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Development of a capillary zone electrophoresis–electrospray ionisation tandem mass spectrometry method for the analysis of fluoroquinolone antibiotics

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Abstract

The applicability of a capillary zone electrophoresis–electrospray ionisation tandem mass spectrometric (CZE–ESI-MS–MS) method for the separation of nine fluoroquinolones was investigated. Method optimisation involved systematic trouble-shooting starting with the type and duration of capillary pre-washing and conditioning, the choice of both the CE run buffer, MS sheath liquid, CE run potential, ESI spray voltage, sheath gas flow-rate, MS capillary voltage and CE capillary and MS capillary temperatures. Another extremely important factor was found to be the degree to which the CE capillary protrudes into the ESI chamber as well as whether or not sheath gas and spray voltage are employed during the CE injection or not. The importance of the latter has, to our knowledge, not been addressed elsewhere. Nine fluoroquinolones have been separated and detected in a single run by this technique.

Keywords: Optimisation; Fluoroquinolones; Quinolones; Antibiotics

1. Introduction

Fluoroquinolones (FQs) are a widely used class of antibiotics, whose utilisation in industrial farming has been one of the main contributing factors towards the emergence of resistant bacteria. The European Union have therefore published directives either banning or setting maximum residue limits (MRLs) for these substances in foodstuffs of animal origin [1]. In order to implement these directives, methods which provide indisputable identification and determination of such multiresidues are clearly needed [2].

Much work has been carried out on these FQs in an attempt to fulfill this need by various modes of capillary electrophoresis both with ultraviolet and fluorescence modes of detection [3–8]; liquid chromatography with ultraviolet detection [9–14]; liquid chromatography with fluorescence detection [15–18]; gas chromatography with mass spectrometric detection [19] and liquid chromatography with tandem mass spectrometric (MS–MS) detection [20–27]. However, the combined advantages of capillary zone electrophoresis (CZE) and electrospray ionisation (ESI) MS–MS in terms of CE's high efficiency, high speed and low sample volume required coupled with the high selectivity of MS–MS detection have until now not been used for the multiresidue separation and detection of fluoroquinolones.

The on-line combination of CZE and ESI-MS–MS has several potentially challenging instrumental as-

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pects which complicate the successful union of these two processes. Recent reviews [28,29] have summarised the challenges involved in using the many electromigration schemes (capillary zone electrophoresis, capillary gel electrophoresis, capillary isoelectric focusing, capillary isotachophoresis, micellar electrokinetic chromatography and capillary electrokinetic chromatography) with MS detection as well as their application to biological matrices. The aim here, however, was to develop a method which would provide electrophoretic separation of nine FQs [danofloxacin (DAN), ofloxacin (OFL), marbofloxacin (MAR), enrofloxacin (ENR), enoxacin (ENX), ciprofloxacin (CIP), norfloxacin (NOR), cinoxacin (CIN) and flumequine (FLU)] within a single run, using a simple volatile buffer compatible with ESI-MS–MS within a reasonable total analysis time and providing repeatable results.

2. Experimental

2.1. Instrumentation

Electrophoretic measurements were carried out using a CE Ultra capillary electrophoresis instrument (Thermo Separation Products, Riviera Beach, FL, USA) coupled to an LCQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA). CE data were collected via Thermoquest's PC1000 system software operating under an OS2 environment, resulting in the need to treat ASCII files of raw data further via Excel in a Windows NT environment. MS data were collected and treated via ThermoFinnigan's Xcalibur software (Windows NT operating system). The un-coated fused-silica capillaries were manufactured by Polymicro Technologies (TSP050375 REEL: 06/14/2001 08:16:37, lot: IKG 02A), each 50 μm I.D. capillary coming from the same 100 m lot. Detection windows were prepared by burning off the polyimide coating using a Micro Solve CE capillary window stripper (Scientific Resources, Gloucester, UK), at 34.2 cm (spanning 34.1–34.3 cm) and total lengths, recorded for each separately, were of the order of 90±2 cm. All pH measurements were carried out using a Metrohm 744 pH meter (Herisau, Switzerland).

2.2. Chemicals, reagents and standards

Ultra pure water of >18 MΩ cm^{-1} resistivity and T.O.C. of <5 μg l^{-1} was obtained from a Milli-Q 185 system (Millipore, France), fed by pure water from a Millipore Elix 5 system, the MQ grade being used to make up all solutions. Analytical reagent-grade ammonium formate (>99% purity), ammonium acetate (>99% purity) and ammonium carbonate (>30% NH_3) were obtained from Fluka and used to prepare the various background electrolytes (BGEs) tested. Ultra pure sodium hydroxide (1 *M*, Fluka), hydrochloric acid (20%, Aldrich), formic acid (98% purity, Fluka) and acetic acid (96% purity, Merck) were used for both capillary washing and/or BGE pH adjustment. Buffers for calibration of the pH meter were Metrohm 4, 7 and 9. HPLC grade ethanol (Merck) was used in the sheath liquid composition and HPLC grade methanol (Fluka) was used for FQ dissolution. Bulk nitrogen (>99.9975%), used as the MS sheath gas and pure bottled helium (>99.999%) used as the ion trap collision gas were obtained from Air Liquide (Charleroi, Belgium). FQ standards, norfloxacin, ofloxacin, cinoxacin and flumequine were purchased from Sigma–Aldrich (St. Louis, MO, USA) while enrofloxacin, enoxacin and ciprofloxacin were obtained from Bayer (Leverkusen, Germany), danofloxacin from Pfizer (Groton, CT, USA) and marbofloxacin from Vetoquinol (Aartselaar, Belgium). Quinine (QUIN), used as an internal standard (I.S.), came from Sigma.

