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Short communication

Analysis of hydroxy-cocaine metabolites as evidence of cocaine consumption: Identification by parent ion search and quantitation by UHPLC-MS/MS in hair sample

Mauro Minoli [a], Sara Casati [a], Ilaria Angeli [a], Alessandro Ravelli [a], Paola Rota [b], Pietro Allevi [b], Marica Orioli [a, *]

[a] Laboratory of Forensic Toxicology, Department of Biomedical, Surgical and Dental Science, University of Milan, 20133, Milan, Italy
[b] Department of Biomedical, Surgical and Dental Sciences, University of Milan, 20133, Milan, Italy

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ABSTRACT

Over the last decade, hair analysis has become a routine procedure in most forensic laboratories and, complementary to blood and urine, hair is a unique biological matrix which gives the opportunity to establish a temporal consumption profile. Despite hair is widely used to identify drug use, environmental contamination continues to represent a challenging factor of this procedure, especially for cocaine (COC). In the last few years several strategies have been proposed in order to distinguish between actual use and external contamination, however the commonly detected COC metabolites probably are insufficient for demonstrating cocaine use through hair testing. Thus, the aim of this study is to develop an ultra high performance liquid cromatography - tandem mass spectrometry (UHPLC-MS/MS) method able to detect and quantify hydroxy-COC metabolites, as specific markers of COC abuse, in hair samples from COC consumers, thus enabling unambiguous evidence of COC consumption. At the beginning, since no commercial reference materials were available, COC-positive hair samples were tested using parent ion scan-based analysis to extract hydroxy COC metabolites target ions. Once identified, the reference materials were synthesized by our analytical laboratory allowing the development of the first UHPLC-MS/MS validated method to quantify p- and m-isomers of hydroxy COC, as well as hydroxy benzoylecgonine (BE) and hydroxy norcocaine (NCOC). The method was successfully applied to a large number of COC-positive hair samples and introduced into a routine procedure for testing drug ingestion in order to evaluate for the first-time hydroxy metabolites of COC ranges in hair and their correlation with COC and BE.

1. Introduction

Interpretation of hair analytical results continues to be a serious challenge in the field of forensic toxicology. Complementary to blood and urine, hair is a unique biological matrix which enables to establish a temporal (chronic or occasional) consumption profile [1]. Moreover, hair analysis offers many advantages such as a non-invasive sampling, an easy storage and a wide time window. In general, both active drugs and their metabolites are retained in the hair shaft, depending on their chemical structure and their affinity with melanin [1]. Testing for drug metabolites in hair analysis is a common practice to differentiate intake from passive contamination. This is especially important for drugs which are commonly sniffed or smoked (e.g. cocaine) because hair can be easier contaminated through smoke or dust. Several studies were performed about the effectiveness of different hair washing procedure at removing COC and its metabolites. Unfortunately, none of these procedures resulted sufficient enough to completely remove them [2,3]. The implementation of extensive washing hair steps is a point of discussion in the scientific community due to the possibility of unintentional and unpredictable drug extraction and/or artefacts formation [4]. Also for this reason, COC hair testing procedures contemplate additional test for its main metabolites, such as BE, ecgonine methyl ester (EME), norcocaine (NCOC) or coca-ethylene (CE), the latter formed if COC has been used in combination with alcohol [5]. Although COC metabolites would appear to be a solution to discriminate consumption from external contamination issue, BE, EME, NCOC have all been detected as impurities in street COC as a by-product of the manufacturing process [6,7]. BE might also be slowly formed from COC within keratinized hair through a non-metabolic process [8] or during sample preparation; NCOC is present as an alkaloid in Erythroxylon coca Lam. leaves [9], but it is mainly formed through COC treatment with potassium permanganate during its illicit production process [7]. Thus, the presence of commonly detected COC metabolites appears to be insufficient for demonstrating unequivocally COC use through hair testing. According to the Substance Abuse and Mental Health Services Administration (SAMHSA), the detection of COC at concentrations higher than 500 pg/mg and of BE at concentrations higher than 50 pg/mg plus a BE/COC ratio greater than 5%, or alternatively,

* Corresponding author.
Email address: marica.orioli@unimi.it (M. Orioli)

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Table 1
MRM parameters: Dwell time, de-clustering potential (DP), collision energy (CE), precursor and product ion transitions for p- and m-OH-COC, p- and m-OH-BE, p- and m-OH-NCOC, m-OH-BE-d3.

