Screening of fluoroquinolones in environmental waters using disk-based solid-phase extraction combined to microplate fluorimetric determination and LC-MS/MS

Patrícia S. Peixoto[a], Ildikó V. Tóth[a], Luisa Barreiros[a,b], Ana Machado[c], Adriano A. Bordalo[c], José L. F. C. Lima[a] and Marcela A. Segundo[a]

[a]LAQV, REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; [b]Núcleo de Investigação e Intervenção em Farmácia (NIIF), Centro de Investigação em Saúde e Ambiente (CISA), Escola Superior de Saúde, Instituto Politécnico do Porto, Porto, Portugal; [c]Department of Population Studies, ICBAS, Institute of Biomedical Sciences, University of Porto, Porto, Portugal and CIIMAR

ABSTRACT

Fluoroquinolones are in the order of the day concerning environmental contamination through anthropogenic activities, resulting in increased risk for antibiotic resistance dissemination. In this context, accessible, low-cost analytical methods are required for implementation of comprehensive surveillance and screening schemes. In this work, we propose a down-scaled disk-based solid-phase extraction system from which the eluate can be first screened by miniaturized fluorimetric reading, followed by individual determination of target fluoroquinolones (ciprofloxacin, norfloxacin, and enrofloxacin) by liquid chromatography combined to tandem mass spectrometry. The fluorimetric measurement is based on the intrinsic fluorescence of fluoroquinolones. Disk-based retention was performed after sample acidification (pH 4.0) by mixed-mode cation exchange using polystyrene divinylbenzene sulphonated sorbent. Sample loading was precisely controlled in a dedicated flow system operating at 4.0 mL min^{-1}. Different eluent compositions were tested, with elution performed by 1.00 mL of methanol-ammonium hydroxide (98:2, v/v), with subsequent reading of eluate in both detectors. Quantification was attained for 2–25 µg L^{-1} range, with LOD values at 1 µg L^{-1}. The proposed approach was successfully applied to estuarine waters from the Douro River, with comparable results to a conventional SPE-LC-MS/MS procedure.

KEYWORDS

Disk-based solid-phase extraction; fluoroquinolones; environmental waters; microplate fluorimetry; tandem mass spectrometry

1. Introduction

Antimicrobial compounds are widely used in human and veterinary medicine, including livestock and aquaculture [1]. However, concern about their harmful impact on balance of natural ecosystems and antimicrobial resistance has been increased nowadays. Antibiotic resistance is rising to alarming levels and spreading worldwide, threatening the capacity to treat infectious diseases. Fluoroquinolones (FQs) are bactericidal

compounds able to suppress bacterial replication by inhibition of topoisomerases II and IV. FQs are frequently determined in the environment. Norfloxacin (NOR), enrofloxacin (ENR) and ciprofloxacin (CIP) are amongst the most usually found FQs in environmental waters at ng to µg per litre levels [1–4]. In fact, the analysis of fluoroquinolone antibiotics in environmental matrices confirmed their widespread use, especially of norfloxacin and ciprofloxacin and their inefficient removal in WWTPs [5,6]. Along with norfloxacin, enrofloxacin is frequently used in animal intensive production, despite recent recommendations from the European Medicines Agency [7]. This occurrence is related to their high levels of use and slow degradation mechanisms. For instance, the persistence of FQs in sludge-treated soils delays their biodegradation [8,9]. Furthermore, photolysis, as the main degradation pathway of FQs in water, is a slow mechanism [10], originating toxic products that act in synergism with parent compounds [4,11].

Recently, different methods have been applied for determination of FQs [12], such as capillary electrophoresis [13], high pressure liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) [14–19], coupled to diode array detection [20,21], or coupled to resonance light scattering [22]. Fluorimetric-based methods have also been developed based on FQs intrinsic fluorescent properties as in the proposed methodology for quantification of CIP in seawater at ng L^{-1} level [23], or in the screening method applied in groundwater samples from intensive livestock production systems [24], or even resorting to automation for preconcentration in molecularly imprinted polymers [25]. Fluorescent labelling of FQs followed by chromatographic-fluorimetric detection has also been recently applied for the determination of CIP and NOR in surface water [26].

Sample pretreatment has been frequently required for accurate FQ analysis in environmental waters, in order to decrease matrix effects and to increase sensitivity. In this context, solid-phase extraction (SPE) has been implemented for sample preparation as it copes with rather large sample volumes (50–100 mL). Sorbents such as polymer-based Strata-X [15], molecularly imprinted polymers [23,25] and also restricted-access material based on bovine serum albumin and C8 [14] have been used for preconcentration of FQs from water samples, mainly in cartridge format.

Disk-based SPE methods for determination of different pollutants in water have been developed [27–29], including fluoroquinolones [30–32]. When compared to cartridge-based sorbents, disks present several advantages, such as higher extraction efficiency, application of higher sample loading flow rates and reduction of eluting solvent volumes, due to increasing of contact surface area between solutions and sorbent particles [33]. Nevertheless, they do not offer an adequate method for environmental screening when disk-based sample treatment is combined to LC-MS/MS where a single run takes more than 25 min [30,32]. Recently, a chemometric-based fluorimetric method was proposed, fostering direct determination of norfloxacin, ofloxacin and enoxacin retained on C-18 disk [31]. Nevertheless, expeditious screening tools for FQs monitoring as environmental contaminants are still required.

