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Impact of coca leaf flour candy consumption on cocaine and benzoylecgonine levels: The role of hygrine and cuscohygrine in distinguishing licit from illicit cocaine use

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ABSTRACT

The consumption of coca leaf products, a traditional practice in several Latin American countries, raises forensic challenges in distinguishing legal consumption from illicit cocaine use. This study investigates the implications of consuming coca flour-based candies on drug detection thresholds in oral fluid (OF) and urine for drug-impaired driving (DUID) and workplace drug testing (WDT) contexts. Three commercial candy brands were analyzed, revealing significant variability in coca alkaloid content. Volunteers consumed these candies under controlled conditions, with biological samples collected at various intervals. Key analytes, including cocaine (COC), benzoylecgonine (BE), ecgonine methyl ester (EME), tropococaine (TRO) and cocaethylene (CE), were quantified alongside coca leaf-specific markers (cuscohygrine, cinnamoylcocaine, and hygrine). Analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with multiple reaction monitoring mode (MRM) transitions to ensure specificity.

Results: indicate that coca leaf markers (CUS, HYG) remain detectable after candy consumption, while traditional cocaine markers (COC, BE) exceeded international cutoffs for DUID and WDT in certain cases, particularly following ingestion of high-alkaloid brands. Coca-flour candies with alcoholic beverages produced detectable CE levels but never exceeded the cutoff values of 8 ng/mL in OF or 20 ng/mL in urine required by DUID guidelines. Analyte variability was influenced by candy brand, quantity consumed, and method of ingestion, with oral fluid showing prolonged detection when candies adhered to teeth. These findings emphasize the need to account for legal coca product consumption, as misinterpreted results could unjustly categorize consumers as illicit drug users, requiring nuanced approaches in forensic toxicology.

1. Introduction

It is already widely known that the consumption of coca leaves is a common and long-standing practice in several Latin American countries. In some of these countries, such as Argentina, it is a legally accepted practice [1]. In many cases, it is difficult to distinguish whether a positive result for cocaine/benzoylecgonine in urine or oral fluid comes from illegal use (inhaling, smoking or injecting) or from legal consumption of coca leaves. It is therefore of particular interest to be able to differentiate the origin of drug use in criminal proceedings in South American countries, as well as in drug-impaired driving (DUID) cases, workplace drug testing (WDT) programs, and anti-doping controls. Hygrine (HYG) and cuscohygrine (CUS) are two alkaloids found in coca leaves that are lost during the illegal production of cocaine [2]. For this reason, they have been proposed, along with cinnamoylcocaine (CIN), as markers of coca leaf consumption [3,4]. Cinnamoylcocaine is considered a secondary marker because, depending on the process used in the illegal production of cocaine, it may also be present in street drugs [5].

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In the Andean region, coca leaf consumption is not limited to chewing coca leaves or drinking coca tea. In fact, coca-based products such as coca candies, chocolates, cereal bars, beverages, and cookies made with coca flour are also sold in markets and specialized shops. These products are consumed not only by the local population but also by tourists visiting landmarks such as the Machu Picchu ruins in Peru. Many of these visitors, upon arrival, may experience altitude sickness, and coca leaves or their derivatives can naturally help alleviate their symptoms. Moreover, some visitors purchase these novel products and take them back to their home countries in Europe, the United States, and elsewhere, often unaware of the potential legal consequences of their use in those countries [6].

Urine is a valuable sample for qualitative analysis in forensic toxicology due to its non-invasive collection, wide drug detection window and high analyte concentrations. However, it is less suitable for quantification compared to blood. Variability in drug excretion rates complicates result interpretation. Factors such as individual metabolism, hydration levels, renal function, and urine pH can affect drug concentrations. For example, hydration can dilute urine, leading to false negatives, while dehydration may cause false positives. Impaired kidney function can extend detection times, while healthy kidneys may clear drugs more rapidly. These factors make it difficult to precisely determine drug use timing or accurately quantify drug intake from urine alone [7, 8].

Oral fluid (OF) is a widely used specimen in forensic toxicology laboratories for applications such as drug-impaired driving investigations, workplace drug testing, and criminal and death investigations, among others. It is easy to collect, non-invasive, and has a shorter detection window compared to urine and hair. Additionally, the parent drug that was consumed can be detected.

In view of the above, the objective of this study was to investigate the possibility of legal implications for an individual consuming candies made with coca flour, considering the cut-off concentration values for cocaine/benzoylecgonine/cocaethylene (COC/BE/CE) currently established by DUID and WDT regulations for oral fluid/urine [9–13]. Furthermore, the concentrations of markers for coca leaf consumption, such as HYG, CUS, and CIN, as well as tropococaine (TRO) and ecgonine methyl ester (EME) (EME is an alkaloid found in coca leaves as well as a metabolite of cocaine), will be measured in urine and oral fluid samples. The study will assess whether the concentrations of alkaloids from the coca leaf and cocaine metabolites (BE and EME) are influenced by factors such as the brand of candy, the amount consumed, and the method of ingestion. Additionally, the feasibility of CE formation (a toxic metabolite synthesized in the liver by individuals who consume cocaine and alcohol simultaneously) will be evaluated when the candies are consumed in conjunction with alcoholic beverages. A total of forty-four (44) biological samples and three commercial candies were analyzed.

