Determination of organic microcontaminants in agricultural soils irrigated with reclaimed wastewater: Target and suspect approaches

Multiresidue analysis of contaminants of emerging concern in agricultural soils irrigated with reclaimed wastewater: target and suspect approaches

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Abstract

Water scarcity is a problem worldwide, affecting specially countries with desert/semi-desert areas and low/irregular rainfall. In this context, reuse of reclaimed wastewater (RWW) for agricultural irrigation is undoubtedly a key strategy to reduce fresh water consumption. It is well-known that current wastewater treatments do not effectively remove contaminants of emerging concern (CECs), and research in water analysis of CECs is extensive. However, the focus on agricultural soils irrigated with RWW as potential recipients of CECs and potential sources of CECs to crops is still in their beginnings. This study aims to apply a target and a suspect approach for the monitoring of CECs in agricultural soils and a soilless subtract, both irrigated with RWW for more than ten years. The study involved, firstly, the development and validation of an extraction method for target analysis of 73 CECs using a QuEChERS-based method and liquid chromatography coupled to quadrupole-linear ion trap mass spectrometry (LC-QqLIT-MS/MS); and secondly, the application of a suspect workflow for the screening of a list of 1300 potential contaminants using LC coupled to quadrupole-time-of-flight MS (LC-QTOF-MS). The results demonstrated the occurrence of 12 CECs in the agricultural soil samples and 27 in the soilless subtract (0.1 to 100 ng g^{-1}, dry weight, d.w.). The suspect analysis leaded to the confirmation of 28 CECs analytes from the list of candidates. The subsequent combination of both strategies (suspect and target) revealed the presence of 11 new CECs which were not previously reported. These results highlight the importance of monitoring soils with RWW-based irrigation and the application of wide-scope approaches for environmental analysis.

Keywords

Contaminants of emerging concern (CECs), soil, wastewater reuse, wastewater irrigation, suspect analysis, target analysis, field conditions

Nowadays, water scarcity for agriculture purposes has become one of the main problems worldwide due to the climate change and raising population. In Mediterranean countries, where low rainfall is unevenly distributed over the year and water resources are limited, reuse of reclaimed wastewater (RWW) for crop irrigation is essential to deal with water shortages. This practice reduces fresh water withdrawals and contributes to an efficient water usage.[1]

Nevertheless, the inefficient removal of contaminants of emerging concern (CECs) in wastewater treatment plants (WWTPs) leads to unpredictable long-term consequences for the environment. In particular, these CECs are released in agricultural fields after repeated RWW irrigation occurrences, being able to accumulate in soils[2,3] and translocate to crops intended for human consumption.[4–6] Their behavior and persistence depend on their different physical-chemical properties, adsorption, conjugation form and charge in the soil-compound system, but also on soil characteristics and agricultural practices.[7] Data about the occurrence/accumulation of CECs in agricultural soils and their possible translocation to the final product are needed to ensure a safe use of RWW and subsequent consumer acceptance.

Considering the large number of CECs commonly found in RWW and their various properties, it is necessary to apply wide scope extraction methodologies to provide a thorough evaluation and, therefore, a better understanding of their behavior and effects. The most frequently extraction methods applied to soil samples are ultrasound-assisted extraction (USE), pressurized-liquid extraction (PLE) and microwave-assisted extraction (MAE).[8] However, the QuEChERS (acronym of quick, easy, cheap, effective, rugged and safe) method, which was primary developed for the determination of pesticides in crops,[9] has been successfully applied to the extraction of microcontaminants (including pesticides, pharmaceuticals, veterinary drugs among others) in different environmental commodities such as sewage sludge,[10,11] water, soil, sediments,[12–14] agricultural fields which were amended with manure or sludge,[15] agricultural soil[16] and vegetables.[4,17] However, in most cases, the scope of the methods is limited and focused on the monitoring of selected groups of compounds, very often in studies conducted under controlled conditions. Nevertheless, a comprehensive evaluation of the occurrence of CECs in real soils, often exposed to long periods of irrigation with RWW and subject to the influence of a large number of pollutants, requires multi-analyte methods able to identify a larger number of compounds, as well as their transformation products (TPs), whose relevance has been previously highlighted.[5]

In addition to the need for multi-residue extraction procedures, the analysis of CECs at trace level in complex environmental commodities is necessarily accomplished by liquid chromatography-tandem mass spectrometry (LC-MS/MS) for target analysis in search of sensitivity and selectivity.[4] Likewise, screening methodologies carried out by high-resolution mass spectrometry (HRMS) using quadrupole time-of-flight (QTOF-MS) and Orbitrap analyzers, have opened a new scenario making possible the identification of CECs out of the scope by non-target and suspect screening strategies.[18,19]

Although the number of studies investigating the presence and accumulation of CECs in soils is increasing in the recent years, evidence in real agricultural fields is scarce, especially when irrigation based on RWW is applied.[3,20] Table S1 compiles some of the most recent studies conducted under field conditions. Although these studies provide valuable information for the understanding of the behavior of CECs in real soils, it is still necessary to expand knowledge about the influence of factors as diverse as the type of soil, type of crop, type of irrigation or the influence of cultivation practices, such as intensive or soilless cultivation. Besides, it is important to notice that the application of a target and a suspect strategy to obtain wide scope occurrence data is very limited. Up to our knowledge, this is the first application of a combined target-suspect analysis for the monitoring of CECs in agricultural soils irrigated with RWW.

