Título artículo / Títol article:	Investigation of degradation products of cocaine and benzoylecgonine in the aquatic environment
Autores / Autors	Bijlsma, Lubertus ; Boix Sales, Clara ; Niessen, Wilfried M.A. ; Ibáñez Martínez, María ; Sancho Llopis, Juan Vicente ; Hernández Hernández, Félix
Revista:	Science of The Total Environment Volume 443, 15 January 2013
Versión / Versió:	Preprint de l'autor
Cita bibliográfica / Cita bibliogràfica (ISO 690):	BIJLSMA, Lubertus, et al. Investigation of degradation products of cocaine and benzoylecgonine in the aquatic environment. Science of the Total Environment, 2013, 443: 200-208.
url Repositori UJI:	http://hdl.handle.net/10234/83146

Investigation of degradation products of cocaine and benzoylecgonine in the aquatic environment

Lubertus Bijlsma[a], Clara Boix[a], Wilfried M.A. Niessen[b], María Ibáñez[a], Juan V. Sancho[a], Félix Hernández[a]*

(a) Research Institute for Pesticides and Water, University Jaume I, Avda. Sos Baynat, E-12071 Castellón, Spain.

(b) hyphen MassSpec, Leiden, the Netherlands

** Corresponding author* felix.hernandez@uji.es, *Tel +34 964 387366, Fax +34 964 387368*

ABSTRACT

In this work, ultra-high-performance liquid chromatography (UHPLC) coupled to a hybrid quadrupole time-of-flight mass spectrometer (QTOF MS) has allowed the discovery and elucidation of degradation products of cocaine and its main metabolite benzoylecgonine (BE) in water. Spiked surface water was subjected to hydrolysis, chlorination and photo-degradation (both ultraviolet irradiation and simulated sunlight). After degradation of cocaine, up to sixteen compounds were detected and tentatively identified (1 resulting from hydrolysis; 8 from chlorination; 7 from photo-degradation), three of which are well known cocaine metabolites (BE, norbenzoylecgonine and norcocaine). Regarding BE degradation, up to ten compounds were found (3 from chlorination; 7 from photo-degradation), including one known metabolite (norbenzoylecgonine). Since reference standards were available for the major metabolites, they could be confirmed using information on retention time and fragment ions. The other degradates resulted from chlorination, dealkylation, hydroxylation and nitration, or from a combination of these processes. Several influent and effluent sewage water, and surface water samples were then screened for the identified compounds (known and unknown) using UHPLC-tandem MS with triple quadrupole. BE, norcocaine and norbenzoylecgonine were identified in these samples as major metabolites. Four previously unreported degradates were also found in some of the samples under study, illustrating the usefulness and applicability of the degradation experiments performed in this work.

Keywords

Cocaine, degradation and transformation products, water, time-of-flight mass spectrometry.

1. INTRODUCTION

Cocaine use has increased during the last decade and is the illicit drug with the second-highest consumption in Europe, behind only cannabis (EMCDDA 2010). After consumption and excretion, cocaine enters the sewage treatment plants (STPs) as the parent drug or as human metabolites (mainly benzoylecgonine (BE)) and may end up in the receiving surface waters as a consequence of incomplete elimination in the STPs. In most studies, if the presence of cocaine in the aquatic environment is reported, only the parent compound and a few relevant metabolites, commonly BE and cocaethylene or ecgonine methyl ester are included (Baker and Kasprzyk-Hordern 2011). Occasionally, in monitoring studies dealing with sewage- and surface water, some minor metabolites have been found, such as norBE and norcocaine (e.g. Chiaia et al. 2008; Zuccato et al. 2008; Bijlsma et al. 2009; Bisceglia et al. 2010). Although concentrations reported in surface water are generally low (i.e. 7 – 60 ng/L for cocaine and 15 – 191 ng/L for BE (Huerta-Fontela et al. 2008; Gheorghe et al. 2008)), there is a potential negative impact of their presence in the aquatic ecosystem (Binelli et al. 2012). Especially, the effects of combined exposure to multiple compounds are of potential concern.

In order to evaluate the hazard in the water cycle, not only removal of the parent compounds and metabolites in the treatment processes must be taken into account, but also the possible formation of degradation/transformation products (TPs). In some countries (e.g. Italy), chlorination is progressively abandoned because of its potential for generating unwanted TPs and replaced by UV irradiation (Antonelli et al. 2008). Furthermore, after incomplete elimination during chlorination (Huerta-Fontela et al. 2008; Boleda et al. 2009), cocaine and BE which ended up in surface water may be exposed to natural sunlight and produce photo-degradation products. The same would occur for cocaine and BE still present in treated wastewater when no tertiary treatment is applied in the STP (e.g. Gheorghe et al. 2008; Huerta-Fontela et al. 2008; Bijlsma et al. 2009; Bisceglia et al. 2010). Despite the fact that some TPs are more persistent or might exhibit similar toxicity than their parent compounds (Farré et al. 2008; Kern et al. 2009; Fatta-Kassinos et al. 2011; Metz et al. 2011), the research on TPs of illicit drugs has received little attention. Nevertheless, investigation of TPs is of importance to know the overall contribution of chemicals in the environment. Information on potential TPs that may be present in the environment can be used to set-up monitoring studies in order to get a wider and more realistic view on the impact of cocaine on the aquatic environment.

The identification of TPs in the aquatic environment, especially unknown ones, is a challenging task for analytical chemists and commonly various techniques and/or analytical reference standards are necessary for a reliable confirmation (Wick et al. 2011). An important analytical tool in the elucidation of TPs is high resolution mass spectrometry (HRMS), with analyzers like Orbitrap and time-of-flight (TOF). The accurate mass full-spectrum acquisition and the possibility to obtain fragment ions by coupling HRMS to ion trap or quadrupole analyzers is highly suitable and helpful for the proposal of convincing molecular structures (Ibañez et al. 2004; Farré et al. 2008; Quintana et al. 2010; Metz et al. 2011).

Laboratory degradation experiments in combination with HRMS are one of the most useful tools to identify TPs that can be formed in the aquatic environment. They have been applied mainly to elucidate pesticide and pharmaceutical TPs formed in water (Ibañez et al. 2004; Hernández et al. 2008; Quintana et al. 2010; Wick et al. 2011). Treatment conditions applied by STPs, *e.g.* chlorination and UV irradiation, can be simulated, as well as natural sunlight. The most important TPs identified can subsequently be included in multi-residue LC tandem MS methods with triple quadrupole. This has allowed the detection of parent compounds and of their related TPs in sewage-, surface- and/or drinking water (Hernández et al. 2008; Quintana et al. 2010; Wick et al. 2011), and illustrates the importance of investigating TPs.

The use of MS^E is an attractive option, which is feasible working with hybrid QTOF MS instruments. Using this approach, information on both (de)protonated molecules and their fragment ions is acquired simultaneously in a single injection (Hernández et al. 2011). The accurate mass measurement of the (de)protonated molecule generally allows the assignment of a highly probable molecular formula. Subsequently, fragment ions as well as neutral losses can be investigated in order to elucidate the structure of the TPs detected. Available software for the detection of metabolites and TPs are usually offered by MS manufacturers. They compare and contrast data of a presumptive positive sample with a control or blank sample. This facilitates data processing and might even detect (low abundant) compounds overlooked by visual inspection.

The objective in this paper was to perform a study on TPs of cocaine and BE that might be found in the aquatic environment. Several laboratory controlled degradation experiments (i.e. hydrolysis, chlorination, and photo-degradation under ultraviolet (UV) irradiation and simulated sunlight) have been carried out and the TPs formed investigated by LC-QTOF under MS^E mode. To the best of our knowledge, several unknown TPs reported in

this study have not previously described in the literature. In a subsequent step, influent and effluent sewage water, and also surface waters, were searched for the identified TPs.

