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RESEARCH

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Validation of a modified QuEChERS method for the extraction of multiple classes of pharmaceuticals from soils

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Abstract

Background: The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method can be employed for multi-residue analyses instead of traditional extraction methods due to its advantages in terms of extraction time and required equipment. A modified version of the QuEChERS method has been developed for quantifying eight pharmaceuticals belonging to different classes in three real soils with different chemical properties. Firstly, the soils have been polluted with all contaminants and the recoveries were determined by liquid chromatography tandem–mass spectrometry. Due to similar recoveries from the three soils, the validation of the method has been carried out only on a soil by determining linearity, recovery, precision, limit of detection (LOD) and limit of quantification (LOQ) values. A matrix-matched calibration for the soil has been adopted in order to avoid the matrix effect and three levels of fortification (50, 100 and 500 $\mu g\ L^{-1}$) were used.

Results: The recovery of all pharmaceuticals, with the exception of tetracycline, from any soil was between 72 and 113%. In the validation procedure, recoveries of fortified samples ranged from 80 to 99%, the relative standard deviations ranged between 1.2 and 11.8%, and the LOQ between 20 and 36.9 $\mu g\ kg^{-1}$.

Conclusion: The results of the present study confirmed the validity of the modified QuEChERS method for the extraction of pharmaceuticals from soils in the range 50–500 $\mu g\ kg^{-1}$.

Keywords: QuEChERS method, Pharmaceutical contaminants, Soil, Liquid chromatography, Mass spectrometry, Validation method

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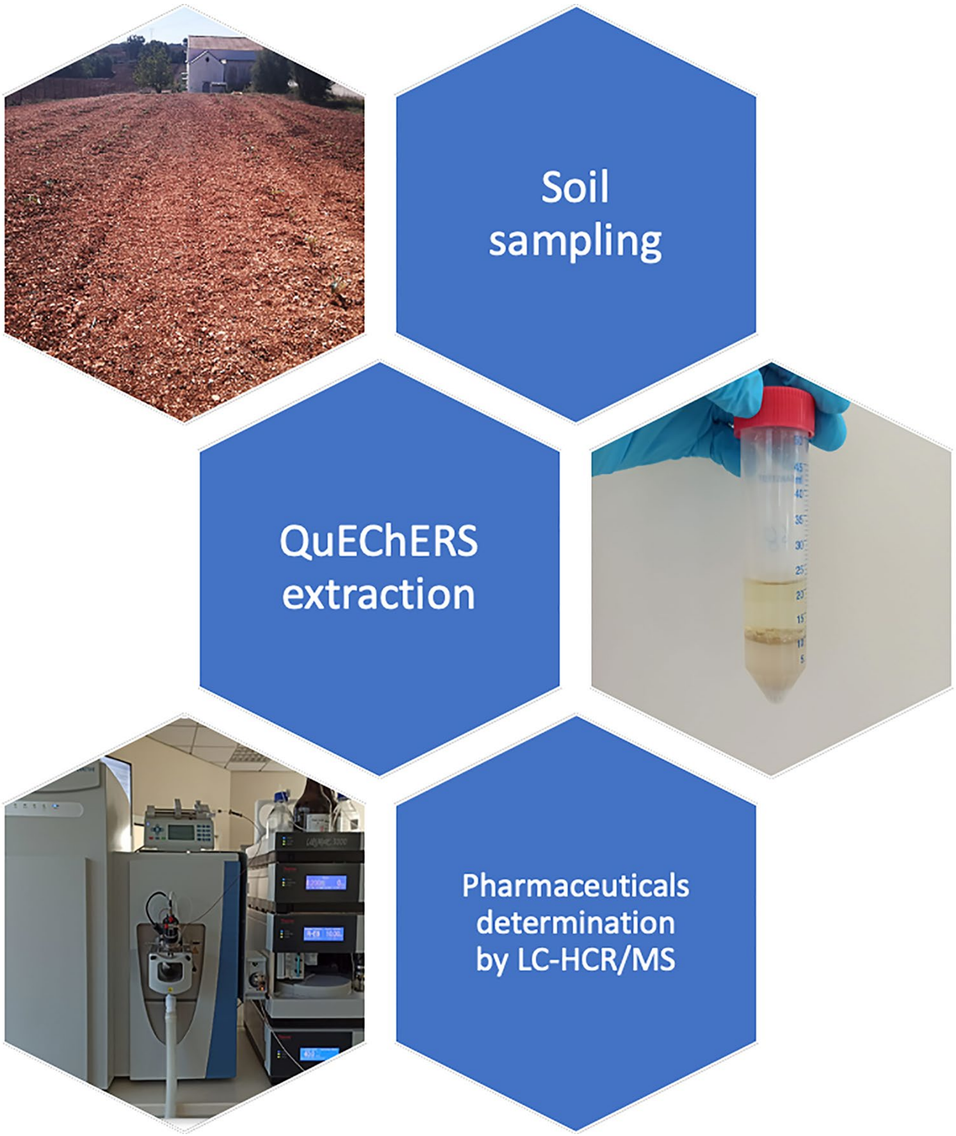
Graphical Abstract



Background

In the last years, contaminants of emerging concern (CECs) have become increasingly widespread because of the more intense anthropogenic activities and higher amount of wastes released into the environment. CECs include personal care products (fragrance, detergents, deodorants, cleaning products), pharmaceuticals (analgesics, anesthetics, antimicrobial, anti-inflammatory), drugs, pesticides, nanomaterials, flame retardants, and hormones. In particular, pharmaceuticals are biologically active substances used in both human and veterinary medicine for therapeutic and preventive purposes, and in husbandry and food production. This kind of contaminants are dangerous due to their persistence in the environment and their potential toxicity for humans, wildlife, and flora [1]. Pharmaceuticals can reach the environment through urine that, even if accounting only for 1% of the conventional wastewater volume, contributes for about 64% of these compounds found in wastewater bodies [2]. Pharmaceuticals occurring in wastewaters can persist even after secondary and tertiary water treatments [3], thus causing environmental contamination when wastewaters are used for irrigation [2].

