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Research Article

Highly sensitive detection of five typical fluoroquinolones in low-fat milk by field-enhanced sample injection-based CE in bubble cell capillary

Fluoroquinolones are a group of synthetic antibiotics with a broad activity spectrum against mycoplasma, Gram-positive, and Gram-negative bacteria. Due to the extensive use of fluoroquinolones in farming and veterinary science, there is a constant need in the analytical methods able to efficiently monitor their residues in food products of animal origin, regulated by Commission Regulation (European Union) no. 37/2010. Herein, field-enhanced sample injection for sample stacking prior the CZE separation was developed inside a bubble cell capillary for highly sensitive detection of five typical fluoroquinolones in bovine milk. Ethylenediamine was proposed as the main component of BGE for the antibiotics separation. The effect of BGE composition, injection parameters, and water plug length on the field-enhanced sample injection-based CE with UV detection was investigated. Under the optimized conditions, described field-enhanced sample injection-based CE-UV analysis of fluoroquinolones provides LODs varying from 0.4 to 1.3 ng/mL. These LOD values are much lower (from 460 to 1500 times) than those obtained by a conventional CE in a standard capillary without bubble cell. The developed method was finally applied for the analysis of fluoroquinolones in low-fat milk from a Swiss supermarket. Sample recovery values from 93.6 to 106.0% for different fluoroquinolones, and LODs from 0.7 to 2.5 μg/kg, were achieved. Moreover, the proposed ethylenediamine-based BGE as volatile and compatible with MS system, enabled the coupling of the field-enhanced sample injection-based CE with a recently introduced electrostatic spray ionization MS via an iontophoretic fraction collection interface for qualitative fluoroquinolones identification.

Keywords:
Bubble cell capillary / CE-electrostatic spray ionization MS / Field-enhanced sample injection / Fluoroquinolones / Milk DOI 10.1002/elps.201400294

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1 Introduction

Fluoroquinolones (FQs) are a group of synthetic antibiotics derived from quinolone nalidixic acid by introduction of a fluorine atom at position 6 and a piperazine moiety at position 7 (Table 1). Since the first introduction of FQs for therapeutic treatment of respiratory and urinary infections in livestock in the mid-1990s [1], they have become the most commonly prescribed antibiotics for food-producing animals because of their broad activity spectrum against mycoplasma and bacteria (both Gram positive and negative) [2]. The extensive utilization of antibiotics has inevitably caused the accumulation of their residues in food, which can lead to the emergence of drug-resistant bacteria and a potential health hazard for humans [3]. Therefore, European Union has fixed a maximum residue limit (MRL) of some FQs in food products of animal origin including bovine milk. Although there is no defined

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Abbreviations: **CIP**, ciprofloxacin; **EDA**, ethylenediamine; **ENR**, enrofloxacin; **ESTASI-MS**, electrostatic spray ionization MS; **FESI**, field-enhanced sample injection; **FLE**, fleroxacin; **FQ**, fluoroquinolone; **LOM**, lomefloxacin; **MRL**, maximum residue limits; **OFL**, ofloxacin; **SEF**, signal enhancement factor; **t-ITP**, transient isotachophoretic stacking

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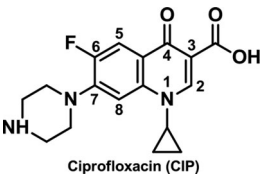
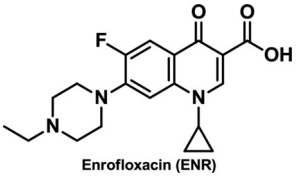
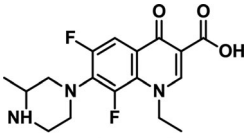
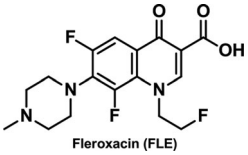
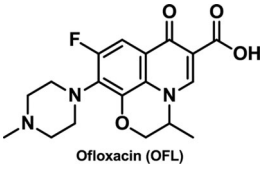
Colour Online: See the article online to view Figs. 1–4 in colour.

Table 1. Molecular structures, M_r, and dissociation constants (pK_a) of the FQs

FQs	M_r	pK_a 1	pK_a 2	Refs.
 Ciprofloxacin (CIP)	331.34	5.90	8.89	[5]
 Enrofloxacin (ENR)	359.39	6.32	8.62	[5]
 Lomefloxacin (LOM)	351.35	5.82	9.30	[6]
 Fleroxacin (FLE)	369.34	5.46	8.00	[7]
 Ofloxacin (OFL)	361.37	5.97	8.28	[5]

MRL for lomefloxacin (LOM), fleroxacin (FLE), and ofloxacin (OFL), the permissible total amount of enrofloxacin (ENR) and its metabolite ciprofloxacin (CIP) is required to be lower than 100 μg/kg [4]. It is necessary to develop analytical methods that are sensitive enough to monitor and determine these antibiotics at such a low level in bovine milk.

