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Determination of cocaine and its derivatives in hair samples by liquid phase microextraction (LPME) and gas chromatography–mass spectrometry (GC–MS)

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ABSTRACT

Hair testing is a recognized approach when it comes to accessing historical drug use. According to the World Drug Report of United Nations Office on Drugs and Crime (UNODC) 2015, Brazil is the largest cocaine (COC) market in South America. New analytical methodologies to detect crack/cocaine analytes in hair samples are highly desirable. Here, a method consisting of a liquid-phase microextraction (LPME) as a clean-up step, followed by gas chromatography–mass spectrometry (GC–MS) analysis has been proposed. The new validated method consisted of a washing step; an overnight incubation with methanol and a quick derivatization with butylchloroformate. Once derivatized, the samples were then submitted to the LPME procedure. Limits of detection (LoD) and quantitation (LoQ) obtained were of 0.1 and 0.5 ng/mg for COC 0.4 and 0.5 ng/mg for anhydroecgonine methyl ester (AEME); 0.03 and 0.05 for cocaethylene (CE), respectively and 0.05 ng/mg for both LoD and LoQ for benzoylecgonine (BZE). All calibration curves were linear over the scope applied, from LoQ up to 20 ng/mg, with a $r^2 > 0.99$. Precision and accuracy assays showed acceptable %RSD values, according to international guidelines. Twelve *postmortem* head hair samples stemming from the Institute of Legal Medicine of Sao Paulo (IML-SP) have been analyzed, from which seven have shown to be positive for COC (0.75–>20 ng/mg) and BZE (0.1–>20 ng/mg). Apart from COC's main metabolite, four samples were also positive for CE (0.1–3.9 ng/mg) and three samples for AEME (0.5–4.9 ng/mg). To conclude, the LPME technique together with GC–MS analysis have shown promising results and were able to meet the demand of the laboratory of analyzing *postmortem* hair samples to look for all four analytes.

1. Introduction

Cocaine (COC) abuse is an overwhelming worldwide problem capable of initiating a series of indirect public health issues. The latest estimates show that there are about 18.8 million COC users in the whole world and Brazil contributes to this number with an astonishing 4.4 million COC users in the country [1].

Consequently, there is an imminent urge for the development of new methodologies together with alternative matrices. Hair analysis is fast growing and it represents a very powerful tool for instance on retrospective investigations of chronic exposure due to its long window of detection or in the case of exhumed bodies as this matrix is extremely resistant to decay and exempt of any peculiar storage conditions other than room temperature, not to mention it does not suffer from *postmortem* redistribution phenomenon [2].

To attend to this demand, classical extractions have been in place for several years, such and liquid–liquid (LLE) or solid-phase (SPE) extractions. However, these are now being replaced by new 'green' procedures, as they are able to highly reduce solvent use, respond rapidly to a great demand and with significantly lower costing. Some of those techniques currently available comprise of solid-phase microextraction (SPME), stir-bar sportive extraction (SBSE), liquid-phase microextraction (LPME) and membrane microextraction with several different adaptations to their original form [3].

During this work, LPME has been employed after methanolic extraction of the compounds of interest for sample clean-up purposes. The technique of LPME itself has previously shown to be

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successful in the case of analyzing benzodiazepines in urine [4], cocaine and its derivatives in human breast milk [5] and even amphetamine [6] or phenobarbital in hair [7], however, the aim of this work was to develop a methodology capable of analyzing benzoilecgonine by means of derivatization prior to LPME clean-up, which is the novelty being proposed.

Therefore, we have aimed to detect COC and its main metabolite (benzoylecgonine—BZE) together with its pyrolysis product (anhydroecgonine methyl ester—AEME) and the product resulting from its concomitant use with ethanol (cocaethylene—CE) by gas chromatography–mass spectrometry (GC–MS) analysis (Fig. 1).

2. Material & methods

2.1. Reagents and reference standards

COC; AEME; CE and BZE solutions (1.0 mg/mL in acetonitrile or methanol) along with their deuterated internal standards COC-d3; CE-d3 and BZE-d3 solutions (100 μg/mL in acetonitrile or methanol) were purchased from Cerilliant Analytical Reference Standards (Round Rock, TX, USA). Butylchloroformate, pyridine, methanol and dihexyl ether were purchased from Sigma-Aldrich (St. Louis, MI, USA). Hydrochloric acid (HCl); acetonitrile; dichloromethane; anhydrous sodium bicarbonate ($NaHCO_3$) and potassium carbonate (K_2CO_3) were purchased from Merck (Darmstadt, Germany).

