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

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ABSTRACT

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

Graphical Abstract (GA)

Keywords

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

1. INTRODUCTION

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

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

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

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

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

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

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

2. METHODS

2.1. Reagents and chemicals

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

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

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

2.2. Instrumentation

2.2.1. LC-ESI-QTOF MS

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

with an orthogonal Z-spray electrospray ionization interface (ESI) operating in both positive- and negative-ion modes and controlled by MassLynx v 4.1 software. The chromatographic separation was performed using an Acquity UPLC BEH C18 100 mm × 2.1 mm, 1.7 μm particle size analytical column (Waters). The mobile phases used were A = H_2O and B = MeOH, both with 0.01% FA. The percentage of organic modifier (B) was changed linearly as follows: 0 min, 10%; 9 min, 90%; 11 min, 90%; 11.1 min, 10%; 14 min, 10%. The flow rate was 0.3 mL/min. The column and sample temperatures were kept at 40 °C and 5 ºC, respectively. For MS^E experiments, two acquisition functions with different collision energies were created: the low-energy (LE) function with a collision energy of 4 eV, and the high energy (HE) function with a collision energy ramp ranging from 15 to 40 eV. The same cone voltage (15 V) and collision energy ramp was used for additional MS/MS experiments. Further details on instrument operating conditions can be found elsewhere (Boix, et al., 2013).

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

2.2.2. LC-ESI-QqQ MS

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

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 ºC (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 ºC
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 ºC. 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 ºC.

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 ºC under a gentle stream of nitrogen and reconstructed in 1 mL of
222 10:90 MeOH:H_2O. 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.

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

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

3.1.3. Photo-degradation (UV)

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

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

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

3.1.4. Photo-degradation (Sunlight simulation)

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

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

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

Different reactions, such as hydroxylation, demethylation, methylation, or a combination of them, would explain the formation of the seven photo-degraded sunlight TPs. Their elemental compositions, retention times, ionization modes, accurate masses, fragment ions, mass errors and DBEs are shown in **Table 5SI**. TP 1PS (*m/z* 185.0814, $C_9H_{13}O_4^-$) could have suffered a hydrogenation to form TP 2PS (*m/z* 187.0970, $C_9H_{15}O_4^-$). The chemical structure of both TPs would correspond to the A ring of THC-COOH, see **Figure 4**. The main fragment 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, 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

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

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

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

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

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

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

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

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FIGURE CAPTIONS

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

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

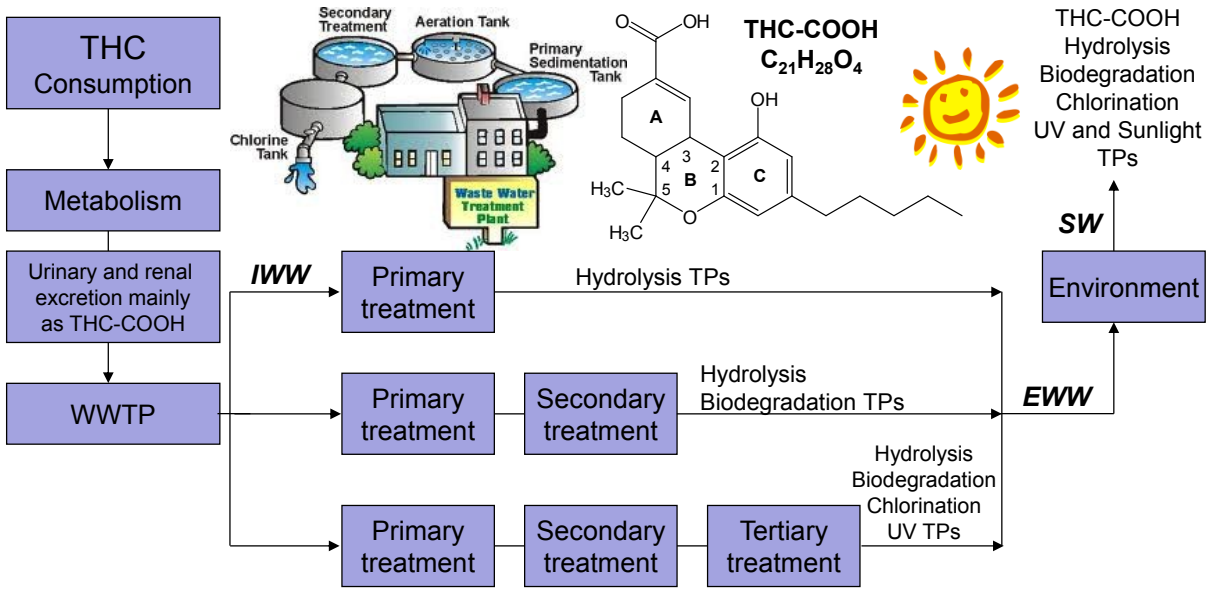
Figure 2. Degradation curves for TPs obtained after THC-COOH chlorination experiments (QTOF under ESI negative ionization mode)