Therefore, in this work, a fluorimetric-microplate based method after disk-based SPE is proposed for screening of FQs in environmental waters. Although disk-based SPE has been proposed before for extraction of FQs, this strategy was not used as a component of a screening technique using direct microplate assay as proposed here. For this, a miniaturized SPE device was assembled with eluate screening in a microplate reader

based on intrinsic fluorescence of FQs. Following the detection of FQs, confirmation and individual determination of target FQs (norfloxacin, ciprofloxacin and enrofloxacin) were pursued by direct transfer from the microplate to HPLC-MS/MS. To the best of our knowledge, this is also the first time that fluorimetry and mass spectrometry are combined for screening and confirmatory analysis of fluoroquinolones.

2. Experimental

2.1. *Chemicals and reagents*

Ciprofloxacin (CIP), norfloxacin (NOR) and enrofloxacin (ENR) were of analytical grade and purchased from Sigma Aldrich (St Louis, MO, USA). Water from arium water purification systems (resistivity >18 MΩ cm, Sartorius, Göttingen, Germany) was used. Acetic acid glacial for HPLC was purchased from VWR (Fontenay-sous-Bois, France).

Individual stock standard solutions of FQs containing 1 g L^{-1} were prepared by dissolving the appropriate mass in 1 mL of water-formic acid (99.9:0.1, v/v). Intermediate stock solutions containing 10 mg L^{-1} of each FQ were prepared by dilution of 10 μL of respective stock solutions in 1 mL of methanol-NH_4OH (98:2, v/v). CIP, NOR and ENR working solutions (15 to 500 μg L^{-1}) were prepared daily by dilution of respective intermediate solution. Acetic acid solution 0.5 M was prepared by appropriate dilution of acetic acid glacial. Thereafter, acetic acid (pH 4; 100 μM) was prepared by dilution of this solution. Eluent solution (methanol-NH_4OH (98:2, v/v)) was prepared by dilution of commercial NH_4OH 25% (w/w) in methanol. SPE standards (2, 5, 15 and 25 μg L^{-1}) were prepared by dilution of intermediate stock solution at 10 mg L^{-1}.

For HPLC-MS/MS analysis, acetonitrile (LiChrosolv LC-MS grade) and formic acid were purchased from Merck (Darmstadt, Germany). The mobile phase components water-formic acid (99.9:0.1, v/v) and acetonitrile-formic acid (99.9:0.1, v/v), were filtered through 0.22 μm Millipore (Billerica, MA) GVWP filter and 0.45 μm Millipore HVHP filter, respectively. Deuterated ciprofloxacin-d_8 (CIP-d_8) was used as internal standard, and acquired from Toronto Research Chemicals Inc. (Toronto, ON, Canada), through LGC standards (Barcelona, Spain). For calibration of HPLC-MS/MS method, solutions containing CIP, NOR and ENR were prepared at 5, 10, 25, 50, 100, 175 and 250 μg L^{-1} in acetonitrile-water-formic acid (30:69.9:0.1, v/v). The internal standard CIP-d_8 was added to each standard solution at 50 μg L^{-1}.

Prior to LC-MS/MS analysis, conventional SPE of target samples was performed using Oasis HLB cartridges (60 μm, 150 mg, 6 mL) from Waters (Milford, MA, USA) for comparison purposes.

2.2. *Disk-based solid-phase extraction procedure*

Miniaturized SPE extraction procedure (Figure 1) consisted in cutting and housing the mixed-mode ion exchange polystyrene divinylbenzene sulphonated (SDB-RPS) disk from Empore™ (Bellefonte, PA, USA), into a polypropylene disk holder, 13 mm diameter (Swinnex® filter holder, SX0001300, Millipore), comprising one extraction unit. C-18 sorbent was also tested and acquired from Empore™. Four units were connected in parallel to propulsion tubes (Tygon®, 2.06 mm i.d.), fitted in a peristaltic pump (Gilson

