Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 332–341

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Hollow fiber-based liquid phase microextraction (HF-LPME) as a new approach for the HPLC determination of fluoroquinolones in biological and environmental matrices

María Ramos Payán, Miguel Ángel Bello López*, Rut Fernández-Torres, Juan Antonio Ocaña González, Manuel Callejón Mochón

Department of Analytical Chemistry, Faculty of Chemistry, University of Seville, Prof. García González, s/n, 41012 Seville, Spain

ARTICLE INFO

Article history:
Received 6 September 2010
Received in revised form 26 January 2011
Accepted 28 January 2011

Keywords:
Hollow fiber liquid phase microextraction
Fluoroquinolones
Bovine urine
Environmental water
HPLC

ABSTRACT

In this paper, a three phase hollow fiber-based liquid phase microextraction (HF-LPME) combined with a HPLC procedure using diode array (DAD) and fluorescence detection (FLD) has been developed for the determination of eight widely used fluoroquinolones: marbofloxacin (MRB), norfloxacin (NRF), ciprofloxacin (CPR), danofloxacin (DNF), enrofloxacin (ENR), gatifloxacin (GTF), grepafloxacin (GRP) and flumequine (FLM). A Q3/2 Accurel PP polypropylene hollow fiber supporting 1-octanol was used between a 2 M Na_2SO_4 aqueous solution (pH 7) as donor phase and aqueous solution (pH 12) as acceptor phase. The microextraction parameters were optimised from an experimental central composite design. The procedure allows very low detection and quantitation limits of 0.3–16 ng L^{-1} and 1–50 ng L^{-1}, respectively. The proposed method was applied to the determination of the analytes in bovine urine and in environmental water samples (surface, tap and wastewater).

1. Introduction

Fluoroquinolones (FQs) are antibacterial agents widely used due to their broad spectrum activity against both gram-positive and gram-negative bacteria through inhibition of their DNA gyrase. They have good oral absorption and are applied as both human and veterinary medicine; at sub-therapeutic levels FQs, like other antibiotics, promote animal's growth. The FQs administered to humans or animals are almost excreted as unchanged compounds in urine, and are mainly effluent from the wastewater treatment plants (WWTPs) [1]. FQs are rather resistant to microbial degradation [2–4], and these compounds might be persisting within environmental waters because of their strong sorption properties. It is well-known that bacteria exposed to antibiotics may acquire resistances [5] and surface waters can be an adequate propagation vector for resistant diseases. For these and other reasons, it is necessary to develop simple and sensitive methods for enabling the determination of these antibiotics at naturally occurring levels and on matrices of variable complexity.

The use of clean-up procedures is an old analytical tool that, in the last years, has suffered very important developments in order to resolve the analytical problems derived from the analysis of complex samples or the quantitation/detection at very low levels. Solid phase extraction (SPE) is the most used clean-up analytical procedure, however, in the last years there has been a highly interest in developing new clean-up procedures.

Liquid–liquid extraction (LLE) is a classical and common technique used for preconcentration and clean-up prior to chromatographic or electrophoretic analysis that requires large organic solvent consumption. It is also tedious and analyte-loss is frequent due to multi-stage operations that cannot be neglected. Liquid-phase microextraction (LPME), based on a droplet of water-immiscible organic solvent hanging at the end of a microsyringe needle (single drop microextraction, SDME) [6,7], is a simple, inexpensive, fast, effective and virtually solvent-free sample pretreatment technique. However, SDME is not very robust, and the droplets may be lost from the needle tip of the microsyringe during extraction.

Audunsson [8] introduced an alternative concept for LPME that was developed by Thordarson et al. [9] and for Pedersen-Bjergaard and Rasmussen [10] based on the use of single, low-cost, disposable, porous, hollow fibers made of polypropylene. In this concept, the analytes of interest are extracted from aqueous samples, through a thin layer of organic solvent (several microlitres) immobilized within the pores of a porous hollow fiber, and into an acceptor solution inside the lumen of the hollow fiber; when acceptor phase is an aqueous phase the procedure is known as three-phase HF-LPME. The disposable nature of the hollow fiber totally eliminates

* Corresponding author. Tel.: +34 954557172; fax: +34 954557168.
E-mail address: mabello@us.es (M.Á. Bello López).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.01.037

Table 1
Structure and IUPAC name of the examined fluoroquinolones.

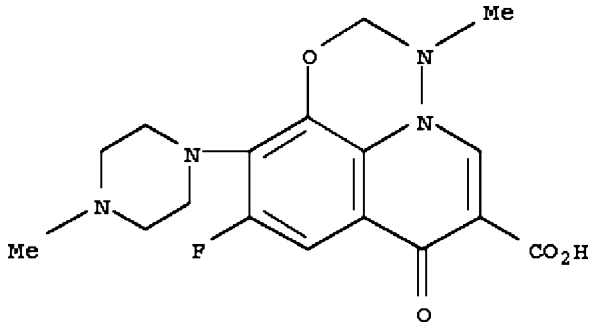
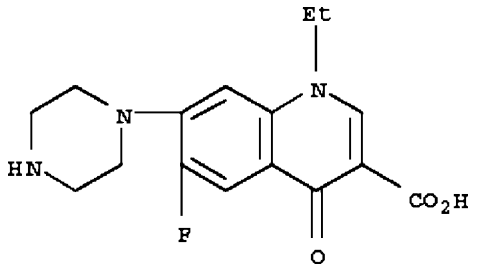
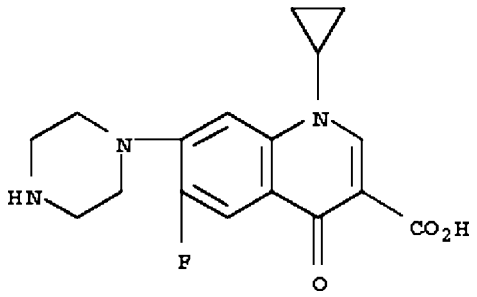
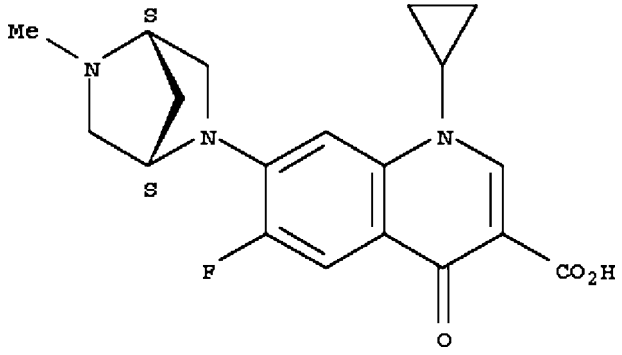
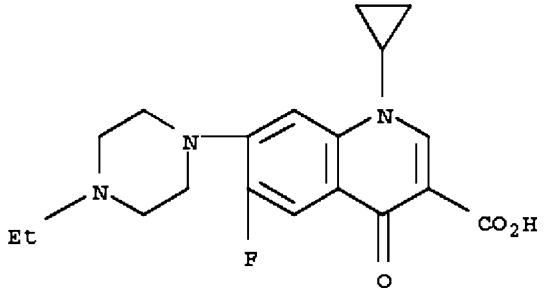
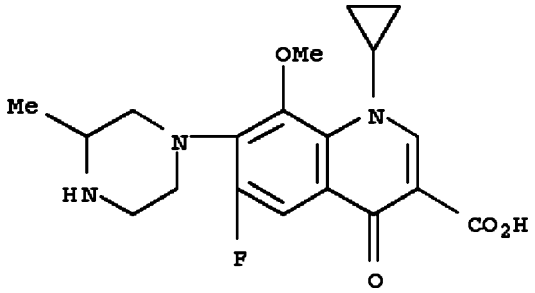
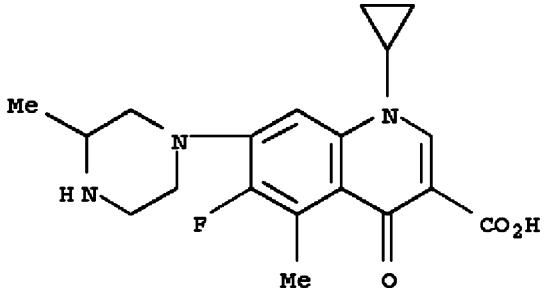
	Marbofloxacin 9-Fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[3,2,1-ij][4,1,2] benzoxadiazine-6-carboxylic acid
	Norfloxacin 1-Ethyl-6-fluoro-1,4-dihydro-7-(1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid
	Ciprofloxacin 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid
	Danofloxacin 1-Cyclopropyl-6-fluoro-1,4-dihydro-7-[(1S,4S)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl]-4-oxo-3-quinolinecarboxylic acid
	Enrofloxacin 1-Cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid

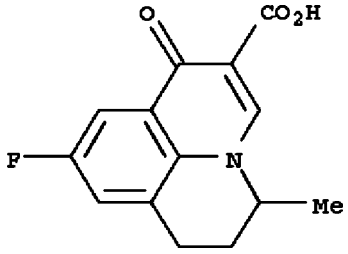
Table 1 (*Continued*)



Gatifloxacin
1-Cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid



Grepafloxacin
1-Cyclopropyl-6-fluoro-1,4-dihydro-5-methyl-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid



Flumequine
9-Fluoro-6,7-dihydro-5-methyl-1-oxo-1H,5H-benzo[ij]quinolizine-2-carboxylic acid

the possibility of sample carryover and ensures reproducibility. In addition, the small pore size prevents large molecules and particles present in the donor solution from entering the accepting phase and, at the same time, most macromolecules do not enter the hollow fiber because they are not soluble in the organic phase present in the pores, thus yielding very clean extracts [11]. Several reviews on hollow fiber-based LPME have been reported [12–15].

Analysis of FQs for the drug monitoring in body fluids samples and their determination in aqueous samples have been carried out mainly by high-performance liquid chromatography (HPLC) with UV [16–18] fluorescence [19–22], electrochemical detection [23], or HPLC coupled to mass spectrometers for detection [19,24–28]. The methods of analysis are coupled with diverse clean-up procedures like off-line SPE [22,25,28–32], on-line SPE [18,24], pressurized liquid extraction [33–35] or molecularly imprinted polymer extraction [26,27].

The aim of this work was the development of a sensitive and environmental friendly HF-LPME combined with HPLC diode array-fluorescence detection (DAD–FLD) for the determination of fluoroquinolones, that can be easily applicable to several matrices like urine or environmental waters (including wastewaters) at the naturally occurring levels. HF-LPME reduces the organic solvents consumption to several microlitres in contrast to another clean-up/preconcentration alternatives like SPE which is according to the current trends to a "Green Chemistry".

Recently a method that uses liquid phase microextraction for the determination of fluoroquinolones has been published by Poliwoda et al. [36]. Authors analysed four fluoroquinolone antibiotics: ciprofloxacin, enrofloxacin, norfloxacin and danofloxacin, in river water samples. The proposed method involves their HPLC determination with UV (270 nm) detection previous extraction using a Q3/2 Accurel PP polypropylene hollow fiber membrane supporting 20% (w/w) di-(2-ethylhexyl) phosphoric acid in di-*n*-hexyl ether as liquid membrane. Aqueous pH 6 and 0.1 M HCl were used as donor and acceptor phases, respectively. Recoveries in the 93–120% range were obtained from diluted (1:1) river water samples with detection limits between 10 and 25 ng L^{-1}.

In this work, a HPLC DAD–FLD method combined with prior HF-LPME was developed for the sensitive determination of eight widely used fluoroquinolones: marbofloxacin (MRB), norfloxacin (NRF), ciprofloxacin (CPR), danofloxacin (DNF), enrofloxacin (ENR), gatifloxacin (GTF), grepafloxacin (GRP) and flumequine (FLM) (Table 1 shows their structures and IUPAC names). The method has been successfully applied to their determination in spiked and urine samples from treated dairy cows (Jersey breed) and on several environmental water samples: wastewaters from the different treatments steps of a WWTP, and water samples from river, lake and water supply network. A previous similar article has been published with the emphasis to develop a method to determine sulfonamides in environmental waters [37].

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical-reagent grade or better. All solutions and dilutions were prepared with ultrapure water from a Milli-Q Plus water purification system (Millipore, Billerica, MA, USA). MRB, NRF, CPR, DNF, ENR, GTF, GRP, FLM, dihexyl ether and 1-octanol were purchased from Fluka–Sigma–Aldrich (Madrid, Spain) and the rest of products were obtained from Merck (Darmstadt, Germany).

Methanolic working solutions of MRB, NRF, CPR, DNF, ENR, GTF, GRP and FLM were daily prepared by adequate dilutions

Table 2
Monitoring wavelengths and retention times.

	DAD			FLD			
	λ max (nm)	t_R (min)	S.D. (min)	λexc (nm)	λem (nm)	t_R (min)	S.D. (min)
MRB	300	3.57	0.006	300	515	4.02	0.009
NRF	274	4.56	0.012	278	445	5.15	0.011
CPR	280	5.15	0.009	280	456	5.79	0.014
DNF	280	6.69	0.014	280	456	7.52	0.016
ENR	280	7.55	0.011	280	456	8.15	0.012
GTF	287	10.61	0.012	292	484	11.03	0.014
GRP	280	13.25	0.013	330	441	13.80	0.012
FLM	315	16.21	0.008	315	368	16.79	0.007

Table 3
Efficiency and selectivity chromatographic parameters for the proposed HPLC procedure (for abbreviation see text).

	$W_{1/2}$ (min)	T	N	k	α	R_s
MRB	0.1365	1.47	3789	2.16	1.41	4.04
NRF	0.1527	1.37	4940	3.04	1.17	2.11
CPR	0.1767	1.46	4705	3.56	1.38	4.72
DNF	0.2083	1.44	5714	4.92	1.15	2.34
ENR	0.2250	1.36	6237	5.68	1.48	6.86
GTF	0.3015	1.47	6860	8.39	1.28	8.25
GRP	0.0760	1.36	168,389	10.73	1.24	21.95
FLM	0.0831	1.00	210,801	13.35	1.24	21.95
Critical values		<1.5	>2000	>2	>1	>1.5

from methanolic 100 (g mL^{-1} stock solutions. Q3/2 Accurel PP polypropylene hollow fiber (600 μm i.d., 200 (m wall thickness and 0.2 (m pore size) was purchased from Membrana (Wuppertal, Germany).

2.2. Chromatographic conditions

The chromatographic separation was performed at 10 °C using a LaChrom® VWR-Hitachi (Barcelona, Spain) with a quaternary L-2130 pump. The injector was a Rheodyne manual injection valve Model 7725i, fitted with a 20-μL sample loop. Separations were carried out using an LichroCART® 75-4 Purosphere® STAR RP-18e 3 μm (75 mm × 4.0 mm i.d.) (VWR, Darmstadt, Germany) preceded by a guard column Kromasil® 100 Å, C18, 5 μm (15 mm × 4.6 mm i.d.) (Scharlab S.L., Barcelona, Spain).

The mobile phase consisted of 0.1% formic acid (pH 2.6) (component A) and acetonitrile (component B) at a flow rate of 0.8 mL min^{-1}. An initial composition 86–14% (A–B) was used in isocratic mode for 8 min and then a linear elution gradient was programmed from 86% to 20% A for another 12 min. Three minutes were waited between injections which allowed re-equilibration of the column to the initial conditions.

