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Simultaneous determination of nine fluoroquinolones in egg white and egg yolk by liquid chromatography with fluorescence detection

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Abstract

A liquid chromatographic (LC) method with fluorescence detection was developed for determination of nine fluoroquinolones (FQs) in egg white and yolk. Egg white samples were deproteinized with acidified ethanol (egg yolk samples with acetonitrile and acidified ethanol), followed by defatting with hexane once (white) or twice (yolk), and extracting FQs into acetonitrile. After acetonitrile was evaporated, the residue was dissolved in mobile phase, and FQs were detected in LC with a fluorescence detector. Recoveries for nine FQs from white and yolk were 74.7–85.6%, 79.1–91.2%, respectively, with excellent relative standard deviations. The limits of quantification were 5–20 ng g^{-1}.

Keywords: Fluoroquinolones; LC; Eggs; Method

1. Introduction

Fluoroquinolones (FQs) are a highly potent group of synthetic antimicrobials used in human and veterinary medicine. They act by inhibiting bacteria DNA-gynase or topoisomerase which leads to cell death. The broad-spectrum antibacterial activity, good absorption after oral administration and extensive tissue distribution make FQs suitable for the therapy of many infections in farmed animals and fish. In China, several FQs, such as enrofloxacin, ciprofloxacin, danofloxacin, sarafloxacin and difloxacin, have been licensed for use in food-producing animals. And the maximum residue limits (MRL) have been fixed for these FQs (Table 1). FQs that are labeled for humans and are of potential interest for veterinary medicine include norfloxacin, ofloxacin, pefloxacin and lomefloxacin. In the last decades, FQs are the most frequently used antimicrobials for the treatment of severe intestinal and respiratory infections in domestic animals and poultry in China. The wide application has raised public health concerns because the presence of FQs residues in meat, milk and eggs may lead to pathogen resistance to FQs in human. Therefore, there is a need for development of simple and sensitive multi-residue methods for determination of FQs in foods like eggs.

Several methods have been reported for analysis the residues of FQs in eggs. Gorla et al. reported an HPLC method with UV detection for determination enrofloxacin and ciprofloxacin in egg yolk or white, but their extraction gave very low recovery (36–50% for ciprofloxacin, 49–85% for enrofloxacin) [1]. To get satisfactory recovery from fortified eggs, Maxell et al. tried ASTED system to isolate sarafloxacin from eggs. The recovery of the method was 87–102%, with the LOQ at 1 ng g^{-1} [2]. And Schneider and Donoghue also developed an HPLC method with fluorescence detection using ASTED system to determinate six FQs in whole eggs, and

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Table 1
Maximum residue limits (MRL) fixed by China

Drug	Species	Target tissues	MRL ($ng\,g^{-1}$ or $ng\,ml^{-1}$)
Difloxacin	Bovine/ovine	Muscle	400
		Skin + fat	100
		Liver	1400
		Kidney	800
	Swine	Muscle	400
		Skin + fat	100
		Liver	800
		Kidney	800
	Chicken/turkey	Muscle	300
		Skin + fat	400
		Liver	1900
		Kidney	600
Sarafloxacin	Chicken	Muscle	10
		Fat	20
		Liver/kidney	80
	Fish	Muscle + skin	30
Enrofloxacin + ciprofloxacin	Bovine/ovine	Muscle	100
		Fat	100
		Liver	300
		Kidney	200
		Milk	100
	Swine/rabbit	Muscle	100
		Fat	100
		Liver	200
		Kidney	300
	Poultry	Muscle	100
		Skin + fat	100
		Liver	200
		Kidney	300
Danofloxacin	Bovine/ovine	Muscle	200
		Fat	100
		Liver	400
		Kidney	400
		Milk	30
	Poultry	Muscle	200
		Skin+ fat	100
		Liver	400
		Kidney	400
	Swine and other animals	Muscle	100
		Skin + fat	50
		Liver	200
		Kidney	200

