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Solvent-free microwave-assisted extraction of fluoroquinolones from soil and liquid chromatography-fluorescence determination

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ABSTRACT

Presented hereafter is a novel method entailing solvent free microwave-assisted extraction (MAE) and HPLC equipped with Fluorimetric Detector (HPLC-FD) for the simultaneous determination at $\mu g\,kg^{-1}$ concentration of eight fluoroquinolone antibiotics (FQs) (Ciprofloxacin, Danofloxacin, Enrofloxacin, Flerofloxacin, Levofloxacin, Marbofloxacin, Norfloxacin and Orbifloxacin) in agricultural soils. The extraction was quantitatively performed, in a single step, by using an aqueous solution containing Mg(II) as complexing agent, thus avoiding consumption of organic solvents. The optimal MAE conditions have been established through a chemometric approach by considering temperature, irradiation time and matrix moisture or solvent, as the most important recognized variables affecting the extraction yield. Satisfying recoveries (69–110%, spikes 0.03–0.5 $mg\,kg^{-1}$) were gained with a single MAE cycle of 20 min, at 80 °C in 20% (w/v) $Mg(NO_3)_2$ solution as leaching agent. MAE-SPE recoveries at 10 $\mu g\,kg^{-1}$, concentration near method quantification limits (MQLs), were in the range 60–85%. Good repeatability and within-lab reproducibility were observed (both in the range 1–16%). The applicability of the method to real samples was assessed on natural contaminated soils. Compared to ultrasonic-assisted extraction (UAE), MAE was shown to be highly competitive in terms of extraction efficacy and analysis speed.

1. Introduction

Over the last decade, advances in sample preparation have resulted in new techniques, such as supercritical fluid extraction (SFE), microwave-assisted extraction (MAE) or pressurized liquid extraction (PLE), which greatly improved the speed and the efficacy of the extraction process from solid matrices [1]. Contrary to SFE, which has been limited by high matrix dependence and difficulty in extracting polar compounds, PLE and MAE are appealing alternatives to conventional techniques [2], as substantiated by the increasing number of papers published in the last years [3,4].

MAE has been applied in the speciation of metals in sediments [5] and a recent review gathered the MAE-based methods for the determination of organic contaminants, such as PCBs, PAHs, flame retardants, surfactants, estrogens, personal care products and pharmaceutical compounds, in solid samples [2]. Fluoroquinolones (FQs), highly useful antibacterial agents, belong to the latter class and are considered emerging pollutants. The great chemical stability of the heterocyclic ring makes these highly persistent contaminants and the relatively solubility increases their environmental diffusion. Indeed, they have been widely determined in surface [6,7], ground [8], and drinking [9] waters, wastewater [10–12] at concentration levels ranging from nanograms to micrograms *per* liter. Up to now, FQs have been frequently detected at 0.05–0.4 $mg\,kg^{-1}$ [3,13–15], up to 9.8, 8.3, 2.42 $mg\,kg^{-1}$ in soil [16], manure [14] and sludge [13], respectively. There is concern about the effects of their mobility, as their behaviour and that of their degradation products are still largely unknown; potential chronic effects of long-term and low-level exposures on environmental organisms and on human health are suspected. Certain is that these cause an increased bacterial resistance, as reported in several studies [[17] and references herein included]. At present no indicative tolerable value of antibiotics has been fixed for the different environmental compartments [18], although in the year 1996 the EMEA (European Agency for the Evaluation of Medicinal products) guideline set a threshold value of 0.1 $mg\,kg^{-1}$ for residues of veterinary pharmaceuticals in soil and 0.1 $mg\,L^{-1}$ in groundwater [19]. A revised guideline on environmental impact assessment for veterinary medicinal products has been published in the year 2008 [20].

In solid matrices, FQs occurrence is accompanied by an enhanced persistence in respect of water – where, instead, they rapidly degraded *via* photochemical paths [21] – due to the strong binding to soil minerals that retards both biodegradation and photodegradation [22] and lessen mobility. At this regard, a decrease in drug concentration was observed as function of soil depth [15].

While an extensive body of literature dealing with FQs determination in aquatic systems is currently available [17], only few

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methods have been developed for FQs quantification in soil and manure, basing on different sample pre-treatment, specifically accelerated solvent extraction (ASE) [13,23], ultrasonic-assisted extraction (UAE) [14,15,24–26], liquid–liquid extraction (LLE) [27] and MAE [3,16]. These methods require one or more extraction cycles [13,15,27] to gain good recovery and, with some exceptions [16,26], organic solvents are usually employed.