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

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

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

574 Graphical abstract



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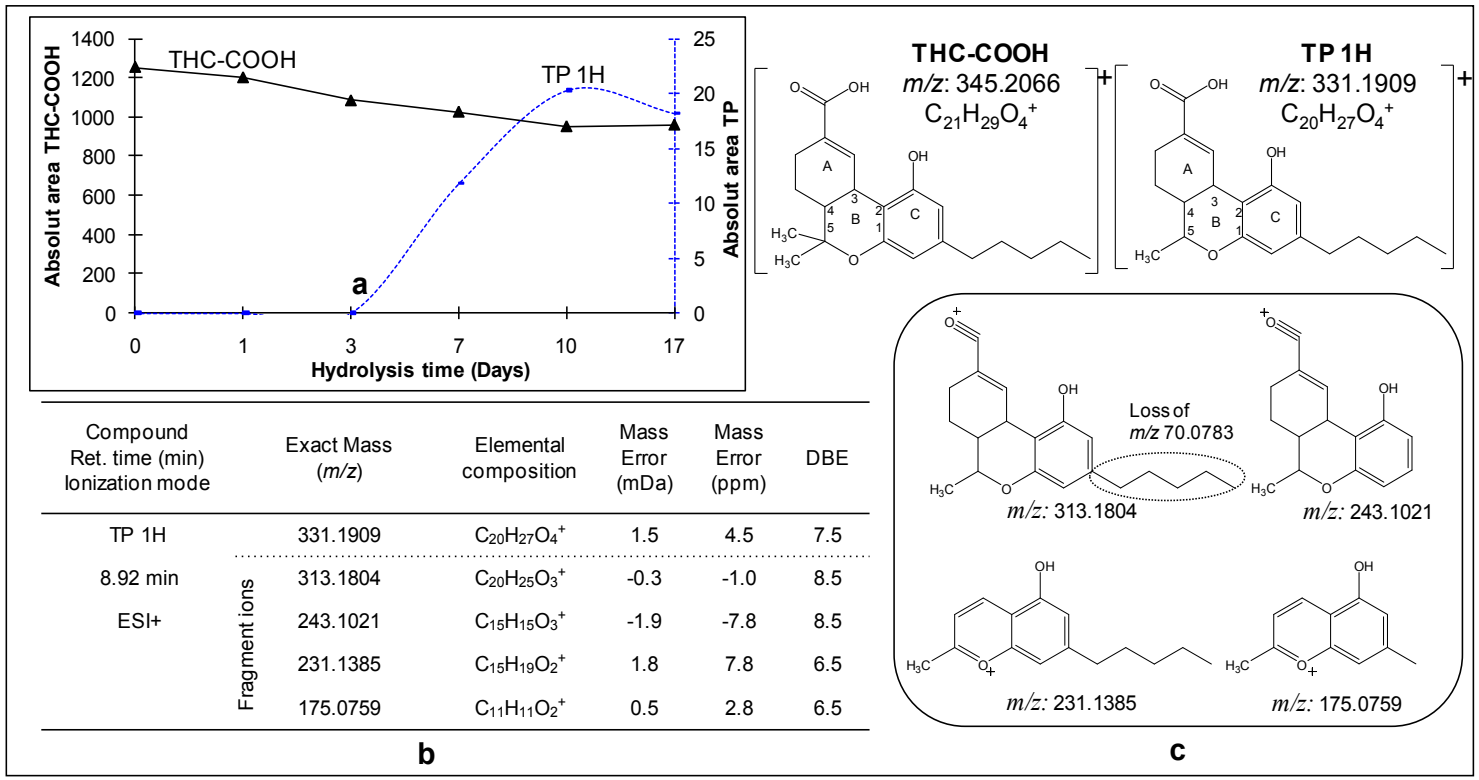
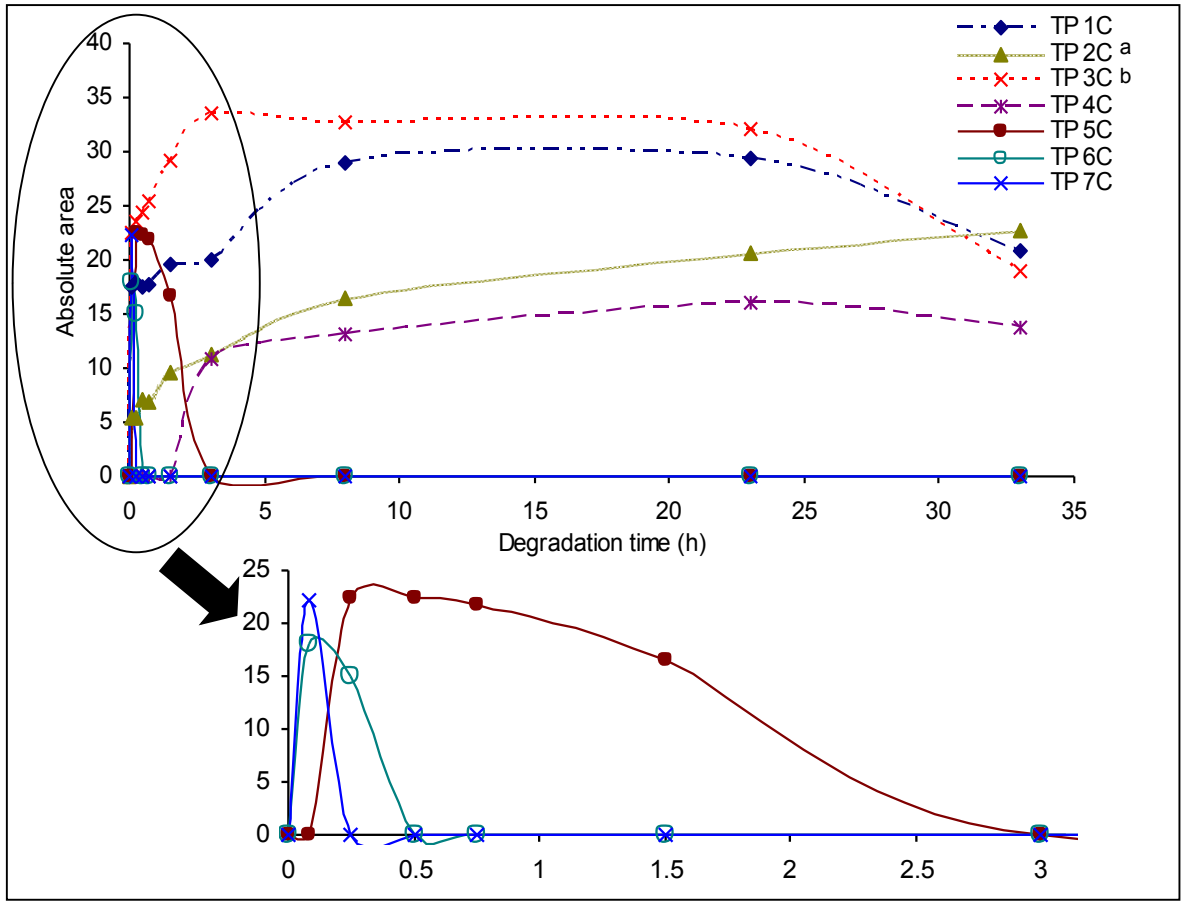


