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1 INVESTIGATION OF CANNABIS BIOMARKERS AND
2 TRANSFORMATION PRODUCTS IN WATERS BY LIQUID
3 CHROMATOGRAPHY COUPLED TO TIME OF FLIGHT AND TRIPLE
4 QUADRUPOLE MASS SPECTROMETRY

5

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11

12 **ABSTRACT**

13 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH) is commonly selected as biomarker
14 for the investigation of cannabis consumption through wastewater analysis. The removal
15 efficiency of THC-COOH in wastewater treatment plants (WWTPs) has been reported to vary
16 between 31-98%. Accordingly, possible transformation products (TPs) of this metabolite
17 might be formed during treatment processes or in receiving surface water under
18 environmental conditions. In this work, surface water was spiked with THC-COOH and
19 subjected to hydrolysis, chlorination and photo-degradation (both ultraviolet and simulated
20 sunlight) experiments under laboratory-controlled conditions. One hydrolysis, eight
21 chlorination, three ultraviolet photo-degradation and seven sunlight photo-degradation TPs
22 were tentatively identified by liquid chromatography coupled to quadrupole time-of-flight
23 mass spectrometer (LC-QTOF MS). In a subsequent step, THC-COOH and the identified TPs
24 were searched in wastewater samples using LC coupled to tandem mass spectrometry (LC-
25 MS/MS) with triple quadrupole. THC-COOH was found in all influent and effluent
26 wastewater samples analyzed, although at significant lower concentrations in the effluent
27 samples. The removal efficiency of WWTP under study was approximately 86%.
28 Furthermore, THC-COOH was also investigated in several surface waters, and it was detected
29 in 50% of the samples analyzed. Regarding TPs, none were found in influent wastewater,
30 while one hydrolysis and five photo-degradation (simulated sunlight) TPs were detected in
31 effluent and surface waters. The most detected compound, resulting from sunlight photo-
32 degradation, was found in 60% of surface waters analyzed. This fact illustrates the importance
33 of investigating these TPs in the aquatic environment.

34 **Graphical Abstract (GA)**

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36

37 **Keywords**

38 Cannabis biomarkers; wastewater; surface water; removal efficiency;
39 transformation/degradation products; time-of-flight mass spectrometry; triple quadrupole
40 mass spectrometry.

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58 1. INTRODUCTION

59 Cannabis is the most widely used illicit drug in Europe (EMCDDA 2010). Its
60 psychoactive compound, Δ^9 -tetrahydrocannabinol (THC), is extensively metabolized leading
61 to low excretion rates as unchanged compound (Postigo, et al., 2010). 11-nor-9-carboxy- Δ^9 -
62 tetrahydrocannabinol (THC-COOH) and its glucuronide are the main metabolites of cannabis
63 in urine (Skopp and Pötsch, 2004; Weinmann, et al., 2001). This fact has led researchers to
64 select THC-COOH as biomarker to estimate cannabis consumption from wastewater analysis
65 (Lai, et al., 2011; van Nuijs, et al., 2011) and also in environmental studies (Berset, et al.,
66 2010; Bijlsma, et al., 2009; Boleda, et al., 2009; Vazquez-Roig, et al., 2010).

67 THC-COOH enters wastewater treatment plants (WWTPs) after the consumption of
68 cannabis. There are several treatment processes that may be performed inside the WWTPs.
69 While primary and secondary treatments are applied in most WWTPs, only some of them use
70 additional processes, such as ozonation, ultraviolet light (UV) or chlorination (EPA 2004).
71 During these treatments, THC-COOH can be removed and/or transformed into different
72 transformation products (TPs) that may be released in receiving surface water (SW).
73 Therefore, the detection and confirmation of cannabinoids in aqueous samples is important
74 from an environmental perspective (Boleda, et al., 2009; Vazquez-Roig, et al., 2010).

75 It is common to report lower concentrations of THC-COOH in effluent wastewater
76 (EWW) than in influent wastewater (IWW) (Bijlsma, et al., 2009; Bijlsma, et al., 2012;
77 Boleda, et al., 2007; Castiglioni, et al., 2006; Postigo, et al., 2010). From these data, it may
78 imply that THC-COOH is partially eliminated in WWTPs. Different percentages of THC-
79 COOH removal efficiency have been reported in the literature, ranging between 31-98%
80 (Bijlsma, et al., 2012; Boleda, et al., 2009; Postigo, et al., 2010). Moreover, some papers
81 reported the detection of this metabolite in surface water at low levels (Boleda, et al., 2007;
82 Postigo, et al., 2010; Vazquez-Roig, et al., 2010). It may be expected that different TPs are

83 generated by transformation/degradation processes in WWTPs but also under environmental
84 conditions in the aquatic ecosystem. The ecotoxic, mutagenic and other potential effects of
85 TPs are mostly unknown and need to be investigated (Fatta-Kassinos, et al., 2011). Only
86 limited data shows that some TPs are as hazardous, or even more so, than the parent
87 compound, producing negative effects on humans and wildlife (Farré, et al., 2008; Fatta-
88 Kassinos, et al., 2011; Gosetti, et al., 2013; Kern, et al., 2009). For these reasons, it is
89 important to investigate the possible presence of THC-COOH TPs in the environment due to
90 the wide consumption of cannabis around the world.

91 The analytical determination of THC-COOH in waters is mostly based on liquid
92 chromatography (LC) coupled to tandem mass spectrometry (MS/MS), a robust and well-
93 established technique for the sensitive determination of illicit drugs in the aquatic
94 environment (Bijlsma, et al., 2009; Boleda, et al., 2007; Castiglioni, et al., 2006; Postigo, et
95 al., 2010; Thomas, et al., 2012). High resolution mass spectrometry (HRMS) instruments,
96 such as Orbitrap (Bijlsma, et al., 2013b; Kern, et al., 2009; Wick, et al., 2011) and Time-of-
97 Flight (TOF) (Bijlsma, et al., 2013a; Ibáñez, et al., 2004; Ibáñez, et al., 2011; Quintana, et al.,
98 2010), are advanced analytical tools for the tentative identification and elucidation of TPs,
99 thanks to the sensitive accurate-mass full-spectrum acquisition provided by these analyzers. In
100 addition, hybrid analyzers, such as (Q)TOF MS, allow data acquisition under MS^E mode
101 (Boix, et al., 2013; Hernández, et al., 2011), obtaining simultaneously the accurate masses of
102 both (de)protonated molecules and the fragment ions in a single injection. This is highly
103 useful for identification/elucidation purposes.

104 The objective of this paper is to perform an investigation on THC-COOH as cannabis
105 biomarker in waters and on the formation of possible TPs, using LC-(Q)TOF MS under MS^E
106 acquisition mode. For this purpose, laboratory controlled degradation experiments
107 (hydrolysis, chlorination and photo-degradation) were first carried out trying to tentatively

108 identify and elucidate the formed TPs using LC-(Q)TOF-MS. In a second step, THC-COOH
109 and the TPs identified in the laboratory experiments were searched by LC-QqQ MS, in both
110 influent and effluent wastewaters, in order to investigate the effect of the treatment processes
111 on generating these TPs in the WWTPs. Several surface water samples were also analysed to
112 know whether the THC-COOH TPs are present in the aquatic environment.

113

114 **2. METHODS**

115 **2.1. Reagents and chemicals**

116 A reference standard of THC-COOH was purchased from the National Measurement
117 Institute (Pymble, Australia). A stock solution of 100 mg/L was prepared in methanol
118 (MeOH). A working solution (10 mg/L) was made by ten times diluting the stock solution
119 with MeOH.

120 HPLC-grade methanol (MeOH), acetonitrile (ACN), sodium hydroxide (NaOH, 99%)
121 and formic acid (FA, 98-100%) were acquired from Scharlau (Barcelona, Spain). A Milli-Q
122 ultra-pure water system from Millipore (Bedford, MA, USA) was used to obtain the HPLC
123 grade water. Leucine enkephalin, used as the lock mass (m/z 556.2771 in positive- and m/z
124 554.2615 in negative-ion mode) was purchased from Sigma-Aldrich.

125 Solid-phase extraction (SPE) cartridges (Oasis-HLB; 3 mL, 60 mg) were purchased from
126 Waters (Milford, MA, USA).

127 **2.2. Instrumentation**

128 **2.2.1. LC-ESI-QTOF MS**

129 An ultra-high-performance liquid chromatography (UHPLC) system (Waters Acquity,
130 Milford, MA, USA) was interfaced to a hybrid quadrupole orthogonal acceleration time-of-
131 flight mass spectrometer (Q-TOF Premier, Waters Micromass, Manchester, UK) equipped

132 with an orthogonal Z-spray electrospray ionization interface (ESI) operating in both positive-
133 and negative-ion modes and controlled by MassLynx v 4.1 software. The chromatographic
134 separation was performed using an Acquity UPLC BEH C18 100 mm × 2.1 mm, 1.7 μm
135 particle size analytical column (Waters). The mobile phases used were A = H₂O and B =
136 MeOH, both with 0.01% FA. The percentage of organic modifier (B) was changed linearly as
137 follows: 0 min, 10%; 9 min, 90%; 11 min, 90%; 11.1 min, 10%; 14 min, 10%. The flow rate
138 was 0.3 mL/min. The column and sample temperatures were kept at 40 °C and 5 °C,
139 respectively. For MS^E experiments, two acquisition functions with different collision energies
140 were created: the low-energy (LE) function with a collision energy of 4 eV, and the high
141 energy (HE) function with a collision energy ramp ranging from 15 to 40 eV. The same cone
142 voltage (15 V) and collision energy ramp was used for additional MS/MS experiments.
143 Further details on instrument operating conditions can be found elsewhere (Boix, et al., 2013).

144 Data were processed using MetaboLynx XS software (within MassLynx).

145 2.2.2. LC-ESI-QqQ MS

146 An ultra-high-performance liquid chromatography system (Waters Acquity, Milford, MA,
147 USA) was interfaced to a triple quadrupole mass spectrometer (Xevo TQS, Waters
148 Micromass, Manchester, UK) equipped with an orthogonal Z-spray electrospray ionization
149 interface (ESI) operating in positive (3.0 kV) and negative (-2.0 kV) ion modes. The
150 chromatographic separation was obtained using the same analytical column and
151 chromatographic conditions as for QTOF analysis. Cone gas as well as desolvation gas was
152 nitrogen (Praxair, Valencia, Spain). The cone gas and the desolvation gas flows were set to
153 250 L/h and 1200 L/h, respectively. For operation in the MS/MS mode, collision gas was
154 argon 99.995% (Praxair, Madrid, Spain) with a pressure of 4×10^{-3} mbar in the collision cell
155 (0.15 mL/min). Source and desolvation temperatures were 150 °C and 650 °C, respectively.
156 Dwell times were automatically selected.

157 Data were processed using TargetLynx software (within MassLynx).

158 **2.3. Degradation experiments**

159 Several degradation experiments were performed trying to simulate environmental
160 conditions (hydrolysis and sunlight photo-degradation) and some processes that can occur in
161 wastewater treatment plants (hydrolysis, chlorination and UV photo-degradation).
162 Biodegradation experiments were not performed because no adequate material is available at
163 our laboratory. The general strategy for identification of TPs using UHPLC-ESI-QTOF MS
164 can be found elsewhere (Acero, et al., 20108; Trovó, et al., 2009; Bijlsma, et al., 2013a;
165 Hernández, et al., 2008; Ibáñez, et al., 2004; Ibáñez, et al., 2006).

