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Discrimination between chewing of coca leaves or drinking of coca tea and smoking of "paco" (coca paste) by hair analysis. A preliminary study of possibilities and limitations

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ABSTRACT

Background: Hair analysis is a suitable way to discriminate between coca chewers and consumers of manufactured cocaine using the coca alkaloids hygrine (HYG) and cuscohygrine (CUS) as markers. In the present preliminary study it was examined whether CUS and HYG can be detected in hair of occasional and moderate coca chewers or coca tea drinkers, whether CUS and HYG appear in hair of PACO consumers (smoking coca paste waste), and whether anhydroecgonine methyl ester (AEME) is a useful cocaine smoking marker in this context.
Method: Three groups were included: 10 volunteers from Buenos Aires with occasional or moderate chewing of coca leaves or drinking coca tea, 20 Argentinean PACO smokers and 8 German cocaine users. The hair samples (1–4 segments) were analyzed by a validated LC–MS/MS method for cocaine (COC), norcocaine (NC), benzoylecgonine (BE), ecgonine methyl ester (EME), cocaethylene (CE), cinnamoylcocaine (CIN), tropacocaine (TRO), AEME, CUS and HYG. For comparison, eight samples of coca leaves or coca tea were analyzed.
Results: Only low concentrations of COC were found in hair of seven occasional users of coca leaves or coca tea (0.010–0.051 ng/mg). For three moderate chewers of coca leaves all compounds were detected including AEME but except TRO. The hair samples of PACO smokers contained much higher concentrations of COC (0.027–341 ng/mg, mean 37.4 ng/mg) and its metabolites. CUS was not found in these samples but traces of HYG were seen in 8 of 37 hair segments. AEME as a marker for coca smoking was detected in hair of 15 smokers. In comparison to COC, the concentrations of EME and CIN were higher for PACO smokers than for German cocaine consumers. AEME (56 ± 20 μg/g) was detected in all coca leave and coca tea samples which explains the detection of this substance in hair of coca chewers. Therefore, its use for differentiation between coca chewers and PACO smokers is limited.
Conclusion: CUS remains to be the most suitable marker in hair for chewing coca leaves or drinking coca tea more frequently than two times per month since it does not appear in hair of Argentinean PACO smokers and German cocaine users. Contrary to a previous proposal, the ratios CIN/COC and EME/COC appeared not to be applicable as criteria for this purpose because of the higher concentration of these alkaloids in hair of PACO smokers. More research is needed to assess the value of AEME in hair of South American coca leave or cocaine users.

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1. Introduction

Chewing of coca leaves and preparing and drinking of coca tea from coca leaves are legal practices and socially integrated in countries like Argentina, Perú, Bolivia or Chile. There is a Federal Law in Argentina that allow this practice [1]. Contrary to coca leaves, all different kinds of cocaine preparations (cocaine hydrochloride, crack or coca paste) are illegal and banned by national laws in all South American countries. Therefore, it is imperative to find an analytical solution in those cases where cocaine was detected in traffic control or in workplace drugs testing, and the accused claims to consume only coca leaves (chewing or drinking coca tea) but not any kind of processed cocaine.

The hair analysis for drugs of abuse proved to be an essential and very helpful tool to obtain evidence about the exposure of drugs in cases of driving ability examination, workplace testing, drug trafficking, criminal liability, child custody [2,3]. The advantage of hair in comparison to blood, urine or oral fluid is the extended time window, allowing to detect chronic consumption up to years. It was shown in previous papers that the coca alkaloids hygrine (HYG) and cuscohygrine (CUS) are suitable markers of coca leave chewing in urine [4], oral fluid [5] and hair [6]. Because of the hydrophilic behavior, these two alkaloids are lost in the illicit cocaine production and are not or only to a minor extent present in manufactured cocaine hydrochloride [7].

