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Short communication

Simultaneous determination of fluoroquinolones in foods of animal origin by a high performance liquid chromatography and a liquid chromatography tandem mass spectrometry with accelerated solvent extraction

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ABSTRACT

A confirmatory and quantitative method based on a high performance liquid chromatography UV detector (HPLC-UV) and a liquid chromatography tandem mass spectrometry (LC–MS/MS) with an extraction procedure of accelerated solvent extraction (ASE) has been developed for simultaneous determination of 15 kinds of fluoroquinolones in various animal origin food samples. The sample preparation procedures consist of an extraction step with acetonitrile and a cleaning-up step with Oasis HLB cartridge. Parameters for extraction pressure and temperature, cycle of ASE, clean-up, and analysis procedure have been optimized systematically. The recoveries of FQNs spiked in the tissues as the muscle, liver, kidney of swine, bovine, chicken and fish at a concentration range of 10–800 μg/kg were found between 70.6% and 111.1% with relative standard deviations (RSD) less than 15% in HPLC. The LOD and LOQ of the HPLC for the 15 FQNs were 3 μg/kg and 10 μg/kg, respectively, and those of the LC–MS/MS were 0.3 and 1 μg/kg, respectively. These rapid and reliable methods can be used to efficiently separate, characterize and quantify the residues of 15 FQNs (Marbofloxacin, Enoxacin, Fleroxacin, Ofloxacin, Pefloxacin, Lomefloxacin, Danofloxacin, Enrofloxacin, Orbifloxacin, Cinoxacin, Gatifloxacin, Sarafloxacin, Difloxacin, Nalidixic Acid, Flumequine) in food of animal origin.

1. Introduction

Fluoroquinolones (FQNs) antibiotics are a group of relatively new and synthetic antibiotics. FQNs, derived from 3-quinolonecarboxylic acid, have a fluorine substituent at the R6 position and show a broad spectrum of microbiological potency as well as rapid absorption following the oral administration route. Their structures are given in Fig. 1.

FQNs were initially used in the treatment of urinary tract infections. In recent years, there has been a growing interest in using FQNS as therapeutic drugs in animal husbandry after the various chemical modifications involving substitution of different functional groups around the quinolone ring, which led to improve both the antimicrobial potency and the pharmacokinetic properties of them. Human exposure to FQNS due to its presence in foods of animal origin can contribute to adverse effects on health. In fact, the presence of FQNs in foods of animal origin can also constitute a resistance selection to the pathogens [1].

It is reported that high performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC–MS) and liquid chromatography tandem mass spectrometry (LC–MS/MS) [2–4] could be applied to detect the residues of FQNs in foods of animal origin. However, the limits of detection (LOD) differed between 1 μg/kg and 40 μg/kg, and the ion pairing reagents and exact pH of the mobile phase were required in order to achieve a better separation [5,6]. Again, triethanolamine were sometimes added to the mobile phase to reduce peak tailing. In addition, the methods reported up to date were just focused on less than ten analytes of structural similarities such as enrofloxacin, ciprofloxacin, norfloxacin and ofloxacin in only one or two tissues. Whereas, when looking at the common procedural scheme, we reasonably suppose that it would be useful and presently feasible to achieve consensus conditions able to acceptably recover most of drugs of FQNs.

Although different sample preparation strategies as liquid to liquid extraction (LLE) [2,7–10], solid-phase micro extraction (SPME) [11–13], and supercritical fluid extraction (SFE) [14] were described for the analysis of FQNs in foods of animal origin, the poor

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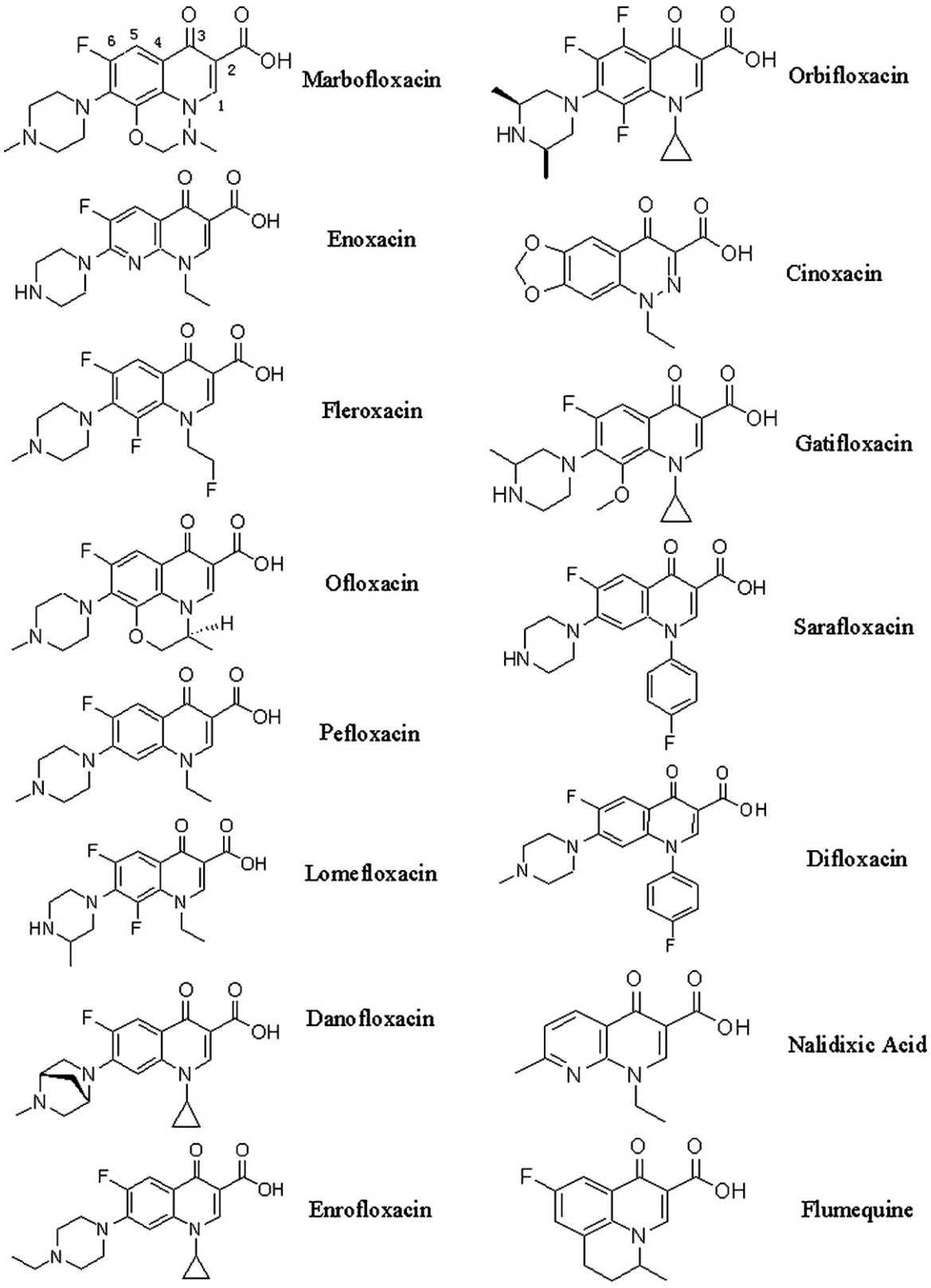

