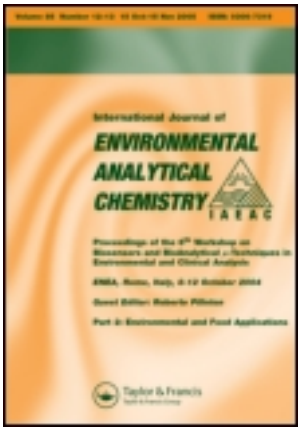

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/geac20

Fluoroquinolones in soils: Assessment of extraction methods

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Available online: 10 Nov 2011

To cite this article: Fernando Gómez-Granados, Rosa Codony, Mercè Granados & M. Dolors Prat (2011): Fluoroquinolones in soils: Assessment of extraction methods, International Journal of Environmental Analytical Chemistry, 91:14, 1353-1366

To link to this article: http://dx.doi.org/10.1080/03067319.2010.500056

Intern. J. Environ. Anal. Chem.
Vol. 91, No. 14, December 2011, 1353–1366

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Fluoroquinolones in soils: Assessment of extraction methods

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(*Received 12 February 2010; final version received 6 June 2010*)

The extraction of six fluoroquinolones from agricultural soils with different physicochemical characteristics (pH, texture and organic carbon content) has been investigated. Different solvents, consisting of hydroorganic mixtures, have been tested, and basic buffer solutions, with either acetonitrile or acetone, have proved to be suitable extraction solvents. Conventional mechanical shaking and microwave-assisted extraction techniques have been evaluated, and mechanical shaking has been selected. Recovery rates from freshly spiked (overnight) soils ranged from 65 to 90%, depending on the quinolone. No relevant dependence on soil characteristics has been observed. The effect of ageing on the extraction behaviour of fluoroquinolones has also been considered. In comparison with freshly spiked soils, extractions from residues aged for some months have resulted in about 20–25% lower recovery rates.

Keywords: fluoroquinolones; soil analysis; environmental analysis; aged residues; antibacterial agents

1. Introduction

Antimicrobial compounds are widely used in many countries worldwide to treat or prevent infectious diseases. Their addition into animal feed to improve growth rate and feed efficiency was banned in the European Union in 2006 [1]. Upon treatment of the animals, these pharmaceuticals may reach the environment, either through direct excretion or when manure is spread on agricultural land as fertilisers. Soil is the environmental compartment primarily exposed to these contaminants, but, depending on their interactions with the soil, they may accumulate there or come into ground or surface water. Little is still known about the environmental impact of these drugs, but their occurrence in the environment is of great concern and they have been identified as emerging environmental contaminants [2–5]. In addition of their potential ecological effects, they can enhance the generation and spread of drug-resistant bacterial stocks.

This study is focused on fluoroquinolones (FQs), one of the groups of antimicrobial compounds used in veterinary medicine. These compounds (Figure 1) contain an acidic carboxylic group and a basic amino group in the heterocyclic ring, with reported pK_a

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ISSN 0306–7319 print/ISSN 1029–0397 online

http://dx.doi.org/10.1080/03067319.2010.500056
http://www.tandfonline.com

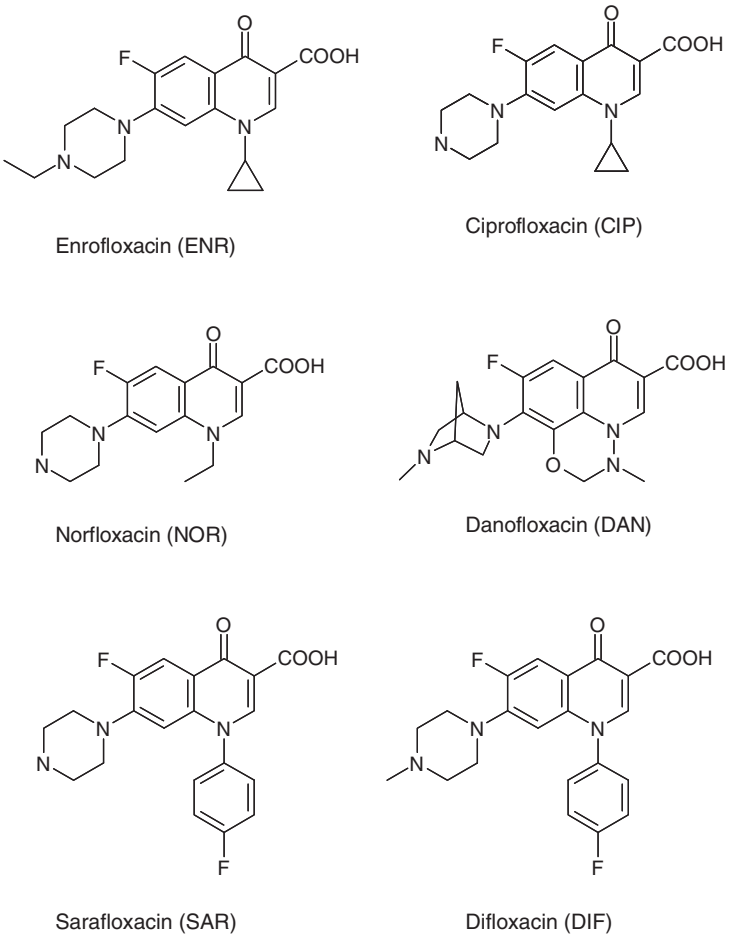

Figure 1. Structural formulas of the fluoroquinolones studied.