In the first study about the discrimination between Argentinean coca chewers and German consumers of manufactured cocaine by hair analysis, some preliminary criteria for coca chewing were proposed based on the detection and concentrations in hair of cocaine (COC), cinnamoylcocaine (CIN), ecgonine methyl ester (EME), HYG and CUS: Concentration ratios CIN/COC > 0.02, CUS/COC > 0.01, EME/COC > 0.015, and HYG (detected, typical range still to be determined) as well as the presence of two cuscohygrine metabolites [6]. These criteria should be used in combination.

An important analytical prerequisite for using such ratios is that the hydrolysis of COC by acidic or basic agents during sample preparation is excluded. However, these criteria cannot exclude the use of cocaine in addition to coca chewing. Evidence against coca chewing can be obtained by detection of typical adulterants such as levamisole or lidocaine. These are a strong indication of using manufactured cocaine in Europe but are not typical for South American countries where smoking of coca paste is a preferred kind of abuse.

Argentina is geographically near to Bolivia and Peru, two main producers of coca leaves. The coca paste is produced in the countries of origin and, in part, is sent to Argentina for finishing the production of cocaine hydrochloride. Paste base is one of the products obtained in the first steps of the extraction of coca leaves. The residue of coca paste is called PACO and is smoked, e.g. in combination with tobacco or cannabis, mainly by the lower social classes. This way to consume illegal cocaine is increasing in Argentina.

Heating cocaine base leads to the pyrolysis product methylecgonidine (anhydroecgonine methyl ester, AEME) [8,9]. Therefore, it was proposed as a marker in hair to differentiate between smoking and cocaine use via other routes of consumption [10–12]. However, this application of AEME seems to be limited since it was detected as an artifact in GC-EI-MS analysis of cocaine [13] and was repeatedly described as a constituent of some varieties of coca leaf [14–17].

The participants of the previous study [6] were residents from the northwest of Argentina with high tradition of almost daily consuming coca leaves. However, the pattern of consuming of coca leaves is variable in the different regions of Argentina. For instance, few people from Buenos Aires consume coca leaves or drink coca tea only occasionally. Furthermore, there were no studies about cocaine in PACO smokers in Argentina found in literature. In order to assess the efficiency of hair analysis for the discrimination between occasional coca chewers or coca tea drinkers and users of manufactured cocaine, it was investigated in the present study (1) whether CUS and HYG are detectable in hair of occasional chewers of coca leaves or drinkers of coca tea, (2) whether CUS and HYG appear in hair of PACO consumers from Argentina, and (3) whether AEME is useful as a cocaine smoking marker in this context.

2. Materials and methods

2.1. Participants of the study

The project was conducted according to the Helsinki ethical principles for medical research involving human subjects of the World Medical Association. A written informed consent was obtained from each individual and personal data as well as test results were anonymized in a way the identification of the person was impossible.

Three groups of volunteers were included in this study. The first group consisted of 10 adults (CC01-CC10) who live in Buenos Aires city and were moderate or occasional coca chewers or coca tea drinkers, with the self-reported frequency of daily, 1–3 times per week, 2 times per month or even less. Personal data as well as self-reported data about the coca-leaf consumption habits were recorded during sampling and are given in Table 1.

The second group consisted of 20 smokers of paste base (PS01-PS20), in Argentina popularly known as PACO. The volunteers were 9 women and 11 men, aged between 18 to 48 years, with problematic use of psychoactive substances who voluntarily attended outpatient withdrawal treatment. Most of the PACO consumers were polydrug-users and had received treatment previously.

Finally, the third group were 8 German cocaine users (GC1–GC8) with positive results for cocaine in a previous test with

Table 1
Personal data, self-reported consumption habits and hair samples of the coca chewers or drinkers of coca tea.