Different strategies have been carried out to monitor residual levels of FQs in bovine milk including immunoassays [8–10], HPLC with optical [11–13] or MS [3, 14] detection, and CE with electrochemical [15], UV [16, 17], or MS [18, 19] detection. Owing to its limitation in sensitivity, CE is not so commonly used as HPLC in the field of multiresidue separation and detection. Therefore, it is important to overcome this limitation of CE because of its advantages like high speed, high efficiency, and low sample consumption.

The limited sensitivity of CE results from its lower loading capacity and shorter detection path length in contrast with those of HPLC. To overcome the issue of loading capacity, various strategies for sample preconcentration have been widely studied. Sample preconcentration can be generally classified into offline [20, 21] and online [22, 23] modes. The online sample preconcentration is considered to be more applicable since it can be conveniently accomplished by manipulations of the experimental parameters before CE separation. Generally, online preconcentration can be realized by either SPE [22, 24] or electrophoretic stacking. Electrophoretic stacking is based on the electrophoretic velocity changes of analytes when they encounter a change of strength in the electrical field at the boundary between the sample matrix and BGE zones. Different approaches for electrophoretic-based online sample preconcentration were developed since it was first proposed in 1992 [25], namely field-amplified sample stacking [26, 27], field-enhanced sample injection (FESI) [23, 28], large-volume sample stacking (LVSS) [29], and transient isotachophoretic stacking (t-ITP) [30, 31]. In general, FESI is suitable for the samples with low conductivity and t-ITP is used for the samples with high conductivity [23].

Extending the length of optical detection path by employing a bubble cell [27, 32, 33] or a longitudinal cell [34, 35] capillary is another way to improve the sensitivity of CE with UV detection. Longitudinal cells, usually prepared by bending of a small section of the capillary column into a Z-shaped flow cell, with a path length of up to a few millimeters parallel to the optical detection, can produce enhanced sensitivity [34], but the loss in resolution prevents this construction from further development. With the bubble cell capillaries, which are commercially available now, an improvement of detection sensitivity based on increasing the id 3–5 times can be obtained without greatly decreased resolution [27].

In this study, we demonstrated a FESI method to enhance the detection sensitivity of five different FQs, including CIP, ENR, LOM, FLE, and OFL, in semi-skimmed bovine milk using a bubble cell capillary. A new effective ethylenediamine (EDA)-based BGE compatible with MS detection was proposed. After optimization of the experimental conditions required for FESI stacking, we coupled FESI-CE with the recently reported electrostatic spray ionization MS (ESTASI-MS) [36]. The CE fractions were spotted on an insulating plate through a homemade robotic system-based iontophoretic fraction collection device [37] for MS identification. At the end, we applied this method to analyze FQs in bovine milk, obtaining satisfying recovery values and LODs.

2 Materials and methods

2.1 Materials

CIP, ENR, LOM, FLE were purchased from TCI Deutschland (Eschborn, Germany), and OFL from Sigma-Aldrich (Schnelldorf, Switzerland). All other chemicals used, like formic acid, acetic acid, EDA, were analytical reagent grade and obtained from Fluka (Buchs, Switzerland) unless specified. All buffers were prepared with deionized water produced by an alpha-Q system (Millipore, Zug, Switzerland).

Standard antibiotic solutions of CIP, ENR, LOM, FLE, and OFL were prepared in 10 mM NaOH with a concentration

of 1 mg/mL for each compound, and diluted with deionized water before use.

2.2 Apparatus

The FESI-CE experiments were carried out with a 7100 A CE apparatus (Agilent, Waldbronn, Germany) equipped with a DAD, and the detection wavelengths were chosen as 270 nm (the wavelength with maximum absorption for CIP and ENR [16]) and 280 nm (the wavelength with maximum absorption for LOM, OFL, and FLE [38]). Unless specified, all electropherograms were shown with a detection wavelength of 280 nm, and the quantitation of LOM, OFL, and FLE was based on the absorbance at 280 nm, while quantitation of CIP and ENR was based on the absorbance at 270 nm. Fused silica capillaries (50/375 μm id/od, 40/48.5 cm effective/total length) were obtained from BGB analytik AG (Böckten, Switzerland), and Extended Light Path (bubble cell) Bare Fused-Silica Capillaries (50/375 μm id/od, 40/48.5 cm effective/total length, bubble cell diameter of 150 μm) were kindly offered by Agilent Technologies (Waldbronn, Germany).