Little is still known about the fate and effects of pharmaceuticals in general [4], and particularly in soil where they can undergo volatilization, microbial degradation and photodegradation [5], and leaching [6]. Furthermore, pharmaceuticals of low hydrophobicity can interact with soil organic matter and be accumulated in soil [7]. For these reasons, monitoring their concentrations in soil is very important.

Traditional methods for the extraction of organic contaminants from different matrices include the use of Soxhlet, pressurized liquids, ultrasound-assistance, solid-phase extraction or microextraction, and dispersive liquid–liquid microextraction [8]. The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method is a simplified version of conventional extraction methods for multi-residue analysis [9] and is suitable for large-scale residues analysis in different matrices [10]. This procedure, developed by Anastassiades et al. [11], was primarily used to identify and quantify pesticide residues in fruits and vegetables [12, 13]. The two official versions of the QuEChERS method are based on the International Official Method 2007.01 by the Association of Official Analytical Collaboration (AOAC) [14], and the European Standard Method EN 15662 (2019) [15]. The two variants differ in the buffer used, i.e., the AOAC uses the acetate buffer while the European standard uses the citrate buffer. Both versions of QuEChERS method feature several advantages: (i) the utilization of acetonitrile as solvent that can be injected into either a gas or a liquid chromatograph [13]; (ii) they are easily adaptable to different conditions [9]; (iii) are eco-sustainable due to the low extraction solvent amount, limited lab space and water requirements with respect to other extraction methods [16], and (iv) simple pretreatments [17]. Therefore, the QuEChERS method can ensure a rapid screening of many pollutants in many samples [18]. The identification and quantification of pollutants after extraction through the QuEChERS method can be performed by gas or liquid chromatography (GC or LC) using mass spectrometry (MS) or tandem mass spectrometry (MS/MS) as the detector.

The QuEChERS method has been used for the extraction of CECs from several matrices, especially the edible parts of crops, while few studies have been conducted for isolating and quantifying CECs from soils. Lesueur et al. [19] were the first researchers to apply this method to analyze various classes of pesticides in soil, obtaining satisfactory results so that the method was considered a promising alternative to traditional ones. Successively, different classes of pesticides were extracted from soils often using modifications of the QuEChERS method [20–22]. For example, Fernandes et al. [23] optimized the extraction of pesticides from soil by introducing sonication in the extraction step to better homogenize the samples, and modifying the adsorbents, and the amount of sample and water added during the extraction. Kaczynskì et al. [24] also modified the buffer and the clean-up used to improve the extraction of several herbicides from soil.

The QuEChERS method has been rarely used for the extraction of pharmaceuticals from soil [25]. In particular, each study was optimized modifying the QuEChERS method as a function of the specific pharmaceutical to be extracted. For example, Bragança et al. [26] tested different sample/solvent ratios, extraction solvents, times and processes to optimize the quantification of ibuprofen in soil using HPLC coupled with a fluorescence detector. Salvia et al. [27, 28] used the QuEChERS method to analyze 14 veterinary products, 11 hormonal steroids and 6 human contaminants in soil, by modifying the clean-up step with the use of two cartridges in tandem, i.e., SAX cartridge and Strata-X cartridge, respectively. Recently, García Valverde et al. [29] tested a modified QuEChERS method on 13 pesticides, 12 pharmaceuticals and 5 transformation products present in soil using anhydrous $MgSO_4$, Na_3 citrate·$2H_2O$, NaCl and Na_2H citrate·$1.5{\cdot}H_2O$ in the extraction phase and C18 columns in the clean-up phases. Successively, Martínez Bueno et al. [30] used the QuEChERS method modified by García Valverde et al. [29] to extract 30 CECs from water irrigation, tomato and soil samples.

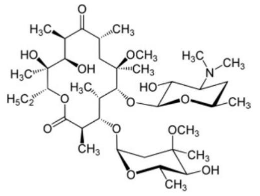
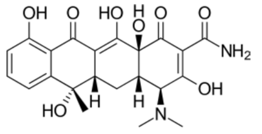
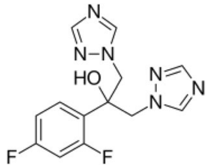
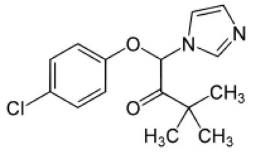
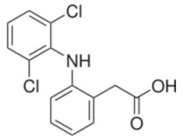
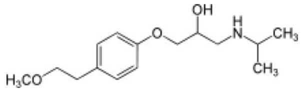
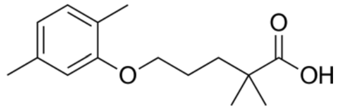
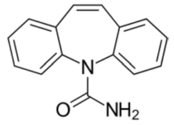
The objective of this work was to validate a modified QuEChERS method applied to the extraction from soils of eight worldwide-consumed scarcely studied, pharmaceuticals of different classes.