Fig. 1. Structures of 15 fluoroquinolones.

extraction efficacy, relatively low recoveries (>50–70%), the inability to simultaneously extract all FQNs, and the tedious and usually time-consuming procedures seriously limited those methods to apply in practice due to the fact that FQNs have diverse physicochemical properties.

Accelerated solvent extraction (ASE) is a new sample preparation method for trace analyte. This technique uses conventional solvents at elevated pressure and temperature to quickly extract solid samples [15–17]. The process takes an advantage of the increasing solubility of analyte at the elevated temperature and

the pressure and, under such conditions, it also increases the diffusion rate and decreases the viscosity and surface tension, so that the kinetic processes for analytes desorbing from the matrix are accelerated. ASE combines the benefits of high-throughput, automation, and favorable environmental impact due to the low solvent consumption, compared to conventional extraction techniques. Pecorelli et al. [18] and Okeke et al. [19] and Díaz-álvarez et al. [20] applied ASE to extract FQNs from feedstuffs and infant foods. Herranz et al. [21] have optimized ASE parameters to extract FQNs from table eggs. Lillenberg et al. [22] used the mixture of 0.35% phosphoric acid and acetonitrile (1:1, v/v) with 0.01 M citric acid monohydrate as extraction solvent to extract ciprofloxacin, norfloxacin and ofloxacin from sewage. Rodriguez et al. [23] have used ACN/*o*-phosphoric acid 50 mM pH 3.0 (80:20, v/v) to extract ciprofloxacin, enrofloxacin, sarafloxacine, danofloxacin and norfloxacin from infant foods with recoveries between 69% and 107%. However, there is no any report on the application of ASE to extract the residues of 15 FQNs in the edible tissues of animals (such as muscle, liver and kidney) where the analytes are in trace and are subjecting to matrix interference.

The present work describes an HPLC and LC–MS/MS method combined with a simple, fast and efficient extraction procedure based on ASE that enable to determine 15 FQNs in different animal tissues. The variables affecting ASE efficiency as extraction solvent, temperature, time and type of dispersion agent were systematically optimized, and the conditions for HPLC separation and for LC–MS/MS detection were meanwhile investigated in order to get satisfactory recoveries and to minimize matrix effects. The method has been successfully applied to the determination of ENRO and its biologically active metabolite CIPRO in incurred muscle of swine.

2. Experimental

2.1. Chemicals and standard solution

Nalidixic Acid, Flumequine, Pefloxacin, Lomefloxacin, Danofloxacin, Fleroxacin, Gatifloxacin, Sarafloxacin, Orbifloxacin, Cinoxacin, Enoxacin, Enrofloxacin, Ofloxacin, Marbofloxacin and Difloxacin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methanol and acetonitrile were obtained from Fisher (Bar-Bel, France). Citric acid, ammonium acetate, acetic acid and ammonia solution (25%) were supplied by Beijing Chemical Company (Beijing, China). The cartridges used for solid-phase extraction were Oasis Hydrophilic–Lipophilic Balance (HLB) cartridges (3 cm^3/60 mg) from Waters (Milford, MA, USA). Filter membranes (0.22 μm) used to filter the extracts before injection into the chromatographic system were provided by Agilent (Palo Alto, CA, USA). Separation columns for HPLC and LC–MS/MS were ZORBAX SB-aq-C18 (150 mm × 4.6 mm I.D., 5 μm) (Agilent Technology, USA), Hypersil Golden (100 mm × 2.1 mm, 3.5 μm) (Thermo scientific, Germany), respectively. Unless indicated otherwise, all reagents used in the present study were analytically pure substances and HPLC-grade. Deionized water (18 MΩ m) was generated by a Milli-Q water-purification system (Millipore, Bedford, MA, USA). Single stock standards were prepared at 1 mg/mL in acetonitrile, mixed intermediate standards at 1000 μg/L in acetonitrile and analytical standards at 10, 20, 50, 100 and 200 μg/L in deionized (DI) water and acetonitrile (1/1 = v/v), analytical standards were prepared daily.

2.2. Sample preparation

The tissues for blank samples as muscle, liver and kidney of swine and bovine, muscle and liver of chicken, and muscle of fish were purchased from a local market. They were homogenized with a high-speed food blender, and stored at below −20 °C until the time of analysis.

2.2.1. Accelerated solvent extraction

ASE was carried out using a Dionex accelerated solvent extractor 200 (Dionex, Sunnyvale, CA, USA) equipped with 11 mL stainless-steel extraction cells. For each cell, 5 g tissues thoroughly mixed and blended with 1.0 g of diatomaceous earth until a complete dispersion was obtained, was placed in cellulose filters (Dionex) which were in turn placed in the stainless steel extraction cells. All the cells were heated in a water bath at 40 °C to improve and facilitate the handling of the mixture, using acetonitrile as solvent. Optimized extraction conditions were obtained by sequentially varying one experimental parameter while all other parameters remained fixed. Final conditions used in the extraction for FQNS were as follows: time of heating cell 2 min, time of solvent in contact with the sample 2 min (static time), pressure at 70 bar, temperature at 65 °C, time of purging with nitrogen to remove residual solvent in the cell 60 s, flushing volume of water related to that of cell: 50%, flushing cycle: two. The extraction solution was evaporated to dryness (under nitrogen flow) at 40 °C and reconstituted in 10 mL of phosphate solution.

2.2.2. Clean-up by solid-phase extraction

SPE column was activated with 3 mL of methanol and 3 mL of water. Then, the solution obtained in the sample extraction step was passed through the column. The cartridge was washed twice with 5 mL of mixture of methanol:water (10:90, v/v) and then dried under vacuum for 1 min. The compounds of interest were eluted with 4 mL of methanol and the fraction was evaporated to dryness (under nitrogen flow) at 40 °C. The residue was dissolved in 1 mL of HPLC original mobile phase. After filtration, this solution was injected into HPLC or LC–MS/MS for analysis.

