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The qualitative and quantitative determination of quinolones of first and second generation by capillary electrophoresis

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Abstract

Capillary electrophoresis (CE) was applied to the study of 10 quinolones of first and second generation — nalidixic acid, oxolinic acid, pipemidic acid, cinoxacin, norfloxacin, ciprofloxacin, ofloxacin, pefloxacin, fleroxacin, and flumequine. Separation was performed on a fused silica capillary (75 μm–60 cm) using a phosphate buffer (pH 7.0, 125 mM). Detection was at 214 nm. Only norfloxacin and ciprofloxacin cannot be separated in this way. Because of the specificity of the method, the identification of the individual quinolones by their migration time was possible. The same system has been applied for the quantitative determination of quinolones in tablets and capsules. Excipients do not adversely affect the results. Some parameters (linearity, precision, accuracy) were validated. Especially the possibility of simultaneous quantification and identification of the active ingredient in the finished product is very attractive.

Keywords: Capillary electrophoresis; Quinolones; Qualitative analysis; Quantitative analysis

1. Introduction

The quinolones and fluoroquinolones comprise a series of synthetic antibacterial agents and are derived from nalidixic acid, a naphthyridine derivative introduced for the treatment of urinary tract infections.

Analogues of nalidixic acid were investigated in the hope of widening the spectrum of action. Structure-activity studies have shown that the 1,4-dihydro-4-oxo-3-pyridinecarboxylic acid moiety is essential for the antibacterial activity [1]. The pyridine system must be annulated with an aromatic ring, but it was possible to alter the heterocyclic ring. Isosteric heterocyclic groupings in this class include the quinolones (e.g. oxolinic acid), the cinnolines (e.g. cinoxacin), the pyridopyrimidines (e.g. pipemidic acid), and the fluoroquinolones (e.g. ciprofloxacin, norfloxacin, ofloxacin, pefloxacin, fleroxacin, flumequine).

The effective antibacterial spectrum of the quinolone class is largely confined to gram-negative bacteria. Newer members of this group pos-

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Table 1
Selection of the internal standard

Substance to be examined	Appropriate internal standard
Ofloxacin	Norfloxacin
	Ciprofloxacin
Norfloxacin	Pipemidic acid
	Ofloxacin
	Fleroxacin
Ciprofloxacin	Pipemidic acid
	Ofloxacin
	Fleroxacin
Fleroxacin	Pefloxacin
	Ofloxacin
	Norfloxacin
	Ciprofloxacin
Pipedimic acid	Pefloxacin
	Fleroxacin
	Norfloxacin
	Ciprofloxacin
Pefloxacin	Fleroxacin
	Pipemidic acid
	Norfloxacin
	Ciprofloxacin
Oxolinic acid	Flumequine
	Cinoxacin
Cinoxacin	Flumequine
	Oxolinic acid
Nalidixic acid	Oxolinic acid
Flumequine	Cinoxacin
	Oxolinic acid

sessing 6-fluoro and 7-piperazinyl substituents exhibit an extended spectrum of activity [1,2].

Until now, high performance liquid chromatography has been a major technique used for the determination of quinolones and fluoroquinolones [3–18]. Almost the system was applied to the quantitative determination of a well-defined quinolone in biological fluids (therapeutic drug monitoring). The proof of the specificity is limited to the separation of the drug and its metabolites [18]. Simultaneous separation of structure-related compounds in pharmaceutical formulations and for the purity control of bulk products was described [3–17]. The same technique was applied in the monograph about ciprofloxacin in the European Pharmacopoeia [19] for the purity control (related substances).

Analysis by means of capillary electrophoresis (CE) has been achieved for different drugs of this class, but the studies have usually been limited to the separation of only a few quinolones and fluoroquinolones [20,21]. One study has reported on the determination of ciprofloxacin and its related impurities [22]. In 1997, Sun and Shen optimized a capillary electrophoretic separation of 14 quinolone antibacterials by using the overlapping resolution mapping scheme [23]. With the simple capillary zone electrophoresis (CZE), four monofluorinated quinolones were completely coalesced and a number of substances were not baseline separated. Only by using micellar electrokinetic chromatography and a mixture of sodium cholate, sodium heptanesulphonate, acetonitrile, and a sodium borate–sodium dihydrogen phosphate buffer pH 7.3, could the problem be solved.

The aim of this study was to develop a selective method able to separate a large number of structurally related quinolones and fluoroquinolones by the simple CZE. The system is appropriate for quantitative determination in different pharmaceutical formulations without specific sample pretreatment. The paper deals with the validation of the most important parameters — specificity, linearity, precision and accuracy.

