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# Comparison of different sample treatments for the analysis of quinolones in milk by capillary-liquid chromatography with laser induced fluorescence detection

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ABSTRACT

A simple and very sensitive capillary-liquid chromatography method coupled with laser induced fluorescence detection has been developed for the simultaneous determination of seven quinolones of veterinary use in milk. Moreover, a comparison between two different sample treatments (QuEChERS and molecularly imprinted polymer, MIP) has been carried out in terms of efficiency of the extraction (number of analytes to be analysed and absence of interferences), throughput, linear dynamic range in matrix-matches calibrations, detection and quantification limits and accuracy (trueness and precision, by means of recovery assays). The results showed that the QuEChERS procedure was more efficient and faster, showing good recoveries, sensitivity and precision for all the studied compounds. Employing this proposed method, very low detection limits, between 0.4 μg/kg for danofloxacin, and 6 μg/kg for sarafloxacin, have been obtained.



## 1. Introduction

Quinolones (Qns) are among the most important groups of antibiotics, whose activity is based on the inhibition of bacterial DNA synthesis. They are used for the treatment of a wide variety of infections in human [1] and in food producing animals, causing residues in foodstuffs [2] that produce adverse reaction in humans, as allergic reactions or antibiotic resistance. Therefore, to ensure food safety, European Union (EU) has set maximum residue limits (MRLs) for eights Qns of veterinary use in different animal products [3]. For instance, in the case of milk, these MRLs range from 30 μg/kg for danofloxacin (DANO) to 100 μg/kg for the sum of enrofloxacin (ENRO) and its metabolite ciprofloxacin (CIPRO), so the determination of these compounds at trace levels requires sensitive analytical methods to comply with current legislation. Most of the works use high performance liquid chromatography (HPLC) [4,5] or ultra high performance liquid chromatography (UHPLC) coupled with UV/vis detection [6–13], conventional fluorescence [8,14–17], or mass spectrometry (MS) [15,18–24] for their quantification in different kind of samples. Capillary electrophoresis (CE) coupled with UV/vis detection [25,26] conventional fluorescence [27] and MS [28–30] has also been proposed as an alternative to chromatographic techniques. In addition, laser induced fluorescence (LIF) has been used coupled with CE as a very sensitive detection method for the determination of different Qns, such as ENRO and CIPRO in chicken muscle [31], CIPRO, DANO, ENRO and sarafloxacin (SARA) in milk and kidney [32] and ofloxacin, lomefloxacin, norfloxacin, DANO, ENRO and SARA in environmental waters [33].

One of the main problems concerning the determination of residues of Qns in foods derived from animals is the sample treatment. Different procedures have been proposed to improve the cleanup process and pre-concentration of the antibiotics from different matrices [5]. Thus, solid–liquid extraction [15], microwave extraction [7], pressurized liquid extraction [29,34], solid-phase extraction (SPE) [6,8,16,17,20,33,35], molecular imprinted-SPE (MISPE) [13,14,32], dispersive SPE [30] and stir rod sorptive extraction (SRSE) [24], have been used depending on the characteristics of the sample and the extracted Qns.

Recently, new methodologies have been proposed for the treatment of samples containing Qns. Among them, a fast and inexpensive method, so-called Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) methodology and MISPE are promising alternatives. The QuEChERS methodology presents some advantages, such as its simplicity, minimum steps, and effectiveness for cleaning-up complex samples [36]. It involves two steps: the first one is an extraction step based on partitioning via salting-out extraction involving the equilibrium between an aqueous and an organic layer, and the second one is a dispersive SPE step that involves further cleanup using combinations of $MgSO_4$ and different sorbents, such as $C_{18}$, to remove interfering substances. Recently, different QuEChERS procedures have been applied in the multidetermination of veterinary drugs residues, including Qns in animal tissue [37], milk [38,39] or eggs [40]. Concerning MISPE, molecularly imprinted polymers (MIPs) are synthetic materials with artificially generated recognition sites able to specifically capture target molecules. Thus, the link between the MIPs and its target molecules makes it ideal for

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the selective extraction of compounds at trace levels, being of special interest for complex matrices [41]. This methodology has been proposed for the determination of Qns in kidney samples [32], baby foods [13] and milk [32,14], respectively.

