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Determination of quinolones in plasma samples by capillary electrophoresis using solid-phase extraction

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Abstract

The potential of capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC) have been investigated for the separation and quantitative determination of 10 quinolone antibiotics. The influence of different conditions, such as the buffer and pH of the electrolyte, the surfactant and the ion-pairing agents added to the electrolyte and the organic modifier were studied. A buffer consisting of 40 m*M* sodium tetraborate at pH 8.1 containing 10% (v/v) methanol was found to be a highly efficient electrophoretic system for separating lomefloxacin, enoxacin, norfloxacin, pipemidic acid, ofloxacin, piromidic acid, flumequine, oxolinic acid, cinoxacin and nalidixic acid. A solid-phase extraction method to remove the sample matrix (pig plasma samples) was developed on a C_{18} cartridge using a mixture of methanol–water (70:30, v/v). The method is specific and reproducible and mean recoveries were in the range 94.0±4.2% and 123.3±4.1% for pig plasma samples over the range used. A linear relationship between concentration and peak area for each compound in pig plasma samples was obtained in the concentration range 5–20 mg l^{-1} and detection limits were between 1.1 and 2.4 mg l^{-1}.

Keywords: Quinolones

1. Introduction

Quinolones are an important group of synthetic antibiotics with bactericidal action. The antibacterial activity of these compounds was shown to result from selective inhibition of bacterial DNA synthesis [1,2]. They all have a carboxylic acid group in position ''4'', so are often referred to as 4-quinolones. These carboxylic acid groups are active against many gram-positive and gram-negative bacteria [3]. Numerous structurally related quinolones have been synthesized and several are in routine clinical use throughout the world. Their antibacterial activity is greatly increased by the addition of 6-fluoro- and 7-piperazinyl groups to the molecule. The more recent introduction of fluorinated quinolones represents a particularly important therapeutic advance, since these agents have broad antibacterial activity and are an effective oral for the treatment for a wide variety of infectious diseases [4]. They are also widely used to treat and prevent of veterinary diseases in food-producing animals [3].

The need to identify quinolones in various biological tissues and fluids is obvious. Numerous techniques have been developed for their analysis in biological fluids and pharmaceutical preparations. Most of the analytical methods published are based on high-performance liquid chromatography (HPLC)

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[3,5–9], and some have been reported to determine them in various biological matrices [3,6–8]. Carlucci [8] wrote a review which included a different HPLC assay which was designed to determine different fluoroquinolones in biological fluids.

Other studies have concentrated on the structure and pK_a values of quinolones. For instance, Barbosa and co-workers [9–12] studied the variation of their dissociation constants and those of other types of compounds like diuretics and peptides in several acetonitrile–water mixed solvents. Different gradient elution HPLC was used to determine impurities and degradation products of some quinolones because of their large differences in the chromatographic behavior [5]. Reverse-phase liquid chromatography (RPLC) was used to quantify structure–retention relationships of different quinolones [10]. All of these studies provide information about the behavior of these compounds.

Capillary electrophoresis (CE) is a highly efficient method of separation which is generally used to determine charged components because it combines high resolution and easy automation with modest sample requirements and low solvent consumption [13–15]. Little attention has been paid, however, to separate quinolone antibiotics.

It should be possible to perform CE analysis [16–20] on quinolones because they have carboxylic acid function in their structures (Fig. 1), and because CE is an attractive resolution technique because of its high efficiency and short analysis times and because it requires smaller volumes of analytes and running solution additives than HPLC. Both capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MEKC) were used.

CE has been used to determine ciprofloxacin in pharmaceutical formulations [16] and for the enantioselective separation of ofloxacin and DU-6859 using either vancomycin [21] or the γ-cyclodextrin–zinc(II)–*o*-phenylalanine mixture [18] as a chiral selector. Sun and Chen [17] performed a capillary electrophoretic separation of 14 antibacterial quinolones using standard solutions. To optimize the separation they employed a triangular overlapping mapping scheme in which three factors relevant to the running buffer composition were chosen: the concentrations of sodium cholate (the micelle-forming surfactant), sodium heptanesulfonate (ion-pairing agent) and the percentage volume of acetonitrile.

Although several reports have shown that CE is suitable for pharmaceutical analysis, only a few have been applied to biological samples [19,20]. They were applied predominantly to human body fluids; the sample was pretreated by deproteinization adding different reagents to the sample. Möller et al. [19] developed a CE method with laser-induced fluorescence to determine moxifloxacin in plasma and microdialysate and the results obtained were cross-validated with an established HPLC method. Pérez-Rubio et al. [20] determined six quinolone antibiotics simultaneously by CZE in serum and urine samples.