Methods

Chemicals and instrumentation

Acetonitrile (ACN), LC–MS grade methanol (MeOH), LC–MS grade water, magnesium sulphate anhydrous ($MgSO_4$), sodium acetate (Na acetate) and primary secondary amine (PSA) were purchased from Sigma-Aldrich. The extraction tube contained 1.5 g of Na acetate and 6 g of $MgSO_4$, (pH = 4.8), while the clean-up tube contained 900 mg of $MgSO_4$ and 150 mg of PSA. The QuE-Lab® Tubes used for the extractions were bought from Lab Instruments (Italy). The analytical standards (purity > 99%) of carbamazepine, clarithromycin, climbazole, diclofenac, fluconazole, gemfibrozil, metoprolol, and tetracycline were supplied from Lab Instruments (Italy), and their characteristics are shown in Table 1.

Table 1 Physicochemical properties of the selected contaminants of emerging concerns (CECs)

CECs	Molecular weight g mol^{-1}	Chemical structure	Chemical class	Water solubility mg L^{-1}	Log K_{OW}	pKa
Clarithromycin	748		Antibiotic	1.693 at 25 °C	3.16	8.99
Tetracycline	444.4		Antibiotic	231 at 25 °C	− 1.37	3.3
Fluconazole	306.27		Antifungal	4.363 at 25 °C	0.25	2.27
Climbazole	292.76		Antifungal	58 at 25 °C	3.76	6.49
Diclofenac	296.1		Anti-inflammatory	2.37 at 25 °C	4.15	4.15
Metoprolol	267.36		Beta-blocker	0.4 at 25 °C	1.88	9.7
Gemfibrozil	250.33		Antilipemic	11 at 25 °C	4.77	4.5
Carbamazepine	236.27		Antidepressant	18 at 25 °C	2.45	13.9

Analytical standards

Stock solutions of each pharmaceutical were prepared by dissolving 10 mg of the pure deuterated standard in 10 mL of acetonitrile or methanol. The multi-compounds stock standard solution was prepared by dissolving 1 mL of each standard in 20 mL of solvent, diluting to 50 mg L^{-1}, and storing at -18 ± 3 °C in the dark.

Soil samples collection and analysis

The soil samples used in this work were collected from three sites in Southern Italy. The first soil was located near a wastewater treatment plant (WWTP) station and cultivated with irrigated vegetables (Soil 1: Noci, 40°79′19″ N, 17°08′14″ E). The second soil was cultivated with irrigated apricots (Soil 2: Turi, 40°91′35″ N, 16°97′82″ E), and no river or WWTP station was nearby. The third soil was collected in the Alta Murgia National Park, in the municipality of Altamura (Soil 3, 41°03′08″ N, 16°30′65″ E), where no river or WWTP station were present. The three soils chosen feature different physicochemical properties that might affect the recovery of the contaminants.

Nine sub-samples of each soil, amounting to about 5 kg, were collected using an auger, at a depth between 0 and 20 cm with a grid sampling scheme. Each composite sample was air-dried at room temperature and sieved at 2 mm before analysis. Particle size was determined by the pipette method. The main physicochemical properties were measured according to conventional analytical methods described in Sparks et al. [31]. In particular, pH was determined in distilled water and KCl using a soil/water ratio of 1:2.5, electrical conductivity (EC) in distilled water using a soil/water ratio of 1:2, organic carbon (OC) content by the Walkley and Black method, total nitrogen (TN) by the Kjeldahl method, and available phosphorous (P_{ava}) by the Olsen colorimetric method.

Extraction

10 mL of MilliQ water was added to 5 g of each dried soil sample into a polypropylene centrifuge tube (50 mL) and shaken vigorously for 1 min by using a Vortex mixer at maximum speed (Vortex Fisher Scientific FB15013 TopMix). Then, 15 mL of ACN, 2 mL of MeOH and an aliquot of 17, 34 or 170 µL of the multi-compounds stock standard solution were added to achieve the concentrations of 50, 100 and 500 µg L^{-1}, respectively, and the tubes were hand-shaken for 1 min. Then, salting-out with acetate buffer (6 g $MgSO_4$, 1.5 g Na acetate) was performed to facilitate the separation between the organic and aqueous phases [32]. Successively, the tubes were immediately manually shaken for 1 min to prevent the formation of $MgSO_4$ conglomerates and centrifuged for 4 min at 3700 rpm. Clean-up step of samples was carried out by transferring an aliquot of 6 mL of the supernatant into a 15-mL centrifuge tube containing 150 mg PSA sorbent and 900 mg $MgSO_4$. The tubes were shaken for 1 min and centrifuged at 3700 rpm for 3 min. The extracts were filtered through a membrane filter (PVDF, 0.22 µm) and aliquots of 1.5 mL were transferred into screw cap vials. The same procedure was applied without the addition of the multi-compounds stock standard solution to check the possible presence of selected contaminants in the soil samples.

Figure 1 shows the scheme of the QuEChERS method used for the extraction of pharmaceuticals from soil samples.

Liquid chromatography tandem–mass spectrometry (LC–HRMS) analysis

A Thermo Scientific™ UltiMate 3000 UHPLC equipped with a degasser, a high-pressure gradient pump, a WPS autosampler and a column oven, and a Q Exactive mass spectrometer were used for data acquisition. 10 µL of each sample were injected in Accucore™ aQ C18 Polar Endcapped (2.6 µm; 100 × 2.1 mm) column (Thermo Fisher Scientific) and maintained at 40 °C. The mobile phases were composed of 4 mM ammonium formate with 0.1% formic acid in LC–MS grade water and 4 mM ammonium formate with 0.1% formic acid in LC–MS grade methanol. The system used a heated electrospray ionization source with the following parameters: sheath gas flow rate, 35 arbitrary units (a.u.), aux gas flow rate, 20 (a.u.), spray voltage, 3.8 kV, capillary temperature, 320 °C, S-lens RF level, 50 and aux gas heater temperature, 220 °C. The Orbitrap instruments run a full scan in positive mode at the following conditions: AGC target, 1e6, maximum IT, 200 ms, scan range, 70–900 m z^{-1}, and instrument resolving power (FWHM at 200 m z^{-1}), 140,000 (set at 70,000 for experimental matrices). Nitrogen was used as the sheath and auxiliary gas. Data were acquired and processed by the Thermo Xcalibur 4.0.27.10, Chromeleon, and Trace Finder 3.3 methods. For each CEC, 5-ppm mass tolerance was used for the extracted ion chromatogram.

