

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

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22 **Abstract**

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

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44

45 **Keywords**

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

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

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

62 Considering the large number of CECs commonly found in RWW and their various
63 properties, it is necessary to apply wide scope extraction methodologies to provide a thorough
64 evaluation and, therefore, a better understanding of their behavior and effects. The most
65 frequently extraction methods applied to soil samples are ultrasound-assisted extraction (USE),
66 pressurized-liquid extraction (PLE) and microwave-assisted extraction (MAE).⁸ However, the
67 QuEChERS (acronym of quick, easy, cheap, effective, rugged and safe) method, which was
68 primary developed for the determination of pesticides in crops,⁹ has been successfully applied to
69 the extraction of microcontaminants (including pesticides, pharmaceuticals, veterinary drugs
70 among others) in different environmental commodities such as sewage sludge,^{10,11} water, soil,
71 sediments,¹²⁻¹⁴ agricultural fields which were amended with manure or sludge,¹⁵ agricultural
72 soil¹⁶ and vegetables.^{4,17} However, in most cases, the scope of the methods is limited and
73 focused on the monitoring of selected groups of compounds, very often in studies conducted
74 under controlled conditions. Nevertheless, a comprehensive evaluation of the occurrence of
75 CECs in real soils, often exposed to long periods of irrigation with RWW and subject to the
76 influence of a large number of pollutants, requires multi-analyte methods able to identify a
77 larger number of compounds, as well as their transformation products (TPs), whose relevance
78 has been previously highlighted.⁵

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

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

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

102

103 **EXPERIMENTAL SECTION**

104 **Chemicals and Reagents.** A total of 73 target compounds (priority substances,
105 pharmaceuticals and TPs) have been selected based on their recurrent identification in WWTP
106 effluents (Table S2).²¹ Reference standards (purity > 98%) were acquired from Sigma-Aldrich
107 (Steinheim, Germany). Acetonitrile (MeCN), methanol (MeOH), glacial acetic acid and formic
108 acid (LC-MS grade) were purchased from Sigma-Aldrich. Ultrapure water was produced using
109 a Milli-Q water purification system from Millipore (Darmstadt, Germany). For QuEChERS
110 extraction method, anhydrous magnesium sulfate (MgSO₄), sodium acetate (NaOAc), sodium
111 chloride (NaCl), sodium citrate tribasic dihydrate (C₆H₅Na₃O₇·2H₂O) and disodium hydrogen
112 citrate sesquihydrate (C₆H₆Na₂O₇·1.5H₂O) were purchased from Sigma Aldrich (all purity >
113 98%). Octadecyl-silyl-modified silica gel (C18) and primary-secondary amine (PSA) were from
114 Supelco (Bellefonte, PA, USA).

115 Stock standard solutions of each analyte were prepared at 1000-2000 mg L⁻¹ in MeOH. The
116 surrogate standards carbamazepine-d¹⁰ and cyclophosphamide-d⁴ were used as internal quality
117 standards for extractions. Multi-compound working solutions were prepared at a concentration
118 of 10 mg L⁻¹ in MeOH by proper dilution of the individual stock solutions. All standard
119 solutions were stored in amber glass vials at -20°C. Daily working solutions, prepared at
120 appropriate concentrations in MeCN:H₂O (10:90, v/v) or in matrix extract, were used for the
121 preparation of the calibration standards and the validation study.

