



Determination study of contaminants of emerging concern at trace levels in agricultural soil. A pilot study

M. García Valverde, M.J. Martínez Bueno, M.M. Gómez-Ramos, A. Aguilera, M.D. Gil García, A.R. Fernández-Alba *

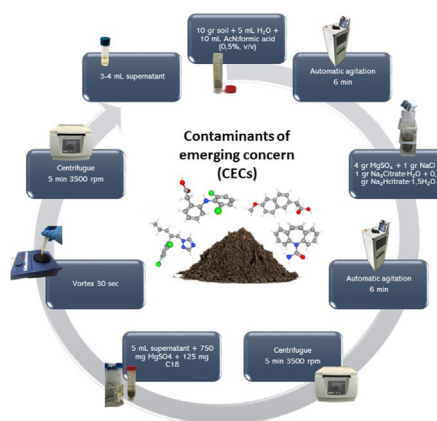
University of Almería, Department of Chemistry and Physics, Agrifood Campus of International Excellence (ceiA3), Ctra. Sacramento s/n, La Cañada de San Urbano, 04120 Almería, Spain



HIGHLIGHTS

- Three different extraction methods were developed and compared.
- All they affordable for routinely control laboratories
- Sensitive quantification of 30 contaminants of emerging concern in agricultural soil
- A modified QuEChERS method showed the best results.
- A pilot study was carried out in real agronomic conditions in a greenhouse.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we aimed to develop and validate a quick, easy, and robust extraction method for the simultaneous determination of 30 organic contaminants of emerging concern (CECs) including some transformation products in soil samples. Three different extraction methods based on an ultrasonic cylindrical probe (UAE), a pressurized liquid extraction (PLE), and a QuEChERS method were compared. Ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC-MS/MS) was used for identification and quantification of the target analytes. A modified QuEChERS method showed the best results in terms of extractability and accuracy. The extraction procedure developed provided adequate extraction performances (70% of the target analytes were recovered within a 70–99% range), with good repeatability and reproducibility (variations below 20%) and great sensitivity (LOQ < 0.1 ng/g in most cases). No matrix effects were observed for 70% of the compounds. Finally, the analytical methodology was applied in a pilot study where agricultural soil was irrigated with reclaimed water spiked with the contaminants under study. Of the 25 CECs added in irrigation water, a total of 13 pesticides and 5 pharmaceutical products were detected at concentration ranges from 0.1 to 1.2 ng/g (d.w) and from 0.1 to 2.0 ng/g (d.w), respectively.

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1. Introduction

Water scarcity is one of the largest issues at a worldwide level. During recent decades, the use of reclaimed water for agricultural irrigation has steadily increased as an alternative to solve the lack of availability of

* Corresponding author.

E-mail address: amadeo@ual.es (A.R. Fernández-Alba).

freshwater (Lavrnić et al., 2017). It is estimated that approximately 32% of reclaimed water is used for agricultural irrigation, 20% for landscape irrigation and 19% for industrial processes (Helmecke et al., 2020). The drawback is that this water type contain many different organic contaminants of emerging concern (CECs) because as it has been displayed by different authors, the removal procedures of these chemicals in water treatment plants are deficient (Calderon-Preciado et al., 2011; Martínez-Bueno et al., 2012). Recently, the European Parliament and the Council has published a new Regulation with regarding the minimum requirements for water quality and control for the safe reuse of treated urban wastewater (Regulation (EU) 2020/741, 2020).

Nowadays, analytical methods are focused on the determination of CECs, such as pesticides, pharmaceuticals or personal care products, in reclaimed water and vegetable grown with this type of water (Beltran et al., 2020; Calderon-Preciado et al., 2013; Wu et al., 2014). Anti-inflammatory drugs (diclofenac, ibuprofen or naproxen), anti-hypertensives (atenolol or furosemide), antiepileptic (carbamazepine), analgesic (acetaminophen) and antibiotics (azithromycin, ciprofloxacin, clarithromycin, ofloxacin and oxytetracycline) have been the pharmaceuticals more analyzed to date (Carmona et al., 2017; Pico et al., 2019). Whereas neonicotinoid insecticides (acetamiprid, imidacloprid or thiacloprid) and fungicides (thiabendazole, azoxystrobin, penconazole or diazinon) have been the pesticides more reported in such matrices (Acosta-Dacal et al., 2021; Perez-Mayan et al., 2020; Pico et al., 2019). Nevertheless, there is a large gap of knowledge on the extent to which terrestrial ecosystems are affected by the use of reclaimed water for agricultural irrigation. CECs come into contact with agricultural soil and those with low hydrophobicity are accumulated in the soil through interactions with organic material (Beltran et al., 2020). Thus, the determination of CECs in agricultural soils irrigated with reclaimed water for assessing potential environmental and human health effects is necessary.

Several extraction techniques have been applied to the analysis of soil samples (Andreu and Picó, 2019; Santana-Mayor et al., 2019). Most of them are based on ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE) or supercritical fluid extraction (SFE). Sonication through the cylindrical probe is more efficient than an ultrasonic bath but this last is more common for its low cost (Lesueur et al., 2008). In PLE the solid sample is extracted with solvent at high pressures and high temperatures. This extraction method has good results in the efficiency of the extraction process because of the solvent with high temperature can penetrate better to solid sample (Perez-Mayan et al., 2020). SFE is often preferred because it is faster, requires less solvent and has a lower risk of sample contamination (Wilga et al., 2008). However, all they often require a further clean-up step to the purification of the extract. In the last years, an extraction procedure called QuEChERS, a method typically used to extract pesticides from food samples, it has been applied to extract organic compounds from soil. The procedure is based on a salting-out extraction with acidified acetonitrile, followed by a dispersive solid-phase extraction (d-SPE). It is rapid, simple, requires low solvent consumption and allows the extraction of a large number of compounds at the same time. This method applied to soil was first reported by Lesueur et al. (2008). The good recoveries obtained in soil samples are turning the QuEChERS method into an interesting alternative to extract CECs from soil (Acosta-Dacal et al., 2021; Benedetti et al., 2020; Masia et al., 2015).

Soil matrices extracts usually have a high content of organic components (such as humic acids), and lipids which increase the viscosity of the sample, and as consequence, also the matrix effects derived from the presence of interfering substances in the injection vial. According to recent reviews, analytical methods for the determination of CECs in soil are scarce and mainly developed for sediments and sewage sludge (Benedetti et al., 2020; Luque-Munoz et al., 2017; Malvar et al., 2020; Martin-Pozo et al., 2019; Ponce-Robles et al., 2017). Also, most of them are only to specific chemical families of contaminants, such as pesticides (Feng et al., 2015; Lesueur et al., 2008; Li et al., 2013) or

pharmaceuticals, veterinary medicines and daily personal care products (García-Galan et al., 2013; Lee et al., 2017; Salvia et al., 2012). Therefore, the development of a multi-residue extraction method that allows the simultaneous analysis of several classes of organic compounds with different physicochemical properties at trace levels in agricultural soil is necessary.

