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AEME production in cocaine positive hair after thermal hair treatment

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ABSTRACT

Introduction: Currently, hair straightening has become a regular hair treatment for women but likewise for men. Several studies have shown that thermal straightening has an influence on the concentration of ethyl glucuronide and of drugs of abuse content in hair. Heat treatment of hair may decrease concentrations of cocaine (COC) and of cocaethylene (CE) in hair and increase concentrations of benzoylecgonine (BZE). The goal of this study was to evaluate the influence of thermal straightening on anhydroecgonine methyl ester (AEME), a known cocaine smoking marker, in hair.
Method: 42 positive COC hair samples were treated in vitro with iron plates heated to 200 °C. During this treatment one lock of hair was put sequentially 30 times in contact with a hair straightener during 2 s, the other lock was not treated. The hair samples were analyzed by a validated GC/MS method for AEME, COC and its metabolites BZE, norcocaine (NC), ecgonine methyl ester (EME) and CE.
Results: After treatment, a median increase of concentrations was observed for AEME (110.3%) and BZE (27.6%) whereas a median decrease was found for COC (56.9%), NC (46.7%), EME (33.3%) and CE (41.7%). The median BZE/COC ratio of 0.6 in not treated hair increased to 1.5 in treated hair.
Conclusion: Regarding our in vitro results, AEME may be produced by thermal hair straightening. Therefore, the presence of AEME in hair should not be used as an irrefutable prove of cocaine smoking. Our study shows that for the interpretation of AEME results in hair, potential heat treatment of hair should be considered. A ratio BZE/COC higher 1 appears to be a good marker to identify thermal treatment of hair before collection. Finally, thermal straightening should be documented during hair collection and should also be considered for the interpretation of COC results in hair.

1. Introduction

Hair straightening represents nowadays a regular used hair treatment especially for women. During straightening hair strands are put in contact with an excessive heat, as iron plates are usually heated between 150–250 °C. Hydrogen and disulfide bonds are broken down, allowing keratin chains to move to a new position that results in straightened hair. After cooling down, the hydrogen and disulfide bonds between the keratin are reformed. As keratin molecules are in a different position, when the bonds are reformed, the hair stays in the straightened shape for a certain amount of time [1]. Thermal hair straightening has shown to influence the stability of ethyl glucuronide, THC, GHB and cocainics in hair [1–3].

Crack cocaine is a free-base cocaine that is smoked and inhaled with its specific volatile pyrolysis product, anhydroecgonine methyl ester (AEME) [4]. The lungs rapidly absorb cocaine (COC) when smoked as crack to reach the brain shortly after. Due to its short-term effects, crack is smoked at higher frequency, which rapidly induces dependency [5]. The main products detected in biological matrices from crack cocaine addicts are COC, benzoylecgonine (BZE), ecgonine methylester (EME), norcocaine (NCOC), AEME and cocaethylene (CE), which is a byproduct of concurrent consumption of alcohol and COC [6]. However, AEME has been shown to be a specific marker of crack usage [4–7].

AEME has also been detected in hair. Very few data has been published on AEME detection in hair and concentrations were generally low in comparison to other cocainics [8].The exact routes of incorporation of AEME in hair still remain to be investigated. Some studies show that its incorporation may be influenced by melanin and that one route of incorporation may be by passive diffusion from the blood to the hair follicle or through sebum [7–10]. Hoelzle et al. [6] concluded that external contamination may play a major role for the sequestration of AEME in hair matrix.

According to former studies [11,12], crack is evaporated at 96–98 °C. Between temperatures from 255 °C to 420 °C the amount of cocaine converted to AEME was ranging between 50–80% and at 650 °C the amount was greater than 80%. During hair straightening, hair strands are usually heated between 150 and 250 °C, so a transformation of COC to AEME in the hair lock is very probable.

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The goal of this study is to evaluate the influence of thermal straightening on the marker of crack use (AEME) and on cocainics (COC, BZE, EME, NCOC and CE).

2. Materials and method

2.1. Chemicals

Solvents were supplied by Biosolve (Valkenswaard, The Netherlands), hydrogen chloride from Merck, (Darmstadt, Germany), potassium hydroxide was obtained from WWR International (Leuven, Belgium) and pentafluoropropionic anhydride/pentafluoropropanol (PFPA/PFPOH) from Sigma Aldrich (Darmstadt, Germany). Reference standards (EME, EME-d3, COC, COC-d3, BZE, BZE-d3, CE, CE-d3, NCOC and NCOC-d3) were purchased from LCG Standards (Molsheim, France). AEME and AEME-d3 were provided by Lipomed AG (Arlesheim, Switzerland).

