Optimization of a Capillary Electrophoresis Method for the Separation of Quinolone Derivatives

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Background: In this work the applicability of capillary zone electrophoresis for the separation of quinolones from different generations has been studied.
Objective: Our aim was to develop a capillary electrophoretic method for the simultaneous separation of four quinolones and also to optimize the analytical conditions.
Material and methods: Capillary electrophoresis (CE) is a family of related techniques that use narrow-bore fused-silica capillaries to perform high efficiency separations of both small and large molecules. For this we chose four quinolones: a naftiridine derivate (nalidixic acid), a pyrido-pyrimidine derivate (pipemidic acid) and two second generation fluoroquinolones with different structures (ciprofloxacin, ofloxacin).
Results: A fast and reliable method has been developed, using a separation buffer composed of 40 mM natrium tetraborate and 5% methanol as organic modifier, with whom we achieved the separation of the studied quinolones in less than 10 minutes.
Conclusions: CE proved to be an efficient tool in the separation of quinolones from different generations.

Keywords: quinolones, fluoroquinolones, optimization of separation, capillary electrophoresis

Introduction

Quinolones are potent synthetic chemotherapeutic antibacterial class. The first generation of the quinolones begins with the introduction of nalidixic acid used in the treatment of urinary infection, but the major break-through came with the introduction in therapy of the fluoroquinolones, compounds with a fluorine atom attached to the quinolone ring system in position C6 [1,2]. Because of their particular mechanism of action, potency, activity and relatively simple chemical structure fluoroquinolones offer greater potency, a broader spectrum of antimicrobial activity, greater efficacy against resistant organisms, and better safety profile than older quinolones [3, 4, 5]. Capillary electrophoresis (CE) is a family of related techniques that use narrow-bore fused-silica capillaries to perform high efficiency separations of both small and large molecules [6]. Although CE is originally the method considered for the analysis of biological macromolecules, but recently has also been utilized for the separation of other compounds with pharmaceutical use including here quinolones derivatives [7, 8, 9]. CE offers a number of advantages when compared with the more frequently used chromatographic techniques: extremely small amount of sample, high separation efficiency and resolution, rapid separation, automated instrumentation, various modes to vary selectivity and simple separation mechanism [8, 10]. Capillary zone electrophoresis (CZE) is the simplest form of CE, the separation mechanism being based on the differences in the charge-to-mass ratio of the analytes. The separation is based on the differences between the own electrophoretic mobility of the analytes [6, 11, 12, 13]. Because fluoroquinolones have very similar structures, they are neutral from electrophoretic point of view and consequently have very similar electrophoretic mobility, probably CZE is not the suitable option for their separation [14]. Micellar electric capillary chromatography (MECC) is probably the best solution for the separation of this kind of substances. This paper wants to prove the applicability of CZE for the separation of quinolones from different generations, quinolones having different structural characteristics. For this we chose four quinolones: a naftiridine derivate (nalidixic acid), a pyrido-pyrimidine derivate (pipemidic acid) and two second generation fluoroquinolones with different structures (ciprofloxacin, ofloxacin) (Figure 1).

Material and method

the four quinolones were purchased from the following sources: ciprofloxacin and ofloxacin from Ranbaxy, nalidixic acid, pipemidic acid from Sigma-Aldrich. During the experimental work the following reagents were used: methanol GR p.a. and acetonitrile GR p.a. (Lach-Ner), boric acid and natrium tetraborate (Merck), natrium hydroxide

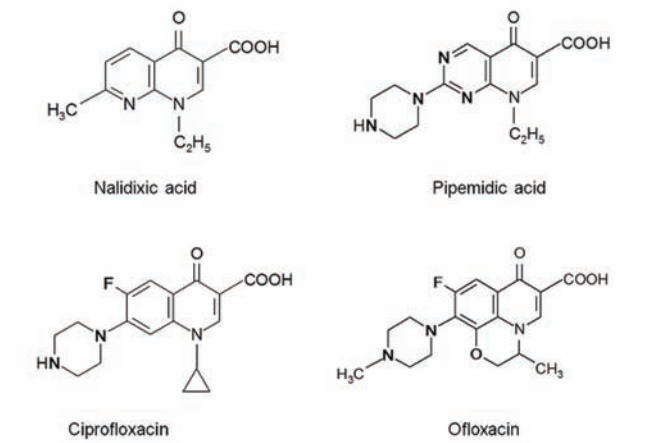

Fig. 1. Chemical structures of the four quinolones studied: nalidixic acid, pipemidic acid, ciprofloxacin and ofloxacin

