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# Development of an Improved Method for Trace Analysis of Quinolones in Eggs of Laying Hens and Wildlife Species Using Molecularly Imprinted Polymers

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**ABSTRACT:** A sensitive, selective, and efficient method was developed for simultaneous determination of 11 fluoroquinolones (FQs), ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, flumequine, marbofloxacin, norfloxacin, ofloxacin, oxolinic acid, pipemidic acid, and sarafloxacin, in eggs by molecularly imprinted polymer (MIP) and column liquid chromatography−electrospray ionization−tandem mass spectrometry (LC−ESI−MS/MS). Samples were diluted with 50 mM sodium dihydrogen phosphate at pH 7.4, followed by purification with a commercial MIP (SupelMIP SPE—Fluoroquinolones). Recoveries for the 11 quinolones were in the range of 90−106% with intra- and interday relative standard deviation ranging from 1 to 6% and from 3 to 8%, respectively. Limits of detection (LODs) were 0.12−0.85 ng/g, and limits of quantification (LOQs) were 0.36 and 2.59 ng/g, whereas the decision limit ($CC_\alpha$) and detection capability ($CC_\beta$) ranged from 0.46 to 3.35 ng/g and from 0.59 to 4.12 ng/g, respectively. The calculated relevant validation parameters are in an acceptable range and in compliance with the requirements of Commission Decision 2002/657/EC. Moreover, a comparison to two other sample treatments [solid-phase extraction (SPE) and solvent extraction] has been carried out. The method was applied to lying hens, Japanese quail, and black-headed gull eggs, in which FQs were not found. The method was also applied to study the depletion of sarafloxacin in eggs.

**KEYWORDS:** *Fluoroquinolones, LC−MS/MS, triple quadrupole, molecularly imprinted polymers, solvent extraction, solid-phase extraction, eggs, wildlife eggs*

## INTRODUCTION

Fluoroquinolones (FQs) are antimicrobial agents used in the treatment of a variety of bacterial infections that act directly on bacterial DNA by inhibiting topoisomerase.[1,2] In addition to the use of these antibacterials in human medicine, a significant increase in their use for the treatment and prevention of veterinary disease was noted over the past decade. They have a very broad clinical application in livestock, poultry, fish, and domestic animals in the treatment and prevention of respiratory, enteric, and urinary tract infections.[1] FQs have a tendency to accumulate as residues in avian eggs and other edible tissues, resulting in inappropriate yolk formation, high rates of mortality, and significant reductions in the mean of wet body weights, crown−rump and anterior−posterior head lengths, and different types of structural anomalies, as well as a delay of chondrogenesis and ossification of the maternally treated chick embryos.[2] Although FQs are included in the Annex of Commission Regulation (EU) No. 37/2010[3] on pharmacologically active substances and their classification regarding maximum residue limits (MRLs) in foodstuffs of animal origin, their use is prohibited in animals producing eggs for human consumption. FQs are also recognized as widespread environmental contaminants,[1,4] and their residues could be detected in wild bird eggs by accumulation during the laying period. This could produce toxicological effects at the developing, individual, or population level.[5] Whole bird eggs also represent a non-invasive matrix to monitor the presence of contaminants and can be used as indicators of pollution.[6] Thus, development of efficient methods to detect and quantitate FQs in eggs is warranted for food and environmental safety.

A number of methods based on enzyme-linked immunosorbent assay (ELISA),[7] biosensors,[8] liquid chromatography (LC) with fluorescence[9−11] or mass spectrometry (MS),[12−18] and capillary electrophoresis (CE) with laser-induced fluorescence[19] or MS[15,16,20,21] have been developed for the determination of FQs. These methods have been extensively reviewed in some papers.[2,22−24] Although several analytical methods to identify and quantify FQs have been successfully elaborated, the biological sample preparation is one of the most crucial and difficult steps in residue analysis.

Eggs, in particular, are a very complex matrix. Lipids and proteins represent 11 and 13% of the egg, respectively. Some FQs bind to lipoproteins, which hinders their extraction, while several organic solvents form emulsions and foams with the matrix. In most cases, the analytes were extracted from the egg matrix by liquid extraction (LE), sometimes combined with a cleanup by either solid-phase extraction (SPE), microdialysis, or in-tube solid-phase microextraction (SPME).[8,10,13,25−28] Other sample preparation techniques used less for the analysis of quinolones in eggs were diphasic dialysis,[29] pressurized liquid extraction (PLE),[9,12,30] and matrix solid-phase dispersion (MSPD).[26] Because of the variation in components of eggs (especially if several bird species are covered), it is difficult to find a suitable cleanup procedure. Some extracts after cleanup

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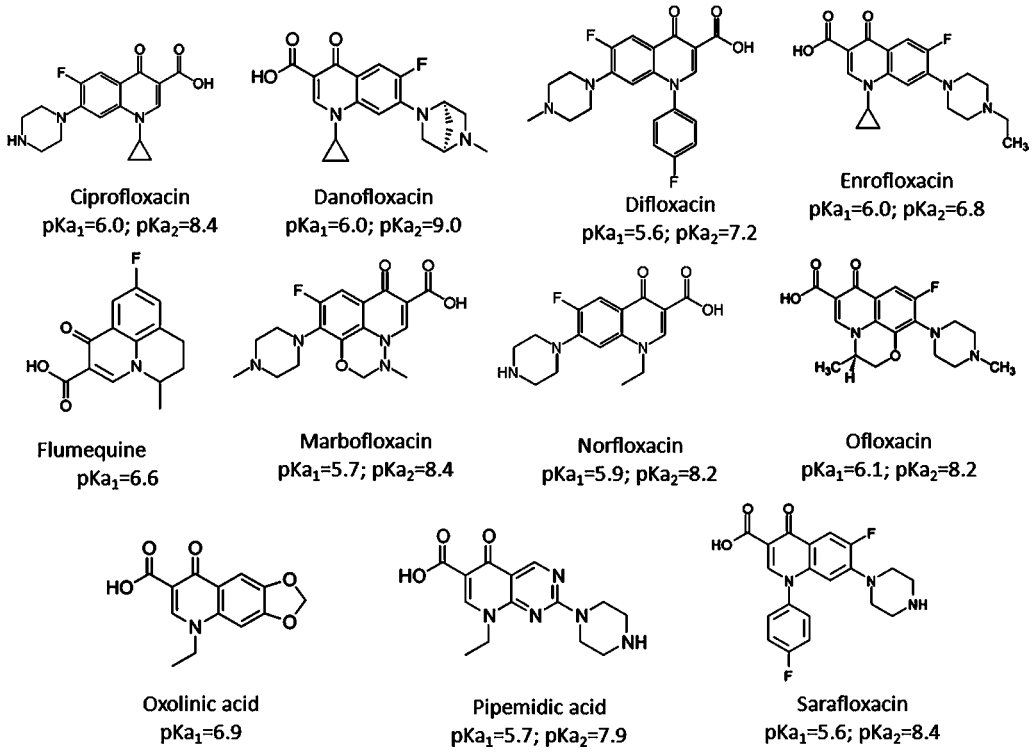


**Figure 1.** Structures and p$K_a$ values of the studied FQs.

using traditional SPE cartridges may not be clean enough for chromatographic analysis, even using sophisticated MS systems.

Nowadays, it is well-known that molecularly imprinted polymers (MIPs) represent a clear alternative to classic methodologies for the extraction and cleanup of target analytes. The use of SPE procedures involving MIPs is an attractive choice for the analysis of quinolone in complex sample matrices. Recently, several MIP sorbents using different FQs as the templates have been reported to analyze water[31] or biological[32] samples, including milk.[33,34] MIPs for FQs are already commercially available, and successful applications have been described in the literature for milk and kidney,[19,35] baby food,[36] and honey.[35] However, little attention has been paid to take advantage of MIP for selective extraction of FQ residues from food eggs.