2.2. Instrumentation

Analyses were done using a gas chromatograph 7890A linked to a mass selective detector 5975C inert XL (Agilent, Waldbronn, Germany). A pulsed splitless injection was done at 250 °C. 2 μL of each sample was injected in GC column VF-5MS (5%-Phenyl Methyl Silox, 12 m × 0.200 mm × 0.33 μm) column (Agilent). Starting temperature was set at 60 °C (2 min hold), increased at 40 °C/min–170 °C (no hold), increased again at 8 °C/min–234 °C (no hold) and finally at 50 °C/min–300 °C (2 min hold).

2.3. Hair samples

Forty-two positive cocaine hair samples from driving ability examination cases were used for this study. Color of hair was documented. Hair segments up to 3 cm were analyzed and analyses were done in triplicate (if sufficient material is available).

2.4. In vitro thermal straightening

The samples were split into two strands. One strand was treated in vitro with iron plates (Philips SalonStraight Pro XL, Amsterdam, The Netherlands) heated at 200 °C. Hair was put in contact with theses plates consequently 30 times for 2 s corresponding approximately to a daily treatment during one month [1]. The other strand of hair was not treated.

In an additional study two different hair samples were treated up to four heating cycles corresponding to 1, 2, 3 or 4 min of total treatment.

2.5. In vitro heating of cocaine in presence or without water

100 ng of cocaine was put in 5 glass vials with 100 μL water and in 5 glass vials without water. The vials were closed, then placed during 0, 1, 2, 3 or 4 min in an oven at 200 °C and then cooled down rapidly. Cocaine, BZE and AEME concentrations were then determined in all vials.

2.6. Cocainics determination in hair

Before pulverization hair samples were washed for approximatively 1 min with water and acetone, pulverized and incubated in phosphate buffer (0.1 M, pH 6.0) for 2 h in an ultrasonic bath [1]. After solid-phase extraction with Clean Screen Columns (UCT), derivatization was performed using pentafluoropropionic anhydride and pentafluoropropanol. Analyses were done by GC/MS in electron impact mode: The selected ions and their corresponding deuterated forms used as internal standards were the following (quantifying ions highlighted in bold): AEME – m/z **152**, 181; AEME-d3 – m/z **155**, 184; EME – m/z **182**, 345; EME-d3 – m/z **185**; BZE – m/z **300**, 421; BZE-d3 – m/z **303**; COC – m/z **182**, 303; COC-d3 – m/z **185**; NCOC – m/z **313**, 435; NCOC-d3 – m/z **316**; CE – m/z **196**, 317; CE-d3 – m/z **199**. The potential formation of AEME in the injector as described by Toennes et al [13] was controlled by the use of a low injection temperature (250°C) and the use of deuterated internal standard AEME-d3.

3. Results and discussion

Validation data for COC, BZE, AEME, CE, EME and NCOC are presented in Table 1.

The concentrations of AEME, COC and its metabolites before and after in vitro thermal straightening of 42 cocaine positive hair samples are shown in Table 2. Thermal straightening produced an important decrease of cocaine concentrations in hair. In all the samples a median decrease of 56.9% of cocaine was observed (range: 0.5% to a total loss). The obtained results show that after thermal treatment of hair cocaine positive hair specimens (above SoHT cut-off levels of 0.5 ng/mg) cocaine concentrations may decrease below this cut-off and thus may produce false-negative results.

In almost all hair specimens (38 of 42 cases), an increase of BZE was found ranging from 0.4% to 170.8% (median 27.6%). In one sample no variation was shown (case 14) and in 3 hair samples a decrease was found ranging from 6.4% to 28.2% (cases 26, 40, 41). Twenty-one untreated hair samples were positive for CE (0.06–1.29 ng/mg). For these samples, a median decrease of 41.7% of CE was determined and a total loss was shown in 4 hair samples. In one sample (case 38), an increase of CE content (14.7%) was found. As the concentration is low, this may increase the potential analytical error range and therefore this increase should not be over interpreted. Furthermore, from a chemical point of view, increase in absence of ethanol is rather improbable.

Concentrations of EME and NC decreased with a median of 33.3% and 46.7% respectively.

An important increase in AEME concentrations for 38 of the hair specimens was shown (see Fig. 1). The increase ranged from 20.8% to 626.7% (median 110.3%).

Table 1
Validation data for the determination of COC, BZE, AEME, EME, CE and NC in hair.

