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1 Investigation on the removal of the major cocaine metabolite (benzoylecgonine) in
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4

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14

15 **Abstract**

16 A microcapillary film reactor (MCF) was adopted to evaluate and compare the removal efficiency
17 of benzoylecgonine (BE), an emerging micropollutant deriving from illicit drug abuse (cocaine), in
18 different aqueous matrices: milliQ water, synthetic and real wastewater and surface water. The
19 removal processes investigated were the direct photolysis with UV radiation at 254 nm, and the
20 advanced oxidative process (AOP) with the same UV radiation and hydrogen peroxide. As a result
21 of the microfluidics approach developed through an innovative experimental apparatus, full
22 conversion of BE was reached within a few seconds or minutes of residence time in the MCF
23 depending on the process conditions adopted. The radiation dose was estimated to be approximately
24 5.5 J cm⁻². The innovative MCF reactor was found to be an effective tool for photochemical studies,
25 especially when using highly priced, uncommon, or regulated substances. The removal efficiency

was affected by the nature of the aqueous matrix, due to the presence of different xenobiotics and natural compounds that act primarily as HO[•] radical scavengers and secondly as inner UV₂₅₄ filters. Moreover, nano-liquid chromatography (LC)-high resolution-mass spectrometry analysis was utilized to identify the main reaction transformation products, showing the formation of hydroxylated aromatics during the photochemical treatment.

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Keywords: microcapillary film photoreactor; advanced oxidation processes; benzoylecgonine; wastewater; surface waters; emerging micropollutants

1. Introduction

1.1. Benzoylecgonine as an emerging pollutant

In the last few decades, non-conventional micropollutant removal is an emerging research focus (Asu et al., 2011; Corominas et al., 2013). The improvement in analytical technologies and the increasing consumption of both illicit and legal drugs by the world population allowed the discovery and identification in wastewater effluents (WW) and surface water (SurW) of new classes of contaminants, such as pharmaceuticals (Zuccato et al., 2009) and personal care products (Huerta-Fontela et al., 2008a; Bartelt-Hunt et al., 2009). Among these contaminants of emerging concern, in the last few years, the scientific literature shows an increasing interest in substances deriving from illicit drug consumption (Richardson et al., 2014; Richardson, 2012). These compounds reach wastewater treatment plants (WWTPs) through the drug users' urine and faeces and then are

52 released into the environment unchanged or as metabolites (Zuccato et al., 2009; Castiglioni et al.,
53 2008). The eco-toxicological effects for most of these compounds are unknown, but their potential
54 hazard cannot be excluded (Huerta-Fontela et al., 2008b; Kasprzyk-Hordern et al., 2008; Repice et
55 al., 2008). Cannabinoids, cocaine, opiates, and amphetamine-like drugs are the most used illicit
56 drugs worldwide (van Nuijs et al., 2011). Cocaine is the second most used illicit drug in Europe
57 (World Drug Report, 2012). In the human body, only 1-9% of consumed cocaine is excreted in
58 urine as the unchanged parent drug, whereas the rest of it is metabolized to benzoylecgonine (45%)
59 and ecgonine methyl ester (40%) (Castiglioni et al., 2008). Consequently, benzoylecgonine (Fig. 1)
60 is the primary metabolite of cocaine, and it was found in almost all the WW and SurW samples in
61 Europe and in other parts of the world at higher levels than its parent compound (Irvine et al., 2011;
62 Yin Lai et al., 2013).

63 Moreover, while cocaine and its metabolite ecgonine methyl ester tend to spontaneously degrade in
64 WW and SurW effluents (more or less rapidly depending on the pH and temperature),
65 benzoylecgonine (BE) concentration is almost constant or slightly increasing, due to its relative
66 stability in aqueous matrices and the partial degradation of cocaine into BE (Castiglioni et al., 2006;
67 Gheorghe et al., 2008; Castiglioni et al., 2011; van Nuijs et al., 2012). Tables S1 a-b in the
68 supplementary information section show the BE levels reported in SurW and WWTPs effluents.
69 The finding of BE in WWTPs effluents attests to the incomplete removal in conventional plants.
70 Because of its relatively high levels in the inlet, even with good removal efficiency ($\geq 80\%$), the
71 levels in the effluents are still higher than other illicit drug contaminants (Huerta-Fontela et al.,
72 2008; Kasprzyk-Hordern et al., 2008; Postigo et al., 2009; Repice et al., 2013; Du et al., 2014).
73 Despite the presence of illicit drug contaminants in WW and SurW, there are only a few studies
74 addressing the eco-toxicological effects of BE on the aquatic environment. The exposure of
75 *Dreissena polymorpha* (a zebra mussel) to BE concentrations of 0.5 and 1.0 $\mu\text{g} \cdot \text{L}^{-1}$, resulted in a
76 3.5 fold increase of the oxidative stress in comparison to the background level and either an
77 increased or inhibited activity of antioxidant and detoxifying enzymes depending on the BE levels