2.2. LPME device

Hollow-fiber Q3/2 Accurel KM polypropylene (600 μm i.d., 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany).

2.3. Instrumentation

The analyses were performed using an Agilent 6850 Network GC System gas chromatograph coupled with an Agilent® 5975 Series quadrupole mass selective detector (MSD) (Wilmington, DE, USA). Chromatographic separation was achieved on a HP-5MS fused-silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness) using helium as the carrier gas at 1.0 mL/min in a constant flow rate mode. Injections were made in the pulsed splitless mode. The MSD was operated by electronic impact (70 eV) in selected ion monitoring (SIM) mode. The temperature of the injector port was 250 °C, and the interface temperature was 280 °C. The oven ramp consisted of starting the oven's temperature at 90 °C for 1 min, increased by 15 °C/min up to 250 °C, held for 2 min and then increased once again by 25 °C/min up to 280 °C and held for 2 min, giving a total run time of approximately 17 min. The chosen qualifying and quantifying ions were the following (quantifying ions highlighted in bold): COC—m/z **182**, 272, 303; COC-d3—m/z **185**, 275, 306; AEME—m/z **152**, 166, 181; CE—m/z **196**, 272, 317; CE-d3—m/z **199**, 275, 320; BZE butyl ester—m/z **224**,

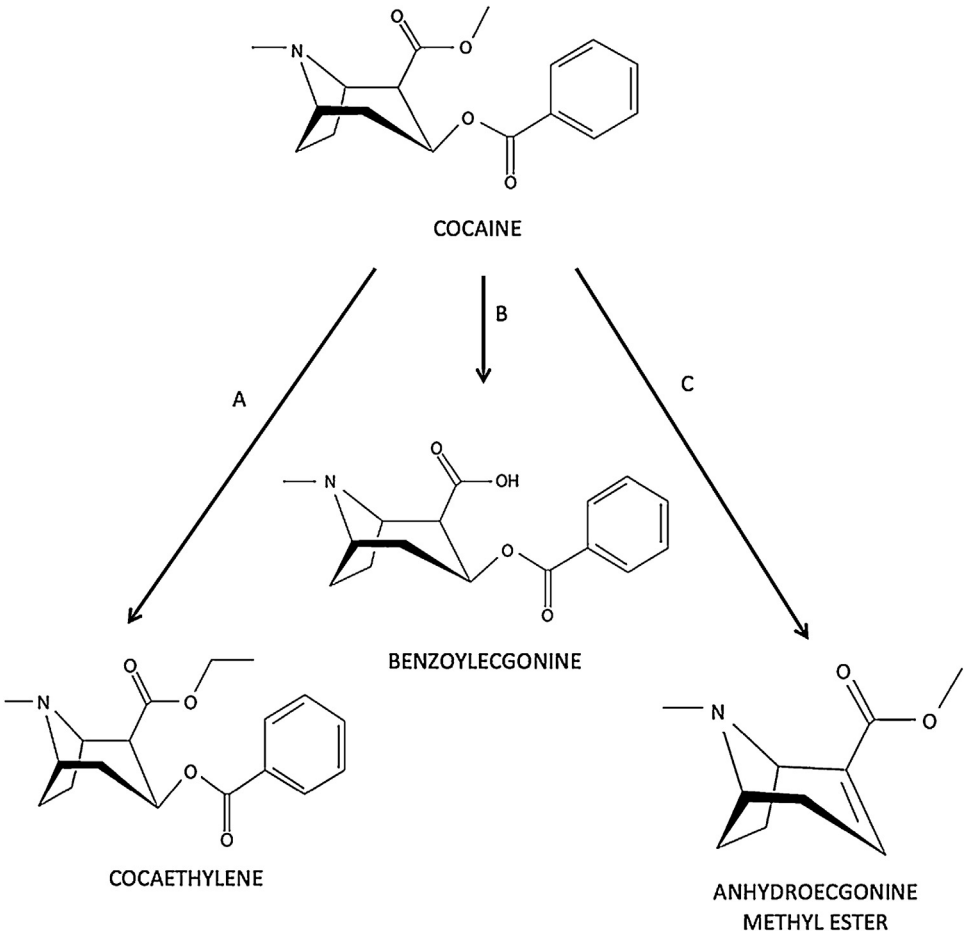

Fig. 1. Chemical structure of COC along with its major metabolite (BZE) and some of its derivatives (CE and AEME). (A) Hepatic transesterification; (B) spontaneous hydrolysis or hydrolysis mediated by carboxylesterases; (C) pyrolysis.