Table 2
Reference solutions for the quantitative determination

Reference substance	Reference solution (mg/100 ml)	Running buffer	Diluted reference solution (μg/ml)
Norfloxacin	±175	Phosphate buffer, pH 7.0	±350
Pipemidic acid	±100	Phosphate buffer, pH 7.0	±200
Flumequine	±60	Phosphate buffer, pH 8.0	±120
Cinoxacin	±90	Phosphate buffer, pH 8.0	±180
Oxolinic acid	±95	Phosphate buffer, pH 8.0	±190

Table 3
Sample preparation for the quantitative determination

	Average mass (mg)	Running buffer	Sample solution (mg powder/100 ml)	Internal standard solution (mg/100 ml)	Diluted sample solution (μg/ml)
Norfloxacin [Zoroxin®] 400 mg tablets	509.3	Phosphate buffer, pH 7.0	±220 mg	Pipedimic acid · $3H_2O$, 140 mg	±345
Pipedimic acid · $3H_2O$ [Pipram®] 235 mg capsules	334.6	Phosphate buffer, pH 7.0	±150 mg	Pefloxacin, 130 mg	±210
Flumequine [Apurone®] 400 mg tablets	524.0	Phosphate buffer, pH 8.0	±80 mg	Cinoxazin, 70 mg	±122
Cinoxacin [Cinobac®] 500 mg capsules	628.2	Phosphate buffer, pH 8.0	±110 mg	Flumequine, 50 mg	±175
Oxolinic acid [Uritrate®] 750 mg tablets	1076	Phosphate buffer, pH 8.0	±135 mg	Flumequine, 50 mg	±188

2. Experimental

2.1. Instruments

The development and validation of the method and the experiments were performed on a Waters Quanta 4000 CE instrument (Millipore, Waters). The capillary used was a 75 μm fused silica capillary (60 cm in total length and 52.5 cm to the detector).

Hydrostatic injections were performed by lifting the sample vial approximately 10 cm above the height of the buffer vial for 10 s. For detection, the absorbance was measured by means of an on-line fixed-wavelength UV detector with a zinc discharge lamp and a 214 nm filter. The running voltage was 22 kV. The data were collected on a Hewlett-Packard Integrator (HP 3396 Series II), which was also used for processing the areas of the peaks.

2.2. Reagents

Sodium hydroxide (p.a.), sodium dihydrogen phosphate monohydrate (p.a.), disodium hydrogen phosphate dihydrate (p.a.), sodium acetate trihydrate (p.a), and acetic acid (99% p.a.) were obtained from E. Merck (Germany). Oxolinic acid (M_r 261.4), pipemidic acid (M_r 303.3), cinoxacin (M_r 262.2), norfloxacin (M_r 319.3), ofloxacin (M_r 361.4), flumequine (M_r 261.3), and sodium lauryl sulphate (99%) were obtained from Sigma (Germany), and nalidixic acid CRS from the European Pharmacopoeia Commission (Strasbourg, France).

Reference solutions for qualitative analysis of pefloxacin, fleroxacin, and ciprofloxacin were prepared from the commercially available drugs (Peflacine®, Quinodis®, and Ciproxine®) by mixing the powder with the phosphate buffer pH 7.0. The suspensions were filtered through a membrane (0.45 μm).

2.3. Running buffers

2.3.1. Sodium borate buffers (100 mM, pH 8.0 and 9.0)

Solutions were made up by dissolving 6.18 g of boric acid in 500 ml of water and adjusting the pH with 1 M sodium hydroxide, before diluting to 1000 ml with water. The solutions were filtered through a membrane (0.45 μm).

2.3.2. Sodium phosphate buffers (100, 120 and 150 mM, pH 7.0)

Solutions were made up by dissolving, respectively, 13.80, 17.25, or 20.70 g of sodium dihydro-

gen phosphate monohydrate in 500 ml of waterand adjusting the pH with 1 M sodium hydroxide, before diluting to 1000 ml with water. The solutions were filtered through a membrane (0.45 μm).

2.3.3. Sodium phosphate buffer (100 mM, pH 8.0)

The buffer was made up by dissolving 13.80 g of sodium dihydrogen phosphate monohydrate in 500 ml of water and adjusting the pH with 1 M sodium hydroxide, before diluting to 1000 ml with water. The solution was filtered through a membrane (0.45 μm).

2.4. Internal standard solutions

Selection of the internal standard had to be made on the basis of the substance to be examined (Table 1). An appropriate amount of the compound (Table 3) was dissolved in 10 ml 0.1 M sodium hydroxide and diluted to 100 ml with the corresponding running buffer (phosphate buffer, pH 7.0 or 8.0).