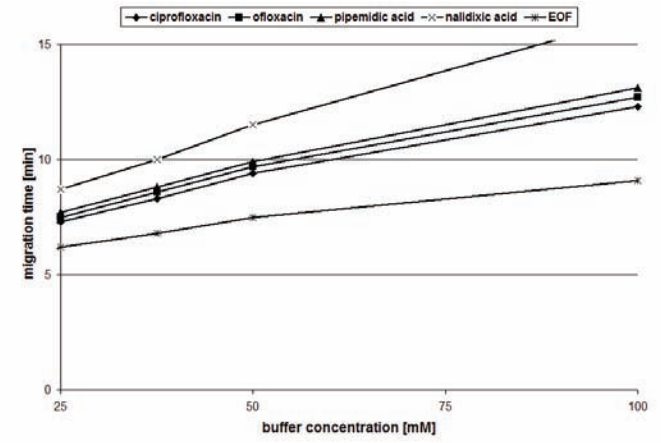

Fig. 2. Variation of the migration times depending on the buffer concentration (separation conditions: capillary 60 cm (52 cm) x 50 µm I.D., natrium tetraborate buffer, pH 9.3, voltage +22 kV, detection: UV absorption at 214 nm, concentration of analytes 10 µg/ml).

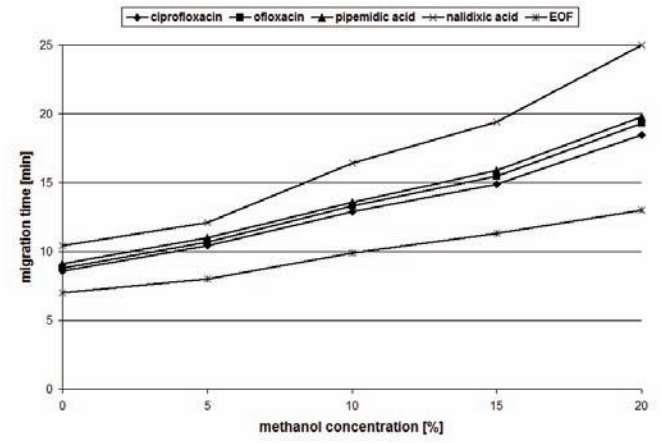

Fig. 3. Variation of the migration times depending on the methanol concentration (separation conditions: capillary 60 cm (52 cm) x 50 µm I.D., buffer 40 mM natrium tetraborate, pH 9.3, voltage +22 kV, detection: UV absorption at 214 nm, concentration of analytes 10 µg/ml).

solution 0.1 N (Agilent). All reagents were of analytical grade. The deionized water was prepared with a Milli-Q system (Millipore). The CE experiments were conducted using an Agilent 6100 capillary electrophoresis system and data was recorded and processed by use of Chemstation software version 7.01 (Agilent). In all measurements hydrodynamic sample injection was used, by applying a pressure of 30 mbar for 8 seconds, the sample solutions being introduced at the anodic end of the capillary. Separations were performed using a fused-silica capillary of 60 cm × 50 µm I.D. (effective length: 52 cm) (Agilent). The applied voltage was +22 kV; the current was kept below 200 µA. The temperature of the capillary was kept at 15 °C. Detection of the analytes was performed using a photodiode array detection system set to 214 nm and 280 nm. Quinolone stock solutions were prepared in methanol at concentrations of 1 mg/ml and later diluted to the appropriate concentrations. At the beginning of each day the capillary was conditioned with NaOH 0.1 N for 5 minutes, water for 5 minutes, and buffer solution for another 5 minutes. The capillary was preconditioned before every measurement with water for 1 minute and then with the buffer electrolyte for 3 minutes [15].

Results

Our aim was not only to elaborate a simple, rapid and efficient capillary electrophoretic method for the separation of the four quinolone derivates, but also the optimization of the separation conditions. For these we studied the influence of buffer concentration, buffer additives, buffer pH, temperature, applied voltage, injection pressure and capillary length on the separation. Migration time increased with the increase of the buffer concentrations, because of the decrease of electroosmotic flow (EOF) with the increase in ionic strength (Figure 2).