Table 2 shows the monitoring wavelengths for DAD and FLD detection, the retention times and the corresponding standard deviations for the analyzed compounds.

2.3. Supported liquid membrane preparation and extraction procedure

Hollow fibers were cut into 27 cm pieces, washed with acetone in an ultrasonic bath and dried. The fiber was soaked with 1-octanol during 10 s to impregnate the pores, and rinsed with water on the outside by placing it into the ultrasonic bath for 30 s in order to remove the excess of organic solvent. The lumen of the prepared fiber piece was filled with 50 μL of acceptor phase (pH 12 aqueous solution) using a HPLC syringe. Both open ends of the fiber were closed by means of a hot soldering tool and a plastic film (Parafilm®, Pechiney Plastic Packaging Company, Chicago, IL, USA). During extraction the membrane portion that contains the acceptor phase was immersed in the 50 mL sample solution (pH 7, Na_2SO_4 2 M) contained into a 50 mL glass beaker. The sample was stirred for 5.5 h by means of a magnetic stirrer ANS-00/1 Science Basic Solutions (Rubí, Barcelona, SPAIN) at 300 rpm. After extraction, the fiber was taken out, one of the ends was cut and the acceptor phase was extracted using a HPLC syringe and injected into the HPLC system.

2.4. Preparation of urine cow samples

Spiked and real urine samples from Jersey cows were directly extracted by the HF-LPME procedure after their 1:50 dilutions (1:1000 to avoid saturation in the FLD detector) in aqueous 2 M Na_2SO_4 solution and NaOH addition just to obtain pH 7. If necessary, urine samples were stored in the dark at 4 °C no more than several hours prior to HF-LPME extraction.

2.5. Preparation of environmental water samples

Wastewater samples were obtained from "Guadalquivir"-ALJARAFESA Wastewater Treatment Plant which is located in Palomares del Río, Seville, Spain. The WWTP essentially receives urban wastewaters. The capacity of this WWTP is 100,000 inhabitants and the discharged flow is 12,433,313 m^3/year (2008 data). Grab samples of the influent (raw water, WWR), after the primary sedimentation tank (WW1), after the aeration tank (WW2) and the effluent (treated water after anaerobic digestion, WWT) were collected in 11th January 2010.

Two samples from Guadalquivir River were analysed. One (RIVER1) from Coria del Río, Seville, 2 km downstream the WWTP previously mentioned and other sample (RIVER2) was taken at the mouth of Guadalquivir River (Sanlúcar de Barrameda, Cádiz) where water has a high seawater proportion. Lake water sample (LAKE) comes from "Lagos del Serrano" (Guillena, Seville). Tap water sample (TAP) was obtained directly from the laboratory tap.

All samples, except tap water, were filtered through a GDU1 glass fibre filter bed (10–1 μm) (Whatman, Mainstone, UK) and through Pall Nylaflo™ nylon membrane filter 0.45 μm (Pall Corporation, Ann Arbor, MI, USA) and adjusted to pH 2 with HCl. Filtered samples were stored in the dark at 4 °C prior to HF-LPME extraction, no more than one week.

Water samples, were directly analysed after Na_2SO_4 addition for a 2 M final concentration; NaOH was added just to obtain pH 7 prior to be submitted to the HF-LPME procedure.

3. Results and discussion

3.1. Chromatographic conditions

Looking for a fast and high resolution separation a LiChroCART® 75-4 Purosphere® STAR RP-18e (3 μm) was selected as working column. This column is a highly packed HPLC column that allows to higher resolution separations using usual flow-rates. The selected column provides good resolution and good peak symmetry.

The mobile phase consisted of 0.1% formic acid and acetonitrile. Different gradient elution conditions were tested in order to save time of analysis without sacrificing peak shape. The gradient elution program described in Section 2.2 was the best option in terms of time of analysis, shape of the peaks and reproducibility.

Fig. 1 shows representative chromatograms from aqueous standards submitted to the HF-LPME procedure.

The efficiency and selectivity chromatographic parameters of the proposed procedure are shown in Table 3, N (number of theoretical plates), T (asymmetry factor), $W_{1/2}$ (peak half-width), k (retention factor), α (selectivity factor), R_s (peak resolution). As it can be seen, all parameters are according to their critical values.

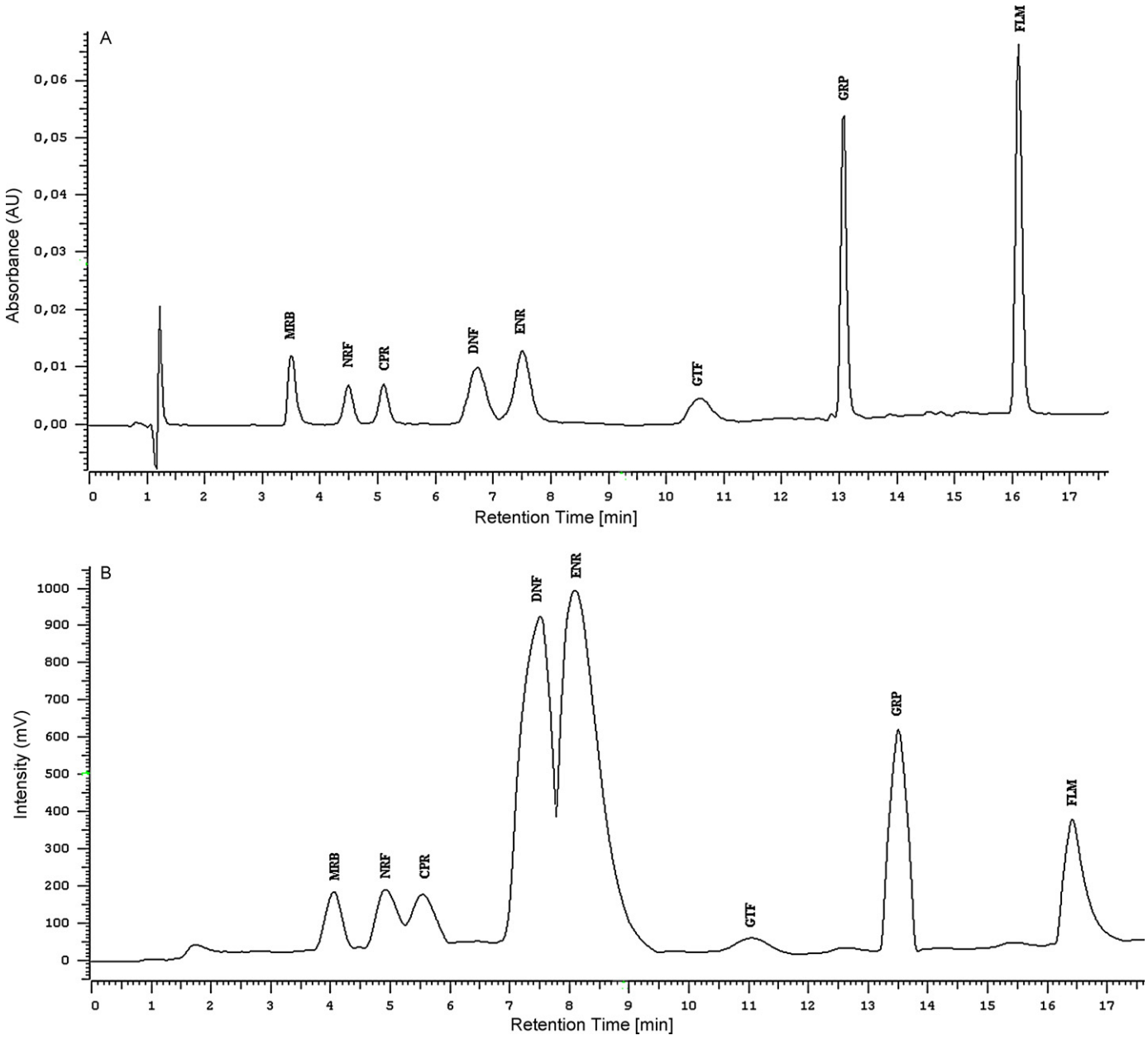


Fig. 1. DAD (a) and FLD (b) chromatograms from standard aqueous solutions (10 and 5 ng L^{-1}, respectively).