2. MATERIALS AND METHODS

2.1. Reagents and chemicals

Cocaine, norcocaine, BE and norbenzoylecgonine (norBE) reference standards were purchased from the National Measurement Institute (Pymble, Australia) and Cerilliant (Round Rock, TX, USA). Standard solutions of cocaine and BE were prepared at 500 mg/L in acetonitrile (ACN) and methanol (MeOH), respectively. Intermediate work solutions (50 mg/L) were made by diluting the solution ten times with MeOH.

HPLC-grade MeOH, ACN and formic acid (FA) were acquired from Scharlau (Barcelona, Spain). Sodium hypochlorite solution (available chlorine 10%) was obtained from Sigma-Aldrich. A Milli-Q ultra-pure water system from Millipore (Bedford, MA, USA) was used to obtain the HPLC grade water. Leucine enkephalin and imazalil were purchased from Sigma-Aldrich and Dr. Ehrenstorfer (Augsburg, Germany), respectively.

Solid-phase extraction (SPE) cartridges (Oasis-HLB; 3mL, 60 mg) were purchased from Waters (Milford, MA, USA). Prior to use, the SPE cartridges were conditioned by washing and rinsing with 3 mL of MeOH and 3 mL of Milli-Q water.

2.2. Degradation experiments

Blank surface water from the Mijares River (Castellón, Spain) was collected in November 2010 and used for all laboratory controlled experiments. Surface water (pH 8.1) was selected in order to simulate reality, as it contains matrix components which may affect degradation.

Surface water used for hydrolysis, chlorination and photo-degradation experiments was spiked with cocaine or BE at a concentration of 0.5 mg/L. This relatively high concentration allowed better evaluation of degradation products, and especially facilitated the detection of minor TPs. Non-spiked surface water samples were subjected to the same degradation processes and used as control samples.

The hydrolysis and chlorination experiments were performed at room temperature and in darkness. Regarding chlorination, 40 µL of ten-fold diluted sodium hypochlorite solution was added to 50 mL of each surface water sample. During the experiment, 2 mL aliquots of the water sample were collected at several time intervals (0, 30 min, 1, 3, 10 h, 1, 3, 7, 11 and

15 days for hydrolysis; and 0, 30 min, 1 and 3 hours for chlorination), after stirring of the water solutions, and were immediately stored at –20 ºC until analysis.

Photo-degradation experiments were carried out under UV irradiation and simulated sunlight. UV irradiation was performed using a mercury lamp with its main output at 254 nm. 250 mL surface water samples were kept in quartz glass vessels at a distance of ~15 cm from the lamp. The experiment was carried out in a fume hood at room temperature over a period of 72 h under constant stirring of the samples. Sunlight was simulated using a solar simulation system (Suntest XLS+, Atlas MTT, Linsengericht, Germany), equipped with a xenon arc lamp as radiation source and a solar light filter allowing a wavelength in the range of 300 - 800 nm. The radiation intensity was set to 500 W/m^2 and the light dose per hour of irradiation to 1.8 MJ/h. In this way, 90 irradiation hours corresponds to 15 days of natural sun light (dose: 288 MJ/m^2). The degradation was performed using 250 mL closed quartz glass vessels and sample temperature was set to 25ºC in order to minimize sample evaporation and possible thermal transformation. Aliquots were sampled after stirring of the water solution. The first 2 mL water aliquots were analysed, prior to the irradiation experiments (t = 0). During irradiation experiments, 2 mL water samples were taken at different time intervals (see hydrolysis experiment), and immediately stored at –20 ºC until analysis.

2.3. Instrumentation

For identification and elucidation of TPs, a Waters Acquity ultra-high-performance liquid chromatography (UHPLC) system was interfaced to a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-TOF Premier, Waters Micromass) equipped with an orthogonal Z-spray electrospray ionization interface (ESI) operating in both positive-ion and negative-ion mode and controlled by MassLynx v 4.1 software. Leucine enkephalin was used as the lock mass (*m/z* 556.2771 in positive-ion and *m/z* 554.2615 in negative-ion mode) ensuring typically mass errors below 2 mDa.

The UHPLC separation was performed using an Acquity UPLC BEH C18, 1.7 µm particle size analytical column, 100 mm × 2.1 mm (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.

For MS^E experiments, two acquisition functions were created and simultaneously used within the same run: the low-energy function (LE) with a collision energy of 4 eV, where mainly the (de) protonated intact molecules are observed, and the high energy (HE) function

with a collision energy ramp ranging from 15 to 40 eV, where fragmentation is promoted. The same collision energy ramp was used for additional MS/MS experiments. The optimized cone voltage (15 V) and collision energy ramp were identical for both cocaine and BE and seemed therefore most adequate for the screening of their corresponding degradation products. Further details on instrument operating conditions can be found elsewhere (Hernández et al. 2011).

For screening of TPs in sewage waters, a TQD triple quadrupole (QqQ) mass spectrometer with electrospray ionization source (Waters) was used. Chromatographic separation was performed using the same analytical column and gradient as used in UHPLC-QTOF analysis. The analysis of surface waters was performed under similar conditions using the TQS (QqQ) mass spectrometer (Waters).

2.4. Elucidation / identification procedure

Waters MetaboLynx software (an application manager within MassLynx) was used to compare accurate mass data of spiked and blank (non-spiked) samples from the laboratory experiments.

The data comparison by MetaboLynx was performed in two ways. First, for expected TPs, (bio)transformation processes reported in the literature were included in the processing settings. These consisted of a mass window ± 10 mDa for extracted ion chromatograms (XICs) of each specific exact mass; peaks with less than 10 area units were eliminated. Second, searching for unexpected TPs was performed by mass spectral comparison of non-spiked *versus* spiked samples. XICs were automatically generated for each sample (spiked and non-spiked) over a range from *m/z* 70 to 550 Da, at 1 Da mass window, and compared.

The most likely elemental compositions of (de)protonated molecules were calculated based on accurate mass LE spectra of the peaks of interest. The accurate mass HE spectra were then used to calculate possible elemental compositions of fragment ions. Assuming that most TPs share similar fragmentation pathways with the parent drug (Wang and Bartlett 1998; Bijlsma et al. 2011), fragmentation was compared to that of cocaine and BE, and the TP structures were proposed.

2.5. Water samples

Five influent and five effluent sewage water samples (24-hour composite) and five surface water grab samples from different locations of the Comunidad Valenciana (Eastern Spain) were collected and immediately stored at –20 ºC. Sewage water was collected from

205 STPs of Castellón and Benicàssim, while surface water was collected from the Albufera
206 national park of Valencia.
207 100 mL of five-fold diluted (with MilliQ) influent wastewater, 100 mL of effluent
208 wastewater or 100 mL surface water was taken for analysis. The samples were loaded onto
209 the HLB cartridges by gravity, and then cartridges were vacuum-dried for 10 min. Analytes
210 were eluted with 5 mL of MeOH. The extracts were evaporated to dryness at 35ºC under a
211 gentle stream of nitrogen and reconstructed in 1 mL of 10:90 MeOH:H_2O. Analyses of
212 cocaine and BE TPs were performed by injecting 20 µL of the final extract into the UHPLC-
213 TQD system (sewage water) or 100 µL in the UHPLC-TQS system (surface water).
214

3. RESULTS AND DISCUSSION

Many known TPs of environmental contaminants share similar fragmentation pathways as their parent molecules. Then, knowledge of structures of fragment ions and basic fragmentation rules are helpful for achieving confident TP structure proposals. Isotope fit, Double Bound Equivalent (DBE), and accurate mass of fragments observed in the HE function were used to discard potential chemical formulas in order to obtain the most plausible structures of TPs.