In this context, the main objective of this study was to develop and validate a quick, easy and robust extraction method for the simultaneous determination of 30 CECs (including pesticides, pharmaceutical products and some of the main transformation products) in agricultural soil. Three different extraction methods based on an UAE, a PLE without clean-up step, and a QuEChERS method were compared. To our knowledge, a multi-residue and inter-family extraction method for agricultural soil analysis has never been proposed.

2. Materials and methods

2.1. Chemicals and reagents

30 of most frequently reported as agricultural and urban organic environmental contaminants in the literature were selected in this study (Martínez-Bueno et al., 2012). They comprise 13 pesticides (acetamiprid, azoxystrobin, carbendazim, diazinon, diuron, fluxapyroxad, imidacloprid, myclobutanil, penconazole, pymetrozine, thiabendazole, thiacloprid, thiamethoxam), 12 pharmaceuticals (acetaminophen, atenolol, caffeine, carbamazepine, ciprofloxacin, codeine, diclofenac, furosemide, gemfibrozil, hydrochlorothiazide, naproxen, ofloxacin) and 5 transformation products (4-methylamino-antipyrine, 4-amino-antipyrine, 4-formylamino-antipyrine, 4,4-acetylamino-antipyrine and carbamazepine-10,11-epoxy). Table 1 shows some of their physicochemical properties. Caffeine-13C,

Table 1
Physicochemical properties of all target compounds selected in this study.

Family	Compound	Log Kow	pKa	Koc	Water solubility
Antibiotic	Ofloxacin	-0.4	5.9	44	28,300
Insecticide	Pymetrozine	-0.2	4.1	246-7875	270
Analgesic	4-AAA*	-0.1	12.4	n.a	40,226
Insecticide	Thiamethoxam	-0.1	0.4	32-237	4100
Stimulant	Caffeine	-0.1	14	741-7762	21,700
Diuretic	Hydrochlorothiazide	0.1	7.9	12	722
b-blocker	Atenolol	0.2	9.6	n.a	13,300
Analgesic	4-FAA*	0.2	12.7	n.a	101,289
Antibiotic	Ciprofloxacin	0.3	6.1	61,000	30,000
Analgesic	Acetaminophen	0.5	9.4	20,844	14,000
Analgesic	4-AA*	0.5	4.1	n.a	727,617
Analgesic	4-MAA*	0.6	n.a	n.a	28,897
Insecticide	Imidacloprid	0.6	11.1	156-800	610
Insecticide	Acetamiprid	0.8	0.7	132-267	4250
Analgesic	Codeine	1.2	8.2	700	<1
Insecticide	Thiacloprid	1.3	0.5	408-1584	185
Fungicide	Carbendazim	1.5	4.2	122-2805	8
Antiepileptic	Epoxide-CBZ*	1.6	n.a	n.a	1340
Diuretic	Furosemide	2.0	3.9	110	73
Herbicide	Diuron	2.3	13.6	55-962	37
Fungicide	Thiabendazole	2.4	4.7	2500-4680	30
Antiepileptic	Carbamazepine (CBZ)	2.4	13.9	510	18
Fungicide	Azoxystrobin	2.5	0.9	210-580	7
Fungicide	Fluxapyroxad	3.1	12.6	496-1424	3
Fungicide	Myclobutanil	3.2	2.3	950	115
Anti-inflammatory	Naproxen	3.2	4.1	330	16
Insecticide	Diazinon	3.3	2.6	191-1842	60
Fungicide	Penconazole	3.7	1.5	786-4120	73
Analgesic	Diclofenac	4.0	4.1	245	2
Lipid regulators	Gemfibrozil	4.8	4.5	430	11

Kow: octanol/water coefficient; pKa value: negative log of the acid dissociation constant; Koc (ml/g): organic carbon sorption constant; Water solubility at 25 °C (mg/L): solubility in water; *Metabolites (Epoxide-CBZ: carbamazepine-10,11-Epoxi; 4-MAA: 4-methylamino-antipyrine; 4-AA: 4-amino-antipyrine; 4-FAA: 4-formylamino-antipyrine; 4-AAA: 4,4-acetylamino-antipyrine); n.a.: not available.

carbendazim-d3, malathion-d10 and dichlorvos-d6 were selected as internal standards to check the extraction efficiency. Dimethoate-d6 was used as injection standards for analytical efficiency. The reference standards with high purity (>98%) were acquired from Sigma-Aldrich (Steinheim, Germany), except codeine that was obtained by pill. Each pill contained 28.7 mg of codeine phosphate hemihydrate. Working solutions of pesticides and pharmaceuticals were prepared by dilution of the individual stock solutions at 10000 mg/L in AcN. Hydrochlorothiazide, acetaminophen and codeine were prepared in methanol at pH = 10 and a mixture water-methanol (50:50, v/v). These solutions were stored at -20 °C in amber screw-capped glass vials. For identification and quantification were daily prepared standard working solution at 1 mg/L.

For the optimization of the chromatographic and mass spectrometer conditions intermediate solutions at 200 µg/L were prepared. HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany) and LC-MS optima grade water from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (purity 98%) was obtained from Fluka (Buchs, Germany). Magnesium sulphate anhydrous (MgSO₄), sodium chloride (NaCl), sodium hydrogenocitrate sesquihydrate (Na₂HCitrate·1,5H₂O) and sodium citrate tribasic dihydrate (Na₃Citrate·2H₂O) were purchased from Sigma-Aldrich (Steinheim, Germany). Primary-secondary amine (PSA) was obtained by Supelco (Bellefonte, PA, USA) and Bondesil-C18 sorbents from Agilent Technologies (Santa Clara, CA, USA).

2.2. Sample collection

A tomato (*Solanum lycopersicum* L.) crop was grown in a greenhouse located in Almeria (Southeast Spain), under controlled agronomic conditions and using reclaimed water to drip irrigation obtained from a wastewater treatment station with an ultrafiltration/chlorination process. The mixture had a nitrate content below 0,04 meq/L, 169 mg/L of chloride and 106 mg/L of sodium, and pH and electrical conductivity values of 8.0 and 536 µS/cm, respectively. The greenhouse's surface (540 m²) was divided into two plots: one irrigated only with reclaimed water (control plot A) and the other with spiked reclaimed water (treatment plot B). No targeted organic contaminant residues were detected in the reclaimed water used to the study. The spiked reclaimed water used in the plot B was prepared to add to each one of the 25 selected CECs at 1 µg/L (considering the worst-case scenario, based on previous results of our research group) (Martínez-Bueno et al., 2012). No transformation products were added to the irrigation water to study the potential degradation of the precursor compounds (carbamazepine and metamizole). This solution was pumped through a dosing pump (3 L/h). The crop/soil was daily irrigated for 25 min. The total amount of spiked reclaimed water used in each plot was 2200 L. Irrigation water samples (100 mL) were taken once a week in clean polypropylene bottles from the exit of the drips at 10 cm depth, in each of the study plots. The agricultural soil contained 15% clay, 20% silt and 65% sand. It had 0.92% organic carbon, 100 ppm of total nitrogen, and pH and electrical conductivity values of 7.7 and 3240 µS/cm, respectively. Soil samples of the upper 10 cm layer were collected in polyethylene bags and transferred to the laboratory where they were sifted with a 2 mm diameter sieve and dehydrated in an oven at 30 °C for 24 h. Finally, samples were frozen and stored at -20 °C until their analysis.