In this work we present a rapid, robust and sensitive method for the multiresidue determination of both veterinary and human-use FQs in agricultural soil, based on a *single* MAE cycle by using an aqueous solution of 20% (w/v) $Mg(NO_3)_2$ as leaching agent – forming chelates with the deprotonated carboxylic acid group of FQs [26] – thus avoiding consumption of organic solvents, followed by reversed-phase HPLC-FD. The variables involved in MAE were investigated through a multivariate experimental factorial design to individuate the optimal conditions to be applied in the achievement of the best extraction efficiency. A comparison with UAE performance is as well presented. Finally, the method developed was applied to the analysis of natural contaminated soils.

2. Experimental

2.1. Instruments and apparatus

Microwave extraction was performed by a Marsxpress microwave system supplied by CEM (CEM s.r.l., Cologno al Serio, Italy) equipped with a 16 PTFE vessels carousel and internal temperature control. The maximum irradiation power was 1600 W.

Ultrasonic extraction was carried out by means of a VWR International (Milan, Italy) Ultrasonic cleaner (USC 200-2600).

A Sigma 2-16P centrifuge (Celbio S.p.a., Pero, Italy) was used after sample extraction.

The HPLC system consisted of a pump Series 200 equipped with vacuum degasser and interfaced with a programmable fluorescence detector (FD) (Perkin Elmer). A 250 mm × 4.6 mm, 5 μm Ascentis RP-Amide (Supelco) coupled with a similar guard-column was used as analytical column.

2.2. Reagents

All the chemicals employed were reagent grade or higher in quality and were used without any further purification. Ciprofloxacin (CIP), Danofloxacin (DAN), Enrofloxacin (ENR), Levofloxacin (LEV), Marbofloxacin (MAR), Norfloxacin (NOR) and Orbifloxacin (ORB) were supplied by Fluka (Sigma–Aldrich), and Flerofloxacin (FLE) by Riedel-de Haën. HPLC gradient grade acetonitrile (ACN) was purchased by VWR, H_3PO_4 (85%, w/w) by Carlo Erba and ultra-pure water (resistivity 18.2 MΩ cm^{-1} at 25 °C) was produced in laboratory by a Millipore Milli-Q system. NaOH anhydrous pellets (97%) were obtained from Carlo Erba. Hexahydrate $Mg(NO_3)_2$ (97%) and ammonia solution (30% v/v) were purchased from Sigma–Aldrich and Carlo Erba.

FQs stock solutions of 300 μg mL^{-1} were prepared in methanol containing 0.1% (v/v) NaOH 1 M and stored in the dark at 4 °C for a maximum of three months. FQs working solutions of 6 μg mL^{-1} in 25 mM H_3PO_4 were renewed weekly. All the laboratory operations involving the use of standard solutions were conducted in the dark under red light.

2.3. Sample preparation

Two typical agricultural soils from South Lombardy plain (Italy) collected in Ferrera Erbognone, F1(45.11024N, 8.891125E) and F2 (45.12648N, 8.87709E), were chosen for the recovery studies and were characterized for their physico-chemical properties and mineralogical composition, as reported in Table 1. Texture, pH and organic carbon are typical of most agricultural soils [26]. After collection, samples were left to dry at room temperature, homogenized and sieved (2 mm). Before spiking, the native FQs content was determined as reported in Section 3.1 and it was negligible (<MDLs) than the higher, though realistic, spikes. Soil samples (0.5–1 g) were then fortified at environment-significant levels (0.01, 0.03, 0.08 and 0.5 mg kg^{-1}) into 5 mL weight-boats and stored overnight at room temperature in the dark, to allow solvent evaporation and FQs adsorption equilibrium to the matrix sites.

Table 1
Physico-chemical characteristics and mineralogical composition of the two soils (F1, F2) collected in Ferrera Erbognone village.

	Sample F1	Sample F2
pH in H_2O	6.4	6.4
pH in KCl	5.2	5.5
Total organic carbon (%)	1.0	1.1
Cation exchange capacity (mequiv./100 g)	13	15
Composition of soil particles (%)		
Sand	46.0	44.8
Clay	12.6	11.6
Silt	41.4	43.0
Mineralogical composition (%)		
Serpentine	7	5
Chlorite	13	8
Mica	16	24
Quartz	23	28
Feldspar	12	8
Plagioclase	19	22
Calcite	n.d.	n.d.
Amphibole	10	5

n.d.: not detectable.

