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Simultaneous Determination of Trace Levels of 10 Quinolones in Swine, Chicken, and Shrimp Muscle Tissues Using HPLC with Programmable Fluorescence Detection

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A HPLC method using a modified sample preparation procedure was optimized and validated for the quantification of 10 quinolones (QNs), including marbofloxacin, ciprofloxacin, norfloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin, difloxacin, oxolinic acid, and flumequine, in swine, chicken, and shrimp tissues. In this method, only a small mass (≤2.0 g) of sample and a relatively small volume of organic reagent (≤4.6 mL) of a nonchlorinated extraction solvent were required. The QNs were analyzed by liquid chromatography in a single run using a gradient elution program and with a programmable fluorescence detector to obtain optimum detection wavelengths. Mean recoveries of 10 drugs from edible animal tissues at a concentration range of 1−100 ng g^{-1} were 72.8−106.8% with relative standard deviations below 11.2%. The limits of quantification for each QN in different muscle tissues ranged from 0.3 to 1.0 ng g^{-1}, which were below the lowest maximum residue limits (10 ng g^{-1}) established in many countries. The method was also applied to the measurement of QN residues in commercial muscle samples. The results showed it was rapid, simple, sensitive, and suitable for use in food surveillance programs.

KEYWORDS: Quinolones; residue; swine; chicken; shrimp; muscle; HPLC; programmable fluorescence detection

INTRODUCTION

Quinolones (QNs) are synthetic antibacterial agents with a broad spectrum of activities. The antimicrobial targets of QNs are bacterial DNA gyrase and topoisomerase IV enzymes essential for DNA replication and transcription (*1*, *2*). They have been widely used in food-producing animals, aquaculture, and humans to treat bacterial infections. As for other antibiotics, their use in food-producing animals as well as aquaculture could result in residues in edible tissues. High levels of residues in food could cause toxic effects and/or allergic reactions in consumers and lead to the development of resistance of human pathogens to QNs (*3*, *4*). To protect the health of consumers, many countries including the People's Republic of China (PRC) have established maximum residue limits (MRLs) for QNs in food-producing animals. The MRLs in swine, chicken, and shrimp tissues established by the European Union (EU) and the PRC (*5*, *6*) range from 100 to 500 ng g^{-1} for most QNs and 10 ng g^{-1} for sarafloxacin (PRC only). The U.S. Food and Drug Administration (FDA) has actually banned the use of enrofloxacin in poultry because of the emergence of resistance of *Campylobacter* to QNs, which may result in ineffective treatment of human diseases by these antibiotics (*7*).

Many chromatography techniques, including several reviews concerning analysis methods (*8*−*10*), had been reported for monitoring quinolones in biological samples. High-performance liquid chromatography (HPLC) with fluorescence, ultraviolet (*11*−*15*), or mass spectrometric (*16*−*19*) detection were the most commonly used analytical methods for the determination of QN antibiotics in foods of animal origin. However, most of these published methods used complicated sample preparation procedures prior to chromatographic analysis and require large amounts of organic solvents (*11*, *17*, *18*, *20*, *21*), especially halogenated solvents (*11*, *13*−*15*, *20*), which could present a greater health hazard than the pesticides or drug residues to be determined (*22*). Moreover, sample preparation is the part of the analytical procedure most likely to contribute to analytical uncertainty. The advent of modern chromatographic instruments with automated injection and routine data handling means that sample preparation is the most time-consuming, labor intensive, and therefore costly part of the procedure when dealing with complex matrices (*23*).

The objective of this study was to improve the sample preparation process and to develop a simple, rapid, and sensitive method for the determination of residual multiple QNs in edible animal tissues without the need for large amounts of organic solvents. The muscle matrices were