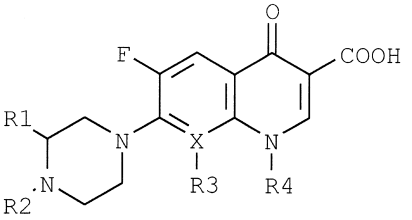
Solid-phase extraction (SPE) has gained popularity over the years in the preparation of samples for a wide range of analytes in complex matrices because of its better selectivity, its simpler operation, and the lower consumption of solvents. However, few reports have employed SPE for extracting antibiotics from plasma samples [22–25]. Manceau et al. [26] used SPE as a pretreatment in the analysis of enrofloxacin and ciprofloxacin in biological fluids by HPLC.

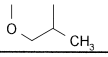
This paper studied the optimization of the separation of 10 antibacterial quinolones (Fig. 1) using CZE and MEKC modes. For this purpose the pH and composition of the electrophoretic buffer and type and proportion of the organic modifier were optimized. The animal plasma was pretreated using a C_{18} SPE and different parameters were optimized. The optimum CZE method is rapid and is useful for determining these 10 antibiotics in animal plasma samples.

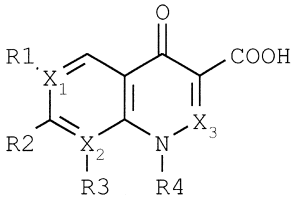
2. Experimental

2.1. Instrumentation

Electrophoretic experiments were performed using a Hewlett-Packard 3D CE instrument (Waldbronn, Germany) equipped with a diode array detector. Data were collected using the software provided with the HP Chemstation version A.03.01 chromatographic data system. The capillary was uncoated fused-silica (64.5 cm×75 μm I.D.) supplied by Supelco (Bellefonte, PA, USA). A detection window was prepared



	R1	R2	R3	R4	X
Enoxacin (1)	H	H	---	Et	N
Lomefloxacin (2)	CH_3	H	F	Et	C
Norfloxacin (3)	H	H	H	Et	CH
Ofloxacin (4)	H	CH_3			C
Ciprofloxacin (5)	H	H	H		CH



	R1	R2	R3	R4	X1	X2	X3
Cinoxacin (6)			H	Et	C	C	N
Flumequine (7)	F	H			C	C	CH
Nalidixic acid (8)	H	CH3	---	Et	C	N	CH
Piromidic acid (9)	---		---	Et	N	N	CH
Oxolinic acid (10)			---	Et	C	N	CH

Fig. 1. Structures of the quinolone antibiotics studied.

by burning off the polyimide coating 56 cm from the capillary inlet.

2.2. Reagents and standards

Enoxacin, lomefloxacin, norfloxacin, ofloxacin, pipemidic acid, cinoxacin, flumequine, nalidixic acid, piromidic acid and oxolinic acid were purchased from Sigma (St. Louis, MO, USA). Standard stock solutions of 1000 mg l^{-1} were prepared in 0.1 *M* NaOH and stored under refrigeration. Standard working solutions were prepared weekly or daily, depending on their concentration, by diluting the standard stock solution with water which had been purified by a Milli-Q system (Millipore, Bedford, MA, USA).

Methanol (MeOH) (Merck, Darmstadt, Germany), acetic acid (Probus, Barcelona, Spain), sodium acetate (Prolabo, Bois, France) and sodium octanesulfonate (OCN) (Sigma) were used to pretreat the sample.

Sodium tetraborate (Fluka, Buchs, Switzerland), potassium dihydrogenphosphate (KH_2PO_4) (Fluka), sodium dodecyl sulfate (SDS) (Sigma), tetrabutylammonium bromide (TBA) (Sigma), OCN, acetonitrile (ACN) (Merck), HCl (Probus) and MeOH were used to prepare the run buffer solution.

2.3. Electrophoretic conditions

The electrophoretic solution was a stock solution of 40 m*M* sodium tetraborate containing 10% MeOH. This running buffer was adjusted to pH 8.1 with 6 *M* HCl. Before use, the capillary was rinsed with 1 *M* NaOH (1000 mbar pressurised flow) for 5 min, then with Milli-Q water for 10 min and finally flushed with running buffer for 3 min successively. The detector was set at 260 nm. The injection was hydrodynamic at a pressure of 50 mbar for 4.8 s. Capillary temperature and separation voltage were 30°C and 30 kV, respectively.