The fragmentation of cocaine and BE has been studied previously by our own group (Bijlsma et al. 2011) and by others (Wang and Bartlett 1998). This has facilitated the elucidation of some of the TPs found in this work. The most abundant fragment ions in the mass spectra of both compounds are *m/z* 105 ($C_6H_5CO^+$) and a fragment due to the neutral loss of benzoic acid (122 Da). Subsequent fragmentation of the resulting ion $[M+H - 122]^+$ can produce fragments with *m/z* 150, 122, 119, 108, 91 and 82, involving a further loss of methanol or water or elimination of part of the bicyclic ring system, followed by hydrogen rearrangement.

Proposed structures for the TPs found in this work are shown in **Figure 1**.

3.1. Hydrolysis

Gheorge et al. (2008) performed a detailed study on the stability of BE and cocaine in surface and wastewater, testing at different temperatures and pH values in order to establish optimal conditions for sample storage. Degradation of cocaine was minimal at -20 °C and pH 2. However, in our study, realistic environmental conditions were chosen for the experiments without any adjustment of pH and temperature. It is therefore likely that besides hydrolysis, potential biodegradation might also occur. To some extent, these processes may yield the same products.

Complete cocaine and some BE degradation was observed in surface water after keeping the solution in darkness at room temperature for 15 days (data not shown). Cocaine was mainly transformed into BE through chemical hydrolysis of cocaine ester bonds. Ecgonine methyl ester (EME), another hydrolytic product reported for cocaine (Postigo et al. 2011), was not observed. EME is presumed to be solely an *in vivo* metabolite as a result of enzymatic hydrolysis and for that reason it is unlikely to be formed during cocaine degradation in water (Klette et al. 2000). Gheorghe et al. (2008) had similar results to the

248 present work, where cocaine and EME degraded in spiked surface water, while BE initially
249 increased owing to the possible chemical hydrolysis of cocaine.
250
251 **3.2. Chlorination**
252 **Table 1** summarizes the TPs of cocaine and BE formed during chlorination. Retention
253 times and experimental *m/z*-values, proposed elemental composition of the protonated TPs
254 and their fragment ions, the mass error in mDa, and the double bond equivalent (DBE) are
255 given.
256 Chlorination TPs of cocaine and BE were investigated under the experimental
257 conditions described in section 2.2. High chlorine concentration (8 mg/L) was used, similar to
258 the conditions employed by STPs for wastewater treatment. In previous studies on acidic
259 pharmaceutical TPs, ascorbic acid was found to be an effective quenching agent to prevent
260 further degradation with chlorine (Quintana et al. 2010). However, in the present study, we
261 observed that it affected the stability of some TPs (the monochlorinated TP-C4, -C7 and -C8
262 were no longer observed after adding ascorbic acid to the sample vials). Therefore, we did not
263 use ascorbic acid addition in our experiments. The sample aliquots taken at different times
264 were frozen, stored and thawed just before analysis. In any case, quenching chlorination
265 seemed not much important in this case, as a fast degradation of cocaine and BE occurred
266 (after 30 minutes neither cocaine nor BE was observed in sample aliquots analyzed).
267 The simultaneous acquisition of accurate mass LE and HE spectra and useful isotopic
268 pattern information (distribution of the ^{37}Cl isotope) obtained in the MS^E mode, allowed the
269 detection and tentative identification of several TPs, in a single injection. Among these TPs,
270 some well-known cocaine metabolites, BE (TP-C1), norBE (TP-C2), and norcocaine (TP-C3),
271 were identified and subsequently confirmed by using reference standards. All TPs were
272 determined and identified in the positive-ion mode. Besides the protonated molecules
273 $[M+H]^+$, their sodium adducts $[M+Na]^+$ were also observed surely owing to the presence of
274 sodium in NaClO. Some TPs that contain a carboxylic group could also be analyzed in
275 negative-ion mode; however, analysis under negative mode did not reveal additional TPs to
276 those observed in positive mode.
277 TP-C4 ($C_9H_{15}ClNO_3^+$, *m/z* 220.0740), with an abundant peak at 4.81 min, may be
278 generated via benzoylester cleavage and chlorination. Initial fragmentation involves losses of
279 water and methanol (to ions with *m/z* 202 and 188, respectively), suggesting that this TP is a
280 secondary product from TP-C3 (norcocaine).

Chlorination of cocaine yielded an intense peak at 8.27 min with $[M+H]^+$ *m/z* 324.1003, named as TP-C8 ($C_{16}H_{19}ClNO_4^+$), corresponding to demethylation of the bridgehead nitrogen and consecutive halogenation (**Figure S1A**). The fragmentation of this TP was comparable to cocaine and its metabolites, where the most abundant fragments ions are *m/z* 105 ($C_6H_5CO^+$) and *m/z* 202 (loss of benzoic acid, 122 Da) (Wang and Bartlett 1998). Secondary fragmentation of the ion *m/z* 202 ($[M+H–122]^+$) involves the loss of either HCl or CH_3OH to ions *m/z* 166 and 170, respectively, the later indicating that initially *N*-demethylation rather than *O*-demethylation occurred. The complete fragmentation pathway for TP-C8 is proposed in **Figure S1B**. The characteristic chlorine isotopic pattern confirms the presence of Cl in the fragment ions with *m/z* 202, 170 and 142, whereas it is absent in the ions with *m/z* 288, 166, 134 and 105.

Another TP of cocaine, TP-C6 ($[M+H]^+$, *m/z* 318.1336) is has the same nominal mass as cocaethylene ($C_{18}H_{24}NO_4^+$, *m/z* 318.1705), but they could be differentiated both chromatographically and by HRMS, as a difference of 36.9 mDa was observed. The most likely molecular formula for TP-C6 is $C_{17}H_{20}NO_5^+$ (*m/z* 318.1341, Δ 0.5 mDa). Thus, TP-C6 would result from oxidation (+O–2H) of cocaine during chlorination experiments, which probably occurs on the bicyclic ring system, since the characteristic fragment ion with *m/z* 105 ($C_6H_5CO^+$) is still present.

Chlorination of BE resulted in TP-C5 ($[M+H]^+$, *m/z* 304.1185) and TP-C7 ($C_{15}H_{17}ClNO_4^+$, *m/z* 310.0846) at retention times of 5.89 min and 7.53 min, respectively (**Table 1**). These compounds show similar fragmentation pathways to TP-C6 and TP-C8, respectively, although with an expected mass shift of -14 in several of the *m/z* values. These TPs were also observed after cocaine chlorination, where BE probably acted as an intermediate.

The most abundant TPs formed after cocaine chlorination corresponded to TP-C8 and TP-C1 (BE), whereas TP-C5 could be considered as minor TP. The abundance of TP-C2 (norBE), solely formed after chlorination of BE, was in the same order of magnitude as TP-C3 (norcocaine),-C4,-C6 and -C7.

The data obtained was not sufficient to predict the exact position of the chlorine or keto group in the unknown TPs (from C4 to C8). The combination of several spectroscopic techniques, such as further analysis by nuclear magnetic resonance (NMR), would be required to definitely elucidate the molecular structure of these compounds. Nevertheless, the information obtained in this study regarding the elemental composition of protonated TPs and their fragment ions will allow screening of these compounds in future monitoring studies.

This is of interest to have more realistic and complete information, as these TPs are not included in environmental studies related with the presence of cocaine.

3.3. Photo-degradation

Photo-degradation of cocaine and BE in aqueous solution under simulated sunlight and/or UV irradiation resulted in eight TPs (**Figure 1**) including two known metabolites: BE (TP-P4) and norBE (TP-P5). The TPs and the data obtained from the QTOF experiments are summarized in **Table 2.** Initially, TP-P2 isomers and TP-P4 were also generated after UV irradiation, but these TPs were effectively removed after 3 and 8 hours, respectively.

TP-P1 ($C_9H_{14}NO_2^+$, *m/z* 168.1025), with an abundant peak at 1.93 min, was generated under simulated sunlight of BE. TP-P1 may be produced via cleavage of the benzoyl ester bond, reduction of the carboxylic acid group and dehydrogenation. Its fragmentation under HE acquisition mode involved the loss of water (*m/z* 150) and subsequent loss of formaldehyde (*m/z* 120) and of the bridgehead nitrogen (*m/z* 93). The fact that there is no loss of both H_2O and CO indicates the absence of a carboxylic acid function in this molecule. No confirmative position of the double-bond could be given. However most likely it would be located in the part away from the hydroxyl and aldehyde group.