Compound Ret. time (min) Ionization mode		Exact Mass (*m/z*)	Elemental composition	Mass Error (mDa)	Mass Error (ppm)	DBE
TP 1H		331.1909	$C_{20}H_{27}O_4^+$	1.5	4.5	7.5
8.92 min	Fragment ions	313.1804	$C_{20}H_{25}O_3^+$	-0.3	-1.0	8.5
ESI+		243.1021	$C_{15}H_{15}O_3^+$	-1.9	-7.8	8.5
		231.1385	$C_{15}H_{19}O_2^+$	1.8	7.8	6.5
		175.0759	$C_{11}H_{11}O_2^+$	0.5	2.8	6.5

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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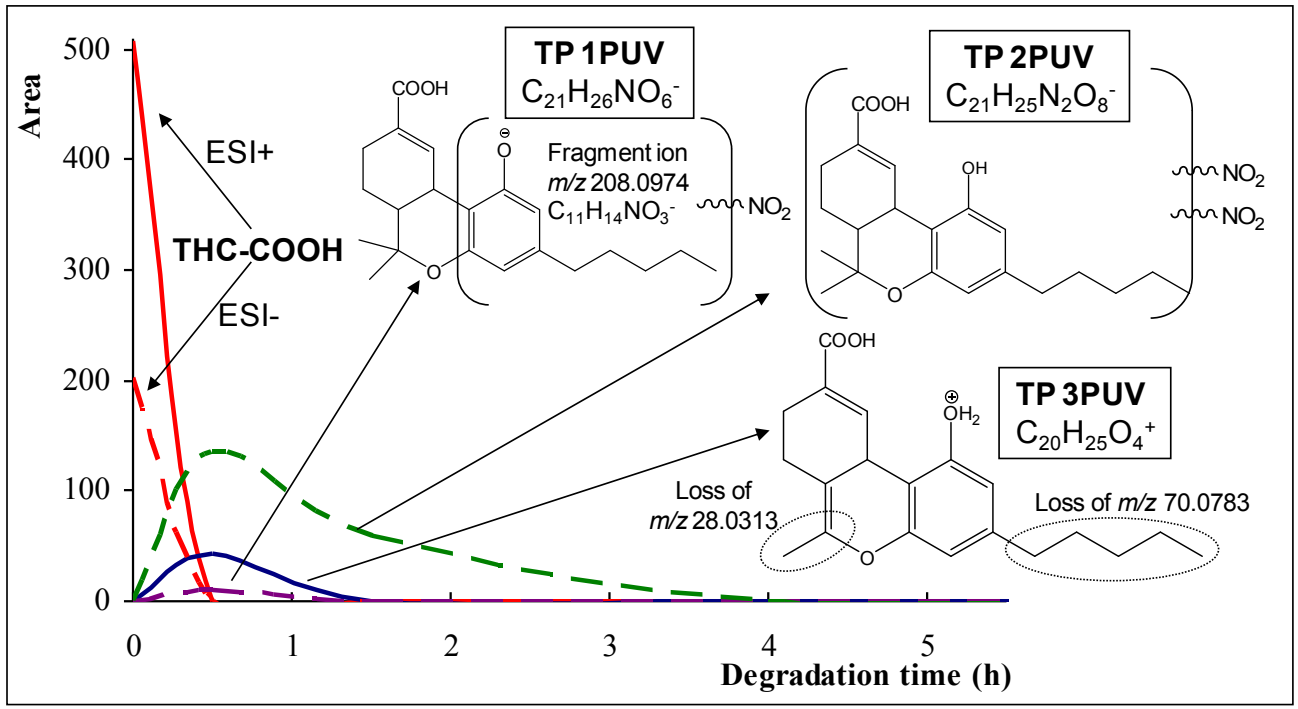

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590 Figure 3

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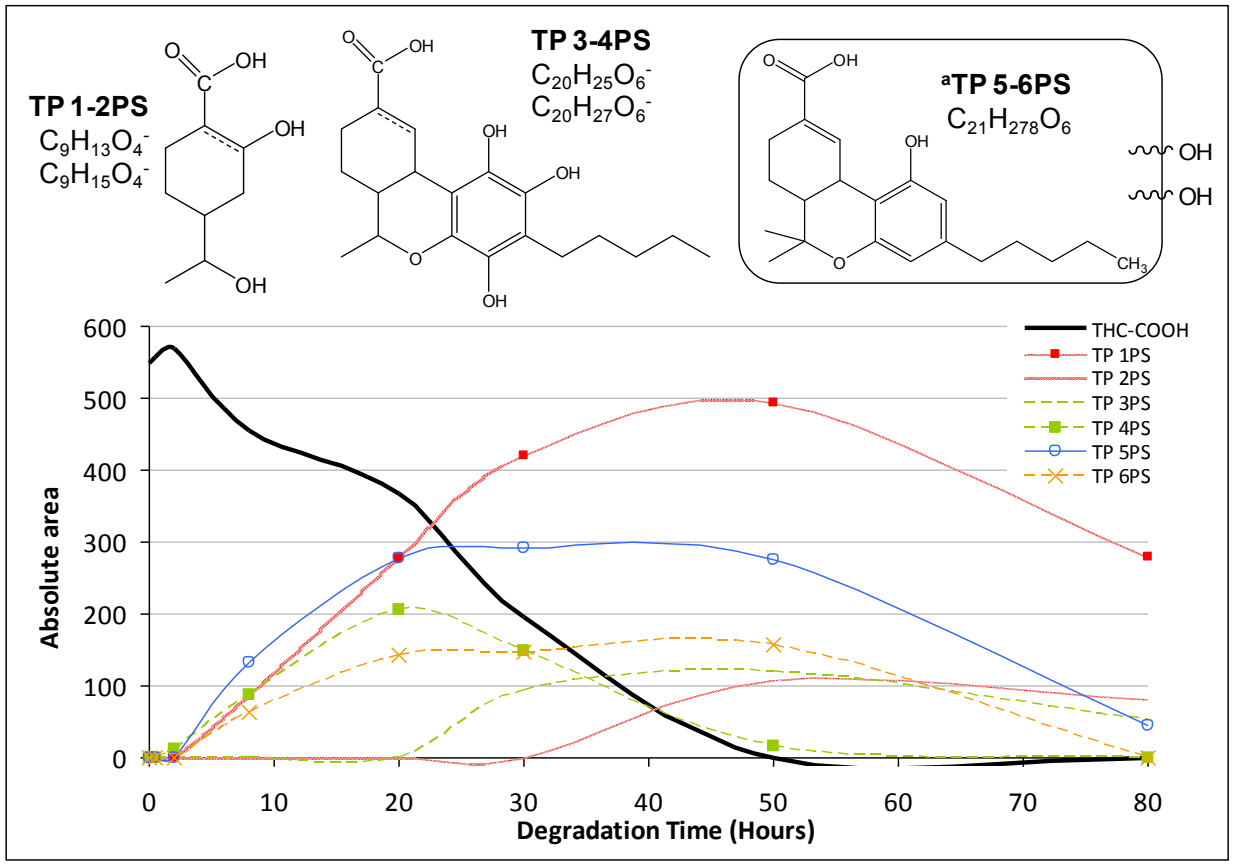