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell time (msec)	DP (eV)	CE (eV)
p-OH-COC	320.3	182.0	70	110	38
		121.1	70	110	38
		200.0	70	110	47
m-OH-COC	320.3	182.0	70	110	38
		121.1	70	110	38
		93.0	70	110	47
p-, m-OH-BE	306.2	168.0	70	85	20
		121.1	70	85	35
		150.1	70	85	29
p-, m-OH-NCOC	306.2	168.0	70	85	20
		136.0	70	85	27
		121.1	70	85	25
m-OH-BE-d3	309.0	171.1	70	85	25
		153.1	70	85	30
		121.1	70	85	38

the detection of NCOC and CE at concentration higher than 50 pg/mg indicate an active COC use [10]. However, if COC, BE, CE and NCOC are currently the main target compounds in routine COC hair-testing analysis, further metabolites have also been detected in several matrices after COC consumption. For example, eleven COC metabolites in urine specimen from COC user were identified by Zhang and Foltz including also m-hydroxy-benzoylecgonine (m-OH-BE), p-hydroxy-cocaine (p-OH-COC), and m-hydroxy-cocaine (m-OH-COC) [11]. Over the last two decades, above-mentioned and other hydroxy cocaine metabolites formed by liver enzymes during body passage have been widely characterized in urine, plasma, blood and oral fluid specimens [12–14]. Considering the diffusion of these hydroxy metabolites from blood to hair during hair fiber formation, their determination could be the starting point to enable unambiguous evidence of COC consumption. First studies in hair have allowed the detection of o-, p- and m-OH-COC [15,16], however there are some evidence of m-OH-COC observed in seized samples [7]. According to the European Guidelines for Workplace Drug and Alcohol Testing in Hair [17] for COC actual use confirmation, testing should cover COC and at least two metabolites among BE, CE, NCOC or EME, and hydroxy metabolites of COC at cut-off concentrations of 0.05 ng/mg hair, respectively. To date, as recently showed by Franz et al., only p- and m-OH-BE as well as p- and m-hydroxy-norcocaine (m-OH-NCOC) could not be identified from seized samples [18].

This study is aimed to set up and validate an UHPLC-MS/MS (ESI) method in Multiple Reaction Monitoring (MRM) mode in order to analyze and quantify the main hydroxy COC metabolites in authentic COC-positive hair specimens. To achieve this goal, as first step we used a parent ion scan-based approach in order to obtain a qualitative profile of hydroxy COC metabolites in hair samples. As second step, we obtained the reference materials by a self-made synthesis performed in our laboratory, to our knowledge for the first time, at least for OH-NCOC. Finally, in order to avoid the risk of false positive in discussed COC-positive cases, the method was introduced into a routine procedure for testing drug ingestion (n = 100) allowing the evaluation of hydroxy COC metabolites ranges in hair and their correlation with COC and BE.

2. Experimental section

2.1. Chemicals and reagents

Ultrapure water, acetonitrile, dichloromethane, isopropanol, methanol, ethyl acetate, n-exane, hydrochloride acid and sodium hydroxide were of analytical grade and purchased from Carlo Erba (Milan, Italy). Formic acid (98%–100%) and cocaine were from Sigma–Aldrich (Milan, Italy). The reference materials m- and p-OH-COC, m- and p-OH-BE, m- and p-OH-NCOC, m-OH-BE-d3 were custom-synthesized by our analytical laboratory. The 96-well plate SPEC MP1 was obtained from Agilent Technologies (Palo Alto, CA, USA).

2.2. Standard solutions, calibrators and quality control samples

Stock solutions of m- and p-OH-COC, m- and p-OH-BE, m- and p-OH-NCOC, m-OH-BE-d3, COC-d3 and BE-d3 were prepared at the final concentration of 0.01 mg/mL in methanol. All the solutions were stored in the dark at – 20 °C. Working solution was prepared from stock solutions by appropriate dilution in methanol and used for the preparation of calibration curves and quality control (QC) samples, at the following concentrations: 200 ng/mL for m- and p-OH-COC, 40 ng/mL for m- and p-OH-BE and 20 µg/mL for m- and p-OH-NCOC.

Calibration standards containing

- 1, 2, 5, 10, 20, 50, 100 and 200 pg per mg hair for m- and p-OH-COC;
- 0.2, 0.4, 1, 2, 4, 10, 20, and 40 pg per mg hair for m- and p-OH-BE;
- 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 pg per mg hair for m- and p-OH-NCOC;
- 100 ng for m-OH-BE-d3 (IS);

were prepared daily for each analytical batch by adding suitable amounts of working solutions to 50 mg of pre-checked drug-free hair pool samples.

