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Research Article

Single-step extraction followed by LC for determination of (fluoro)quinolone drug residues in muscle, eggs, and milk

In this study, a simplified method for the extraction and determination of seven fluoroquinolone residues (danofloxacin, difloxacin, enrofloxacin, marbofloxacin, orbifloxacin, ofloxacin, and sarafloxacin) and three quinolones (oxolinic acid, flumequine, and nalidixic acid), in porcine muscle, table eggs, and commercial whole milk, which required no cleanup step, was devised. This procedure involves the extraction of analytes from the samples *via* liquid-phase extraction, and the subsequent quantitative determination was accomplished *via* LC-fluorescence detection. Analyte separation was successfully conducted on an XBridge-C_{18} column, with a linear gradient mobile phase composed of acetonitrile and 0.01 M oxalic acid buffer at pH = 3.5. The one-step liquid-liquid extraction method evidenced good selectivity, precision (RSDs = 0.26–15.07%), and recovery of the extractable analytes, ranging from 61.12 to 115.93% in matrices. The LOQs ranged from 0.3 to 25 μg/kg. A survey of ten samples purchased from local markets was conducted, and none of the samples harbored fluoroquinolone residues. This method is an improvement over existing methodologies, since no additional cleanup was necessary.

Keywords: Egg / Fluoroquinolones / Milk / Porcine muscle / Residue analysis
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1 Introduction

Fluoro(quinolones) are a synthetic group of antibacterials whose activity is related to the inhibition of bacterial DNA gyrase, which leads to cell damage and death [1]. Owing to their broad spectrum of bactericidal activity against a broad range of bacteria, their use is not restricted to human medicine, but also finds wide application in the treatment and prevention of veterinary diseases, and even as growth-promoting agents [2, 3]. It is, therefore, anticipated that the misuse of fluoroquinolones can leave residues in edible animal meats and may give rise to public health concerns, including development of resistant bacterial strains, allergic hypersensitivity reactions, and even toxic effects [4]. In order to mitigate these hazards, the European Union has regulated the use of quinolones in food-producing animals [5]. Additionally, the European Union has established the specific Decision 2002/657/EC, which concerns procedures for the validation of analytical methods, to ensure the quality and comparability of analytical results and the interpretation of results in the official control of residues in food-producing animals [6]. Overall, the analysis of residual quantities of drugs in foods of animal origin is crucial for the quality control of consumer products.

A number of analytical methods have been validated for the multiresidue analysis of fluoroquinolone antibiotics, including HPLC [1, 7–9] LC-MS/MS [10–13], and CZE-MS [14]. MS/MS allows for the multiresidue determination of quinolones in different matrices with the possibility of confirming the presence of these compounds by means of fragment abundance ratios at rather low concentration levels. In fact, the equipment required for this is quite expensive and requires skilled personnel; in actuality only a few laboratories can currently afford to acquire these instruments. Furthermore, the majorities of the analytical methods (using LC or LC-MS/MS) thus far developed for the multiresidue determination of fluoroquinolone residues in milk [8, 10, 13, 15–20], eggs [21–23], and muscle [24, 25] utilized SPE as a cleanup and/or preconcentration step.

The principal objective of this study, therefore, was to develop a practical, accurate, and precise method for the

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Abbreviations: DANO, danofloxacin; **DIFLO,** difloxacin; **ENRO,** enrofloxacin; **FLUME,** flumequine; **IS,** internal standard; **MARBO,** marbofloxacin; **OFLO,** ofloxacin; **ORBI,** orbifloxacin; **OXO,** oxolinic acid; **SARA,** sarafloxacin

rapid extraction and quantitation of fluoroquinolone residues (marbofloxacin (MARBO), ofloxacin (OFLO), danofloxacin (DANO), enrofloxacin (ENRO), orbifloxacin (ORBI), difloxacin (DIFLO), sarafloxacin (SARA), cinoxacin (CINO) (IS), oxolinic acid (OXO), nalidixic acid (NALI), and flumequine (FLUME)) in porcine muscle, table eggs, and whole milk. All fluoroquinolones were analyzed in a single chromatographic run. In this study, liquid-phase extraction was employed without additional cleanup procedures, followed by LC with fluorescence detection.

2 Materials and methods

2.1 Chemical and reagents

Standard DIFLO, MARBO, and SARA were supplied by Dr. Ehrenstorfer (Augsburg, Germany). DANO (99.9%) and ORBI were purchased from Riedel-de Haen (Sigma-Aldrich GmbH, Seelze, Germany). ENRO, FLUME, NALI, OFLO, OXO, and CINO (IS) were supplied by Sigma-Aldrich (St Louis, MO, USA). Acetonitrile, *n*-hexane, and methanol HPLC-grade solvents were provided by J.T. Baker (Griesheim, Germany). Sodium sulfate was obtained from Junsei Chemical (Kyoto, Japan). Trichloroacetic acid and oxalic acid were acquired from Sigma-Aldrich. All other chemicals and solvents were of analytical grade, unless stated otherwise. Solutions prepared for HPLC were passed through a 0.45 μm PVDF syringe filter (Whatman, Maidstone, England) before use. Porcine muscle, table eggs, and commercial whole milk were purchased from large markets in which organic products were sold. Preliminary analyses demonstrated that they were analyte-free.