166 Surface water from the Mijares River (Castellón, Spain) was collected in November 2011
167 and used for all laboratory controlled experiments (**Table 1SI** shows the main physic-
168 chemical characteristics of the water used in laboratory experiments). The samples used for
169 hydrolysis, chlorination and photo-degradation experiments were spiked with THC-COOH at
170 1 mg/L (for UV experiments 0.5 mg/L). This relative high concentration allowed better
171 detection and evaluation of TPs, and facilitated the detection of minor TPs. Non-spiked
172 surface water samples were subjected to the same degradation processes and used as control
173 samples.

174 *Hydrolysis* experiments were performed in darkness at room temperature. 2-mL aliquots
175 were sampled at different time intervals (0, 1d, 3d, 7d, 10d and 17 days) and immediately
176 stored at -20 °C.

177 *Chlorination* experiments were performed adding 40 µL of commercial NaClO 1% w/v
178 solution to 50 mL of spiked sample. To study degradation kinetics in time, 2-mL aliquots at
179 different time intervals (0, 5min, 15min, 30min, 45min, 1.5h, 3h, 8h, 23h and 33 hours) were
180 sampled and stored at -20 °C (Bijlsma et al. 2013a).

181 *Photo-degradation* experiments were carried out under UV radiation and simulated
182 sunlight. UV radiation was performed using a mercury lamp with its main output at 254 nm.
183 The 250 mL surface water samples were kept in quartz glass vessels at a distance of ~15 cm
184 from the lamp. The experiment was carried out in a fume hood at room temperature over a
185 period of 72 h under constant stirring of the samples (0, 30min, 1.5h, 4h, 7h, 20h, 30h, 53h
186 and 72h). Sunlight was simulated using a solar simulation system (Suntest XLS+, Atlas MTT,
187 Linsengericht, Germany), equipped with a xenon arc lamp as radiation source and a solar light
188 filter allowing a wavelength in the range of 300 - 800 nm. The radiation intensity was set to
189 500 W/m^2 and the light dose per hour of irradiation to 1.8 MJ/h. In this way, 90 irradiation
190 hours corresponds to 15 days of natural sun light (dose: 288 MJ/m^2). The degradation was
191 performed using 250-mL closed quartz glass vessels and sample temperature was set to 25 °C
192 in order to minimize sample evaporation and possible thermal transformation. Aliquots were
193 sampled after stirring of the water solution. During irradiation, 2-mL water samples were
194 taken at different time intervals (0, 30min, 2h, 8h, 20h, 30h, 50h and 80h) and immediately
195 stored at -20 °C. In the photo-degradation experiments, evaporation of the samples was
196 observed and assessed by correcting the peak-areas as a function of the measured volume.

197 **2.4. Data processing**

198 MetaboLynx XS application manager (Micromass v 4.1) was used to process QTOF MS
199 data obtained from degradation studies. This software compares eXtracted Ion
200 Chromatograms (XICs) of a positive/degraded sample to a control sample for detecting,
201 identifying and reporting differential ions/chromatographic peaks which would correspond, in
202 principle, to transformation products (Boix, et al., 2013; Ibáñez, et al., 2006).

203 TargetLynx (also from Micromass v 4.1) was employed for automatically processing data
204 from triple quadrupole analysis. For confirmation of positive findings, the acquisition of two
205 selected reaction monitoring (SRM) transitions per compound together with the agreement in

206 both retention time and Q/q ratio deviation were required (Commission Decision 2002).
207 Reference Q/q ratios were obtained from TPs identified in degradation experiments as the
208 ratio between the most abundant transition (Q, quantitative) and the other/s measured
209 transition/s (q, confirmation) (Boix, et al., 2013).

210 **2.5. Water samples**

211 Ten 24-h composite wastewater samples (five IWW and five EWW) were collected from
212 a WWTP located in Castellón (Eastern Spain), which main characteristics are summarized in
213 **Table 2SI**. Ten grab surface samples were collected from four sites of Albufera Natural Park
214 of Valencia (Eastern Spain) and from six sites of two Dutch rivers (Rhine and Meuse). All
215 samples were taken from March to May in 2012, and immediately stored at $-20\text{ }^{\circ}\text{C}$.

216 **2.6. Sample treatment**

217 A SPE step was applied prior analysis to pre-concentrate the sample. SPE Oasis HLB
218 cartridges were conditioned with 3 mL MeOH and 3 mL Milli-Q water. After loading 50 mL
219 water sample (IWW were previously 4-fold diluted with Milli-Q water), cartridges were
220 vacuum dried for 5 min. Analytes were eluted with 5 mL MeOH. The extracts were
221 evaporated to dryness at $35\text{ }^{\circ}\text{C}$ under a gentle stream of nitrogen and reconstructed in 1 mL of
222 10:90 MeOH:H₂O. UHPLC- MS/MS QqQ analyses were performed by injecting 50 μL of the
223 final extract into the system.

224

225 **3. RESULTS AND DISCUSSION**

226 **3.1. Degradation experiments**

227 **3.1.1. Hydrolysis**

228 **Figure 1a** shows the hydrolytic degradation of THC-COOH during 17 days in darkness at
229 room temperature. THC-COOH was transformed (around 20%) into TP 1H, which appeared
230 on the third day reaching its maximum concentration after 10 days.

231 Information on elemental composition, retention time, fragment ions, mass errors and
232 double bond equivalent (DBE) obtained for TP 1H is shown in **Figure 1b**. This TP was
233 detected in positive ionization mode, eluting at 8.92 min. Its elemental composition,
234 $C_{20}H_{27}O_4^+$, implies the loss of one methyl group from the THC-COOH molecule. The
235 demethylation could have occurred in two sites of the original structure: in ring B or in
236 aliphatic chain. The loss observed at 70.0783 Da might correspond to the aliphatic chain
237 (C_5H_{10} , +1.1 mDa). This would indicate that TP 1H structure would be more feasible if the
238 demethylation from THC-COOH occurred in the B ring (pos. 5). A possible structure of the
239 protonated molecule of this TP and structures of the fragment ions are shown in **Figure 1c**.

240 3.1.2. Chlorination

241 The study of THC-COOH TPs was performed using a chlorine concentration commonly
242 applied in a tertiary treatment in WWTP (0.8 mg/L) (Bijlsma, et al., 2013a). Under these
243 conditions THC-COOH was completely degraded after 5 min. Eight TPs were detected, four
244 being intermediate compounds and the other four showing higher persistence along the
245 experiment. As an example, **Figure 2** illustrates the profiles for the seven TPs detected in
246 negative ionization mode 33 hours after the chlorination experiment. TP 3C showed the
247 highest absolute area followed by TP 1C, TP 2C and TP 4C, which were still all present in the
248 water sample after 33 h. On the contrary, three of these TPs (5C, 6C and 7C) might act as
249 intermediate compounds, as they were observed after 5 min of chlorination but quickly
250 disappeared to probably yield more persistent TPs. Regarding investigation of real-world
251 water samples, persistent TPs seem to be the most relevant compounds as they may be present
252 in EWW and/or SW with a possible negative impact in the environment.

253 In addition to the seven TPs detected in negative mode, there was another chlorination TP
254 (8C) observed under positive mode. This compound disappeared after 30 min of chlorination.

255 **Figure 2SI** shows the narrow mass window eXtracted Ion Chromatograms (nw-XICs,
256 0.02 Da mass window width) corresponding to the (de)protonated molecules obtained after 5
257 min (TPs 5C, 6C, 7C and 8C) and after 33 h (TPs 1C, 2C, 3C and 4C) of chlorination. Note
258 that for TPs 2C and 3C, the main fragments at m/z 315.1596 and 397.1418 are shown, as they
259 were more sensitive than the deprotonated molecule. **Table 3SI** summarizes the information
260 obtained for THC-COOH TPs formed after chlorination. Retention times and exact masses,
261 proposed elemental composition for the (de)protonated TPs and their fragment ions, mass
262 errors (mDa and ppm) and DBE are given. All chlorination TPs contained chlorine atoms,
263 except TP 1C, which was the result of oxidation from the original THC-COOH molecule,
264 consistent with the oxidant properties of NaClO. The number of chlorine atoms (between 1-4)
265 was assigned based on the isotopic pattern observed in the mass spectrum.

266 **Figure 2SIa** shows intermediate compounds TP 6C and TP 7C containing four chlorine
267 atoms, some of which could be replaced by hydroxyl groups [OH] yielding the persistent TP
268 2C and TP 3C. Chemical structures could not be proposed for these compounds, as there were
269 too many possibilities to locate the positions of chlorine and hydroxyl groups. Additional
270 analytical techniques, such as NMR or H/D exchange, could be applied for obtaining extra
271 structural information.

272 3.1.3. Photo-degradation (UV)

273 Trying to simulate another tertiary treatment in WWTP, the ultraviolet photo-
274 degradation of THC-COOH was studied. **Figure 3** shows that THC-COOH was quickly
275 degraded after 30 minutes of UV exposure, yielding three TPs (TP 1PUV, 2PUV and 3PUV)
276 which also disappeared completely after 4 hours. These results illustrate that UV disinfection
277 process would be an effective removal treatment for THC-COOH in WWTPs. As previously
278 stated, persistent TPs seem to be the most relevant compounds from an environmental point
279 of view. For this reason, in some countries, e.g., Italy, chlorination is being progressively

280 abandoned because of its potential for generating unwanted TPs, and replaced by UV
281 irradiation (Antonelli, et al., 2008).

282 Among the three TPs found after photo-degradation experiments with UV, one was observed
283 in positive mode while the other two were in negative ionization mode. Elemental
284 compositions, retention times, fragment ions, mass errors and DBEs are summarized in **Table**
285 **4SI**. TP 1PUV (m/z 388.1760, $C_{21}H_{26}NO_6^-$, -1.0 mDa) and TP 2PUV (m/z 433.1611,
286 $C_{21}H_{25}N_2O_8^-$, +0.2 mDa) eluted at 9.16 and 9.83 minutes, respectively. Their elemental
287 compositions differed in one and two nitro groups (NO_2) from THC-COOH molecule,
288 respectively. The NO_2 group could easily be introduced since the photo-degradation
289 experiments were performed in surface water from the Mijares River (Castellón province),
290 where relatively high nitrate concentrations are usual due to the wide use of fertilizers in this
291 agricultural area (Bijlsma, et al., 2013a; Hernández, et al., 2008; Wick, et al., 2011). Based on
292 fragmentation information, the nitration could have occurred on the benzene ring for the TP
293 1PUV (see fragment ion at m/z 208.0974, $C_{11}H_{14}NO_3^-$, **Figure 3**). For TP 2PUV further
294 information would be necessary to tentatively assign a chemical structure, as the fragment
295 ions available not provide enough information. The photo-degradation product TP 3PUV
296 showed less polarity than the two nitrated TPs, and eluted at 10.13 minutes. This TP was only
297 detected in positive ionization mode (m/z 329.1763). According to the accurate mass data
298 obtained, the elemental composition was assigned to $C_{20}H_{25}O_4^+$ ($\Delta mDa=+0.2$), which would
299 result from the loss of CH_4 from the THC-COOH molecule. The neutral losses observed in
300 HE mass spectrum (m/z 28.0313 C_2H_4 and m/z 70.0783 C_5H_{10} , **Figure 3**), would imply a
301 demethylation and dehydrogenation in B ring (pos. 5) instead of the aliphatic ring of THC-
302 COOH. The structures proposed for ultraviolet TPs are shown in **Figure 3**.