2.3. HPLC analysis

The HPLC system consists of a Waters 2695 separations module and 2487 dual λ absorbance detector (Waters, USA). A Zorbax SB-Aq-C18 (250 mm × 4.6 mm I.D., 5 μm) HPLC column was used for separating FQNs. The operating temperature of the column was set at 35 °C. The injection volume was 50 μL. The mobile phase used was a three gradient system with methanol/acetonitrile/0.02 M citric acid and 0.03 M ammonium acetate. The starting mobile phase composition at 0 min was 10/10/80, it was switched to 11/13/76 after 20 min, and then it was switched to 10/30/60 and maintained for 10 min. The flow rate was 0.8 mL/min. The wavelength of UV detector was set at 278 nm.

2.4. LC–MS/MS analysis

Analysis was carried out using a Thermo-electron TSQ Quantum Access triple quadrupole mass spectrometer (the ion mode was positive) coupled with a Surveyor LC pump and an autosampler. The separation was achieved by a Hypersil Golden (150 mm × 2.1 mm, 3.5 μm). Mobile phase A was methanol, B was acetonitrile, and C was 5 mM ammonium acetate and 0.2% formic acid aqueous solution. The gradient composition of mobile phase was initially 20/2/78, and then programmed to 20/5/75 at 4 min, and finally switched to 20/10/70 at 20 min and maintained for 5 min. The flow rate was 0.25 mL/min and injection volume was 10 μL. The source parameters were optimized by monitoring the MS/MS spectra of the analytes. Selected reaction monitoring (SRM) was performed on each of the analyte protonated molecular ions using the parameters: source voltage 5 kV, capillary temperature at 350 °C, sheath gas (nitrogen) 35, auxiliary gas 5, Q1 peak width 0.70 amu, Q3 peak width 0.50 amu, collision gas (argon) 1.50 mTorr, scan width

1–2 amu, and scan time 0.3–0.5 s. Collision energies were set at the maximum for each transition, and ranged from 18 to 30 eV. These parameters were optimized for matrix extracts to confirm analyte residues. Using positive ion electrospray ionization, triple quadrupole mass spectrometer parameters were optimized. Selected reaction monitoring (SRM) was used to measure the transitions from the protonated molecular ions to product ions that have been described in previous electrospray LC–MS/MS methods for these compounds [2–4].

3. Results and discussion

3.1. HPLC-separation

Since 15 FQNs were included in our study, the chromatographic conditions were influenced by the physic-chemical properties of different drugs, such as solubility, polarity and UV absorption. Several columns such as Inertsil ODS-3, Agilent ZORBAX SB-C18, Agilent ZORBAX Aq-C18 and Waters XBridge C18 have been used, since Agilent ZORBAX Aq-C18 was acid proof and residual silanol endcapping, it was finally selected. In our study, no ion pairing reagent was used and acceptable peak shapes were still obtained without tailing by using the reversed-phase column. The optimum mobile phase was methanol/acetonitrile/0.02 M citric acid and 0.03 M ammonium acetate with a gradient elution program. In order to improve specificity and minimize interferences from matrices or solvent system that may occur at lower wavelengths, we performed the analysis at 278 nm. Under the described conditions, all of the analytes were well resolved with a resolution factor greater than 2.0 with a run time.

3.2. Optimization of ASE condition

For ASE, parameters that significantly affect recoveries are the extraction solvent, the temperature, the pressure, the static extraction time, and the number of cycles. In this study, each parameter has been optimized separately.

For our study, seven representative fluoroquinolones (Gatifloxacin, danofloxacin, sarafloxacin, difloxacin, dfloxacin, flumequine, enrofloxacin) were included and different solvents have been investigated, the results revealed that pure acetonitrile was the best (see Table 1).

To evaluate the effect of temperature, different temperatures ranged from 45 °C to 85 °C (45, 55, 65, 75 and 85 °C) have been investigated. The decrease in recovery was also observed at

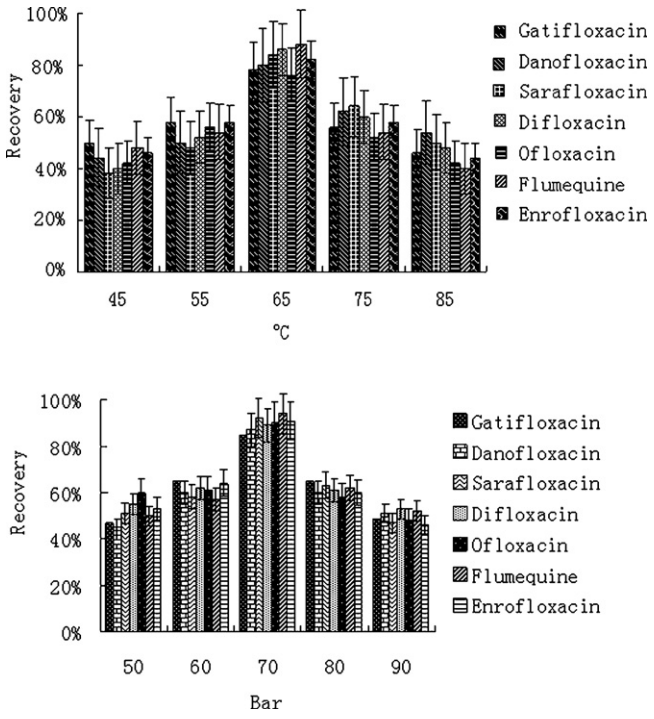

Fig. 2. Influence of temperature (a) and pressure (b) in the recovery.

temperatures exceeding 65 °C (Fig. 2a), resulting either from thermal degradation or from loss in method selectivity due to the more efficient extraction of interfering matrix components. The effect of pressure on extraction efficiency of antibiotics was studied in a range of 50–90 bar. A pressure of 70 bar was selected (Fig. 2b).

In view of the different parameters (e.g. solvent volume, temperature, pressure and number of extraction cycles) affecting the ASE extraction process together with a desirability function, a multivariate DOE has been used to optimize the values of the significant parameters to achieve the highest global recovery for all representative fluoroquinolones. The optimized ASE conditions were further applied for method development and validation. The fractional factorial designs (FFD) were applied: Flush volume (%), temperature (T), pressure (P) and number of extraction cycles (C). A fractional factorial design was defined by an experimental domain constituted by a central point and two levels corresponding to the maximum and the minimum values for each extraction parameter.

