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Determination of some quinolones in tablets, human plasma and urine by differential-pulse polarography

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Abstract

A differential pulse polarographic method was developed for the determination of norfloxacin, cinoxacin and pipemidic, oxolinic and piromidic acids in tablets and biological fluids. Well defined peaks, useful for an accurate and precise assay, were observed in the appropriate supporting electrolyte (Britton-Robinson and phosphate buffers), depending on both the kind of preparation (tablet, plasma or urine) and the quinolone investigated. The analysis of quinolones in biological fluids requires a prior clean-up procedure (treatment with acetonitrile and 2 M potassium hydroxide for plasma and solid-liquid extraction for urine) while common excipients were found not to interfere in the tablet assay. In each of the above situations (tablet, plasma or urine), good precision of the method evaluated as the CV, was found.

Key words: Quinolone determination; Differential-pulse polarography; Pharmaceutical dosage form; Human plasma; Urine

1. Introduction

Quinolones, a series of nalidixic acid analogues, have become a major class of synthetic antibacterial agents. Their high concentration in urine after oral administration have made them useful for the treatment of urinary tract infections. These substances have a similar primary chemical structure but belong either to the quinolones, naphthyridines or to pyridopyrimidines. The polarographic behaviour of some quinolones has already been described and two distinct mechanisms have been proposed for their polarographic reduction, each involving a two-electron transfer (Staroscik et al., 1974; Grubb, 1979; Van Oort et al., 1983; Smyth, 1992).

Since the polarographic determination of norfloxacin, cinoxacin and piromidic, pipemidic and oxolinic acids has been studied neither in biological fluids nor, with the exception for pipemidic acid, in pharmaceutical preparations, it seemed of interest to develop a precise, accurate and sensitive method for the differential pulse (DP) polarographic determination of the above-mentioned quinolones in complex matrices.

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The procedures are described in the present work.

2. Materials and methods

2.1. Materials

Methanol (E. Merck), acetonitrile (E. Merck) acetic acid (Carlo Erba) and all other chemicals used for the preparation of the buffers and other supporting electrolytes were of analytical reagent grade. Water was purified by using a Milli-Q system (Millipore).

pH 8.9 Britton Robinson buffer was prepared by adding 7.75 ml of 0.13 M sodium diethylbarbiturate methanol-water (20:80, v/v) solution to 2.25 ml of 0.111 M hydrochloric acid methanol-water (20:80, v/v) solution.

Pure piromidic and pipemidic acids (BTS Industria Chimica), oxolinic acid (OHEMI), cinoxacin (Eli Lilly Italia) and norfloxacin (Merck Sharp and Dohme) were used as received.

Samples of quinolone tablets were locally purchased in pharmacies. Plasma was obtained from the Polyclinic Hemotransfusional Center of the Faculty of Medicine, Siena University. Urine was obtained from healthy male volunteers.

2.2. Apparatus and polarographic conditions

Polarograms were obtained with an EG&G (PAR) model 264A polarographic analyser, together with an EG&G PAR model 303A mercury drop electrode (DME) with a silver/silver chloride reference electrode and platinum auxiliary electrode connected with an EG&G PAR RE0073 model *x-y* recorder. The instrument was operated in the differential pulse mode (pulse amplitude, 25 mV), using a scan rate of 2 mV s^{-1}. Scans were performed from -0.5 to -1.2 V and from -0.8 to -1.5 V vs silver/silver chloride for cinoxacin, piromidic and pipemidic acids and for norfloxacin and oxolinic acid, respectively.

Controlled-potential coulometry was performed using an Amel model 552 potentiostat and a model 563 current integrator. The experiments were carried out in the absence of oxygen in a 20 ml cell with a mercury pool as the working electrode; a platinum as auxiliary electrode and a fixed potential of -1.0 and -1.7 V vs SCE for cinoxacin, piromidic and pipemidic acids and for norfloxacin and oxolinic acid, respectively, were used.

2.3. Preparation of calibration graph and analysis of tablets

0.01 M stock solutions were prepared by dissolving each sample in 0.04 M KOH. Standard solutions were obtained by making up to 100 ml with water 1 ml of stock solutions. A known volume (10 ml) of the supporting electrolyte (acetic acid in water-methanol 80:20, v/v, for cinoxacin and piromidic, acid and pH 8.9 Britton Robinson buffer for norfloxacin and oxolinic acid) was added to the cell and de-aerated with nitrogen for 10 min. For each sample, excepting oxolinic acid, a calibration graph was prepared by micropipetting a series of aliquots of the appropriate quinolone standard solution into the polarographic cell containing the supporting electrolyte. For oxolinic acid the additions were made using the stock solution directly.