303 3.1.4. Photo-degradation (Sunlight simulation)

304 Laboratory experiments simulating natural sun light were also performed in order to
305 investigate the possible degradation of THC-COOH in water under environmental conditions.
306 Seven TPs were detected in this case. **Figure 4** shows the degradation rate of the TPs detected
307 in negative ionization mode. As can be seen, THC-COOH was completely degraded after 50 h
308 of irradiation by simulation suntest reactor (equivalent to 8 days of natural sun irradiation).
309 Six TPs were formed, although some of them started to decrease in abundance after 50 h. The
310 most abundant TP, in terms of absolute area, was the TP 1PS which presented its maximum
311 abundance after 45 h of photo-degradation. This TP might suffer a hydrogenation to form the
312 TP 2PS which was still present after 75 h. Similar hydrogenation was observed for TPs 3PS
313 and 4PS. TP 3PS started to increase in abundance after 30 h, just when TP 4PS started to
314 decrease. Finally, TP 5PS and TP 6PS also showed significant maximum intensities, ~300 and
315 ~150 a.u., respectively, after 20 h (**Figure 4**).

316 Another TP was observed only under positive mode. TP 7PS, appeared at 50 h, showing
317 its maximum after 80 h.

318 It is important to consider these sunlight photo-degradation TPs when investigating the
319 presence of cannabis derivatives in the aquatic environment, as they might be present in
320 environmental waters exposed to solar radiation.

321 Different reactions, such as hydroxylation, demethylation, methylation, or a combination
322 of them, would explain the formation of the seven photo-degraded sunlight TPs. Their
323 elemental compositions, retention times, ionization modes, accurate masses, fragment ions,
324 mass errors and DBEs are shown in **Table 5SI**. TP 1PS (m/z 185.0814, $C_9H_{13}O_4^-$) could have
325 suffered a hydrogenation to form TP 2PS (m/z 187.0970, $C_9H_{15}O_4^-$). The chemical structure of
326 both TPs would correspond to the A ring of THC-COOH, see **Figure 4**. The main fragment
327 ions at m/z 141.0921 ($C_8H_{13}O_2^-$) and m/z 143.1069 ($C_8H_{15}O_2^-$) for TP 1PS and TP 2PS,
328 respectively, indicate a typical loss (CO_2) from a carboxylic acid group. Similar

329 hydrogenation was observed in another two photo-degradation products: TP 4PS (8.06 min,
330 $C_{20}H_{26}O_6$) and TP 3PS (7.45 min, $C_{20}H_{28}O_6$). As **Figure 4** shows, both TPs were detected in
331 negative as well as positive ion modes. Their chemical structures differ from the THC-COOH
332 molecule in two hydroxylations on the benzene ring plus one demethylation on
333 cyclohexanoate ring. TP 5PS and TP 6PS were photo-degradation TPs sharing the same exact
334 mass but eluting at different retention times (8.64 and 8.80 min, respectively). They were
335 detected in positive (m/z 377.1964) and negative (m/z 375.1808) ionization modes too. The
336 chemical structure ($C_{21}H_{28}O_6$) of these isomers might be the result of a double hydroxylation
337 of THC-COOH. However, the positions of the hydroxyl groups were difficult to predict with
338 the information available on fragment ions.

339 To the authors' best knowledge, these cannabis TPs have not yet been reported in
340 scientific literature.

341 **3.2. Analysis of real-world samples**

342 Five effluent and five influent wastewaters were analyzed by LC-(ESI)-MS/MS (QqQ).
343 Sample treatment consisted of pre-concentration by SPE with Oasis HLB, which has been
344 widely used in the analysis of many different organic pollutants with a wide polarity range.
345 The LC conditions were the same as used in the degradation experiments, in order to obtain
346 comparable retention times. Nineteen TPs and the metabolite THC-COOH were included as
347 target analytes in the method (**Table 6SI**). For each compound, two SRM transitions were
348 selected based on fragment ions observed by QTOF MS in the degradation experiments. All
349 compounds measured under electrospray positive and negative ionization mode were
350 determined simultaneously in just one injection. In some particular cases, a pseudo-transition
351 was included, i.e. for TPs 1C, 1PS and 2PS, as only one fragment ion (or additional fragment
352 ions of low intensity) was observed in QTOF mass spectra. For the reliable identification of
353 the compounds detected in the samples, the compliance of retention time and Q/q ratios

354 within maximum tolerances allowed was required (Commission Decision 2002). As reference
355 standards of TPs were unavailable, the sample vial obtained in the degradation experiments
356 with the highest concentration of analyte was used instead. The use of Q/q ratios for
357 confirmation was problematic for some TPs, as the value was above 10, which means that the
358 second product ion had very low abundance, making the use of the second transition less
359 useful. This was reflected in the analysis of some samples as indicated bellow.

360 In addition to wastewater samples, several surface waters collected from Spain and The
361 Netherlands were also analysed.

362 A summary of the positive findings in IWW, EWW and SW is shown in **Table 1**. THC-
363 COOH was detected in all IWW (mean concentration 56 ng/L) and also in EWW, although at
364 a significantly lower levels (mean concentration 8 ng/L). This is in agreement with the
365 literature, as this major cannabis metabolite is frequently detected in IWW as a result of the
366 wide cannabis consumption (Thomas, et al., 2012; Bijlsma, et al., 2012; Boleda, et al., 2007;
367 Postigo, et al., 2010). The presence of this metabolite in EWW suggests that a partial removal
368 takes place in the WWTPs. No TPs were found in influent wastewater, which might be
369 explained by the fact that still not any treatment has been applied for contaminants removal.
370 However, several TPs were detected in effluent wastewaters. The hydrolysis TP 1H and five
371 sunlight photo-degradation TPs (2PS, 3PS, 4PS, 6PS and 7PS) were detected in effluent
372 samples (between 40-80% of positive samples). These TPs were detected using the most
373 abundant transition (Q); however, the second transition was not clearly observed, surely due
374 to low concentration levels of these TPs in the samples. In relation to surface water, the major
375 metabolite THC-COOH was found in 5 out of 10 the samples analyzed (the four samples
376 collected from Spain and one sample from The Netherlands). It must be noticed that the
377 Spanish samples were collected in an area (Albufera lake) very close to Valencia. This can
378 explain the presence of this metabolite in all the samples analysed.

379 Regarding cannabis TPs, five of these compounds were detected and confirmed in several
380 surface samples: the hydrolysis TP 1H and four sunlight photo-degradation TPs (1PS, 2PS,
381 5PS and 6PS). The most frequent compound was TP 1PS (m/z 185) which was found in 6 out
382 of 10 surface waters analyzed. However, its identity could not be fully confirmed in 2 samples
383 as Q/q ratio deviation exceeded the maximum tolerance allowed ($\pm 30\%$ for ion ratios between
384 5-10) (Commission Decision 2002). A similar situation was observed for TP 2PS (m/z 187),
385 which was detected in 4 samples. It must be noticed that a pseudo MS/MS transition was used
386 for TPs 1PS and 2PS; this would make confirmation using Q/q ratios more troublesome, due
387 to the lower specificity of these transitions (i.e. the same precursor and product ion selected).

388 In order to illustrate the confirmation of positive findings, **Figure 5** shows LC-MS/MS
389 chromatograms for TP 1H detected in a surface water sample from Spain; and for TPs 1PS,
390 2PS, 5PS and 6PS detected in a surface water sample from The Netherlands. As can be seen,
391 experimental Q/q ratios were within or close to the maximum deviations and R_t were in
392 agreement with the reference compounds. This allowed us to confirm the identity of the
393 compounds detected, with only the exception of TP 2PS. As previously stated, this might be
394 due to the poor selectivity of the pseudo-SRM transitions used for this compound making the
395 presence of interfering compounds more likely. It is worth noting that non-compliance of Q/q
396 ratio might lead to false negatives in the case that a matrix-interferent was sharing one of the
397 transitions, thereby producing ion intensity ratios different than expected.

398

399 **4. CONCLUSIONS**

400 This work reports the behaviour of THC-COOH, the major urinary metabolite of
401 cannabis, subjected to hydrolysis, chlorination and photo-degradation (sunlight and UV)
402 experiments under controlled conditions at the laboratory.

403 Altogether, nineteen THC-COOH TPs (one hydrolysis, eight chlorination, three UV and
404 seven sunlight photo-degradation) were identified by LC-QTOF MS. To the best of our
405 knowledge, most of these TPs have not been reported in the scientific literature yet.

406 The results from laboratory degradation suggest that UV treatment could be an effective
407 way for removal of THC-COOH in WWTPs, better than chlorination, which generates
408 unwanted TPs.

409 Analysis of wastewater samples (five IWW and five EWW) and surface waters has
410 allowed us to estimate the removal efficiency of the WWTPs under study as well as to
411 evaluate the presence of cannabis TPs in the aquatic environment. The metabolite THC-
412 COOH was detected in all IWW and EWW samples analysed, and a removal efficiency of
413 around 86% was estimated. Regarding TPs, none of them were found in influent wastewater,
414 while one hydrolysis and five sunlight photo-degradation TPs were found in effluent
415 wastewaters. In surface waters, THC-COOH was detected in 50% of samples analyzed. The
416 most detected compound (60% of samples) was the sunlight photo-degradation TP 1PS with
417 *m/z* 185.0814.

418 The results obtained in the present paper illustrate the importance of investigating TPs in
419 the aquatic environment. Some of the TPs reported in this article, particularly those resulting
420 from sunlight photo-degradation, would need to be investigated in more detail to have a
421 realistic overview of cannabinoids impact in the aquatic environment. Reference standards
422 would be required to unequivocally confirm the identity of these compounds, and to develop
423 analytical methodologies able to accurately quantify their concentration levels in waters.

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448 **References**

- 449 Acero, J.L., Benítez, F.J., Real, F.J., González, M., 2008. Chlorination of organophosphorus
450 pesticides in natural waters. *J. Hazard. Mater.* 153, 320-328.
- 451 Antonelli, M., Mezzanotte, V., Nurizzo, C., 2008. Wastewater disinfection by UV irradiation:
452 Short and long-term efficiency. *Environ. Eng. Sci.* 25, 363-373.
- 453 Berset, J.-., Brenneisen, R., Mathieu, C., 2010. Analysis of illicit and illicit drugs in waste,
454 surface and lake water samples using large volume direct injection high performance liquid
455 chromatography - Electrospray tandem mass spectrometry (HPLC-MS/MS). *Chemosphere.*
456 81, 859-866.
- 457 Bijlsma, L., Boix, C., Niessen, W.M.A., Ibáñez, M., Sancho, J.V., Hernández, F., 2013a.
458 Investigation of degradation products of cocaine and benzoylecgonine in the aquatic
459 environment. *Sci. Total Environ.* 443, 200-208.
- 460 Bijlsma, L., Emke, E., Hernández, F., de Voogt, P., 2013b. Performance of the linear ion trap
461 Orbitrap mass analyzer for qualitative and quantitative analysis of drugs of abuse and relevant
462 metabolites in sewage water. *Anal. Chim. Acta.* 768, 102-110.
- 463 Bijlsma, L., Emke, E., Hernández, F., De Voogt, P., 2012. Investigation of drugs of abuse and
464 relevant metabolites in Dutch sewage water by liquid chromatography coupled to high
465 resolution mass spectrometry. *Chemosphere.* 89, 1399-1406.
- 466 Bijlsma, L., Sancho, J.V., Pitarch, E., Ibáñez, M., Hernández, F., 2009. Simultaneous ultra-
467 high-pressure liquid chromatography-tandem mass spectrometry determination of
468 amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis
469 metabolite in surface water and urban wastewater. *J. Chromatogr. A.* 1216, 3078-3089.