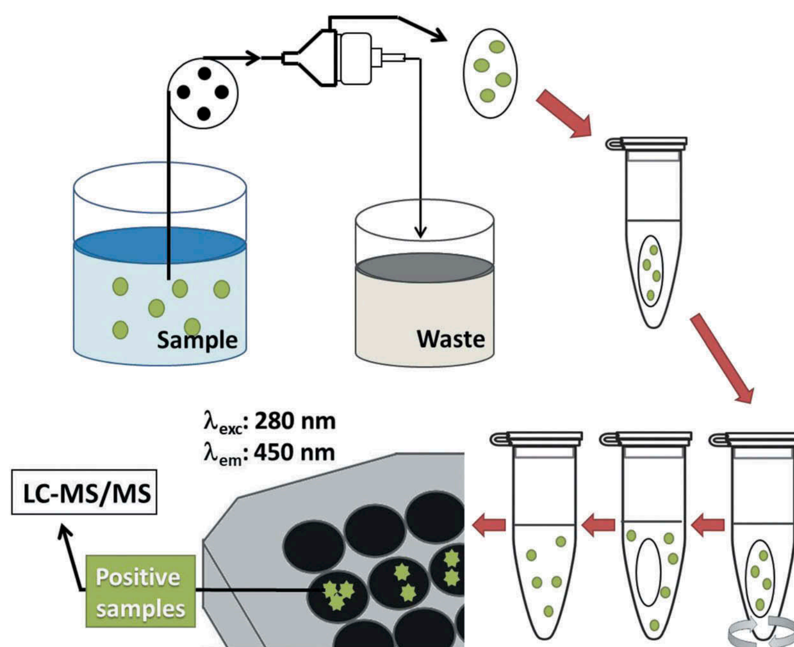

Figure 1. Schematic representation of µSPE and detection workflow, using SDB-RPS disks for extraction and preconcentration of fluoroquinolones (green circles). Sample is pumped through the µSPE disk inserted in a flow-based device. After extraction, the disk is removed from the device and soaked in eluent. After stirring, the disk is discarded and eluate is analysed by microplate fluorimetry. Positive samples are reanalysed by LC-MS/MS.

Minipuls 2, Villiers-le-Bel, France). FQs extraction was performed by loading 25 mL of sample acidified with acetic acid (pH 4.0) at 4.0 mL min^{-1} through one of the extraction units. After extraction, disks were dried during 6 min (4 mL min^{-1}) by passage of air. FQs elution was performed by removing each disk from the holder to a tube, and by adding 1.00 mL of methanol-NH_4OH (98:2, v/v). Next, the tubes were stirred in an orbital shaker 3005 (GFL® Gesellschaft für Labortechnik mbH, Burgwedel, Germany) at 500 rpm during 10 min, followed by a 5 min stop period. Then, disks were removed, and eluates were acidified with 50 µL of formic acid. Eluates were centrifuged at 9000 × g during 8 min at 4°C, following analysis under microplate format (250 µL).

For HPLC-MS/MS analysis, the aliquots of 200 µL of eluate placed in the microplate were transferred to vials, and 2 µL of internal standard (to reach final concentration of 50 µg L^{-1} of CIP-d_8) were added.

2.3. *Microplate procedure*

Fluorescence measurements were performed using a 96-well microplate reader Cytation™3 (BioTek Instruments, Winooski, VT, USA) with a Xenon Flash as light source. Two-hundred and fifty µL of SPE extracts or samples were placed in triplicate at a flat bottom 96-well microplates Microfluor® 1 black (Ref. 735-0527, Thermo Scientific, Waltham, MA USA) as depicted in Figure S1. Fluorescence measurement was performed

using 280 nm as excitation wavelength and 450 nm as emission wavelength 5 min after addition of standards and samples to the microplate.

2.4. *HPLC-MS/MS method*

Chromatographic analysis was performed in a Nexera X2 UHPLC system comprising two LC-30AD pumps, a DGU-20A5R degassing unit, a SIL-30AC autosampler and a CTO-20AC oven (Shimadzu Corporation, Kyoto, Japan). The MS/MS system was a triple quadrupole LCMS-8040 mass spectrometer equipped with an electrospray ionization source (ESI) (Shimadzu Corporation).

Chromatographic separation was established by using the method proposed by Paíga *et al*. [34]. A reverse phase Mediterranea sea C18 column (3 µm, 100 × 2.1 mm; Teknokroma, Barcelona, Spain) was applied and elution was performed in gradient mode with water–formic acid (99.9:0.1, v/v) as solvent A, and acetonitrile-formic acid (99.9:0.1, v/v) as solvent B, at total flow rate of 0.3 mL min^{-1}. The column eluate was directed to ESI-MS/MS interface between 2.50 and 4.25 min of run time.

The mass spectrometer was operated in positive ionization mode (ESI+), and data were acquired in multiple reaction monitoring (MRM) mode (NOR, m/z 320.10 > 302.15, m/z 320.10 > 276.20; CIP, m/z 332.10 > 314.10, m/z 332.10 > 231.10; ENR, m/z 360.00 > 316.25, m/z 360.00 > 342.15). Deuterated CIP (CIP-d_8) was monitored at m/z transitions 340.20 > 322.20 and 340.20 > 235.10. Retention time was similar for these compounds, and reliable assessment was assured by monitoring different transitions for quantification and identification for each compound.

The following parameters were used for analysis: nebulizing gas (N_2) flow rate at 2.6 L min^{-1}, drying gas (N_2) flow rate at 15 L min^{-1}, desolvation line temperature at 300ºC, heat block temperature at 425ºC, detector voltage at 1.88 kV, collision gas (argon) at 230 kPa. The injection volume was 5 µL. Peak detection and quantification were performed using LabSolutions software version 5.60 SP2 (Shimadzu Corporation).

