Food Sci. Biotechnol. Vol. 18, No. 4, pp. 978 ~ 984 (2009)

Food Science and Biotechnology

Multiresidue Determination of Quinolones in Porcine, Chicken, and Bovine Muscle Using Liquid Chromatography with Fluorescence Detection

Sanghee Lee, Yousin Shim, Hyunju Kim, and Dongbin Shin*

Korea Food Research Institute, Seongnam, Gyeonggi 463-746, Korea

Abstract An analytical method for the simultaneous determination of 9 quinolones (QNs) in porcine, chicken, and bovine muscles was developed and validated using liquid chromatography-fluorescence detector (LC-FLD). The samples were extracted using a liquid-liquid extraction (LLE) process. Chromatographic separation was achieved on a reverse phase C_8 column with a gradient elution using a mobile phase of 200 mM ammonium acetate buffer (pH 4.5) and acetonitrile (ACN). The proposed method was validated according to the Food and Drug Administration (FDA) guideline for bioanalytical assay procedures. Recoveries of QNs were 83.1-111.9% with relative standard deviations (RSDs) below 15%. Linearity within a range of 30-500 μg/kg was obtained with the correlation coefficient (R^2) of 0.9967-0.9999. The limits of detection (LOD) were 1-16 μg/kg. These values were lower than the maximum residues limits (MRLs) established by the European Union (EU). The present method was successfully applied to determine QNs in edible muscles.

Keywords: quinolone, bovine muscle, chicken muscle, porcine muscle, high pressure liquid chromatography-fluorescence detector (HPLC-FLD), validation

Introduction

Quinolones (QNs) are synthetic antimicrobial agents with an excellent activity against a broad spectrum of Gram-negative and Gram-positive bacteria by inhibiting DNA gyrase or type IV topoisomerase used in human and veterinary medicines (1,2). Due to the excellence of activity, QNs have been approved for use in treating and preventing animal diseases. However, the misuse of QNs in veterinary practice might impact the consumer's health (3). Therefore, to ensure food safety, the European Union (EU) has established maximum residue limits (MRLs) for QNs in animal foodstuffs (4,5). The MRLs established by the EU range from 100 to 400 mg/kg for most QNs in porcine, poultry, and bovine muscles, while there is no prescribed MRLs for sarafloxacin (SAR) except salmon muscle.

Several published reports are available for screening QNs based on microbiological or immunoassay techniques (4,6) in biological samples. However, serious defects are found in the techniques with poor sensitivity and selectivity. Liquid chromatography (LC) is the most commonly used tool for the confirmation and quantitation of residual QNs. Among LC techniques, high pressure liquid chromatography (HPLC) coupled with ultraviolet (UV)(7,8), fluorescence (FL) (9-13) or mass spectrometric detection (MS) (14-16) is the most widely used technique, considering the specificity, rapidity, and selectivity of multiresidue analysis of QNs in animal foodstuffs.

The optimization of the conditions for extracting analytes from the animal muscles is the most crucial step to determine the residues of QNs, because it takes much time and efforts. In most cases, clean up and preconcentration steps using solid phase extraction (SPE) are carried out to reduce the matrix interference from samples before the chromatographic analysis (8,17,18), but the SPE procedure requires long time and high costs. Moreover, in some cases, the SPE procedures may result in low recovery. Therefore, liquid-liquid extraction (LLE), which minimizes the matrix effects, would be the most economic extracting method if it is developed.

Consequently, the objective of this present work was to develop a fast, precise, and accurate performance liquid chromatography-fluorescence detector (HPLC-FLD) method for simultaneous determination of 9 QNs [marbofloxacin, ciprofloxacin, norfloxacin (IS), danofloxacin, enrofloxacin, sarafloxacin, difloxacin, oxolinic acid, and flumequine] in porcine, chicken, and bovine muscles.

Materials and Methods

Reagents and chemicals Norfloxacin (NOR; internal standard, IS), danofloxacin (DAN), ciprofloxacin (CIP), difloxacin (DIF), enrofloxacin (ENR), oxolinic acid (OXO), and flumequine (FLU) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Marbofloxacin (MAR) and sarafloxacin (SAR) were obtained from Abbott Lab. (Abbott Park, IL, USA). Acetonitrile (ACN) and hexane were purchased from Fisher Scientific (Fair Lawn, NJ, USA), and ammonium acetate was obtained from Sigma-Aldrich. Water was purified with a Milli-Q system (Millipore, Bedford, MA, USA). All chemicals and solvents were used in LC grade.

