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Determination of Furazolidone in Urine by Square-Wave Voltammetric Method

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A fast, simple and sensitive square-wave voltammetric (SWV) method for the determination of trace amounts of furazolidone (FZ) in urine is reported. A three-electrode system containing stationary mercury dropping (SMDE) working electrode, Pt auxiliary electrode and Ag/AgCl reference electrode was used throughout. Briton-Rabinson buffer solution is used as both pH adjusting agent and supporting electrolyte. The calibration graph showed good linearity in the concentration range of 20-900 ng ml^{-1} of furazolidone with a regression coefficient of 0.9996. The equation $\Delta(i) = 0.0095C_{FZ} + 0.234$ was used for calculation of furazolidone concentration in the sample solution, where C_{FZ} is the concentration of furazolidone in ng ml^{-1} and $\Delta(i)$ is the difference between voltammogram peak currents of sample and blank solution. The RSD for 8 replicate measurements of a 60 ng ml^{-1} solution and LOD of the proposed method were found to be 2.2% and 5.2 ng ml^{-1}, respectively. The procedure was successfully applied to the determination of furazolidone in urine samples.

Keywords*:* Furazolidone, SW Voltammetry, Urine, Determination

INTRODUCTION

Furazolidone and other nitrofuran derivatives have been used for more than 30 years in medicine for the treatment of gastrointestinal infections in animals and humans. The main pharmaceutical uses of nitro aromatic compounds (RNO_2) are as antibacterial and anticancer agents [1,2]. It has been reported that nitro compounds generate a reversible one electron process due to the formation of the nitro radical anion ($RNO_2^{\bullet -}$) and an irreversible three electrons process corresponding to the formation of the hydroxylamine (RNHOH) in aprotic media [3-5]. Furazolidone (FZ) is an antibiotic used to treat diarrhea and enteritis caused by bacteria or protozoan infections (tiny, one-celled animals). Furazolidone is marketed by Roberts Laboratories under the brand name Furoxone [6].

Furazolidone (FZ), 3-(5-nitrofurfurylidene-amino)-2-oxazolidinone, is a chemically stable, yellow, crystalline compound. FZ is one of the rare examples of a drug developed against a parasite which has since gained broad use as an antibacterial agent. The drug is well concentrated in the urine, *i.e.*, 75% of the dose is rapidly metabolized by the liver, but 25% of the dose is excreted in the urine unchanged.

Thus, there is an increasing demand for rapid and simple methods for the determination of FZ in urine and pharmaceutical preparations. Methods based on HPLC and classical volumetric titrations are recommended by the Pharmacopoeias for the direct determination of FZ [7]. Although the HPLC method is daily practiced in many routine analysis laboratories, it is time consuming and very tedious as it is based on the use of acetic anhydride. Various research studies have been devoted to the development of new high performance alternative methods for determination of the drug in dosage forms using different techniques such as voltammetry [8,9], chromatography [10-12] and different spectrophotometric methods [13-17]. However, most of these

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methods suffer from large solvent consumption and/or small dynamic range of concentration [7-17].

The proposed SWV method proposed in this paper shows clear advantages such as short period of real time of drug analysis, no need for pre-treatment or time-consuming extraction steps prior to the drug analysis and no need for gas purging process. The method has large linear dynamic range and lower detection limits for determination of the drug in biological fluids compared with most of the reported methods [7-17].

Table 1. Recovery Test of FZ in Spiked Urine Sample Solutions

FZ added to urine (ng ml^{-1})	FZ found (ng ml^{-1})[a]	%Recovery	RSD (%)
0.0	0.0	-	-
40.0	39.0	97.5	3.0
100.0	98.0	98.0	2.0
200.0	203.0	101.5	1.0
500.0	495.0	99.0	0.6

[a]Average of three determinations.

EXPERIMENTAL

Reagents and Solutions

All chemicals were of analytical grade from Merck. Pure FZ was obtained from Sigma. Double distilled water was used throughout.

A furazolidone (FZ) stock solution (1000 μg ml^{-1}) was prepared by dissolving 0.1000 g of the drug in 50 ml of acetonitrile and diluting it to 100 ml with distilled water in a 100 ml volumetric flask. Working solutions were prepared by appropriate dilution of the stock solution with distilled water.