values in the 5.5–6.6 range for the carboxylic acid and in the 7.2–8.9 range for the protonated amino group [6]. Because of the zwitterionic character of FQs, the deprotonated carboxylic group prevails at the typical soil pH range of 5–9, and it is assumed to be responsible for the high sorption coefficients of these compounds into soil materials [7–11]. Studies on the occurrence of FQs in soils fertilised with animal manure report concentrations in the range 0.01–0.4 mg Kg^{-1} [12,13]. Moreover, FQs persist in soils several months after their introduction into this environmental compartment [13,14].

Appropriate analytical methods are required to determine the concentration levels at which these pharmaceuticals accumulate in the different environmental compartments and to study the fate of veterinary drugs released in the environment. A large number of analytical methods have been proposed for the analysis of quinolones in food [15–18], but studies devoted to the determination of FQ in solid environmental samples are still scarce [14,18–26], and most of them refer to first-generation quinolones. The methods usually reported for food samples, based on liquid chromatography (LC) with either fluorimetric

or mass spectrometry detection, are also suitable for environmental samples, but the extraction of FQs from the soil matrix is a crucial step in the analytical procedure and still requires further research work.

The few extraction methods reported for the extraction of FQs in solid environmental samples [11,14,19,20] use organic polar solvents in combination with acidic or basic aqueous solutions. There is no consensus about the most efficient solvent for FQs. Golet *et al*. [14] chose acetinitrile in acidic medium to extract CIP and NOR from sludge and soil matrices, whereas Turiel *et al*. [19] concluded that acetone in basic medium is the most successful extractant. Pressurised liquid extraction (PLE), microwave assisted extraction or ultrasonic extraction have been used as extraction techniques, but no comparative data between these exhaustive extraction techniques and simple shake extraction has been reported.

Extraction efficiency not only depends on the extraction solvent and the methodology used. The soil physicochemical characteristics may also play a strong influence on the interaction between FQs and the soil matrix and, therefore, on the extraction [11]. Moreover, physical or chemical ageing processes, such as diffusion throughout soil micropores or the formation of covalent bindings with some soil components, may result in an adverse effect on extraction [27,28].

The aim of this work is to develop a robust and efficient method for the extractions of six FQs from soil samples for its application to the residue analysis of these compounds in agricultural soils. The study is mainly focused in the selection of a suitable extraction solvent and the best conditions for the extraction of FQs from soils, accounting for the effect of ageing and soil characteristics on extraction behaviour. Several soils, covering a wide range of physicochemical properties and different ageing times, have been used.

2. Experimental

2.1 *Apparatus*

Chromatographic analysis was performed in an Agilent 1100 system (Palo Alto, CA, USA), consisting of an HP 1100 quaternary pump, a Rheodyne 7725 injector with a 25µl injection loop, and an Agilent 1100 fluorimetric detector. The analytical column was an Inertsil C_8 (250 × 4.6 mm), 5 µm (Alltech, Deerfield, IL, USA) equipped with a similar guard-column.

MAE was carried out using an *ETHOS E* closed-vessel system (1000W) supplied by Milestone (Sorisole, Italy). The system is designed for extraction with organic solvents and able to hold twelve 100-mL extraction vessels.

The pH was measured on a CRISON GLP 21 pH-meter (Alella, Barcelona, Spain), equipped with a CRISON 52-02 combined glass electrode. A Heraeus Christ centrifuge (Osterode am Harz, Germany) was used to carry out the extractions.

For SPE preconcentration a Rapid Trace Workstation (Caliper LifeSciences, Inc. Massachusetts, USA) was used.

2.2 *Chemicals and solutions*

Ciprofloxacin hydrochloride and enrofloxacin were supplied by Fluka (Buchs, Switzerland), Spain), norfloxacin by Boral Química (Barcelona, Spain), danofloxacin mesilate by Pfizer (Sandwich, NJ, USA), difloxacin hydrochloride and sarafloxacin

hydrochloride by Abbott (North Chicago, IL, USA) and marbofloxacin by Vétoquinol (Lure, France). Individual primary stock standard solutions (200 mg L^1) of all FQs were prepared monthly by dissolving the compounds in 0.01 mol L^{-1} aqueous nitric acid and were stored in dark glass bottles at 4°C. Secondary stock standard solutions containing 1 and 20 mg L^{-1} of each FQ were prepared weekly by mixing the primary stock solutions of the six FQ and diluting with a pH 4 oxalic buffer. Working solutions for spiking and calibration were prepared daily by dilution of stock standard solutions with pH 4 oxalic buffer.

