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Electrochemical determination of gatifloxacin, moxifloxacin and sparfloxacin fluoroquinolonic antibiotics on glassy carbon electrode in pharmaceutical formulations

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Three fluoroquinolones; gatifloxacin (GAT), moxifloxacin (MOX) and sparfloxacin (SPAR) were electrochemically studied in various buffer systems at different pH values, using a glassy carbon electrode. The three fluoroquinolones were electrochemically oxidized at potential range (0.65–1.1 V) *vs* Ag-AgCl-KCl. The oxidation was irreversible and exhibited adsorption-controlled process behavior at all pH values and buffers studied. An electroanalytical methodology based on the adsorptive behavior of fluoroquinolones on glassy carbon electrode (GCE) and according to the linear relation between peak current and concentration using differential pulse voltammetry (DPV) method was successfully applied to the determination of the three fluoroquinolones in bulk and tablets. The proposed methods were statistically in agreement with that obtained by spectrophotometric comparison method. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: adsorptive stripping voltammetry; glassy carbon electrode; electrooxidation; fuoroquinolones; tablets

Introduction

The quinolones comprise a series of broad-spectrum synthetic antibacterial agents derived from nalidixic acid. They were discovered casually in 1962 and since then have essentially been used in the treatment of a wide range of infectious diseases.[1] The fluoroquinolones are quinolones with fluorine at position 6 of naphthyridine ring. Published structure-activity data show that the fluorine atom broadens their activity spectrum against both Gram-negative and Gram-positive pathogens. Several analytical methods for quantitative determination of fluoroquinolones in pharmaceutical formulations are reported, like capillary electrophoresis,[2] UV spectrophotometry,[3–5] spectrofluorimetry,[6,7] and high performance liquid chromatography (HPLC). The majority of HPLC methods were applied in determination of fluoroquinolones in biological fluids, edible animal products, feed, and to a lesser extent, in pharmaceutical formulations.[8–10] Most of the reported methods involve troublesome mobile phase (buffers) and difficult detection methods (fluorescence or mass detectors).

Electrochemical methods have proved to be sensitive and reliable for the determination of numerous electroactive drug components in pharmaceutical dosage forms and biological fluids.[11] The presence of carbonyl and carboxylic acid groups within the fluoroquinolones molecules has initiated several polarographic studies.[12,13] Electrochemical stripping techniques proved highly suitable tools for fluoroquinolones analysis. The strong tendency of fluoroquinolones to accumulate at the electrode surface offers a possibility of their highly sensitive determination by stripping voltammetry. Stripping voltammetry techniques have been used for determination of several fluoroquinolones, such as norfloxacin,[14,15] fleroxacin,[16] pefloxacin,[17,18] lomefloxacin,[19] trovafloxacin,[20] ofloxacin,[21,22] enrofloxacin,[23] levofloxacin,[24,25] gatifloxacin,[26] danofloxacin, and orbifloxacin[27] with sensitivity equivalent to that of HPLC techniques.

The objective of the present study was to develop and validate a simple, precise, accurate, and economic electroanalytical method for the quantitative determination of the three fluoroquinolones: gatifloxacin (GAT), moxifloxacin (MOX) and sparfloxacin (SPAR) in tablets. No extraction step was involved in the proposed methods, thereby decreasing time and error in quantitation. The data were compared with those obtained by spectrophotometric method, chosen as reference for validation of the method proposed.