3.2. Optimization and evaluation of experimental conditions for HF-LPME extraction

First, several tests with donor phases pH 5–7 and acceptor phases pH 10–12 were carried out in order to choose the more adequate liquid supported on the polypropylene membrane; dihexyl ether and 1-octanol were checked and the best results were obtained with 1-octanol, so this was the liquid supported selected.

On the other hand, donor phases solutions containing NaCl (2–6 M) or Na_2SO_4 (0.5 M to saturation) were checked and, in general, salting out allows an increase in the extraction efficiency with the salt concentration that is more pronounced with Na_2SO_4 so aqueous saturated (approx. 2 M) Na_2SO_4 solutions were selected as optimum donor phases.

In order to optimise the experimental extraction parameters an experimental design was applied. The objective of the experimental designs is to get as much information as possible with the least number of experiences. To apply the experimental design, the fundamental objectives of the experimental planning are to identify controllable factors that significantly influence the outcome of the experiment, minimizing the effects of uncontrollable factors and secondly to optimize the objective function to get the best response.The influence of the experimental variables (called factors in experimental design), namely pH donor phase (X_1), pH acceptor phase (X_2) and time stirring (X_3) has been considered to find the best conditions for the eight compounds studied in this work. The optimization has been carried out by using a central composite design (CCD) for three factors at two levels. These designs account for the main factors and binary interactions that influence the signal, with a low number of assays.

The design matrix corresponds to three factors and twenty experiments. The design consists of three distinct sets of experimental runs: eight runs on the basis of levels +1 or −1, four runs

Table 4
Real and coded factors.

Factors	Levels				
	−1.68	−1	0	1	1.68
X_1 (pH)	5	5.8	7	8.2	9
X_2 (pH)	11	11.4	12	12.6	13
X_3 (time)	2 h	3 h	4.5 h	6 h	7 h

on the basis of levels +1.68 or −1.68 and six runs at the center of design (see supplementary electronic material).

Table 4 shows the coded levels of selected factors (−168, −1, 0, 1, 1.68), as well as their uncoded values.The computer program used on the experimental design was ECHIP ver. 6.4.1 (Velocity Pointe, Wilmington, DE, USA). The result is contour maps and response surfaces for each of the compounds, and the optimal combination for all of them in both cases. The figures of contour maps and surface response for each of the compounds, the contour map for the optimum combination for all the compounds and the three-factor response surface for all compounds are included as supplementary electronic material.

After a scrutiny of the optimal conditions, it was considered a slight modification of the optimal level of factor X_3 (stirring time) in order to favor the extraction for those less sensitive compounds (analytical optimal conditions). So, the optimal conditions used were those described in Section 2.3.

3.3. Linearity, sensitivity, precision and robustness for the HF-LPME extraction

Linearity of the response function was studied from external calibration. A 10-point (in triplicate) calibration curve, was constructed using a least-square linear regression analysis of standards mixtures of the analytes at different concentrations. Using the selected HF-LPME conditions, aqueous pH 7 solutions with different analytes concentrations were submitted to the liquid microextraction procedure and analysed according to the described HPLC procedure. Peak areas were proportional to concentrations in the donor phase. A linear relationship was obtained with correlation coefficients $r \geq 0.999$ and the calibration curves obtained showed no changes over the course of one month. Detection and quantitation limits were calculated as the minimum concentration of an analyte giving peaks whose signal-to-noise ratio is 3 and 10, respectively. Enrichment factor was calculated as the increase in the concentration between the aqueous external donor phase and the internal (acceptor) phase. Table 5 shows the corresponding data and, as can be seen, the high enrichment values obtained for all the analytes allow the detection and determination of very lows levels. In fact, detection and quantitation limits were of the same, even better, magnitude order that other methods that use mass spectrometry detection [19,21,22,24,26,35].

Table 5
Detection limits, linear ranges, % linearity and enrichment factors for the HPLC method (DAD and FLD detection) combined with prior HF-LPME.

	DAD			FLD			Enrichment
	LOD[a]	Linear range[a]	R^2[b]	LOD[a]	Linear range[a]	R^2[b]	
MRB	16	50–10^6	0.9997	16	50–10^5	0.9996	95
NRF	20	60–10^6	0.9998	5	15–10^5	0.9997	60
CPR	10	30–10^6	0.9996	3	8–10^5	0.9996	50
DNF	7	20–10^6	0.9997	0.7	2–10^3	0.9996	200
ENR	7	20–10^6	0.9997	1.3	4–10^3	0.9997	200
GTF	20	60–10^6	0.9996	13	40–10^5	0.9996	100
GRP	13	40–10^6	0.9997	1.7	5–10^4	0.9997	600
FLM	7	20–10^6	0.9997	0.3	1–10^3	0.9997	900

[a] ng L^{-1}.
[b] Correlation coefficient.

Table 6
Recoveries (%) using the proposed HF-LPME/HPLC method from spiked bovine urine samples (average of three determinations ± standard deviation).

	Urine spiked level (mg L^{-1})		
	0.025	0.5	5
MRB	95.7 ± 1.0	96.4 ± 0.9	100.2 ± 1.0
NRF	99.6 ± 0.9	100.2 ± 1.0	99.9 ± 1.0
CPR	99.8 ± 1.1	100.5 ± 1.4	99.7 ± 1.5
DNF	99.7 ± 0.5	99.9 ± 1.9	99.9 ± 0.9
ENR	99.0 ± 0.6	99.9 ± 1.0	99.9 ± 1.4
GTF	99.4 ± 2.1	99.7 ± 0.6	99.9 ± 0.9
GRP	98.1 ± 1.1	99.3 ± 0.9	99.3 ± 0.6
FLM	76.0 ± 1.9	76.2 ± 1.5	79.0 ± 2.4

To evaluate the repeatability and the intermediate precision, aqueous samples at three concentrations levels 10^2, 10^3 and 10^5 ng L^{-1} (in triplicate) were subjected to the entire analytical procedure and measured in one single day and one day per week during two months, respectively. Intermediate precision was performed using the prediction of actual concentrations from the validation standards selected for the analytical assay in the $m \times p \times n$ design (m = analytical levels, p = days and n = replications). From the corresponding ANOVA, the intermediate precision was computed [38]. The repeatability, expressed as relative standard deviation, was in the range 0.6–1.2%. Intermediate precision also expressed as relative standard deviation, was in the range 1.0–1.8%.

The robustness study is based on a landmark procedure suggested by Youden [39]. A design matrix (two factors in eight experiments) was used (see supplementary electronic material). The levels +1 or −1 correspond to high and low pH values, 7.5 and 6.5 for donor phase and 12.5 and 11.5 for the acceptor phase. Stirring time is not considered as a variable for robustness study due to its high optimum value (5.5 h) and the fact that variations in the order of minutes do not have significant effects in the extraction efficiency.

The effect of a given factor, say x_i can be estimated as the difference of result averages at levels +1 and −1:

$$D(x_i) = \frac{1}{4}\left[\sum R_{(x_i=+1)} - \sum R_{(x_i=-1)}\right]$$

when R is the corresponding experimental result obtained.

A significance t-test is used [40] to determine whether variations have a significant effect on the result,

$$t(x_i) = \frac{\sqrt{2}|D(x_i)|}{S_{IP}}$$

where S_{IP} is the standard deviation of the intermediate precision, evaluated in the precision study. The $t(x_i)$ values were compared with the corresponding critical t values (n = 4) at 5% significance level and three degrees of freedom. Results obtained (see supplementary electronic material) that t values calculated for each factor are lower than the tabulated one (3.18), so the procedure can be considered robust against the considered factors for all the analysed compounds.