2.3. Preparation and storage of standards

Stock solutions of ~100 μg g^{-1} of each FQ and I.S. were prepared by weighing 2 mg FQ and making up to 20 g with pure methanol in 25 ml Duran bottles (previously cleaned with successive 3% Mucasol, pure water and methanol rinsing). All were then placed in an ultrasonic bath (Branson 5510) and sonicated for 10 min. Ciprofloxacin, marbofloxacin and flumequine all needed a further 10 min sonication before being fully dissolved in the methanol. Each FQ was then sub-divided into three clean glass vials, two being stored at −30 °C (3 month usage period), the other being stored at 5 °C for immediate use (2 week usage period). Once removed from

−30 °C the vial contents were then stored at 5 °C and used within 2 weeks. Mixtures of all 9 FQ plus I.S. were prepared by taking 2 ml each giving ~10 μg g^{-1} concentration of each. Measurement mixtures were then prepared by taking 900 μl of the 10 μg g^{-1} mixture and adding 300 μl relevant BGE i.e. 7.5 μg g^{-1} FQs present in 25% BGE. Mixtures of 6 FQs were prepared by taking 500 μl each 100 μg g^{-1} stock and adding 1 ml BGE resulting in 12.5 μg g^{-1} FQs present in 25% BGE. All CE vial contents were sonicated for 1 min before initial measurement.

2.4. Preparation and storage of buffers

The buffers were made up using stock solutions, filtered though 0.45 μm nylon filters and sonicated for 5 min before use. The stock solutions were kept at 5 °C for maximum 1 month. pH adjustments were carried out using either formic or acetic acid. The ammonium formate (AF)–formic acid (FA) buffers were made by taking suitable aliquots of a 200 m*M* AF stock solution and adjusting with FA to achieve the desired pH.

- 10 m*M* AF–477 m*M* FA: 1 ml of 200 m*M* AF+184 μl FA+9 ml pure water; pH 2.55
- 20 m*M* AF–86 m*M* FA: 1.5 ml of 200 m*M* AF+50 μl FA+13.5 ml pure water; pH 3.11
- 20 m*M* AF–84 m*M* FA: 2.0 ml of 200 m*M* AF+65 μl FA+18 ml pure water; pH 3.14

The ammonium acetate (AmAc)–acetic acid (AA) buffer was made by taking a suitable aliquot of a 100 m*M* AmAc stock solution and adjusting with AA to achieve the desired pH.

- 40 m*M* AmAc–1.04 *M* AA: 8 ml of 100 m*M* AmAc+1.25 ml AA+12 ml pure water; pH 3.25

The ammonium carbonate (AC) buffers were made directly as follows:

- 83 m*M* directly i.e. 0.801 g/100 ml pure water; pH 9.13
- 100 m*M* directly i.e. 0.961 g/100 ml pure water; pH 9.20
- 120 m*M* directly i.e. 1.152 g/100 ml pure water; pH 9.12

2.5. Washing, conditioning and drying of CE capillary

If working with a buffer in a low pH region then daily washing, in the un-coupled mode[1], was carried out at 30 °C as follows: 0.1 *M* HCl washed through for 3 min; BGE (special rinsing vial) washed through for 10 min; BGE (front electrode vial) washed through for 2 min. If working with a buffer in a high pH region then the daily washing procedure was the same as for a low pH buffer except that 0.1 *M* NaOH replaced the 0.1 *M* HCl and the relevant BGE was used. Conditioning the CE capillary was carried out at either of two potential ranges as follows, in the un-coupled mode and with the sheath liquid flowing around the CE capillary outlet at 1 μl per min, 27 kV with a 9 s step were applied for 7 min at 30 °C for the low pH buffer and 21 kV/9 s; 7 min at 30 °C for the high pH buffer. Vial (1.2 ml) replenishment of the BGE rinsing vial and the BGE front electrode vial was carried out after every three runs and the capillary was washed for 3 min at the beginning of every run with the BGE. At the end of every day the CE capillary was washed and dried at 30 °C as follows: 0.1 *M* HCl or 0.1 *M* NaOH (depending on pH range of separation) washed through for 3 min at 30 °C, followed by a pure water rinse of 6 min and a final drying step of 3 min. The UV diagnostics of the dry capillary were then recorded, at two wavelengths, for comparison of the day to day variations in the state of the detection window and the sample: reference beam ratios.

3. Results and discussion

Since the purpose of this work was to optimise a CZE–ESI-MS–MS method for the simultaneous analysis of nine fluoroquinolones, each optimisation step will now be described.