2.3. Extraction method

Three extraction methods were assessed and compared in this work: (i) an ultrasonic extraction method with a cylindrical probe (UAE), (ii) a new pressurized liquid extraction method without clean-up step (PLE) and (iii) a QuEChERS method. In order to compare the results, solvent composition used in all they were the same. Prior to extraction step, 10 µL of a mix of internal extraction standards at 10 mg/L was added to check the extraction efficiency (caffeine-13C, carbendazim-d3, and dichlorvos-d6). Before injection, 100 µL of the final extract was evaporated to dryness under a nitrogen stream and reconstituted with 90 µL

of AcN:water solution (1:9, v/v) and 10 µL of dimethoate-d6 in all methods.

2.3.1. Pressurized liquid extraction (PLE) method

PLE experiments were carried out with an automated extraction system (EDGE®, CEM Corporation, Charlotte, NC, USA). The experimental conditions used in this study were based on a previously reported method by our research group to pesticide residue analysis in dry food commodity samples (Díaz-Galiano et al., 2021). Briefly, samples were extracted with 10 mL of AcN (0.5% v/v, formic acid) at 40 °C and 25 psi. Soil samples (10 g d.w) were introduced into an aluminium Q-Cup sample holder. A thin 0.3 µm glass fibre filter (G1 Q-Discs®) and two cellulose filters (C9 Q-Disc®) were used to provide structural support and to filter the sample before analysis. Q-Cups and Q-Discs® (G1 and C9 varieties) were also provided by CEM Corporation. The Q-Cup was then placed into the EDGE instrument alongside a 50 mL PTFE falcon tube to collect the sample extract. Total time of extraction was 6 min for the sample. In comparison with other reported procedures based on PLE, no clean-up sorbents have been used in this study. A diagram of the procedure is presented in Fig. 1.

2.3.2. Ultrasonic accelerated extraction (UAE) method

UAE experiments were carried out with a Sonoplus HD 3100 ultrasonic system (Badelin Electronic GmbH & Co., KG, Germany). The apparatus was equipped with a GM3100 high-intensity generator (100 W), a UW 3100 ultrasonic converter, an SH 70G standard horn, and a 3 mm-diameter titanium MS73 probe for 2–50 mL volumes. Pre-treated soil samples (10 g d.w) were hydrated with 5 mL of distilled water (vortexed for 30 s and left for 10 min) and extracted with 10 mL of AcN (0.5% v/v, formic acid) by sonication at a 75% amplitude for 2 min with an ultrasonic probe (seven extraction cycles of 15 s each plus a 2 s pause during this 120 s). Set-up conditions used in this study were based on a previously reported method by our research group to pesticide residue analysis in dry food commodity (Gil-García et al., 2018). After that, the tubes were centrifuged at 3500 rpm for 10 min. Then, 5 mL of supernatant was transferred to a 15 mL polyethylene tube with 750 mg of anhydrous MgSO₄, 120 mg of PSA and 120 mg of C18. Next, tubes were shaken with vortex for 30 s and centrifuged at 3500 rpm for 5 min. An operational scheme of the procedure is shown in Fig. 1.

2.3.3. Modified QuEChERS method

Sample extraction was based on a previously published method by our research group (García Valverde et al., 2021). Briefly, dried soil samples (10 g d.w) were rehydrated with 5 mL distilled water. Then the samples were vortexed for 30 s and left for 10 min. Subsequently, 10 mL of acidified AcN (0.5% v/v, formic acid) was added. The samples were shaken in an automatic axial extractor (AGITAX®, CirtaLab.S.L., Spain) for 6 min at 25 °C. Next, 4 g of anhydrous MgSO₄, 1 g of Na₃Citrate·2H₂O, 1 g of NaCl and 0.5 g of Na₂HCitrate·1,5H₂O were added and shaken once more. The samples were centrifuged at 3500 rpm for 5 min. Then, 5 mL of supernatant was transferred to a 15 mL polyethylene tube with 750 mg of anhydrous MgSO₄, and 125 mg of C18. The tubes were vortexed for 30 s and centrifuged at 3500 rpm for 5 min. Fig. 1 shown a scheme of the QuEChERS procedure used for the soil sample extraction.

2.4. Liquid chromatography-tandem mass spectrometry analysis

A Sciex Exion HPLC coupled to a Sciex 6500+ TripleQuand-LC-MS/MS from Sciex was used for the analysis. Chromatographic separation was performed on a Zorbax Eclipse Plus C8 of 1.8 µm × 2.1 mm × 100 mm (Agilent). Mobile phases were 0.1% formic acid in water optima (solvent A) and AcN (solvent B) at a constant flow rate of 0.3 mL/min. The optimized gradient program was: 10% of B (initial conditions) for 0.5 min, after a linear gradient up to 100% of B in 11.5 min; kept at