2.2 Standard solutions

Stock solutions of 100 μg/mL of CINO, DANO, DIFLO, ENRO, FLUME, NALI, MARBO, ORBI, OXO, OFLO, and SARA were prepared in methanol. The stock solutions were stored at 4°C for no longer than a couple of months, with the exception of FLUME, NALI, and OXO, which were stable only for a couple of weeks [6]. The working solutions for HPLC injections were prepared daily from the stock solution in a solvent mixture of acetonitrile and 0.01 M oxalic acid (17:83, v/v).

Table 1. Gradient condition used for the separation of the tested analytes

Compound	Mobile phase			Excitation/Emission wavelength		
	Time (min)	A (%)	B (%)	Time (min)	Ex (nm)	Em (nm)
MARBO, OFLO	1	17	83	1	290	495
DANO, ENRO, ORBI DIFLO, SARA, Cino (IS)	20	17	83	7	278	455
OXO, Nali, FLUME	21–30	40	60	21–30	327	369
	32–35	17	83	32–35	290	495

A (%): acetonitrile, B (%): 0.01 M oxalic acid (pH 3.5).

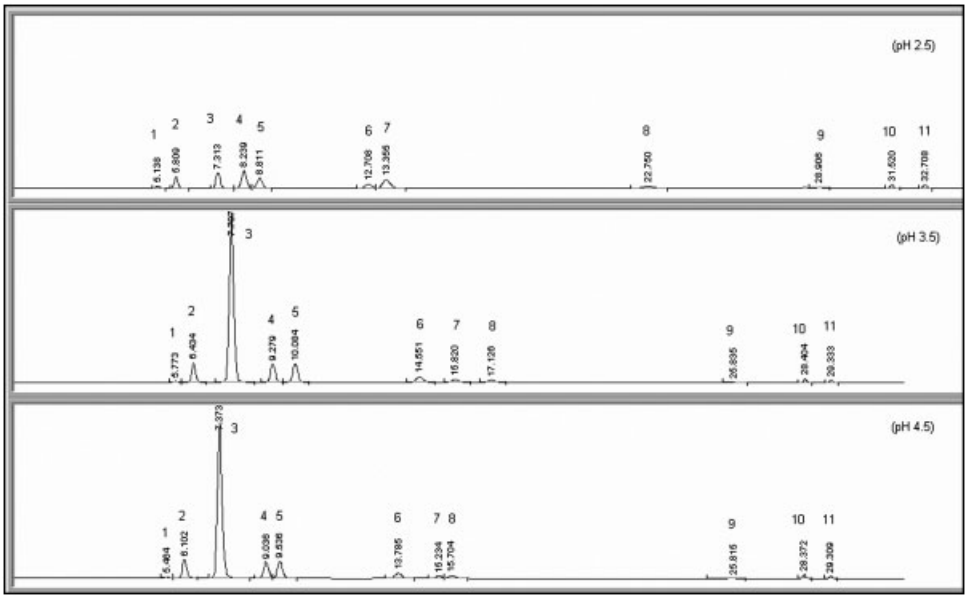

Figure 1. Effect of various pH values on the chromatographic separation of the tested (fluoro)quinolones. (1) MARBO, (2) OFLO, (3) DANO, (4) ENRO, (5) ORBI, (6) SARA, (7) DIFLO, (8) CINO, (9) OXO, (10) NALI, and (11) FLUME.

2.3 Sample preparation

Blank or spiked porcine samples were chopped and whole table eggs and commercial milk were homogenized. All samples were stored at −20°C. A measure of 2 g (approximately 2 mL of milk) of blended samples were transferred to a 50 mL polypropylene centrifuge tube and mixed with a specific volume (200 μL) of internal standard (IS) (CINO, 4 μg/mL) [26]. After a 10 min equilibration period, 7 mL of 2.5% trichloroacetic acid/acetonitrile (25/75, v/v) was added, vortex-mixed (15 s), and left undisturbed for 10 min. A quantity of 2 g of anhydrous sodium sulfate (4 g in milk) was added thereafter, thoroughly mixed for 15 s, and maintained for 5 min. The samples were centrifuged at 2700 × *g* for 20 min. The samples were then extracted once again with 7 mL of 2.5% trichloroacetic acid/acetonitrile (25:75, v/v), and maintained at room temperature for 10 min. The organic layers were recovered after 20 min centrifugation at 2700 × *g*. *n*-Hexane (10 mL) was then added to the combined supernatant. After 15 s vortex mixing, the upper layer was discarded after 10 min of centrifugation at 2700 × *g*. The lower layers were transferred to glass tubes and concentrated under a stream of nitrogen at 45–50°C. The concentrated residues were then dissolved in 2 mL of mobile phase (0.01 M oxalic acid/acetonitrile (83:17, v/v),

