



Identification of transformation products of carbamazepine in lettuce crops irrigated with Ultraviolet-C treated water

Martínez-Piernas, A. B., Nahim-Granados, S., Polo-López, M. I., Fernández-Ibáñez, P., Murgolo, S., Mascolo, G., & Agüera, A. (2019). Identification of transformation products of carbamazepine in lettuce crops irrigated with Ultraviolet-C treated water. *Environmental Pollution*, 247, 1009-1019. <https://doi.org/10.1016/j.envpol.2019.02.001>

[Link to publication record in Ulster University Research Portal](#)

Published in:
Environmental Pollution

Publication Status:
Published (in print/issue): 01/04/2019

DOI:
[10.1016/j.envpol.2019.02.001](https://doi.org/10.1016/j.envpol.2019.02.001)

Document Version
Author Accepted version

Document Licence:
CC BY-NC-ND

General rights

The copyright and moral rights to the output are retained by the output author(s), unless otherwise stated by the document licence.

Unless otherwise stated, users are permitted to download a copy of the output for personal study or non-commercial research and are permitted to freely distribute the URL of the output. They are not permitted to alter, reproduce, distribute or make any commercial use of the output without obtaining the permission of the author(s).

If the document is licenced under Creative Commons, the rights of users of the documents can be found at <https://creativecommons.org/share-your-work/licenses/>.

Take down policy

The Research Portal is Ulster University's institutional repository that provides access to Ulster's research outputs. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact pure-support@ulster.ac.uk

1 **Identification of transformation products of carbamazepine in lettuce**
2 **crops irrigated with Ultraviolet-C treated water**

3
4 A.B. Martínez-Piernas¹, S. Nahim-Granados², M.I. Polo-López², P. Fernández-Ibáñez³,
5 S. Murgolo⁴, G. Mascolo⁴, A. Agüera¹

6 ¹ *CIESOL, Joint Centre University of Almeria–CIEMAT, Carretera de Sacramento s/n,*
7 *E-04120, Almeria, Spain*

8 ² *Plataforma Solar de Almería–CIEMAT, Carretera Senés km 4, 04200 Tabernas,*
9 *Almería, Spain*

10 ³ *Nanotechnology and Integrated BioEngineering Centre, School of Engineering,*
11 *University of Ulster, Newtownabbey, Northern Ireland, United Kingdom*

12 ⁴ *CNR, Istituto di Ricerca Sulle Acque, Via F. de Blasio 5, 70132, Bari, Italy*

13

14 **Abstract**

15 Transformation of organic microcontaminants (OMCs) during wastewater treatments
16 results in the generation of transformation products (TPs), which can be more persistent
17 than parent compounds. Due to reuse of reclaimed wastewater (RWW) for crop
18 irrigation, OMCs and TPs are released in soils being capable to translocate to crops.
19 Furthermore, OMCs are also susceptible to transformation once they reach the soil or
20 crops. The recalcitrant antiepileptic carbamazepine (CBZ) and some of its frequently
21 reported TPs have been found in agricultural systems. However, there is no knowledge
22 about the fate in reuse practices of multiple CBZ TPs that can be formed during
23 wastewater treatment processes. For the first time, this work presents a study of the
24 behavior of CBZ TPs generated after a conventional Ultraviolet-C (UVC) treatment in
25 an agricultural environment. The UVC-treated water was used for the irrigation of
26 lettuces grown under controlled conditions. The latter was compared to the fate of TPs
27 generated in the peat and plant by irrigation with non-treated water containing CBZ. A
28 suspect screening strategy was developed to identify the TPs using liquid
29 chromatography coupled to quadrupole-time-of-flight (LC-QTOF-MS). The results
30 revealed the presence of 24 TPs, 22 in UVC-treated water, 11 in peat and 9 in lettuce
31 leaves. 4 of the TPs identified in peat (iminostilbene, TP 271B, TP 285A-B); and 3 in
32 leaves (10-11 dihydrocarbamazepine, TP 271A-B) were not previously reported in soils
33 or edible parts of crops, respectively. Comparing the TPs found in peat and lettuces
34 derived from both irrigation conditions, no significant differences regarding TPs
35 formation or occurrence were observed. UVC treatment did not contribute to the
36 formation of different TPs than those generated by transformation or metabolism of
37 CBZ in peat or plant material. This research improves the current knowledge on the fate
38 of CBZ TPs in agricultural systems as a consequence of reuse practices.

39

40 **Keywords:** Carbamazepine, transformation products, LC-QTOF-MS, wastewater
41 reuse, suspect screening

42

43 1. Introduction

44 Nowadays, standard treatment processes applied in wastewater treatment plants
45 (WWTPs) do not remove efficiently a large variety of organic microcontaminants
46 (OMCs) as pharmaceuticals, personal care products or pesticides (Campos-Mañas et al.,
47 2017). With OMCs, several recalcitrant transformation products (TPs), generated during
48 the treatments, are continuously discharged in WWTP effluents (Schollée et al., 2015).
49 As agricultural practices demand a large amount of water, reuse of reclaimed
50 wastewater (RWW) has become a common practice in many dry areas to deal with
51 water shortages. Consequently, OMCs and TPs have been reported in agricultural soils
52 at concentrations up to $\mu\text{g g}^{-1}$ (Chen et al., 2011; Christou et al., 2017; Kinney et al.,
53 2006; Koba et al., 2016). Due to their physical-chemical properties, some of these
54 compounds have the potential to be uptaken via plant roots (Wu et al., 2015). Once
55 compounds have entered the plant, a subsequent translocation toward other parts of
56 plants, including the edible part of crops, can take place resulting in the possible
57 introduction of undesirable substances into the food chain. Although the number of
58 studies dedicated to soil accumulation and plant uptake of OMCs is steadily increasing
59 in recent years (Carter et al., 2018; Larivière et al., 2017; Martínez-Piernas et al.,
60 2018a), little information is available regarding TPs behavior in soil/plant systems.
61 These TPs often present similar or even greater concentration levels than their parent
62 compounds in WWTP effluents (Bahlmann et al., 2014). Additionally, they can be also
63 generated in soils from biotic/abiotic transformations and in crops as a consequence of
64 the metabolism of plants (Huynh et al., 2018; Riemenschneider et al., 2017).
65 Considering that some TPs have analogous or even more severe biological activity than
66 parent compounds (Brezina et al., 2017), their fate and ecotoxicological and human
67 health risks merit further research.

68

69 Generally, TPs show very diverse physical-chemical properties due to their different
70 structures. For this reason, broad spectrum extraction methodologies are required to
71 obtain efficient recoveries in a wide range of compounds. QuEChERS (Quick,
72 Easy, Cheap, Effective, Rugged and Safe) and pressurized liquid extraction (PLE) have
73 demonstrated to be good alternatives even in these complex environmental matrices
74 (Martínez-Piernas et al., 2018b) (Jelić et al., 2009). Besides, the application of screening
75 methodologies accomplished by liquid chromatography coupled to high resolution mass

76 spectrometry (HRMS), have undoubtedly improved the identification of unexpected or
77 not previously validated compounds by the application of non-target and suspect
78 screening approaches (Martínez-Piernas et al., 2018a).

79 Carbamazepine (CBZ) is one of the most frequently detected OMCs in WWTP effluents
80 due to its recurrent prescription for neuropsychiatric disorders (Ambrósio et al., 2002)
81 and its low removal by the application of standard wastewater treatment processes
82 (Zhang et al., 2008). Because of its persistence and ubiquitous occurrence, it has been
83 proposed as an appropriate indicator for the evaluation of anthropogenic impact on the
84 aquatic environment (Kinney et al., 2008). According to the criteria established by
85 Council Directive 92/32/EEC, CBZ has been classified as potentially harmful
86 compound for aquatic organisms (Fent K., 2008). In addition, the formation of several
87 of its TPs has been reported by the application of different wastewater treatments, which
88 are presented in Table S1. CBZ undergoes transformation to various aldehydes, ketones
89 and hydroxylated derivatives and known ecotoxic compounds as acridine and acridone
90 (Donner et al., 2013). However, information about presence and fate of CBZ TPs in
91 soils and crops is still scarce. Riemenschneider et al. (Riemenschneider et al., 2017)
92 investigated the formation and translocation of CBZ TPs through the different parts of
93 tomato plants irrigated with a spiked solution of CBZ under hydroponic conditions.
94 Regarding soils, Koba et al. (Koba et al., 2016) evaluated the stability of CBZ in
95 different soils, identifying in samples up to three TPs after an incubation process with
96 CBZ. Nevertheless, to our knowledge, no data is available about the fate of CBZ TPs
97 produced after standard tertiary treatments in agricultural systems. Regarding tertiary
98 treatments, advanced oxidation processes (AOPs), which are characterized by the
99 formation of powerful oxidizing species, have been proved to be effective in the
100 degradation of organic contaminants (Malato et al., 2009). Among available AOPs,
101 ultraviolet treatment is one of the most extended processes for drinking and wastewater
102 purification and, in particular, degradation of CBZ by UV and UV-based AOP has been
103 widely reported in literature (Dai et al., 2012; Deng et al., 2013; Ghasemian et al.,
104 2017).

