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Simultaneous Determination of (Fluoro)quinolone Antibiotics in Kidney, Marine Products, Eggs, and Muscle by Enzyme-Linked Immunosorbent Assay (ELISA)

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A direct competitive enzyme-linked immunosorbent assay (ELISA) was developed to detect a broad range of (fluoro)quinolones in various matrices. In the optimized generic test, anti-sarafloxacin antibodies in combination with norfloxacin conjugate showed 50% binding inhibition at 0.21 ng mL^{-1} for sarafloxacin in buffer. Screening for this class of antibiotics is accomplished using a simple, rapid extraction carried out with a 1:1 mixture of methanol and phosphate-buffered saline adjusted to pH 7.4. This common extraction was able to detect 15 (fluoro)quinolone residues such as sarafloxacin, norfloxacin, difloxacin, ciprofloxacin, pefloxacin, ofloxacin, cinoxacin, danofloxacin, enrofloxacin, marbofloxacin, lomefloxacin, enoxacin, flumequine, oxolinic acid, and nalidixic acid in pig kidney, poultry muscle, egg, fish, and shrimp. The assay's detection capabilities (CCβ) for most of these compounds were <10 $\mu g\ kg^{-1}$ except for the sarafloxacin-, oxolinic acid-, flumequine-, and cinoxacin-spiked matrices, the estimated CCβ values of which were <4, <25, <100, and <200 $\mu g\ kg^{-1}$, respectively.

KEYWORDS: Antibiotic; fluoroquinolones; ELISA; detection; residues

INTRODUCTION

(Fluoro)quinolones are a synthetic class of antibiotics. They are widely used for both the prevention and treatment of various diseases in animal husbandry and aquaculture, as well as in humans. (Fluoro)quinolones act via inhibition of DNA-gyrase, abolishing its activity by interfering with the DNA rejoining reaction. Because gyrase is an essential enzyme in prokaryotes, but not found in eukaryotes, bacteria are an ideal target for these antibiotics (*1*−*3*).

The original quinolones have only modest activity against Enterobacteriaceae and some other facultative Gram-negative bacteria. Fluorinated quinolones, called fluoroquinolones, were developed from the original quinolones (derived from nalidixic acid). The development of fluoroquinolones extended the spectrum of antibiotic activity to include *Pseudomonas aeruginosa* and some Gram-positive bacteria and substantially increased activity against other Gram-negative bacteria (*1*, *3*, *4*). The widespread use of (fluoro)quinolones in agriculture and aquaculture has resulted in the potential presence of these compound residues in foodstuffs of animal origin. In parallel to the exposure to low levels of these compounds, an increase of resistant human pathogens constituting a public health hazard, primarily through the increased risk of treatment failures, has been observed. Therefore, the chemical variety of (fluoro)-quinolones and the possibility of trace level residues in food made it necessary to develop a sensitive multiresidue screening method.

Within the framework of its policy on consumer health protection, the European Union (EU) established maximum residue levels (MRLs) for various classes of antibiotics including (fluoro)quinolones in different food matrices of animal origin and from various species (*5*, *6*). The European Agency for the Evaluation of Medicinal Products (EMEA) established these MRLs (**Table 1**).

Most determination techniques are based on a liquid chromatography (LC) separation. Gas chromatography (GC) and high-performance thin-layer chromatography (HPTLC) are used in only a small proportion of the methods reviewed. Some analytical techniques, such as luminescence or immunological methods, are used without prior chromatographic separation (*7*). Only immunochemical methods based on ELISA are discussed under Results (*8*−*13*).

This paper describes the development of an immunochemical-based multiresidue screening method (ELISA) aimed at the determination of (fluoro)quinolone residues in foods (**Figure 1**). The immunochemical approach was explored because it may

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Table 1. MRL Values Established by the EU for (Fluoro)quinolones of Veterinary Use

compound	species	MRL value (μg kg^{-1}) muscle	kidney	egg
danofloxacin	all species other than hereafter	100[a]	200	*b*
	bovine, ovine, caprine	200	400	
	poultry	200	400	
enrofloxacin + ciprofloxacin	all species other than hereafter	100[a]	200	*b*
	bovine, ovine, caprine	100	200	
	porcine, rabbit	100	300	
	poultry	100	300	
flumequine	all species other than hereafter	200	1000	*b*
	bovine, ovine, caprine, porcine	200	1500	
	poultry	400	1000	
	fish	600[a]		
sarafloxacin	fish (salmonidae)	30[a]		
oxolinic acid	bovine	100	150	*b*
	porcine	100	150	
	chicken	100	150	
	fish	100[a]		
difloxacin	all species other than hereafter	300[a]	600	*b*
	bovine, ovine, caprine	400	800	
	porcine	400	800	
	poultry	300	600	
marbofloxacin	porcine, bovine	150	150	

