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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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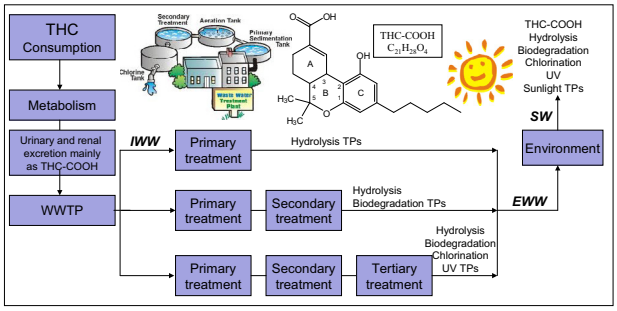
HIGHLIGHTS

- Degradation assays (hydrolysis, photo-degradation and chlorination) were performed.
- LC-QTOF MS allowed identifying up to 17 TPs under laboratory controlled conditions.
- THC-COOH was detected in 100% of wastewater and in 50% of surface water analyzed.
- 1 Hydrolysis and 5 photo-degradation TPs were detected in the water samples.
- Some of these transformation products have not been reported in the literature yet.

GRAPHICAL ABSTRACT

Schematic overview for THC-COOH presence and behavior in WWTPs and in the environment.



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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% and 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.

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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 (Weinmann et al., 2001; Skopp and Pötsch, 2004). 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 (Bijlsma et al., 2009; Boleda et al., 2009; Berset et al., 2010; 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) (Castiglioni et al., 2006; Boleda et al., 2007; Bijlsma et al., 2009, 2012; 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% and 98% (Boleda et al., 2009; Postigo et al., 2010; Bijlsma et al., 2012). 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., 2011a,b). 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., 2011a,b; Gosetti et al., submitted for publication; 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 (Castiglioni et al., 2006; Boleda et al., 2007; Bijlsma et al., 2009; Postigo et al., 2010; Thomas et al., 2012). High resolution mass spectrometry (HRMS) instruments, such as Orbitrap (Kern et al., 2009; Wick et al., 2011; Bijlsma et al., 2013b) and time-of-flight (TOF) (Ibáñez et al., 2004, 2011; Quintana et al., 2010; Bijlsma et al., 2013a), 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 (Hernández et al., 2011; Boix et al., 2013), 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 analyzed 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^{-1} was prepared in methanol (MeOH). A working solution (10 mg L^{-1}) 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^{-1}. The column and sample temperatures were kept at 40 °C and 5 °C, 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