78 and the exposure time (Parolini et al., 2013). In addition, an increased peroxidation of lipids and
79 protein carbonylation, DNA damage, and cellular apoptotic death were found in experiments carried
80 out up to 14 days (Binelli et al., 2013). Even more detrimental damage cannot be excluded at
81 longer exposure times and in concert with the effects of other WW and SurW micropollutants.
82 The kinetic behaviour of BE during a disinfection stage for water reuse was previously investigated
83 (Russo et al., 2016) treating contaminated milliQ water under UV₂₅₄ radiation only.
84 The purpose of this study is to investigate the effect of an advanced oxidation process (AOP) with
85 hydrogen peroxide and UV₂₅₄ radiation on the removal and transformation of BE in four different
86 aqueous matrices: milliQ water, synthetic wastewater (SWW), real wastewater (RWW) and SurW.
87 *1.2. Microcapillary photoreactor*
88 The degradation experiments were carried out in a novel microcapillary film (MCF) array photo-
89 reactor (Reis and Li Puma, 2015) consisting of 10 microcapillaries with a mean diameter of about
90 200 µm transparent to UVC light. A schematic of the reactor is reported in fig. S1 in the
91 supplementary information section. This experimental apparatus allows the use of very small
92 volumes of reacting solution compared to the usual batch photo-reactors, leading to economic and
93 environmental benefits. A significant benefit is the reduction of the volume of the experimental
94 waste solution and decreased usage of costly border controlled substances, including the adoption of
95 illicit drugs as the tested compounds. The cost of the low power (8 W) UV lamp necessary for this
96 kind of reactor is also very low. Moreover, as described elsewhere (Reis and Li Puma, 2015; Russo
97 et al., 2016), the extremely small reactor volume and optical path length allows for complete
98 conversion of the contaminant in a few seconds or a few minutes depending on the treatment
99 adopted. This is possible as a result of the high surface/volume ratio achievable in a microcapillary
100 reactor and high photon irradiance, which is realized even in optically thick fluids. Therefore, the
101 MCF photoreactor represents a powerful tool for the rapid determination of photodegradation
102 kinetics of contaminants in fluids at a laboratory scale. The wider impact of this study will be
103 maximized by the integration of MCF with ultra-rapid analytics for the determination of

104 transformation products of contaminants of emerging concern which are often available only in
105 minute quantities.

106

107 **2. Materials and methods**

108 *2.1. Materials*

109 Hydrogen peroxide (30% v/v), benzoylecgonine ($\geq 99\%$ w/w), acetonitrile ($\geq 99.9\%$ v/v), formic
110 acid ($\geq 95\%$ v/v), sodium hydroxide, and sulfuric acid were purchased from Sigma-Aldrich. The
111 milli-Q water was prepared by a Millipore Elix water purification system.

112 Synthetic wastewater was prepared following the OECD Guidelines (Organisation for Economic
113 Cooperation and Development, 1999) using milliQ water and peptone (32 ppm), meat extract (22
114 ppm), urea (6 ppm), K_2HPO_4 (28 ppm), $CaCl_2 \cdot H_2O$ (4 ppm), NaCl (7 ppm) and Mg_2SO_4 (0.6 ppm).
115 These substances were purchased from Sigma-Aldrich. Real wastewater was sampled from the
116 sewage water treatment plant of Festival Drive, Loughborough, Leicestershire (UK). Surface water
117 was collected from the Grand Union Canal in the same zone. All the real water samples were
118 filtered through Whatman nylon filters (0.45 μm), to avoid clogging of the MCF and of the high
119 performance liquid chromatography (HPLC) tubing. Furthermore, this allowed the direct evaluation
120 of the degradation of BE in the different aqueous matrices without considering the effect of the
121 suspended solid matter on the absorption of radiation and sorption of BE and its transformation
122 products (TPs).