2.4. Procedures

2.4.1. Microwave-assisted extraction

0.5–1 g of spiked soil was transferred in the PTFE vessel. 8 mL of an aqueous solution 20% (w/v) $Mg(NO_3)_2{\cdot}6H_2O$ and 2% (v/v) NH_3 was added. After microwave irradiation (20 min, 80 °C), the vessel was left air-cool, the extract transferred in 50 mL PP tube and centrifuged for 10 min at 4500 rpm. The limpid solution was acidified with H_3PO_4 (1:5) before HPLC analysis.

2.4.2. Chromatographic determination

After an equilibration period of 5 min, 50 μL of each sample were injected into the HPLC system. The FD excitation/emission wavelengths selected were 297/507 nm for MAR, 280/450 nm for CIP, DAN, ENR, FLE, NOR, ORB and 280/500 nm for LEV. Eluent A was a 25 mM H_3PO_4 and eluent B was ACN. Isocratic elution with 13% B was performed for 11.3 min, and then a 1 min linear gradient to 12% B was applied, followed by a 17 min isocratic elution. Afterwards, the initial conditions were reestablished by a 2 min linear gradient, followed by an equilibration time of 5 min. Analyses were performed at a flow rate of 1 mL min^{-1}. A FD chromatogram from a FQs standard mixture in phosphate buffer is shown in Fig. 1.

2.4.3. Method validation

Since FQs are polar and highly fluorescent compounds, reversed-phase liquid chromatography with fluorimetric detection is a suitable technique for their determination. Peak identification was performed by comparing fluorescence spectra and retention time of each sample with those of FQs standards. Considering that FQs are detected as native compounds and not as derivates, the information provided by fluorescence spectra are quite valuable in terms of selectivity. In order to achieve the maximum sensitivity, detection was performed by wavelength programming so that each analyte was determined at its optimal wavelength.

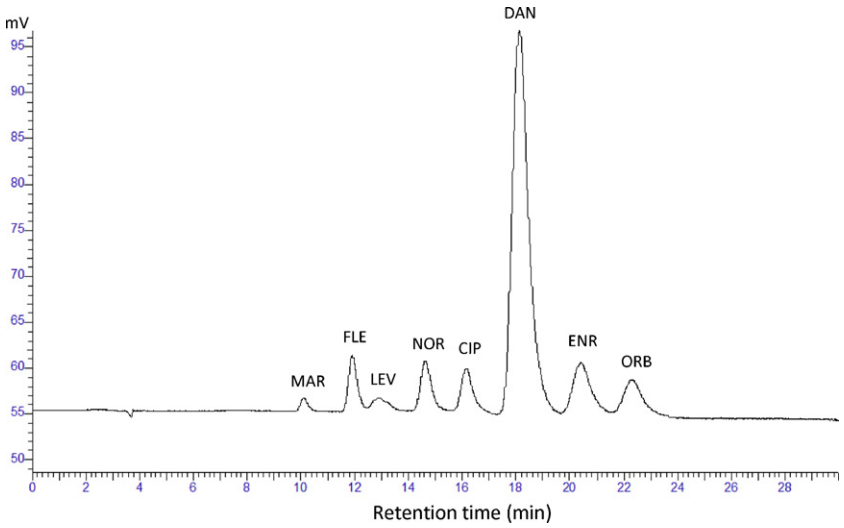

Fig. 1. FD chromatogram obtained for the FQs standard mixture at a concentration of 22.5 μg L^{-1}.

Four point calibration curves were generated for each analyte in the range 2–50 μg L^{-1} corresponding to 0.016–0.4 mg kg^{-1} in soil, by analysis in triplicate in phosphate buffer (H_3PO_4 25 mM), in extracting solution 20% (w/v) $Mg(NO_3)_2{\cdot}6H_2O$/2% NH_3 and in extracted agricultural soil blank (matrix-matched calibration). Linearity range is indeed wider [7], but here limited to 2–50 μg L^{-1} to more accurately evaluate recovery at the most critical concentrations that are at the same time also the most common in soils [3,13–15].

The matrix effects were evaluated from the respective slopes [4] in phosphate buffer, extracting and matrix-matched solutions.

