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Improved capillary electrophoretic separation of nine (fluoro)quinolones with fluorescence detection for biological and environmental samples

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Abstract

A capillary electrophoretic method has been developed for the separation of nine (fluoro)quinolones. Detection is done by fluorescence measurement with broad wavelength band excitation between 240 and 400 nm. Best separation is achieved in a carrier electrolyte containing 50 mM H_3PO_4 adjusted to pH 7.55–acetonitrile (60:40, v/v). Detection limits are in the low $\mu g\,l^{-1}$ range. The suitability to real samples has been demonstrated by analyzing blood samples and surface water samples. Sample preconcentration and sample clean-up can easily be done by solid-phase extraction. Different phases based on alkyl- or phenyl-modified silica as well as on polymers have been investigated for this purpose. The method should also be useful for determination of residues of (fluoro)quinolones in food or other matrices.

Keywords: Fluoroquinolones; Quinolones; Antibiotics

1. Introduction

Quinolones and fluoroquinolones are antibiotics belonging to the family of gyrase inhibitors. They are used for animals (poultry, bovine, porcine, rabbits, deer, fish) as well as for humans. To prevent the emergence of resistant bacteria it is necessary to monitor the concentration of quinolones and fluoroquinolones in different media comprising muscle, fat, skin, liver, kidney, milk, eggs and even surface water of fishponds. Determination in human blood is required for pharmacokinetic studies. Therapeutic drug monitoring is necessary if the drug is taken chronically, if there are toxic side effects in case of an overdose, and if there is only a narrow therapeutic range. Moreover, pharmacological studies demonstrated a much better correlation between the clinical effects of (fluoro)quinolones and their plasma concentration than just the daily intake [1].

The maximum residue limits for these substances in food are rather low wherefore analytical techniques of high sensitivity are required. Several HPLC methods with fluorescence detection [2–9] and UV detection [10–12] have been reported in the literature. HPLC suffers from a serious drawback since it is not possible to separate all different (fluoro)quinolones in one run. Yorke and Froc [2] used three different HPLC conditions to quantify a wide range of these analytes. The problem of incomplete separation could be compensated by using MS as detector, but so far MS was rather used for positive identification of the (fluoro)quinolones than for quantitation [4,6]. Golet et al. managed to separate nine (fluoro)quinolones on an RP-Amido C_{16} column at 50 °C [13]. Hernandez-Arteseros et al. gave an overview on analysis of quinolone residues in edible animal products including extraction methods, clean-up and determination techniques which also comprise a GC method and immunoassays for enrofloxacin and ciprofloxacin [14].

Recently, a series of capillary zone electrophoresis (CZE) methods for analyzing (fluoro)quinolone antibiotics has been

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published. Many of them suffer from poor sensitivity since they are not combined with fluorescence detection [15–22]. In various cases resolution was not sufficient [15,16,18,21,23] or the focus was put just on three or even less analytes [19–21,24–26]. To enhance sensitivity, laser-induced fluorescence (LIF) detection [24,26,27] or hyphenation with MS have been applied [23].