470 Boix, C., Ibáñez, M., Sancho, J.V., Niessen, W.M.A., Hernández, F., 2013 Investigating the
471 presence of omeprazole in waters by liquid chromatography coupled to low and high
472 resolution mass spectrometry (i): Degradation experiments. *J. Mass Spectrom.* DOI
473 10.1002/jms.3260.

474 Boleda, M.R., Galceran, M.T., Ventura, F., 2009. Monitoring of opiates, cannabinoids and
475 their metabolites in wastewater, surface water and finished water in Catalonia, Spain. *Water*
476 *Res.* 43, 1126-1136.

477 Boleda, M.R., Galceran, M.T., Ventura, F., 2007. Trace determination of cannabinoids and
478 opiates in wastewater and surface waters by ultra-performance liquid chromatography-tandem
479 mass spectrometry. *J. Chromatogr. A.* 1175, 38-48.

480 Castiglioni, S., Zuccato, E., Crisci, E., Chiabrando, C., Fanelli, R., Bagnati, R., 2006.
481 Identification and measurement of illicit drugs and their metabolites in urban wastewater by
482 liquid chromatography-tandem mass spectrometry. *Anal. Chem.* 78, 8421-8429.

483 EPA. Washington, DC (2004). "Primer for Municipal Waste water Treatment systems."
484 Document no. EPA 832-R-04-0012

485 European Monitoring Centre for Drugs and Drug Addiction. The state of the drugs problem in
486 Europe. EMCDDA Annual Report. 2010 Available from [URL:http://www.emcdda.europa.eu/](http://www.emcdda.europa.eu/)
487 (accessed January 2012)

488 Commission Decision 2002/657/CE of 12 August 2002, **Implementing Council Directive**
489 **96/23/EC Concerning the Performance of Analytical Methods of Interpretation of**
490 **Results.**

491 Farré, M.I., Pérez, S., Kantiani, L., Barceló, D., 2008. Fate and toxicity of emerging
492 pollutants, their metabolites and transformation products in the aquatic environment. *TrAC*
493 *Trends Anal. Chem.* 27, 991-1007.

494 Fatta-Kassinos, D., Vasquez, M.I., Kümmerer, K., 2011. Transformation products of
495 pharmaceuticals in surface waters and wastewater formed during photolysis and advanced
496 oxidation processes - Degradation, elucidation of byproducts and assessment of their
497 biological potency. *Chemosphere.* 85, 693-709.

498 Fatta-Kassinos, D., Meric, S., Nikolaou, A., 2011. Pharmaceutical residues in environmental
499 waters and wastewater: Current state of knowledge and future research. *Anal. Bioanal. Chem.*
500 399, 251-275.

501 Gosetti, F., Mazzucco, E., Gennaro, M.C., Marengo, E., 2013. The challenge of non-target
502 uhplc/ms analysis for the identification of emerging contaminants in water. *Environmental*
503 *Chemistry for a Sustainable World*, submitted for publication.

504 Hernández, F., Bijlsma, L., Sancho, J.V., Díaz, R., Ibáñez, M., 2011. Rapid wide-scope
505 screening of drugs of abuse, prescription drugs with potential for abuse and their metabolites
506 in influent and effluent urban wastewater by ultrahigh pressure liquid chromatography-
507 quadrupole-time-of-flight-mass spectrometry. *Anal. Chim. Acta.* 684, 87-97.

508 Hernández, F., Ibáñez, M., Pozo, Ó.J., Sancho, J.V., 2008. Investigating the presence of
509 pesticide transformation products in water by using liquid chromatography-mass spectrometry
510 with different mass analyzers. *J. Mass Spectrom.* 43, 173-184.

511 Ibáñez, M., Sancho, J.V., Pozo, O.J., Hernández, F., 2011. Use of quadrupole time-of-flight
512 mass spectrometry to determine proposed structures of transformation products of the
513 herbicide bromacil after water chlorination. *Rapid Commun. Mass Spectrom.* 25, 3103-3113.

514 Ibáñez, M., Sancho, J.V., Pozo, Ó.J., Hernández, F., 2006. Use of liquid chromatography
515 quadrupole time-of-flight mass spectrometry in the elucidation of transformation products and
516 metabolites of pesticides. Diazinon as a case study. *Anal. Bioanal. Chem.* 384, 448-457.

517 Ibáñez, M., Sancho, J.V., Pozo, Ó.J., Hernández, F., 2004. Use of Quadrupole Time-of-Flight
518 Mass Spectrometry in Environmental Analysis: Elucidation of Transformation Products of
519 Triazine Herbicides in Water after UV Exposure. *Anal. Chem.* 76, 1328-1335.

520 Kern, S., Fenner, K., Singer, H.P., Schwarzenbach, R.P., Hollender, J., 2009. Identification of
521 transformation products of organic contaminants in natural waters by computer-aided
522 prediction and high-resolution mass spectrometry. *Environ. Sci. Technol.* 43, 7039-7046.

523 Lai, F.Y., Ort, C., Gartner, C., Carter, S., Prichard, J., Kirkbride, P., Bruno, R., Hall, W.,
524 Eaglesham, G., Mueller, J.F., 2011. Refining the estimation of illicit drug consumptions from
525 wastewater analysis: Co-analysis of prescription pharmaceuticals and uncertainty assessment.
526 *Water Res.* 45, 4437-4448.

527 Postigo, C., López de Alda, M.J., Barceló, D., 2010. Drugs of abuse and their metabolites in
528 the Ebro River basin: Occurrence in sewage and surface water, sewage treatment plants
529 removal efficiency, and collective drug usage estimation. *Environ. Int.* 36, 75-84.

530 Quintana, J.B., Rodil, R., López-Mahía, P., Muniategui-Lorenzo, S., Prada-Rodríguez, D.,
531 2010. Investigating the chlorination of acidic pharmaceuticals and by-product formation aided
532 by an experimental design methodology. *Water Res.* 44, 243-255.

533 Skopp, G., Pötsch, L., 2004. An Investigation of the Stability of Free and Glucuronidated 11-
534 Nor- Δ^9 -Tetrahydrocannabinol-9-carboxylic Acid in Authentic Urine Samples. *J. Anal.*
535 *Toxicol.* 28, 35-40.

536 Thomas, K.V., Bijlsma, L., Castiglioni, S., Covaci, A., Emke, E., Grabic, R., Hernández, F.,
537 Karolak, S., Kasprzyk-Hordern, B., Lindberg, R.H., Lopez de Alda, M., Meierjohann, A., Ort,
538 C., Pico, Y., Quintana, J.B., Reid, M., Rieckermann, J., Terzic, S., van Nuijs, A.L.N., de
539 Voogt, P., 2012. Comparing illicit drug use in 19 European cities through sewage analysis.
540 *Sci. Total Environ.* 432, 432-439.

541 Trovó, A.G., Nogueira, R.F.P., Agüera, A., Sirtori, C., Fernández-Alba, A.R., 2009.
542 Photodegradation of sulfamethoxazole in various aqueous media: Persistence, toxicity and
543 photoproducts assessment. *Chemosphere.* 77, 1292-1298.

544 van Nuijs, A.L.N., Castiglioni, S., Tarcomnicu, I., Postigo, C., de Alda, M.L., Neels, H.,
545 Zuccato, E., Barcelo, D., Covaci, A., 2011. Illicit drug consumption estimations derived from
546 wastewater analysis: A critical review. *Sci. Total Environ.* 409, 3564-3577.

547 Vazquez-Roig, P., Andreu, V., Blasco, C., Picó, Y., 2010. SPE and LC-MS/MS determination
548 of 14 illicit drugs in surface waters from the Natural Park of L'Albufera (València, Spain).
549 *Anal. Bioanal. Chem.* 397, 2851-2864.

550 Weinmann, W., Goerner, M., Vogt, S., Goerke, R., Pollak, S., 2001. Fast confirmation of 11-
551 nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH) in urine by LC/MS/MS using
552 negative atmospheric-pressure chemical ionisation (APCI). *Forensic Sci. Int.* 121, 103-107.

553 Wick, A., Wagner, M., Ternes, T.A., 2011. Elucidation of the transformation pathway of the
554 opium alkaloid codeine in biological wastewater treatment. *Environ. Sci. Technol.* 45, 3374-
555 3385.

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558 **FIGURE CAPTIONS**

559 **Graphical Abstract (GA).** Schematic overview for THC-COOH presence and behaviour in
560 WWTPs and in the environment

561 **Figure 1.** (a) Degradation of THC-COOH under hydrolysis conditions and TP formed, (b)
562 Elemental composition, retention time, fragments ions, mass errors and DBE. (c) Proposed
563 structures for TP 1H and for its fragment ions

564 **Figure 2.** Degradation curves for TPs obtained after THC-COOH chlorination experiments
565 (QTOF under ESI negative ionization mode)

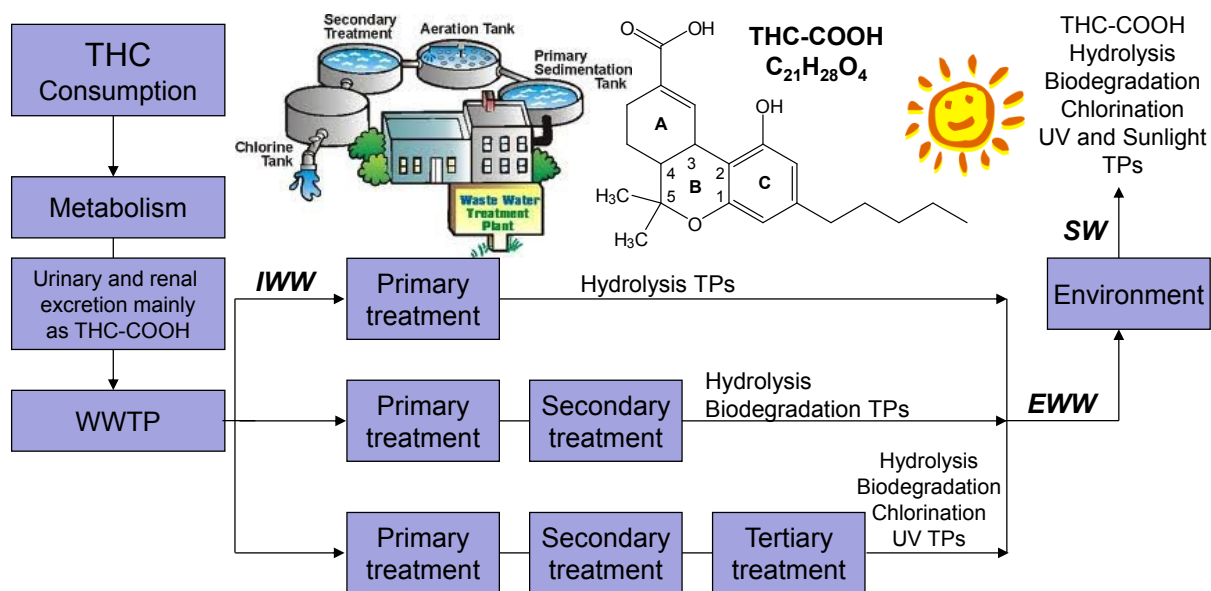
566 **Figure 3.** Degradation curves for TPs 1PUV, 2PUV and 3PUV, detected in positive and
567 negative ion mode, and proposed structures. Ultraviolet photo-degradation experiments