2.4. Solid-phase extraction as pretreatment of the sample

Waters Sep-Pak cartridges Plus (360 mg, C_{18}) were used to pretreat the sample. The cartridge was activated with 6 ml of MeOH followed by 1 ml of 0.7 *M* acetic acid–0.04 *M* sodium acetate buffer (pH 3–4) and 1 ml of 5 m*M* OCN, at a flow-rate of 1–2 ml min^{-1} using water aspirator as a vacuum source connected to the cartridge pack. A 2-ml volume of sample which contained 5 m*M* OCN was passed through the cartridge. The cartridge was washed with 1 ml of extraction buffer and 0.5 ml of Milli-Q water. The compounds of the sample were eluted from the cartridge with 2 ml of MeOH–water (70:30, v/v).

3. Results and discussion

3.1. Optimization of capillary electrophoresis separation

The aim of this paper is to develop a simple and rapid method of separating and identifying 10 quinolones. These compounds are characterized by their structure. We can divided them into two groups: compounds with piperazinyl substituent (group I) and compounds without (group II). Group I comprises: lomefloxacin (1), enoxacin (2), norfloxacin (3), pipemidic acid (4) and ofloxacin (5); group II comprises: piromidic acid (6), oxolinic acid (7), flumequine (8), cinoxacin (9) and nalidixic acid (10). The pK_a values of the compounds of group I range from 7.5 to 8.5 for the ammonium form and from 5.5 to 6.0 for the carboxylic function. The pK_a values of the compounds of group II is due to carboxylic function [27].

Because of these pK_a values it is reasonable to start with a buffer system with a pH range of 6.5–7.5 using the CZE mode, since at this pH quinolones of group I are neutral because they are in zwitterionic or unionized form at their isoelectric points (between pH 6.5–7.5), whereas quinolones of group II are negatively charged because they are purely acid and their pK_a values are due to carboxylic function. The optimization of the separation was carried out using standard solutions of 25 mg l^{-1} of these 10 quinolones. The running buffer used was 18 m*M* sodium dihydrogenphosphate (adjusting pH to 7.3 with 50 m*M* sodium tetraborate). Under these conditions the 10 quinolones could not be separated, and the electropherogram (Fig. 2) shows that the five piperazinyl-carrying quinolones migrated as a group

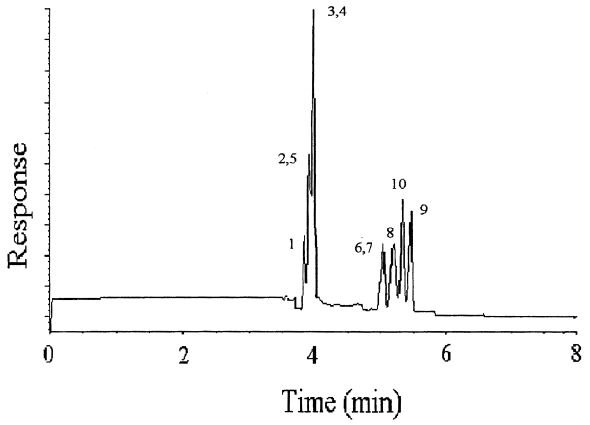


Fig. 2. CZE electropherogram of 25 mg l^{-1} of quinolone antibiotics from standard solution. Electrophoretic conditions: 18 m*M* sodium dihydrogenphosphate adjusted to pH 7.3 with 50 m*M* sodium tetraborate; injection, 50 mbar for 4.8 s; separation voltage, 30 kV; capillary temperature, 30°C; UV detection at 260 nm. Compound identities as shown in Fig. 1.

ahead of the five piperazinyl non-carrying quinolones, which formed another group of somewhat better-separated peaks.

MEKC is generally employed when the simple CZE mode cannot separate all compounds. First of all we studied the addition of a surfactant to the running buffer (the same running buffer as before). Sun and Chen [17] studied different bile salts to separate a group of quinolone antibiotics and the results were good. However, in this paper we used SDS because this surfactant is widely used in MEKC and also because of economic considerations. The working range was set at 8–25 m*M* of SDS. With greater concentrations of this surfactant separation was worse. Under these conditions results were best when 20 m*M* SDS was used, and there were seven peaks (Fig. 3a). However, without adding any modifiers, several peaks were still partially merged. Under such circumstances, the ion-pairing reagent sodium octanesulfonate (5 m*M*) was added (Fig. 3b) to improve selectivity and peaks shapes according to the literature [17], but there was only a slight improvement. We had to improve the resolution and efficiency of the separation, so we studied the addition of another ion-pairing reagent, TBA. Results were best when 5 m*M* of TBA was added to the