Standard solution Individual QN stock solutions (100

*Corresponding author: Tel: +82-31-780-9126; Fax: +82-31-709-9280
E-mail: shindb@kfri.re.kr
Received February 12, 2009; Revised April 14, 2009;
Accepted April 21, 2009

μg/mL) of MAR, NOR, CIP, DAN, ENR, SAR, and DIF were prepared in ammonium acetate buffer containing 15% ACN. Individual QN stock solutions (100 μg/mL) of OXO and FLU were prepared in ammonium acetate buffer containing 50% ACN. These solutions were stored at 4°C in brown volumetric flasks. Mixed working solutions (1 μg/mL) were prepared by diluting the stock solutions in LC buffer on the day of the experiment. The working standard solution, used to spike the muscle samples or construct the calibration curves, prepared by diluting the mixed stock solution (1 μg/mL) to concentration 30, 50, 100, 250, and 500 ng/mL with LC buffer solution. A internal standard (IS) solution was prepared at a concentration of 100 μg/mL.

Instrumentation and analytical conditions The HPLC system was consisted of a Waters Alliance 2695 quaternary solvent delivery system with a 2475 fluorescence detector (Waters Co., Milford, MA, USA). Chromatographic separation of the QNs was performed on a Zorbax Eclipse XDB-C_8 column (150×4.6 mm i.d., 5 μm particle size) from Agilent (Palo Alto, CA, USA). The mobile phase consisted of 200 mM ammonium acetate buffer (pH 4.5; mobile phase A) and ACN (mobile phase B) was run at a flow rate of 1.5 mL/min with a gradient program as follows: 10% B (0-8 min), 10-17% B (3 min), 17% B (2 min), 17-45% B (4 min), 45% B (3 min), 45-90% B (1 min), and 90% B (2 min). After completing the chromatographic elution, the mobile phase was programmed to its initial condition within 1.5 min, and during the 6 min reconditioning time was set before next injection. The injection volume was 10 μL, and the column temperature was maintained at 35°C. The fluorescence excitation/emission wavelengths were programmed at 297/515 nm for MAR from 0.0 to

