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Multiresidue determination of (fluoro)quinolone antibiotics in chicken by polymer monolith microextraction and field-amplified sample stacking procedures coupled to CE-UV

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ABSTRACT

Simultaneous determination of 9 (fluoro)quinolone antibiotics (FQs) was accomplished by capillary electrophoresis-ultraviolet (CE-UV) based on poly(methacrylic acid-co-ethylene glycol dimethacrylate) (MAA-EGDMA) monolith microextraction (PMME) coupled with on-line preconcentration technique of field-amplified sample stacking (FASS). The effects of composition of the acid and organic solvent in the sample solution, sampling time, and voltage on the efficiency of the sample stacking have been systematically investigated. Several parameters that influence extraction efficiency for PMME such as pH of sample solution, extraction volume, and wash and desorption conditions were optimized. In the proposed method, a substantial increase in sensitivity for all the FQs tested was achieved by the combination of PMME procedure with on-line preconcentration of FASS prior to CE analysis. Good linearities were obtained for the 9 tested FQs with the correlation coefficients (R) above 0.9954. The limits of detection (S/N = 3) were found to be 2.4–34.0 ng g^{-1} and the recoveries ranged from 81.2 to 100% with relative standard deviations less than 11.3%. The proposed PMME–FASS–CE method was applied to the determination of FQs residues in chicken samples.

1. Introduction

As one of the most important class of synthetic antibacterials in human and veterinary medicines, FQs have been used as medicines for humans and as veterinary drugs for animals worldwide [1,2]. Usually, the administration of FQs to animals has been applied mainly on food-producing animals such as pig, chicken, turkey and fish [3]. The extensive administration of such antibiotics to animals, destined for human consumption, has become a serious problem because their residues are a source of concern due to the emergence of drug-resistant bacteria and also a potential health hazard for consumers [4–7]. To ensure that consumers of the food are not exposed to residues at potentially harmful concentrations, the European Union (EU) has set tolerance levels (maximum residue limits, MRLs) for various classes of antibiotics among which including FQs, in foodstuffs of animal origin in different animal tissues [8]. The MRLs in chicken muscle for FQs ranges between 100 ng g^{-1} for oxolinic acid (OXO), and 400 ng g^{-1} for flumequine (FLU), respectively (as shown in Table 1). Since the beginning of year 2000, a MRL is required for every new substance that might be commercialised in the EU for veterinary use. The great chemical variety of FQs and trace level MRLs values, made it necessary to develop sufficiently sensitive multiresidue analytical methods for screening the residues possibly present in edible animal products.

Methods published in the literatures concerning the multiresidue analysis of FQs in edible animal products such as chicken muscle mainly involve HPLC [6,9–14]. As a powerful complementary separation technique to HPLC, capillary electrophoresis (CE) has been proven useful in the separation and determination of series of FQs because of its high resolution, speed, the extremely small sample volume required and high versatility in terms of separation modes [15,16]. However, as is well known, although excellent separation efficiency is easily obtained, CE, particularly when applied to biological samples with UV absorbance, suffers from limited concentration sensitivity due to the short optical pathlength inherent in the in-capillary detection. Although using more sensitive detection schemes, such as laser-induced fluorescence [17–19] and MS detector [20,21], can improve the limit of quantification (LOQ) of analytes in CE, they are not widespread due to rather expensive instrumentation for a common laboratory. Therefore, as alternative analytical approaches to enhance sensitivity in CE using UV detection, several electrophoretic-based techniques of

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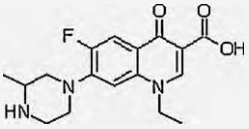
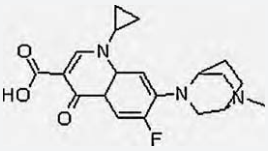
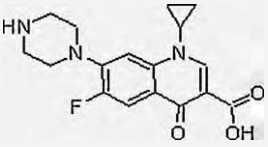
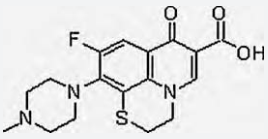
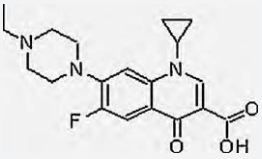
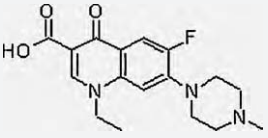
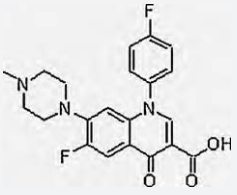
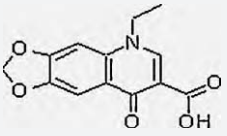
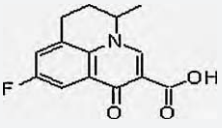
Table 1
Structures, pK_a values and MRLs (EU) of the studied FQs.

