

Analysis of cocaine and its principal metabolites in waste and surface water using solid-phase extraction and liquid chromatography–ion trap tandem mass spectrometry

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Abstract A validated method based on solid-phase extraction (SPE) and liquid chromatography–ion trap tandem mass spectrometry (LC-MS/MS) is described for the determination of cocaine (COC) and its principal metabolites, benzoylecgonine (BE) and ecgonine methyl ester (EME), in waste and surface water. Several SPE adsorbents were investigated and the highest recoveries (95.7 ± 5.5 , 91.8 ± 2.2 and $72.5 \pm 5.3\%$ for COC, BE and EME, respectively) were obtained for OASIS HLB[®] cartridges (6 mL/500 mg) using 100 mL of waste water or 500 mL of surface water. Extracts were analysed by reversed-phase (RP) or hydrophilic interaction

(HILIC) LC-MS/MS in positive ion mode with multiple reactions monitoring (MRM); the latter is the first reported application of the HILIC technique for drugs of abuse in water samples. Corresponding deuterated internal standards were used for quantification. The method limits of quantification (LOQs) for COC and BE were 4 and 2 ng L⁻¹, respectively, when RPLC was used and 1, 0.5 and 20 ng L⁻¹ for COC, BE and EME, respectively, with the HILIC setup. For COC and BE, the LOQs were below the concentrations measured in real water samples. Stability tests were conducted to establish the optimal conditions for sample storage (pH, temperature and time). The degradation of COC was minimal at -20 °C and pH=2, but it was substantial at +20 °C and pH=6. The validated method was applied to a set of waste and surface water samples collected in Belgium.

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Introduction

In contemporary society, illicit drugs are extensively used, and moreover, cocaine is one of the preferred drugs of abuse. The use of cocaine has direct physiological effects, such as central nervous system over-stimulation, lifetime risk of heart attack, pulmonary complications, and altered serotonin levels [1]. The recreational use of cocaine has increased dramatically over the last 25 years as a result of increased availability and lowering prices. The trends of drug abuse are currently estimated indirectly from population surveys, consumer interviews, individual medical records and crime statistics. However, these general indicators do not accurately estimate the local use [2].

In humans, only a fraction of cocaine is excreted in urine as parent compound, whereas the largest amount is excreted as benzoylecgonine (BE), the most common metabolite, and as ecgonine methyl ester (EME), which are used to obtain evidence of cocaine use (Fig. 1) [3]. Consequently, the abuse of cocaine has been monitored through the analysis of cocaine and its metabolites in various biological matrices e.g. serum/whole blood [4] or urine [5]. However, these analyses are generally performed on persons with a high degree of intoxication or on suspected drug users. Nevertheless, the extent of the cocaine use by the general population remains largely unknown.

Recently, more direct and realistic approaches have been proposed based on the measurement of urinary excreted drugs and metabolites, in local waste and surface water. These approaches are similar to those proposed for therapeutic drugs and other pharmaceuticals [6]. Only recently, cocaine and its metabolites have been measured in urban waste water and surface water and their environmental levels were used to estimate local cocaine consumption [2, 7–10].

In gas chromatography–mass spectrometry (GC-MS), a technique widely used for confirmation of cocaine use, the sample pretreatment is generally laborious. Due to the need to measure polar metabolites (BE and EME), derivatisation prior to the GC analysis is carried out to improve chromatographic performance [11, 12]. Approaches based on liquid chromatography–mass spectrometry (LC-MS) have only recently emerged for the analysis of cocaine and metabolites [5, 13, 14]. LC-MS has several advantages over the GC-MS approach: it does not require derivatisation, it is more versatile, and the mobile phase is compatible with the sample pretreatment [14].

Up to now, only a few methods have been reported for the analysis of cocaine and its principal metabolites in waste and surface water [7–9]. In these methods, solid-phase extraction

(SPE) of COC and metabolites has not been extensively investigated, the only tested adsorbents until now being Oasis MCX® [7] and Oasis HLB® [8, 9]. Moreover, these methods have all been based on reversed-phase (RP) LC, on which the more polar metabolite, EME, has a limited chromatographic retention and was therefore seldom investigated.

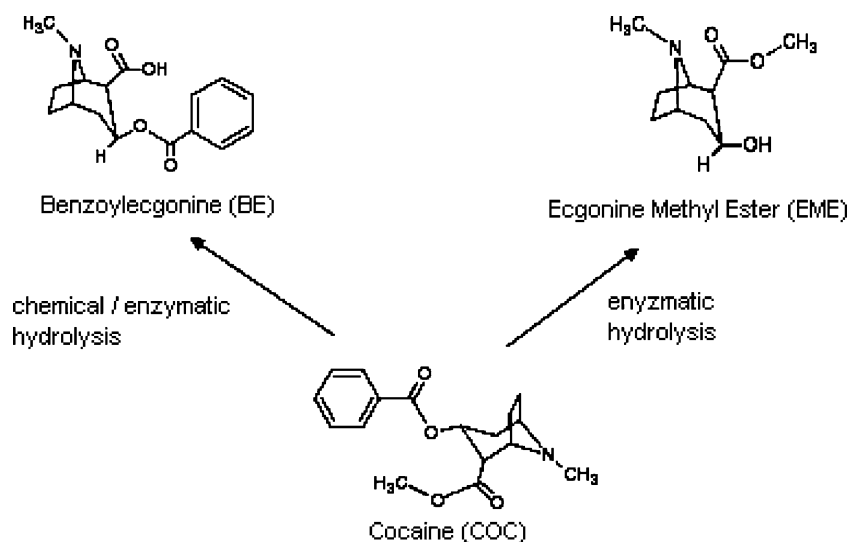
The principal aim of this work was to optimize and validate an analytical procedure for the determination of cocaine and its principal metabolites in waste and surface water. The first aim was to enlarge the range of tested SPE cartridges towards their suitability in extraction efficiency for cocaine and metabolites. Secondly, hydrophilic interaction liquid chromatography (HILIC) has been investigated for the separation of polar compounds, such as EME. Thirdly, several conditions (pH, temperature and duration of storage) were explored in order to provide maximum stability of cocaine and its metabolites during sample collection, transport and short-term storage. The method presented here will constitute the working platform for a large scale Belgian monitoring project for COC and metabolites in waste and surface water. Together with data on pharmacokinetics, it will serve as basis for the estimation of the COC consumption by the general population.