After photo-degradation of both cocaine and BE, three isomeric products named as TP-P2 ($C_{16}H_{20}NO_5^+$, *m/z* 306.1341) were detected at retention times of 2.78, 3.07 and 3.91 min (**Figure 2A, left**). They would correspond to a hydroxylation product of BE. During photo-degradation of cocaine, BE was formed and readily transformed afterwards acting as a photo-intermediate (**Figure 3**). In MS^E, the three TP-P2 isomers showed similar fragmentation with fragment ions *m/z* 168 (loss of 138 Da, i.e., benzoic acid+O) and *m/z* 121 ($C_7H_5O_2^+$, corresponding to $C_6H_5CO^+$ + O, *m/z* 105+16), indicating that hydroxylation occurs at the phenyl ring. Accordingly, the TP-P2 isomers were presumably *ortho*-, *meta*- and *para*-hydroxy-BE. *In vivo*, cocaine metabolizes to BE and then to norBE and/or to *meta*-hydroxyl-BE and *para*-hydroxyl-BE (Klette et al. 2000). The suggested hydroxylation products here are similar to the monohydroxylated cocaine products generated by hydrogen peroxide treatment (Tanaka et al. 2002), as a result of solar photo-degradation using a catalyst (titanium dioxide, TiO_2), or by a photo-Fenton reaction (Postigo et al. 2011). In our work, the three isomers seemed to be formed in the photo-degradation experiments as the XIC at the $[M+H]^+$ exact mass (*m/z* 306.1341) revealed. All the three isomers gave fragment ions with *m/z* 168, 150 and 121, whereas only two compounds generated the ion with *m/z* 186 (**Figure 2A**). This is probably due to the fact that the loss of 120 Da ($C_7H_4O_2$) results in resonance-stabilized

neutrals only for the *ortho*- and *para*-analogues (**Figure 2B**). Thus, the peak at 3.07 min should be the *meta*-hydroxy-BE. Combining this information with literature data on the elution order of *para*- and *meta*-hydroxy-BE (Pichini et al. 2005; Bisceglia et al. 2010), one can conclude that the isomer at 2.78 min is the *para*- isomer and the one with 3.90 min the *ortho*- isomer.

NorBE (named as TP-P5 in this section) co-eluted with one of the hydoxylated derivatives (Rt = 3.96 and 3.91, respectively) and as a consequence overlapping spectra were obtained in MS^E. In this case, additional product ion MS/MS experiments were performed to obtain "clean" accurate mass spectra of both compounds to confirm their identities.

The accurate mass of two other, less abundant but interesting, unknown isomeric TPs with retention times 2.94 and 3.54 min was determined to be *m/z* 351.1180 (TP-P3) (**Figure S2**). The most likely molecular formula is $C_{16}H_{18}N_2O_7$ (mass error –1.2 mDa). Therefore, TP-P3 is suggested to be generated via hydroxylation (+ OH) and nitration (+ NO_2) of BE. The incorporation of a NO_2 group is feasible, since the photo-degradation experiments were carried out using surface water of the Mijares River (Castellón province) , where relatively high nitrate concentrations (around 10 mg/L) are normally present owing to the wide use of fertilizers in this agricultural area (Hernández et al. 2008). The presence of the common fragment ions with *m/z* 168, 150, 119 and 82, indicated that hydroxylation and nitration did not take place on the bicyclic ring system, but on the phenyl ring. This could be confirmed by the presence of a major fragment ion with *m/z* 166.0140 ($C_7H_4NO_4^+$) corresponding to $C_6H_5CO^+ + OH + NO_2 – H_2$ (*m/z* 105 + 17 + 46 – 2). The positions of the NO_2 and OH group could not be definitively determined. From a structural point of view, one might expect more than two chromatographic peaks, since there are various possible combinations regarding the positions of NO_2 and OH. Supposedly, hydroxylation takes place first, because a possible TP corresponding to the nitration of BE ($C_{16}H_{20}NO_4^+ + NO_2$) with *m/z* 335 was not observed, whereas hydroxyl-BE was in fact found, as previously discussed. Based on the effect of a hydroxyl-group on electrophilic substitution, the entrance of NO_2 is probably *ortho*- and *para*- orientated (Morrison and Boyd 1992). Together with the three possible hydroxy-BE structures, this would result in six conceivable combinations (four *ortho*- and two *para*-orientated). Nevertheless, only two chromatographic peaks were observed at 2.94 and 3.54 min (**Figure S2**). Possibly, the small differences in polarity allowed co-elution of the *ortho*- and of the *para*- orientated isomers. Owing to interaction via intra-molecular H-bonding of the neighbouring -NO_2 and -OH groups in the *ortho*- position the overall polarity of the molecule decreases. As an example, *o*-nitrophenol is retained stronger than *p*-nitrophenol

using a reversed-phase analytical column (Masqué et al. 2000). The presence of nitrated derivates indicates influence of the matrix on the degradation of the parent compound.

The use of UHPLC allowed decreasing analysis time with excellent chromatographic resolution. These characteristics are important in terms of sample throughput, separation efficiency and sensitivity (Wilson et al. 2005). In **Figure 2** and **Figure S2**, XICs of common fragments show several chromatographic peaks resulting from different TPs. The chromatographic separation of these TPs was important in order to avoid overlapping of spectra acquired using the MS^E approach and to facilitate a reliable identification. Furthermore, the inherent increased sensitivity favoured the detection of less abundant TPs.

3.4. Screening of water samples

Screening of cocaine TPs has been performed in several sewage and surface water samples, including the highest number of TPs reported until now. To this aim an UHPLC-MS/MS (QqQ) system was used for the screening of the above suggested TPs in the water samples. This technique is especially suited for target screening in complex matrices as high sensitivity can be achieved in selected reaction monitoring (SRM) mode. SPE was applied to five influent, five effluent sewage waters and five surface waters in order to pre-concentrate and clean-up the samples (see Section 2.5). Hydrophilic and lipophilic balanced (HLB) cartridges were selected, which demonstrated good efficiency for cocaine, BE and other drugs, pharmaceuticals and metabolites with different physical and chemical characteristics (Gheorghe et al. 2008; Baker and Kasprzyk-Hordern 2011; Gracia-Lor et al. 2011). The precursor and product ions, i.e. the MS/MS transitions acquired, were selected (**Table 3**) on the basis of the main ions observed in previous QTOF MS analysis performed along the degradation experiments. A more sensitive QqQ analyzer (i.e. TQS) was used for surface water analysis, due to the lower concentrations expected in comparison with sewage water.

Besides the known metabolites (norcocaine, BE and norBE), TPs of cocaine and BE have been detected, for the first time, in water samples. TP-P1 was found in one influent, four effluents and four surface waters, and TP-P2 isomers were present in four influent and two effluent sewage waters and four surface waters. The TPs had been elucidated after photo-degradation of cocaine and BE by simulated sunlight and/or UV irradiation. Therefore, their presence in influents might be noticed as remarkable, as influent sewage water is normally not exposed to sunlight or UV irradiation. As previously discussed, TP-P2 isomers are suggested to be *ortho*-, *meta*- and *para*-hydroxy-BE. *Meta*-hydroxy-BE and *para*-hydroxy-BE have been reported as *in vivo* metabolites and might therefore be present in influent sewage water

as a consequence of excretion. Nevertheless, *ortho*-hydroxy-BE and TP-P1 were also present in influents. Thus, other processes (e.g. bacterial decomposition) in the sewage system might occur and also play a role in their formation. The presence of TP-P1 and TP-P2 in effluent could be caused by incomplete elimination in the STP or degradation of cocaine and BE during treatment. Furthermore, these TPs were not only found for the first time in influent and effluent sewage waters, but also in surface waters. TPs might enter surface waters by releasing sewage effluents or be formed by photo-degradation via natural sunlight. **Figure 4** shows a positive finding of TP-P1 and TP-P2 isomers in effluent sewage water and in surface water. Although their reference standards were not available, the fact that all the three SRM transitions acquired and that relative retention times to BE (RT_{-TP}/RT_{-BE}) were in good agreement (< 0.01 min) with the TPs identified in degradation experiments give reliability to these findings.