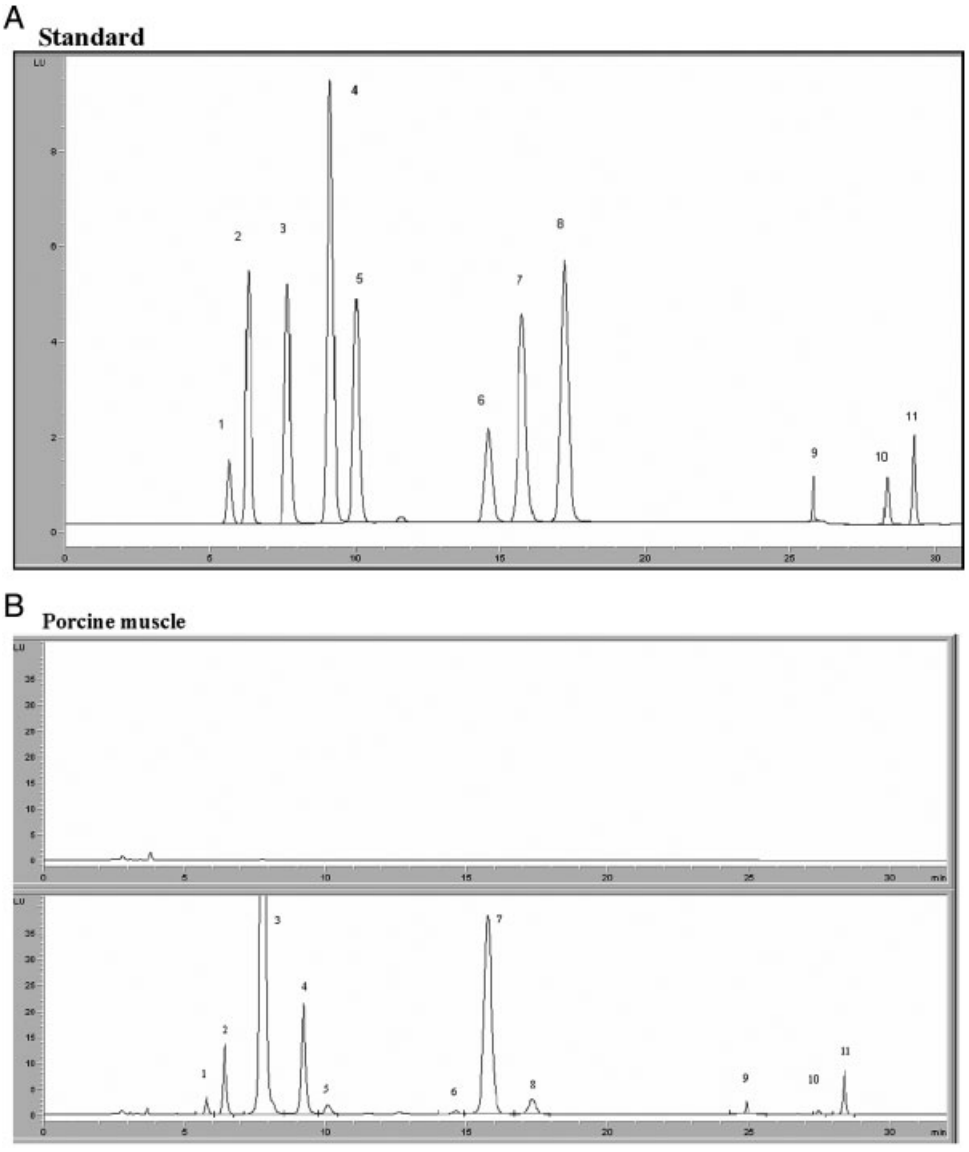

Figure 2. Representative chromatogram of: (A) a standard mixture of the tested (fluoro)quinolones; (B) unspiked porcine muscle (top) and spiked porcine muscle (bottom); (C) unspiked table eggs (top) and spiked table eggs (bottom); (D) unspiked whole milk (top) and spiked whole milk (bottom); and (E) real samples. (1) MARBO, (2) OFLO, (3) DANO, (4) ENRO, (5) ORBI, (6) SARA, (7) DIFLO, (8) CINO, (9) OXO, (10) NALI, and (11) FLUME.

vortex mixed (15 s), and filtered through a 0.45 μm PVDF syringe filter (Whatman) after 20 min centrifugation at 15 290 × *g* (4°C). The residues were stored at 4°C until injection at room temperature into a high-performance liquid chromatograph equipped with a fluorescence detector [9].