272, 345 and BZE butyl ester-d3—m/z **227**, 275, 348. The acceptance criteria used for qualification were based on retention times within 2% of those from reference standards analyzed under the same batch as well as a significantly satisfactory visual match when comparing their mass spectra and finally the ion ratio [8].

2.4. Head hair samples

Blank head hair samples were obtained from volunteers who reported not to have used any of the substances under this study. They have been used to carry out the development and further validation of the method. Real case *postmortem* head hair samples were obtained from Institute of Legal Medicine of Sao Paulo (IML-SP) through collection of a pencil-thickness lock of hair, near the scalp and from the upper vertex region. Samples have been packed using a foil sheet and stored in paper envelopes correctly labeled with their respective reference number. Collection of real case samples has been approved by both the Faculty of Pharmaceutical Sciences Ethics Committee of the University of Sao Paulo (USP) (Ethics Protocol Approval no.1.613.511) and the Faculty of Medicine Ethics Committee of USP (Ethics Protocol Approval no.1.671.423) as well as the Scientific Commission of IML-SP.

2.5. Sample extraction, derivatization and clean-up by LPME

After collection of head hair samples, they have been cut into extremely small pieces and for that matter, a hair segment of 4 cm from proximal to distal has been used. Furthermore, aliquots of 50 mg were separated and decontaminated by washing each aliquot with a mild detergent and water followed by 2.0 mL of dichloromethane for 15 min at 37 °C. Once dried, 2.0 mL of methanol were added to the falcon tubes along with the respective internal standards (COC-d3, CE-d3 and BZE-d3) at a concentration of 2.0 ng/mg. The tubes were then completely sealed with *parafilm M* for further incubation of the samples at 50 °C for 18 h to enhance drug liberation from the matrix. Once cooled, all the volume from the tube has been transferred to a 4 mL glass vial and contents were then evaporated under nitrogen (N_2) flow at 50 °C [7].

Once dried out, the residue obtained was submitted to derivatization with 100 μL of acetonitrile, 2.0 μL of pyridine and 2.0 μL of butylchloroformate under ultrasonic bath for 6 min. After that, the solution had its pH adjusted to 9–10 through the addition of 1.5 mL of deionized water and 30 mg of solid buffer ($NaHCO_3$: K_2CO_3—2:1) [9].

After derivatization, the contents were transferred to 2 mL eppendorf tubes and subjected to the LPME technique. This extraction process involved the immersion of a 9 cm propylene hollow fiber in dihexyl ether (organic phase) for about 15 s. After that, the lumen of the fiber was filled with about 50–70 μL of 0.05 M HCl (acceptor phase) using an electrophorese pipette tip. Both ends of the fiber were then closed up using a smooth jaw plier followed by insertion of the fiber into the solution on the eppendorf tube (see Fig. 2).

The system was then stirred by means of multi-tube horizontal shaker for 10 min at 2400 rpm. After that, the extract was then taken out, evaporated at 40 °C under N_2 flow and reconstituted with 50 μL of ethyl acetate. Finally, 1.0 μL of the extracted solution was injected into the GC–MS system.

2.6. Method validation

The proposed method has been validated according to the recommended international parameters. For that matter the following guidelines have been consulted: Scientific Working Group for Forensic Toxicology [10]: 'Standard Practices for Method Validation in Forensic Toxicology'; Society of Hair Testing [11]: 'Society of Hair Testing guidelines for drug testing in hair'; The European Workplace Drug Testing Society [12]: 'Guidelines for European workplace drug and alcohol testing in hair' and United Nations Office on Drugs and Crime [8]: 'Guidance for the Validation of Analytical Methodology and Calibration of Equipment used for Testing of Illicit Drugs in Seized Materials and Biological Specimens'.

The following parameters have been evaluated: selectivity; limit of detection (LoD); limit of quantitation (LoQ); linearity; precision (intra and inter-day); accuracy and carryover.