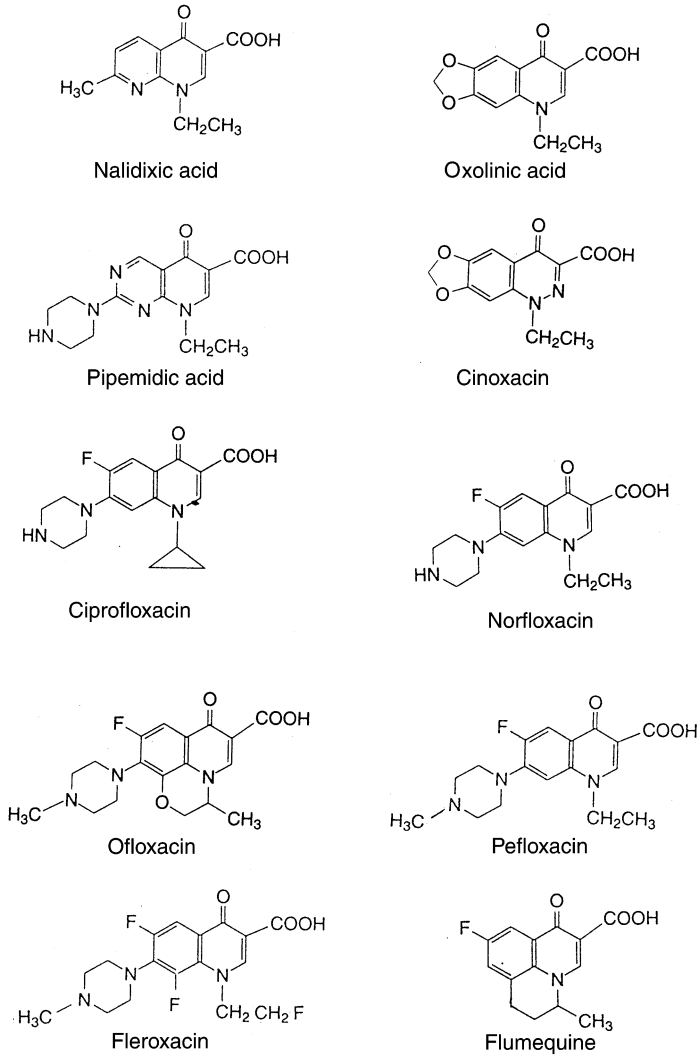

Fig. 1. The chemical structures of the quinolones and the fluoroquinolones.