Fierens et al. [18] used phosphate and borate carrier electrolytes within a pH-range from 7 to 8 in order to separate ofloxacin, norfloxacin, ciprofloxacin, flerofloxacin, pipedimic acid, pefloxacin, oxolinic acid, cinoxacin, nalidixic acid and flumequine. Best results were achieved with a phosphate buffer pH 7.0, although a complete separation was not realized. Bannefeld et al. [26] developed a CE method for the determination of ciprofloxacin and desethyleneciprofloxacin with LIF detection using a phosphate/borate buffer at pH 2.3. The reported limit of quantification (LOQ) was 20 $\mu g\,l^{-1}$. Awadallah et al. [25] succeeded in separating the enantiomers of ofloxacin with a phosphate buffer pH 3 reaching an LOQ of 11.4 $\mu g\,l^{-1}$. Schmitt-Kopplin et al. [27] used CZE as well as micellar electokinetic chromatography (MEKC) with carrier electrolytes at pH 9.2. MEKC allowed a separation of norfloxacin, enrofloxacin, ofloxacin and ciprofloxacin/danofloxacin. For enrofloxacin limits of detection (LODs) of 280 $\mu g\,l^{-1}$ (UV detection) and 37 $\mu g\,l^{-1}$ (LIF detection) were achieved. McCourt et al. [23] developed a CZE method for the separation of nine fluoroquinolones (danofloxacin, ofloxacin, marbofloxacin, enrofloxacin, enoxacin, ciprofloxacin, norfloxacin, cinoxacin and flumequine) with electrospray ionization (ESI) MS detection using carrier electrolytes with ammonium formate, acetate and carbonate buffers within a pH range from 2.55 to 9.20. Complete resolution of signals was not achieved, but is of less importance with MS detection. Barron et al. [15] investigated carrier electrolytes within the pH range from 7 to 9 and the addition of acetonitrile. Best results were achieved with a phosphate buffer pH 8.00 containing 10% (w/w) of acetonitrile leading to a separation of danofloxacin, marbofloxacin, enrofloxacin, difloxacin and flumequine, whereas norfloxacin, ciprofloxacin and sarafloxacin migrate in one zone and cannot be separated. Hernandez et al. [21] analyzed ciprofloxacin, enrofloxacin and flumequine in plasma samples using isotachophoresis (ITP)–CZE with a phosphate buffer pH 9 as a carrier electrolyte achieving LODs of 50–85 $\mu g\,l^{-1}$ after appropriate sample pre-treatment. Barron et al. determined enrofloxacin and ciprofloxacin [20] or difloxacin and sarafloxacin [19] in biological materials using a diethylmalonate buffer pH 8.22. LODs between 10 and 25 $\mu g\,l^{-1}$ were reported. Using a borate carrier electrolyte pH 8.1 containing 10% (v/v) methanol, Hernandez et al. [28] achieved nearly a baseline separation of nalidixic acid, cinoxacin, flumequine, oxolinic acid, piromidic acid, ofloxacin, pipemidic acid and lomefloxacin. Nevertheless, norfloxacin and enoxacin did not show good resolution. Hernandez et al. [16] also examined non-aqueous capillary electrophoresis for separation of difloxacin, enrofloxacin, danofloxacin, marbofloxacin and ciprofloxacin. A separation was possible in a carrier electrolyte of ammonium acetate, hexadimethrine bromide and acetic acid in methanol–acetonitrile (50:50, v/v).

Sample clean-up and pre-concentration of (fluoro)quinolones has been done mainly by solid-phase extraction (SPE) [1,3,6,8,9–11,13,14,18,24,26,28]. C_{18}-modified silica and poly(styrene-divinylbenzene)-based materials as well as mixtures with strong cation exchangers have been used. In the case of simultaneous determination of a wide range of different (fluoro)quinolones it is obvious that optimization of SPE conditions must lead to a compromise. SPE cartridges containing C_{18} material turned out to be the best choice when using samples at a pH of 4 or slightly higher [10]. Recoveries better than 85% have been reported. Nevertheless, Golet et al. [13] decided in favour for mixed phase cation exchange SPE cartridges with sample solutions at pH 3 achieving recoveries higher than 80%. When considering only one or just a few (fluoro)quinolones, the choice of SPE material and conditions like pH of the analyte solution can be adapted to the accordant analytes resulting in better recoveries. Hernandez et al. [21] used Oasis HLB SPE cartridges giving recoveries of about 100% for enrofloxacin and about 90% for ciprofloxacin and flumequine. Holtzapple et al. [3] used high-performance immunoaffinity chromatography for online sample clean-up instead of SPE.

In this paper, a CZE method with fluorescence detection is presented for nine (fluoro)quinolones shown in Fig. 1. This method aims at sufficient sensitivity and superior separation selectivity that would make it orthogonal to existing HPLC methods. Instead of a laser, a continuum light source was used in the fluorescence detector that allows the selection of a proper excitation wavelength range best suited for the analytes. Two matrices (pond water and human blood) were examined in order to show applicability to complex samples. Especially in water analysis no reports on determination of (fluoro)quinolones by CE exist in the current literature. The (fluoro)quinolones selected in the present study were those that are currently on the market in Austria for treatment of humans or animals. Both groups have been included in order to develop a general method for broad applicability.