The purpose of this work is to develop a sensitive and selective method for the determination of seven Qns of veterinary use, namely CIPRO, DANO, ENRO, SARA, difloxacin (DIFLO), oxolinic acid (OXO) and flumequine (FLUME) (see structures in Fig. 1), using capillary HPLC-LIF detection as an alternative to quantify traces of these analytes in milk. Capillary HPLC is a miniaturized technique in which columns of internal diameter of typically 500 μm and flow rates up to 20 μl $min^{-1}$ are used. This technique shows several advantages compared to analytical HPLC, such as better resolution, lower detection limits and lower solvent consumption, being more environmentally friendly than conventional HPLC. It is recommended when sample volume is limited, and especially to gain sensitivity, which can be greatly increased when LIF detection is coupled as detection technique. These veterinary compounds have been selected considering that some of them such as DIFLO, SARA and OXO, are not allowed in animals producers of milk for human consumption, and for the rest, MRLs have been established by EU legislation [3]. Furthermore, a comparison between two different sample treatments (QuEChERS and MISPE) has been carried out, in terms of efficiency, recovery, sensitivity and precision.

## 2. Experimental

### 2.1. Reagents and materials

Solvents were HPLC grade, reagents were analytical grade and Qns were analytical standard grade. Ultrapure water (Milli-Q Plus system, Millipore Bedford, MA, USA) was used throughout the work. Methanol (MeOH), acetonitrile (ACN), ammonium hydroxide (30%), sodium dihydrogen phosphate monohydrate and phosphoric acid (85%) were obtained from Panreac-Química (Barcelona, Spain). Acetic acid, formic acid and citric acid were supplied by Merck (Darmstadt, Germany). DANO, SARA and DIFLO were supplied by Riedel-de Haën (Seelze, Germany), FLUME by Sigma (St. Louis, MO, USA) and CIPRO, ENRO and OXO by Fluka (Steinheim, Germany).

Stock standard solutions (100 mg/L) of each Qn were prepared by dissolving the appropriate amount of each analyte in ACN, and were stored in the dark at 4 °C. Under such conditions, they were stable for at least 1 month. Working solutions (containing all Qns) were prepared diary from the individual stock solutions and diluted with Milli-Q water.

Kits SampliQ QuEChERS (kindly supplied by Agilent Technologies Inc., Wilmington, DE, USA) consisted on 50 mL buffered QuEChERS extraction tubes (4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate, 0.5 g disodium citrate sesquihydrate) and dispersive tubes (15 mL, 150 mg $C_{18}$ and 900 mg $MgSO_4$). Extraction cartridges containing MIPs (SupelMIP Qns SPE Column, 25 mg, 3 mL, Supelco, Bellefonte, PA, USA) were used in the MISPE treatment.

Filters of 13 mm with 0.2 μm nylon membrane (Bulk Acrodisc®, Pall Corp., MI, USA), were used for filtration of the final extracts before analysis. The mobile phase was filtered before use in 47 mm filters with 0.2 μm nylon membranes (Supelco, Bellefonte, PA, USA).

### 2.2. Instrumentation

Separation was performed on a 1200 Series Capillary LC System from Agilent Technologies, coupled to a LIF detector (Zetalif Evolution model LIF UV-01, Picometrics S.A., Ramonville, France), with a He–Cd laser with an excitation wavelength of 325 nm. Data were collected using the software provided with the HP ChemStation version A.09.01. A fused-silica capillary (75 μm I.D.) from Polymicro Technologies (Phoenix, AZ, USA) was used to couple the LC and the LIF detector. Different chromatographic columns were tested for separation of Qns, namely: Luna $C_{18}$, 150 mm × 0.3 mm, 5 μm; Luna $C_{18}$, 150 mm × 0.5 mm, 5 μm; and Luna $C_8$, 150 mm × 0.3 mm, 3 μm (all of them from Phenomenex, Torrance, CA, USA).