122 **Sample Collection and Preparation.** Soil samples from three greenhouses (intensive
123 production, 13000–25000 m²) in Almeria province (Spain) were selected to monitor the
124 occurrence and accumulation of the target CECs in the agricultural soil. These greenhouses
125 were dedicated to the cultivation of two tomato varieties (retinto and ramyle) and have been
126 irrigated with RWW for at least ten years. A fourth greenhouse was an experimental soilless
127 culture of tomato (cherry variety) grown in pots filled with perlite substrate, which was selected
128 as a reference of a different type of cultivation. RWW was supplied by a regeneration plant
129 which treats WWTP secondary effluents by filtration (sand and anthracite filters) and
130 chlorination (NaClO) and ensures the quality of the water in accordance with the Spanish
131 regulations on water regeneration. Drip irrigation was used in all cases. Two sampling
132 campaigns were carried out in two consecutive years (June 2016 and June 2017), coinciding
133 with the end of tomato cultivation event. The different physical-chemical soil properties are
134 summarized in Table S3. Samples (500 g) were composed of five soil cores taken following a
135 W route in the greenhouse and sampling at a depth of 10-15 cm next to the root of the plant
136 (which was often next to the irrigation line). The subsamples were then mixed to form a
137 composite sample which was thoroughly homogenized, sieved, freeze dried until constant
138 weight and grinded. Finally, samples were kept in the dark at -20°C until their analysis. For
139 CECs quantification, each sample was extracted per triplicate. Non-spiked greenhouse soil
140 samples (GH2) were used as “blank” samples for method optimization and validation. Perlite
141 substrate from the soilless culture was submitted to the same treatment as the soil samples.

142 **Sample Extraction.** Two versions of the QuEChERS method were compared in this work
143 (Figure S1): (i) based on the AOAC Official Method 2007.07²² and (ii) based on the European

144 Standard Method EN Code 15662 (EN) published by CEN (European Committee for
145 Standardization).²³ For both, 1 g of sample was weighed in a 50-ml polypropylene tube. After
146 that, 4 mL of Milli-Q H₂O were added, then shaken in a vortex for 30 s and left for 15 min. For
147 the AOAC version, 10 mL of 1% acetic acid in MeCN and 20 μ L of the extraction surrogate
148 standard solution at 1000 μ g L⁻¹ were added to the sample and the tube was shaken for 5 min.
149 Following this, 5 g of anhydrous MgSO₄ and 1.5 g of NaOAc were added and the tube was
150 shaken again (5 min) and centrifuged (3500 rpm, 2054g) for 5 min. The EN involved the use of
151 10 mL of MeCN. The same volume of extraction quality control solution as in the AOAC
152 method was added. After shaking the mixture for 5 min, 5 g of anhydrous MgSO₄, 1 g of NaCl,
153 1 g of sodium citrate tribasic dihydrate and 0.5 g of disodium hydrogen citrate sesquihydrate
154 were added. The tube was then shaken for 5 min and centrifuged at 3500 rpm (5 min).
155 Furthermore, three different d-SPE clean-up mixtures were tested for both extraction methods
156 (Figure S1). To this aim, a 5-ml aliquot of the upper organic phase of the extract was transferred
157 to a 15 mL centrifuge tube and cleaned up by addition of three sets of sorbents consisting of: (i)
158 750 mg of anhydrous MgSO₄, 125 mg of C18 and 125 mg of PSA; (ii) 750 mg of anhydrous
159 MgSO₄ and 125 mg of C18; and (iii) 750 mg of anhydrous MgSO₄ and 125 mg of PSA. The
160 tubes were shaken vigorously for 30 s in a vortex and centrifuged (3500 rpm) for 5 min. After
161 that, the upper layers were transferred to screw-cap vials adding 40 μ L of MeCN at 1% of
162 formic acid. At last, 100 μ L of the final extract was evaporated to dryness under a gentle N₂
163 stream, reconstituted in 100 μ L of MeCN:H₂O (10:90, v/v) and injected in the LC-MS/MS
164 system.