2. Experimental

2.1. Reagents and chemicals

All standard solutions were prepared from stock standards (1 mg/mL dissolved in acetonitrile) of COC, BE, EME, t-CIN, CE and TRO supplied by LGC Standards, S.L.U. (Barcelona Spain). CUS (10 mg) was obtained from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Deuterated analogous standard solutions (0.1 mg/mL) of cocaine-d3 (COC-d3), benzoylecgonine-d3 (BE-d3), ecgonine-d3 methyl ester (EME-d3) and cocaethylene-d3 (CE-d3) were supplied by LGC Standards, S.L.U. and cuscohygrine-d6 (CUS-d6) (0.1 mg/mL) was obtained from Toronto Research Chemicals Inc. HYG was not available at the time of the start of this study, and it was unambiguously identified by the MS spectrum obtained from coca leaves after extraction and further identification based on m/z (precursor ion) / m/z (product ion) transitions after coca leaf extract infusion and mass spectral recording. HYG could not be quantified in lack of a reference substance and only the peak area ratio HYG/COC-d3 was used as a relative measure of the concentration. This procedure has been described in detail in a prior publication [14]. Acetonitrile and methanol (LC-MS grade) were purchased from Riedel-deHaën (Seelze, Germany), formic acid (98 %) from Panreac (Barcelona, Spain) and ammonium formate (99 %) from Fluka Analytical (Steinheim, Germany). Ultrapure water was obtained from a MilliQ-A10 system (Millipore, Bedford, MA, USA). The Waters Oasis® HLB extraction cartridge of 3 cm^3 /60 mg came from Waters (Spain). Cellulose acetate syringe filters (0.20 μm) were from Labbox Labware S. L. (Barcelona, Spain).

2.2. Working solutions

Working standard calibrations were prepared in acetonitrile from stock solutions at concentrations of 0.05; 0.1; 0.5; 1.0; 2.0 and 10 μg/mL for COC, BE, EME, t-CIN, TRO, CE and CUS. A mix of deuterated analogues was prepared in acetonitrile from stock solutions at a concentration of 1.0 μg/mL (COC-d3, BE-d3, EME-d3, CE-d3) plus 10 μg/mL (CUS-d6). All working standards were stored at −20 °C when not in use.

2.3. Calibrators and quality control (QC)

Six drug-free OF samples, each with a volume of 1.0 mL, were spiked with drugs at concentrations of 1, 5, 10, 50, 100, and 200 ng/mL. Internal standards were added at a concentration of 10 ng/mL for COC-d3, BE-d3, EME-d3, and CE-d3, and at 50 ng/mL for CUS-d6. Quality control samples at concentrations of 10, 20, and 50 ng/mL were also prepared, containing the internal standards. Before being spiked, OF samples were centrifuged at 10000 rpm for 10 min. The spiked OF mixtures were subjected to the SPE procedure using Waters Oasis® HLB extraction cartridges, according to previous publication validation method [15].

For urine analysis, eight analyte concentrations were prepared by spiking 5.0 mL of drug-free urine, previously centrifuged at 3500 rpm, at concentrations of 200, 150, 100, 50, 20, 10, 2, and 1 ng/mL, with deuterated internal standards at a concentration of 4 ng/mL for COC-d3, BE-d3, EME-d3, and CE-d3, and 20 ng/mL for CUS-d6. The fortified urine samples were extracted using Waters Oasis® HLB extraction cartridges [16]. The selection of urine cutoff values considered the recommended cutoff levels established by international guidelines for cocaine and benzoylecgonine (Table 1). In addition, the concentration of 1.0 ng/mL was also considered for establishing the sensitivity of the method since the expected low concentration of coca alkaloids in urine and OF after coca flour candy consumption.

For the analysis of the three commercial coca flour candies (for the purposes of this study will be labeled as Am, Ac, and Ao), four standards with deuterated internal standards (COC-d3, EME-d3, CUS-d6) were prepared in mobile phase in a 92:8 ratio (4:1 acetonitrile: methanol): 20 mM ammonium formate/formic acid buffer (pH 4.2), at concentrations of 0.25, 0.5, 1, and 2 ppm.

The candy samples, as well as the spiked OF and the urine of the volunteers, were analyzed in duplicate on two separate days. The main validation parameters from our previous publication [15] are briefly summarized as follows: LOD values were less than 1.0 ng/mL for COC, BE, CE, EME, TRO and CIN, and 10 ng/mL for CUS. The LOQs were 1.0 ng/mL for COC, BE and CE, and 5.0 ng/mL for EME, TRO and CIN. The matrix effect (suppression or enhancement) did not exceed 25 %, except for EME which showed a matrix effect of −37 % (OF). The linear working range was 1.0–200 ng/mL for COC, BE, CE, TRO, CIN and EME, and 5.0–200 ng/mL for CUS. In this study, CE was added to the analytical panel.

2.4. Instrumental analysis and measurement

2.4.1. Thermo Scientific INDIKO analyzer

An automated Thermo Scientific INDIKO analyzer for drugs of abuse was employed, capable of detecting the presence of benzoylecgonine (a

Table 1
Guidelines on regulatory cutoff levels for cocaine, benzoylecgonine, and cocaethylene in urine and oral fluid.