achieved good sensitivity and satisfactory recovery for the six FQs (65–110%) [3]. But the ASTED system restricted the method to a few laboratories. Chu et al. were able to simultaneously extract ciprofloxacin, enrofloxacin and sarafloxacin from egg white or yolk using acetonitrile, with recovery more than 80%, CV less than 11% [4]. Using hydrochloride acid for extraction and Sep-Pak C18 cartridge for clean-up, Gigosos et al. developed an HPLC method with diode array detector for simultaneous determination of five FQs in bovine kidney, muscle and egg. The recovery, relative standard deviation and limit of detection were satisfactory [5]. Rose et al. reported good recoveries for nine FQS except for danofloxacin (49% at 10 $ng\,g^{-1}$ level) and enoxacin (55–56% at 50 $ng\,g^{-1}$ level), but the method was unable to simultaneously extract the nine drugs, and unable to simultaneously determine more than four FQs in one run [6]. Shim et al. reported a good recovery (83–96%) from fortified eggs of four FQs using supercritical fluid extraction (SFE); however, it required special SFE equipment to perform SFE [7]. Schneider and Donoghue developed a novel LC method with fluorescence

detector for quantitation of eight FQs in mixed eggs, egg white and egg yolk, and confirmation with MS^n which was connected to the output from the fluorescence detector [8]. The acetonitrile extraction followed by hexane defatting gave a general good recovery for seven of the eight FQs in fortified mixed eggs (62–92%), but the extraction procedure gave low recovery for desethylene ciprofloxacin (46.0–65.4%) and norfolxacin (55.6–75.9%) in fortified egg yolk samples.

Since there are nine FQs (norfloxacin, enrofloxacin, danofloxacin, sarafloxacin and difloxacin, pefloxacin, lomefloxacin, ciprofloxacin and ofloxacin) available for using food producing-animals in China, a multi-residue method to simultaneously determine all these FQs in eggs is needed. Here, an effective and simple LC method was presented for determination of nine FQs in eggs. In this method, nine FQs in egg yolk or egg white were extracted in a single extraction procedure and determined by LC with fluorescence detector in a single run.

2. Experimental

2.1. Materials and reagents

Ciprofloxacin hydrochloride (99.8%), pefloxacin methanesulphonate (99.6%), ofloxacin (99.4%), norfloxacin (99.6%), enrofloxacin (100%), sarafloxacin (99.6%) standards were purchased from China Institute of Veterinary Drug Control (Beijing, China). Lomefloxacin hydrochloride (90.0%) was obtained from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China), and danofloxacin methanesulphonate (99.7%) from Guangdong Yantang Veterinary Medicine Factory (Guangzhou,