568 **Figure 4.** TPs identified after THC-COOH sunlight photo-degradation experiments (QTOF
569 under ESI negative ionization mode)

570 **Figure 5.** LC- MS/MS QqQ chromatograms for (a) TP 1H in Spanish surface water; (b) TP
571 1PS, (c) TP 2PS and (d) TP 5PS and 6PS detected in The Netherlands surface water

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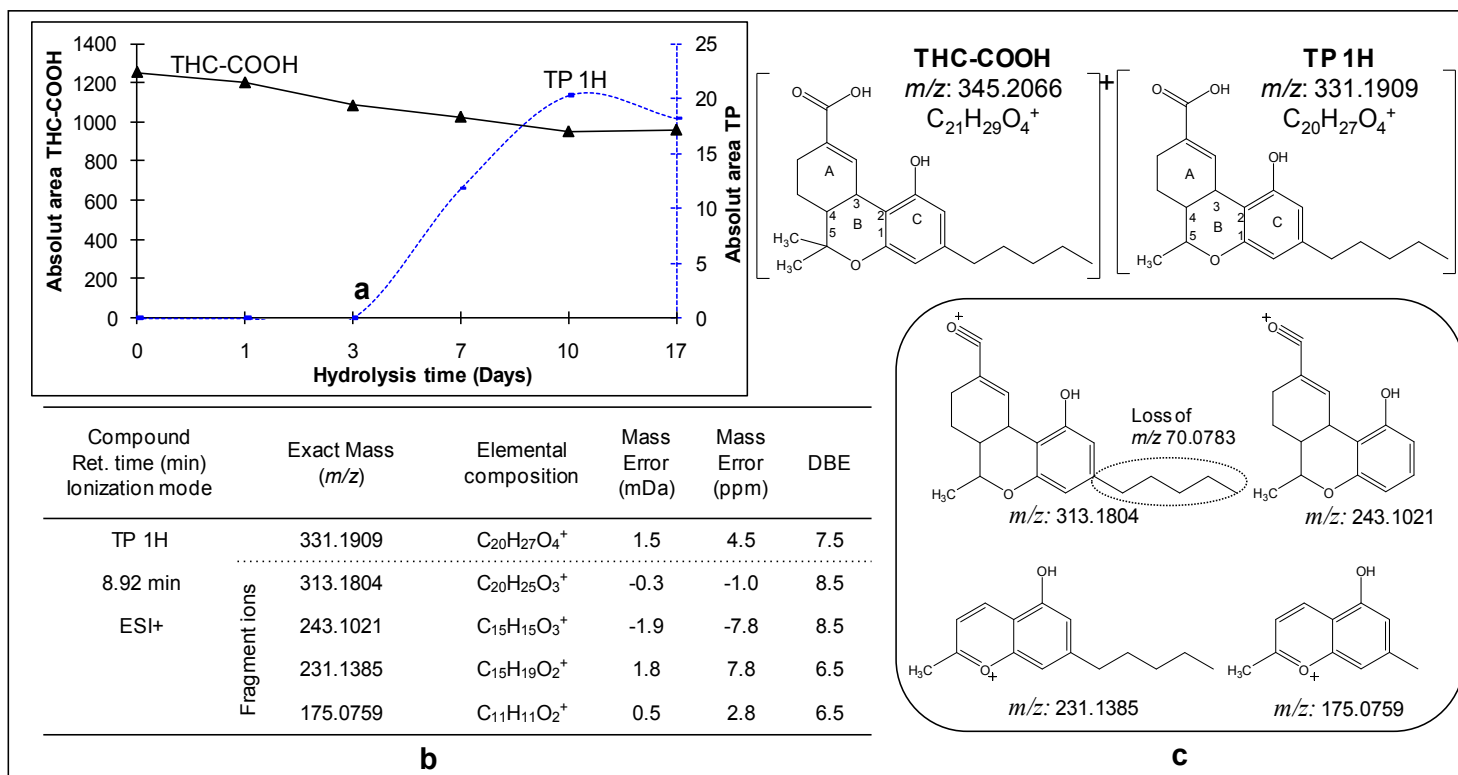
574 Graphical abstract



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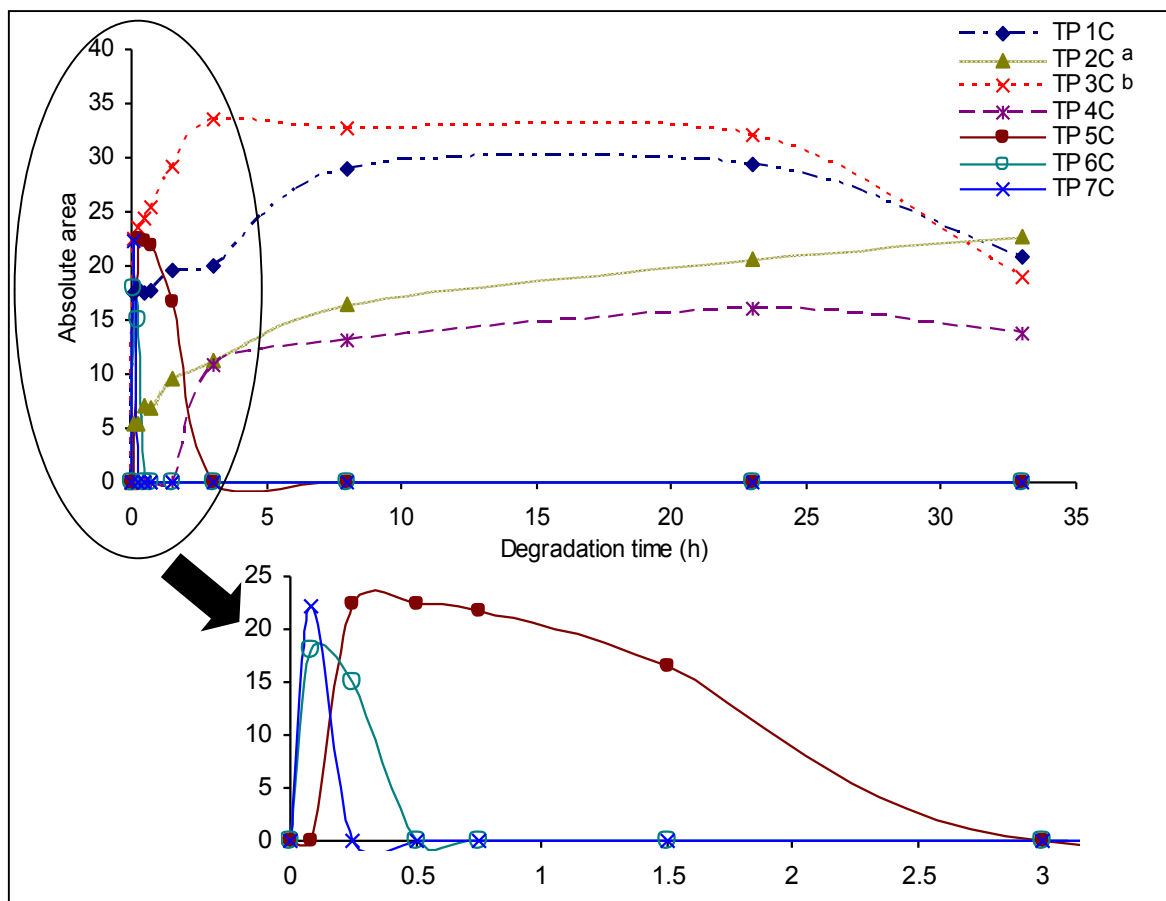


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579 Figure 1

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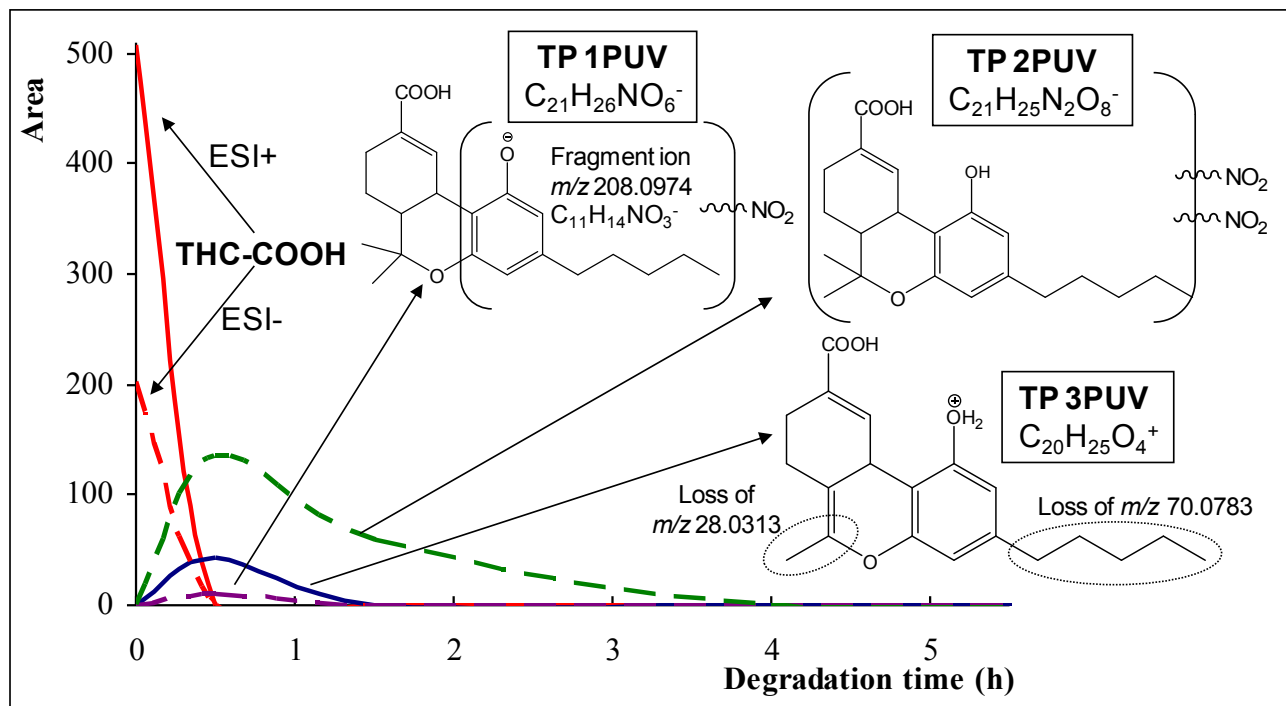
584 ^a For TP 2C, main fragment at m/z 315.1596 was more sensitive than the deprotonated molecule (m/z 459.1422)

585 ^b For TP 3C, main fragment at m/z 397.1418 was more sensitive than the deprotonated molecule (m/z 477.1083)