In future studies, additional degradation experiments should be performed in wastewaters in order to address the presence of some of the identified TPs in influents analyzed. Moreover, reference standards of the discovered TPs are required in order to report concentration levels. Subsequently, extended monitoring studies should be set up analyzing paired wastewater and receiving surface waters. This will give more insight in the environmental fate of cocaine, its metabolites and TPs.

4. CONCLUSIONS

Data on the presence of TPs of organic contaminants in the aquatic environment are required nowadays to have a realistic overview of water quality. UHPLC-QTOF MS has been demonstrated in this work as a valuable tool for the identification of TPs of cocaine and its main metabolite BE in water. After laboratory-controlled hydrolysis, chlorination and photo-degradation experiments, the structures of several TPs have been tentatively established. The applicability of these studies has been demonstrated by analyses of sewage water (influent and effluent) and surface water where, in addition to well-known cocaine metabolites, other TPs identified in laboratory experiments have been found and reported for the first time. The relevance of TPs should not be neglected, as it might be necessary to take them into account in future monitoring studies. Other knowledge gaps, such as ecotoxicity and the effects of multiple compound exposure, need to be adressed in order to perform well-founded environmental risk assessment. This implies that lot of research is still required by environmental scientists and analytical chemists, especially when dealing with emerging contaminants.

ACKNOWLEDGEMENTS

The authors are very grateful to the Serveis Centrals d'Instrumentació Científica (SCIC) of University Jaume I for using the mass spectrometers.

This work has been developed under financial support provided by the University Jaume I-Fundación Bancaixa (Project reference: P1 1B2007-13) and the Spanish Ministry of Education and Science (Projects reference: CTQ2009-12347). The authors also acknowledge the financial support of Generalitat Valenciana, as research group of excellence PROMETEO/2009/054.

SUPPLEMENTARY DATA

In this section, two figures, one reporting the identification of cocaine chlorination TP-C8 (Figure S1) and the other including the identification of cocaine photo-degradation TP-P3 (Figure S2), are included to have supportive visual information on the written text.

REFERENCES

Antonelli M, Mezzanotte V, Nurizzo C. Wastewater disinfection by UV irradiation: short long term-efficiency. Environ Eng Sci 2008; 25: 363-373

Baker DR, Kasprzyk-Hordern B. Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography-mass spectrometry. J Chromatogr A 2011; 1218: 8036-8059

Bijlsma L, Sancho JV, Hernández F, Niessen WMA Fragmentation pathways of drugs of abuse and their metabolites based on QTOF MS/MS and MS^E accurate-mass spectra. J Mass Spectrom 2011; 46: 865 – 875

Bijlsma L, Sancho JV, Pitarch E, Ibáñez M, Hernández F. Simultaneous ultra-high-pressure liquid chromatography-tandem mass spectrometry determination of amphetamine and amphetamine-like stimulants, cocaine and its metabolites, and a cannabis metabolite in surface water and urban wastewater. J Chromatogr A 2009; 1216: 3078-3089

Binelli A, Pedriali A, Riva C, Parolini M. Illicit drugs as new environmental pollutants: Cyto-genotoxic effects of cocaine on the biological model *Dreissena polymorpha*. Chemosphere 2012; 86: 906-911

Bisceglia KJ, Roberts AL, Schantz MM, Lippa KA. Quantification of drugs of abuse in municipal wastewater via SPE and direct injection liquid chromatography mass spectrometry. Anal Bioanal Chem 2010; 398: 2701-2712

Boleda MR, Galceran MT, Ventura F. Behavior of pharmaceuticals and drugs of abuse in a drinking water treatment plant (DWTP) using combined conventional and ultrafiltration and reverse osmosis (UF/RO) treatments. Environ Pollut 2011; 159: 1584 - 1591

Chiaia AC, Banta-Green C, Field J. Eliminating solid phase extraction with large-volume injection LC/MS/MS: analysis of illicit and legal drugs and human urine indicators in US wastewater. Environ Sci Technol 2008; 42: 8841-8848

European Monitoring Centre for Drugs and Drug Addiction. The state of the drugs problem in Europe. EMCDDA Annual Report 2010. Available from URL: http://www.emcdda.europa.eu/ publications/annual-report/2010. Accessed 10 July 2012

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

Fatta-Kassinos D, Meric S, Nikolaou A. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. Anal Bioanal Chem 2011; 399: 251-257

Gheorghe A, van Nuijs A, Pecceu B, Bervoets L, Jorgens PG, Blust R, Neels H, Covaci A. Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography-ion trap tandem mass spectrometry. Anal Bioanal Chem 2008; 391: 1309-1319

Gracia-Lor E, Sancho JV, Hernández F. Multi-class determination of around 50 pharmaceuticals, including 26 antibiotics, in environmental and wastewater samples by ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr A 2011; 1218: 2264-2275

Hernández F, Bijlsma L, Sancho JV, Díaz R, Ibáñez M. Rapid wide-scope screening of drugs of abuse, prescription drugs with potential for abuse and their metabolites in influent and effluent urban wastewater by ultrahigh pressure liquid chromatography-quadrupole-time-of-flight-mass spectrometry. Anal Chim Acta 2011; 684: 96-106

Hernández F, Ibáñez M, Pozo OJ, Sancho JV. Investigating the presence of pesticide transformation products in water by liquid chromatography-mass spectrometry with different mass analyzers. J Mass Spectrom 2008; 43: 173-184

Huerta-Fontela M, Galceran MT, Ventura F. Stimulatory drugs of abuse in surface waters and their removal in a conventional drinking water treatment plant. Environ Sci Technol 2008; 42: 6809-6816

Ibañez M, Sancho JV, Pozo OJ, Hernández F. Use of quadrupole time-of-flight mass spectrometry in environmental analysis: elucidation of transformation products of traizine herbicides in water after UV exposure. Anal Chem 2004; 76: 1328-1335

Kern S, Fenner K, Singer HP, Schwarzenbach RP, Hollender J. Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. Environ Sci Technol 2009; 43: 7039-7046

526 Klette KL, Poch GK, Czarny R, Lau CO. Simultaneous GC-MS analysis of meta- and para-
527 hydroxybenzoylecgonine and norbenzoylecgonine: A secondary method to corroborate
528 cocaine ingestion using nonhydrolytic metabolites. J Anal Tox 2000; 24: 482-488

529 Masqué N, Marcé RM, Borull F, Cormack PAG, Sherrington DV. Synthesis and evaluation of
530 a molecularly imprinted polymer for selective on-line solid-phase extraction of 4-
531 nitrophenol from environmental water. Anal Chem 2000; 72: 4122–4126

532 Metz DH, Meyer M, Dotson A, Beerendonk E, Dionysiou DD. The effect of UV/H_2O_2
533 treatment on disinfection by product formation potential under simulated distribution
534 system conditions. Water Res 2011; 45: 3969 - 3980

535 Morrison RT, Boyd RN. Electrophilic aromatic substitution. In: Organic chemistry, 6th ed.
536 Prentice-Hall Inc., Englewood Cliffs (NJ), ISBN: 0-13-643669-2; 1993; p. 517-548

537 Pichini S, Marchei E, Pacifici R, Pellegrini M, Lozano J, García-Algar O. Application of a
538 validated high-performance liquid chromatography-mass spectrometry assay to the
539 analysis of m- and p-hydroxybenzoylecgonine in meconium. J Chromatogr B 2005; 820:
540 151-156