Substance	Accuracy (% bias)		Precision (% bias)			
	Within-day	Between-day	Within-day	Between-day	Recovery (%)	LLOQ (pg/mg)
COC	2.6	3.2	6.1	0.8	91.7	3.2
BZE	1.6	3.3	1.8	1.6	103.6	9.1
AEME	4.9	6.4	1.8	3.6	102.4	6.4
EME	6.4	4.3	1.9	2.0	100	5.4
CE	2.1	10.0	3.6	5.4	90.2	12.5
NC	9.4	5.8	3.0	0.7	94.2	16.6

Validation performed at a concentration of 500 pg/mg hair.

Table 2
Effect of thermal treatment on COC, BZE, AEME, CE, EME and NC content in hair.

Case	Hair colour	COC			BZE			AEME			CE			EME			NC		
		NT	T	D	NT	T	D	NT	T	D	NT	T	D	NT	T	D	NT	T	D
1	B	0.7	0.4	−47	0.5	0.5	4.3	0.10	0.15	49.3	<	<	–	<	<	–	0.05	0.05	−15.8
2	B	0.5	0.3	−47	0.6	0.6	1.1	0.10	0.12	20.8	<	<	–	<	<	–	<	<	–
3	B	0.6	0.5	−19.7	0.6	0.7	15.8	0.10	0.16	61.2	0.07	0.06	−20.5	<	<	–	<	<	–
4 †	B	1.7	0.7	−60	0.5	1.0	91	0.14	0.54	281	0.20	0.16	−21.7	<	<	–	0.19	0.09	−52.4
5	B	202.9	55.1	−73	25.4	38.2	50	12.20	22.02	80.5	0.13	0.12	−11.6	0.46	0.30	−33.3	<	<	–
6	B	1.0	0.6	−42	0.7	0.9	23	0.14	0.24	71.6	0.10	0.06	−35.5	0.12	0.11	−3.2	<	<	–
7	B	6.0	2.4	−60	2.9	3.2	11	0.59	1.40	135	1.29	0.69	−46.6	<	<	–	<	<	–
8	B	3.5	0.7	−80	0.8	1.5	76	0.10	0.44	340	0.18	0.08	−53.2	<	<	–	0.09	0.02	−81.6
9	Ba	12.7	3.6	−72	16.2	17.1	5.2	0.77	2.94	280	0.09	0.05	−43.9	0.39	<	−100.0	0.18	0.08	−55.9
10	B	2.2	0.8	−64	0.8	0.9	15	0.13	0.39	211	<	<	–	0.07	0.05	−34.4	0.06	<	−100
11	B	0.5	0.4	−23.9	0.3	0.5	46.9	0.10	0.16	62.4	<	<	–	<	<	–	<	<	–
12	B	5.6	2.4	−58	5.3	5.5	4.7	0.36	1.12	214	<	<	–	0.07	0.06	−14.7	0.18	0.14	−23.9
13	B	21.4	1.4	−93	2.4	4.1	70	0.98	1.70	74.5	0.08	<	−100	0.27	<	−100.0	0.31	0.12	−61.2
14	Ba	1.9	0.7	−63	2.2	2.2	0.0	0.24	0.51	114	0.18	0.14	−21.4	<	<	–	0.15	0.05	−62.3
15	B	3.0	1.2	−58	2.6	3.4	32	0.36	1.07	196	0.23	0.12	−48.6	0.10	<	−100.0	0.13	0.12	−9.1
16 †	B	26.9	9.4	−65	12.7	16.1	26	2.12	7.04	233	0.09	0.05	−41.9	1.31	0.77	−41.5	1.74	0.81	−53.1
17	Ba	0.1	<	−100	0.1	0.1	20	0.10	0.19	94	<	<	–	<	<	–	<	<	–
18*	B	1.2	0.6	−45	0.7	0.9	39	0.19	0.29	48.8	<	<	–	<	<	–	<	<	–
