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Adsorptive Stripping Voltammetric Determination of Fluoroquinolones in Pharmaceuticals

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ABSTRACT

Sensitive cathodic stripping voltammetric methods have been developed for two fluoroquinolone antibacterial drugs, norfloxacin and enoxacin. Dimethylformamide (DMF) and 0.1 M hydrochloric acid were used as media for norfloxacin and enoxacin respectively. Potassium chloride was used as base electrolyte. A reduction wave was observed for norfloxacin within the range of -1.02 to -1.13 V and for enoxacin within -0.93 to -1.07 V. Linear calibration ranges for norfloxacin and enoxacin were observed within 20-100 μg/mL and 1-40 μg/mL with detection limits of 10 μg/mL and 50 ng/mL respectively. Relative standard deviations (RSD) for the analysis of 10 μg/mL norfloxacin and 10 μg/mL enoxacin (n = 5) were observed 1.1% and 0.2%. The presence of glucose, lactose, sorbitol, gum arabic, starch, magnesium stearate, methylparaben and propylparaben did not affect the determination. The methods were used for the analysis of pharmaceutical preparations and quantitative recoveries were obtained with relative deviation within 1.6-4.75%.

Key words: fluoroquinolone, norfloxacin, enoxacin, adsorptive stripping voltammetry (AdSV)

INTRODUCTION

Norfloxacin (1-ethy-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl) quinoline-3-carboxylic acid) and enoxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid) are broad spectrum antibiotics of the class fluoroquinolone. They are effective against both gram positive and gram negative organisms and are used in the treatment of urinary tract and respiratory tract infections, gastro-intestinal and sexually transmitted diseases[1-2].

A number of analytical methods are reported for the fluoroquinolones based on mainly spectrofluorometry[3], spectrophotometry[4-5], liquid chromatography[6], atomic absorption[7] and voltammetric method[8]. Liquid chromatographic methods are more reported[9-14].

Voltammetric methods are simple and the required sensitivity and selectivity for drug analysis are easily achieved. Jabber and Lounici have reported voltammetric methods for norfloxacin using direct current (DC), differential pulse polarography and adsorptive differential pulse stripping voltammetry[15-16]. However similar voltammetric methods are not available for enoxacin. The present work examines cathodic stripping voltammetry for norfloxacin and enoxacin, with better detection limit for enoxacin. The determination of norfloxacin has been carried out at slightly modified conditions than reported[16].

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MATERIALS AND METHODS

Reagent grade dimethylformamide (DMF) and hydrochloric acid (37%) were purchased from E. Merck, Germany. Potassium chloride was from Sigma, Switzerland. The base electrolytes potassium chloride, potassium nitrate, lithium chloride, sodium acetate and potassium dihydrogen phosphate tested were from E. Merck, Germany. Freshly prepared doubly distilled deionized water was used throughout the study. Norfloxacin was obtained from Novartis (Pvt.) Ltd. Jamshoro, Pakistan and enoxacin from Abbot Laboratories (Pvt.) Ltd. Karachi, Pakistan. The percent purity of drugs was 99.9%. The drugs were used without further purification. The drugs indicated better solubility in DMF and 0.1 M HCl and the stock solutions of norfloxacin (1 mg/mL) and enoxacin (1 mg/mL) were prepared in DMF and 0.1 M HCl respectively. Further dilutions were carried out in the same solvent as required. Potassium chloride (0.1 M) was prepared in distilled water.

Voltammetric studies were performed with an automatic 746-VA Trace Analyzer equipped with 747-VA Stand (Metrohm, Switzerland). The 747-VA stand includes a three electrode system, an Ag/AgCl (3 M KCl) reference electrode, a platinum wire as an auxiliary electrode and hanging mercury drop electrode as a working electrode. A printer (Epson LX300) attached with the instrument was used for printing purposes. A medium size mercury drop was employed as the Hanging Mercury Drop Electrode (HMDE). The solutions were stirred by a PTFE - coated

stirring bar rotated by a magnetic stirrer during the preconcentration step.