The optimum buffer concentration was set at 40 mM. In order to increase the selectivity of the separation organic solvents (methanol, acetonitrile) were used as buffer additives. The aim was to modify the electrophoretic mobilities of the analytes. Increasing the organic solvent concentra-

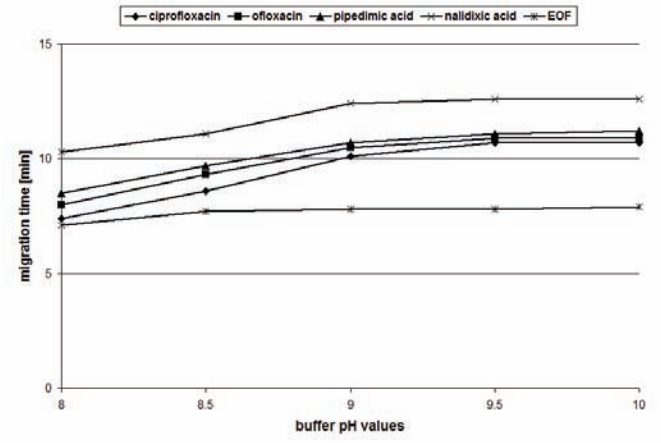

Fig. 4. Variation of the migration times depending on the buffer pH (separation conditions: capillary 60 cm (52 cm) x 50 µm I.D., buffer 40 mM natrium tetraborate + 5% methanol, voltage +22 kV, detection: UV absorption at 214 nm, concentration of analytes 10 µg/ml).

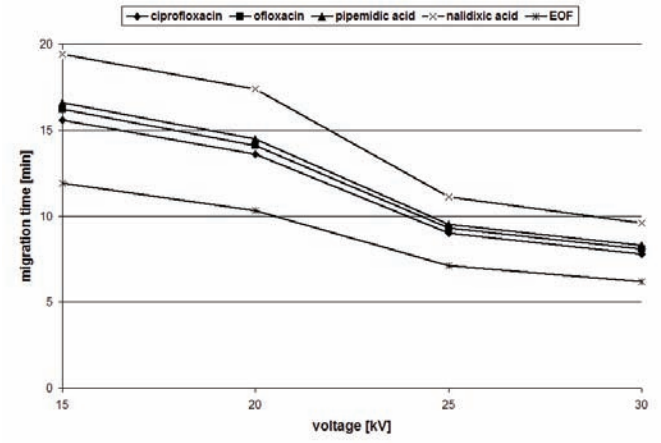

Fig. 5. Variation of the migration times depending on the applied voltage (separation conditions: capillary 60 cm (52 cm) x 50 µm I.D., buffer 40 mM natrium tetraborate + 5% methanol, pH – 9.3, detection: UV absorption at 214 nm, concentration of analytes 10 µg/ml).

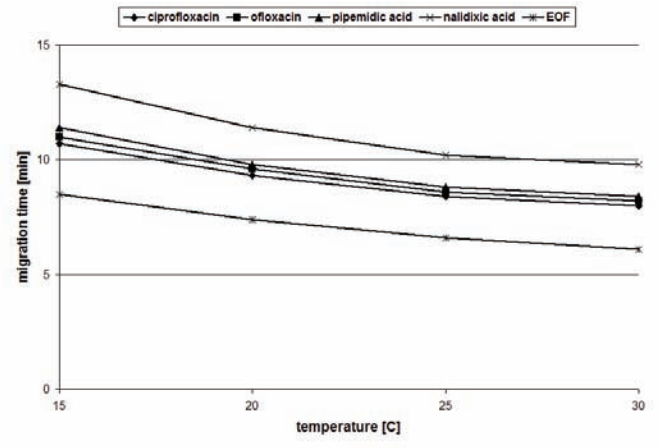

Fig. 6. Variation of the migration times depending on the temperature of the system (separation conditions: capillary 60 cm (52 cm) x 50 µm I.D., buffer 40 mM natrium tetraborate + 5% methanol, pH 9.3, voltage +22 kV, detection: UV absorption at 214 nm, concentration of analytes 500 µg/ml)

tion in the buffer solutions reduced the electroosmotic flow (EOF), consequently increasing migration time but also the resolution of the separation (Figure 3).

Methanol proved to be more efficient than acetonitrile. Also methanol reduced EOF more so than acetonitrile. The optimum methanol concentration was set at 5% methanol in the running buffer. The migration time is also influenced by the pH values. pH values were manipulated by adding boric acid and natrium hydroxide to the buffer solution. The migration time increased with the increase of pH values, but low pH values proved to be ineffective for the separation, the EOF being greatly reduced, due to less silanol ionization of the capillary wall. High pH values increased the EOF, but this can lead to incomplete separation. We obtain an appropriate separation at a pH around 9 (interval pH values 8–10) (Figure 4).

The migration times decreased with the increase of the applied voltage, the limiting factor here being the Joule heating (Figure 5).

The optimal voltage was set at + 22 kV and determined by performing runs at increasing voltages until the deterioration in the separation resolution was observed. Migration times increased with the decrease of the temperature (Figure 6) and slowly decreased with the increase of the injection pressure of the sample.