The objective of the present work was to develop a multiresidue method for the analysis of 11 FQ residues, whose names and structures are shown in Figure 1, in eggs of several species. The species covered are laying hens (*Gallus gallus domesticus*), Japanese quail (*Coturnix japonica*) and black-headed gull (*Chroicocephalus ridibundus*). The method was fully validated according to the provisions of Council Decision 2002/657 in eggs from laying hens. Furthermore, different sample treatments [liquid extraction, SPE, and molecularly imprinted SPE (MISPE)] have been compared for recovery, sensitivity, and precision.

## EXPERIMENTAL SECTION

**Chemicals.** Formic acid (reagent grade), dichloromethane, acetonitrile, and methanol (gradient grade for LC) were from Merck (Darmstadt, Germany). Ammonia and sodium dihydrogen phosphate were from Panreac (Barcelona, Spain). High-purity water was prepared using a Milli-Q water purification system (Millipore, Milford, MA). The isotope-labeled internal standards (ISs) ciprofloxacin-$d_3$ and ofloxacin-$d_8$ as well as the 11 quinolones studied (ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, flumequine, marbofloxacin, norfloxacin, ofloxacin, oxolinic acid, pipemidic acid, and sarafloxacin) were purchased from Sigma-Aldrich (Madrid, Spain). Individual standard solutions were prepared in methanol and methanol acidified at the concentration of 1000 $\mu$g/mL, and the working standard solution was prepared by mixing the appropriate amounts of the individual standard solutions and diluting with methanol to a final concentration of 1 $\mu$g/mL. Working solutions were prepared daily by diluting this solution with water. All solutions were stored in amber glass bottles at 4 °C in the dark. Solutions of ISs were diluted to a concentration of 1 $\mu$g/mL with methanol. Appropriate volumes of the ISs were added to egg samples to obtain concentrations of 10 ng/mL in the extracts ready to be injected in the LC−MS equipment. Extracts were filtered through a 0.45 $\mu$m membrane polypropylene filter (Pall, Ann Arbor, MI), and samples and mobile phases were filtered through a 0.22 $\mu$m membrane polypropylene filter (Pall, Ann Arbor, MI).

A Chromabond SPE vacuum manifold with 12 ports and a self-cleaning dry vacuum system Laboport SH (Bonsai Advanced Technologies S.L., Madrid, Spain) were used for loading the surface samples and drying the cartridges. The SupelMIP SPE—Fluoroquinolones column (25 mg/3 mL) was from Supelco (Bellefonte, PA), and the Oasis hydrophilic−lipophilic balance (HLB) cartridge (60 mg sorbent/3 mL) was from Waters (Milford, MA).

Centrifuge Eppendorff 05810PR 25 vials were purchased from Eppendorff (Hamburg, Germany). Vortex Atomixen was from Boitron (Barcelona, Spain). Multisample Turbovap LV evaporator was from Zymark (Hopkinton, MA).

**Chromatographic and Detection Conditions.** The chromatographic instrument was a HP1200 series LC, an automatic injector, a degasser, a quaternary pump, and a column oven coupled to an Agilent 6410 triple quadrupole (QQQ) mass spectrometer, equipped with an electrospray ionization (ESI) interface (Agilent Technologies, Waldbronn, Germany). Data were processed using MassHunter Workstation Software for qualitative and quantitative analyses (A GL Sciences, Tokyo, Japan). Chromatographic separation of quinolones was performed on a Xterra MS C18 LC column (100 × 2.1 mm inner diameter, 3.5 $\mu$m) protected by a Securityguard cartridge $C_{18}$ (4 × 2 mm inner diameter), both from Waters (Millipore, Bedford, MA). The flow rate was 0.4 mL/min. The injection volume

**Table 1. LC−MS/MS Parameters**

| compound | $T_r$ (min) | SMR1 transition (quantifier) | CE (V) | SMR2 transition (qualifier) | CE (V) | fragment | dwell time | SMR2/SMR2 (qualifier relative response %)[a] |
|---|---|---|---|---|---|---|---|---|
| pipemidic acid | 5.14 | 304 → 286 | 14 | 304 → 217 | 18 | 112 | 20 | 56 (67.7%) |
| marbofloxacin | 5.50 | 363 → 72 | 29 | 363 → 345 | 17 | 122 | 20 | 28.1 (129.1%) |
| ofloxacin-$d_3$ | 5.72 | 365 → 321 | 18 | 365 → 261 | 26 | 142 | 20 | 84.8 (95.3%) |
| ofloxacin | 5.72 | 362 → 318 | 18 | 362 → 261 | 26 | 140 | 20 | 75 (97.4%) |
| norfloxacin | 6.26 | 320 → 302 | 17 | 320 → 231 | 41 | 122 | 20 | 22.6 (103.7%) |
| cipropfloxacin-$d_8$ | 5.83 | 340 → 322 | 17 | 340 → 235 | 41 | 132 | 20 | 80.2 (105.7%) |
| cipropfloxacin | 5.83 | 332 → 314 | 17 | 332 → 231 | 41 | 132 | 20 | 69.5 (105.5%) |
| enrofloxacin | 6.79 | 360 → 342 | 18 | 360 → 316 | 14 | 132 | 20 | 100.3 (118.3%) |
| danofloxacin | 6.80 | 358 → 340 | 21 | 358 → 82 | 45 | 142 | 20 | 29.8 (99.9%) |
| difloxacin | 7.04 | 400 → 356 | 17 | 400 → 382 | 21 | 142 | 20 | 83.3 (125.9%) |
| sarafloxacin | 7.44 | 386 → 368 | 21 | 386 → 299 | 25 | 150 | 20 | 62.6 (75.7%) |
| oxolinic acid | 8.38 | 262 → 244 | 13 | 262 → 160 | 41 | 114 | 20 | 5.5 (107%) |
| flumequine | 10.80 | 262 → 244 | 33 | 262 → 202 | 33 | 104 | 20 | 27.2 (102%) |

[a]Mean value of the seven calibration standards.

was 5 $\mu$L. The column temperature was maintained at 25 °C. The mobile phase consist of methanol−water, both of them acidified with formic acid (0.01%). The gradient program started with 10% methanol that is increased linearly to 95% from 0 to 15 min, maintained at 95% from 15 to 18 min, and re-equilibrated to the initial conditions in 10 min. The injection volume was 5 $\mu$L.

Ionization and fragmentation settings were optimized by direct injection of pesticide standard solutions. tandem mass spectrometry (MS/MS) was performed in the selected reaction monitoring (SRM) mode using ESI in positive mode. For each compound, two characteristic fragmentations of the protonated molecule $[M + H]^+$ were monitored. The first and most abundant fragment was used for quantification, while the second fragment was used as a qualifier. Collision energy and cone voltage were optimized for each pesticide (Table 1). Nitrogen was used as collision, nebulizing, and desolvation gas. The ESI conditions were as follows: capillary voltage, 4000 V; nebulizer, 15 psi; source temperature, 300 °C; and gas flow, 3 L/min. To maximize sensitivity, dynamic multiple reactions monitoring (MRM) was used, with $MS_1$ and $MS_2$ at unit resolution and a cell acceleration voltage of 7 eV for all of the compounds.

**MIP-Based Extraction Procedure.** The procedure using MIPs was derived from that described by Supelco for kidney: 2 g of spiked egg samples was homogenized with 20 mL of 50 mM $NaH_2PO_4$ at pH 7.4 and centrifuged for 10 min at 5000 rpm. The resulting supernatant was filtered using a 0.45 $\mu$m filter and processed using the SupelMIP SPE—Fluoroquinolones (25 mg/3 mL). The SPE cartridges were preconditioned by 1 mL of methanol, followed by 2 mL of 50 mM $NaH_2PO_4$ at pH 7.4. An extract aliquot of 2 mL was trapped through the SPE tube, without any previous pH adjustment, at a flow rate of 0.5 mL/min. Afterward, the cartridge was washed with 3 mL of Milli-Q water and 1 mL of acetonitrile and dried under vacuum for 15 min. FQs were eluted from the sorbent with 3 mL of a 2% ammonium hydroxide and methanol/water mixture (75:25, v/v). The extract was evaporated in a TurboVap under nitrogen at 35 °C to dryness and, finally, reconstituted with 100 $\mu$L of methanol.