2.5. *Sample collection and conventional SPE for comparison purposes*

Surface water samples from the Douro River estuary (NW Iberian Peninsula), were collected in triplicate using 500 mL acid-cleaned polyethylene bottles, along a salinity gradient at rising high tide. The exact position of each sampling site was obtained by means of GPS (Magellan 600, San Dimas, CA, USA). Key physical and chemical parameters, namely temperature, salinity, pH, oxygen saturation and turbidity (Table S1) were measured in situ using a YSI6920 CTD multiparameter probe (YSI Inc., Yellow Springs, OH, USA). All samples were kept in the dark, refrigerated in ice chests until further analysis. Samples were filtered through 1.2 µm glass microfibre filters (VWR International, Leuven, Belgium) followed by 0.22 µm Millipore GVWP filters.

Conventional SPE was performed using Oasis HLB (Waters), with sorbent conditioning, sample loading (50 mL), and matrix removal conducted as previously established by Paiga *et al*. [34], with minor modifications. Therefore, analyte elution was performed with 10 mL of methanol-NH_4OH (99.5:0.5, v/v) instead of pure methanol, followed by acidification with formic acid. Subsequently, extracts were evaporated to dryness, and

resuspended in 500 µL of acetonitrile-water-formic acid (30:69.9:0.1, v/v). Prior to HPLC-MS/MS analysis, 5 µL of CIP-d_8 (internal standard) was added in order to reach the final concentration of 50 µg L^{-1}.

3. Results and discussion

3.1. *Implementation of fluorimetric determination of FQs under microplate format*

Fluorescence properties of fluoroquinolones are based on the common aromatic structure, making it suitable for development of screening methods based on a common feature that can be evaluated for several compounds belonging to the same group. Fluoroquinolones are weak acids, and their fluorescence spectra depend on the degree of protonation. Considering that molecule charge will also have an effect on solid-phase extraction, regarding both adsorption to sorbent and elution, spectra of ciprofloxacin as model FQ was evaluated in different solvents (methanol and methanol:water), and different pH values. Formic acid and NH_4OH were chosen as acid and basic modifiers, respectively, for their compatibility with mass spectrometry detection.

Increase of formic acid content up to 2% (v/v) in methanol enhanced both excitation and emission intensity without changing spectra features, providing a sensitivity value two-times higher when compared to methanolic media (Table S2). Regarding methanol: water ratio (tested with a constant concentration of formic acid at 2% (v/v)), no changes of spectra features were observed. However, lower sensitivity values were attained with increasing water content, reaching less than half value when water content is increased up to *ca*. 50% (v/v) when compared to methanolic media (Table S2). Finally, addition of NH_4OH provided no fluorescence, which was recovered upon addition of formic acid, nevertheless showing lower sensitivity (Table S2). In fact, a strong dependence of quantum yields with pH for both ciprofloxacin and norfloxacin has been observed before, with the highest value at pH 3.5, and a decrease to one-hundredth at pH 11 [35]. This can be correlated with protonation of both FQs because their net charge is positive from protonation of the amine group in the piperazine ring for pH values <4. For pH values >10, the net charge is negative from loss of a proton at the carboxylic group attached to the quinolone moiety. This is in accordance with a previous report [36] where cationic FQs exhibited the highest fluorescence intensity, while the emission of anionic species was highly variable.

3.2. *Implementation of disk-based SPE procedure*

Disk-based extraction of fluoroquinolones has been reported before using syringes operated manually [31], or vacuum-based manifolds [30,32]. In both situations, it is not easy to maintain a constant flow rate. Considering the reproducible conditions attained in flow-based SPE extractions [29,37] using dedicated devices for supporting the disk membrane, commercially available holders aimed for filtration of small volumes were selected, and connected to a multi-channel pump for simultaneous processing of several samples. In this way, sample loading volume and flow rate would be precisely

controlled through pump operation [38]. Moreover, removal of water residue can also be performed using the same apparatus by pumping air after sample loading.

Concerning analyte elution, eluent could also be sent through the disk by the pump. Nevertheless, suitable pumping tubing should be selected, and transport lines would have to be filled with organic solvent, increasing the amount spent per determination. Therefore, a different approach is proposed (Figure 1), where extraction and disk drying take place under flow conditions, but elution of retained analytes is performed by soaking the disk in a minimum amount of solvent.

For pH values >2, FQs will always have charged groups, either only positive (pH <5), or only negative (pH >10), or both at the same time as a zwitterion species. Hence, extraction based on the reverse-phase mechanism may not be adequate for achieving high recoveries. Even though, C-18 disks were tested, using a water sample surrogate (10 mL), containing ciprofloxacin at 50 µg L^{-1}. To guarantee that all species were positively charged, sample pH was adjusted to 4 before SPE using acetic acid. Elution was tried using methanol:NH_4OH (98:2, v/v), causing species charge to change from positive to negative. Recovery was <20%, even after acidification of eluate before fluorescence measurement against standards prepared in the same solvent.