[a] For fin fish, this MRL relates to "muscle + skin in natural proportions". [b] Not for use in animals from which eggs are produced for human consumption.

offer support for the generation of rapid screening techniques as well as simple and cost-effective sample cleanup methods.

MATERIALS AND METHODS

Standards. Sarafloxacin, ofloxacin, flumequine, norfloxacin, ciprofloxacin hydrochloride, oxolinic acid, cinoxacin, lomefloxacin, enoxacin, and nalidixic acid were provided by Sigma (St. Louis, MO). Enrofloxacin was obtained from Bayer (Leverkusen, Germany), difloxacin hydrochloride was from Chemos GmbH (Regenstauf, Germany), pefloxacin was from Rhone Poulenc (Vitry sur Seine, France), danofloxacin was from Pfizer Inc. (Cambridge, MA), and marbofloxacin was kindly provided by UBE Europe (Lure, France).

Immunogen and Enzyme Conjugate Synthesis for the Generic ELISA. Haptens of norfloxacin and sarafloxacin containing a carboxylic acid group on the piperazinyl ring were synthesized and bound to carrier proteins to prepare immunogenic agents. The norfloxacin and sarafloxacin haptens were coupled to horseradish peroxidase via the newly introduced carboxylic acid group to form the enzyme conjugate required for the competitive ELISA. These chemical syntheses were described in detail in Tittlemier et al. (*13*).

Immunization. Immunogen emulsions were injected subcutaneously into four sites on the animal. Rabbits were immunized every 28 days with 200 μg of immunogen, and blood samples were taken from the marginal vein of the ear 10 days after each immunization (from the third immunization onward).

Generic ELISA Procedure. Ninety-six-well microtiter plates (Nunc, Roskilde, Denmark) were coated beforehand with purified sheep antibody directed against rabbit IgG (Zentech, Liège, Belgium). The wells were filled with 50 μL of working standard prepared in assay buffer, 50 μL of diluted sample, or assay buffer (B_0). The assay buffer composition was as follows: 0.15 M NaCl, 0.056 M $Na_2HPO_4{\cdot}2H_2O$, 0.009 M $NaH_2PO4{\cdot}2H_2O$, 0.2% gelatin, 0.05% Tween 20, 0.01% 8-anilino-1-naphthalenesulfonic acid ammonium salt, and 0.0028 M ascorbic acid. Two additional wells were filled with 150 μL of assay buffer and were dedicated to nonspecific binding (NSB). Then the antiserum was diluted to an optimal concentration (1 part in 40000) in assay buffer, and 100 μL of dilute antiserum was dispensed into all wells. The microtiter plate was incubated for 1 h at 4 °C. Afterward, 100 μL of norfloxacin enzyme conjugate diluted 1:50000 in assay buffer was added to each well (except for the two wells corresponding to NSB), and the plate was again incubated for 1 h at 4 °C. The wells were then emptied and washed three times with washing buffer (0.15 M NaCl from VWR International, Leuven, Belgium; 0.05% Tween 20 from Merck, Darmstadt, Germany). A 150 μL aliquot of chromogen mixture [(3,3′,5,5′-tetramethylbenzidine) (TMB) and enzyme substrate H_2O_2 (v/v)] was added to each well. Both TMB and H_2O_2 were obtained from KPL (Gaithersburg, MD). The enzyme reaction was stopped by the addition of 6 M sulfuric acid (50 μL). The absorbance of the solution at 450 nm was read within 30 min. The average optical density (OD_{450}) of B_0 wells, containing all components except the (fluoro)quinolone competitor, was taken and represents 100% activity. The test wells (i.e., those wells containing working standards or samples) were normalized to the 100% activity wells, and percent inhibition was calculated as

$$\frac{(OD_{450,test} - OD_{450,NSB})}{(OD_{450,B_0} - OD_{450,NSB})} \times 100$$

Sample Preparation. Samples (5 g) were homogenized with 5 mL of extraction solvent consisting of a 1:1 (v/v) mixture of methanol (VWR International) and phosphate-buffered saline (PBS: 9 g of NaCl, 7.78 g of $Na_2HPO_4{\cdot}2H_2O$, and 0.75 g of KH_2PO_4 in 1000 mL of H_2O, from VWR International) adjusted to pH 7.4 with 7.5 N HCl.