A pH-meter (Crison model pH 2000, Barcelona, Spain) with a resolution of ±0.01 pH unit, a centrifuge (Universal 320R from Hettich Zentrifugen, Tuttlingen, Germany), a VisiprepTM DL vacuum manifold for SPE (Supelco) and a vortex-2 Genie (Scientific Industries, Bohemia, NY, USA) were also used.

### 2.3. Chromatographic conditions

The mobile phase consisted on 10 mM aqueous citric acid solution pH 4.75 (pH adjusted with ammonium hydroxide) (eluent A) and 10 mM citric acid in ACN (eluent B). The gradient elution started with 11% B followed by a linear gradient to 20% B (6 min) and to 90% B (11 min). The initial conditions were reestablished by a 5 min linear gradient, followed by an equilibration time of 10 min. Analyses were performed at a flow rate of 15 μL/min and a column temperature of 35 °C, using an injection volume of 8 μL. Half an hour before starting the measurements, the laser was switched on and the room temperature was kept constant at approximately 19 °C.

### 2.4. Sample treatment

In this study we have tested two different methodologies for extraction and cleanup of whole cow milk samples (UHT milk): QuEChERS and MISPE.

#### 2.4.1. Use of QuEChERS for treatment of milk samples

The QuEChERS procedure was adapted from that described by Agilent Technologies for the determination of Qns in bovine liver [42]. Samples of 2 g of milk were spiked at different concentration levels of Qns using the working standard solutions. They were placed into 50 mL centrifuge tubes and homogenized in vortex. Then 8 mL of 30 mM $NaH_2PO_4$ buffer pH 7.0 was added, shaking by hand for 10 s. Subsequently, 10 mL of 5% formic acid in ACN was added to the tube, shaking by hand for 30 s. Agilent SampliQ EN QuEChERS extraction kit was added and the tube was shaken vigorously for 1 min. After that, the sample was centrifuged at 9000 rpm for 5 min and 4 mL of the upper ACN layer was transferred to another tube containing the dispersive SPE ($C_{18}$ and $MgSO_4$) and stirred in vortex for 1 min. The tube was centrifuged at 9000 rpm for 5 min. Then, 1 mL of supernatant was transferred to a vial, dried at 35 °C under a stream of nitrogen and reconstituted with 1 mL of 10 mM aqueous citric acid solution pH 4.75 (pH adjusted with ammonium hydroxide). Finally, the samples were filtered with a 0.2 μm filter before injection.

#### 2.4.2. Use of MISPE for treatment of milk samples

Samples of 10 g of milk were spiked at different concentration levels using the working standard solutions. After homogenizing in vortex, samples were diluted with 10 mL of 10 mM ammonium acetate buffer pH 5.0, shaken for 5 min and then centrifuged for 5 min at 5000 rpm. The pH of the supernatant was adjusted to 7.0 with 3% ammonium hydroxide solution, and 10 mM ammonium acetate buffer pH 7.0 was added to obtain a final volume of 25 mL. For MIP cartridge conditioning, 1 mL of methanol, 2 mL of water and 0.5 mL of 10 mM ammonium acetate buffer pH 7, were successively applied. An aliquot of 1 mL of the final sample solution was charged into the preconditioned cartridge at a flow rate of approximately 0.2 mL/min. After that, the cartridge was washed with 3 mL of water at a flow rate lower than 0.5 mL/min and vacuum (400 mbar) was applied for 2 min. The elution was achieved with 3 mL MeOH/$H_2O$