105 The objective of this study was to increase the current knowledge on the fate of CBZ
106 TPs in an agricultural system based on a lettuce crop grown in peat under controlled
107 conditions. The presence and fate of TPs in these two commodities were compared

108 considering two different TP sources: i) TPs formed after conventional Ultraviolet-C
109 (UVC) treatment applied to the irrigation water, and ii) TPs formed in the soil-plant
110 system by the transformation of CBZ introduced by the irrigation water. The
111 identification of CBZ TPs was carried out by the application of a suspect screening
112 strategy by LC-QTOF-MS, which included up to 47 TPs commonly found after
113 different decontamination or biological processes.

114 **2. Materials and methods**

115 2.1 Chemicals

116 Carbamazepine (CBZ), carbamazepine 10,11-epoxide (EPOX), acridone (ACRO),
117 acridine (ACRI), oxcarbazepine (OX) and carbamazepine-d₁₀ (CBZ-d₁₀) analytical
118 standards (purity grade $\geq 98\%$) were purchased from Sigma Aldrich (Steinheim,
119 Germany). Iminostilbene, 9-acridinecarboxylic acid, 9-acridine-carboxaldehyde and 10-
120 11 dihydrocarbamazepine (all purity $\geq 98\%$) identified by suspect screening analysis
121 and acquired for confirmatory purposes were also purchased from Sigma Aldrich. LC-
122 MS grade acetonitrile (MeCN), methanol (MeOH), water, formic acid and acetic acid
123 were purchased from Sigma Aldrich. For QuEChERS, magnesium sulfate (MgSO₄),
124 sodium acetate (NaOAc), octadecyl silica (C18) and primary-secondary amine (PSA)
125 were purchased from Sigma Aldrich. Hydromatrix was provided by Thermo Fisher
126 Scientific (Waltham, USA).

127 Stock standard solutions were prepared in MeOH at a concentration of 1000 mg L⁻¹. A
128 mixed working solution containing all standards was prepared at 10 mg L⁻¹ in MeOH by
129 proper dilution of each stock standard solution. All solutions were prepared in amber
130 glass vials and stored at -20 °C. CBZ-d₁₀ was used as extraction quality control check.

131 2.2 Experimental set-up

132 2.2.1 Experimental lettuce cultivation

133 Seeds of lettuce (*Lactuca sativa*) obtained from a local provider were cultivated under
134 controlled conditions of temperature and humidity in an experimental greenhouse
135 described by Martínez-Piernas et al., 2018b. 90 propylene pots (9 × 9 × 10 cm) were
136 filled with sterilized peat (autoclaved using autoclave-bags at 121 °C during 15 min in

137 batches of 5 kg of peat). The peat was a mixture of blond peat, black peat, coconut
138 fibers and perlite containing N, P, and K in a ratio (w/v) of 13–14–13 g L⁻¹,
139 respectively, pH 7, organic matter dry matter ratio of 80%, apparent density of 0.38 kg
140 L⁻¹ and 120 mS m⁻¹ of conductivity, according to the manufacturer. The growing crops
141 was not done under sterile conditions. The growing period was conducted from May to
142 July 2016, a total of 10 weeks. Three experimental conditions (30 pots each) were
143 performed separately to avoid any cross-contamination: a) control samples irrigated
144 with synthetic water (SW); b) samples irrigated with SW spiked with 1 mg/L of CBZ
145 (SW+CBZ); and c) samples irrigated with SW spiked with 1 mg L⁻¹ of CBZ and treated
146 by UVC (SW+CBZ+UVC). Pots were irrigated every two days. The experimental setup
147 for the three cultivations of lettuce crops is shown Figure S1. The sampling strategy was
148 designed to evaluate potential presence and accumulation of CBZ and metabolites/TPs
149 in peat and lettuce leaves. A total of five sampling events occurred. Samples were taken
150 every two weeks from the second week of growth until the tenth week (harvest). In each
151 sampling event, ten pots randomly selected were taken (leaves and peat) and combined
152 to form a homogenized composite sample which was extracted per triplicate. The final
153 size of lettuce leaves was 15 cm in the last sampling event.

154 2.2.2 Irrigation water

155 SW was prepared following the recipe published in (American Public Health
156 Association, American Water Works Association, 2012) under the “standard
157 moderately-hard freshwater” nomenclature, based on the characteristics of groundwater
158 in Almería province (Spain). For the irrigation of crops with CBZ, SW was spiked with
159 the appropriate amount of pure CBZ standard to reach a final concentration of 1 mg L⁻¹.
160 Before each irrigation event, a fresh solution of CBZ was prepared to avoid the possible
161 formation of undesirable TPs. For the irrigation tests with treated-CBZ, UVC treatments
162 were carried out in a pilot plant previously described by Miralles-Cuevas et al., 2017.
163 Briefly, it consists of three independent low-pressure UVC lamps (254 nm peak
164 wavelengths, 230 W and 40 mJ cm⁻² of UV dose or fluence) serially connected to
165 holding tank. The volume of each lamp-camera is 5 L. In this work, the system was
166 operated with one UVC lamp in recirculating batch mode at 30 L/min of flow. The tank
167 was filled with 80 L of SW and spiked with CBZ (1 mg L⁻¹). After 10 min of mixing in
168 the dark, a Time 0 was taken out and the UVC lamp was switched on. From this time,

169 samples were taken every 2 min during the first 20 min, and every 5 min till the end of
170 the treatment (60 min total exposure time). The treated water (ca. 60 L) was stored at
171 4°C and used for crop irrigation during one week. The same procedure was repeated
172 weekly during the irrigation period (a total of 10 weeks) in order to use fresh-batches
173 and avoid possible fluctuations of TPs during storage. Irrigation events occurred every
174 two-three days depending on plant water demand, with 50 mL of water/pots. All water
175 batches were analyzed by LC-QTOF-MS before irrigation to verify the absence of any
176 compound in control water (SW), the absence of TPs in water spiked with CBZ
177 (SW+CBZ) and possible fluctuations in the formation of TPs in treated water
178 (SW+CBZ+UVC).

179 2.3 Sample preparation

180 2.3.1 Lettuce extraction

181 Leaves of lettuce samples were washed with tap water, chopped and stored in the dark
182 at -20°C until their analysis. Samples were extracted by a QuEChERS-based extraction
183 method including a dispersive solid-phase extraction (d-SPE) clean-up step (Martínez-
184 Piernas et al., 2018b). Briefly, a representative aliquot of 10 g of previously
185 homogenized sample was weighed in a 50 mL PTFE centrifuge tube. 10 mL of MeCN
186 at 1% of acetic acid and 50 µL of CBZ-d₁₀ (400 µg L⁻¹), used as internal quality control,
187 were added and the tube was shaken for 5 min. After that, 6 g of MgSO₄ and 1.5 g of
188 NaOAc were added and the tube was vigorously shaken for 5 min and centrifuged at
189 3500 rpm (2054 g) for 5 min. Then, a 5 mL aliquot of the organic layer was transferred
190 to a 15 mL centrifuge tube containing 125 mg of PSA, 125 mg of C18 and 750 mg of
191 anhydrous MgSO₄. The tube was then shaken for 30 s in a Vortex and centrifuged again
192 (3500 rpm, 5 min). After that, the extracts (4 mL) were transferred to screw cap vials
193 and 10 µL of MeCN 1% formic acid per mL of extract were added. Finally, an aliquot
194 of 150 µL of the extract was evaporated until dryness and reconstituted with the same
195 volume of MeCN:H₂O (10:90, v/v) before the injection in the LC-QTOF-MS/MS
196 system.

197 2.3.2 Peat extraction

198 Peat samples were homogenized, freeze dried and finally grinded using a Mixer Mill
199 MM 301 equipped with two cells of 35mL made of ZrO₂. Samples were extracted by

200 PLE following the protocol described in (Jelić et al., 2009) using an ASE 300
201 accelerated solvent extractor followed by a solid-phase extraction (SPE) clean-up step. 1
202 g of homogeneous freeze-dried peat was placed in a stainless steel extraction cell of 11
203 mL, which was filled with hydromatrix. The extraction solvent consisted of a mixture of
204 MeOH:H₂O (1:2, v/v). Optimized PLE parameters chosen were: a temperature 100 °C, a
205 preheating period of 5 min, a total of 3 static cycles (5 min each), and total flush volume
206 of 100% of the cell with 60 s of nitrogen purge. PLE extract (about 40 mL) was diluted
207 in 500 ml of H₂O and cleaned-up by SPE using Oasis HLB cartridges (200 mg, 6 mL).
208 The cartridges were conditioned with 5 mL of MeOH followed by 5 mL of H₂O at
209 neutral pH. The elution of compounds was carried out with 8 mL of MeOH. Then, SPE
210 extracts were evaporated under nitrogen stream and reconstituted in 1 mL of
211 MeCN:H₂O (10:90, v/v) before LC-QTOF-MS/MS injection.