FQs	Chemical structure	pK_{a1}	pK_{a2}	MRL(EU) (ng g^{-1})[a]
LOM		6.56[b]	8.47[b]	Not established
DAN		6.07[b]	8.56[b]	200
CIP		5.86[b]	8.24[b]	100 (CIP + ENR)
RUF		6.00[c]	8.70[c]	Not established.
ENR		6.81[b]	8.04[b]	100 (CIP + ENR)
PEF		6.68[b]	7.83[b]	Not established
DIF		5.66[b]	7.24[b]	300
OXO		6.88[d]	–	100
FLU		6.65[d]	–	400

[a] MRLs of some (fluoro)quinolones in chicken muscle according to Drug Administration and European Union, Ref. [8].
[b] Ref. [41].
[c] Ref. [42].
[d] Ref. [43].

on-line preconcentration prior to CE such as normal stacking, field-amplified sample stacking (FASS), pH junctions, isotachophoresis (ITP) and sweeping [22–25] have been proposed. These techniques take advantage of differences in mobility and conductivity between sample and buffer to preconcentrate the analytes, and are widely employed because no special devices are required. The bibliography includes reports of systems in which an ITP preconcentration is combined with CE for analysis of marbofloxacin [26], and ITP and large volume sample stacking (LVSS) for ciprofloxacin, enrofloxacin and flumequine [27] in pig plasma samples, respectively. As a matter of fact, in comparison to ITP, LVSS and other on-line preconcentration techniques, FASS has been most commonly used in CE, since it is quite simple and easy to implement and can provide a greatly improved concentration sensitivity without adverse effects

on peak shape and resolution [28–30]. The FASS is based on a mismatch between the electric conductivity of the sample and that of the running buffer, in which when voltage is applied to the two ends of the capillary, charged analytes prepared in a more diluted buffer or solvent will experience a higher electric field strength because of the low conductivity of sample matrix and move faster than the ions inside the background electrolyte (BGE), and this produces a sharpening of the analyte zone at the boundary with the BGE. The most important prerequisite for achieving high sensitivity associated with FASS is that samples have to be free of electrolytes. Considering the strong matrix effects that are present in biological samples, extract clean-up is essential prior to FASS–CE, to remove as many interfering compounds as possible from the complex matrices and also to preconcentrate the analytes of interest and thus to increase the sensitivity.

Sample clean-up and preconcentration of FQs in edible animal products have always been carried out by solid-phase extraction (SPE) and liquid–liquid extraction (LLE) [6,7]. However, these conventional methods need to evaporate and to reconstitute the dry residue in a suitable solvent prior to the quantitative CE determination. These steps are tedious, time-consuming, and also prone to loss of analytes by evaporation and adsorption. As a consequence, research activities have been oriented toward the development of economy, efficient and miniaturized sample pretreatment methods. Solid-phase microextraction (SPME) has been shown to be an attractive alternative preconcentration method since it is solvent-waste-free, simple to operate, fast and extremely affordable to combine on-line or off-line with CE [31–33]. In recent years, our group has developed a poly(methacrylic acid-*co*-ethylene glycol dimethacrylate) (MAA-EGDMA) monolith microextraction (PMME) method which has been successfully combined off-line with CE for sensitive analysis of basic drugs, angiotensin II receptor antagonists, and sulfonamides in biological matrices [34–38]. This monolithic material exhibited a high extraction capacity as well as excellent biocompatibility in dealing with biological samples.

In this paper, we proposed a sensitive CZE-UV method by a combination of the PMME procedure with the online stacking method (FASS) to determinate and quantify simultaneously very low quantities (at the ng g^{-1} level) of 9 FQs (lomefloxacin, danofloxacin, ciprofloxacin, rufloxacin, enrofloxacin, pefloxacin, difloxacin, oxolinic acid and flumequine) in chicken muscle. Although lomefloxacin, rufloxacin, pefloxacin are not regulated drugs, the main interest in their determinations arise in that we aim to provide a new strategy for sample treatment and multi-component screening simultaneously in biomatrix. As far as we know, the FASS has not yet been used to analyse FQs, and the PMME–FASS–CZE method was the first time employed for monitoring the multiresidue of FQs in edible animal tissue.

2. Experimental

2.1. Reagents and materials

Ethylene dimethacrylate (EGDMA, 98% pure) was purchased from Acros (Sweden). Methacrylic acid (MAA), azobisisobutyronitrile (AIBN), dodecanol, toluene, and dichloromethane were obtained from Shanghai General Chemical Reagent Factory (Shanghai, China) and were of analytical reagent grade. The poly(MAA-*co*-EGDMA) monolithic capillary was synthesized by a polymerization method described previously [39].

Sodium hydroxide (NaOH), hydrochloric acid (HCl), disodium hydrogenphosphate (Na_2HPO_4), sodium phosphate (NaH_2PO_4), phosphoric acid (H_3PO_4), sodium tetraborate (Na_3BO_3), boric acid (H_3BO_3), acetonitrile (MeCN), formic acid (FA), acetic acid (HOAc) and trifluoroacetic acid (TFA) were also purchased from Shanghai General Chemical Reagent Factory and were of analytical reagent grade. Double distilled water was used throughout the experiments.

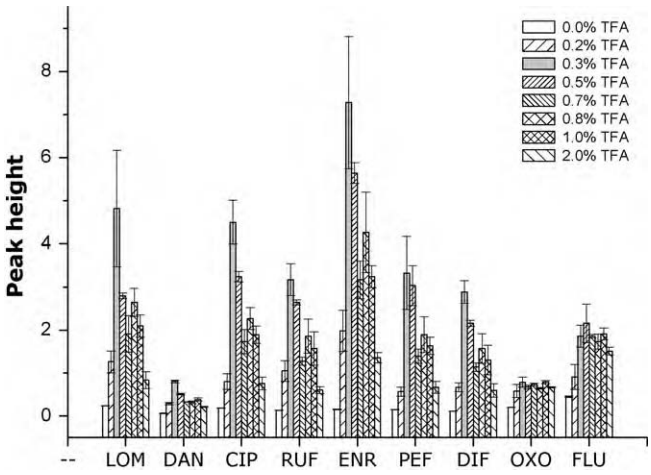


Fig. 1. Effects of TFA content in sample solution on detection sensitivity. Separation buffer electrolyte, 125 mM Na_2HPO_4 (adjusted to pH 8.7 with 1 mol L^{-1} H_3PO_4); Separation voltage, 18 kV; UV detection at 220 nm; FASS procedure, electrokinetic injection of sample for 12 s at 15 kV; The 9 FQs at a concentration of 5 μg mL^{-1} diluted with pure MeCN from the stock solutions, and different contents of TFA (v/v) was added. Other experimental conditions are outlined in Section 2.