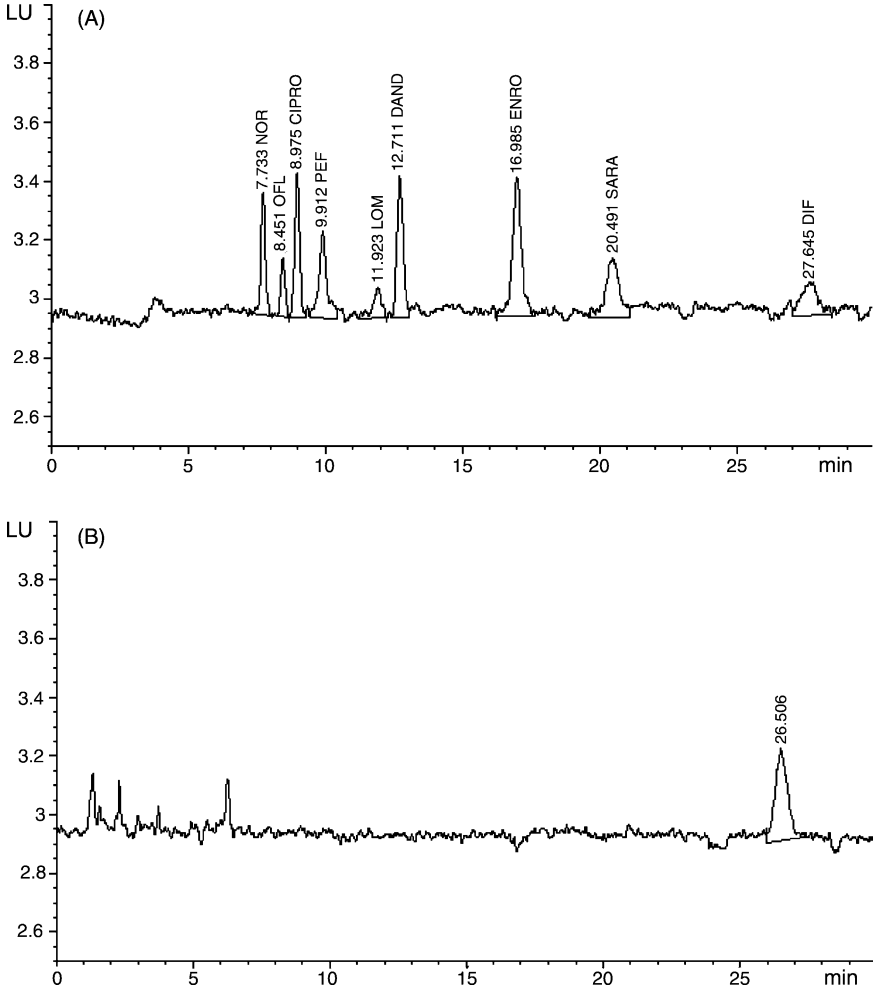

Fig. 1. Typical liquid chromatograms: (A) nine FQs in LC working standard solution (20 ng g^{-1} for norfloxacin, ofloxacin, ciprofloxacin, enrofloxacin, sarafloxacin, 10 ng g^{-1} for pefloxacin, lomefloxacin, difloxacin, 5 ng g^{-1} for danofloxacin); (B) an extract of blank egg white; (C) an extract of egg white fortified with nine FQs (20 ng g^{-1} for norfloxacin, ofloxacin, ciprofloxacin, enrofloxacin, sarafloxacin, 10 ng g^{-1} for pefloxacin, lomefloxacin, difloxacin, 5 ng g^{-1} for danofloxacin); (D) an extract of blank egg yolk; (E) an extract of egg yolk fortified with nine FQs (20 ng g^{-1} for norfloxacin, ofloxacin, ciprofloxacin, enrofloxacin, sarafloxacin, 10 ng g^{-1} for pefloxacin, lomefloxacin, difloxacin, 5 ng g^{-1} for danofloxacin).