19*	B	6.7	3.1	−54	3.4	3.6	4.3	0.34	0.82	141	0.63	0.39	−38.1	<	<	–	<	<	–
20*	DB	0.4	0.2	−50	0.2	0.2	9.8	<	<	–	<	<	–	0.07	0.02	−68.2	<	<	–
21*	B	8.1	3.7	−54	7.4	7.4	0.4	0.59	1.18	100	<	<	–	0.06	0.06	−4.1	<	<	–
22 †	DB	5.7	0.9	−84	0.7	1.3	73.4	0.44	0.71	61.0	0.06	<	−100.0	0.08	0.03	−64.1	<	<	–
23	B	0.4	0.2	−39.4	0.3	0.5	60.2	0.10	0.14	38.7	<	<	–	<	<	–	<	<	–
24 †	B	3.1	1.0	−69.3	2.7	2.8	3.1	0.51	0.76	50.1	<	<	–	0.06	0.04	−29.6	0.25	0.10	−57.9
25*	B	0.2	0.1	−53.5	0.1	0.2	49.8	<	<	–	<	<	–	0.30	0.27	−11.7	<	<	–
26*	B	20.2	8.8	−56.6	22.0	20.6	−6.4	1.70	4.52	165.6	0.33	0.16	−52.0	0.17	<	−100.0	0.48	0.22	−54.9
27 †	B	66.7	26.0	−60.9	34.2	39.6	15.9	5.72	15.36	168.4	0.16	0.10	−41.6	1.00	0.39	−61.4	1.11	0.59	−46.7
28*	B	1.1	0.7	−36.7	1.1	1.4	31.5	0.14	0.24	77.2	<	<	–	0.05	0.04	−14.0	<	<	–
29	Ba	2.8	1.2	−58.2	1.8	2.6	47.8	0.31	1.09	251.4	0.18	0.11	−41.7	0.09	0.07	−21.1	<	<	–
30 †	DB	4.7	0.7	−84.9	1.1	1.4	28.8	0.26	0.78	203.3	<	<	–	<	<	–	0.10	0.05	−51.4
31 †	B	208.1	89.0	−57.2	38.8	69.4	78.9	6.53	18.25	179.6	<	<	–	0.31	0.25	−21.1	0.83	0.71	−14.8
32	DB	16.1	16.0	−0.5	8.3	18.4	120.6	0.37	2.72	626.7	<	<	–	<	<	–	0.30	0.50	69.0
33*	B	20.7	9.3	−55.0	5.7	5.8	1.5	0.66	1.52	130.4	<	<	–	0.13	0.11	−15.3	0.22	0.17	−23.2
34*	DB	4.8	3.3	−30.9	1.1	1.6	51.5	0.18	0.33	83.3	0.20	0.15	−22.9	<	<	–	0.09	0.09	−2.6
35	B	6.1	4.3	−30.2	5.9	7.8	33.3	0.25	0.71	180.4	<	<	–	<	<	–	0.19	0.16	−16.8
36 †	DB	6.3	1.7	−72.5	0.6	1.5	170.8	0.18	0.37	106.3	<	<	–	<	<	–	<	<	–
37	DB	2.6	1.7	−32.9	0.9	1.3	48.0	0.13	0.27	100.2	0.35	0.26	−26.5	<	<	–	0.08	0.06	−14.5
38	Ba	39.2	26.8	−31.5	17.3	31.2	80.1	1.81	7.96	339.3	0.30	0.35	14.7	0.63	0.53	−15.1	0.32	0.41	28.2
39	B	0.3	0.2	−22.3	0.2	0.3	69.1	<	<	–	<	<	–	<	<	–	<	<	–
40	B	0.7	0.4	−43.8	0.5	0.5	−13.7	0.10	0.13	33.6	0.06	<	−100.0	<	<	–	<	<	–
41 *	Bo	0.2	0.05	−79.6	0.12	0.09	−28.2	<	<	–	0.08	<	−100.0	<	<	–	0.05	<	−100.0
42	LB	1.6	0.6	−60.4	1.7	1.8	4.6	0.14	0.20	49.0	<	<	–	0.04	0.03	−39.8	0.07	0.05	−27.4