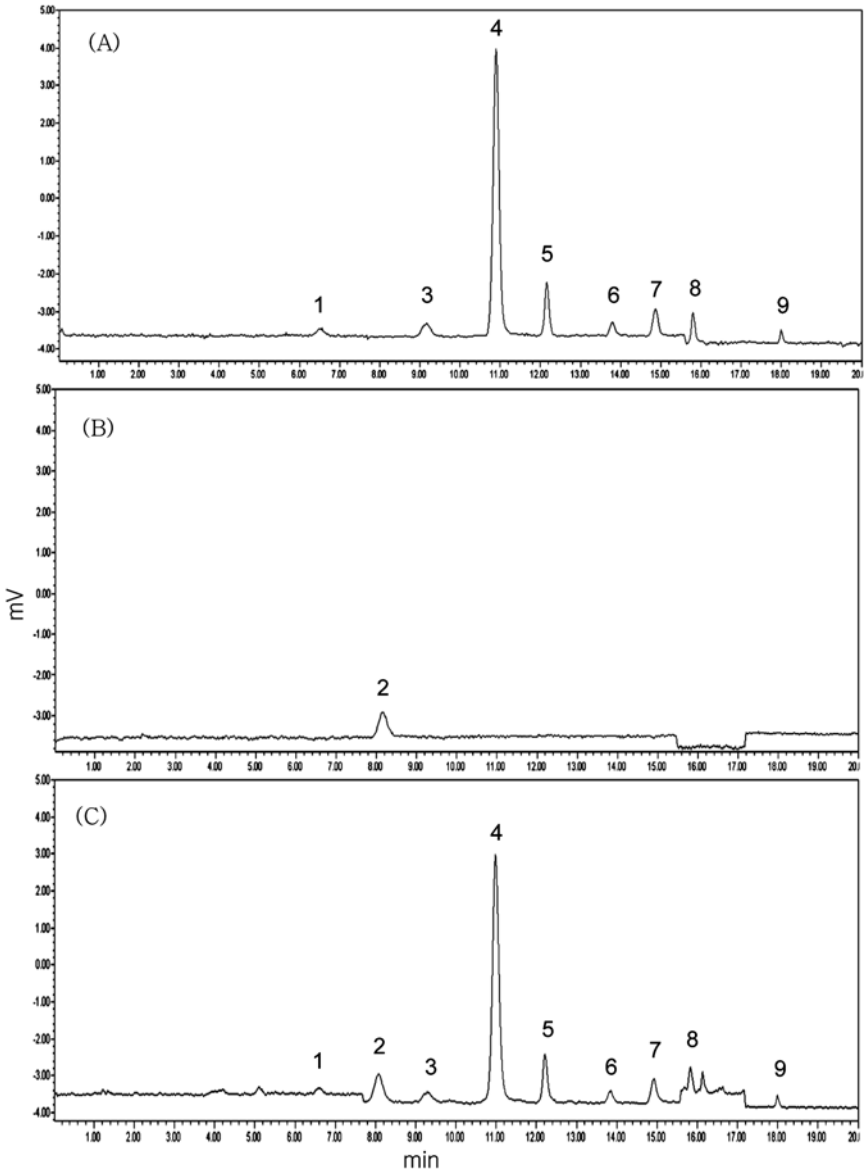

Fig. 1. HPLC chromatograms of (A) a standard mixture of the selected QNs, (B) an unspiked porcine muscle, and (C) a spiked porcine muscle at 100 μg/kg of QNs. Peaks identifications: (1) MAR, (2) NOR (IS), (3) CIP, (4) DAN, (5) ENR, (6) SAR, (7) DIF, (8) OXO, and (9) FLU.

7.0 min, at 280/450 nm for NOR, CIP, DAN, ENR, SAR, and DIF from 7.0 to 15.3 min, and at 260/365 nm for OXO from 15.3 to 17.1 min, and at 325/365 nm for FLU from 17.1 to 19.0 min.

Sample preparation One g of minced muscle was placed in a 50-mL polypropylene centrifuge tube containing 1 mL of 100 μg/kg solution of IS and 1 mL of 1 M trichloroacetic acid (TCA). Then, about 2 g of anhydrous sodium sulfate was added, thoroughly mixed for 15 sec, and left for 5 min. In order to extract QNs from animal muscle, 2 sequential volumes (8 and 5 mL) of ACN were used. After shaking for 5 min, 10 mL of *n*-hexane was added. The mixture was vortexed for 15 sec and centrifuged at 2,000×g for 20 min. The upper layers were discarded. The lower layers were transferred into a glass tube and dried at 50°C. The residue was reconstituted in 0.5 mL ACN and 0.5 mL mobile phase A. The sample was passed through a 0.45-μm PVDF syringe filter (Whatman, Maidstone, UK) before injection to the HPLC system.

Method validation After selection of the optimum conditions for the sample preparation step and the LC-FD measurements, validation of method was performed according to FDA guideline for bioanalytical assay procedures (19).

The selectivity of the method was assessed by investigating the presence or absence of any interference during the same chromatographic run as the examined QNs, with the established method for porcine, chicken, and bovine muscles.

Linearity was examined using a calibration curve, obtained by analyzing blank edible muscles spiked with IS at 100 μg/kg and QNs at 5 different concentrations (30, 50, 100, 250, and 500 μg/kg), with 3 replicates/concentration. Linear