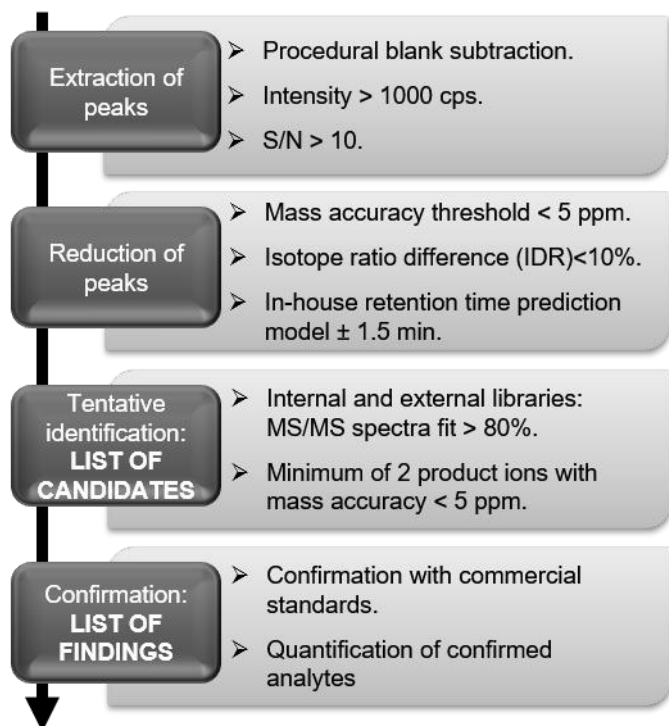
165 **Sample Spiking Tests.** To determine how the time elapsing between spiking and sample
166 analysis can affect the performance of the extraction, four diverse spiked-to-extraction times (1
167 h, 24 h, 48 h and 6 days) were tested. For trials, the spiking procedure was as follows (Figure
168 S2): aliquots of 1 g of freeze dried soil samples were placed in 50-mL propylene tubes and
169 spiked with 100 μ L of a working solution (200 μ g L⁻¹) in MeOH, then samples were shaken in a
170 vortex for 30 s and the residual solvent was evaporated under N₂ stream for 15 min. Finally, the
171 sample was kept at room temperature without the cap to remove possible remaining MeOH
172 during the spiked-to-extraction time. The volume added was prepared by proper dilution of
173 working solutions to obtain a final concentration of 20 ng g⁻¹ in soil (d.w.). The samples were
174 extracted with the AOAC version followed by a d-SPE (MgSO₄/C18) as described in the
175 previous section.

176 **Liquid Chromatography-Mass Spectrometry. Target Analysis.** Analysis of target
177 compounds was carried out with an Agilent 1200 LC system (Agilent Technologies, Foster
178 City, CA, USA). The analytical column was a XDB C18 (15 x 4.6 mm; 1.8 μ m particle size,
179 Agilent Technologies, Palo Alto, CA, USA) operated at a constant flow rate of 0.4 mL min⁻¹
180 and using an injection volume of 10 μ L. Eluent A was 0.1% formic acid in water and eluent B
181 was MeCN. Elution started with 10% B, which was kept constant for 1 min, increased to 50%
182 within 4 min, to 100% within 10 min, kept constant for 4 min and reduced to 10% in 0.1 min.
183 The total analysis run time was 14.1 min and the post-run equilibration time 4 min. The LC
184 system was coupled to a hybrid quadrupole-linear ion trap-mass spectrometer (QqLIT) 5500
185 QTRAP® from Sciex Instruments (Foster City, CA, USA) equipped with an electrospray (ESI)
186 source (TurboIon Spray), operating in positive and negative polarities. The source settings were:
187 ionspray voltage, 5000V; curtain gas, 25 (arbitrary units); GS1, 50 psi, GS2, 40 psi; and
188 temperature, 500 °C. N₂ served as nebulizer, curtain and collision gas. Compounds were
189 analyzed by MRM using the protonated or deprotonated molecular ion as precursor and two
190 MS/MS transitions. To increase the sensitivity of the analytical method, the Schedule MRM™
191 Algorithm was applied with a retention time window of 40 sec per transition. The optimal mass