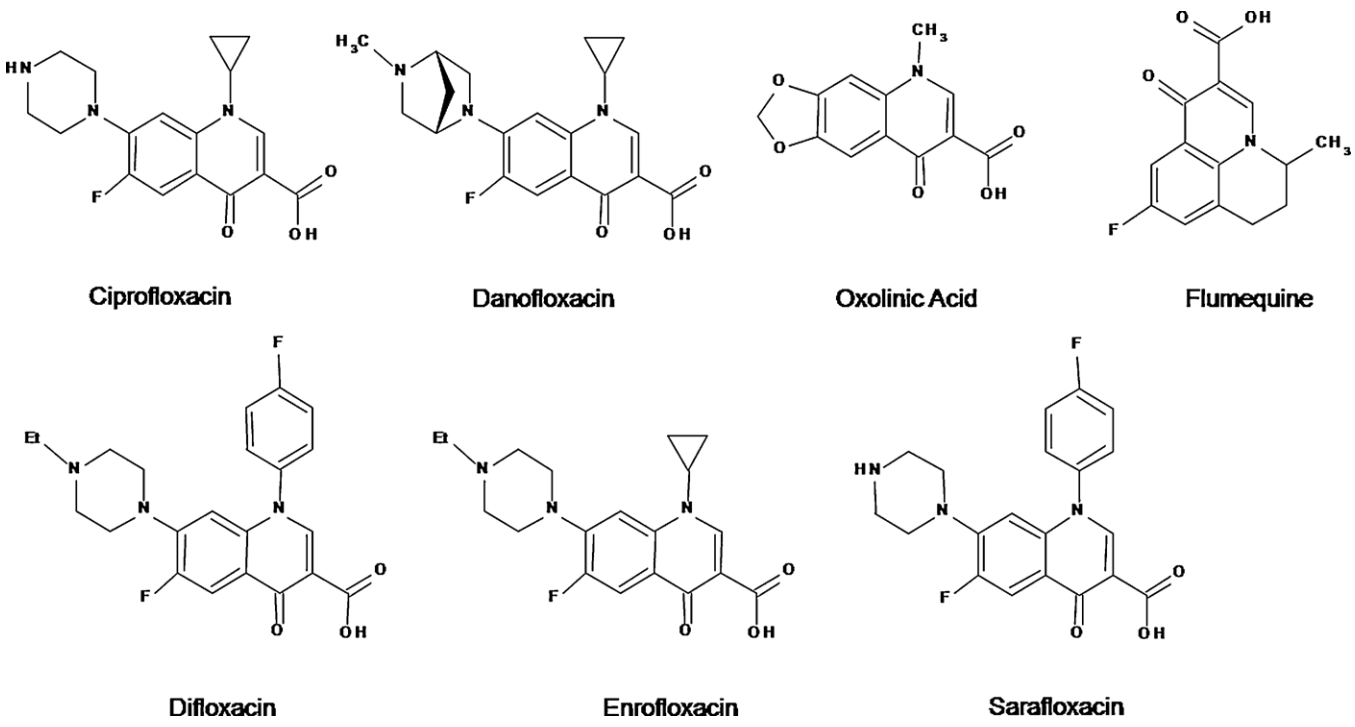


**Fig. 1.** Chemical structures of the selected Qns.

(50/50, v/v) with 3% ammonium hydroxide. This extract was evaporated to dryness at 35 °C under a stream of nitrogen and the residue was reconstituted in 1 mL of 10 mM citric acid pH 4.75, filtered and analysed by the proposed method.

## 3. Results and discussion

### 3.1. Optimization of the chromatographic separation

Qns have been commonly analysed by HPLC using citric acid and ACN as mobile phase [6,7]. So, using these solvents the separation method was optimized. First of all, the pH of the mobile phase was studied between 2.5 and 5.5, using a concentration of 0.01 M citric acid in both, the aqueous and organic phases (A and B, respectively), and a Luna $C_8$ 150 mm × 0.3 mm, 3 μm column. Best compromise between signal intensity and resolution was found when pH 4.75 was used.

Concerning the chromatographic columns, usually $C_8$ and $C_{18}$ have been used to get the best separation for Qns [6]. Thus, three columns were tested, namely: (1) Luna $C_8$ 150 mm × 0.3 mm, 3 μm; (2) Luna $C_{18}$ 150 mm × 0.3 mm, 5 μm; and (3) Luna $C_{18}$ 150 mm × 0.5 mm, 5 μm. Narrow peaks and better resolutions, especially between SARA and DIFLO, were obtained when $C_{18}$ columns (2 and 3) were used. Slightly higher peaks were obtained for most of the analytes when column 3 was used. However, the separation took place shortly when the smaller diameter columns (1 and 2) were used. Finally, column 2 was selected as a compromise between resolution and analysis time.