Lomefloxacin (LOM), danofloxacin (DAN), ciprofloxacin (CIP), enrofloxacin (ENR), difloxacin (DIF), oxolinic acid (OXO) and flumequine (FLU) were purchased from Laboratories of Dr. Ehrenstorfer (Augsburg, Germany). Rufloxacin (RUF), and pefloxacin (PEF) were supplied by the State Food and Drug Administration of Wuhan Municipality (Wuhan, China). Individual stock solutions of CIP, ENR, RUF, PEF, DIF, and FLU, were prepared in a concentration of 500 μg mL^{-1} stock solution in MeCN. Individual stock solution of OXO was prepared in MeCN at a concentration of 200 μg mL^{-1}, and LOM and DAN were prepared as 500 μg mL^{-1} stock solutions in water. The working standard solution was diluted with MeCN by adding different contents of water and different acids (TFA, HOAc, and FA) or phosphate solution at different pHs to the desired concentration for experiments (see legends to Figs. 1–5 for further details). Ephedrine was employed as the internal standard (I.S.) and

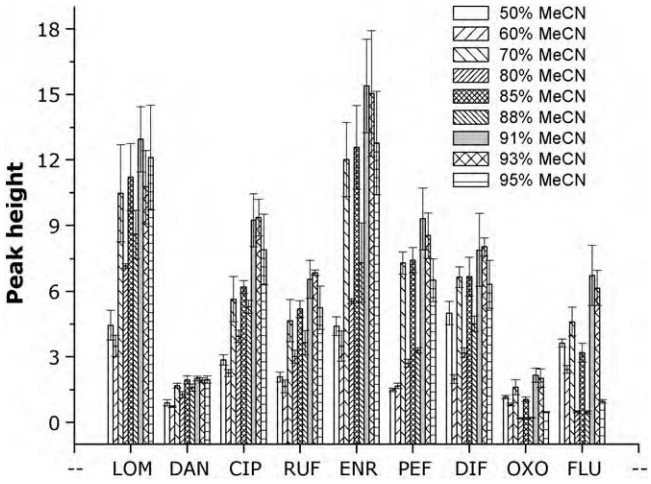


Fig. 2. Effects of MeCN content in sample solution on detection sensitivity. The 9 FQs at a concentration of 5 μg mL^{-1} diluted with MeCN at various volume percent water from the stock solutions, and 0.3% TFA (v/v) was added. Other experimental conditions are as Fig. 1.

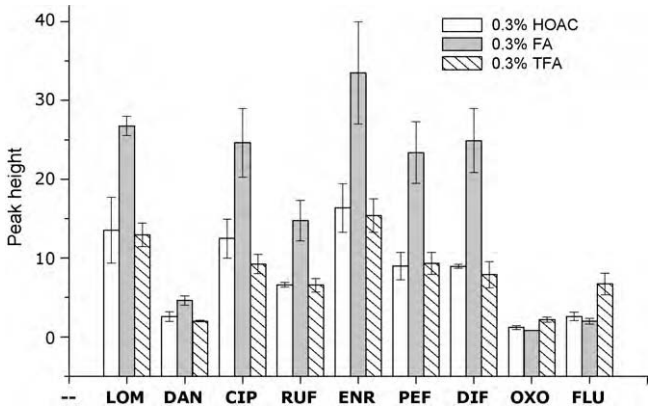


Fig. 3. Effects of different acids, TFA, HOAc, and FA, in sample solution on detection sensitivity. The 9 FQs at a concentration of 5 μg mL^{-1} diluted with 91% (v/v) MeCN from the stock solutions, and 0.3% TFA (v/v), 0.3% HOAc (v/v), and 0.3% FA (v/v) were added, respectively. Other experimental conditions are as Fig. 1.

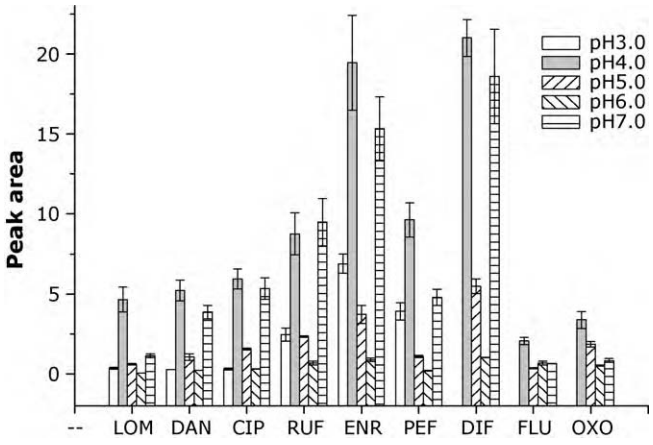


Fig. 4. Optimization of the pH of the sample solution. Standard sample solutions of 9 FQs spiked at 0.5 μg mL^{-1} were prepared with 25 mM phosphate solution at pH 3.0–7.0. PMME conditions: 1.0 mL of the sample solution was pumped through the monolithic capillary at 0.15 mL min^{-1} for the sample loading. Other extraction conditions and CE conditions were outlined in Sections 2.2 and 2.4.

