JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effect of Protein on the Detection of Fluoroquinolone Residues in Fish Meat

Huifang Li, Jungang Yin,* Yongming Liu, and Jian Shang*

College of Chemistry and Chemical Engineering, Yantai University, Yantai 264005, People's Republic of China

ABSTRACT: Using fish serum albumin (FSA) as the model protein, molecular fluorescence spectrometry and high-performance liquid chromatography (HPLC) were applied to study the effect of protein on the extraction of fluoroquinolone (FQ) residues in fish meat. There was a strong interaction between FQs and protein through hydrogen bonds, which could be broken as protein degenerated with 60−100% (v/v) acetonitrile acid solution, and FQs bound with protein were released in various degrees. On the basis of the results, a novel sample preparation procedure loosely based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) methodology was developed for the determination of FQ residues in fish muscle samples, using 90% (v/v) acetonitrile acid solution as the extractant, combined with a dispersive solid-phase extraction (DSPE) cleanup step. Mean recoveries of four FQs from spiked samples at a concentration range of 50−200 ng g^{-1} were 73.3−95.9% with relative standard deviations (RSD) lower than 10.7%.

KEYWORDS: *molecular fluorescence spectrometry, liquid chromatography, fluoroquinolones, protein, fish*

INTRODUCTION

Fluoroquinolones (FQs) are synthetic antimicrobials available for treatment of animals, poultry, and fish in many countries. The increased use of FQs has led to increasing resistance to these antimicrobials, with rates of resistance that vary by both organism and geographic region. In addition, antimicrobial resistance is the most prominent human health risk associated with intensive animal farming and antibiotic use.[1] Therefore, effective control of food safety is necessary to ensure that they are not present at levels that may pose health risks to consumers.

So far, several pretreatment methods have been reported for the determination of FQ residues in animal tissues.[2−13] Most of them require a sample treatment, including deproteinization and subsequent cleanup steps by solid-phase extraction (SPE).[2−11] Because the procedure consisted of many experimental steps, it was time-consuming and not suitable for routine analysis. In the related literature regarding the analysis of FQs in fish and meat samples, Stubbings and Bigwood[12] used a new sample preparation procedure employing acetonitrile extraction and dispersive solid-phase extraction (DSPE) based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) methodology,[14] but the result was unsatisfactory. McMullen and Schenck[13] got a better recovery with a similar method, but the muscle tissues had to be extracted two times and the study of the effect of protein on the extraction of drug residues in fish sample was not performed. On the basis of the previous research, a novel, simpler, and more effective sample pretreatment method for FQ determination in fish meat samples is expected.

As reported by Cakmakci et al., fluoroquinolones such as fleroxacin penetrats well into muscle tissue, but not into fat tissue.[15] To detect FQ residues in fish meat to the greatest extent, the effect of protein should be considered and eliminated in the extraction process of drug residues. Thus, the study on the interaction of FQs and protein is the basis of extraction of FQs in fish muscle samples. To our knowledge, no published study has carried out theoretical research on the extraction of veterinary drug residues in fish meat. So far, the study on the interaction of FQs with protein has focused on human serum albumin,[16,17] bovine serum albumin,[18] trypsin,[19] and so on. Due to the serious veterinary drug residue of farmed fish, we chose fish serum protein as the researched object, which has not been studied before.

One of the important methods to determine the interactions of protein with a variety of substances is to measure the quenching of the protein fluorescence.[16−18] The phenomenon of the quenching of fish serum fluorescence strongly related to the albumin fraction, as the albumin is the principal carrier for drugs, especially for concentrations lower than 10 μM.[20] As the most abundant protein in vertebrate plasma, serum albumin has an important role in drug disposition and efficacy. Most drugs bind reversibly to albumin, and the interaction between albumin and drugs can induce conformational changes in protein structure.[21−23] Studies on the accessibility of quenchers to albumin's fluorophore groups help to illuminate albumin's binding mechanisms to drugs and provide clues to the nature of the binding phenomenon.

Our work emphasized the interaction mechanism of four FQs (ciprofloxacin HCl, enrofloxacin, norfloxacin, and noxacin) binding with protein and the effect of acetonitrile on the structure of protein. On the basis of the above results, an appropriate acetonitrile acid solution was selected as extractant, combined with DSPE purification to satisfy the fast and effective detection of FQ residues in fish meat.