2.6.1. Selectivity

Selectivity assay was evaluated through the analysis of six zero samples (containing only internal standard) and two blank

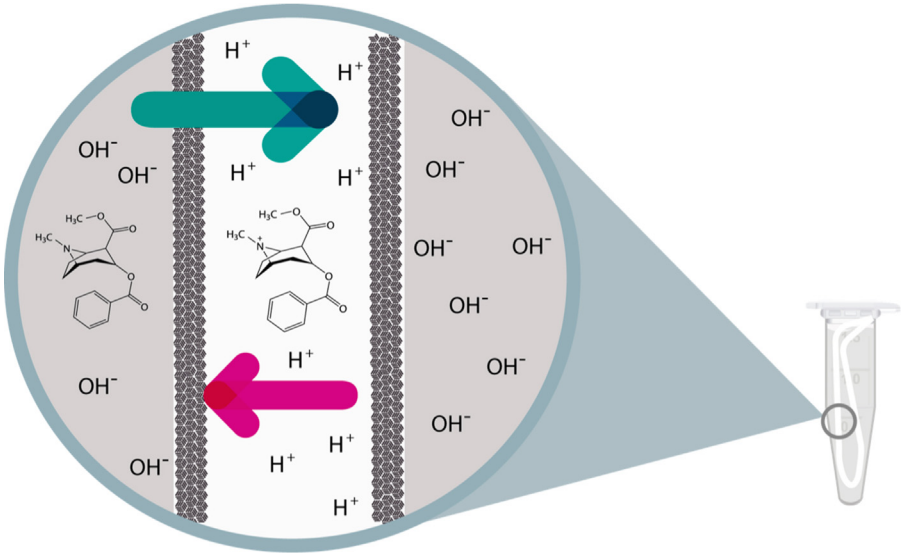

Fig. 2. LPME illustration showing the chemical structure of COC migrating into the hollow fiber and once it becomes ionized due to the acidic environment, it can not travel back to the solution, therefore being trapped inside the fiber.

samples (no substance added). The presence or absence of any interfering peaks (endogenous substances) near the retention time of the analytes has been assessed.

2.6.2. Limit of detection (LoD) and limit of quantitation (LoQ)

The LoD was estimated by using three different blank head hair samples, analyzed in duplicate over three runs. The mean and standard deviation of all negative samples were calculated. Likewise, spiked hair samples with decreasing concentrations were also evaluated. The lowest concentration of a spiked hair sample capable of producing a signal greater than the average of the negative signal samples (X) plus 3.3 times the standard deviation (s) was identified as being the LoD:

$$LoD = X + 3.3\,s$$

For the determination of LoQ, three samples of a known concentration were analyzed over three runs in order to show that all the necessary criteria for detection; identification; precision and accuracy have been reached (relative standard deviation—% RSD < 15%). Both LoD and LoQ obtained retention times with a maximum variation of ±2% and mass spectra with the same appearance and ion proportion.

2.6.3. Linearity

The study of linearity was estimated by the analysis of extracted samples obtained from aliquots of spiked hair, in five replicates, with an internal standard concentration of 2 ng/mg. Calibration curve linear ranges for COC and AEME were 0.5–20 ng/mg. For CE and BZE quantification two curves were constructed at low (0.05–1.0 ng/mg) and high (1.0–20 ng/mg) concentrations. The phenomenon of heteroscedasticity was evaluated by application of the *F-test*.

2.6.4. Intra and inter-day precision

Intra-assay precision has been accessed by the analysis of three quality control (QC) levels in five replicates on a single day, while inter-assay precision has been determined by the analysis of three QC levels in five replicates for three consecutive days.

The results obtained from this experiment are expressed as % RSD and were calculated through ANOVA single factor's function in Microsoft Excel®. The three QC levels studied were: low (LQC); medium (MQC) and high (HQC). Considering that LQC concentrations shall be approximately three times the lowest end of the working range of the method and HQC concentrations shall be within approximately 80% (or more) of the highest end of the working range of the method while MQC concentrations shall be near the midpoint of the low and high concentrations [10]. The acceptance criteria used was of 20% for LQC and 15% for MQC and HQC.

2.6.5. Preparation of calibrators and quality control (QC) samples

Quality control samples have been prepared by spiking the appropriate solutions at concentrations of 1.5 ng/mg for COC and AEME as their LQC and 0.2 ng/mg for the remaining CE and BZE.As for the MQC and HQC, solutions have been spiked at 9.0 and 18.0, respectively, for all four analytes of interest, as stated in Table 1.