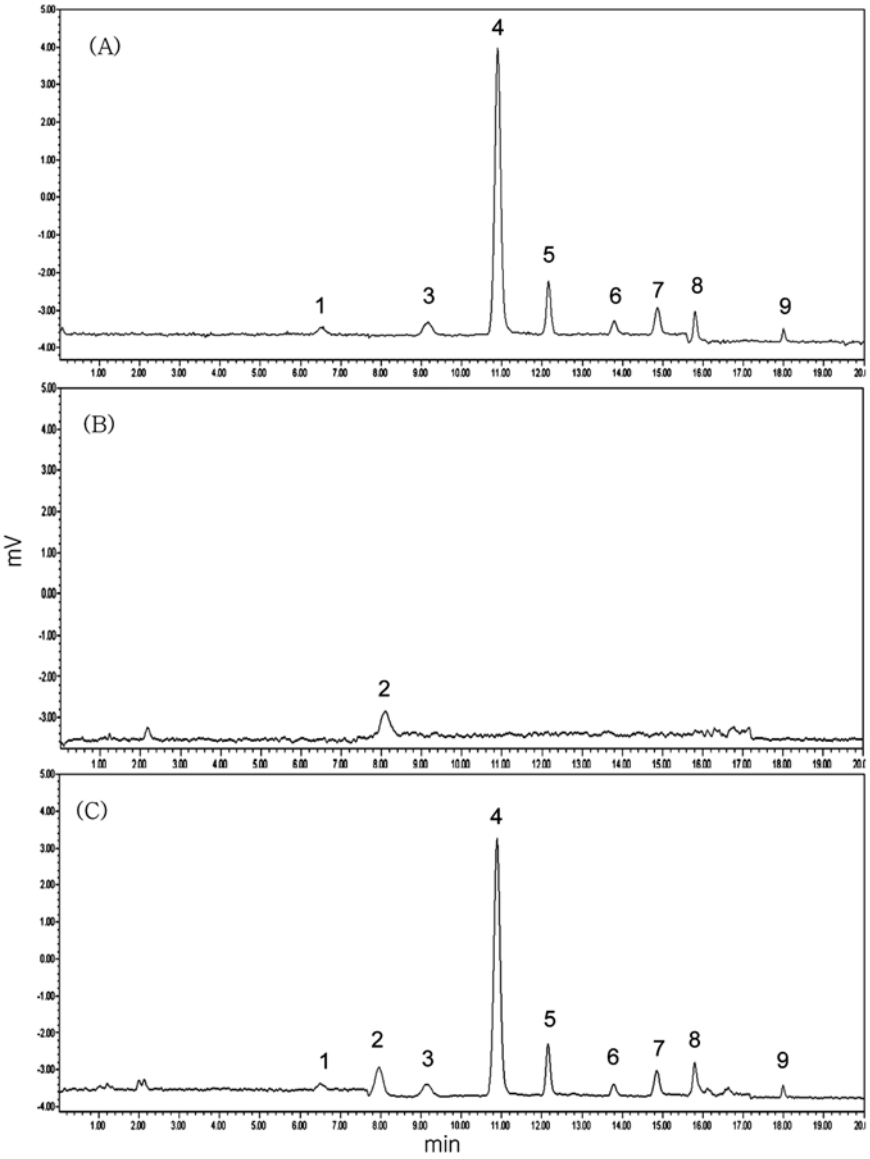

Fig. 2. HPLC chromatograms of (A) a standard mixture of the selected QNs, (B) an unspiked chicken muscle, and (C) a spiked chicken muscle at 100 μg/kg of QNs. Peaks identifications: (1) MAR, (2) NOR (IS), (3) CIP, (4) DAN, (5) ENR, (6) SAR, (7) DIF, (8) OXO, and (9) FLU.

regression analysis was performed using the ratio of analyte peak area/internal standard peak area vs. analyte concentration.

The limit of detection (LOD) and the limit of quantification (LOQ) of the method as 3.3 σ/S and as 10 σ/S, respectively were based on the standard deviation (SD), which was y-intercepts of regression analysis (σ) and the slope (S) of the calibration curve (20).

Precision and accuracy were assessed by analyzing spiked porcine, chicken, and bovine muscles at concentration levels of 50, 100, and 500 μg/kg. The 3 independent analyses were conducted each day for repeatability tests (n=3, intra-day precision) at 3 concentration levels (50, 100, and 500 μg/kg). This was repeated for 3 consecutive days for the purposes of the reproducibility test (n=9, inter-day precision) at same concentration levels.

Results and Discussion

Optimization of the chromatographic analysis Separation is usually performed with silica-based reversed-phase column, mainly packed with C_{18} or C_8 particles (8). As with the previous results (8), this study reports the use of a Zorbax Eclipse XDB-C_8 column for QNs determination, because this column permits a good separation and a short retention time of all analytes investigated in a single LC run. Also, the pH of the mobile phase was a critical factor in achieving the chromatographic separation of the QNs. In this study, citrate buffer-, formic acid-, and ammonium acetate aqueous solution-ACN was tested as the mobile phase, and the increased concentration of these aqueous solution were investigated (8,21). From 10 to 100 mM citrate buffer, 0.02%-0.1% formic acid, and 20-100 mM