[a] For TTPs 5-6PS, detection of its main fragment at *m/z* 331.1909 was more sensitive than the deprotonated molecule at *m/z* 375.1808

Figure 4

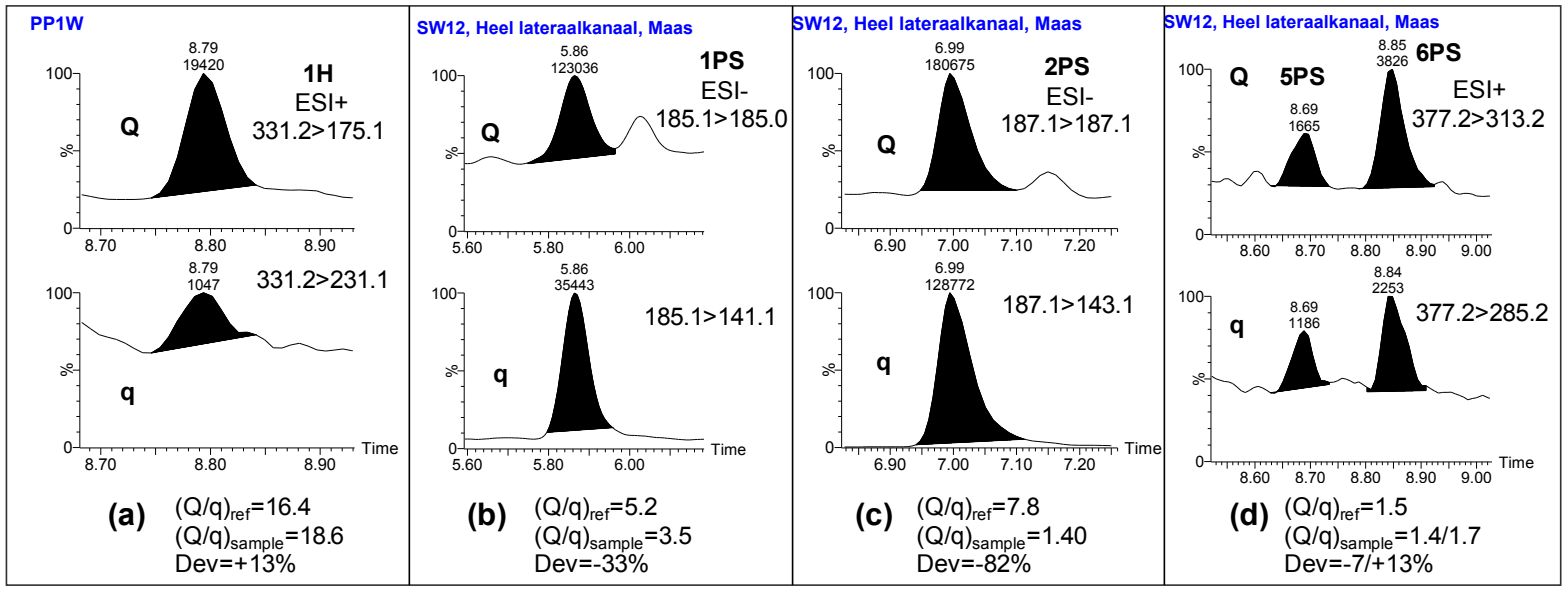
PP1W
8.79
19420
Q
1H
ESI+
331.2>175.1
8.79
1047
331.2>231.1
q
Time
(a)
(Q/q)ref=16.4
(Q/q)sample=18.6
Dev=+13%
SW12, Heel lateraalkanaal, Maas
5.86
123036
Q
1PS
ESI-
185.1>185.0
5.86
35443
185.1>141.1
q
Time
(b)
(Q/q)ref=5.2
(Q/q)sample=3.5
Dev=-33%
SW12, Heel lateraalkanaal, Maas
6.99
180675
Q
2PS
ESI-
187.1>187.1
6.99
128772
187.1>143.1
q
Time
(c)
(Q/q)ref=7.8
(Q/q)sample=1.40
Dev=-82%
SW12, Heel lateraalkanaal, Maas
Q
5PS
8.69
1665
8.85
3826
6PS
ESI+
377.2>313.2
8.69
1186
8.84
2253
377.2>285.2
q
Time
(d)
(Q/q)ref=1.5
(Q/q)sample=1.4/1.7
Dev=-7/+13%