123 *2.2. Analytical methods*

124 The concentrations of hydrogen peroxide and BE in the samples were evaluated by HPLC analysis.
125 The HPLC (1100 Agilent) apparatus was equipped with a Gemini C18 (Phenomenex) reverse phase
126 column and a diode array UV/VIS detector ($\lambda = 232$ nm). The mobile phase consisted of a mixture
127 of formic acid aqueous solution (25 mM) (A) and acetonitrile (B) flowing at 0.6 mL min^{-1} . The
128 following gradient was adopted: 7% B to 28% B in 9 min, to 50% B in 5 min, constant for 2 min,
129 and then to 35.7% in 3 min, and finally returned to the initial conditions in 5 min. The retention

130 times of H₂O₂ and BE were 4.9 min and 13.8 min, respectively. The pH of the reacting solutions
131 was measured with an Accumet Basic AB-10 pH-meter. A Perkin Elmer UV/VIS spectrometer
132 (Lambda 35) was used to determine the absorbance spectra of the aqueous matrices. The
133 conductivity and the total organic carbon (TOC) of the water samples were respectively measured
134 with a WPA Linton Cambridge CM35 conductivity meter and a TOC-5000A TOC analyzer
135 (Shimadzu). The nitrate and nitrite content were measured by means of ion chromatography
136 (Metrohm 761 Compact). Table 1 summarizes the main water features.

137 2.3. *Experimental apparatus and procedures*

138 2.3.1. *Apparatus and treatment*

139 The spiked solutions were prepared by adding the solid BE to each filtered aqueous matrix. The
140 initial concentration of BE in the solutions was kept in the range $2.8 \cdot 10^{-5} \pm 4.0 \cdot 10^{-6}$ mol L⁻¹. This
141 value is much greater than the usual level of BE found in RWW and SurW (Tab. S1); however, it
142 was necessary to keep the concentration at a higher level for a better evaluation and comparison of
143 the removal kinetics and for the identification of the transformation byproducts.

144 In order to study the degradation of BE with the UV₂₅₄/H₂O₂ process, a fluorinated ethylene-
145 propylene (FEP-Teflon) MCF photoreactor containing 10 capillaries with a mean hydraulic
146 diameter of 195 µm was used. The description and the validation of the microreactor has been
147 described in detail elsewhere (Reis and Li Puma, 2015; Russo et al., 2016). As previously reported,
148 the microreactor was coiled around a 8 W (nominal power) UV monochromatic lamp (Germicidal
149 G8T5) emitting radiation at 254 nm. The segments not coiled were covered with aluminum foil to
150 avoid UV radiation reaching these sections. Experiments were performed in continuous flow
151 through the reactor. The residence time (i.e., the reaction time) was changed by varying the length
152 of the reactor exposed to the UV₂₅₄ radiation. Samples at different residence times were collected
153 from the outlet of the MCF after reaching the steady state condition (i.e., about 5 times the reactor
154 volume processed) and rapidly analyzed. The concentration of BE in the inlet to the reactor did not
155 vary during the experimental runs. All experiments were carried out at room temperature (~ 25 °C).

156 It was verified that thermostatic control was not necessary because at the outlet of the reactor, the
157 temperature of the solution did not vary significantly. The pH of the reacting solutions was adjusted
158 with dilute solutions of NaOH and H₂SO₄. At the end of each experiment, the pH of the solutions
159 was re-measured and the results were always close to the initial value.

160 2.3.2. Analysis of transformation products

161 Aqueous reaction mixtures and untreated controls were extracted using solid phase extraction (SPE)
162 and analyzed using LC-mass spectrometry (MS)/MS. Water samples (150 mL) were passed through
163 SPE cartridges (Waters Oasis HLB, 6cc) at a rate of approximately 5 mL/min and were eluted with
164 8 mL of methanol, in 4 mL increments, using gravity elution. The eluted solutions were stored in
165 the freezer throughout the duration of the experiments. They were either used as is or blown down
166 using nitrogen to make a more concentrated solution.