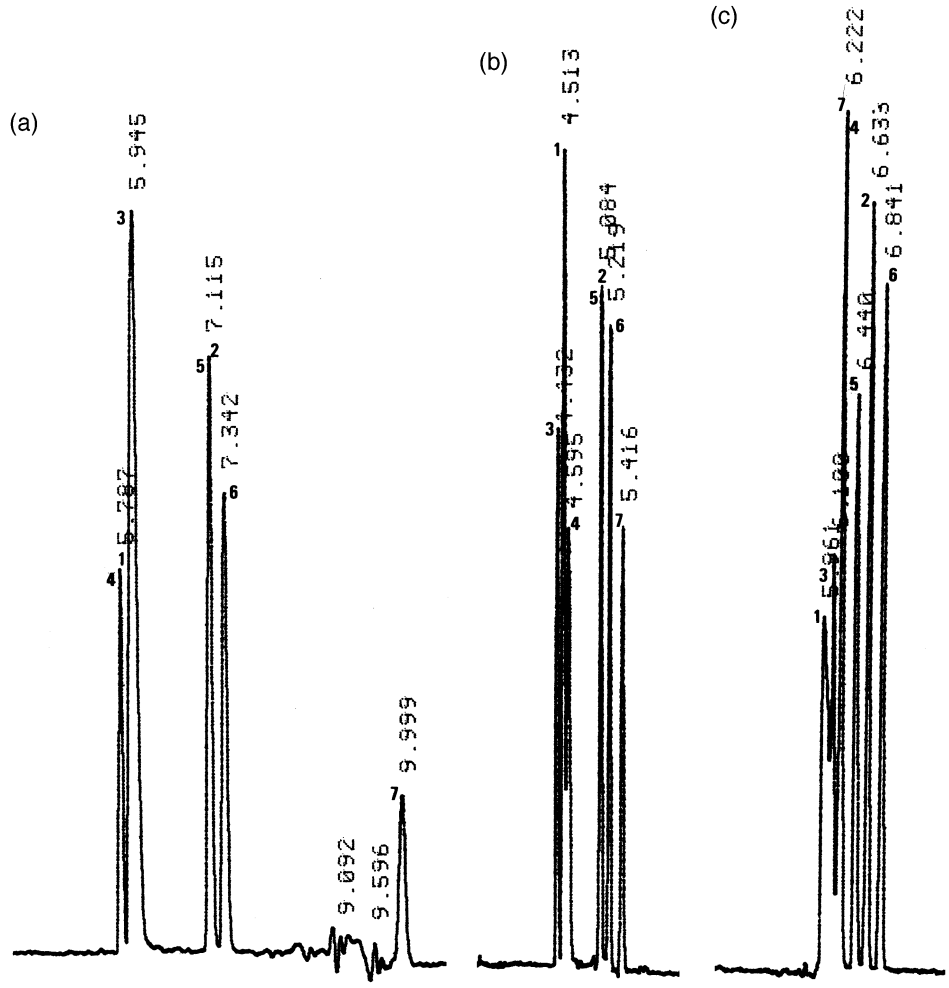

Fig. 2. Electropherogram of a mixture of several quinolones and fluoroquinolones when using a different running buffer. (A) Sodium phosphate buffer (pH 5.5, 125 mM); (B) sodium phosphate buffer (pH 6.0, 125 mM); (C) sodium phosphate buffer (pH 6.5, 125 mM). Peaks: (1) norfloxacin; (2) pipemidic acid; (3) ofloxacin; (4) cipofloxacin; (5) flexoacin; (6) pefloxacin; and (7) formamide.

2.5. Reference solutions

Reference solutions were prepared by weighing accurately an appropriate amount of the corresponding reference substance, dissolving in 10 ml 0.1 M sodium hydroxide and diluting to 100 ml with the corresponding running buffer (Table 2). Using a buffer of pH 7.0, the solutions of flumequine, cinoxacin, and oxolinic acid became turbid due to low solubility. For these substances, a phosphate buffer at pH 8.0 was necessary. An amount of 5 ml of the solution was mixed with 5 ml of th-e internal standard solution and diluted to 25 ml with the buffer solution (Table 2).

2.6. Sample preparations

Ten or 20 tablets or the contents of 10 or 20 capsules were weighed, ground, and mixed. An appropriate amount of the powder (Table 3) was mixed with 10 ml of 0.1 M sodium hydroxide and diluted to 100 ml with the corresponding running buffer (phosphate buffer, pH 7.0 or 8.0). The sample was filtered through a membrane (0.45 μm). An amount of 5 ml of the filtrate was mixed with 5 ml of the appropriate internal standard solution (Table 3) and diluted to 25 ml with the running buffer.

3. Results and discussion

3.1. Optimization of the method

Structures of the quinolones and the fluoroquinolones determined in the pharmaceutical formulations and of those of the corresponding standards are shown in Fig. 1.

The first class (nalidixic acid, oxolinic acid, cinoxacin, and flumequine) is mainly characterized by the presence of an ionizable carboxyl group. The pK_a values fall in the range 5.6–6.4. These high pK_a values, relative to the pK_a value of pyridine-3-carboxylic acid (4.8), are attributed to the acid weakening effect of hydrogen bonding of the 3-carboxyl group to the 4-carbonyl group [1].