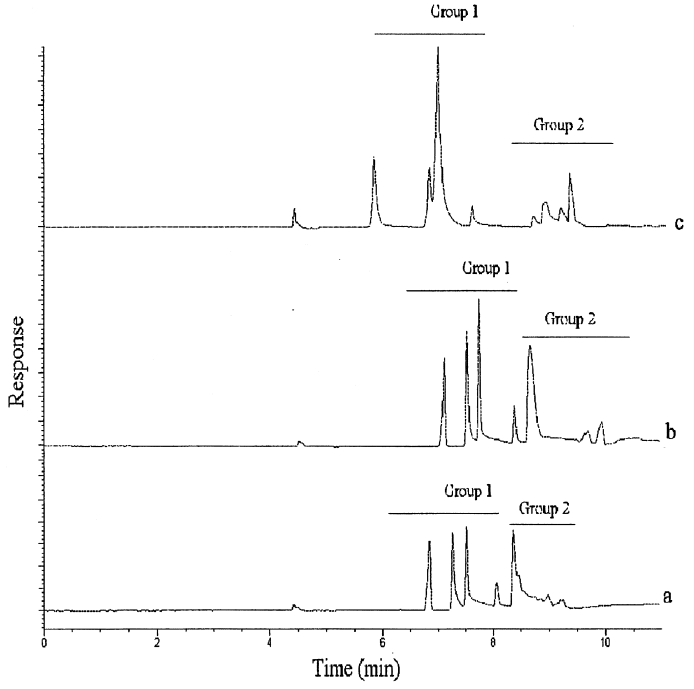


Fig. 3. MEKC electropherograms of 25 mg l^{-1} of quinolone antibiotics from standard solution with (a) 20 m*M* SDS, (b) 20 m*M* SDS and 5 m*M* OCN and (c) 20 m*M* SDS and 5 m*M* TBA in the running buffer. For other conditions see Fig. 2.

running buffer (Fig. 3c) but separation still had to be improved. We also studied a different gradient voltages, however it did not increase the resolution.

After all these analyses, separation had only improved a little. We therefore decided to change the pH of the running buffer to ionize, at least partly, all the quinolones in this study and so simplify the method. In this case the running buffer was 40 m*M* sodium tetraborate and the pH was studied between 9.2 and 8.1 adjusted with diluted HCl. Fig. 4 shows that the best result was obtained at pH 8.1. Under these conditions we can see 10 peaks. From here onwards it is only necessary to improve the resolution of the peaks. We also considered adding organic solvents to the electrophoretic buffer because they affect several variables, including viscosity, dielectric constant and zeta potential. The presence of acetonitrile or methanol increased the migration time of all the compounds. Increasing the concentrations of the organic solvents up to approximately 10% (v/v) improved the separation of the 10 quinolones. The best results were obtained when 10% MeOH was added (Fig. 5a). As we can see, the quinolones in group I were eluted first, followed by those in group II. This was because the compounds in group I are bulkier and because all quinolones of group II are charged negatively and quinolones of group I are in the unionized form or negatively charged depending of the value of aminogroup pK_a. If its pK_a is lower than 8.1 these quinolones are negatively charged, but if it is higher then they are in the unionized form.