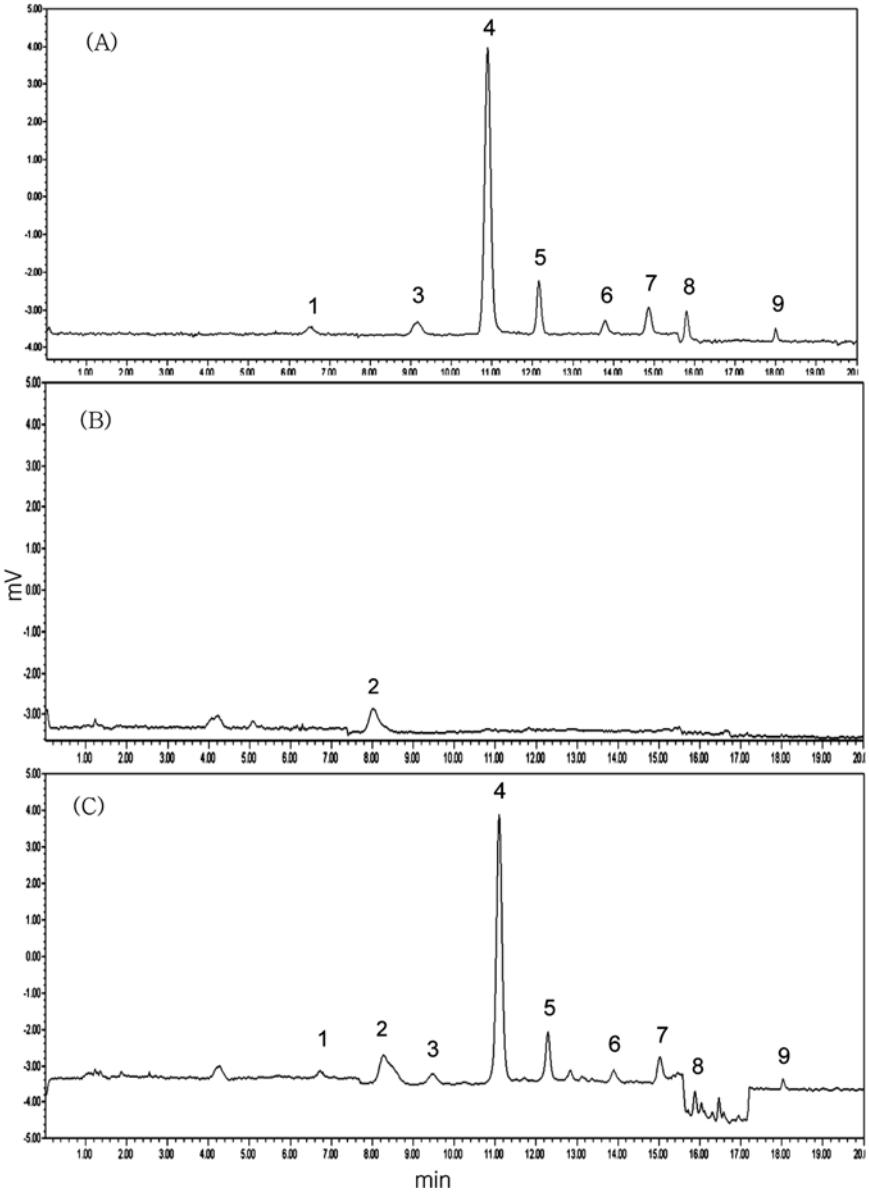

Fig. 3. HPLC chromatograms of (A) a standard mixture of the selected QNs, (B) an unspiked bovine muscle, and (C) a spiked bovine muscle at 100 μg/kg of QNs. Peaks identifications: (1) MAR, (2) NOR (IS), (3) CIP, (4) DAN, (5) ENR, (6) SAR, (7) DIF, (8) OXO, and (9) FLU.

ammonium acetate aqueous solution-ACN used as the mobile phase could lead to severely tailing peaks. For the best resolution of analytes, as in this study, 200 mM ammonium acetate aqueous solution-ACN was chosen as the mobile phase. The chromatogram obtained with the optimized mobile phase is shown in Fig. 1-3. As is shown in Fig. 1-3, no interference was observed in porcine, chicken, and bovine muscle blank samples.

Optimization of the sample preparation For the optimal extraction procedure, general extraction was carried out with ACN or MeOH-water mixtures containing metaphosphoric acid (HPO_3), trifluoroacetic acid (TFA), acetic acid, hydrogen chloride (HCl), TCA, sodium hydroxide (NaOH) or phosphate buffer, and then was purified using SPE (2,17,21,22). But, this extraction procedure was in some cases produced low recoveries and required expert skills (8,21). Moreover the most critical fault of SPE process is taking a too long in the time of analysis. Therefore, in order to improve these disadvantages, we tried to find out an optimal LLE process. The LLE procedure was based on previous method reported by Choi *et al*. (23). Consequently, the best recoveries (≥83%) were achieved according to sample preparation to determine the QNs from porcine, chicken, and bovine muscle.

Method validation selectivity As is shown in Fig. 1-3, the proposed method for 3 different blank muscle samples to verify the selectivity showed no interference around the retention time of the 9 QNs in any of the samples analyzed.