Recovery of the MAE procedure for each FQ was determined by spiking at three different concentrations (0.03, 0.08 and 0.5 mg kg^{-1}), and that of the entire procedure (MAE-SPE-LC-FD) at a concentration near MQLs (0.01 mg kg^{-1}).

Method detection and quantification limits (MDLs and MQLs, respectively) were calculated on the basis of the instrumental detection and quantification limits (IDLs and IQLs, respectively) evaluated from linear regression parameters.

The precision of the method was evaluated by three measurements for each analyte *per* day during three days: 0.5 g of soil spiked with 0.5 mg kg^{-1} of each compound was used under optimal working conditions.

3. Results and discussion

3.1. Optimization of MAE

Most methods described for FQs determination in solid matrices, *viz.* soil, sediment, sludge and manure, are based on UAE [14,15,26] and ASE [13,23]. The method proposed by Turiel et al. [26] was initially chosen because of its simplicity and possibility of avoiding the use of organic extracting solvents. To gain reasonable extraction efficiencies, this was modified by increasing the number of extraction cycles. Two typical agricultural soils located in South Lombardy plain (Italy) spiked with 0.2–0.5 mg kg^{-1} of ENR and MAR were analyzed. Acceptable recoveries, 75–86% (RSD 5–14%) for MAR and ENR, respectively were obtained exclusively performing a minimum of three cycles. Fig. 3b shows the results obtained on sample F1 spiked with 0.5 mg kg^{-1} of ENR and MAR: relatively low recovery (around 60%) was achieved with a 30 min cycle and only performing additionally extractions (2× 30 min) was possible to reach acceptable values. MAE was then adopted in view of its ability to reduce extraction times, possibility of performing multiple extractions [2], and to improve recovery. Pure organic solvents and aqueous mixtures in combination with organic modifiers were not deliberately tested, preferring the aqueous solution 20% (w/v) $Mg(NO_3)_2$ with 2% NH_3 similarly to that already used for the preliminary experiments by UAE and by Turiel et al. [26]. Indeed, it is well known that FQs are able to form stable complexes with Mg(II) and these are better extracted from soil when they are in the anionic form [26].

Optimization of MAE conditions is rather easy owing to the low number of influential parameters [2], namely matrix moisture, time, and temperature, as compared to other extraction techniques such as SFE. For evaluating the significance of the variables involved in the extraction on the recovery rate, *viz.* temperature, microwave irradiation time and Mg(II) amount, a multivariate experimental factorial design (2^3) was applied to the fortified soil sample F1 (0.5 mg kg^{-1}). The experimental domain set up comprised of different levels of temperature (80 and 120 °C), different irradiation times (10 and 20 min) and different $Mg(NO_3)_2$ concentrations (1 and 20%, w/v). The plot of the coefficients of the model elaborated on the basis of the responses (FQs recoveries) is shown in Fig. 2. The significance of each coefficient is evaluated according to usual convention: $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.0001$, while error bars represent the confidence intervals at $p = 0.05$. Interactions between variables were not significant, as evident from their corresponding coefficients indicated as 4, 5, 6 in Fig. 2. The results showed that the extraction yield was strongly influenced by Mg(II) percentage and in a lesser extent, although significant, by irradiation time. In particular, high Mg(II) concentration led to a better extraction, whereas long extraction time disadvantaged. Temperature did not influence the extraction process significantly.

The factorial design was initially applied to ENR, one of the most prescribed FQ antibiotic in the world [28] and largely used in the high livestock concentration area between Pavia and Milan (Italy). This was satisfactorily extracted (yield 96%, RSD 4%) under the following conditions: 80 °C, 10 min, 20% (w/v) $Mg(NO_3)_2$ solution.

The same conditions were then applied to the others FQs investigated, obtaining poorer recoveries, as reported in the second column of Table 2. Being extraction time a significant variable, it has been prolonged to 20 min, achieving an increased FQs yield. The FQs responses had similar trends because the overall sorption interaction of compounds in FQs family is likely to be controlled by their base structure, with little effect of substituent groups [29].

Two extractive cycles at 80 °C, 10 min and 20% (w/v) $Mg(NO_3)_2$ were further carried out: the FQs fraction from the second extrac-

Table 2
Recovery rate of FQs from fortified soils (0.5 mg kg^{-1}) at different MAE conditions and mean extraction yields obtained under optimized conditions (0.01–0.5 mg kg^{-1}).