Figure 5

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+)	-	3[a]	1
TP 1PS (ESI-)	-	-	6[b]
TP 2PS (ESI-)	-	4	4[b]
TP 3PS (ESI-)	-	3	-
TP 4PS (ESI-)	-	2	8
TP 5PS (ESI+)	-	-	1
TP 6PS (ESI+)	-	2	1
TP 7PS (ESI+)	-	3	-

610 [a] : underlined, the number of samples where only one transition was observed
611 [b]: Q/q ratio deviation >50%
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SUPPLEMENTARY INFORMATION

THC-COOH is reported in positive (*m/z* 345.2066) and negative (*m/z* 343.1909) ionization modes. The **Figure 1SI** shows its HE mass spectra and the main fragment ions. In positive ionization mode, the most intense fragment ion at *m/z* 299.2001 corresponds to the loss of formic acid. The other fragments have been justified in our previous works about fragmentation (Bijlsma, et al., 2011). Regarding negative acquisition mode, four low-intense fragment ions were observed. The ion at *m/z* 299 corresponds to the CO_2 loss. The fragment ion at *m/z* 191.1082 ($C_{12}H_{15}O_2^-$, +1.0 mDa) corresponds to the positive 193.1229 ($C_{12}H_{17}O_2^+$, 0.3 mDa). Other fragments ions observed in negative mode at *m/z* 245.1559 ($C_{16}H_{21}O_2^-$, +1.7 mDa) and *m/z* 179.1084 ($C_{11}H_{15}O_2^-$, +1.2 mDa) are illustrated in **Figure 1SI**.