MATERIALS AND METHODS

Materials and Preparation of Solutions. Carp samples were purchased from the local market. Blood samples were taken from the

Received: August 28, 2011
Revised: January 18, 2012
Accepted: January 23, 2012
Published: January 23, 2012

 © 2012 American Chemical Society

hearts of live fish using nonheparinized syringes. The serum was immediately centrifuged at 4000 rpm for 8 min at 4 °C, and the supernatant was taken and stored at 4 °C. The total serum protein concentration determined by the Coomassie brilliant blue method was 38.77 mg mL^{-1}. The concentration of fish serum albumin (FSA) at a molecular mass of 66 kDa determined by SDS-PAGE method was 3.85 mg mL^{-1}. The FSA working solutions were obtained by appropriate dilution of the stock solution with Tris-HCl buffer (pH 7.4) containing different concentrations of acetonitrile, respectively. Fish muscle samples were taken from fish back, homogenized, and preserved at 0 °C in a refrigerator.

Bondesil sorbents (PSA, NH_2, and C18) were obtained from Agela (Tianjin, China). Ciprofloxacin HCl (CPF HCl), enrofloxacin (ERF), norfloxacin (NRF), and enoxacin (EO) were purchased from Zhejiang Guobang Pharmaceutical Co., Ltd. Stock standard solutions of the four FQs in 5 mM HCl were 100 μg mL^{-1}, respectively. Working standard solutions for the calibration curves and the fortifications assays were prepared by suitable dilution.

Apparatus and Chromatographic Conditions. Fluorescence measurements were carried on a Cary Eclispse fluorescence spectrophotometer equipped with a Peltier accessory (temperature control), and a 1.00 cm quartz cell was used. Ultraviolet light at 280 nm with a slit width of 5 nm was used to excite albumin's fluorophore group, triggering the emission of intrinsic fluorescence. The resonance Rayleigh scattering spectrum was obtained by synchronous scanning at $\lambda_{ex} = \lambda_{em}$ ($\Delta\lambda = 0$) with a slit width of 10 nm.

The LC method described here was developed using an Agilent 1100 series HPLC system with a quaternary pump and a diode array detector (DAD). A Chromatorex C18 HPLC column (4.6 mm × 150 mm, 5 μm) from Replete (Dalian, China) was used. The fluorescence excitation/emission wavelengths were programmed at 280/360 nm. The mobile phase of isocratic elution consisted of 0.05 mol L^{-1} citric acid, adjusted to pH 3.5 with triethylamine (TEA), and acetonitrile (82:18). The flow rate was 1 mL min^{-1}. The injection volume was 20 μL, and the column temperature was maintained at 30 °C.

Spectroscopic Measurements. Some studies were carried out to select optimum protein and FQ concentrations for FQ–FSA interaction. FSA concentration was fixed at 0.16 μM, and FQ concentrations varied from 0.1 to 1.0 μM. Fluorescence measurements were performed at different temperatures (20, 25, and 30 °C) in the range of 290–480 nm. Resonance Rayleigh scattering of FSA in different concentrations of acetonitrile–water solution were studied by synchronous fluorescence scanning in the range of 200–600 nm at 25 °C.

Sample Extraction and Cleanup Procedure. The 50, 60, 75, 90, and 100% acetonitrile acid solutions were selected as extractants. Fish meat was fortified at a concentration of 100 ng g^{-1} for each analyte by the addition of spiking solution (25 μL of 10.0 μg mL^{-1} drug standard) to the muscle tissue (2.5 g). Each subsample was homogenized with 10 mL of extractant for 1 min, and the mix was centrifuged at 4000 rpm for 8 min. After standing, 200 mg of PSA was added to the supernatant and centrifuged. All of the elutes were collected and evaporated to dryness under a stream of nitrogen at 50 °C. The residue obtained was dissolved in mobile phase (2 mL) for injection into the LC system after filtration with a 0.45 μm micropore filter membrane.

Method Validation. Recovery studies were carried out with blank muscle tissue samples spiked with standard solutions at three concentration levels, 50, 100, and 200 ng g^{-1}. They were allowed to stand in the dark for 30 min at room temperature to enable total interaction between the antibiotics and sample before the analysis. The validation of the analytical procedure was performed over three days. The linearity of the analytical procedure was evaluated by plotting the detector response (peak area) versus the nominal concentration of FQs present in the tissue sample. The precision (relative standard deviation (RSD)) of the analytical procedure was evaluated by determining the intra- and interday RSDs. The intraday precision is referred to as the repeatability of the assay, whereas the interday precision is referred to as the intermediate precision of the assay.

RESULTS AND DISCUSSION

FSA–FQ Interaction. *Fluorescence Quenching Mechanism.* Fluorescence spectra of FSA in the presence of increasing amounts of FQs (enrofloxacin, for example) are shown in Figure 1. At the excitation wavelength of 280 nm, ERF caused a concentration-dependent quenching of the intrinsic fluorescence of FSA.