	Recovery (%)						
	80 °C, 10 min, 20% (w/v) $Mg(NO_3)_2$	80 °C, 10 min, 30% (w/v) $Mg(NO_3)_2$	120 °C, 10 min, 20% (w/v) $Mg(NO_3)_2$	80 °C, 20 min, 20% (w/v) $Mg(NO_3)_2$			
Spike (mg kg^{-1})	0.5 $n=3$	0.5 $n=3$	0.5 $n=3$	0.5 $n=4$	0.08 $n=3$	0.03 $n=3$	0.01[a] $n=3$
CIP	80 (7)	96 (9)	91 (11)	106 (9)	81 (8)	70 (9)	66 (10)
DAN	65 (7)	72 (6)	71 (8)	78 (5)	79 (9)	70 (6)	69 (7)
ENR	96 (4)	96 (4)	96 (13)	92 (2)	77 (4)	80 (7)	63 (8)
FLE	86 (9)	–	89 (7)	92 (10)	86 (8)	109 (10)	60 (9)
LEV	79 (8)	–	77 (9)	95 (11)	96 (9)	77 (8)	70 (11)
MAR	74 (4)	76 (2)	81 (2)	78 (1)	78 (5)	77 (5)	85 (13)
NOR	83 (9)	–	86 (10)	91 (12)	110 (10)	69 (12)	63 (11)
ORB	106 (11)	–	107 (9)	105 (6)	89 (9)	71 (8)	67 (9)

[a] MAE-SPE-LC-FD recoveries.

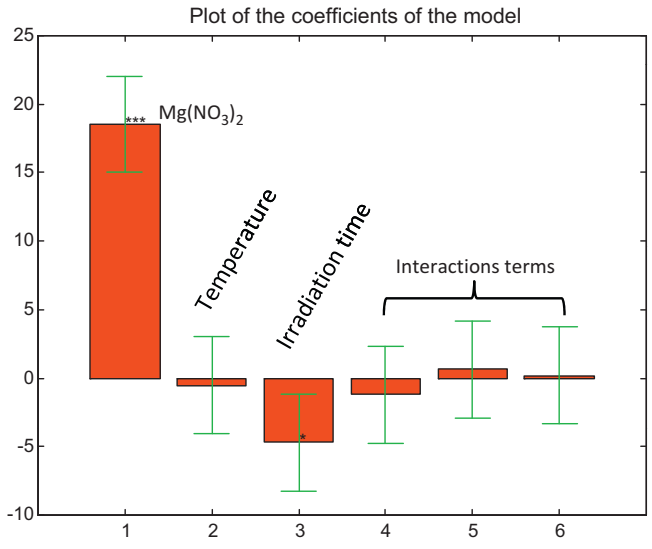

Fig. 2. Plot of the coefficients of the model reporting the significance of the variables investigated in the experimental design study. As explained in the text, stars represent the significance of the coefficients and error bars the confidence intervals at $p=0.05$.

tion was around 5% for all drugs, therefore a single step was convenient to achieve satisfactory recoveries. As for ENR, temperature had no pronounced effect on FQs extraction (Table 2), hence the optimized temperature was 80 °C. An additional extraction has been made at 30% (w/v) $Mg(NO_3)_2$ (Table 2, third column) with results not significantly different ($p=0.05$, $n=3$).

As a result of compromise, the final selected conditions were 80 °C, 20 min and 20% (w/v) $Mg(NO_3)_2$ (Table 2, last four columns). Analytical recoveries were investigated on the same soil sample spiked with concentration (0.03 and 0.08 mg kg^{-1}) near to FQs amounts generally detectable in South Lombardy District and quantifiable without SPE. The results from this series of experiments are presented in Table 2, sixth and seventh columns.

As well, additional recovery experiments have been carried out at lower spiked concentration level (0.01 mg kg^{-1}), near MQLs [4]; extraction, in this case necessarily followed by SPE procedure [7], gave satisfying results, as shown in Table 2 (eighth column). It is important to remark that after sample loading on HLB cartridge, the washing step must be avoided to prevent loss of analytes, due to FQs affinity to Mg(II), present at very high concentration.

It is interesting to make a comparison between the method here proposed for FQs extraction and UAE. MAR and ENR were chosen as target antibiotics as they were subject of our previous researches regarding veterinary FQs [7,21], being the first a new generation veterinary FQ and the second the most used in Italy and already investigated [26]. As it can be appreciated in Fig. 3, the great advantages offered by MAE in terms of efficacy and time saving are evident: a single MAE extraction gives a greater yield for both FQs by using a lower Mg(II) concentration, with respect to that obtained with the three sequential UAE steps.