NT not –treated (ng/mg); **T** treated (ng/mg); **D** difference (%); **B** (brown), **DB** (dark brown), **Ba** (black), **Bo** (blond), **LB** (light brown); Hair segments were analyzed in triplicate († duplicate; * one analysis); < less than the lower limit of quantification (LLOQ).

3.1. Effect of thermal straightening on COC and BZE content in hair

The median BZE/COC ratio increased from 0.6 to 1.5 in treated hair (Fig. 2). The obtained results confirm previous findings that a BZE/COC ratio higher than 1 may be proposed as an indication of frequent heat treatment before hair collection [1]. The fact that for 6 of the 42 hair samples, the ratio BZE/COC was higher than 1 (range: 1.09–1.44) before treatment may be explained that these samples may have been heat straightened before hair collection.

The decrease of COC and the increase of BZE may be explained by a chemical conversion of COC into BZE in presence of heat. To confirm this an in vitro heating study of cocaine (Fig. 3) was done. The obtained results show that COC deteriorates very quickly under the effect of heat: more than 70% of the amount of cocaine decrease after one minute of heating at 200 °C in presence of water or without water. Furthermore, BZE is produced after 1 min of heating, but only in the vial with water and then decreases rapidly.

This in vitro study highlight that cocaine may be hydrolyzed by heat and in presence of water to BZE and that, the duration of heat treatment has a major impact on the BZE/COC ratio.

3.2. Effect of thermal straightening on AEME content in hair

Thirty-eight samples were positive for AEME before hair treatment. The concentration of AEME varied in a wide range from 0.10 ng/mg to 6.53 ng/mg (mean 1.03 ng/mg, median 0.25 ng/mg). After heat treatment, a mean increase of 148.8% was shown and the AEME concentrations of the treated hair samples varied between 0.12 ng/mg to 22.02 ng/mg (mean 2.59 ng/mg, median 0.71 ng/mg). In 4 hair samples AEME was not detectable before and after treatment. It should be noted that for these 4 samples only relatively low COC concentration (range 0.2-0.4 ng/mg) were determined possibly explaining why no increase could be detected.

Regarding our findings the presence of AEME in hair cannot be used as an irrefutable prove of cocaine smoking.

It is interestingly to mention that the obtained median AEME concentration (0.71 ng/mg) after treatment reveals to be higher than the median concentration (0.53 ng/mg) of smokers of coca paste base in South America [14].

The influence of thermal hair straightening on the AEME/COC ratio was also studied. In the present study, our data show that after treatment this ratio increased in all samples (median ratio increase of 553%) (Fig. 4). The median ratio (0.45) after treatment