All homogenates, aside from those containing egg samples, were mixed on a vortex mixer for 30 s and then vigorously shaken for 30 min. The tubes containing egg samples were gently mixed for 30 min to avoid foam and emulsion.

All tubes were centrifuged at 4100*g* for 10 min, and supernatants were transferred into new glass tubes. Just before application to the microtiter plate, samples were again centrifuged at 2300*g* for 10 min and diluted by a factor of 10 in the assay buffer.

RESULTS AND DISCUSSION

Antibody/Conjugate selection. Each rabbit serum was tested using the two peroxidase conjugates produced. Four combinations are possible; therefore, the antibodies may be used in homologous (i.e., identical compounds were used to produce the antibody and peroxidase-conjugate) or heterologous (i.e., different compounds were used to produce the antibody and peroxidase conjugate) assay format. Four competitive ELISAs were used to evaluate the displacement of the conjugate by free standards (sarafloxacin, norfloxacin, difloxacin, ciprofloxacin, pefloxacin, ofloxacin, cinoxacin, danofloxacin, enrofloxacin, marbofloxacine, lomefloxacin, enoxacin, flumequine, oxolinic acid, and nalidixic acid). The assay format used was the same as the one described above, except that the antibodies and peroxidase conjugate were added simultaneously and incubated for one night at 4 °C. The most efficient peroxidase conjugate/antibody combination was selected according to the antibody sensitivity and specificity (**Table 2**). The assay sensitivity was evaluated using the concentration of residue necessary to displace 50% of the peroxidase conjugated from binding to antibodies (ID_{50}). The degree of antibody specificity within the (fluoro)quinolone family (the percent cross-reactivity) was calculated by using the formula

$$\frac{ID_{50\ \text{fluoroquinolone used to raise antibodies}}}{ID_{50\ \text{fluoroquinolone}}} \times 100$$

Analysis of these results shows that antibodies perform best when used in the heterologous format. Among these formats, a percentage of cross-reactivity ≥ 10 was reached for 7 of the 15 compounds tested in the case of anti-norfloxacin/sarafloxacin conjugate and for 11 of them in the case of anti-sarafloxacin/norfloxacin conjugate.