I. *Determination of Norfloxacin (Analytical Procedure)*

DMF solution of norfloxacin (3 mL) containing 20-100 μg and 7 mL of potassium chloride (0.1 M) was transferred into polarographic vessel. The sample was purged for 200 sec with oxygen-free nitrogen (British Oxygen Company, BOC, Karachi). The preconcentration potential measured against Ag/AgCl reference electrode was applied to the fresh mercury drop for 60 sec (tacc = 60 sec) while the solution was stirred. The stirring was then stopped for a period of 10 sec (equilibration time = 10 sec). The voltammogram was then recorded by applying a cathodic differential pulse scan with pulse amplitude of -50 mV. The adsorptive stripping voltammograms were recorded in triplicate for each run automatically by the instrument. The peak heights were assessed on the basis of the difference between the peak height of the analyte and that of base electrolyte alone recorded under the same conditions. The quantitation was carried out by calibration curve and standard addition technique. Voltammogram recorded for norfloxacin using standard addition method is given in Figure 1.

II. *Determination of Norfloxacin in Pharmaceutical Preparations*

Eight tablets of Noroxin (MSD, Merck Sharp & Dohme of Pakistan Ltd.) and Uritac (Wilson's Pharmaceuticals, Islamabad, Pakistan) were separately ground to fine powder. A quantity equivalent to one tablet (400 mg of norfloxacin) of each was weighed, dissolved in DMF, transferred to a 100-mL volumetric flask and diluted to the mark with DMF. This solution was slightly turbid but no further treatment was made. Further dilution was made with DMF to 50 ppm. Three milliliter of this solution and 7 mL of 0.1 M potassium chloride were transferred to polarographic cell and analysis was carried out by the following analytical procedure.

III. *Determination of Enoxacin (Analytical Procedure)*

A solution (9 mL) of enoxacin in 0.1 M hydrochloric acid containing 10-400 μg and 0.1 M potassium chloride (1 mL) were transferred to polarographic vessel and analytical procedure was followed as for norfloxacin. The quantitations were carried out by calibration and standard addition techniques. Voltammogram recorded for enoxacin using standard addition method is given in Figure 2.

IV. *Determination of Enoxacin in Pharmaceutical Preparations*

Eight tables of Enoxabid (Abbot Laboratories (Pvt.) Ltd., Karachi) and Enobact (Adamjee (Pvt.) Ltd., Karachi) were ground to very fine powders. A quantity equivalent to one tablet (400 mg) was weighed and diluted in hydrochloric acid (0.1 M). The final volume was adjusted to 100 mL with 0.1 M hydrochloric acid. The solution was further diluted to 50 ppm and the above analytical procedure was followed.

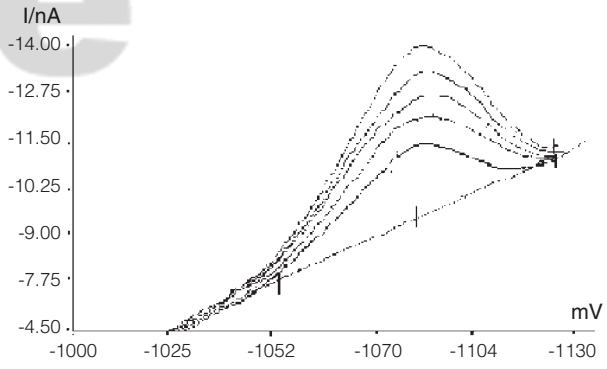

Figure 1. Adsorptive stripping voltammogram for norfloxacin indicating potential (mV) versus current (nA) in 30% DMF and 70% 0.1 M KCl, pH 8.2, peak potential 1.1 V, concentrations 20, 40, 60, 80 and 100 μg/mL^{-1} norfloxacin.