Experimental

Reagents and materials

All investigated compounds (COC, BE and EME), and their deuterated analogues, used as internal standards, were of analytical grade (purity >98%) and were purchased from Cerilliant (Round Rock, TX, USA) at concentrations of $100 \text{ ng } \mu\text{L}^{-1}$ in methanol (MeOH). Further dilutions and working mixtures with concentrations ranging from 0.01 to $10 \text{ ng } \mu\text{L}^{-1}$ were prepared in MeOH. LC-grade acetonitrile

Fig. 1 Simplified major pathways for transformation of cocaine in the human body



(AcN) and MeOH were obtained from Merck (Darmstadt, Germany). LC-grade water was obtained by purifying demineralised water in a Milli-Q system (Millipore, Bedford, MA, USA). Hydrochloric acid (HCl) and ammonium hydroxide (NH₄OH) were also obtained from Merck. A Supelco Visiprep™ SPE Vacuum Manifold with 12 ports and a self-cleaning dry vacuum system™ Welch 2023 was used for the loading of water samples onto the SPE cartridges, the drying of the cartridges and the elution of analytes.

Samples and sample pretreatment

Influent samples were collected from five waste water treatment plants (WWTPs) in the region of Flanders, Belgium. For each location, samples were collected on different dates, when possible, before and after a weekend. Surface water samples were collected from three small rivers in Belgium. WWTP samples were 24-h composite samples, while the surface water samples were grab samples. All samples (1.5 L for waste water and 2.5 L for surface water) were collected in 1-L glass bottles, were adjusted to pH=2 with 37% HCl and were stored at -20 °C until analysis. Before analysis, the water samples were preliminary filtered over a qualitative filter paper (ϕ 125 mm, Whatman, Kent, UK), then over a glass microfibre filter (ϕ 125 mm, Macherey-Nagel, Düren, Germany). The solid suspended particles retained on the filters after the filtration of 100 mL of waste water or 500 mL of surface water were also analysed. After drying, the filter papers with the retained particles were extracted in an ultrasonic bath with 5 mL MeOH (5 min) and, after concentration, the methanolic extracts were injected into the LC-MS/MS system (see below). The deuterated internal standards (COC-*d*₃, BE-*d*₃ and EME-*d*₃) used for quantification were added after the filtration step.

Solid-phase extraction

To quantitatively extract the investigated analytes and to eliminate the influence of matrix components, different types of SPE cartridges (Oasis HLB®, Oasis MCX®, Isolute ENV+®, Isolute C18 (EC)®, Isolute PH®, Bond Elut Certify® and Chromabond Easy®) were tested. Each cartridge was processed following the most appropriate protocol found in the literature.

Oasis HLB® (500 mg/6 mL)

This divinylbenzene/*N*-vinylpyrrolidone copolymer with hydrophilic and lipophilic properties (Waters, New Bedford, MA, USA) was used following different protocols:

1. Protocol 1: conditioning with 3 mL of MeOH and 3 mL of Milli-Q water, sample loading at pH=6, washing with 3 mL of a 5% MeOH in Milli-Q water, drying

under vacuum for 15 min and eluting with 2×4 mL of MeOH [6].

2. Protocol 2: differs from protocol 1 in the conditioning step, which was done with 5 mL of hexane, 5 mL of ethyl acetate, 10 mL of MeOH and 1 mL of Milli-Q water, and in the washing step done with 5 mL of Milli-Q water [15].

Oasis MCX® (500 mg/6 mL)

This mixed mode, polymeric sorbent with strong cation-exchange sulfonic acid groups located on the surface of a poly (divinylbenzene-*co*-*N*-vinylpyrrolidone) copolymer (Waters) was used as follows: conditioning with 6 mL of MeOH, 3 mL of Milli-Q water and 3 mL of Milli-Q water at pH=2, sample loading at pH=2, washing with 3 mL of Milli-Q water at pH=2, drying for 15 min under vacuum and eluting with 6 mL of MeOH and 6 mL of 5% NH₃ in MeOH [7].

Isolute ENV+® (500 mg/6 mL)

This hydrophobic sorbent with hydroxylated polystyrene-divinylbenzene copolymer (IST, Mid Glamorgan, UK) was used as follows: conditioning with 2 mL of MeOH and 6 mL of Milli-Q water, sample loading at pH=6, washing with 6 mL of 5% MeOH in water, drying under vacuum for 15 min and eluting with 2×4 mL of 5% NH₃ in acetone, modified from ref. [16].

Isolute C18(EC)® (500 mg/6 mL)

This strongly apolar and lipophilic sorbent based on octadecylsilica with end-capping of free silanol groups (IST) was used as follows: conditioning with 2 mL of MeOH and 6 mL of Milli-Q water, loading of the water samples at pH=6, washing with 6 mL of a 5% MeOH aqueous solution, drying under vacuum for 15 min, and eluting of COC and metabolites with 2×4 mL of 5% NH₃ in acetone, modified from ref. [16].

Isolute PH® (1000 mg/6 mL)

This silica treated with phenyl groups in which free silanol groups are end-capped (IST) was used as follows: conditioning with 2 mL of MeOH and 6 mL of milli-Q water, sample loading at pH=6, washing with 6 mL of 5% MeOH in water, drying under vacuum for 15 min and eluting with 2×4 mL of 5% NH₃ in acetone, modified from ref. [16].