GUIDELINES		Analyte	Initial test cutoff	Confirmatory test cutoff
SAMHSA_worplace [10]	Urine	Benzoylecgonine	150 ng/mL	100 ng/mL
European Guidelines_Workplace [12]	Urine	Benzoylecgonine	150 ng/mL	100 ng/mL
Logan. Et al. (DUID) [13]	Urine	Cocaine	-	20 ng/mL
		Benzoylecgonine	150 ng/mL	50 ng/mL
		Cocaethylene	-	20 ng/mL
SAMHSA_worplace [9]	Oral Fluid	Cocaine/Benzoylecgonine	15 ng/mL	8 ng/mL_8ng/mL
European Guidelines_Workplace [11]	Oral Fluid	Cocaine+metabolites/Cocaine_Benzoylecgonine	30 ng/mL	8 ng/mL_8ng/mL
Logan. Et al. (DUID) [13]	Oral Fluid	Cocaine	15 ng/mL	8 ng/mL
		Benzoylecgonine	15 ng/mL	8 ng/mL
		Cocaethylene	-	8 ng/mL

cocaine metabolite) in urine samples by means of a homogeneous enzyme immunoassay (CEDIA). A standard cutoff concentration of 300 ng/mL was applied, consistent with routine protocols for forensic sample analysis in our laboratory. Benzoylecgonine concentrations determined by the analyzer as below the cutoff value of 300 ng/mL were considered as preliminary estimates, pending confirmation via LC-MS/MS.

2.4.2. Liquid chromatography mass spectrometry (LC-MS/MS)

Determinations were carried out with a 3200 Q TRAP LC-MS/MS system (ABSciex, Concord, Canada), equipped with a Flexar FX15 UHPLC binary chromatographic pump (PerkinElmer, Waltham, MA, USA), and a Flexar UHPLC autosampler (PerkinElmer). Analyst 1.6 software (ABSciex) was used for system control and data acquisition. MultiQuant 2.1 software (ABSciex) was used for data processing. Separations were performed with an Infinity LabPoroshell 120 Hilic (2.7 mm, 2.10 × 100 mm) from Agilent Technologies (Santa Clara, CA, USA). Temperature control of the column was performed with a GECKO 2000 column heater (temperature control from 30 to 80 °C) from Amchro GmbH (Hattersheim, Germany), a positive electrospray ionization (ESI) mode was used. Measurements were carried out under similar conditions as in our previous works [15,17]. The gradient elution used was 20 mM ammonium formate in ultrapure water (pH 4.2) (A) and an acetonitrile/methanol (4:1) mixture (B) as a mobile phase for compound separation. The gradient was run as the following scheme: from sample injection until 0.1 min, gradient elution to 8 % (A), flow rate 300 (µL/min); from 0.1 to 2 min, a linear gradient to 9 % (A), flow rate 300 (µL/min); from 2 to 6 min, a linear gradient to 15 % (A), flow rate 300 (µL/min); from 6 to 11 min, a linear gradient to 60 % (A), flow rate 500 (µL/min); from 11 to 12 min, isocratic elution with 60 % (A), flow rate 500 (µL/min); from 12 to 13 min, a linear gradient to 40 % (A), flow rate 500 (µL/min); from 13 to 14 min, a linear gradient reduces to 8 % (A), flow rate 300 (µL/min); and from 14 to 20 min, isocratic elution back to 8 % (A), flow rate 300 (µL/min). Chromatographic separations were performed at 40 °C and the chromatographic total run time was 20 min.

The criteria that were used on the data acquisition parameters for each transition of the multiple reaction monitoring mode (MRM) is listed in Table 2. At least two precursor ions to product ion transitions were monitored for each analyte to ensure the specificity of the measurements (the presence of an analyte was confirmed when all qualifying MRM transitions in each chromatographic series were identified). The MRM transitions that offered the most sensitive MRM transitions were finally used for quantification. The optimized ion source parameters (positive ionization) were set at 400 °C for temperature, 5000 V for voltage, 20 psi for curtain gas (N_2), 15 psi for nebulizer gas (N_2), and HIGH mode for collision gas (N_2).

Table 2
MRM transitions, internal standards and retention times of coca alkaloids ad cocaine metabolites.

Analyte name	Precursor ion (amu)	Product ion (amu)	ISTD	RT (min)
BE	290.1	**168.2**	BE-d3	2.71
		105.1		
		77.0		
COC	304.1	**182.0**	COC-d3	2.87
		105.1		
		82.0		
		77.0		
EME	200.1	182.2	EME-d3	4.10
		82.2		
		67.1		
		41.1		
tCIN	330.2	**182.1**	COC-d3	2.94
		103.1		
		77.1		
		51.1		
TRO	246.1	**124.1**	COC-d3	3.05
		77.1		
		67.1		
		51.1		
CUS	225.1	**84.1**	CUS-d6	5.98
		42.1		
HYG	142.1	**84.1**	-	3.55
		42.1		
CE	318.2	**196.1**	CE-d3	2.78
		82.1		
		77.1		
BE-d3	293.1	**171.2**		2.64
		80.0		
COC-d3	307.1	**185.1**		2.78
		85.1		
EME-d3	203.1	185.1		4.21
		85.1		
CE-d3	321.2	**199.1**		2.77
		85.1		
CUS-d6	231	**87**		6.00
		45		

amu: atomic mass unit; ISTD: internal standard; RT: retention time

2.5. Samples

2.5.1. Urine and oral fluid samples

Urine and OF samples were collected from twelve volunteers attending a social gathering, where they consumed mint candies from three commercial brands containing coca flour and other typical candy ingredients. Volunteers were offered three candies from the commercial brands, labeled as Am, Ac, Ao, which they selected and consumed *ad libitum* within a time range. In the study, participants were allowed to freely choose the amount and type of commercial candy to eat after dinner. Previously, all volunteers were informed about the upcoming experience, which involved consuming candies from three commercial brands obtained from retail outlets in Peru, made with coca flour, containing low levels of cocaine and there was no risk to health. Before starting the study, and after providing all the necessary information to the participants, all signed an informed consent document. Based on previous studies [15,18], participants were recommended to take the OF