2.3. Hair sampling procedure and preparation

Pre-checked drug-free human hair samples were collected from 10 volunteers and analyzed to exclude any source of chromatographic interferences and then mixed to obtain a homogeneous pool of blank hair to be used for calibration standards and quality control samples. Hair samples (at least 3 cm length each) for this study were collected from male and female cocaine users (n = 100), tested for drugs chronic abuse in our Laboratory from 2017 to 2018. Diagnosis of chronic drugs abuse was requested for different purposes, such as driving license renewal, adoptions or child custody. All hair samples (calibration standards, quality control and real samples) were reduced in short cuts (2–4 mm) and 50 mg were washed with 3 mL dichloromethane and then dried. Hair extraction was performed in accordance to an internal validated procedure [19]. In brief, samples were hydrolyzed overnight at 45 °C in 2 mL of 0.1 M HCl. The aqueous solution was then transferred into test tubes and placed in an automated SPE workstation for the extraction step.

2.4. UHPLC-MS/MS conditions and parameters

Analyses were performed on 1290 Infinity UHPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled to a Q Trap 5500 triple quadrupole linear ion trap mass spectrometer (Sciex, Darm-

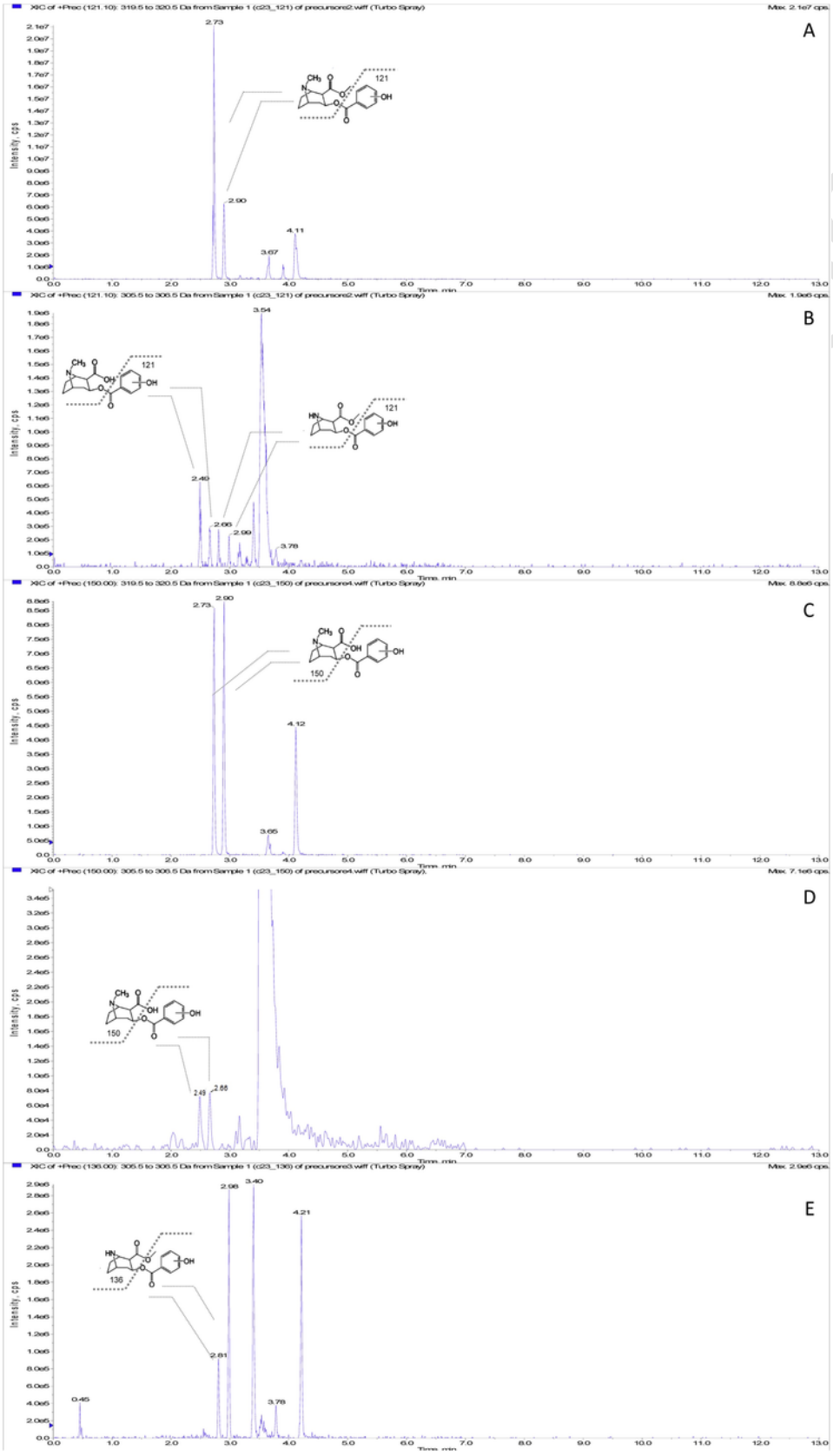
A
B
C
D
E
Intensity, cps
Time, min
121
150
136