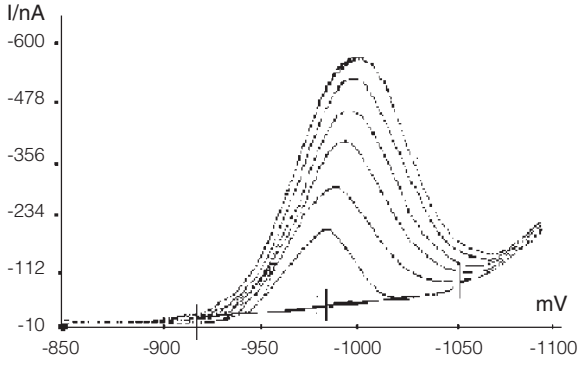

Figure 2. Adsorptive stripping voltammogram for enoxacin indicating potential (mV) versus current (nA) in 0.1 M HCl, pH 1.8, peak potential 0.98 V, concentrations 1-30 μg/mL^{-1} enoxacin.

RESULTS AND DISCUSSION

Adsorptive stripping voltammetry was examined for the determination of norfloxacin and enoxacin. The peak potential is dependent upon pH and base electrolyte which were initially examined. The pH for maximal response for norfloxacin was observed at 8.2, but for enoxacin at pH 1.8. Jaber and Lounici have determined norfloxacin in the pH range 6.0-8.5 in DMF-aqueous medium[16]. The observation of well defined peak of enoxacin at pH 1.8 may be due to the better solubility of enoxacin in 0.1 M HCl resulted from the protonation of additional nitrogen within aromatic ring. The enoxacin contains naphthyridine instead of quinoline group in norfloxacin. The determination of norfloxacin has been reported in the final concentration of 7%

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DMF. The concentration range of norfloxacin investigated in the present work indicated some difficulties, may be due to the low solubility of the drug in the solvent system. Thus, poor reproducibility was observed. However, when DMF was increased to 30%, better reproducibility was observed with coefficient of variation within 1.1% (n = 5). Enoxacin was examined in aqueous-hydrochloric acid solution. The norfloxacin exists in different protonated forms H_2A^+, HA and A^- depending upon the pH of the solution. The species HA (Figure 3A) could be the predominant one within the pH under study, because one of the nitrogen atoms in piperazine moiety is more basic than the carboxalate ion[15-16]. However enoxacin in acidic medium (0.1 M HCl) could be expected to be H_2A^+ and $H_3A_2^+$ species (Figure 3B-C). Base electrolytes, potassium chloride, potassium nitrate, lithium chloride, sodium acetate and potassium dihydrogen phosphate were investigated, but potassium chloride gave better response for both norfloxacin and enoxacin and was used. Using the conditions one reduction wave was observed for norfloxacin and enoxacin within the range -1.02 to -1.13 V and -0.93 to -1.07 V respectively. Linear calibration curves were obtained for 20-80 μg/mL of norfloxacin and 1-30 μg/mL of enoxacin with coefficients of determination (r^2) of 0.9964 (Figure 4) and 0.990 (Figure 5) respectively. The detection limits measured, as three times the background noise, were observed 10 μg/mL and 50 ng/mL for norfloxacin and enoxacin, respectively. The interfering effects of common additions present in pharmaceutical preparations were examined. The common additives like, glucose, lactose, sorbitol, gum arabica, starch, magnesium stearate, methylparaben and propylparaben were added as five times the concentration of norfloxacin or enoxacin. A change in the response of more than 5% was considered as evidence for interference. However these additions did not affect the determination of norfloxacin and enoxacin.

Two pharmaceutical preparations Uritac and Noroxin were analyzed for the content of norfloxacin and Enobact and Enoxabid were analyzed for the contents of enoxacin. The results obtained are summarized in Table 1 and there is close correlation between the expected values and the observed value with relative % deviation within 1.6 to 4.7%.