**SPE.** For comparison, Oasis HLB SPE (60 mg of sorbent/3 mL) was also used for extracting FQs from eggs, as previously described for MIPs.

**Solvent Extraction.** The method used was already reported.[37] Briefly, an aliquot (1 g) of whole egg spiked with the 11 quinolones was placed in a 10 mL Falcon tub containing 5 $\mu$L of 1 $\mu$g/mL ISs and then 250 $\mu$L of concentrated ammonia was added to the mixture. After the mixture (about 5 s) was shaken on a vortex mixer, 2 mL of acetonitrile was added. The mixture was vortexed for about 10 s at high speed and centrifuged for 5 min at 4000 rpm. The supernatant was decanted into another 10 mL Falcon tube, and 4 mL of dichloromethane was added. The upper, aqueous layer was transferred into an auto-sampler vial using a Pasteur pipet and directly analyzed by LC−MS/MS using the conditions described above.

**Egg Samples.** Sarafloxacin was orally administered at a dose of 10 mg/kg/day to two laying hens for 5 consecutive days. Eggs were collected for 20 days after the initial drug treatment. Analysis of each egg sample was performed in triplicate.

Furthermore, commercial laying hens (*G. gallus domesticus*) (15 samples) and quail (*C. japonica*) (15 samples) eggs were purchased in several supermarkets from Valencia. These samples were stored in their original containers (cardboard or plastic egg cups) at room temperature in the dark. Two lyophilized samples of black-headed gull (*C. ridibundus*) eggs were kindly provided by some colleagues. These lyophilized samples were reconstituted (600 mg of eggs and 1400 $\mu$L of water) for the analysis.

*Validation.* Method validation was performed to meet the criteria specified by the European Commission Decision 2002/657/EC.

Analyses of 20 blank samples from various origins and various species, to check the ruggedness of the method, were performed on different days. Selectivity of the methods was assessed by studying the absence of any interference in the same chromatographic run as the examined quinolone.

Blank extracts were fortified with the analytes at five concentration levels to construct calibration curves, which permitted estimation of the linearity and sensitivity of the method. Calibration curves for each FQ, with the respective correlation coefficient, were calculated by least-squares linear regression analysis of the peak area ratio of each analyte to IS of the respective results versus the analyte concentration. Calibration curves were also calculated for standards prepared in methanol to evaluate the matrix effect.

The calculations for the limits of detection (LODs) were based on the standard deviation of $y$ intercepts of regression analysis ($\sigma$) and the slope ($S$), using the following equation: LOD = 3.3 $\sigma/S$. In turn, the limits of quantitation (LOQs) were calculated by the following equation: LOQ = 10 $\sigma/S$. For the measurement of decision limit ($CC_\alpha$) values, samples were spiked at the respective LOQ level of each method as well as the concentration of MRL for those quinolones with specified permitted limits. The $CC_\alpha$ values were calculated as the mean values of the found concentrations plus 1.64 times the corresponding standard deviations. The detection capability ($CC_\beta$) values were obtained after spiking the samples at the $CC_\alpha$ levels by adding 1.64 times the corresponding standard deviations. Statistical analysis for $CC_\alpha$ and $CC_\beta$ was performed at the 95% confidential level, and the number of replicate analyses was 20.

Five egg samples from different origins were analyzed after spiked them with targeted FQs and the IS. This series of analyses was repeated on two other different occasions over 2 weeks under different environmental conditions and by different operators. As suggested by the 2002/657/EC European Decision, one spiked level used was $CC_\beta$

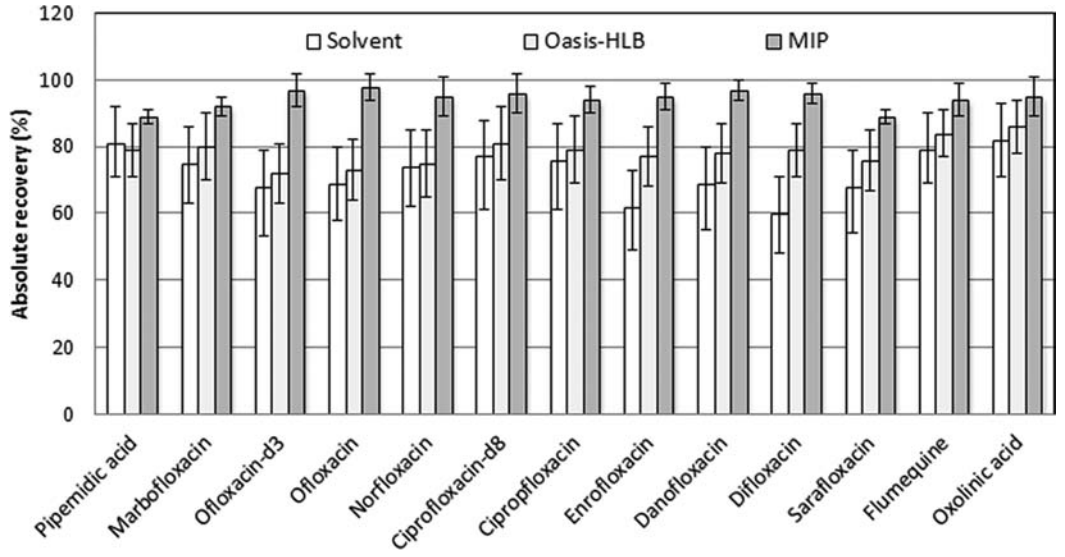


**Figure 2.** Chromatogram of extract obtained by MISPE from eggs (a) spiked with FQs at 10 ng/g and (b) non-spiked.

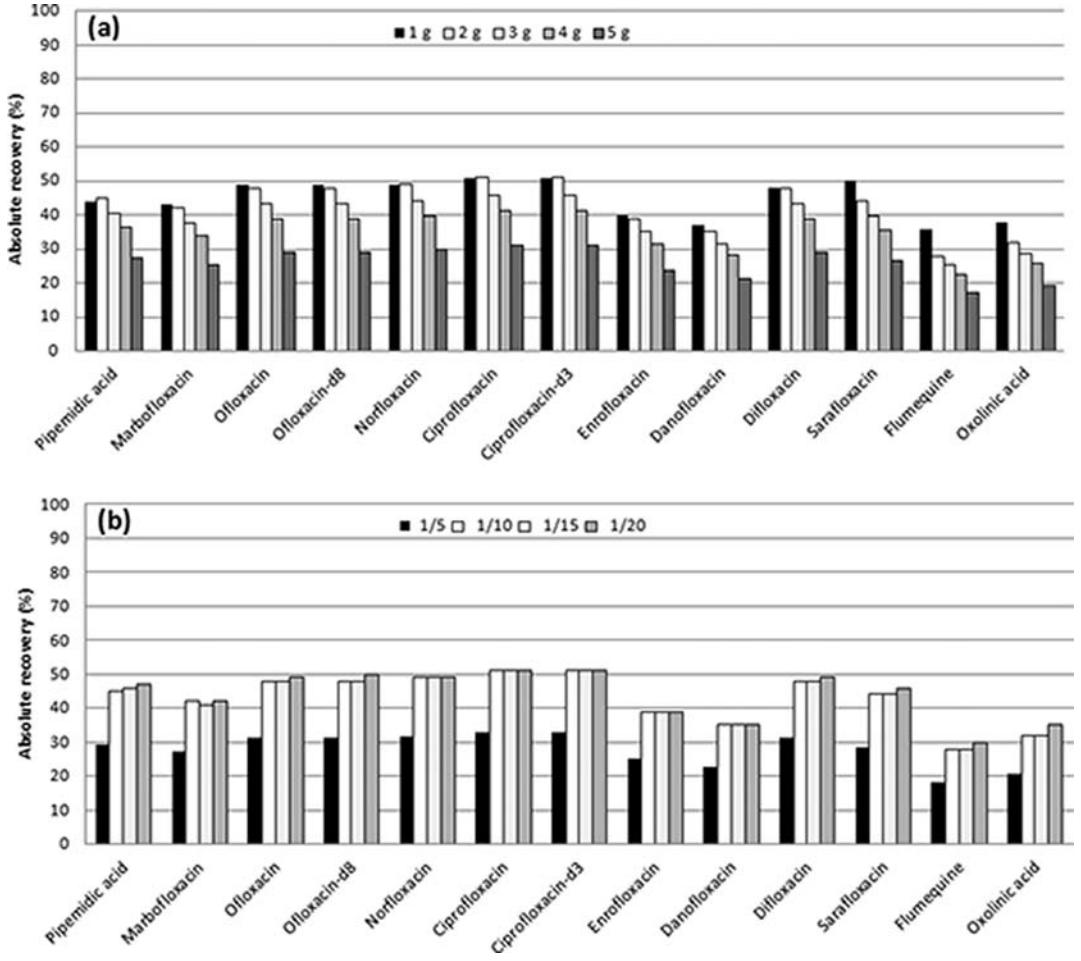


**Figure 3.** Absolute extraction recoveries (%) obtained from spiked (50 ng/g) eggs ($n$ = 3) (a) with different amounts of samples diluted $^{1}/_{30}$ with 50 mM phosphate buffer (pH 7.4) and (b) with 2 g of sample and different dilution factors.