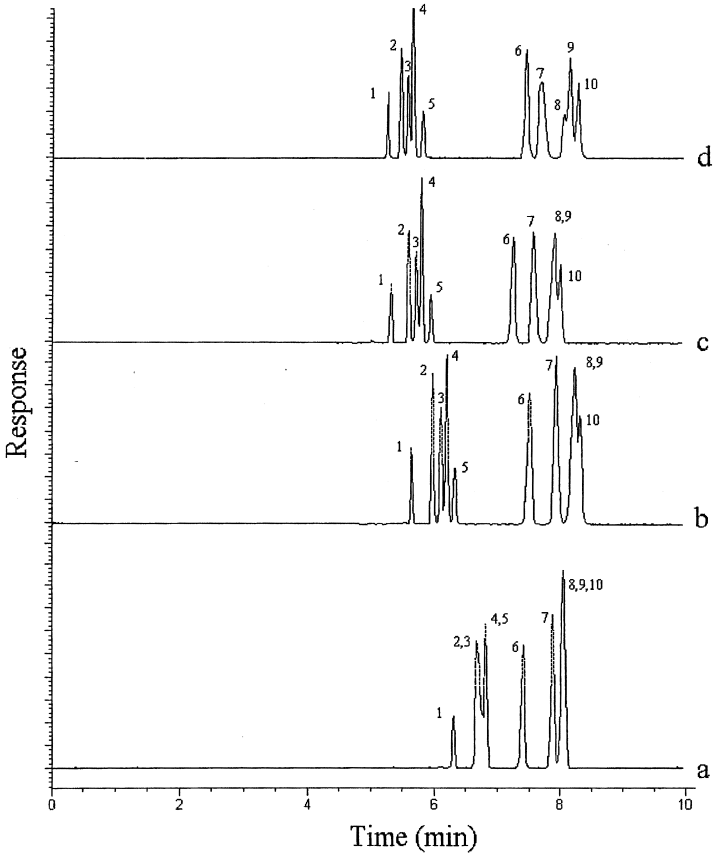


Fig. 4. CZE electropherograms of 25 mg l^{-1} of quinolone antibiotics from standard solution with a running buffer of 40 m*M* sodium tetraborate at (a) pH 9.2, (b) pH 8.5, (c) pH 8.3 and (d) pH 8.1 adjusted with diluted HCl. For other conditions see Fig. 2.

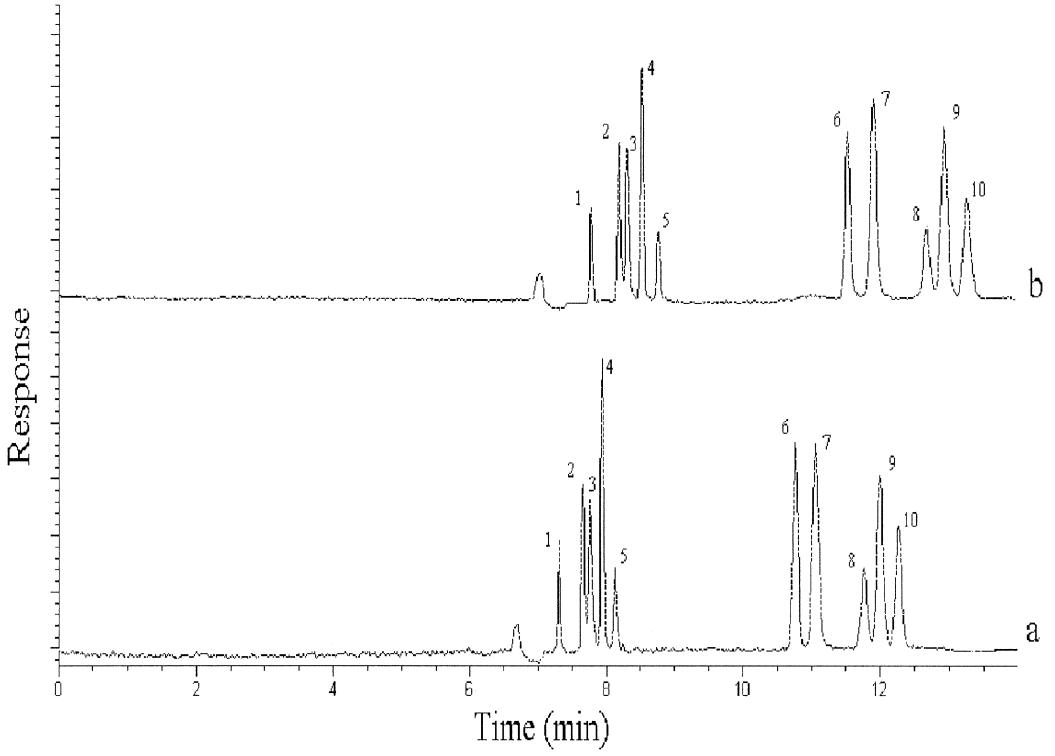


Fig. 5. CZE electropherograms of (a) 5 mg l^{-1} of quinolone antibiotics from standard solution and (b) pig plasma sample with standard addition of 5 mg l^{-1} of quinolone antibiotics after SPE. Running buffer of 40 m*M* sodium tetraborate at pH 8.1 and 10% (v/v) methanol. For other conditions see Fig. 2.

3.2. Optimization of solid-phase extraction method in biological samples

To analyze quinolones in plasma samples by CE it is necessary to remove interferences which are in the background of the sample. This paper proposes using SPE to pretreat the sample. The present application of SPE demonstrates the potential usefulness of nonpolar C_{18} cartridges for a group of quinolone antibiotics. This pretreatment was carried out at a pH of between 3 and 4 since at this pH quinolones in group I are charged positively and those in group II are in the unionized form because of their pK_a values. To carry out SPE using C_{18} cartridges OCN must be added to the sample to neutralize the positive charge of quinolones of group I.