Volunteer	Age, gender	Occupation	Duration of use, years	Frequency	Amount per use, g	Alkaline additive	Hair color	Hair length [cm]
CC01	28f	Attorney	13 (chewing)	4/year	–	No	Brown	16
CC02	28m	Interior designer	1 (tea)	Occasionally	–	–	Brown	39
CC03	36m	Biochemist	15 (chewing)	2/month	5–10	Yes	Brown	6.5
CC04	28m	Attorney	10 (chewing)	1–2/week	5–10	No	Brown	4
CC05	27m	University student	10 (chewing)	Daily	5	No	Brown	4
CC06	36m	Tourism manager	15 (chewing)	3/week	5–10	Occas.	Black	9.5
CC07	28f	Biologist	14 (chewing)	2/month	5	Yes	Brown	53
CC08	36m	Physician	22 (chewing)	2/month	5	No	Black	4.5
CC09	29m	Public servant	15 (chewing)	6/year	5	No	Brown	2.5
CC10	28m	University student	16 (chewing)	15/year	5–10	Yes	Black	6

forensic background. The hair samples were reanalyzed for comparison in these investigations.

2.2. Preparation of hair samples

Scalp hair samples were collected at the vertex posterior region as close as possible to the skin. The samples with a length below 6 cm were investigated in full length. Longer hair samples were analyzed in 1–4 segments of 6 cm depending on the total length.

The sample preparation was performed according to the method optimized and described in detail in previous papers [6,18]. Briefly, the hair samples were washed with water and acetone, dried and cut to pieces of 1–2 mm length. Between 10–50 mg (depending on available sample amount) were exactly weighed in a 1.5 ml Eppendorf vial. After addition of the deuterated standards, the hair were 2-times extracted for 18 h with 0.5 ml of a mixture of methanol/acetonitrile/2 mM ammonium formate (25:25:50, v/v/v) with gentle shaking at 37 °C. The liquid phase was separated from both extractions, united and evaporated in a nitrogen stream to a residue of 0.5 ml in order to remove most of the organic solvents. Five microlitres of the residue were injected for LC–MS/MS.

2.3. Preparation of coca leaves and coca tea samples

Each four different samples of coca leaves from Argentina and of coca tea bags from Argentina, Bolivia and Columbia were analyzed for comparison with the hair results and particularly in order to confirm the presence of AEME. 50 mg coca leaves were cut to small pieces and extracted with 1 ml methanol by shaking for 24 h at 40 °C. The solutions were stored at −18 °C. For the measurement, a 1:100 dilution was prepared by mixing 10 μl of the extract with 10 μl of internal standards solution and 980 μl acetonitrile.

For the preparation of coca tea, one bag (about 1 g coca leaves) was steeped for 5 min with 200 ml 95 °C hot water. 50 μl of the tea were diluted with 45 μl 10 mM ammonium formate, 400 μl acetonitrile and 5 μl of internal standard solution (1 μg/ml) and stored at −18 °C till measurement.

2.4. Analytical methods

The solvents, reagents, reference substances, internal standards, analytical instrumentation and LC–MS/MS method on a HILIC column used in this study as well as the optimization and validation of the methods were described in detail in a previous paper [6]. The basic validation was based on international guidelines and included calibration, determination of linear range and linearity (R^2) and determination of the limits of detection (LOD) and of quantification (LOQ) as well as of the matrix effects [19,20]. All samples were analyzed for cocaine (COC), norcocaine (NC), benzoylecgonine (BE), ecgonine methyl ester (EME), cocaethylene (CE), cinnamoylcocaine (CIN), anhydroecgonine methyl ester (AEME), hygrine (HYG), cuscohygrine (CUS) and tropacocaine (TRO). HYG was unambiguously identified by the MS spectrum but could not be quantified in lack of a reference substance and only the peak area ratio HYG/COC was used as a relative measure of the concentration. The calibration of CIN was performed with a reference substance of trans-CIN and was used for quantification of the sum of *cis*- and *trans*-CIN as described previously [6]. AEME was not included in the original method and was added in a re-evaluation for the present study. The limits of quantification LOQ of all analytes in hair were in the range of 10–30 pg/mg.