Fig. 1. Extracted ion current chromatograms at 320.0 *m/z* ([M + H]+ OH-COC) (A) and 306.2 *m/z* ([M + H]+ OH-BE and OH-NCOC) (B) using a parent ion scan at *m/z* 121 corresponding to p-hydroxybenzoate fragment, at 320.0 *m/z* ([M + H]+ OH-COC) (C) and 306.2 *m/z* ([M + H]+ OH-BE and OH-NCOC) (D) using a parent ion scan at *m/z* 150 corresponding to tropane fragment and at 306.2 *m/z* ([M + H]+ OH-NCOC) (E) using a parent ion scan at *m/z* 136 corresponding to nor tropane fragment, obtained from a representative sample.

stadt, Germany). Compounds were separated on a Kinetex UHPLC XB-C18 column (100 mm x 2.1 mm i.d, 2.6 p.s.) (Phenomenex, CA, USA) using 0.5% formic acid in water containing 1 mM ammonium acetate (mobile phase A) and 0.5% formic acid in acetonitrile with 1 mM ammonium acetate (mobile phase B). Solvent A and B were 95% and 5% at 1.00 min, respectively. Solvent B was increased to 55% from 1.00 to 7.00 min, then increased to 98% from 7.00 to 7.50 min, held at 98% from 7.50 to 9.00 min, and then decreased back to 5% from 9.40 to 9.50 min and held at 5% from 9.50 to 11.00 min for re-equilibration. The flow rate was 0.60 mL/min, and the injection volume was 1 μL. The column thermostatic oven was kept at 38 °C. The working conditions and parameters of the MS were optimized as follows: the ion source was ESI operated in positive mode; resolution of Q-1 and Q-3 was 0.7 ± 0.1 amu; the curtain gas, ion source gas 1, and ion source gas 2 were set at 25, 45 and 10 psi, respectively; the source temperature was 550 °C; the ionization voltage was 5500 eV; the entrance potential was 10 eV; dwell time was fixed 70 msec for each MRM transitions. MS acquisition was performed by both precursor-ion scan and MRM mode, at the same LC and MS instrumental condition. For parent ion scan analysis, the following product ions were selected: *m/z* 121, 150 and 136. MS parameters for precursor-ion scan mode were set as follow: the de-clustering potential was 110, the collision energy was 20–45 eV, the scan range was from 200 to 600 *m/z* and the dwell time was fixed 400 msec for each precursor-ion scan. The MRM conditions and parameters including ion transitions, de-clustering potential (DP) and relative collision energy (CE) are provided in Table 1.

The data acquisition and processing were performed using Analyst®1.6.2 and MultiQuant®2.1.1 software (Sciex, Darmstadt, Germany), respectively.

2.5. Validation procedure

The method was fully validated on hair samples according to the Society of Hair Testing (SoHT) Guidelines for Drug Testing in Hair [2].

To evaluate the carry-over caused by sample processing, a matrix blank sample was processed immediately after the injection of a high concentration sample. The high concentration sample was prepared by fortifying a blank hair sample fortified with all the analytes at concentration 2.5 times higher than the upper limit of quantification (ULOQ).

3. Results and discussion

3.1. Precursor ion monitoring for qualitative analysis of COC-positive hair specimens

Since no commercial reference materials were available in the beginning of our study, the first step was to analyze COC-positive hair samples by precursor ion scanning technique, a valuable tool for the rapid confirmation of targeted compounds or for the nontargeted detection of compounds bearing a common moiety. The aim of this approach was to qualitative identify the OH-COC metabolite, without standards available. First, we used the p-hydroxybenzoate fragment at *m/z* 121 as the well-known common fragment ion of OH-COC metabolites to generate precursor ion spectra in order to screen COC-positive hair samples for the presence of the above-mentioned metabolites. The precursor ion mass spectra were evaluated in order to identify the *m/z* of the precursor ions. Fig. 1 reports the extracted ion current chromatograms at 320.0 *m/z* ([M + H]+ OH-COC; a) and 306.2 *m/z* ([M + H]+ OH-BE and OH-NCOC; b), scanned in a range between 200 and 600 *m/z*, obtained from a representative sample. In the chromatogram reported in Fig. 1a, we can observe the presence of two [M + H]+ signals with retention times of 2.73 and 2.90 min. These signals were tentatively assigned to the p- and m-OH-COC isomers, respectively. In the chromatogram reported in Fig. 1b, we can observe the presence of six [M + H]+ signals with retention times of 2.49, 2.66, 2.81, 2.99, 3.16 and 3.40 min. These signals were tentatively assigned to p-,m-,o-OH-BE (2.49, 2.66, 3.16 min, respectively) and p-,m-,o-OH-NCOC (2.81, 2.99, 3.40 min, respectively). The same approach was used by selecting, as common fragment ion, the tropane moiety at *m/z* 150, peculiar of OH-COC and OH-BE (Fig. 1c and 1d), and the nor tropane moiety at *m/z* 136, peculiar of OH-NCOC. (Fig. 1e). As expected, the XICc relative to the precursor ion a *m/z* 320 (M + H]+ OH-COC; Fig. 1c) reports the same pattern observed using as product ion the fragment at 121 *m/z* (Fig. 1a), while the [M + H]+ signals relative to the three OH-NCOC isomers were found to be absent in the XICc relative to the precursor ion a *m/z* 306 ([M + H]+ OH-BE and OH-NCOC; Fig. 1d) and only the signals relative to OH-Be were detected. In the same way, the precursor ion scan using the ion at *m/z* 136 (nor-tropane moiety) as product ion reports only the presence of the three [M + H]+ signals relative to OH-NCOC isomers (Fig. 1e). The confirmation of the peaks assignment to the different molecules were then achieved by the precursor ion scan analysis, in the same conditions described above, of a standard mix solution (Fig. 2), performed once obtained the metabolites synthesis (see paragraph 3.2).