167 Mass spectrometry analyses of TPs were carried out using a high resolution Thermo Orbitrap Velos
168 Pro (Thermo Scientific, Bremen, Germany). A nano-LC was coupled to the Orbitrap for
169 chromatographic separation. The flow rate was 0.300 μ L/min. Initial conditions were 2% organic
170 (acetonitrile) and were held for 5 min. The percent organic was ramped linearly to 40% over the
171 next 10 min, then ramped linearly to 95% over the next 40 min, held at 95% for 10 min, and then
172 returned to initial conditions. The capillary voltage was 1.8 kV. The column used was a picochip
173 C18 reversed phase column (10 cm x 75 μ m; 3 μ m; New Objective; Woburn, MA). The injection
174 volume was 1 μ L and the column temperature was held at room temperature. Full-scan and data-
175 dependent acquisition (DDA) MS/MS spectra were obtained using positive ion electrospray
176 ionization (ESI). The resolution was 30,000 for full-scan MS, and 7500 for DDA. The mass range
177 was m/z 100-800.

178 A fraction containing two transformation product isomers (TP305) was also collected via
179 continuous fractionation with preparative LC-MS using a Waters 2000 series LC-mass spectrometer
180 with a C18 column and single quadrupole detector. An acetonitrile/water gradient was used, with a
181 100 μ L injection volume and 1.0 mL/min flow rate. The fractions were collected based on retention

182 times. After collection, the sample was freeze dried and dissolved in deuterated methanol (d₄) and
183 analyzed using ¹H nuclear magnetic resonance (NMR) with a Bruker Avance III-HD 400 MHz
184 NMR spectrometer.

185 In this study, TPs are named using a number referring to their molecular mass (e.g., TP 305 refers to
186 a TP with a molecular mass of 305 Da).

187

188 **3. Results and Discussion**

189 *3.1. Degradation Absorbance Characteristics*

190 Absorbance spectra were acquired to characterize and compare the different aqueous matrices. Fig.
191 2a. shows the characteristic absorption peak of BE in milliQ water at the wavelength of 232 nm. No
192 significant differences were observed in the spectra in the pH range 4-8. This was confirmed by the
193 mean reported values of pK_{a1} and pK_{a2} of BE, that are 3.35 and 10.45, respectively (Jeanville et al.,
194 2003; Marchi et al., 2009). The spectra of filtered SWW, RWW, and SurW are also displayed in
195 Fig. 2b. The absorbances were nearly coincident for the real wastewater and surface water samples,
196 whereas the synthetic wastewater showed very low absorbance at 254 nm. This can be ascribed to
197 the higher concentration of the aromatic compounds dissolved in the real wastewater and surface
198 water samples compared to the lower concentrations of these in the synthetic wastewater, which is
199 also supported by the TOC data in Table 1. This difference does not significantly affect the BE
200 removal, as further discussed, because of the different nature of the organic compounds.

201 Control experiments performed in pH range 4-8 with direct photolysis and with the UV₂₅₄/H₂O₂
202 process showed insignificant differences in the removal of BE (data not shown). Consequently, all
203 further experiments were run at pH = 6.0 in milliQ water and at natural pH for the other different
204 aqueous matrices (Table 1). Moreover, no BE degradation was recorded in the presence of
205 hydrogen peroxide ([H₂O₂]/[BE] ~ 500) without UV₂₅₄ radiation (data not shown).

206 *3.2. Degradation Kinetics*

207 Figure 3 shows the removal percentage of BE by direct UV₂₅₄ photolysis at different residence
208 times in the MCF and with the different aqueous matrices that were examined, compared to
209 previously published removal in milliQ water. Even though UV treatment is not often adequate for
210 chemical removal, it is important to consider that in the case of water reuse, UV treatment is
211 indicated as a method to reduce the presence of pathogens. During this process a contemporary
212 transformation of chemicals cannot be excluded.