Acetonitrile (ACN) HPLC gradient grade (Merck, Darmstadt, Germany) and doubly de-ionised water (Milli-Q, Millipore, Molsheim, France) with a resistivity of 18.2 MΩ cm^{-1} were used throughout. All other reagents were of analytical reagent grade.

Buffer solutions at pH 2, 4 and 12 were prepared from phosphoric acid/sodium hydroxide, oxalic acid/sodium hydroxide and sodium hydrogenphosphate/sodium phosphate, respectively.

Glassware used for experiments was previously soaked in 10% nitric acid for 24 h and rinsed with ultrapure water.

2.3 *Samples*

Four uncontaminated soil samples (S1–S4) and a contaminated one (S5) were collected from agricultural fields in Catalonia (Spain). Sample S5 was a soil intentionally contaminated about 2 months before collection with manure from pigs medicated with ENR. Samples were air-dried, sieved (2 mm), irradiated with γCo^{60} to destroy microbial activity and stored in the dark at −20°C. Before extraction experiments, samples were thawed and then shaken to avoid segregation. Table 1 shows parameters referring to the texture and other basic properties of the soil samples.

2.4 *Procedures*

2.4.1 *Spiking*

Samples for recovery studies were spiked by adding 0.25 mL of a spiking solution containing 0.2–2 mg L^{-1} of each FQ to 1.0 g of dry soil sample. This volume moistens the sample sufficiently without there being an excess of water. The mixture was

Table 1. Physico-chemical characteristics of the studied soil samples.

	S1	S2	S3	S4	S5
pH	7.2	7.7	8.4	6.6	7.0
Organic carbon (%)	2.6	7.2	1.8	6.1	6.3
SiO_2 (%)	79.2		43.1	56.0	49.8
CaO (%)	0.9	3.9	20.6	1.7	13.6
Sand (%) 2–0.05 mm[a]	75.7	24.5	30.8	37.3	43.9
Silt (%) 0.05–0.002 mm[a]	13.3	53.1	41.0	48.0	40.0
Clay (%) < 0.002mm[a]	11.0	22.3	28.1	14.7	16.1
USDA classification	Sandy-loam	Silt-loam	Clay-loam	Loam	Loam

[a]Particle diameter (mm).

equilibrated by shaking for 15 min and then left standing overnight at room temperature in the dark before the analysis, or stored at 4°C for several weeks. Spiking solutions (0.2–2 mg L^{-1}) were prepared by diluting the 20 mg L^{-1} standard solution with water

2.4.2 *Extraction*

Extraction was performed by adding 12 mL of the extraction solvent to 1.0 g of spiked sample.

For mechanical shaking, the mixture, in a 30 ml glass-extraction tube, was shaken (end-over-end) at room temperature for about 1.5 hours and then centrifuged (3500 rpm, 20 min). When no SPE was applied, the supernatant was transferred to a 50-ml volumetric flask. The residue was then rinsed with 2 mL water, and the combined extracts adjusted to volume with water; 1 mL of this solution was transferred to a 5 mL volumetric flask, diluted to volume pH 4 oxalic buffer, filtered through a 0.45 μm nylon membrane and injected into the chromatograph. Alternatively, extracts were kept for further clean-up.

For microwave-assisted extraction, the mixture, in a 100-mL PTFE extraction vessel, was irradiated for 20 min at 80°C, air-cooled inside the microwave to under 40°C, transferred to a 30 mL glass tube and centrifuged (20 min, 3500 rpm), and the above-mentioned procedure was applied.

2.4.3 *Clean-up*

The combined extract was transferred to a Turbovap glass tube and the organic solvent was evaporated under a nitrogen stream (about 40 min, 40°C). The aqueous solution was adjusted to pH about 7.0, with hydrochloric acid, diluted to about 10 ml with water and cleaned up with 100 mg Bond Elut C_{18} cartridges. Cartridges were conditioned with 5 mL of methanol followed by 10 ml water and 5 mL phosphate buffer solution pH 7.0. After loading, the cartridge was rinsed with 10 ml water, dried and eluted with 2.5 mL 1M NH_3-acetonitrile (1 : 1). The eluate was evaporated (N_2 stream, 40°C) to about 1.5 ml, diluted to 5 ml with mobile phase A and injected into the LC system.