Under this scenario, the main objectives of this work have been: i) the development and validation of a QuEChERS-based method for the multi-analyte analysis of CECs (73 analytes) in agricultural soils and their analysis by LC-MS/MS; ii) the development of a suspect screening strategy able to identify new CECs out of the target analysis by LC-QTOF-MS; and iii) the application of both, target and suspect approaches, to soils of intensive agriculture, which have been constantly irrigated with RWW for a long period.

EXPERIMENTAL SECTION

Chemicals and Reagents. A total of 73 target compounds (priority substances, pharmaceuticals and TPs) have been selected based on their recurrent identification in WWTP effluents (Table S2).[21] Reference standards (purity > 98%) were acquired from Sigma-Aldrich (Steinheim, Germany). Acetonitrile (MeCN), methanol (MeOH), glacial acetic acid and formic acid (LC-MS grade) were purchased from Sigma-Aldrich. Ultrapure water was produced using a Milli-Q water purification system from Millipore (Darmstadt, Germany). For QuEChERS extraction method, anhydrous magnesium sulfate ($MgSO_4$), sodium acetate (NaOAc), sodium chloride (NaCl), sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) and disodium hydrogen citrate sesquihydrate ($C_6H_6Na_2O_7 \cdot 1.5H_2O$) were purchased from Sigma Aldrich (all purity > 98%). Octadecyl-silyl-modified silica gel (C18) and primary-secondary amine (PSA) were from Supelco (Bellefonte, PA, USA).

Stock standard solutions of each analyte were prepared at 1000-2000 mg L^{-1} in MeOH. The surrogate standards carbamazepine-d^{10} and cyclophosphamide-d^4 were used as internal quality standards for extractions. Multi-compound working solutions were prepared at a concentration of 10 mg L^{-1} in MeOH by proper dilution of the individual stock solutions. All standard solutions were stored in amber glass vials at -20°C. Daily working solutions, prepared at appropriate concentrations in MeCN:H_2O (10:90, *v/v*) or in matrix extract, were used for the preparation of the calibration standards and the validation study.

Sample Collection and Preparation. Soil samples from three greenhouses (intensive production, 13000–25000 m^2) in Almeria province (Spain) were selected to monitor the occurrence and accumulation of the target CECs in the agricultural soil. These greenhouses were dedicated to the cultivation of two tomato varieties (retinto and ramyle) and have been irrigated with RWW for at least ten years. A fourth greenhouse was an experimental soilless culture of tomato (cherry variety) grown in pots filled with perlite substrate, which was selected as a reference of a different type of cultivation. RWW was supplied by a regeneration plant which treats WWTP secondary effluents by filtration (sand and anthracite filters) and chlorination (NaClO) and ensures the quality of the water in accordance with the Spanish regulations on water regeneration. Drip irrigation was used in all cases. Two sampling campaigns were carried out in two consecutive years (June 2016 and June 2017), coinciding with the end of tomato cultivation event. The different physical-chemical soil properties are summarized in Table S3. Samples (500 g) were composed of five soil cores taken following a W route in the greenhouse and sampling at a depth of 10-15 cm next to the root of the plant (which was often next to the irrigation line). The subsamples were then mixed to form a composite sample which was thoroughly homogenized, sieved, freeze dried until constant weight and grinded. Finally, samples were kept in the dark at -20°C until their analysis. For CECs quantification, each sample was extracted per triplicate. Non-spiked greenhouse soil samples (GH2) were used as "blank" samples for method optimization and validation. Perlite substrate from the soilless culture was submitted to the same treatment as the soil samples.

Sample Extraction. Two versions of the QuEChERS method were compared in this work (Figure S1): (i) based on the AOAC Official Method 2007.07[22] and (ii) based on the European