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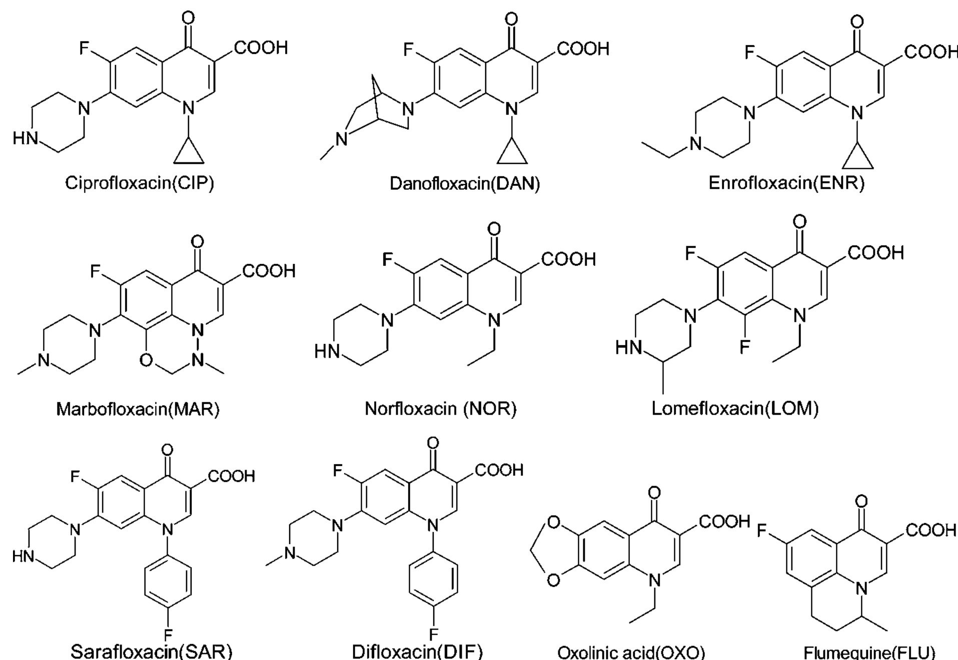

Figure 1. Chemical structures of quinolones.

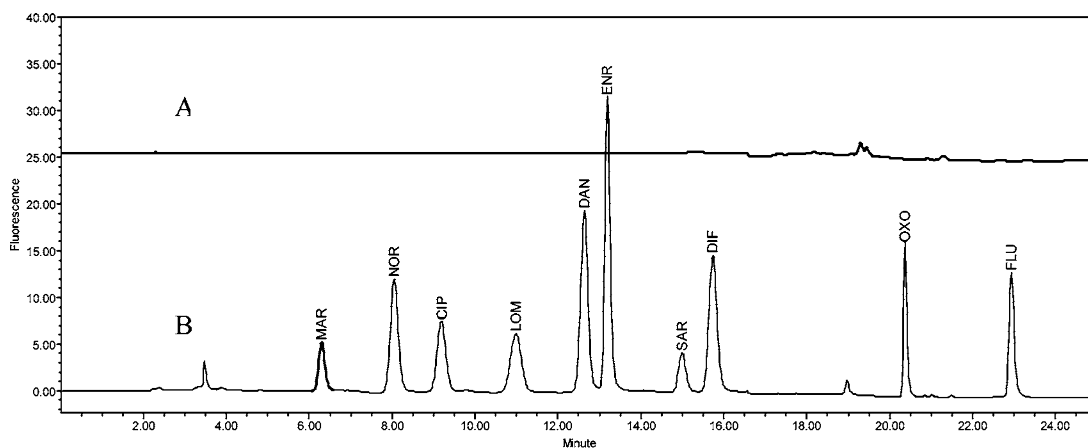

Figure 2. Chromatograms of (**A**) blank swine muscle and (**B**) spiked swine muscle at 100 ng g^{-1} except for DAN at 30 ng g^{-1}.

Table 1. Calibration Equation, LOD, and LOQ for Each QN Drug

drug	concentration range (ng mL^{-1})	calibration equation[a]	r^2 ($n = 3$)	LOD (ng g^{-1})	LOQ (ng g^{-1})
MAR	0.3–1000	$Y = (5.7 \times 10^3)C - 1.3 \times 10^5$	>0.99	0.3	1.0
NOR	0.3–1000	$Y = (7.1 \times 10^3)C - 1.1 \times 10^5$	>0.99	0.3	1.0
CIP	0.3–1000	$Y = (5.3 \times 10^3)C - 9.5 \times 10^3$	>0.99	0.3	1.0
LOM	0.3–1000	$Y = (4.2 \times 10^3)C - 6.0 \times 10^3$	>0.99	0.3	1.0
DAN	0.1–1000	$Y = (1.2 \times 10^4)C - 2.8 \times 10^4$	>0.99	0.1	0.3
ENR	0.3–1000	$Y = (1.1 \times 10^4)C - 7.6 \times 10^2$	>0.99	0.3	1.0
SAR	0.3–1000	$Y = (4.4 \times 10^3)C - 4.5 \times 10^3$	>0.99	0.3	1.0
DIF	0.3–1000	$Y = (7.6 \times 10^3)C - 6.8 \times 10^3$	>0.99	0.3	1.0
OXO	0.3–1000	$Y = (3.0 \times 10^3)C - 2.4 \times 10^3$	>0.99	0.3	1.0
FLU	0.3–1000	$Y = (3.8 \times 10^3)C - 3.9 \times 10^3$	>0.99	0.3	1.0