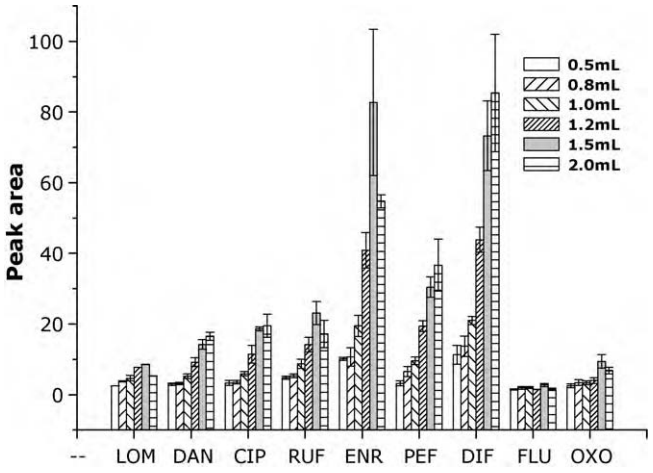


Fig. 5. Extracted sample volume profile of FQs for PMME. Standard sample solutions of 9 FQs spiked at 0.5 μg mL^{-1} were prepared with 25 mM phosphate solution at pH 4.0. PMME conditions and CE conditions were outlined in Sections 2.2 and 2.4.

purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). A solution of 600 μg mL^{-1} of ephedrine was prepared in MeCN–water–FA (91/9/0.3, v/v/v) solution. All of the above solutions were maintained under refrigerator at 4 °C. Chemical structures of the test quinolones in this study are shown in Table 1.

2.2. Instrumental and analytical conditions

The poly(MAA-EGDMA) monolith consists of a regular plastic syringe (1 mL), the capillary (2 cm × 530 μm i.d.; Yongnian, Hebei, China) and a plastic pinhead (one part of the whole syringe). One end of the pinhead coupled seamlessly with the syringe barrel, a metallic needle was removed from the other end of the pinhead, and replaced by a 2 cm monolithic capillary (cut from the prepared monolithic capillary) with adhesive [35,37].

The CE analysis was performed on a CE-L1 system (CE Resources, Singapore) equipped with a Linear UVIS 200 detector (Alltech, Deerfield, IL, USA). Data collection and manipulation were carried out using CSW17 software for chromatography (DataApex, Prague, Czech). Separations were carried out in a bare fused-silica capillary (Yongnian Fiber Plant, Hebei, China) of 50 μm i.d. and an effective length of 65 cm (total length 75 cm). Prior to running for separations, the new capillary was rinsed with 1 mol L^{-1} NaOH for 30 min, water for 15 min, 1 mol L^{-1} HCl for 30 min and water for 15 min in sequence, followed by conditioning with buffer for 15 min. Between runs, the capillary was successively flushed with 1 mol L^{-1} NaOH, water and running buffer at 2 min intervals. The wavelength of UV was set at 220 nm. The CZE system was operated using "normal" polarity (the cathode was located on the detector side). FASS was performed by applying a voltage of 15 kV for 12 s. And all the separations were carried out at a constant voltage of 18 kV using a running buffer of 125 mM Na_2HPO_4 (adjusted to pH 8.7 with 1 mol L^{-1} H_3PO_4) under optimized electrophoretic conditions. Temperature was maintained at 25 °C for the CZE experiments. Before use, the buffer solution was filtered through a membrane filter (0.45 μm) and degassed in an ultrasonic bath for 10 min.

Ephedrine solution was added before CE analysis to minimize the variation resulted from the electrokinetic injection and the fluctuation of the electroosmotic flow. Triplicate injections of the sample were performed and relative peak areas (analyte area/ephedrine area) were used for quantification.

2.3. Sample preparation

Chicken muscle tissues were purchased from a retail local market. These samples were minced and were stored at −4 °C until analysis. 1.5 g of thawed and homogenized chicken samples were accurately weighed and placed into a 10 mL centrifuge tube. Fortified samples were prepared by adding appropriate volumes of stock solutions of FQs (2000 ng mL^{-1}, from 10 μL to 1000 μL). The spiked sample was placed in an ultrasonic bath for 5 min to ensure appropriate distribution of FQs in the matrix. Afterwards, the sample was mixed with 5 mL of acetone by a rotary shaker and the FQs were extracted within 5 min. After being placed in a refrigerator at 0 °C for about 20 min, the samples were centrifuged for 5.0 min at 4000 rpm to achieve a complete phase separation (TDL 60-B, Jingke Scientific Instrument Co., Hunan, China). The supernatant collected was evaporated to dryness at 45 °C under a gentle stream of nitrogen, and then was redissolved with 100 μL of MeCN and 1900 μL of 25 mM phosphate solution (pH 4.0) and again refrigerated at 0 °C for about 20 min. After centrifugation for 5.0 min at 4000 rpm, the sample extract (final volume of 2.0 mL) was filtered through a membrane filter (0.45 μm) and stored in a vial prior to PMME procedure. Blank samples were prepared in the same way as mentioned above but without the compound-spiking step.