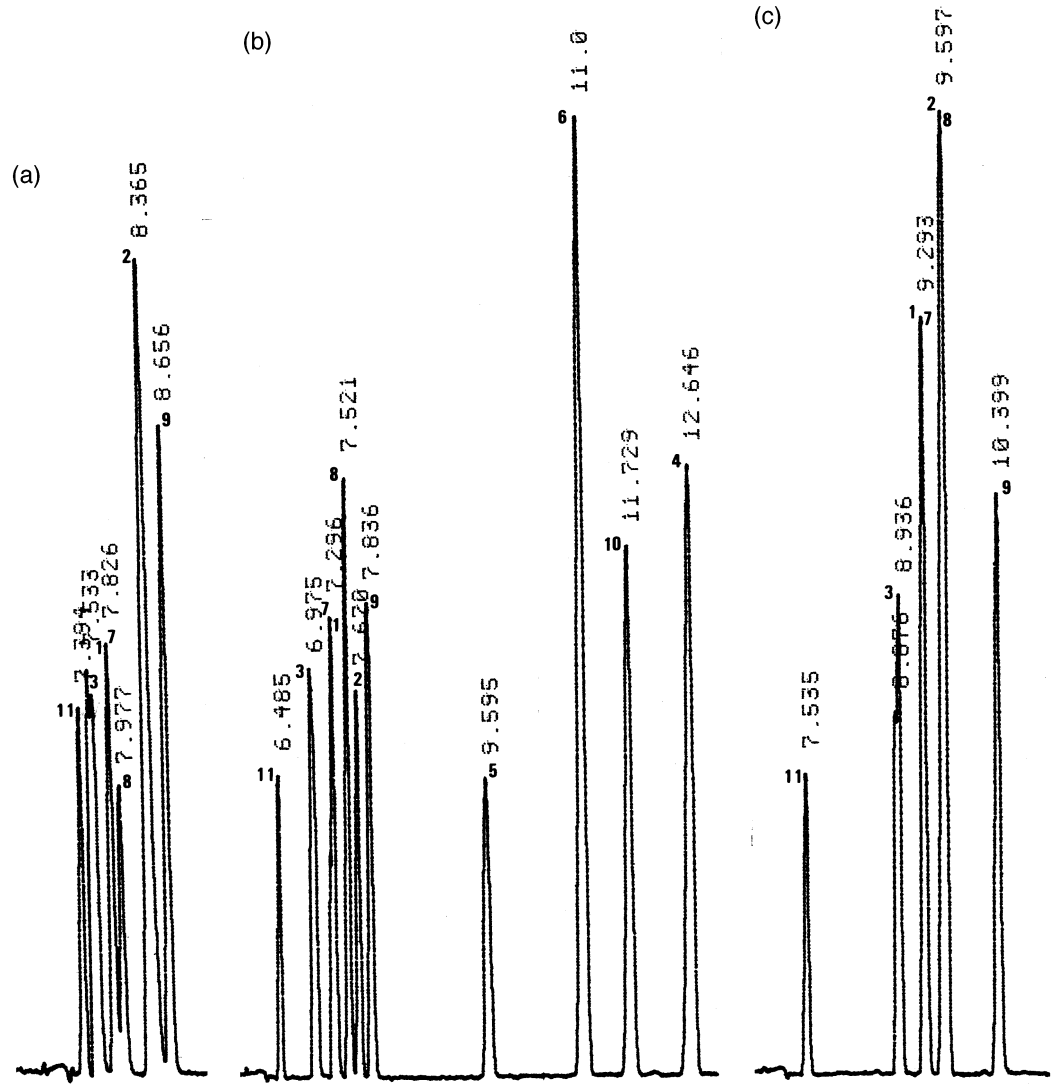

Fig. 3. Electropherogram of a mixture of several quinolones and fluoroquinolones when using a different running buffer. (A) Sodium phosphate buffer (pH 6.75, 125 mM); (B) sodium phosphate buffer (pH 7.0, 125 mM); (C) sodium phosphate buffer (pH 7.5, 125 mM). Peaks: (1) norfloxacin; (2) pipemidic acid; (3) ofloxacin; (4) flumequine; (5) oxolinic acid; (6) cinoxacin; (7) ciprofloxacin; (8) fluroxacin; (9) pefloxacin; (10) nalidixic acid; and (11) formamide.

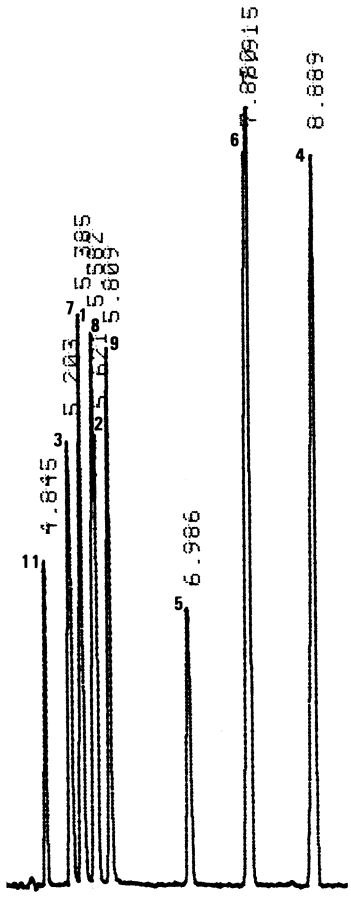

Fig. 4. Electropherogram of a mixture of several quinolones and fluoroquinolones when using a sodium phosphate buffer (pH 7.0, 100 mM) as running buffer. Peaks: (1) norfloxacin; (2) pipemidic acid; (3) ofloxacin; (4) flumequine; (5) oxolinic acid; (6) cinoxacin; (7) ciprofloxacin; (8) fluroxacin; (9) pefloxacin; (10) nalidixic acid; and (11) formamide.