With the SPE method we had to optimize a number of factors: the volume of elution, the percentage of MeOH in the MeOH–water mixture for elution and the sample volume to pass through the cartridge. All of these studies were performed using 5 ml of a standard solution of 25 mg l^{-1} of 10 quinolones. First we studied the elution volume [1 and 2 ml of a mixture of MeOH–water (70:30)], and the results were best with 2 ml. We also studied different mixtures of 60:40 (v/v) and 70:30 (v/v) MeOH–water to make a recovery study. With a 75:25 mixture we have a few problems with the current in the electrophoresis system and it is impossible to make the analysis. Table 1 shows the recovery values (% *R*) of the different quinolones over the percentage of MeOH and their relative standard deviations (RSDs). In all cases the recovery for piromidic acid (6) is very low, which could be due to its structure. The best results were obtained using a mixture of 70:30 with recoveries nearly to 100% and reproducibility RSDs in measured areas were between 2.3 and 15.1%. We then studied the

Table 1
Recovery values (% *R*) and relative standard deviations (RSDs) of quinolones using SPE with different percentages of MeOH–water (%, v/v)[a]

Compound	60:40		70:30	
	% *R*	% RSD (area)	% *R*	% RSD (area)
1	27	4.0	103	3.3
2	26	7.1	127	4.4
3	29	8.2	111	3.1
4	45	5.7	114	3.1
5	35	3.6	128	12.0
6	2	14.1	39	3.3
7	16	17.1	93	2.3
8	51	7.7	125	15.1
9	25	8.2	103	3.9
10	59	6.0	131	10.0

[a] Studies performed using a 5 ml sample volume of a standard solution of 25 mg l^{-1} and a 2 ml elution volume.

volume of sample to pass through the cartridge. These were 2, 5, 10 and 15 ml. Recovery values and their RSDs are shown in Table 2. The recoveries obtained up to 5 ml of sample are good for all compounds except for piromidic acid (6), as we verified earlier. When we used sample volumes upper to 5 ml the recoveries were lower.

To evaluate the calibration graph for each compound, a linear regression was performed with concentrations of calibration standards against measured peak areas using the optimum conditions. All solutions were prepared in MeOH–water (70:30, v/v) as this is the solvent composition introduced to the CE system after solid-phase treatment. The results are shown in Table 3. These solutions were injected five times starting with the least concen-

Table 2
Recovery values (% *R*) obtained with different sample volumes[a]

Compound	2 ml		5 ml		10 ml		15 ml	
	% *R*	% RSD (area)	% *R*	% RSD (area)	% *R*	% RSD (area)	% *R*	% RSD (area)
1	112	7.7	103	3.3	90	12.5	65	15.0
2	112	2.7	127	4.4	121	7.6	92	12.8
3	109	3.4	111	3.1	89	3.9	70	19.6
4	111	1.3	114	12.0	109	3.9	88	7.6
5	89	3.4	128	3.3	121	7.6	96	5.4
6	27	11.5	39	15.1	24	15.4	–	15.7
7	90	3.7	93	2.3	71	15.6	57	14.4
8	94	2.6	125	5.4	142	7.5	96	5.7
9	93	2.9	103	3.9	81	7.0	65	6.4
10	125	5.7	131	10.0	89	10.1	74	17.8

[a] Studies performed using 2 ml of MeOH–water (70:30, v/v).

Table 3
Calibration data and precision for the 10 quinolone antibiotics studied with standard solutions

Compound	Linearity (mg l^{-1})	Correlation coefficient (r^2)	LOD (mg l^{-1})	% RSD[a] (area)	% RSD[a] (time)
1	0.8–45	0.9978	0.4	5.3	2.9
2	1.0–45	0.9957	0.3	4.3	3.1
3	0.8–45	0.9954	0.2	6.2	3.2
4	0.8–45	0.9973	0.2	5.1	3.3
5	0.8–45	0.9971	0.4	5.9	3.3
6	0.8–60	0.9944	0.3	6.2	2.1
7	0.8–35	0.9973	0.4	7.6	3.6
8	0.8–60	0.9986	0.1	6.7	3.4
9	0.8–35	0.9998	0.3	13.0	3.7
10	1.0–35	0.9991	0.2	15.7	4.0

[a] Calculated for 10 consecutive runs at 25 mg l^{-1}.

trated and ending with the most. The area values obtained were successively analyzed using ULC (univariate linear calibration) software [28] to evaluate the correlation coefficient (r^2), RSD and limit of detection (LOD). As we can see in Table 3, the linearities and correlation coefficients of the different compounds were good. The LOD was calculated by Winefordner and Long's method [29] using the ULC program with K equal to 3. The LOD values for quinolones were between 0.1 and 0.4 mg l^{-1}. The RSD of the areas and of the migration time were calculated for 10 repeated injections of standard solutions of 25 mg l^{-1}. A fresh buffer was used after each sequence of three injections. The migration time RSDs are between 2.1 and 4%, while the area RSDs are between 4.3 and 15.7%.