Britton-Robinson buffer solutions (pH = 3.0-12.0) were prepared by adding appropriate amounts of 0.5 M sodium hydroxide solution into solutions containing a mixture of 0.1 M of boric, acetic and phosphoric acids.

Apparatus

All voltammetric measurements were carried out with a SMDE working electrode in a three-electrode arrangement. A platinum wire was used as auxiliary electrode together with a silver-silver chloride reference electrode (Ag/AgCl), using 3 M KCl as electrolyte with a porous membrane.

The measurements were carried out on a Princeton Applied Research (EG & G 273 A) electrochemical device. Electrodes and electrochemical vessels were parts of SMDE 303A EG & G PARC which were controlled by the mentioned device. A Pentium 4 computer controlled all settings and data processing of the system.

Procedure

The general procedure adopted for obtaining square-wave voltammograms was as follows. Into a 10 ml volumetric flask were added 3.0 ml of Britton-Robinson buffer solution of pH 11.0 and appropriate amount of standard FZ solution (10 μg ml^{-1} followed by dilution to the mark with distilled water. The solution was transferred into the electrochemical cell, and after applying 5 s equilibrium time, the SW voltammogram was recorded by applying a negative-going potential scan over the range of -0.25 to -0.48 V. A well defined cathodic peak at -0.40 V was obtained for FZ which is used for further processing. All measurements were made at room temperature.

In the case of biological applications, fresh urine samples are collected from a 33 years old healthy volunteer. Spiked urine samples were obtained by treating 5 ml aliquots of the urine sample with appropriate amount of furazolidone working standard solution. A 20 μl aliquot of spiked urine was transferred into a 10 ml volumetric flask containing 3.0 ml Britton-Robinson buffer (pH = 11.0) and made up to the volume with distilled water. Table 1 shows the results of recovery tests for FZ determination performed on spiked urine samples.

RESULTS AND DISCUSSION

In order to obtain a well defined peak with maximum peak current for the determination of trace amounts of FZ, different chemical and instrumental parameters such as pH, ionic strength, buffer volume, equilibration time (ET), pulse height, frequency, scan increment and mercury drop size were optimized using the one at the time procedure. In the

investigation process, each variable was changed while the others were kept constant.

The effect of pH of the test solution on the peak current of FZ voltammograms was studied by applying Britton-Robinson buffer solutions (Fig. 1). Among the various investigated Britton-Robinson buffers in a pH range 3.0-12.0, the best voltammetric signal in terms of sensitivity (peak height) was obtained at pH 11.0. The optimum volume of the buffer solution was found to be 3 ml.

The effect of salt concentration on the voltammograms peak current of FZ was investigated by adding different concentrations of potassium nitrate salt to the test solution. The concentration of KNO_3 was changed in the range of 0.0-1.0 M in the test solution. The results revealed that the peak current and sensitivity of the method decreases with increasing salt concentration. Thus, further works were performed without addition of any salt.

Equilibration time (ET) which controls a variable delay during the cell performance was studied. Equilibration times of 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 s were applied to the electrodes and the corresponding voltammograms were recorded. The results showed no measurable increase in peak current at ET values greater than 5 s; thus, a time period of 5 s was chosen as optimum ET value.

The effect of scan increment, which determines the amount of potential changes between two data points in the experiment, was investigated. Scan increment values in the range of 1-12 mV (with 1 mV intervals) were applied to the electrodes and the corresponding voltammograms were recorded. The resulting peak currents showed that, by increasing the scan increment values, the voltammograms peak current will also increase steadily; so 6 mV was chosen as the optimum scan increment.

The effect of frequency changes on the voltammograms peak current (-0.40 V) of FZ was studied between the range of 5-300 Hz and 160 Hz was chosen as optimum frequency value (Fig. 2).

The effect of pulse height variation on the peak current of FZ voltammograms was studied in the range of 10-160 mV. The obtained results showed that pulse heights more than 90 mV have no effect on the peak current of FZ. So the optimum pulse height value is 90 mV.