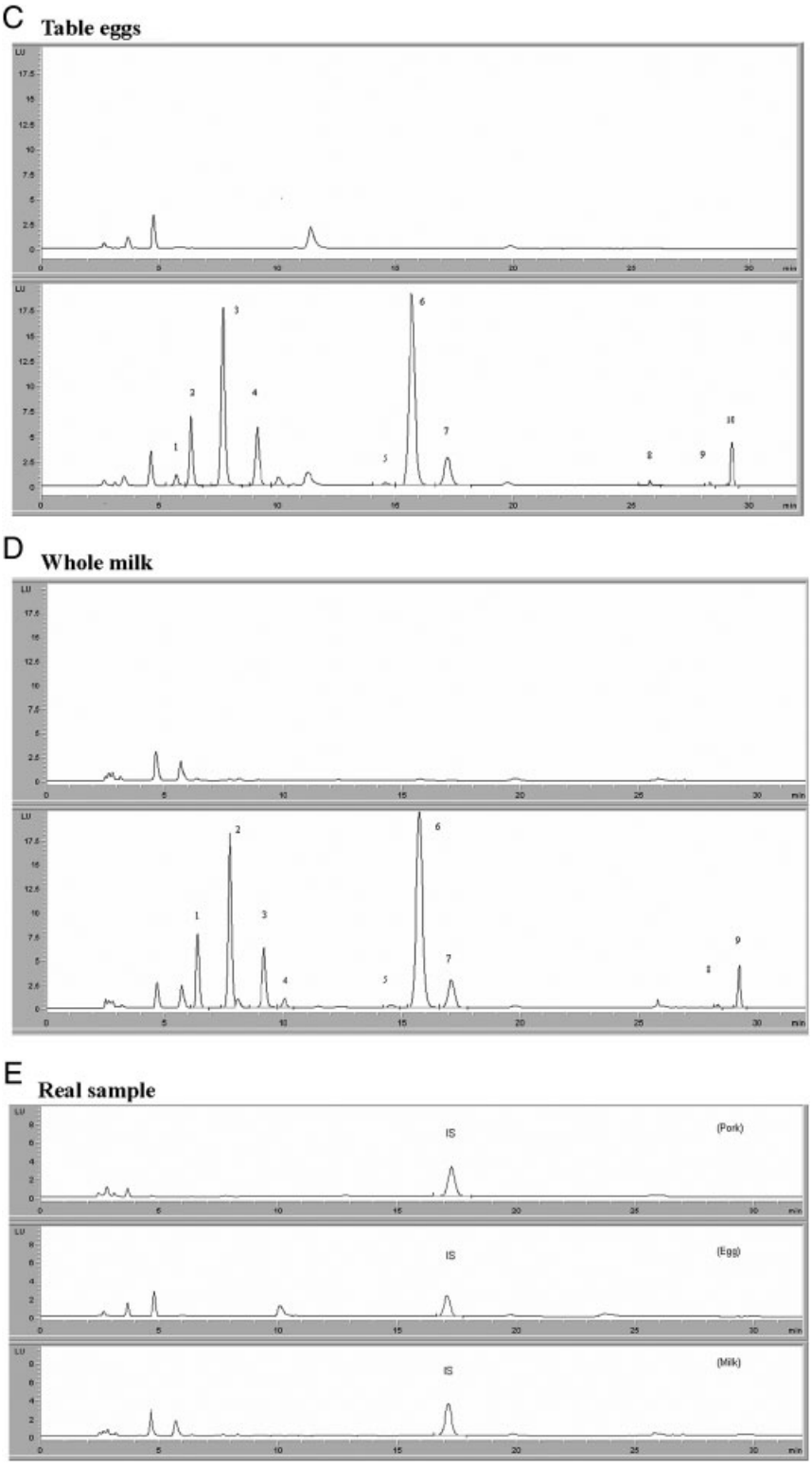

Figure 2. Continued.

2.4 Chromatographic quantification

Fluoroquinolone residue levels were quantitated using an HPLC (Agilent series 1100, Palo Alto, CA, USA) equipped with a quaternary pump (G1311A), a degasser (G1322A), an autosampler (G1313A) containing a 100-μL-sample loop, a colcom (G1316A), and a programmable fluorescence detector (G1321A) operated at appropriate excitation and emission wavelengths for each compound [8]. The samples were separated on a C_{18} stainless column (XBridge, 4.6 mm id × 250 mm, particle size: 5 μm, Waters, Milford, MA, USA), and eluted with a mobile phase consisting of a mixture of acetonitrile and 0.01 M oxalic acid buffer at pH = 3.5. The mobile phase and fluorescence detector were programmed as summarized in Table 1. The gradient mode at a flow rate of 1.0 mL/min and 20 μL aliquots were injected onto the column. The column temperature was maintained at 25°C.

2.5 Method validation

The method was validated in order to fulfill the criteria specified by the CODEX guidelines for specificity, linearity, LOD and LOQ, precision, accuracy, and recovery. Blank samples (porcine muscle, whole table eggs, and commercial milk) were evaluated for matrix interference. Standard solutions of (fluoro)quinolones were analyzed at different temperatures (25, 35, and 45°C) and mobile phase pH values (2.5, 3.5, and 4.5).