The use of a longer capillary can result in a better resolution of the separation but also in longer analysis times.

Table I. Analytical parameters of the separation of quinolones (n = 6, c = 25 µg/ml)

Substance	Migration time (min)	RSD migration time (%)	RSD peak area (%)	Electrophoretic mobility (cm^2/kV min)
Ciprofloxacin	7.66	0.085	0.388	- 5.16
Ofloxacin	7.85	0.027	0.432	- 5.59
Pipemidic acid	8.06	0.078	0.151	- 6.02
Nalidixic acid	9.47	0.147	0.175	- 8.76

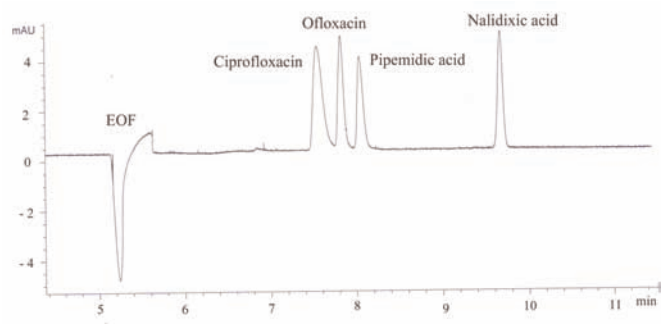

Fig. 7. Electropherogram of the quinolone separation (separation conditions: capillary 60 cm (52 cm) x 50 µm I.D., buffer 40 mM natrium tetraborate + 5% methanol, pH 9.3, voltage +22 kV, detection: UV absortion at 214 nm, concentration of analytes 10 µg/ml).

Discussions

We achieved the best separation using a buffer solution containing 40 mM natrium tetraborate and 5% methanol as buffer additive, at a pH 9.3, with which we separated the four quinolones in less than 10 minutes, the order of separation being: ciprofloxacin, ofloxacin, pipemidic acid, nalidixic acid (Figure 7) [7, 8, 14].

The separation can be explained by taking in consideration the structural characteristics, molecular mass and pKa values of the analytes [9, 11, 12]. The method was evaluated by calculation of standard deviation for migration time and peak area of the components, and also by checking up the linearity of the signal with concentration. Very similar migration times and peak areas were obtained for six repeated measurements of the four quinolones, the RSD values for the migration times and peak areas were smaller than 1% indicating that the precision of the method is good. As it is usual, the precision of migration times was a bit better than that of peak areas (Table I).

We also calculated the own electrophoretic mobility of the analyte which offers a better understanding of the electrophoretic behavior of the analytes. The calibration graphs for the four compounds are showed in figure 8.

We also calculated the individual linear regression equation and the correlation coefficient for each quinolone injecting six solutions with different concentrations in a specific range and three replicates per concentration (Table II).

Conclusions

CZE proved to be an efficient tool in the separation of quinolones from different generations. Even if a separation of fluoroquinolones by CZE is difficult to achieve, the method proved to be useful for the separation of quinolones with different structural characteristics. By manipulating differ-

Table II. Linearity regression data for the four quinolones (concentration range: 10–100 µg/ml)

Substance	Equation	Correlation coefficient
Ciprofloxacin	Y = 0.8167X + 0.1317	0.9954
Ofloxacin	Y = 0.7843X + 0.0415	0.9952
Pipemidic acid	Y = 0.4913X + 0.278	0.9962
Nalidixic acid	Y = 1.0354X + 2.1171	0.9938

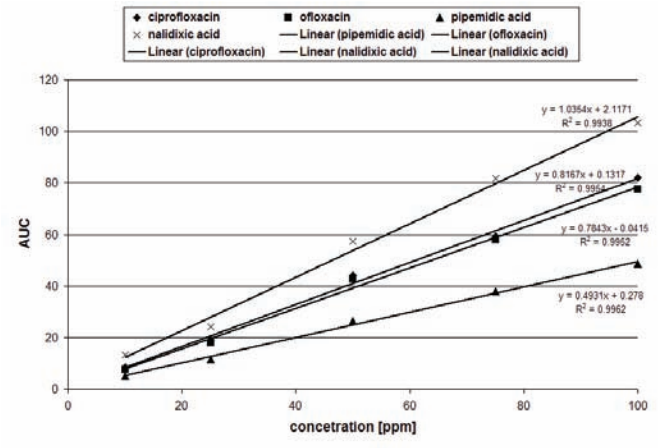

Fig. 8. Calibration graphs

ent analytical parameters we developed and optimize an efficient capillary electrophoresis method for the separation of four quinolones.

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