2.4. PMME procedure

The whole PMME process was just the same as that described in our previous reports [35,37], which included preconditioning, sampling, washing and desorption. A programmable syringe pump (WZB-50, Jianyuan Medical Technology Co., Changsha, China) was employed for providing the driving force for PMME. Firstly, the monolithic capillary was preconditioned first with 0.3 mL of MeCN and then 0.2 mL of phosphate buffer (25 mM, pH 4.0) at a velocity of 0.1 mL min^{-1}. Then 1.5 mL of the sample solution was pumped through the monolithic capillary at 0.15 mL min^{-1} for the sample loading. After that, the monolithic column was washed with water for 1 min in the same way. The residual water in the pinhead and monolithic capillary was pushed out with air using an empty and clean syringe. Subsequently, 0.05 mL of MeCN–water–FA (91:9:0.3, v/v/v) was used for the desorption of the analytes from the monolithic at 0.1 mL min^{-1}. The final eluate was collected into a microvial for the subsequent analysis by CZE. In order to avoid contamination, special syringe was used for each step individually.

3. Results and discussion

3.1. Optimization of CZE separations

As was previously reported and according with the pK_a values [49–51], basic pH around 8 provides better separation of FQs than acidic or stronger alkaline pHs by CZE mode [40–44]. Thus, in the present work, the optimization of the separation was carried out using the common basic buffer system: sodium phosphate covering the pH range of 7.0–9.0 and the concentration range between 75 and 125 mM. All the 9 selected FQs were effectively separated by using a phosphate buffer at pH 8.73. The optimized buffer concentration was 125 mM as it was found to provide a good compromise among peak shape, resolution, and electrical current intensity. The addition of organic modifiers such as acetonitrile or methanol led to poor separation selectivity of the FQs, therefore, they were not employed. In addition, according to the basic principles of FASS, better sample enrichment should be obtained with buffer electrolytes of higher ionic strength [45,46]. Thus 125 mM phosphate electrolyte (pH 8.73) were chosen as the separation buffer in the subsequent discussion in this study. A 18 kV voltage was applied to the buffer because higher voltages increased the electric current intensity up to values higher than 130 μA and no significant improvement with regard to the analysis time and resolution. Finally, the detection wavelength was set at 220 nm to obtain the detector response for most of the selected FQs as high as possible in this work. At this pH, the 9 FQs were all negatively charged and were detected after the EOF.

3.2. Optimization of FASS

In this procedure, sample enrichment is obtained by electrokinetically injecting a sample of lower conductivity compared with the background electrolyte (BGE). In this case, only charged analytes or neutral analytes associated to micelles can be concentrated. Sample solutions of low pH and conductivity tend to give the highest sensitivity. Because of the strongest acidity of TFA among most of organic acids, the effects of the TFA volume percentage in the sample solution (MeCN as matrix) on the sensitivity of field-amplified sample injection were firstly investigated. As demonstrated in Fig. 1, the sensitivity of all the FQs was increased sharply with the TFA content up to a maximum near 0.3–0.5% (v/v) in the sample solution, after which a decline in sensitivity (except for OXO and FLU) was observed. When the 9 FQs was injected as a 0.3% (v/v) TFA solution, the sensitivity was enhanced by 12–47-fold for the former 7 FQs (LOM, DAN, CIP, RUF, ENR, PEF, DIF) compared to the pure MeCN system. According to the pK_a values as listed in Table 1, the presence of a small amount of acid in the sample solution can facilitate the protonation of the former 7 FQs and enhance their stacking efficiency, while OXO and FLU can be present as neutral forms and can hardly be preconcentrated electrokinetically under acidic conditions. This is confirmed by the control experiments in our current study: the electric current is found to be zero when just FLU and OXO (i.e. sample solution without the presence of the other 7 FQs) were injected as a 0.3% (v/v) TFA solution using both the "normal" and "reversed" polarity way. Unexpectedly, as illustrated in Fig. 1, both OXO and FLU were found to be stacked and the sensitivity improvement obtained is around 4-fold compared to the pure MeCN system when they were electrokinetically injected together with the other 7 FQs, indicating that when in the presence of the other 7 FQs, OXO and FLU may be "carried" into the capillary due to the hydrogen bond interactions between the atom F and carboxylic group in their structures. Therefore, it is reasonable that the stacking efficiency was limited for OXO and FLU compared to the other 7 FQs. In this study, an optimal TFA content of 0.3% (v/v) in the sample solution was employed for further investigation.

On the basis of the criterion that addition of organic solvents to a sample solution is a straightforward approach to reduce its conductivity and to enhance sample stacking with low molecular weight polar compounds [28,47], pure MeCN was anticipated as better sample matrix than MeCN–water binary system. Using pure MeCN as sample matrix, however, was not always suitable as a result of current breakdown sometimes occur in the sample solution. Surprisingly, using MeCN–water instead of 100% MeCN as sample matrix was found to keep a stable current at the specified TFA volume percentages in our study. Concerning the effects of MeCN content in sample solution on the detection sensitivity, a more exhaustive study of MeCN concentration in aqueous MeCN–0.3% TFA (v/v) was performed, documenting that the optimal volume fraction of MeCN was 91% (v/v) (as shown in Fig. 2). It is worthy noting that, compared to the pure MeCN system, a more than 2-fold sensitivity enhancement for all of the FQs was achieved when the sample was injected as a 91% (v/v) MeCN solution. This may be the consequence of the charged FQs having the higher mobility in the MeCN–water system, which in turn results in a higher stacking efficiency.