541 Postigo C, Sirtori C, Oller I, Malato S, Maldonado MI, López de Alda M, Barceló D. Solar
542 transformation and photocatalytic treatment of cocaine in water: Kinetics, characterization
543 of major intermediate products and toxicity evaluation. Appl Catal B: Environm 2011;
544 104: 37-48

545 Quintana JB, Rodil R, López-Mahía P, Muniategui-Lorenzo S, Prada-Rodríguez D.
546 Investigating the chlorination of acidic pharmaceuticals and the by-product formation
547 aided by an experimental design methodology. Water Res 2010; 44: 243-255

548 Tanaka S, Lio R, Chinaka S, Takayama N, Hayakawa K. Analysis of reaction products of
549 cocaine and hydrogen peroxide by high-performance liquid chromatography/mass
550 spectrometry. Biomed Chromatogr 2002; 16: 390-394

551 Wang P-P, Bartlett MG. Collision-induced dissociation mass spectra of cocaine, and its
552 metabolites and pyrolysis products. J Mass Spectrom 1998; 33: 961-967

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

556 Wilson ID, Nicholson JK, Castro-Perez J, Granger JH, Johnson KA, Smith BW, Plumb RS
557 High resolution “ultra performance” liquid chromatography coupled to oa-TOF mass
558 spectrometry as a tool for differential metabolic pathway profiling in functional genomic
559 studies. J Proteome Res 2005; 4: 591–598

560 Zuccato E, Castiglioni S, Bagnati R, Chiabrando C, Grassi P, Fanelli R Illicit drugs, a novel
561 group of environmental contaminants. Water Res 2008; 42: 961-968
562

Table 1: Proposed elemental composition of protonated TPs and their fragments ions obtained during chlorination of cocaine and BE, retention time (min), accurate mass (*m/z*), mass error (mDa) and double bond equivalent (DBE).

Compound (area)[a]	Retention time (min)	Accurate mass (*m/z*)	Chemical formulae	Mass Error (mDa)	DBE
TP-C1[b, c]	3.74	290.1400	$C_{16}H_{20}NO_4$	+0.8	7.5
BE		168.0979	$C_9H_{14}NO_2$	-4.6	3.5
(2059)		150.0896	$C_9H_{12}NO$	-2.3	4.5
		119.0478	C_8H_7O	-1.9	5.5
		105.0325	C_7H_5O	-1.5	5.5
		82.0658	C_5H_8N	+0.1	2.5
TP-C2[b]	3.95	276.1229	$C_{15}H_{18}NO_4$	-0.7	7.5
norBE		154.0853	$C_8H_{12}NO_2$	-1.5	3.5
(680)		136.0744	$C_8H_{10}NO$	-1.8	4.5
		105.0332	C_7H_5O	-0.8	5.5
TP-C3[c]	4.31	290.1391	$C_{16}H_{20}NO_4$	-0.1	7.5
norcocaine		168.0998	$C_9H_{14}NO_2$	-2.7	3.5
(491)		136.0750	$C_8H_{10}NO$	-1.2	4.5
		105.0340	C_7H_5O	0.0	5.5
TP-C4[c]	4.81	220.0729	$C_9H_{15}ClNO_3$	-1.1	2.5
(532)		202.0629	$C_9H_{13}ClNO_2$	-0.6	3.5
		188.0475	$C_8H_{11}ClNO_2$	+0.3	3.5
		120.0210	C_4H_7ClNO	-0.6	1.5
		114.0103	C_5H_5ClN	-0.8	3.5
TP-C5[b]	5.89	304.1193	$C_{16}H_{18}NO_5$	+0.8	8.5
(18)		286.1080	$C_{16}H_{16}NO_4$	+0.1	9.5
		182.0823	$C_9H_{12}NO_3$	+0.6	4.5
		154.0855	$C_8H_{12}NO_2$	-1.3	3.5
		136.0740	$C_8H_{10}NO$	-2.2	4.5
		105.0337	C_7H_5O	-0.3	5.5
TP-C6[c]	6.45	318.1336	$C_{17}H_{20}NO_5$	-0.5	8.5
(270)		286.1061	$C_{16}H_{16}NO_4$	-1.8	9.5
		196.0951	$C_{10}H_{14}NO_3$	-2.3	4.5
		168.1002	$C_9H_{14}NO_2$	-2.3	3.5
		136.0743	$C_8H_{10}NO$	-1.9	4.5
		105.0325	C_7H_5O	-1.5	5.5
TP-C7[b]	7.53	310.0837	$C_{15}H_{17}ClNO_4$	-0.9	7.5
(607)		274.1046	$C_{15}H_{16}NO_4$	-3.3	8.5
		188.0493	$C_8H_{11}ClNO_2$	+1.5	3.5
		170.0352	C_8H_9ClNO	-2.1	4.5
		152.0700	$C_8H_{10}NO_2$	-1.2	4.5
		142.0417	C_7H_9ClN	-0.7	3.5
		134.0592	C_8H_8NO	-1.4	5.5
		105.0329	C_7H_5O	-1.1	5.5
TP-C8[c]	8.27	324.0988	$C_{16}H_{19}ClNO_4$	-1.5	7.5
(3765)		288.1216	$C_{16}H_{18}NO_4$	-2.0	8.5
		202.0598	$C_9H_{13}ClNO_2$	-3.7	3.5
		170.0351	C_8H_9ClNO	-2.2	4.5
		166.0838	$C_9H_{12}NO_2$	-3.0	4.5
		142.0406	C_7H_9ClN	-1.8	3.5
		134.0587	C_8H_8NO	-1.9	5.5
		105.0328	C_7H_5O	-1.2	5.5

[a] Maximum absolute area observed (initial area for parent compounds around 4700)
[b] Also detected in negative ionization mode (-V)
[c] TP only observed from cocaine

570 **Table 2:** Proposed elemental composition of protonated TPs and their fragments ions
571 obtained during photo-degradation of cocaine and BE, retention time (min),
572 accurate mass (*m/z*), mass error (mDa) and the double bond equivalent (DBE).

Compound (area)[a]	Retention time (min)	Sun / UV[d]	Accurate mass (*m/z*)	Chemical formulae	Mass error (mDa)	DBE
TP-P1[b]	2.05	Sun	168.1009	$C_9H_{14}NO_2$	-1.6	3.5
(81)			150.0902	$C_9H_{12}NO$	-1.7	4.5
			120.0792	$C_8H_{10}N$	-2.1	4.5
			100.0747	$C_5H_{11}NO$	-1.5	1.5
			93.0686	C_7H_9	-1.8	3.5
TP-P2[c]	2.78	Sun /	306.1332	$C_{16}H_{20}NO_5$	-0.9	7.5
hydroxy-BE	3.07	UV	186.1103[e]	$C_9H_{16}NO_3$	-2.7	2.5
(111)	3.91		168.1014	$C_9H_{14}NO_2$	-1.1	3.5
(46)			150.0903	$C_9H_{12}NO$	-1.6	4.5
(88)			121.0265	$C_7H_5O_2$	-2.5	5.5
			82.0665	C_5H_8N	+0.8	2.5
TP-P3	2.94	Sun	351.1180	$C_{16}H_{19}N_2O_7$	-1.2	8.5
(22)	3.54		168.1004	$C_9H_{14}NO_2$	-2.1	3.5
(39)			166.0118	$C_7H_4NO_4$	-2.2	6.5
			150.0902	$C_9H_{12}NO$	-1.7	4.5
			119.0487	C_8H_7O	-1.0	5.5
			82.0660	C_5H_8N	+0.3	2.5
TP-P4[c]	3.74	Sun /	290.1397	$C_{16}H_{20}NO_4$	+0.5	7.5
BE		UV	272.1304	$C_{16}H_{18}NO_3$	+1.7	8.5
(6189)			168.1047	$C_9H_{14}NO_2$	+2.2	3.5
			150.0946	$C_9H_{12}NO$	+2.7	4.5
			119.0514	C_8H_7O	+1.7	5.5
			105.0357	C_7H_5O	+1.7	5.5
			82.0672	C_5H_8N	+1.5	2.5
TP-P5[c]	3.96	Sun	276.1227	$C_{15}H_{18}NO_4$	-0.9	7.5
norBE			154.0850	$C_8H_{12}NO_2$	-1.8	3.5
(20)			136.0738	$C_8H_{10}NO$	-2.4	4.5
			108.0799	$C_7H_{10}N$	-1.4	3.5
			105.0335	C_7H_5O	-0.5	5.5