Validation procedure

To test the recovery of each contaminant from the three soils, preliminary experiments were performed on the samples spiked with the multi-compounds stock standard solution up to the concentration of 100 µg L^{-1}. Simultaneously, other tubes were spiked with aliquots of each contaminant stock solution up to the same concentration and checked whether the multi-compounds stock solution would show any interference among contaminants during the recovery.

The validation method used was that reported by Caldas et al. [8]. In detail, the recovery was done using three levels of fortification for each contaminant, i.e., 50, 100 and 500 µg L^{-1}, and six replicates for each of them. The calibration curves for each compound in the matrix were obtained by plotting the peak area against the concentration of the corresponding calibration standards at the three calibration levels. The linearity of the calibration curves was evaluated using the coefficient of determination (r^2) of the analytical curves. Recoveries were calculated comparing the response of the analytes in the spiked samples and in the matrix extracts. The relative standard deviation (RSD%) of each concentration represented the precision of the validation method. The

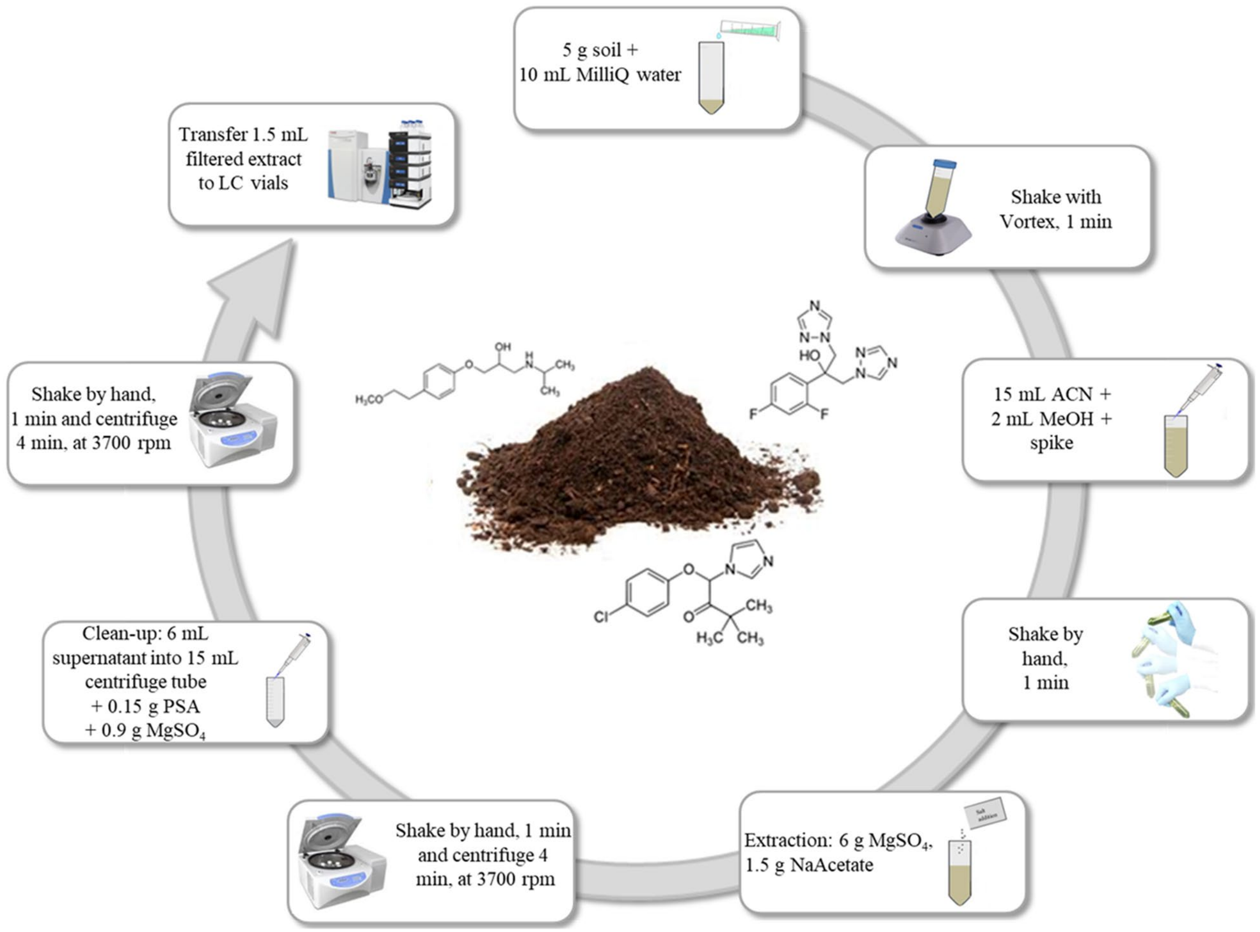


Fig. 1 Schematic presentation of the QuEChERS method for the extraction of pharmaceuticals from soils