Moreover, the method here developed proved to be valid and reliable for processing extremely complex matrices such as a soil characterized by high cation exchange capacity (CEC). In this regard, FQs sorption to matrix sites was shown to be heavily strengthened by increasing CEC values [29], thus hamper-

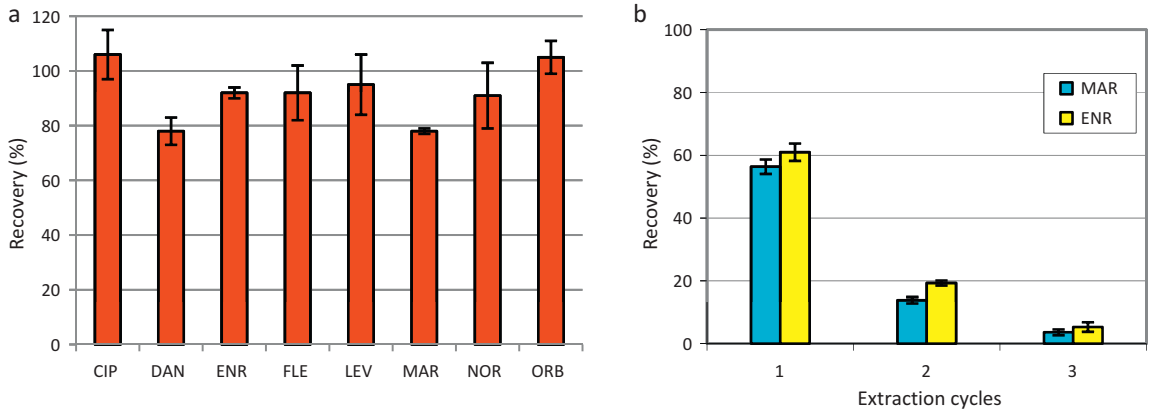

Fig. 3. Extraction yields of FQs after a single MAE cycle of 20 min (a) and after multiple sequential UAE of 30 min each (b) on soil sample F1 fortified with 0.5 mg kg^{-1} of drugs.

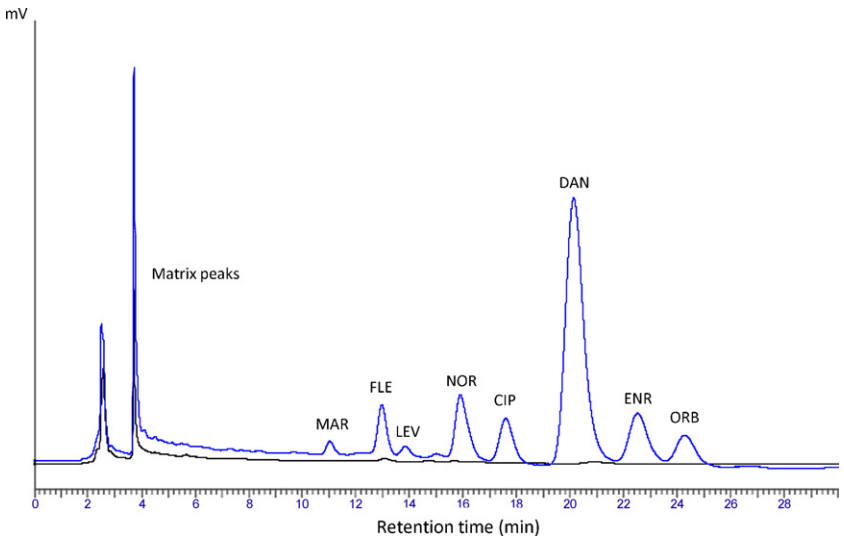

Fig. 4. FD chromatograms overlay of a blank soil extract (black line) and a soil extract spiked with 10 μg L^{-1} of FQs (blue line) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article).

Table 3
Linearity equations, method detection and quantification limits (MDLs, MQLs) repeatability, reproducibility.