Standard Method EN Code 15662 (EN) published by CEN (European Committee for Standardization).[23] For both, 1 g of sample was weighed in a 50-ml polypropylene tube. After that, 4 mL of Milli-Q H_2O were added, then shaken in a vortex for 30 s and left for 15 min. For the AOAC version, 10 mL of 1% acetic acid in MeCN and 20 µL of the extraction surrogate standard solution at 1000 µg L^{-1} were added to the sample and the tube was shaken for 5 min. Following this, 5 g of anhydrous $MgSO_4$ and 1.5 g of NaOAc were added and the tube was shaken again (5 min) and centrifuged (3500 rpm, 2054*g*) for 5 min. The EN involved the use of 10 mL of MeCN. The same volume of extraction quality control solution as in the AOAC method was added. After shaking the mixture for 5 min, 5 g of anhydrous $MgSO_4$, 1 g of NaCl, 1 g of sodium citrate tribasic dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate were added. The tube was then shaken for 5 min and centrifuged at 3500 rpm (5 min). Furthermore, three different d-SPE clean-up mixtures were tested for both extraction methods (Figure S1). To this aim, a 5-ml aliquot of the upper organic phase of the extract was transferred to a 15 mL centrifuge tube and cleaned up by addition of three sets of sorbents consisting of: (i) 750 mg of anhydrous $MgSO_4$, 125 mg of C18 and 125 mg of PSA; (ii) 750 mg of anhydrous $MgSO_4$ and 125 mg of C18; and (iii) 750 mg of anhydrous $MgSO_4$ and 125 mg of PSA. The tubes were shaken vigorously for 30 s in a vortex and centrifuged (3500 rpm) for 5 min. After that, the upper layers were transferred to screw-cap vials adding 40 µL of MeCN at 1% of formic acid. At last, 100 µL of the final extract was evaporated to dryness under a gentle N_2 stream, reconstituted in 100 µL of MeCN:H_2O (10:90, *v/v*) and injected in the LC-MS/MS system.

Sample Spiking Tests. To determine how the time elapsing between spiking and sample analysis can affect the performance of the extraction, four diverse spiked-to-extraction times (1 h, 24 h, 48 h and 6 days) were tested. For trials, the spiking procedure was as follows (Figure S2): aliquots of 1 g of freeze dried soil samples were placed in 50-mL propylene tubes and spiked with 100 µL of a working solution (200 µg L^{-1}) in MeOH, then samples were shaken in a vortex for 30 s and the residual solvent was evaporated under N_2 stream for 15 min. Finally, the sample was kept at room temperature without the cap to remove possible remaining MeOH during the spiked-to-extraction time. The volume added was prepared by proper dilution of working solutions to obtain a final concentration of 20 ng g^{-1} in soil (d.w.). The samples were extracted with the AOAC version followed by a d-SPE ($MgSO_4$/C18) as described in the previous section.

Liquid Chromatography-Mass Spectrometry. *Target Analysis.* Analysis of target compounds was carried out with an Agilent 1200 LC system (Agilent Technologies, Foster City, CA, USA). The analytical column was a XDB C18 (15 x 4.6 mm; 1.8 µm particle size, Agilent Technologies, Palo Alto, CA, USA) operated at a constant flow rate of 0.4 mL min^{-1} and using an injection volume of 10 µL. Eluent A was 0.1% formic acid in water and eluent B was MeCN. Elution started with 10% B, which was kept constant for 1 min, increased to 50% within 4 min, to 100% within 10 min, kept constant for 4 min and reduced to 10% in 0.1 min. The total analysis run time was 14.1 min and the post-run equilibration time 4 min. The LC system was coupled to a hybrid quadrupole-linear ion trap-mass spectrometer (QqLIT) 5500 QTRAP® from Sciex Instruments (Foster City, CA, USA) equipped with an electrospray (ESI) source (TurboIon Spray), operating in positive and negative polarities. The source settings were: ionspray voltage, 5000V; curtain gas, 25 (arbitrary units); GS1, 50 psi, GS2, 40 psi; and temperature, 500 °C. N_2 served as nebulizer, curtain and collision gas. Compounds were analyzed by MRM using the protonated or deprotonated molecular ion as precursor and two MS/MS transitions. To increase the sensitivity of the analytical method, the Schedule MRM™ Algorithm was applied with a retention time window of 40 sec per transition. The optimal mass

spectrometric parameters for each compound are summarized in Table S4. Sciex Analyst version 1.6.2 software was used for data acquisition and processing and MultiQuant 3.0.1 software for quantification purposes.

Suspect Analysis. LC-QTOF-MS was used to carry out the suspect screening. Chromatographic separation was performed in an Agilent 1260 Infinity system equipped with a Poroshell 120 EC-C18 (50 x 4.6 mm, 2.7 μm particle size) column. Water (0.1% formic acid, eluent A) and MeCN (eluent B) were used as mobile phases. An injection volume of 20 μL and a 0.5 mL min^{-1} flow rate were set. The chromatographic gradient went from 90% A (1 min) to 0% in 10 min and kept constant for 4 min before returning to initial conditions. The total run time was 22 min. The LC system was connected to a QTOF mass analyzer Triple TOF 5600+ (Sciex Instruments) with a dual source consisting on an ESI interface for sample injection and an atmospheric-pressure chemical ionization interface (APCI) for calibrant delivery. Both ESI+ and ESI− modes were considered. The ESI source settings were: ionspray voltage, 4500 V; curtain gas, 25 (arbitrary units); GS1, 60 psi; GS2, 60 psi; and temperature, 575°C. Nitrogen served as nebulizer, curtain and collision gas. The equipment worked via TOF MS survey scan followed by four IDA (Information Dependent Acquisition) TOF MS/MS scans within a *m/z* range from 100 to 2000 at a resolving power of 30000. An accumulation time of 250 ms for TOF and 100 ms for IDA were used in each scan. IDA criteria considered dynamic background subtraction. Collision energy of 30 eV with a ±15 eV spread was used in MS/MS fragmentation. Diverse Sciex software (Analyst TF 1.5, PeakView™ 2.2 and MasterView 1.1) were used to record and process LC-QTOF-MS/MS data.