Table 2 Main physicochemical properties of soil samples

Sample	pH		EC	OC	OM	P_{ava}	TN	Particle size fraction			Classification
	H_2O	KCl	µS cm^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	g kg^{-1}	Sand	Silt	Clay	USDA
								%			
Soil 1	8.2 (0.03)	7.9 (0.18)	205 (10.6)	10.6 (0.25)	18.3	30.2 (0.67)	1.1 (0.05)	10.5 (0.70)	44.9 (1.35)	44.6 (0.15)	Silty clay
Soil 2	8.1 (0.07)	7.1 (0.0)	194 (15.6)	15.7 (0.38)	27.1	44.7 (2.43)	1.5 (0.05)	17.3 (0.80)	35.6 (1.80)	47.1 (0.9)	Clay
Soil 3	7.9 (0.02)	7.4 (0.04)	244 (5.0)	28.7 (7.6)	49.5	150.0 (0.79)	2.4 (0.01)	33.1 (1.25)	49.7 (0.70)	17.2 (0.20)	Silty loam

Values in parentheses are the standard deviations

EC electrical conductivity, *OC* organic carbon, *OM* organic matter, P_{ava} available phosphorous, *TN* total nitrogen

linearity, LODs and LOQs were determined according to the ISO 11843-2 [33] referring to the calibration curve.

Results and discussion

Soil properties

The main physicochemical properties of the soil samples examined are referred in Table 2.

The three soils were slightly alkaline and showed different textures (silty clayey, clayey and silty-loamy) according to the USDA classification [34]. Furthermore, they differed for OC, TN and P_{ava} contents, which ranged between 10.6 and 28.7 g kg^{-1}, 1.1 and 2.4 g kg^{-1} and 30.2 and 150.0 mg kg^{-1}, respectively.

Parameters of the extraction method

With respect to the original method, the amount of sample weighed was reduced from 10–15 to 5 g [23, 35], so that the quantities of solvent, the waste produced, storage, labor required and time of analysis were reduced [36]. Water was added to soil in order to obtain a matrix with a high water percentage, similar to food matrices for which the method was originally implemented [25], and achieve pores more accessible to the solvent [36], so improving the extraction efficiency. The ACN has been chosen as the solvent because it was able to extract compounds with different polarities and was easily miscible with water permitting a relatively easy separation of phases by adding salts [37, 38]. Since soils show a stable pH due to their buffer capacity, ACN was not acidified, as reported by Mei et al. [39]. According to the AOAC 2007.01 method, the acetate buffer is used to reduce the effect of the matrix-pH on the ionization and/or degradation of organic compounds during the extraction phase. For salting-out, 6 g of $MgSO_4$ were added to facilitate the separation between the organic and aqueous phases [32].

A time of extraction of 4 min was chosen according to Bragança et al. [26], who found the maximum recovery for ibuprofen and its metabolites from soil after 4 min. The PSA was used in the clean-up step because it possesses a high chelating effect due to its structure composed by primary and secondary amines [40]. No C_{18} sorbent was combined with PSA as soils contained low amount of lipids and polar compounds that generally were retained in the matrix of PSA. With respect to the original method, 150 mg of PSA and 900 mg of $MgSO_4$ were used in the clean-up step instead of 50 mg and 150 mg, respectively.

LC–HRMS determination of pharmaceuticals residues

No detectable pharmaceuticals residues were found in soil samples prior to the experiments. The recoveries of the different pharmaceuticals from the three soils as average of three replicates are reported in Fig. 2, while the chromatograms of spiked samples from SOIL 1 (concentration 100 μg L^{-1} for each pharmaceutical) are reported in Fig. 3. All recoveries comprised between 72 and 113%, with the only exception of tetracycline, whose recovery was lower (about 46%). Vera et al. [40] reported that the application of the QuEChERS extraction method provided good results for different kinds of compounds isolated from various matrices, regardless their polarity. The non-significant differences observed among the three soils in terms of recoveries were in agreement with the results of Pinto et al. [36], who concluded that the binding of compounds to soil was not a determining parameter in the extraction process, because soil with different properties, such as the ones utilized in the present study, determined the same recovery of contaminants.

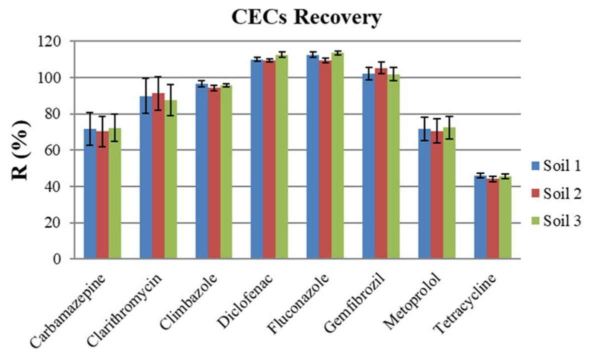


Fig. 2 Percentages of the extraction recoveries of pharmaceuticals

The low recovery of tetracycline may be ascribed to the use of $MgSO_4$, which may cause a strong decrease in the extraction of tetracycline due to its ability to complex with Mg^{2+} [41, 42]. Bourdat-Deschamps et al. [43] found a recovery of tetracycline lower than 9% with the use of $MgSO_4$ as desiccant agent. Orlando and Simionato [42] found that the mass of PSA used to eliminate interferences also determined a reduction of the extraction efficiency for tetracycline, so they used lower quantities of PSA (25 mg) in the clean-up step. Thus, in this study, the use of 150 mg of PSA might have hindered the extraction of tetracycline from soils. Furthermore, the use of acetate buffer might have reduced the recovery of tetracycline. In this regard, da Silva et al. [44] reported better results when the acetate buffer is replaced by the citrate–phosphate buffer. Finally, tetracycline features a very high water solubility and a K_{ow} below 1, which might have influenced negatively its recovery due to a high repartition in the water phase and a low concentration in the organic phase [40].