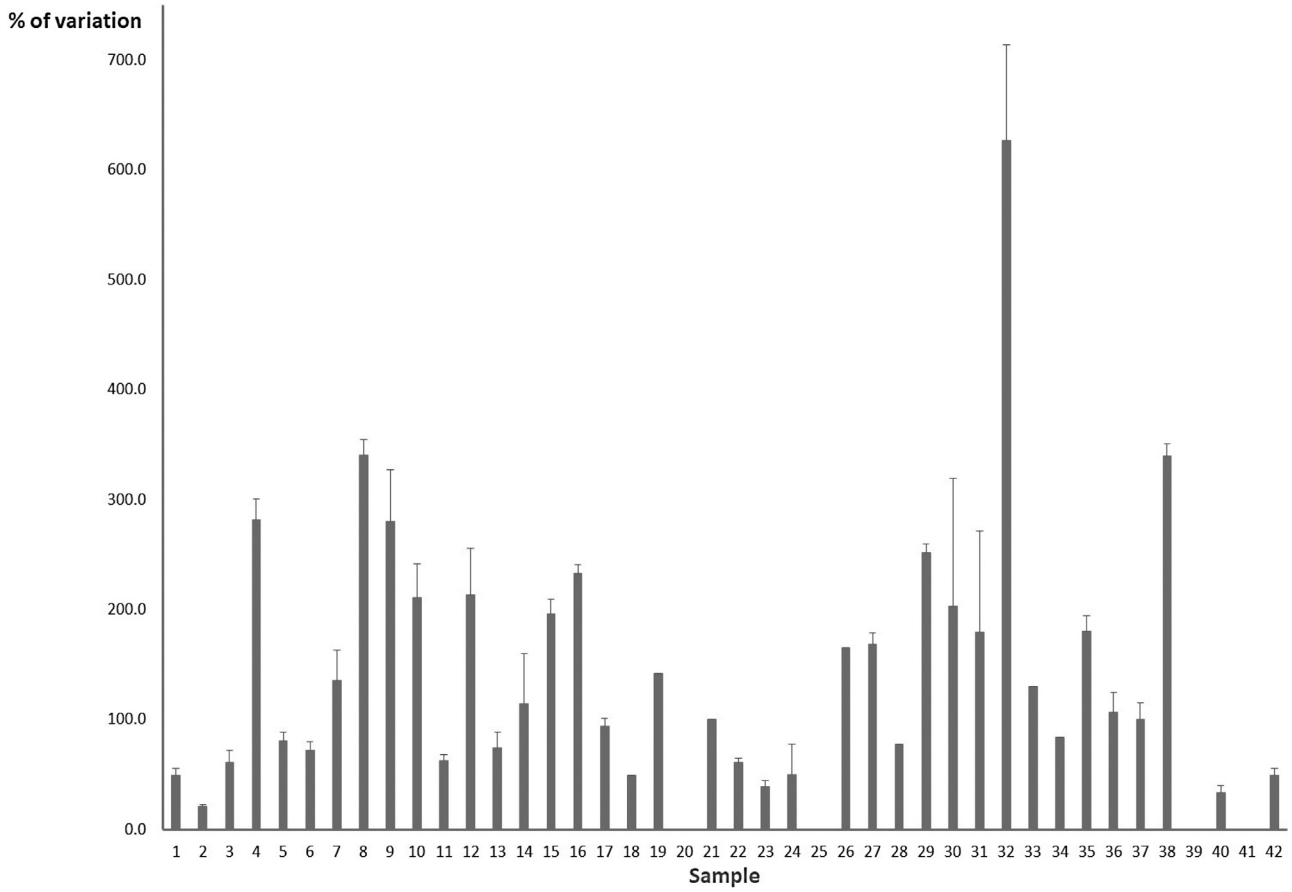

Fig. 1. Percentage of variation of AEME concentrations in not-treated vs treated hair.

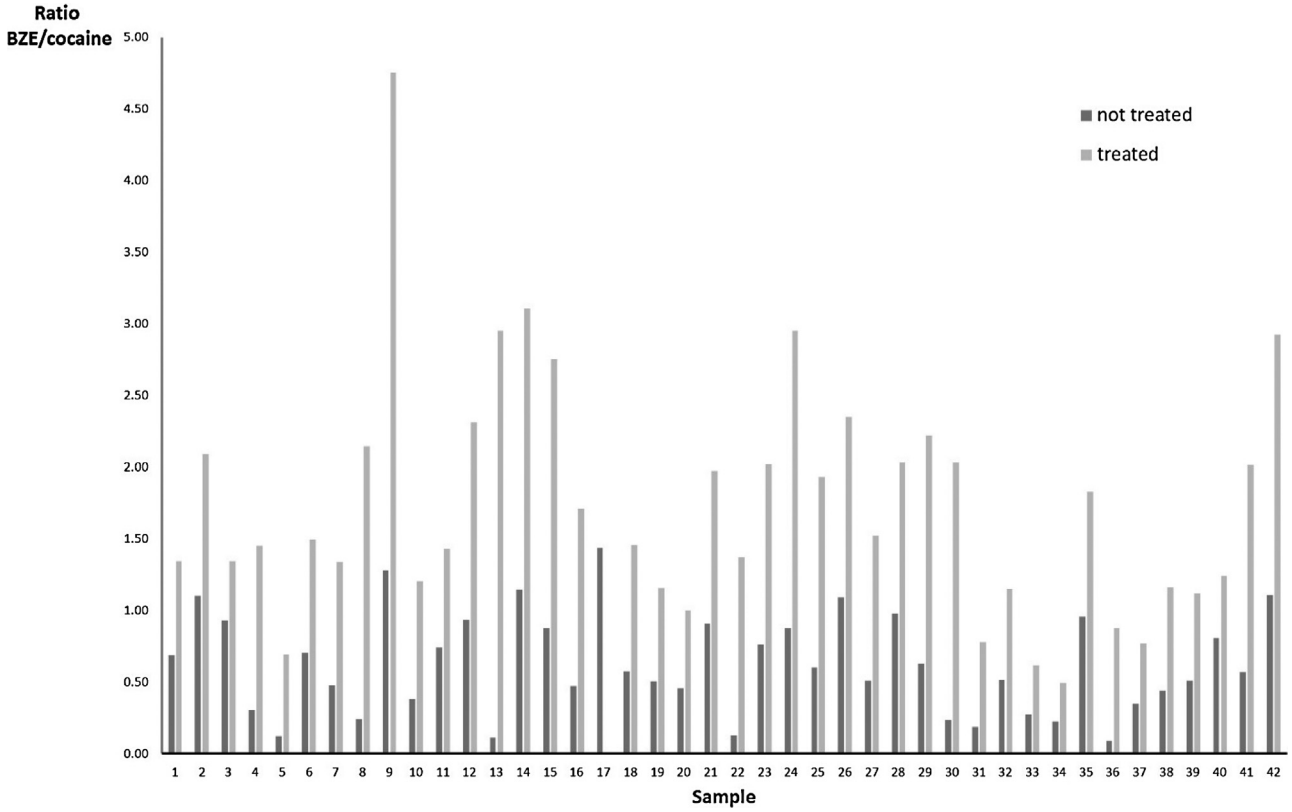

Fig. 2. BZE / Cocaine ratio in not treated *vs* treated hair.