Table 1
Solvent influence on the extraction recovery of studied FQNs from edible tissues.

Extraction solvent	Sample	Recovery (%RSD)						
		Gatifloxacin	Danofloxacin	Sarafloxacin	Difloxacin	Ofloxacin	Flumequine	Enrofloxacin
Acetonitrile	Swine muscle	83 (4)	87 (7)	89 (2)	80 (6)	85 (6)	84 (6)	84 (6)
	Swine liver	91 (3)	80 (5)	91 (3)	90 (4)	84 (6)	90 (2)	90 (4)
	Bovine muscle	89 (5)	88 (4)	82 (6)	92 (4)	92 (3)	85 (3)	87 (4)
	Bovine liver	85 (5)	81 (5)	90 (7)	80 (5)	65 (11)	71 (7)	79 (7)
Acetonitrile/TCA (1:1)	Swine muscle	88 (7)	82 (6)	80 (5)	81 (7)	76 (9)	54 (10)	58 (9)
	Swine liver	63 (5)	47 (2)	50 (5)	52 (6)	58 (4)	62 (9)	69 (6)
	Bovine muscle	78 (10)	77 (6)	62 (6)	71 (8)	65 (8)	80 (5)	78 (5)
	Bovine liver	70 (5)	63 (8)	53 (10)	47 (5)	58 (10)	49 (5)	71 (6)
Acetonitrile/TCA (2:1)	Swine muscle	43 (8)	56 (11)	52 (7)	47 (6)	55 (7)	71 (11)	62 (8)
	Swine liver	46 (4)	60 (5)	58 (9)	41 (6)	58 (12)	70 (4)	75 (4)
	Bovine muscle	72 (6)	55 (3)	61 (3)	60 (7)	52 (5)	67 (6)	60 (10)
	Bovine liver	68 (15)	65 (13)	46 (6)	51 (17)	44 (11)	51 (17)	65 (10)
Acetonitrile/TCA (4:1)	Swine muscle	65 (13)	48 (9)	81 (8)	73 (15)	56 (8)	46 (11)	70 (12)
	Swine liver	60 (9)	57 (7)	82 (6)	80 (9)	59 (9)	61 (13)	80 (9)
	Bovine muscle	46 (6)	65 (10)	73 (15)	61 (14)	60 (17)	58 (4)	41 (9)
	Bovine liver	52 (4)	57 (14)	61 (11)	61 (6)	60 (9)	52 (4)	57 (12)

Table 2
Experimental domain and fractional factorial designs design matrix.

Parameter	Code	Level		
		Minimum	Central	Maximum
Flush volume (%)	V	40	50	60
Temperature (°C)	T	55	65	75
Pressure (bar)	P	60	70	80
Number of cycles	C	1	2	3

Recovery (%) ± RSD (%)

Experiment	V	T	P	C	Gatifloxacin	Danofloxacin	Sarafloxacin	Difloxacin	Ofloxacin	Flumequine	Enrofloxacin
1	40	55	60	1	52 ± 18	51 ± 13	59 ± 19	58 ± 10	62 ± 12	60 ± 9	49 ± 10
2	40	65	70	2	71 ± 11	65 ± 9	69 ± 7	72 ± 14	75 ± 10	66 ± 14	52 ± 17
3	50	75	80	3	62 ± 13	59 ± 11	66 ± 12	62 ± 9	62 ± 10	68 ± 14	65 ± 10
4	50	75	60	2	68 ± 11	60 ± 8	70 ± 13	71 ± 7	79 ± 8	61 ± 10	71 ± 7
5	50	65	70	2	86 ± 3	91 ± 2	89 ± 5	84 ± 6	87 ± 1	90 ± 2	87 ± 5
6	50	65	60	3	74 ± 6	79 ± 7	72 ± 8	78 ± 3	75 ± 6	66 ± 7	68 ± 8
7	60	55	80	2	53 ± 11	62 ± 8	55 ± 13	60 ± 10	63 ± 12	51 ± 16	60 ± 11
8	50	75	70	2	70 ± 8	63 ± 6	78 ± 8	75 ± 9	79 ± 7	70 ± 5	72 ± 7
9	60	65	80	1	61 ± 9	64 ± 10	51 ± 12	58 ± 11	62 ± 9	61 ± 14	50 ± 15

The experimental domain and the resulting FFD design matrix are shown in Table 2. The design consisted of 8 experiments performed by duplicate and 5 replicates for the central point. The results, in terms of average recoveries, are collected in Table 2. The average recoveries collected in Table 2 show that, the best extraction yields were obtained using solvent acetonitrile, 65 °C, 70 bar, two extraction cycles. The flush volume was kept constant at 50% of the cell volume. The optimized PLE conditions were further applied for method development and validation. Using the sample preparation procedure described, the blank chromatogram for all the matrices did not contain peaks at the retention times corresponding to any of the FQNs (see Fig. 3).

3.3. Method validation

Analytes in tissue sample extract were quantified by calibration curves obtained from extract spiked with standard solutions. The calibration parameters are calculated and appropriate linearity was observed. For HPLC, the calibration graphs showed excellent linearity in the studied concentration ranges (the correlation coefficients were more than 0.9990 for all the analytes), while RSD are below 20% in the low level and below 10% in high concentration range. The slope of the equations was very similar for meat extracts, liver or kidney of both animal species, indicating that this factor does not influence the results, and only a few intercept values were negative.