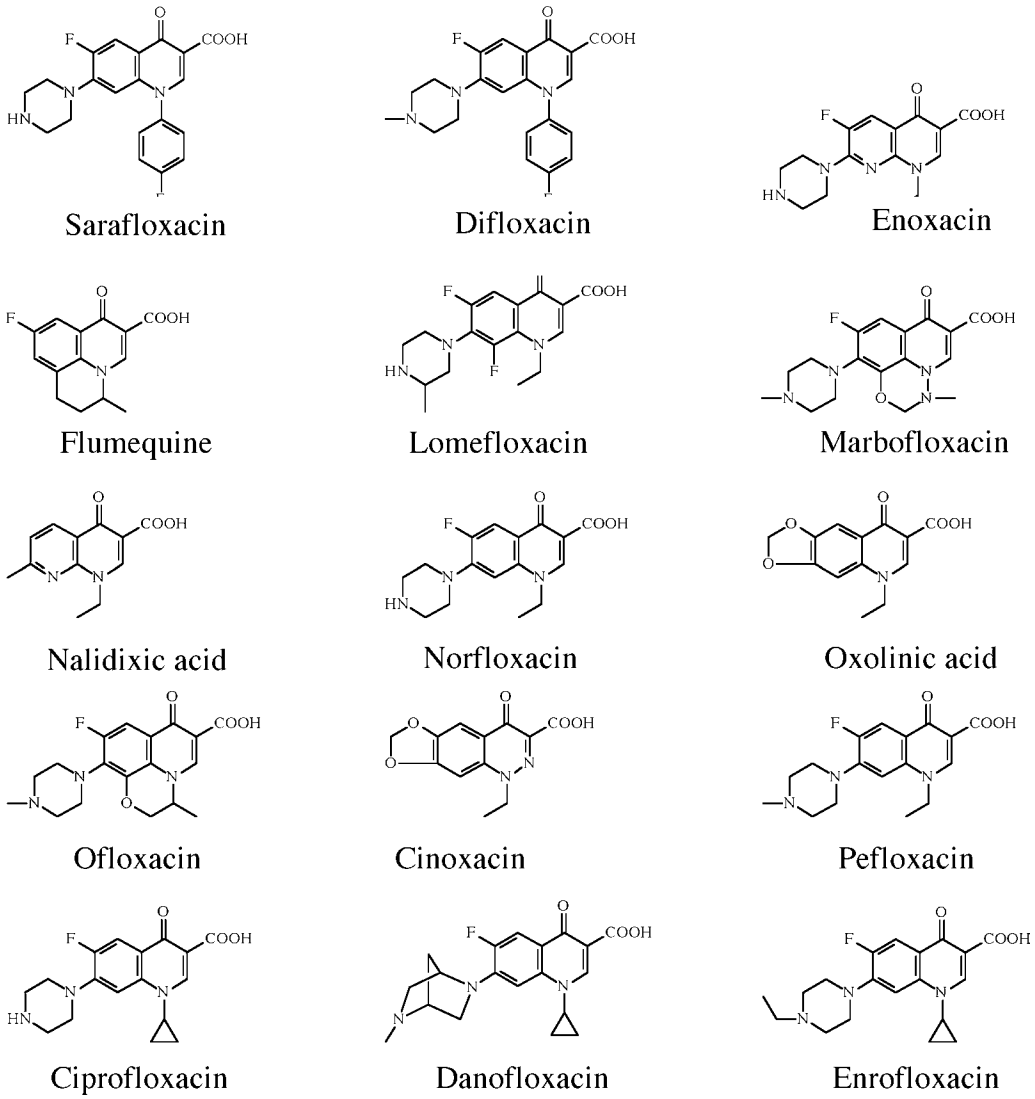

Figure 1. Structures of the (fluoro)quinolones considered in this study.

Table 2. Comparison of (Fluoro)quinolone ID_{50} Values and Cross-Reactivity Profiles (CR) for the Four Possible Antibody/Peroxidase Conjugate Combinations

	AB sarafloxacin/ norfloxacin conjugate		AB sarafloxacin/ sarafloxacin conjugate		AB norfloxacin/ norfloxacin conjugate		AB norfloxacin/ sarafloxacin conjugate	
	ID_{50} (ng mL^{-1})	CR (%)	ID_{50} (ng mL^{-1})	CR (%)	ID_{50} (ng mL^{-1})	CR (%)	ID_{50} (ng mL^{-1})	CR (%)
sarafloxacin	0.12	100	1.0	100	1.29	9	2.22	10
norfloxacin	0.31	39	76.8	1	0.12	100	0.23	100
difloxacin	0.18	67	2.1	46	0.96	13	2.98	8
ciprofloxacin	0.43	28	78.8	1	0.24	50	0.32	72
pefloxacin	0.56	21	50.5	2	0.13	92	0.16	144
ofloxacin	0.77	16	91.0	1	2.61	5	0.99	23
flumequine	12.1	1	255	<1	784	<1	>1000	<1
cinoxacin	43.4	<1	560	<1	>1000	<1	>1000	<1
oxolinic acid	4.71	3	741	<1	111	<1	111	<1
danofloxacin	0.39	31	42.1	2	1.97	6	2.53	9
enrofloxacin	0.33	36	14.1	7	0.09	133	0.04	288
marbofloxacin	0.66	18	101	1	8.02	2	2.09	11
lomefloxacin	1.05	11	112	1	6.75	2	9.37	3
enoxacin	0.62	19	54.7	2	3.19	4	2.64	9
nalidixic acid	2.55	5	>1000	<1	>1000	<1	>1000	<1

Because the latter combination gave better results, it was selected and investigated further.