Subsequently, different buffers were tested, namely: citric acid, formic acid and acetic acid (adjusting the pH with ammonium hydroxide solution in all cases) and citric acid/$Na_2HPO_4$. Using acetic acid, the complete separation between SARA and DIFLO was not achieved, while with the other buffers good and very similar results were obtained. Citric acid was finally selected for the rest of the work. Then, the gradient was optimized to get a satisfactory separation of the Qns. It was set at 11% B (0 min) followed by a linear gradient to 20% B (6 min) and to 90% B (11 min). The flow rate was increased up to 15 μL/min in order to reduce the analysis time.

The temperature of the column was also studied in the range of 30–45 °C. In all cases resolution was good enough and no significant differences were observed. Finally 35 °C was selected. Finally, injection volumes between 1 and 8 μL were tested and 8 μL was selected in order to get the maximum sensitivity. Under these optimum conditions, the separation of the seven compounds was achieved in less than 14 min.

### 3.2. Optimization of sample treatments

The aim of this work was the development of a sensitive method for the simultaneous determination of seven Qns in whole cow milk combined with an optimum extraction procedure based on more recent strategies such as, QuEChERS and MISPE methodologies, so an adequate optimization study was carried out.

#### 3.2.1. Optimization of the QuEChERS procedure

The QuEChERS procedure described by Agilent technologies for the determination of Qns in bovine liver [42] has been adapted in this work for milk samples. This procedure is clearly described in Section 2.4.1. In our case, the final reconstitution step consisted on 1 mL of 10 mM aqueous citric acid solution (pH 4.75 adjusted with ammoniun hydroxide). Fig. 2 shows both chromatograms of a blank and a spiked milk sample treated following the QuEChERS procedure. As can be seen, no interference peaks were found co-migrating with the analytes.

#### 3.2.2. Optimization of the MISPE procedure

A procedure previously developed in our laboratory for the MISPE of CIPRO, DANO, ENRO and SARA, was used as starting point for MISPE treatment [32]. This procedure involved two washing steps of the cartridge containing the MIP, the first using 3 mL of water and the second one using 1 mL of ACN. However, when

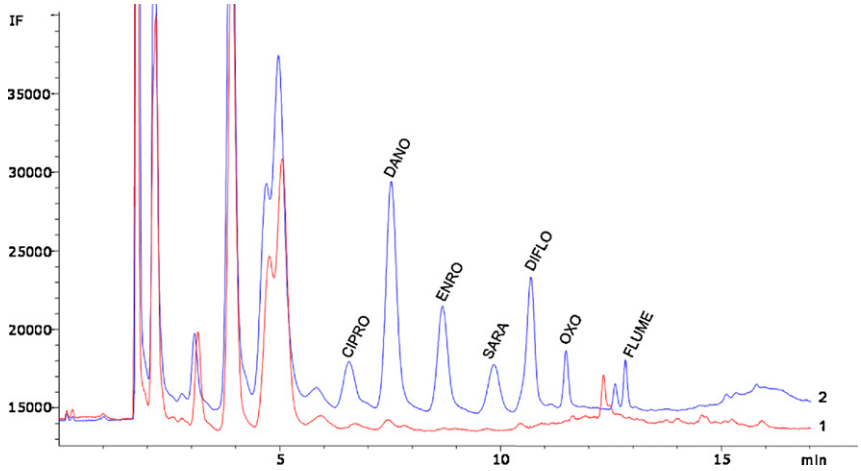


**Fig. 2.** Chromatograms of a blank (1) and a spiked (2) milk sample (25 μg/kg for DANO, OXO and FLUME and 50 μg/kg for the others Qns) extracted with QuEChERS and analysed by the proposed the capillary HPLC-LIF method (IF, fluorescence intensity).