212 2.4 Analysis by liquid chromatography tandem mass spectrometry

213 Chromatographic separation was carried out using a HPLC 1260 Infinity (Agilent
214 Technologies, Palo Alto, CA, USA) system provided with an Eclipse C18 (4.6 x 150
215 mm, 5µm particle size) column (Agilent Technologies). The mobile phases were 0.1%
216 formic acid in water (solvent A) and pure MeCN (solvent B). The injection volume was
217 20 µL and the flow rate was 0.5 mL min⁻¹. The initial proportion of solvent B was 10%,
218 which was kept constant for 2 min, increased to 100% within 38 min, kept constant for
219 10 min and reduced to 10% in 0.1 min. The post-run equilibration time was 15 min.

220

221 For HRMS, a TripleTOF[®] 5600+ System (Sciex, Foster City, CA, USA) equipped with
222 a dual source was used. ESI interface was employed for sample injection and the
223 atmospheric-pressure chemical ionization interface (APCI) for calibrant delivery. The
224 ESI source was operated in positive mode. The parameters applied were 60 psi of gas 1
225 and 2,; 30 psi of curtain gas,; an ionspray voltage of 4500 V; a declustering potential of
226 80 V and a temperature of 575 °C. Nitrogen was used as nebulizer, curtain and collision
227 gas. The acquisition method consisted in a full-scan survey (TOF-MS) followed by four
228 TOF-MS/MS scans carried out by Information Dependent Acquisition (IDA) of the four
229 more intense ions in each TOF-MS scan. Scanned mass range was from 50 to 1000 *m/z*,
230 either in TOF-MS (resolving power of 30000) or TOF-MS/MS experiments. An
231 accumulation time of 250 ms was applied in TOF-MS and 100 ms for IDA scan. IDA

232 criteria considered dynamic background subtraction. Collision energy of 30 eV with a \pm
233 15 eV spread was used in MS/MS fragmentation. Data acquisition was carried out by
234 Analyst TF 1.5, and data processing by PeakViewTM 2.2 and MasterView 1.1.

235 2.5 Suspect screening strategy

236 A suspect list including 47 possible transformation and biotransformation products of
237 CBZ was built according to the previously reported TPs in literature generated by
238 diverse decontamination wastewater treatments and biological processes (Table S1). As
239 first step of data processing, a reduction of the number of peaks for a reliable
240 identification was carried out by applying a peak intensity threshold ≥ 1000 cps, a S/N
241 ratio ≥ 10 and the absence of the mass in the control sample (blank matrix). After that,
242 the criteria adopted for a tentative identification was a mass accuracy error ≤ 5 ppm of
243 the precursor ion and an isotope ratio difference $\leq 10\%$. The MS/MS information was
244 compared with spectra reported in literature, MassBank (“MassBank Database,” n.d.)
245 and ChemSpider (“ChemSpider Database,” n.d.) databases; for which a minimum score
246 of 80% and presence of at least two fragments with an accurate mass error ≤ 5 ppm
247 were considered acceptable. Final confirmation of tentative identified compounds was
248 adopted when the retention time (Rt) of the standard in matrix differed less than ± 0.1
249 min and the MS/MS spectra matched.

250

251 The TPs tentatively identified were grouped according to the confidence levels
252 proposed by Schymanski et al. (Schymanski et al., 2014). Level 4 included TPs for
253 which enough MS/MS fragmentation information was not acquired and, consequently,
254 no structure could be suggested. Level 3 was adopted for those compounds whose
255 MS/MS information matched with literature or libraries, but different structures could
256 be proposed. In Level 2 were accommodated compounds with enough MS/MS and
257 experimental context data to propose a unique probable structure. Finally, Level 1 was
258 considered for TPs confirmed by the unequivocal information of Rt and MS/MS
259 fragmentation of the purchased analytical standard.

260 2.6 Methods validation

261 Both QuEChERS-based and PLE+SPE, procedures applied in this study for the
262 extraction of CBZ and TPs in plant material and peat were validated for a set of 5

263 compounds: CBZ, EPOX, ACRI, ACRO and OX. The validation was carried out in
264 terms of linearity, limits of quantification (LOQs), trueness (recoveries) and precision
265 (relative standard deviations, RSD). For validation purposes, control samples of lettuce
266 and peat were used as blanks.

267 Linearity was studied by spiking matrix blank extracts at 11 different concentrations
268 ranging from 0.1 to 200 ng g⁻¹. Adequate determination coefficients (R²) were
269 considered acceptable when R² ≥ 0.990. Recoveries and precision (n=3) were evaluated
270 by spiking blank samples (20 ng g⁻¹ in peat and 10 ng g⁻¹ in lettuce). LOQs were
271 experimentally calculated as the lowest concentration level spiked in blank matrix
272 extract which fulfill the requirements of analyte confirmation. In Table S2 is compiled
273 the information of both validation methodologies.

274 **3. Results and Discussion**

275 3.1 Identification of CBZ TPs by suspect screening

276 Following the suspect screening approach described above (Section 2.5.), a total of 24
277 TPs out of the 47 included in the suspect list were tentatively identified in some of the
278 analyzed samples (irrigation water, peat or lettuces). The list of candidates and
279 chromatographic and identification information is presented in Table 1.

280

281 One of the main difficulties regarding TPs identification is the differentiation between
282 isomers or compounds with closely related structures, which can show the same
283 accurate mass and elemental composition and even very similar MS/MS fragmentation
284 due to their related structures. This can lead to flimsy or erroneous tentative
285 identifications, even when an adequate chromatographic separation is carried out. This
286 is the case of the 12 TPs classified in Level 3, for which varied structures could be
287 proposed in each case.

288

289 An example regarding difficulties on appropriate identification of TPs in absence of
290 standards was the allocation of structures for TPs 253A-D, with extraction mass *m/z*
291 253.0971 for the [M+H]⁺. The elemental composition, C₁₅H₁₂N₂O₂, corresponded to the
292 addition of one oxygen atom to the structure of CBZ and all of them presented the same
293 fragmentation pattern. Fig. 1 shows the extracted ion chromatogram (XIC: 253.0971
294 *m/z*) and MS/MS spectra of TPs 253B-D in an irrigation water sample, where the

295 similarity of the MS/MS spectra for the three compounds can be appreciated. Among
296 the structures found according to the proposed formula (Table S1), the fragmentation
297 pattern matched well with the spectra of the isomers typically produced by the
298 monohydroxylation of CBZ (OH-CBZ) at different positions, as proposed by several
299 authors (Ahmed and Chiron, 2014; Brezina et al., 2017; Hübner et al., 2014; Jelic et al.,
300 2013; Li et al., 2013; Liu et al., 2016; Zhang et al., 2015; Zhu et al., 2016). Up to three
301 isomers have been reported with hydroxylation at positions 2, 3 and 10. However,
302 analysis of the available analytical standards allowed confirmation (Level 1) of TP
303 253C as EPOX (Ahmed and Chiron, 2014; Hübner et al., 2014; Li et al., 2013; Liu et
304 al., 2016; Zhang et al., 2015; Zhu et al., 2016) and TP 253D as OX (Brezina et al.,
305 2017), as it is shown in Fig. 1 . In this case, only the R_t comparison with the analytical
306 standard allowed to distinguish both compounds. 253B was then tentatively assigned as
307 a monohydroxy derivative, but the R_t and spectral information available was not enough
308 to clarify the position of the hydroxylation in the ring. Therefore, TP 253B was kept in
309 Level 3. TP 253A, also presented the same molecular formula and isotopic profile and
310 could be tentatively proposed as a second OH-CBZ isomer, but due to its low intensity,
311 not enough product ions with significant intensities and exact mass data were acquired.
312 For this reason, it was considered in identification Level 4.

313

314 A similar situation was observed for TP 269 (m/z 269.0920, $C_{15}H_{12}N_2O_3$). Up to five
315 peaks, TPs 269 A-E were detected. TP 269B remained in Level 4 by the same reason
316 already exposed for TP 253A. The other four peaks presented similar characteristic
317 fragments at m/z 251.0815, m/z 208.0757 and m/z 180.0808. The elemental
318 composition, with two additional oxygen atoms with respect to CBZ, was in accordance
319 with the formation of dihydroxy-CBZ derivatives proposed by Hübner et al (Hübner et
320 al., 2014), and TP 269A and 269C were tentatively proposed as dihydroxylated isomers.
321 However, other structures with alike fragments have also been reported in literature by
322 Ahmed and Chiron (2014), Jelic et al., (2013) and Zhu et al., 2016(see Table S1). The
323 hydroxylation of OX intermediate proposed by Jelic et al. (Jelic et al., 2013) match with
324 the mass spectrum of TP 269D (Fig. S2A), which shows the characteristic fragment at
325 m/z 196.0757, corresponding to the formation of hydroxyl acridine ($C_{13}H_9NO$).
326 However, some differences in the spectrum reported for 11-OH-OX (Jelic et al., 2013)
327 can be explained by the different position of the OH group, as it is proposed in Fig. S2.
328 On the other hand, the absence of the diagnostic fragment at m/z 196.0757 in the mass

329 spectrum of TP 269E (Fig. S2B) suggested a different structure for this compound. The
330 alternative proposed by Ahmed and Chiron corresponds to the hydroxylation of 9-
331 formylacridine-10(9H)-carboxamide (Ahmed and Chiron, 2014), which can be plausible
332 and supported by the successive losses of CHNO (m/z 226.0863), H₂O (m/z 208.0757)
333 and CO (m/z 180.0808) observed in the mass spectra (Fig. S2B). A subsequent
334 hydroxylation of TP 269E would be consistent with the formation of dihydroxy
335 derivatives also identified as TP 285A and B (Fig. S3).