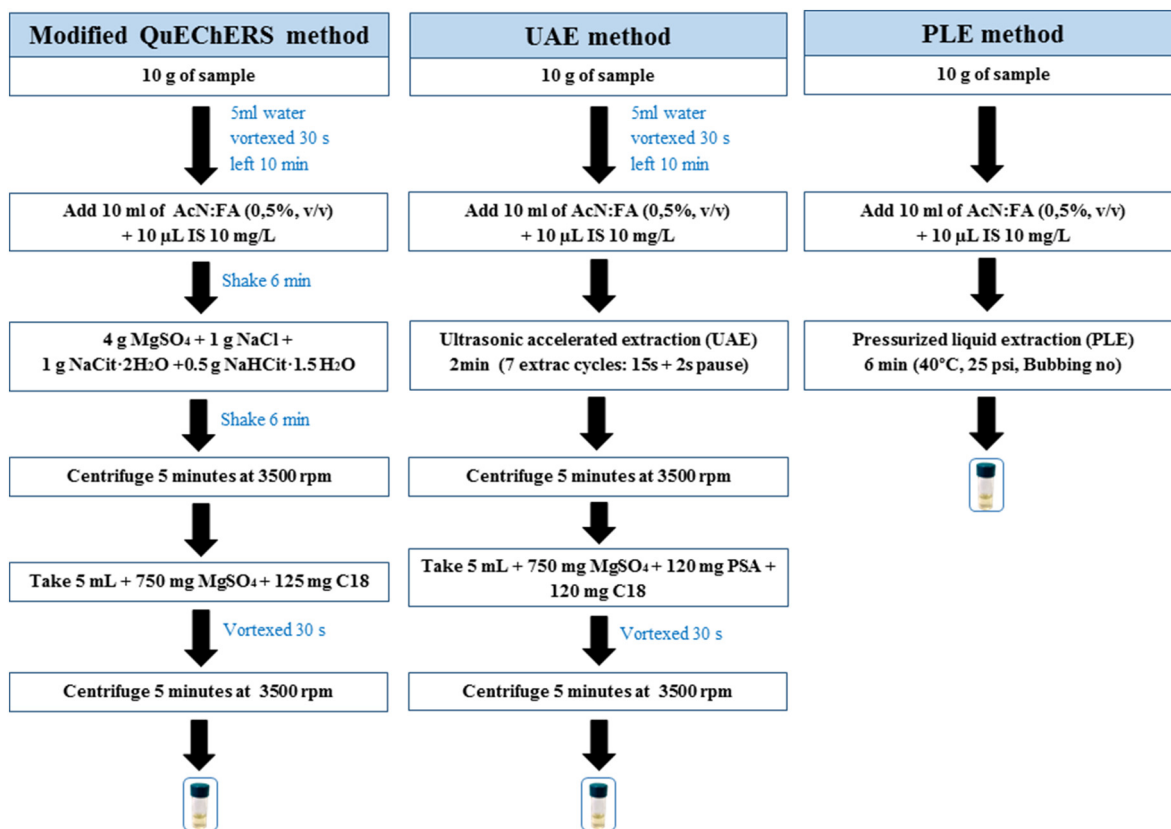


Fig. 1. Diagram of the three procedures used for the soil sample extraction.

100% of B for 4 min and finally, the mobile phase came back to the initial conditions (10% B). The total run time was 18 min and the injection volume was 5 μ L. The HPLC was coupled to a QqQ-MS/MS with an ESI source (turbo spray iondrive), operating with positive and negative ionization modes. The ionization settings used were: curtain gas, 20 (arbitrary units); GS1, 50 psi; GS2, 40 psi; and temperature, 500 $^{\circ}$ C. The ionspray voltages were set at 5000 and -4500 V in positive and negative ionization mode. Nitrogen was used as the nebulizer gas and collision gas.

For the optimization of the chromatographic and mass spectrometer conditions individual pesticides and drugs standard solutions at 200 μ g/L were used. The solutions were infused directly into the MS system in full-scan mode. The most intense ion was chosen as the precursor ion. Next, in product-ion mode the optimal collision energy (CEs) values for the two most intense transition were selected; the most intense ion was selected as the quantifier ion (SRM1) and the second ion as the qualifier ion (SRM2). The optimal mass spectrometric parameters for each target compound are presented in Table S1 as supplementary material. In the SCIEX OS acquisition and quantitative software (SCIEX) was applied schedule SRM with a retention time window of 0.4 min.

2.5. Analytical performance and quality control

The validation of the analytical approaches was performed according to the SANTE European Guideline 12682/2019 (SANTE/12682/2019, 2019). The analytical parameters evaluated were sensitivity, linearity, matrix effect, trueness (in terms of recovery), precision (in terms of method repeatability) and selectivity. The more demanding requirements regarding mass spectrometric confirmation currently set by EU regulations were taken into account for identification and confirmation of the target compounds (Directive 96/23/EC, 2002). These criteria were: The quantification transition (SRM1) with $s/n \geq 10$; the detection transition (SRM2) with $s/n \geq 3$; retention time ± 0.1 min with reference

to standard and comparing of fragment ion area with precursor ion area (ion ratio) with a value $\pm 30\%$.

In order to ensure quality measurements, each day before analysis a control standard mixture (2 ng/L) containing targeted analytes was injected to check the performance of the HPLC, analytical column, and QqQ-MS/MS system. Continuous monitoring of the quality of the analytical procedure was carried out through the inclusion of blanks (solvent) during the day-work sequence. No target analyte was detected in solvent blanks.

3. Results and discussion

3.1. Optimization of the extraction methods

Optimization of three proposed methodologies was carried out using blank soil sample (10 g d.w) spiked at 500 ng of each standard by triplicate. Soil samples acquired from control plot A were analyzed. No targeted compound residues were found in them. Therefore, they were selected as blank material. The spiked soil samples were stored 24 h at 25 $^{\circ}$ C until the total evaporation of the solvent and the correct balance between analytes/matrix before their extraction. The results of the recovery experiments were used to determine the effectivity of the extraction.

Firstly, PLE method optimisation was focused on the extraction solvent. The experimental conditions applied were based on a previously published method by our research group (Diaz-Galiano et al., 2021). In that work, satisfactory recoveries for the pesticide extraction from dried samples were obtained using AcN and a temperature of 40 $^{\circ}$ C. In this occasion, better results for pharmaceutical products were found when extraction organic solvent contained 0.5% of formic acid because a slightly acidic pH favours the extraction of basic compounds. The recoveries of diclofenac, furosemide, naproxen, gemfibrozil, and two transformation products of metamizole (4-AAA and 4-FAA) were 58%,

39%, 54%, 42%, 40%, and 33%, respectively, using AcN:0.5%FA. Values below 15% were obtained for all of them using only AcN as extraction solvent. Data included in Table S2 in supplementary material.

Secondly, UAE method was assessed. The parameters used to the extraction were previously optimized by our group (Gil-García et al., 2018). According to results reported, we decided to select 75% amplitude by 120 s (seven extraction cycles of 15 s with a 2 s of pause) because recoveries are better when the number of cycles decreases and extraction time per cycles increases. Thus, in this work, UAE method optimisation was focused on the influence of clean-up sorbents. In the beginning, the UAE method was done without a clean-up step. The results were slightly higher for all target compounds when a mixture of clean-up sorbents (MgSO₄, PSA and C18) was used. For example, the insecticide thiacloprid and the metabolite of the antiepileptic carbamazepine (carbamazepine-10,11-epoxide) were recovered a 68% without clean-up step. Instead, they were recovered a 74% using clean-up sorbents. Data included in Table S3 in supplementary material.

Finally, citrate QuEChERS method was evaluated in terms of the extraction organic solvent and clean-up sorbents. Two of the most commonly used extraction organic solvents were compared (AcN vs MeOH). No significant differences were observed in the results obtained among them. Taking into account that MeOH produces a greater matrix effect due to the co-extraction of a greater number of matrix interferences, AcN:0.5% FA was finally selected as the extraction solvent (Annesley, 2007). In relation to clean-up sorbents, we examined the use of PSA salt. As expected, the results showed that PSA had a high chelating effect, retaining fatty acids and other polar compounds from the organic extract (Caldas et al., 2011). For example, diclofenac, furosemide and naproxen showed recovery values below 22% using the conventional QuEChERS clean-up with PSA. However, their recoveries were 99%, 66% and 93%, respectively, when PSA was removed. Therefore, PSA sorbent was eliminated from the clean-up step. Data of each experiment can be seen in Table S4 in supplementary material. Last, the influence of an additional clean-up step was also tested. For that, the final extracts were filtered with a 0.45 µm syringe filter. No significant differences in the results were observed. Thus, the filtration step was not included in the final method.