3.1. CZE optimisation

There were several constraints in choosing a suitable buffer system as it had not only to provide

[1]Note: in this text the ‘‘uncoupled’’ mode describes the situation where the ESI chamber is open and so the CE system is un-coupled from the MS system, allowing the CE capillary outflow and sheath liquid to go to waste while the ‘‘coupled’’ mode describes the situation where the ESI chamber is closed, the sheath liquid is flowing but the sheath gas, spray voltage and MS acquisition may or may not be applied, depending on programme segment (described in more detail later in text).

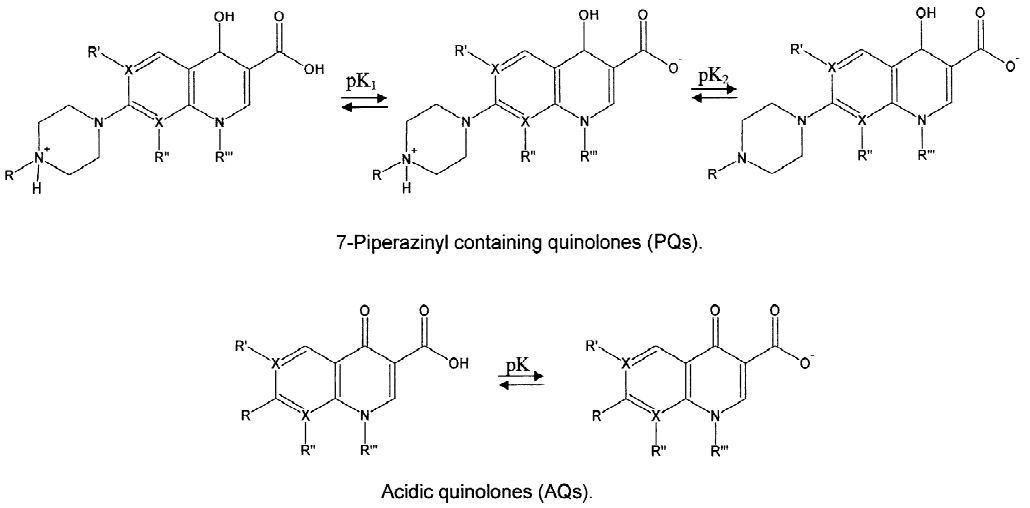

Fig. 1. Acid–base equilibria for the quinolones (re-drawn from J.A. Hernández-Arteseros et al. [20]).

adequate CE separation of the FQs but also to be volatile so as to enhance electrospray ionisation at the ESI-MS interface. As a starting point the pK_a values for nearly all of the FQs were consulted [27] so as to partly determine what buffers might be suitable. Nearly all of the FQs studied here, except flumequine and cinoxacin, have two pK_a values, pK_1 ranging from 5.6 to 6.6 and pK_2 ranging from 7.7 to 8.6. Furthermore, all, except flumequine and cinoxacin, have a piperazinyl group as well as a car-

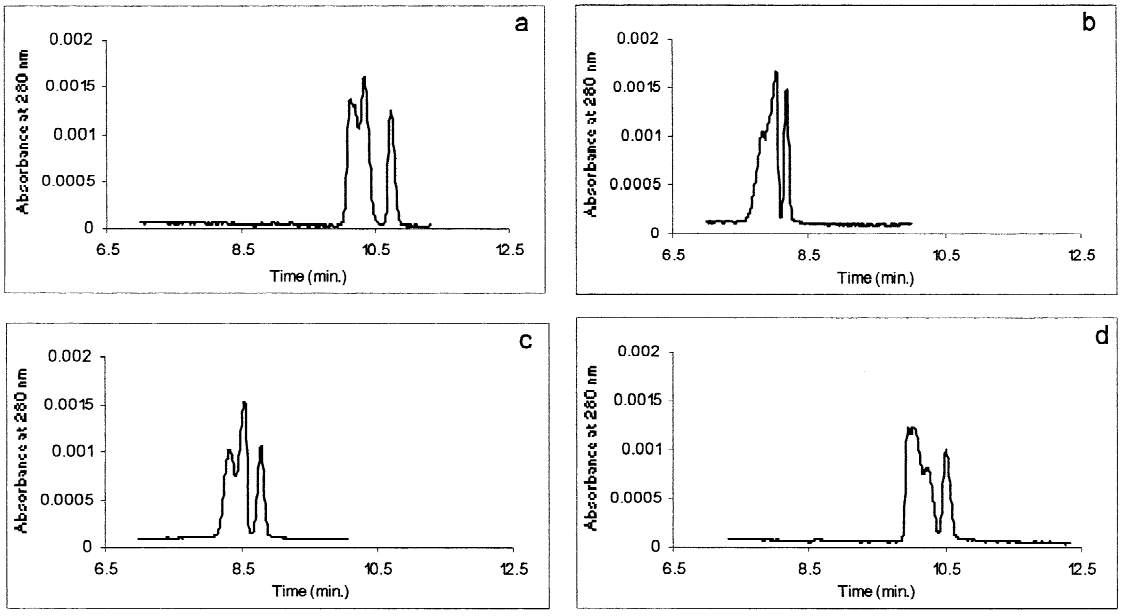

Fig. 2. CZE separation of six FQs (DAN, OFL, MAR, ENR, CIP, NOR) using buffers in the acidic pH region; (a) 10 m*M* AF–477 m*M* FA pH 2.55; (b) 20 m*M* AF–86 m*M* FA pH 3.11; (c) 20 m*M* AF–84 m*M* FA pH 3.14; (d) 40 m*M* AmAc–1.04 *M* AA pH 3.25 (12.5 μg ml^{-1} sample concentration; 10 s*6.89 kPa h.d.i.; 27 kV; 30 °C; uncoupled mode).

boxylic acid group, the piperazinyl moiety also including additional amine groups which thus account for the existence of three different species, cationic, zwitterionic and anionic in aqueous solution while the other two can only be neutral or anionic. For clarity the acid–base equilibria of the piperazinyl containing quinolones (PQs) and the purely acidic quinolones (AQs) are shown in Fig. 1.