2.4.4 *Chromatographic conditions*

The analysis of the extracts was performed by LC/fluorescence using a previously reported binary gradient elution method [29]. Mobile phase A was 10^{-3} mol L^{-1} oxalic acid buffer at pH 4 and mobile phase B was ACN. Both solvents were separately filtered through a 0.22 μm nylon membrane prior to use. The elution profile was as follows: 12% ACN for 12 min, gradient elution from 12% to 26% ACN in 10 min and a post-time of 5 min to go back to the initial conditions. The mobile phase flow rate was set at 1.5 mL min^{-1} and the separation was carried out at room temperature. Excitation/emission wavelengths were 280/450 nm, respectively.

2.4.5 *Recoveries*

The determination of the recovery rates was carried out from spiked samples. Absolute recoveries were determined wit the help of an external calibration curve by comparing peak areas. Calibration solutions, in the ranges 0.25–2.5 μg L^{-1} or 2–50 μg L^{-1}, were

prepared daily by dilution of stock standard solutions with mobile phase A. ANOVA and t test (signification level = 0.05) were used to compare mean recoveries.

3. Results and discussion

3.1 *Extraction in acidic medium*

Initial extraction experiments were performed with ACN/water mixtures (1 : 1) in acid media (pH 2) using MAE and mechanical shaking. This extraction media was selected on the basis of the study of Golet *et al*. on the determination of CIP and NOR [14] in sludges and sludge-treated soils. Since some of the assayed soils are rather basic and contain large amounts of carbonate, relatively concentrated acids or buffer solutions are required to keep solutions at the desired pH value. Therefore, to ensure acidic conditions during extraction, several acid solutions were assayed. Results showed that the addition of 2 mL of 1 mol L^{-1} pH 1.8–2 phosphoric acid buffer to 1 g soil led to the desired value (pH 2). Less concentrated buffer solutions have not a high enough buffer capacity to keep the pH at this value for basic soils.

Prior to the extraction studies, the effect of microwave irradiation on the stability of FQs, as well as the effect of the matrix components on the chromatographic determination, was investigated. Standard solutions of FQs in ACN/phosphoric buffer and in extracts from blank soil S1 were irradiated for times ranging from 10 to 35 min at temperatures from 70 to 110°C. After dilution with water, solutions were directly injected into the chromatographic system. These chromatograms were compared with those obtained from non-irradiated standards in both pure solvent and blank soil extract. Results show that FQ are stable under microwave conditions up to almost 100°C, whereas temperatures higher than 100°C resulted in some degradation of the compounds. Irradiation times higher than 25 min can also lead to some losses of FQs, unless the temperature was kept below 80°C. These experiments also proved that the diluted extracts could be directly analysed by LC-Fl, as the chromatograms thus obtained were quite clean, with no interfering peaks at the retention times of the analytes. Moreover, peak areas obtained from standard solutions of the analytes in pure solvent did not significantly differ from those obtained from chromatograms obtained by addition of the analytes to a soil extract (matrix matched standards), which indicates that external standards in pure solvent can be used for calibration.

The influence of some experimental parameters on FQs extraction efficiency was investigated in soil sample S1. The studied parameters were composition and volume of the extraction solvent, extraction time (microwave irradiation or shaking) and temperature (for MAE). The optimum temperature and irradiation time for MAE were found in the ranges 70–90°C and 10–20 min, respectively. There were no noticeable variations within these ranges, whereas further increasing either the temperature or the irradiation time resulted in a decrease on extraction recovery, probably because of FQs degradation under microwave irradiation. For end-over-end mechanical shaking, the extraction efficiency clearly increased with time up to about 1 h, slightly increased from 1 h to 2 h and no improvement on extraction recoveries was obtained at longer shaking times. The effect of solvent composition (ACN/water ratio and pH of the aqueous solution) on the extraction efficiency was investigated using 12 mL of mixtures in the range 20–90% ACN at pH values from 1.5 to 4: the conclusion was that pH had no effect on extraction in the range 1.5–3 whereas recoveries decreased at higher pH values. The effect of ACN content

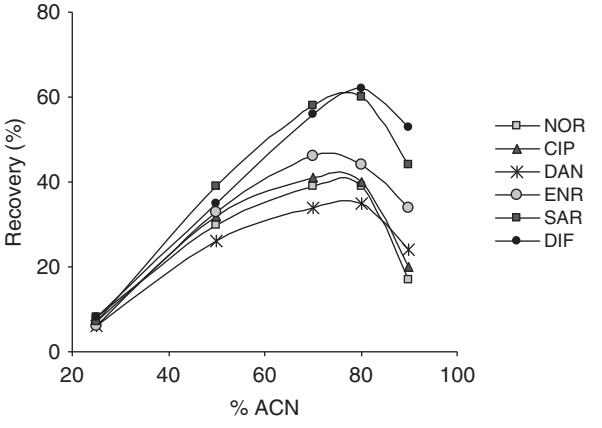

Figure 2. Effect of acetonitrile/water ratio on extraction of FQs from soil S1.

Table 2. Absolute recovery rates (%) in ACN/buffer pH 2 (75/25).