Suspect Screening Workflow. A suspect list composed of 1300 CECs frequently found in WWTP effluents was built on the basis of an investigation about reported CECs in literature and the so-called NORMAN Suspect List Exchange.[24] NORMAN is a network of all interested stakeholders dealing with emerging substances within the framework of the European Commission. The criteria for positive tentative candidates and the suspect workflow are shown in Figure 1. After an adequate procedural blank subtraction, these requirements consisted of an intensity threshold higher than 1000 cps, a S/N ratio higher than 10, a mass accuracy error below 5 ppm for the precursor ion ($[M+H]^+$ for ESI+ mode and $[M-H]^-$ for ESI- mode), an isotope ratio difference below 10%, a difference of ±2 min with an in-house retention time (RT) prediction model, a MS/MS spectral fit higher than 80% when spectra was compared with at least one of three different libraries used (namely Sciex MS/MS Spectral Library, ChemSpider[25] and MassBank[26]) and presence of two MS/MS fragments with an error lower than 5 ppm. Predicted RTs were obtained using a linear correlation between the measured RTs and reported log $K_{O/W}$ values ($RT=0.9676\times\log K_{O/W}+4.1906$ obtained from 100 reference standards analyzed in the same conditions). An error window of ±2 min was assumed considering a compromise between reliability requirements and the inherent limitations of the method.[27] Final confirmation of tentatively identified compounds was achieved by the acquisition and analysis of the correspondent analytical standard, when the RT of the standard differed in ±0.1 min.

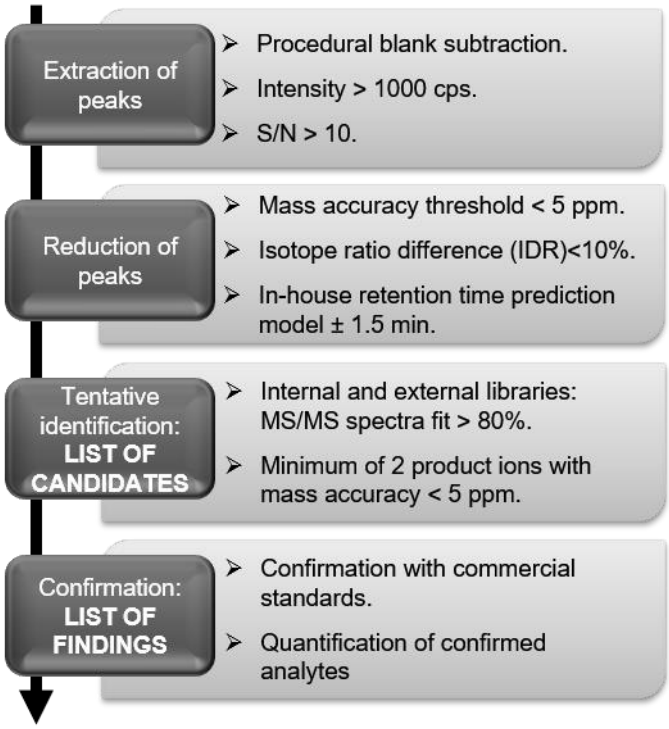

Figure 1. Suspect screening workflow.

Target Method Validation. A validation study was carried out to verify the performance of the proposed method according to relevant parameters, such as linearity, method quantification limits (MQLs), trueness (in terms of recovery) and precision (expressed as relative standard deviation, RSD) under repeatability conditions. Moreover, matrix effect was estimated to evaluate the effect on analytes response.

The linearity in the response was assessed by using matrix-matched calibration standards at six concentration levels, ranging from 0.1 to 100 ng g^{-1} in dry sample (ten times lower in the instrument). Calibration curves were obtained by least-squares linear regression analysis of the peak area versus concentration. Satisfactory linearity was assumed when the determination coefficients (R^2) were ≥0.990. The evaluation of matrix effect (ME) was carried out by comparing the slope of the calibration curves prepared in pure solvent and in matrix extract, according to the following equation: ME (%)= ((slope of calibration curve in matrix / slope of calibration curve in solvent) -1) x 100. Suppression effect was considered when negative values of ME where obtained, and enhancement in case of positive values. Three different ranges were adopted for considering low, medium and strong ME, <20%, 20-50% and >50%, respectively.

Recoveries were calculated per triplicate using spiked samples at five concentration levels: 0.1, 0.5, 1.0, 5.0 and 20 ng g^{-1}, to provide information on analytical performance over a range of concentrations. Acceptable values were considered when recoveries were in the range 70-120%, and RSDs≤20%, following the recommendations of the European Union SANTE guidelines.[28]

The MQLs were experimentally calculated as the minimum concentration of the analyte that yielded a S/N ratio of 10 for the quantification transition with acceptable accuracy and precision (recovery 70–120% and RSD≤20%, *n*=3). When these criteria were not met, the lowest point of the calibration curve was considered as limit of quantification (LOQ). At these values, identification was assured in all cases by the presence of the confirmation transition at a *S*/*N*>3 when the whole method was applied.