ESTASI-MS experiments were carried out using a linear ion trap mass spectrometer (Thermo LTQ Velos, Thermo Fisher Scientic, San Jose, USA). The commercial ESI source was replaced by a home-designed electrostatic spray ionization system, which is described in details elsewhere [36]. A pulsed square wave high voltage was produced by amplifying signals from a function generator (TG513, Thurlby Thandar Instruments, Huntingdon, England) with a high voltage amplifier (10 HVA24-P1, HVP High Voltage Products, Martinsried/Planegg, Germany) to induce ESTASI.

2.3 FESI-CE with UV detection

Before the first use, the capillaries were conditioned by flushing with 1 M NaOH for 30 min and subsequently with deionized water for 15 min. A 40 mM EDA (pH 8.0, adjusted by formic acid) was used as BGE. To ensure the run-to-run reproducibility, each separation was preceded by flushing with 0.2 M NaOH for 1 min, followed by deionized water for 1.5 min and BGE for 2 min. Standard antibiotic mixtures were diluted to required concentrations with deionized water. The procedure of FESI-CE analysis is described below. First, the capillary was prefilled with BGE. Then a plug of deionized water was injected by a low pressure (40 mbar) for 50 s. After that, a negative high voltage (–8 kV) was applied for 4 min to introduce sample into the capillary. With the sample injected and stacked, a positive separation voltage (30 kV) was applied to separate the antibiotic samples during 5–10 min depending on the experimental conditions.

2.4 FESI-CE coupling with ESTASI-MS

The coupling of FESI-CE with ESTASI-MS was realized through a homemade automated fraction collection interface [36]. About 10 cm of the 48.5 cm-long capillary was first painted with silver ink from Ercon (Wareham, MA, USA) at one end of the standard capillary, and then heated at 90°C for 60 min. The capillary was placed in a CE-MS cassette with its painted extremity left outside the CE apparatus and fixed in a ceramic holder. This holder is an integrated part of the homemade robotic system capable to move in all three axes above the insulating plate positioned on the stage. The movements of the capillary and fraction collection procedure were controlled via a Labview program (National Instruments, Austin, TX). During the FESI-CE for concentration and separation, the painted end of the capillary was always grounded and dipped into a droplet (5 μL) of BGE that was previously deposited on the collection plate. The plate was a polyethylene terephthalate covered with a layer of paraffin film (Parafilm®, Pechiney Plastic Packaging Company, Chicago, USA), which has a highly hydrophobic surface advantageous in keeping the current stable during direct CE fraction collection and avoiding sample dispersion during the droplet drying under ambient conditions in dark.

Before ESTASI-MS analysis, 1 μL of ionization solution (50% methanol, 1% acetic acid) was spotted above each droplet to dissolve the dried sample. The droplet on the insulating plated was placed between the ground MS inlet (ion transfer capillary) and an electrode connected to the pulsed square wave high voltage source. Ions produced by ESTASI were then analyzed by the ion trap mass spectrometer.

2.5 Milk sample pretreatment

UHT milk with 1.5% fat was purchased from a local supermarket. Before FESI-CE analysis, the milk samples were treated as follows. 0.5 g aliquot of milk sample (spiked with 2.5 μL FQs at a concentration of 20 μg/mL, 15 μg/mL, 10 μg/mL, 5 μg/mL, 2 μg/mL, or 1 μg/mL to make the milk sample containing 100 μg/kg, 75 μg/kg, 50 μg/kg, 25 μg/kg, 10 μg/kg, or 5 μg/kg FQs, respectively) was shaken with 0.5 mL of saturated ammonium sulfate (pH 7.4, adjusted by ammonia) and centrifuged in a MiniSpin centrifuge (Eppendorf, Germany) at 13 000 rpm for 2 min to remove milk proteins [39]. The supernatant was collected, while the precipitate was mixed with another 0.5 mL of saturated ammonium sulfate, shaken, and centrifuged again. The collected and combined supernatant was then filtered through a 0.45 μm cellulose nitrate membrane filters (Sartorius, Göttingen, Germany). Subsequently, the aqueous solution was purified using a Pierce C18 spin column (Thermo Scientific, Rockford, USA) to remove the salts. To ensure a rapid evaporation of solvent, the compounds remaining in the column resin were eluted by pure methanol (50 μL for three times) instead of a recommended elution solution composed of 70% methanol and 30% deionized water. The effluent was dried in a flow of

nitrogen and redissolved in 0.5 mL diluted NaOH (1 μM) to make the analytes negatively charged for the following FESI stacking.