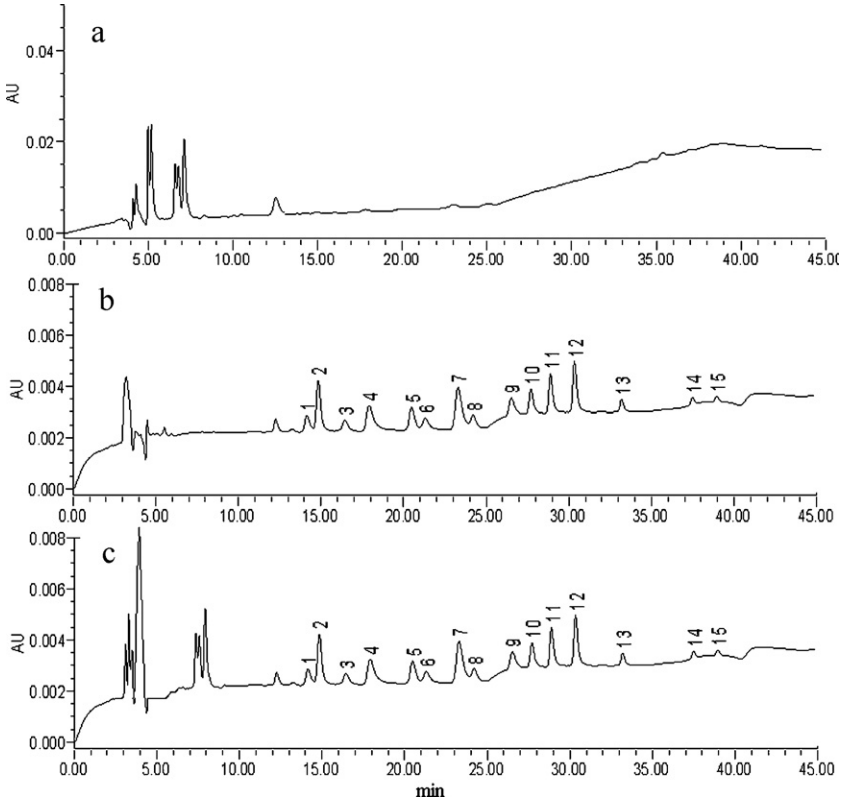

Fig. 3. HPLC chromatograms of blank sheep kidney (a), standard solution (b) and blank sheep kidney spiked with 15 FQNS of each concentration at 10 μg/kg (c).

Table 3
Recovery and reproducibility of 15 drugs in edible tissues of swine.

Drug	Swine muscle			Swine Liver			Swine kidney		
	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)
Marbofloxacin	10	70.6 ± 2.9	4.2	10	96.4 ± 3.1	3.2	10	87.8 ± 3.1	3.5
	150	94.4 ± 8.5	9.0	150	77.2 ± 3.6	4.7	150	87.3 ± 6.8	7.8
	300	92.1 ± 2.8	3.1	300	83.0 ± 3.5	4.2	300	88.0 ± 4.1	4.7
Enoxacin	10	95.7 ± 5.4	5.7	10	64.1 ± 4.0	6.2	10	87.1 ± 6.3	7.2
	20	84.4 ± 5.5	6.6	20	78.3 ± 6.0	7.7	20	86.9 ± 7.6	8.8
	40	94.1 ± 8.3	8.8	40	94.3 ± 7.4	7.9	40	87.5 ± 7.6	8.7
Fleroxacin	10	91.6 ± 8.9	9.8	10	79.8 ± 7.6	9.5	10	87.3 ± 9.3	10.7
	20	91.7 ± 9.5	10.4	20	83.7 ± 9.2	10.9	20	87.2 ± 4.6	5.3
	40	80.1 ± 1.8	2.2	40	89.2 ± 7.6	8.5	40	87.4 ± 2.3	2.7
Ofloxacin	10	97.2 ± 6.8	7.0	10	97.3 ± 6.7	6.8	10	87.1 ± 6.5	7.5
	20	92.8 ± 5.0	5.4	20	97.2 ± 4.6	4.8	20	87.3 ± 4.6	5.3
	40	94.9 ± 8.6	9.1	40	96.5 ± 7.6	7.8	40	87.3 ± 7.5	8.6
Pefloxacin	10	92.4 ± 11.3	12.2	10	96.7 ± 11.7	12.1	10	87.4 ± 10.1	11.6
	20	90.7 ± 1.3	1.4	20	96.9 ± 0.3	0.3	20	87.5 ± 0.3	0.4
	40	107.4 ± 1.4	1.3	40	98.4 ± 1.0	1.0	40	87.7 ± 0.9	1.1
Lomefloxacin	10	90.9 ± 8.5	9.3	10	97.9 ± 0.8	0.8	10	95.3 ± 0.9	1.0
	20	71.6 ± 4.6	6.5	20	103.8 ± 8.5	8.2	20	96.5 ± 1.4	1.4
	40	86.1 ± 2.2	2.6	40	97.9 ± 5.9	6.0	40	92.7 ± 2.8	3.0
Danofloxacin	10	109.1 ± 4.2	3.8	10	80.0 ± 3.1	3.9	10	91.3 ± 5.9	6.4
	100	84.8 ± 1.1	1.3	200	85.7 ± 1.8	2.1	200	96.4 ± 3.9	4.1
	200	82.2 ± 4.0	4.9	400	81.1 ± 2.5	3.1	400	96.6 ± 4.1	4.2
Enrofloxacin	10	97.9 ± 4.2	4.3	10	90.7 ± 15.2	16.8	10	95.5 ± 3.4	3.5
	100	86.4 ± 3.5	4.1	200	94.4 ± 2.0	2.2	300	96.4 ± 6.6	6.9
	200	91.0 ± 6.8	7.5	400	85.8 ± 4.5	5.2	600	97.0 ± 6.9	7.1
Orbifloxacin	10	89.5 ± 7.1	7.9	10	79.6 ± 3.9	5.1	10	97.3 ± 6.6	6.8
	20	80.9 ± 6.8	8.4	20	87.2 ± 6.9	7.9	20	96.7 ± 1.3	1.3
	40	87.2 ± 5.4	6.2	40	90.4 ± 6.8	7.6	40	96.8 ± 5.2	5.4
Cinoxacin	10	90.4 ± 4.2	4.6	10	91.7 ± 7.2	7.9	10	87.2 ± 7.4	8.5
	20	91.7 ± 7.2	7.6	20	91.4 ± 1.5	1.7	20	96.9 ± 6.9	7.2
	40	91.5 ± 10.8	11.8	40	87.9 ± 5.6	6.4	40	98.7 ± 10.5	10.6
Gatifloxacin	10	87.7 ± 3.4	3.9	10	86.1 ± 4.2	5.5	10	86.4 ± 9.3	10.8
	20	86.9 ± 6.6	7.6	20	96.4 ± 4.0	4.2	20	88.3 ± 10.3	11.6
	40	86.7 ± 6.9	7.9	40	87.6 ± 7.4	8.4	40	81.5 ± 2.3	2.8
Sarafloxacin	10	86.9 ± 6.6	7.6	10	87.8 ± 9.0	10.3	10	90.1 ± 6.5	7.2
	20	86.6 ± 3.9	4.5	20	87.6 ± 4.0	4.6	20	93.3 ± 4.5	4.8
	40	87.5 ± 6.9	7.8	40	97.2 ± 3.0	3.1	40	92.0 ± 7.4	8.0
Difloxacin	10	96.8 ± 9.2	9.5	10	92.3 ± 8.0	8.7	10	86.5 ± 7.6	8.7
	400	96.6 ± 10.6	11.0	800	101.6 ± 8.8	8.7	800	99.4 ± 0.9	0.9
	800	96.7 ± 2.4	2.5	1600	102.7 ± 9.2	9.0	1600	86.9 ± 10.6	5.2
Nalidixic Acid	10	87.2 ± 8.5	9.7	10	93.3 ± 6.5	7.0	10	92.5 ± 11.4	8.3
	20	87.0 ± 5.9	6.8	20	102.6 ± 3.9	3.8	20	89.5 ± 0.3	0.3
	40	86.6 ± 3.1	3.5	40	87.6 ± 5.8	6.7	40	79.1 ± 0.9	1.2
Flumequine	10	86.4 ± 1.8	2.1	10	85.2 ± 7.2	8.5	10	87.1 ± 0.8	0.9
	200	86.6 ± 4.1	4.7	500	91.4 ± 7.4	8.1	1500	87.1 ± 7.2	8.3
	400	86.4 ± 6.1	7.1	1000	93.2 ± 8.9	9.6	3000	88.2 ± 7.4	8.4