this procedure was tested on spiked milk samples, low recoveries for OXO and FLUME were obtained, as well as some interferents co-migrating with DIFLO and OXO in the chromatogram were observed. In order to avoid both interferences and losses of analytes, different washing steps were tested, namely: (a) 3 mL of water; (b) 3 mL of water and 1 mL of ACN; and (c) 3 mL of water, 1 mL of ammonium hydroxide 0.1% in water and 1 mL of ACN. Unfortunately, none of these alternatives was useful to avoid these problems and finally only 3 mL of water was used to wash the cartridge containing MIP so as to get a compromise between a clean extract with an adequate recovery of compounds. From this fact, we conclude that MISPE procedure could be useful for the determination of CIPRO, DANO, ENRO and SARA, due to the high losses of OXO and FLUME during the treatment and the impossibility of removing interferences that overlaps with DIFLO and OXO. Fig. 3 shows the chromatograms of a blank and a spiked milk sample extracted with MISPE. The low recoveries of OXO and FLUME could be due to a weaker specific retention of both compounds to the MIP, as their structures (Fig. 1) differs from the basic structure of the others Qns more effectively retained, and this fact could produce a lower degree of interaction with the specific cavities created into the MIP in the synthesis procedure.

### 3.3. Comparison of the proposed extraction procedures

The QuEChERS and MISPE procedures were compared in terms of efficiency of the extraction (number of analytes to be analysed and absence of interferences) throughput, linear dynamic range in matrix-matches calibrations, detection and quantification limits and accuracy (trueness and precision, by means of recovery assays).

*Extraction efficiency*: As can be observed in Figs. 2 and 3, cleaner extracts were obtained when the MISPE procedure was used, but overlapping peaks with DIFLO and OXO were found, being impossible their quantification. Moreover, low recoveries were observed for OXO and FLUME. Thus, it can be concluded that QuEChERS is more efficient for the quantification of these seven Qns in milk samples.

*Throughput*: Due to the adjustment of pH, conditioning of the MIP cartridge, elution and evaporation steps, MISPE procedure is adequate for the analysis of only four compounds and required at least 3 h for the same number of samples than the QuEChERS procedure, which required less than 1 h, being possible in addition the analysis of seven compounds, thus, QuEChERS is more than three times faster than MISPE procedure.

*Linear ranges, detection and quantification limits*: Matrix-matched calibration curves for whole cow milk samples free of

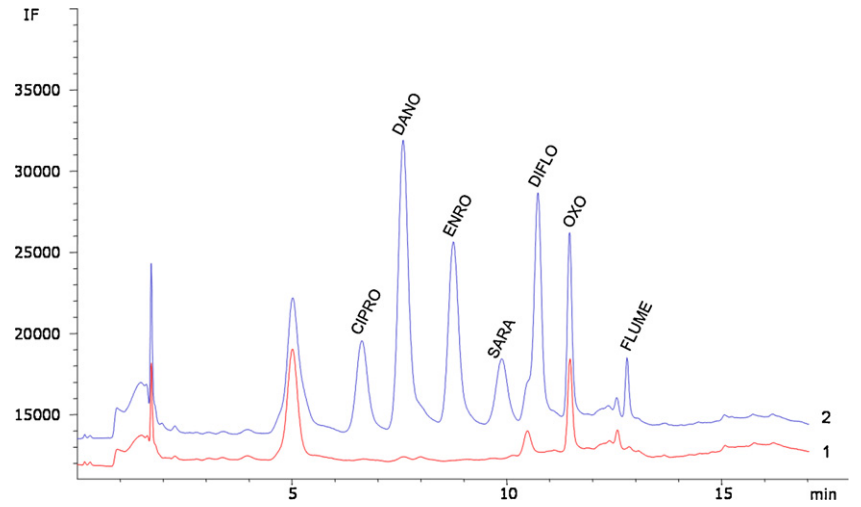


**Fig. 3.** Chromatograms of a blank (1) and a spiked (2) milk sample (25 μg/kg for DANO, OXO and FLUME and 50 μg/kg for the others Qns) extracted with MISPE and analysed by the proposed the capillary HPLC-LIF method (IF, fluorescence intensity).