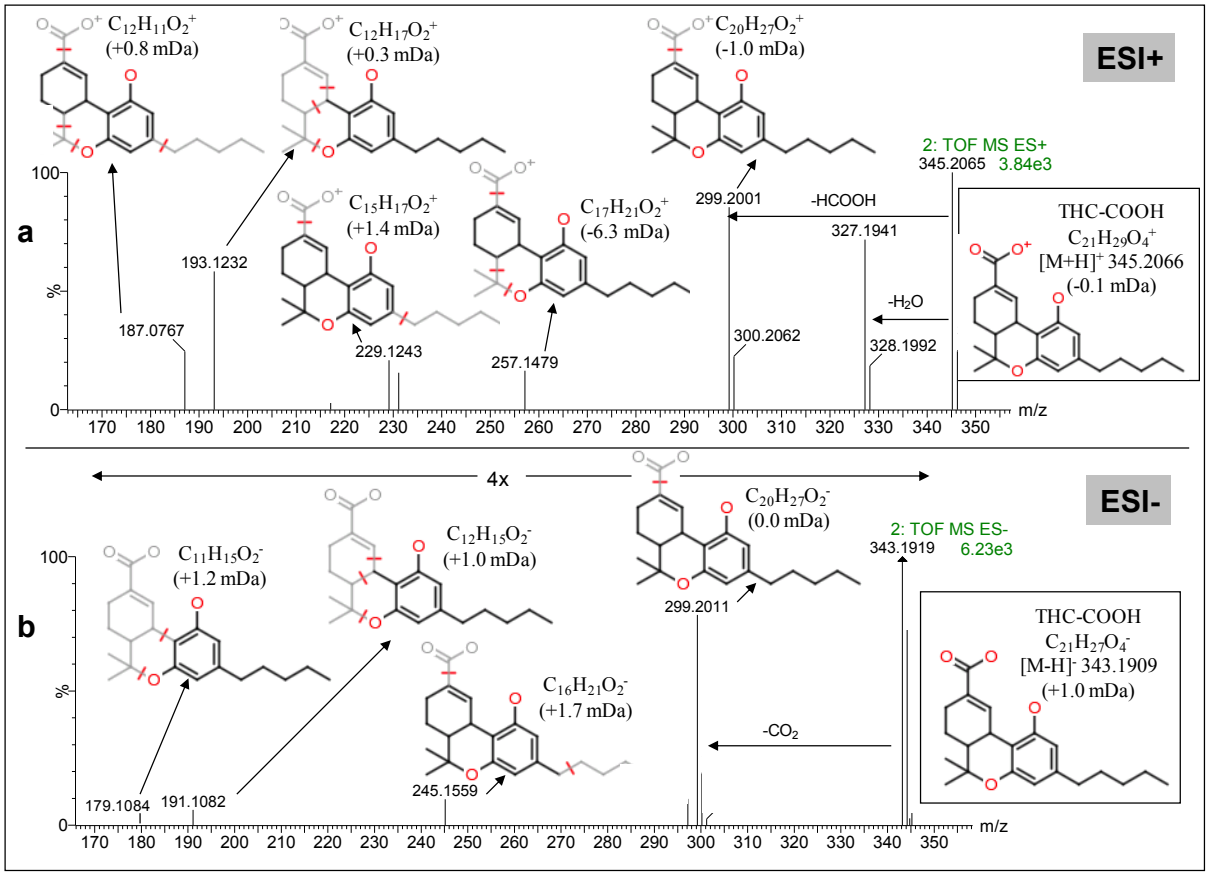

Figure 1SI. QTOF HE spectra of THC-COOH in (a) positive and (b) negative ionization mode; fragment ion structures proposed by MassFragment

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

Characteristics	Values
Temperature (ºC, *in situ*)	15.1
pH (*in situ*)	8.5
Conductivity (µS/cm, *in situ*)	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

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	ESI-	167.0708	$C_9H_{11}O_3^-$	0.1	0.6	4.5
6.17		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	ESI-	459.1422	$C_{21}H_{28}O_9Cl^-$	-2.0	-4.4	7.5
7.15		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	ESI-	477.1083	$C_{21}H_{27}O_8Cl_2^-$	-0.4	-0.8	7.5
7.42		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	ESI-	481.1032	$C_{20}H_{27}O_9Cl_2^-$	1.4	2.9	6.5
7.56		427.1160	$C_{20}H_{24}O_8Cl^-$	2.0	4.7	8.5
		317.1753	$C19H_{25}O_4^-$	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	ESI+	481.0508	$C_{21}H_{25}O_4Cl_4^+$	0.6	1.2	7.5
10.29		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	ESI+	499.0613	$C_{21}H_{27}O_5Cl_4^+$	-1.0	-2.0	6.5
10.33		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
		375.0088	$C_{14}H_{19}O_3Cl_4^+$	0.4	1.1	3.5
	ESI-	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	ESI+	513.0769	$C_{22}H_{29}O_5Cl_4^+$	-4.2	-8.2	6.5
10.57		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	ESI+	447.0897	$C_{21}H_{26}O_4Cl_3^+$	0.1	0.2	7.5
10.71		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

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Table 4SI. Proposed elemental composition, retention time (min), accurate mass (*m/z*), mass error (mDa, ppm) and double bound equivalent (DBE) of (de)protonated TPs of THC-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	ESI-	388.1760	$C_{21}H_{26}NO_6^-$	-1.0	-2.6	9.5
9.16		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	ESI-	433.1611	$C_{21}H_{25}N_2O_8^-$	0.2	0.5	10.5
9.83		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	ESI+	329.1753	$C_{20}H_{25}O_4^+$	0.2	0.6	8.5
10.13		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

693 **Table 5SI.** 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	ESI-	185.0814	$C_9H_{13}O_4^-$	-0.9	-4.9	3.5
5.83		371.1706	$C_{18}H_{27}O_8^-$	1.1	3.0	5.5
		141.0921	$C_8H_{13}O_2^-$	-0.5	-3.5	2.5
TP 2PS	ESI-	187.0970	$C_9H_{15}O_4^-$	-1.4	-7.5	2.5
6.52		375.2019	$C_{18}H_{31}O_8^-$	1.8	4.8	3.5
		169.0865	$C_9H_{11}O_3^-$	2.2	13.0	3.5
		143.1069	$C_8H_{15}O_2^-$	0.3	2.1	1.5
		125.0966	$C_8H_{11}O^-$	2.4	19.2	2.5
TP 3PS	ESI+	365.1964	$C_{20}H_{29}O_6^+$	0.5	1.4	6.5
7.45		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	ESI+	363.1808	$C_{20}H_{27}O_6^+$	1.6	4.4	7.5
8.06		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	$C_6H_{11}O^+$	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	$C_8H_{11}O_2^-$	-5.7	-41.0	3.5
		119.0497	$C_8H_8O^-$	-7.7	-64.7	1.5
TP 5-6PS	ESI+	377.1964	$C_{21}H_{29}O_6^+$	-1.4	-3.7	7.5
8.64 - 8.80		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	$C_{10}H_9O_2^-$	-7.3	-45.3	6.5
TP 7PS	ESI+	395.2070	$C_{21}H_{31}O_7^+$	0.2	0.5	6.5
8.56		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