It has been demonstrated that the acidity properties of sample solution have significant impact on the sensitivity of positively chargeable substances in FASS [28,48]. TFA (pK_a = 0.53), HOAc (pK_a = 4.79) and FA (pK_a = 3.74) have been used for the field-amplified sample injection of sulfonamides in our previous study [38], where TFA was found to be the best for the FASS process. As the strongest acid among the three acids examined, at given concentration, the addition of TFA to the sample solution produced the lowest pH and thus relatively effectively protonated of analytes. Nevertheless, as discussed above, the lowest pH may induce the increase in conductivity of sample solution and therefore deterioration in stacking for some analytes. As presented in Fig. 3, unlike our previous optimization studies for sulfonamides, FA was more effective than TFA and HOAc in enhancing the detection sensitivity with most of the tested FQs except OXO and FLU that characterized by their structure without piperazinyl substituent. This could be explained by considering the relatively strong hydrogen bond interactions in the presence of TFA compared to the other two acids. It should be pointed out that the 91% (v/v) MeCN was further confirmed to be best sample matrix with the addition of any of the three acids. As a compromise, the FQs were dissolved in MeCN–water–FA (91:9:0.3, v/v/v) for the subsequent experiment.

As the last optimization step of the FASS process, the influence of electrokinetic sample injection voltage and time on stacking was also investigated. In principle, application of a higher voltage and a

Table 2
Figures of merit of the CE-UV analysis of 9 (fluoro)quinolones under study using FASS as preconcentration procedures.[a]

FQs	FASS		
	Rs(n,n+1)	NTP[b]	Fold[c]
LOM		203702	127
DAN	1.7	35933	80
CIP	2.9	157350	164
RUF	2.5	165259	155
ENR	4.6	180567	221
PEF	6.0	201518	197
DIF	1.7	174702	252
OXO	20.1	147325	5
FLU	5.1	145101	5

[a] Data given for 5 μg mL^{-1}.
[b] Number of theoretical plates of column, which was determined from peak widths at half height ($w_{0.5}$) using the formula $N = 5.54\,[tR/w_{0.5}]^2$.
[c] Enhancement factor in terms of sensitivity compared to the same conditions without stacking (pressure injection of sample solution at 0.3 psi for 5 s).

longer injection time period should result in more solute injected [46,28]. In practice, when the applied voltage was higher than 20 kV, the electric current frequently dropped to zero. This could caused by excessive Joule heating and bubble formation under the high electric field strength conditions. From practical interest, the injection time was further optimized at an injection voltage of 15 kV, and it was found that the best injection time was 12 s, further increase in injection time would induce current breakdown. Therefore, 12 s of electrokinetic injection at 15 kV of the sample was selected to achieve an efficient sample stacking as well as acceptable repeatability.

Table 2 shows the resolution, number of theoretical plates (NTP), and the enhancement factor for FQs obtained under the optimum electrophoretic conditions. It can be seen that the resolutions are greater than 1.7 and the NTP are above 35,933 for all the FQs, which are appropriate to an accurate determination. Compared to traditional hydrodynamic injection methods, a significant improvement in sensitivity was generally achieved, giving an enhancement factor ranges from 80 to 252 for 7 of the 9 FQs tested, and 5-fold sensitivity enhancement for FLU and OXO.

3.3. Optimization of PMME conditions

Several factors that influence extraction efficiency for PMME such as pH of sample solution, extraction equilibrium profiles, and wash and desorption conditions were optimized. The optimization experiments were performed with 25 mM phosphate matrix solutions spiked with 0.5 μg mL^{-1} of each FQs.

As referenced in Table 1, the pK_a values of 7 quinolones studied here range from 7.24 to 8.70 for the ammonium form and from 5.66 to 6.81 for the carboxylic function [49,50]. The pK_a values of FLU and OXO of are due to carboxylic function [51]. In view of the pK_a values of FQs, the pH optimization was conducted in phosphate matrix solution over the pH 3.0–7.0 range. The pH-dependent of extraction efficiency for the FQs was illustrated in Fig. 4. As it was shown in Fig. 4, the responding signals (presented as peak areas) of nearly all FQs (except RUF) reached maximums at pH 4.0, which may be ascribed to the strong ion-exchange interactions between the cationic analytes and the negatively charged extraction material at a lower pH value. The lower extraction efficiency at pH 3.0 could result from the weak ion-exchange interactions due to the protonated of both the analytes and extraction phase, wherein DIF, OXO and FLU were even found to be hardly "*catched*" by the extraction material. With increasing pH, the ionization degree of the analytes decreased accordingly, leading to decreased ion-exchange interactions for the sample, and the extraction efficiency showed decreases for most analytes. The higher extraction efficiency obtained when the sample solution was at pH 7.0 could attributed to the strengthened hydrophobic interaction at higher pH. Therefore, a sample solution with pH 4.0 was applied to extract the FQs in real samples, which is identical with that recently reported by our group [52].