sample at 0 minutes (immediately after finishing the coca candy), as well as at 30, 60, and/or 90 min, while urine samples could be collected between 1.5 and 36 hours. However, the number of samples collected was left to the participants discretion to prevent any discomfort during the sampling process. The volunteers then collected urine and OF samples in containers provided by our laboratory and recorded the brand, quantity of candies consumed, and the manner of candy consumption, i.e., whether they sucked on the candy until it dissolved or chewed and swallowed it, for example. Participants were also required to report whether they consumed alcohol during the party, type of beverage and provide the time of oral fluid and urine sample collection. All OF and urine samples, including those obtained after the event, were transported to the laboratory the following day and stored at −20°C until processing. The details of the volunteers, sample types, collection times among others are shown in Table 3. The concentration of coca leaf alkaloids (cocaine (COC) and its metabolite BE, EME—an alkaloid of the coca leaf and a cocaine metabolite in biological samples—HYG, CUS, TRO, CIN, and CE were measured in the collected OF (Table 4) and urine samples (Table 5).

Volunteer 5 participated in the meeting, agreed to provide urine and OF samples, but did not consume candies or alcohol due to being responsible for driving a vehicle. The OF and urine samples from volunteer 5 were considered blank samples.

In order to assess the magnitude of the excretion of coca alkaloids in urine, originating from coca flour candies, and to determine whether the excreted levels could exceed the cutoffs recommended by international guidelines for WDT and DUID (TABLE 1), the following criteria were applied: 1) All urine samples were analyzed using INDIKO (Thermo Scientific), an CEDIA immunoassay with a cutoff of 300 ng/mL (benzoylecgonine). 2) Subsequently, the samples were analyzed using LC-MS/MS with cutoff values set at 200, 150, 100, 50, 20, 10, 2, and 1 ng/mL.

OF samples were analyzed using a method previously validated in this laboratory [15,17]. The concentration range investigated for the analytes was between 1 and 200 ng/mL, considering that international guidelines establish a cutoff of 8 ng/mL for COC, BE and CE (TABLE 1). For the remaining alkaloids of the coca leaf (TRO, CUS, HYG, CIN and EME), no proposed cutoff values are available.

A total of 44 biological samples were analyzed (26 oral fluid samples and 18 urine samples) from twelve volunteers. The concentration of the analytes under study (COC and its metabolite BE, EME -an alkaloid of the coca leaf and a cocaine metabolite in biological samplesHYG, CUS, TRO, CIN), and CE was measured in the collected urine and oral fluid samples.

2.5.2. *Candies samples*

Three different commercial brands of candies Am, Ac, Ao were analyzed. The weight of a single candy per brand was determined: 2.4 g, 4.0 g and 3.4 g, respectively. A candy from each commercial brand was ground using a mortar, and two aliquots were taken from each sample. The candy aliquots were extracted for 1 hour using an ultrasonic bath with 2.0 mL of a solvent-buffer mixture in a 95:5 ratio (acetonitrile: Methanol 4:1): 20 mM ammonium formate/formic acid buffer. The samples were then centrifuged, and the supernatant was separated and passed through a 0.22 μm cellulose filter. One mL of the filtrate was spiked with a deuterated standard mixture at a concentration of 100 ng/mL for COC-d3, BE-d3, and EME-d3, and 1 μg/mL for CUS-d6.

3. Results and discussion

Table 6 shows the results of the concentrations of coca leaf alkaloids in three commercial candies from the Andean region of South America. The candy wrappers list the ingredients as follows: sugars, coca essence, and coca flour for Ac and Ao candies, while Am candies do not list coca flour as an ingredient but instead mention “muña” flour (from the plant *Minthostachys mollis*, known as “muña”). However, analysis revealed that Am candies contain coca leaf alkaloids, like the other candies analyzed (Fig. 1). The quantities of each ingredient are not specified. Coca flour is obtained by pulverizing the dried leaves of the coca plant: *Erythroxylum coca* and *Erythroxylum novogranatense* (a green-colored solid). The concentration of coca leaf alkaloids in coca flour depends on factors such as the coca plant variety, cultivation type, the leaf

Table. 3
Description of oral fluid (OF) and urine (U) sampling.

Volunteers	Brand Candy/ Number of Candy/Beverage consume	Code	Sampling Time (min)	Code	Sampling Time (h)	Observation
1	Am-Ac/2/YES_wine	OF_1	30	U_1	1.5	Suck the candies until they dissolved in your mouth
2	Am-Ac/2/YES_wine	OF_2	30	U_2	1.5	Suck the candies until they dissolved in your mouth
3	Am-Ac/4/YES_wine	OF_3	45	U_3a	1.5	Chewed and swallowed candies
4	Am-Ac/2/YES_wine	OF_4	30	U_4	1.5	Suck the candies until they dissolved in your mouth
5	Not consume candy	OF_5	30	U_5	1.5	———
6	Ao/2/NO	OF_6a	0	U_6	1.5	Suck the candies until they dissolved in your mouth
		OF_6b	60			
		OF_6c	90			
7	Ao/2/NO	OF_7a	0	U_7a	4	Suck the candies until they dissolved in your mouth
		OF_7b	60	U_7b	12	
		OF_7c	90			
8	Ao/1/NO	OF_8a	0	U_8a	2	Suck the candy until it dissolved in your mouth
		OF_8b	60	U_8b	8	
		OF_8c	90			
9	Ao/1/YES_wine	OF_9a	0	U_9a	2	Suck the candy until it dissolved in your mouth
		OF_9b	60	U_9b	30	
		OF_9c	90			
10	Ao/1/YES_wine	OF_10a	0	U_10a	2	Suck the candy until it dissolved in your mouth
		OF_10b	60	U_10b	30	
		OF_10c	90			
11	Ao/2/YES_"Brandy"	OF_11a	0	U_10a	2	Chewed and swallowed candies
		OF_11b	60	U_10b	30	
		OF_11c	90			
12	Ao/1/NO	OF_12a	0	U_10a	1.5	Chewed and swallowed candies
		OF_12b	60			
		OF_12c	90			
Total samples: **44**		**26**		**18**		