FQ	Equation[a]	Linearity (r^2)	MDL[b] (μg kg^{-1})	MQL[b] (μg kg^{-1})	Repeatability (RSD%), $n=3$	Reproducibility (RSD%), $n=9$
CIP	$y=99{,}280(1174)x+32{,}608(13{,}507)$	0.9997	4.3	13.0	2.4	2.8
DAN	$y=1{,}075{,}334(7935)x+50{,}620(91{,}257)$	0.9999	2.7	8.1	1.8	1.3
ENR	$y=124{,}581(657)x+18{,}707(7763)$	0.9999	2.0	5.9	2.3	2.2
FLE	$y=75{,}772(793)x+36{,}600(9125)$	0.9998	3.8	11.5	1.6	10.9
LEV	$y=63{,}328(401)x+29{,}523(4613)$	0.9999	2.3	6.9	0.9	11.6
MAR	$y=15{,}991(163)x-307(1876)$	0.9998	3.8	11.1	1.3	1.3
NOR	$y=123{,}030(224)x+39{,}013(2580)$	0.9999	0.7	2.0	1.7	16.5
ORB	$y=90{,}070(525)x-586(6034)$	0.9999	2.1	6.4	1.2	1.9

[a] Calculated as peak areas vs. concentration. In parentheses slope and intercept errors obtained by OLLSR.
[b] Calculated by OLLSR parameters.

ing analyte desorption. This was also experimentally confirmed by Golet et al., proving that six ASE cycles in ACN-phosphoric acid were necessary to ensure a quantitative recovery from soils [13].

3.2. Method validation

3.2.1. Specificity and selectivity

The specificity and selectivity of the method have been evaluated on blank soil extracts chromatograms where no peaks have been evidenced at the retention times of the eight FQs, as well shown in Fig. 4. This excludes the presence of any matrix interfering substances accountable for false positive signals.

3.2.2. Linearity and matrix effects

The linear regression equations, mean of three independent calibration lines, obtained in phosphate buffer, extracting solution and blank matrix extract, showed a good linearity in the range 2–50 μg L^{-1} for the eight FQs and their slopes were not significantly different ($p=0.05$) (see Tables 3 and 4).Thus, reversed-phase liquid chromatography with fluorimetric detection is a suitable technique for FQs determination in complex matrices such as soil, avoiding internal standard correction [4]. Moreover, the method developed minimizes co-extraction of organic matter, accountable for matrix interferences that can influence both sensitivity and selectivity [12,17]. As shown in Fig. 5, high-quality chromatograms were obtained, with good baselines and no interfering peaks at analytes retention times.

Table 4
Linearity equations in phosphate buffer, extracting solution and blank soil extracts.

FQ	Phosphate buffer (25 mM)		MAE extracting solution[b]		Blank soil extract[c]	
	Equation[a]	Linearity (r^2)	Equation[a]	Linearity (r^2)	Equation[a]	Linearity (r^2)
CIP	$y=99{,}280(1174)x+32{,}608(13{,}507)$	0.9997	$y=95{,}680(1243)x+62{,}486(14{,}292)$	0.9996	$y=115{,}571(9089)x-63{,}439(120{,}234)$	0.9939
DAN	$y=1{,}075{,}334(7935)x+50{,}620(91{,}257)$	0.9999	$y=1{,}128{,}461(9906)x-51{,}473(113{,}924)$	0.9998	$y=9{,}992{,}220(19{,}243)x-111{,}150(254{,}562)$	0.9996
ENR	$y=124{,}581(657)x+18{,}707(7763)$	0.9999	$y=120{,}157(2943)x+36{,}317(33{,}842)$	0.9998	$y=121{,}256(5562)x-20{,}449(73{,}576)$	0.9986
FLE	$y=75{,}772(793)x+36{,}600(9125)$	0.9998	$y=74{,}185(987)x+83{,}742(11{,}345)$	0.9996	$y=74{,}426(2867)x+11{,}428(37{,}925)$	0.9985
LEV	$y=63{,}328(401)x+29{,}523(4613)$	0.9999	$y=63{,}236(1306)x+16{,}896(15{,}021)$	0.9991	$y=56{,}044(515)x-23{,}722(6809)$	0.9999
MAR	$y=15{,}991(163)x-307(1876)$	0.9998	$y=15{,}784(491)x+1082(5640)$	0.9981	$y=15{,}937(575)x+9191(7609)$	0.9989
NOR	$y=123{,}030(224)x+39{,}013(2580)$	0.9999	$y=122{,}644(414)x+83{,}204(4767)$	0.9999	$y=121{,}539(11{,}717)x+252{,}304(154{,}995)$	0.9908
ORB	$y=90{,}070(525)x-586(6034)$	0.9999	$y=91{,}935(317)x-15{,}449(3646)$	0.9999	$y=97{,}047(3706)x-123{,}780(49{,}023)$	0.9985

[a] Calculated as peak areas vs. concentration. In parentheses slope and intercept errors obtained by OLLSR.
[b] 20% (w/v) $Mg(NO_3)_2$, 2% (v/v) NH_3 acidified with H_3PO_4 (1:5) before HPLC injection.
[c] Acidified with H_3PO_4 (1:5) before HPLC injection.