3. Results and discussion

3.1. Consumers of coca leaves and coca tea

The concentrations of cocaine, its metabolites and other coca alkaloids in the hair segments of the 10 occasional or moderate users of coca leaves or coca tea are shown in Table 2. COC was detected in all samples with a vague relationship to the self-reported consumption frequency. For CC01, CC02, CC09 and CC10 with the lowest frequency COC was below 0.06 ng/mg whereas for CC05 and CC06 with the highest consumption rate 2.02 and 1.83 ng/mg were measured. The metabolites and the other coca alkaloids were found for CC05, CC06 and CC08 with COC concentrations of 0.61–2.02 ng/mg and only partly for CC04 and CC07. TRO was generally not detected in these samples. The preliminary criteria for coca chewing concerning the concentration ratios in hair EME/COC > 0.015, CIN/COC > 0.02, CUS/COC > 0.01, detection of HYG, which were proposed in [6] are fulfilled as far as the substances were quantified but the two metabolites of CUS were not identified.

CE as a marker of combined cocaine and alcohol consumption was detected in hair from eight volunteers with CE/COC ratios between 0.039 and 0.62 (mean 0.29, median 0.30). This high

Table 2
Concentrations of cocaine, cocaine metabolites and other coca alkaloids in hair of moderate users of coca leaves.

Volunteer	Consumption frequency	Segment length, cm	COC [ng/mg]	NC [ng/mg]	BE [ng/mg]	EME [ng/mg]	AEME [ng/mg]	CE [ng/mg]	CIN [ng/mg]	CUS [ng/mg]	HYG/COC peak area ratio[a]	TRO [ng/mg]
CC01	4/year	0–6	0.022	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		6–12	0.015	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		12–16	0.021	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CC02	Occasionally	0–6	0.0078	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		6–12	0.0099	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		12–18	0.025	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CC03	2/month	0–6.5	0.0097	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CC04	1–2/week	0–4	0.10	n.d.	0.27	0.019	n.d.	0.026	n.d.	n.d.	n.d.	n.d.
CC05	Daily	0–4	2.02	0.11	0.64	0.22	0.092	0.10	0.15	n.d.	0.031	n.d.
CC06	3/week	0–6	1.83	0.061	0.37	0.17	pos.	0.71	0.18	pos.	0.023	n.d.
		6–9.5	1.36	0.027	0.30	0.026	pos.	0.44	0.15	0.097	0.0062	n.d.
CC07	2/month	0–6	0.069	n.d.	0.018	0.084	n.d.	0.020	pos.	pos.	0.038	n.d.
		6–12	0.025	n.d.	n.d.	n.d.	n.d.	0.0089	pos.	n.d.	n.d.	n.d.
		12–18	0.011	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		18–24	0.0084	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CC08	2/month	0–4.5	0.61	0.012	0.085	0.039	pos.	0.024	0.029	0.13	0.0098	n.d.
CC09	6/year	0–2.5	0.039	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CC10	15/year	0–6	0.051	n.d.	n.d.	n.d.	n.d.	0.032	n.d.	n.d.	n.d.	n.d.

n.d. = not detected, pos. = detected, <LOQ.
[a] Identified by mass spectrum in comparison to coca leaves, no reference substance available.

occurrence of CE in hair of coca chewers was also found in the previous study [6] and can be explained by the fact that the habit to chew coca leaves, in general, is after lunch or dinner when it is common to drink alcoholic beverages, most frequently wine.

Surprisingly, 0.092 ng/mg AEME was detected in hair of CC05 with daily coca chewing, and traces of AEME were also identified in the samples from CC06 and CC08. Since the volunteers of this group denied smoking of any coca products, this result can only be explained by the presence of AEME in coca leaves or by its formation during storage or chewing. The formation of AEME as an artifact in the LC–MS/MS analysis after injection of cocaine or ecgonine methyl ester was excluded in this LC–MS/MS study. AEME as a constituent of coca leaves or coca tea was described previously by Novak et al. [14], Jenkins et al. [15], Zuanazzi et al. [16] and Casale et al. [17]. The latter authors determined AEME to be a primary constituent and not an analytical artifact. Furthermore, Reichardt detected AEME in coca tea as well as in oral fluid immediately after drinking of coca tea in her thesis [21]. The presence of AEME in samples of coca leaves and coca tea was also confirmed in the present study (section 3.4).