Still considering the role of charges, disks containing polystyrene divinylbenzene sulphonated (SDB-RPS), which allows the interaction with analytes by reverse-phase and cation exchange mechanisms, were tried as the sorbent anionic groups ($–SO_3^-$) would help to retain target FQs in acidic media, and also to improve elution upon pH increase. Considering that better sensitivity was attained for methanol solutions containing increasing concentrations of formic acid, elution was tried using all solvents mentioned in the previous section, with variable amounts of water and formic acid. Nevertheless, recoveries were <5%, indicating that reverse-phase and a possible competition mechanism with formic acid are not suitable for ciprofloxacin elution. Hence, elution using methanol:NH_4OH (98:2, v/v) was applied, fostering also a cationic exchange mechanism, providing a recovery value of *ca*. 70%. Recovery improvement was not observed when NH_4OH content in the elution solvent was increased up to 5% (v/v).

One of the main steps for attaining good recoveries is the passage of analytes retained in the solid phase to the eluting solution. The contact time between eluent and disk was fixed at 15 min and, initially, stirring was promoted by vortex shaking during 60 s. Orbital shaking during 10 min was also tested, and recovery values improved to *ca*. 80%, with application of this step in following experiments. During the sample loading step, the contact time was defined by the applied flow rate. Values between 1.6 and 4.0 mL min^{-1} were tested. Differences <2% were found for recovered mass, therefore, the highest flow rate was selected for improved sample throughput.

3.3. *Figures of merit of SPE-microplate method*

Calibration curves for each FQ were determined based on fluorimetric readings under microplate format. Linearity was obtained in the range of 15–500 µg L^{-1} for all FQs, without preconcentration, providing the calibration curve parameters indicated in Table 1. After establishing the SPE conditions, calibration curves for each FQ were also determined using 25 mL as sample volume (Table 1). Linearity was obtained in the

Table 1. Parameters of calibration curves obtained from fluorescence intensity vs. concentration (μg L^{-1}), established for ciprofloxacin, norfloxacin and enrofloxacin under microplate format with direct analysis of solutions or after disk-based SPE.

	Direct analysis[b, c]		Analysis after disk-based SPE[c, d]		
Compound[a]	Slope	Intercept	Slope	Intercept	Slope ratio disk-based SPE/direct analysis
NOR	12.9 (±0.2)	59 (±34)	166 (±3)	798 (±34)	12.9
CIP	11.3 (±0.3)	72 (±64)	250 (±6)	670 (±77)	22.1
ENR	17.9 (±0.2)	108 (±35)	224 (±4)	859 (±56)	12.5

[a] NOR: norfloxacin; CIP: ciprofloxacin; ENR: enrofloxacin.
[b] Solvent is methanol–NH_4OH–formic acid (93:2:5, v/v); calibration range: 15–500 μg L^{-1}.
[c] $R^2 > 0.994$.
[d] Solvent is methanol–NH_4OH–formic acid (93:2:5, v/v); sample loading volume: 25 mL; calibration range: 2–25 μg L^{-1}.

range of 2–25 μg L^{-1} (Table 1), and enrichment factors were between 12.5 and 22.1 times, based on the slope ratio. LOD and LOQ values were calculated based on signal to noise ratio. For all compounds tested, values were 1 and 2 μg L^{-1}, respectively. Concerning repeatability, RSD values <5% were attained (*n*= 3).

Regarding the time necessary for sample preconcentration, it takes *ca*. 30 min to preconcentrate 4 samples. Therefore, considering the extraction procedure, eight samples can be processed per hour. Furthermore, as depicted in the proposed microplate scheme (Figure S1), up to 27 samples can be applied simultaneously to the same microplate. Hence, about 50 samples can be processed per day for screening at low μg L^{-1} level with an estimated cost of less than 4 euros per sample.

3.4. *Analysis of samples*

Estuarine water samples were acidified with acetic acid and spiked with NOR, CIP and ENR, at two concentration levels for each target analyte (5 and 25 μg L^{-1}) according to EMA recommendations [39]. They were processed using the proposed disk-based SPE method, and analysed using fluorimetric detection and HPLC-MS/MS (Table 2, Table S3 and Figure 2). For comparison purposes, samples were also analysed according to Païga *et al*. [34] using conventional, packed-sorbent SPE followed by HPLC-MS/MS analysis. Recoveries in fortified samples varied from 43% to 63% for disk-based SPE, and from

Table 2. Values for total FQs (μg L^{-1}, mean ± sd) in estuarine water samples following analysis by disk-based SPE + direct fluorimetry, by disk-based SPE + HPLC-MS/MS, and by conventional SPE + HPLC-MS/MS.