Therefore, the use of an alkaline running buffer may be considered. The second class, represented by the fluoroquinolones and pipemidic acid, possess in addition to the 3-carboxylic acid group, a basic piperazino functionality at the 7-position and a 6-fluoro substituent. The pK_a values for the basic nitrogen fall in the range 8.1–9.3 [1]. As a consequence of the pH of the medium, the substance is either mainly negatively or positively charged. This offers the possibility of using either an acidic or an alkaline running buffer.

With sodium borate at pH 9.0 as a running electrolyte, the selectivity is inadequate. Only the separation between the two classes is possible. In the presence of sodium lauryl sulphate the separation becomes better, but many components display peak tailing. This problem cannot be resolved by lowering the pH to 8.0. An acetate buffer (pH 3.75–5.5) can only be used for the fluoroquinolones and pipemidic acid. In an acidic medium, nalidixic acid, oxolinic acid, cinoxacin, and flumequine are not ionized. The neutral components migrate in a single zone with a velocity determined by the electro-osmotic flow.

Better results were obtained by using a phosphate buffer with a pH between 5.5 and 7.5. In particular, the separation between the amphoteric substances (fluoroquinolones and pipemidic acid) depends on the pH and the molarity of the running electrolyte. The influence of the pH by con-

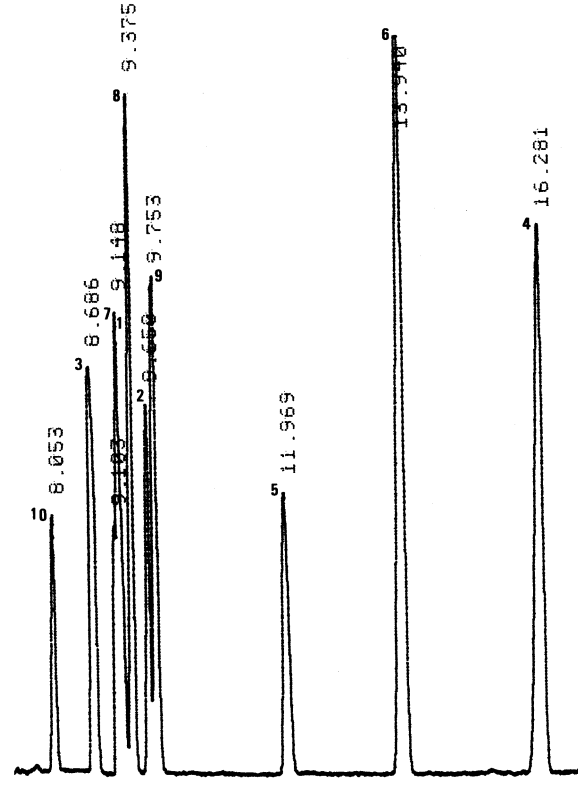

Fig. 5. Electropherogram of a mixture of several quinolones and fluoroquinolones when using a sodium phosphate buffer (pH 7.0, 100 mM) as running buffer. Peaks: (1) norfloxacin; (2) pipemidic acid; (3) ofloxacin; (4) flumequine; (5) oxolinic acid; (6) cinoxacin; (7) ciprofloxacin; (8) fluroxacin; (9) pefloxacin; (10) nalidixic acid; and (11) formamide.