and the other was 50 ng/g. This series of analyses permitted assessment of the within-laboratory reproducibility through the overall relative standard deviation (RSD) of the ion current profiles normalized to the IS signal amplitude.

## RESULTS AND DISCUSSION

**Optimization of LC−MS/MS.** As previously reported[12,25,26] and according to the p$K_a$ values of the different analytes indicated in Figure 1, the separation of quinolones by reversed-phase LC was achieved acidifying the mobile phase.

**Table 2. Effect of the Nature and Number of Washing Steps and the Elution Solvent in the Absolute Recoveries (%) of the FQs from Spiked (50 ng/g) Eggs ($n$ = 3)**

| | recovery (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | number of washing steps (according to the Supelco protocol)[35] | | | | | elution step | | |
| | 1−4[a] | 1−3[b] | 1−2[c] | 1[d] | 0 | Supelco protocol[35] [e] | 2% HCOOH in 75:25 methanol/water | 2% $NH_4OH$ in 75:25 methanol/water |
| pipemidic acid | 52 | 64 | 71 | 68 | 65 | 71 | 84 | 89 |
| marbofloxacin | 49 | 65 | 73 | 70 | 66 | 73 | 86 | 92 |
| ofloxacin | 56 | 68 | 76 | 71 | 67 | 76 | 91 | 97 |
| ofloxacin-$d_8$ | 55 | 69 | 76 | 71 | 67 | 76 | 92 | 98 |
| norfloxacin | 56 | 64 | 71 | 70 | 70 | 71 | 89 | 95 |
| ciprofloxacin | 60 | 65 | 72 | 70 | 65 | 72 | 90 | 96 |
| ciprofloxacin-$d_3$ | 60 | 63 | 71 | 69 | 69 | 71 | 88 | 94 |
| enrofloxacin | 46 | 64 | 71 | 69 | 67 | 71 | 89 | 95 |
| danofloxacin | 40 | 65 | 73 | 70 | 69 | 73 | 91 | 97 |
| difloxacin | 62 | 64 | 71 | 69 | 69 | 71 | 90 | 96 |
| sarafloxacin | 52 | 59 | 66 | 65 | 63 | 66 | 84 | 89 |
| flumequine | 32 | 62 | 69 | 65 | 64 | 69 | 88 | 94 |
| oxolinic acid | 37 | 62 | 69 | 65 | 64 | 69 | 89 | 95 |

[a](i) Ultrapure water, (ii) acetonitrile, (iii) 0.5% acetic acid in acetonitrile, and (iv) 0.5% ammonia in water. [b](i) Ultrapure water, (ii) acetonitrile, and (iii) 0.5% acetic acid in acetonitrile. [c](i) Ultrapure water and (ii) acetonitrile. [d](i) Ultrapure water. [e]Ammonium hydroxide (2%) in methanol.

Figure 2 shows an example of ultrahigh-pressure liquid chromatography (UHPLC)−MS/MS chromatograms of extracts from eggs non-spiked and spiked with quinolones at 10 μg/kg obtained by MISPE. Flumequine and oxolinic acid have a common precursor → product ion transition, but with the LC conditions used, they were separated and could be fully identified by the qualifier precursor → product ion transition (Table 1). These compounds are more hydrophobic than the other FQs, requiring the highest percentage of methanol. The described LC conditions led to a good separation of the 11 quinolones studied in a chromatographic run of 15 min.

The optimization of the MS parameters (fragmentor voltage and collision energy) was assisted by Optimizer software, which automatically determines the precursor ion, the optimal fragmentor, and collision energy and proposes a number of product ions. The most sensitive precursor → product ion transition was used for quantification purposes, and the other transitions were employed for analyte confirmation (Table 1). In this work, four identification points (1 point for the precursor ion and 1.5 points for each ion transition) are proposed for detecting quinolones in eggs. Therefore, this method is fully in compliance with the 2002/657/EC European Decision that requires four identification points for confirmation of contaminants that do not have MRLs in food of animal origin.

**Optimization of the MISPE Procedure.** Initially, the protocols proposed by Supelco for the SupelMIP SPE—Fluoroquinolones columns for the analysis of FQs in milk and kidney samples were tested for the 11 studied FQs in eggs. Both protocols are only different in the treatment of samples (milk was dissolved in an equal amount of 10 mM ammonium acetate, whereas kidney was mixed with 30 mL of 50 mM $Na_2PO_4$). The commercially proposed protocol for milk was not successful for eggs because the extract obstructs the MIP cartridge and does not pass through it. Although a higher degree of centrifugation and/or filtration of the extract through a 0.45 μm glass filter prior to MISPE was also checked, both the MIP column and the filter were always clogged. However, the same optimized MISPE protocol for kidney samples was successfully applied to egg samples.

On the basis of the commercially proposed protocol, a reoptimization of the MISPE was carried out to adapt it to the matrix (egg) and the LC−MS/MS method. First, the sample treatment was optimized to improve the extraction efficiency of the 11 FQs from eggs while keeping maximum sensitivity. The sample amount (1, 2, 3, 4, and 5 g), dilution with 50 mM $NaH_2PO_4$ at pH 7.4 ($^1/_5$, $^1/_{10}$, and $^1/_{20}$), and volume of this solution loaded to the cartridge (1, 2, and 3 mL) were tested. The results compiled in Figure 3 (absolute recoveries) show that recoveries are always inversely proportional to the amount of sample and the dilution factor. A good agreement between efficiency and sensitivity was considered to use 2 g of sample and mix them with 20 mL of 50 mM $NaH_2PO_4$ at pH 7.4. Nevertheless, recovery values were still poor (<58%) (Figure 3). The maximum volume of extract that could be loaded into the cartridge is 2 mL as the protocol recommends because higher volumes provided lower recoveries.

Second, the effectiveness of the protocol recommended by the manufacturer for MIP extraction has been checked. Several studies already indicated that the preconditioning, washing, and elution steps recommended by the commercial protocol did not achieve quantitative elution of the FQs. A significant increase in the recovery percentages, above 15% for all FQs and higher than 30% for danofloxacin, was observed, preconditioning the cartridge with 50 mM phosphate buffer (pH 7.4), instead of water. Similar results have already been reported for kidney samples by Lombardo-Agüi et al.,[19] who proposed use of this solution as an additional step after the water preconditioning.

The commercially proposed protocol suggested four washing steps with different solvents after loading of 2 mL of the supernatant from the sample pretreatment: (i) ultrapure water, (ii) acetonitrile, (iii) 0.5% acetic acid in acetonitrile, and (iv) 0.5% ammonia in water. However, Rodriguez et al.[36] only applied the first three washing steps, avoiding the use of ammonium hydroxide that favored FQ elution from the polymeric sorbent. Lombardo-Agüi et al.[19] observed that satisfactory recoveries were obtained with only the first and second washing steps. As observed in Table 2, an increase of the recoveries is obtained after the first and second washing steps, whereas the third and fourth steps caused a marked

decrease in the recoveries. MS/MS detection applied in this study is a more selective procedure than fluorescence applied in the previous study. However, because the matrix effect, for the moment, was not taken into account, probably decreases in recoveries observed when the first and second washing steps are suppressed could be caused by matrix compounds causing a matrix effect.