2.6.6. Accuracy

Accuracy assay was performed by the quantification of five replicates for each QC level by using a previous calibration curve. The results obtained from these experiments were expressed as a percentage of the known concentration value:

$$\frac{mean\ \ concentration\ \ measured\ -\ \ nominal\ \ concentration}{nominal\ \ \ concentration} \times 100$$

Table 1
Low, medium and high QC level values used within the method for the determination of COC and its metabolites in *postmortem* head hair samples, all expressed as ng/mg. For COC and AEME the LQC was 1.5 ng/mg while for CE and BZE this value was of 0.2 ng/mg. As for MQC and HQC the values obtained were of 9.0 and 18.0 ng/mg, respectively, for all four analytes.

Analytes	LQC (ng/mg)	MQC (ng/mg)	HQC (ng/mg)
COC	1.5	9.0	18.0
AEME	1.5	9.0	18.0
CE	0.2	9.0	18.0
BZE	0.2	9.0	18.0

The acceptance criteria used was of 20% for LQC and 15% for MQC and HQC.

2.6.7. Carryover

Carryover has been tested through the analysis of three blank samples injected into the GC–MS system immediately after the highest concentrated sample in the calibration curve.

3. Results and discussion

3.1. Sample preparation

3.1.1. Incubation

Several research groups have defended overnight incubation of hair as being favorable for drug removal from the specimen [13–17]. The use of methanol is significantly well established in the literature for the extraction of various analytes such as COC and its metabolites. It is known to be effective in the extraction of drugs from the hair matrix and also, it is readily evaporated, which aids on the steps to follow of the procedure which require a dry residue for derivatization purposes [13].

3.1.2. Derivatization step

Typically, BZE is extracted by mixed-phase SPE technique, which allows both the acidic and the basic part of the molecule to be extracted as we are dealing with an amphoteric compound [18].

In the presented methodology, BZE goes through a derivatization step prior to extraction. This particular part of the procedure was based on the method developed by Toledo et al. in which the dry residue is treated with a mixture of pyridine, acetonitrile and butyl-chloroformate prior to extraction by SPME [9]. This derivatization agent has been used as it is capable of remaining stable under the aqueous conditions being proposed.

Butylchloroformate reacts with the carboxylic acid from BZE forming a mixed anhydride derivative of BZE, which will then suffer decarboxylative esterification to yield a final product named butyl-BZE. Meanwhile, pyridine is used as a catalyst to aid in the reaction (see Fig. 3) [19].

In fact, chloroformates have been previously used for derivatization and they have produced satisfactory results [20]. To be even more specific, BZE has been derivatized using chloroformate compounds in previous analytical works [21].

On a more recent note, Chericoni et al. presented a method to determine BZE in urine by GC–MS using liquid–liquid extraction (LLE) where they have successfully used propyl-chloroformate as a derivatization agent [22]. Not only this process makes it able to extract BZE through other techniques rather than mixed-phase SPE but also it increases sensitivity of the method.

3.2. Clean-up by LPME

LPME has proven to be fit-for-purpose and able to attend to the demand of the laboratory. Also, this is a very versatile technique,

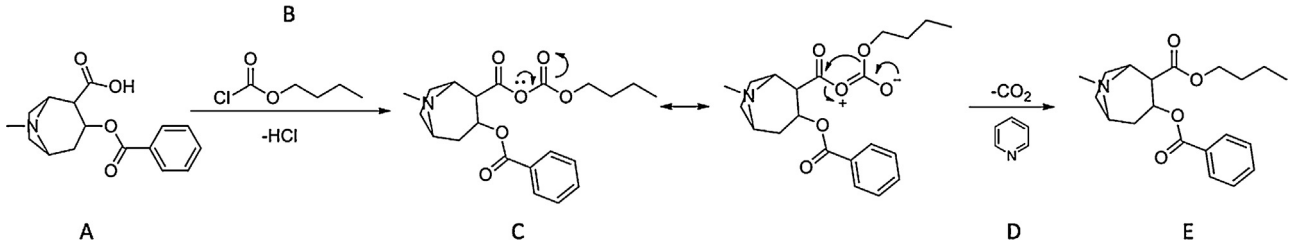

Fig. 3. Chemical reaction showing the mecanism for the formation of butyl-BZE. (A) BZE; (B) butylchloroformate; (C) mixed anhydride derivative of BZE; (D) pyridine; (E) butyl-BZE.

capable of extracting a variety of substances in different types of matrices and substances as previously described in the literature [4–7].

The LPME employed in this work is used to clean-up the methanolic extracts and it is operated in its three-phase mode, which means that the analytes are transferred from an aqueous sample (donor phase) through an organic solvent immobilized in the pores of the hollow-fiber, and into a new aqueous phase inside the lumen of the hollow-fiber serving as the acceptor phase [23].