As previously reported, few studies have been conducted on the recoveries of pharmaceuticals from soils through the QuEChERS method, and few or none of this concern the pharmaceuticals considered in the present study. The results of previous works are summarized in Table 3.

For example, Manasfi et al. [46] used the QuEChERS method to extract diclofenac, metoprolol, clarithromycin, climbazole and carbamazepine from soils after irrigation with treated wastewater spiked with 10 μg L^{-1} of each contaminant. These authors found concentration levels in soil in the 1–30 ng g^{-1} (d.w.) range, however they did not report results in terms of recoveries and validation of the extraction method. Salvia et al. [38] tested a modified QuEChERS extraction method consisting of tandem SPE clean-up using both SAX and Strata-X cartridges, for the recovery of

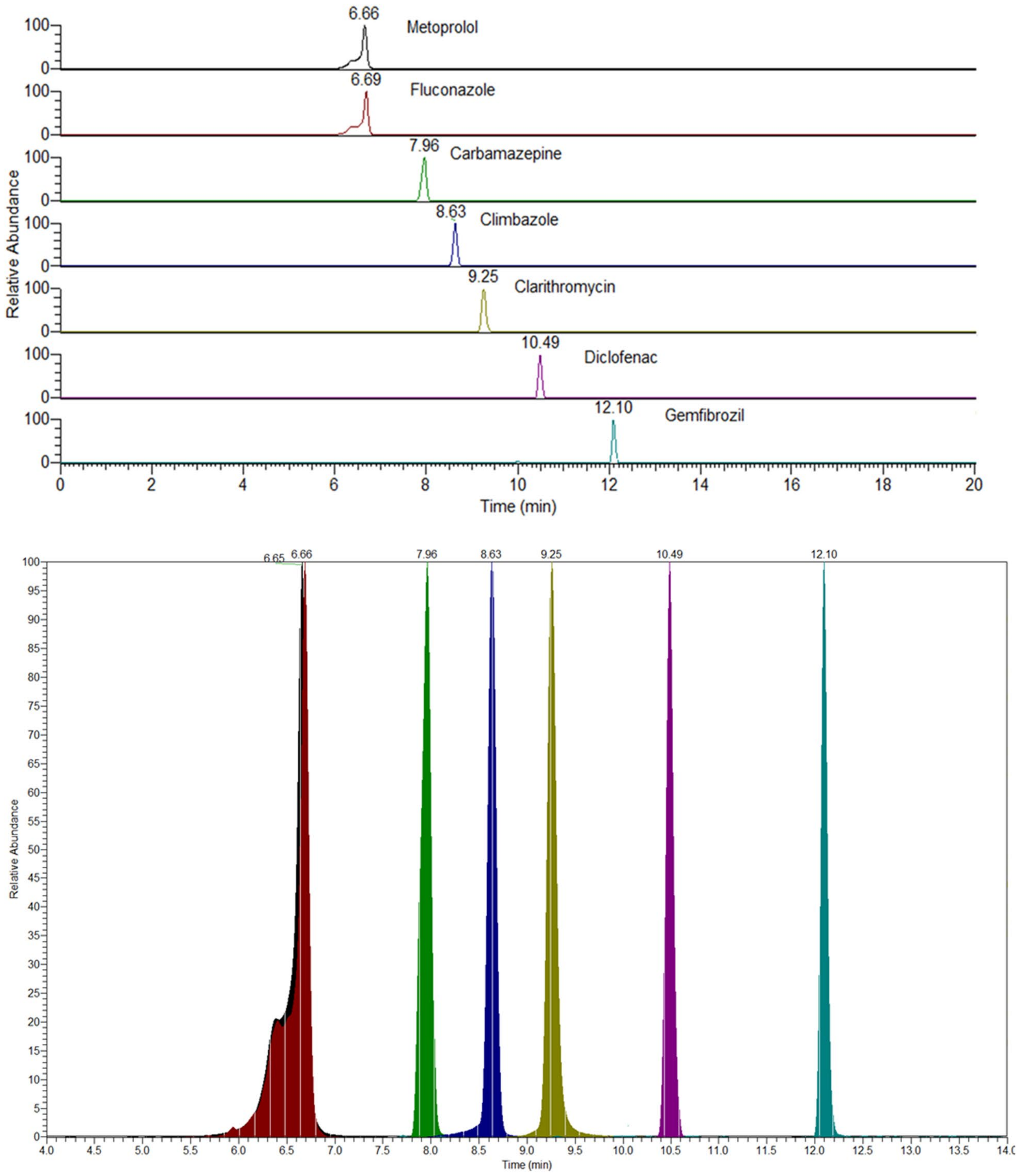


Fig. 3 Liquid chromatography–high-resolution mass spectrometry (LC–HRMS) chromatograms of soil "1" samples spiked with individual standard solutions (top) or multi-standards solution (bottom) up to the concentration of 100 µg L^{-1} of each pharmaceutical

31 compounds, including carbamazepine, from a clay loam soil and found recoveries comprised between 60 and 90%. da Silva et al. [44] tested a modified version of QuEChERS method with different combinations of solvents and salts to extract antibiotics from soils, achieving recoveries comprised between 90.2 and 103.1% for