[a] *Y*, chromatographic peak area; *C*, drug concentration (ng mL^{-1}).

simply vortexed (10 s) and then centrifuged for 5 min at 3500 rpm with phosphate buffer as extraction solution, and these steps were repeated. The supernatant was percolated into a SPE cartridge for cleanup prior to HPLC analysis.

MATERIALS AND METHODS

Reagents and Apparatus. The vortex mixer (model HQ-60) was from North-Biotechnology Co. (Beijing, China), and the centrifuge was purchased from Hettich Co. (Kirchlengern, Germany). The 12-sample nitrogen evaporator (N-EVAP-111) with a heating bath was from

Table 2. Comparison of Different Preparations of Samples with the Procedure in This Study

matrix	drug	sample preparation[a]	detection	LOD (ng g^{-1})	recovery (RSD)(%)	ref
chicken, swine, and shrimp muscles	CIP, ENR, SAR, DIF, MAR, DAN, NOR, LOM, OXO, FLU	(1) 10 mL of PB, vortex for 10 s, 3500 rpm for 5 min, repeat (2) SPE HLB 2 mL of MeOH condition 2 mL of MeOH/10% NH_4OH (19:1) eluting	LC-FLD	0.3–1.0	72–107 (0.7–11.2)	this study
chicken muscles	CIP, ENR, SAR, DIF, MAR, DAN, OXO, FLU, NAL	(1) 200 μL of ACN Ult 20 s, 800 μL of ACN rinse (2) 17000*g* for 3 min at 5 °C (3) Ev + 500 μL of Tris + 300 μL of hexane, 17000*g* for 3 min at 5 °C	three runs LC-FLD	0.5–35	59–77 (4.2–15.5)	*2*
fish and pork muscle	CIP, ENR, SAR, OXO, FLU	(1) 10 mL of PB, Ult 10 min, 4000*g* for 10 min, repeat (2) SPE Dsc-18 3 mL of MeOH condition 5 mL of MeOH/NH_4OH (3:1) eluting	two runs LC-FLD	5–10	73–86 (1.3–14.1)	*12*
chicken muscles	CIP, ENR, SAR, DIF, DAN, OXO, FLU	(1) 10 mL of CH_2Cl_2 shaking 5 min, 366.5 rad/s 5 min (2) 20 mL of CH_2Cl_2 repeat (3) 2 mL of NaOH 209.4 rad/s 5 min, repeat (4) SPE SDB-RPS 2 mL of MeOH condition 2 mL of TFA/ACN (1:3) eluting	LC-UV	16–30	66–91 (4–15)	*13*
chicken muscle	CIP, ENR, SAR, DIF, NOR, DAN, MAR, NAL, OXO, FLU	8 mL of 5% TCA, vortex for 1 min, mix for 10 min, 14000*g* for 5 min at 4 °C	LC-FLD	4–11	29–68 (3.8–26.7)	*14*
pig muscle	CIP, ENR, SAR, DIF, DAN, MAR, OXO, FLU	(1) 25 mL of HPO_3/ACN (3:1) 3500 rpm 5 min (2) 10 mL of HPO_3/CAN (3:1) repeat (3) +75 mL of H_2O (4) SPE ENV 2 mL of MeOH condition, 7.5 mL of hexane defat, 5 mL of 2% TFA/ACN (1:3) and 1 mL of ACN eluting	LC-UV	9–12	81–99 (1–12)	*21*
poultry muscle	CIP, ENR, SAR, DIF, DAN, NOR, OXO, FLU	(1) 5 mL of 0.3% HPO_3/ACN (3:1) 3000 rpm, 10 min (2) 10 mL of 0.3% HPO_3/MeCN (3:1) repeat (3) +77 mL of H_2O (4) SPE ENV 2 mL of MeOH condition, 5 mL of hexane defat, 5 mL of 2% TFA/ACN (1:3) and 1 mL of ACN eluting	LC-UV	5–20	70–85 (3–6)	*25*
chicken livers	SAR, OXO, FLU	(1) AM/ACN (1:6) vortex for 1 min, homogenize for 1 min, 3000*g* for 2 min (2) repeat (3) +NaCl/Hex/DIE defat, 3000*g* for 1 min (4) 2000*g* for 5 min (5) ASTED	LC-FLD	0.2	87–97 (3.6–4.2)	*26*
milk	CIP, OFL, PEF, DIF, ENR, SAR, LOM, DAN, NOR, OXO, FLU	(1) 5 mL of 0.3% TFA/ACN (9:1) mixed Ult 5 min (2) 8000*g* for 6 min (3) SPE 3 mL of MeOH condition, 3 mL of 10% MeOH flushing, 3 mL of MeOH eluting	LC-FLD	1–23	69–88 (1.1–14)	*27*