Sample[a]	Concentration added/μg L^{-1}	Disk-based SPE + direct fluorimetry	Disk-based SPE + HPLC-MS/MS	Conventional SPE + HPLC-MS/MS
A	–	<LOD	<LOD	<LOQ
	15[b]	8.4 ± 0.2	8.3 ± 0.2	11.8 ± 0.3
	75[c]	46.1 ± 1.2	33.2 ± 0.1	40.6 ± 0.4
B	–	<LOD	<LOD	<LOQ
	15[b]	8.3 ± 0.1	7.7 ± 0.1	10.7 ± 0.2
	75[c]	32.1 ± 0.5	31.3 ± 0.2	41.9 ± 0.3
C	–	<LOD	<LOD	<LOQ
	15[b]	9.4 ± 0.2	8.0 ± 0.2	10.6 ± 0.2
	75[c]	37.5 ± 0.7	33.6 ± 0.2	47.1 ± 0.2

[a] Sample volume: 25 mL.
[b] Corresponding to 5 μg L^{-1} of each target FQ.
[c] Corresponding to 25 μg L^{-1} of each target FQ.

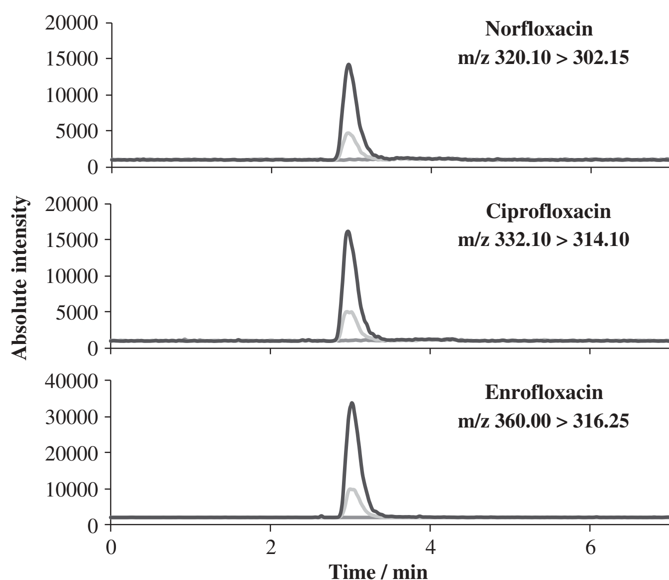

Figure 2. Multiple reaction monitoring chromatograms of norfloxacin, ciprofloxacin and enrofloxacin in estuarine water sample C (dark grey), and after fortification with 5 or 25 µg L^{-1} of each fluoroquinolone (light grey and black, respectively), following analysis by disk-based SPE + HPLC-MS/MS.

54% to 76% for conventional SPE, probably due to FQs depletion by other species present in tested waters.

Compared to the conventional procedure using packed-sorbent, relative deviations between −27% and 14% (mean deviation of −14%) were found, which were within the prediction of the Horwitz function for determinations performed at µg L^{-1} range [40]. Results also show the main advantage of the proposed disk-based SPE procedure to be applied as a screening technique, where positive samples can be reanalysed by HPLC-MS/MS, providing the profile of FQs present in the sample (Table S3). Despite the existence of several methods targeting the determination of fluoroquinolones, this is the first time that a single SPE approach is developed for screening and quantification in positive samples.

3.5. *Comparison to other methods based on SPE*

When comparing the proposed method with recently developed methods for the determination of fluoroquinolones in water, the present method offers higher sample-throughput and simplicity as the main advantages. Moreover, most of the screening methodologies with lower LOD values require sample pretreatment with conventional SPE cartridges combined only with expensive techniques, as LC-MS/MS [15,18,19], MS/MS [14] or LC-FD [23,26].

Conventional SPE schemes that were previously described for extraction of FQs require at least 50 min [16,18,30] or more than 3 h [17,32] for sample loading and drying of solid-phase (Table S4), which are clearly excessive when compared to the 30 min required in the procedure proposed in this work. Moreover, the following analysis

by chromatographic techniques requires the evaporation of the elution solvent and reconstitution in mobile phase [15–17,26,32], which are tedious processes that can decrease the accuracy and precision of results. In the proposed method, the elution solvent is analysed after contacting with the SPE membrane, following simple operations of addition of acid and centrifugation, performed in parallel for several samples simultaneously.

The time taken in sample loading and post-elution operations are minimized by automated [25] or online SPE [14] schemes, but both strategies require dedicated equipment and trained technicians. Concerning the amount of solvent required for elution of FQs retained in the solid sorbent, the approach presented in this work is clearly more environmentally friendly when compared to previous work, requiring only 1 mL of eluent, while 2.5 to 10 mL of eluent are required (Table S4) in most of the conventional SPE strategies proposed before.

Other methods using non-conventional sample pretreatment, such as stir-based sorptive extraction, also required chromatographic-spectrophotometric methodologies [20]. When compared to other proposed methods based on disk-based SPE [30–32], the proposed miniaturized SPE procedure presents a higher LOD. However, it is simpler, as sorbent conditioning and matrix removal steps are not required, and yields a higher sample throughput suitable for screening higher levels at environmental samples. As the present methodology includes a clean-up/preconcentration step, better LOD was observed compared to the microplate fluorimetric method recently proposed [24].

4. Conclusion

Application of the proposed method to real water samples was performed successfully. Attained LOD values allowed the screening of fluoroquinolones in contaminated water samples using a high-throughput procedure fit for purpose. Confirmatory analysis by HPLC-MS/MS was performed only for eluate from samples presenting fluorescence, with a significant economy of time and lab resources, showing its applicability on environmental monitoring.