ELISA Optimization and Comparison to Other Methods. Once selected, the sarafloxacin antibody was evaluated for its ability to bind to other (fluoro)quinolones because the aim was to develop a generic immunoassay with a broad-spectrum recognition of (fluoro)quinolones. Several parameters were tested to determine the optimal conditions described under Generic ELISA Procedure, notably incubation temperature and incubation time. These parameters were evaluated by comparing

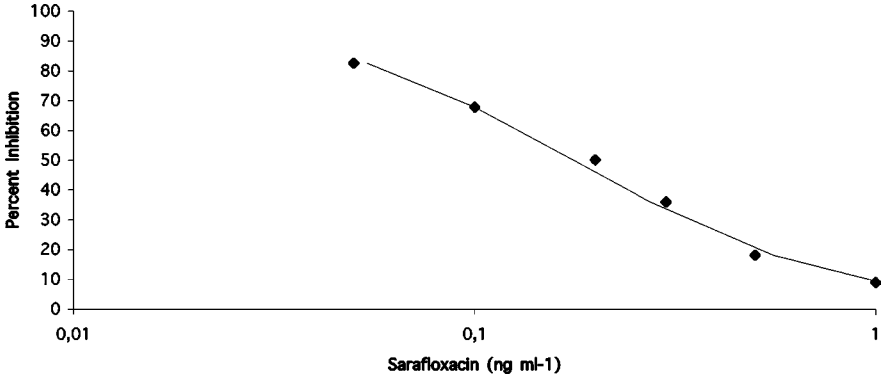

Figure 2. Representative inhibition curve using sarafloxacin as competitor in optimal conditions.

Table 3. ID_{50} Values and Cross-Reactivity Profile of Anti-sarafloxacin Antibody with a Range of (Fluoro)quinolones Using the Optimized ELISA Format

	ID_{50} (ng/mL)	CR (%)
sarafloxacin	0.21	100
norfloxacin	0.20	105
difloxacin	0.33	64
ciprofloxacin	1.22	17
pefloxacin	0.7	30
ofloxacin	0.38	55
flumequine	5.09	4
cinoxacin	25.00	1
oxolinic acid	6.26	3
danofloxacin	0.24	88
enrofloxacin	0.32	66
marbofloxacin	0.47	45
lomefloxacin	0.86	24
enoxacin	0.78	27
nalidixic acid	1.52	14

the antibody sensitivity and specificity (as explained above) while considering the practical considerations required by potential users. First, the results obtained at two incubation times, overnight or 2 h, were compared (incubation was at 4 °C in both cases). The sensitivity was better for the overnight incubation, but as it was quite good even after 2 h and as the cross-reactivity profile was slightly better after 2 h, a 2-h incubation time was chosen.

Second, the results obtained at two incubation temperatures, 4 and 37 °C (2 h) were compared. Both the sensitivity and the specificity of the assay were clearly better when the incubation temperature was 4 °C (data not shown). A representative curve for sarafloxacin obtained with the optimized ELISA is shown in **Figure 2**. This assay exhibited an ID_{50} of 0.21 ng mL^{-1}.

A set of (fluoro)quinolone standards was used with the optimized ELISA to determine more accurate values of ID_{50} for each compound. **Table 3** shows these values and the cross-reactivity of the sarafloxacin antibody toward a variety of (fluoro)quinolone compounds. All (fluoro)quinolone compounds showed a cross-reactivity relative to sarafloxacin $\geq 14\%$, except flumequine, cinoxacin, and oxolinic acid. It is not surprising that the sarafloxacin antibody exhibits lower affinitiy for these three compounds, because they all lack the piperazinyl moiety contained in sarafloxacin and the other compounds having cross-reactivities $\geq 14\%$.

The ELISA described in this paper displays greater sensitivity and applicability to a wider range of (fluoro)quinolones than previously published fluoroquinolone ELISA methods. Bucknall et al. (*11*) have developed a screening procedure for detecting a range of (fluoro)quinolones by immunoassay. The antibody used was reported to recognize fewer compounds and with lower affinity. The norfloxacin antibody cross-reactivity at 50% binding in their generic ELISA was estimated to be lower than 15% for enrofloxacin, flumequine, ciprofloxacin, and cinoxacin; however, only 10 compounds were tested in Bucknall et al.'s work.

Holtzapple et al. (*8*) have reported the production of six sarafloxacin monoclonal antibodies and developed an indirect immunoassay for six compounds. The antibody that exhibited the highest affinity for sarafloxacin showed a value of ID_{50} estimated at 7.3 ng mL^{-1}, compared to 0.21 ng mL^{-1} observed in the current assay. Their antibody was able to recognize with equal affinity difloxacin and trovafloxacin but had some difficulties recognizing norfloxacin, enrofloxacin, and nalidixic acid (cross-reactivities were $<3\%$). Watanabe et al. (*10*) have also recently published a description of a monoclonal-based ELISA for enrofloxacin in biological matrices. The working curve for enrofloxacin in buffer gave an ID_{50} value of 1 ng mL^{-1}, and the chosen antibody is highly specific for enrofloxacin but did not exhibit cross-reactivity toward ciprofloxacin, benofloxacin, danofloxacin, or ofloxacin.