20 tablets were weighed and finely powdered. An accurately weighed quantity corresponding to the average weight was transferred into a 20 ml test-tube containing 10 ml of 0.04 M potassium hydroxide in methanol/water (1:1). The test-tube was shaken with a reciprocating shaker for 20 min and the resulting suspension was transferred into a 100 ml volumetric flask and made up to volume with water (A). After homogenization, the solution was allowed to stand for 30 min and an aliquot of the solution was diluted to 100 ml with water according to the following scheme: piromidic acid 1.0 ml, cinoxacin 1.5 ml, norfloxacin 3.0 ml. 250 μl of the resulting solution were directly transferred into the polarographic cell containing 10 ml of the appropriate supporting electrolyte and the polarogram was recorded using the above-mentioned parameters. For oxolinic acid the analysis was carried out adding into the polarographic cell 250 μl of the concentrated solution (A).

2.4. Analysis of quinolones in human urine

10 ml of urine were spiked with 600 μl of 2×10^{-3} M quinolone standard solution and acidified to pH 2.9 with 85% phosphoric acid. The urine was subjected to solid-phase extraction onto a series of two in-series Supelclean LC-8 3 ml cartridges previously conditioned as follows: the columns were placed on the vacuum manifold (Visiprep Supelco), 9 ml of methanol and 9 ml of pH 2.9 0.2 M phosphate buffer were sequentially passed through them. The urine sample was slowly drawn through the columns and then the electroactive residues of the urine matrix were washed passing through the columns 40 ml of pH 4 0.2 M phosphate buffer. The columns were dried at full vacuum for 1 min and then the drug was eluted by passing 9 ml of methanol. The urine methanolic extract was concentrated to 2 ml under nitrogen.

In the case of cinoxacin, piromidic and pipemidic acids, 2 ml of urine methanolic extract were transferred into the cell. The cell was completed with the supporting electrolyte (6 ml of distilled water, 1.85 ml of 2 M potassium chloride and 0.15 ml of 85% phosphoric acid; resulting pH, 1.3). The polarographic solution was briefly stirred and deoxygenated for 10 min and then the DP polarographic scan was performed.

In the case of oxolinic acid and norfloxacin, 2 ml of urine methalonic extract were transferred into the polarographic cell which was completed with the supporting electrolyte (5 ml of pH 8.5 0.2 M phosphate buffer, 2 ml of 2.5 M potassium chloride and 1 ml of distilled water). The above-described procedure was then used.

Quantitation of quinolones was then performed by means of a calibration graph prepared by adding to the polarographic cell, containing the appropriate supporting electrolyte and 2 ml of the unspiked urine methanolic extract, aliquots of the appropriate quinolone sufficient for achieving a final concentration of 8–550 μg ml^{-1} in the polarographic cell.

2.5. Analysis of quinolones in human plasma

1 ml of plasma was spiked with 100 μl of 2×10^{-3} M quinolone solution and vortexed for 20 min. After this period, 20 μl of 2 M potassium hydroxide and 2 ml of acetonitrile were added and vortexed. After centrifugation for 15 min at approx. 3500 rpm, 2 ml of the surnatant were transferred into the polarographic cell which was completed as described above for urine analysis.

Quantitation of quinolones was then performed by means of a calibration graph prepared by adding to the polarographic cell, containing

Table 1
Data of calibration curves and some experimental parameters for DP polarographic determination of quinolones in tablets, human plasma and urine

	Sample	Concentration range (μg ml^{-1})	Slope (nA ml μg^{-1})	Intercept (nA)	Correlation coefficient	pH	E_p (V)
Tablet	piromidic acid	3 – 20	24.21	4.520	0.9999	3.0	−0.75
	cinoxacin	10 – 30	62.03	−330.2	0.9984	3.0	−0.80
	norfloxacin	15 – 50	30.97	−58.83	0.9998	8.9	−1.50
	oxolinic acid	200 –1400	18.03	−167.0	0.9992	8.9	−1.76
Urine	piromidic acid	8.25– 504.6	89.16	94.32	0.9997	1.3	−0.69
	cinoxacin	20.79– 456.3	102.48	93.67	0.9996	1.3	−0.74
	norfloxacin	29.7 – 563.2	66.52	71.62	0.9998	8.5	−1.42
	pipemidic acid	15.7 – 527.2	78.25	85.69	0.9997	1.3	−0.76
	oxolinic acid	23.15– 554.14	70.65	70.79	0.9998	8.5	−1.68
Plasma	piromidic acid	0.83– 8.96	103.5	96.78	0.9996	1.3	−0.66
	cinoxacin	0.67– 8.3	86.18	85.49	0.9992	1.3	−0.71
	norfloxacin	0.66– 10.24	88.75	90.33	0.9993	8.5	−1.40
	pipemidic acid	0.89– 9.60	85.77	70.52	0.9987	1.3	−0.73
	oxolinic acid	0.66– 8.32	72.55	85.76	0.9989	8.5	−1.66

the appropriate supporting electrolyte and 2 ml of the unspiked plasmatic methanolic extract, aliquots of the appropriate quinolone sufficient for achieving a final concentration of 0.7–10 μg ml^{-1}.