2. Experimental

2.1. Instrumentation

A HP ^{3D}CE instrument (Agilent Technologies, Waldbronn, Germany) was used for all electrophoretic experiments. It was equipped with a capillary cartridge containing a fused silica capillary of 70 cm total length (55 cm effective length from inlet to detection window) × 75 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA). The capillary was coupled to an Argos 250B fluorescence detector (Flux Instruments, Basel, Switzerland) containing a mercury-xenon lamp. Best results were achieved with a broad band filter

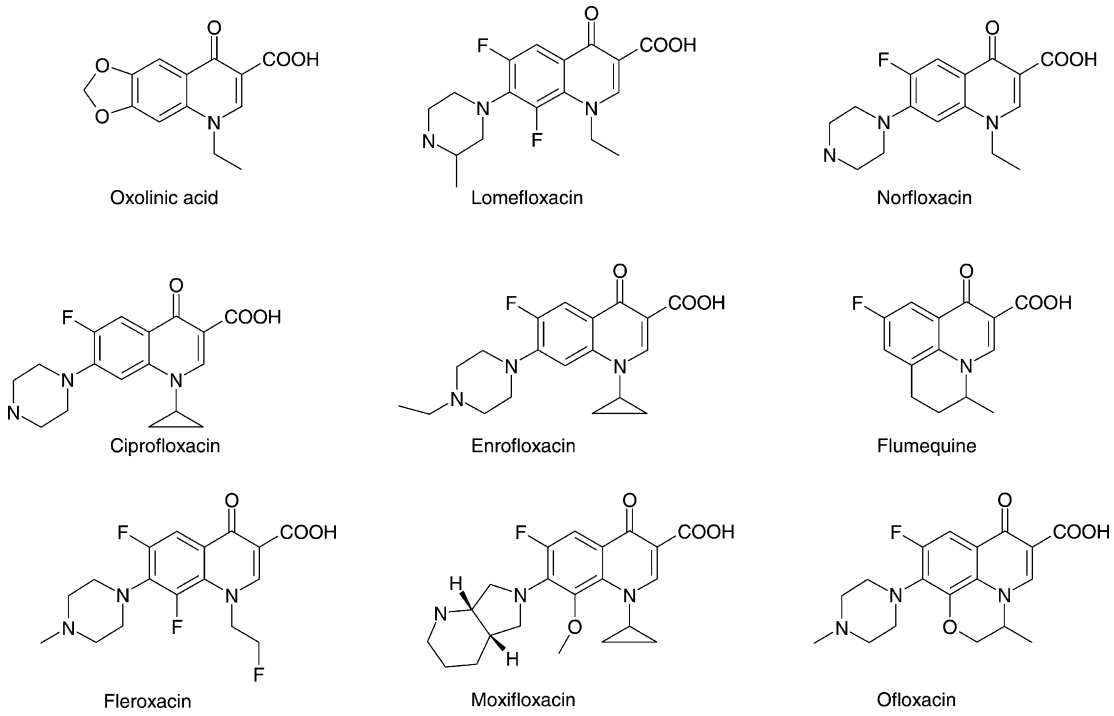

Fig. 1. Structures of the investigated (fluoro)quinolones.

(240–400 nm) for excitation and a 435 nm cut-off filter for emission. The signal from the detector was processed by Agilent ChemStation after digitalization by an Agilent 35900E A/D converter. Injection was done in the hydrodynamic mode at 50 mbar for 8 s.

For SPE the following materials were investigated: Oasis HLB (200 mg, 6 ml) from Waters (Milford, MA, USA); Chromabond Tetracycline (500 mg, 6 ml) from Machery–Nagel (Düren, Germany); Chromabond C_8 (200 mg, 3 ml) from Machery–Nagel; Bakerbond Phenyl (500 mg, 3 ml) from J.T. Baker (Phillipsburg, NJ, USA); Isolute ENV+ (200 mg, 3 ml) from IST (Hengoed, UK); LiChrolut EN (200 mg, 3 ml) from Merck (Darmstadt, Germany).