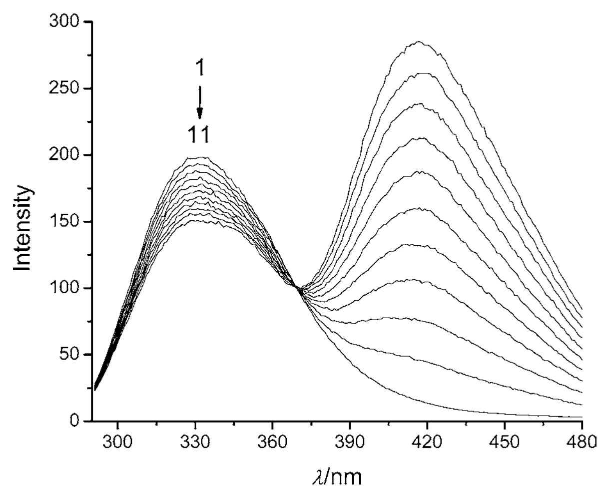

Figure 1. Effect of enrofloxacin on fluorescence spectra of FSA at 25 °C. $\lambda_{ex}/\lambda_{em}$ = 280/333 nm; C_{FSA} = 0.16 μM; C/μM curves 1–11: 0, 0.422, 0.843, 1.26, 1.69, 2.11, 2.53, 2.95, 3.37, 3.79, and 4.22.

The fluorescence quenching data are usually analyzed according to the Stern–Volmer equation[24]

$$F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F are the steady-state fluorescence intensities in the absence and presence of FQs, respectively, K_q is the bimolecular quenching rate constant (when K_q is $>10^{12}$, it is considered to be a static quenching procedure), τ_0 is the fluorescence lifetime in the absence of quencher ($\tau_0 \approx 10^{-8}$s), K_{SV} is the Stern–Volmer quenching constant, and [Q] is the FQ concentration. As shown in Figure 2, F_0/F had a good linear relationship with the concentration of ERF. The values of K_{SV} and K_q for the binding interaction of FQs with FSA at

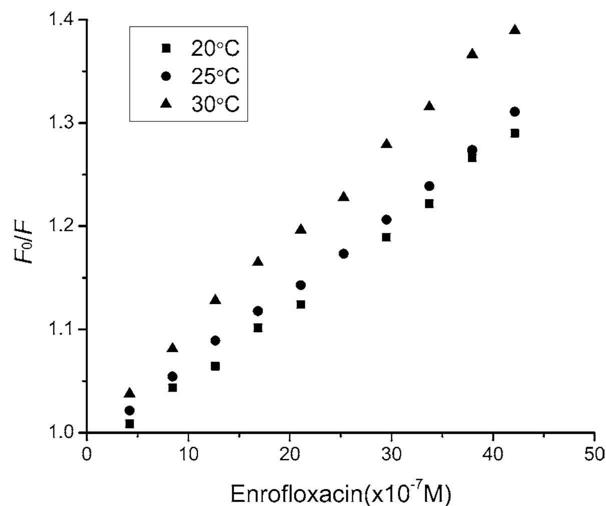

Figure 2. Stern–Volmer plots of FSA titrated against enrofloxacin at 20, 25, and 30 °C.

different temperatures are listed in Table 1. These results indicated that the probable quenching mechanism of fluorescence of FSA by FQs is a static quenching procedure.

Analysis of Binding Equilibrium. For static quenching, the equilibrium between free and bound molecules is given by the equation[25,26]

$$\log[(F_0 - F)/F] = \log K + n \log[\mathrm{Q}] \quad (2)$$

where K and n are the binding constant and the number of binding sites, respectively. When plotted as log $[(F_0 - F)/F]$ with log[Q], a good linear relationship is shown in Figure 3 (ERF, for example). From the figure we know that the binding constant increases with increasing temperature. The values of K and n at different temperatures are listed in Table 1. The result showed that the FQs interact strongly with FSA through one binding site.