3.2. OH-COC metabolites synthesis

The m-OH-BE and p-OH-BE were prepared (see Supporting Information) through literature protocols starting from ecgonine methyl ester (**1**) obtained by controlled hydrolysis of cocaine. Separate condensation of (**1**) with m- and p-benzyloxybenzoyl chloride afforded the m-benzyloxycocaine (**2**) and the p-benzyloxycocaine (**3**) respectively. Deprotection by hydrogenolysis to the corresponding m- and p-hydroxycocaine (**4**) and (**5**) and subsequent methyl ester hydrolysis afforded the m-OH-BE and the p-OH-BE. The literature unreported m-OH-NCOC and p-OH-NCOC were prepared starting from the protected compounds (**2**) or (**3**), by demethylation to m-benzyloxynorcocaine (**6**) or to the p-benzyloxynorcocaine (**7**) using 1-chloroethyl chloroformate and their subsequent hydrogenolytic deprotection. Finally the deuterated internal standard (m-OH-BE-d_3) was prepared by treatment of m-benzyloxynorcocaine (**6**) with CD_3I to afford the deuterated compound (**8**) which was debenzylated to [N-CD_3]-m-hydroxycocaine (**9**) to give, after controlled hydrolysis of methyl ester, the m-OH-BE-d_3 (Fig. S9).

3.3. MRM analysis for quantitative analysis of Hydroxy-COC metabolites

Once m- and p-OH-COC, m- and p-OH-BE, m- and p-OH-NCOC reference compounds were available, an UHPLC–MS/MS procedure