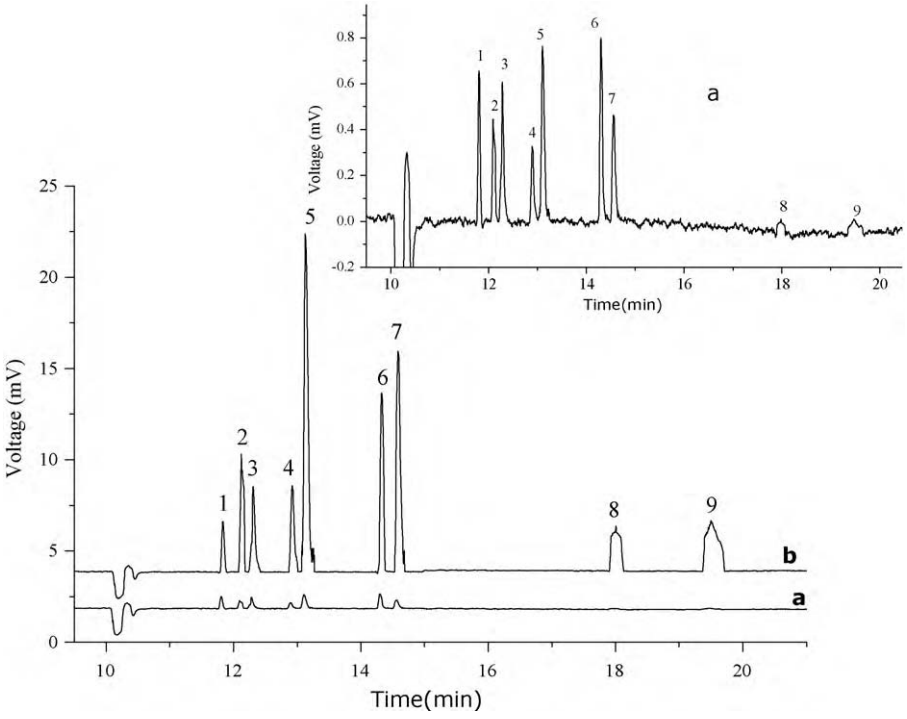


Fig. 6. Electropherograms of FQs standard sample obtained by direct CE analysis (a) and PMME-CE (b). Peaks: (1) LOM, (2) DAN, (3)CIP, (4) RUF, (5) ENR, (6) PEF, (7) DIF, (8) OXO, and (9) FLU. All the FQs were 0.5 μg mL^{-1}. PMME conditions and CE conditions were outlined in Sections 2.2 and 2.4.

To investigate the extraction capacity of the monolithic capillary, the extraction volume profiles of nine FQs were monitored by increasing sampling volume for a 0.5 μg mL^{-1} standard solution. The extraction flow-rate was kept at 0.15 mL min^{-1}, and the sampling volume was increased from 0.5 to 2 mL. As shown in Fig. 5, although increasing the sample volume might be beneficial to increase the extraction amounts of some FQs (presented as peak areas), 1.5 mL of sample solution was selected in our studies to achieve sufficient detection sensitivity together with rapid analysis to make the strategy more applicable to real sample analysis.

To ensure direct compatibility with the on-line CE preconcentration, sample solution optimized in FASS, i.e. MeCN–water–FA (91:9:0.3, v/v/v), was used as desorption solution. It was found that a thorough desorption could be achieved with 0.05 mL of the solvent. No peak was detected in the following blank analysis. Subsequently, 0.2 mL of water was used to eliminate the residual buffer which was unacceptable in FASS. Then the residue of water was pushed out by injecting air into the monolithic capillary before desorption with an empty and clean syringe.

The electropherogram of FQs obtained by PMME-CE under the optimized conditions are shown in Fig. 6B. In comparison with the electropherogram of direct injection (Fig. 6A), a significant peak height enhancement was observed after extraction. The enrichment factors calculated by comparing the peak area obtained with PPME and without preconcentration to be 4–26 for 9 FQs, indicating the remarkable preconcentration ability of the monolithic column.

3.4. Application to chicken matrix samples

To evaluate the viability of the PMME–FASS–CZE method, the established system was employed to the analysis of FQs in chicken samples. None of the studied FQs was detected in the blank chicken sample treated as the proposed sample clean-up and enrichment procedure, suggesting there is no interferences from the matrix that influenced the quantification of the analytes. Fig. 7 shows the electropherogram obtained by PMME–FASS–CZE with UV detection of the chicken sample spiked with 9 FQs at a concentration of 50 ng g^{-1}. As a result, under the optimized conditions, a substantial increase in sensitivity achieved by PMME-coupled to FASS allowed the detection of FQs in the trace level concentration range

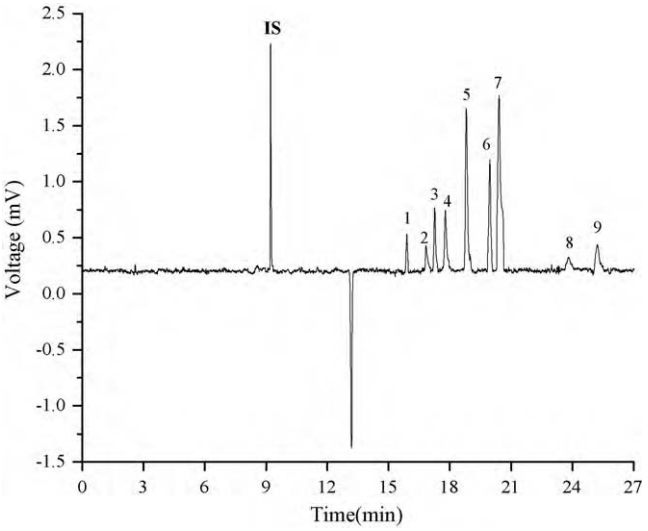


Fig. 7. Electropherogram of chicken sample spiked with 50 ng g^{-1} of each FQs by PMME – FASS – CE method. Peaks: (1) LOM, (2) DAN, (3)CIP, (4) RUF, (5) ENR, (6) PEF, (7) DIF, (8) OXO, and (9) FLU. IS was 10 μg mL^{-1}. Extraction conditions and CE conditions were outlined in Sections 2.2 and 2.4.

Table 3
Calibration curves of 9 (fluoro)quinolones in chicken samples using the PMME–FASS–CZE method.

