to the mark with methanol. The sample solution thus prepared was diluted with methanol to get the solution containing 25:62.5 µg/ml ofloxacin:nitazoxanide.

After optimizing all the experimental parameters, standard addition method was used for polarographic determination of ofloxacin and nitazoxanide at pH-8.36. For this, 15 ml of supporting electrolyte Britton-Robinson buffer of pH-8.36 was taken in sample cell, purged for 300 s with N_2 gas and the polarogram was recorded in the voltage range of -0.05 mV to -1.45 mV for blank measurement in DPP mode. Then appropriate amount of binary mixture solution was added to the same sample cell containing 15ml of supporting electrolyte Britton-Robinson buffer of pH-8.36 and scanned in the same range in DPP mode. In same manner five readings were obtained with two replications. Validation parameters like precision, accuracy, LOQ and robustness were studied as per ICH guidelines[29].

Nitazoxanide has nitro group in its structure (fig. 1). Study of nitazoxanide through electrochemical behaviour was possible due to reduction of nitro group to amino group. Ofloxacin (fig. 1) has been a subject of several electrochemical investigations. Belal *et al*. [17] have recently studied ofloxacin electrochemical behaviour by means of differential pulse polarography (DPP). They found that electrochemical activity of ofloxacin is due to the reduction of the carbonyl C=O group and it proceeds in two steps (fig. 2).

The effect of supporting electrolytes like 0.1 mM potassium chloride, acetic acid-sodium acetate buffer of pH-6, ammonia-ammonium chloride of pH-8, disodium hydrogen phosphate-potassium dihydrogen phosphate of pH-8 and Britton-Robinson buffer (pH-4 and pH-8.36) on the current was examined.

The experimental result shows that the peaks were not obtained for both the compounds in case of ammonia-ammonium chloride of pH-8 and disodium hydrogen phosphate-potassium dihydrogen phosphate of pH-8 while in other cases cathodic reduction peaks for both the compounds were obtained. However, peaks were more sensitive and clear in case of Britton-Robinson buffer of pH-8.36 (fig. 3).

Working parameters of DPP method were established and effect of deposition potential, pulse amplitude