Chromatography was performed on a 1100 HPLC System (Agilent Technologies, Waldbronn, Germany) equipped with a vacuum degasser, quaternary pump, autosampler (50 μl injection volume) and a 821-FP fluorescence detector (Jasco, Tokyo, Japan). The separation column was a 250 mm × 4.6 mm I.D. YMC-Pack Pro C_{18}, 3 μm particle size, 12 nm pore size, obtained from YMC (Schermbeck, Germany). 50 mM formic acid–methanol (70:30, v/v) at a flow rate of 0.8 ml min^{-1} was used for isocratic elution. The excitation wavelength for fluorescence detection was 278 nm, emission was measured at 445 nm.

2.2. Chemicals

Reagent grade phosphoric acid, acetic acid, potassium hydroxide, and EDTA disodium salt were purchased from Merck (Darmstadt, Germany). Acetonitrile and methanol (HPLC gradient grade) and ethyl acetate (ultra resi-analyzed quality) were purchased from J.T. Baker (Deventer, The Netherlands). 18 MΩ water obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout the work.

Flumequine and oxolinic acid were obtained from Sigma (Steinheim, Germany), enrofloxacin and ciprofloxacin from Fluka (Buchs, Switzerland). Stock solutions of 100 mg l^{-1} were prepared in acetonitrile–water (50:50, v/v). All other fluoroquinolones were not available as pure standards but were extracted from the following commercially available drug formulations (film tablets): Uniquin 400 mg (lomefloxacin), Biochemie (Vienna, Austria); Quinodis 200 mg (fleroxacin), Grünenthal (Vienna, Austria); Floxacin 400 mg (norfloxacin), STADA Arzneimittel (Vienna, Austria); Oflox 200 mg (ofloxacin), STADA Arzneimittel; Avelox 400 mg (moxifloxacin), Bayer Austria (Vienna, Austria). Eventual small deviations of the contents from the declared values given for the pharmaceutical formulations were neglected within this work. For extraction of the active agents from the film tablets they were finely ground and an appropriate amount was weighed and mixed with acetonitrile–water (50:50, v/v) to give a stock solution of 100 mg l^{-1} of each fluoroquinolone. The suspension was treated in an ultrasonic bath for 10 min and filtered through a syringe filter of 0.45 μm pore size. In order to check the quantitative performance of the extraction process, the recovery of ciprofloxacin from a 500 mg film tablet (Hexal Pharma, Vienna, Austria) was determined. For this purpose, HPLC with fluorescence detection was used. A calibration curve was established with standard solutions in acetonitrile–water (10:90, v/v) prepared

from pure ciprofloxacin. The extract from the tablet was diluted with water to an acetonitrile concentration of 10% (v/v) and injected into the HPLC system. The content of the tablet was found to be 100.3% of the declared value. Spiking the ground tablet with ciprofloxacin followed by the extraction process proved a quantitative recovery.

All stock solutions were stored at 4 °C. Standard solutions were prepared from the stock solutions by dilution with acetonitrile–water (50:50, v/v).

2.3. Preparation of carrier electrolytes

A series of carrier electrolytes was prepared from 250 mM phosphate solutions of different pH values (7.00, 7.35, 7.55) by dilution with water and adding different amounts of organic solvents.

Each carrier electrolyte had a concentration of 50 mM phosphate and contained methanol or acetonitrile in a range between 0 and 50% (v/v).

2.4. Sample pretreatment

In the case of blood samples, 1 ml of EDTA-treated human blood was thoroughly mixed with 2 ml of protein precipitation solution consisting of 0.1 M $ZnSO_4$ in methanol–water (30:70, v/v). After centrifugation (4000 rpm, 10 min) the liquid layer was diluted with 50 ml of water containing 100 mg EDTA disodium salt adjusted to pH 4.00 with acetic acid.

Sample clean-up was done by SPE. The SPE cartridges were conditioned with ethyl acetate, methanol and aqueous 0.2% EDTA (pH 4.00). After the application of the deproteinized blood sample a washing step with water containing 0.2% EDTA (pH 4.00) was included. Elution was carried out with 2 ml methanol–water (75:25, v/v) and 2 ml methanol. The eluate was brought to dryness by a stream of nitrogen and reconstituted with 0.5 ml acetonitrile–water (50:50, v/v). Sample solutions were stored at 4 °C.