Detection of (Fluoro)quinolones in Various Matrices. For use in the ELISA performance evaluation, four different matrices were chosen according to several criteria, such as their presence in the table of established MRLs or as not allowed substances (e.g., eggs), if many papers refer to these matrices, whether they are involved in the metabolism or elimination of (fluoro)-quinolones in the body. The performance of the extraction method was evaluated for egg, marine products (fish and shrimp), pig kidney, and chicken muscle. Kidney tissue was chosen because renal excretion is the primary route of excretion for most (fluoro)quinolones (*3*). However, some (fluoro)-quinolones bear both an acidic group (carboxylic acid) and a basic group (tertiary amine), which results in amphoteric properties; therefore, their distribution in the body is more homogeneous. Marine products were used because (fluoro)-quinolones have been widely used in aquaculture since 1987 (*14*).

ELISA for (Fluoro)quinolones in Egg, Marine Product, Kidney, and Muscle. For each matrix, known negative samples without fortification (blank) and spiked with (fluoro)quinolones at various concentrations were extracted and analyzed using the optimized ELISA procedure. (Fluoro)quinolones are soluble in polar organic solvents but not in nonpolar ones, such as hexane and toluene. They are soluble in hydro-organic or aqueous acidic and basic media (*7*). The first results showed that a common extraction was able to detect all tested compounds (sarafloxacin, norfloxacin, difloxacin, ciprofloxacin, pefloxacin, ofloxacin, cinoxacin, danofloxacin, enrofloxacin, marbofloxacin, lomefloxacin, enoxacin, flumequine, oxolinic acid, and nalidixic acid) in all matrices. However, the ELISA was influenced by the

Table 4. Ratio Binding B/B_0 for a Range of (Fluoro)quinolones Extracted from Chicken Muscle, Pig Kidney, Marine Product, and Egg ($n = 2$)

		B/B_0 (%)			
compound	fortification level (μg kg^{-1})	muscle	kidney	marine product	egg
blank	0	102	86	97	118
sarafloxacin	10	41	27	40	29
norfloxacin	10	47	40	42	40
difloxacin	10	49	34	43	36
ciprofloxacin	10	53	46	42	41
pefloxacin	10	56	54	51	40
ofloxacin	10	59	50	55	62
flumequine	100	55	55	47	58
cinoxacin	200	62	64	60	68
oxolinic acid	25	68	68	65	67
danofloxacin	10	53	48	50	38
enrofloxacin	10	49	45	43	48
marbofloxacin	10	56	43	48	44
lomefloxacin	10	72	57	57	61
enoxacin	10	68	49	51	50
nalidixic acid	10	74	66	71	70

components in biological matrices; thus, sample extracts were diluted 10-fold in assay buffer before application onto the 96-well microtiter plates.

The data obtained from blank and fortified samples were calculated as explained previously and are shown in **Table 4**. The column labeled "fortification level" gives the amount in micrograms per kilogram of each compound used to spike the samples before the extraction procedure. Most samples spiked with (fluoro)quinolones at 10 μg kg^{-1} showed a percent inhibition lower than those obtained with blank samples. This means that most fluoroquinolones can be detected with this assay when present at 10 μg kg^{-1}, the exceptions being cinoxacin, flumequine, and oxolinic acid. Samples must be fortified with cinoxacin at 200 μg kg^{-1}, with flumequine at 100 μg kg^{-1}, and with oxolinic acid at 25 μg kg^{-1} to observe a percent inhibition lower than those obtained with blank samples. Such results were expected because the sarafloxacin antibody exhibited a lower affinity in buffer for flumequine, cinoxacin, and oxolinic acid. The percent inhibition values for each (fluoro)quinolone were similar from one matrix to another, and all negative samples displayed a percent inhibition near those recorded for the 100% activity (B_0) samples. Thus, this generic ELISA has been proven to be capable of detecting simultaneously sarafloxacin, norfloxacin, difloxacin, ciprofloxacin, pefloxacin, ofloxacin, danofloxacin, enrofloxacin, marbofloxacin, lomefloxacin, enoxacin, nalidixic acid, cinoxacin, flumequine, and oxolinic acid in egg, muscle, marine product (fish and shrimp), and kidney.