Linearity, limit of detection (LOD), and limits of quantitation (LOQ) Calibration curves were obtained by least-squares linear regression analysis using the ratio analyte peak area/internal standard peak area against analyte concentration.

All calibration data, as well as LOD and LOQ are presented in Table 1. Linearity is evaluated using concentration levels of 30, 50, 100, 250, and 500 μg/kg. Regression analysis revealed a correlation coefficient (R^2) of between 0.9982 and 0.9999 for porcine muscle, 0.9978-0.9998 for chicken muscle, and 0.9967-0.9998 for bovine muscle. The LODs of the various QNs extracted from porcine muscle ranged from 1 to 15 μg/kg, whereas in chicken muscle these values ranged from 1 to 16 μg/kg, and in bovine muscle, 1 to 12 μg/kg. The LOQs obtained from porcine, chicken, and bovine muscles are below the MRLs established in the Council Regulation 2377/90 of the EU (5).

Precision and accuracy The precision based on intra-day repeatability was assessed by replicate (n=3) measurements from 3 spiked muscle at 3 different concentration levels of 50, 100, and 500 μg/kg. Statistical evaluation revealed

Table 1. Linearity and sensitivity data of the developed method for the determination of QNs in spiked animal muscle

Analytes	Concentration range (mg/kg)	Slope (mg/kg)	Intercept	R^2	LOD (mg/kg)	LOQ (mg/kg)
Porcine muscle						
MAR	30-500	0.0018±0.0001	0.0327±0.0063	0.9997	11	34
CIP	30-500	0.0048±0.0005	-0.0640±0.0119	0.9991	8	25
DAN	30-500	0.0645±0.0062	0.1511±0.0212	0.9998	1	3
ENR	30-500	0.0100±0.0003	-0.0178±0.0076	0.9999	3	8
SAR	30-500	0.0024±0.0001	0.0293±0.0090	0.9991	12	37
DIF	30-500	0.0056±0.0004	-0.0001±0.0129	0.9989	8	23
OXO	30-500	0.0041±0.0004	-0.0095±0.0135	0.9982	11	33
FLU	30-500	0.0015±0.0001	0.0169±0.0070	0.9992	15	46
Chicken muscle						
MAR	30-500	0.0016±0.0001	0.0167±0.0065	0.9978	13	40
CIP	30-500	0.0039±0.0001	-0.0051±0.0059	0.9993	5	15
DAN	30-500	0.0573±0.0012	0.2283±0.0171	0.9998	1	3
ENR	30-500	0.0081±0.0002	0.0240±0.0074	0.9997	3	9
SAR	30-500	0.0021±0.0001	0.0205±0.0100	0.9996	16	48
DIF	30-500	0.0053±0.0001	0.0198±0.0124	0.9991	8	24
OXO	30-500	0.0037±0.0002	0.0978±0.0123	0.9989	11	34
FLU	30-500	0.0014±0.0001	0.0065±0.0029	0.9995	7	20
Bovine muscle						
MAR	30-500	0.0015±0.0001	0.0443±0.0049	0.9967	11	33
CIP	30-500	0.0037±0.0001	0.0237±0.0068	0.9993	6	18
DAN	30-500	0.0627±0.0002	0.1702±0.0093	0.9998	1	2
ENR	30-500	0.0087±0.0001	0.0067±0.0037	0.9990	1	4
SAR	30-500	0.0022±0.0001	0.0165±0.0053	0.9986	8	24
DIF	30-500	0.0054±0.0002	0.0004±0.0114	0.9994	7	21
OXO	30-500	0.0035±0.0004	-0.0519±0.0098	0.9990	9	28
FLU	30-500	0.0014±0.0001	0.0074±0.0051	0.9990	12	35

Table 2. Intra-day (*n*=3) and inter-day (over a period of 3 consecutive days) precision and accuracy data for the determination of QNs in spiked animal muscle