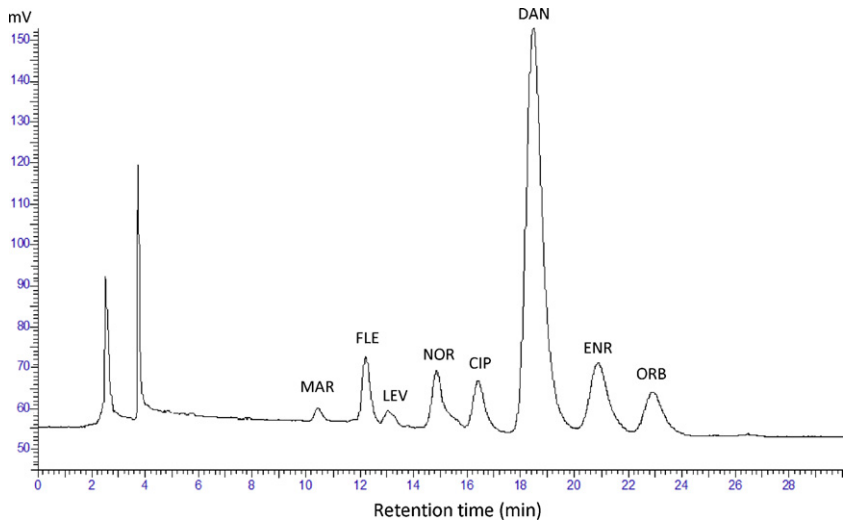

Fig. 5. FD chromatogram obtained from a fortified soil sample (0.08 mg kg^{-1}).

3.2.3. MDLs and MQLs

As reported in Table 3, MDLs and MQLs calculated from the linear regression parameters were in the range 1–5 μg kg^{-1} and 2–13 μg kg^{-1}, respectively. A concentration of 10 μg kg^{-1} near MQLs, provides recovery in the range 60–85% (Table 2, eighth column). The procedure here developed is hence suitable also for the determination of FQs in soils at concentration of a few micrograms *per* kilogram.

3.2.4. Recovery and precision

MAE recoveries were determined at three concentration levels (0.03, 0.08 and 0.5 mg kg^{-1}) and total MAE-SPE-LC-FD recoveries at 0.01 mg kg^{-1} (see Table 2). These were in the range 69–110% and 60–85%, respectively, and the different values are only due to the pharmaceuticals and not to the matrix. The repeatability (0.9–2.4%) and reproducibility (1.3–16.5%), listed in Table 3, are comparable with those reported for pharmaceuticals [4,8].

4. Determination of FQs in contaminated soils

The method was then applied to the analysis of agricultural soils collected around Pavia, for assessing its applicability to environmental matrices. Soil was sampled from the top layer (0–5 cm depth) of a field located in Belgioioso village close to a swine farm regularly employing ENR and MAR antibiotics. After MAE and pre-concentration on WAX-HLB cartridges [7], 20(3) μg kg^{-1} of ENR and 11.3(5) μg kg^{-1} of MAR, mean values of three replicates on independent sub-samples, were found. These results show that the common practice of using animal manure as fertilizer, as a sustainable principle of nutrient recycling, is an effective *route* for these drugs to enter into the soil compartment.

5. Conclusions

This work presents a simple, fast, highly selective and sensitive solvent-free MAE procedure for the simultaneous extraction of several FQs in soil samples, and their subsequent quantification by HPLC-FD. In a single extraction run this method allows to achieve excellent recoveries (69–110%), competitive detection limits (1–5 μg kg^{-1}) in respect to the literature [16,26], good repeatability and reproducibility, also at the very low concentrations (μg kg^{-1}) at which FQs can be present in soils. Low concentrations near MQLs are accurately determined by SPE after extraction, indeed the entire procedure MAE-SPE-LC-FD recoveries were in the range 60–85%. The proposed procedure was developed on agricultural soil samples spiked with antibiotics at realistic concentration levels; the method has been suitably validated and applied to the analysis of agricultural fields located near swine and cattle farms in South Lombardy plain.

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