336

337 Identification of TPs 208A and 208B (m/z 208.0756; C₁₄H₉NO), could not be carried
338 out. Two structures could fit with this m/z corresponding to 9-acridine-carboxaldehyde
339 (Hübner et al., 2014; Liu et al., 2016; Seiwert et al., 2015; Zhang et al., 2015; Zhu et al.,
340 2016), which was discarded by the analysis of the analytical standard, and the human
341 metabolite CBZ iminoquinone (Brezina et al., 2017), which shared a characteristic
342 product ion at m/z 152.0495 (C₁₁H₅N), with TP 208B. However, this evidence was too
343 weak for the allocation of the CBZ iminoquinone structure and both compounds
344 remained in Level 4.

345

346 TPs 224A (Rt 4.7 min) and 224B (Rt 22.7 min) were detected at m/z 224.0706
347 (C₁₄H₉NO₂). Both presented the same MS/MS fragments. Some authors have associated
348 this formula to varied structures (Brezina et al., 2017; Hübner et al., 2014; Jelic et al.,
349 2013; Li et al., 2013; Riemenschneider et al., 2017), describing common product ions in
350 many cases (Table S1). One of the most plausible ones for TP 224A, due to its polar
351 chromatographic behavior, was 9-acridinecarboxylic acid, which was confirmed by the
352 analytical standard. The retention time behavior of TP 224B could match with varied
353 structures. However, based on the MS/MS spectrum, the compound was tentatively
354 proposed as acridone-N-carbaldehyde, according with Li et al. (Li et al., 2013) in a
355 study about identification and kinetic of metabolites of CBZ in soil. The similarity with
356 the fragmentation pattern of acridone after the loss of -CO (m/z 196.0750; C₁₃H₉NO)
357 supported the proposal of this structure at Level 3.

358

359 In the case of TPs 267A and 267B (m/z 267.0764; C₁₅H₁₀N₂O₃), only the first one was
360 tentatively proposed as 11-keto oxcarbazepine, based on the MS/MS fragmentation
361 pattern reported by Jelic et al. (2013) and Koba et al. (2016). Many other structures
362 have been reported for TPs with m/z 267.0764, however not enough evidences have

363 been found that support a structure assignation. Consequently, TP 267B was not
364 assigned (Level 4).

365

366 TPs 271A and B, also presented very similar fragmentation, which matched well with
367 that proposed by Jelic et al. (Jelic et al., 2013) and Hübner et al. (Hübner et al., 2014)
368 for 10,11-dihydro-10,11-dihydroxycarbamazepine. Li et al. (Li et al., 2013) also
369 confirmed this compound by comparing with an authentic standard and reported the
370 presence of the cis and trans stereoisomers, which could correspond with the two peaks
371 observed. The assignation of this structure was also reinforced with the identification of
372 TP 287 (m/z 287.1026; $C_{15}H_{14}N_2O_4$), which could correspond with a further
373 hydroxylation of the benzene ring in TP 271.

374

375 Despite of the general absence of TPs MS/MS spectra in spectral libraries and
376 databases, attributable to the scarce availability of commercial analytical standards,
377 some of the TPs under study could be identified by this way. This was the case of TP
378 194 (m/z 194.0964, $C_{14}H_{11}N$), which matched with iminostilbene structure (94% score)
379 in MassBank, or TP 239 (m/z 239.1179, $C_{15}H_{14}N_2O$), which revealed a 90% of spectral
380 score match with 10,11-dihydrocarbamazepine in ChemSpider database. Both were
381 confirmed by subsequent standard acquisition and analysis.

382

383 Although spectral and context evidences pointed out a tentative structure proposal for
384 most of the compounds investigated, a definite confirmation by analytical standard
385 (Level 1) was only obtained for 7 TPs namely ACRI, ACRO, EPOX, OX, TP 194
386 (iminostilbene), TP 224A (9-acridinecarboxylic acid) and TP 239 (10-11
387 Dihydrocarbamazepine).

388

389 3.2 Identification of CBZ TPs in irrigation water, peat and lettuce leaves

390

391 It is already known that CBZ is a recalcitrant compound whose removal is not efficient
392 by conventional treatments, leading to its constant detection in WWTP effluents
393 (Campos-Mañas et al., 2017). In this study, the removal of CBZ in water after UVC
394 treatment described in the experimental section was about 20 %, as it is shown in Fig.
395 S4. However, despite its persistence, a total of 22 CBZ TPs were identified in the
396 treated water (SW+CBZ+UVC), which was used in the irrigation assays (see Table 2).

397 In general, peak areas were comparable in each irrigation batch, showing a repetitive
398 pattern of TPs formation. The most abundant TP found in treated water was EPOX
399 followed by TP 253B (OH-CBZ), TP 194 (iminostilbene) and ACRI, which were
400 detected from the second minute of treatment (Fig. S5). Overall, TP abundances
401 increased with treatment time due to the persistence of CBZ. Alternatively, no TPs were
402 detected in the irrigation water containing CBZ, which had not undergone any
403 treatment.

404

405 For peat and lettuces irrigated with SW, neither CBZ nor any of its TPs were observed.
406 Regarding samples irrigated with SW+CBZ and SW+CBZ+UVC, the results showed
407 that almost identical TPs were detected in both irrigation experiments. As can be seen in
408 Table 2, 10 TPs were identified in peat irrigated with untreated water containing CBZ
409 and 11 in peat irrigated with the treated water, while 9 TPs were found in lettuces
410 regardless of the water used for irrigation. These data suggest that UVC treatment did
411 not contribute to the presence of different TPs with respect to those formed by the
412 transformation of CBZ in peat or lettuce. TPs formation was also possible by the only
413 presence of CBZ in irrigation water. This is in agreement with the results found by
414 Riemenschneider et al. (Riemenschneider et al., 2017) for tomato plants cultivated
415 under hydroponic conditions irrigated with a nutrient solution containing only CBZ.

416

417 In regard to the abundances of the identified TPs, these were higher, in general, in peats
418 irrigated with UVC-treated water since the treatment promotes the TPs formation. Fig. 2
419 shows the evolution on the abundances of the CBZ TPs detected in lettuce and peat
420 samples during the plant growth. Higher differences were observed in TPs 271C and
421 285B. This pattern was also observed when concentrations of EPOX, ACRI and ACRO
422 were quantitatively evaluated. As shown in Table 3, slightly higher TP concentration
423 values were obtained from peat irrigated with treated water, although this correlation
424 was not always observed in lettuce. For the vegetable, the CBZ TP concentrations were
425 higher in samples irrigated with SW+CBZ (Table 3 and Fig. 2). This demonstrates the
426 necessity to develop efficient wastewater treatments able to completely remove
427 recalcitrant compounds as CBZ in order to prevent their plant metabolization, which
428 may lead to the detection of TPs in edible parts of crops. TP concentrations followed the
429 order EPOX > ACRO > ACRI in every commodity and experimental irrigation test.
430 EPOX has been reported as the most abundant TP in soils (Koba et al., 2016) and

431 tomato plants (Riemenschneider et al., 2017) exposed to CBZ for long periods. In
432 general, TPs did not show biodegradability but a clear accumulation along sampling
433 events was detected (Table 3), highlighting the accumulation of EPOX in both type of
434 samples and irrigation tests. This accumulation can be explained in part by the increase
435 in the transpiration rate associated with the growth of the lettuce plant (Dodgen et al.,
436 2015), although other factors, such as the plant's physiology, environmental conditions
437 and TPs physicochemical properties (i.e. lipophilicity and electrical charge), can also
438 contribute to this behavior (Christou et al., 2017).

439

440 In Table 2, it is also shown two TPs (TP 285 A and B) detected in peat samples and not
441 in water. Therefore, it can be hypothesized that the formation of these TPs can be
442 attributed to the metabolism of CBZ in peat since none of them was previously
443 identified in UVC-treated irrigation water. The absence of TP 285A in peat irrigated
444 with SW+CBZ could be attributable to a lower formation of this isomer. Concerning
445 lettuce samples, TP 269C and 271B were found only in lettuce leaves. They could be
446 supposedly generated by the metabolization of CBZ in plant material. Furthermore,
447 their absence in peat samples may also be attributed to their further mineralization or
448 degradation to other TPs in peat.