Bond Elut Certify® (300 mg/6 mL)

This sorbent with lipophilic and strongly cationic properties (Varian, Walnut Creek, Ca, USA) was used as follows:

conditioning of the cartridges with 3 mL of MeOH and 3 mL of Milli-Q water, loading samples at pH=6, washing with 2 mL of Milli-Q water at pH=2 and 3 mL of MeOH, drying for 15 min under vacuum and eluting with 2×4 mL of 80:20 DCM/isopropanol mixture with 2% NH₃ [17].

Chromabond Easy[®] (500 mg/6 mL)

This bifunctional polystyrene–divinylbenzene copolymer (Macherey-Nagel, Düren, Germany) was used with protocol 2 presented for the Oasis HLB[®] cartridges.

LC-MS/MS

Separation of COC, BE and EME was carried out on an Agilent 1100 series HPLC system with binary pump and auto sampler (Agilent technologies, Palo Alto, Ca, USA). The HPLC system was coupled to an Agilent 1100 Series LC-MSD ion trap with electrospray ionisation (ESI) equipped with an HP Chemstation for MS control and spectral processing. The ESI interface was operated in positive ionization mode. MS analyses were performed in multiple reaction monitoring (MRM) mode by measuring the fragmentation products of the protonated pseudomolecular ions of COC, BE and EME and their internal standards in three different fragmentation windows (Table 1). The ESI voltage was set at 3,500 V. The averages were set at 3 and the maximum accumulation time at 100 ms. Parameters were optimised by injection of known amounts of the six compounds and evaluating retention times, analyte response and peak widths and shapes.

Reversed-phase liquid chromatography–tandem mass spectrometry (RPLC-MS/MS)

For RPLC, a C₁₈ reversed-phase column, Zorbax Extended C₁₈ (2.1 mm×50 mm×3.5 μm) (Agilent Technologies), equipped with a guard column (2.1 mm×12.5 mm×5 μm) containing the same stationary phase as the RP column, was

Table 1 Fragmentation pattern of COC, BE and EME and their internal standards

Compound	Retention time RPLC (min)	Retention time HILIC (min)	Precursor ion (<i>m/z</i>)	Product ion (<i>m/z</i>)
COC	7.6	9.3	304	182
COC- <i>d</i> ₃	7.6	9.3	307	185
BE	6.4	4.7	290	168
BE- <i>d</i> ₃	6.4	4.7	293	171
EME	nm	13.6	200	182
EME- <i>d</i> ₃	nm	13.6	203	185

nm not measured

used. The optimum binary mobile phase was solvent A: water/AcN 98:2, 10 mM formate buffer, pH=3; solvent B: AcN. The optimized gradient was as follows: starting eluent containing 97% solvent A and 3% solvent B; 0–6 min 3% to 40% solvent B; 6–12 min 40% to 90% solvent B; 12–22 min equilibration of the column with the starting eluent. The flow rate was kept constant at 250 μL min⁻¹. The injected volume was 2 μL. Nitrogen was used as nebulising gas at a pressure of 40 psi. Dry gas debit and temperature were set at 8 L min⁻¹ and 280 °C, respectively.

Hydrophilic interaction chromatography–tandem mass spectrometry (HILIC-MS/MS)

For HILIC, the separation of COC and its principal metabolites was carried out on a ZORBAX Rx-SIL column (2.1 mm×150 mm×5 μm) (Agilent) using a guard column (2.1 mm×12.5 mm×5 μm) containing the same stationary phase as the HILIC column. The optimum mobile phase comprised solvent A: ammonium acetate 2 mM/acetic acid buffer (pH=4.5); solvent B: ACN. The gradient was as follows: 0–1 min 80% solvent B; 1–10 min 80% to 40% solvent B; 10–18 min constant 40% solvent B; 21–31 min: equilibration of the column with the starting eluent containing 80% ACN. The flow rate was kept constant at 250 μL min⁻¹. The injected volume was 2 μL for waste water extracts and 5 μL for surface water extracts. Nitrogen was used as nebulising gas at a pressure of 25 psi. Dry gas debit and temperature were set at 12 L min⁻¹ and 350 °C, respectively.

Analytical parameters

Quantification of COC, BE and EME was performed with the help of deuterated corresponding internal standards (COC-*d*₃, BE-*d*₃, EME-*d*₃). A seven-point calibration curve was generated for each analyte by injecting standard solutions with different amounts of each compound and fixed amounts of internal standards. Linearity was obtained for the following ranges of absolute amounts of analytes injected on the column (35–3,300 pg for BE, 10–870 pg for COC and 15–780 pg for EME). Correlation coefficients were 0.9946, 0.9982 and 0.9968 for COC, BE and EME, respectively, and relative standard deviations (RSDs) ranged from 2.2 to 6.1% for five consecutive injections.

Instrumental limits of quantification, expressed as picograms injected, were measured for S/N=10. If we extrapolate these values and consider the analytical procedure (100 mL water extracted and final extract reconcentrated to 150 μL), the method limits of quantification (LOQ) were 4 and 2 ng L⁻¹ for COC and BE, respectively, when RPLC was used, and were 1, 0.5 and 20 ng L⁻¹ for COC, BE and EME, respectively, with the HILIC setup.

For all tested SPE columns, the recoveries of COC and metabolites were calculated based on the following experiments: 20.6 ng COC, 84.1 ng BE and 19.1 ng EME were added to 100 mL of tap water and each cartridge was then used according to its own protocol (see above). EME was added only for Oasis HLB[®] adsorbent. After elution in a glass tube, 20.9 ng COC-*d*₃, 86.5 ng BE-*d*₃ and 19.3 ng EME-*d*₃ were added to the eluates, the mixtures were vortexed and after evaporation under a gentle nitrogen stream, the dried residue was redissolved in 150 μ L MeOH/solvent A (see above) (1:1, v/v) for the RPLC system and in 150 μ L AcN/MeOH (3:1, v/v) for the HILIC system, vortexed, centrifuged and transferred into a glass vial to be injected into the LC-MS/MS system.