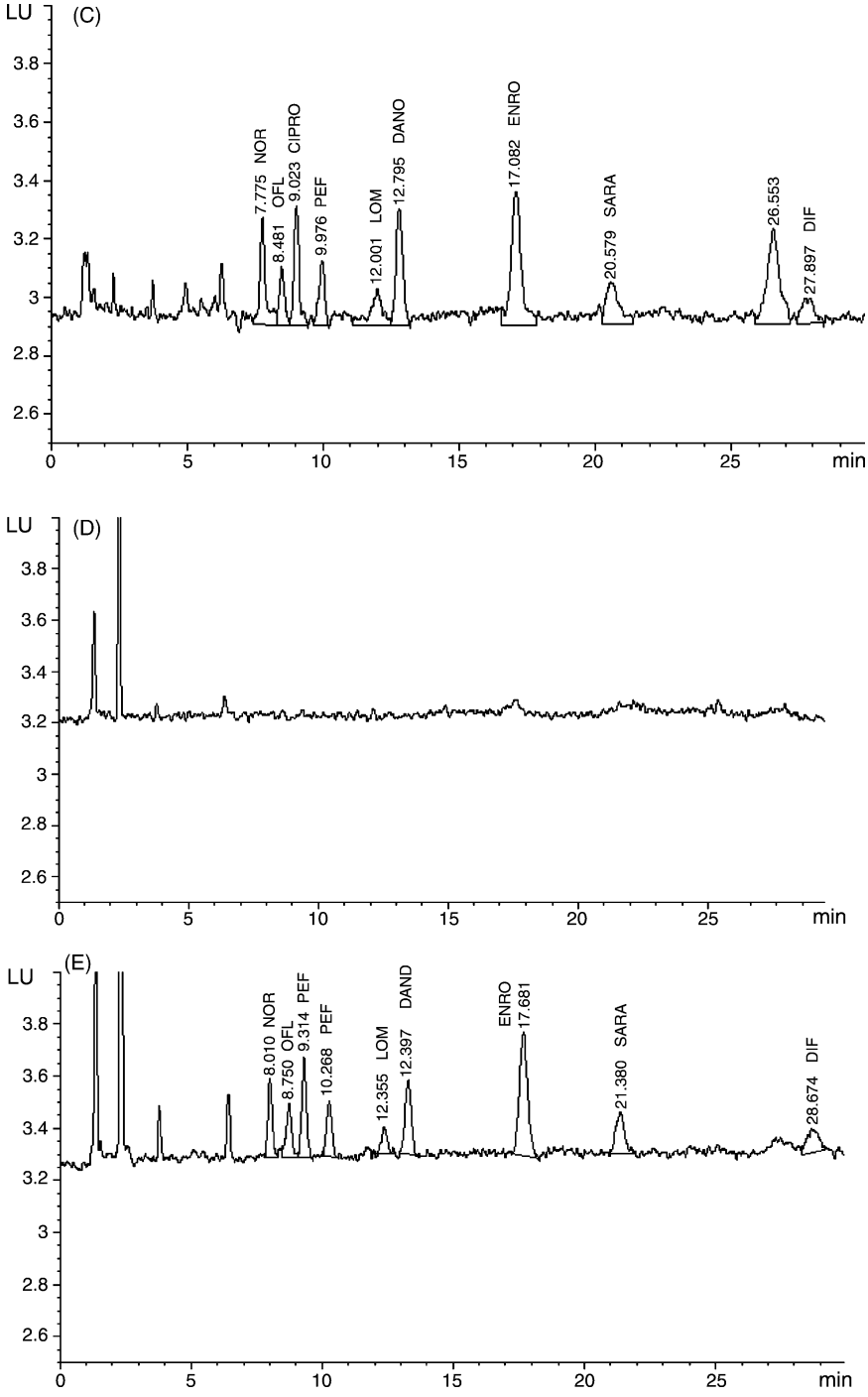

Fig. 1. (*Continued*).

Guangdong, China). Difloxacin hydrochloride (100.6%) was a gift of Guangzhou Huihua Animal Health Products Limited Co. (Guangzhou, Guangdong, China).

Double distilled water was used in preparing all solutions. Acetonitrile from Fisher Scientific was HPLC-grade. Methanol, ethanol, hexane, triethylamine, acetic acid, citric acid, hydrochloride acid, trichloroacetic acid, and ammonium acetate from Guangzhou Chemical Reagent Factory (Guangzhou, Guangdong, China) were analytical reagent grade.

2.2. Standard solutions

Individual stock standard solutions (0.1 mg ml^{-1}) of FQs were prepared by dissolving 10.0 mg of each norflorxacin, enrofloxacin and sarafloxacin, 10.1 mg of ofloxacin, 11.1 mg

of lomefloxacin hydrochloride in 2 ml of 10% acetic acid, 11.7 mg of ciprofloxacin hydrochloride, 12.3 mg of pefloxacin methanesulphonate, 12.75 mg of danofloxacin methanesulphonate, 10.8 mg of difloxacin hydrochloride in 2.0 ml of purified water and adjusting to 100 ml with methanol. These solutions were stored at 4 °C and stable for at least 6 months.

Working standard mixture solutions of nine FQs were prepared by mixing desired volume of individual stock standard solutions and serially diluting to different levels with methanol. These solutions were stored at 4 °C and stable for at least 1 month.

2.3. Sample preparation

Eggs were obtained from laying hens without antibiotics. After collecting, egg yolk or white was separated, homogenized, and stored at 4 °C for no longer than 24 h until analysis. 0.5 g of homogenate of egg yolk or white were accurately weighted into a 15 ml polypropylene centrifuge tube. For calibration and recovery studies, 100 μl of working standard mixture solution were added and vortex-mixed at 1500 rpm. After standing for 30 min, 4 ml of acetic acid–absolute ethanol (1:99, v/v) were added into egg white, and vortex-mixed vigorously. For egg yolk extraction, 0.25 ml of acetonitrile were added and vortex-mixed, followed by adding 4 ml of acetic acid–absolute ethanol (1:99, v/v) and vigorous mixing. Then both yolk and white samples were shaken for 30 min on a shaker and stood for 5 min, followed by centrifugation for 15 min at 5500 × *g* at 4 °C. The supernatant was moved into a 10 ml centrifuge tube, and dried at 78 °C under nitrogen stream. 0.5 ml of acetonitrile were added to dissolve the residue. After mixing on a vortexer at 2000 rpm, 2 ml of hexane were added and vortex-mixed at 1000 rpm. After standing for 5 min, the upper layer was pipetted out and discarded. For egg yolk, the hexane defatting step was repeated by adding another 2 ml of hexane into the low layer. Then the low layer from yolk or white sample was evaporated to dryness at 78 °C under nitrogen stream, and the residue was dissolved in 0.5 ml mobile phase. The resulting solution was transferred to a 2 ml microcentrifuge tube and centrifuged for 10 min at 6600 × *g*. The supernatant was filtered through 0.2 μm nylon filter and 20 μl of the filtered solution were injected into LC for analysis.