		% Recovery[a]					
	Sample	NOR	CIP	DAN	ENR	SAR	DIF
MAE	S1	39 ± 5	40 ± 6	35 ± 5	44 ± 3	61 ± 6	62 ± 5
	S2	31 ± 5	36 ± 3	30 ± 3	37 ± 6	52 ± 5	58 ± 6
Mechanical shaking	S1	32 ± 3	31 ± 4	30 ± 4	42 ± 3	60 ± 5	64 ± 4
	S2	28 ± 5	32 ± 5	31 ± 4	37 ± 5	53 ± 5	58 ± 6

[a]Percentage recovery mean ± standard deviation. $N = 4$.

on extraction (Figure 2) showed a similar pattern for all FQ, with a maximum in the 70–80% ACN range. Finally, several volumes of extracting solution (6, 12 and 20 mL) were tested: using 6 mL of extraction solvent instead of 12 mL resulted in a relevant decrease of extraction recoveries, whereas little improvement was observed when solvent volume was increased from 12 to 20 mL.

From these results, 3 mL of a pH 2 aqueous buffer solution and 9 mL of ACN were selected as optimum conditions. Recovery rates obtained from two soil samples (S1 and S2) with these conditions and using both MAE and mechanical shaking for extraction are shown in Table 2: it is clear that recovery rates after a single extraction step were rather low, ranging from 30 to 65%. Recovery clearly depends on the analyte, the more polar analytes, NOR and CIP, being the less extracted FQs. Only two different soil samples were tested at this stage, but no noticeable differences were observed between them. In a second extraction with 12 mL of the same solvent, about 30% of NOR, CIP and DAN and 50% of SAR and DIF still remaining in the soil were extracted. These results are not significantly different from those obtained in the first extraction, and give evidence that these low recoveries are due neither to degradation processes nor to non-extractable irreversibly sorbed compounds, but to the distribution coefficients between the two phases. Two sequential extractions led to overall recovery rates of: 50–60% for NOR, CIP and DAN and 70–80% for SAR and DIF. From a practical viewpoint, a procedure based

on two extractions is tedious and time consuming, especially when further steps are not compatible with the organic solvent and this must be evaporated.

Differences between MAE and end-over-end shaking were not significant, except for NOR and CIP in soil S1 (α about 0.04), which is rather surprising, as it is a general assumption that MAE provides higher extraction efficiency than conventional extraction techniques. PLE has not been tested in this work, but results from the other extraction techniques are in good agreement with those from Golet *et al.* [14] who, using PLE and a similar solvent for the extraction of CIP and NOR from sludge-treated soils, reported recoveries of about 40% after one extraction cycle (15 mL) and 60% after two sequential extractions (15 mL each).

Other extractant solutions, such as acetone, methanol and basic media, were tested as an attempt to improve extraction. Results obtained with other organic solvents in acidic medium did not improve extraction data with respect to ACN: ethanol/water mixtures showed very low recoveries, and acetone/water mixtures led to values similar to those obtained with ACN. Data obtained in basic media, however, were promising, and were carefully investigated.

3.2 *Extraction in basic media*

ACN/water, acetone/water and methanol/water mixtures at pH values about 12 were tested, with mechanical shaking. Initial experiments proved that methanol again led to very low extraction recoveries, whereas ACN and acetone gave good extraction efficiencies, with recovery rates much higher than those obtained in acidic conditions, this effect being more noticeable for the less recovered analytes. For ACN/water mixtures, it was verified that, as was the case in acidic media, the highest recoveries were obtained with solutions containing 70–80% ACN. For acetone/water mixtures, maximum recoveries were obtained in the 50–80% range (Figure 3), and the selected value was 60% acetone. Some extraction tests using ammonia and phosphate buffer solutions showed that a high pH value is required to achieve high extraction efficiencies: 2 mol L^{-1} ammonia and 1 mol L^{-1} phosphate buffer solution were assayed to adjust the pH of the aqueous solution

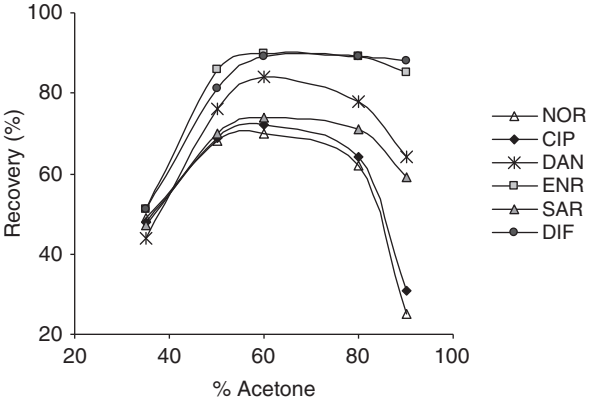

Figure 3. Effect of acetone/water ratio on extraction of FQs from soil S1.

to 12, and better precision was obtained with phosphate buffer. Extraction efficiency did not increase with extraction time from 1 h to overnight, and 1.5 h was selected.