Thermodynamic Parameters between FSA and Four FQs. The acting forces between biomolecule and an organic compound mainly include hydrophobic effects, hydrogen bonds, van der Waals forces, electrostatic attraction, and so on. To estimate the binding modes of FSA–FQs, the thermodynamic parameters ΔH, ΔS, and ΔG can be calculated from the following equations, based on the binding constants at different temperatures:

$$\Delta G = -RT \ln K = \Delta H - T\Delta S \quad (3)$$

$$\ln(K_2/K_1) = \Delta H/R(1/T_1 - 1/T_2) \quad (4)$$

$$\Delta S = (\Delta H - \Delta G)/\mathrm{T} \quad (5)$$

Where K is the binding constant, R is the gas constant, and ΔH, ΔS, and ΔG are the enthalpy change, entropy change, and free energy change, respectively. ΔH, ΔS, and ΔG at different temperatures were calculated and are listed in Table 1. The ΔG values of all four interactions were negative, showing that the binding processes were spontaneous. According to the relationship between the signs of the thermodynamic parameters and protein–ligand interactions,[27] it was deduced that the acting forces of FSA–FQs were mainly hydrogen bonds, because the ΔH and ΔS of the procedure were negative. As protein is the dominating matrix in fish muscle tissues, hydrogen bonds between drugs and protein must be eliminated to get good recoveries in the extraction of FQ residues.

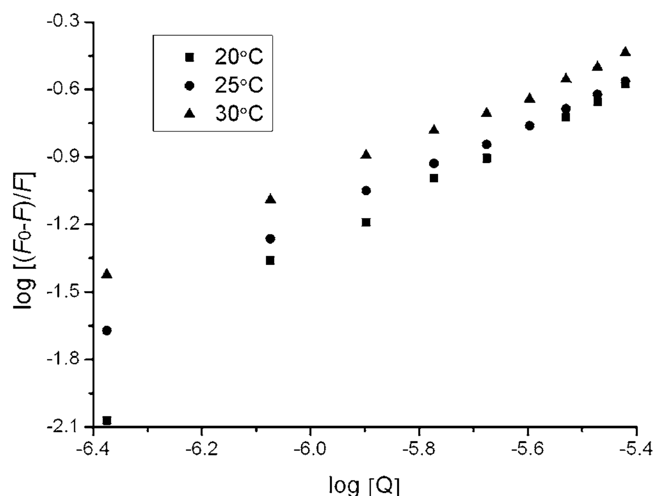

Figure 3. Plots of $\log[(F_0 - F)/F]$ versus log[Q] at 20, 25, and 30 °C.

Effect of Acetonitrile on Protein. It was reported that protein is more denatured in aqueous–organic mixtures than in the corresponding pure organic solvents.[28] The FSA fluorescence in different concentrations of acetonitrile–water solution was determined at 25 °C (Figure 4). When acetonitrile concentration increased from 45 to 100% (v/v), the FSA fluorescence intensity kept decreasing, indicating that as protein denatured and precipitated, the solubility of protein reduced and reached its minimum in pure acetonitrile.

A further study on the effect of acetonitrile on protein was carried out by resonance Rayleigh scattering, which could reflect structural changes of the protein molecules and the degree of protein denaturation in the low protein concentration regime.[29] As shown in Figure 5, when the acetonitrile concentration was 50%, Rayleigh scattering intensity began to increase and achieved its maximum at 60%, indicating that supramolecular aggregation was occurring, resulting in strong scattering interaction. The hydrogen bonds that maintain protein secondary structure had been broken and hydrophobic groups exposed outside. Protein molecules changed from ordered, curled, and tight structure into unordered, loose, and

Table 1. Parameters of the Bindings between Four FQs and FSA[a]

FQ	T (K)	$K_{SV}/10^4$ (L mol^{-1})	$K_q/10^{12}$ (L mol^{-1} s^{-1})	$K/10^5$ (L mol^{-1})	n	$\Delta G/10^4$ (kJ mol^{-1})	$\Delta H/10^5$ (J mol^{-1})	ΔS (J K^{-1} mol^{-1})
CPF HCl	293	8.9	8.9	2.0	1.07	−3.0	−0.5	−62.6
	298	8.8	8.8	1.3	1.03	−2.9		
	303	9.8	9.8	1.0	1.08	−2.9		
ERF	293	7.4	7.4	190	1.44	−4.1	−3.9	−1188.5
	298	7.5	7.5	3.6	1.12	−3.2		
	303	9.3	9.3	0.95	1.00	−2.9		
NRF	293	7.8	7.8	5.8	1.1	−3.2	−1.1	−258
	298	10.6	10.6	3.2	1.09	−3.1		
	303	7.5	7.5	1.3	1.05	−3.0		
EO	293	9.9	9.9	10	1.19	−3.4	−2.1	−588.2
	298	13.6	13.6	6.4	1.13	−3.3		
	303	9.9	9.9	0.63	0.96	−2.8		

[a] K_{SV}, Stern–Volmer quenching constant; K_q, bimolecular quenching rate constant; K, binding constant; n, number of binding sites; ΔH, ΔS, and ΔG, enthalpy, entropy, and free energy change, respectively.