In this case, the donor phase containing the sample had its pH adjusted (9–10) so that the analytes could remain in their molecular form and therefore being able to travel through the organic phase (dihexyl ether) into a new acidic environment (hydrochloric acid) thereby being retained in the fiber lumen in their ionized form (see Fig. 2).

The cautious pH adjustment, in between 9 and 10, not only allows the analytes to travel to within the lumen of the fiber, but also, it is a crucial step to diminish the possibility of fast COC hydrolysis to BZE, ecgonine methyl ester (EME) and ecgonine under highly alkaline environments. This is accomplished through the addition of a buffer solution.

This technique has proven to be much cheaper when compared to conventional LLE (0.98 USD/per extraction) or SPE (1.72 USD/per extraction) procedures and even SPME (2.04 USD/per extraction) as the price is of approximately 0.36 USD per LPME extraction. Although SPME is also known as a microextraction technique, it comprises of many other disadvantages apart from a costly apparatus, such as its short life and high likelihood of carryover.

LPME is both an environmental and user-friendly technique due to the scarce volume of hazardous solvents used, it is easy to perform and it definitely avoids the risk of carryover as all hollow fibers are disposable. However, the major drawback of LPME is still the absence of devices for automation.

3.3. Method validation

Method validation has shown to be successful. The method showed no interfering peaks at the retention times of interest, therefore being selective for the all analytes.

The LoD values obtained in this method were of 0.1; 0.4; 0.03 and 0.05 ng/mg while the LoQ values achieved were of 0.5; 0.5; 0.05 and 0.05 ng/mg for COC; AEME; CE and BZE, respectively. These are considered to be suitable for this study as they follow the recommendations from the Society of Hair Testing (SoHT) for cut-off values of COC (0.5 ng/mg) and its metabolites (0.05 ng/mg) [11]. As AEME does not have any pre-established cut-off value, considering its usage pattern, the values obtained seemed rather adequate.

The linear range studied started at the LoQ, up to 20 ng/mg for COC and AEME. For CE and BZE, two calibration curves have been done, as the magnitude range between the calibration points used was too distant. Therefore, an initial curve from LoQ to 1.0 ng/mg was in place, followed by a second one from 1.0 to 20 ng/mg [15].

The method has shown to be linear for all analytes with r^2 values above 0.99. Heteroscedasticity phenomenon was observed through the *F-test* and the following Table 2 presents the data for the LoD and LoQ values, concentration ranges, calibration curve equations, weighing factors and coefficient of determination after proper correction weights have been placed. The x and y letters represent concentration and peak area, respectively.

Both intra and inter-day precision have met the criteria established by international guidelines and the results for these assays can be seen in Table 3.

Values from intra-day precision have ranged from 1.5 to 11.9% while inter-day precision showed a variation range between 2.1 and 14.4% for all three levels. As it can be seen, the highest values refer to AEME and this is most likely due to the fact that it is the only substance without an analog deuterated standard of its own and for this purpose CE-d3 has been used.

Accuracy has also proven to be within the accepted international criteria for method validation and the values are shown in Table 4.

Overall, the lowest value obtained was of 84.5% (LQC of BZE) and the highest 99.6% (HQC of COC) which shows that the range is within acceptable values.

The method has shown no carryover through the absence of peaks at the retention times of analytes on all three consecutive blank samples, injected straight after the highest calibration point.

Table 2
Linearity results from the method for determination of COC and its metabolites in *postmortem* head hair samples for all analytes and their respective calibration curves. Values being shown are: LoD and LoQ; concentration ranges; calibration curve's equations; weighing factors applied and resulting r^2 after adequate correction using the statistical tool '*F-test*'.

Analytes	LoD (ng/mg)	LoQ (ng/mg)	Concentration range	Calibration curve equation	Weight factor applied	r^2
COC	0.1	0.5	LoQ to 20 ng/mg	y = 0.5505x – 0.0052	1/x	0.999
AEME	0.4	0.5	LoQ to 20 ng/mg	y = 0.5765x + 0.009	Homocedastic	0.995
CE	0.03	0.05	LoQ to 1.0 ng/mg	y = 0.5804x + 0.015	Homocedastic	0.999
			1.0–20 ng/mg	y = 0.5405x – 0.0357	$1/x^2$	0.999
BZE	0.05	0.05	LoQ to 1.0 ng/mg	y = 0.5758x + 0.021	Homocedastic	0.997
			1.0–20 ng/mg	y = 0.5306x – 0.3647	$1/y^2$	0.997

Table 3
Precision results from the method for determination of COC and its metabolites in *postmortem* head hair samples for all QC levels and their respective intra and inter-day precision values expressed as relative standard deviation (%).