In conclusion, a simple and fast screening method using disk-based SPE with fluorimetric quantification of three target fluoroquinolones in environmental waters was developed. Application to other fluoroquinolones present in other environmental water samples (pond water, groundwater, biogas digester water, lagoon wastewater) is envisioned.

Acknowledgments

This work received financial support from the European Union (FEDER funds through COMPETE POCI-01-0145-FEDER-031756 and POCI-01-0145-FEDER-007265) and National Funds (FCT/MEC, Fundação para a Ciência e Tecnologia and Ministério da Educação e Ciência) through project PTDC/CTA-AMB/31756/2017 and under the Partnership Agreement PT2020 UID/QUI/50006/2013. L. Barreiros thanks FCT and POCH (Programa Operacional Capital Humano) for her post-doc grant (SFRH/BPD/89668/2012). P.S. Peixoto acknowledges funding from CAPES, Ciencia sem Fronteiras program under grant BEX 9532/13-7.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Fundação para a Ciência e a Tecnologia [PT2020 UID/QUI/50006/2013,PTDC/CTA-AMB/31756/2017,SFRH/BPD/89668/2012]; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior [BEX 9532/13-7]; European Regional Development Fund [POCI-01-0145-FEDER-007265,POCI-01-0145-FEDER-031756].

References

[1] X. Zhang, H. Zhao, J. Du, Y. Qu, C. Shen, F. Tan, J. Chen and X. Quan, Environ. Sci. Pollut. Res. Int. **24**, 16478 (2017). doi:10.1007/s11356-017-9296-7.

[2] L. Riaz, T. Mahmood, A. Kamal, M. Shafqat and A. Rashid, Environ. Toxicol. Pharmacol. **52**, 14 (2017). doi:10.1016/j.etap.2017.03.002.

[3] N. Janecko, L. Pokludova, J. Blahova, Z. Svobodova and I. Literak, Environ. Toxicol. Chem. **35**, 2647 (2016). doi:10.1002/etc.3552.

[4] X. Van Doorslaer, J. Dewulf, H. Van Langenhove and K. Demeestere, Sci. Total Environ. **500**, 250 (2014). doi:10.1016/j.scitotenv.2014.08.075.

[5] M. Gros, M. Petrovic, A. Ginebreda and D. Barcelo, Environ. Int. **36**, 15 (2010). doi:10.1016/j.envint.2009.09.002.

[6] A.J. Watkinson, E.J. Murby, D.W. Kolpin and S.D. Costanzo, Sci. Total Environ. **407**, 2711 (2009). doi:10.1016/j.scitotenv.2008.11.059.

[7] European Medicines Agency, 2018, Questions and Answers on Use of Enrofloxacin-Containing Veterinary Medicines Administered via Drinking Water to Chickens and Turkeys - Follow-Up Assessment after the Referral under Article 35 of Directive 2001/82/EC (EMEA/V/A/089), https://www.ema.europa.eu/documents/referral/enrofloxacin-article-35-referral-questions-answers-use-enrofloxacin-containing-veterinary-medicines_en.pdf

[8] P. Sukul and M. Spiteller, *Fluoroquinolone antibiotics in the environment*, in *Reviews of Environmental Contamination and Toxicology*, G. Ware, H. Nigg and D. Doerge, eds., Springer, New York, 2007, p. 131.

[9] W.Q. Cao, J. Song and G.P. Yang, Environ. Chem. **14**, 350 (2017). doi:10.1071/en16188.

[10] A. Shrivastava, A.K. Singh, N. Sachdev, D.R. Shrivastava and S. Prasad, Environ. Chem. **14**, 231 (2017). doi:10.1071/en17034.

[11] M. Sturini, A. Speltini, F. Maraschi, L. Pretali, E.N. Ferri and A. Profumo, Chemosphere **134**, 313 (2015). doi:10.1016/j.chemosphere.2015.04.081.

[12] P.S. Peixoto, I.V. Toth, M.A. Segundo and J.L.F.C Lima, Int. J. Environ. Anal. Chem. **96**, 185 (2016). doi:10.1080/03067319.2015.1128539.

[13] A.M. Garcia-Campana, L. Gamiz-Gracia, F.J. Lara, M.D. Iruela and C. Cruces-Blanco, Anal. Bioanal. Chem. **395**, 967 (2009). doi:10.1007/s00216-009-2867-9.