Linearity was evaluated for each of the evaluated samples (porcine, whole table eggs, and commercial milk) using spiked samples at six concentration levels (0.5, 1, 2, 3, 4, and at 5 times the permitted limit (MRL)). Each sample was analyzed five times. Calibration curves were calculated *via* least-squares linear regression analysis of the peak area ratio of each analyte to the IS. The LODs and the LOQs were calculated as the lowest analyte concentration required to generate a response at a signal/noise ratio of 3 and a signal/noise ratio of 10, respectively.

Precision and accuracy for intra-day ($n = 5$) and inter-day ($n = 5$) were determined at four different concentrations (0.5, 1, 2, and 5 times the permitted limit). Recovery was performed on fortified samples at concentrations of 0.5, 1, 2, and 5 times the permitted limit. Six samples were prepared for each concentration level. The responses of the (fluoro)-quinolones added to blank samples before extraction were compared with those in which (fluoro)quinolones were added after extraction (control).

3 Results and discussion

3.1 Chromatography

In order to optimize the separation of the analytes, the constituents and the relative percentages of the mobile phase were tested. The linear gradient mobile phase consisting of acetonitrile/0.01 M oxalic acid was used. OXO, NALI, and FLUME were eluted at very slow retention times under isocratic conditions of 0.01 M oxalic acid/acetonitrile (83:17, v/v, pH = 3.5). The organic gradient is required for the elution of the less polar substances. Adequate chromatographic separation of the ten (fluoro)-quinolones was conducted from 0 to 20 min with 0.01 M oxalic acid/acetonitrile (83:17, v/v, pH = 3.5), from 20 to 30 min with 0.01 M oxalic acid/acetonitrile (60:40, v/v, pH = 3.5).

The excitation/emission wavelengths used for the fluorescence detector were 290/495 nm for MARBO and OFLO, 278/455 nm for DANO, ENRO, ORBI, SARA, DIFLO, and CINO (IS) and 327/369 nm for OXO, NALI, and FLUME [27] (Table 1).

Column temperatures were tested at 25, 35, and 45°C. All peaks were well resolved at 25°C. Adjacent peaks between OFLO and DANO were noted and OXO peak was detected somewhat less prominently at 35 and 45°C than at 25°C.

pH is one of the most powerful tools for optimizing the separation of analyte mixtures [2]. To optimize the pH of the mobile phase, three different values (2.5, 3.5, and 4.5) were utilized. Good separations were obtained at pH 3.5. Adjacent peaks between SARA and DIFLO were not resolved at pH 2.5. Peaks between ENRO and ORBI overlapped at pH 4.5 (Fig. 1).

A typical chromatogram corresponding to a standard mixture of the selected antibiotics is shown in Fig. 2A. The separation of the analytes was achieved in less than 30 min. The retention times were 5.5 min for MARBO, 6.1 min for OFLO, 7.4 min for DANO, 9.3 min for ENRO, 10.1 min for ORBI, 14.6 min for SARA, 15.8 min for DIFLO, 17.1 min for CINO (IS), 25.8 min for OXO, 28.4 min for NALI, and 29.3 min for FLUME.

CINO was selected as an IS for LC quantification, as this quinolone was extracted efficiently from all matrices and did not coelute with any of the evaluated (fluoro)-quinolones.

3.2 Optimization of sample preparation

The majority of the methods reported for (fluoro)quinolone residues in tissue involve protein precipitation with an organic solvent in the presence of either an acid or a base. The most commonly employed organic solvents are acetonitrile, ethanol, and methanol, with acetonitrile being the most frequently used [28]. The analytes were extracted from a complex matrix after deproteinization with a mixture of trichloroacetic acid and acetonitrile [8, 9, 29]. This mixture proved effective in the deproteinization of porcine tissue, whole table eggs, and commercial milk, as well as isolation of analytes from spiked samples. Reductions in acetonitrile content did not adversely influence recovery, while

minimizing the isolation of co-extractive components from matrices [24].

3.3 Method validation

3.3.1 Specificity

The specificity of the method utilized for each sample was evaluated by analyzing blank samples. None of them evidenced any interference from porcine tissues, *i.e.* all analytes were extracted successfully from porcine muscle (Fig. 2B). Interference peaks from whole table eggs at the retention time of ORBI and commercial milk at the retention times of MARBO and OXO were observed. Put another way, nine and eight (fluoro)quinolones were isolated successfully in table eggs and commercial whole milk, respectively (Fig. 2C and D). It should be noted that previous studies dealt only with one matrix or similar matrices, simply because each matrix required a specific development method.