3.4. Cow urine analysis

3.4.1. Recovery assays on spiked urine

A urine pool from two cows was spiked with the studied fluoroquinolones at three concentration levels (0.025, 0.5 and 5 (g mL^{-1}) and submitted to the HF-LPME procedure described in Section 2. Representative DAD and FLD chromatograms can be observed in Fig. 2 including the corresponding blank chromatograms. As it can be seen, the peaks have good resolution and good baselines were

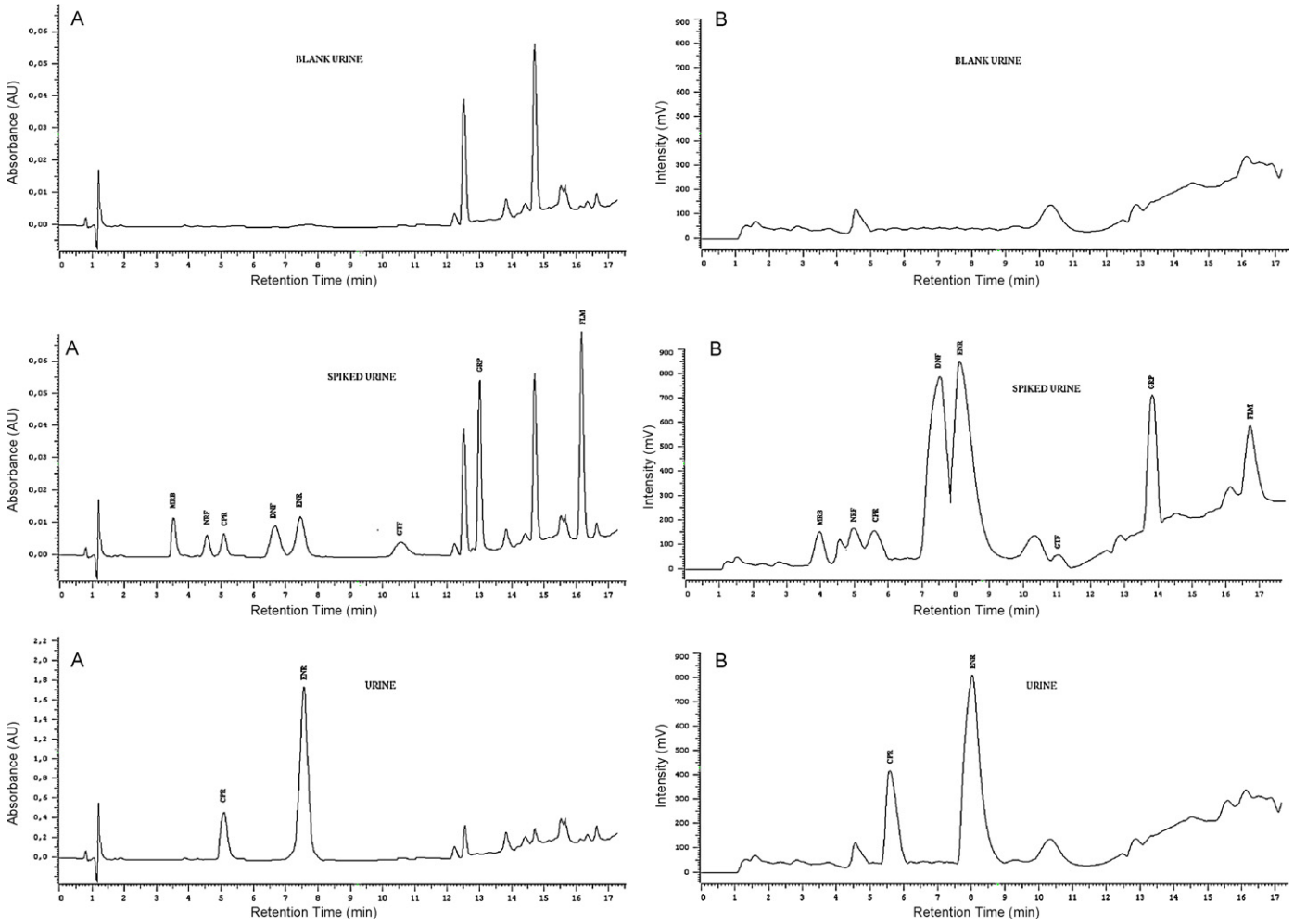


Fig. 2. DAD (a) and FLD (b) chromatograms from blank and spiked (0.5 μg L^{-1}) cow urine samples and from one urine sample obtained after the administration of enrofloxacin submitted to the proposed HF-LPME procedure (for details see text).

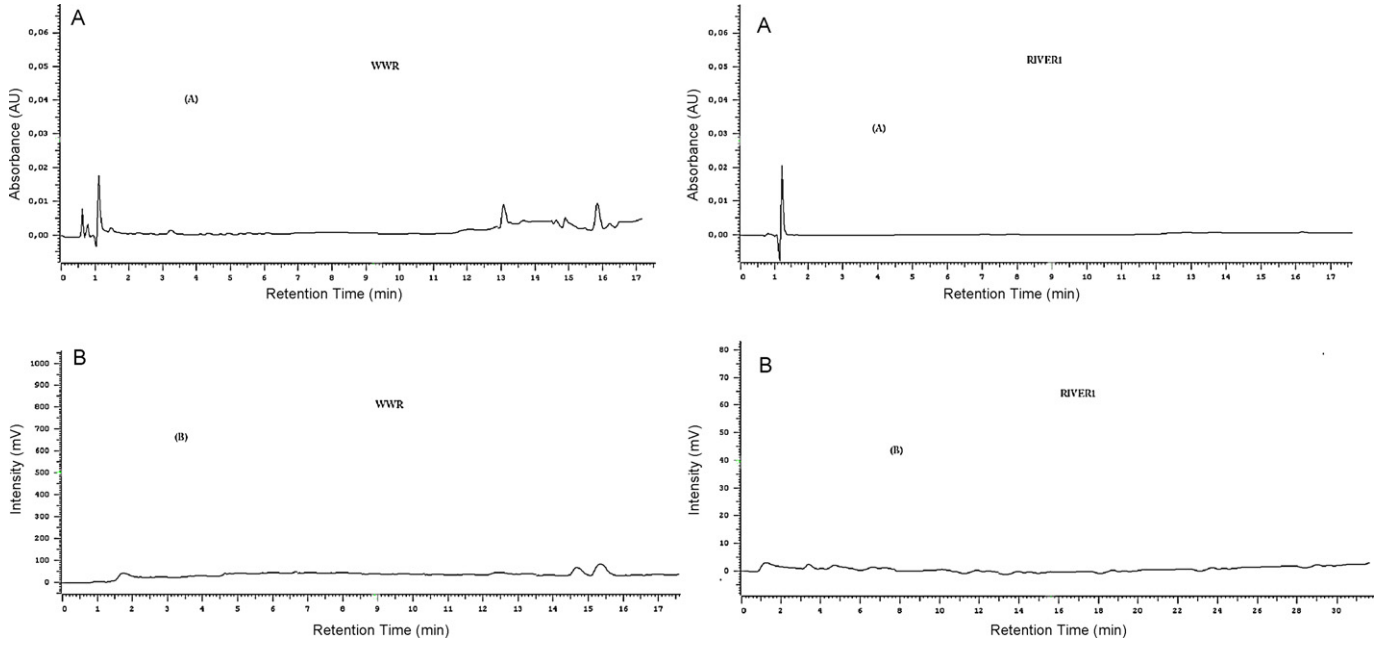


Fig. 3. DAD (a) and FLD (b) chromatograms of blank wastewater (WWR) and river (RIVER1) samples submitted to the proposed HF-LPME procedure.