Data in Figure 4 show the recoveries obtained for a single 1.5 h end-over-end shaking extraction applied to S1 soil sample using ACN/water (75/25) and acetone/water (60/40) in both acidic (pH 2) and basic (pH 12) media. Extraction efficiency is clearly better in basic medium for all FQ, with recovery rates in the range 65–90. Acetone and ACN give similar recovery rates, and both may be considered to be suitable extractants. Besides extraction efficiency for the target compounds, other factors such as the amount of co-extracted soil compounds in sample extracts should be evaluated; ACN extracts were slightly cleaner, but differences were not significant.

Table 3 summarises extraction recovery rates obtained from a set of four soils with ACN and acetone based mixtures. Soils had different textures, and the organic carbon content ranged from 1.8 to 7.2%. Results showed good extraction efficiency for all analytes and samples under the selected conditions. In general, no significant differences were found between recoveries from different soils (one-way ANOVA). Regarding

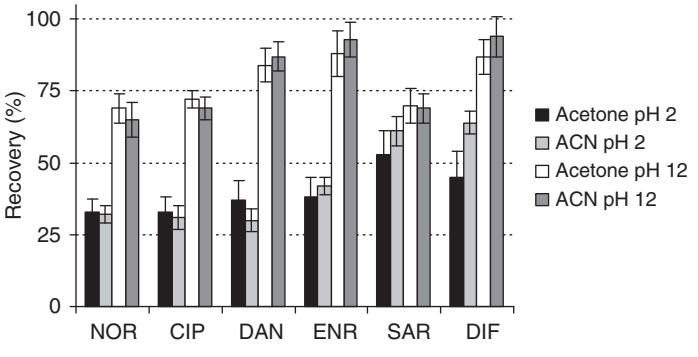

Figure 4. Effect of extraction solvent. Recovery rates after 1.5 hours end-over-end shaking extraction. Soil S1 spiked overnight with 500 ng g^{-1} of each FQ.

Table 3. Extraction recovery rates from diferent soils using acetone/buffer pH 12 (60/40) and ACN/buffer pH 12 (75/25). Mechanical shaking (1.5 h).

		% Recovery[a]					
	Sample	NOR	CIP	DAN	ENR	SAR	DIF
Acetone	S1	69 ± 5	72 ± 3	79 ± 6	80 ± 8	70 ± 6	85 ± 6
	S2	66 ± 5	70 ± 5	81 ± 7	89 ± 5	75 ± 3	91 ± 7
	S3	71 ± 7	71 ± 7	80 ± 6	81 ± 7	70 ± 5	80 ± 5
	S4	64 ± 6	67 ± 6	84 ± 5	87 ± 4	74 ± 4	92 ± 6
ACN	S1	64 ± 6	69 ± 4	84 ± 5	93 ± 6	69 ± 5	94 ± 8
	S2	68 ± 7	69 ± 5	79 ± 6	85 ± 5	71 ± 6	87 ± 5
	S3	73 ± 8	74 ± 6	82 ± 7	79 ± 4	66 ± 6	80 ± 7
	S4	67 ± 4	68 ± 3	80 ± 6	84 ± 3	71 ± 4	88 ± 4

[a]Percentage recovery mean ± standard deviation. $N = 3$.

individual FQ, some differences between analytes can be observed, NOR, CIP and SAR showing lower recoveries (65–75%) than DAN, ENR and DIF (about 80–90%). Two sequential extractions led to overall recovery rates of: about 80% for NOR, CIP and SAR and over 95% for ENR, DAN and DIF – but also increased extraction time and solvent consumption.

3.3 *Extraction of aged FQ residues and manure amended soils*

Two different approaches were considered in this study to ascertain whether extraction efficiencies for incurred FQ residues can be extrapolated from those obtained with overnight spiked soils: the effect of soil ageing on recovery extraction rates was investigated and the different methods tested were applied to the manure amended soil S5.

To study the ageing effect, acetone and ACN, both in basic media, were used to extract laboratory-aged spiked soil samples. Spiked S1 and S3 samples were left standing overnight at room temperature in the dark, or kept at 4°C for up to three months. Results from sample S1, obtained after extraction with acetone/pH 12 buffer (60/40) are given in Figure 5: no significant differences were observed between overnight spiked samples and those extracted 5 days after spiking, with recoveries in the range 70–90% in all cases; for three months aged FQ residues, recovery rates decreased to 55–70%. Data from sample S3 showed similar trends, with an average decrease in FQ recoveries of about 10% for one-month-aged residues; extraction with the ACN system led to comparable results. The existence of some ageing effect is clear but, in contrast to other antibacterial compounds, such as SAs [30,31], the formation of strongly sorbed non-extractable fractions is not dramatic.