The three commercial brands of coca flour based candies used in this study were arbitrarily labeled as: Ac, Am, and Ao.

Table 4
Concentration of coca leaf alkaloids and cocaine metabolites in oral fluid (OF) after ingestion of different coca flour based candies.

Volunteers	Code	Sampling Time (min)	COC (ng/mL)	BE (ng/mL)	EME (ng/mL)	CUS (ng/mL)	HYG R=area $_{(HYG)}$/IS$_{_COCd3}$	CIN (ng/mL)	TRO (ng/mL)	CE (ng/mL)
1	OF_1	30	10	n.d.	n.d.	n.d.	n.d.	5	n.d.	n.d.
2	OF_2	30	125	38	6	20	0.13	26	n.d.	n.d.
3	OF_3	45	31	3	n.d.	n.d.	0.02	5	n.d.	n.d.
4	OF_4	30	n.d.	45	n.d.	10	0.18	27	n.d.	n.d.
5	OF_5	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	OF_6a	0	1185	354	444	1264	3.59	462	n.d.	n.d.
	OF_6b	60	24	3	41	48	0.09	14	n.d.	n.d.
	OF_6c	90	n.d.	1	38	33	0.06	4	n.d.	n.d.
7	OF_7a	0	3186	1207	1983	5604	4.39	935	n.d.	n.d.
	OF_7b	60	368	54	148	292	0.37	128	n.d.	n.d.
	OF_7c	90	112	15	84	185	0.17	61	n.d.	n.d.
8	OF_8a	0	2621	416	491	3725	3.02	390	n.d.	n.d.
	OF_8b	60	35	2	12	22	0.05	10	n.d.	n.d.
	OF_8c	90	14	6	31	41	0.08	6	n.d.	n.d.
9	OF_9a	0	1275	310	466	1013	3.00	500	n.d.	n.d.
	OF_9b	60	14	1	9	16	0.04	6	n.d.	n.d.
	OF_9c	90	n.d.	n.d.	5	14	0.02	1	n.d.	n.d.
10	OF_10a	0	1085	334	308	1190	3.12	306	n.d.	n.d.
	OF_10b	60	29	4	9	29	0.05	11	n.d.	n.d.
	OF_10c	90	n.d.	n.d.	5	13	0.01	1	n.d.	n.d.
11	OF_11a	0	1016	282	333	577	2.61	319	n.d.	3
	OF_11b	60	242	25	29	84	0.13	96	n.d.	4
	OF_11c	90	58	11	22	69	0.05	26	n.d.	3
12	OF_12a	0	1109	233	450	730	2.17	392	n.d.	n.d.
	OF_12b	60	2	1	6	76	0.01	2	n.d.	n.d.
	OF_12c	90	13	2	13	73	0.03	8	n.d.	n.d.

n. d.: Not detected n.d. (COC, BE, CIN, TRO, CE) < 1 ng/mL n.d. (EME) < 5 ng/mL n.d. (CUS) < 10 ng/mL n.d. (HYG) when relation S/N < 3
Cells exceeding the cutoff (COC/BE: 8 ng/mL) according to DUID [13] and WDT [11] are highlighted.

Table 5
Concentration of coca leaf alkaloids and cocaine metabolites in urine after consumption of different coca flour based candies.

Volunteers	Code	Sampling time (h)	COC (ng/mL)	BE (ng/mL)	EME (ng/mL)	CUS (ng/mL)	HYG R=area $_{(HYG)}$/IS$_{_COCd3}$	CIN (ng/mL)	TRO (ng/mL)	CE (ng/mL)	Screening (INDIKO) (ng/mL)
1	U_1	1.5	1	6	n.d.	n.d.	0.11	n.d.	n.d.	n.d.	1.1
2	U_2	1.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0
3	U_3a	1.5	n.d.	1	n.d.	n.d.	0.09	n.d.	n.d.	n.d.	0
	U_3b	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0
4	U_4	1.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0
5	U_5	1.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0
6	U_6	1.5	10	47	43	28	0.52	1	n.d.	n.d.	34.3
7	U_7a	4	n.d.	232	40	245	3.82	2	n.d.	n.d.	250.8
	U_7b	12	n.d.	52	33	10	0.44	n.d.	n.d.	n.d.	79.7
8	U_8a	2	32	256	n.d.	133	0.65	2	n.d.	n.d.	294.1
	U_8b	8	5	220	n.d.	122	0.08	n.d.	n.d.	n.d.	226.9
9	U_9a	2	14	27	61	57	0.59	2	n.d.	n.d.	27.5
	U_9b	30	23	20	23	35	0.46	n.d.	n.d.	n.d.	9.4
10	U_10a	2	1	49	29	110	0.62	n.d.	n.d.	n.d.	39.1
	U_10b	30	2	10	37	171	0.39	n.d.	n.d.	n.d.	5.4
11	U_11a	2	38	115	67	16	2.62	2	n.d.	14	141.4
	U_11b	12	n.d.	50	25	35	0.62	n.d.	n.d.	n.d.	62.2
12	U_12a	1.5	n.d.	16	21	n.d.	0.41	n.d.	n.d.	n.d.	19.1

n.d.: not detected n.d. (COC, BE, CIN, TRO, CE) < 1 ng/mL n.d. (EME) < 5 ng/mL n.d. (CUS) < 10 ng/mL n.d. (HYG) when relation S/N < 3
Cells exceeding the cutoff according to DUID [13] (COC 20 ng/mL; BE: 50 ng/mL) and according to WDT [12] (BE 150 ng/mL) are highlighted.