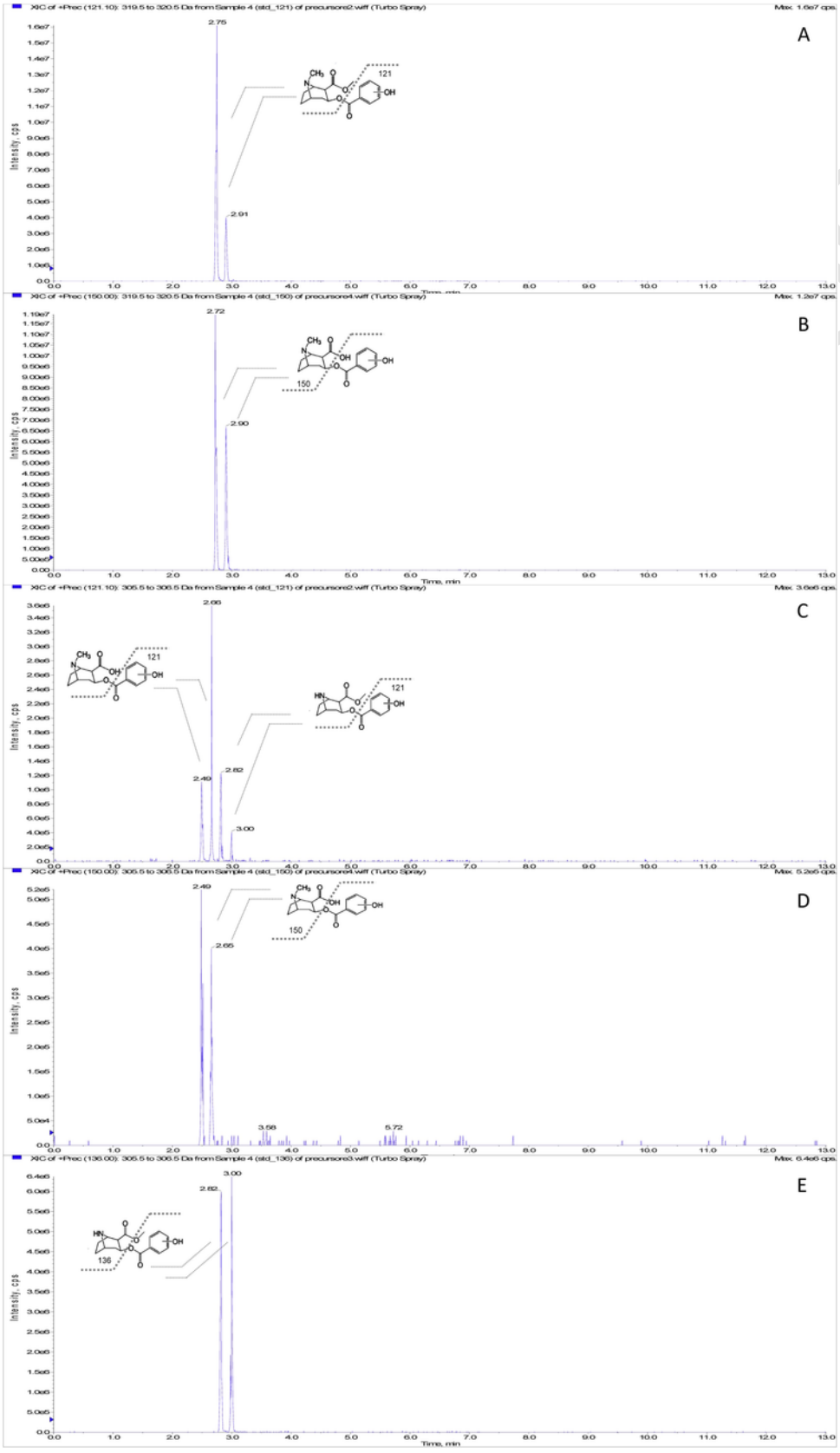
A
B
C
D
E
Intensity, cps
Time, min
2.75
2.91
2.72
2.90
2.66
2.49
2.82
3.00
3.58
5.72
121
150
136

Fig. 2. Extracted ion current chromatograms at 320.0 *m/z* ([M + H]+ OH-COC) (A,B) and 306.2 *m/z* ([M + H]+ OH-BE and OH-NCOC) (C, D, E), obtained from a standard mix solution, using parent ion scan at *m/z* 121, 150 and 136 corresponding to p-hydroxybenzoate, tropane and nor tropane fragment, respectively.

Table 2
Calibration parameters for selected analytes.

Compound	r^2	CV (%)	Analytical range (pg/mg)	LOD (pg/mg)	LOQ (pg/mg)
m-OH-COC	0.993	9.10	1-200	0.21	0.71
p-OH-COC	0.997	9.28	1-200	0.26	0.87
m-OH-BE	0.992	3.79	0.2-40	0.05	0.17
p-OH-BE	0.998	4.89	0.2-40	0.05	0.16
m-OH-NCOC	0.999	4.61	0.1-20	0.03	0.09
p-OH-NCOC	0.998	6.31	0.1-20	0.03	0.09

(by MRM scan mode) was developed and validated for hydroxy-COC metabolites quantitation, according to the Society of Hair Testing (SoHT) Guidelines for Drug Testing in Hair [2].

3.4. Method validation

3.4.1. Calibration range and linearity

Standard calibration curves (n = 6) were obtained by fortifying 50 mg drug-free hair aliquots with standard solutions, as described at paragraph 2.2. The calibration ranges were set as follow:

- m- and p-OH-COC: 1–200 pg/mg;
- m- and p-OH-BE: 0.2–40 pg/mg;
- m- and p-OH-NCOC: 0.1–20 pg/mg.

All calibration curves showed good linearity ($r^2 > 0.993$) for all the analyzed compounds over the entire investigated range when using linear correlation. The mean %CV were 9.10 and 9.28 for m- and p-OH-COC, 3.79 and 4.89 for m- and p-OH-BE and 4.61 and 6.31 for m- and p-OH-NCOC, respectively. LOD and LOQ have been calculated for all analytes and are listed in Table 2.

Table 3
Intra-day and inter-day measured, precision, accuracy and recovery for m-, p-OH-COC; m-, p-OH-BE; m-, p-OH-NCOC.