It was therefore decided to try two pH ranges, either below pK_1 using ammonium formate–formic acid mixtures ranging in pH from 2.5 to 3.2 or by using an ammonium acetate–acetic acid mixture (pH 3.25) or else above the pK_2 value by using ammonium carbonate as a buffer. The initial stage of the optimisation (low pH buffers) was carried out on mixtures of six FQs, namely, DAN, OFL, MAR, ENR, CIP and NOR while the rest of the optimisation procedure involved mixtures of the nine FQs (six mentioned above and ENX, FLU and CIN). The acidic pH used in both the ammonium formate–formic acid mixtures and the ammonium acetate–acetic acid mixture would have meant that of the six PQs in the standard mixture, the cationic species should be pre-dominant while the basic pH of the ammonium carbonate should have shifted the equilibrium to the right, leaving the anionic species predominant for both the AQs and PQs. As can be seen in Fig. 2, neither the ammonium acetate–acetic acid buffer mixture nor the ammonium formate–formic acid mixtures (pH<3.58) provided reasonable electrophoretic separation possibly due to interactions on the interior capillary wall of the PQ's predominantly cationic form and lack of an electroosmotic flow (EOF). Due to the lack of adequate separation the order of migration is not proposed for these acidic BGEs. However, when changing to the ammonium carbonate buffer systems of pH >9.12, and thus to the anionic form of the FQs (PQs plus AQs), separation of all nine FQs was achieved (Fig. 3). Increasing the ionic strength of the buffer from 83 m*M* to 120 m*M* slowed down the EOF, giving even better resolution but naturally longer migration

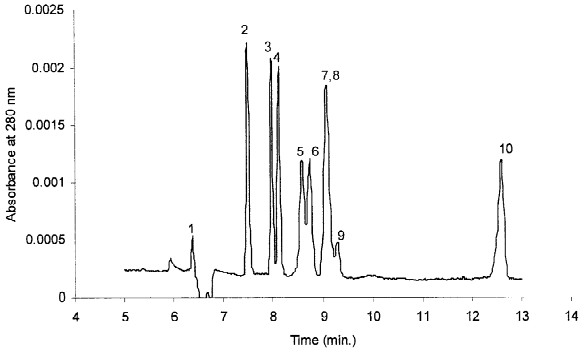

Fig. 3. CZE separation of nine FQs using a buffer in the basic pH region (120 m*M* ammonium carbonate pH 9.12, 7.5 μg ml^{-1} sample concentration in CHR.4.18; BGE; 10 s*6.89 kPa h.d.i.; 21 kV; 30 °C; uncoupled mode). Peaks labelled as follows: QUIN as 1; DAN as 2; OFL as 3; MAR as 4; ENR as 5; ENX as 6; CIP as 7; NOR as 8; CIN as 9 and FLU as 10.

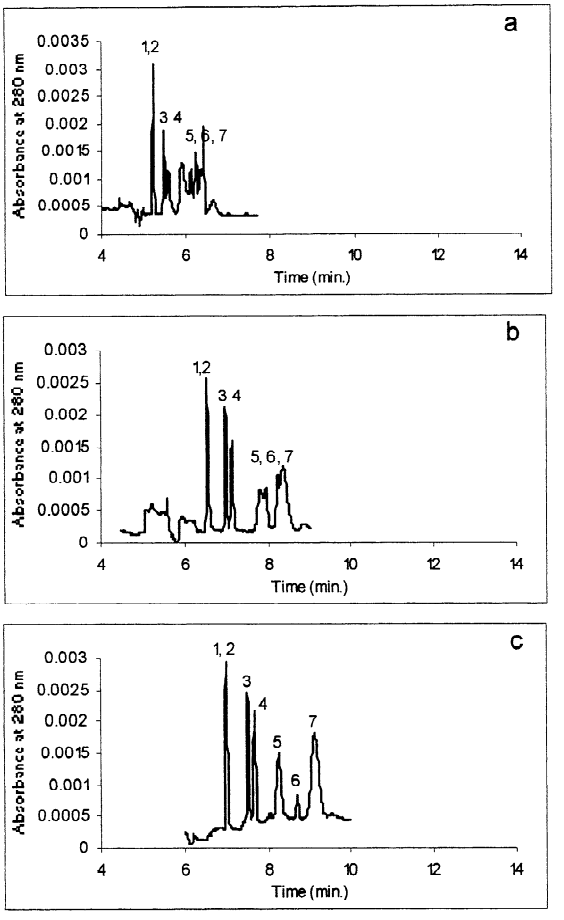

Fig. 4. Effect of varying the ammonium carbonate buffer concentration on the CZE–ESI-MS separation of six FQs and I.S.; (a) 83 m*M*; (b) 100 m*M*; (c) 120 m*M*. The order of migration is QUIN 1, DAN 2, OFL 3, MAR 4, ENR 5, CIP 6, NOR 7 for a 10.72 μg ml^{-1} sample concentration in 1/4 BGE; 10 s*6.89 kPa h.d.i.; 27 kV; 30 °C; coupled mode.