449

450 Some of the CBZ TPs investigated in this study as ACRI, ACRO, EPOX and TP 267A,
451 have already been reported in agricultural soils and soilless cultures (Koba et al., 2016;
452 Li et al., 2013; Martínez-Piernas et al., 2018a). As well, ACRI, ACRO, EPOX, OX, TP
453 224 A (9-acridinecarboxylic acid), TP 239 (10-11 dihydrocarbamazepine), TP 253 A/B,
454 TP 271 A/B have been identified in plant tissues as roots, stems or leaves (Martínez-
455 Piernas et al., 2018b; Riemenschneider et al., 2017, 2016), the suspect screening
456 approach applied has allowed the tentative identification of new TPs not previously
457 found neither in agricultural substrate or soils nor in vegetable matrices. To the authors'
458 knowledge, this study reports for the first time the identification of TP 194
459 (iminostilbene), TP 271B and TPs 285A/B in an agricultural substrate as peat, as well as
460 TP 239 and TPs 271A/B in a lettuce crop.

461

462 One of the main challenges regarding reuse of WW for agricultural purposes is having
463 more knowledge about the formation and occurrence of TPs more toxic than parent
464 compounds. Some of the TPs identified in lettuce samples in this study, as ACRI and

465 ACRO have exhibited more toxicity, when both analytes were found mixed, than CBZ
466 itself across multiple trophic levels (Donner et al., 2013). Besides, in a genotoxicity
467 prediction study carried out by Brezina et al. (Brezina et al., 2017), CBZ derivatives such
468 as 9-acridinecarboxylic acid (TP 224A) showed higher toxicological relevance than
469 CBZ. On the other hand, EPOX has potential genotoxic carcinogenicity (Houeto et al.,
470 2012). In this outline, it is necessary not only a toxicological evaluation of parent
471 compounds but also taking into account mixture toxicities to evaluate human health and
472 environmental impacts derived from reuse of RWW in agriculture.

473 **4. Conclusions**

474 This work presents the first evaluation of the behavior of CBZ TPs formed by a
475 conventional UVC water treatment in an agricultural system. The UVC-treated water
476 was used to irrigate a lettuce crop grown in peat under controlled conditions. The fate of
477 TPs in the latter was compared to the TPs generated due to CBZ degradation processes
478 in both commodities. For TPs identification, a rapid and semi-automatic suspect
479 screening approach was applied to peat and lettuce samples by LC-QTOF-MS. The
480 suspect screening strategy revealed the presence of up to 11 CBZ TPs in peat and 9 in
481 lettuce leaves, showing the potential of the suspect screening approach. No substantial
482 differences regarding TPs formation or fate were found derived from the diverse
483 irrigations. In any case, TPs were likely to reach the edible parts of crops, so
484 highlighting the need for efficient wastewater treatments able to remove OMC to avoid
485 their translocation to plant tissues. This study has contributed to a better understanding
486 of the fate of CBZ TPs and results obtained can serve as a basis to extend the study of
487 these TPs to field crops, grown under diverse conditions. As a general remark, more
488 knowledge regarding OMC TPs structure and behavior must be obtained in order to
489 fully assess the risk associated with their discharge in the environment and human
490 consumption due to reuse practices in agriculture.

491

492 **5. Acknowledgments**

493 The authors acknowledge the COST Action ES1403 NEREUS “New and emerging
494 challenges and opportunities in wastewater reuse”, supported by COST (European
495 Cooperation in Science and Technology). A. B. Martínez-Piernas gratefully
496 acknowledge the financial support she received during a Short-Term Scientific Mission

497 (STSM) offered by the COST Action ES1403 and the Cooperation agreement between
498 the University of Almería and PSA-CIEMAT for the financial support of her PhD
499 scholarship.

500

501 **6. References**

- 502 Ahmed, M.M., Chiron, S., 2014. Solar photo-Fenton like using persulphate for
503 carbamazepine removal from domestic wastewater. *Water Res.* 48, 229–236.
504 <https://doi.org/10.1016/j.watres.2013.09.033>
- 505 Ambrósio, A.F., Soares-da-Silva, P., Carvalho, C.M., Carvalho, A.P., 2002.
506 Mechanisms of action of carbamazepine and its derivatives, oxcarbazepine, BIA 2-
507 093, and BIA 2-024. *Neurochem. Res.* 27, 121–130.
508 <https://doi.org/10.1023/A:1014814924965>
- 509 American Public Health Association, American Water Works Association, W.P.C.F.,
510 2012. Standard methods for the examination of water and wastewater / prepared
511 and published jointly by American Public Health Association, American Water
512 Works Association, Water Pollution Control Federation ; joint editorial board 25
513 editions of this work Thumbnail Title, Author, Edition Date[Sorted decending],
514 22nd ed. American Public Health Association, Washington, D.C.
- 515 Bahlmann, A., Brack, W., Schneider, R.J., Krauss, M., 2014. Carbamazepine and its
516 metabolites in wastewater: Analytical pitfalls and occurrence in Germany and
517 Portugal. *Water Res.* 57, 104–114. <https://doi.org/10.1016/j.watres.2014.03.022>
- 518 Brezina, E., Prasse, C., Meyer, J., Mückter, H., Ternes, T.A., 2017. Investigation and
519 risk evaluation of the occurrence of carbamazepine, oxcarbazepine, their human
520 metabolites and transformation products in the urban water cycle. *Environ. Pollut.*
521 225, 261–269. <https://doi.org/10.1016/j.envpol.2016.10.106>
- 522 Campos-Mañas, M.C., Plaza-Bolaños, P., Sánchez-Pérez, J.A., Malato, S., Agüera, A.,
523 2017. Fast determination of pesticides and other contaminants of emerging concern
524 in treated wastewater using direct injection coupled to highly sensitive ultra-high
525 performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A*
526 1507, 84–94. <https://doi.org/10.1016/j.chroma.2017.05.053>
- 527 Carter, L.J., Williams, M., Martin, S., Kamaludeen, S.P.B., Kookana, R.S., 2018.
528 Sorption, plant uptake and metabolism of benzodiazepines. *Sci. Total Environ.*
529 628–629, 18–25. <https://doi.org/10.1016/j.scitotenv.2018.01.337>

530 ChemSpider Database [WWW Document], n.d. URL <http://www.chemspider.com/>

531 Chen, F., Ying, G.G., Kong, L.X., Wang, L., Zhao, J.L., Zhou, L.J., Zhang, L.J., 2011.

532 Distribution and accumulation of endocrine-disrupting chemicals and

533 pharmaceuticals in wastewater irrigated soils in Hebei, China. *Environ. Pollut.* 159,

534 1490–1498. <https://doi.org/10.1016/j.envpol.2011.03.016>

535 Christou, A., Karaolia, P., Hapeshi, E., Michael, C., Fatta-Kassinos, D., 2017. Long-

536 term wastewater irrigation of vegetables in real agricultural systems: Concentration

537 of pharmaceuticals in soil, uptake and bioaccumulation in tomato fruits and human

538 health risk assessment. *Water Res.* 109, 24–34.

539 <https://doi.org/10.1016/j.watres.2016.11.033>

540 Dai, C.M., Zhou, X.F., Zhang, Y.L., Duan, Y.P., Qiang, Z.M., Zhang, T.C., 2012.

541 Comparative study of the degradation of carbamazepine in water by advanced

542 oxidation processes. *Environ. Technol. (United Kingdom)* 33, 1101–1109.

543 <https://doi.org/10.1080/09593330.2011.610359>

544 Deng, J., Shao, Y., Gao, N., Xia, S., Tan, C., Zhou, S., Hu, X., 2013. Degradation of the

545 antiepileptic drug carbamazepine upon different UV-based advanced oxidation

546 processes in water. *Chem. Eng. J.* 222, 150–158.

547 <https://doi.org/10.1016/j.cej.2013.02.045>

548 Dodgen, L., Ueda, A., Wu, X., Parker, D., Gan, J., 2015. Effect of transpiration on plant

549 accumulation and translocation of PPCP/EDCs. *Environ. Pollut.* 198, 144–153.

550 <https://doi.org/10.1016/j.envpol.2015.01.002>

551 Donner, E., Kosjek, T., Qualmann, S., Kusk, K.O., Heath, E., Revitt, D.M., Ledin, A.,

552 Andersen, H.R., 2013. Ecotoxicity of carbamazepine and its UV photolysis

553 transformation products. *Sci. Total Environ.* 443, 870–876.

554 <https://doi.org/10.1016/j.scitotenv.2012.11.059>

555 Fent K., 2008. Effects of Pharmaceuticals on Aquatic Organisms, in: Kümmerer K.

556 (Ed.), *Pharmaceuticals in the Environment*. Springer, Heidelberg, pp. 175–203.

557 https://doi.org/10.1007/978-3-540-74664-5_12

558 Ghasemian, S., Nasuhoglu, D., Omanovic, S., Yargeau, Y., 2017. Photoelectrocatalytic

559 degradation of pharmaceutical carbamazepine using Sb-doped Sn_{80%}-W_{20%}-oxide

560 electrodes. *Sep. Purif. Technol.*, 188, 52–59.