Altogether, it follows from the results in Table 2 and the literature that the discrimination between coca chewers and consumers of manufactured cocaine is not possible for occasional and low consumption frequency and that the detection of AEME in hair of coca chewers does not unambiguously prove smoking of cocaine products but can be explained by its presence in coca leaves or its formation during storage of the leaves or during tea preparation.

3.2. PACO smokers

Hair testing results of paste base smokers were not reported previously. The results of the group of 20 smokers of PACO (paste base) in withdrawal treatment are shown in Table 3. The concentration of COC varies in a wide range from 0.027 ng/mg for PS05 to 341 ng/mg for PS19. The metabolites NC, BE and EME were detected and could be quantified in almost all hair samples. With the exception of PS08 and two segments of PS 05 with the lowest COC concentrations, CE was also found in all samples too, showing that combined alcohol consumption played a role also for PACO smokers.

AEME was detectable in hair of 15 volunteers of this group with 0.07–9.4 ng/mg (mean 1.14 ng/mg, median 0.53 ng/mg, 0.7–10.8% of the COC concentration). Besides the samples with lowest COC concentrations from PS05 and PS08, AEME was also not found in hair of PS12, PS14 and PS17 with 1.72–4.28 ng/mg COC. These concentrations are in the same range or slightly lower than the data about AEME in hair of cocaine consumers in Europe and America reported by Kintz et al. (7 positive out of 65, 0.2–2.4 ng/mg [10]), Tsanaclis and Wicks (635 positive out of 7146, 0.1–80.9 ng/mg, median 0,8 ng/mg [22]), Cognard et al. (0–45 ng/mg, mean 7.3 ng/mg, median 2.5 ng/mg [23]) and Pego et al. (5 positive out of 7, 0,2–4.9 ng/mg [24]). However, the analysis in these previous investigations was performed by GC/MS and increased results by formation of AEME as an artifact at high GC temperatures cannot be excluded [13]. Nevertheless, the relative high abundance of 75% positive AEME results in hair of the PACO smokers in

Table 3
Concentrations of cocaine, cocaine metabolites and other coca alkaloids in hair of PACO smokers in withdrawal treatment.