Stability tests

Surface water from a pond located at the University of Antwerp, for which preliminary measurements have shown that COC and BE were not present, was spiked to a given concentration of 103 ng L⁻¹ COC and 420 ng L⁻¹ BE. Aliquots of 100 mL were prepared and the following parameters were modified: pH (2 and 6), temperature (-20 °C, +4 °C and +20 °C) and storage time (1, 3 and 5 days). Each batch of samples contained a water sample from the pond.

Results and discussion

SPE optimisation

Mixed-phase sorbents, such as Bond Elut Certify[®], have been used with success in the preconcentration of cocaine and its metabolites in biological fluids [17]. Recently, new sorbents based on polymeric phases, such as Oasis HLB[®] or Oasis MCX[®], have been investigated for the analysis of cocaine in biological samples [18]. However, the few methods described until now for the analysis of cocaine and metabolites in waste water and surface water have not investigated other adsorbents [7–9].

In the present study, specific parameters, such as improved recoveries and ability of delivering clean extracts, were investigated for several SPE adsorbents. Additionally, the stability of the cartridges to pH and dryness, the volume and type of solvents used, were also regarded as important factors. The average recoveries and standard deviations of COC and metabolites for different tested SPE cartridges are shown in Table 2. Good recoveries were obtained for Oasis HLB[®] (protocols 1 and 2) and for Isolute C18 (EC)[®]. However, the latter cartridge was not stable at low pH values and cannot run dry during conditioning or sample loading. Surprisingly, the use of Oasis MCX[®] resulted in lower recoveries for BE (Table 2), while higher recoveries have been reported elsewhere [7]. It is possible that the amount of adsorbent used in the present study (500 mg) was too high and leading thus to incomplete elution of BE. Castiglioni et al. [7] have used cartridges containing less adsorbent (60 mg). However, such low amounts of adsorbents have a low breakthrough volume and therefore cannot be used for the loading of large sample volumes (e.g. 500 mL surface water). Since the protocol 1 with Oasis HLB[®] uses less solvents and less time for the sample preparation, this was further chosen for the processing of the waste (100 mL) and surface (500 mL) water samples.

LC-MS/MS optimisation

Different parameters (binary mobile phase composition, injection volume and gradient) have been optimised by injection of known amounts of COC and metabolites and corresponding deuterated internal standards and by evaluating the retention times, peak intensities, peak widths and peak shapes.

RPLC

For the LC optimisation, the influence of the organic solvent (MeOH or AcN), the concentration of formate buffer (10, 25 or 50 mM), as well as the pH of the formate buffer (2.5, 3.0 and 4.0), were evaluated. The chosen

Table 2 Recoveries \pm standard deviations for COC, BE and EME on different SPE cartridges

SPE cartridge	N	Recovery COC (%)	Recovery BE (%)	Recovery EME (%)
Oasis HLB [®] protocol 1	3	95.7 \pm 5.5	91.8 \pm 2.2	72.5 \pm 5.3
Oasis HLB [®] protocol 2	3	99.8 \pm 8.0	102.3 \pm 6.6	nm
Oasis MCX [®]	1	107.4	66.7	nm
Isolute C18(EC) [®]	3	86.0 \pm 8.8	80.6 \pm 4.3	nm
Isolute ENV+ [®]	3	95.4 \pm 5.8	7.2 \pm 1.6	nm
Isolute PH [®]	1	83.9	11.1	nm
Bond Elut Certify [®]	3	103.1 \pm 0.1	50.7 \pm 7.1	46.6 \pm 1.7
Chromabond Easy [®]	1	67.4	23.1	nm

nm not measured

organic solvent was AcN, based on the observation that separations with AcN proceeded faster than with MeOH. The preferred pH of the formate buffer was 3, because at pH=4 lower peak intensities were obtained, whereas at pH=2.5 the buffer does not have its optimal buffer capacity [19]. The buffer concentration seems to play a less important role on the peak characteristics in this chromatographic system. Consequently, the best separation conditions were obtained for the following mobile phase: solvent (A) water/AcN 98:2, 10 mM formate buffer, pH=3; solvent (B) AcN. A typical chromatogram can be seen in Fig. 2.

Different injection volumes (1, 2, 5 and 10 μL) were also tested. Broad and tailing peaks resulted from injection volumes of 5 and 10 μL , while low peak intensities were obtained when 1 μL of reconstituted extract was injected. An injection volume of 2 μL produced the best chromatograms in terms of acceptable peak intensity and peak shape for all target compounds.

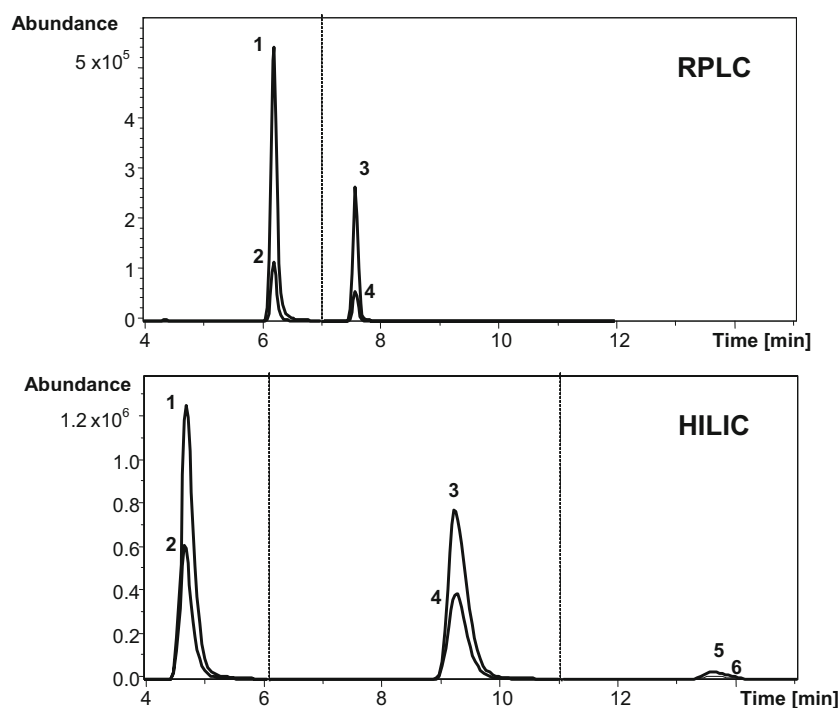
HILIC

The high polarity of cocaine and its metabolites justifies the application of the newly introduced HILIC technique. On a reversed-phase (RP) column, polar compounds (such as BE and EME) are poorly retained and elute early in the chromatogram, together with other non-retained components of the matrix, thus causing a decreased ionisation efficiency in the ion source [20, 21]. By using a