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

195 *Suspect Analysis.* LC-QTOF-MS was used to carry out the suspect screening.
196 Chromatographic separation was performed in an Agilent 1260 Infinity system equipped with a
197 Poroshell 120 EC-C18 (50 x 4.6 mm, 2.7 μm particle size) column. Water (0.1% formic acid,
198 eluent A) and MeCN (eluent B) were used as mobile phases. An injection volume of 20 μL and
199 a 0.5 mL min^{-1} flow rate were set. The chromatographic gradient went from 90% A (1 min) to
200 0% in 10 min and kept constant for 4 min before returning to initial conditions. The total run
201 time was 22 min. The LC system was connected to a QTOF mass analyzer Triple TOF 5600+
202 (Sciex Instruments) with a dual source consisting on an ESI interface for sample injection and
203 an atmospheric-pressure chemical ionization interface (APCI) for calibrant delivery. Both ESI+
204 and ESI- modes were considered. The ESI source settings were: ionspray voltage, 4500 V;
205 curtain gas, 25 (arbitrary units); GS1, 60 psi; GS2, 60 psi; and temperature, 575 $^{\circ}\text{C}$. Nitrogen
206 served as nebulizer, curtain and collision gas. The equipment worked via TOF MS survey scan
207 followed by four IDA (Information Dependent Acquisition) TOF MS/MS scans within a m/z
208 range from 100 to 2000 at a resolving power of 30000. An accumulation time of 250 ms for
209 TOF and 100 ms for IDA were used in each scan. IDA criteria considered dynamic background
210 subtraction. Collision energy of 30 eV with a ± 15 eV spread was used in MS/MS fragmentation.
211 Diverse Sciex software (Analyst TF 1.5, PeakViewTM 2.2 and MasterView 1.1) were used to
212 record and process LC-QTOF-MS/MS data.

213 **Suspect Screening Workflow.** A suspect list composed of 1300 CECs frequently found in
214 WWTP effluents was built on the basis of an investigation about reported CECs in literature
215 and the so-called NORMAN Suspect List Exchange.²⁴ NORMAN is a network of all interested
216 stakeholders dealing with emerging substances within the framework of the European
217 Commission. The criteria for positive tentative candidates and the suspect workflow are shown
218 in Figure 1. After an adequate procedural blank subtraction, these requirements consisted of an
219 intensity threshold higher than 1000 cps, a S/N ratio higher than 10, a mass accuracy error
220 below 5 ppm for the precursor ion ($[\text{M}+\text{H}]^+$ for ESI+ mode and $[\text{M}-\text{H}]^-$ for ESI- mode), an
221 isotope ratio difference below 10%, a difference of ± 2 min with an in-house retention time (RT)
222 prediction model, a MS/MS spectral fit higher than 80% when spectra was compared with at
223 least one of three different libraries used (namely Sciex MS/MS Spectral Library, ChemSpider²⁵
224 and MassBank²⁶) and presence of two MS/MS fragments with an error lower than 5 ppm.
225 Predicted RTs were obtained using a linear correlation between the measured RTs and reported
226 $\log K_{O/W}$ values ($\text{RT}=0.9676 \times \log K_{O/W} + 4.1906$ obtained from 100 reference standards analyzed
227 in the same conditions). An error window of ± 2 min was assumed considering a compromise
228 between reliability requirements and the inherent limitations of the method.²⁷ Final confirmation
229 of tentatively identified compounds was achieved by the acquisition and analysis of the
230 correspondent analytical standard, when the RT of the standard differed in ± 0.1 min.



231 **Figure 1.** Suspect screening workflow.