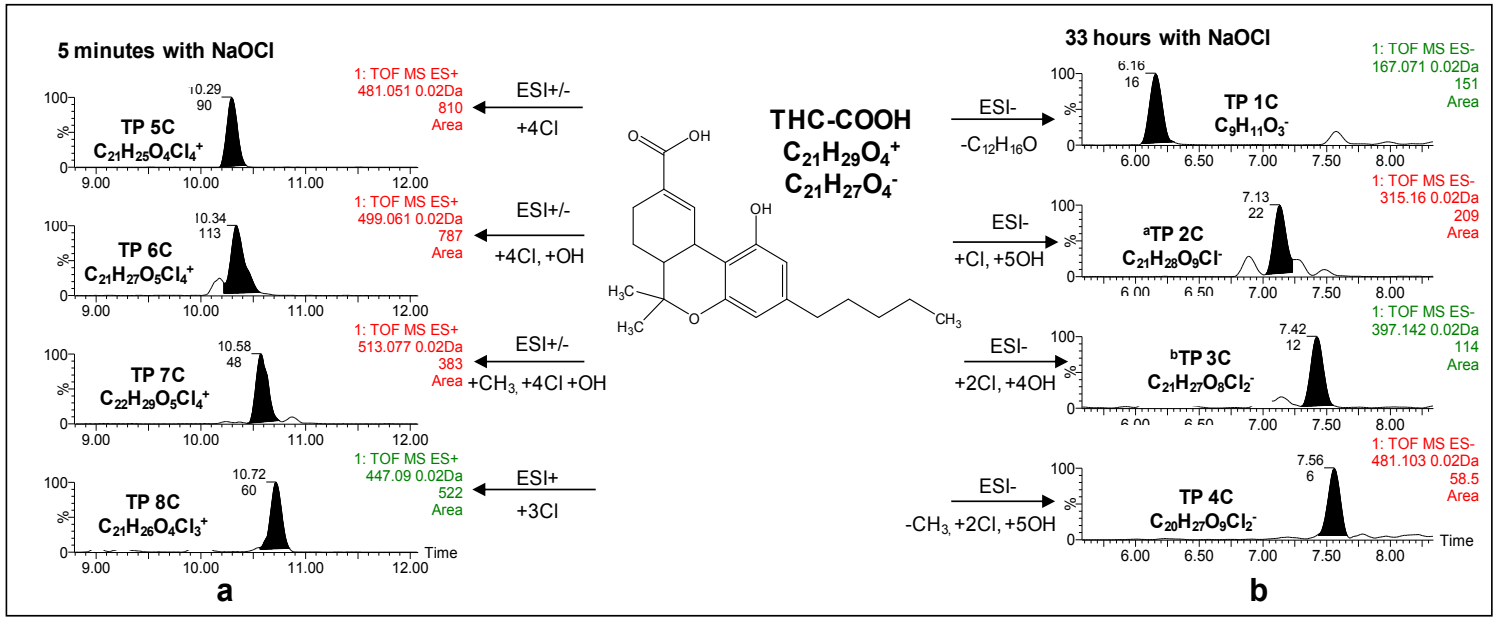
	Ionization mode	Retention time (min)	Parent compound	Product ion 1(Q)	CE[a] (eV)	Product ion 2 (q)	[a]CE (eV)	Ion ratio Q/q
THC-COOH	ESI +	9.86	345.0	327.3	15	299.3	20	1.6
	ESI -		343.2	299.2	20	245.2	20	19.8[b]
Hydrolysis								
TP 1H	ESI +	8.80	331.2	175.1	20	231.1	20	16.4
Chlorination								
TP 1C	ESI -	6.17	167.1	*167.1*[c]	5	95.1	20	25.1
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	>100
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	10.7
	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	*185.1*[c]	10	141.1	20	5.2
TP 2PS	ESI -	6.68	187.1	*187.1*[c]	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	90.9
TP 6PS	ESI +	8.84	377.2	313.2	20	285.2	20	1.5
	ESI -		331.2	287.2	20	161.1	20	68.4
TP 7PS	ESI +	8.64	395.2	313.2	20	285.1	20	58.0

[a] CE: collision energy

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

[c] Pseudo MS/MS transition

[b] Fo



[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