It should be mentioned that the dissolved oxygen has no

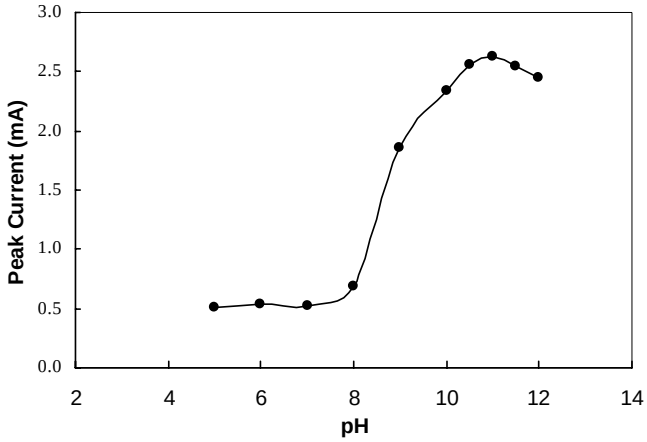

Fig. 1. Effect of buffer pH on the peak currents of FZ. Conditions: FZ, 400 ng ml^{-1}; buffer volume, 3.0 ml; purge time, 0 s; equilibrium time, 5 s; drop size, large; frequency, 200 Hz; pulse height, 90 mV; scan increment, 6 mV.

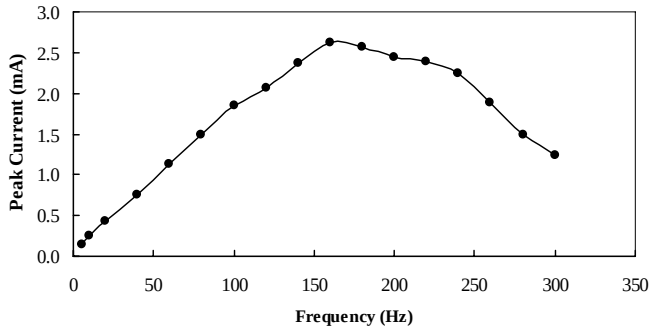

Fig. 2. Effect of Frequency on the peak currents of FZ. Conditions: FZ, 400 ng ml^{-1}; buffer volume, 3.0 ml; purge time, 0 s; equilibrium time, 5 s; drop size, large; pulse height, 90 mV; scan increment, 6 mV.

effect on the resulting peak currents of FZ. As it is seen in the Fig. 3, the blank chromatogram (doted line) is taken without any nitrogen gas purge for oxygen removal. Thus, all measurements were performed without any gas purging pre-treatment process.

The optimized chemical and instrumental parameters thus obtained are as follows. pH, 11.0; buffer volume, 3 ml; equilibrium time (ET), 5 s; scan increment, 6 mV; frequency,

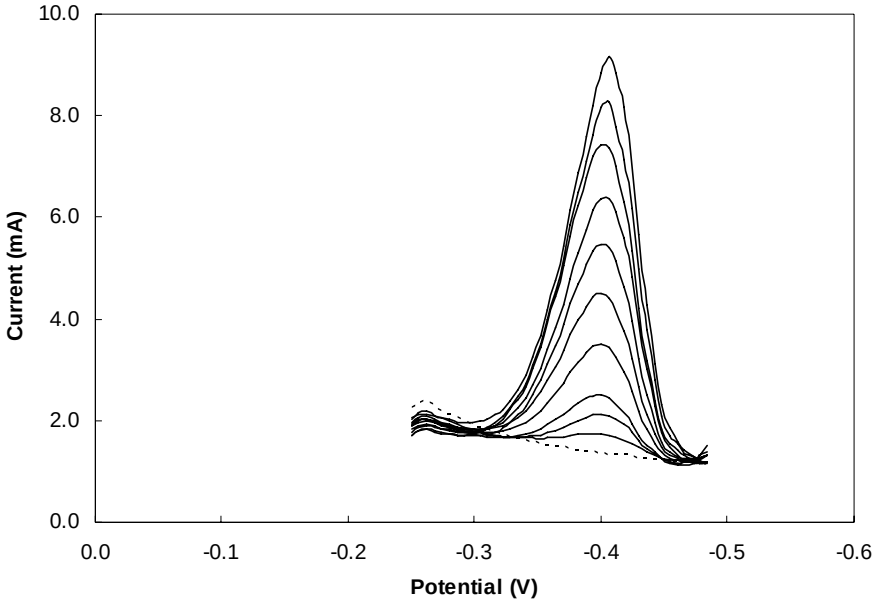

Fig. 3. Square-wave voltammograms for different concentrations of FZ (20-900 ng ml^{-1}) under optimum conditions. The broken line is for blank solution and other voltammograms are for different concentrations of FZ.