2.4. Instrumentation and chromatographic conditions

The chromatographic system was HP-1100 series high performance liquid chromatograph from Agilent Technologies (Palo Alto, CA, USA) equipped with quaternary pump, on-line degasser, autosampler, column heater, and fluorescence detector (G1321A mode) connected on-line. Chromatographic separation of the nine FQs was achieved on a C_{18} Hypersil-BDS (250 mm × 4.6 mm, 5 μm) column from Agilent Technologies (Palo Alto, CA, USA). The LC mobile phase consisted of acetonitrile/aqueous solution

Table 2
Regression data[a] and correlation coefficients (r^2) for standard curves of nine FQs in egg white and yolk

Drug	Tested range (ng g^{-1})	Egg white		Egg yolk	
		Slope (±S.D.)	Intercept (±S.D.)	Slope (±S.D.)	Intercept (±S.D.)
Norfloxacin	20–2000	2.891×10^{-4} ($\pm 8.172 \times 10^{-6}$)	4.865×10^{-3} ($\pm 3.355 \times 10^{-3}$)	3.287×10^{-4} ($\pm 9.501 \times 10^{-6}$)	-5.186×10^{-3} ($\pm 9.178 \times 10^{-3}$)
Ofloxacin	20–2000	4.237×10^{-4} ($\pm 2.845 \times 10^{-5}$)	6.967×10^{-3} ($\pm 6.086 \times 10^{-3}$)	4.346×10^{-4} ($\pm 1.335 \times 10^{-5}$	3.472×10^{-3} ($\pm 6.318 \times 10^{-3}$)
Ciprofloxacin	20–2000	2.824×10^{4} ($\pm 1.420 \times 10^{-5}$)	8.752×10^{-3} ($\pm 4.029 \times 10^{-3}$)	3.262×10^{-4} ($\pm 9.220 \times 10^{-6}$)	2.270×10^{-3} ($\pm 8.506 \times 10^{-3}$)
Pefloxacin	10–1000	1.549×10^{-4} ($\pm 8.420 \times 10^{-6}$)	2.093×10^{-3} ($\pm 4.788 \times 10^{-3}$)	1.486×10^{-4} ($\pm 3.374 \times 10^{-6}$)	-1.426×10^{-3} ($\pm 3.238 \times 10^{-3}$)
Lomefloxacin	10–1000	4.777×10^{-4} ($\pm 3.083 \times 10^{-5}$)	-1.764×10^{-3} ($\pm 5.999 \times 10^{-3}$)	4.663×10^{-4} ($\pm 1.322 \times 10^{-5}$)	-4.200×10^{-3} ($\pm 3.200 \times 10^{-3}$)
Danofloxacin	5–500	0.2938×10^{-4} ($\pm 1.738 \times 10^{-6}$)	-2.254×10^{-3} ($\pm 3.253 \times 10^{-3}$)	2.556×10^{-5} ($\pm 2.881 \times 10^{-7}$)	-1.520×10^{-3} ($\pm 1.704 \times 10^{-3}$)
Enrofloxacin	20–2000	1.547×10^{-4} ($\pm 8.528 \times 10^{-6}$)	1.1776×10^{-2} ($\pm 4.327 \times 10^{-3}$)	1.342×10^{-4} ($\pm 2.604 \times 10^{-6}$)	5.478×10^{-3} ($\pm 5.359 \times 10^{-3}$)
Sarafloxacin	20–2000	3.836×10^{-4} ($\pm 1.932 \times 10^{-5}$)	1.1037×10^{-2} ($\pm 2.179 \times 10^{-3}$)	3.797×10^{-4} ($\pm 1.537 \times 10^{-5}$)	-1.307×10^{-3} ($\pm 8.767 \times 10^{-3}$)
Difloxacin	10–1000	2.304×10^{-4} ($\pm 1.116 \times 10^{-5}$)	-9.991×10^{-3} ($\pm 7.262 \times 10^{-3}$)	1.968×10^{-4} ($\pm 4.546 \times 10^{-6}$)	1.497×10^{-2} ($\pm 3.718 \times 10^{-3}$)

[a] Seven points data for each curve average from five replicates of fortified egg white and egg yolk.

(9:91,v/v). The aqueous solution contained 50 mM citric acid and 100 mM ammonium acetate, adjusted to pH 4.0 with triethylamine, and filtered through a 0.45 μm nylon filter. Detection was performed with a fluorescence detector, and the excitation/emission wavelengths were 278/465 nm. The flow-rate of the mobile phase was 2.2 ml min^{-1}, the injection volume was 20 μl, and the column temperature was maintained at 50 °C.