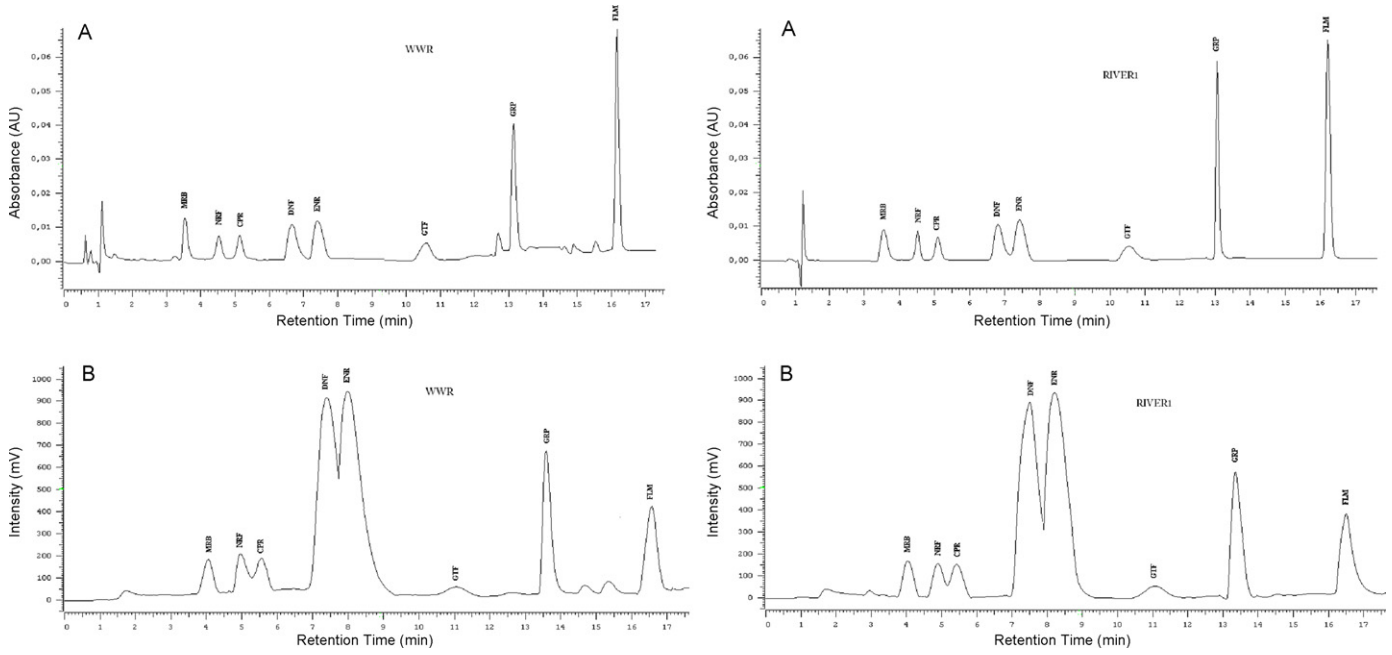


Fig. 4. DAD (a) and FLD (b) chromatograms from spiked (500 ng L^{-1}) wastewater (WWR) and river (RIVER1) samples submitted to the proposed HF-LPME procedure.

obtained; however some peaks appear in the blank samples that not interfere with the corresponding to the analysed fluoroquinolones. Table 6 shows the corresponding recoveries obtained and, as it can be seen, the values obtained were within the range 96–100% for practically all the analysed compounds, only FLQ shows recoveries between 76 and 79%. The data obtained demonstrates that the proposed HF-LPME procedure could be adequate for cow urine samples analysis.

Table 7
Recoveries (%) using the proposed HF-LPME/HPLC method from water spiked samples (average of three determinations ± standard deviation).

Spiked level[a]	Water sample[b]							
	WWR	WW1	WW2	WWT	RIVER1	RIVER2	LAKE	TAP
MRB								
150	86.7 ± 0.5	87.3 ± 1.0	87.1 ± 0.8	88.2 ± 1.1	99.1 ± 0.5	99.6 ± 4.0	100.0 ± 0.7	100.0 ± 1.0
500	90.0 ± 1.0	91.3 ± 0.8	91.1 ± 0.7	92.3 ± 0.7	99.4 ± 0.6	99.8 ± 1.0	100.1 ± 0.5	99.9 ± 0.3
5000	91.2 ± 0.5	91.5 ± 0.7	91.9 ± 0.8	92.7 ± 0.8	100.1 ± 0.5	99.9 ± 1.2	99.9 ± 0.6	100.0 ± 0.5
NRF								
150	96.9 ± 1.5	97.2 ± 0.5	97.9 ± 1.2	98.5 ± 1.1	99.8 ± 0.8	99.6 ± 0.5	99.9 ± 1.0	100.1 ± 0.6
500	99.0 ± 1.6	98.9 ± 1.0	99.2 ± 0.7	100.1 ± 1.0	99.8 ± 0.8	99.9 ± 0.5	100.0 ± 1.3	100.0 ± 0.8
5000	99.1 ± 0.5	99.2 ± 0.6	99.1 ± 0.6	99.5 ± 0.7	99.9 ± 1.1	99.8 ± 0.6	100.0 ± 2.0	100.1 ± 0.5
CPR								
150	96.4 ± 1.0	96.5 ± 0.7	97.2 ± 1.0	98.3 ± 0.7	99.0 ± 0.7	98.9 ± 1.6	100.2 ± 1.2	99.9 ± 0.7
500	99.4 ± 1.1	99.5 ± 1.1	99.6 ± 0.7	99.8 ± 0.7	99.0 ± 0.8	99.2 ± 1.2	99.6 ± 0.7	100.1 ± 1.1
5000	99.5 ± 0.9	99.4 ± 0.6	99.7 ± 0.6	99.9 ± 0.6	100.0 ± 0.7	99.2 ± 1.1	99.9 ± 0.7	100.1 ± 0.5
DNF								
150	98.8 ± 0.6	98.9 ± 1.2	98.9 ± 0.7	99.2 ± 1.3	99.3 ± 0.8	99.6 ± 1.0	99.9 ± 0.8	99.9 ± 0.3
500	99.9 ± 0.6	99.9 ± 1.1	99.8 ± 0.5	100.0 ± 0.6	99.4 ± 0.7	99.3 ± 0.7	99.9 ± 1.5	100.0 ± 0.5
5000	100.0 ± 0.5	99.9 ± 0.5	99.9 ± 0.6	99.9 ± 1.2	100.0 ± 0.7	99.8 ± 0.5	99.9 ± 0.7	100.0 ± 0.7
ENR								
150	96.7 ± 0.6	96.4 ± 1.1	97.6 ± 0.6	97.6 ± 1.2	99.4 ± 1.3	99.7 ± 0.6	100.0 ± 1.1	99.9 ± 1.0
500	98.6 ± 0.7	99.6 ± 0.5	99.8 ± 0.7	99.9 ± 0.8	99.4 ± 0.6	100.0 ± 0.5	99.9 ± 1.2	100.1 ± 0.9
5000	98.9 ± 0.5	99.90 ± 0.6	99.1 ± 0.6	99.5 ± 0.7	99.8 ± 0.7	100.1 ± 0.6	99.95 ± 1.0	99.9 ± 0.6
GTF								
150	98.7 ± 0.7	98.9 ± 0.6	98.9 ± 0.5	99.2 ± 1.3	99.8 ± 0.8	99.8 ± 0.6	99.9 ± 0.7	100.0 ± 0.8
500	99.1 ± 0.7	99.1 ± 1.1	99.5 ± 0.6	99.8 ± 0.6	95.8 ± 0.3	96.6 ± 0.8	99.8 ± 1.2	100.0 ± 0.7
5000	99.3 ± 0.7	99.9 ± 0.5	99.6 ± 1.0	99.8 ± 0.5	99.8 ± 0.5	98.9 ± 0.3	100.1 ± 0.7	99.9 ± 0.7
GRP								
150	98.6 ± 0.6	98.8 ± 1.0	99.0 ± 1.2	99.6 ± 0.6	99.4 ± 0.7	100.0 ± 0.7	100.1 ± 1.3	99.9 ± 0.8
500	99.1 ± 1.0	99.8 ± 0.5	99.6 ± 1.0	99.9 ± 0.7	99.3 ± 0.6	99.0 ± 0.9	99.9 ± 1.0	100.1 ± 0.5
5000	99.3 ± 0.5	99.8 ± 0.5	99.7 ± 0.6	99.9 ± 0.5	99.9 ± 0.6	99.8 ± 0.9	99.9 ± 1.0	100.1 ± 0.6
FLM								
150	91.6 ± 0.7	92.2 ± 0.6	92.6 ± 1.2	95.3 ± 0.8	99.6 ± 0.7	99.8 ± 1.0	99.8 ± 1.1	99.9 ± 0.6
500	93.0 ± 0.3	92.8 ± 0.7	93.9 ± 0.6	95.2 ± 0.8	99.7 ± 0.6	99.7 ± 0.7	99.9 ± 0.8	100.0 ± 0.6
5000	93.5 ± 0.5	93.6 ± 0.5	94.2 ± 1.0	96.9 ± 0.7	100.0 ± 0.5	99.8 ± 1.5	99.9 ± 0.5	100.0 ± 1.1