Compound	Concentration level (pg/mg)	Precision (%)		Accuracy (%)		Recovery (%)	Matrix effect (%)
		Intraday	Interday	Intraday	Interday		
m-OH-COC	1	6.56	8.64	6.56	11.4	93.4	−10.9
	10	5.04	5.57	7.66	5.63	74.1	−5.59
	200	6.62	7.48	7.48	11.6	94.2	−4.76
p-OH-COC	1	6.61	13.2	5.51	9.01	92.1	−10.4
	10	3.11	4.14	4.72	6.38	80.6	−6.37
	200	4.43	6.20	11.6	9.19	96.1	−4.86
m-OH-BE	0.2	5.85	8.58	3.05	5.75	52.1	−8.87
	2	1.89	2.94	2.54	4.13	39.8	−6.40
	40	3.91	4.44	5.53	2.85	39.3	−2.08
p-OH-BE	0.2	3.44	12.7	4.56	7.91	49.9	−12.7
	2	5.17	5.77	5.48	8.47	43.2	−6.73
	40	1.79	3.28	4.82	3.56	43.8	−8.23
m-OH-NCOC	0.1	4.23	4.24	3.30	8.94	93.8	−7.44
	1	4.98	5.95	5.61	7.95	75.5	−4.18
	20	1.51	2.43	3.83	2.54	73.4	−8.75
p-OH-NCOC	0.1	3.83	5.18	2.95	7.77	77.7	−11.1
	1	3.43	6.42	8.21	8.28	80.2	0.54
	20	2.09	2.99	7.09	5.19	83.1	−2.97

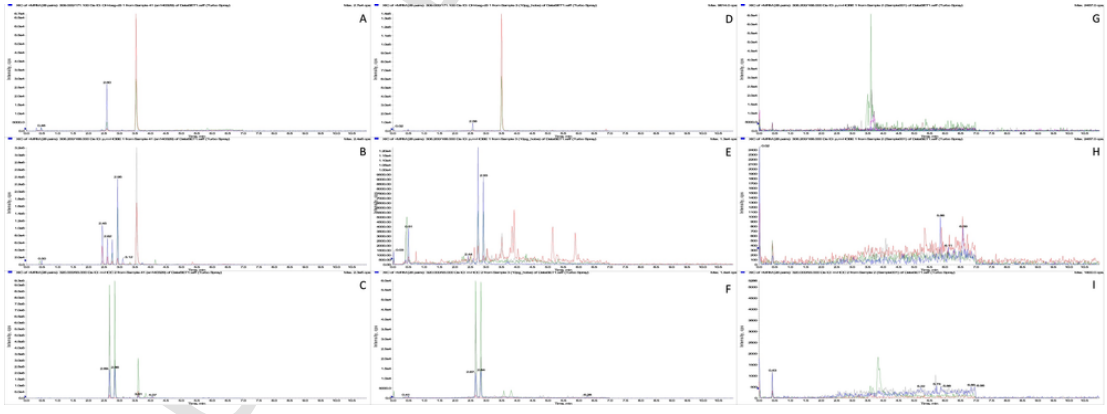

Fig. 3. MRM chromatograms from a COC-positive sample (A -> C), from a spiked sample at LLOQ (D -> F) and from a blank sample (G -> I). A, D and G: MRM transitions relative to SI (OH-BE-d_3); B, E and H: MRM transitions relative to OH-NCOC and OH-BE; C, F and I: MRM transitions relative to OH-COC.

3.4.2. Precision and accuracy

Regarding precision and accuracy, the method showed good performance in terms of both repeatability and reproducibility, showing CV values below 14%. The same results were obtained for accuracy studies. Both intra- and inter-day precision and accuracy for all the analytes were within acceptable limits, as shown in Table 3.

3.4.3. Extraction efficiency and matrix effect

The mean extraction efficiency was satisfactory, being over to 39.3% for all the analytes, as reported in Table 3. Matrix effect ranged from – 12.7% to + 0.54%. These results indicate an acceptable extraction recovery and a negligible effect from the matrix on the ionization of p- and m-OH-COC, p- and m-OH-BE, p- and m-OH-NCOC.

3.4.4. Carry-over

Carry-over was not observed for any of the analytes in a blank sample which was immediately processed after a fortified sample containing 500 pg/mg of p- and m-OH-COC, 100 pg/mg of p- and m-OH-BE, 50 pg/mg of p- and m-OH-NCOC, respectively.

3.4.5. MRM analysis of COC-positive hair specimens

The MRM method was then applied, with the aim to obtain a quantitative distribution, for the first time through a validated method, of cocaine hydroxy metabolites in COC-positive hair specimens. Fig. 3 reports the MRM chromatograms relative to a COC-positive sample (A->C), a spiked sample at LLOQ (D -> F) and a blank sample (G -> I). The MRM chromatograms A, D and G are relative to SI (m-OH-BE-d_3) transitions; chromatograms C, F and I are relative to OH-COC transitions and chromatograms B, E and H to OH-BE and OH-NCOC transitions.