dx.doi.org/10.1021/jf2034658 | J. Agric.Food Chem. 2012, 60, 1722–1727

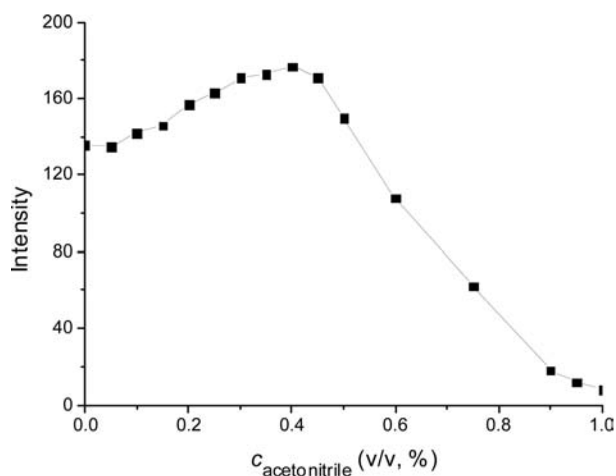

Figure 4. FSA fluorescence intensity in different acetonitrile concentration solutions at 25 °C. $\lambda_{ex}/\lambda_{em}$ = 280/333 nm; C_{FSA} = 0.16 μM.

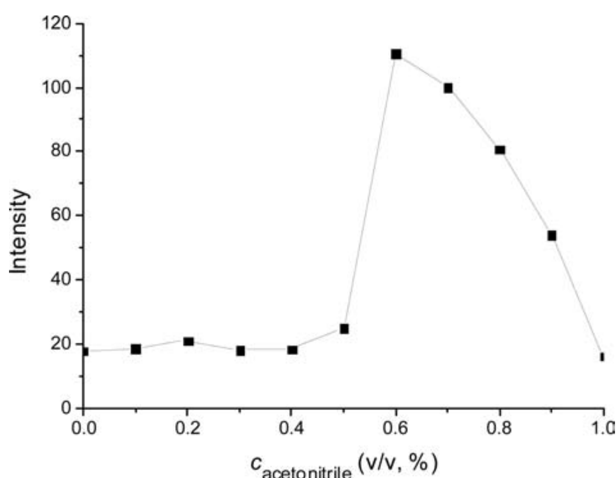

Figure 5. Rayleigh scattering intensity of FSA in different acetonitrile concentration solutions at 25 °C. λ_{ex} = λ_{em} = 480 nm; C_{FSA} = 0.014 μM.

extended structure. As the concentration of acetonitrile kept increasing, the protein molecules started to precipitate and the scattering intensity kept reducing.

Extraction and Cleanup Procedure. On the basis of the results above, the concentration of acetonitrile solution would be an important factor for the extraction of fish drug residues. Extractants containing 50, 60, 75, 90, and 100% acetonitrile were applied to extract the FQ residues at a fortified level of 100 ng g^{-1} in fish meat samples. The recoveries of FQs with different extractants are summarized in Figure 6 and Table 2. When protein was precipitated with 90% acetonitrile solution, the recoveries of four FQs were >80%. It was deduced that the hydrogen bonds were destroyed completely as matrix protein precipitated, and then the drugs bound with protein changed into a free state and dissolved in the extract. When pure acetonitrile was used, the recovery rate was low. This is because in the pure organic solvent, the secondary structure of protein remains essentially intact;[28] hydrogen bonds could not be destroyed, and drugs were packaged as protein quickly gathered together. When in 50, 60, and 75% acetonitrile solutions, the hydrogen bonds between protein and drugs were not destroyed

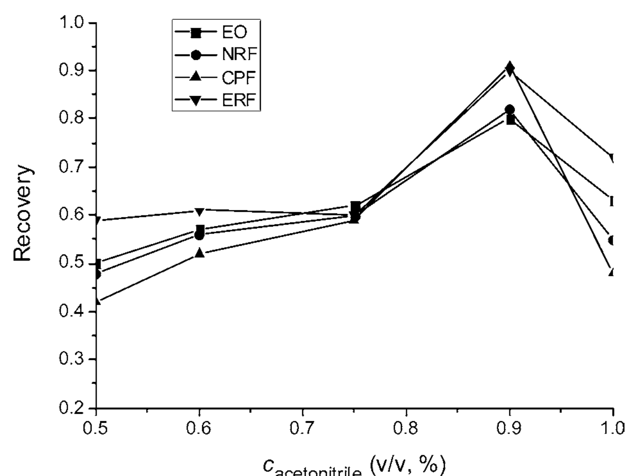

Figure 6. Effect of acetonitrile on the recoveries of FQs at a fortified level of 100 ng g^{-1} in fish meat samples.

completely (Figures 4 and 5) and the drugs could not be well released.