In case of surface water, 500 ml sample were filtrated through a 0.1 μm filter. Afterwards, 1 g EDTA disodium salt was added and the pH was adjusted to pH 4.00 with acetic acid. The subsequent SPE procedure was the same as described above for blood samples.

3. Results

The problem of electrophoretic separation methods reported so far in the literature for more than just three or four (fluoro)quinolones is proper resolution. Separation of the structurally very similar analytes like norfloxacin and ciprofloxacin, which differ only by an ethyl respectively an cyclopropyl side group, is challenging.

In the present work, a series of phosphate carrier electrolytes was investigated within a relatively narrow pH segment (7.00, 7.35, 7.55). Neutral carrier electrolytes lead to complete dissociation of the carboxylic acid group of the analytes, whereas the different extent of protonation of a nitrogen group within the different (fluoro)quinolones allows an electrophoretic separation. The pK_B values of the investigated (fluoro)quinolones range from 3.2 to 10.6; six out of nine (fluoro)quinolones give pK_B values between 5.1 and 7.2. Barbosa et al. [29] published pK_B values in different water-acetonitrile mixtures (e.g. 40% acetonitrile (v/v): norfloxacin 5.27, ciprofloxacin 5.54, ofloxacin 5.69 and fleroxacin 6.00). Therefore, neutral buffer solutions as carrier electrolytes seem to be adequate to cause different net charge of the analytes and to allow electrophoretic separation.

Fully aqueous carrier electrolytes did not result in a complete separation of all analytes, so that the addition of methanol or acetonitrile was investigated. Carrier electrolytes containing methanol led to poor separation selectivity as well as to very low sensitivity. It is to assume that methanol causes fluorescence quenching. Generally, a decrease of sensitivity with increasing content of the organic modifier, even with acetonitrile, was observed. The data for carrier electrolytes of different content of organic modifiers at pH 7.00 are displayed in Table 1 to demonstrate the behaviour of the critical pair ciprofloxacin and norfloxacin with respect to migration time, resolution and sensitivity.

Systematic variation of the carrier electrolyte composition resulted in best separations with 40% (v/v) acetonitrile. pH was slightly varied for that composition (pH 7.00, 7.35 and 7.55). The following carrier electrolyte was found to serve best our requirements: 50 mM phosphate buffer pH 7.55 in acetonitrile–water (40:60, v/v). At pH 7.55 the migration times are considerably longer than at pH 7.00. Nevertheless, this pH was chosen with respect to sufficient resolution for real samples and sufficient time between the EOF signal and the analyte signals. Fig. 2 shows the optimized CE separation of a standard solution of all nine (fluoro)quinolones at a concentration of 400 μg l^{-1} each. The order of migration agrees with the pK_B values calculated by Barbosa et al. [29] for ofloxacin, norfloxacin, ciprofloxacin and fleroxacin.

Table 1
Resolution, migration time and relative signal height (relative to an aqueous carrier electrolyte) of norfloxacin and ciprofloxacin

Organic modifier	Migration times (min)		Relative signal heights	Resolution
	Norfloxacin	Ciprofloxacin		
None	9.5	9.5	100.0	No resolution
10% ACN	10.92	11.45	67.3	1.03
20% ACN	12.42	13.12	57.0	1.06
30% ACN	14	14.61	53.0	1.47
40% ACN	15.31	15.99	45.5	1.68
50% ACN	16.51	17.52	45.2	1.18
10% MeOH	12.4	12.4	65.0	No resolution
20% MeOH	17.86	19.19	40.3	0.55
30% MeOH	25.6	28.12	32.7	0.50
40% MeOH	36.76	39.33	18.7	1.07
50% MeOH	51.03	56.23	7.7	1.19

Fused silica capillary, 65 cm (effective length 50 cm) × 75 μm I.D. Carrier electrolyte: 50 mM H_3PO_4, pH 7.00, with different content of organic modifier. Separation voltage: 20 kV.