Table 6
Concentration of coca leaf alkaloids in coca flour based candies.

CANDY BRAND	Weight/candy (g)	COC (mg/candy)	EME (mg/candy)	CUS (mg/candy)	HYG R=area$_{(HYG)}$/area$_{(IS_COCd3)}$	CIN (mg/candy)	TRO (mg/candy)
Am	2.4	0.0003	0.00005	0.00006	0.05	0.00006	0.00001
Ao	4.0	0.098	0.0358	0.0358	7.25	0.01427	0.0005
Ac	3.4	0.003	0.00012	0.00002	1.17	0.00015	0.00003

The three commercial brands of coca flour based candies used in this study were arbitrarily labeled as: Am, Ao, and Ac.

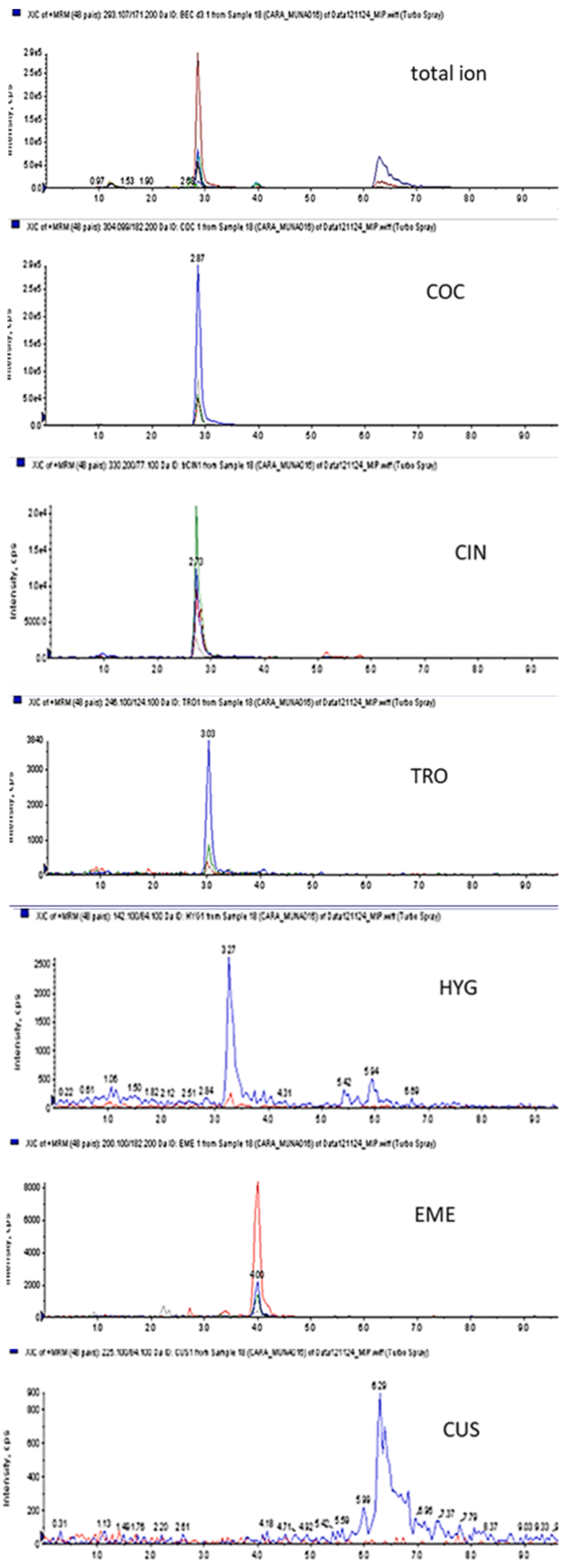

Fig. 1. Total ion chromatogram and ion extracted chromatogram of alkaloids from coca flour based candy in brand candy "Am".

maturation time, and the amount of coca flour added to the candies, among others. Consequently, the expected coca leaf alkaloid content in the analyzed candies will vary, as shown in Table 6.

The concentration of alkaloids in Ao brand candy was higher than the concentration present in the other brands analyzed.

Tables 4 and 5 list the concentrations of coca leaf alkaloids—COC, EME, TRO, CIN, and CUS—and COC metabolites—BE, EME, and CE—in ng/mL of OF and/or urine. A commercial reference standard for HYG was not available at the time of this study; its identification was conducted based on retention time and its two transitions at 84 and 42, using coca leaf samples and coca-flour candies as references. HYG is reported as a ratio of areas, calculated by dividing the area of HYG by the area of the deuterated internal standard COC-d3. HYG was identified using a 6530 Accurate-Mass QTOF-MS instrument (Agilent Technologies, Santa Clara, USA), as we previously reported [14].