pentafluorophenylpropyl-silica stationary phase, Needham et al. [20] have shown that a higher proportion of AcN in the mobile phase improves the desolvation process and gives better LOQs. The EME signal was enhanced by a factor of 16 in comparison with that obtained with a RP column. This improvement in the signal of polar analytes on HILIC columns is in agreement with other studies [22].

The present work investigated the applicability of HILIC and high organic mobile phase for a more sensitive analysis of polar metabolites of cocaine in waste water. HILIC has already been successfully reported as a viable alternative to RPLC [22, 23] and in particular for the quantitative analysis of COC, BE and EME in body fluids and tissue [24, 25]. Figure 2 shows the chromatographic separation of COC, BE and EME on the HILIC column. As a consequence of the different retention mechanism, EME eluted later in the chromatogram and, therefore, can be measured in an easier way than by using RPLC. Since EME is more retained on the HILIC column, it is not subject to ion suppression near the void (as in RPLC) and therefore a reduction in analytical signal is minimised. The increased sensitivity for EME using HILIC is probably a combination of signal enhancement (through desolvation) from the more organic mobile phase together with the greater retention on the HILIC phase and thus, less ion suppression. It is expected that the HILIC approach would lead to a lesser influence of the matrix effects on the analytical signals. The possibility of measuring EME (and eventual other polar metabolites) is very important since this will allow better calculations for

Fig. 2 Chromatograms of COC and metabolites obtained by RPLC (*top*) and HILIC (*bottom*): 1 BE- d_3 , 2 BE, 3 COC- d_3 , 4 COC, 5 EME- d_3 , 6 EME



the equivalents of cocaine present in the analysed water samples.

Quantification and method validation

Quantification of COC, BE and EME was performed with the help of deuterated internal standards (COC- d_3 , BE- d_3 , EME- d_3). A multilevel calibration curve was generated for each analyte by plotting the ratio between the peak areas of analyte and the corresponding deuterated standard versus the ratio between the absolute injected amounts. The linearity of the analytical response was tested on the calibration curves. The following tests were performed: r^2 (> 0.995), t -test on intercept, t -test on slope, homoscedasticity and ANOVA. All calibration curves passed these tests and it has been concluded that the curves were linear in the studied range. Correlation coefficients (r^2) were > 0.995 for COC, BE and EME in both RPLC and HILIC systems.

The intermediate precision was determined by triplicate experiments with tap water spiked with COC and BE (206 and 841 ng L⁻¹, respectively), and this procedure was repeated for 3 days. The intraday RSDs were 4.6 and 8.0% for COC and BE, respectively, while the interday RSDs were 4.2 and 8.7%, respectively.

The trueness was determined based on similar experiments ($n=6$) as described above using tap water samples spiked with COC and BE at two different concentration levels. The trueness was calculated by comparing the mean analyte value recovered from the spiked samples with the target (spiked) concentration. The obtained trueness values were 94.7 ± 7.6 and $93.6 \pm 4.3\%$ for BE and COC, respectively.

The LOQs were determined by injection of low concentrations of analytes (10.8 ng L⁻¹ BE, 5.7 ng L⁻¹ COC and 47.8 ng L⁻¹ EME). The LOQ was calculated from following formula: $LOQ = 10 \times h_{\max} \times R$, where h_{\max} = maximal amplitude of the noise and R = concentration of compound (in ng L⁻¹)/peak height [26]. For the RPLC-MS/MS system, LOQs were 4 and 2 ng L⁻¹ for COC and BE, respectively. For the HILIC method, the LOQ values were 0.5, 1 and 20 ng L⁻¹ BE, COC and EME, respectively.

Ion suppression

To evaluate potential matrix effects, the signal suppression for each analyte was calculated as percentage decrease in the signal intensity recorded in the sample (waste, surface or tap water) versus the intensity in Milli-Q water, as described by Hernando et al. [27]. For the waste water extract, the signal suppression was between 30 and 48%, while for surface and tap water, it was considerably lower (22% and 12%, respectively). However, the signal suppression decreased under 10% for each of the three matrices investigated when centrifugation followed by filtration of

extracts has been performed. The improvement was obvious for surface water (when 500 mL was used) and for waste water (which is a complex matrix even if only 100 mL was used). Jeanville et al. [21] also indicated that filtration and centrifugation of the extract obtained from urine were needed to prevent the presence of endogenous interferences which were the main cause for ion suppression, while Dams et al. [28] concluded that matrix effects should also be investigated when less laborious or minimal sample preparation is included in the method. The simple dilution of the extract as suggested by Fatta et al. [29] is not a viable option since this would lead to a decrease in detectability. Unfortunately, matrix effects have not yet been reported in the literature for the analysis of drugs of abuse in waste and surface water, indicating that this issue should be closely investigated in the future.

Stability of cocaine and metabolites in waste water analytical implications

The presence of COC and metabolites in urban waste water is mainly a direct consequence of their urinary excretion. Therefore, adequate knowledge of the metabolism of COC in the human body and the excretion of metabolites (BE and EME) is required for a correct assessment of their environmental concentrations.