[a] PB, phosphate buffer; Ult, ultrasonic probe; MeOH, methanol; ACN, acetonitrile; AM, ammonium aqueous; TFA, trifluoroacetic acid; TCA, trichloroacetic acid; CH_2Cl_2, dichloromethane; Hex, hexane; DIE, diethyl ether; NAL, nalidixic acid; OFL, ofloxacin; PEF, pefloxacin.

Organomation Associates Inc. (Berlin, MA). The HPLC system consisted of a Waters Alliance 2695 quaternary solvent delivery system with a 2475 fluorescence detector (Waters Co., Milford, MA). The reverse phase analytical column was a Symmetry C18 (250 mm × 4.5 mm i.d., 5 μm) from Waters Co. Acetonitrile and methanol (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ). The OASIS HLB solid-phase extraction cartridge (3 mL, 60 mg) was from Waters Co. Water was purified with a Milli-Q system (Millipore, Bedford, MA). All other reagents were of analytical grade.

Quinolone Standards. Ciprofloxacin hydrochloride (CIP, 99.8%), norfloxacin (NOR, 100.1%), lomefloxacin (LOM, 99.1%), enrofloxacin (ENR, 99.3%), sarafloxacin hydrochloride (SAR, 99.1%), oxolinic acid (OXO, 99.9%), and flumequine (FLU, 99.5%) were purchased from the China Institute of Veterinary Drug Control (Beijing, China). Danofloxacin (DAN, 99%) was a gift from Dr. Fangyang He (College of Veterinary Medicine, China Agricultural University). Difloxacin hydrochloride (DIF, 99.0%) was purchased from Sigma Chemical Co. (St. Louis, MO). Marbofloxacin (MAR, 99.0%) was sourced from Dr. Ehrenstorfer (Augsburg, Germany). Chemical structures of the QNs used in this study are shown in **Figure 1**.

Individual QN stock solutions (100 mg mL^{-1}) were prepared in methanol containing 2% of 0.03 mol L^{-1} sodium hydroxide and stored at 4 °C in brown volumetric flasks. Mixed working standards (1000 ng mL^{-1}) were prepared by diluting the stock solutions in methanol. The working solution was used to spike muscle samples or further diluted with phosphate buffer (PB) solution for the construction of calibration curves.

Chromatographic Conditions. The analysis of standards, fortified samples, and market samples was performed using a HPLC-programmable fluorescence detection system. The mobile phase consisted of aqueous formic acid solution (0.02%, pH 2.8) and acetonitrile and was run at a flow rate of 1.0 mL min^{-1} with a gradient program as follows: Acetonitrile was 9% for 8 min, increased 12% by 1 min, and maintained for 4 min. Then the organic solvent phase was increased by 45% from 13 to 17 min and maintained for 5 min. The analytical column was eluted using 90% acetonitrile during each analysis run. All of the analytes were eluted within 24 min, and a 9 min post time allowed reequilibration of the column. The injection volume was 100 mL, and the column temperature was maintained at 35 °C.