[14] M. Denadai and Q.B. Cass, J. Chromatogr. A **1418**, 177 (2015). doi:10.1016/j.chroma.2015.09.066.
[15] M. Gbylik-Sikorska, A. Posyniak, T. Sniegocki and J. Zmudzki, Chemosphere **119**, 8 (2015). doi:10.1016/j.chemosphere.2014.04.105.
[16] N. Dorival-Garcia, A. Zafra-Gomez, S. Cantarero, A. Navalon and J.L. Vilchez, Microchem J **106**, 323 (2013). doi:10.1016/j.microc.2012.09.002.
[17] M. Wagil, J. Kumirska, S. Stolte, A. Puckowski, J. Maszkowska, P. Stepnowski and A. Bialk-Bielinska, Sci. Total Environ. **493**, 1006 (2014). doi:10.1016/j.scitotenv.2014.06.082.
[18] H. Ziarrusta, N. Val, H. Dominguez, L. Mijangos, A. Prieto, A. Usobiaga, N. Etxebarria, O. Zuloaga and M. Olivares, Anal. Bioanal. Chem. **409**, 6359 (2017). doi:10.1007/s00216-017-0575-4.
[19] N.F. Tetzner and S. Rath, Int. J. Environ. Anal. Chem. 2018. doi:10.1080/03067319.2018.1531395
[20] M. Mei and X. Huang, J. Sep. Sci. **39**, 1908 (2016). doi:10.1002/jssc.201600232.
[21] M. Gao, J. Wang, X.K. Song, X. He, R.A. Dahlgren, Z.Z. Zhang, S.G. Ru and X.D. Wang, Anal. Bioanal. Chem. **410**, 2671 (2018). doi:10.1007/s00216-018-0942-9.
[22] Z.Y. Pan, J.D. Peng, Y. Chen, X. Zang, H.J. Peng, L.L. Bu, H. Xiao, Y. He, F. Chen and Y. Chen, Microchem J. **136**, 71 (2018). doi:10.1016/j.microc.2017.01.009.
[23] Z. Lian and J. Wang, Mar. Pollut. Bull. **111**, 411 (2016). doi:10.1016/j.marpolbul.2016.07.034.
[24] S.V. Kergaravat, R.L. Althaus and S.R. Hernandez, Int. J. Environ. Anal. Chem. **98**, 1063 (2018). doi:10.1080/03067319.2018.1520227.
[25] P.S. Peixoto, E.M.P. Silva, M.V. Osório, L. Barreiros, J.L.F.C. Lima and M.A. Segundo, Anal. Methods **10**, 2180 (2018). doi:10.1039/c8ay00327k.
[26] B. Prutthiwanasan, C. Phechkrajang and L. Suntornsuk, Talanta **159**, 74 (2016). doi:10.1016/j.talanta.2016.05.080.
[27] C. Aguilar, F. Borrull and R.M. Marce, J. Chromatogr. A. **754**, 77 (1996). doi:10.1016/s0021-9673(96)00222-1.
[28] C. Erger and T.C. Schmidt, Trac-Trends Anal. Chem **61**, 74 (2014). doi:10.1016/j.trac.2014.05.006.
[29] M. Manera, M. Miro, J.M. Estela, V. Cerda, M.A. Segundo and J.L.F.C. Lima, Anal. Chim. Acta **600**, 155 (2007). doi:10.1016/j.aca.2007.01.040.
[30] E.M. Golet, A.C. Alder, A. Hartmann, T.A. Ternes and W. Giger, Anal. Chem. **73**, 3632 (2001). doi:10.1021/ac0015265.
[31] D. Gonzalez-Gomez, F. Canada-Canada, A.D. Campiglia, A. Espinosa-Mansilla, A.M. de la Pena and J.S. Jeong, J. Water. Chem. Technol. **38**, 280 (2016). doi:10.3103/s1063455x16050064.
[32] H. Nakata, K. Kannan, P.D. Jones and J.P. Giesy, Chemosphere **58**, 759 (2005). doi:10.1016/j.chemosphere.2004.08.097.
[33] J. Płotka-Wasylka, N. Szczepańska, M. de la Guardia and J. Namieśnik, Trac-Trends Anal. Chem **77**, 23 (2016). doi:10.1016/j.trac.2015.10.010.
[34] P. Paiga, L.H. Santos and C. Delerue-Matos, J. Pharm. Biomed. Anal. **135**, 75 (2017). doi:10.1016/j.jpba.2016.12.013.
[35] P. Bilski, L.J. Martinez, E.B. Koker and C.F. Chignell, Photochem. Photobiol. **64**, 496 (1996). doi:10.1111/j.1751-1097.1996.tb03096.x.
[36] A.P. Vilches, M.J. Nieto, M.R. Mazzieri and R.H. Manzo, Molecules **5**, 398 (2000). doi:10.3390/50300398.
[37] M. Miro, H.M. Oliveira and M.A. Segundo, Trac-Trends Anal. Chem **30**, 153 (2011). doi:10.1016/j.trac.2010.08.007.
[38] P.S. Peixoto, I.V. Toth, S. Machado, L. Barreiros, A. Machado, A.A. Bordalo, J.L.F.C. Lima and M.A. Segundo, Anal. Methods **10**, 690 (2018). doi:10.1039/c7ay02624b.
[39] European Medicines Agency, Guideline on Bioanalytical Method Validation, EMEA/CHMP/EWP/192217/2009. Rev. 1 Corr. 2 (2011).
[40] Statistical Subcommittee of the Analytical Methods Committee – Royal Society of Chemistry, The amazing Horwitz function, AMC Technical Brief No. 17, 2004.