The confirmation of the analytes in the samples was performed based on the EU SANTE/11813/2017 guidelines,[28] which require the presence of two SMR transitions at the correct LC RT and with the correct ion ratio, expressed as relative to the most intense ion used for identification. The RT of the analyte in the extract should correspond to that of the calibration standard with a tolerance of ±0.1 min and the ratios of selected ions, should not deviate more than 30%.

RESULTS AND DISCUSSION

Extraction and clean-up optimization. In order to investigate the influence on recoveries of some experimental parameters, different extraction pH values and d-SPE sorbents were evaluated (Figure S1). Two variants of the QuEChERS method (based on AOAC official method and EN method) were compared. Both procedures were applied to the freeze-dried soil samples after rehydration with 4 mL of water, as usual in matrices of low water content. In the AOAC method, the acetate buffer provided a nominal pH of 4.8 while the EN method, using a citrate buffer, gave a higher pH of 5-5.5.[29] The clean-up step was evaluated comparing different mixtures of $MgSO_4$, C18 and PSA (Figure S1). $MgSO_4$ is used to remove water excess, C18 eliminates non-polar matrix interferences, and PSA is commonly used to retain polar organic acids and pigments. To simplify, all the experiments were performed per triplicate at a single concentration (20 ng g^{-1}). Figure 2 shows the results obtained under all the assayed conditions. The extraction pH is a critical parameter and slight variations can affect the efficiency of the method, mainly for acidic and basic compounds.[9] A higher percentage of the total number of compounds was successfully extracted in all cases (recoveries between 70 and 120%, RSD≤20%, n=3) when more acidic conditions (AOAC method) were applied, which is in agreement with the results reported by Salvia et al.[15] Regarding the clean-up, the best results for 81% of the compounds were obtained when the AOAC extracts were purified with the $MgSO_4$+C18 mixture.

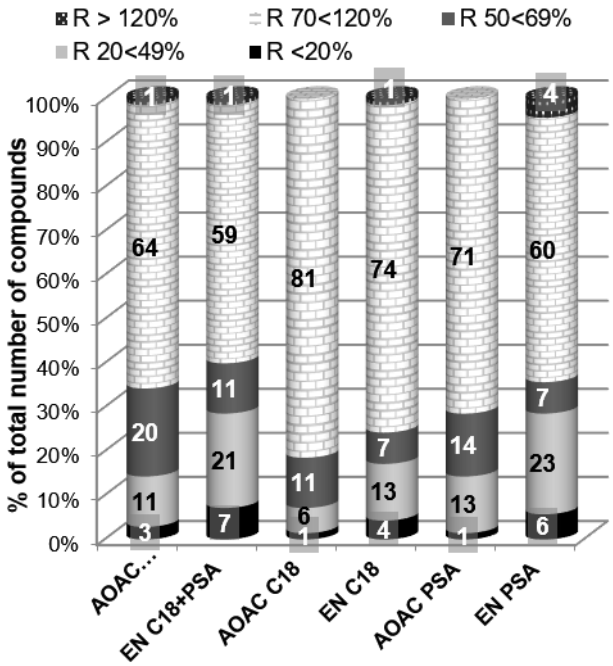

Figure 2. Summary of recovery results from the different QuEChERS and d-SPE conditions tested.

Considering the compounds presenting better recoveries with the combination AOAC/$MgSO_4$+C18 with respect to EN/MgSO4+C18, we can indicate lincomycin (77% versus 45%) and loratadine (100% versus 63%). The same behavior was observed for sulfonamide antibiotics (sulfadiazine, sulfamethazine, sulfamethoxazole, sulfapyridine and sulfathizole) with improved recoveries in the range 52-70% compared with the low recoveries (21% to 29%) obtained with the EN method (RSD values ≤ 20% in all cases). This behavior is related to the amphoteric character of sulfonamides, which plays an important role for their extraction from soil, since their partitioning is pH-dependent. More acidic conditions also improved sulfonamide extraction in the study carried out by Young-Jun Lee et al.,[16] who compared the efficiencies of the AOAC and EN methods for a group of ten CECs in agricultural soil, obtaining better recoveries with the AOAC method. No or limited effects were observed for the rest of the target analytes.

Higher variation in the recoveries was found during the clean-up study by d-SPE. The combination $MgSO_4$+C18 yielded better results under both buffered conditions, while presence of PSA reduced extraction efficiency in all cases. This can be explained considering that PSA acts as chelating agent with acidic compounds, as it has been previously reported.[30] Clofibric acid, furosemide, indomethacin, ketoprofen, ketorolac, mefenamic acid and methylprednisolone, showed significant lower recovery values in presence of PSA, decreasing a 50% in some cases (Figure S3). These results agree with those published by De Carlo et al.[14] According to the results obtained and in order to find a compromise due to the diverse physical-chemical properties of the analytes under study, the AOAC method followed by d-SPE with $MgSO_4$+C18 was chosen for subsequent validation.