Table 1. Results of analysis for norfloxacin and enoxacin in pharmaceutical preparations

Name of tablet	Reported amount (mg/tablet)	Amount found (mg/tablet)	Relative deviation (%)
Uritac (Wilson's)	400	393.5	-1.625
Noroxin (MSD)	400	381	-4.75
Enobact (Abbott Lab)	400	419	4.75
Enoxabid (Admjee)	400	384	-4

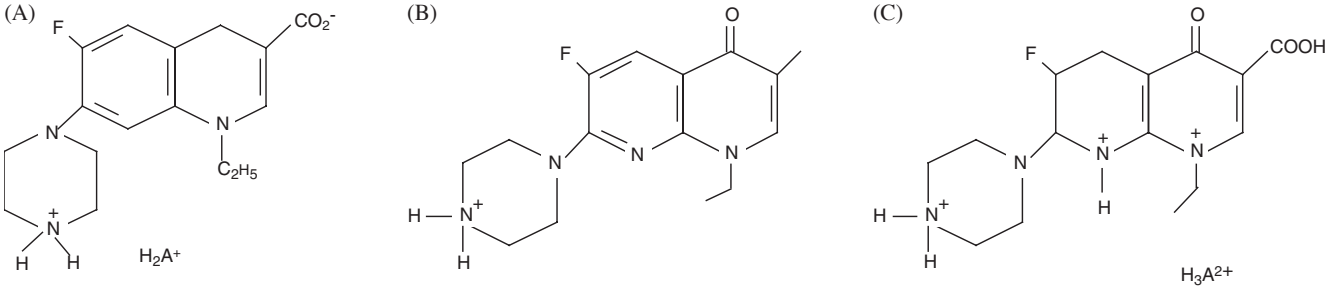

Figure 3. (A) Protonated species of norfloxacin. (B and C) Protonated species of enoxacin.

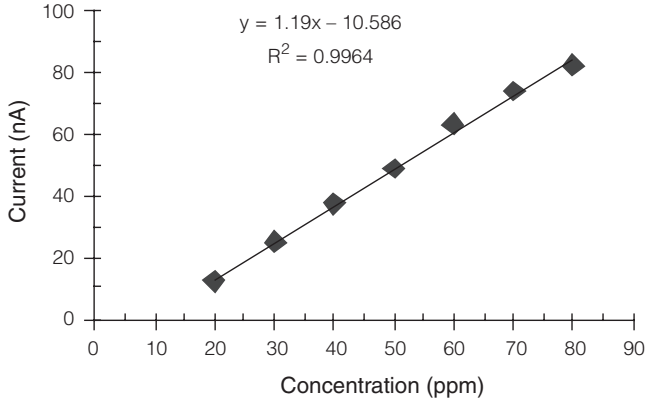

Figure 4. Calibration curve for norfloxacin conditions as Figure 1.

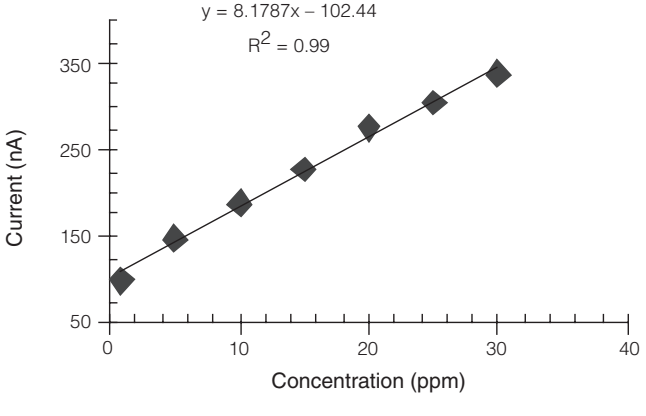

Figure 5. Calibration curve for enoxacin conditions as Figure 2.

CONCLUSIONS

Simple cathodic stripping voltammetric methods have been developed for norfloxacin and enoxacin in aqueous-DMF and aqueous-hydrochloric acid systems. Potassium chloride was used as base electrolyte. Norfloxacin and enoxacin showed well defined peaks at pH 8.2 and 1.8 with detection limits of 10 μg/mL and 0.05 μg/mL respectively. The methods were applied for analysis of norfloxacin and enoxacin in pharmaceutical preparations with relative % deviation of 1.6-4.7% from the expected values.

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