3.2. Comparison of extraction methods

Fig. 1 shown a flow chart of the three proposed methodologies to quickly compare the operational parameters of each one of them. A summary of the main experimental conditions tested with each method is presented in Table 2. The composition and volume of organic solvent used for the extraction of analytes from the matrix (10 mL AcN:0.5%FA) as well as the amount of soil sample treated (10 g d.w) were similar for the three proposed methods.

Before UAE and QuEChERS extraction, dried soil samples were rehydrated with 5 mL of distilled water, in agreement with the DG-SANTE. The SANTE document recommends the addition of water to

dry matrices before their extraction to improve the extraction efficiency (SANTE/12682/2019, 2019). This step makes sample pores more accessible to the extraction solvent improving the extraction efficiency of polar compounds. Nevertheless, the sample hydration was avoided in the PLE method since higher energy extraction conditions were applied in this approach (Díaz-Galiano et al., 2021).

PLE method was the fastest approach (6 min by sample), whereas UAE and QuEChERS methods required two steps, one for sample extraction (7 min and 17 min, respectively by sample) and other for a clean-up step (5.5 min). Nevertheless, the QuEChERS method allows the simultaneous and automatized extraction of up to 12 samples by run. PLE method also allows the automatized extraction but requires high-cost equipment. Our extraction method based on PLE was faster than ones reported by Pérez-Mayan et al. (2020) and Masia et al. (2015) for the determination of pesticides in agricultural soils or by Malvar et al. (2020) for the determination of pharmaceuticals in sludge. Both methods required a total extraction time for 16 min. Pérez-Mayan et al. (2020) required two extractions with two different solvents and temperatures whereas Masia et al. (2015) required an extraction for 7 min of heated. On the other hand, QuEChERS method proposed in this paper was less time consuming than that proposed by Salvia et al. (2012) and Malvar et al. (2020) to extract organic contaminants from soil and sludge, respectively. Salvia et al. (2012) reported an extraction in just 7 min but then, two clean-up steps by solid-phase extraction were required. In the approach published by Malvar et al. (2020) the agitation steps were done with a vortex, whereas we used an automatic agitation system.

Recoveries were calculated comparing the response of the analytes in spiked samples and the response in matrix extracts after the extraction process. Fig. 2 shows the recovery results obtained to selected CECs depending on the extraction method. As can be seen, (two transformation products of analgesic metamizole (4-AA and 4-MAA) could not be recovered with any approach. Only the proposed QuEChERS method allowed the recovery of the antibiotics ofloxacin, and ciprofloxacin, but below to 5% in both cases. The repeatability was similar for the three proposed method (RSD below 6%). Recoveries obtain by PLE had a range from 9 to 89% with a mean value of 46% (RSD 46%). Recovery values were lower than 50% for 12 of 26 recovered compounds using PLE. UAE method It presented recoveries ranged from 12 to 101% with a mean value of 62% (RSD 39%). The ultrasonic cylindrical probe allowed to enhance the efficiency of the extraction process and to duplicate the recovery values of three pharmaceuticals (acetaminophen, codeine, and carbamazepine-10,11-epoxide) and two pesticides (carbendazim and thiabendazole). The recoveries obtained by Malvar et al. (2020) to carbamazepine and its main metabolite carbamazepine-10,11-epoxide were 72% and 56%, respectively using an ultrasonic bath. In the present study, values of 87% to carbamazepine and 74% for its metabolite were obtained. Similar results were reported by Lesueur et al. (2008). They achieved recovery values of 50% to carbendazim and 60% to diuron using an ultrasonic cylindrical probe while their recoveries were 62%

Table 2
Experimental conditions evaluated with each extraction method.

Sample amount (g)	PLE		UAE		QuEChERS		
	10	10	10	10	10	10	10
Hydration (mL)	–	–	5	5	5	5	5
Extraction solvent	AcN	AcN 0.5%FA	AcN 0.5%FA	AcN 0.5%FA	AcN 0.5%FA	MeOH 0.5%FA	AcN 0.5%FA
Total volumen (mL)	10	10	10	10	10	10	10
Clean-up	–	–	–	MgSO ₄ , PSA, C18	MgSO ₄ , PSA, C18	MgSO ₄ , PSA, C18	MgSO ₄ , C18
Total extraction time (min, per sample)	6	–	2 + 5 ^a	–	12 + 5 ^a	–	–
Total clean-up time (min, per sample)	–	–	–	0.5 + 5 ^a	0.5 + 5 ^a	–	–
Total time of method (min, per sample)	6	–	7	12.5	22.5	–	–
Total number of samples by run	12	–	1	–	12	–	–
Total time of method (min, per 12 samples)	72	–	84	150	22.5	–	–

^a Centrifugation step.

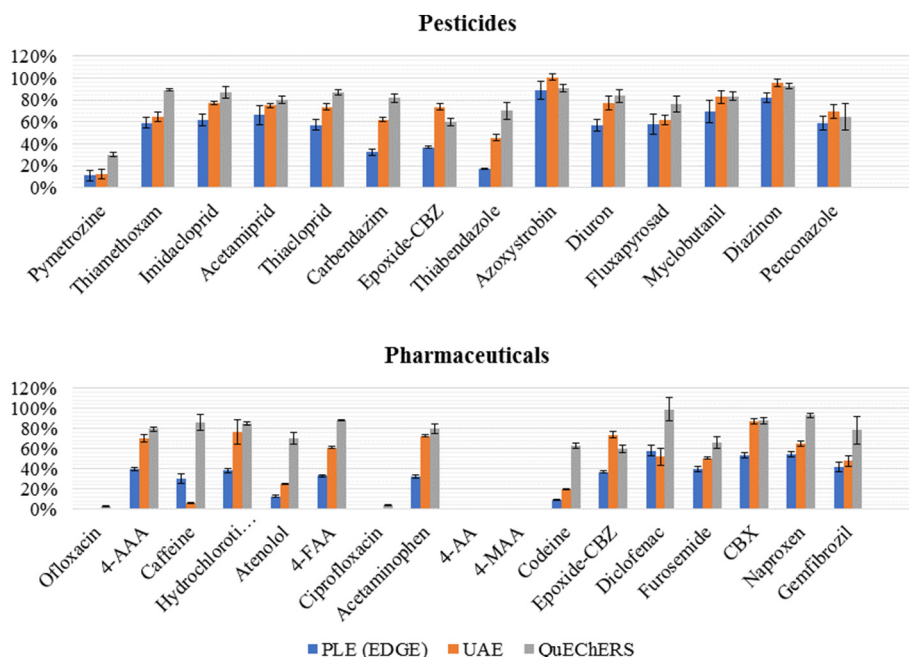


Fig. 2. Average recovery values (%) of the CECs at 50 ng/g from soil with the three extraction methods studied (error bars representing the standard deviation).