To demonstrate how useful the CE method is for determining quinolones, pig plasma samples were analyzed. Samples were spiked with different quantities of the compounds up to a concentration range of between 5 and 20 mg l^{-1} before SPE treatment and 2 ml of sample was passed through the cartridge. The correlation coefficients are as good as in standard solutions. The LODs of these quinolones are between 1.1 and 2.4 mg l^{-1} with the method set out in this paper. The results are summarized in Table 4. The limit of quantification (LOQ) in pig plasma samples is the lowest concentration from which it is possible to quantify the analyte with reasonable statistical certainty. The LOQ was calculated by Widefordner and Long's method [29] using the ULC program [28] with K equal to 6. The LOQ for pig plasma estimated by measuring the response of 10 drug-free pig plasma sample was determined to be in the range 2.2 to 4.8 mg l^{-1}. The LOQ estimated by the smallest validated concentration within the limits of precision and accuracy set for the method was determined to be 5 mg l^{-1} for all quinolones. Repeatability of the method did not exceed 19.0% measured in peak are and 5.0% measured in migration time.

The extraction efficiency (recovery) was determined by comparity peak areas from drug-free samples spiked with known amounts of drugs (in the range of concentrations of the calibration curves) and standard solutions injected directly in the electrophoresis system. Each sample was determined in triplicate.

The recoveries obtained for pig plasma (Table 5) were stable over the range used (5–20 mg l^{-1}). They varied from 90.0 to 129.3%, except for piromidic acid. The mean recovery varied from 94.0±4.2 to 123.3±4.1% for the three tested concentrations representing the entire range of the calibration curve (low, medium and high concentrations).

Fig. 5b shows the separation of these quinolones from a pig plasma sample spiked with a standard solution of 5 mg l^{-1}.

4. Conclusions

Our results suggest that 40 mM of borate buffer at pH 8.1 containing 10% (v/v) methanol is a very efficient running buffer for separating quinolone

Table 4
Calibration data and precision for the 10 quinolone antibiotics studied with pig plasma sample after SPE with standard addition

Compounds	Linearity (mg l^{-1})	Correlation coefficient (r^2)	LOD (mg l^{-1})	% RSD[a] (area)	% RSD[a] (time)
1	5–20	0.9998	1.2	15.3	4.5
2	5–20	0.9990	2.0	6.7	2.5
3	5–20	0.9950	1.8	9.3	3.5
4	5–20	0.9970	2.2	8.2	2.8
5	5–20	0.9998	2.2	9.6	2.6
6	5–20	0.9918	1.2	19.0	3.8
7	5–20	0.9994	1.6	14.4	4.9
8	5–20	0.9964	2.3	17.4	4.0
9	5–15	0.9942	1.1	15.9	5.0
10	5–20	0.9970	2.4	16.0	3.4

[a] Calculated for 10 consecutive runs at 5 mg l^{-1} spiked with a standard of 10 quinolones.

Table 5
Recovery (% *R*) study and repeatability (% RSD) from pig plasma samples at different spiked level preconcentrations[a]

Compound	Low concentration, 5 ppm		Medium concentration, 10 ppm		High concentration, 20 ppm		Mean recovery (%)
	Mitjana (% *R*)	% RSD	Mitjana (% *R*)	% RSD	Mitjana (% *R*)	% RSD	
1	104.3	4.1	120.5	2.2	106.7	5.2	110.5±8.7
2	99.9	0.1	119.6	3.9	106.2	1.8	108.6±10.1
3	119.9	4.2	122.3	2.8	127.8	2.5	123.3±4.1
4	98.0	9.6	110.8	5.2	119.3	2.2	109.4±10.7
5	97.7	13.8	115.1	8.2	91.7	7.1	101.5±12.2
6	68.0	8.7	63.4	15.6	73.0	17.0	68.1±4.8
7	90.2	7.5	98.5	14.7	93.4	9.0	94.0±4.2
8	107.7	8.4	126.0	6.4	128.7	2.5	120.8±11.4
9	100.9	8.2	102.5	12.8	107.5	13.6	103.6±3.4
10	106.7	15.6	123.9	7.6	124.7	8.3	118.4±10.2

[a] Calculated for 10 consecutive runs at each concentration spiked with a standard of 10 quinolones.

antibiotics. This method in which SPE treatment was used as a clean-up step for obtaining a background free of interferences in the electropherograms of real samples, successfully separated and determined the ten quinolones in pig plasma samples.