Decision Limits and Detection Capabilities. The last step was the determination of decision limits (CCα) and detection capabilities (CCβ) of the newly developed qualitative screening method. First, 20 representative blank samples (for each matrix) were extracted and analyzed using the optimized ELISA procedure. The concentration of each blank sample was calculated, and the decision limit of each matrix was then estimated by the mean ($n = 20$) plus 3 times the standard deviation. CCα values correspond to 0.5 μg kg^{-1} for chicken muscle, 0.6 μg kg^{-1} for pig kidney, 0.7 μg kg^{-1} for marine product (fish and shrimp), and 0.3 μg kg^{-1} for egg.

The detection capability is defined as the lowest concentration that can be determined with an error probability of β ($\leq$5%) (*15*). Theoretically, if 19 of the 20 fortified samples are declared to be noncompliant, then the CCβ equals the level of fortification. If 18 or fewer of the fortified samples are declared to be noncompliant, then the CCβ is greater than the level of fortification. If all of the fortified samples are declared to be noncompliant, then the CCβ is lower than the level of fortification. In practice, the CCβ was chosen as the lowest tested fortification level giving no negative result in an analysis of 20 spiked blanks; this decision should avoid the problem of false negatives. The CCβ values obtained in this way for muscle samples are listed in **Table 5**. Because no notable difference were observed among matrices when the different samples were fortified with all (fluoro)quinolones (**Table 4**), the CCβ values in the three other matrices were expected to be the same as those estimated for muscle. The CCβ values for sarafloxacin and flumequine were, however, estimated in the three other matrixes. For the sarafloxacin- and flumequine-spiked matrices, the estimated detection capabilities were <4 and <100 μg kg^{-1}, respectively.

Table 5. Detection Capabilities (CCβ) in Muscle Matrix

CCβ (μg kg^{-1})	compound
<4	sarafloxacin
<10	norfloxacin, difloxacin, ciprofloxacin, pefloxacin, ofloxacin, danofloxacin, enrofloxacin, marbofloxacin, lomefloxacin, enoxacin, and nalidixic acid
<25	oxolinic acid
<100	flumequine
<200	cinoxacin

The team of Watanabe et al. (*10*) has developed an ELISA for enrofloxacin in chicken liver, chicken muscle, and cow's milk, but the inability of their procedure to detect ciprofloxacin residues renders the assay unsuitable for routine use in Europe.

Holtzapple et al. (*8*) have described a procedure for extracting sarafloxacin from chicken liver. The smallest amount of sarafloxacin added to samples and detected by the assay was 10 μg kg^{-1}.

Bucknall et al. (*11*) have developed generic and specific immunoassays for bovine milk and sheep kidney. The assay sensitivities for kidney tissue (concentration corresponding to the value of $B_0 - 3 \times$ standard deviation) were <6 μg kg^{-1} for all residues tested in a generic assay (norfloxacin, nalidixic acid, enrofloxacin, flumequine, ciprofloxacin, ofloxacin, oxolinic acid, enoxacin, marbofloxacin, cinoxacin, and pipemidic acid). Some of the standard curves were not parallel, however; this could be due to a competitive effect. Dose−response curves for specific assays in a kidney homogenate yielded ID_{50} values for ciprofloxacin (12 μg kg^{-1}), enrofloxacin (14 μg kg^{-1}), flumequine (29 μg kg^{-1}), and nalidixic acid (31 μg kg^{-1}).

An indirect ELISA for ciprofloxacin has recently been described (*12*), featuring a sensitivity of 0.32 ng mL^{-1} (3.2 μg kg^{-1} assuming a 100 g L^{-1} homogenate), 70% cross-reactivity with enrofloxacin, and 45% cross-reactivity with norfloxacin.

It can be concluded that this generic ELISA has been shown to be capable of detecting simultaneously 15 (fluoro)quinolone residues in four matrices (egg, chicken muscle, pig kidney, and marine product (fish and shrimp). As the generic assay cannot identify individual drugs or distinguish the components of a mixture, a noncompliant result would require a confirmatory assay (LC-MS/MS). Because of concerns about drug residues entering the food chain and contributing to bacterial resistance, MRLs (**Table 1**) have been set for several (fluoro)quinolones. The results of the ELISA performed in the matrices (except in egg) demonstrate that this screening assay was able to detect all of these residues at levels lower than the established MRLs.

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