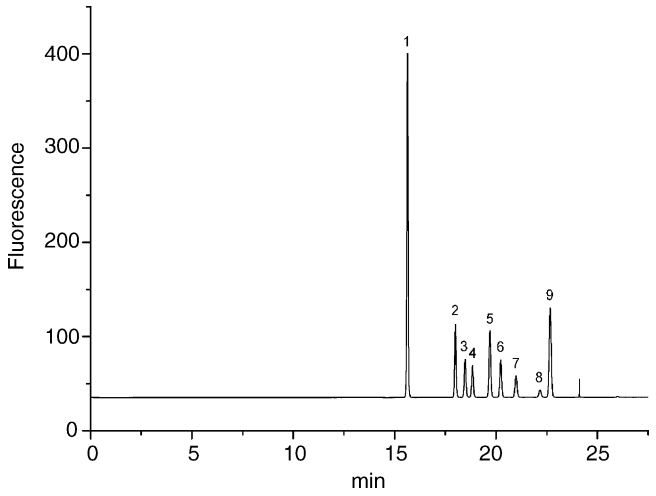

Fig. 2. Electropherogram of a standard solution of nine (fluoro)quinolones (400 μg l^{-1} each). Peaks: 1 = moxifloxacin, 2 = lomefloxacin, 3 = norfloxacin, 4 = ciprofloxacin, 5 = ofloxacin, 6 = enrofloxacin, 7 = oxolinic acid, 8 = flumequine, 9 = fleroxacin. Fused silica capillary, 70 cm (effective length 55 cm) × 75 μm I.D. Carrier electrolyte: 50 mM H_3PO_4, pH 7.55, 40% acetonitrile. Separation voltage: 20 kV. Fluorescence detection ex 240–400 nm filter, em 435 nm cut-off filter.

SPE was employed for clean-up and preconcentration of real samples. To find out the most appropriate SPE material, six different phases were examined. Generally, an SPE material was required that would give good recoveries even for relatively polar substances. Three different polymer-based reversed-phase materials (Oasis HLB, Isolute ENV+, LiChrolut EN) and three different silica-based reversed-phase materials (Chromabond C_8, Chromabond Tetracycline, Bakerbond Phenyl) were included in the investigations.

In a series of experiments it turned out that proper adjustment of the pH of the sample was essential. Recoveries could be improved tremendously when adding EDTA and adjusting the pH to 4 instead of no buffering. Besides adjusting the pH of the sample to 4, also the water used for washing the SPE material after sample application had to be buffered to a pH of 4 to avoid loss of analytes. Elution was first tried with methanol resulting in very low recoveries for the polar (fluoro)quinolones. The use of methanol–water (75:25, v/v) improved the recoveries of these (fluoro)quinolones. Nevertheless, the less polar substances like flumequine and oxolinic acid were not eluted under such conditions. Therefore, the finally used elution process consisted of two steps, first using methanol–water (75:25, v/v) and then using pure methanol.

Detailed experiments with sample solutions at pH 4 indicated that the different SPE materials provide significantly different suitability for (fluoro)quinolones in general and varying selectivity for each single analyte. Chromabond Tetracycline (a C_{18} modified silica which is recommended for preconcentration of residues of tetracycline antibiotics) turned out to be the most adequate SPE material, closely followed by Oasis HLB. All other tested SPE materials resulted in good recoveries only for oxolinic acid and flumequine. Table 2 shows the recoveries obtained with Chromabond Tetracycline and Oasis HLB for blood, surface water, and an aqueous standard. With the other SPE materials (Chromabond C_8, Isolute ENV+, LiChrolut EN and Bakerbond Phenyl) only aqueouss standard solutions and blood

Table 2
Recoveries (%) for solid-phase extraction of (fluoro)quinolones in a standard solution (50 ml, 4 μg l^{-1} each), in pond water (500 ml, 400 ng l^{-1} each), and in blood (1 ml spiked with 200 μg l^{-1} each, diluted to 50 ml) using different SPE materials