OF samples were collected from volunteers 1, 2, 4, and 5 at 30 min. Volunteer 3 provided an OF sample at 45 min while volunteers 6–12 provided samples at 0 min (immediately after finishing the candy consumption), 60 and 90 min (Table 4). It is important to note that, in general, OF samples collected mainly at time 0 had a greenish hue due to the color of the candies, justifying the elevated concentrations found for the analytes COC, BE, EME, CUS, CIN, and HYG (area ration) (Fig. 2). Furthermore, it should be considered that these candies are solid, sticky products that, if chewed, can adhere to teeth, potentially extending the detection window in OF sample analysis.

Only one urine sample was collected from volunteers 1–6 and volunteer 12 at 1.5 h; for the remaining volunteers (7, 8, 9, 10, and 11), two urine samples were collected between 2 hours and 30 hours post-consumption. Details are shown in Table 5.

Analyte concentrations below the lowest point used in the calibration curve for OF (COC, BE, CIN, TRO, CE: 1 ng/mL; EME: 5 ng/mL; and CUS: 10 ng/mL) and below the final cutoff for urine (COC, BE, CIN, TRO, CE: 1 ng/mL; EME: 2 ng/mL; and CUS: 10 ng/mL) are reported as nd (not detected). Analytes such as COC, CE, BE, and TRO can be detected below 1 ng/mL, but it was considered that concentrations below 1 ng/

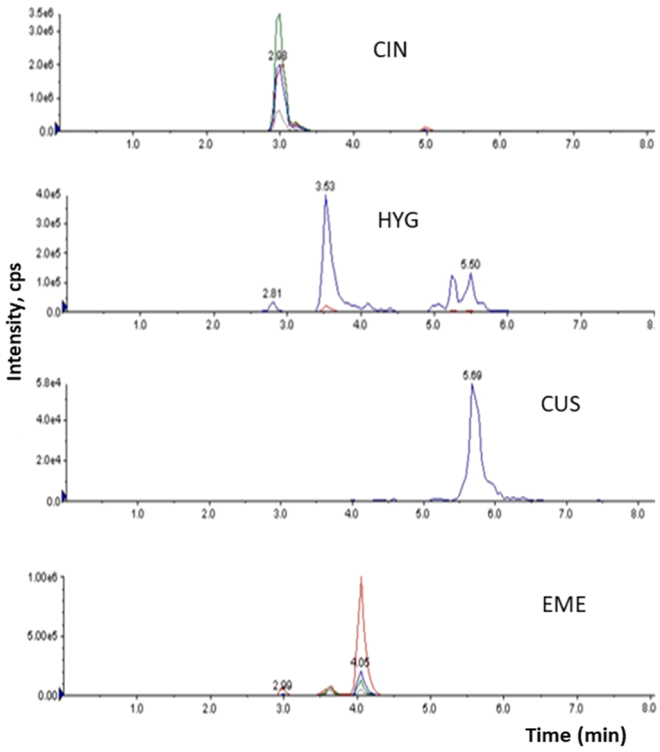

Fig. 2. Ion extracted chromatograms of CIN, HYG, CUS and EME in oral fluid from volunteer 12 (sampling time 0 min).