213 The removal rate of BE in the SWW sample was slower than in milliQ water. This rate difference
214 could be easily justified by the presence of several compounds in SWW that may act as inner filters
215 (optical competitors absorbing part of the radiation) since the absorption of the SWW at 254 nm
216 (Fig. 2) was slightly higher than the absorbance of milliQ represented by the zero line in the
217 absorbance spectrum. In spite of this, as reported elsewhere (De Laurentiis et al., 2012), while the
218 chromophoric dissolved organic matter would inhibit the direct photolysis of BE by an inner-filter
219 effect, it could simultaneously act as a producer of oxidant species that could promote BE
220 degradation. Therefore, the degradation of BE showed in fig. 2 must be considered as a result of the
221 balance of the effects described above.

222 Moreover, the BE removal percentage and rate in SurW is very close to the removal in milliQ
223 water, whereas in RWW effluents is significantly faster. These phenomena occur despite both SurW
224 and RWW samples having considerably greater absorbances than the milliQ water. This evidence
225 could suggest that the inner filter effect from the dissolved species was fully or more than offset by
226 the chemical nature of the organic compounds present in these water matrices. Specifically, the
227 photolysis of nitrates are a supplementary source of hydroxyl radicals (Mack and Bolton, 1999) and
228 since the nitrate content of the RWW sample was double that of the SurW sample, (Table 2), the
229 removal of BE in the RWW water matrix could be expected to be higher than in the SurW sample
230 (Fig. 3). This effect has also been reported for the removal of other water contaminants (Alif and
231 Boule, 1991; Pamela et al., 1998; Goldstein and Rabani, 2008; Keen et al., 2012; Ji et al., 2012; Li

et al., 2014; Zaviska et al., 2014). The nitrate photolysis follows the pathways below (Mack and Bolton, 1999):

HO[•] radicals increase the removal rate of BE in the RWW sample by oxidizing the organic contaminant (Fig. 3). The possibility that the photolysis of nitrite could contribute to the generation of hydroxyl radicals is not considered because of their negligible content compared to nitrates concentration (Tab. 1).

Once the effects of different aqueous matrices on the indirect photolysis of BE were investigated, the removal of BE by UV₂₅₄/H₂O₂ process was studied. The photolysis of the H₂O₂ molecule, with radiation at 254 nm, yields two strongly reactive HO[•] radicals. The reaction pathways can be exemplified as follow:

Reaction (10) describes the scavenging effect of the organic compounds naturally or artificially present in the water. This reaction was not considered for the process in milliQ water due to the absence of organic contaminants. However, $\text{HO}^\bullet$ radical production via reactions 1-4 was considered where necessary. Mack and Bolton (1999) report that, using UV and nitrate, hydroxyl radical generation is not as efficient as the $\text{HO}^\bullet$ production with hydrogen peroxide. The reduced UV/nitrate efficiency is due to the nitrate's low hydroxyl radical quantum yield in the 250 nm to 350 nm range compared to the quantum yield of direct photolysis of hydrogen peroxide $\Phi_{254}^{\text{H}_2\text{O}_2} = 0.5 \text{ mol ein}^{-1}$ (Nicole et al., 1990). Consequently reaction (5) is faster than (4) in the presence of nitrates in the water.

Fig. 4 displays the effect of different $[\text{H}_2\text{O}_2]_0/[\text{BE}]_0$ ratios on the removal efficiency in milliQ water, where $[\text{H}_2\text{O}_2]_0$ and $[\text{BE}]_0$ are respectively the initial concentrations of hydrogen peroxide and BE. Higher $[\text{H}_2\text{O}_2]_0/[\text{BE}]_0$ ratios resulted in greater percentages of removal. Fig. 5 shows the BE percentage of removal at different $[\text{H}_2\text{O}_2]_0/[\text{BE}]_0$ ratios for the four types of water matrices at three significant reaction times.

However, the gain in the removal percentage gradually lessens with increased $[\text{H}_2\text{O}_2]_0/[\text{BE}]_0$ ratio, such that the removal degree at a fixed reaction time reached a plateau for all aqueous matrices investigated (Fig. 5). In effect, an increase of the relative hydrogen peroxide concentration results also in increased rate of reaction (8) so that a smaller than expected amount of hydroxyl radicals is effective in the degradation. The high $[\text{H}_2\text{O}_2]_0/[\text{BE}]_0$ ratios adopted in this experiment can be justified because in real wastewater samples collected worldwide, BE is just one of many micropollutants found in effluents and in surface waters. A greater hydrogen peroxide concentration may be necessary for efficient degradation of these different mixtures of xenobiotics normally present in water bodies.