Table 4
Recovery and repeatability of 15 drugs in edible tissues of bovine.

Drug	Bovine muscle			Bovine Liver			Bovine kidney		
	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)
Marbofloxacin	10	97.5 ± 2.8	2.9	10	96.8 ± 3.1	3.2	10	87.1 ± 4.0	4.6
	150	102.5 ± 1.8	1.8	150	96.6 ± 7.3	7.6	150	87.4 ± 6.2	7.1
	300	103.3 ± 3.1	3.0	300	96.4 ± 4.0	4.1	300	87.4 ± 7.6	8.6
Enoxacin	10	103.6 ± 5.6	5.4	10	86.5 ± 7.6	8.7	10	96.7 ± 6.0	6.2
	20	104.8 ± 5.3	5.1	20	96.1 ± 7.4	7.7	20	86.4 ± 9.3	10.8
	40	104.8 ± 8.3	7.9	40	96.9 ± 7.6	7.8	40	86.9 ± 10.6	12.2
Fleroxacin	10	104.6 ± 9.1	8.7	10	86.8 ± 6.2	7.2	10	104.0 ± 1.9	1.9
	20	100.5 ± 8.9	8.9	20	96.6 ± 10.6	10.9	20	86.6 ± 4.0	4.6
	40	106.2 ± 2.1	2.0	40	96.3 ± 2.4	2.5	40	96.9 ± 9.2	9.5
Ofloxacin	10	102.9 ± 7.2	7.0	10	87.0 ± 10.6	12.2	10	87.0 ± 7.6	8.7
	20	101.9 ± 5.0	4.9	20	96.7 ± 4.6	4.8	20	96.5 ± 7.4	7.7
	40	102.2 ± 8.6	8.5	40	97.1 ± 7.6	7.8	40	86.9 ± 9.3	10.7
Pefloxacin	10	101.4 ± 13.2	13.0	10	87.2 ± 2.4	2.7	10	97.8 ± 6.7	6.8
	20	96.3 ± 1.0	1.1	20	97.9 ± 0.3	0.3	20	97.0 ± 11.7	12.1
	40	102.6 ± 1.0	0.9	40	97.7 ± 1.0	1.0	40	86.8 ± 6.6	7.6
Lomefloxacin	10	97.3 ± 3.1	3.1	10	92.3 ± 8.0	8.7	10	97.3 ± 2.4	2.5
	20	97.2 ± 10.2	10.7	20	101.6 ± 8.8	8.7	20	97.2 ± 6.6	6.8
	40	96.2 ± 4.0	4.2	40	102.7 ± 9.2	9.0	40	96.2 ± 4.6	4.8
Danofloxacin	10	87.0 ± 7.5	8.7	10	86.6 ± 7.0	8.1	10	97.3 ± 7.6	7.8
	200	97.3 ± 6.0	6.1	400	104.6 ± 7.0	6.7	400	96.5 ± 11.7	12.2
	400	97.1 ± 7.6	7.8	800	105.9 ± 3.1	2.9	800	97.1 ± 0.3	0.3
Enrofloxacin	10	96.8 ± 9.2	9.5	10	105.2 ± 19.0	18.1	10	96.8 ± 1.0	1.0
	100	96.6 ± 10.6	11.0	300	98.8 ± 3.1	3.1	200	96.6 ± 0.8	0.8
	200	96.7 ± 2.4	2.5	600	110.7 ± 5.5	5.0	400	96.7 ± 6.0	6.2
Orbifloxacin	10	97.5 ± 6.6	6.8	10	105.3 ± 5.2	5.0	10	97.5 ± 7.4	7.6
	20	97.2 ± 4.6	4.8	20	101.5 ± 5.1	5.0	20	97.2 ± 7.6	7.8
	40	97.1 ± 7.6	7.8	40	103.0 ± 8.6	8.3	40	97.1 ± 9.2	9.5
Cinoxacin	10	97.0 ± 11.7	12.1	10	101.4 ± 13.3	13.1	10	97.0 ± 10.6	10.9
	20	98.1 ± 7.5	7.7	20	91.9 ± 1.2	1.3	20	98.1 ± 3.1	3.1
	40	97.3 ± 11.6	11.9	40	100.6 ± 1.0	1.0	40	97.3 ± 7.2	7.6
Gatifloxacin	10	86.8 ± 3.1	3.5	10	74.1 ± 8.8	11.9	10	86.5 ± 6.2	7.2
	20	86.7 ± 8.6	9.9	20	98.1 ± 9.2	9.4	20	97.1 ± 7.6	7.8
	40	86.9 ± 4.0	4.6	40	92.4 ± 1.9	2.0	40	97.5 ± 9.2	9.4
Sarafloxacin	10	86.3 ± 7.6	8.8	10	102.4 ± 7.3	7.1	10	86.6 ± 5.3	6.1
	20	97.1 ± 7.4	7.6	20	101.1 ± 5.1	5.1	20	97.0 ± 2.4	2.5
	40	86.8 ± 7.5	8.7	40	94.6 ± 8.6	9.1	40	97.3 ± 6.7	6.8
Difloxacin	10	85.6 ± 9.4	10.7	10	97.6 ± 13.2	13.6	10	86.9 ± 10.6	12.1
	400	97.0 ± 4.6	4.8	1400	95.8 ± 1.1	1.2	800	96.9 ± 7.6	7.8
	800	86.6 ± 2.5	2.7	2800	103.0 ± 1.2	1.2	1600	97.8 ± 10.6	10.8
Nalidixic Acid	10	86.3 ± 4.6	5.3	10	102.9 ± 2.3	2.3	10	86.5 ± 6.6	7.6
	20	97.7 ± 2.4	2.4	20	98.9 ± 3.9	3.9	20	97.5 ± 6.6	6.8
	40	86.1 ± 3.1	3.6	40	96.8 ± 3.0	3.0	40	97.1 ± 4.6	4.7
Flumequine	10	86.7 ± 5.4	6.2	10	98.3 ± 5.7	5.8	10	97.0 ± 7.6	7.8
	200	97.2 ± 10.6	10.9	500	90.4 ± 5.5	6.1	1500	96.9 ± 1.9	2.1
	400	86.6 ± 1.0	1.1	1000	88.1 ± 8.1	9.2	3000	97.4 ± 6.0	6.1

Table 5
Recovery and reproducibility of 15 drugs in edible tissues in chicken and fish.