3.3.2 Linearity

The chromatographic method was shown to be linear at six concentration levels (0.5, 1, 2, 3, 4, and 5 times the permitted limit) ($r \geq 0.993$). All calibration data, as well as LODs and LOQs are listed in Table 2. The LOQs ranged from 0.3 to 25 μg/kg in porcine muscle, whole table eggs, and commercial milk. The LODs and LOQs were in all cases lower than the maximum residue limits suggested by the Korea Food and Drug Administration [30] for these compounds in the tested matrices.

3.3.3 Precision and accuracy

To characterize the reproducibility, precision, and accuracy of the method presented herein, all matrices were analyzed

Table 2. (a) Correlation values of (fluoro)quinolones in porcine muscle; (b) correlation values of (fluoro)quinolones in table eggs; (c) correlation values of (fluoro)quinolones in commercial whole milk

Analyte	Range (μg/kg)	Slope	Intercept	r^2	LOD (μg/kg)	LOQ (μg/kg)
(a)						
MARBO	75–750	1.5695 ± 0.1467	0.0051 ± 0.0124	1.000	5	15
OFLO	50–500	9.5799 ± 0.8472	0.0018 ± 0.0544	0.999	0.3	1
DANO	50–500	174.3879 ± 12.8818	1.4354 ± 1.0446	0.995	0.1	0.3
ENRO	50–500	19.8750 ± 1.2705	0.0312 ± 0.1419	0.999	0.3	1
ORBI	10–100	8.8854 ± 0.3597	0.0091 ± 0.0162	1.000	0.6	2
SARA	5–50	7.2297 ± 0.5318	−0.0002 ± 0.0052	0.999	2	5
DIFLO	200–2000	13.9648 ± 0.9269	0.1215 ± 0.3038	0.999	0.6	2
OXO	50–500	1.1093 ± 0.1156	0.0121 ± 0.0101	0.997	0.8	25
NALI	15–150	1.2921 ± 0.0950	0.0059 ± 0.0048	0.993	2.5	7.5
FLUME	100–1000	2.7287 ± 0.1770	0.0149 ± 0.0305	0.999	3	10
(b)						
MARBO	37.5–375	1.1874 ± 0.0705	0.0770 ± 0.0068	0.988	5	15
OFLO	50–500	9.2394 ± 0.8369	−0.0277 ± 0.0829	0.999	0.3	1
DANO	15–150	83.5289 ± 7.2782	0.0277 ± 0.1892	0.999	0.1	0.3
ENRO	25–250	18.5066 ± 1.8039	−0.0514 ± 0.1264	0.996	0.3	1
SARA	5–50	6.5612 ± 0.6294	0.0017 ± 0.0107	1.000	2	5
DIFLO	200–2000	13.6370 ± 1.1158	0.0645 ± 0.3964	0.999	0.6	2
OXO	25–250	0.8724 ± 0.0478	0.0068 ± 0.0049	0.997	0.8	25
NALI	15–150	0.6494 ± 0.0552	−0.0008 ± 0.0023	0.998	2.5	7.5
FLUME	100–1000	2.5562 ± 0.2433	−0.0107 ± 0.0193	0.999	3	10
(c)						
OFLO	50–500	9.1744 ± 0.3365	0.0312 ± 0.0252	0.999	0.3	1
DANO	15–150	89.1617 ± 2.6866	0.0458 ± 0.0705	0.999	0.1	0.3
ENRO	25–250	19.6029 ± 0.6188	0.0234 ± 0.0326	0.999	0.3	1
ORBI	10–100	7.9893 ± 0.1618	0.0063 ± 0.0063	0.999	0.6	2
SARA	5–50	7.1639 ± 0.5411	−0.0033 ± 0.0051	0.997	2	5
DIFLO	200–2000	13.0313 ± 0.5856	0.0022 ± 0.1469	0.999	0.6	2
NALI	15–150	1.1591 ± 0.0585	0.0005 ± 0.0036	0.996	2.5	7.5
FLUME	100–1000	2.4765 ± 0.1082	0.0110 ± 0.0164	0.998	3	10

Table 3. (a) Precision, accuracy, and recoveries of (fluoro)quinolones in fortified porcine muscle; (b) precision, accuracy, and recoveries of (fluoro)quinolones in fortified table eggs; (c) precision, accuracy, and recoveries of (fluoro)quinolones in fortified milk samples