160 Hz; pulse height, 90 mV.

Under the obtained optimum conditions, the calibration graph for determination of FZ is obtained in the concentration range of 20-900 ng ml^{-1} of FZ (voltammograms for different concentrations of FZ are shown in Fig. 3) with a regression equation $\Delta(i) = 0.0095C_{FZ} + 0.234$ (n = 8) and a correlation coefficient of 0.9996. On the basis of 8 replicates tracing of calibration curve, the values of 0.0095 ± 0.0003 and 0.234 ± 0.0004 were also obtained for slope and intercept, respectively. The RSD for 8 replicate measurements and LOD of the proposed method were obtained as 2.2% and 5.2 ng ml^{-1}, respectively [18]. Limit of quantitation (LOQ) of the proposed method is 18 ng ml^{-1}. The repeatability and reproducibility evaluations for peak current and peak potential of FZ were carried out. For 16 measurements of peak current and peak potential of a 60 ng ml^{-1} solution of FZ by the proposed SWV method on one day, the RSD values of 3.13% and 1.07% are obtained, respectively. 16 measurements of peak current and peak potential were also carried out on different solutions containing the same amount of FZ (60 ng ml^{-1}) on different days and RSD values of 2.80% and 3.70% were obtained, respectively.

Interference Study

One of the striking points of any new method is its interfering limit of the potential interferences. In order to evaluate the tolerance limit of different interfering species for the proposed method, the interferences due to several cations and anions that may be commonly present in pharmaceutical and biological samples were studied in detail. This study was carried out under the optimum conditions for 60 ng ml^{-1} of FZ. The starting point was 1000 times of interfering ions *vs*. FZ; A relative error of 5% with respect to the signal of FZ was considered tolerable. Under the optimized experimental conditions at pH 11.0, some cations were precipitated and, after centrifugation of solutions and separation of precipitate, the voltammogram of the clear sample solutions were recorded. The results are shown in Table 2.

Application

In order to investigate the matrix effect of a complex sample on the proposed method, experiments were performed to determine the FZ content in spiked urine samples. Recovery studies were conducted with samples spiked with 20, 60, 120 and 400 ng ml^{-1} of FZ. The results of the recovery studies are

Table 2. Effect of Foreign Species on Determination of FZ

Foreign species	Tolerance ratio
CH_3COO^-, Ba^{2+}, Br^-, Ca^{2+}, CO_3^{2-}, F^-, K^+, NO_2^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, Cl^-, urea, sucrose, glucose, fructose	1000
ClO_4^-, Zn^{2+}, Mg^{2+}, Starch, citrate, tartarate, urea	800
Fe^{3+}, Mn^{2+}	300
NH_4^+	200
Al^{3+}, I^- [a]	100
Thiourea, acetaminophene	40
Caffeine	5

[a]The interfering ion precipitated by Ag^+.

summarized in Table 1. As seen, excellent recoveries were observed indicating that the constituents of the urine samples (matrix) do not interfere in any way with the detection of FZ. Therefore, the proposed voltammetric method could be used for the determination of FZ in urine samples.

CONCLUSIONS

In the present proposed procedure, square-wave voltammetry was used for the determination of Furazolidone in human urine samples. The procedure showed clear advantages such as short period of real time of drug analysis. Besides, no pre-treatment or time-consuming extraction steps were required prior to the drug analysis and also there was no need for gas purging process. In addition, the proposed procedure offered lower detection limits for determination of the drug in biological fluids compared with most of the reported methods [7-17]. The optimized procedure is simple, very rapid, specific, reproducible and sensitive enough for the drug assay at trace analysis.

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