The major metabolic pathways of COC described in the literature are the chemical hydrolysis to BE, which accounts for 45% of the transformed COC and the enzymatic hydrolysis to EME, which accounts for a similar percentage or slightly less [30, 31]. Only a small percentage (1–5%) COC is excreted unchanged in urine after 24 h [31, 32]. However, the amount of COC found in human urine after a certain time and the detection of the principal metabolites, BE and EME, depends on (i) the amount of cocaine used; (ii) the route of administration; (iii) individual characteristics of the consumer (age, health status, metabolism, pH of urine) [32, 33].

Thus, it is perfectly justified to use BE and EME as the main indicators of cocaine consumption and to investigate their presence in environmental (aqueous) samples. However, less is known about the stability of COC and metabolites in aqueous matrices, other than biological specimens (e.g. blood and urine) currently investigated in clinical and forensic toxicology. For whole blood and plasma, degradation of COC, BE and EME increased with increasing storage time (up to 15 days) and temperatures between 4 and 40 °C [34]. The reduction of COC and EME by approximately 15–20% and the increase in BE by approximately 10% have been reported for whole blood samples stored at -10 °C for 6 months. These changes were most likely caused by the hydrolysis of COC to BE and of EME to ecgonine [14].

Minor metabolisation pathways involve the aromatic *m*- and *p*-hydroxylation of COC, followed by partial hydrolysis to the corresponding HO-BE isomers [35]. Several other metabolites of COC (ecgonidine, norecgonidine methyl ester, nor-EME, *m*-OH-BE, ecgonine, ecgonidine methyl ester, nor-COC, *p*-OH-COC and *m*-OH-COC) are possibly formed in the human body [36], but at much lower rates than BE and EME. These metabolites have not yet been evidenced in environmental samples. Although it is expected that all COC metabolites would be further metabolised, the analysis of the end product (ecgonine) is difficult due to its high polarity and its poor retention on RPLC columns [24]. However, preliminary tests have shown that ecgonine can be separated using the HILIC approach (retention time ca. 10 min), but was not detectable in the waste water samples.

In the stability experiments performed in the present study, a high stability was evidenced for BE during the time interval investigated (up to 5 days), for both pH values (2 and 6) and at all three storage temperatures ($-20\text{ }^{\circ}\text{C}$, $+4\text{ }^{\circ}\text{C}$ and $+20\text{ }^{\circ}\text{C}$) (Fig. 3). For COC, a similar stability was observed for all three temperatures at pH=2, during the time interval of 5 days (Fig. 3). In contrast, at $+4\text{ }^{\circ}\text{C}$ and

pH=6, the degradation of COC was 22% after 3 days and 35% after 5 days. Castiglioni et al. [7] have reported that COC was degraded by 36% after 3 days storage at $4\text{ }^{\circ}\text{C}$. However, in this case, the pH of the water sample was not adjusted and presumably the pH was > 6 (normal pH of waste water), at which degradation occurs faster than in conditions described in the present study. A dramatic degradation of COC has been observed at $+20\text{ }^{\circ}\text{C}$, when ca. 75% of the present COC was degraded after only 1 day. Such behaviour is similar to that observed for COC and BE during storage of whole blood at various temperatures [4].

To investigate further the fate of COC and metabolites in conditions close to those present in the environment (ambient temperature, pH=6), a short-term stability experiment was performed. It was found that more than 40% of COC and EME were degraded after 5 h, while BE remained constant or even increased (probably due to additional formation from the degradation of COC). After 24 h, almost 90% of COC and EME were degraded (Fig. 4), which is in good agreement with the high degradation of COC seen in the medium-term stability tests (see above).

These findings are of uttermost importance for two principal reasons. Firstly, for sample storage, it is recom-

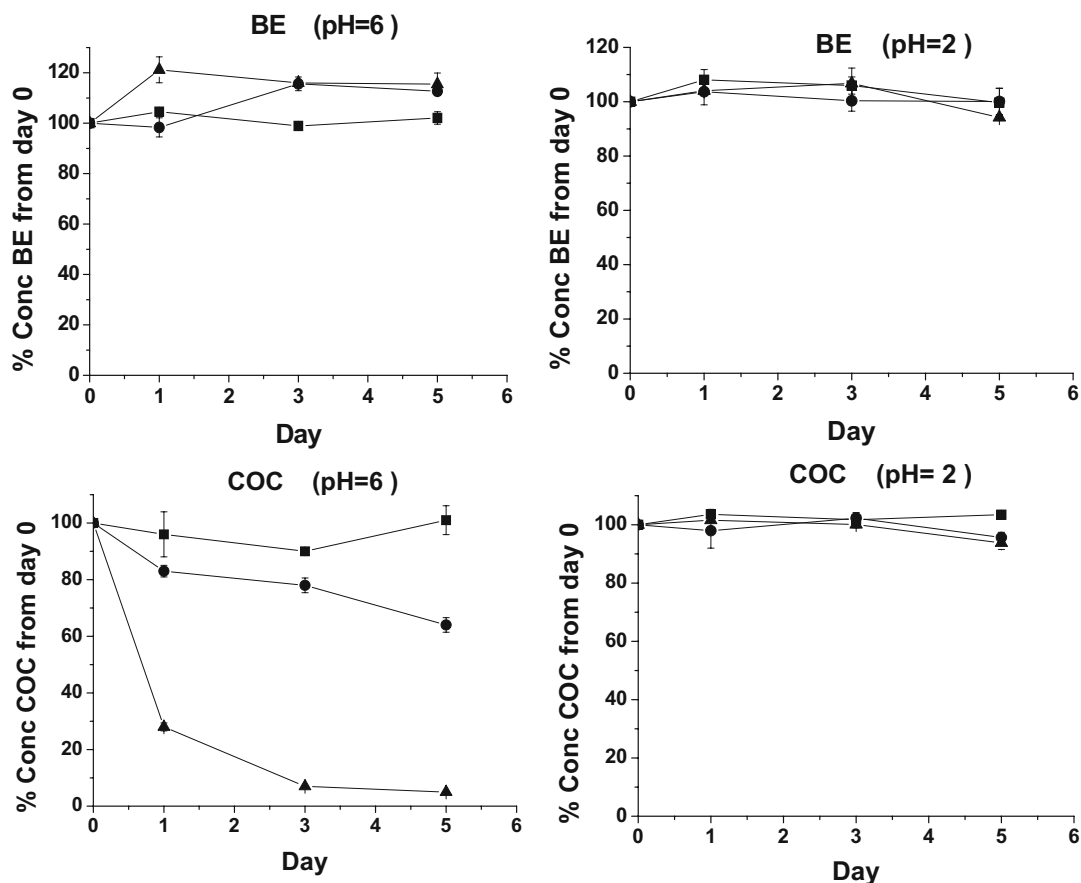
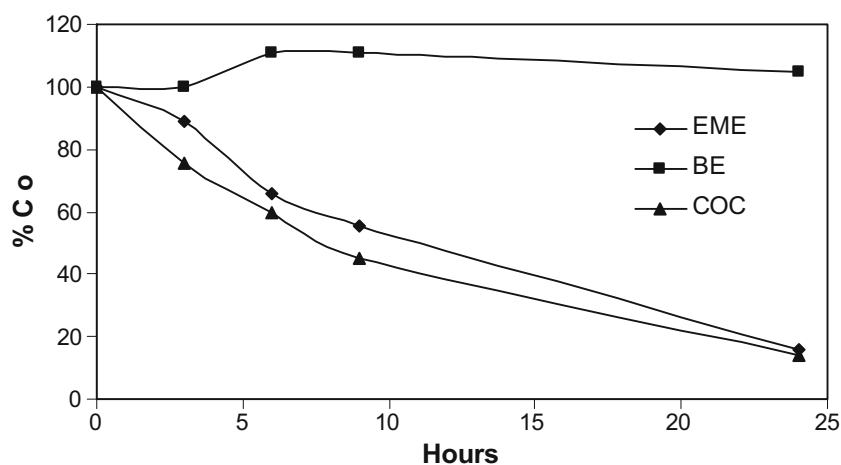