The purification ability of dispersive sorbents C18, NH_2, and PSA to the supernatant resulting from extraction procedures and their adsorption to FQs was also studied. The recovery data (Table 3) showed that C18 has certain adsorption to FQs, but this could be negligible for NH_2 and PSA. Otherwise, the purification effect of NH_2 is not as good as that of PSA according to experiment phenomena, and the residual impurities had a negative influence on the recovery. Thus, PSA was the best choice of purification agent in this experiment.

On the basis of refs 8, 9, 12, and 13 and our repeated experiments, 90% (v/v) acetonitrile (1% (v/v) acetic acid) in HCl (0.15 M) solution was selected to extract the FQ residues in fish, and the extraction procedures performed best with cleanups using PSA sorbent in dispersive format; the recovery was better than reported.[12]

Method Validation. Calibration curves were obtained by linear least-squares regression, plotting peak area versus concentration. CPF HCl, ERF, NRF, and EO had good linear response within the range from 20 to 300 ng mL^{-1}. The mean values of correlation coefficients (R^2) were >0.999 for all FQs. The limits of quantification (LOQs) of these FQs, expressed as the lowest tested level with acceptable RSD, were 8 ng g^{-1} for CPF HCl and 5 ng g^{-1} for ERF, NRF, and EO. The recovery and precision of the method were evaluated by the analysis of six samples spiked with four FQs at three different fortification levels, 50, 100, and 200 ng g^{-1}, on three different days (n = 18). Under our conditions, the estimated extraction recoveries from spiked fish muscle tissues for the four FQs reached high values, ranging from 73.3 to 95.9% (Table 4). The intra- and interday precisions were established for six measurements of spiked fish meat samples with the four analytes at three fortification levels, carried out within the same day and on three different days, respectively. The RSD for the three spiking concentrations was <10.7%, demonstrating the good precision of the method (Table 4).

Application to Real Samples. The effectiveness of the optimized methodology was checked by analyzing fish muscle from carp samples, which were orally medicated with a daily dose of 30 mg kg^{-1} CPF HCl for 6 days. As shown in Figure 7, the peak of CPF was detected and there was no interfering peak

Table 2. Recovery Data of FQs for Different Extractants by Adding FQs in Fish Meat (n = 3)[a]

FQ	fortification level (ng g^{-1})	recovery ± SD				
		extract a1	extract a2	extract a3	extract a4	extract a5
EO	100	63 ± 1	80 ± 1	62 ± 1	57 ± 1	50 ± 1
NRF	100	55 ± 2	82 ± 1	60 ± 0	56 ± 0	48 ± 2
CPF HCl	100	48 ± 1	91 ± 1	59 ± 0	52 ± 1	42 ± 2
ERF	100	72 ± 4	90 ± 1	60 ± 1	61 ± 0	59 ± 1

[a]Extracts: a1, pure acetonitrile; a2, 90% (v/v) acetonitrile acidic solution; a3, 75% (v/v) acetonitrile acidic solution; a4, 60% (v/v) acetonitrile acidic solution; a5, 50% (v/v) acetonitrile acidic solution.

Table 3. Recovery Data of FQs for Different Sorbents by Using 90% (v/v) Acetonitrile Acidic Solution as the Extractant (n = 3)

FQ	fortification level (ng g^{-1})	PSA recovery ± SD		NH_2 recovery ± SD		C18 recovery ± SD	
		blank	fish	blank	Fish	blank	fish
EO	100	96 ± 1	80 ± 1	97 ± 0	78 ± 2	87 ± 0	82 ± 2
NRF	100	96 ± 1	82 ± 1	95 ± 2	79 ± 3	87 ± 3	83 ± 2
CPF HCl	100	95 ± 3	90 ± 1	93 ± 1	83 ± 1	88 ± 0	85 ± 1
ERF	100	98 ± 1	91 ± 1	98 ± 2	85 ± 3	87 ± 1	88 ± 1

Table 4. Accuracy and Precision (Intraday and Interday) of the Proposed Method

FQ	T_r (min)	fortification level (ng g^{-1})	mean recovery (%)	intraday precision (%) RSD	interday precision (%) RSD
EO	3.8	50	75.4	3.5	5.4
		100	80.7	3.1	6.2
		200	84.3	5.4	6.1
NRF	4.3	50	73.3	8.3	8.5
		100	82.7	3.2	3.6
		200	83.5	2.7	2.8
CPF HCl	4.8	50	80.2	8.9	10.7
		100	86.5	5.7	7.2
		200	85.9	5.8	9.8
ERF	6.8	50	81.9	5.7	6.0
		100	92.7	4.4	5.9
		200	95.9	4.1	6.6