Analytes	Range (μg/kg)	Precision RSD (%)		Accuracy (%)		Recovery Mean ± RSD (%)
		Intra-day	Inter-day	Intra-day	Inter-day	
(a)						
MARBO	75	9.06	5.42	110.40	103.44	89.53 ± 10.63
	150[a)]	10.64	7.35	105.79	101.13	94.05 ± 10.91
	300	4.59	9.68	102.10	98.13	97.13 ± 8.23
	750	5.77	10.67	101.74	99.86	90.59 ± 5.87
OFLO	50	10.47	8.43	111.60	103.22	89.67 ± 9.40
	100[a)]	5.20	5.67	103.53	99.99	90.86 ± 5.82
	200	3.46	8.60	104.43	99.59	95.95 ± 7.24
	500	2.56	8.07	101.32	97.13	88.74 ± 6.75
DANO	50	4.86	11.39	77.07	88.51	87.95 ± 9.93
	100[a)]	3.69	6.38	90.25	95.53	90.73 ± 6.05
	200	5.71	6.06	93.02	99.74	96.32 ± 8.29
	500	5.17	5.70	90.42	95.61	88.20 ± 6.57
ENRO	50	3.93	10.43	88.63	97.13	91.83 ± 9.18
	100[a)]	3.05	7.44	95.29	98.61	95.26 ± 5.31
	200	5.05	6.91	95.67	99.00	100.46 ± 7.91
	500	4.81	4.76	97.64	97.72	91.99 ± 6.38
ORBI	10	5.71	11.42	109.09	90.89	115.39 ± 9.95
	20[a)]	6.46	7.79	105.47	97.93	99.93 ± 4.25
	40	5.85	7.57	103.42	98.28	101.00 ± 7.91
	100	3.13	3.68	100.17	100.47	93.82 ± 6.48
SARA	5	3.52	8.54	86.93	102.57	95.33 ± 14.39
	10[a)]	7.63	5.97	89.65	103.59	96.42 ± 8.37
	20	11.99	12.96	90.08	99.03	95.75 ± 10.22
	50	4.28	3.99	97.68	98.10	98.86 ± 9.27
DIFLO	200	10.11	9.19	105.85	95.53	95.17 ± 8.53
	400[a)]	4.11	7.10	101.36	98.60	88.43 ± 6.26
	800	2.59	7.96	103.08	100.96	88.82 ± 4.41
	2000	1.30	6.38	99.04	97.60	83.72 ± 5.61
OXO	50	3.02	8.44	87.70	94.71	108.92 ± 7.22
	100[a)]	3.99	13.67	93.98	99.36	109.82 ± 3.13
	200	5.58	13.56	104.46	106.01	110.68 ± 5.46
	500	3.73	9.25	98.54	99.24	98.49 ± 5.77
NALI	15	9.13	7.37	70.01	71.87	103.71 ± 10.44
	30[a)]	6.64	6.69	86.38	86.08	111.41 ± 8.41
	60	10.13	4.66	96.37	100.20	105.29 ± 6.41
	150	6.57	3.88	95.52	101.20	97.29 ± 10.95
FLUME	100	3.17	8.46	88.36	96.10	97.94 ± 7.33
	200[a)]	4.35	5.57	95.89	99.14	101.39 ± 3.14
	400	4.26	6.59	96.78	100.77	107.13 ± 6.69
	1000	3.71	5.69	96.21	98.44	96.54 ± 5.80
(b)						
MARBO	37.5	8.89	6.08	64.56	74.46	95.00 ± 2.78
	75[a)]	6.98	13.30	95.75	85.69	74.63 ± 4.88
	150	1.54	4.84	100.39	100.42	72.59 ± 1.15
	375	3.41	13.71	98.99	91.27	78.53 ± 4.54
OFLO	50	4.29	4.11	109.52	108.16	80.81 ± 3.79
	100[a)]	1.81	3.78	100.36	107.53	73.67 ± 2.35
	200	3.97	3.26	95.70	102.44	73.38 ± 3.58
	500	2.12	7.92	103.25	100.35	80.61 ± 6.10