573 [a] Maximum absolute area observed (initial area for parent compounds around 7800)

574 [b] TP only observed from BE

575 [c] Also detected in negative ionization mode (-V)

576 [d] TP as a result of irradiation under simulated sunlight (Sun) and/or ultraviolet (UV)

577 [e] This fragment ion is not present in the *meta*-hydroxyBE (Tr= 3.07min)

578

579 **Table 3:** UHPLC-MS/MS parameters established for the SRM acquisition mode.

580

Compounds	Retention time (min)	Precursor ion (*m/z*) $[M + H]^+$	CV[a] (V)	CE[b] (eV)	Product ion (m/z)
TP-C1/TP-P4 (BE)	3.74	290.1	40	20	168.2
				30	105.0
				30	82.0
TP-C2/TP-P5 (norBE)	3.95	276.2	45	15	154.1
				20	136.1
				30	105.0
TP-C3 (norcocaine)	4.31	290.1	40	20	168.2
				25	136.1
				30	105.0
TP-C4	4.81	220.1	35	30	188.0
				25	120.0
				30	114.0
TP-C5	5.89	304.1	30	25	154.1
				25	136.0
				30	105.3
TP-C6	6.45	318.1	30	25	286.1
				20	196.2
				25	136.1
TP-C7	7.53	310.1	30	25	188.0
				25	152.0
				35	105.0
TP-C8	8.27	324.1	35	25	288.1
				25	166.1
				25	105.0
TP-P1	2.05	168.1	35	30	150.1
				25	120.1
				30	93.0
TP-P2	2.78	306.1	35	20	168.1
	3.07			15	186.1
	3.91			30	121.0
TP-P3	2.94	351.1	35	35	119.0
	3.54			15	168.1
				35	82.1

581 [a]CV, cone voltage; [b]CE, collision energy

582

583

584

FIGURE CAPTIONS

Figure. 1 Structure proposals for the identified TPs (TP-Cx were observed after chlorination and TP-Px were observed after photo-degradation).

[a] TP only observed from photo-degradation of BE.

[b] TP detected in sewage and environmental waters

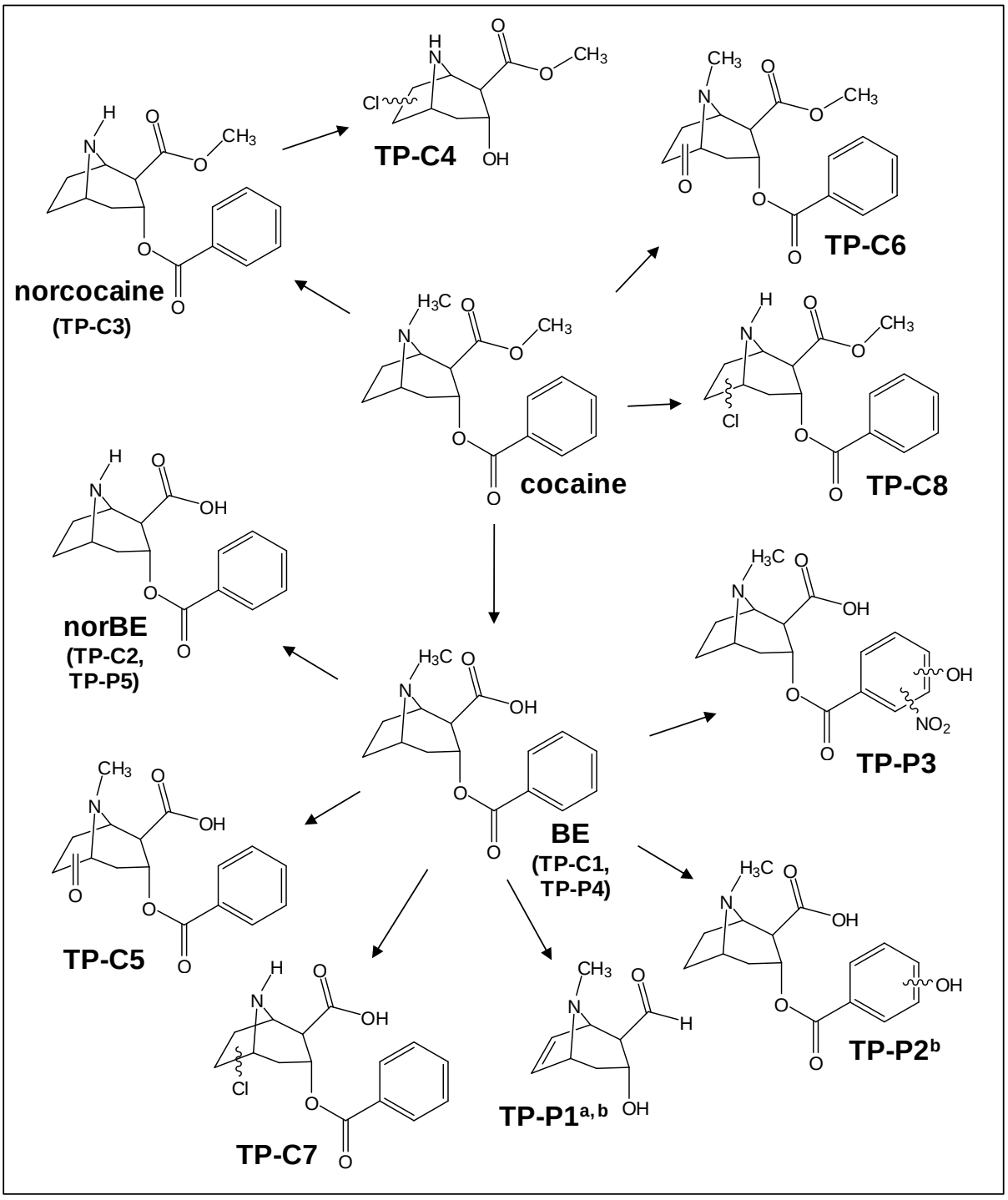
Figure. 2 Detection and identification of cocaine and BE photo-degradation TP-P2 by UHPLC-QTOF MS operating under MS^E. (A) narrow-window XICs (± 10 mDa) of TP-P2 and structures suggested for $[M+H]^+$ of TP-P2 and its fragment ions. (B) Structure proposals for the neutral loss of 120 Da ($C_7H_4O_2$).

Notice the presence of other chromatographic peaks (marked with *), supporting that other TPs share the same fragmentation.

Figure. 3 Photo-degradation of cocaine where BE acts as photo-intermediate in the formation of TP-P2.

Figure. 4 UHPLC-MS/MS chromatograms corresponding to the positive finding of TP-P1 and TP-P2 in (top) effluent sewage water (analyzed using TQD, December 2011) and (bottom) surface water (analyzed using TQS, April 2012). Retention times of BE were 3.75 min (TQD) and 3.84 (TQS).