Drug	Chicken muscle			Chicken liver			Fish muscle		
	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)	Spiked level (μg/kg)	Overall recovery (%)	RSD (%)
Marbofloxacin	10	87.3 ± 3.1	3.5	10	78.9 ± 7.0	8.9	10	75.4 ± 3.2	4.2
	20	86.8 ± 7.1	8.2	20	95.3 ± 5.0	5.3	20	84.0 ± 1.9	2.3
	40	88.7 ± 4.0	4.5	40	89.6 ± 8.5	9.5	40	86.1 ± 4.2	4.9
Enoxacin	10	86.9 ± 6.2	7.2	10	102.6 ± 3.3	3.0	10	87.2 ± 7.4	8.5
	20	86.5 ± 7.6	8.8	20	95.4 ± 3.2	3.3	20	87.6 ± 7.4	8.4
	40	87.0 ± 7.5	8.7	40	94.0 ± 4.9	5.2	40	92.8 ± 1.0	1.0
Fleroxacin	10	87.0 ± 9.3	10.7	10	86.8 ± 7.6	8.2	10	92.9 ± 2.8	3.0
	20	94.5 ± 3.0	3.1	20	92.5 ± 5.4	5.9	20	88.8 ± 0.8	0.9
	40	87.0 ± 2.3	2.7	40	106.2 ± 5.3	5.0	40	93.0 ± 2.6	2.8
Ofloxacin	10	95.3 ± 8.2	8.6	10	87.0 ± 6.6	7.5	10	92.0 ± 3.6	4.0
	20	87.1 ± 4.6	5.3	20	98.9 ± 8.9	9.0	20	88.8 ± 3.4	3.8
	40	97.2 ± 3.0	3.1	40	97.1 ± 9.4	9.7	40	87.0 ± 7.5	8.7
Pefloxacin	10	87.0 ± 9.6	10.4	10	87.6 ± 0.3	0.4	10	87.0 ± 7.5	8.7
	20	96.4 ± 1.8	1.8	20	108.0 ± 1.0	0.9	20	88.9 ± 2.3	2.6
	40	86.6 ± 1.0	1.1	40	114.5 ± 1.1	1.0	40	80.6 ± 10.6	11.2
Lomefloxacin	10	87.1 ± 6.3	7.2	10	80.4 ± 3.2	3.9	10	99.8 ± 3.1	3.1
	20	86.8 ± 7.6	8.7	20	95.5 ± 9.4	9.8	20	87.9 ± 4.8	5.5
	40	93.9 ± 3.3	3.1	40	91.7 ± 1.8	1.9	40	87.8 ± 9.0	10.3
Danofloxacin	10	86.8 ± 9.3	10.7	10	100.5 ± 7.0	6.9	10	87.6 ± 4.0	4.6
	200	86.6 ± 7.6	9.2	400	95.0 ± 5.0	5.3	100	87.2 ± 3.5	4.0
	400	87.0 ± 2.3	2.7	800	95.4 ± 8.5	9.0	200	92.3 ± 8.0	8.7
Enrofloxacin	10	87.0 ± 3.1	3.5	10	88.2 ± 7.6	8.6	10	90.7 ± 1.6	1.8
	100	86.7 ± 7.0	7.6	200	94.2 ± 1.0	1.1	100	89.5 ± 2.0	2.2
	200	90.1 ± 9.4	10.4	400	101.3 ± 1.1	1.1	200	86.9 ± 4.0	4.6
Orbifloxacin	10	86.6 ± 6.5	7.5	10	86.4 ± 4.6	5.3	10	91.0 ± 5.6	6.1
	20	94.6 ± 8.9	9.2	20	98.6 ± 3.0	3.0	20	90.7 ± 7.6	8.4
	40	90.5 ± 9.1	10.0	40	97.2 ± 5.4	5.6	40	86.2 ± 7.6	8.8
Cinoxacin	10	86.9 ± 11.6	13.4	10	96.7 ± 5.3	5.5	10	95.8 ± 3.8	4.0
	20	87.4 ± 0.3	0.4	20	111.1 ± 8.2	7.4	20	77.6 ± 2.9	3.7
	40	86.7 ± 1.0	1.1	40	109.1 ± 8.9	8.2	40	84.2 ± 4.8	5.7
Gatifloxacin	10	87.3 ± 3.1	3.5	10	87.2 ± 7.8	9.5	10	77.1 ± 2.6	3.4
	20	97.4 ± 3.4	3.5	20	97.4 ± 6.1	6.3	20	90.8 ± 6.6	7.3
	40	88.1 ± 4.0	4.5	40	96.7 ± 2.3	2.4	40	90.7 ± 4.0	4.5
Sarafloxacin	10	87.2 ± 6.2	7.1	10	96.5 ± 3.5	3.6	10	95.6 ± 5.2	5.5
	300	86.1 ± 7.6	8.8	1900	97.7 ± 5.0	5.2	300	97.6 ± 8.7	8.9
	600	87.3 ± 7.5	8.6	3800	97.0 ± 3.5	3.6	600	88.6 ± 7.9	8.9
Difloxacin	10	86.8 ± 9.3	10.7	10	86.8 ± 2.3	2.7	10	73.0 ± 0.9	1.2
	20	96.6 ± 6.6	6.6	100	97.4 ± 7.2	7.4	20	97.2 ± 4.8	5.0
	40	97.8 ± 4.0	4.1	200	97.1 ± 12.4	12.7	40	97.6 ± 2.9	3.0
Nalidixic Acid	10	73.2 ± 1.9	2.6	10	78.2 ± 1.5	1.9	10	75.2 ± 1.5	2.0
	20	86.7 ± 4.6	5.3	20	86.7 ± 2.3	2.7	20	89.4 ± 10.2	11.4
	40	87.0 ± 7.5	8.7	40	97.7 ± 2.6	2.7	40	90.6 ± 5.2	5.7
Flumequine	10	86.8 ± 10.6	12.2	10	97.7 ± 3.6	3.7	10	86.8 ± 6.6	7.6
	400	87.4 ± 0.3	0.4	800	97.5 ± 0.7	0.7	600	92.3 ± 10.8	11.7
	800	86.7 ± 1.0	1.1	1600	97.5 ± 2.7	2.7	1200	92.6 ± 2.1	2.3