times (Fig. 4). At this high ionic strength, undesirably high currents causing joule heating were encountered at potentials of >24 kV (coupled mode). The optimised run potential for the 120 m*M* ammonium carbonate buffer was thus found to be 23 kV in the coupled mode (Fig. 5) and 21 kV in the uncoupled mode, the difference in applied potential being due to the effect of the spray voltage in the coupled mode. Therefore in order to achieve similar run currents in the coupled and uncoupled modes, an extra 2–3 kV has to be applied across the CE capillary to compensate for the 4 kV spray voltage at the ESI interface. Temperatures lower than 30 °C were found to increase migration times and deleteriously affect analyte peak resolution and so 30 °C was taken as optimum. Optimisation of both the injection duration and pressure, using the hydrodynamic mode of injection (h.d.i.), resulted in 10 s at 6.89 kPa being chosen.

3.2. ESI-MS optimisation

Individual tune files for each of the FQs were optimised via direct infusion at 10 μl min^{-1}. A tune file to suit all FQs was then composed and tested at a sheath gas of 6.3 l h^{-1} (7 units), to mimic the CE–MS situation. MS–MS on all individual FQs was then carried out to establish the necessary collision energy for characteristic fragmentation patterns—found to be 35%. The LCQ MS relies upon a spray cone of a triple coaxial design where the CE capillary is allowed to protrude slightly from the stainless steel tip of the cone, at which point the sheath gas and sheath liquid all mix. The MS instrument manufacturers also provide an option of reducing the distance between the spray and the heated capillary, which when positioned in the closest way leaves 1.5 cm to the capillary inlet, as opposed to 2.5 cm in direct infusion mode, thus greatly increasing the amount of ions entering the MS capillary in the CE–MS mode. Getting the right balance between capillary tip protrusion, found here to be optimal at 0.25 mm, sheath gas flow, found here to be optimal at 6.3 l h^{-1} and sheath liquid composition and flow-rate is crucial to successful CE–MS measurements. So as to reduce the amount of unnecessary dilution of the CE out-flow, the flow-rate of the sheath liquid (SL) was kept to a

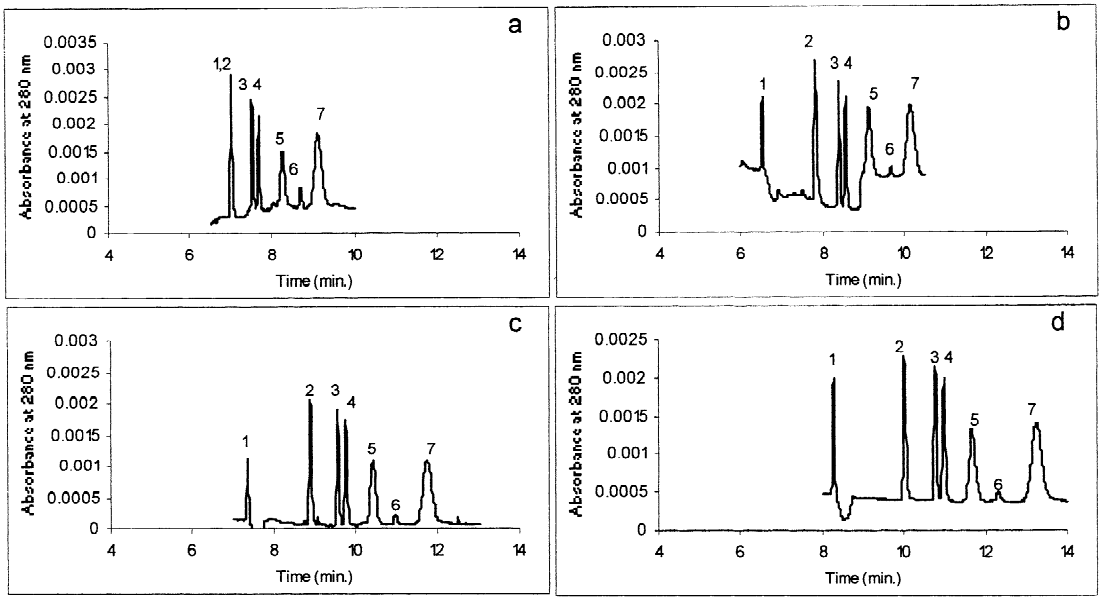

Fig. 5. Effect of varying the CE capillary potential on the CZE–ESI-MS separation of six FQs and I.S.: (a) 27 kV; (b) 25 kV; (c) 23 kV and (d) 21 kV. The order of migration is QUIN 1; DAN 2, OFL 3, MAR 4, ENR 5, CIP 6, NOR 7 for a 10.72 μg ml^{-1} sample concentration in 1/4 BGE; 10 s*6.89 kPa h.d.i.; 120 m*M* ammonium carbonate, pH 9.12; 30 °C; coupled mode.