3 Results and discussion

3.1 Effect of ion strength of BGE on FESI stacking

The effect of BGE on FESI-CE for FQs analysis was firstly studied since the properties of BGE, including constituent, pH and concentration, have crucial impacts on efficiency, resolution, and sensitivity of FESI-CE.

As reported in the literature, basic BGE is usually selected for FQs separation [16, 17]. Considering its compatibility with MS, the BGE should be composed of volatile salts, for example ammonium carbonate [40]. However, the chosen FQs cannot be separated completely by using ammonium carbonate (data not shown). BGE based on EDA with pH 8.0 adjusted by formic acid resulted in a good separation resolution for the considered five FQs. Moreover, no suppression in ESTASI-MS signal of FQs was found when a mixture of EDA and formic acid was used for direct ESTASI-MS analysis of chosen antibiotics, meaning that this BGE was compatible with ESTASI-MS. Hence, BGE containing EDA and formic acid at pH 8.0 was used for the subsequent optimization.

To the best of our knowledge, if the Joule heating remains negligible, increasing of the BGE ionic strength usually leads to higher sample preconcentration ability of FESI, which is based on the fact that each given analyte displays very different velocities in the high-conductivity BGE zone and low-conductivity sample zone. To be more precise, the local electric field existing in the low-conductivity sample zone is much higher than in the BGE, which determines the following phenomenon: the analytes move rapidly from the sample vial to the inlet of the capillary and slow down once they reach the high-conductivity BGE zone. As a consequence, a stacking process happens at the boundary of sample matrix and BGE. From this point of view, the sample stacking efficiency increases with the increase of BGE ionic strength. In our study, EDA (pH 8.0) solutions in deionized water at concentrations from 20 to 60 mM were tested providing the results shown in Fig. 1. By increasing the BGE concentration from 20 mM to 40 mM, improvements of separation resolution and stacking ability were both observed. At ionic strengths higher than 40 mM, no further increase in FESI stacking ability occurred supposedly because of peak broadening resulted from an excessive Joule heating. As result, 40 mM ionic strength EDA buffer at pH 8.0 was thus used as an optimized BGE for the following analyses. It is worth mentioning that even the BGE containing only 20 mM of EDA (Fig. 1A) could produce signal enhancement factors (SEF, peak height achieved by FESI divided by peak height obtained with conventional CZE without any preconcentration step (e.g. Fig. 1F), and the quotient multiplied by the dilution factor) [41] from 50 to 100, showing the importance of FESI stacking.

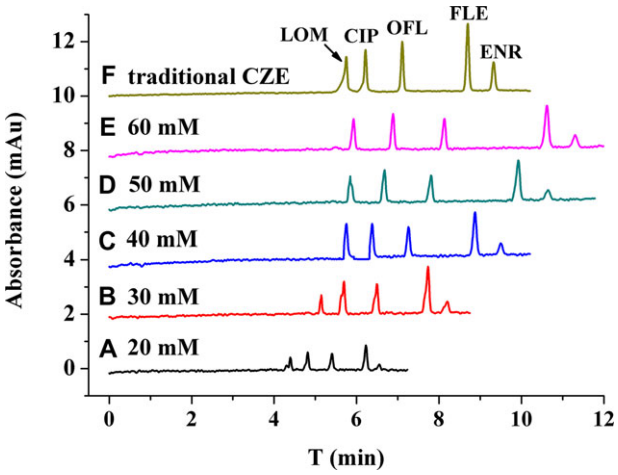


Figure 1. (A)–(E) Effect of BGE concentration ranging from 20 to 60 mM on the FESI stacking and CZE separation. (F) Conventional CZE without any sample stacking. FESI-CZE conditions: a standard capillary with total/effective length of 48.5/40 cm and id/od of 50/365 μm for separation, water plug injected by a low pressure of 40 mbar for 50 s, sample mixture of five FQs at a concentration of 25 ng/mL for each compound was injected by a high voltage of –8 kV for 4 min, separated at 30 kV and detected at 280 nm. Conventional CZE conditions: sample of FQs at a concentration of 5 μg/mL and injected by a pressure of 35 mbar for 40 s, others same with those of FESI-CE.

3.2 Optimization of FESI parameters

The amount of FQs injected into the capillary and the efficiency of sample stacking depend on the time duration and voltage of FESI step, as well as the length of water plug introduced before the electrokinetic injection.