**Table 1**
Matrix-matched calibration curves and performance characteristics of the capillary HPLC-LIF method using QuEChERS and MISPE sample treatments for the analysis of Qns in milk samples.

| | QuEChERS procedure | | | | MISPE procedure | | | | MRL (μg/kg) |
|---|---|---|---|---|---|---|---|---|---|
| | Linear dynamic range (μg/kg) | $R^2$ | LOD (μg/kg) | LOQ (μg/kg) | Linear dynamic range (μg/kg) | $R^2$ | LOD (μg/kg) | LOQ (μg/kg) | |
| CIPRO | 16.8–250.0 | 0.994 | 5.0 | 16.8 | 8.7–250.0 | 0.994 | 2.6 | 8.7 | 100 |
| DANO | 1.4–250.0 | 0.992 | 0.4 | 1.4 | 1.9–250.0 | 0.996 | 0.5 | 1.9 | 30 |
| ENRO | 10.0–250.0 | 0.991 | 3.0 | 10.0 | 4.6–250.0 | 0.992 | 1.4 | 4.6 | 100 |
| SARA | 20.0–250.0 | 0.992 | 6.0 | 20.0 | 11.7–250.0 | 0.990 | 3.4 | 11.7 | [b] |
| DIFLO | 6.2–250.0 | 0.991 | 1.9 | 6.2 | [a] | [a] | [a] | [a] | [c] |
| OXO | 9.0–250.0 | 0.990 | 2.7 | 9.0 | [a] | [a] | [a] | [a] | [c] |
| FLUME | 6.5–250.0 | 0.992 | 1.9 | 6.5 | [a] | [a] | [a] | [a] | 50 |

[a] Not calculated due to existence of overlapping peaks or poor recoveries.
[b] MRL not established for milk samples.
[c] Not allowed for use in animals from which milk is produced for human consumption.

analytes and spiked with different concentration levels of Qns (25, 50, 100, 150 and 250 μg/kg for CIPRO, ENRO, SARA and DIFLO and 10, 25, 50, 150 and 250 μg/kg for DANO, OXO and FLUME) were established, considering peak areas as analytical signal. Each concentration level was prepared by triplicate, submitted to the QuEChERS or MISPE procedure and analysed by the proposed capillary HPLC-LIF method. In the case of DIFLO, OXO and FLUME, the calibration curve was not established with the MISPE treatment, due to the existence of overlapping peaks or poor recoveries. Limits of detection (LODs) and limits of quantification (LOQs) were considered as 3 × S/N ratio and 10 × S/N ratio, respectively. Lower LOD and LOQ were obtained for CIPRO, ENRO and SARA when MISPE procedure was used and nearly the same results were obtained for DANO with both procedures. In all cases, the LOQs were lower enough for the quantification of the analytes below their MRLs. Table 1 summarizes the results obtained for the analytes that could be properly quantified with both sample treatments.

*Trueness:* Samples were spiked at two concentration levels: 25 μg/kg for DANO, OXO and FLUME and 50 μg/kg for the others Qns (Level 1) and 150 μg/kg of each Qns (Level 2). Three samples were prepared at each concentration level, submitted to the QuEChERS or MISPE procedure and injected by triplicate in the capillary HPLC-LIF system, following the proposed method. As can be observed in Table 2, higher recoveries were obtained with the QuEChERS extraction procedure, although slightly better relative standard deviations (RSD) were obtained with the MISPE procedure.

### 3.4. Precision study of the QuEChERS extraction procedure

From the previous study, we deduced that the QuEChERS procedure can be considered as the most suitable approach for the simultaneous extraction of the selected compounds. For such reason, in order to complete the validation of the proposed method

**Table 2**
Recovery percentages obtained for the analysed Qns in milk samples using the capillary HPLC-LIF method with QuEChERS and MISPE sample treatments ($n = 9$).