Volunteer	Age, gender	Segment, cm	COC [ng/mg]	NC [ng/mg]	BE [ng/mg]	EME [ng/mg]	AEME [ng/mg]	CE [ng/mg]	CIN [ng/mg]	CUS [ng/mg]	Area ratio[a] HYG/COC	TRO [ng/mg]
PS01	18f	0–6	2.27	0.088	0.9785	0.047	0.099	0.030	0.22	n.d.	n.d.	n.d.
		6–12	4.50	0.040	2.08	0.031	0.18	0.017	0.24	n.d.	n.d.	n.d.
		12–18	5.62	0.026	2.25	0.033	0.21	0.012	0.25	n.d.	n.d.	n.d.
		18–24	5.78	n.d.	2.24	0.043	0.18	0.011	0.30	n.d.	n.d.	n.d.
PS02	29f	0–6	92.0	1.12	14.1	1.07	0.89	6.1	3.97	n.d.	n.d.	n.d.
		6–12	96.3	0.55	15.8	0.70	1.04	2.46	4.95	n.d.	n.d.	n.d.
PS03	29f	0–6	66.6	2.97	19.4	4.33	1.81	2.05	10.6	n.d.	n.d.	n.d.
		6–12	71.9	1.07	34.7	3.98	1.59	0.74	7.8	n.d.	n.d.	n.d.
		12–18	59.2	0.41	34.5	3.65	1.17	0.24	5.4	n.d.	n.d.	n.d.
		18–24	57.5	0.36	32.3	2.91	1.20	0.17	6.3	n.d.	n.d.	n.d.
PS04	30f	0–9	7.80	0.67	4.46	0.14	0.41	0.089	0.93	n.d.	n.d.	n.d.
		9–18	9.47	0.14	8.47	0.20	0.40	0.032	0.62	n.d.	n.d.	n.d.
PS05	35f	0–6	0.083	0.0073	0.080	pos.	n.d.	0.0074	n.d.	n.d.	n.d.	n.d.
		6–12	0.12	0.0052	0.12	0.0054	n.d.	pos.	n.d.	n.d.	n.d.	n.d.
		12–18	0.035	pos.	0.046	pos.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		18–24	0.027	pos.	0.016	pos.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PS06	36f	0–9	3.73	0.084	1.14	0.073	0.073	0.45	0.47	n.d.	n.d.	n.d.
		9–18	7.97	0.044	2.08	0.32	0.13	0.098	0.559	n.d.	n.d.	n.d.
PS07	37f	0–8	43.8	3.16	10.4	3.21	2.05	0.38	3.2	n.d.	n.d.	n.d.
		8–16	65.8	3.58	23.6	2.38	3.44	0.35	5.0	n.d.	n.d.	n.d.
PS08	38f	0–6	0.080	pos.	0.021	0.005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		6–12	0.42	0.014	0.078	0.0089	n.d.	n.d.	0.046	n.d.	n.d.	n.d.
		12–30	0.079	pos.	0.019	0.0048	n.d.	n.d.	0.012	n.d.	n.d.	n.d.
PS09	44f	0–6	52.1	0.56	8.8	0.43	0.53	0.72	2.61	n.d.	0.00025	n.d.
		6–12	32.6	0.28	9.96	0.35	0.38	0.42	2.12	n.d.	n.d.	n.d.
		12–18	18.7	0.065	2.89	0.226	0.14	0.067	1.22	n.d.	n.d.	n.d.
PS10	21m	0–3	14.6	0.82	2.27	1.26	1.57	0.18	2.76	n.d.	0.0017	n.d.
PS11	28m	0–2.5	51.3	0.70	5.86	1.92	0.36	0.90	1.68	n.d.	0.0012	n.d.
PS12	29m	0–2.5	1.72	0.039	0.36	1.49	n.d.	0.065	n.d.	n.d.	n.d.	n.d.
PS13	30m	0–7	169	4.56	39.3	12.7	2.0	4.7	6.17	n.d.	0.00020	n.d.
PS14	30m	0–3.5	4.28	0.067	0.53	0.176	n.d.	0.10	0.17	n.d.	0.0049	n.d.
PS15	30m	0–4.5	23.7	0.76	5.19	0.67	0.57	0.47	0.86	n.d.	n.d.	n.d.
PS16	31m	0–5	30.6	1.24	3.68	1.99	0.39	1.98	1.16	n.d.	0.00039	n.d.
PS17	35m	0–5.5	2.67	0.020	0.23	0.021	n.d.	1.11	0.099	n.d.	0.0033	n.d.
PS18	36m	0–2	4.60	0.021	0.32	0.076	0.093	0.072	0.16	n.d.	n.d.	n.d.
PS19	37m	0–3	341	5.74	6.92	6.75	9.4	1.69	45.1	n.d.	n.d.	n.d.
PS20	48m	0–8	19.5	0.73	8.30	0.38	0.61	0.010	0.96	n.d.	0.00035	n.d.

n.d. = not detected, pos. = detected, <LOQ.
[a] Identified by mass spectrum in comparison to coca leaves, no reference substance available.

comparison to the literature data supports its origin from pyrolysis of paste base.

Application of the preliminary criteria for coca chewing described in Ref. [6] to these samples led to the following results: Only the criterion CUS/COC > 0.01 is not fulfilled and excludes coca chewing since CUS was not detected in all samples. However, the ratio CIN/COC of 0.033–0.189 (mean 0,077, median 0.065) exceeds the proposed lower limit for chewing of 0.02 for all samples. The ratio EME/COC is 0.006–0.087 (mean 0.033, median 0.021) and exceeds the proposed lower limit of 0.015 in 23 of the analyzed 39 hair segments, and traces of HYG were detected in 8 hair segments. That means that from these criteria only the absence of CUS excludes coca chewing as the reason of the positive cocaine results for all participants in this group.