Optimization of the sample spiking procedure. Spiking is a key procedure for the evaluation of method efficiency. In general, the analysis of environmental commodities such as soil, sediments, sewage sludge or manure, implies the fortification of the dry sample which is commonly carried out by adding small volumes of a multi-compound standard solution in organic solvent followed by an evaporation step. It is well-known that the time elapse between spiking the samples and starting the analysis is crucial to achieve the optimum adsorption equilibrium and consequently, to avoid overestimation on recoveries.[8] Some recent expert opinions have highlighted the lack of information about the spiking procedures and how realistic are recovery results in comparison with concentrations found in real samples.[31] In this study, diverse spiked-to-extraction time periods were tested: 1 h, 24 h, 48 h and 6 days. The results showed that most of target compounds rapidly reached the adsorption equilibrium in soil, and their recoveries remained stable under all tested conditions. However, some compounds showed significant differences in the recoveries with the time (Table S5). Thus, recoveries of acetaminophen, furosemide, methylprednisolone and salbutamol decreased after spiked-to-extraction time periods of 48 h, while betamethasone, ranitidine, terbutaline and sulfonamide antibiotics already experimented a drastic reduction at 24 h. Although dissipation because of the TPs cannot be fully excluded, it seems clear that sorption or other interactions with the soil system play an important role in the increment of non-extractable amount of the compounds with time,[32] remaining their recoveries stable after this time. To apply more realistic conditions as well as to reach a compromise for the largest part of the compounds, a spiked-to-extraction time of 48 h was selected for method validation.

Validation study. To test the efficiency of the proposed method, recovery tests at 5 concentration levels were carried out: 0.1, 0.5, 1.0, 5.0 and 20 ng g^{-1} (dry weight). The results obtained are summarized in Table S6. Considering the large number of compounds studied and their different properties, the results for the proposed method were satisfactory. This approach achieved to extract a total of 53 over 73 compounds (73%) with recoveries in the range 70-

120% and RSD≤20%. For most compounds, reproducible recovery values were also obtained between the diverse concentrations tested. For 20 compounds the methodology showed recovery rates out of the acceptable range, but with RSD≤20%, which means that the method was still repetitive and reliable for their analysis. Lower precision was observed for acetaminophen, clotrimazole, fenofibrate, flumequine and pravastatin, with recovery values that differed more than 20% among concentrations or even for the same concentration level. Despite these analytes do not fulfill the proposed acceptability criteria, they were kept in the study as they can be considered for qualitative or semi-quantitative purposes.

Linearity was investigated in the range from 0.1 to 100 ng g^{-1}. All analytes showed R^2 values higher than 0.995 (Table S6). Average ME for each compound was also evaluated: 63 targets out of 73 showed low ME (ME<20%), which proves the efficiency of the purification step avoiding undesirable co-extractive matrix substances. The predominant effect observed was signal suppression for the 59% of the compounds. Only clotrimazole showed a strong ME (-52%). These results can explain in part the low recoveries and lack of precision observed for this compound (Table S6).

MQLs ranged from 0.1 to 5 ng g^{-1} (Table S6), with 89% of compounds presenting values below 1.0 ng g^{-1}. These values are in the same range as those reported by other authors, using different QuEChERS approaches[16,33] or even with other methodologies as USE or PLE.[34,35] However, no previous data are available in literature for many of the analytes included in this study, because in most cases the reported methods are focused on a limited number of target compounds.

Occurrence of CECs in field samples irrigated with RWW. *Target screening*. To verify the applicability of the method and evaluate the exposure of agricultural soils to the target compounds in real farming conditions, the proposed method was applied to the analysis of three agricultural soils which had been irrigated with reclaimed water for long periods. A substrate (perlite) from a soilless culture was also evaluated to assess the influence of this agricultural practice on the availability of CEC for crops (more details in the Experimental Section).

Table 1 summarizes the results found for the three soils sampled (GH1-GH3) and the soilless perlite substrate (SP1) during the two sampling events. Up to 12 compounds were found at concentrations ranging from 0.10 to 17 ng g^{-1} in the soils (Note: concentrations in real samples always in d.w.). In general terms, no clear trend was observed in CEC concentrations detected in the GHs during the two years of the survey. In most cases, the concentrations detected were comparable, which suggests that the presence of the CECs in soils is more due to the continuous introduction of the contaminants by the irrigation than to an accumulation because of their persistence in the soil. Six compounds, namely caffeine, its metabolite paraxanthine, carbamazepine, citalopram, hydrochlorothiazide and clarithromycin, were found in all samples at significant concentrations, thus indicating that these analytes are capable to be retained/accumulated, indistinctly of soil properties (Table S3). In contrast, the SP1 perlite substrate accumulated a largest number of CECs, up to 27 compounds compared to 12 in GH2, or 7 in GH1 and GH3. Besides, the highest detected concentration in all samples was also found in the perlite, up to 100 ng g^{-1} for citalopram. Perlite is an inert, porous and lightweight material widely used in soilless cultures since provides adequate aeration and proper water retention and drainage capabilities. These properties, together with an expected reduction in the interaction of the CECs with the substrate compared to the soil, can increase their availability for the plant and thus pose a higher risk of translocation to the fruits. Although positive effects of RWW irrigation in soilless systems have been reported on saving ordinary irrigation water and commercial fertilizers,[36] there is no evidence of the impact that these practices can have on the

presence of CECs in crops. Therefore, more research is needed to increase data and knowledge about this issue.