To investigate the effect of the sample injection time on the stacking efficiency of FESI, the FQs mixture was injected into the capillary using a time duration varying from 1 to 5 min, while the injection voltage was fixed at –8 kV and water plug was injected by 40 mbar during 50 s. As depicted in Fig. 2A, the peak heights of different compounds increased with the injection time from 1 to 4 min, and stayed at slightly changed levels with further time duration extension. Therefore, the time duration for sample injection was chosen as 4 min.

Subsequently, the voltage for injection was optimized with a given time duration for sample injection (4 min) and a given length of water plug (injected by 40 mbar for 50 s). According to the pK_a values indicated in Table 1, the FQs dissolved in diluted NaOH are negatively charged, and thereby negative voltage was applied for the FESI step. Voltage ranging from –2 to –9 kV was studied and the results obtained are shown in Fig. 2B. When the voltage was varied from –2 to –6 kV, the peak heights of all five antibiotics increased sharply and achieved the maximum with the injection voltage of –7 kV; whereas, further increase of injection voltage would result in the decrease of the peak height for some of the compounds. Thus, –7 kV was used for sample injection in the subsequent FESI-CE analysis.

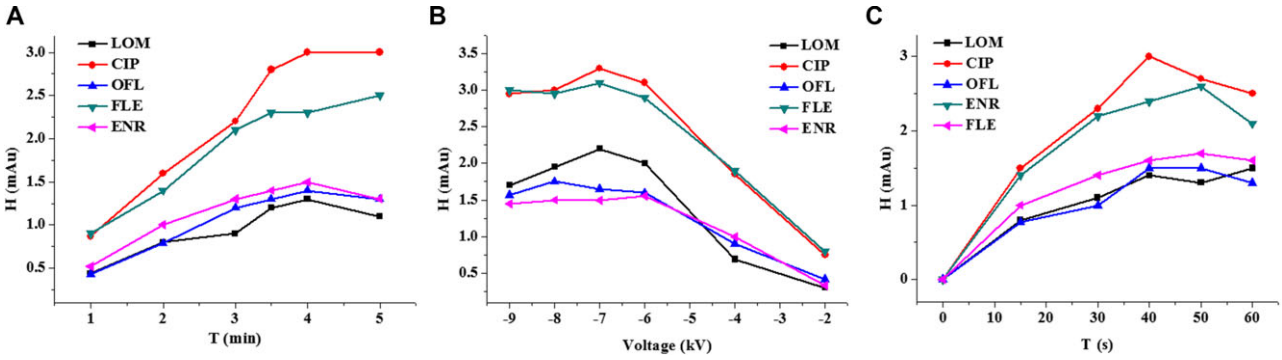


Figure 2. Optimization of FESI-CE-UV parameters. Dependence of the FQs peak heights (H) on variations of: (A) time for sample injection, (B) voltage for injection at 4 min of sample injection, (C) time duration for injecting a water plug at 40 mbar, 4 min of sample injection and –7 kV of injection voltage. Other experimental conditions are the same to the ones indicated in Fig. 1.

The length of water plug is another significant parameter for effective preconcentration of the analytes by FESI, because it provides an enhanced electric field at the inlet of the capillary. In the presented work, water was injected into the capillary by a pressure of 40 mbar, and the time duration for water injection ranging from 0 to 60 s was investigated with optimized conditions for other sample injection parameters (–7 kV for 4 min). When the water plug was shorter than the optimum length, the sample injected into the capillary would be removed out of the capillary from the inlet by a reverse EOF. Longer water plug was beneficial for FESI, but a too long water plug may result in a serious peak broadening and current dropping since it was not easy to be removed from the capillary during the electrokinetic injection. Consequently, the optimized time duration for water injected at 40 mbar, obtained from Fig. 2C, was 40 s.

3.3 Repeatability, linearity, and sensitivity of the method

Five replicated experiments were conducted under the optimized conditions to study the repeatability of the developed FESI-CE in a bubble cell capillary for the analysis of FQs at a concentration of 25 ng/mL for each compound. The RSDs of migration times and peak heights were calculated for the five FQs. The results shown in Table 2 indicate that the run-to-run repeatability of FQs analyses under optimized FESI conditions are excellent with largest RSD of 3.1% for migration time and 8.0% for peak height.

To study the linearity of this proposed FESI-CE, the mixtures of five FQs at different concentrations prepared and analyzed using both a bubble cell and standard capillaries. The dependence of FQs peak heights on their concentrations in the sample were fitted linearly and presented in Table 2 (regression lines with confidence interval of the parameters included presented in Supporting Information Table S2). The calibration curve equations depict that slope of the linear regression lines obtained in bubble cell capillary were higher than those in standard capillary as expected. Thus, FESI-CE in bubble cell capillary provides a good way to improve sensitivity, compared with that in a standard capillary. The SEF obtained by this method in comparison with conventional CE without any sample stacking performed in a standard capillary was calculated to be 460–1500.