Table 3. Continued

Analytes	Range (μg/kg)	Precision RSD (%)		Accuracy (%)		Recovery Mean ± RSD (%)
		Intra-day	Inter-day	Intra-day	Inter-day	
DANO	15	6.36	8.52	101.62	102.44	81.74 ± 5.12
	30[a)]	1.70	0.80	96.54	102.53	75.90 ± 2.35
	60	3.96	7.37	94.75	102.25	73.38 ± 3.58
	150	3.49	8.97	102.66	100.09	80.61 ± 6.10
ENRO	25	6.23	11.83	113.21	107.84	80.34 ± 5.70
	50[a)]	2.41	7.48	103.99	114.52	81.45 ± 3.18
	100	1.51	9.14	97.38	101.40	80.41 ± 1.27
	250	4.06	6.62	100.19	100.94	85.27 ± 6.21
SARA	5	11.94	13.49	102.85	99.35	61.22 ± 14.39
	10[a)]	8.37	16.91	103.35	89.33	62.36 ± 12.39
	20	2.59	10.17	103.34	93.05	77.48 ± 14.85
	50	2.62	16.87	96.94	84.03	74.23 ± 4.78
DIFLO	200	6.68	6.57	103.53	110.24	87.13 ± 5.48
	400[a)]	0.93	2.10	99.73	105.79	77.82 ± 2.45
	800	1.70	5.18	97.43	103.84	80.26 ± 2.41
	2000	3.93	6.29	103.18	100.52	88.65 ± 6.02
OXO	25	2.49	4.97	100.24	103.94	96.87 ± 5.88
	50[a)]	5.35	3.63	103.25	99.62	97.37 ± 8.44
	100	2.24	9.55	107.20	107.71	88.53 ± 3.52
	250	1.79	5.94	93.30	95.30	91.65 ± 5.32
NALI	15	0.88	14.99	101.94	88.66	82.56 ± 7.09
	30[a)]	3.58	10.34	96.30	99.28	80.14 ± 2.80
	60	6.88	6.85	97.12	94.18	82.12 ± 5.62
	150	6.49	8.89	83.33	96.02	89.55 ± 5.96
FLUME	100	1.51	1.38	102.45	103.28	81.74 ± 1.76
	200[a)]	5.20	1.91	98.04	106.03	77.28 ± 4.76
	400	3.63	4.57	95.48	99.20	84.51 ± 3.79
	1000	7.19	11.81	99.42	97.72	94.20 ± 7.05
(c)						
OFLO	50	1.99	3.37	97.64	98.15	89.42 ± 2.85
	100[a)]	0.75	4.95	98.93	102.97	88.39 ± 6.15
	200	3.34	3.05	101.15	99.31	90.85 ± 9.15
	500	0.78	3.11	102.87	99.14	95.91 ± 0.79
DANO	15	3.01	1.87	96.68	93.33	85.67 ± 2.95
	30[a)]	6.74	3.84	102.23	102.21	85.65 ± 596
	60	10.83	2.27	106.13	100.66	86.04 ± 9.81
	150	0.75	2.72	103.66	100.86	94.01 ± 0.06
ENRO	25	1.73	2.83	101.75	100.23	95.30 ± 3.89
	50[a)]	1.42	3.84	103.17	101.66	87.71 ± 2.69
	100	0.86	1.98	104.32	99.87	87.84 ± 2.93
	250	0.78	4.43	108.06	101.32	95.92 ± 5.09
ORBI	10	9.63	4.86	103.34	100.00	104.26 ± 8.03
	20[a)]	6.84	5.66	102.60	98.81	94.81 ± 6.44
	40	5.55	3.00	102.38	96.10	92.53 ± 4.95
	100	2.34	3.62	103.05	98.55	97.47 ± 2.10
SARA	5	7.28	11.82	92.99	107.56	86.02 ± 7.32
	10[a)]	7.68	9.84	92.80	100.75	90.43 ± 10.00
	20	8.97	9.80	90.71	101.02	84.50 ± 8.32
	50	6.43	3.09	92.83	94.35	88.06 ± 6.86
DIFLO	200	1.48	1.91	100.45	100.09	92.02 ± 2.47
	400[a)]	1.80	4.16	102.02	100.91	90.12 ± 2.64
	800	0.91	3.37	102.30	97.87	90.26 ± 1.81
	2000	0.26	4.36	105.17	98.74	95.50 ± 7.45

Table 3. Continued

Analytes	Range (μg/kg)	Precision RSD (%)		Accuracy (%)		Recovery Mean ± RSD (%)
		Intra-day	Inter-day	Intra-day	Inter-day	
NALI	15	7.44	15.07	113.27	112.19	103.89 ± 6.59
	30[a)]	9.32	12.52	101.43	100.19	92.76 ± 8.99
	60	5.72	7.08	104.80	103.49	95.08 ± 5.34
	150	2.93	6.10	105.70	98.00	89.43 ± 7.09
FLUME	100	1.24	3.78	96.98	99.05	87.23 ± 3.10
	200[a)]	2.23	7.51	99.89	98.67	87.27 ± 3.85
	400	1.70	11.16	100.79	97.56	88.42 ± 3.18
	1000	0.65	4.79	105.42	99.23	94.69 ± 7.89

a) Denoting the MRL suggested by KFDA.

at concentrations of 0.5, 1, 2, and 5 times the limits permitted by the Korea Food and Drug Administration. Intra-day ($n = 5$) and inter-day ($n = 5$) precisions (expressed as RSD%) and accuracy (expressed as %) are summarized in Table 3.

3.3.4 Recovery

The recoveries of (fluoro)quinolones were almost identical between the porcine muscle and commercial whole milk. The extraction of analytes from table eggs is somewhat more difficult than for the porcine muscle or milk, as could be observed from the relatively low recovery rate (from 72.59 ± 1.15 to 97.37 ± 8.44%). The least efficient extraction was that of SARA from table eggs.

3.4 Method application

The proposed method was applied to the determination of possible (fluoro)quinolones in ten different porcine muscle, table eggs, and commercial whole milk samples purchased from large markets in Seoul. In all cases, the (fluoro)quinolone contents in the assayed samples were below the LODs (Fig. 2E).

4 Concluding remarks

In this study, an analytical protocol involving a one-step extraction followed by LC was successfully developed. In terms of accuracy, no extract cleanup was deemed necessary. The developed method was characterized by acceptable accuracy, precision, and recovery. This procedure is simple and allows for the determination of the residues of these compounds in different matrices with a high degree of sensitivity.

The authors have declared no conflict of interest.

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