Table 3 QuEChERS extraction of pharmaceuticals from soils

Type of soil and location	Analyte	Extraction procedure	Clean-up procedure	Analytical methods	Recovery	References
Sandy silty clay soil, local field, Hérault, France	Diclofenac Carbamazepine Metoprolol Clarithromycin Climbazole	The extraction was in accordance with Montemurro et al. [45] that tested different levels of acidification of the extraction solvent with formic acid (0.5 and 1%) or ammonium acetate	50 mg PSA, 150 mg C18, 900 mg MgSO4. Acidification was also evaluated after the cleaning phase by adding 0.05% formic acid	SCIEX ExionLC™ AD system coupled with the SCIEX X500R QTOF system (Sciex, Redwood city, CA, U.S.) with Turbo V™ source and Electrospray Ionization (ESI) operating in positive and negative mode	1–30 ng g^{-1}	[46]
Clay-loam soil not treated with manure or sludge, location not specified	Carbamazepine[a]	5 g of soil were spiked at 50 ng/g with MeOH solution containing 31 contaminants. 15 mL of ACN were added to the tube and the AOC acetate buffer was used in the extraction phase	Two steps of clean-up: SAX cartridge and Strata-X cartridge after conditioning the cartridges themselves with MeOH and 0.04 M citric acid	HP1100 chromatographic system. The LC system was coupled to a triple-stage quadrupole mass spectrometer 3200 QTrap with an electrospray ion (ESI) source (TurboV, AB Sciex)	> 90%	[28, 38]
Agricultural soils, location not specified	Clarithromycin Tetracycline	1 g of soil was extracted with ACN acidified or not with acetic acid; 6 g of Na_2SO_4 as separation salt with or without NaOAc	50 mg PSA, 150 mg C18 sorbent and 900 mg of Na_2SO_4	Ultra-High-Performance-Liquid Chromatography (UHPLC) Acquity system coupled to a 5500 QTRAP hybrid quadrupole-linear ion trap tandem mass spectrometer (QqLIT AB Sciex, Foster City, CA, USA)	90.2–103.1% for clarithromycin n.r.-144.7% for tetracycline according to the different solvents and salts adopted	[44]
Soils collected in 29 different points homogenously distributed along the Turia River (Central East of Spain)	Diclofenac Gemfibrozil Carbamazepine Clarithromycin Metoprolol Tetracycline Diclofenac Gemfibrozil	1 g of soil was extracted with ACN, and 6 g $MgSO_4$, 1.5 g NaCl, 1.5 g $C_6H_5Na_3O_7$ * 2 H_2O, and 0.75 g [HOC(COOH)($CH_2COONa)_2$ 1.5 H_2O] were added as salts	50 mg PSA, 150 mg anhydrous $MgSO_4$, and 50 mg of C18	1260 Infinity Ultra High-Performance Liquid Chromatograph (UHPLC) system coupled to a 6410 Triple Quad Mass Spectrometry from Agilent Technologies (Santa Clara, CA, USA) to perform tandem mass spectrometry (MS/MS) and an electrospray ionization source in both ion modes	Between 31 and 88%	[47]
Agricultural soils (tomatoes), Almeria (Spain)	Carbamazepine[a] Gemfibrozil[a] Diclofenac[a]	10 g of soil were extracted with acidified ACN (0.5% v/v, formic acid). 4 g of anhydrous $MgSO_4$, 1 g of $Na_3Citrate·2H_2O$, 1 g of NaCl and 0.5 g of $Na_2HCitrate·1,5H_2O$ were added as salts	750 mg of anhydrous $MgSO_4$, and 125 mg of C18	A Sciex Exion HPLC coupled to a Sciex 6500 + Triple Quad-LC–MS/MS from Sciex	79–88% for carbamazepine, 76–79% for gemfibrozil, 86–99% for diclofenac, according to the different concentrations	[29, 30]

Table 3 (continued)

Type of soil and location	Analyte	Extraction procedure	Clean-up procedure	Analytical methods	Recovery	References
Agriculture field (0–20 cm) in Tianjin, China	Clarithromycin[a]	5 g of soils were extracted with Na_2EDTA, ACN, and phosphate, acetate or citrate buffer, and NaCl as salt	d-SPE adsorbents (25 mg of PSA,10 mg of C18, and 100 mg of MgSO4)	UHPLC–MS/MS consisted of a Waters Acquity UPLCTM (Waters, USA) equipped with an ACQUITY UPLC BEHTMC18column (1.8 µm; 2.1 mm × 100 mm)	103.9–113.8%, according to the different buffers	[49]

[a] Authors validated the method

clarithromycin, whereas no or lower recoveries were achieved for tetracyclines using four QuEChERS methodologies, and a good recovery was obtained only with the use of citrate–phosphate buffer at pH 7.0, acetonitrile as solvent and Na_2SO_4 as desiccant salt.

The method presented in this study showed better results for some CECs in comparison to other modified QuEChERS methods. Applying a modified QuEChERS method based on the use of a mixture of salts in the extraction phase and the cartridge Strata-X 33U Polymeric Reversed Phase in the clean-up step, Carmona et al. [47] found recoveries lower than 70% for diclofenac, gemfibrozil and carbamazepine from soil samples. Malvar et al. [48] achieved a recovery below 55% for carbamazepine, using methanol as solvent, a mixture of $MgSO_4$ and NaCl as salts, and PSA + C18 in the clean-up step. García Valverde et al. [29] tested a modified QuEChERS method, based on acidifying the solvent, using a mixture of salts in the extraction phase, and the $MgSO_4$ + C18 in the clean-up step, to extract various CEC_s from soils and achieved recoveries of about 80% and 100% for gemfibrozil and diclofenac, respectively.

Validation of the extraction method

The results of the validation method are shown in Table 4.