Suspect screening. To expand the scope of the proposed method to additional compounds for which reference standards are not available in our laboratory, a suspect screening approach was applied according to the workflow shown in Figure 1. A compiled suspect list containing 1300 contaminants was used to scan the soil samples; this list includes pharmaceuticals, antibiotics or TPs. Samples were processed using the MasterView™ software, which provides automated peak-picking algorithms to find chromatographic features according to preestablished criteria (Figure 1). Only $[M+H]^+$ and $[M-H]^-$ ions, above a S/N and peak intensity threshold and significantly differentiated from the control sample (procedural blank), were considered. The list of potential positives was also reduced assuming mass accuracy, isotope ratio and RT filters. Finally, additional data to support identification was obtained by comparison of the acquired MS/MS spectra with MS/MS libraries (namely, Sciex Library, MassBank and ChemSpider). A score >80% and presence of at least two product ions with mass accuracy < 5ppm were set as criteria for a reliable structure allocation. Up to 33 candidates were identified by the suspect screening approach, this means 2.2% of the initial suspect list (Figure 3).

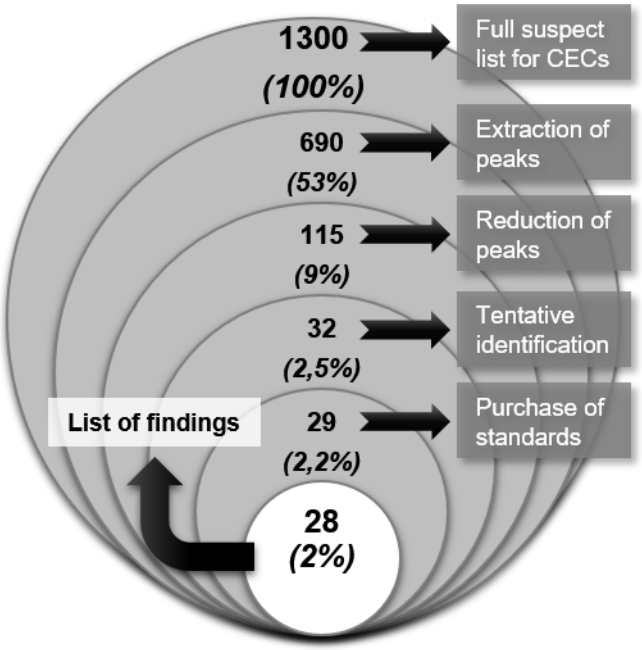

Figure 3. Reduction of peaks from the suspect analysis related to each step of the workflow.

Table S7 shows the list of candidates as well as the values obtained for the criteria proposed. For unequivocal confirmation, analytical standards were purchased for 29 of them, obtaining positive confirmation for 28 candidates by comparison of the RT and MS/MS spectra obtained under the same analytical conditions as the soil samples. These results confirm the usefulness of this analytical strategy and the validity of the criteria applied (Figure 1). Only the metabolite tramadol-N-oxide could not be confirmed. The prediction of the RT was not considered as a conclusive criterion, because of the limitations of the procedure applied. The error window selected was too strict and was considered only as a support of the rest of the criteria rather than as exclusion criterion. The set of compounds confirmed mainly included drugs related to cardiac diseases (hypertension, arrhythmias); for the treatment of Alzheimer's disease, antidepressants/antipsychotics, antihistamines and opioids (Figure 4), among others.