Fig. 3 Stability profiles of COC and BE at pH=6 and pH=2 during a 5-day interval for the following temperatures: ■ $-20\text{ }^{\circ}\text{C}$, ● $4\text{ }^{\circ}\text{C}$, ▲ $20\text{ }^{\circ}\text{C}$

Fig. 4 Stability profiles of COC, BE and EME at pH=6 during 24 h at 20 °C



mended to acidify the samples to pH=2 immediately after sampling and store them at -20 °C. In this way, minimal degradation of COC and metabolites occurs. Secondly, the extent of degradation for COC and metabolites plays a key role in the calculation of equivalents of cocaine used per day from the concentrations of COC and metabolites in waste water.

However, the previously described stability tests have several limitations which have to be considered when additional tests are planned: (i) the experiments were not conducted in a comparable wastewater matrix (which will contain a different suite of organics and inorganics in

contrast to the pond water); (ii) intermediate temperatures between 4 and 20 °C, corresponding to actual temperatures in the sewage system, should also be tested; (iii) the pH range should also include values above 6, which are frequently measured in waste and surface water samples. The experiments are still quite valuable in predicting the “potential” stability of COC, BE and EME in wastewater and highlight the important of acidification for storage.

Additionally, tests have been carried out to estimate the possible adsorption of COC and metabolites on the suspended material. COC and BE could not be measured in the analysed particles, not even in solids obtained from

Table 3 Concentrations (ng L^{-1}) of COC and BE in influent WWTPs and surface water from Belgium, Italy, Spain and Switzerland

Sampling site	Sampling date	Type water	Method	Concentration COC (ng L^{-1})	Concentration BE (ng L^{-1})	Reference
Grote Molenbeek	22 Jun 2007	Surface	HILIC	26	191	This study
Demer	26 Jul 2007	Surface	HILIC	13	44	This study
Senette	27 Jul 2007	Surface	HILIC	7	53	This study
Lier	5 Sept 2007	Waste	HILIC	74	94	This study
Tessenderlo	5 Sept 2007	Waste	HILIC	22	82	This study
Lier	6 Feb 2006	Waste	RPLC	294	1,187	This study
Lier	17 Feb 2006	Waste	RPLC	25	164	This study
Lier	5 Apr 2007	Waste	RPLC	275	1,005	This study
Hoboken	6 Feb 2006	Waste	RPLC	457	1,607	This study
Hoboken	17 Feb 2007	Waste	RPLC	61	705	This study
Hoboken	5 Apr 2007	Waste	RPLC	678	1,898	This study
Mol	5 Apr 2007	Waste	RPLC	48	230	This study
Lommel	5 Apr 2007	Waste	RPLC	112	553	This study
Po (Ita)	Apr–Sept 2006	Surface	RPLC	1.2	25	[6]
Llobregat (Spa)	Feb 2006	Surface	RPLC	6	77	[9]
Nosedo (Ita)	Apr–Sept 2006	Waste	RPLC	421	1,132	[7]
Lugano (Swi)	Mar 2006	Waste	RPLC	218	547	[7]
Catalonia (Spa)	Feb 2006	Waste	RPLC	79	810	[9]

Ita Italy, Spa Spain, Swi Switzerland

water samples with high dissolved concentrations of COC and BE. This supports the hypothesis that hydrophilic analytes, such as COC and metabolites, which have a low K_{ow} of 2.3 and 1.3 for COC and BE, respectively [10], are predominantly present in the dissolved aqueous phase and adsorb poorly or not at all onto solid particles. Indeed, Beausse [37] has indicated that pharmaceutical compounds need a certain degree of lipophilicity to adsorb onto solid particles and thus to be present in the sewage sludge results from waste water treatment. It is not yet clear how this affects the removal of COC and metabolites in WWTPs.