minimum, found to be 1 μl min^{-1}. Matching the SL with the CE BGE was not found to be necessary due to the vast difference in flow-rates i.e. the flow-rate from the capillary during a run is of the order of a few nl min^{-1} while the sheath liquid flow-rate, even when set to its minimum, accounts for a considerable dilution of the BGE/sample. Therefore a SL of ethanol–pure water–formic acid (60:39:1) (degassed for 5 min before filling 500 μl Hamilton syringe) was used and provided good ionisation of the FQs. Varying the spray voltage on the electrospray needle was also found to seriously affect the spray current and stable currents were found at a potential setting of +4 kV. The MS capillary temperature was held at 275 °C throughout (previously optimised) and the potential applied on this capillary was 16 V (compromise setting obtained from the various individual FQ tune files).

CZE–ESI-MS data acquisition was carried out in full scan mode from 220 to 400 m/z (centroid mode) using a maximum injection time of 200 ms and performing 3 microscans. The CZE–ESI-MS–MS data acquisition was performed in the full scan MS–MS mode, in the 90–400 m/z region using a maximum injection time of 400 ms and performing 2 microscans. A collision energy of 35%, using helium as collision gas, was used for all FQs and the isolation widths varied between 0.8 and 1 m/z.

3.3. CZE–ESI-MS–MS optimised conditions

An important factor which seriously affects the injection in CE–multiple MS (MS^n) runs and until now, not mentioned in the literature, is that if the application of spray voltage and sheath gas are not delayed until after the CE injection has taken place, then the injection fails entirely (Fig. 6). Taking this into account allows completely automatic CE–MS^n runs to be carried out as long as the MS programme is split into two segments, the first segment lasting long enough (see Table 1) for the CE injection to have taken place and during which the spray voltage and sheath gas are set to zero, followed by the second segment when optimal spray voltage and sheath gas settings are used to enhance the FQ ionisation process at the interface. Another aspect that can be problematic while working in an automatic way with this coupled system is the build up of

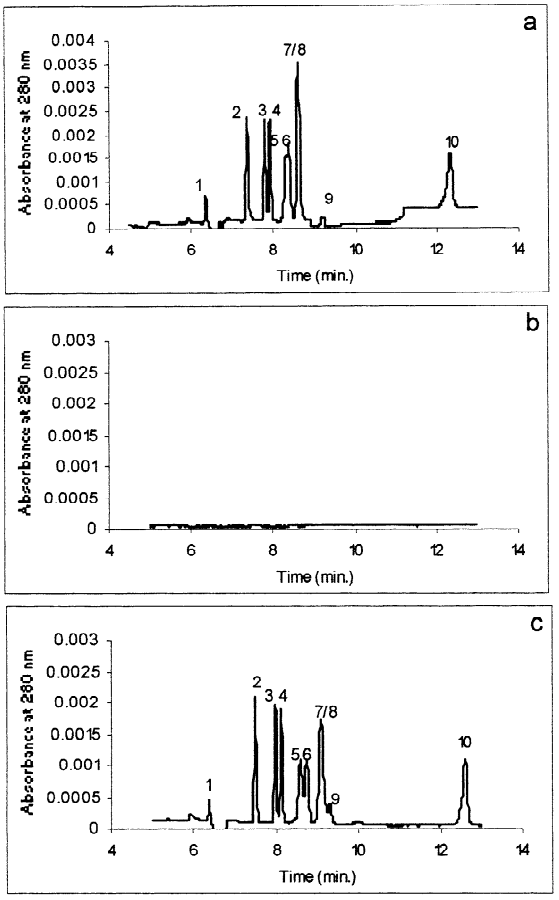

Fig. 6. Effect of applying spray voltage and sheath gas in the ESI chamber **after** the CE injection phase: (a) uncoupled run at 19 kV; (b) coupled run at 21 kV with spray voltage and sheath gas employed throughout and (c) coupled run at 21 kV with delay in employment of spray voltage and sheath gas until after the CE injection had taken place. The order of migration is QUIN 1; DAN 2; OFL 3; MAR 4; ENR 5; ENX 6; CIP 7; NOR 8; CIN 9 and FLU 10 for a 7.5 μg ml^{-1} FQ and I.S. mixture in 1/4 BGE; 10 s*6.89 kPa h.d.i.; 120 m*M* ammonium carbonate, pH 9.12; 30 °C.

droplets on the tip of the CE capillary which cannot be avoided during the pre-washing stage of the run. So as to avoid a surge into the ion trap of the ionised components of this build-up, a 2 min delay before MS data acquisition begins is incorporated. This remedy has, to our knowledge, not been published before.