561 <https://doi.org/10.1016/j.seppur.2017.07.007>

562 Houeto, P., Carton, A., Guerbet, M., Mauclaire, A.C., Gatignol, C., Lechat, P., Masset,

563 D., 2012. Assessment of the health risks related to the presence of drug residues in

564 water for human consumption: Application to carbamazepine. *Regul. Toxicol.*
565 *Pharmacol.* 62, 41–48. <https://doi.org/10.1016/j.yrtph.2011.11.012>

566 Hübner, U., Seiwert, B., Reemtsma, T., Jekel, M., 2014. Ozonation products of
567 carbamazepine and their removal from secondary effluents by soil aquifer
568 treatment - Indications from column experiments. *Water Res.* 49, 34–43.
569 <https://doi.org/10.1016/j.watres.2013.11.016>

570 Huynh, K., Banach, E., Reinhold, D., 2018. Transformation, Conjugation, and
571 Sequestration Following the Uptake of Triclocarban by Jalapeno Pepper Plants. *J.*
572 *Agric. Food Chem.* 66, 4032–4043. <https://doi.org/10.1021/acs.jafc.7b06150>

573 Jelic, A., Michael, I., Achilleos, A., Hapeshi, E., Lambropoulou, D., Perez, S., Petrovic,
574 M., Fatta-Kassinos, D., Barcelo, D., 2013. Transformation products and reaction
575 pathways of carbamazepine during photocatalytic and sonophotocatalytic
576 treatment. *J. Hazard. Mater.* 263, 177–186.
577 <https://doi.org/10.1016/j.jhazmat.2013.07.068>

578 Jelić, A., Petrović, M., Barceló, D., 2009. Multi-residue method for trace level
579 determination of pharmaceuticals in solid samples using pressurized liquid
580 extraction followed by liquid chromatography/quadrupole-linear ion trap mass
581 spectrometry. *Talanta* 80, 363–371. <https://doi.org/10.1016/j.talanta.2009.06.077>

582 Kinney, C.A., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner,
583 S.L., Bossio, J.P., Benotti, M.J., 2008. Bioaccumulation of pharmaceuticals and
584 other anthropogenic waste indicators in earthworms from agricultural soil amended
585 with biosolid or swine manure. *Environ. Sci. Technol.* 42, 1863–1870.
586 <https://doi.org/10.1021/es702304c>

587 Kinney, C.A., Furlong, E.T., Werner, S.L., Cahill, J.D., 2006. Presence and distribution
588 of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water.
589 *Environ. Toxicol. Chem.* 25, 317–326. <https://doi.org/10.1897/05-187R.1>

590 Koba, O., Golovko, O., Kodesova, R., Klement, A., Grabic, R., 2016. Transformation of
591 atenolol, metoprolol, and carbamazepine in soils: The identification, quantification,
592 and stability of the transformation products and further implications for the
593 environment. *Environ. Pollut.* 218, 574–585.
594 <https://doi.org/10.1016/j.envpol.2016.07.041>

595 Larivière, A., Lissalde, S., Soubrand, M., Casellas-Français, M., 2017. Overview of
596 Multiresidues Analytical Methods for the Quantitation of Pharmaceuticals in
597 Environmental Solid Matrixes: Comparison of Analytical Development Strategy

598 for Sewage Sludge, Manure, Soil, and Sediment Samples. *Anal. Chem.* 89,
599 453–465. <https://doi.org/10.1021/acs.analchem.6b04382>

600 Li, J., Dodgen, L., Ye, Q., Gan, J., 2013. Degradation kinetics and metabolites of
601 carbamazepine in soil. *Environ. Sci. Technol.* 47, 3678–3684.
602 <https://doi.org/10.1021/es304944c>

603 Liu, N., Lei, Z.D., Wang, T., Wang, J.J., Zhang, X.D., Xu, G., Tang, L., 2016.
604 Radiolysis of carbamazepine aqueous solution using electron beam irradiation
605 combining with hydrogen peroxide: Efficiency and mechanism. *Chem. Eng. J.* 295,
606 484–493. <https://doi.org/10.1016/j.cej.2016.03.040>

607 Malato, S., Fernández-Ibáñez, P., Maldonado, M.I., Blanco, J., Gernjak, W., 2009.
608 Decontamination and disinfection of water by solar photocatalysis: Recent
609 overview and trends. *Catal. Today* 147, 1–59.
610 <https://doi.org/10.1016/j.cattod.2009.06.018>

611 Martínez-Piernas, A.B., Plaza-Bolaños, P., García-Gómez, E., Fernández-Ibáñez, P.,
612 Agüera, A., 2018a. Determination of organic microcontaminants in agricultural
613 soils irrigated with reclaimed wastewater: Target and suspect approaches. *Anal.*
614 *Chim. Acta* 1030, 115–124. <https://doi.org/10.1016/j.aca.2018.05.049>

615 Martínez-Piernas, A.B., Polo-López, M.I., Fernández-Ibáñez, P., Agüera, A., 2018b.
616 Validation and application of a multiresidue method based on liquid
617 chromatography-tandem mass spectrometry for evaluating the plant uptake of 74
618 microcontaminants in crops irrigated with treated municipal wastewater. *J.*
619 *Chromatogr. A* 1534, 10–21. <https://doi.org/10.1016/j.chroma.2017.12.037>

620 MassBank Database [WWW Document], n.d. URL <https://massbank.eu/>

621 Miralles-Cuevas, S., Darowna, D., Wanag, A., Mozia, S., Malato, S., Oller, I., 2017.
622 Comparison of UV/H₂O₂, UV/S₂O₈²⁻, solar/Fe(II)/H₂O₂ and
623 solar/Fe(II)/S₂O₈²⁻ at pilot plant scale for the elimination of micro-contaminants
624 in natural water: An economic assessment. *Chem. Eng. J.* 310, 514–524.
625 <https://doi.org/10.1016/j.cej.2016.06.121>

626 Riemenschneider, C., Al-Raggad, M., Moeder, M., Seiwert, B., Salameh, E., Reemtsma,
627 T., 2016. Pharmaceuticals, Their Metabolites, and Other Polar Pollutants in Field-
628 Grown Vegetables Irrigated with Treated Municipal Wastewater. *J. Agric. Food*
629 *Chem.* 64, 5784–5792. <https://doi.org/10.1021/acs.jafc.6b01696>

630 Riemenschneider, C., Seiwert, B., Moeder, M., Schwarz, D., Reemtsma, T., 2017.
631 Extensive Transformation of the Pharmaceutical Carbamazepine Following Uptake

632 into Intact Tomato Plants. *Environ. Sci. Technol.* 51, 6100–6109.
633 <https://doi.org/10.1021/acs.est.6b06485>

634 Schollée, J.E., Schymanski, E.L., Avak, S.E., Loos, M., Hollender, J., 2015. Prioritizing
635 Unknown Transformation Products from Biologically-Treated Wastewater Using
636 High-Resolution Mass Spectrometry, Multivariate Statistics, and Metabolic Logic.
637 *Anal. Chem.* 87, 12121–12129. <https://doi.org/10.1021/acs.analchem.5b02905>

638 Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J.,
639 2014. Identifying small molecules via high resolution mass spectrometry:
640 Communicating confidence. *Environ. Sci. Technol.* 48, 2097–2098.
641 <https://doi.org/10.1021/es5002105>

642 Seiwert, B., Golan-Rozen, N., Weidauer, C., Riemenschneider, C., Chefetz, B., Hadar,
643 Y., Reemtsma, T., 2015. Electrochemistry Combined with LC-HRMS: Elucidating
644 Transformation Products of the Recalcitrant Pharmaceutical Compound
645 Carbamazepine Generated by the White-Rot Fungus *Pleurotus ostreatus*. *Environ.*
646 *Sci. Technol.* 49, 12342–12350. <https://doi.org/10.1021/acs.est.5b02229>

647 Stein, K., Ramil, M., Fink, G., Sander, M., Ternes, T.A., 2008. Analysis and sorption of
648 psychoactive drugs onto sediment. *Environ. Sci. Technol.* 42, 6415–6423.
649 <https://doi.org/10.1021/es702959a>

650 Wu, X., Dodgen, L.K., Conkle, J.L., Gan, J., 2015. Plant uptake of pharmaceutical and
651 personal care products from recycled water and biosolids: A review. *Sci. Total*
652 *Environ.* 536, 655–666. <https://doi.org/10.1016/j.scitotenv.2015.07.129>

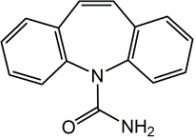
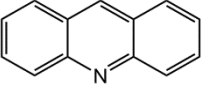
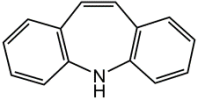
653 Zhang, Q., Chen, J., Dai, C., Zhang, Y., Zhou, X., 2015. Degradation of carbamazepine
654 and toxicity evaluation using the UV/persulfate process in aqueous solution. *J.*
655 *Chem. Technol. Biotechnol.* 90, 701–708. <https://doi.org/10.1002/jctb.4360>

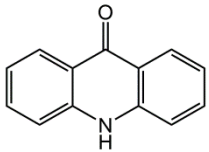
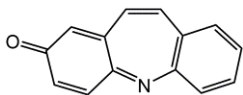
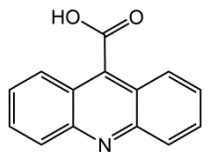
656 Zhang, Y., Geißen, S.U., Gal, C., 2008. Carbamazepine and diclofenac: Removal in
657 wastewater treatment plants and occurrence in water bodies. *Chemosphere* 73,
658 1151–1161. <https://doi.org/10.1016/j.chemosphere.2008.07.086>