and 77%, respectively, with the proposed method in this work. This slight difference in the results can be explained by the diameter of the probe used in the extraction. Lesueur et al. (2008) used a probe with a diameter of 12.7 mm while in our study a smaller probe (3 mm) was used. That allows better penetration of the solvent into the matrix. The modified QuEChERS extraction method without PSA provided the highest extraction recoveries ranged from 24 to 120% (mean value 79%, RSD 33%). The recoveries were higher than 70% for all recovered analytes, except to pymetrozine (30%), codeine (63%), carbamazepine epoxide (60%), furosemide (66%), and penconazole (65%). These values were higher than other investigation previously published. According to the research of Carmona et al. (2017) diclofenac, gemfibrozil, naproxen and carbamazepine were recovered in values below 70% from soil samples. Malvar et al. (2020) reported recovery values below 55% for caffeine, carbamazepine and carbamazepine-10,11-epoxide. However, our extraction method also based on QuEChERS method recovered these compounds above 79%. The results obtained to some pesticides were also better than those reported by Fernandes et al. (2013). For example, azoxystrobin, diazinon or myclobutanil were recovered below 80% while values ranged from 82% to 93% were obtained with our method. In all proposed methods, the recoveries of the selected internal standards (caffeine-13C, carbendazim-d3, malathion-d10, dichlorvos-d6 and dimethoate-d6) were between 70 and 120%.

In summary, average recoveries obtained using UAE and QuEChERS were better than PLE, whose values were higher than 70% for many of the analytes studied. At last, the QuEChERS extraction method was selected based on the results obtained (higher recoveries) and taking into account the extraction times and tedious protocol associated with the UAE technique.

3.3. Method validation

Linearity, matrix effect, limit of quantification (LOQs), precision (repeatability) and trueness (recovery) were evaluated according to the European Union quality control guidance document (SANTE/12682/2019, 2019). A modified QuEChERS method using AcN:FA (0.5% v/v) without PSA was chosen as the best extraction method. Table 3 summarized the validation results obtained using the modified QuEChERS method proposed in this study.

The sensibility of the method was evaluated in terms of limit of quantification (LOQs). LOQs were estimated as the lowest concentration level in the matrix with a signal to noise ratio (S/N) of 10 to the first transition (quantitation transition) and 3 to the second transition (confirmation transition). As can be seen in Table 3, more than 75% of the target CECs (23/30) showed LOQ of 0.05 ng/g. The diuretic furosemide, the analgesics acetaminophen, the antibiotic ciprofloxacin and 4-FAA (a metabolite of metamizole) presented the highest LOQs (0.5 ng/g), followed by the anti-inflammatory naproxen, the antibiotic ofloxacin and the metabolite 4-AAA (0.1 ng/g). These values were lower than those published in the literature to soil samples. Acosta-Dacal et al. (2021) reported LOQs to diazinon, imidacloprid, myclobutanil, penconazole, thiacloprid and thiamethoxam between 0.5 and 2.5 ng/g, Lesueur et al. (2008) reported a LOQ of 0.08 ng/g for carbendazim and Carmona et al. (2017) obtained LOQ values between 15 and 20 ng/g to diclofenac, gemfibrozil, carbamazepine and codeine.

The linearity and matrix effect were evaluated using areas obtained of calibration curves prepared in matrix and solvent at seven concentration levels from 0.05 to 50 ng/g (range from LOQ to one hundred times more). Satisfactory results were observed because correlation coefficients (r^2) were higher than 0.99 in all the cases (see Table 3). Matrix effect (ME) was studied comparing the slope of the calibration curve in the matrix with the slope of the calibration curve in the solvent. Results showed that 70% of the targeted CECs not presented matrix effect ($\leq 20\%$), 26% shown intermediate matrix effect (between 20 and 50%) and only one, ofloxacin had strong effect matrix (62%). These results were better than other previously published in the literature (Acosta-Dacal et al., 2021; Fernandes et al., 2013; Masia et al., 2015).

Recovery studies were evaluated from spiked samples at 10 and 50 ng/g by triplicate. Recoveries were calculated comparing the response of the analyte in spiked samples and the response in matrix extracts after the extraction process at the concentration level previously mentioned. Two transformation products of analgesic metamizole (4-AA and 4-MAA) were not recovered, whereas two antibiotics (ofloxacin and ciprofloxacin) were extracted below 5%. An explanation for these low recoveries could be related to the fact of all them present very high water solubility values (≥ 25 mg/L), the pKa ranged from 4 to 6, and the Log Kow was below 1, suggesting a possibly high repartition in the water phase and as a consequence, a low concentration in the analyzed organic phase, which explains the low recoveries obtained (Vera

Table 3
Validations results using the modified QuEChERS method for soil samples.

Compound	Log Kow	LOQ (ng/g)	Linearity (r2)	ME (%)	Recovery (%)		Inter/intraday (%)
					10 ng/g	50 ng/g	
Ofloxacin	-0.4	0.1	0.999	-65%	-	3%	7/18
Pymetrozine	-0.2	0.05	1.000	-6%	30%	30%	1/12
4-AAA	-0.1	0.1	0.994	-7%	73%	79%	4/19
Thiamethoxam	-0.1	0.05	0.999	-9%	87%	89%	1/6
Caffeine	-0.1	0.05	1.000	-6%	72%	86%	1/16
Hydrochlorothiazide	0.1	0.05	1.000	1%	85%	85%	5/18
Atenolol	0.2	0.05	0.998	-25%	73%	75%	3/4
4-FAA	0.2	0.5	0.999	0%	81%	89%	3/4
Ciprofloxacin	0.3	0.5	0.999	-29%	-	4%	15/18
Acetaminophen	0.5	0.5	0.999	-5%	76%	80%	5/20
4-AA	0.5	0.05	0.999	-2%	-	-	4/10
Imidacloprid	0.6	0.05	1.000	-16%	86%	87%	2/15
4-MAA	0.6	0.05	1.000	-20%	-	-	3/19
Acetamiprid	0.8	0.05	0.998	-11%	83%	80%	8/10
Codeine	1.2	0.05	0.991	-7%	50%	63%	2/5
Thiacloprid	1.3	0.05	0.998	-20%	82%	87%	3/6
Carbendazim	1.5	0.05	0.998	-17%	70%	82%	4/4
Epoxide-CBZ	1.6	0.05	0.995	-13%	68%	60%	3/10
Furosemide	2.0	0.5	1.000	2%	63%	66%	15/7
Diuron	2.3	0.05	0.998	-11%	79%	83%	2/6
Thiabendazole	2.4	0.05	0.992	-15%	62%	70%	7/19
CBZ	2.4	0.05	1.000	-26%	79%	88%	6/3
Azoxystrobin	2.5	0.05	0.992	-33%	71%	90%	5/6
Fluxapyroxad	3.1	0.05	0.999	-21%	73%	76%	4/3
Myclobutanil	3.2	0.05	0.999	-17%	81%	83%	15/15
Naproxen	3.2	0.1	1.000	-17%	82%	93%	3/9
Diazinon	3.3	0.05	1.000	-34%	84%	93%	4/6
Penconazole	3.7	0.05	0.999	-30%	73%	65%	7/19
Diclofenac	4.0	0.05	0.997	-18%	86%	99%	15/10
Gemfibrozil	4.8	0.05	0.999	-33%	76%	79%	9/13