Using the optimised conditions, summarised in Table 1, a full separation and identification of all

Table 1
Summary of the optimum conditions found for the separation and detection of 9 FQs by CZE–ESI-MS–MS (further details in text)

CZE	ESI-MS–MS	
• Untreated fused-silica, 90.03 cm (effective length 34.2 cm)×50 μm I.D. • Buffer: 120 m*M* ammonium carbonate, pH 9.12, filtered through 0.45 μm nylon filters and sonicated for 5 min. • Programme for coupled operation: 30 °C, 3 min. BGE pre-wash; 10 s, 6.89 kPa h.d.i.; 38 min run at 21 kV.	• Sheath liquid: 60% EtOH, 39% pure water, 1% formic acid,1 μl min^{-1}. • CE capillary tip protrusion of 0.25 mm. • Programme divided into two segments, the first applied just before and during the CE injection, the second applied immediately after and maintained throughout the rest of the run.	
	Segment One • Duration 2 min. • Capillary temperature 275 °C. • 0 kV spray voltage (pos. mode). • 0 units sheath gas flow. • 16 V capillary voltage. • No acquisition	*Segment Two* •Duration 36 min. •Capillary temperature 275 °C. • 4 kV spray voltage (pos. mode). • 7 units sheath gas flow. • 16 V capillary voltage. • 11 Scan events (first delayed by 2 min). • Full MS 220–400 *m/z* • 10 MS–MS events at 35% collision energy.

nine FQs was possible (Fig. 7). Table 2 shows the predominant daughter ions found for each FQ and their relative abundance. It is this kind of data which can provide indisputable identification of such compounds when found in biological matrices.

3.4. Repeatability

The repeatability (see Table 3 and Fig. 8) of the method was tested within-day for the CZE UV data (migration time, peak areas and peak heights) and the

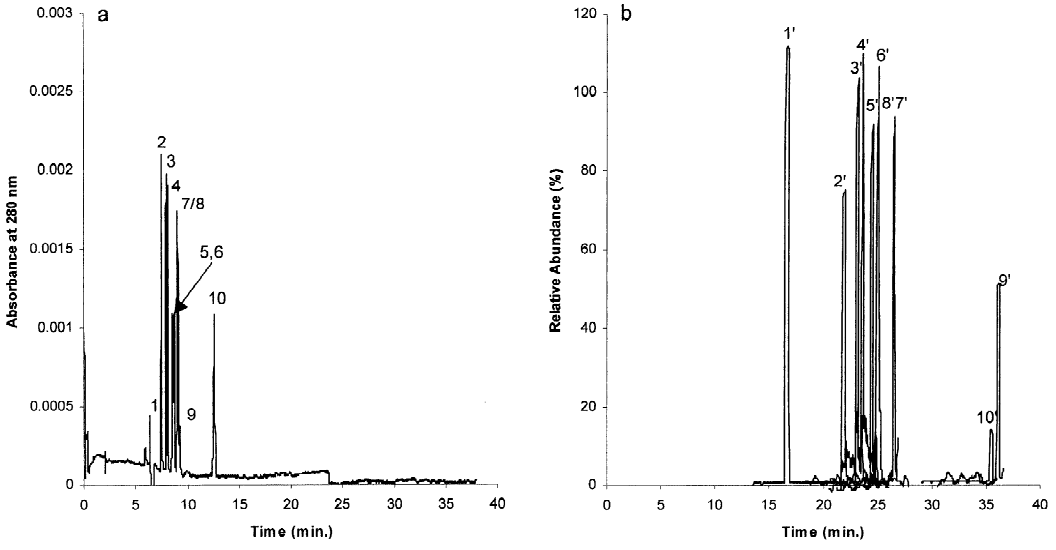

Fig. 7. Electropherogram (a) and corresponding MS signal intensity (b) for 9 FQs and quinine (I.S.). 7.5 μg ml^{-1} standard mixture in 1/4 BGE; 10 s*6.89 kPa h.d.i.; 120 m*M* ammonium carbonate, pH 9.12; 21 kV; coupled mode. Peaks labelled as follows: QUIN as 1,1′; DAN as 2,2′; OFL as 3,3′; MAR as 4,4′; ENR as 5,5′; ENX as 6,6′; CIP as 7,7′; NOR as 8,8′; CIN as 9,9′ and FLU as 10,10′.

Table 2
Parent and product ions of the FQs and I.S.

Name	Migration time and ion detection times (min) RSD<1%, $n=3$	MS spectra: parent ions m/z (% relative abundance)	MS^n spectra: product ions m/z (% relative abundance)
Quinine (QUIN)	6.42 and 16.82	325.3 (100)	307.2 (100); 160.2 (20)
Danofloxacin (DAN)	7.53 and 22.58	358.2 (100); 340.3 (5)	340.2 (95); 314.2 (25)
Ofloxacin (OFL)	8.02 and 23.96	362.20 (100); 344.3 (5)	318.2 (100)→261.2 (100)
Marbofloxacin (MAR)	8.16 and 24.05	363.20 (100); 345.3 (5)	345.1 (100); 320.0 (60)
Enrofloxacin (ENR)	8.61 and 25.37	360.20 (100); 342.4 (12)	316.2 (100)→245.2 (100)
Enoxacin (ENX)	8.75 and 26.06	321.13 (100); 303.3 (10)	303.2 (100)→275.1 (70)
Ciprofloxacin (CIP)	9.06 and 27.09	332.20 (100); 314.3 (15)	288.2 (35)→245.2 (50)
Norfloxacin (NOR)	9.06 and 27.07	320.10 (100); 302.3 (8)	276.2 (75)→233.2 (45)
Cinoxacin (CIN)	9.40 and 35.96	263.12 (50); 285.1 (100)	245.1 (100); 217.3 (50)
Flumequine (FLU)	12.78 and 35.87	262.13 (100); 244.1 (20)	244.1 (100); 202.0 (20)

Note: In the MS^n spectra column a semicolon between m/z indicates two product ions as a result of MS^2 of the parent ion, while an arrow indicates that MS^3 on the MS^2 product ion was necessary to obtain useful and characteristic transitions.