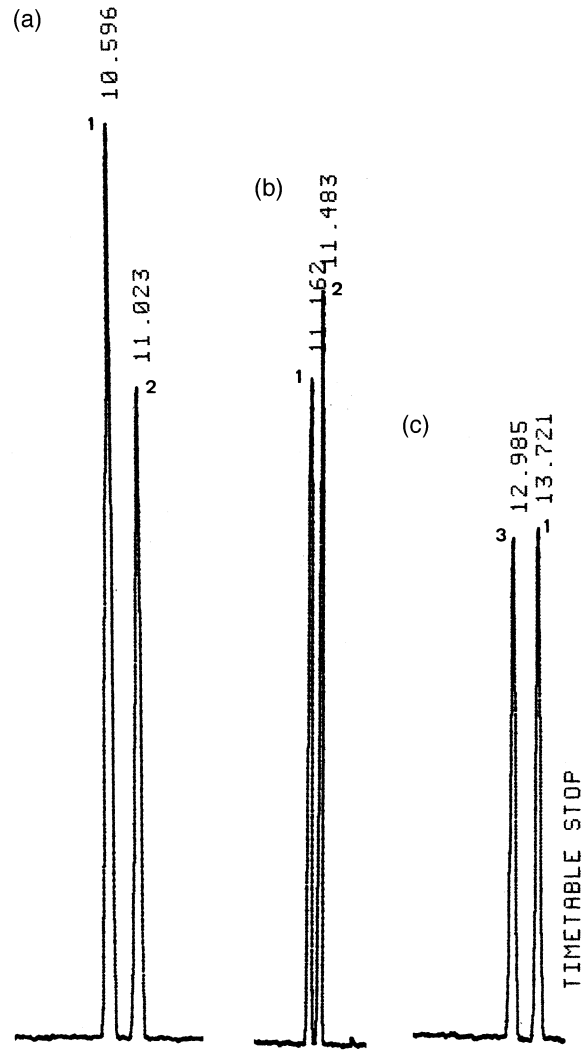

Fig. 6. Electropherogram of the quantitative determination of flumequine (A), cinoxacin (B) and oxolinic acid (C). Running buffer: sodium phosphate (pH 8.0, 100 mM). Peaks: (1) flumequine; (2) cinoxacin; and (3) oxolinic acid.

stant molarity (125 mM) is clearly demonstrated in Figs. 2 and 3. The selectivity depends not only on the pH, but also on the ionic strength of the running buffer. At a concentration of 100 mM phosphate, pipemidic acid co-migrates with fleroxacin (Fig. 4). Only by using a phosphate concentration of 125 mM is pipemidic acid baseline separated from fleroxacin (Fig. 3B). At 150 mM, the selectivity decreases resulting in an inadequate separation between pipemidic acid and pefloxacin (Fig. 5). Norfloxacin and ciprofloxacin cannot be separated in this way. In spite of the lower solubility of the acidic components (oxolinic acid, cinoxacin, nalidixic acid, and flumequine), a phosphate buffer at pH 7.0 (125 mM) is mainly appropriate for qualitative analysis or identification (Fig. 3B).

3.2. Quantitative determination in pharmaceutical formulations

The same system (sodium phosphate buffer, pH 7.0, 125 mM) may be applied for the quantitative

Table 4
Specificity

	Migration time	Relative migration time
Formamide (marker)	6.485	1.000
Ofloxacin	6.975	1.076
Norfloxacin	7.296	1.125
Ciprofloxacin	7.296	1.125
Fleroxacin	7.521	1.160
Pipemidic acid	7.620	1.175
Pefloxacin	7.836	1.208
Oxolinic acid	9.595	1.480
Cinoxacin	11.000	1.696
Nalidixic acid	11.729	1.809
Flumequine	12.646	1.958

determination of quinolones and fluoroquinolones in tablets and capsules. Because of the low solubility of flumequine, cinoxacin, oxolinic acid, and nalidixic acid, it was necessary to use a phosphate buffer at pH 8.0 for the quantitative determination (Fig. 6). The migration times of the different components decreased, but the selectivity remained sufficient. Nalidixic acid and flumequine were not baseline separated and an appropriate choice of the internal standard was necessary.

Table 7
Repeatability of 10 consecutive injections of the same sample

Sample solution	Relative standard deviation (%)
Flumequine	0.75
Cinoxacin	0.92
Oxolinic acid	0.59
Norfloxacin	1.91
Pipemidic acid	1.25

Table 8
Accuracy

	Placebo + 80%	Placebo + 100%	Placebo + 120%
Flumequine	97.6%	94.5%	97.0%
Cinoxacin	100.6%	100.1%	101.1%
Oxolinic acid	97.8%	99.0%	100.5%
Norfloxacin	100.2%	98.4%	99.9%

By means of different placebo mixtures it was demonstrated that the following excipients do not adversely affect the results — lactose, maize starch, microcrystalline cellulose, magnesium stearate, silicon dioxide, macrogol, talc, pregela-

Table 5
Linearity

	Concentration range (μg/ml)	Correlation coefficient (r^2)	Regression equations
Flumequine	35–175	0.99986	$y = 0.08306x + 0.01182$
Cinoxacin	50–250	0.99992	$y = 0.03705x + 000459$
Oxolinic acid	55–275	0.99984	$y = 0.05850x + 0.01037$
Norfloxacin	100–500	0.99891	$y = 0.07080x + 0.02473$
Pipemidic acid · 3 H_2O	60–300	0.99928	$y = 0.03836x + 0.00791$

Table 6
Precision (repeatability) of the total analysis of 10 replicate samples

Substance to be examined	Theoretical amount (mg)	Amount found	Relative standard deviation (%)
Flumequine [Apurone®]	400	403.7 ± 4.27 mg or 100.9%	1.06
Cinoxacin [Cinobac®]	500	489.8 ± 5.00 mg or 98.0%	1.02
Oxolinic acid [Uritrate®]	750	758.5 ± 10.08 mg or 101.1%	1.33
Norfloxacin [Zoroxin®]	400	405.7 ± 7.59 mg or 101.4%	1.87
Pipedimic acid · $3H_2O$ [Pipram®]	235	239.2 ± 3.26 mg or 101.8%	1.36

tinized starch, macrogol 6000, croscarmellose sodium, hypromellose, or titanium dioxide.