This investigation has shown the method developed is an alternative to HPLC methods in terms to short analysis time (13 min) and simple clean-up system, and the LODs obtained are in accordance to other methods developed to analyze quinolones with a capillary electrophoresis system using UV–visible detection but a little higher when quinolones are analyzed by HPLC.

We will continue working to decrease detection limits and we will study different types of SPE cartridges. We will also try to increase the sensitivity of the CE method with different preconcentration techniques.

Testing of the suitability of the method for the analysis the same molecules in plasma of other species or in the other tissues is in progress in our laboratory. The method could be successfully applied to monitor structurally related novel fluoroquinolone antibiotics in plasma.

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References

[1] G.L. Mandell, W.A. Petri Jr., in: J.G. Hardman, A.G. Gilman, L.E. Limbird (Eds.), The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, 9th ed., 1996, p. 1065.
[2] V.T. Andriole, The Quinolones, Academic Press, London, 1988.
[3] D.A. Volmer, B. Mansoori, S.J. Locke, Anal. Chem. 69 (1997) 4143.
[4] D. Currie, L. Lynas, D.G. Kennedy, W.J. McCaughey, Food Addit. Contam. 15 (1998) 651.
[5] L. Elrod Jr., C.L. Linton, J. Morley, T.G. Golich, C. Gay, Chromatographia 41 (1995) 141.
[6] G. Carlucci, P. Mazzeo, G. Palumbo, Chromatographia 43 (1996) 261.
[7] I.N. Papadoyannis, V.F. Samanidou, K.A. Georga, Anal. Lett. 31 (1998) 1717.
[8] G. Carlucci, J. Chromatogr. A 812 (1998) 343.
[9] J. Barbosa, R. Bergés, V. Sanz-Nebot, J. Chromatogr. A 823 (1998) 411.
[10] J. Barbosa, G. Fonrodona, I. Marqués, V. Sanz-Nebot, I. Toro, Anal. Chim. Acta 351 (1997) 397.
[11] J. Barbosa, I. Marqués, G. Fonrodona, D. Barrón, R. Bergés, Anal. Chim. Acta 347 (1997) 385.
[12] J. Barbosa, I. Marqués, G. Fonrodona, D. Barrón, V. Sanz-Nebot, Trends Anal. Chem. 16 (1997) 140.
[13] M.G. Khaledi, High Performance Capillary Electrophoresis – Theory, Techniques and Applications, Wiley, New York, 1998.
[14] K.D. Altria, Analysis of Pharmaceuticals by Capillary Electrophoresis, Chromatographia CE Series, Vieweg, Braunschweig/Wiesbaden, 1998.

[15] S.M. Lunte, D.M. Radzik, Pharmaceutical and Biomedical Applications of Capillary Electrophoresis, Elsevier, UK, 1996.
[16] K.D. Altria, Y.L. Chanter, J. Chromatogr. A 652 (1993) 459.
[17] S. Sun, L. Chen, J. Chromatogr. A 766 (1997) 215.
[18] T. Horimai, M. Ohara, M. Ichinose, J. Chromatogr. A 760 (1997) 235.
[19] J.-G. Möller, H. Staβ, R. Heinig, G. Blaschke, J. Chromatogr. B 716 (1998) 325.
[20] T. Pérez-Rubio, C. Martínez-Lozano, A. Sanz, E. Bravo, Chromatographia 49 (1999) 419.
[21] T. Arai, N. Nimura, T. Kinoshita, J. Chromatogr. A 736 (1996) 303.
[22] H.J. Nelis, J. Van den Branden, B. Verhaeghe, A. De Kruif, D. Mattheeuws, A.P. de leenheer, Antimicrob. Agents Chemother. 3 (1992) 1606.
[23] J.O. Boison, G.O. Korsrud, J.D. MacNeil, L. Keng, J. Chromatogr. 576 (1992) 315.
[24] W.J.J. Krauwinkel, N.J. Volkers-Kamermans, J. Van Zijveld, J. Chromatogr. 617 (1993) 334.
[25] M. Hernández, F. Borrull, M. Calull, J. Chromatogr. B 731 (1999) 309.
[26] J. Manceau, M. Gicquel, M. Laurentie, P. Sanders, J. Chromatogr. B 726 (1999) 175.
[27] K. Takács-Novák, B. Noszál, G. Keresztúri, B. Podányi, G. Szász, J. Pharm. Sci. 79 (1990) 1023.
[28] R. Boqué, F.X. Rius, D.L. Massart, J. Chem. Educ. (Computer Ser.) 71 (1994) 230.
[29] J.D. Winefordner, G.L. Long, Anal. Chem. 55 (1983) 712A.