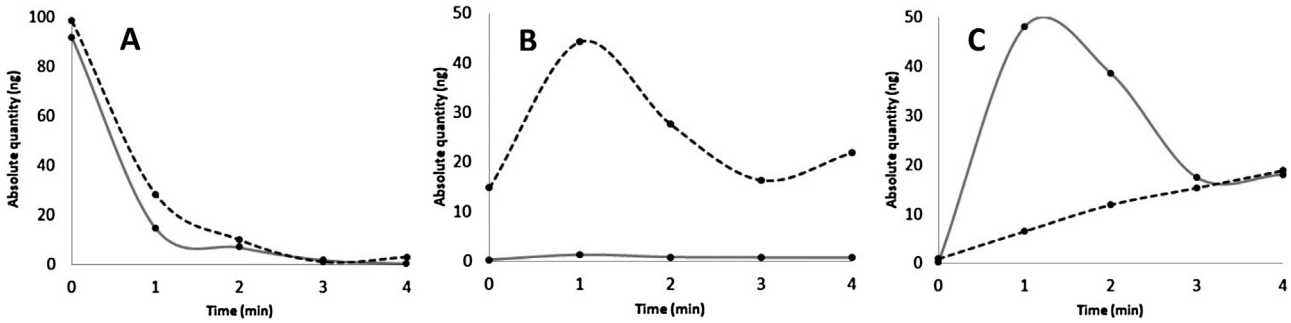

Fig. 3. Stability of COC incubated at 200 °C in presence of water or without water. 100 ng of COC was put in two vials with 100 μL water or without water. COC (**A**), BZE (**B**) and AEME (**C**) concentrations were measured after different times (min) in presence of water (dash line) or without water (solid line).

was much higher than the median ratio (0.02) from coca paste base smokers [14].

The increase of AEME after treatment with heating plates may be explained by an in situ thermic transformation of COC to AEME in the hair matrix. Fig. 5 shows the kinetics of COC and AEME concentrations in two hair samples after increasing total time of contacts (1, 2, 3 or 4 min). After 1 min COC amount decreases to 50%, whereas the AEME amount significantly increases. Between