3.3. Validation of the method

3.3.1. Specificity

The specificity of the method was demonstrated by the good separation of nine of the 10 different quinolones and fluoroquinolones (Table 4 and Fig. 3B). Only ciprofloxacin migrated with the same velocity as norfloxacin and could not be separated in this way.

3.3.2. Linearity

The detector responses were found to be linear for the different components in the concentration range as mentioned in Table 5. The regression analysis data for the calibration curves were calculated using peak areas.

3.3.3. Precision

The precision (repeatability) was determined by the total analysis of 10 replicate samples under the same operating conditions, by the same analyst, and on the same day. The mean value of the concentration and the relative standard deviation are summarized in Table 6.

The error of the equipment, the electrophoretic separation, and the relative standard deviation were determined by performing 10 consecutive injections of the same sample (Table 7).

3.3.4. Accuracy

The accuracy of the method was determined by investigating the recovery of each component at three levels ranging from 80 to 120% of the theoretical concentration from placebo mixtures spiked with the active substance (Table 8).

4. Conclusion

The determination of different quinolones of first and second generation by capillary electrophoresis has been achieved. The study demonstrates that CE can be successfully applied to the qualitative and quantitative analysis in pharmaceutical formulations. Due to the high selectivity of the method, identification is also possible on the basis of the migration time. The possibility of simultaneous quantification and identification of the active ingredient is very important by the quality control of the finished product.

References

[1] A.R. Martin, in: J.N. Delgado, W.A. Remers (Eds.), Textbook of Organic Medicinal and Pharmaceutical Chemistry, 10th ed., Lippincott-Raven, Philadelphia, New York, 1998, pp. 196–202.
[2] W. Sneader, Drug Prototypes and their Exploitation, Wiley, Chicester, England, 1996.
[3] S.K. Lee, Y.H. Park, C.J. Yoon, D.W. Lee, J. Microcolumn Sep. 10 (1998) 133–139.
[4] Y.P. Chen, C.Y. Shaw, B.L. Chang, Yaowu Shipin Fenxi 4 (1996) 155–164.
[5] K. Borner, E. Borner, H. Hartwig, H. Lode, Methodol. Surv. Biochem. Anal. 20 (1990) 131–144.
[6] A.P. Argekar, S.U. Kapadia, S.V. Raj, S.S. Kunjir, Indian Drugs 33 (1996) 261–266.
[7] L. Elrod Jr., T.G. Golich, D.I. Shaffer, B.P. Shelat, J. Liq. Chromatogr. 15 (1992) 451–466.
[8] J.F. Bauer, L. Elrod Jr., J.R. Fornnarino, D.E. Heathcote, S.K. Krogh, C.L. Linton, B.J. Norris, J.E. Quick, Pharm. Res. 7 (1990) 1177–1180.
[9] N.H. Foda, J. Liq. Chromatogr. 18 (1995) 4135–4147.
[10] R.T. Sane, D.V. Patel, S.N. Dhumal, V.R. Nerurkar, P.S. Mainkar, D.P. Gangal, Indian Drugs 27 (1990) 248–250.
[11] R.T. Sane, V.G. Nayak, V.R. Bhate, M.D. Joshi, S.M. Purandare, V.G. Nayak, Indian Drugs 26 (1989) 497–499.
[12] P.M. Lacroix, N.M. Curran, R.W. Sears, J. Pharm. Biomed. Anal. 14 (1996) 641–654.
[13] R. Jain, C.L. Jain, LC-GC 10 (1992) 707–708.
[14] M. Cordoba-Borrego, M. Cordoba-Diaz, D. Cordoba-Diaz, J. Pharm. Biomed. Anal. 18 (1999) 919–926.
[15] G. Carlucci, P. Mazzeo, T. Fantozzi, Anal. Lett. 26 (1993) 2193–2201.
[16] V.M. Shinde, B.S. Desai, N.M. Tendolkar, Indian Drugs 35 (1998) 715–717.
[17] W. Backe, Arch. Pharm. 320 (1987) 1093–1094.
[18] G. Carlucci, J. Chromatogr. A 812 (1998) 343–367.