[a] ng L^{-1}.
[b] Average recovery (%) ± standard deviation ($n = 3$).

3.4.2. Analysis of urine samples from cows under veterinary treatment

A urine sample from a Jersey cow of approximately 800 kg weight submitted to an enrofloxacin treatment with injectable ALSIR® 5% (Laboratorios Esteve Veterinaria, Spain) at 1 mL/20 kg day doses during five days was collected and submitted to the HF-LPME procedure described in Section 2. Fig. 2 shows the corresponding DAD and FLD chromatograms. Urine contents of 9.4 ± 0.01 and 17.6 ± 0.02 ($g\,mL^{-1}$ for CPR and ENR were measured, respectively. The presence of ciprofloxacin in the urine sample is due to it is the enrofloxacin metabolite.

3.5. Environmental water samples analysis

The different water samples were selected taking into account the maximum variability with respect to provenance and matrix composition. First, the different water samples were submitted to the HF-LPME proposed procedure and analysed. None of the fluoroquinolones were detected in the water analysed samples.

In order to check the suitability of the proposed procedure spiked samples at three concentration levels: 150, 500 and 5000 $ng\,L^{-1}$, were analysed. Results obtained are shown in Table 7. As can be seen in all cases excellent recoveries were obtained with values in the 97–100% range, only MRB and FLM show recoveries of 87–92% and 91–96% for the urban wastewater samples that are traditionally complex samples from the analytical point of view.

Figs. 3 and 4 show representative DAD and FLD chromatograms obtained from blank and spiked (500 $ng\,L^{-1}$) wastewater (WWR) and river (RIVER1) samples. These samples have been selected as the more complex wastewater (raw wastewater) and surface water. As it can be seen, RIVER1 blank chromatograms show excellent baselines. Spiked WWR and RIVER1 chromatograms only show well defined peaks corresponding to the added substances.

In general, excellent recoveries were obtained with values of practically 100%, only MRB and FLM show slightly lower values for some of the analysed samples, but in any case in the 87–97% range. These recoveries were at least of the same, but frequently better, magnitude order that other previously published methods [16,17,19–21,24,26,30,32]. The excellent recoveries, preconcentrations and clean-up obtained imply a great advantage over other sample treatment procedures which justifies the proposed HF-LPME extraction for its use in environmental water analyses.

4. Conclusions

This study presents a hollow fiber-based liquid phase microextraction (HF-LPME) method combined with an HPLC (DAD–FLD) determination using a highly packed chromatographic column that allows a simple, low-cost, accurate, high sensitive and selective methodology for the determination of eight widely used fluoroquinolones. The proposed extraction procedure has a very low (few μL) organic solvent consumption. The excellent preconcentration and clean-up obtained implies a great advantage over other sample treatment procedures. Additionally, the use of two detectors brings additional selectivity for the method, which is important for the analyses of environmental samples.

Despite the long extraction time used, it is a procedure with little handling and completely unassisted unlike other extraction procedures. Additionally, the cost by sample analysed is practically null compared with other existing extraction alternatives like SPE, mixed-phase cation exchange (MPC) [19,20], magnetic molecularly imprinted polymer (MMIP) [26] or pressurized liquid extraction (PLE) [33–35].

The proposed procedure has been demonstrated adequate for the determination of the analytes in cow urine and environmental water samples, including urban wastewaters that usually require tedious clean-up and preconcentration steps, obtaining, in general, recoveries around 100% for all the analysed compounds.

Acknowledgments

This work was supported by the Project CTM2009-12858-C02-01 from the "Dirección General de Investigación y Gestión del Plan Nacional de I+D+i (Ministerio de Educación y Ciencia, SPAIN)".

MRP is grateful to University of Seville for personal funding through the "Fundación Cámara" program.

We are grateful to Inmaculada Rodríguez Maqueda from ALJARAFESA for kindly supplying the analysed wastewater samples from "Guadalquivir" Wastewater Treatment Plant located in Palomares del Río, Seville, Spain.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.01.037

References

[1] C.G. Daughton, T.A. Ternes, Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107 (1999) 907.
[2] A. Al.Almad, F.D. Daschner, K. Kummerer, Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of wastewater bacteria, Arch. Environ. Contam. Toxicol. 37 (1999) 158.
[3] F. Ingerslev, B.H. Sorensen, Biodegradability properties of sulfonamides in activated sludge, Environ. Toxicol. Chem. 19 (2000) 2467–2473.
[4] K. Kummerer, A. Al.Almad, V. Mersch-Sundermann, Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test, Chemosphere 40 (2000) 701–710.
[5] K. Pedersen, A. Wedderkopp, Resistance to quinolones in *Campylobacter jejuni* and *Campylobacter coli* from Danish broilers at farm level, J. Appl. Microbiol. 94 (2003) 111–119.
[6] M.A. Jeannot, F. Cantwell, Solvent microextraction into a single drop, Anal. Chem. 68 (1996) 2236–2240.
[7] H.G. Liu, P.K. Dasgupta, Analytical chemistry in a drop solvent extraction in a microdrop, Anal. Chem. 68 (1996) 1817–1821.
[8] G. Audunsson, Aqueous/aqueous extraction by means of a liquid membrane for sample cleanup and preconcentration of amines in a flow system, Anal. Chem. 58 (1986) 2714–2723.
[9] E. Thordarson, S. Pálmarsdóttir, L. Mathiasson, A. Jönsson, Sample preparation using a miniaturized supported liquid membrane device connected on-line to packed capillary liquid chromatography, Anal. Chem. 68 (1996) 2559–2563.
[10] S. Pedersen-Bjergaard, K.E. Rasmussen, Liquid–liquid–liquid microextraction for sample preparation of biological fluids prior to capillary electrophoresis, Anal. Chem. 71 (1999) 2650–2656.
[11] M. Ramos-Payán, M.A. Bello, R. Fernández-Torres, J.L. Pérez-Bernal, M. Callejón, HPLC determination of ibuprofen, diclofenac and salicylic acid using hollow fiber-based liquid phase microextraction (HF-LPME), Anal. Chim. Acta 653 (2009) 184–190.
[12] E. Psillakis, N. Kalogerakis, Developments in liquid-phase microextraction, Trends Anal. Chem. 22 (2003) 565–574.
[13] K.E. Rasmussen, S. Pedersen-Bjergaard, Developments in hollow fibre-based, liquid-phase microextraction, Trends Anal. Chem. 23 (2004) 1–10.
[14] S. Pedersen-Bjergaard, K.E. Rasmussen, Bioanalysis of drugs by liquid-phase microextraction coupled to separation techniques, J. Chromatogr. B 817 (2005) 3–12.
[15] S. Pedersen-Bjergaard, K.E. Rasmussen, Liquid-phase microextraction with porous hollow fibers, a miniaturized and highly flexible format for liquid–liquid extraction, J. Chromatogr. A 1184 (2008) 132–142.
[16] M. Ferdig, A. Kaleta, T.D. Vo, W. Buchberger, Improved capillary electrophoretic separation of nine (fluoro)quinolones with fluorescence detection for biological and environmental samples, J. Chromatogr. A 1047 (2004) 305–311.
[17] E. Turiel, G. Bordin, A.R. Rodríguez, Trace enrichment of (fluoro)quinolone antibiotics in surface waters by solid-phase extraction and their determination by liquid chromatography–ultraviolet detection, J. Chromatogr. A 1008 (2003) 145–155.
[18] H. Jing-Fang, L. Bo, Y. Qiong-Wei, F. Yu-Qi, Determination of fluoroquinolones in eggs using in-tube solid-phase microextraction coupled to high-performance liquid chromatography, Anal. Bioanal. Chem. 384 (2006) 1228–1235.
[19] H. Nakata, K. Kannan, P.D. Jones, J.P. Giesy, Determination of fluoroquinolone antibiotics in wastewater effluents by liquid chromatography–mass spectrometry and fluorescence detection, Chemosphere 58 (2005) 759–766.
[20] E.M. Golet, A.C. Alder, A. Hartmann, T.A. Ternes, W. Giger, Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase

extraction and liquid chromatography with fluorescence detection, Anal. Chem. 73 (2001) 3632–3638.
[21] A. Pena, J. Pina, L.J.G. Silva, L. Meisel, M. Lino, Fluoroquinolone antibiotics determination in piggeries environmental waters, J. Environ. Monit. 12 (2010) 642–646.
[22] A.V. Herrera-Herrera, J. Hernandez-Borges, M.A. Rodriguez-Delgado, Fluoroquinolone antibiotic determination in bovine, ovine and caprine milk using solid-phase extraction and high-performance liquid chromatography-fluorescence detection with ionic liquids as mobile phase additives, J. Chromatogr. A 1216 (2009) 7281–7287.
[23] M.I. Rodriguez, A. Guiberteau, T. Galeano, M.A. Martinez-Canas, Simultaneous determination of quinolones for veterinary use by high-performance liquid chromatography with electrochemical detection, J. Chromatogr. B 878 (2010) 398–402.
[24] K. Mitami, H. Kataoka, Determination of fluoroquinolones in environmental waters by in-tube solid-phase microextraction coupled with liquid chromatography–tandem mass spectrometry, Anal. Chim. Acta 562 (2006) 16–22.
[25] M. Li-Li, G. Chang-Sheng, H. Wei, S. Jian, Z. Xing-Wang, R. Yue-Fei, W. Yu-Qiu, Simultaneous extraction and determination of eighteen fluoroquinolone, tetracycline and sulfonamide antibiotics from soils using solid-phase extraction and liquid chromatography–tandem mass spectrometry, Fenxi Huaxue 38 (2010) 21–26.
[26] C. Ligang, Z. Xiaopan, X. Yang, D. Xiaobo, S. Xin, S. Lei, W. Hui, Z. Qi, Y. Aimin, Z. Hanqi, Determination of fluoroquinolone antibiotics in environmental water samples based on magnetic molecularly imprinted polymer extraction followed by liquid chromatography–tandem mass spectrometry, Anal. Chim. Acta 662 (2010) 31–38.
[27] S. Hanwen, L. Guangyu, Q. Fengxia, Determination of fluoroquinolone antibiotics in environmental water samples based on magnetic molecularly imprinted polymer extraction followed by liquid chromatography–tandem mass spectrometry, Shipin Kexue (Beijing, China) 30 (2009) 151–154.
[28] S. Jianzhong, G. Liming, X. Fei, R. Qinxiong, X. Xi, L. Xiaowei, D. Shuangyang, Simultaneous determination of fluoroquinolones tetracyclines and sulfonamides in chicken muscle by UPLC–MS–MS, Chromatographia 71 (2010) 383–388.
[29] Q. Tang, T. Yang, X. Tan, J. Luo, Simultaneous determination of fluoroquinolone antibiotic residues in milk sample by solid-phase extraction–liquid chromatography–tandem mass spectrometry, J. Agric. Food Chem. 57 (2009) 4535–4539.
[30] M. Lombardo-Aguei, L. Gamiz-Gracia, A.M. Garcia-Campana, C. Cruces-Blanco, Sensitive determination of fluoroquinolone residues in waters by capillary electrophoresis with laser-induced fluorescence detection, Anal. Bioanal. Chem. 396 (2010) 1551–1557.
[31] S. Wang, H. Mu, Y. Bai, Y. Zhang, H. Liu, Multiresidue determination of fluoroquinolones, organophosphorus and *N*-methyl carbamates simultaneously in porcine tissue using MSPD and HPLC–DAD, J. Chromatogr. B 877 (2009) 2961–2966.
[32] M. Sturini, A. Speltini, L. Pretali, E. Fasani, A. Profumo, Solid-phase extraction and HPLC determination of fluoroquinolones in surface waters, J. Sep. Sci. 32 (2009) 3020–3028.
[33] E. Rodriguez, F. Villoslada, M.C. Moreno-Bondi, M.D. Marazuela, Optimization of a pressurized liquid extraction method by experimental design methodologies for the determination of fluoroquinolone residues in infant foods by liquid chromatography, J. Chromatogr. A 1217 (2010) 605–613.
[34] M. Lillenberg, S. Yurchenko, K. Kipper, K. Herodes, V. Pihl, K. Sepp, R. Lohmus, L. Nei, Simultaneous determination of fluoroquinolones, sulfonamides and tetracyclines in sewage sludge by pressurized liquid extraction and liquid chromatography electrospray ionization–mass spectrometry, J. Chromatogr. A 1216 (2009) 5949–5954.
[35] V. Carretero, C. Blasco, Y. Pico, Multi-class determination of antimicrobials in meat by pressurized liquid extraction and liquid chromatography–tandem mass spectrometry, J. Chromatogr. A 1209 (2008) 162–173.
[36] A. Poliwoda, M. Krzyzak, P.P. Wieczrorek, Supported liquid membrane extraction with single hollow fiber for the analysis of fluoroquinolones from environmental surface water samples, J. Chromatogr. A 1217 (2010) 3590–3597.
[37] M. Ramos, M.A. Bello, R. Fernández-Torres, M. Villar, M. Callejón, Hollow fiber-based liquid phase microextraction (HF-LPME) for a highly sensitive HPLC determination of sulfonamides and their main metabolites, J. Chromatogr. B 879 (2011) 197–204.
[38] A.G. Gonzalez, M.A. Herrador, A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles, Trends Anal. Chem. 26 (2007) 227–238.
[39] W.Y. Youden, Statistical Techniques for Collaborative Tests, AOAC, Washington, DC, 1967.
[40] Y. Vander Heyden, K. Luypaert, C. Hartmann, D.L. Massart, J. Hoogmartens, J. De Beer, Ruggedness tests on the high-performance liquid chromatography assay of the United States Pharmacopeia XXII for tetracycline hydrochloride A comparison of experimental designs and statistical interpretations, Anal. Chim. Acta 312 (1995) 245–262.