Analytes	Added (mg/kg)	Porcine muscle			Chicken muscle			Bovine muscle		
		Recovery (%)	Intra-day (RSD%)	Inter-day (RSD%)	Recovery (%)	Intra-day (RSD%)	Inter-day (RSD%)	Recovery (%)	Intra-day (RSD%)	Inter-day (RSD%)
MAR	50	108.5	8.5	14.9	95.5	7.8	9.5	101.0	13.1	12.2
	100	93.6	9.4	11.6	98.0	13.2	10.6	99.9	2.4	5.1
	500	100.8	1.1	1.6	99.2	2.2	2.1	100.8	2.1	1.6
CIP	50	111.9	3.1	6.7	99.7	11.7	9.0	100.9	8.6	13.7
	100	96.6	8.4	10.9	101.0	5.6	4.2	99.6	4.4	3.6
	500	100.6	1.7	1.7	100.2	1.7	1.0	99.8	1.4	1.6
DAN	50	103.5	2.8	6.4	88.7	4.2	9.1	96.8	5.4	7.9
	100	98.3	4.8	5.4	103.2	4.9	5.7	100.2	9.5	6.5
	500	100.3	0.7	0.9	99.4	1.0	0.6	99.8	1.0	0.9
ENR	50	100.5	6.2	9.2	93.6	3.6	9.6	96.2	13.6	12.6
	100	96.3	6.1	5.6	99.5	2.6	2.4	101.7	2.6	4.8
	500	99.9	0.7	0.9	99.5	0.6	0.7	100.0	1.2	1.2
SAR	50	104.9	6.6	7.3	97.7	3.4	8.9	102.0	8.4	6.8
	100	96.3	2.5	5.8	98.6	3.2	4.8	96.3	4.2	7.6
	500	99.9	2.2	2.1	99.7	0.5	1.3	99.0	0.4	1.3
DIF	50	99.1	10.1	14.4	103.1	5.7	7.4	99.5	6.8	6.7
	100	98.0	3.5	5.2	95.6	2.5	3.3	100.8	4.2	4.4
	500	100.2	0.4	1.1	99.7	0.1	0.9	100.0	1.1	0.7
OXO	50	108.6	6.6	9.3	83.1	11.1	14.8	107.8	6.1	10.2
	100	98.1	6.7	6.7	102.3	3.5	5.2	98.6	3.1	5.6
	500	100.3	0.5	0.9	99.2	0.2	1.3	100.8	1.2	1.4
FLU	50	110.6	11.2	11.5	91.0	7.8	9.7	98.2	6.8	4.9
	100	95.1	10.9	8.4	102.9	3.8	6.7	101.4	8.9	5.2
	500	100.4	1.9	1.6	98.6	0.9	2.3	100.5	2.8	2.2

relative standard deviations (RSDs %) at different values in the range of 0.4-11.2% for porcine muscle, 0.1-13.2% for chicken muscle, and 0.4-13.6% for bovine muscle (Table 2). The inter-day precision was established using edible muscle samples at the same concentration range as above. A triplicate determination of each concentration over a period of 3 consecutive days was followed by same experimental procedures. RSD values for all the examined QNs were reported between 0.9 and 14.9% for porcine muscle, 0.7 and 14.8% for chicken muscle, and 0.7 and 13.7% for bovine muscle (Table 2).

The measurements for the recovery were performed on spiked samples of porcine, chicken, and bovine muscles at the LOQ concentration, as well as 2 higher concentrations (50, 100, and 500 μg/kg). The recoveries for porcine muscle varied between 93.6 and 111.9%, for chicken muscle between 83.1 and 103.2%, and for bovine muscle between 96.2 and 107.8%. The mean precision and accuracy for the limits value was found to be less than the maximum tolerable RSDs of 15% and within 80-120% in accordance with FDA guidance for bioanalytical validation (19).

The present study used a simple extraction procedure based on LLE without additional clean-up steps and was found to be selective and sensitive and was successfully applied to porcine, chicken, and bovine muscles.

References

1. Drlica K, Zhao X. DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol. Mol. Biol. R. 61: 377-392 (1997)
2. Hernández-Arteseros JA, Barbosa J, Compañó R, Prat MD. Analysis of quinolone residues in edible animal products. J. Chromatogr. A 945: 1-24 (2002)
3. Use of quinolones in food animals and potential impact on human health. Report of a WHO Meeting. Geneva, Switzerland, WHO/EMC/ZDI/98.10, http:/www.who.int/emc. Accessed Aug. 12, 2008.
4. Okerman L, Noppe H, Cornet V, De Zutter L. Microbiological detection of 10 quinolone antibiotic residues and its application to artificially contaminated poultry samples. Food. Addit. Contam. 24: 252-257 (2007)
5. Commission Regulation (EC) 1181/2002, Amending Annexes I of Council Regulation (EEC) no. 2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (text, with EEA relevance). Off. J. Eur. Commun. L221: 8-36 (2002)
6. Watanabe H, Satake A, Kido Y, Tsuji A. Monoclonal-based enzyme-linked immunosorbent assay and immunochromatographic assay for enrofloxacin in biological matrices. Analyst 127: 98-103 (2002)
7. Christodoulou EA, Samanidou VF, Papadoyannis IN. Validation of an HPLC-UV method according to the European Union Decision 2002/657/EC for the simultaneous determination of 10 quinolones in chicken muscle and egg yolk. J. Chromatogr. B 859: 246-255 (2007)
8. Bailac S, Ballesteros O, Jiménez-Lozano E, Barrón D, Sanz-Nebota V, Navalón A, Vílchez JL, Barbosa J. Determination of quinolones