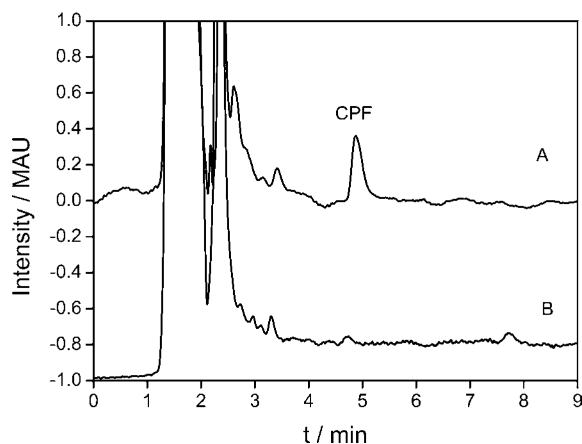

Figure 7. HPLC chromatogram obtained by the proposed method from fish orally medicated with a daily dose of 30 mg kg^{-1} CPF HCl for 6 days (A) and analyte-free fish sample (B).

in the resulting chromatogram. The mean CPF contamination level found in fish muscle samples was 29.1 ng g^{-1}.

Conclusions. This study researched the effect of protein on the extraction of FQ residues in fish meat. In 90% (v/v) acetonitrile acid solution, the hydrogen bonds between protein and FQs were destroyed completely as matrix protein precipitated, and the FQs bound with protein could be released to the highest extent. On the basis of the results, we developed a new sample pretreatment method using 90% (v/v) acetonitrile acid solution as the extractant, followed by a DSPE cleanup step. The developed method has good analytical performance and was suitable for the fast and effective detection of the FQ residues in fish meat tissues.

AUTHOR INFORMATION

Corresponding Author

*(J.Y.) Phone: +86-535-6902401. Fax: +86-535-6902401. E-mail: jungangyin@163.com. (J.S.) Phone: +86-535-6902700. Fax: +86-535-6902078. E-mail: shangjian.@126.com.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) World Health Organization (WHO). *Use of Quinolones in Food Animals and Potential Impact on Human Health*; report WHO/EMC/ZDI/98; Geneva, Switzerland, 1998.

(2) Samanidou, V.; Evaggelopoulou, E.; Trötzmüller, M.; Guo, X. H.; Lankmayr, E. Multi-residue determination of seven quinolones antibiotics in gilthead seabream using liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2008**, *1203*, 115−123.

(3) Fernandez-Torres, R.; Bello Lopez, M. A.; Olias Consentino, M.; Callejon Mochon, M.; Ramos Payan, M. Enzymatic-microwave assisted extraction and high-performance liquid chromatography−mass spectrometry for the determination of selected veterinary antibiotics in fish and mussel samples. *J. Pharm. Biomed. Anal.* **2011**, *54*, 1146−1156.

(4) Yorke, J. C.; Froc, P. Quantitation of nine quinolones in chicken tissues by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr., A* **2000**, *882*, 63−77.

(5) Johnston, L.; Mackay, L.; Croft, M. Determination of quinolones and fluoroquinolones in fish tissue and seafood by high-performance