LOQs: Limits Of Quantification; Linearity expressed by the correlation coefficient; ME: Matrix effect; Inter/Intra repeatability expressed as relative standard deviation (RSD, in parenthesis).

et al., 2013). Considering only the analytes that were recovered above 5%, more than 80% of targeted CECs were recovered above 70%, four were between 60% and 66%, and only one was extracted below 50% (see Table 3). The results were compared with those from previous research. Masia et al. (2015) reported recoveries lower than 80% to carbendazim, diazinon, diuron, imidacloprid and thiabendazole, whereas we obtained values above 83% to all them. Malvar et al. (2020) obtained average recoveries to caffeine (25%), carbamazepine (53%), or carbamazepine 10,11-epoxide (48%) lower than our results, which were higher than 70% for the above-named.

Intra and inter-day precision (repeatability/reproducibility) were calculated for each analyte from results obtained of the recovery study in terms of relative standard deviations (RSD, %). The repeatability was acceptable with values ranged from 0% to 19%. Finally, the specificity/selectivity of the analytical methodology was assessed through the analysis of three blank samples extracted by the proposed method. No other significant peaks ($S/N > 3$) were found at the specific retention times of the target pesticides.

3.4. Application of the method to real samples

The developed analytical method was applied to agricultural soil samples obtained from a pilot study carried out under controlled real conditions in an experimental farm located in Almería (Spain) for sixteen weeks in 2020. Some examples of chromatograms of the identified compounds in the agricultural soil samples analyzed are shown in the Fig. 3. The recoveries of each internal standard in all samples (extraction and injection) were between 75 and 100%.

The distribution and average concentration of the CECs detected in the irrigation water samples (16 samples) and in the agricultural soil samples (3 samples) irrigate with contaminated reclaimed water can be seen in Fig. 4. The measured concentrations of CECs in contaminated reclaimed water were generally between 0.9 µg/L and 1.1 µg/L, except to the diuretics furosemide (0.2 µg/L) and hydrochlorothiazide (0.4 µg/L),

and the insecticide diazinon (0.4 µg/L). These low concentrations can be related to losing in the irrigation system and/or the reservoir tank used for storing the contaminated recycled water solution in the field, due to degradation processes. As it has been found in literature, both furosemide, hydrochlorothiazide, and diazinon can be degraded by exposure to strong UV light (Cies et al., 2015; Mansour et al., 1997). The results could suggest that furosemide was highly sensitive to UV exposure with more than 30% of the compound degraded after 3 h of treatment, whereas diazinon was 50% degraded after 5 days of exposure to sunlight.

Out of the 25 CECs added compounds in irrigation water, a total of 13 pesticides and 5 pharmaceutical products were detected in soil samples irrigated with contaminated reclaimed water. None of the selected transformation products was found in the agricultural soil samples analyzed. The pesticides concentration levels ranged from 0.1 to 1.2 ng/g (d.w.), whereas pharmaceuticals were found between 0.1 and 2.0 ng/g (d.w.). As can be seen in Fig. 4, in general, CECs with low and medium polarity ($\text{Log Kow} \geq 2.5$) shown a greater accumulation rate in soil. The fungicides penconazole and myclobutanil were the target compounds detected at the highest concentrations (2.0 ng/g d.w.). Considering the amount of pesticide added of each and the irrigation time, their accumulation rates were 62%, and 49%, respectively. Their high octanol-water partition coefficient ($\text{Kow} > 3.0$) as well as their soil sorption coefficient ($\text{Koc} > 500$) implying a strong binding to soil and therefore low mobility in soil (see Table 1). Similar concentrations to both substances were found by Acosta-Dacal et al. (2021) in different types of agricultural soil samples (up to 3.9 ng/g to penconazole and 2.4 ng/g to myclobutanil). Pymetrozine also showed a high accumulation rate (38%). Despite its low Kow (-0.2), it presents a high Koc, which explains that this compound can firmly be fixed in the organic matter of the soil and tends to accumulate in it. Acetamiprid, diuron and diazinon were the pesticides found at the lowest concentration levels in the soil samples, 0.1, 0.4 and 0.4 ng/g d.w, respectively.

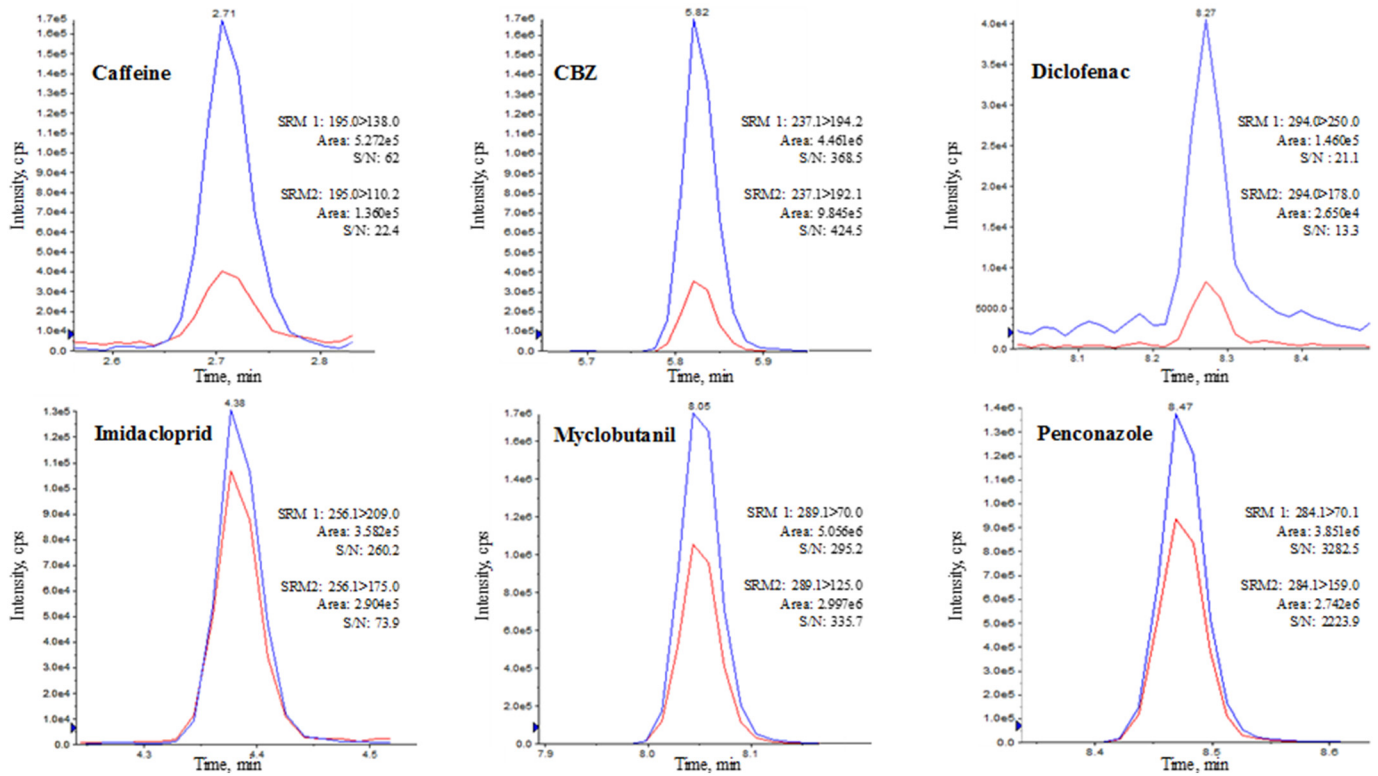


Fig. 3. Selected Ion Chromatogram (XIC) of some of the CECs detected in agricultural soil samples irrigated with reclaimed water.