Afterwards, the LODs and LOQs of the developed FESI-CE for FQs detection in standard and bubble cell capillaries were calculated as 3 S/N and 10 S/N respectively, and summarized in Table 2. The LODs and LOQs for different FQs were improved almost by three to four times with the use of a bubble cell capillary for optical detection. It is worth mentioning that in general the developed method was more sensitive to the CIP and FLE analyses due to their relatively high absorptivity at the given wavelengths and the discrimination of electrodynamic injection.

The obtained results demonstrate high sensitivity of the developed FESI-CE-UV analysis in comparison with other techniques like LC-MS [3] and ELISA [8] with LODs of 1.0 and 2.4 ng/mL, respectively. The presented method is suitability for milk sample analysis, as the MRL values for FQs in milk are lower than 100 μg/kg.

3.4 Fraction identification by coupling FESI-CE with ESTASI-MS

FESI-CE with UV detection provides a sensitive way to quantify FQs, while MS spectra can perform the direct qualitative control of antibiotics. Herein, the developed FESI-CE was coupled with a home-designed ESTASI-MS via a robotic system-based fraction collection device for the fraction identification. Compared with online hyphenation of CE with MS, the offline coupling of FESI-CE with ESTASI-MS via a robotic system for fraction collection suffers from a significant sensitivity decrease, which results from the contradiction between the sample concentration during CE separation and the sample dilution during the offline fraction collection on the plate for ESTASI-MS. Moreover, the amount of sample injected into the capillary was at a level of 10–100 nL, while the volume of ionization buffer for ESTASI-MS was at least 1 μL.

Table 2. Repeatability (RSD) of migration time (T) and peak height (H), calibration curves, LODs, and LOQs of FQs with the optimized procedure for FESI-CE-UV

FQs	RSD (%)[a] ($n = 5$)		Calibration curve[b]		LOD/LOQ (ng/mL)	
	T	H	Bubble cell capillary	Standard capillary	Bubble cell capillary	Standard capillary
LOM	3.1	6.9	$y = 0.120x - 0.07$, $R^2 = 0.990$	$y = 0.049x - 0.15$, $R^2 = 0.989$	1.3/4.4	3.4/11.4
CIP	1.8	6.0	$y = 0.24x - 0.28$, $R^2 = 0.987$	$y = 0.061x + 0.09$, $R^2 = 0.964$	0.8/2.5	2.1/7.0
OFL	1.4	7.1	$y = 0.143x - 0.11$, $R^2 = 0.986$	$y = 0.045x + 0.08$, $R^2 = 0.961$	1.1/3.7	3.0/10.1
FLE	0.6	8.0	$y = 0.37x - 0.42$, $R^2 = 0.990$	$y = 0.115x - 0.30$, $R^2 = 0.970$	0.4/1.3	1.4/4.8
ENR	1.0	3.4	$y = 0.18x - 0.45$, $R^2 = 0.984$	$y = 0.037x + 0.20$, $R^2 = 0.951$	1.0/3.4	2.1/6.9

a) Data are given for the sample of 25 ng/mL for each compound inside a bubble cell capillary. Results of repeatability study obtained by using one point with low concentration and one with high concentration are displayed in Supporting Information Table S1.
b) The higher concentration for the dynamic range is 100 ng/mL.