265 A comparison of the time scale of UV₂₅₄ direct photolysis against UV₂₅₄/H₂O₂ removal of BE
266 shows significantly different characteristic kinetics of the two processes. The UV₂₅₄ radiation
267 treatment (Fig. 3) requires up to two orders of magnitude longer time for the degradation of BE
268 compared to the UV₂₅₄/H₂O₂ process (Fig. 4). The difference in magnitude suggests that direct
269 photolysis is a negligible process during the AOPs and that it would be highly inefficient on its own
270 for BE degradation.

271 As shown in Fig. 5, BE removal is higher in milliQ water once all of the operating conditions are
272 standardized between the different aqueous matrices. This phenomenon is due to the absence of
273 organic molecules acting as scavengers of hydroxyl radicals. However, the presence of scavengers
274 alone cannot justify the differences in rates between RWW effluent and natural SurW. The rate
275 differences can be related to the balance between the contrasting effects of reactions 1-4. In almost
276 all of the experiments, the removal percentages in RWW samples are slightly but significantly
277 higher than in SurW. Similarly to what was reported for direct photolysis, this last evidence is in
278 agreement with RWW having a higher nitrate concentration with respect to SurW since the
279 absorbance of the two aqueous matrices is comparable (Fig 2).

280 3.3. Transformation Products

281 Several transformation products (TPs) resulting from UV photolysis and UV/H₂O₂ treatment of BE
282 were observed. These products increased in abundance with increasing reaction time, and they were
283 tentatively identified using their accurate masses (Orbitrap-MS) and MS/MS fragmentation. Fig. 6
284 shows some of the most prominent TPs found in UV/H₂O₂ reactions of BE, in which 40% of BE
285 was degraded (~ 0.5 s reaction time). These products were also present at 90% and 100%
286 degradation of BE, as well as in samples treated with UV only.

287 Three of these TPs (TP 305a, 305b, and 305c, fig. 6a) appear to be isomers, each having an
288 observed accurate mass of m/z 306.1348 (as the (M+H)⁺ ion), and eluting at 20.56, 21.18, and 23.05
289 min, respectively. Their accurate mass suggests a molecular formula of C₁₆H₁₉NO₅ (theoretical
290 mass of m/z of 306.1336). These TPs are likely monohydroxylated isomers, meta-, para-, and ortho-

hydroxybenzoylecgonine. The MS/MS product ion spectra support these structural assignments. At a CE of 40 eV, two distinct fragments (m/z 168.1023 and 121.0287, fig. 7) matched viable fragmentation on the two bonds on either side of the ester oxygen (theoretical m/z of 168.1024 and 121.0289, respectively, for these two fragment ions). In particular, the m/z 121.0287 fragment ion, with a formula of $C_7H_5O_2$, indicates that hydroxyl radical addition occurs on the benzene ring, which is consistent with UV and UV/H₂O₂ chemistry (Andreozzi et al., 2003).

Two of these TPs were isolated using preparative LC-MS, and NMR data supported the assignment of the ortho- and para-hydroxylated TPs. Despite low levels collected from these reaction mixtures, there was strong evidence for the ortho-hydroxy-BE [H6 d (J = 5.5 Hz), δ = 8.208 ppm; H4 m, δ = 7.740 ppm; H3 d (J = 8.04 Hz), δ = 7.630 ppm; H5 t (J = 8.04 Hz), δ = 7.478 ppm]. The para-hydroxy-BE assignment was also suggested by NMR data [H2,6 broad singlet, δ = 7.831 ppm; H3,5 broad singlet δ = 7.207 ppm], but it was not as definitive, due to lower levels of this TP isolated relative to the ortho isomer. These TPs were also reported as by-products formed during two solar photocatalytic treatments (TiO₂ photocatalysis and photo-Fenton) of cocaine in water (Postigo et al. 2011).