Experimental

Apparatus and Reagents

Voltammetric measurements were performed using CHI610C Electrochemical Analyzer controlled by CHI Version 9.09 (CH Instruments, Austin, TX USA). A three-electrode system was composed of a glassy carbon (BAS model MF-2012, $\Phi = 3$ mm) working electrode, an Ag/AgCl/3 M KCl (BAS model MF-2063)

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reference electrode and a platinum wire (BAS model MW-1032) counter electrode. Before measurements, the glassy carbon electrode was polished manually with aqueous slurry of 0.05 mm alumina powder on a smooth polishing cloth. For analytical application, the following parameters were employed – DPV conditions: pulse amplitude 50 mV, pulse width 50 ms, interval time 0.2 s; CV conditions: initial and final potential were variable, depending on the pH value and the cut of the electrolyte. Scan rate measurements in the range 5–500 mV/s were carried out. A Perkin Elmer UV–VIS double beam spectrophotometer equipped with a PC for data processing (UV WinLab-ver 2.80.03, Perkin Elmer, MA USA) was used for recording absorption spectra. The pH was measured using CG 808 digital pH-meter with glass combination electrode (Mainz Schott Gerate, Germany). All measurements were carried out at ambient temperature of the laboratory ($22\,^{\circ}$C).

The three fluoroquinolones – GAT, MOX and SPAR contained in Gatilox®, Avalox®, and Spara® tablets as 400 mg, 400 mg, and 200 mg respectively, were obtained from a local pharmacy. The stock solution of fluoroquinolone (1×10^{-2} molL^{-1}) was prepared in water; stock solution of SPAR was prepared in methanol. Standard solutions were prepared by dilution of the stock solution with Millipore water. All chemicals were of analytical reagent grade. Phosphate buffer (0.1 molL^{-1}, pH 2.0–11.9) were used as supporting electrolytes.

Analysis of Tablets Formulations

Five tablets were accurately weighed and finely powdered using a pestle and mortar. A weighed portion of this powder equivalent to 100 mg of the drug was transferred into a small conical flask and about 50 ml of Millipore water was added. The flask contents were stirred for 10 min, and completed to volume with Millipore water. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte in order to obtain the analyzed solution. The amount of drug was calculated using the linear regression equation obtained from the calibration curve of drug. To study accuracy and precision, and to check the interference from excipients used in dosage forms, known amounts of pure drug were added to different pre-analyzed formulations of the studied fluoroquinolones, and the mixtures were analyzed by the proposed method.

Results and Discussion

Electrochemical Behavior of Fluoroquinolones GAT, MOX and SPAR

The cyclic voltammetric behavior of the three fluoroquinolones – GAT, MOX, and SPAR – at glassy carbon electrode in phosphate buffer (pH 7.0) is shown in Figure 1. The electrochemical behavior of the fluoroquinolones at this electrode over the pH range 2.0–11.9 yielded an oxidation process in the potential window of (0.85–0.91) V. The fact that no peaks were observed in the anodic branch scans suggests that the process is irreversible. Scan rate studies were also carried out to assess whether the process on glassy carbon electrode was under diffusion or adsorption control. A linear dependence of the peak intensity upon the scan rate was found, demonstrating adsorption behavior. A plot of logarithm of peak current versus logarithm of scan rate gave a straight line with a slope of 0.878, which is very close to the theoretical value of 1.0 that is expressed for an ideal reaction for an adsorption-controlled electrode process.[28] The peak potential shifts to more positive values on increasing the scan rate, which confirms the irreversibility of the oxidation process.

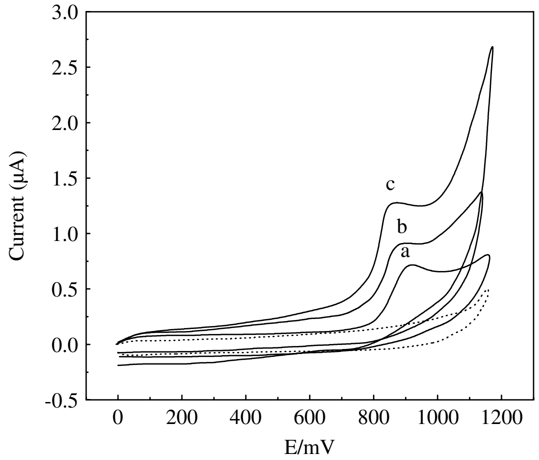

Figure 1. Cyclic voltammograms for 5.0×10^{-5} molL^{-1} (A) GAT, (B) MOX, and (C) SPAR at glassy carbon electrode in phosphate buffer (0.2 molL^{-1}, pH 7.0) and scan rate = 50 mV s^{-1}. The dotted line represents the blank scan.