232

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

238 The linearity in the response was assessed by using matrix-matched calibration standards at
 239 six concentration levels, ranging from 0.1 to 100 ng g⁻¹ in dry sample (ten times lower in the
 240 instrument). Calibration curves were obtained by least-squares linear regression analysis of the
 241 peak area versus concentration. Satisfactory linearity was assumed when the determination
 242 coefficients (R²) were ≥0.990. The evaluation of matrix effect (ME) was carried out by
 243 comparing the slope of the calibration curves prepared in pure solvent and in matrix extract,
 244 according to the following equation: ME (%) = ((slope of calibration curve in matrix / slope of
 245 calibration curve in solvent) - 1) x 100. Suppression effect was considered when negative values
 246 of ME were obtained, and enhancement in case of positive values. Three different ranges were
 247 adopted for considering low, medium and strong ME, <20%, 20-50% and >50%, respectively.

248 Recoveries were calculated per triplicate using spiked samples at five concentration levels:
 249 0.1, 0.5, 1.0, 5.0 and 20 ng g⁻¹, to provide information on analytical performance over a range of
 250 concentrations. Acceptable values were considered when recoveries were in the range 70-120%,
 251 and RSDs ≤ 20%, following the recommendations of the European Union SANTE guidelines.²⁸

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

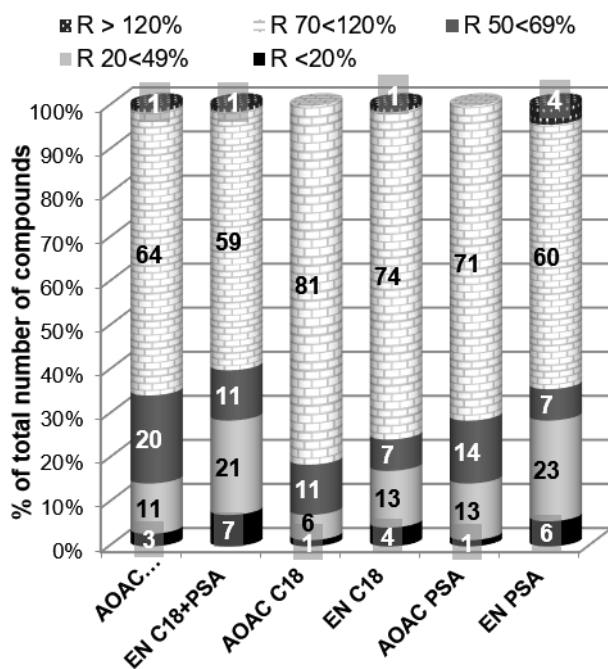
258 The confirmation of the analytes in the samples was performed based on the EU
 259 SANTE/11813/2017 guidelines,²⁸ which require the presence of two SMR transitions at the
 260 correct LC RT and with the correct ion ratio, expressed as relative to the most intense ion used
 261 for identification.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

264

265 RESULTS AND DISCUSSION

266 **Extraction and clean-up optimization.** In order to investigate the influence on recoveries of
 267 some experimental parameters, different extraction pH values and d-SPE sorbents were
 268 evaluated (Figure S1). Two variants of the QuEChERS method (based on AOAC official
 269 method and EN method) were compared. Both procedures were applied to the freeze-dried soil
 270 samples after rehydration with 4 mL of water, as usual in matrices of low water content. In the
 271 AOAC method, the acetate buffer provided a nominal pH of 4.8 while the EN method, using a
 272 citrate buffer, gave a higher pH of 5-5.5.²⁹ The clean-up step was evaluated comparing different
 273 mixtures of MgSO₄, C18 and PSA (Figure S1). MgSO₄ is used to remove water excess, C18
 274 eliminates non-polar matrix interferences, and PSA is commonly used to retain polar organic
 275 acids and pigments. To simplify, all the experiments were performed per triplicate at a single
 276 concentration (20 ng g⁻¹). Figure 2 shows the results obtained under all the assayed conditions.
 277 The extraction pH is a critical parameter and slight variations can affect the efficiency of the
 278 method, mainly for acidic and basic compounds.⁹ A higher percentage of the total number of
 279 compounds was successfully extracted in all cases (recoveries between 70 and 120%,
 280 RSD \leq 20%, n=3) when more acidic conditions (AOAC method) were applied, which is in
 281 agreement with the results reported by Salvia et al.¹⁵ Regarding the clean-up, the best results for
 282 81% of the compounds were obtained when the AOAC extracts were purified with the
 283 MgSO₄+C18 mixture.