It is also quite well-established that the elution step proposed in the commercial protocol did not produce a complete elution of the analytes; therefore, different aqueous mixtures of methanol (25−90%) were studied in the presence of a 2% ammonium hydroxide solution or 2% formic acid because both acid and basic solutions could be appropriate to elute FQs. Acidified solutions were discarded because, in any case, MIP leakage was observed after percolation of such acidic solutions through the cartridge and selecting a 2% ammonium hydroxide and methanol/water mixture (75:25, v/v) as optimum. Different percentages (1−5%) of ammonium hydroxide solution in the mixture were studied, and the best results were obtained using 2%. Finally, different elution volumes (1−4 mL) were tested to increase the recovery percentages. The highest recoveries were obtained with a volume of 3 mL. This final extract was evaporated under gentle nitrogen stream, and the residue was recomposed in 100 μL.

**Validation of the Method.** *Ruggedness and Specificity.* Analyses of 20 blank egg samples from different origins indicated that no interferences for each of the two ion transitions selected for any individual analyte were present (see panels a and b of Figure 2). Over the time period employed for developing this method, ratios of the retention times of the analytes to that of the IS never exceeded 2.5%. Over 4 months, the tolerance of the relative ion abundances did not vary more than 20%. This value is in compliance with the maximum permitted tolerance for relative ion transitions (see section 2.3.3.2 of the 2002/657/EC European Decision).

*Calibration Curves, Detection, and Quantification Limits.* A pool of 20 blank egg extracts was spiked with the analytes at seven concentration levels, 5, 10, 25, 50, 100, 250, and 500 ng/g, for all quinolones, except flumequine and oxolinic acid, which were spiked at 2 ng/g instead of 5 ng/g and analyzed. Each point of the calibration curves was the average of three replicates. In any case, the signal amplitude of each analyte was normalized to that of the IS. The regression coefficients ranged between 0.9990 and 0.9997, demonstrating good linearity ($r$ > 0.999) for all of the FQs within the studied concentration range (see Table 3 for full results).

To estimate the matrix effect, calibration curves were also constructed by adding quinolones to methanol at the same concentration levels as reported above (data not shown here). A comparison of the slopes relative to water- and matrix-based calibration curves indicated that the ion signal intensities of the studied FQs were not affected by either a positive or negative matrix effect.

Table 4 shows the obtained LOD, LOQ, $CC_\alpha$, and $CC_\beta$ for each FQ, which ranged from 0.12 to 0.85 ng/g, from 0.36 to 2.59 ng/g, from 0.46 to 3.35 ng/g, and from 0.59 to 4.12 ng/g, respectively. In comparison to other previously reported procedures, the values reported in this work are more than 10-fold lower for the same antibiotics, involving less sample manipulation.[7−19]

*Recovery and Precision Study.* The trueness of the proposed methodology was checked carrying out recovery experiments in egg samples spiked at two concentration levels of $CC_\beta$ and 50 ng/g. The results obtained, expressed as relative recoveries, are reported in Table 5. Mean recoveries ranged between 92 and 103% with intraday RSDs <6%. Mean recoveries of the ISs ofloxacin-$d_3$ and ciprofloxacin-$d_8$ at the spiked level (10 ng/g) were 94 ± 4 and 98 ± 3%, respectively. Within-laboratory reproducibility was determined by extracting and analyzing the same samples by triplicate on 3 different days. Mean recoveries ranged between 90 and 106% for the different FQs, with RSD values lower than 8%. Mean recoveries of the ISs ofloxacin-$d_3$ and ciprofloxacin-$d_8$ at the spiked level (10 ng/g) were 95 ± 7 and 99 ± 6%, respectively. These results confirm the good reproducibility of the optimized method.

The recoveries provided by this method at the $CC_\beta$ level were also checked in the three different egg species analyzed during this study. Figure 4 shows the absolute recoveries and the RSDs obtained. Recoveries of 90−99% were obtained in eggs of laying hens (*G. gallus domensticus*). Recoveries of 87−99% were obtained in eggs of quails (*C. japonica*). Recoveries of 89−98% were obtained in eggs of black-headed gulls (*C. ridibundus*). The RSDs were always lower than 9%. Mean recoveries of the IS at the spiked level (10 ng/g) for the three species were 98 ± 1% for ofloxacin-$d_3$ and 97 ± 2% for ciprofloxacin-$d_8$. Thus, apparent differences were not observed between eggs of different animal species.

*Comparison to Other Extraction Methods.* A comparison of the MIP method to two other extraction procedures (SPE and solvent extraction) was performed. Figure 5 shows the

**Table 3. Calibration Curves on Matrix-Matched Standards Prepared Using the MIP Extraction Method for the Determination of the 11 Examined FQs Using Ofloxacin-$d_8$ (First Four FQs) and Ciprofloxacin-$d_8$ (Next Seven FQs) at 10 ng/g as ISs**

| | range (μg/kg) | slope | intercept | $R^2$ |
|---|---|---|---|---|
| pipemidic acid | 5−500 | 0.522 ± 0.062 | 0.188 ± 0.048 | 0.9997 |
| marbofloxacin | 5−500 | 0.625 ± 0.032 | 0.129 ± 0.052 | 0.9993 |
| ofloxacin | 5−500 | 0.316 ± 0.039 | 0.285 ± 0.045 | 0.9994 |
| norfloxacin | 5−500 | 0.242 ± 0.028 | 0.099 ± 0.046 | 0.9995 |
| cipropfloxacin | 5−500 | 0.252 ± 0.045 | 0.329 ± 0.051 | 0.9991 |
| enrofloxacin | 5−500 | 0.638 ± 0.076 | 1.761 ± 0.154 | 0.9993 |
| danofloxacin | 5−500 | 0.784 ± 0.082 | 1.851 ± 0.203 | 0.9995 |
| difloxacin | 5−500 | 0.901 ± 0.107 | 1.893 ± 0.194 | 0.9990 |
| sarafloxacin | 5−500 | 0.911 ± 0.093 | 0.866 ± 0.104 | 0.9991 |
| flumequine | 2−500 | 2.745 ± 0.284 | 0.229 ± 0.098 | 0.9993 |
| oxolinic acid | 2−500 | 1.179 ± 0.125 | 0.224 ± 0.089 | 0.9996 |

**Table 4. LOD, LOQ, $CC_\alpha$, and $CC_\beta$ of the MIP-Based Method for Detecting 11 FQs in Eggs**

| | LODs (ng/g) | LOQs (ng/g) | $CC_\alpha$ (ng/g) | $CC_\beta$ (ng/g) |
|---|---|---|---|---|
| pipemidic acid | 0.30 | 0.92 | 1.16 | 1.45 |
| marbofloxacin | 0.27 | 0.83 | 1.05 | 1.29 |
| ofloxacin | 0.47 | 1.42 | 1.80 | 2.21 |
| norfloxacin | 0.63 | 1.90 | 2.49 | 3.07 |
| cipropfloxacin | 0.67 | 2.02 | 2.62 | 3.22 |
| enrofloxacin | 0.80 | 2.41 | 3.09 | 3.80 |
| danofloxacin | 0.85 | 2.59 | 3.35 | 4.12 |
| difloxacin | 0.71 | 2.15 | 2.72 | 3.34 |
| sarafloxacin | 0.38 | 1.14 | 1.50 | 1.84 |
| flumequine | 0.12 | 0.36 | 0.46 | 0.59 |
| oxolinic acid | 0.25 | 0.75 | 0.97 | 1.22 |