Table 3. Accuracy and Precision of Quinolones in Fortified Tissue Samples

		swine muscle			chicken muscle			shrimp tissue		
			RSD[a] (%)			RSD (%)			RSD (%)	
drug	fortification (ng g^{-1})	recovery (%)	intra-day	inter-day	recovery (%)	intra-day	inter-day	recovery (%)	intra-day	inter-day
MAR	1	89.3	3.5	4.2	90.2	3.2	5.1	88.5	0.9	1.9
	10	91.2	2.9	5.1	95.2	1.0	2.1	92.5	4.1	3.6
	50	92.5	1.1	3.2	93.2	2.9	8.2	91.4	4.5	4.9
	100	88.9	3.2	6.9	87.8	4.1	4.7	91.4	2.2	4.3
NOR	1	76.6	5.6	6.2	79.1	3.2	8.8	75.2	3.9	5.8
	10	79.3	3.1	3.9	73.8	3.9	5.5	76.5	4.1	6.3
	50	77.6	1.6	4.5	75.5	2.1	5.2	79.3	6.3	9.9
	100	78.3	1.8	1.6	74.1	3.3	4.9	78.9	0.5	4.1
CIP	1	83.1	7.3	5.9	75.8	1.7	6.1	84.8	2.3	8.7
	10	86.2	4.2	6.2	79.4	2.0	3.6	87.9	0.8	2.9
	50	81.0	1.7	3.8	75.2	2.9	4.9	79.9	1.1	2.1
	100	79.2	2.0	2.9	72.8	3.2	3.8	82.5	4.3	5.9
LOM	1	83.9	2.5	6.6	90.5	0.5	1.7	84.7	3.2	5.0
	10	86.1	1.3	4.1	90.5	4.1	3.6	88.6	3.7	6.1
	50	93.2	1.8	3.9	94.4	3.5	4.2	95.1	2.1	3.8
	100	91.1	0.7	1.6	93.4	4.2	4.3	94.2	0.8	2.6
DAN	0.3	92.5	3.7	5.3	87.9	2.5	2.0	93.3	5.9	8.8
	10	90.8	2.9	6.8	89.0	5.2	6.7	89.4	7.4	11.2
	50	80.8	1.5	7.2	86.2	3.2	2.6	90.7	4.9	9.9
	100	78.8	1.2	1.6	88.9	2.6	3.4	87.4	4.3	6.7
ENR	1	106.8	7.4	9.2	99.5	5.2	7.5	104.6	1.2	6.0
	10	95.3	5.9	7.8	99.8	1.1	6.8	98.9	3.0	8.9
	50	93.3	0.8	3.9	96.7	2.5	3.7	93.9	2.3	3.4
	100	91.5	1.4	3.4	98.5	1.3	8.4	95.8	0.8	7.5
SAR	1	86.5	3.6	6.6	73.7	6.7	8.8	79.4	1.1	6.8
	10	83.9	1.2	8.3	80.3	2.6	3.9	80.1	2.9	6.9
	50	84.1	2.1	6.9	76.1	3.0	4.2	78.9	1.5	4.2
	100	81.9	1.6	2.0	83.6	3.5	7.1	79.8	2.9	7.4
DIF	1	100.4	4.8	10.3	91.8	5.1	5.5	99.0	7.8	10.2
	10	97.3	4.2	4.9	97.1	3.2	4.5	93.9	4.1	4.9
	50	92.8	1.8	3.7	97.2	2.2	7.3	97.2	1.7	3.6
	100	91.5	1.1	1.5	98.3	2.6	6.2	98.3	2.9	6.9
OXO	1	73.6	2.8	8.8	88.0	5.3	7.9	87.4	5.3	7.9
	10	87.8	1.8	6.9	95.1	3.9	9.9	92.1	2.2	4.9
	50	86.8	1.1	6.5	99.2	2.7	2.0	91.7	1.6	6.3
	100	85.4	1.2	2.4	102.1	3.3	1.8	95.3	3.2	6.4
FLU	1	93.5	4.6	9.8	93.1	1.9	6.6	94.2	3.6	7.1
	10	92.5	2.7	6.5	103.2	3.6	4.9	98.5	4.9	5.2
	50	87.1	1.3	7.8	96.6	3.7	5.9	94.6	1.2	7.4
	100	86.2	1.3	2.3	99.2	1.6	2.0	95.4	3.2	3.9

[a] Relative standard deviation (intra-day, $n = 5$; inter-day, $n = 3$).

The fluorescence excitation/emission wavelengths were programmed at 297/515 nm for MAR from 0.0 to 6.8 min, at 280/450 nm for NOR, CIP, LOM, DAN, ENR, SAR, and DIF from 6.8 to 16.5 min, and at 320/365 nm for OXO and FLU from 16.5 to 35.0 min.