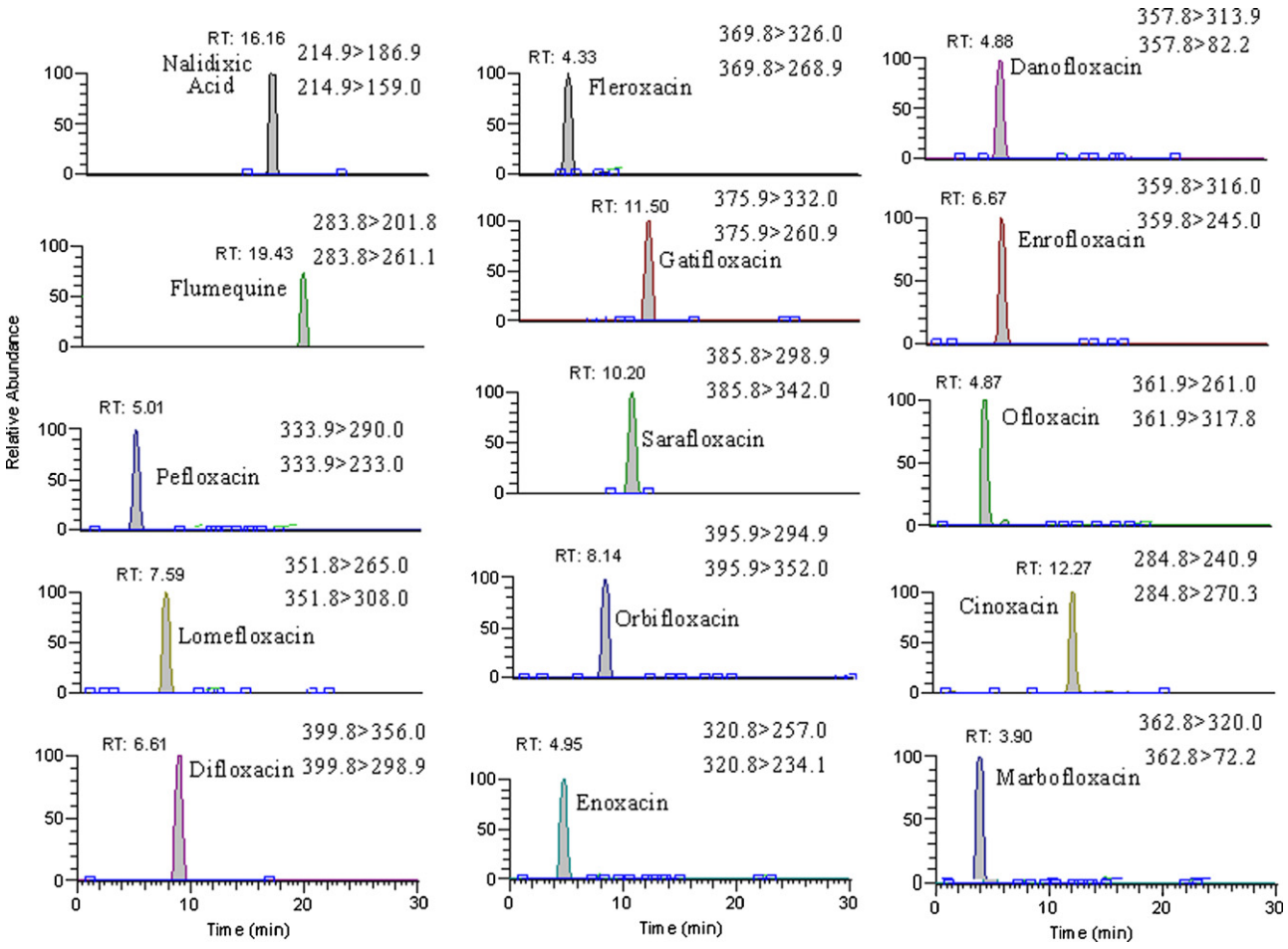

Fig. 4. LC–MS/MS chromatograms of 15 FQNS standard solution (5 μg/L).

Typical chromatograms of blank and fortified tissue samples are shown in Fig. 4. The obtained chromatograms did not show any interference, as no detectable matrix peaks were eluted in the retention time of the target FQNS.

Under the instrumental conditions reported in the experimental section, the calibration curves presented good linearity (r for all curves were >0.999). To evaluate the accuracy and precision of the method, blank and spiked tissue sample were analyzed using the optimized analytical method. The results are collected in Tables 3–5. Recoveries of 76.1–89.9% were obtained for the target FQNs at all fortification levels with RSDs lower than 9%.

For HPLC method, the LOD for 15 FQNs in all tissues was 3 μg/kg, the LOQ for 15 FQNs was 10 μg/kg. All performances are improved with tandem LC–MS/MS, the LOD was 0.3 μg/kg for each drug in edible tissues, the LOQ was 1 μg/kg for each drug by LC–MS/MS.

3.4. Stability

Stabilities of 15 analytes in different solutions as stock solutions, standard working solution (100 mg/L) and resulting extracts (Blank swine liver sample was spiked at levels of 50 μg/kg and then extracted) were studied. Using the same calibration set, after comparing with the background noise in various matrices, the results demonstrated that, there were no interfering peaks that could be detected on the expected retention time for these target analytes (within 2.5%). Consequently, stock solutions were found to be stable for at least 3 months in plastic tube at −20 °C, standard working solution for 2 week (except lomefloxacin, it was stable for 2 months), and resulting extracts for 1 week at 4 °C.

4. Conclusion

This work reports for the first time the application of ASE to the extraction of 15 FQNs from foods of animal origin. The method allows obtaining higher recoveries compared to the most commonly used traditional extraction methods and increasing sample throughput due to the high ASE automation grade. The optimized ASE procedure provided good accuracy and precision values by choosing acetonitrile as solvent. The LODs and LOQs of the HPLC method were found to be within the range 3–10 μg/kg, showing that the method could be useful for the determination of FQNs residues in contaminated foods of animal origin and screening of illegal use of FQNs in husbandry. The method was successfully applied to the analysis of real samples from supermarket and confirmation of such residues has been performed by LC–MS/MS analysis.

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