Compound	Chromabond Tetracycline			Oasis HLB			Chromabond C_8		
	Blood	Surface water	Standard	Blood	Surface water	Standard	Blood	Surface water	Standard
Moxifloxacin	97.7	95.3	92.4	90.4	102.8	88.4	<5	nd	<5
Lomefloxacin	101.2	96.4	107.0	85.5	104.9	90.6	<5	nd	<5
Norfloxacin	80.5	83.2	71.8	55.7	97.1	60.7	<5	nd	<5
Ciprofloxacin	84.0	82.7	74.2	63.4	92.9	63.1	<5	nd	<5
Ofloxacin	97.8	98.9	97.1	70.4	107.0	75.5	<5	nd	<5
Enrofloxacin	115.3	91.9	100.7	78.3	95.4	91.8	<5	nd	<5
Oxolinic acid	112.4	106.7	115.4	108.6	104.2	106.9	115	nd	116
Flumequine	108.2	101.7	127.5	93.8	101.6	108.0	112	nd	117
Fleroxacin	99.6	101.9	101.4	77.1	108.9	100.5	<5	nd	<5
	Bakerbond Phenyl			Isolute ENV+			LiChrolut EN		
	Blood	Surface water	Standard	Blood	Surface water	Standard	Blood	Surface water	Standard
Moxifloxacin	<5	nd	<5	<5	nd	<5	<5	nd	<5
Lomefloxacin	<5	nd	<5	<5	nd	<5	<5	nd	<5
Norfloxacin	<5	nd	<5	<5	nd	<5	<5	nd	<5
Ciprofloxacin	<5	nd	<5	<5	nd	<5	<5	nd	<5
Ofloxacin	<5	nd	<5	<5	nd	<5	<5	nd	<5
Enrofloxacin	<5	nd	<5	<5	nd	<5	<5	nd	<5
Oxolinic acid	78	nd	84	88	nd	96	98	nd	101
Flumequine	80	nd	81	86	nd	92	92	nd	99
Fleroxacin	<5	nd	<5	<5	nd	<5	<5	nd	<5

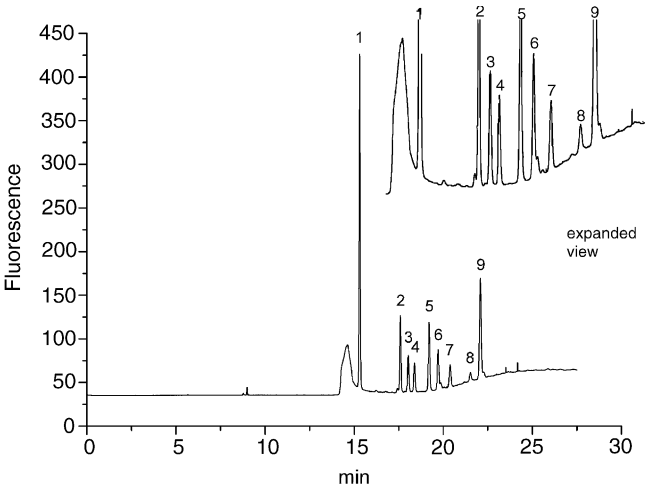

Fig. 3. Electropherogram of a spiked surface water sample (concentration of each (fluoro)quinolone 400 ng l^{-1}) after purification and preconcentration with SPE (Chromabond Tetracycline). Electrophoretic conditions and migration order as in Fig. 2.

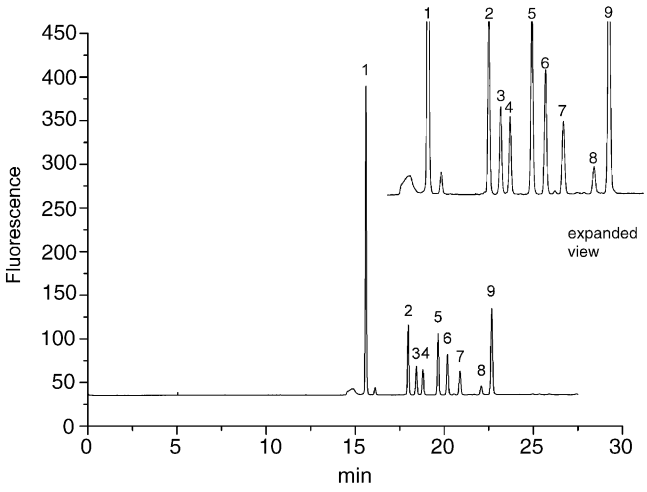

Fig. 4. Electropherogram of a spiked blood sample (concentration of each (fluoro)quinolone 200 μg l^{-1}) after purification and preconcentration with SPE (Chromabond Tetracycline). Electrophoretic conditions and migration order as in Fig. 2.