MS data (ion detection time). Migration time and MS ion detection time precision ($n=3$) was <1% for nearly all FQs and peak area and peak height precisions ($n=3$) were less than 5% excluding Quinine and Cinoxacin (low UV signal).

4. Conclusions

The coupling of CZE to ESI-MS–MS has been successfully optimised to allow the separation, using 120 m*M* ammonium carbonate (pH of 9.12) and identification of nine fluoroquinolones within a single run. Successful measurements strongly depend on delaying the employment of sheath gas and spray voltage until after the CE injection as well as delaying MS acquisition by some minutes after the sheath gas and spray voltage have been applied to avoid a surge into the ion trap. Under these conditions reproducible measurements can be carried out. Furthermore the fragmentation patterns achieved provide indisputable identification for antibiotics

Table 3
Migration (UV det.) time (min), MS ion detection (MS det.) time (min) and peak area (UV) data for the 7.5 μg ml^{-1} FQ standard mixture in 1/4 BGE; 10 s*6.89 kPa h.d.i.; 120 m*M* ammonium carbonate, pH 9.12; 23 kV 30 °C; coupled mode

	QUIN (I.S.)	DAN	OFL	MAR	ENR	ENX	CIP/NOR	CIN	FLU
UV det. ($n=3$)	6.424	7.530	8.020	8.162	8.607	8.750	9.056	9.401	12.780
SD	0.048	0.046	0.040	0.038	0.021	0.011	0.022	0.102	0.165
RSD (%)	0.75	0.61	0.50	0.47	0.25	0.12	0.25	1.08	1.29
MS det. ($n=3$)	16.82	22.58	23.96	24.05	25.37	26.06	27.09/27.07	35.96	35.87
SD	0.325	0.127	0.190	0.104	0.313	0.098	0.031/0.031	0.300	0.359
RSD (%)	1.93	0.56	0.79	0.43	1.24	0.38	0.11/0.11	0.83	1.00
Area (UV) ($n=3$)	1260	7441	6947	6994	6876	5840	13 360	1364	8902
SD	334	216	478	544	457	98	275	291	314
RSD (%)	26.53	2.91	6.89	7.78	6.64	1.68	2.06	21.34	3.53

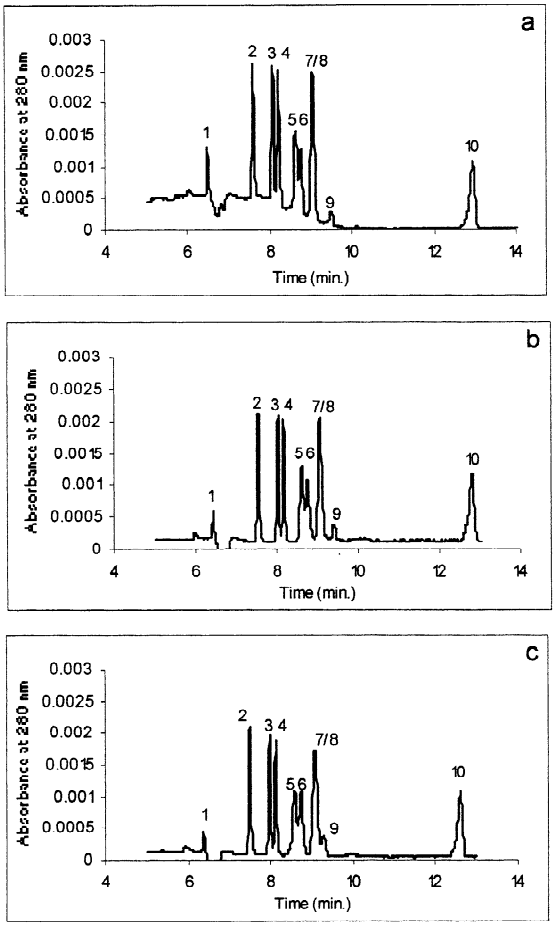

Fig. 8. Three repeat (a, b, c) measurements (same and unreplenished BGE vial) of 7.5 μg ml^{-1} FQ and I.S. mixture (QUIN as 1; DAN as 2; OFL as 3; MAR as 4; ENR as 5; ENX as 6; CIP as 7; NOR as 8; CIN as 9 and FLU as 10.) in 1/4 BGE; 10 s, 6.89 kPa h.d.i.; 120 m*M* ammonium carbonate, pH 9.12; 23 kV; 30 °C; coupled mode.

such as these with legally restricted residue amounts. The method therefore could be very promising for its application to banned or limited fluoroquinolones in foodstuffs of animal origin. Further development of this method in terms of achieving best detection and quantification limits for the FQs will now be undertaken as well as optimising its application to bovine, porcine and poultry samples such as urine, liver, kidney, etc.

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References

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