Analytes	Precision (%RSD)					
	Intra-day			Inter-day		
	LQC	MQC	HQC	LQC	MQC	HQC
COC	5.9	1.5	3.4	7.5	4.2	4.2
AEME	6.2	6.9	11.9	12.9	14.4	12.1
CE	2.7	3.3	4.0	2.6	3.9	5.0
BZE	2.2	2.1	4.1	2.1	2.8	4.8

Table 4
Accuracy results from the method for determination of COC and its metabolites in *postmortem* head hair samples for all QC levels and their respective values expressed as a percentage (%).

Analytes	Accuracy (%)		
	LQC	MQC	HQC
COC	99.6	94.3	97.6
AEME	93.5	96.3	95.6
CE	94.3	93.1	97.2
BZE	84.5	97.2	98.2

3.4. Method application

After adequate validation of the proposed method, 12 real case *postmortem* head hair samples have been analyzed, from which seven are highly likely to be positive for COC and BZE along with four samples for CE and three samples for AEME. Table 5 shows all positive samples and their respective values for all analytes of interest.

It is known that interpretation of drug hair concentrations still remains one of the most critical matters in forensic analysis. This is due to external contamination, especially with smoked drugs such as crack as its likelihood of polluting one's hair is considerably higher [24].

Table 5
Postmortem head hair samples results for all analytes under study.

Samples	Analytes			
	COC (ng/mg)	CE (ng/mg)	BZE (ng/mg)	AEME (ng/mg)
1	4.7	0.0	0.6	1.6
2	>20	0.0	7.7	4.9
3	>20	3.9	>20	0.2
4	9.1	0.2	0.9	0.2
5	15.2	0.1	1.6	0.5
6	0.7	0.0	0.1	0.0
7	1.4	0.9	0.4	0.0

Washing procedures have been in place for several years and many authors consistently support them as an essential step to avoid this external contamination [24]. However, this topic is constantly under debate as some studies do consider this wash step inefficient.

Indeed, washing procedures are considered not to be sufficient to fully eliminate external contamination of hair [24–27]. For this reason, wash to hair ratios (W/H), proposed by Tsanaclis and Wicks have been taken into account [28].

Moreover, other positivity criteria have been applied, such as the cut-off levels proposed by SoHT as well as the presence of endogenous metabolites in order to avoid wrongful results when interpreting data from real *postmortem* cases.

For the matter of this work, wash waters have been both extracted and further analyzed under the same conditions as those proposed. All COC-positive samples have presented W/H ratios <0.1, indicating likely drug use [28]. Considering these ratios are most frequently used in the case that no other metabolite has been found, in this study, all COC-positive samples have also been positive for BZE.

Finally, all drug concentrations encountered did obey to the cut-off levels proposed by the SoHT. The following Fig. 4 shows the chromatograms of a real *postmortem* case sample (sample number 5 under Table 5) and Fig. 5 shows the chromatograms of a spiked sample at the lowest calibration point containing the different LoQ for all analytes under study.