586 Figure 2

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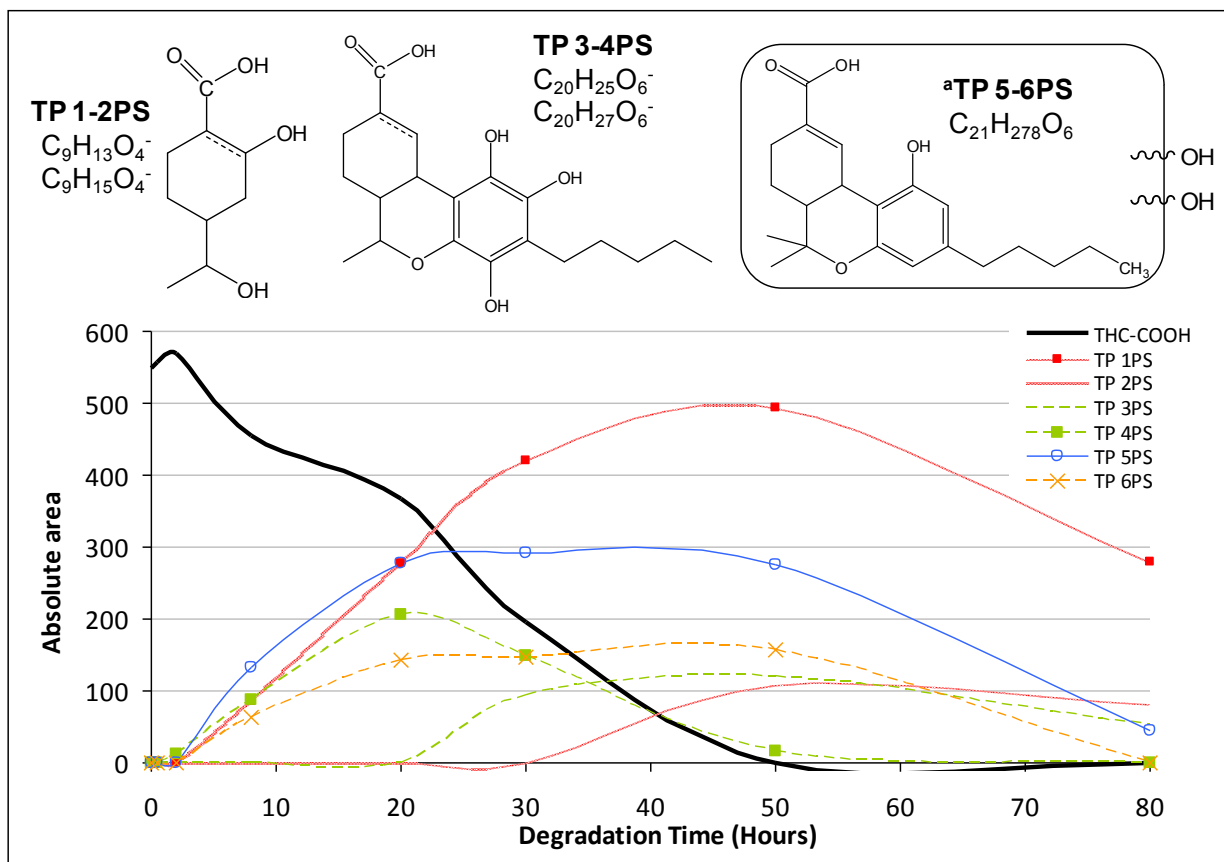
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590 Figure 3

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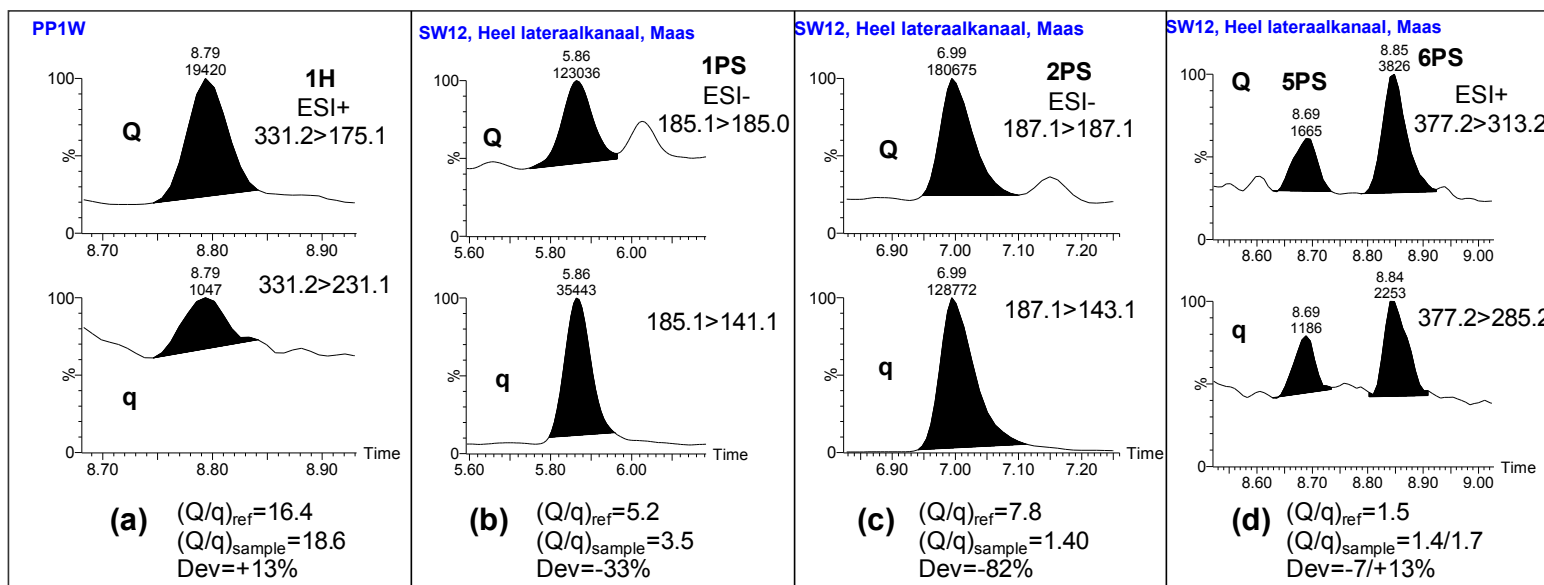


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595 ^a For TTPs 5-6PS, detection of its main fragment at m/z 331.1909 was more sensitive than the deprotonated molecule at m/z 375.1808

596 Figure 4

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599 Figure 5

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607 **Table 1.** Main cannabis metabolite (THC-COOH) and TPs detected in IWW, E
608 samples by LC-MS/MS

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	Number of positive findings		
	IWW (n=5)	EWW (n=5)	SW (n=10)
THC-COOH (ESI-/+)	5	5	5
TP 1H (ESI+)	-	<u>3</u> ^a	1
TP 1PS (ESI-)	-	-	<u>6</u> ^b
TP 2PS (ESI-)	-	<u>4</u>	<u>4</u> ^b
TP 3PS (ESI-)	-	<u>3</u>	-
TP 4PS (ESI-)	-	<u>2</u>	<u>8</u>
TP 5PS (ESI+)	-	-	1
TP 6PS (ESI+)	-	<u>2</u>	1
TP 7PS (ESI+)	-	<u>3</u>	-

610 ^a : underlined, the number of samples where only one transition was observed

611 ^b: Q/q ratio deviation >50%

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618 **SUPPLEMENTARY INFORMATION**

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620 THC-COOH is reported in positive (m/z 345.2066) and negative (m/z 343.1909)

621 ionization modes. The **Figure 1SI** shows its HE mass spectra and the main fragment ions. In

622 positive ionization mode, the most intense fragment ion at m/z 299.2001 corresponds to the

623 loss of formic acid. The other fragments have been justified in our previous works about

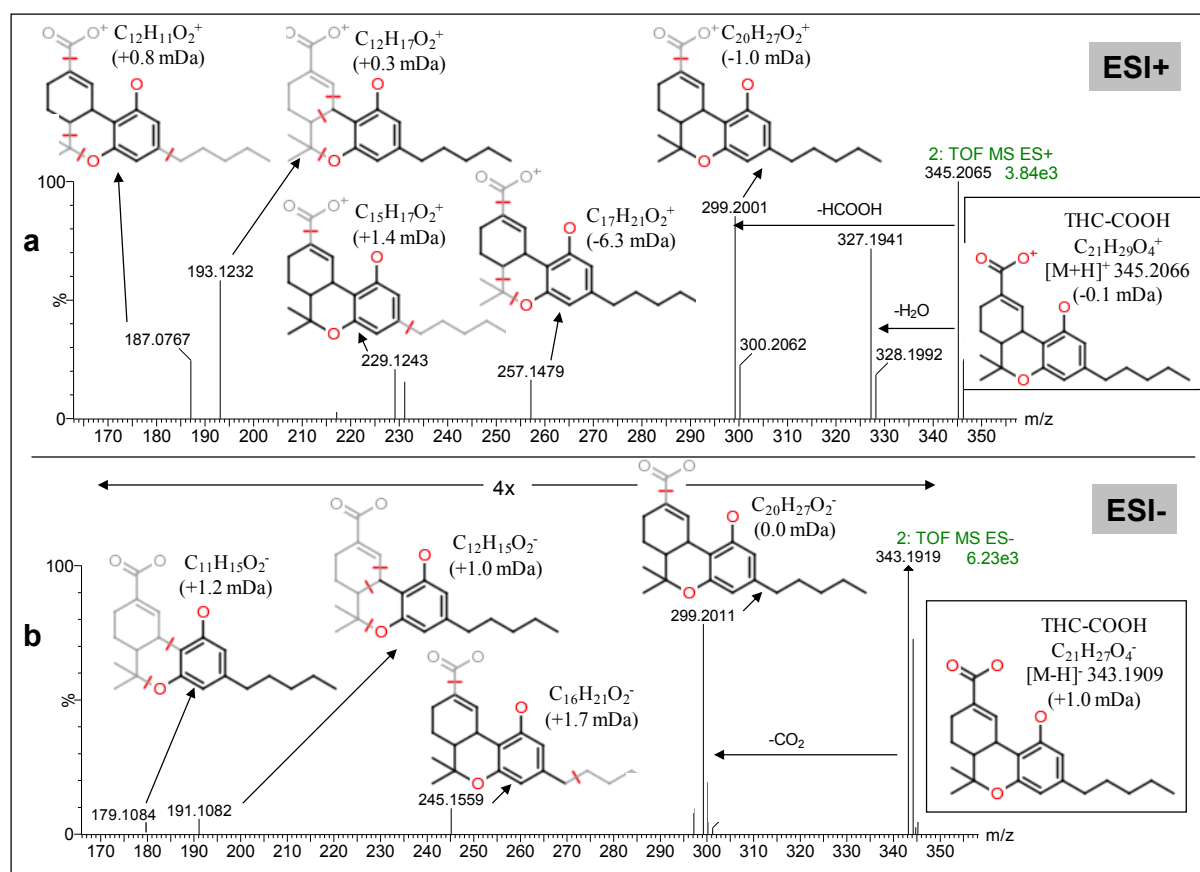
624 fragmentation (Bijlsma, et al., 2011). Regarding negative acquisition mode, four low-intense

625 fragment ions were observed. The ion at m/z 299 corresponds to the CO_2 loss. The fragment

626 ion at m/z 191.1082 ($\text{C}_{12}\text{H}_{15}\text{O}_2^-$, +1.0 mDa) corresponds to the positive 193.1229 ($\text{C}_{12}\text{H}_{17}\text{O}_2^+$,

627 0.3 mDa). Other fragments ions observed in negative mode at m/z 245.1559 ($\text{C}_{16}\text{H}_{21}\text{O}_2^-$, +1.7

628 mDa) and m/z 179.1084 ($\text{C}_{11}\text{H}_{15}\text{O}_2^-$, +1.2 mDa) are illustrated in **Figure 1SI**.