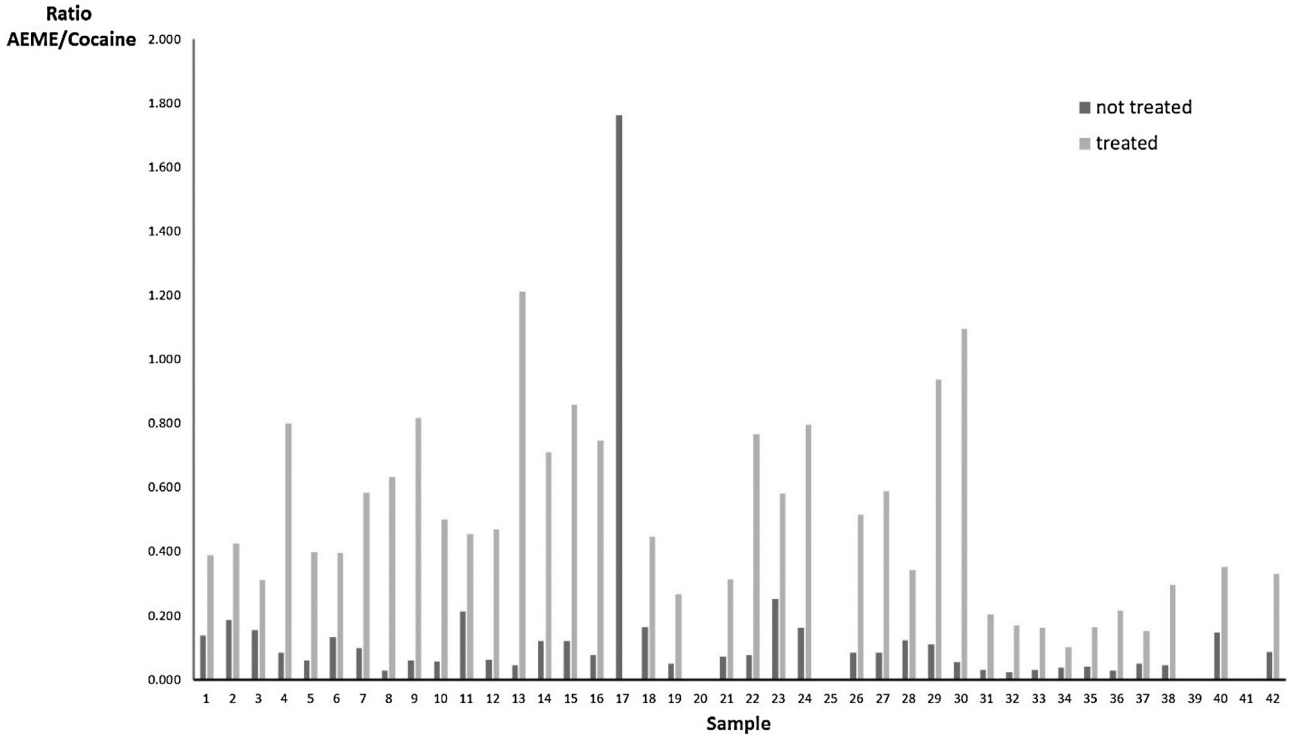

Fig. 4. AEME / Cocaine ratio in not treated hair *vs* treated hair.

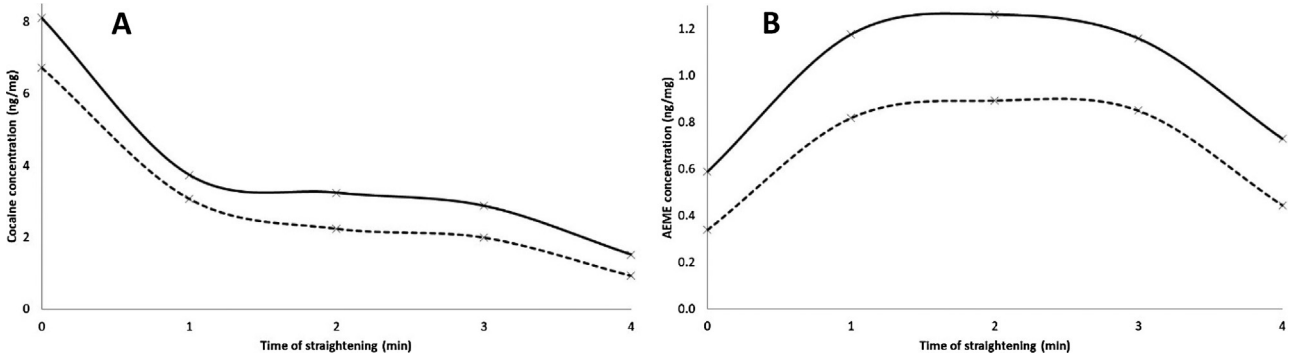

Fig. 5. Kinetics of COC (**A**) and AEME (**B**) concentrations in two hair samples (solid and dash line respectively) after sequentially treating several times on contact with heated iron plates at 200 °C during 2 s corresponding to a total time of contact of 0, 1, 2, 3 or 4 min.

1–3 min, the AEME amount remains stable, and then decrease to approximatively the initial value.

The in vitro heating study of cocaine shows that the thermic transformation of COC to AEME is more important in absence of water than in presence with water during the first 3 min (Fig. 3c).

These findings show that the production AEME after heat treatment may depend on the duration of hair treatment, but also on the humidity of the treated hair.

4. Conclusions

Former studies have shown that AEME in hair is a marker of crack use. As AEME may also be produced in cocaine positive hair after heat treatment, the presence of AEME in hair cannot be used as an irrefutable prove of cocaine smoking.

Therefore, for the interpretation of AEME results in hair, potential heat treatment of hair should be considered. Even if the ratio BZE/COC may be influenced by other factors, a ratio above 1 may be proposed to be an indication of thermic hair straightening before hair collection.

Our study also shows that the production of BZE vs AEME after heat treatment may depend on the duration of hair treatment. In the thermal decomposition of COC process in hair there are competing hydrolysis in presence of H2O to BZE and elimination of benzoic acid to AEME (formation of double bond in AEME) and finally loss by evaporation.

Additional studies are needed on controlled cocaine users where crack use can be ruled out in order to evaluate if the ratio AEME/COC in hair may also be interesting to differentiate between crack use and thermal straightening.

Our study shows the importance of documenting thermal straightening during hair collection and that it is mandatory to consider this parameter for the interpretation of cocaine results in hair.

CRediT authorship contribution statement

Nicolas Gambier: Conceptualization, Formal analysis, Methodology, Validation, Supervision, Writing - original draft, Writing - review & editing. **Jenny Warling:** Formal analysis, Methodology, Writing - original draft. **Nicolas Van Elsue:** Formal analysis, Writing - original draft. **Michel Yegles:** Methodology, Validation, Writing - original draft, Writing - review & editing, Supervision.

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