Finally, soil S5, which had previously been amended with manure from animals treated with ENR, was extracted with acetone and ACN in both acidic and basic media and the results were compared. ENR is easily metabolised to CIP: for this reason, both FQs existed in the manure and, consequently, in the soil. Results, given in Table 4, show that the pattern observed with freshly spiked soils were reproduced in this contaminated soil: extraction in basic medium is much more effective (about 2.5 times) than in acidic

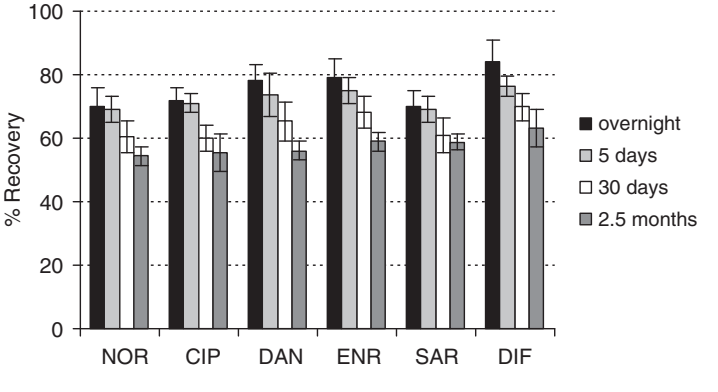

Figure 5. Effect of aging on extraction. Recovery rates obtained from freshly to up 3 moths spiked soil S1. 1.5 hours end-over-end shaking with acetone/buffer pH 12.

conditions for both FQs, and no significant differences were found between data obtained with acetone and ACN in basic medium.

3.4 *Application to soil analysis*

The previously described extraction studies were performed with soils spiked at a relatively high level (500 ng g^{-1}). It allows us to simply dilute the obtained extracts before injection, thus avoiding a clean-up step. For soils containing FQ at lower concentrations (50–100 ng g^{-1}) dilution prevents detection of most FQ, and thus a clean-up step was included. A SPE method, based on reversed phase cartridges, adapted from a previously reported method for the analysis of quinolones in water samples [32] was first assayed. To achieve good FQ retention, the hydroorganic extract must be evaporated to remove most of the ACN and the pH of the loading solution must be adjusted in the range 5–7. Since the extract consists of a pH 12 phosphate buffer solution, addition of hydrochloric acid permits easy adjustment of the pH to about 7.0. In the original procedure, elution was performed with a mixture based on acetonitrile/aqueous sodium hydroxide. A highly basic solution is essential for the elution of FQs but, in view to a further detection by MS, the use of ammonia instead of sodium hydroxide is preferred, and an elution solvent consisting of 1M NH_3/ACN (1 : 1) was found suitable.

Recoveries obtained when the SPE procedure described under procedures was applied to standard solutions of FQ were in the range 82–95%, but results obtained from spiked extracts of blank soils lead to lower recoveries. Moreover, extracts obtained from non-spiked soils S3 and S4 showed some small peaks close to the retention times of the analytes. Other SPE conditions were then assayed. These included mixed-mode cation-exchange phases to retain the cationic analytes from acidified extracts, as well as the use of several washing solvents to remove interfering compounds. None of the assayed conditions resulted in cleaner extracts. Regarding SPE recoveries, they were highest in the case of C_{18}, and comparable with those obtained with Oasis HLB. Recovery rates obtained from spiked extracts of the studied soils in a C_{18} cartridge, which was the finally selected, ranged from about 65% for NOR and CIP to about 85% for DIF.

Once the experimental conditions of the clean-up were established, the whole sample treatment procedure (i.e. extraction plus clean-up) was applied to soil samples S1 and S3. To assess the applicability of the method, samples were spiked at four levels in the range 50–500 ng g^{-1} for each FQ. Chromatograms obtained from blank soils and soil S1 spiked at 50 ng g^{-1} are shown in Figure 6. The straight lines obtained when plotting the amount of analytes found against the amounts added (Figure 7), with $R^2 > 0.98$ in all cases, demonstrate that recovery rates do not depend on analyte concentration in the range

Table 4. Extracted amounts of ENR and CIP from a soil contaminated with manure of pigs medicated with ENR.

Extraction solvent	ENR (ng g^{-1})	CIP (ng g^{-1})
Acetone/pH 12	875 ± 67	192 ± 25
ACN/pH 12	935 ± 54	210 ± 22
ACN/pH 2	340 ± 26	73 ± 19
ACN/pH 2 (MAE)	425 ± 38	90 ± 17

[a]Mean value ± standard deviation.