In particular, the peak at 3.5 min corresponds to SI, the peaks at 2.8 and 2.9 at p- and m-OH-NCOC and the peaks at 2.5 and 2.6 min at p- and m OH-BE.

Results obtained from analysis of a pool of COC-positive hair (n = 100), as described at paragraph 2.3, have shown a concentration range between 0.63–491 pg/mg for m-OH-COC (mean 58.5 pg/mg, median 15.9 pg/mg) and 1.28–466 pg/mg for p-OH-COC (mean 59.5 pg/mg, median 17.5 pg/mg); 0.23–49.64 pg/mg for m-OH-BE (mean 8.15 pg/mg, median 2.32 pg/mg) and 0.23–39.5 pg/mg for p-OH-BE (mean 5.02 pg/mg, median 1.78 pg/mg) and between 0.28–28.7 ng/mg for m-OH-NCOC (mean 5.52 pg/mg, median 1.14 ng/mg) and 0.11–17.4 pg/mg for p-OH-NCOC (mean 2.27 pg/mg, 0.39 pg/mg). The same pool was analyzed by a routine internal validated GC–MS method for COC and BE showing a concentration range between 0.18–110 ng/mg for COC (mean 19.2 ng/mg, median 3.40 ng/mg) and 0.2–32.8 ng/mg (mean 4.06 ng/mg, median 0.78 ng/mg) for BE. Currently, a cut-off for p- and m-OH-COC, p- and m-OH-BE and p- and m-OH-NCOC is still missing, therefore in this preliminary phase we may consider the samples positive for hydroxy metabolites when their concentrations are higher than the respective defined LOQ values. Taking into consideration this evaluation, the frequency of positive findings in COC-positive cases (n = 100) was 83.0% for p-OH-COC, 92.5% for m-OH-COC, 88.7% for p-OH-BE, 92.5% for m-OH-BE, 75.5% for p-OH-NCOC and 83.0% for m-OH-NCOC.

The data here reported are very similar to those reported by Musshoff et al [3] at least for p-OH-COC and m-OH-COC, obtained by them through a validated method.

Data relative to hydroxy BE and NCOC levels are instead pretty different, but the explanation could be in the fact that Musshoff could not apply a validated quantitative method for their analysis, since they did not have available standards for these metabolites.

Finally, in the paper of Franz et al. [19] criteria for the interpretation of COC findings from hair samples were suggested. In detail, hair samples with a concentration > 0.1 ng COC/mg hair are reported as positive due to COC ingestion if positive findings for p- or m-OH-BE or p- or m-OH-NC were obtained and/or the metabolic ratio based on the peak areas for p- or m- OH-COC exceeds the thresholds of 0.1% and 0.2%, respectively. According to these criteria, our data obtained by a quantitative validation procedure were found in 83% of samples (> 0.1 ng COC/mg hair) positive for p- or m-OH-BE or p- or m-OH-NC and the ratio based on the concentration for p- and m-OH-COC exceeds the thresholds defined above in 82% and 68% of samples (> 0.1 ng COC/mg hair), respectively.

On the other side, the percentage value of hair samples with a BE/COC concentration ratio ranging from 10% to 30%, was found to be 73.2%.

4. Conclusions

Currently, COC and its main metabolite BE are the principal targets of conventional hair analysis, in order to proof the COC consumption [20]. However, it's well known that BE, a hydrolysis product of COC, is also present in street COC samples and in addition it is slowly formed from COC within the keratinized hair or during sample preparation. For these reasons, it cannot be used to certain proof COC use. Several studies have started to focus attention on different COC metabolites [11,12]; this in order to have an unquestionable proof of COC consumption and therefore to discriminate between use and external contamination. Among these, to our best knowledge, p-OH-BE, m-OH-BE, p-OH-NCOC and m-OH-NCOC have been never found in street seized samples These hydroxy metabolites were detected for the first time in hair samples from COC abusers by Musshoff et al. [3] but without performing the method validation, due to the lack of available standards. The present work fills this gap, firstly by developing, for the first time, the synthesis of OH-BE and OH-NCOC (p/m) and therefore performing the full validation of the UHPLC-MS/MS method for their analysis (also for OH-COC, even though it's not a specific metabolite of COC consumption). In addition, we apply a specific mass analysis technique, the parent ion scan mode, in order to obtain a qualitative profile of all the considered hydroxy COC metabolites in hair samples.

In summary, the availability of a validated quantitative method for the COC hydroxy metabolites determination in hair may enable a definite proof of drug ingestion. This could be the starting point in order to include hydroxylated cocaine species analysis into future COC hair testing guidelines. Further studies will be necessary to elucidate the possible correlation between hydroxy metabolites of cocaine and their precursor in hair.

Conflict of interest disclosure

The authors declared no competing financial interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2019.04.028.

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