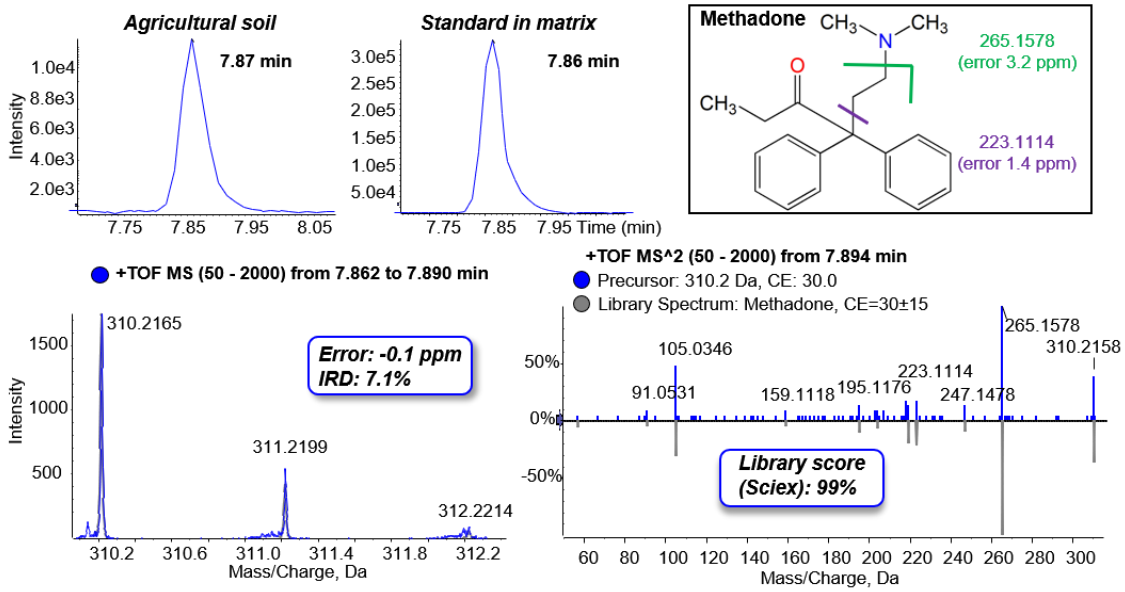

Figure 4. Detection of methadone in agricultural soil by suspect analysis.

Also remarkable is the presence of the metabolites N-desmethylcitalopram, o-desmethyltramadol, acridine and acridone (reported metabolites of the antiepileptic carbamazepine) and EDDP (metabolite of the opioid analgesic methadone). Although a complete validation of the identified compounds has not been carried out, a quantitative estimation was obtained by preparing matrix-matched calibration curves. The concentrations calculated are shown in Table 2. Again, the substrate SP1 accumulated the largest number and concentration of compounds. Only 10 were detected in the soil samples. From them, nicotinamide, the anti-arrhythmia agent flecainide and the antihypertensive telmisartan were detected in all samples, and at the higher concentrations, which ranged from 14 ng g^{-1} to 25 ng g^{-1} d.w. The eventual identification of lamotrigine in GH2 is also of interest, because of the reported risk associated to the presence of this compound in vegetables.[37]

Reference to the presence of CECs in soils irrigated with WW under field conditions has been reported in previous studies. Table S1 shows some examples. In most cases carbamazepine and caffeine are the compounds more frequently reported, probably because they are the most studied. Also reference to hydrochlorothiazide, clarithromycin, lamotrigine, diazepam, venlafaxine, fluoxetine and the metabolites acridine, acridone and carbamazepine epoxide has been described. However, to our knowledge, no information is available in literature about the fate under real conditions of a large list of CECs studied in this work. Such is the case of citalopram and its metabolite N-desmethylcitalopram, azithromycin, paraxanthine, theophylline, flecainide, irbesartan, nicotinamide, methadone (Figure 4), sulpiride or telmisartan, for which more information is required regarding presence, fate and risk associated.

Concerning the results obtained in the perlite substrate, it seems clear that the accumulation of contaminants and availability for the plants is higher when wastewater is applied in soilless cultures. Thus, studies on the potential intake of these compounds by crops are necessary if these practices are applied in crops intended for consumption. The fact that some compounds such as 4-formylaminoantipyrine, citalopram, fluoxetine, hydrochlorothiazide and venlafaxine among others, reached 10 to 100 times higher concentrations than the rest of the compounds. These levels could be explained due to their recurrent presence and elevated concentrations reported in WWTP effluents.[21] These data highlight the necessity of having broad-spectrum

analytical methods that allow a comprehensive evaluation of the fate of CECs in agriculture soils usually present in the irrigation water.

CONCLUSIONS

The application of a workflow combining target and suspect screening which has been applied to the determination of CECs in agricultural soils and perlite substrate irrigated with RWW has demonstrated the occurrence of non-previously reported analytes. The developed and optimized QuEChERS-based method for the target analysis of 73 CECs showed the presence of 12 CECs. The proposed suspect analysis revealed the occurrence of up to 28 new compounds (from an initial list of 1300), 11 of them not previously reported (as methadone, a well-known opioid). These results indicate that focus must be paid to agricultural soils irrigated with RWW from the point of view of the possible levels of CECs and not only RWW quality. More research is necessary with alternative substrate such as soilless substrate since it shows a different behavior when compared to real soil in terms of potential accumulation of CECs. Furthermore, a following step to understand the full process should be the study of possible translocations of CECs to the final products and at which levels may take place.

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ASSOCIATED CONTENT

Supporting Information Available

The Supporting Information is available free of charge on the ACS Publication Site (http://pubs.acs.org).

Additional information about reported CEC monitoring in soils; further experimental details (list of target analytes, list of suspect CECs confirmed; physical-chemical properties of the soil samples; LC-QqLIT-MS/MS details; aging experiments; recovery and validation data; list of candidates of the suspect analysis; and extraction schemes (PDF).

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552 **FIGURE CAPTIONS**

553 **Figure 1.** Suspect screening workflow.

554 **Figure 2.** Summary of recovery results from the different QuEChERS and d-SPE conditions
555 tested.

556 **Figure 3.** Reduction of peaks from the suspect analysis related to each step of the workflow.

557 **Figure 4.** Detection of methadone in agricultural soil by suspect analysis.