The recovery percentages achieved were between 80 and 99% at any fortification level, and the recoveries of selected internal standards were between 70 and 120%. García Valverde et al. [29] obtained similar recoveries, i.e., 86% and 76% for diclofenac and gemfibrozil, respectively. Meng et al. [49] achieved recoveries between 103.9 and 113.8 for clarithromycin in soils spiked with 10–200 μg kg^{-1} of the contaminant.

Generally, the precision of the method for each analyte is calculated in terms of RSD % of the recovery, which must be in the range 0 to 19% for its validation [29]. The values of RSD % achieved in the present study by the modified QuEChERS method proposed ranged from 1.2 to 11.8%, thus the current RSD% validation condition is respected. The selectivity of the method was assessed by the analysis of three blank samples extracted with the same method, where no residues of contaminants were detected. Therefore, similarly to previous studies [50–52], matrix-matched calibration was used to compensate the matrix effect, as soil complexity may suppress or enhance some signals. The linearity of the calibration curves was evaluated by using the peak areas. The response of the detector was linear for each compound in the range considered, with r^2 higher than 0.9967 (Table 3). These results can be considered satisfactory because the correlation coefficients (r^2) were higher than 0.99 in all cases. In general, pharmaceuticals show good linearity in the range 1.5–500 μg kg^{-1} [26].

The LOD values ranged from 10 to 18.4 μg kg^{-1}, and the LOQ values from 20 to 36.9 μg kg^{-1} (Table 3). As the lowest spike concentration was 50 μg kg^{-1} and the values of LOD and LOQ were always lower than this value, the method can be validated in the range 50–500 μg kg^{-1}. The values of LOD and LOQ available in the literature are variable. For soil samples contaminated by pesticides, Caldas et al. [8] found LODs and LOQs in the ranges 4–17 μg kg^{-1} and 10–50 μg kg^{-1}, respectively. Values of LOQ between 0.5 and 2.5 μg kg^{-1} have been reported for diazinon, imidacloprid, myclobutanil, penconazole, thiacloprid and thiamethoxam [50], between 11.41 to 79.23 g kg^{-1} for organochlorine pesticides [51], and of 0.08 μg kg^{-1} for carbendazim [18]. Similar to the results of this work, LOQ values between 15 and 20 μg kg^{-1} have been reported for diclofenac, gemfibrozil, carbamazepine and codeine [47].

Table 4 Parameters used to validate the method

CEC	r^2	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Level of fortification					
				50 μg L^{-1}		100 μg L^{-1}		500 μg L^{-1}	
				R%	RSD	R%	RSD	R%	RSD
Gemfibrozil	0.9993	12.7	25.4	92.8	3.0	84.7	1.5	93.4	4.1
Clarithromycin	0.9993	12.6	25.3	79.8	3.4	80.8	4.3	87.7	3.1
Metoprolol	0.9982	15.3	30.6	85.6	3.2	83.2	2.9	86.1	2.8
Fluconazole	0.9993	10.8	21.6	92.7	6.2	86.3	7.5	95.0	6.7
Diclofenac	0.9982	18.4	36.9	89.0	11.8	82.7	11.8	94.1	6.7
Climbazole	0.9967	15.6	31.3	79.5	1.9	83.2	2.3	88.4	1.8
Carbamazepine	0.9984	10.0	20.0	93.9	1.3	93.1	1.2	99.1	11.4

LOD limit of detection, *LOQ* limit of quantification, *R* recovery percentage, *RSD* relative standard deviation

Conclusions

The proposed modified QuEChERS method can replace the traditional methods to identify and quantify residues of clarithromycin, fluconazole, climbazole, diclofenac, metoprolol, gemfibrozil, and carbamazepine in soil. This method shows many advantages including the possibility to extract a large number of samples simultaneously, the limitation of pre-treatments, the reduction of solvent and glassware uses and, therefore, the reduction of costs. In addition, this method is simple and fast and features a good recovery and LODs in the range of μg kg^{-1}.

To our knowledge, no other QuEChERS method has been validated for the quantification of metoprolol, fluconazole and climbazole extracted from soils, and results of this study may be useful for further studies on the fate of these contaminants in soils. Additional studies are needed for tetracycline, whose recovery was insufficient for the validation of the method. Finally, further studies appear necessary to implement the extraction method for contaminants with K_{ow} lower than 1.

Abbreviations

QuEChERS: Quick, easy, cheap, effective, rugged, and safe; LOD: Limit of detection; LOQ: Limit of quantification; CEC: Contaminant of emerging concern; AOAC: Association of Official Analytical Collaboration; GC: Gas chromatography; LC: Liquid chromatography; MS: Mass spectrometry; MS/MS: Tandem mass spectrometry; HPLC: High-performance liquid chromatography; SAX: Strong anion exchange; ACN: Acetonitrile; MeOH: Methanol; PSA: Primary secondary amine; UHPLC: Ultra high-performance liquid chromatography; R^2: Coefficient of determination; RSD: Relative standard deviation; USDA: United States Department of Agriculture; LC–HRMS: Liquid chromatography–high-resolution mass spectrometry.

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Author contributions

GB, CCo, FDM and AT conceived and designed the experimental strategies and manuscript. FDM, FM, CCa and AM performed all experiments and analyses. AT, FDM and FM organized all data to insert in the publication. GB and CCo supervised all experiments and analyses. AT, FDM and FM performed the statistical analysis. AT, FDM, FM wrote the original draft of manuscript. GB and CCo revised and validated the results. All authors read and approved the final manuscript.

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Availability of data and materials

The dataset used and/or analyzed during the current study is available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This manuscript is an original research and has not been published or submitted in other journals.

Consent for publication

All authors agreed to publish in the journal.

Competing interests

The authors declare that they have no competing interests.

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