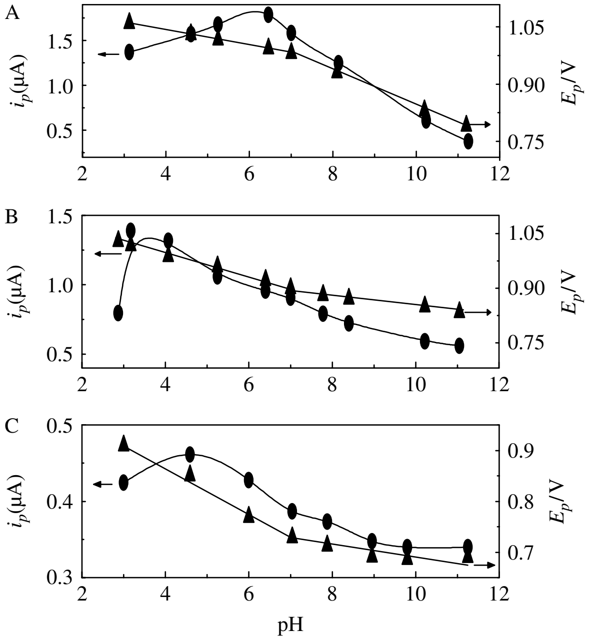

Figure 2. Effect of pH on (a) peak potential and (b) peak current for 5.0×10^{-5} molL^{-1} (A) GAT, (B) MOX, and (C) SPAR solutions in phosphate buffer by means of cyclic voltammetry at glassy carbon electrode, scan rate = 50 mVs^{-1}.

Effect of pH

The influence of the solution phosphate buffer (pH 2.0–11.9) on the response of the peak current and potential was analyzed. The dependence of peak potential (E_p) and peak current (i_p) on pH for phosphate buffer solution is shown in Figure 2. The best results with respect to enhancement, shape and reproducibility of the peak current were obtained in 0.2 mol l^{-1} phosphate buffer solution pH 6.5, 3.2 and 4.5 for GAT, MOX and SPAR respectively.

These solutions were selected as supporting electrolytes. For E_p *vs* pH, it is observed that the peak potential decrease as the increase of the pH from 2.0–11.2, which could indicate the presence of chemical reaction (proton-transfer reaction) precedes the electrode process.

Effect of Accumulation

The interfacial accumulation of fluoroquinolones on a glassy carbon electrode is indicated from the cyclic voltammograms of 1×10^{-5} molL^{-1} fluoroquinolones in 0.2 M phosphate buffer (pH 6.4–9.8) recorded before and after preconcentration time. The short preconcentration time results in a large anodic peak. Figure 3 shows the dependence of the adsorptive peak current on the preconcentration time of fluoroquinolones: GAT, MOX, and SPAR. The peak current increases with increasing preconcentration time, indicating enhancement of fluoroquinolone concentration at the electrode surface. As the accumulation time increases, the peak current tends to level off, showing that the adsorptive equilibrium is reached. Thus, considerable increase in sensitivity can be achieved by the application of adsorptive-stripping voltammetry to determination of fluoroquinolones. The effect of the accumulation potential on peak intensity was also evaluated for M fluoroquinolone solution following 60 s preconcentration time over the range 0.0 to 0.6 V and open circuit potential OCP. No significant effect of accumulation potential on peak intensity was observed. Therefore, in all cases an accumulation potential of OCP was chosen for analytical process.