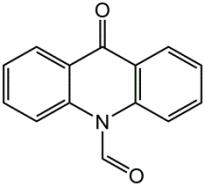
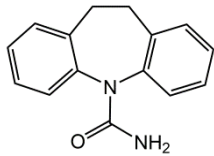
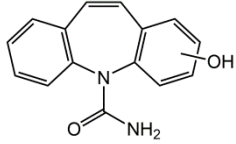
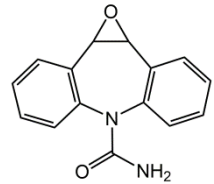
659 Zhu, Z., Chen, Y., Gu, Y., Wu, F., Lu, W., Xu, T., Chen, W., 2016. Catalytic
660 degradation of recalcitrant pollutants by Fenton-like process using
661 polyacrylonitrile-supported iron (II) phthalocyanine nanofibers: Intermediates and
662 pathway. *Water Res.* 93, 296–305. <https://doi.org/10.1016/j.watres.2016.02.035>

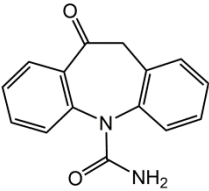
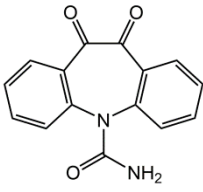
663
664

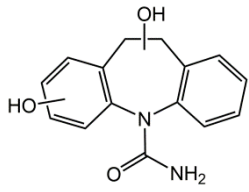
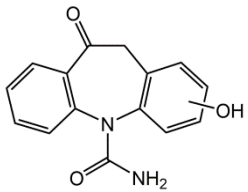
665 **Tables**666 **Table 1.** List of CBZ TPs identified in samples, accurate mass and chromatographic information.

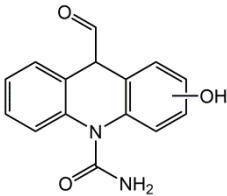
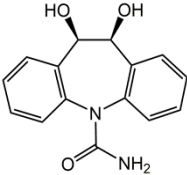
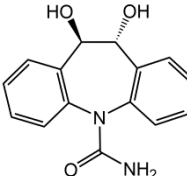
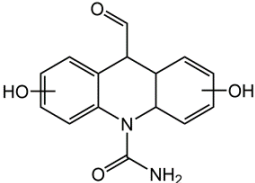
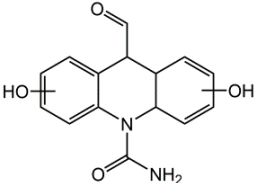
Compound	Structure	Molecular formula	[M+H] ⁺ (<i>m/z</i>)	Error (ppm)	Rt (min)	Product ion (PI)	Assigned formula	PI Error (ppm)	Identification level	Criteria	Reference
CBZ		C ₁₅ H ₁₂ N ₂ O	237.1022	-0.2	21.2	194.0964 192.0808 179.0730	C ₁₄ H ₁₁ N C ₁₄ H ₉ N C ₁₃ H ₉ N	-0.1 -2.5 0.3	L1	Standard	
TP 180 (ACRI)		C ₁₃ H ₉ N	180.0807	-0.8	12.0	178.0651 154.0651 153.0699	C ₁₃ H ₇ N C ₁₁ H ₇ N C ₁₂ H ₈	2.7 -4.7 -4.9	L1	Standard	(Ahmed and Chiron, 2014; Li et al., 2013; Liu et al., 2016; Zhang et al., 2015; Zhu et al., 2016)
TP 194 (Iminostilbene)		C ₁₄ H ₁₁ N	194.0964	-3.7	31.8	179.0730 167.0730 152.0621	C ₁₃ H ₉ N C ₁₂ H ₉ N C ₁₂ H ₈	-2.0 -9.9 -3.6	L1	Standard	(Liu et al., 2016)

TP 196 (ACRO)		C ₁₃ H ₉ NO	196.0756	-1.3	19.8	178.0651 167.073 139.0542 115.0542	C ₁₃ H ₇ N C ₁₂ H ₉ N C ₁₁ H ₆ C ₉ H ₆	-0.7 -0.3 -1.6 2	L1	Standard	(Brezina et al., 2017; Hübner et al., 2014; Liu et al., 2016; Zhu et al., 2016)
TP 208A	No proposal	C ₁₄ H ₉ NO	208.0756	-0.6	26.0	190.0651 180.0808 178.0651 154.0651 153.0699	C ₁₄ H ₇ N C ₁₃ H ₉ N C ₁₃ H ₇ N C ₁₁ H ₇ N C ₁₂ H ₈	0.4 4.0 -1.3 4.4 3.4	L4		(Hübner et al., 2014; Seiwert et al., 2015)
TP 208B (CBZ iminoquinone)		C ₁₄ H ₉ NO	208.0756	-0.6	28.7	180.0808 178.0651 152.0495	C ₁₃ H ₉ N C ₁₃ H ₇ N C ₁₁ H ₅ N	-4.9 -6.3 -4.4	L4	MS/MS spectra and RT reported	(Brezina et al., 2017; Liu et al., 2016)
TP 224A (9- acridinecarboxy- lic acid)		C ₁₄ H ₉ NO ₂	224.0706	-1.2	4.7	196.0757 180.0808 167.0730	C ₁₃ H ₉ NO C ₁₃ H ₉ N C ₁₂ H ₉ N	4.6 2.4 1.5	L1	Standard	(Brezina et al., 2017; Hübner et al., 2014; Jelic et al., 2013; Li et al., 2013; Riemenschneider et al., 2017)

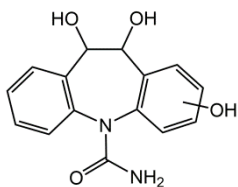
TP 224B (Acridone-N-carbaldehyde)		C ₁₄ H ₉ NO ₂	224.0706	4.0	22.7	196.0757 180.0808 167.0730	C ₁₃ H ₉ NO C ₁₃ H ₉ N C ₁₂ H ₉ N	0.6 2.9 -2.1	L3	MS/MS spectra	(Li et al., 2013)
TP 239 (10-11 Dihydrocarbamazepine)		C ₁₅ H ₁₄ N ₂ O	239.1179	0.5	21.5	196.1121 194.0964 180.0808	C ₁₄ H ₁₃ N C ₁₄ H ₁₁ N C ₁₃ H ₉ N	-5.0 -1.7 0.7	L1	Standard	(Stein et al., 2008)
TP 253A		C ₁₅ H ₁₂ N ₂ O ₂	253.0971	-3.8	13.5	No MS/MS			L4		
TP 253B (OH-CBZ)		C ₁₅ H ₁₂ N ₂ O ₂	253.0971	1.4	17.3	236.0706 210.0913 208.0757 182.0964 180.0808 167.0730	C ₁₅ H ₉ NO ₂ C ₁₄ H ₁₁ NO C ₁₄ H ₉ NO C ₁₃ H ₁₁ N C ₁₃ H ₉ N C ₁₂ H ₉ N	4.5 -0.7 4.0 -4.0 -6.5 -2.7	L3	MS/MS spectra reported	(Jelic et al., 2013)
TP 253C (EPOX)		C ₁₅ H ₁₂ N ₂ O ₂	253.0971	0.4	18.2	236.0706 210.0913 208.0757 182.0964 180.0808 167.0730	C ₁₅ H ₉ NO ₂ C ₁₄ H ₁₁ NO C ₁₄ H ₉ NO C ₁₃ H ₁₁ N C ₁₃ H ₉ N C ₁₂ H ₉ N	-6.4 -1.6 -1.9 -2.9 -0.4 0.9	L1	Standard	(Ahmed and Chiron, 2014; Hübner et al., 2014; Li et al., 2013; Liu et al., 2016; Zhang et al., 2015; Zhu et al.,