609



610
611
612 **Figure 1**
613
614

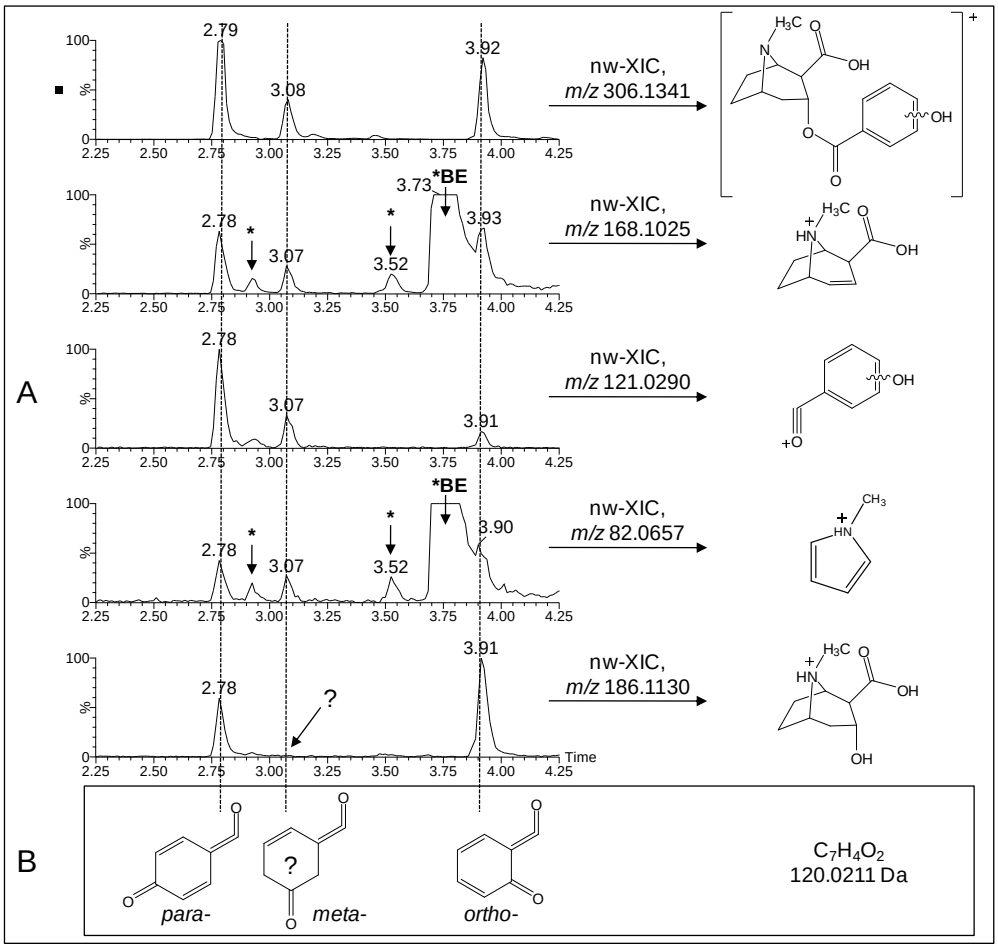
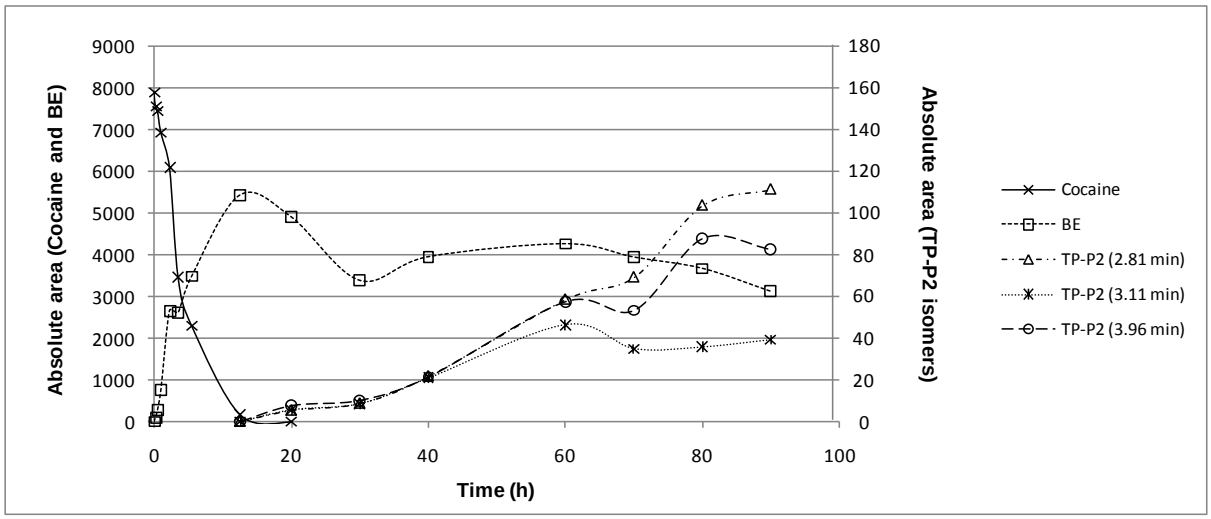

A
B
nw-XIC,
m/z 306.1341
nw-XIC,
m/z 168.1025
nw-XIC,
m/z 121.0290
nw-XIC,
m/z 82.0657
nw-XIC,
m/z 186.1130
2.79
3.08
3.92
3.73
*BE
3.93
2.78
3.07
3.52
3.91
3.90
?
Time
para-
meta-
ortho-
C7H4O2
120.0211 Da

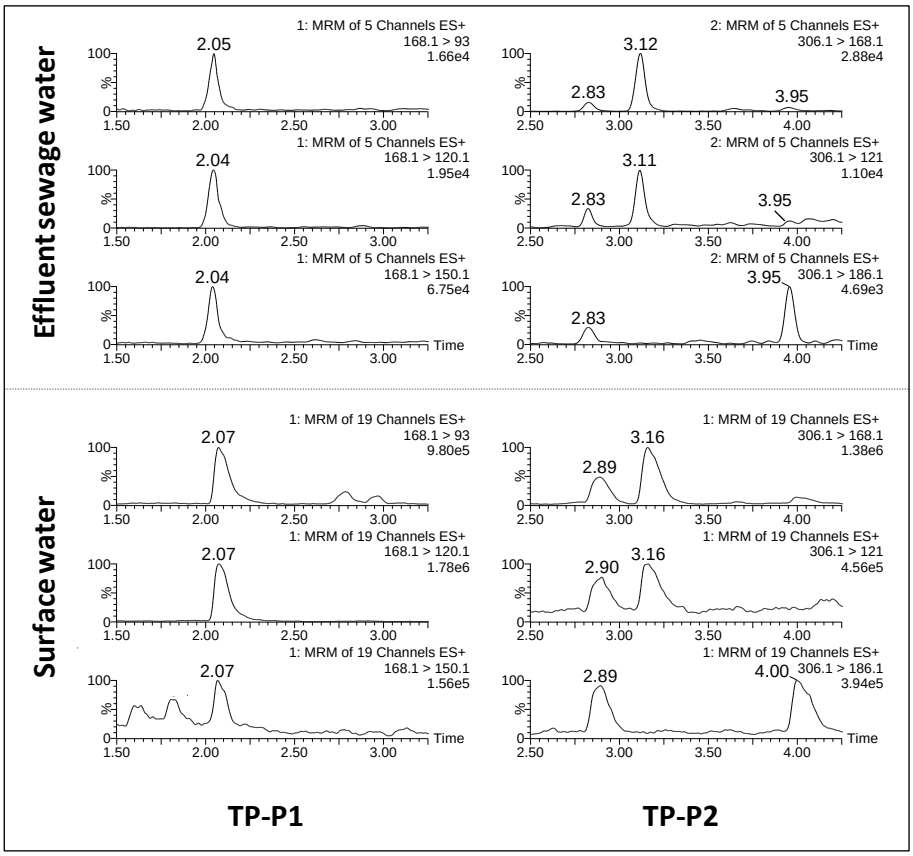
Figure 2

620



621
622
623 **Figure 3**
624

625



626
627
628 **Figure 4**