**Table 5. Within-Day ($n$ = 5) and Between-Day (over a Period of 6 Consecutive Days) Precision and Accuracy Data for the Determination of FQs in Eggs**

| | | | within-day | | | between-days | |
|---|---|---|---|---|---|---|---|
| analytes | added (ng/g) | found ± SD ($\mu$g/kg) | RSD (%) | recovery (%) | found ± SD ($\mu$g/kg) | RSD (%) | recovery (%) |
| pipemidic acid | 1.45 | 1.33 ± 0.04 | 3 | 92 | 1.36 ± 0.07 | 5 | 94 |
| | 50 | 46.50 ± 0.93 | 2 | 93 | 46.00 ± 1.84 | 4 | 92 |
| marbofloxacin | 1.29 | 1.19 ± 0.05 | 4 | 92 | 1.19 ± 0.07 | 6 | 92 |
| | 50 | 46.00 ± 1.38 | 3 | 92 | 47.00 ± 1.88 | 4 | 94 |
| ofloxacin | 2.21 | 2.28 ± 0.09 | 4 | 103 | 2.34 ± 0.16 | 7 | 106 |
| | 50 | 50.00 ± 1.00 | 2 | 100 | 50.50 ± 2.02 | 4 | 101 |
| norfloxacin | 3.07 | 2.98 ± 0.12 | 4 | 97 | 3.04 ± 0.18 | 6 | 99 |
| | 50 | 49.50 ± 1.49 | 3 | 99 | 48.50 ± 1.94 | 4 | 97 |
| cipropfloxacin | 3.22 | 3.03 ± 0.18 | 6 | 94 | 2.90 ± 0.23 | 8 | 90 |
| | 50 | 48.00 ± 1.92 | 4 | 96 | 47.00 ± 2.82 | 6 | 94 |
| enrofloxacin | 3.8 | 3.61 ± 0.18 | 5 | 95 | 3.50 ± 0.24 | 7 | 92 |
| | 50 | 47.50 ± 2.38 | 5 | 95 | 48.50 ± 1.94 | 4 | 97 |
| danofloxacin | 4.12 | 4.08 ± 0.12 | 3 | 99 | 4.20 ± 0.21 | 5 | 102 |
| | 50 | 50.50 ± 0.51 | 1 | 101 | 52.00 ± 1.56 | 3 | 104 |
| difloxacin | 3.34 | 3.11 ± 0.12 | 4 | 93 | 3.04 ± 0.18 | 6 | 91 |
| | 50 | 49.00 ± 0.98 | 2 | 98 | 47.00 ± 1.88 | 4 | 94 |
| sarafloxacin | 1.84 | 1.69 ± 0.05 | 3 | 92 | 1.66 ± 0.12 | 7 | 90 |
| | 50 | 46.00 ± 0.92 | 2 | 92 | 46.50 ± 1.40 | 3 | 93 |
| flumequine | 0.59 | 0.57 ± 0.02 | 3 | 96 | 0.56 ± 0.04 | 8 | 95 |
| | 50 | 49.00 ± 1.47 | 3 | 98 | 49.50 ± 2.48 | 5 | 99 |
| oxolinic acid | 1.22 | 1.17 ± 0.07 | 6 | 96 | 1.13 ± 0.09 | 8 | 93 |
| | 50 | 47.00 ± 1.88 | 4 | 94 | 48.50 ± 1.94 | 4 | 97 |

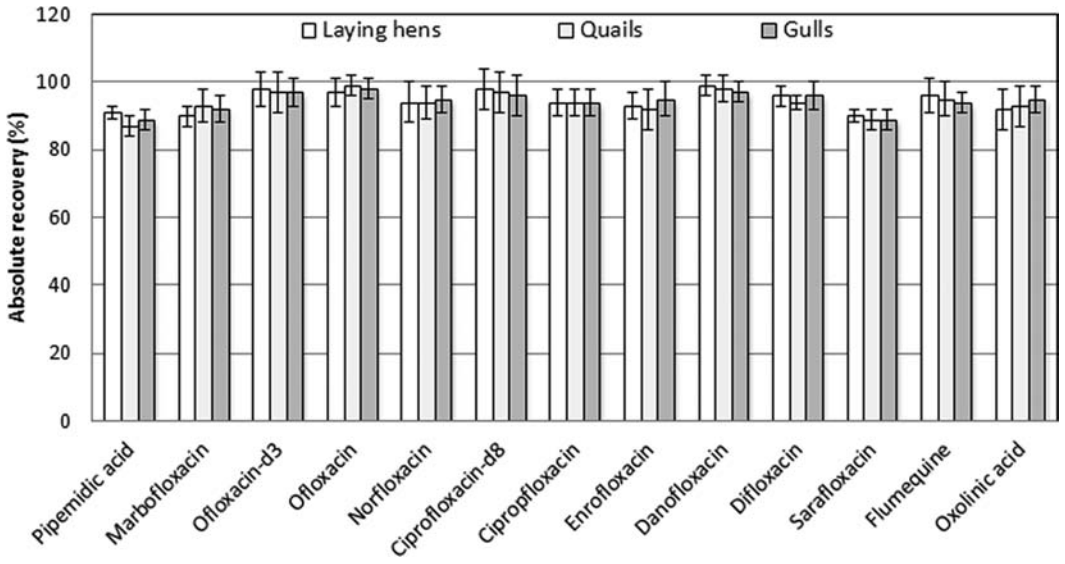


**Figure 4.** Absolute extraction recoveries (%) obtained in spiked (at the $CC_\beta$) eggs of different bird species.

recoveries and RSDs for each FQ at a concentration of 10 ng/g, and Table 6 shows a summary of the main characteristics and parameters of each method. MIP recoveries were, at least, 10% higher than those obtained by SPE and 20% higher than those obtained by solvent extraction. This last procedure also showed better sensitivity than SPE (between 5 and 20 times) or solvent extraction (between 6 and 30 times), accuracy (recoveries were 89−98% in front of 72−86% for SPE and 60−82% for solvent), and precision (repeatability RSDs < 6% and reproducibility RSDs < 8%). When both SPE methods were compared to solvent extraction, the main advantage is the avoidance of toxic organic solvent, such as dichloromethane.

The linearity of the calibration curves constructed from the analysis of spiked samples was good in all three procedures, with correlation coefficients always greater than 0.99, even though it was slightly better by MISPE. Matrix effects were studied for the three procedures, comparing these calibration curves to those obtained for standard solution. Using solvent extraction, an important suppression in the response, owing to the matrix effect, is observed for the majority of the FQs, whereas using SPE also, a slight enhancement of the response (ranging from 0 to 25%) dependent upon the compound is noted. The amount of matrix per milliliter in the final extract was 1 g/mL for the three methods. When this is taken into

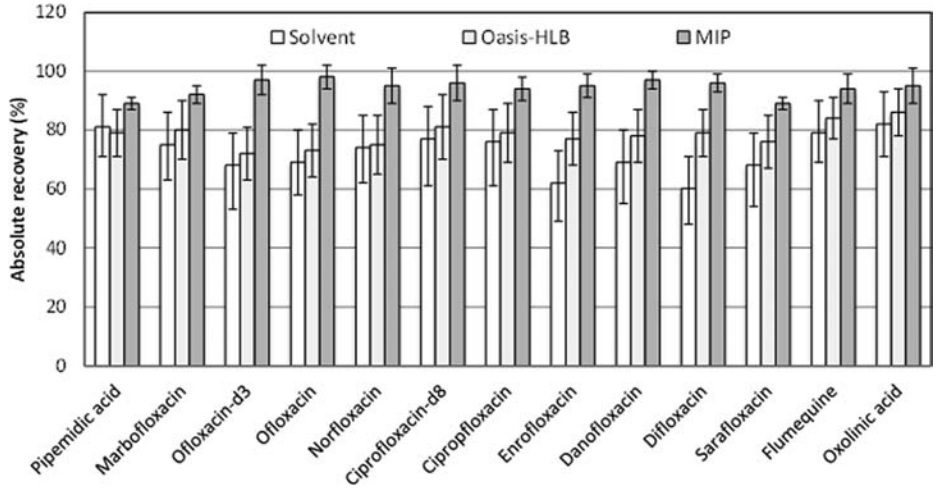


**Figure 5.** Absolute extraction recoveries (%) from spiked (at 10 ng/g) eggs obtained using the MIP, Oasis HLB, and solvent extraction for 11 FQs spiked in laying hen eggs at 50 ng/g.