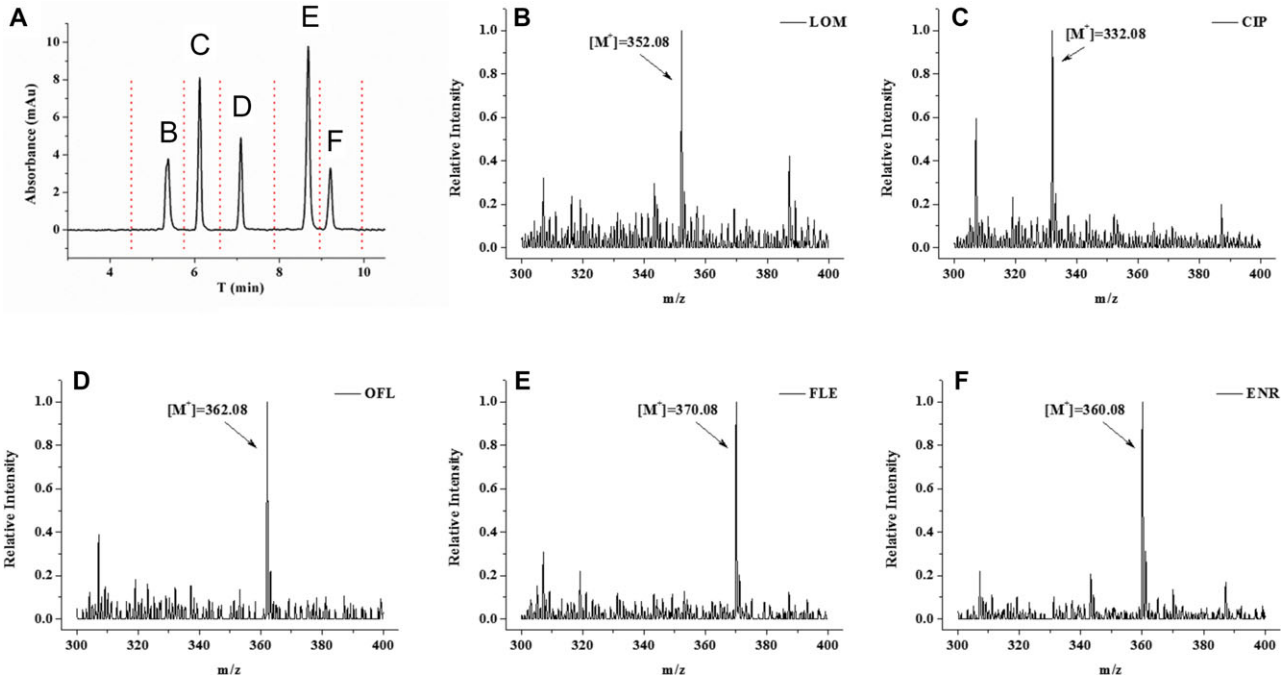


Figure 3. FESI-CE-UV and FESI-CE-ESTASI-MS for a FQs mixture (100 ng/mL for each compound): (A) FESI-CE-UV electropherogram with indication of corresponded fractions collected on collection plate; (B)–(F) ESTASI-MS spectra for five different compounds collected during FESI-CE. FESI-CE conditions: sample injected by –8 kV for 4 min, water plug injected by 40 mbar for 50 s, separation in 40 mM EDA (pH 8.0) at 30 kV in a standard capillary with total/effective length of 48.5/40 cm and id/od of 50/365 μm, UV at 280 nm. ESTASI-MS: 1 μL ionization solution (1% acetic acid and 50% methanol in water) and a pulsed high voltage of 10 kV and 20 Hz for the ionization of each spot.

The dilution limited the sensitivity of coupling of FESI-CE with ESTASI-MS, even when a highly hydrophobic plate prepared from a plastic film was used to minimize the dilution effect. Therefore, coupling of FESI-CE with ESTASI-MS was mainly applied for qualitative monitor.

Electropherogram of FESI-CE with UV detection and the corresponding ESTASI-MS spectra are presented in Fig. 3. Due to the difference of effective lengths of the capillaries used in FESI-CE-UV and FESI-CE-ESTASI-MS, the collection time program was well calculated with respect to distance, time and velocity. The time for changing the collection spot by moving the capillary above the collection plate was always fixed at the midpoint of the migration time of two adjacent compounds to avoid the possible mixture of two different fractions. The MS spectra corresponding to the sample spots collected are shown in Fig. 3B–F, confirming the feasibility of the collection method and peaks identification from the UV electropherogram in Fig. 3A. The MS spectra indicated that the peaks in Fig. 3A corresponded to LOM, CIP, OFL, FLE, and ENR, respectively. In spite of the fact that the limited sensitivity of such a CE-MS coupling hindered its application for quantitative analysis of sample in low concentrations, it provided a novel interface for easy offline hyphenation of CE with MS equipped with an ambient ionization source.

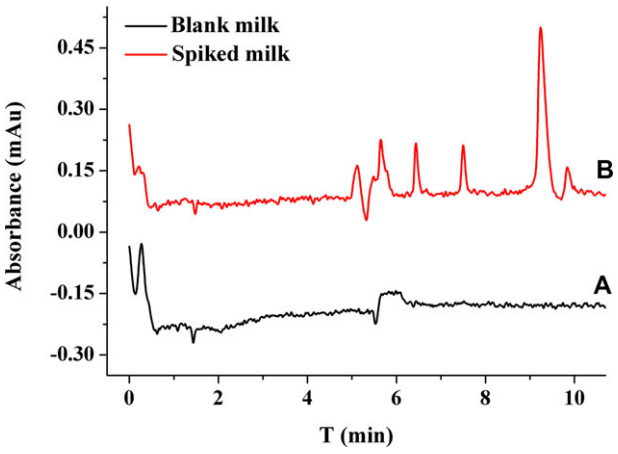


Figure 4. Electropherogram of (A) pretreated blank milk sample and (B) pretreated milk sample spiked with 5 μg/kg of FQs.