3. Results and discussion

3.1. Optimization of the LC conditions

Typical chromatograms of working standard mixture, blank white, fortified white, blank yolk and fortified yolk are shown in Fig. 1A–E. Nine FQs could be effectively separated in a single run under the specified LC conditions. From the Fig. 1B and D, it was apparent that there was no obvious interference at the retention times of the nine FQs. several sources of control eggs were tested. There was no interference background in the control egg (both white and yolk) samples when the eggs were from the laying hens without antibiotic treatment.

A variety of mobile phases were used for FQs separation. Usually acetonitrile or methanol was used as organic phase, orthophosphoric acid, tetrabutyl ammomium bromide, ammonium acetate, citric acid, formic acid or acetic acid as aqueous phase. In this study, nine FQs with very similar structure have to be separated in one run, therefore different concentrations of aqueous components (orthophosphoric acid, formic acid or acetic acid) with organic components (acetonitrile or methanol) of the mobile phase were tried to elute the drugs, but the peaks of norfloxacin and ofloxacin could not be completely separated from each other. A good separation of nine FQs was achieved combining 50 mM citric acid and 100 mM ammonium acetate as aqueous component of mobile phase and acetonitrile as organic component. And the retention times of FQs were greatly influenced by the percentage of acetonitrile in mobile phase: the higher percentage of acetonitrile in mobile phase, the short retention times of the FQs. However, the peaks of norfloxacin and ofloxacin, and the peaks of ciprofloxacin and pefloxacin could not be separated when acetonitrile in mobile phase was more than 9%. So the mobile phase was set as acetonitrile/aqueous solution (9:91). When the normal flow-rate (1.0 ml min^{-1}) of the mobile phase was used, the retention time of the last drug (difloxacin) was almost at 100 min. Therefore, a high flow-

Table 3
Average recovery and precision of FQs from fortified egg white

FQs	Fortified level (ng g^{-1})	Average recovery (%) and (R.S.D., %) (n = 5)				Overall recovery (%) (n = 20)	Inter-day (R.S.D., %) (n = 20)
		Day 1	Day 2	Day 3	Day 4		
Norfloxacin	20	88.5 (12.4)	78.4 (10.9)	69.4 (18.3)	75.6 (3.5)	77.2	14.5
	200	76.3 (6.8)	79.7 (3.2)	74.5 (6.1)	75.4 (9.3)	76.5	13.7
	2000	75.8 (3.2)	82.2 (5.8)	72.9 (11.3)	74.8 (5.6)	76.4	11.3
Ofloxacin	20	83.4 (10.3)	74.8 (2.1)	84.6 (5.9)	78.7 (5.9)	80.4	13.7
	200	77.0 (2.4)	82.8 (2.4)	80.2 (12.9)	70.7 (4.1)	77.7	13.4
	2000	76.3 (2.2)	86.9 (6.7)	80.0 (4.6)	70.8 (2.1)	78.5	10.6
Ciprofloxacin	20	80.0 (13.6)	82.8 (7.2)	76.5 (2.8)	86.9 (7.9)	81.6	11.3
	200	76.8 (5.9)	82.1 (3.9)	76.3 (5.5)	75.3 (4.9)	77.6	5.8
	2000	81.0 (3.0)	88.7 (6.3)	80.0 (11.7)	78.5 (2.4)	82.1	8.9
Pefloxacin	10	78.1 (6.1)	85.8 (1.9)	77.3 (2.5)	74.4 (4.9)	78.9	6.7
	100	77.0 (3.5)	80.9 (2.2)	76.0 (1.5)	70.8 (5.5)	76.2	5.7
	1000	78.3 (2.0)	83.2 (5.7)	77.1 (4.1)	77.1 (4.0)	78.9	5.0
Danofloxacin	5	72.7 (10.9)	83.5 (2.9)	83.6 (6.4)	64.4 (10.8)	77.5	8.2
	50	82.4 (9.0)	84.2 (4.8)	79.6 (7.5)	64.0 (4.6)	78.1	5.6
	500	78.2 (5.0)	80.8 (6.4)	80.2 (12.5)	68.1 (5.9)	85.6	6.3
Enrofloxacin	20	74.0 (6.7)	77.8 (4.2)	74.3 (9.2)	83.7 (7.1)	74.7	12.7
	200	79.6 (5.7)	81.2 (2.4)	79.2 (2.1)	72.3 (2.7)	77.7	6.9
	2000	86.5 (2.5)	89.8 (5.6)	85. 7 (3.2)	80.3 (2.5)	82.2	14.0
Sarafloxacin	20	67.9 (8.2)	68.8 (2.5)	79.0 (11.1)	83.1 (12.4)	79.2	13.6
	200	78.2 (6.6)	81.1 (3.5)	79.0 (3.4)	72.3 (6.3)	77.8	10.9
	2000	79.2 (3.2)	86.6 (5.3)	78.9 (9.9)	83.9 (3.0)	80.8	7.2
Difloxacin	10	77.2 (8.9)	73.3 (14.6)	84.7 (6.8)	81.6 (4.5)	79.5	14.2
	100	78.6 (3.2)	91.0 (2.6)	67.3 (6.0)	74.3 (5.6)	75.3	13.1
	1000	78.5 (2.3)	88.4 (6.0)	80.5 (5.0)	75.8 (2.9)	80.8	14.4
Lomefloxacin	10	76.3 (7.4)	81.0 (14.9)	79.5 (20.6)	81.0 (15.0)	76.1	13.5
	100	80.3 (11.6)	78.8 (2.7)	72.9 (1.7)	69.3 (1.1)	77.6	13.2
	1000	84.9 (7.4)	86.3 (8.4)	75.0 (10.2)	76.8 (1.1)	76.8	10.2