284



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

287

288 Considering the compounds presenting better recoveries with the combination
289 AOAC/MgSO₄+C18 with respect to EN/MgSO₄+C18, we can indicate lincomycin (77% versus
290 45%) and loratadine (100% versus 63%). The same behavior was observed for sulfonamide
291 antibiotics (sulfadiazine, sulfamethazine, sulfamethoxazole, sulfapyridine and sulfathizole) with
292 improved recoveries in the range 52-70% compared with the low recoveries (21% to 29%)
293 obtained with the EN method (RSD values ≤ 20% in all cases). This behavior is related to the
294 amphoteric character of sulfonamides, which plays an important role for their extraction from
295 soil, since their partitioning is pH-dependent. More acidic conditions also improved
296 sulfonamide extraction in the study carried out by Young-Jun Lee et al.,¹⁶ who compared the
297 efficiencies of the AOAC and EN methods for a group of ten CECs in agricultural soil,
298 obtaining better recoveries with the AOAC method. No or limited effects were observed for the
299 rest of the target analytes.

300 Higher variation in the recoveries was found during the clean-up study by d-SPE. The
301 combination MgSO₄+C18 yielded better results under both buffered conditions, while presence
302 of PSA reduced extraction efficiency in all cases. This can be explained considering that PSA
303 acts as chelating agent with acidic compounds, as it has been previously reported.³⁰ Clofibric
304 acid, furosemide, indomethacin, ketoprofen, ketorolac, mefenamic acid and methylprednisolone,
305 showed significant lower recovery values in presence of PSA, decreasing a 50% in some cases
306 (Figure S3). These results agree with those published by De Carlo et al.¹⁴ According to the
307 results obtained and in order to find a compromise due to the diverse physical-chemical
308 properties of the analytes under study, the AOAC method followed by d-SPE with MgSO₄+C18
309 was chosen for subsequent validation.

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

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

336 120% and $RSD \leq 20\%$. For most compounds, reproducible recovery values were also obtained
337 between the diverse concentrations tested. For 20 compounds the methodology showed
338 recovery rates out of the acceptable range, but with $RSD \leq 20\%$, which means that the method
339 was still repetitive and reliable for their analysis. Lower precision was observed for
340 acetaminophen, clotrimazole, fenofibrate, flumequine and pravastatin, with recovery values that
341 differed more than 20% among concentrations or even for the same concentration level. Despite
342 these analytes do not fulfill the proposed acceptability criteria, they were kept in the study as
343 they can be considered for qualitative or semi-quantitative purposes.

344 Linearity was investigated in the range from 0.1 to 100 ng g⁻¹. All analytes showed R² values
345 higher than 0.995 (Table S6). Average ME for each compound was also evaluated: 63 targets
346 out of 73 showed low ME (ME < 20%), which proves the efficiency of the purification step
347 avoiding undesirable co-extractive matrix substances. The predominant effect observed was
348 signal suppression for the 59% of the compounds. Only clotrimazole showed a strong ME (-
349 52%). These results can explain in part the low recoveries and lack of precision observed for
350 this compound (Table S6).