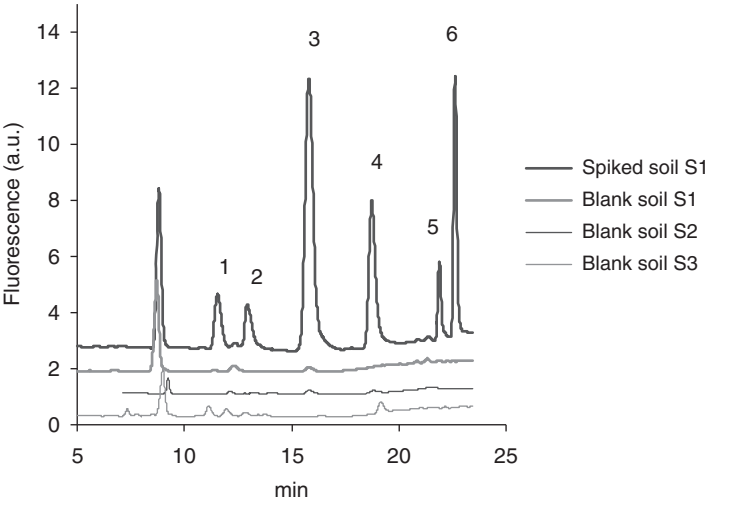

Figure 6. Chromatograms of extracts from blank soils S1, S2 and S3 and from soil S1 spiked with 50 ng g^{-1} of each FQ. Peaks: 1: NOR, 2: CIP, 3: DAN, 4: ENR, 5: SAR, 6: DIF.

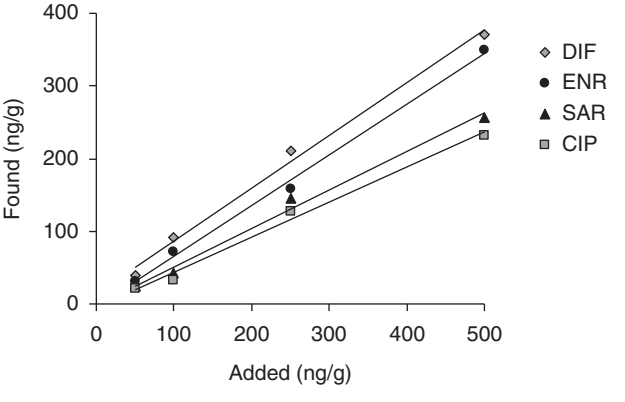

Figure 7. Overall recovery rates obtained from soils S1 and S3 spiked with 50–500 ng g^{-1} of each FQ.

studied. Mean extraction recovery rates, expressed as the slopes of these straight lines, are listed on Table 5. Since the FQs least extracted from soils, CIP and NOR, are also the least recovered in the SPE step, the mean overall recovery rates obtained for these FQs are somewhat low (about 50%). However, since they are reproducible (RSD $<$ 10%), data can be corrected for recovery. The limits of quantification were established from 10 times the signal to noise ratio in chromatograms of blank soils extracts, and were found to be 15 ng g^{-1} (NOR), 20 ng g^{-1} (CIP), 2 ng g^{-1} (DAN), 5 ng g^{-1} (ENR), 8 ng g^{-1} (SAR) and 2 ng g^{-1} (DIF). The highest values for NOR and CIP are mainly due to the fact that chromatograms are more noisy at the time window corresponding to NOR and CIP peaks, and also to their lower recoveries. Therefore, the quantification of lower concentration levels requires the use of a more specific detection system such as mass spectrometry.

Table 5. Mean overall recoveries from soils S1 and S4 spiked at 50–500 ng g^{-1}.

	Recovery[a] (%)					
Sample	NOR	CIP	DAN	ENR	SAR	DIF
S1	40 ± 4	42 ± 4	57 ± 5	62 ± 4	48 ± 4	71 ± 3
S3	47 ± 5	48 ± 4	66 ± 4	70 ± 3	53 ± 4	74 ± 2

[a]Slope (%) ± standard deviation.

4. Conclusions

Acetonitrile/water and acetone/water mixtures in basic media are suitable solvents for the extraction of fluoroquinolones in soils. Extraction of the cationic species, at low pH values, result in much lower recovery rates. MAE slightly improves extraction efficiency in comparison with simple shaking extraction, but the latter is recommended, since MAE introduces some complexity into the procedure and differences in recovery rates are not significant. Extraction of aged FQs in soils reveals some decrease (20–25%) of extraction recovery from freshly to months-old residues. The proposed extraction method can be applied to the analysis of FQs in soils at the ng g^{-1} level. Although fluorescence detection is highly sensitive for FQs, a detection system based on mass spectrometry is supposed to be more suitable for the analysis of such complex samples.

Acknowledgements

Financial support from the Spanish Ministerio de Ciencia y Tecnología (Project CTQ2007-62773) is gratefully acknowledged. The authors acknowledge M. Aran from *Applus + Agroambiental* (Sidamon, Lleida, Spain) for providing soil samples. F.A. Gómez also thanks ALBAN Programe for a grant.

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