Validation of the Analytical Procedure

The determinations of fluoroquinolones GAX, MOX, and SPAR were carried out in aqueous media at a glassy carbon electrode using electrochemical techniques. We selected differential pulse voltammetry (DPV) excitation mode as the DPV has well-established advantages, including good discrimination against background current, and low detection and determination limits.[29]

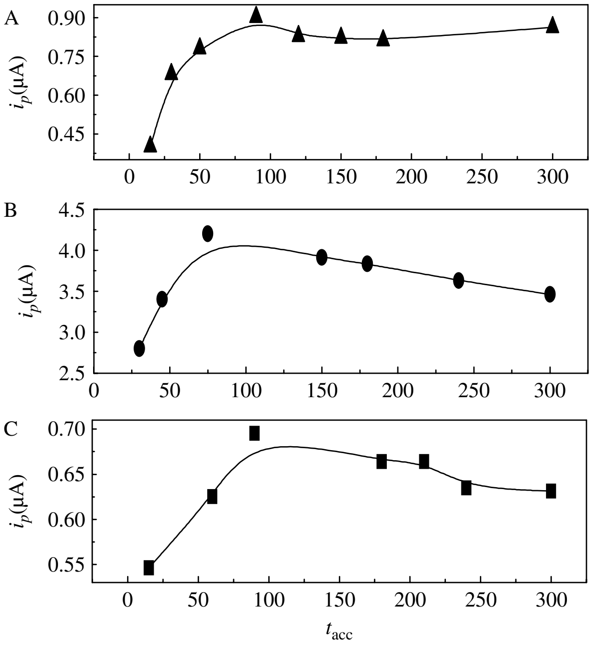

Figure 3. Effect of the accumulation time on the peak current for 1.0×10^{-5} molL^{-1} (A) GAT, (B) MOX and (C) SPAR solutions in phosphate buffer (0.2 molL^{-1}, phosphate buffers of pH 6.5; 3.2 and 4.5, respectively. Scan rate = 50 mV s^{-1}.

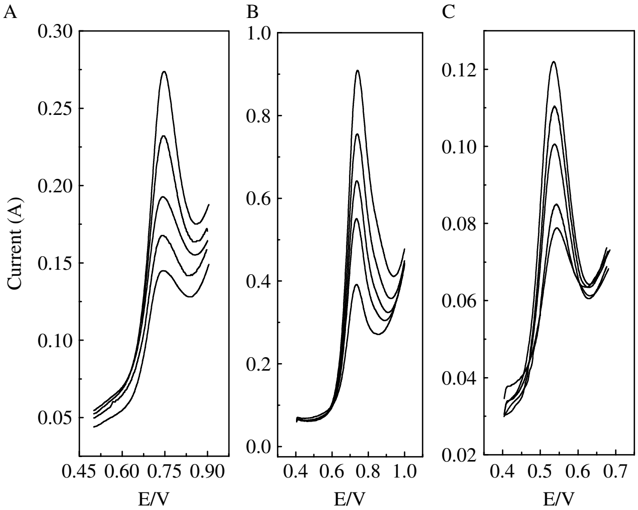

Figure 4. Adsorptive stripping voltammograms for increasing drug concentrations from 0.5 to 1.5 μM (A) GAT, (B) MOX, and (C) SPAR following accumulation for 60 min at OCP potential in 0.2 mol l^{-1} phosphate buffer solution pH 6.5, 3.2 and 4.5 for GAT, MOX, and SPAR, respectively.

Quantitative evaluation is based on the dependence of the peak current on the fluoroquinolone GAT, MOX, or SPAR concentration. Well-defined differential pulse anodic peaks were observed. The peak current increased linearly with increasing amounts of fluoroquinolone by AdSDPV method. Figure 4 shows the dependence of the peak current on pure drug concentration following an accumulation time of 60 s at open circuit potental in phosphate buffers. The calibration equations from the standard solutions of studied fluoroquinolones, according to procedures previously described, were constructed using DPV technique. The calibration plots characteristics are collected in Table 1. The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equation,[30] LOD = 3 s/m; LOQ = 10 s/m where s, the noise estimate, is the standard deviation of the peak current. The LOD and LOQ values, summarized in Table 1, indicate the higher sensitivity of the proposed procedure. It was found that the obtained values are lower than that obtained by spectrophotometric method.[3–5] The values obtained for SPAR at glassy carbon electrode are comparable with that using a β-cyclodextrin modified carbon paste electrode.[31] The values obtained for GAT, SPAR[32] and MOX[13] are higher than that reported at hanging mercury drop electrode, but, an alternate way to toxic mercury is the use of the glassy carbon electrode.