**Table 6. MISPE, Oasis HLB SPE, and Solvent Extraction Performance Comparison**

| | MISPE | Oasis HLB SPE | solvent |
|---|---|---|---|
| spiking concentrations (ng/g) | $CC_\beta$ and 50 | $CC_\beta$ and 50 | $CC_\beta$ and 50 |
| accuracy (% absolute recovery) | 89–98 | 72–86 | 60–82 |
| repeatability (RSD, %) | <6 | <10 | <14 |
| reproducibility (RSD, %) | <8 | <16 | <18 |
| linearity ($r^2$) | >0.999 | >0.999 | >0.999 |
| matrix effect (%) | 0 | 0–25 | oct–45 |
| selectivity | high | low | low |
| sensitivity | | | |
| (LOQ, ng/g) | 0.36–2.59 | 5.42–10.29 | 6.27–12.87 |
| ($CC_\beta$, ng/g) | 0.59–4.12 | 15.23–30.82 | 18.25–36.29 |
| time (min) per sample | 60 | 60 | 30 |
| organic solvent | | | |
| (mL) | ~15 | ~15 | ~4 |
| type[a] | MeOH/AcN | MeOH/AcN | AcN/DCM |
| cost (€/sample) | 15 | 10 | 4 |

[a]AcN, acetonitrile; DCM, dichloromethane; and MeOH, methanol.

account, the absence of the matrix effect is an interesting feature of MISPE.

However, solvent extraction and SPE present some advantages with respect to MIPs, such as low cost and, in the case of solvent extraction, simplicity (it avoids the tedious evaporation step). Commercially MIPs are more expensive than Oasis HLB cartridges. Both SPE method results are much more expensive than solvent extraction, which only requires a few microliters of a base and a few milliliters of acetonitrile and methanol. However, the results presented indicate the potential of MIPs for determining FQs in eggs in terms of sensitivity and selectivity.

*Application.* To evaluate the feasibility of the method for the analysis of real samples, eggs from hens treated with sarafloxacin were analyzed. Sarafloxacin was detected in eggs on the second day of dosing and reached a maximum at 24 h after drug withdrawal. Thereafter, the FQ levels in eggs declined rapidly and were undetectable 7 days after the last dose (see Figure 6). The error bars represent the RSD for each daily sample set, which are related to the differences in residue concentrations in eggs collected from individual laying hens on a specific day. Although statistical assessment is not possible

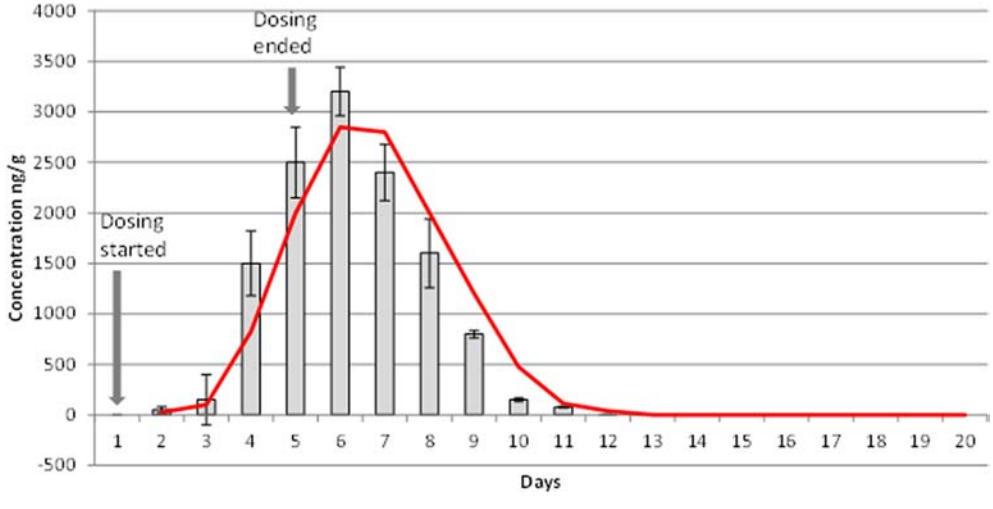


**Figure 6.** Dosing (5 days) and withdrawal (15 days) bar graph for sarafloxacin in whole eggs from treated hens (dose of 10 mg/kg).

because of the limited number of samples, the results shown in Figure 6 reveal the trend of elimination of these drugs in whole eggs. This trend is in agreement with previous data reported for other FQs, such as enrofloxacin.[8,9,12]

Residues of the studied FQs were not found in the commercial samples of eggs from laying hens, quails, and kindly donated samples of black-headed gulls analyzed. This result was expected because the use of FQ is prohibited in animals producing eggs for human consumption, since years ago, environmental concentrations are still low, and the exact data on the origin of the gull eggs were not available.

In summary, a simple, selective, and sensitive strategy for the determination of 11 FQs in eggs has been developed, showing the usefulness of MIPs as a powerful tool for extraction and sample cleanup. The MISPE procedure developed has been reduced in comparison to those commercially proposed for kidney, honey, and milk, with an increase in the recovery percentages. As seen, a very clean extract was obtained using this MISPE procedure in such a complex matrix, being a very selective and efficient analyte extraction method. This advantage in combination with the high sensitivity of the MS/MS detection provided a very useful method for the analysis of these 11 FQs in this kind of sample of animal origin. The proposed method also provided satisfactory results in terms of trueness and precision; therefore, the accuracy for the analysis of these samples was demonstrated.

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### Notes

The authors declare no competing financial interest.

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## REFERENCES

(1) Pico, Y.; Andreu, V. Fluoroquinolones in soil—Risks and challenges. *Anal. Bioanal. Chem.* **2007**, *387*, 1287−1299.

(2) Andreu, V.; Blasco, C.; Pico, Y. Analytical strategies to determine quinolone residues in food and the environment. *TrAC, Trends Anal. Chem.* **2007**, *26*, 534−556.

(3) European Commission.. Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *J. Off. Eur. Comm.* **2010**, *L15*, 1−72.

(4) da Silva, B. F.; Jelic, A.; Lopez-Serna, R.; Mozeto, A. A.; Petrovic, M.; Barcelo, D. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. *Chemosphere* **2011**, *85*, 1331−1339.

(5) Lemus, J. A.; Blanco, G.; Arroyo, B.; Martinez, F.; Grande, J. Fatal embryo chondral damage associated with fluoroquinolones in eggs of threatened avian scavengers. *Environ. Pollut.* **2009**, *157*, 2421−2427.

(6) Chobtang, J.; de Boer, I. J. M.; Hoogenboom, R. L. A. P.; Haasnoot, W.; Kijlstra, A.; Meerburg, B. G. The need and potential of biosensors to detect dioxins and dioxin-like polychlorinated biphenyls along the milk, eggs and meat food chain. *Sensors* **2011**, *11*, 11692−11716.

(7) Scortichini, G.; Annunziata, L.; Di Girolamo, V.; Buratti, R.; Galarini, R. Validation of an enzyme-linked immunosorbent assay screening for quinolones in egg, poultry muscle and feed samples. *Anal. Chim. Acta* **2009**, *637*, 273−278.

(8) Huet, A.; Charlier, C.; Weigel, S.; Godefroy, S. B.; Delahaut, P. Validation of an optical surface plasmon resonance biosensor assay for screening (fluoro)quinolones in egg, fish and poultry. *Food Addit. Contam.* **2009**, *26*, 1341−1347.

(9) Jimenez, V.; Companyo, R.; Guiteras, J. Validation of a method for the analysis of nine quinolones in eggs by pressurized liquid extraction and liquid chromatography with fluorescence detection. *Talanta* **2011**, *85*, 596−606.

(10) Rambla-Alegre, M.; Collado-Sanchez, M.; Esteve-Romero, J.; Carda-Broch, S. Quinolones control in milk and eggs samples by liquid chromatography using a surfactant-mediated mobile phase. *Anal. Bioanal. Chem.* **2011**, *400*, 1303−1313.