Regarding pharmaceutical products, the antiepileptic carbamazepine was the compound detected at the highest levels in the soil samples, up to 1.2 ng/g d.w, whereas atenolol was found at lowest concentrations (0.1 ng/g d.w). Similar data were found by Beltran et al. (2020). They explained the low concentrations found to atenolol based on its high solubility in water (30,000 mg/L) and its low

Kow (0.2). Caffeine was detected at a concentration of 0.9 ng/g which suppose an accumulation percent in the soil of 21%. Caffeine has been one of the pharmaceutical most detected in soil (Pico et al., 2019). Diclofenac should be the most accumulated compound in the soil due to its low solubility in water and its high Kow value (4.0), however, it showed an accumulation rate of 10%. Carter et al.

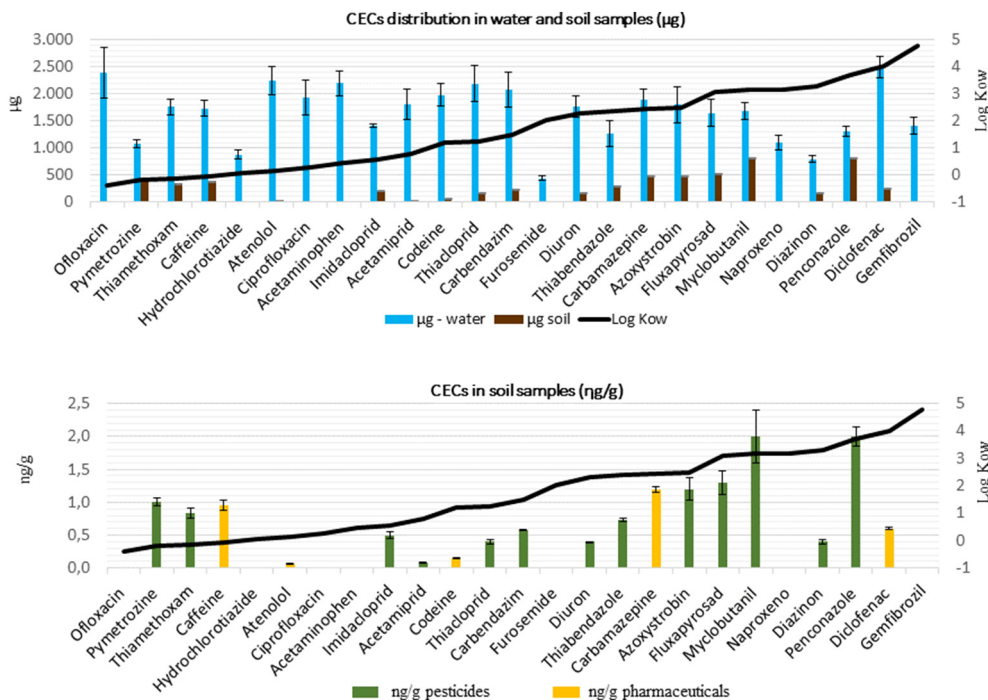


Fig. 4. Average concentration of the CECs detected in water and agricultural soil samples irrigate with spiked reclaimed water at 1 µg/L of each selected compound (error bars representing the standard deviation).

(2014) reported that diclofenac was easily biodegraded, which can explain its low value.

4. Conclusions

The modified QuEChERS method developed in this study presented the highest recoveries (between 29 and 99%, average 79%), followed by the UAE (between 12 and 101%, average 62%) whereas the PLE showed the lowest recoveries (between 9 and 89%, average 46%) of the three proposed methods at a 50 ng/g fortification level. In none of the tested methods, 4-AA, 4-MAA, ciprofloxacin and ofloxacin, compounds with a water solubility higher than 25×10^3 mg/L, moderate acidity ($4 \leq \text{pKa} \leq 6$), and very low polarity ($\text{Log Kow} \leq 1$) could be extracted properly. However, the modified QuEChERS method was the most efficient method with around 80% of the extracted target CECs with recoveries range from 70 to 99%. Codeine and pymetrozine presented low recoveries in all the methods. The proposed QuEChERS method demonstrated to be a useful tool for the simultaneous extraction of multiclass organic contaminants with different physicochemical properties from soil samples. It enabled to obtain higher recoveries than those found in the literature to date, for most compounds included in this study. Therefore, this method can be an alternative to techniques more used recently to analyze soil, sludge and sediments such as PLE or UAE.

Finally, the validated method was applied to agricultural soil samples obtained from an experimental farm of UAL-ANECOOP located in Almería (Spain). The total amount of pesticides released during the irrigation was 41×10^3 µg and the CECs total load detected in the soil samples was 5872 ng/g (d.w). The accumulation rates of pesticides in the soil samples ranged from 2% to 62%, whereas pharmaceuticals ranged from 1% to 26%. It means a final concentration that ranged from 0.1 to 1.2 ng/g (d.w) in pesticides and from 0.1 to 2.0 ng/g (d.w) in pharmaceutical products. The simulated reclaimed water was spiked at similar levels than those typically found in reclaimed water for most of the CECs selected in this study. The results obtained under greenhouse conditions highlight the importance to carry out analysis of soil irrigated with reclaimed water to evaluate the long-term accumulation process of these substances in agricultural soil. Future research about the application of the analytical methodology proposed in this study to agricultural soil samples with different content of organic matter is needed.

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CRedit authorship contribution statement

- 1. guarantor of integrity of the entire study:** MMG; ARF
- 2. study concepts and design:** MJM; MMG; AA; ARF
- 3. literature research:** MGV; MJM; AA; ARF
- 4. laboratory work:** MGV; MJM;
- 5. data analysis:** MGV; MJM; MDG
- 6. statistical analysis:** MGV; MJM; MDG; ARF
- 7. manuscript preparation:** MGV; MJM; AA; MGD; ARF
- 8. manuscript editing:** MJM; ARF

Declaration of competing interest

The authors declare no conflict of interest. This is an independent research. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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