Sample Extraction. The following sample extraction was modified on the basis of a previous method (*12*). Briefly, 2 g of thawed and minced muscle tissues was weighed and placed in a 50-mL polypropylene centrifuge tube and spiked with the standard working solution. Ten milliliters of phosphate buffer solution (PBS, 0.01 M, pH 7.0) was added to the samples. The samples were allowed to stand for 15 min at room temperature, and then they were vortex mixed (about 10 s) before centrifugation for 5 min at 3500 rpm (2300*g*). The supernatant was collected, and the extraction was repeated. Ten milliliters of the combined extraction was percolated thorough an HLB SPE cartridge, which was preconditioned with 2 mL of methanol and 2 mL of HPLC grade water. After the cartridge had been washed with 3 mL of water/methanol (4:1, v/v), the compounds were eluted with 2 mL of 10% ammonia hydroxide aqueous solution/methanol (1:19, v/v). The collected eluate was evaporated to dryness under a gentle stream of N_2 at ca. 45 °C and reconstituted in 1 mL of PBS before injection to the HPLC system.

RESULTS AND DISCUSSION

Method Validation. The chromatogram of the mixed standard solution of 10 QNs is presented in **Figure 2**. The 10 QNs were well separated by the HPLC method in a single run with a programmable fluorescence detector and a gradient elution program. Standard calibration curves were constructed with standard solutions of 0, 0.3, 1, 5, 10, 50, 100, and 1000 ng mL^{-1}. The calibration curve plotting the peak area against concentration for each drug was linear at the concentration range of 0.3−1000 ng mL^{-1}, with correlation coefficients (r^2) of >0.99. The equation for each drug is shown in **Table 1**.

The limit of detection (LOD) and the limit of quantification (LOQ) were defined as a signal-to-noise ratio (S/N) of 3:1 or 10:1, respectively (*24*). As shown in **Table 1**, the LODs were 0.1 ng g^{-1} for DAN and 0.3 ng g^{-1} for all other QNs (MAR, NOR, CIP, LOM, ENR, SAR, DIF, OXO, and FLU). The LOQs were 0.3 ng g^{-1} for DAN and 1.0 ng g^{-1} for other QNs. The LODs were also lower than those reported for other studies, which are shown in **Table 2**, ranging from 6 to 30 ng g^{-1} (*13*)

Table 4. Determined Concentrations (Nanograms per Gram) of Quinolone Residues in Market Swine, Chicken, and Shrimp Samples in China

species	sample code	MAR	NOR	CIP	LOM	DAN	ENR	SAR	DIF	OXO	FLU
swine	BJ006	–[a]	–	–	–	–	2.5	–	–	–	–
	BJ028	–		–	–	–	5.1	–	–	–	–
	CD009	–	–	36.2	–	–	–	–	–	–	–
	NC003	–	2.3	4.7	–	–	18.2	–	–	–	–
	NC023	–	5.2	22.6	–	–	86.3	–	–	–	–
	NC024	–	43.2	73.6	–	–	160.9	–	–	–	–
	NC026	–	1.2	–	–	–	2.3	–	–	–	–
	NC028	–	–	34.6	–	–	3.4	–	–	–	–
	SH016	–	–	–	29.3	–	–	–	–	–	–
	SH017	–	–	–	13.9	–	–	–	–	–	–
	XA001	–	1.1	1.0	–	–	2.0	–	–	–	–
	XA021	–	2.1	48.7	–	–	82.2	–	–	–	–
chicken	BJ005	–	5.6	3.5	–	–	41.0	–	–	–	–
	BJ015	–	–	59.3	–	–	85.3	–	–	–	–
	BJ018	–	–	1.5	–	–	–	–	45.2	–	–
	BJ023	–	–	–	15.9	–	17.3	–	–	–	–
	BJ024	–	12.5	–	–	–	–	6.9	–	–	–
shrimp	BJ005	–	–	21.8	–	–	14.8	–	–	5.1	81.0
	BJ011	–	–	15.9	–	–	–	–	–	56.3	–
	BJ014	–	–	8.9	–	–	55.9	–	–	54.1	12.8

[a] –, below the limit of detection.

and from 4 to 11 ng g^{-1} (*14*). Similar LODs for several quinolones in chicken muscles were obtained by Yorke and Froc (*2*) (0.5−35 ng g^{-1}) and Ramos et al. (*12*) (5−10 ng g^{-1}), but these authors used two or three different HPLC conditions instead of a single run for all quinolones.