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630 **Figure 1SI.** QTOF HE spectra of THC-COOH in (a) positive and (b) negative ionization
631 mode; fragment ion structures proposed by MassFragment

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634 **Table 1SI.** Characteristics of the surface water used in the degradation experiments

Characteristics	Values
Temperature (°C, <i>in situ</i>)	15.1
pH (<i>in situ</i>)	8.5
Conductivity (μS/cm, <i>in situ</i>)	810.7
Hardness (expressed as ppm CaCO ₃)	316.8
Alkalinity (expressed as ppm HCO ₃ ⁻)	131.6
Chlorides (expressed as ppm Cl ⁻)	44.2
Nitrates (expressed as ppm NO ₃ ⁻)	3.8
Phosphate (expressed as ppm PO ₄ ³⁻)	2.8
Organic matter (expressed as ppm O ₂)	2.2
Sulphates (expressed as ppm SO ₄ ²⁻)	218.7

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659 **Table 2SI.** Some characteristics of the Castellon WWTP

Castellon WWTP	
Population served	32,000
Origin	urban and mixed urban and industrial
Average flow rate (m ³ /d)	8250
EWW samples	Treatment^a
3 samples	Primary step, biological
1 sample	Primary step, biological, removal Chlorination
1 sample	Primary step, biological, removal UV

660 ^a Primary step: physical treatment (inc. grit removal). Secondary step: biological treatment
 661 (activated sludge). Tertiary step: removal by chlorination or ultraviolet treatment.

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685 **Table 3SI.** Proposed elemental composition, retention time (min), accurate mass (m/z), mass
686 error (mDa, ppm) and double bound equivalent (DBE) of (de)protonated TPs of THC-COOH
687 and their fragments ions obtained in chlorination experiments

Compound name Retention time (min)	Ionization mode	Exact mass (m/z)	Elemental composition	Mass Error (mDa)	Mass Error (ppm)	DBE
TP 1C 6.17	ESI-	167.0708	$C_9H_{11}O_3^-$	0.1	0.6	4.5
		123.0810	$C_8H_{11}O^-$	0.3	2.4	3.5
		95.0861	$C_7H_{11}^-$	1.5	15.8	2.5
TP 2C 7.15	ESI-	459.1422	$C_{21}H_{28}O_9Cl^-$	-2.0	-4.4	7.5
		395.1342	$C_{19}H_{23}O_9^-$	-2.1	-5.3	8.5
		315.1596	$C_{19}H_{23}O_4^-$	0.5	1.6	8.5
		253.1592	$C_{18}H_{21}O^-$	2.6	10.3	8.5
TP 3C 7.42	ESI-	477.1083	$C_{21}H_{27}O_8Cl_2^-$	-0.4	-0.8	7.5
		433.1185	$C_{20}H_{27}O_6Cl_2^-$	0.9	2.1	6.5
		397.1418	$C_{20}H_{26}O_6Cl^-$	1.3	3.3	7.5
		361.1651	$C_{20}H_{25}O_6^-$	1.4	3.9	8.5
		259.1334	$C_{16}H_{19}O_3^-$	1.6	6.2	7.5
		231.1385	$C_{15}H_{19}O_2^-$	-0.5	-2.2	6.5
		179.0708	$C_{10}H_{11}O_3^-$	0.2	1.1	5.5
TP 4C 7.56	ESI-	481.1032	$C_{20}H_{27}O_9Cl_2^-$	1.4	2.9	6.5
		427.1160	$C_{20}H_{24}O_8Cl^-$	2.0	4.7	8.5
		317.1753	$C_{19}H_{25}O_4^-$	1.8	5.7	7.5
		199.0970	$C_{10}H_{15}O_4^-$	0.2	1.0	3.5
		167.0708	$C_9H_{11}O_3^-$	1.9	11.4	4.5
		139.0395	$C_7H_7O_3^-$	1.5	10.8	4.5
		95.0497	$C_6H_7O^-$	1.9	20.0	3.5
TP 5C 10.29	ESI+	481.0508	$C_{21}H_{25}O_4Cl_4^+$	0.6	1.2	7.5
		374.9958	$C_{16}H_{14}O_4Cl_3^+$	-1.3	-3.5	8.5
		332.9488	$C_{13}H_8O_4Cl_3^+$	-0.2	-0.6	8.5
		314.9383	$C_{13}H_6O_3Cl_3^+$	-0.6	-1.9	9.5
		276.9590	$C_{11}H_8O_2Cl_3^+$	0.6	2.2	6.5
	ESI-	479.0350	$C_{21}H_{21}O_4Cl_4^-$	0.4	0.8	8.5
		407.0817	$C_{21}H_{21}O_4Cl_2^-$	1.6	3.9	10.5
		399.0685	$C_{20}H_{22}O_2Cl_3^-$	-2.0	-5.0	8.5
		363.0919	$C_{20}H_{21}O_2Cl_2^-$	0.3	0.8	9.5
		TP 6C 10.33	ESI+	499.0613	$C_{21}H_{27}O_5Cl_4^+$	-1.0
456.9932	$C_{21}H_{17}O_3Cl_4^+$			-1.0	-2.2	11.5
314.9383	$C_{13}H_6O_3Cl_3^+$			3.4	10.8	9.5
428.9830	$C_{16}H_{17}O_5Cl_4^+$			-4.2	-9.8	6.5
395.0736	$C_{21}H_{22}OCl_3^+$			3.3	8.4	9.5
ESI-	375.0088		$C_{14}H_{19}O_3Cl_4^+$	0.4	1.1	3.5
	497.0456		$C_{21}H_{25}O_5Cl_4^-$	1.7	3.4	7.5
	461.0689		$C_{21}H_{24}O_5Cl_3^-$	2.3	5.0	8.5
	427.1079		$C_{21}H_{25}O_5Cl_2^-$	-0.1	-0.2	8.5
	425.0923		$C_{21}H_{23}O_5Cl_2^-$	2.2	5.2	9.5
381.1024	$C_{20}H_{22}O_3Cl_2^-$	1.5	3.9	8.5		
TP 7C 10.57	ESI+	513.0769	$C_{22}H_{29}O_5Cl_4^+$	-4.2	-8.2	6.5
		445.0740	$C_{21}H_{24}O_4Cl_3^+$	4.7	10.6	8.5
		395.0817	$C_{20}H_{21}O_4Cl_2^+$	0.3	0.8	9.5
		374.9958	$C_{16}H_{14}O_4Cl_3^+$	-1.5	-4.0	8.5
		356.9852	$C_{16}H_{12}O_3Cl_3^+$	-1.3	-3.6	9.5
	314.9383	$C_{13}H_6O_3Cl_3^+$	3.9	12.4	9.5	
	ESI-	511.0613	$C_{22}H_{27}O_5Cl_4^-$	1.5	2.9	7.5
		395.1181	$C_{21}H_{25}O_3Cl_2^-$	1.8	4.6	8.5
TP 8C 10.71		ESI+	447.0897	$C_{21}H_{26}O_4Cl_3^+$	0.1	0.2
	393.1024		$C_{21}H_{23}O_3Cl_2^+$	-0.5	-1.3	9.5
	351.0685		$C_{16}H_{22}O_2Cl_3^+$	0.4	1.1	4.5
	325.0398		$C_{16}H_{15}O_3Cl_2^+$	-1.2	-3.7	8.5
	165.0916		$C_{10}H_{13}O_2^+$	0.3	1.8	4.5
123.0446	$C_7H_7O_2^+$	0.4	3.3	4.5		

689 **Table 4SI.** Proposed elemental composition, retention time (min), accurate mass (m/z),
 690 mass error (mDa, ppm) and double bound equivalent (DBE) of (de)protonated TPs of THC-
 691 COOH and their fragments ions obtained during UV photo-degradation of experiments

Compound name Retention time (min)	Ionization mode	Exact mass (m/z)	Elemental Composition	Mass Error (mDa)	Mass Error (ppm)	DBE
TP 1PUV 9.16	ESI-	388.1760	$C_{21}H_{26}NO_6^-$	-1.0	-2.6	9.5
		344.1862	$C_{20}H_{26}NO_4^-$	4.0	11.6	8.5
		284.0559	$C_{15}H_{10}NO_5^-$	2.8	9.9	11.5
		236.0923	$C_{12}H_{14}NO_4^-$	0.5	2.1	6.5
		208.0974	$C_{11}H_{14}NO_3^-$	0.9	4.3	5.5
TP 2PUV 9.83	ESI-	433.1611	$C_{21}H_{25}N_2O_8^-$	0.2	0.5	10.5
		389.1713	$C_{20}H_{24}N_2O_6^-$	2.4	6.2	9.5
		256.0974	$C_{15}H_{14}NO_3^-$	2.7	10.5	9.5
TP 3PUV 10.13	ESI+	329.1753	$C_{20}H_{25}O_4^+$	0.2	0.6	8.5
		301.1440	$C_{18}H_{21}O_4^+$	-1.1	-3.7	8.5
		231.0657	$C_{13}H_{11}O_4^+$	2.5	10.8	8.5
		215.0708	$C_{13}H_{11}O_3^+$	1.2	5.6	8.5
		201.0552	$C_{12}H_9O_3^+$	-1.9	-9.5	8.5

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693 **Table SSI.** Proposed elemental composition, retention time (min), accurate mass (m/z), mass
694 error (mDa, ppm) and double bound equivalent (DBE) of (de)protonated TPs of THC-COOH
695 and their fragments ions obtained during sunlight photo-degradation experiments