Analysis of Belgian waste and surface waters

To test the reliability of the optimised method, several influent waste water samples and surface water samples were analysed following the described protocol. Waste water samples were analysed using the RPLC-MS/MS (four WWTPs) and the HILIC-LC-MS/MS (two WWTPs) systems, while surface water samples were analysed using the more sensitive HILIC-LC-MS/MS system (Table 3). Blank water samples were obtained from a small, local water stream.

All WWTP samples contained COC and BE at concentrations higher than the LOQs. There were clear differences in the concentrations for COC and BE between the different WWTPs, but also between water samples collected from the same WWTP at different dates. Several parameters may account for these observed differences: (i) the water debit through the WWTP; (ii) the day of collection, since the use of COC might be higher during weekends; (iii) the number of people which are served by the WWTP; (iv) seasonal effects (e.g. summer–winter variation, rainfall and temperature).

The obtained concentrations for both waste water and surface water are in the same range as recent results from the literature [7–10]. As originally proposed by Daughton and Ternes [38] and later by Zuccato et al. [2], the measured concentrations of COC and metabolites (and illicit drugs in general) may serve to estimate the illicit drug usage at both the community and urban level. Furthermore, based on differences between profiles of COC and metabolites in urine and waste waters, the origin of COC in waste and surface waters, as well as the administration route, can be estimated.

Conclusion

This study is a part of a larger project which aims at monitoring cocaine and metabolites in the Belgian urban and surface water systems and which is a starting point for the estimation of the COC use of the general population.

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References

- Lakoski JM, Galloway MP, White FJ (1991) Cocaine: pharmacology, physiology and clinical strategies. CRC Press, Boca Raton
- Zuccato E, Chiabrando C, Castiglioni S, Calamari D, Bagnati R, Schiarea S, Fanelli R (2005) *Environ Health* 4:14–20
- Warner A, Norman AB (2000) *Therap Drug Monit* 22:266–270
- McCurdy HH, Callahan LS, Williams RD (1989) *J Forensic Sci* 34:858–870
- Dams R, Murphy CM, Lambert WE, Huestis MA (2003) *Rapid Commun Mass Spectrom* 17:1665–1670
- Weigel S, Kallenborn R, Huhnerfuss H (2004) *J Chromatogr A* 1023:183–195
- Castiglioni S, Zuccato E, Crisci E, Chiabrando C, Fanelli R, Bagnati R (2006) *Anal Chem* 78:8421–8429
- Hummel D, Löffler D, Fink G, Ternes TA (2006) *Environ Sci Technol* 40:7321–7328
- Huerta-Fontela M, Galceran MT, Ventura F (2007) *Anal Chem* 79:3821–3829
- Bones J, Thomas KV, Paull B (2007) *J Environ Monit* 9:701–707
- Moeller MR, Steinmeyer S, Kraemer T (1998) *J Chromatogr B* 713:91–109
- Van Bocxlaer JF, Clauwaert KM, Lambert WE, Deforce DL, Van den Eeckhout EG, De Leenheer AP (2000) *Mass Spectrom Rev* 19:165–170
- Gaillard Y, Pepin G (1997) *Forensic Sci Intern* 86:49–59
- Johansen SS, Bhatia HM (2007) *J Chromatogr B* 852:338–344
- Akre C, Fedeniuk R, MacNeil JD (2004) *Analyst* 129:145–149
- Biotage. Technical note 126; Sample preparation by non-polar SPE using Isolute® SPE sorbents; (<http://www.biotage.com>)
- Wylie FM, Torrance H, Seymour A, Buttress S, Oliver JS (2005) *Forensic Sci Intern* 150:199–204
- Rook EJ, Hillebrand MJX, Rosing H, van Ree JM, Beijnen JH (2005) *J Chromatogr B* 824:213–221
- Tindall GW (2002) *LC GC North America* 20:1114–1118
- Needham SR, Jeanville PM, Brown PR, Estape ES (2000) *J Chromatogr B* 748:77–87
- Jeanville PM, Estape ES, Needham SR, Cole MJ (2000) *J Am Soc Mass Spectrom* 11:257–263
- Grumbach ES, Wagrowski-Diehl DM, Mazzeo JR, Alden B, Iraneta PC (2004) *LC GC North America* 22:1010–1023
- Hemstrom P, Irgum K (2006) *J Sep Sci* 29:1784–1821
- Giroud C, Michaud K, Sporkert F, Eap C, Augsburger M, Cardinal P, Mangin P (2004) *J Anal Toxicol* 28:464–474
- Pizzolato TM, Lopez de Alda MJ, D Barcelo (2007) *Trends Anal Chem* 26:609–624
- Armbruster DA, Tillman MD, Hubbs LM (1994) *Clin Chem* 40:1233–1238
- Hernando M, Heath E, Petrovic M, Barceló D (2006) *Anal Bioanal Chem* 385:985–991

28. Dams R, Huestis MA, Lambert WE, Murphy CM (2003) *J Am Soc Mass Spectrom* 14:1290–1294
29. Fatta D, Nikolaou A, Achilleos A, Meric S (2007) *Trends Anal Chem* 26:515–533
30. Ambre J (1985) *J Anal Toxicol* 9:241–245
31. Reese TJ (1997) Pharmacokinetics of cocaine: considerations when assessing cocaine use by urinalysis. *NIDA Res Monogr* 175: 221–234
32. Baselt RC (2004) *Disposition of toxic drugs and chemicals in man*, 7th edn. Biomedical, Foster City, CA
33. Cone EJ, Sampson-Cone AH, Darwin WD, Huestis MA, Oyler JM (2003) *J Anal Toxicol* 27:386–401
34. Skopp G, Klingmann A, Pötsch L, Mattern R (2001) *Ther Drug Monit* 23:174–181
35. Maurer HH, Sauer C, Theobald DS (2006) *Ther Drug Monit* 28:447–453
36. Zhang JY, Foltz RL (1990) *J Anal Toxicol* 14:201–205
37. Beausse J (2004) *Trends Anal Chem* 23:753–761
38. Daughton CG, Ternes TA (1999) *Environ Health Perspect* 107 (suppl 6):907–938