FQs	Linearity range (ng g^{-1})	Calibration curve			LOD (ng g^{-1})	LOQ (ng g^{-1})
		Intercept	Slope	R		
LOM	50–1000	0.4036	0.0023	0.9967	10.4	34.7
DAN	50–1000	0.4087	0.0038	0.9954	16.4	54.7
CIP	50–1000	0.2548	0.0059	0.9986	5.0	16.8
RUF	50–1000	0.2487	0.0069	0.9991	5.8	19.4
ENR	50–1000	0.9754	0.0231	0.9971	1.4	4.6
PEF	50–1000	0.3263	0.0091	0.9970	2.5	8.4
DIF	50–1000	1.6417	0.0212	0.9977	2.4	7.8
OXO	50–1000	0.1270	0.0008	0.9965	34.0	113.3
FLU	50–1000	0.1191	0.0033	0.9958	13.3	44.4

Table 4
Intraday and interday precision of relative peak areas obtained by analyzing 9 (fluoro)quinolones spiked at three different concentrations in chicken samples with the proposed PMME–FASS–CZE method.[a]

FQs	RSD (%)					
	50 ng g^{-1}		100 ng g^{-1}		1000 ng g^{-1}	
	Intraday[b]	Interday[c]	Intraday[b]	Interday[c]	Intraday[b]	Interday[c]
LOM	4.7	3.9	1.1	4.7	3.6	9.8
DAN	4.3	9.5	6.8	8.0	5.8	8.6
CIP	3.0	8.0	1.2	5.2	3.5	7.0
RUF	2.1	4.1	3.9	8.3	4.5	3.9
ENR	3.4	5.3	3.2	4.8	3.2	3.4
PEF	3.0	6.5	2.9	6.9	6.7	6.0
DIF	7.4	10.4	9.6	12.2	8.2	12.6
OXO	7.6	11.8	4.6	6.8	7.5	8.0
FLU	9.9	14.1	8.2	12.4	7.9	13.6

[a] Extraction conditions and CE conditions outlined in Section 2.
[b] Analysis of three independent extracts obtained from a chicken sample spiked at the specified concentration over a day.
[c] Analysis of five independent extracts obtained from a chicken sample spiked at the specified concentration for consecutive 5 days.

Table 5
Recoveries (%) of 9 (fluoro)quinolones spiked at different levels in chicken samples using the PMME–FASS–CZE method.

FQs	Recovery (%, RSD%), intraday (n = 3)		
	50 ng g^{-1}	100 ng g^{-1}	1000 ng g^{-1}
LOM	98.8(5.8)	98.1(2.7)	96.7(3.9)
DAN	81.2(9.3)	93.7(8.2)	91.3(10.1)
CIP	93.6(2.3)	98.4(1.1)	93.4(2.0)
RUF	96.6(4.2)	97.9(2.9)	95.4(5.4)
ENR	93.4(5.9)	98.6(3.3)	84.9(3.8)
PEF	99.3(2.5)	97.6(2.9)	85.2(5.7)
DIF	91.3(7.6)	97.5(10.4)	93.4(6.1)
OXO	81.3(5.8)	92.8(2.6)	100(4.0)
FLU	84.7(11.3)	96.1(6.5)	94.1(4.2)

be readily realized, indicating a remarkable applicability of the proposed method for sensitive analysis of the FQs in chicken samples.

The developed method was further validated using chicken muscle tissue samples fortified with several levels of standard FQs mixture, and subjected to the entire clean-up and enrichment procedure. Before CE analysis, a constant amount of 12.0 μg mL^{-1} ephedrine was added as IS. Linear regression analyses were performed using ratios of peak areas of FQs to that of IS against the respective FQs concentration. Detection limits (LODs) and quantification limits (LOQs) were defined, respectively, as the signal corresponding to 3 and 10 times the baseline noise standard deviation. The repeatability (intraday precision) was calculated from consecutive analyses of three independently prepared extractions from the spiked chicken samples over a day. And the reproducibility (day-to-day or interday precision) was determined by extracting five independently prepared chicken samples for 5 consecutive days. As summarized in Table 3, good linearity was observed in the concentration range of 50–1000 ng g^{-1}, with a correlation coefficient better than 0.9954 for the spiked chicken samples, indicating the suitability of the method for quantitative analysis. And the proposed PMME–FASS–CE method is sufficiently sensitive to analyse these FQs in chicken because the LOQs (4.6–113.3 ng g^{-1}) obtained were far below the MRL values (100–400 ng g^{-1}) established for these drugs by the EU [8]. Table 4 shows the intraday and interday precisions at three concentrations involving the low, medium and high level of the calibration curve range, which are lower than 9.9% and 14.1%, respectively. The accuracy of this method was examined using recovery studies by comparing the extraction efficiency obtained by extracting spiked chicken samples to that of the standard sample, with the results listed in Table 5. Good recoveries were obtained for all of the nine quinolones tested, giving the values in the range of 81.2–100%. The RSDs (intraday), summarized in Table 5, also showed satisfactory levels. These parameters do not show dependence on the concentration of FQs spiked in the sample, demonstrating that the established PMME–FASS–CZE method were acceptable for routine monitoring of FQs in chicken samples.

4. Conclusion

In the present study, a PMME–FASS–CE method for multiresidue determination of 9 FQs in chicken samples was developed. Satisfactory results were obtained with regard to selectivity, linearity, accuracy, and precision. LOQs much lower than the MRLs (EU) were attained. As the extractants by PMME can be directly analysed using CZE with UV detection, and the sensitivity offered by PMME–FASS–CZE was more than enough to determine the trace amount of FQs in chicken samples, the method developed may find applications for analyzing such analytes residues in other edible animal tissues. In conclusion, the PMME–FASS–CE method once again proved to offer a sensitive, easy, fast and low-cost method for trace analysis in complex matrix, to be proposed as an alternative to other more expensive methods for multiresidue determination of FQs in food quality and safety controls.

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