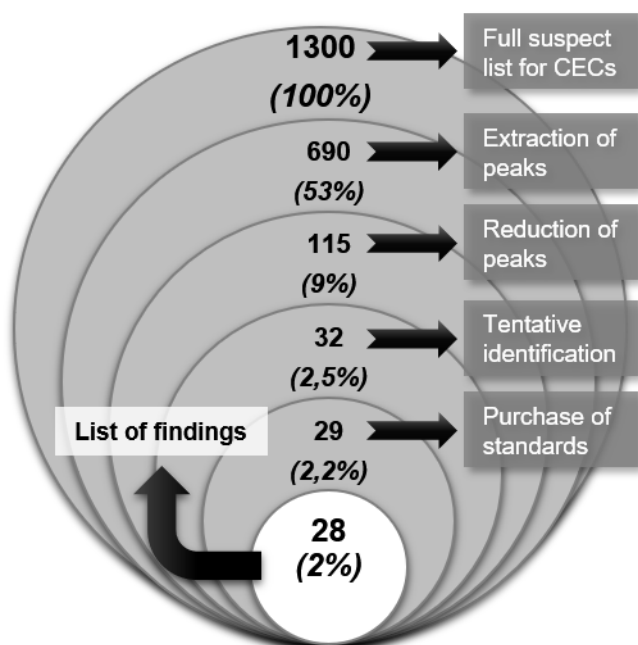
351 MQLs ranged from 0.1 to 5 ng g⁻¹ (Table S6), with 89% of compounds presenting values
352 below 1.0 ng g⁻¹. These values are in the same range as those reported by other authors, using
353 different QuEChERS approaches^{16,33} or even with other methodologies as USE or PLE.^{34,35}
354 However, no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

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

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

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

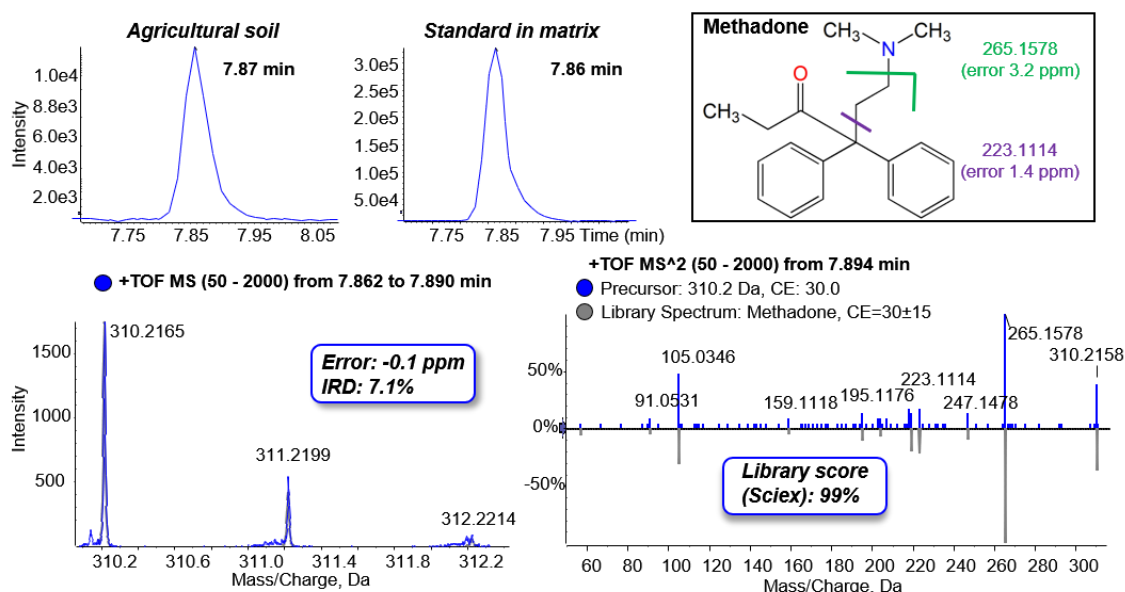
385 *Suspect screening.* To expand the scope of the proposed method to additional compounds for
386 which reference standards are not available in our laboratory, a suspect screening approach was
387 applied according to the workflow shown in Figure 1. A compiled suspect list containing 1300
388 contaminants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



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

400

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



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

414

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

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

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

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

446

447 **CONCLUSIONS**

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

460

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470

471 **ASSOCIATED CONTENT**

472 **Supporting Information Available**

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

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

479

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551

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.