rate (2.2 ml min^{-1}) of the mobile phase and a high column temperature (50 °C) were used to reduce the run time. Under these conditions, the typical operating back-pressure was 150–155 bar, and the columns could last 2 months.

3.2. Optimization of the sample treatment procedure

Eggs are difficult food matrix for residue analysis because of significant binding between the lipoprotein matrices of eggs and drugs, resulting in poor extraction and isolation of FQs [7]. To deproteinize of egg white and yolk, we tried different concentrations of hydrochloride (1.0 and 1.5 M), trichloroacetic acids (5 and 15%), or mixture of methanol with different concentration of trichloroacetic acids. But all these solutions could not precipitate egg proteins completely. A mixture of acetonitrile and acetic acid could precipitate protein, but a very low recovery (<15%) was achieved. Finally, acetic acid–absolute ethanol (1:99) was able to precipitate proteins of egg white, and a good recovery was obtained for white. For yolk extraction, acetic acid–absolute ethanol (1:99) only achieved 17–50% recovery. When a little (0.25 ml) acetonitrile was added into yolk and mixed before adding 4 ml of acetic acid–absolute ethanol (1:99), satisfactory recovery (>80%) was obtained for the nine FQs.

3.3. Calibration

In order to establish the standard calibration curves, seven-level of the standard samples were prepared in replicates of five by extracting the spiked egg white (or yolk) as described in sample preparation, and detected in HPLC. The concentrations of samples ranged 20–2000 ng g^{-1} for norfloxacin, ofloxacin, ciprofloxacin, enrofloxacin and sarafloxacin, 10–1000 ng g^{-1} for pefloxacin, difloxacin and lomefloxacin, 5–500 ng g^{-1} for danofloxacin. The standard calibration curve in egg white (or yolk) of each FQs was calculated by line regression of the measured peak areas of LC chromatograms and the corresponding concentrations of the standard calibration samples. The slope of the calibration curve of each FQs and its standard deviation (S.D.), the intercept and its S.D. from egg white and yolk were given in Table 2. The calibration curve of each fluoroquinolone showed good linearity with correlation coefficient (r^2) more than 0.998, indicating good correlations between FQs concentrations and peak areas. As there is no interference background for the nine FQs, the limits of quantification (LOQs) expressed as the lowest concentration point in standard curve, were 20.0 ng g^{-1} for norfloxacin, ofloxacin, ciprofloxacin, enrofloxacin and sarafloxacin, 10.0 ng g^{-1}