3.3. German cocaine consumers

For comparison, hair samples of 8 German cocaine consumers (GC1–GC8), who were tested positive previously in different context, were included in the study. The results are shown in Table 4. The COC concentration ranged from 0.89 to 89.5 ng/mg, and NC, BE and EME were quantifiable in all samples. CE was detected in five samples. Three samples were positive for AEME (0.17–2.85 ng/mg), indicating consumption by smoking. CUS was negative in all samples but traces of HYG were detected in two samples in a similar range as for the PACO smokers (Table 3). CIN was found in two samples but the ratio CIN/COC (0.0007–0.010) was below the typical range for coca chewers (>0.02). However, the ratio EME/COC (0.007–0.21) exceeds the proposed lower limit for chewing of 0.015 in five of the eight samples. Since EME is a hydrolytic metabolite of cocaine it can be formed from COC in the same way as BE by reaction with water within the hair matrix and outside the hair root. Because of this possible hydrolytic formation of EME, the ratio EME/COC appears not to be a specific criterion for coca chewing.

3.4. Anhydroecgonine methyl ester (AEME) in coca leaves and coca tea

In order to explain the detection of AEME in hair of three coca chewers in Table 2 and to confirm previous reports of its presence as a constituent in coca leaves [14–17,21], each four different samples of coca leaves and of coca tea were analyzed as described in section 2.3. The percentage of the alkaloids in the dry plant material is given in Table 5. The results of all 8 samples are in a similar range with COC, CIN, EME and CUS as the main components. HYG was only qualitatively detected. There is no essential difference between the methanolic extraction of the coca leaves and the aqueous extraction with hot water of the tea bags.

The mean content of AEME is 0.0056 ± 0.0020% (56 ± 20 μg/g). In previous papers, AEME in coca leaves was only qualitatively identified [14–17]. A quantitative determination was only performed in the PhD thesis of Reichardt who found 51–66 ng/ml in 5 samples of tea brewed from 1 g of Erythroxylum coca in 250 ml water [21]. Recalculation to the amount of coca leaves leads to 0.0014% AEME at about the same COC content of 0.42% (data given for comparison in Table 5), that is about four times lower than in the present study.

It follows from these results that AEME in hair of coca chewers can originate from its content in coca leaves. Therefore, a positive hair test of AEME in such cases cannot be used as an unambiguous prove of cocaine smoking. It is conceivable that a cut-off of AEME or of its ratio to COC or to EME might be helpful to exclude coca chewing as a reason of a positive AEME result. However, further investigations with a larger number of regular and heavy coca chewers are necessary to verify this possibility. In the present study, the ratio AEME/COC varies too much and is not sufficiently different between the positive samples from 15 PACO smokers (0.007–0.052), four German cocaine consumers (0.022–0.16) and three coca chewers (0.045 or smaller) and is in a similar range as in coca leaves or coca tea 0.0033–0.016).

Table 4
Concentrations of cocaine, cocaine metabolites and other coca alkaloids in hair of German cocaine users.

Volunteer	Age, gender	Segment, cm	COC [ng/mg]	NC [ng/mg]	BE [ng/mg]	EME [ng/mg]	AEME [ng/mg]	CE [ng/mg]	CIN [ng/mg]	CUS [ng/mg]	Area ratio[a] HYG/COC	TRO [ng/mg]
GC1	39m	0-5	2.20	0.0068	0.61	0.019	n.d.	n.d.	n.d.	n.d.	0.0079	n.d.
GC2	29f	0–6	0.89	0.035	0.42	0.041	n.d.	0.62	n.d.	n.d.	n.d.	n.d.
GC3	30m	0–1	89.5	2.01	16.6	0.72	2.85	1.42	0.060	n.d.	n.d.	pos.
GC4	29m	0–6	7.9	0.16	1.08	0.054	0.17	n.d.	n.d.	n.d.	n.d.	n.d.
GC5	28f	0–6	5.12	0.082	2.54	0.26	n.d.	0.44	n.d.	n.d.	n.d.	n.d.
GC6	22m	0–5	3.89	0.025	0.80	0.061	n.d.	0.19	n.d.	n.d.	n.d.	n.d.
GC7	32m	0–6	1.53	0.0096	0.18	0.033	0.25	pos.	n.d.	n.d.	0.0011	n.d.
GC8	25f	0–6	1.94	0.047	0.68	0.052	n.d.	n.d.	0.019	n.d.	n.d.	n.d.

n.d. = not detected, pos. = detected. <LOQ.
[a] Identified by mass spectrum in comparison to coca leaves, no reference substance available.