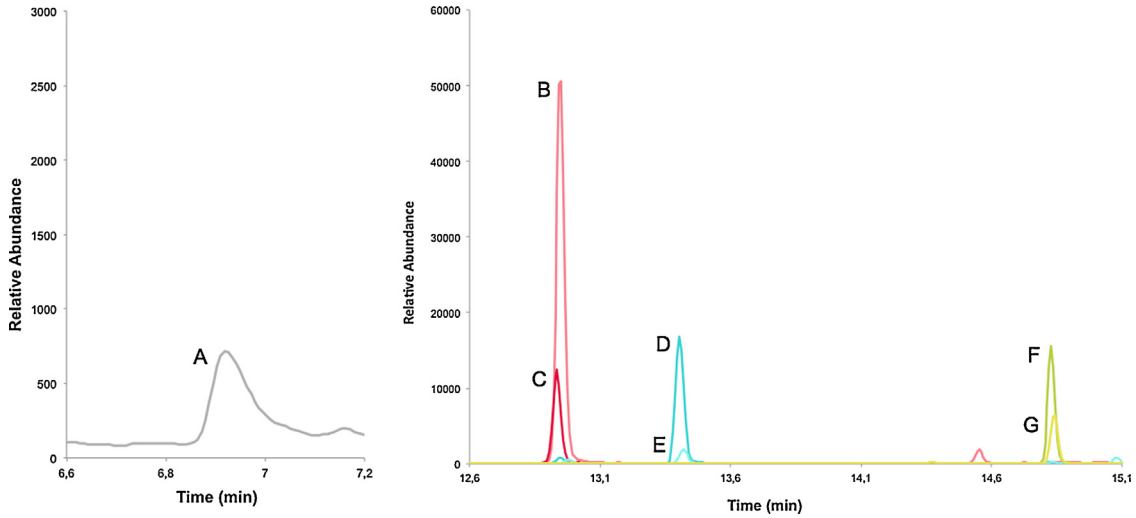

Fig. 4. GC–MS chromatogram from the analysis of a real *postmortem* head hair sample from IML-SP, obtained through the application of the LPME method, where A—AEME (m/z 152); B—COC (m/z 182); C—COC-d3 (m/z 185); D—CE (m/z 196); E—CE-d3 (m/z 199); F—BZE butyl ester (m/z 224) and G—BZE butyl ester-d3 (m/z 227). The resulting concentrations found for all analytes were: A—0.5 ng/mg; B—15.2 ng/mg; E—0.1 ng/mg and G—1.6 ng/mg, while their respective deuterated internal standards (C, D and F) were added at a concentration of 2 ng/mg.

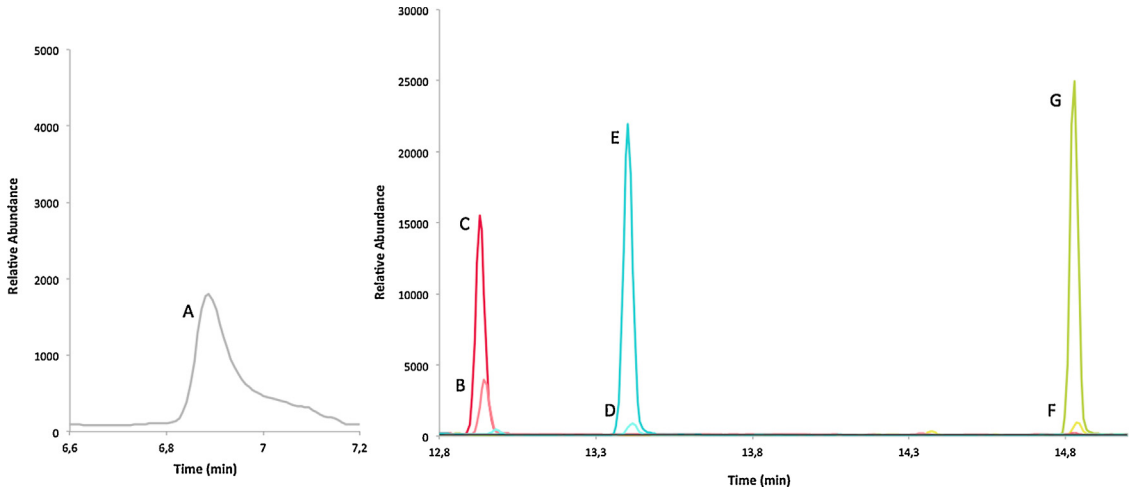

Fig. 5. GC–MS chromatogram of a spiked head hair sample at the lowest calibration point, obtained through the application of the LPME method, where A—AEME (m/z 152): 0.5 ng/mg; B—COC (m/z 182):0.5 ng/mg; C—COC-d3 (m/z 185):2 ng/mg; D—CE (m/z 196):0.05 ng/mg; E—CE-d3 (m/z 199):2 ng/mg; F—BZE butyl ester (m/z 224):0.05 ng/mg and G—BZE butyl ester-d3 (m/z 227):2 ng/mg.

4. Conclusion

In this present work, a new method for the determination of COC and its derivatives in hair specimens was developed. The method has been successfully validated following international guidelines and both extraction technique (LPME) and chromatographic analysis (GC–MS) have shown to be suitable and fit-for-purpose. Real case samples have been effectively analyzed and quantified, therefore, it is possible to affirm that the proposed method is capable of being applied to different situations, as needed. To the best of our knowledge this is the first method for the analysis of hair samples where benzoylecgonine can be detected by means of a derivatization step prior to the LPME clean-up step.

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Conflict of interest

All authors report no conflict of interest.

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