3.5 Milk sample analysis

The proposed method was used for the analysis of FQs spiked milk samples. Prior to FESI-CE, FQs spiked milk samples were centrifuged after the addition of ammonium sulfate to remove the milk proteins. Besides, C18 spin columns were applied for desalting, considering the fact that FESI-CE is based on the conductivity difference between sample and BGE and the salts in sample may significantly decrease the stacking efficiency of FESI. Figure 4A and B show the FESI-CE electropherograms of a blank milk sample and milk sample containing five FQs (5 μg/kg each). It can be observed from the analysis of blank milk sample that the FQs are not present in UHT milk from local market, and no interfering compounds appear at the migration times of the analytes that demonstrated that the sample treatment was satisfactory.

Analyses were performed for milk samples spiked with different concentrations of FQs (100 μg/kg and 25 μg/kg), each in three repetitions, for recovery study. As displayed in Supporting Information Table S3, the recoveries of FQs in spiked milk calculated by the calibration curves in standard FQs solutions (shown in Table 2) ranged from 55.2 to 72.0%. Since there was nearly no matrix interference found in the electropherogram of FESI-CE (Fig. 4), the low recovery values could be resulted from the loss of analytes during the sample pretreatment caused by coprecipitation with milk proteins and photodegradation [42] during the long time duration for C18 cleanup. To ensure the accuracy of quantitation, calibration curves were further demonstrated in bovine milk spiked with FQs at different concentrations, considering that the repeatability of the sample pretreatment is satisfactory (RSD values between 3.9 and 12.6%, shown in Table 3). The dependence of FQs peak heights on their concentrations spiked in the milk were fitted linearly and the regression equations were presented in Table 3. LODs of FQs in spiked milk were obtained from 0.7 to 2.5 μg/kg, which were much lower than the permissible MRL established by European Union, and comparable with or much better than those of reported HPLC-based methods [3,11], immunoassay [9,10], and CE-based methods [15, 17, 19]. Based on the newly obtained calibration curves in milk, the recovery values were recalculated to be from 93.6 to 106.0% for 25 μg/kg FQs spiked milk and from 101.7 to 105.3% for 100 μg/kg FQs spiked milk (Table 3), which presented better efficiency of the sample recovery, sufficient for the correct performance of the FQs determination in milk.

4 Concluding remarks

The presented work shows the development of FESI-CE-UV analysis using a bubble cell capillary to determine five typical FQs in semi-skimmed bovine milk purchased from a supermarket in Switzerland. A new volatile EDA-based BGE was proposed for efficient FQs separation. Developed analytical system displays the sensitivity improved by 1200-fold in comparison with traditional CZE performed in a standard capillary without any preconcentration step. The proposed method was demonstrated to be simple, sensitive, and rapid, showing the usefulness of FESI staking step and bubble cell capillary applied together to achieve low LODs with the CE-UV analysis for FQs monitoring in milk samples. The obtained LODs are in the range from 0.7 to 2.5 μg/kg, far below the permissible MRL established by European Union. Presented methods can be easily extended to the analysis of the whole milk by adding another centrifugation step prior to the sample deproteinization. Moreover, the developed FESI-CE technique was coupled with ESTASI-MS in order to perform the

Table 3. Recovery, RSDs, calibration curves, LODs, and LOQs of selected FQs in spiked milk sample analyzed by FESI-CE-UV

FQs	Spiked milk samples				Calibration curve	LOD/LOQ(μg/kg)
	100 μg/kg		25 μg/kg			
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)		
LOM	103.1	8.2	106.0	5.7	$y = 0.068x + 0.031$, $R^2 = 0.984$	2.5/8.3
CIP	105.1	4.2	98.0	3.9	$y = 0.166x - 0.45$, $R^2 = 0.972$	1.2/3.9
OFL	104.5	10.4	94.4	4.4	$y = 0.098x - 0.44$, $R^2 = 0.982$	2.3/7.5
FLE	105.3	5.4	101.1	12.3	$y = 0.223x - 0.70$, $R^2 = 0.984$	0.7/2.3
ENR	101.7	12.6	93.6	4.9	$y = 0.129x - 0.55$, $R^2 = 0.992$	2.0/6.8

qualitative control of FESI-CE performance and direct antibiotic identification, which illustrated a new interface for off-line hyphenation of CE with an open ion source-based MS.

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