The precision (inter- and intra-day) and accuracy of the method were assessed using porcine and chicken muscle and shrimp samples spiked with QNs at 1, 10, 50, and 100 ng g^{-1} on three different days. Good recoveries were obtained for each QN at all fortification levels as shown in **Table 3**. The mean recoveries were between 73.6 and 106.8% in swine muscle with relative standard deviations (RSD) of 0.7−10.3%. In chicken tissue, recoveries were 72.8−103.2% for each analyte with RSD values of $<$9.9%. The recoveries of the 10 QNs in shrimp tissue ranged from 75.2 to 104.6% with RSD values of 0.8−11.2%. The recoveries and precision were better than or comparable to those achieved by the published methods with recoveries of 29−77% and RSD values of up to 15 or 26% (*2*, *14*).

Chromatograms of blank and fortified tissues are shown in **Figure 2**. The 10 QNs were well separated in a single run with a programmable fluorescence detector. No significant interfering peaks were found in the control sample, indicating high specificity and selectivity of the extraction and chromatographic method.

Sample Preparation. Because QNs are soluble in polar organic solvents, dichloromethane (*11*, *13*), acetonitrile (*11*, *17*, *18*, *20*, *21*, *25*−*27*), and trichloroacetic acid (*14*, *15*, *20*, *27*) were used as extraction solutions in most of the previously reported methods. Although good recoveries were obtained, the extraction process involved complex and time-consuming treatments and the use of large amounts of organic solvents, especially chlorinated solvents such as dichloromethane and trichloroacetic acid.

In this study, satisfactory recoveries ($>$72% from swine, chicken, and shrimp muscle tissues) were obtained using phosphate buffer as the extraction solution followed by solid-phase extraction. During the optimization of the sample preparation procedure, different vortex and centrifuging times were studied to obtain the best recovery and least preparation time. It was found that there was no difference with mixing for 10 s, 2 min, or longer and centrifugation for 5 min or longer. Therefore, homogenized tissues were extracted with phosphate buffer by vortexing for 10 s and centrifugation for 5 min and repeated before the extract was cleaned up on a SPE cartridge. Compared with the reported extraction procedures (including protein precipitation, defatting, and other complex treatments) listed in **Table 2**, the sample preparation procedure in this method used less organic solvents and fewer steps, but good recoveries, accuracy, LOD, and LOQ were obtained. Each sample extraction process used only 4.6 mL of organic solvent (methanol), and 40 samples were easily processed within 4 h; however, in many previous procedures, 10−25 mL of organic reagents, even chlorinated solvents, was used.

Determination of QN Residues in Market Samples. One hundred and fifty swine muscle samples randomly collected from the markets of five cities (Beijing, Shanghai, Xi'an, Chengdu, and Nanchang) in China 24 chicken muscles and 14 shrimp samples from the markets in Beijing were analyzed by the HPLC method. Twenty of 188 samples were found to contain one or multiple QNs at concentrations from 1.0 to 160.9 ng g^{-1} (**Table 4**). NOR, CIP, ENR, LOM, SAR, and DIF were determined in swine and chicken samples and OXO and FLU in shrimp.

Conclusions. In this work, an improved HPLC method was developed for the determination of 10 QNs from swine, chicken, and shrimp samples using phosphate aqueous solution prior to SPE cleanup. The high selectivity and sensitivity of the HPLC method achieved a quantification limit 10−100-fold lower than the MRLs established by the EU and PRC. The optimized procedure was suitable for use in practice.

ABBREVIATIONS USED

QNs, quinolones; MRLs, maximum residue limits; HPLC, high-performance liquid chromatography; FLD, fluorescence detection; MAR, marbofloxacin; NOR, norfloxacin; CIP, ciprofloxacin; LOM, lomefloxacin; DAN, danofloxacin; ENR, enrofloxacin; SAR, sarafloxacin; DIF, difloxacin; OXO, oxolinic acid; FLU, flumequine; S/N, signal-to-noise ratio; LOD, limit of detection; LOQ, limit of quantification; RSD, relative standard deviations; r^2, correlation coefficient.

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