(11) Takeda, N.; Gotoh, M.; Matsuoka, T. Rapid screening method for quinolone residues in livestock and fishery products using immobilised metal chelate affinity chromatographic clean-up and liquid chromatography−fluorescence detection. *Food Addit. Contam.* **2011**, *28*, 1168−1174.

(12) Bogialli, S.; D'Ascenzo, G.; Di Corcia, A.; Lagana, A.; Tramontana, G. Simple assay for monitoring seven quinolone antibacterials in eggs: Extraction with hot water and liquid chromatography coupled to tandem mass spectrometry. Laboratory validation in line with the European Union Commission Decision 657/2002/EC. *J. Chromatogr., A* **2009**, *1216*, 794−800.

(13) Deng, X. J.; Yang, H. Q.; Li, J. Z.; Song, Y.; Guo, D. H.; Luo, Y.; Du, X. N.; Bo, T. Multiclass residues screening of 105 veterinary drugs in meat, milk, and egg using ultra high performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry. *J. Liq. Chromatogr. Relat. Technol.* **2011**, *39*, 2286−2303.

(14) Junza, A.; Amatya, R.; Barron, D.; Barbosa, J. Comparative study of the LC−MS/MS and UPLC−MS/MS for the multi-residue analysis of quinolones, penicillins and cephalosporins in cow milk, and validation according to the regulation 2002/657/EC. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2011**, *879*, 2601−2610.

(15) Hermo, M. P.; Nemutlu, E.; Barbosa, J.; Barron, D. Multiresidue determination of quinolones regulated by the European Union in bovine and porcine plasma. Application of chromatographic and capillary electrophoretic methodologies. *Biomed. Chromatogr.* **2011**, *25*, 555−569.

(16) Heymo, M. P.; Nemutlu, E.; Kir, S.; Barron, D.; Barbosa, J. Improved determination of quinolones in milk at their MRL levels using LC−UV, LC−FD, LC−MS and LC−MS/MS and validation in line with regulation 2002/657/EC. *Anal. Chim. Acta* **2008**, *613*, 98−107.

(17) Hermo, M. P.; Barron, D.; Barbosa, J. Determination of multiresidue quinolones regulated by the European Union in pig liver samples: High-resolution time-of-flight mass spectrometry versus tandem mass spectrometry detection. *J. Chromatogr., A* **2008**, *1201*, 1−14.

(18) Hermo, M. P.; Nemutlu, E.; Ku, S.; Barron, D.; Barbosa, J. Improved determination of quinolones in milk at their MRL levels using LC−UV, LC−FD, LC−MS and LC−MS/MS and validation in line with regulation 2002/657/EC. *Anal. Chim. Acta* **2008**, *613*, 98−107.

(19) Lombardo-Agüi, M.; Garcia-Campaña, A. M.; Gámiz-Gracia, L.; Cruces Blanco, C. Laser induced fluorescence coupled to capillary electrophoresis for the determination of fluoroquinolones in foods of animal origin using molecularly imprinted polymers. *J. Chromatogr., A* **2010**, *1217*, 2237−2242.

(20) Juan-Garcia, A.; Font, G.; Pico, Y. Determination of quinolone residues in chicken and fish by capillary electrophoresis−mass spectrometry. *Electrophoresis* **2006**, *27*, 2240−2249.

(21) Juan-Garcia, A.; Font, G.; Pico, Y. Simultaneous determination of different classes of antibiotics in fish and livestock by CE−MS. *Electrophoresis* **2007**, *28*, 4180−4191.
(22) Blasco, C.; Torres, C. M.; Pico, Y. Progress in a antibacterials analysis of residual in food. *TrAC, Trends Anal. Chem.* **2007**, *26*, 895−913.
(23) Pico, Y.; Barcelo, D. The expanding role of LC−MS in analyzing metabolites and degradation products of food contaminants. *TrAC, Trends Anal. Chem.* **2008**, *27*, 821−835.
(24) Malik, A. K.; Blasco, C.; Pico, Y. Liquid chromatography−mass spectrometry in food safety. *J. Chromatogr., A* **2010**, *1217*, 4018−4040.
(25) Cho, H. J.; Yi, H.; Cho, S. M.; Lee, D. G.; Cho, K.; Abd El-Aty, A.; Shim, J. H.; Lee, S. H.; Jeong, J. Y.; Shin, H. C. Single-step extraction followed by LC for determination of (fluoro)quinolone drug residues in muscle, eggs, and milk. *J. Sep. Sci.* **2010**, *33*, 1034−1043.
(26) Garrido Frenich, A.; del Mar Aguilera-Luiz, M.; Martinez Vidal, J. L.; Romero-Gonzalez, R. Comparison of several extraction techniques for multiclass analysis of veterinary drugs in eggs using ultra-high pressure liquid chromatography−tandem mass spectrometry. *Anal. Chim. Acta* **2010**, *661*, 150−160.
(27) Shen, J.; Li, H.; Jiang, H.; Zhou, D.; Xu, F.; Li, J.; Ding, S. Simultaneous determination of 13 quinolones in eggs using column high-performance liquid chromatography/electrospray ionization-tandem mass spectrometry and depletion of pefloxacin methanesulfonate in eggs. *J. AOAC Int.* **2008**, *91*, 1499−1506.
(28) Zheng, M. M.; Ruan, G. D.; Feng, Y. Q. Evaluating polymer monolith in-tube solid-phase microextraction coupled to liquid chromatography/quadrupole time-of-flight mass spectrometry for reliable quantification and confirmation of quinolone antibacterials in edible animal food. *J. Chromatogr., A* **2009**, *1216*, 7510−7519.
(29) Pulgarin, J. A. M.; Molina, A. A.; Munoz, S. R. Rapid chemiluminescent determination of enrofloxacin in eggs and veterinary drugs. *Anal. Lett.* **2011**, *44*, 2194−2208.
(30) Jimenez, V.; Rubies, A.; Centrich, F.; Companyo, R.; Guiteras, J. Development and validation of a multiclass method for the analysis of antibiotic residues in eggs by liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2011**, *1218*, 1443−1451.
(31) Benito-Pena, E.; Urraca, J. L.; Sellergren, B.; Cruz Moreno-Bondi, M. Solid-phase extraction of fluoroquinolones from aqueous samples using a water-compatible stochiometrically imprinted polymer. *J. Chromatogr., A* **2008**, *1208*, 62−70.
(32) Benito-Pena, E.; Martins, S.; Orellana, G.; Moreno-Bondi, M. C. Water-compatible molecularly imprinted polymer for the selective recognition of fluoroquinolone antibiotics in biological samples. *Anal. Bioanal. Chem.* **2009**, *393*, 235−245.
(33) Liu, P. Y.; Shen, J.; Gao, L.; Liu, L.; Li, R.; Li, Q. Determination of fluoroquinolones in milk by high-performance liquid chromatography using mixed-templates imprinted polymer extraction. *Asian J. Chem.* **2010**, *22*, 6275−6288.
(34) Zheng, M. M.; Gong, R.; Zhao, X.; Feng, Y. Q. Selective sample pretreatment by molecularly imprinted polymer monolith for the analysis of fluoroquinolones from milk samples. *J. Chromatogr., A* **2010**, *1217*, 2075−2081.
(35) Supelco. *SupelMIP SPE—Fluoroquinolones*; Sigma-Aldrich: St. Louis, MO, 2008.
(36) Rodriguez, E.; Moreno-Bondi, M. C.; Marazuela, M. D. Multiresidue determination of fluoroquinolone antimicrobials in baby foods by liquid chromatography. *Food Chem.* **2011**, *127*, 1354−1360.
(37) Hassouan, M. K.; Ballesteros, O.; Taoufiki, J.; Vilchez, J. L.; Cabrera-Aguilera, M.; Navalon, A. Multiresidue determination of quinolone antibacterials in eggs of laying hens by liquid chromatography with fluorescence detection. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* **2007**, *852*, 625−630.