TP 253D (OX)		$C_{15}H_{12}N_2O_2$	253.0971	0.6	18.9	236.0706 210.0913 208.0757 182.0964 180.0808 167.0730	$C_{15}H_9NO_2$ 0.2 $C_{14}H_{11}NO$ -1.6 $C_{14}H_9NO$ 0.1 $C_{13}H_{11}N$ -4.0 $C_{13}H_9N$ -4.3 $C_{12}H_9N$ -4.5	L1	Standard	(Brezina et al., 2017)
TP 267A (11-Keto oxcarbazepine)		$C_{15}H_{10}N_2O_3$	267.0764	1.3	15.3	239.0815 224.0706 196.0757 168.0808 212.0706	$C_{14}H_{10}N_2O_2$ 3.6 $C_{14}H_9NO_2$ 3.6 $C_{13}H_9NO$ 4.9 $C_{12}H_9N$ 8.5 $C_{13}H_9NO_2$ 8.5	L3	MS/MS spectra reported	(Ahmed and Chiron, 2014; Brezina et al., 2017; Hübner et al., 2014; Jelic et al., 2013; Koba et al., 2016; Li et al., 2013; Zhu et al., 2016)
TP 267B	No proposal	$C_{15}H_{10}N_2O_3$	267.0764	0.3	18.8	224.0706 222.0550 206.0600 196.0757 167.0730	$C_{14}H_9NO_2$ -0.5 $C_{14}H_7NO_2$ 2.0 $C_{14}H_7NO$ -1.2 $C_{13}H_9NO$ 0.6 $C_{12}H_9N$ 5.7	L4		(Ahmed and Chiron, 2014; Brezina et al., 2017; Hübner et al., 2014; Jelic et

											al., 2013; Koba et al., 2016; Li et al., 2013; Zhu et al., 2016)
TP 269A		$C_{15}H_{12}N_2O_3$	269.0920	3.8	12.4	251.0815 208.0757 180.0808	$C_{15}H_{10}N_2O_2$ -0.4 $C_{14}H_9NO$ -4.8 $C_{13}H_9N$ -3.8	L3	MS/MS spectra reported	(Ahmed and Chiron, 2014; Hübner et al., 2014; Jelic et al., 2013)	
TP 269B		$C_{15}H_{12}N_2O_3$	269.0920	2.1	15.1	No MS/MS		L4			
TP 269C		$C_{15}H_{12}N_2O_3$	269.0920	0.8	16.8	251.0815 208.0757 180.0808	$C_{15}H_{10}N_2O_2$ 0.8 $C_{14}H_9NO$ -0.4 $C_{13}H_9N$ 2.4	L3	MS/MS spectra reported	(Ahmed and Chiron, 2014; Hübner et al., 2014; Jelic et al., 2013)	
TP 269D		$C_{15}H_{12}N_2O_3$	269.0920	-2.5	17.1	251.0815 208.0757 196.0757 180.0808	$C_{15}H_{10}N_2O_2$ -0.8 $C_{14}H_9NO$ -5.7 $C_{13}H_9NO$ -7.1 $C_{13}H_9N$ 4.6	L3	MS/MS spectra reported	(Ahmed and Chiron, 2014; Hübner et al., 2014; Jelic et al., 2013)	

TP 269E		C ₁₅ H ₁₂ N ₂ O ₃	269.0920	1	17.5	251.0815 226.0863 208.0757 180.0808	C ₁₅ H ₁₀ N ₂ O ₂ -9.2 C ₁₄ H ₁₁ NO ₂ 4.2 C ₁₄ H ₉ NO -2.4 C ₁₃ H ₉ N -5.4	L3	MS/MS spectra reported	(Ahmed and Chiron, 2014; Hübner et al., 2014; Jelic et al., 2013)
TP 271A/B		C ₁₅ H ₁₄ N ₂ O ₃	271.1077	-1.3	14.0	253.0971 236.0706 210.0913 208.0757 180.0808	C ₁₅ H ₁₂ N ₂ O ₂ 1.8 C ₁₅ H ₉ NO ₂ -5.1 C ₁₄ H ₁₁ NO 2.7 C ₁₄ H ₉ NO 1.0 C ₁₃ H ₉ N -0.4	L3	MS/MS spectra reported	(Hübner et al., 2014; Jelic et al., 2013; Li et al., 2013)
		C ₁₅ H ₁₄ N ₂ O ₃	271.1077	-3.54	14.8	236.0706 210.0913 180.0808	C ₁₅ H ₉ NO ₂ -6.4 C ₁₄ H ₁₁ NO 1.2 C ₁₃ H ₉ N 1.7	L3	MS/MS spectra reported	(Hübner et al., 2014; Jelic et al., 2013)
TP 285A		C ₁₅ H ₁₂ N ₂ O ₄	285.0867	0.2	7.4	267.0764 249.0659 239.0815 221.0709 212.0706	C ₁₅ H ₁₀ N ₂ O ₃ 5.0 C ₁₅ H ₈ N ₂ O ₂ -7.4 C ₁₄ H ₁₀ N ₂ O ₂ 0.4 C ₁₄ H ₈ N ₂ O -0.2 C ₁₃ H ₉ NO ₂ -7.9	L3	MS/MS spectra reported	(Ahmed and Chiron, 2014)
TP 285B		C ₁₅ H ₁₂ N ₂ O ₄	285.0867	-0.5	13.2	267.0764 239.0815 193.0760	C ₁₅ H ₁₀ N ₂ O ₃ -4.9 C ₁₄ H ₁₀ N ₂ O ₂ -5 C ₁₃ H ₈ N ₂ -3.8	L3	MS/MS spectra reported	(Ahmed and Chiron, 2014)

TP 287



$C_{15}H_{14}N_2O_4$

287.1026

0.6

15.6

236.0706

223.0866

210.0913

180.0808

$C_{15}H_9NO_2$

4.2

$C_{14}H_{10}N_2O$

0.9

$C_{14}H_{11}NO$

-4

$C_{13}H_9N$

0.7

L3

Elucidatio
n

667

668 **Table 2.** List of the CBZ TPs detected per commodity during the vegetable growth (“X”
 669 indicates presence and “-” absence of the TPs in the studied matrices).

Compound	Irrigation conditions				
	SW ^a +CBZ ^b		SW+CBZ+UVC ^c		
	Lettuc e	Peat	Water	Lettuce	Pea t
CBZ	X	X	X	X	X
ACRI	X	X	X	X	X
TP 194	-	X	X	-	X
ACRO	X	X	X	X	X
TP 208A	-	-	X	-	-
TP 208B	-	-	X	-	-
TP 224A	X	X	X	X	X
TP 224B	-	X	X	-	X
TP 239	X	X	X	X	X
TP 253A	-	-	X	-	-
TP 253B	X	X	X	X	X
EPOX	X	X	X	X	X
OX	-	-	X	-	-
TP 267A	-	-	X	-	-
TP 267B	-	-	X	-	-
TP 269A	-	-	X	-	-
TP 269B	-	-	X	-	-
TP 269C	X	-	X	X	-
TP 269D	-	-	X	-	-
TP 269E	-	-	X	-	-
TP 271A	X	-	X	X	-
TP 271B	X	X	X	X	X
TP 285A	-	-	-	-	X
TP 285B	-	X	-	-	X
TP 287	-	-	X	-	-

670 ^aSW, Synthetic water; ^bCBZ, Carbamazepine; ^cUVC, Ultraviolet-C treatment
 671

672 **Table 3.** Concentrations (ng g⁻¹) of CBZ and the validated TPs found in peat and lettuce
 673 samples in both irrigation experiments.

Peat^a / Lettuce^b irrigated with SW^c+CBZ^d				
Week of plant growth	ACRI	ACRO	EPOX	CBZ
2	ND ^e (-) / ND (-)	28 ± 1.2 / ND (-)	15 ± 1.9 / 34 ± 3.6	1844 ± 154 / 658 ± 26
4	4.4 ± 1 / ND (-)	23 ± 6.7 / ND (-)	40 ± 2.7 / 85 ± 7.5	1795 ± 598 / 803 ± 42
6	2.8 ± 0.8 / ND (-)	23 ± 3.1 / 1.3 ± 0.26	43 ± 10 / 82 ± 4.7	2264 ± 357 / 1112 ± 23
8	3.2 ± 1.2 / ND (-)	27 ± 10 / 1.7 ± 0.69	59 ± 4.4 / 103 ± 3.9	2260 ± 308 / 1090 ± 27
10	9.8 ± 1.1 / 0.65 ± 0.1	28 ± 3.3 / 5.1 ± 1.2	85 ± 6.7 / 187 ± 6.1	3097 ± 377 / 1749 ± 49
Peat / Lettuce irrigated with SW+CBZ+UVC^f				
Week of plant growth	ACRI	ACRO	EPOX	CBZ
2	13 ± 1.7 / ND (-)	42 ± 1.3 / ND (-)	23 ± 0.94 / 24 ± 1.1	1000 ± 13 / 419 ± 9
4	8.3 ± 5.5 / ND (-)	36 ± 6.7 / ND (-)	36 ± 1.8 / 37 ± 1.6	1381 ± 42 / 556 ± 16
6	15 ± 4.9 / ND (-)	39 ± 3.1 / 0.76 ± 0.23	51 ± 2.4 / 57 ± 3.5	1655 ± 16 / 828 ± 68
8	21 ± 2.3 / ND (-)	39 ± 10 / 1.9 ± 0.26	66 ± 1.6 / 79 ± 6.1	1945 ± 76 / 889 ± 69
10	23 ± 1.4 / 0.33 ± 0.05	44 ± 3.3 / 2.5 ± 0.56	92 ± 2.9 / 100 ± 11	2265 ± 22 / 1018 ± 100

674 ^aPeat concentrations in dry weight, d.w.; ^bLettuce concentrations in wet weight, w.w.; ^cSynthetic water;

675 ^dCarbamazepine; ^eNot Detected; ^fUltraviolet-C treatment

676

677

678 **Figure captions**

679 **Figure 1.** Extracted ion chromatogram (XIC) and MS/MS spectra of EPOX, OX and TP
680 253B from a UVC treated irrigation water sample. Comparison of the MS/MS spectra
681 of EPOX and OX with the analytical standard.

682 **Figure 2.** Evolution on the abundances of the CBZ TPs detected in lettuce and peat
683 samples during the plant growth.