liquid chromatography with electrospray ionisation tandem mass spectrometric detection. *J. Chromatogr., A* **2002**, *982*, 97−109.
(6) Clemente, M.; Hermo, M. P.; 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* **2006**, *1135*, 170−178.
(7) Hermo, M. P.; Barrón, D.; Barbosa, J. Development of analytical methods for multiresidue determination of quinolones in pig muscle samples by liquid chromatography with ultraviolet detection, liquid chromatography−mass spectrometry and liquid chromagraphy−tandem mass spectrometry. *J. Chromatogr., A* **2006**, *1104*, 132−139.
(8) Maraschiello, C.; Cusidó, E.; Abellán, M.; Vilageliu, J. Validation of an analytical procedure for the determination of the fluoroquinolone ofloxacin in chicken tissues. *J. Chromatogr., B* **2001**, *754*, 311−318.
(9) Pena, A.; Silva, L. J. G.; Pereira, A.; Meisel, L.; Lino, C. M. Determination of fluoroquinolone residues in poultry muscle in Portugal. *Anal. Bioanal. Chem.* **2010**, *397*, 2615−2621.
(10) Dufresne, G.; Fouquet, A. Multiresidue determination of quinolone and fluoroquinolone antibiotics in fish and shrimp by liquid chromatography/tandem mass spectrometry. *J. AOAC Int.* **2007**, *90*, 604−612.
(11) Zhang, H.; Chen, S.; Lu, Y. B.; Dai, Z. Y. Simultaneous determination of quinolones in fish by liquid chromatography coupled with fluorescence detection: comparison of sub-2 lm particles and conventional C18 columns. *J. Sep. Sci.* **2010**, *33*, 1959−1967.
(12) Stubbings, G.; Bigwood, T. The development and validation of a multiclass liquid chromatography tandem mass spectrometry (LC−MS/MS) procedure for the determination of veterinary drug residues in animal tissue using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach. *Anal. Chim. Acta* **2009**, *637*, 68−78.
(13) Mcmullen, S. E.; Schenck, F. J. Rapid method for the determination and confirmation of fluoroquinolone residues in catfish using liquid chromatography/fluorescence detection and liquid chromatography-tandem mass spectrometry. *J. AOAC Int.* **2009**, *92*, 1233−1240.
(14) Anastassiades, M.; Lehotay, S. J.; Stajnbaher, D.; Schenck, F. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412−431.
(15) Cakmakci, M.; Gossweiler, L.; Schilling, J.; Schlumpf, R.; Geroulanos, S. Penetration of fleroxacin into human lung, muscle, and fat tissue. *Drugs Exp. Clin. Res.* **1992**, *18*, 299−302.
(16) Zhang, L. W.; Wang, K.; Zhang, X. X. Study of the interactions between fluoroquinolones and human serum albumin by affinity capillary electrophoresis and fluorescence method. *Anal. Chim. Acta* **2007**, *603*, 101−110.
(17) Seedher, N.; Agarwal, P. Complexation of fluoroquinolone antibiotics with human serum albumin: a fluorescence quenching study. *J. Lumin.* **2010**, *130*, 1841−1848.
(18) Kamat, B. P. Study of the interaction between fluoroquinolones and bovine serum albumin. *J. Pharm. Biomed. Anal.* **2005**, *39*, 1046−1050.
(19) Huang, Y. B.; Liu, B. Z.; Yu, Z.; Zi, Y. Q. Luminescence quenching effect for the interaction of prulifloxacin with trypsin−Britton−Robinson buffer solution system. *J. Lumin.* **2010**, *130*, 360−364.
(20) Silva, D.; Moreira, M. C.; Bastos, V. C.; Bastos, J. C.; Cortez, C. M. The interaction of methyl-parathion with serum and albumin of the neo-tropical fish *Piaractus mesopotamicus*. *Ecotoxicol. Environ. Saf.* **2010**, *73*, 32−37.
(21) Kragh-Hansen, U. Molecular aspects of ligand binding to serum albumin. *Pharmacol. Rev.* **1981**, *33*, 17−53.
(22) Theodore, P. *All about Albumin, Biochemistry, Genetics, and Medical Applications*; Academic Press: New York, 1996.
(23) Hushcha, T. O.; Luik, A. I.; Naboka, Yu. N. Conformation changes of albumin in its interaction with physiologically active compounds as studied by quasi-elastic light scattering spectroscopy and ultrasonic method. *Talanta* **2000**, *53*, 29−34.
(24) Lakovicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic/Plenum: New York, 1999.
(25) Kandagal, P. B.; Ashoka, S.; Seetharamappa, J.; Shaikh, S. M. T.; Jadegoud, Y.; Ijare, O. B. Study of the interaction of an anticancer drug with human and bovine serum albumin: spectroscopic approach. *J. Pharm. Biomed. Anal.* **2006**, *41*, 393−396.
(26) Feng, X. Z.; lin, Z.; Yang, L. J.; Wang, C.; Bai, C. L. Investigation of the interaction between acridine orange and bovine serum albumin. *Talanta* **1998**, *47*, 1223−1229.
(27) Ross, P. D.; Subramanian, S. Thermodynamics of protein association reactions: forces contributing. *Biochemistry* **1981**, *20*, 3096−3102.
(28) Griebenow, K.; Klibanov, A. M. On protein denaturation in aqueous-organic mixtures but not in pure organic solvents. *J. Am. Chem. Soc.* **1996**, *118*, 11695−11700.
(29) Vetri, V.; D'Amico, M.; Fodera, V.; Leone, M.; Ponzoni, A.; Sberveglieri, G.; Militello, V. Bovine serum albumin protofibril-like aggregates formation: solo but not simple mechanism. *Arch. Biochem. Biophys.* **2011**, *508*, 13−24.