TP 321 was observed at m/z 322.1300 ($(M+H)^+$ ion). This TP is likely dihydroxybenzoylecgonine, which has a theoretical m/z of 322.1285 for the $(M+H)^+$ ion. This TP eluted at 19.36, 21.84 min, and 25.12 min, and likely has both hydroxyl substituents on the benzene ring, consistent with TP 305a, 305b, and 305c, and consistent with hydroxyl radical chemistry. It is currently not known which isomer this TP represents (6 isomers are possible). MS/MS data provide evidence that the three chromatographic peaks in Fig. 6B indicate three isomers of dihydroxybenzoylecgonine, all of which have the two hydroxyl substituents on the aromatic ring. When the TP was fragmented using CID (27 eV), the resulting fragmentation pattern matches the half of the parent molecule with the bridged ring and no evidence of the hydroxyl groups. The half with the aromatic ring (and hydroxyl groups) breaks off as a neutral molecule and is not observed by the detector. Three chromatographic peaks for TP 321 were seen in the MS/MS data, and all of them yielded the same fragmentation

317 pattern as discussed above. Work continues to identify other less predominant TPs, as well as to
318 collect additional individual TP fractions (using preparative-LC-MS) for nuclear magnetic
319 resonance (NMR) analysis and confirmation of proposed chemical structures. NMR is particularly
320 helpful for determining substituent positions, and can aid in the identification of specific isomers for
321 TPs for cases where MS spectra are not definitive (Wendel et al., 2014; Luft et al., 2014). Further
322 chemical characterization of transformation products will be done using 2-dimensional NMR
323 spectroscopy on transformation products isolated with preparative-LC, and further mass
324 spectrometry analyses will be performed using targeted MS/MS on selected degradation products.

325

326

327 **4. Conclusions**

- 328 • The microfluidics approach adopted in this study allowed a full investigation on the removal of
329 benzoylecgonine by photolysis and the UV₂₅₄/H₂O₂ process using minute quantities (100 mg) of
330 this border controlled substance.
- 331 • Benzoylecgonine, the primary human urinary metabolite of cocaine, was efficiently removed by
332 UV₂₅₄ photolysis coupled with H₂O₂ in a microcapillary film photoreactor at approximately neutral
333 pH. The removal efficiency was affected by the nature of the aqueous matrix.
- 334 • Mass spectrometry analyses showed a significant decrease of benzoylecgonine and subsequent
335 increase in transformation products. The potential toxicity of these compounds is as yet unknown.

336

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340

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Water matrix	pH	Conductivity (µS)	TOC (ppm)	Nitrates (mg/L)	Nitrites (mg/L)
MilliQ	6.0	2.5	0.248	1.0	0
SWW	6.0	80	20.79	1.0	0
RWW	7.6	960	43.25	52.4	0,17
SurW	7.8	930	46.37	25.8	0,04

Tab. 1. Measured parameters of different aqueous matrices. MilliQ (milliQ water); SWW (synthetic wastewater); RWW (real wastewater); SurW (surface water).

Fig. 1. Molecular structures of cocaine and benzoylecgonine.

black and white in print is required.

Fig. 2.

Fig. 2a. Extinction coefficient of BE, nitrate and hydrogen peroxide in milliQ water and spectra irradiance of the UV₂₅₄ lamp.

Fig. 2b. Absorbance of the different aqueous matrices.

Black and white in print is required

Fig. 3. Removal degree of BE through direct photolysis process. Effect of aqueous matrix. SWW (Synthetic Waste Water) – RWW (Real Waste Water) – SurW (Surface Water). (MilliQ Water, pH = 6.0, data from Russo et al., 2015).

Black and white in print is required.

Fig. 4. Removal degree of BE through UV_{254}/H_2O_2 process in milliQ water: effect of $[H_2O_2]_0/[BE]_0$ ratio at pH = 6.0.

Black and white in print is required.

Fig. 5. BE removal degree in different aqueous matrices at various $[\text{H}_2\text{O}_2]_0 / [\text{BE}]_0$ ratios and contact time during UV/ H_2O_2 treatment.

Color in print is required.

Fig. 6. Extracted ion LC-MS chromatograms for: A) TP 305a, TP 305b, and TP 305c; and B) TP 321, resulting from UV/H₂O₂ reactions with BE (~ 0.5 s reaction time, 40% degradation of BE).

Color in print is required

Fig. 7. Product ion MS/MS spectrum of m/z 306.1343 ($(M+H)^+$ ion of TP 305) with proposed fragmentation sites.

Color in print is required.

Supplementary data (figure and tables)

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