Table 5
Alkaloid content in dry coca leaves and coca tea bags.

Sample[a]	COC, %	CIN, %	EME, %	AEME,%	CUS, %	BE, %
Coca tea 1	0.31	0.21	0.067	–[b]	0.038	0.012
Coca tea 2	0.38	0.048	0.15	0.0029	0.14	0.0051
Coca tea 3	0.41	0.089	0.061	0.0059	0.14	0.024
Coca tea 4	0.36	0.091	0.18	0.0088	0.24	0.027
Coca leaves 1	0.30	0.104	0.11	0.0067	0.11	0.016
Coca leaves 2	0.38	0.067	0.10	0.0041	0.16	0.035
Coca leaves 3	0.25	0.023	0.038	0.0037	0.088	0.012
Coca leaves 4	0.40	0.051	0.093	0.0072	0.16	0.031
Mean	0.35 ± 0.05	0.085 ± 0.05	0.10 ± 0.04	0.0056 ± 0.002	0.13 ± 0.06	0.020 ± 0.01
Coca tea [13]	0.42	–	0.15	0.0014	–	0.15

[a] Tea 1: Coca Nasa-Nasa Esh's, Industria Indígena, Columbia, 1.02 g per bag; tea 2: unlabeled, 1.23 g per bag; tea 3: trimate mate, Windsor "*es compatir*", 1.34 g per bag, Bolivia; tea 4: coca mate, Windsor, "*es compatir*", 0.77 g per bag, Bolivia. Coca leaves from different sources in North Argentina.
[b] Analytical error, value excluded.

4. Conclusions and limitations of the study

Concerning the main reason of this study, the discrimination between coca chewers and users of manufactured cocaine in South America by hair analysis, and in completion of the previous report [6], the following conclusions can be drawn from the results: Caused by the sensitivity of the present routine LC–MS/MS methods in hair analysis with a lower limit of quantification of about 0.01 ng/mg for most drugs and metabolites, the analytical proof of coca chewing is limited to samples with a COC concentration above 0.5 ng/mg and cannot be applied to rare or occasional consumption.

The main marker for coca chewing remains CUS which was not found in hair of the Argentinean PACO smokers and of the German cocaine consumers. The second marker HYG can be used for confirmation, but traces of this marker were also detected in hair of some of the PACO smokers as well as some German cocaine users, showing that it is not completely removed during the illegal cocaine production [7].

Until now, hair results of South American PACO smokers were not described in literature. In comparison to the German cocaine consumers of this and the previous study [6], they provide higher concentrations of EME and CIN in comparison to COC. Obviously, these two coca alkaloids are still contained to a higher degree in PACO, or EME is formed to a higher extent from cocaine as a metabolite or by hydrolysis in hair. Therefore, the preliminary criteria of CIN and EME for coca chewing (CIN/COC > 0.02 and EME/COC > 0.015) proposed in Ref. [6] are not applicable to hair samples from Argentinean PACO smokers.

AEME appears not to be a specific marker for smoking of cocaine base in South America since it is a minor constituent of coca leaves and can occur in hair of coca chewers without smoking. However, this does not concern its use as a crack marker in Europe or North America. In summary, the discrimination between using of coca leaves and manufactured cocaine in forensic cases by hair analysis is only to some extent possible and must be supported by other evidence.

It is a limitation of this preliminary study that, due to the lack of a reference substance, only qualitative results for HYG could be given. Furthermore, no heavy coca chewers were included, particularly for determination of AEME, which was not measured in the first report [6]. Generally, the results must be corroborated by further investigations with more participants and with reliable data about frequency and special manners of coca chewing or PACO smoking in order to elucidate additional factors such as use of basic additives or color and cosmetic treatment of hair.

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