Table 4
Average recovery (%) and precision (%) of FQs from fortified egg yolk

FQs	Fortified level (ng g^{-1})	Average recovery (%) and (R.S.D., %) (n = 5)				Overall recovery (%) (n = 20)	Inter-day (R.S.D., %) (n = 20)
		Day 1	Day 2	Day 3	Day 4		
Norfloxacin	20	86.8 (4.3)	77.2 (5.7)	84.0 (2.3)	70.0 (5.5)	79.5	9.4
	200	79.0 (4.2)	82.9 (4.4)	82.4 (4.0)	72.2 (5.9)	79.1	7.3
	2000	88.1 (3.5)	77.6 (4.9)	83.7 (6.0)	77.5 (3.1)	81.7	7.0
Ofloxacin	20	81.3 (5.4)	86.1 (13.9)	94.1 (8.4)	78.3 (5.2)	85.0	11.4
	200	81.6 (3.6)	86.4 (1.4)	86.6 (2.9)	82.5 (4.9)	84.3	4.1
	2000	92.7 (2.9)	89.3 (2.0)	91.5 (6.0)	89.3 (4.0)	90.6	4.0
Ciprofloxacin	20	86.6 (3.1)	83.4 (10.1)	96.2 (13.0)	77.1 (3.3)	85.8	11.8
	200	85.8 (4.4)	88.5 (4.0)	88.74 (4.2)	82.0 (6.2)	86.3	5.7
	2000	89.6 (2.9)	80.0 (3.5)	85.2 (4.9)	80.0 (2.1)	83.7	5.9
Pefloxacin	10	87.1 (3.5)	84.3 (2.0)	88.6 (1.7)	82.0 (4.4)	85.5	4.1
	100	85.0 (3.5)	83.6 (1.5)	82.6 (2.6)	78.0 (10.4)	82.3	3.9
	1000	85.8 (2.2)	81.4 (1.8)	83.5 (4.4)	81.1 (1.0)	82.8	3.4
Danofloxacin	5	85.0 (4.5)	88.0 (8.7)	85.8 (2.9)	82.8 (3.9)	85.2	7.0
	50	86.2 (3.7)	86.6 (2.5)	85.1 (3.4)	82.2 (1.6)	91.2	3.9
	500	82.5 (2.5)	78.7 (1.8)	80.5 (5.3)	78.7 (2.1)	81.0	3.3
Enrofloxacin	20	87.9 (8.7)	84.7 (8.6)	86.1 (3.3)	82.1 (3.4)	86.8	8.3
	200	93.8 (3.7)	92.7 (1.6)	92.1 (1.6)	86.2 (1.6)	82.8	5.1
	2000	83.9 (2.3)	79.4 (1.8)	81.6 (3.8)	79.2 (1.0)	88.0	4.3
Sarafloxacin	20	94.0 (6.5)	86.1 (8.6)	88.1 (10.8)	79.0 (6.2)	83.7	14.3
	200	81.7 (4.1)	84.8 (3.1)	81.4 (3.3)	83.1 (8.1)	86.8	8.8
	2000	92.5 (2.9)	85.6 (2.3)	87.5 (4.5)	86.2 (2.1)	81.5	2.7
Difloxacin	10	86.4 (2.6)	90.2 (4.9)	73.0 (7.9)	85.0 (11.3)	87.7	14.9
	100	90.8 (2.6)	84.0 (9.4)	89.6 (6.2)	82.9 (3.4)	89.1	10.0
	1000	83.7 (2.1)	82.7 (10.5)	78.8 (4.1)	80.9 (2.0)	82	4.5
Lomefloxacin	10	84.8 (3.5)	90.1 (7.0)	85.1 (5.8)	90.8 (10.8)	85.4	5.6
	100	88.5 (12.4)	91.4 (2.3)	91.3 (3.2)	85.3 (2.8)	85.0	3.3
	1000	86.3 (2.7)	79.2 (2.3)	83.1 (4.1)	79.4 (1.2)	80.1	3.6

for pefloxacin, lomefloxacin and difloxacin, 5.0 ng g^{-1} for danofloxacin.

3.4. Recoveries and repeatability

For the studies of recovery and repeatability, blank egg white or yolk was spiked with the nine FQs at three levels (5–2000 ng g^{-1}), and extracted as described in sample preparation. Five replicated samples of each level were prepared and analyzed under the same experimental conditions, during the same day and on 4 different days, respectively. The recoveries of fortified white and yolk were shown in Tables 3 and 4, respectively. A good recovery for the nine FQs was obtained from both white and yolk, indicating the extraction procedures described in sample preparation were very effective. And the average recoveries of nine FQs from fortified yolk samples (79.1%–91.2%) at three fortified levels were higher than those from fortified egg white (74.7%–85.6%). The inter-day relative standard deviations (R.S.D.) from both egg white and yolk were less than 15%, most of the intra-day R.S.D. were less than 10%, indicating that the method developed had an acceptable precision.

4. Conclusions

A simple and sensitive LC-fluorescence method was developed for determination of norfloxacin, ofloxacin, ciprofloxacin, pefloxacin, lomefloxacin, danofloxacin, enrofloxacin, sarafloxacin, difloxacin in egg white and yolk. The LOQs achieved by the methods were 5–20 ng g^{-1}.

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