in chicken tissues by liquid chromatography with ultraviolet absorbance detection. J. Chromatogr. A 1029: 145-151 (2004)
9. Zhao S, Jiang H, Li X, Mi T, Li C, Shen J. Simultaneous determination of trace levels of 10 quinolones in swine, chicken, and shrimp muscle tissues using HPLC with programmable fluorescence detection. J. Agr. Food. Chem. 55: 3829-3834 (2007)
10. Verdon E, Couedor P, Roudaut B, Sandérs P. Multiresidue method for simultaneous determination of ten quinolone antibacterial residues in multimatrix/multispecies animal tissues by liquid chromatography with fluorescence detection: Single laboratory validation study. J. AOAC. Int. 88: 1179-1192 (2005)
11. Idowu OR, Peggins JO. Simple, rapid determination of enrofloxacin and ciprofloxacin in bovine milk and plasma by high-performance liquid chromatography with fluorescence detection. J. Pharm. Biomed. Anal. 35: 143-153 (2004)
12. Chu PS, Wang RC, Chu HV. Liquid chromatographic determination of fluoroquinolones in egg albumen and egg yolk of laying hens using fluorometric detection. J. Agr. Food. Chem. 50: 4452-4455 (2002)
13. Schneider MJ, Donghue DJ. Multiresidue analysis of fluoroquinolone antibiotics in chicken tissue using liquid chromatography-fluorescence-multiple mass spectrometry. J. Chromatogr. B 780: 83-92 (2002)
14. Bailac S, Barrón D, Barbosa J. New extraction procedure to improve the determination of quinolones in poultry muscle by liquid chromatography with ultraviolet and mass spectrometric detection. Anal. Chim. Acta 580: 163-169 (2006)
15. Schneider MJ, Reyes-Herrera I, Donoghue DJ. Evaluation of serum as a potential matrix for multiresidue determination of fluoroquinolone antibiotics in chicken using liquid chromatography-fluorescence-mass spectrometry(n). J. AOAC. Int. 90: 1716-1723 (2007)
16. Yorke JC, Froc P. Quantitation of nine quinolones in chicken tissues by high-performance liquid chromatography with fluorescence detection. J. Chromatogr. A 882: 63-77 (2000)
17. Christodoulou EA, Samanidou VF, Papadoyannis IN. Development and validation of an HPLC confirmatory method for residue analysis of ten quinolones in tissues of various food-producing animals, according to the European Union Decision 2002/657/EC. J. Sep. Sci. 30: 2676-2686 (2007)
18. Jimenez-Lozano E, Roy D, Barron D, Barbosa J. Effective sorbents for solid-phase extraction in the analysis of quinolones in animal tissues by capillary electrophoresis. Electrophoresis 25: 65-73 (2004)
19. US Department of Health and Human Services, Food and Drug Administration, Center Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM): Guidance for Industry, Bioanalytical Method. Available from: http://www.fda.gov/cder/guidance/index.htm. Accessed Jun. 20, 2008.
20. ICH. Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology. ICH-Q2B (1996)
21. Clemente M, Hermo MP, Barrón D, Barbosa J. Confirmatory and quantitative analysis using experimental design for the extraction and liquid chromatography-UV, liquid chromatography-mass spectrometry, and liquid chromatography-mass spectrometry/mass spectrometry determination of quinolones in turkey muscle. J. Chromatogr. A 1135: 170-178 (2006)
22. Christodoulou EA, Samanidou VF, Papadoyannis IN. Development of an HPLC multi-residue method for the determination of ten quinolones in bovine liver and porcine kidney according to the European Union Decision 2002/657/EC. J. Sep. Sci. 31: 119-127 (2008)
23. Choi HJ, Abd El-Aty AM, Goudah A, Sung GM, Yi H, Seo DC, Kim JS, Shim JH, Jeong JY, Lee SH, Shin HC. Monitoring of fluoroquinolone residual levels in chicken eggs by microbiological assay and confirmation by liquid chromatography. Biomed. Chromatogr. 22: 92-99 (2008)