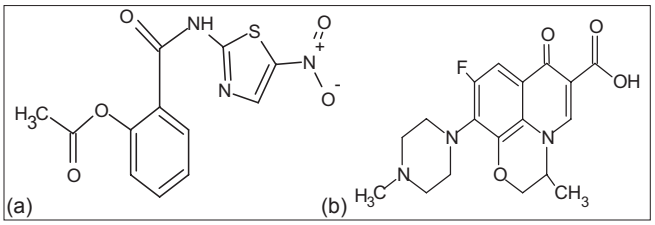

Fig. 1: Chemical structures of (a) nitazoxanide and (b) Ofloxacin

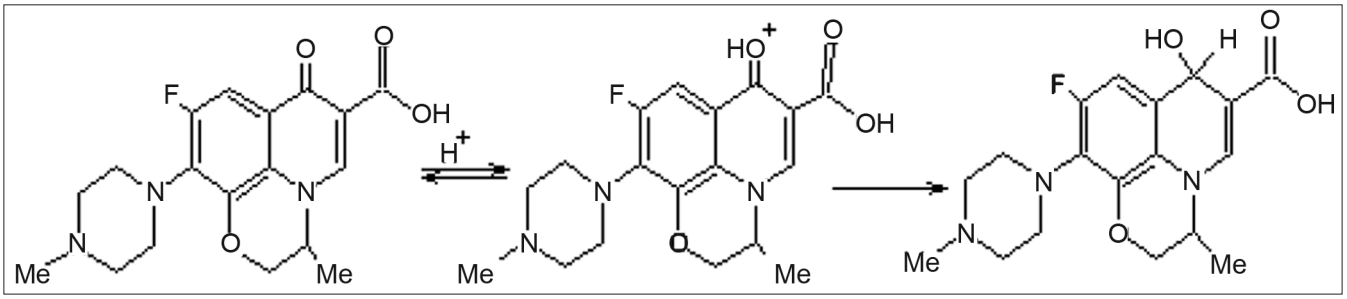

Fig. 2: Chemical reaction for reduction of Ofloxacin

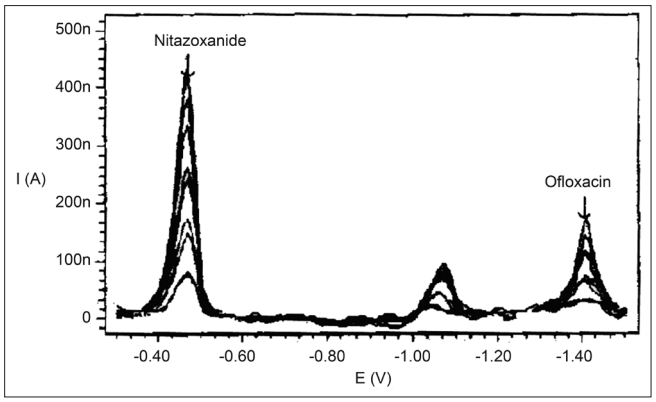

Fig. 3: Differential pulse polarogram of binary mixture of nitazoxanide and ofloxacin

voltage step time was studied. The best polarogram was obtained with deposition potential -1.3 V, deposition time 60 s, drop size 4, equilibrium time 10 s, voltage step 0.009 V, pulse amplitude 0.06 V and voltage step time 0.2 s (Table 1).

Interday and intraday precision for Differential Pulse Polarography method was measured in terms of % RSD. The experiment (preparation of calibration curve) was repeated five times in a day for intra-day and on five different days for inter-day precision (Table 2).

Accuracy of the proposed method was studied by recovery studies. Recovery studies were carried out by addition of known of standard drugs solution of nitazoxanide and ofloxacin to preanalysed tablet solution. The resulting solution was analyzed by proposed method. Recovery studies were carried out at the 80%, 100% and 120% level of the label claim. Results of recovery studies and percentage recovery were found to be satisfactory (Table 2). The minimum concentrations of nitazoxanide and ofloxacin which could be quantified were 0.083 μg/ml and 0.208 μg/ml, respectively.

The robustness of the method was determined by using different solvents for the preparation of stock solution of standard drug. The drug stock solution was prepared in methanol and 0.1M methanolic NaOH. The average value of %RSD of the responses for determination of ofloxacin and nitazoxanide were less than 2% reveals the robustness of the method.

Applicability of the proposed DPP method was determined by analyzing the commercially available tablets containing nitazoxanide and ofloxacin and

TABLE 1: WORKING CONDITIONS FOR DIFFERENTIAL PULSE POLAROGRAPHIC ANALYSIS OF NITAZOXANIDE AND OFLOXACIN

Parameter	Optimized value
Electrode	HMDE
Cleaning potential	-0.3 V
Deposition potential	-1.5 V
Deposition time	60 s
Voltage step	0.004 V
Pulse amplitude	0.04 V
Pulse time	0.05 s
Voltage step time	0.2 s
Equilibrium time	10 s
Drop size	4

TABLE 2: SUMMARY OF VALIDATION PARAMETERS FOR PROPOSED DPP METHOD

Parameter	Ofloxacin	Nitazoxanide
Precision (%RSD)		
Intraday(n=5)	1.141	0.603
Interday(n=5)	1.378	0.458
Accuracy	98.39±0.77	98.39±1.17
LOQ	0.208μg/ml	0.083 μg/ml
Robustness(%RSD)	0.603	0.4996

LOQ = Limit of quantification. RSD = Relative standard deviation.

TABLE 3: ASSAY RESULTS FOR COMBINED DOSAGE FORM USING PROPOSED DPP METHOD

Formulation	Drugs	Labeled claim mg/ tablet	Amount* found (mg)	% Found±SD
F1* DT	Nitazoxanide	500	492.80	98.56±0.61
	Ofloxacin	200	196.41	98.20±0.94

*Average of five determinations SD = Standard deviation

results were found to be in good agreement with the label claim (Table 3).

The obtained results show that the proposed DPP method is simple, accurate, precise as well as sensitive and can be used for the quantitative determination of ofloxacin and nitazoxanide alone or in mixture without any prior separation of individual drug. The method was found to be more sensitive than the HPLC method reported for the simultaneous estimation of nitazoxanide and ofloxacin. The value of %RSD for intraday and interday precision was found less than 2. This value confirms that method is precise. The value of % recovery greater than 98% for this method shows that the method is accurate and free from the interference of excipients used in formulation.

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