| | QuEChERS: % recovery (% RSD) | | MIPs: % recovery (% RSD) | |
|---|---|---|---|---|
| | Level 1[a] | Level 2[b] | Level 1[a] | Level 2[b] |
| CIPRO | 83.4 (5.4) | 85.5 (1.4) | 74.8 (0.8) | 77.4 (2.2) |
| DANO | 92.1 (2.7) | 98.3 (1.9) | 66.5 (0.8) | 74.5 (1.0) |
| ENRO | 97.6 (2.2) | 99.5 (3.1) | 70.4 (2.7) | 76.0 (1.4) |
| SARA | 92.6 (3.1) | 103.0 (3.2) | 72.0 (5.0) | 77.3 (2.4) |
| DIFLO | 103.9 (1.5) | 99.9 (2.6) | [c] | [c] |
| OXO | 99.8 (8.9) | 97.3 (2.7) | [c] | [c] |
| FLUME | 92.5 (6.4) | 98.2 (2.3) | <37 | <34 |

[a] Level 1: 25 μg/kg for DANO, OXO and FLUME and 50 μg/kg for the others Qns.
[b] Level 2: 150 μg/kg for each Qn.
[c] Not calculated due to the existence of overlapping peaks.

**Table 3**
Precision of the capillary HPLC-LIF method using QuEChERS sample treatment in milk samples.

| | Intraday precision, RSD (%) ($n = 15$) | | Interday precision, RSD (%) ($n = 15$) | |
|---|---|---|---|---|
| | Level 1[a] | Level 2[b] | Level 1[a] | Level 2[b] |
| CIPRO | 5.6 | 8.3 | 7.5 | 9.0 |
| DANO | 5.8 | 6.3 | 11.2 | 9.7 |
| ENRO | 6.4 | 7.6 | 10.2 | 7.5 |
| SARA | 10.5 | 8.3 | 11.2 | 11.7 |
| DIFLO | 5.4 | 6.3 | 7.0 | 6.2 |
| OXO | 8.3 | 9.1 | 7.4 | 7.9 |
| FLUME | 5.8 | 10.6 | 10.4 | 5.9 |

[a] Level 1: 25 μg/kg for DANO and 50 μg/kg for the other Qns.
[b] Level 2: 150 μg/kg for every Qn.

QuEChERS-capillary HPLC-LIF for the Qns analysis, the precision of the method has only been estimated using the QuEChERS extraction procedure. The precision of the method has been evaluated in terms of repeatability (intraday precision) and intermediate precision (interday precision). Repeatability was assessed by means of repetitive application of the whole procedure to five whole milk samples (experimental replicates) spiked at two concentration levels: 25 μg/kg for DANO, OXO and FLUME and 50 μg/kg for the rest of Qns (Level 1) and 150 μg/kg of each Qn (Level 2). Each sample was injected by triplicate (instrumental replicates) on the same day. Intermediate precision was assessed with a similar procedure, but the samples were analysed in five different days. The results, expressed as RSD of peak areas, are given in Table 3. As can be observed, very good results were obtained in all cases.

## 4. Conclusions

LIF has been proposed as a very attractive detection technique in capillary HPLC for the analysis of Qns, showing high sensitivity and selectivity. However, the simultaneous extraction of different Qns from milk presents several difficulties due to the complexity of the matrix. Relatively recent strategies, such as QuEChERS and MISPE methodologies have been compared, and although MISPE provides cleaner extracts, QuEChERS has shown to be a good alternative for the simultaneous extraction of a great number of Qns from milk, reducing extraction time, increasing sample throughput and providing good recoveries and precisions, showing the suitability of this procedure for the monitoring of Qns residues in foodstuff and being environmentally friendly. The obtained LOQs for the whole proposed method were in the very low μg/kg level and could be easily improved to ng/kg range by just decreasing the solvent volume in the reconstitution step used in the QuEChERS procedure. This method is one of the most sensitive proposed for the analysis of these compounds in comparison to conventional fluorescence,

being able to determine seven analytes simultaneously. In addition, to our knowledge, this is the first time that capillary-liquid chromatography is coupled to LIF for the determination of Qns. This study shows interesting perspectives of the application of QuEChERS for the monitoring of antibiotics in other food samples and with different analytical methodologies.

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