Moreover, several excipients used in the pharmaceutical formulations were investigated for their interference for the determination of 1×10^{-4} molL^{-1} studied drug. The tolerance limit was taken as the concentration causing an error +5%. Excipients contained within a tablet mass, as given by the manufacturer; did not disturb the determination of the main constituent at optimal conditions.

Assay of Fluoroquinolones in Tablets

The proposed method was successfully applied to the analysis of fluoroquinolones in tablets without the need for any pretreatment step prior to analysis. Five tablets were analyzed by the procedure described in the experimental section. The amounts of drug in

Table 1. Characteristics of fluoroquinolones calibration plots

	Gatifloxacin	Moxifloxacin	Sparfloxacin
Linearity range/(M)	$5 \times 10^{-6} - 1.5 \times 10^{-5}$	$5 \times 10^{-6} - 1.5 \times 10^{-5}$	$5 \times 10^{-6} - 1.5 \times 10^{-5}$
Slope/(μA/mM)	0.013	0.049	0.004
Intercept/μA	−0.022	−0.031	0.006
Correlation coefficient	0.993	0.995	0.993
SD of slope	8.222×10^{-4}	0.00271	2.932×10^{-4}
SD of intercept	0.00872	0.02870	0.00311
LOD/(M)	5.75×10^{-7} M	4.37×10^{-7} M	3.54×10^{-7} M
LOQ/(M)	1.90×10^{-6} M	1.45×10^{-6}M	1.18×10^{-6} M

Table 2. Application of the proposed voltammetric method to the determination of fluoroquinolones in Tablets

	Voltammetric method			Spectrophotometric method		
	Gatifloxacin	Moxifloxacin	Sparfloxacin	Gatifloxacin	Moxifloxacin	Sparfloxacin
Labeled amount/mg	400 mg	400 mg	200 mg	400 mg	400 mg	200 mg
N	5	5	5	5	5	5
$\overline{X}$	408	404	199	412	408	200
SD	14.28	11.31	3.78	11.94	8.56	5.00
RSD (%)	3.5	2.8	1.9	2.9	2.1	2.5
t-test of significance	0.48	0.63	0.35	$\{t(P = 0.05)\} = 2.31$	$\{t(P = 0.05)\} = 2.31$	$\{t(P = 0.05)\} = 2.31$
F-test of significance	1.43	1.74	1.75	$\{F(P = 0.05)\} = 6.388$	$\{F(P = 0.05)\} = 6.388$	$\{F(P = 0.05)\} = 6.388$

tablets were calculated by reference to the appropriate calibration plots. The fluoroquinolones in bulk and pharmaceutical dosage forms were also determined with spectrophotometric method, which are proposed for comparison with the DPV technique. The results obtained for the tablets dosage forms by electroanalytical and spectrophotometric methods are listed in Table 2. The results show no significant difference between spectrophotometric and proposed methods with regard to precision and accuracy, but voltammetric assays are more sensitive, simple, and rapid than spectrophotometric methods, without the need for any pretreatment step prior to analysis.

Conclusion

An adsorptive-stripping voltammetry AdSDPV has been developed for the determination of GAT, MOX, and SPAR fluoroquinolones in tablet dosage forms. The developed determination method is based on the adsorptive and electrochemical behavior of the drugs on glassy carbon electrode. The analyses were performed without any interference from additives present in the tablets. The method is simple, fast, sensitive, and does not require the expensive grades of solutions that are needed for HPLC procedures.

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