samples were tested. Due to the poor recoveries achieved, preconcentration of surface water was not investigated with these materials. The blood samples used for investigation of the recoveries were spiked at a level of 200 μg l^{-1} of each of the (fluoro)quinolones, whereas the surface water samples were spiked at a level of 400 ng l^{-1} of each analyte. The recoveries given in Table 2 were calculated with the help of an external calibration curve established with standards in the appropriate concentration range. The use of an internal standard might be appropriate, but such an approach has not yet been investigated in the present study.

Turiel et al. [10] proposed to wash the SPE cartridges after sample application with an acetate buffer (pH 4) containing 15% of acetonitrile. According to the authors this procedure minimizes the interferences from matrix components. Unfortunately, in the present work the use of organic solvents in the washing liquid resulted in significantly decreased recoveries so that totally aqueous washing solutions were preferred.

Electropherograms of a spiked blood sample and a spiked surface water sample after SPE treatment with Chromabond Tetracycline are shown in Figs. 3 and 4. Regarding peaks from matrix components, SPE with Oasis HLB yielded virtually the same electropherograms as Chromabond Tetracycline. These electropherograms indicate that despite the broad wavelength range from 240 to 400 nm used for excitation the detection selectivity is quite good. Some experiments using just a narrow excitation wavelength band at 278 nm corresponding to the maximum of the excitation spectrum of most fluoroquinolones yielded sensitivities far too poor for trace analysis.

Linearity was verified with standard solutions in the range from 100 to 5000 μg l^{-1} for each component, linearity in the matrix was demonstrated by spiking samples in a range that yielded concentrations between 100 and 5000 μg l^{-1} after preconcentration. The correlation coefficient was better than 0.999 in all cases. Method repeatability was investigated with standard solutions at a level of 400 μg l^{-1} as well as with spiked blood and pond water samples at a level of 200 μg l^{-1} and 400 ng l^{-1}, respectively. In each case, six measurements were carried out giving R.S.D.s below 3%. LODs and LOQs are given in Table 3.

Table 3
LODs and LOQs for fluorescence detection of (fluoro)quinolones in different matrices

Compound	Standard solution		Surface water, pre-concentration factor 1000		Blood, pre-concentration factor 2	
	LOD (μg l^{-1}) S/N = 3	LOQ (μg l^{-1}) S/N = 10	LOD (ng l^{-1}) S/N = 3	LOQ (ng l^{-1}) S/N = 10	LOD (μg l^{-1}) S/N = 3	LOQ (μg l^{-1}) S/N = 10
Moxifloxacin	0.7	2	1	3	0.5	1.5
Lomefloxacin	3.5	10	5	15	2	6
Norfloxacin	6.0	15	10	30	3	10
Ciprofloxacin	7.5	20	10	30	4	12
Ofloxacin	3.5	10	5	15	2	6
Enrofloxacin	6.0	15	10	30	3	10
Oxolinic acid	11.0	30	15	45	7	20
Flumequine	30.0	100	50	150	15	45
Fleroxacin	2.5	7.5	5	15	1.5	5

4. Conclusions

The investigations presented in this paper demonstrate that fluorescence detection with broad wavelength band excitation is an attractive technique in capillary electrophoresis and may be a powerful alternative to laser-induced fluorescence detection. This instrumentation allowed the development of a simple, yet sensitive and selective method for the determination of nine (fluoro)quinolones in different matrices. The limits of quantification were in the low $\mu g\,l^{-1}$ range but can be easily improved to the $ng\,l^{-1}$ range if solid-phase extraction is used for preconcentration. The SPE procedure also leads to sufficient sample clean-up, so that no seriously interfering peaks were encountered in the electropherograms of blood and surface water samples. It should be kept in mind that this work should just give a general demonstration of the feasibility of capillary electrophoresis and fluorescence detection for trace analysis of various (fluoro)quinolones. There may be a much wider range of applications in the area of food and other kinds of samples. Such investigations and proper validation of the methods will be the subject of future work.

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