Compound name Retention time (min)	Ionization mode	Exact mass (m/z)	Elemental composition	Mass Error (mDa)	Mass Error (ppm)	DBE	
TP 1PS 5.83	ESI-	185.0814	C ₉ H ₁₃ O ₄ ⁻	-0.9	-4.9	3.5	
		371.1706	C ₁₈ H ₂₇ O ₈ ⁻	1.1	3.0	5.5	
		141.0921	C ₈ H ₁₃ O ₂ ⁻	-0.5	-3.5	2.5	
TP 2PS 6.52	ESI-	187.0970	C ₉ H ₁₅ O ₄ ⁻	-1.4	-7.5	2.5	
		375.2019	C ₁₈ H ₃₁ O ₈ ⁻	1.8	4.8	3.5	
		169.0865	C ₉ H ₁₁ O ₃ ⁻	2.2	13.0	3.5	
		143.1069	C ₈ H ₁₅ O ₂ ⁻	0.3	2.1	1.5	
		125.0966	C ₈ H ₁₁ O ⁻	2.4	19.2	2.5	
TP 3PS 7.45	ESI+	365.1964	C ₂₀ H ₂₉ O ₆ ⁺	0.5	1.4	6.5	
		347.1858	C ₂₀ H ₂₇ O ₅ ⁺	0.8	2.3	7.5	
		329.1753	C ₂₀ H ₂₅ O ₄ ⁺	1.8	5.5	8.5	
		319.1910	C ₁₉ H ₂₇ O ₄ ⁺	2.0	6.3	6.5	
		301.1804	C ₁₉ H ₂₅ O ₃ ⁺	0.6	2.0	7.5	
		283.1698	C ₁₉ H ₂₃ O ₂ ⁺	1.1	3.9	8.5	
		213.1279	C ₁₅ H ₁₇ O ⁺	-0.9	-4.2	7.5	
		199.1123	C ₁₄ H ₁₅ O ⁺	-1.7	-8.5	7.5	
		ESI-	363.1808	C ₂₀ H ₂₇ O ₆ ⁻	0.5	1.4	7.5
	319.1909		C ₁₉ H ₂₇ O ₄ ⁻	-0.3	-0.9	6.5	
	275.2011		C ₁₈ H ₂₇ O ₂ ⁻	1.7	6.2	5.5	
	223.0970		C ₁₂ H ₁₅ O ₄ ⁻	-3.7	-16.6	5.5	
	191.1072		C ₁₂ H ₁₅ O ₂ ⁻	0.2	1.0	5.5	
	TP 4PS 8.06		ESI+	363.1808	C ₂₀ H ₂₇ O ₆ ⁺	1.6	4.4
		265.1076		C ₁₄ H ₁₇ O ₅ ⁺	0.4	1.5	6.5
247.0970		C ₁₄ H ₁₅ O ₄ ⁺		0.4	1.6	7.5	
229.0865		C ₁₄ H ₁₃ O ₃ ⁺		-0.3	-1.3	8.5	
205.0865		C ₁₂ H ₁₃ O ₃ ⁺		-1.7	-8.3	6.5	
187.0759		C ₁₂ H ₁₁ O ₂ ⁺		-1.2	-6.4	7.5	
179.1072		C ₁₁ H ₁₅ O ₂ ⁺		-1.2	-6.7	4.5	
159.0810		C ₁₁ H ₁₁ O ⁺		-1.2	-7.5	6.5	
99.0810		C ₆ H ₁₁ O ⁺		0.0	0.0	1.5	
ESI-		361.1651	C ₂₀ H ₂₅ O ₆ ⁻	1.2	3.3	8.5	
		343.1545	C ₂₀ H ₂₃ O ₅ ⁻	2.5	7.3	8.5	
		299.1647	C ₁₉ H ₂₃ O ₃ ⁻	-0.7	-2.3	8.5	
		243.0657	C ₁₄ H ₁₁ O ₄ ⁻	-7.7	-31.7	9.5	
		221.0814	C ₁₂ H ₁₃ O ₄ ⁻	0.6	2.7	6.5	
		177.0916	C ₁₁ H ₁₃ O ₂ ⁻	1.0	5.6	5.5	
		139.0759	C ₈ H ₁₁ O ₂ ⁻	-5.7	-41.0	3.5	
119.0497	C ₈ H ₈ O ⁻	-7.7	-64.7	1.5			
TP 5-6PS 8.64 - 8.80	ESI+	377.1964	C ₂₁ H ₂₉ O ₆ ⁺	-1.4	-3.7	7.5	
		359.1858	C ₂₁ H ₂₇ O ₅ ⁺	-0.3	-0.8	8.5	
		331.1909	C ₂₀ H ₂₇ O ₄ ⁺	0.2	0.6	7.5	
		313.1804	C ₂₀ H ₂₅ O ₃ ⁺	-1.0	-3.2	8.5	
		285.1855	C ₁₉ H ₂₅ O ₂ ⁺	-1.7	-6.0	7.5	
		243.1385	C ₁₆ H ₁₉ O ₂ ⁺	-1.5	-6.2	7.5	
	ESI-	375.1808	C ₂₁ H ₂₇ O ₆ ⁻	2.2	5.9	8.5	
		331.1909	C ₂₀ H ₂₇ O ₄ ⁻	-2.5	-7.5	7.5	
		287.2011	C ₁₉ H ₂₇ O ₂ ⁻	-4.8	-16.7	6.5	
		161.0603	C ₁₀ H ₉ O ₂ ⁻	-7.3	-45.3	6.5	
TP 7PS 8.56	ESI+	395.2070	C ₂₁ H ₃₁ O ₇ ⁺	0.2	0.5	6.5	
		377.1964	C ₂₁ H ₂₉ O ₆ ⁺	3.2	8.5	7.5	
		359.1858	C ₂₁ H ₂₇ O ₅ ⁺	-1.6	-4.5	8.5	
		331.1910	C ₂₀ H ₂₇ O ₄ ⁺	0.1	0.3	7.5	
		313.1804	C ₂₀ H ₂₅ O ₃ ⁺	0.9	2.9	8.5	
		285.1855	C ₁₉ H ₂₅ O ₂ ⁺	-0.4	-1.4	7.5	
		243.1385	C ₁₆ H ₁₉ O ₂ ⁺	0.2	0.8	8.5	
		187.6759	C ₁₂ H ₁₁ O ₂ ⁺	1.1	5.9	7.5	

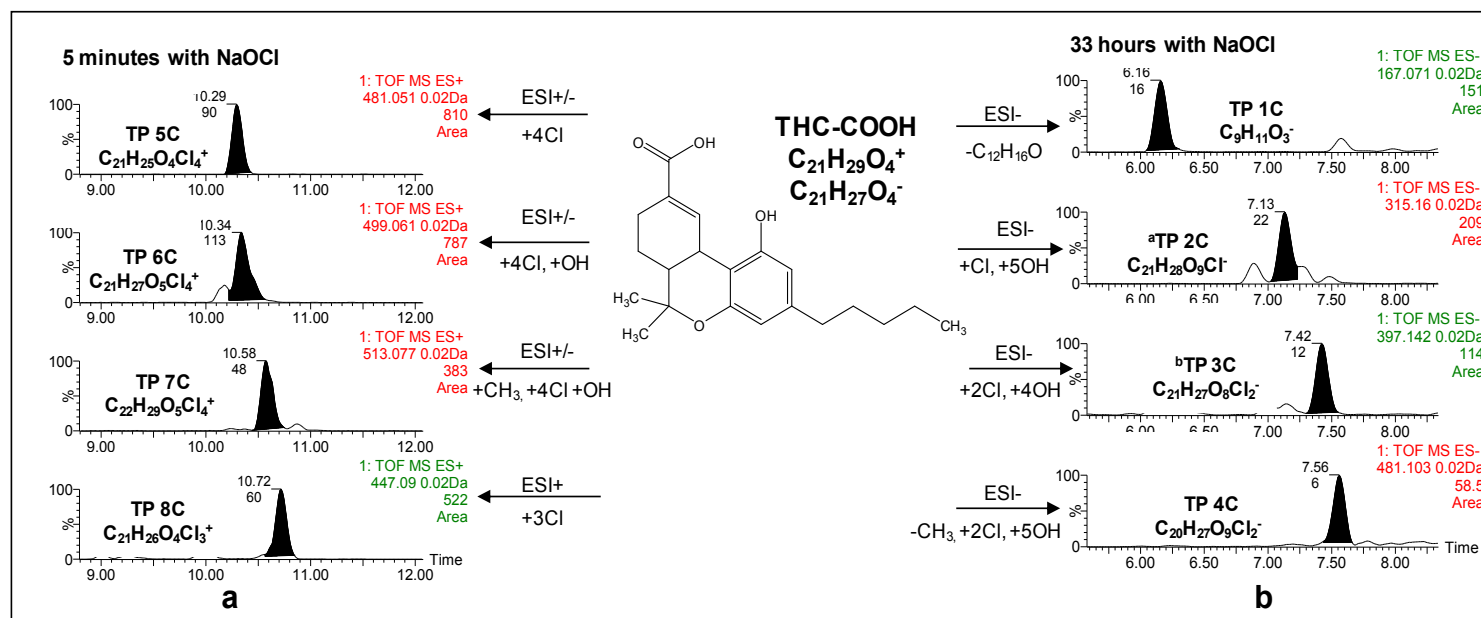
Table 6SI. MS/MS parameters selected for analysis of TTPs by QqQ MS

	Ionization mode	Retention time (min)	Parent compound	Product ion 1(Q)	CE ^a (eV)	Product ion 2 (q)	^a CE (eV)	Ion ratio Q/q
THC-COOH	ESI +	9.86	345.0	327.3	15	299.3	20	1.6
	ESI -		343.2	299.2	20	245.2	20	<u>19.8^b</u>
Hydrolysis								
TP 1H	ESI +	8.80	331.2	175.1	20	231.1	20	<u>16.4</u>
Chlorination								
TP 1C	ESI -	6.17	167.1	<i>167.1^c</i>	5	95.1	20	<u>25.1</u>
TP 2C	ESI -	6.43	395.1	315.2	20	253.2	20	2.6
TP 3C	ESI -	7.80	477.1	361.2	20	231.1	20	8.8
TP 4C	ESI -	7.75	481.1	199.1	20	427.1	20	<u>>100</u>
TP 5C	ESI +	10.40	481.1	277.0	20	315.0	20	1.7
	ESI -		479.0	399.1	20	363.1	20	1.0
TP 6C	ESI +	10.30	499.0	375.0	20	395.1	20	2.8
	ESI -		497.0	461.1	20	381.1	20	0.8
TP 7C	ESI +	10.68	513.1	375.0	20	395.1	20	<u>10.7</u>
	ESI -		511.1	395.1	20	375.0	20	2.7
TP 8C	ESI +	10.71	447.1	393.1	20	123.0	20	1.2
Photo-degradation								
UV								
TP 1PUV	ESI -	9.16	388.1	236.1	20	284.1	20	-
TP 2PUV	ESI -	9.83	433.2	389.2	20	256.1	20	-
TP 3PUV	ESI +	10.13	329.2	231.1	20	215.1	20	-
Photo-degradation								
Sunlight								
TP 1PS	ESI -	5.84	185.1	<i>185.1^c</i>	10	141.1	20	5.2
TP 2PS	ESI -	6.68	187.1	<i>187.1^c</i>	10	143.1	20	7.8
TP 3PS	ESI +	7.53	365.2	283.2	20	301.2	20	3.8
	ESI -		363.2	319.2	20	275.2	20	1.5
TP 4PS	ESI +	8.18	363.1	247.1	20	265.1	20	4.3
	ESI -		361.2	177.1	20	221.1	20	2.3
TP 5PS	ESI +	8.68	377.2	313.2	20	285.2	20	1.5
	ESI -		331.2	287.2	20	161.1	20	<u>90.9</u>
TP 6PS	ESI +	8.84	377.2	313.2	20	285.2	20	1.5
	ESI -		331.2	287.2	20	161.1	20	<u>68.4</u>
TP 7PS	ESI +	8.64	395.2	313.2	20	285.1	20	<u>58.0</u>

697 ^aCE: collision energy

698 ^b Underlined ion ratios are above 10. These transitions are difficult to observe in the samples due to the
699 low abundance of the product ion 2. In these cases, compliance of Q/q ratio in the samples is rather
700 problematic

701 ^c Pseudo MS/MS transition702 ^b Fo



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^a For TP 2C, main fragment at m/z 315.1596 was more sensitive than the deprotonated molecule (m/z 459.1422)

^b For TP 3C, main fragment at m/z 397.1418 was more sensitive than the deprotonated molecule (m/z 477.1083)

Figure 2SI. nw-XICs for the (de)protonated TP molecule and suggested elemental composition obtained. Chlorination experiments after (a) 5 minutes and (b) 33 hours

References