3. Results and discussion

The calibration curve parameters determined by least-squares fitting of the experimental data and the results of quinolone polarographic analysis in urine, plasma and tablets are summarized in Tables 1 and 2 which also list the characterizing parameters (E_p, pH) of the polarographic reduction.

Coulometric analysis confirmed that two electrons per molecule are involved in the electrochemical process, due to the hydrogenation of the ethylenic bond in the azinone ring (Van Oort et al., 1983.) or to the reduction of the carbonyl group on C_4 of the same ring (Staroscik et al., 1974; Grubb, 1979; Smyth, 1992).

The polarographic study performed at different pH values allowed the distinction into two groups of the quinolones examined. The first includes piromidic and pipemidic acids and cinoxacin. For these compounds peak potential varies quite linearly with pH within the range 1–12 (Fig. 1). The second group includes the other two molecules studied (norfloxacin and oxolinic acid) for which no peaks were observed for pH < 6. The distinction also reflected the structural differences between the two classes, the second being differing from the first in the lack of basic nitrogen atoms in the condensed ring. In all cases, the shift of the peak potential to more

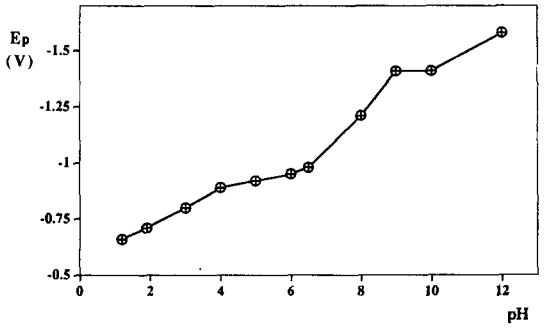

Fig. 1. Effect of pH on cinoxacin peak potential. Supporting electrolyte: 0.22 M phosphoric acid and 0.37 M potassium chloride.

Table 2
Results for quinolone assay in tablets, human plasma and urine

	Sample	Claimed content per tablet (mg)	% average recovery (n = 5)	CV
Tablet	piromidic acid	500	98.6	1.06
	cinoxacin	500	98.6	1.12
	norfloxacin	400	98.0	0.71
	oxolinic acid	750	98.1	0.96
		Detection limit (μg ml^{-1} in cell)		
Urine	piromidic acid	2.5	93.4	1.8
	cinoxacin	6.3	95.6	2.0
	norfloxacin	9.0	93.0	1.9
	pipemidic acid	4.8	94.7	1.4
	oxolinic acid	7.0	94.7	2.3
Plasma	piromidic acid	0.25	89.7	4.5
	cinoxacin	0.20	91.7	3.8
	norfloxacin	0.20	90.5	4.1
	pipemidic acid	0.27	91.8	4.7
	oxolinic acid	0.20	90.9	5.1

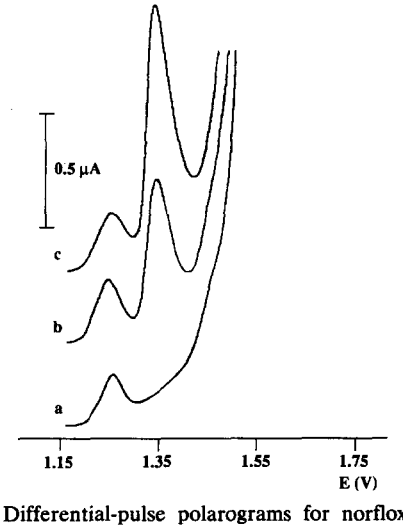

Fig. 2. Differential-pulse polarograms for norfloxacin plasmatic methanolic extract in 0.1 M pH 8.5 phosphate buffer and 0.5 M potassium chloride. (a) Blank; (b,c) additions of 80 and 160 μl, respectively, of 2×10^{-3} M norfloxacin solution.

negative values with increasing pH confirmed that hydrogen ions were consumed in the reduction.

The application of the above-described conditions to the analysis of quinolone formulated tablets and of samples of human plasma and urine gave rise to the results reported in Table 2. Quantitation was performed by means of the calibration graph method achieving, for each analysis, the specified percent recoveries. Good reproducibilities, evaluated in terms of coefficient of variation, were determined by making five replicate scans of solutions at about the 1×10^{-4} M level.

A typical DP polarographic curve of one of the quinolones examined, in plasma (norfloxacin) is reported in Fig. 2 as representative for the experimental results of quinolones in biological fluids.

With a view to increasing the sensitivity of the analysis, further electroanalytical work is in progress in this laboratory, adsorptive stripping voltammetry being one possibility.

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