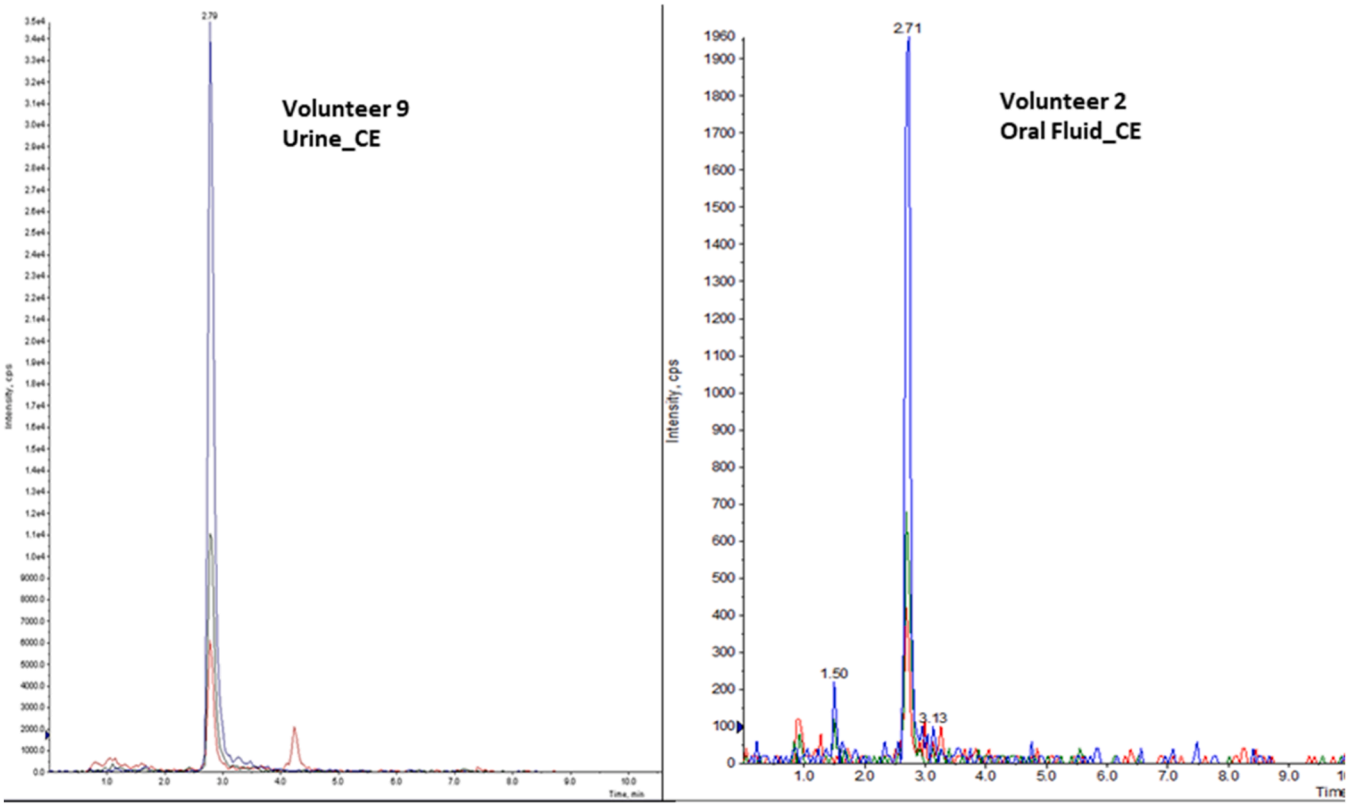

Fig. 3. Ion extracted chromatograms of CE (concentration lower than 1 ng/mL) in urine and oral fluid from two volunteers (U_9; OF_2).

mL do not provide significant value to be reported. Therefore, some samples, such as OF_1, OF_2, OF_3, OF_9a y 9b, OF_10a, 10b y 10c were possible to detect all transitions for CE below 1 ng/mL, as well as in the urine of the same volunteers. (Fig. 3)

Benzoylecgonine was initially determined in urine using the INDIKO system by Thermo Scientific. As shown in Table 5, none of the urine samples analyzed exceeded this cutoff value of 300 ng/mL. Although the BE concentrations reported using the INDIKO are below the cutoff value and may not be as reliable as values above the cutoff, they fall within the range observed for the same analyte using LC-MS/MS. (Table 5)

As expected, OF and urine samples from volunteers 1, 2, 3, and 4, who consumed candies from the commercial brands Am and Ac, showed lower concentrations and undetected analytes compared to volunteers who consumed Ao candies. These findings are in good agreement with the alkaloid content observed in the tested candies Table 6.

In Tables 4 and 5, the concentrations of COC and BE are highlighted, as they exceed the recommended values specified by international guidelines namely for DUID/WDT in OF 8 ng/mL for COC and BE; in urine, for DUID: 20 ng/mL for COC and 50 ng/mL for BE and 100 ng/mL for BE in urine for WDT (TABLE 1). CE concentrations remained below the international recommended threshold of 8 ng/mL in OF. Notably, in only one case (volunteer 11), CE was detected at concentrations above 1 ng/mL in all three oral fluid samples and in the 2-hour urine sample. Volunteer 11 consumed a distilled beverage with a higher alcohol concentration than the wine consumed by volunteers 1, 2, 3, 4, 9, and 10 (36 % vol vs. 14 % vol), which may account for the higher CE formation. However, additional samples are needed to confirm this isolated observation. TRO was not detected in any of the analyzed OF and urine samples, in line with its low concentrations in coca leaves and the commercial candies analyzed.

When comparing the simple oral fluid (OF) samples from volunteers who chewed and swallowed the candies with those from volunteers who allowed the candies to dissolve entirely, we observe:

A) OF_3 (who chewed and swallowed four (4) candies: 2 Ac and 2 Am) compared to the group of volunteers OF_1, OF_2, and OF_4, who only allowed two (2) candies of the brands Ac and Am to dissolve, shows (OF_3 sample) no relevant increase in the concentrations of the analyzed analytes; in fact, some analytes, such as BE, EME, CUS, HYG, and CIN, are even lower in concentration or undetectable.

B) When comparing volunteer 11, who chewed and swallowed two (2) Ao candies, with volunteer 7, who consumed the same quantity and brand of candies but allowed them to dissolve completely, a significant difference is observed in the concentrations of all analyzed analytes across the three OF samples (0 min, 60 min, and 90 min), especially in the OF sample at time 0 min. Volunteer 6, who also ingested two (2) Ao candies and reportedly allowed them to dissolve, exhibits higher OF concentrations at time 0 min for COC, BE, EME, CUS, CIN, and HYG (relation area) than volunteer 11. These preliminary findings suggest that the manner of candy consumption may affect the concentrations of the analyzed alkaloids/metabolites, at least in samples collected in the first few minutes following candy consumption.

Regarding the results obtained in urine (Table 5), drawing conclusions is more challenging due to the variability in excretion rates of different compounds, influenced by factors such as metabolism, hydration, renal function, drug properties, and individual differences, the aim was to obtain an approximate estimation of concentration ranges.

It is noteworthy that in both oral fluid and urine samples analyzed, while the traditional analytes used to monitor cocaine consumption (COC and BE) in DUID and WDT are positive and exceed the cutoff values required by control systems, the coca leaf consumption markers CUS and HYG remain detectable, highlighting their predictive value as indicators of legal coca use. Conversely, CIN, which is used as a secondary marker of coca leaf consumption, proved useful in the analyzed OF samples (Table 4) but was less effective in urine samples, where many results were non-detectable for CIN (Table 5).

4. Conclusion

Surprisingly, consuming a candy containing coca leaf flour as an ingredient can cause an individual to test positive in urine and/or oral fluid, exceeding currently established cutoffs for traffic control (DUID) or in the workplace (WDT) for cocaine/benzoylecgonine/cocaethylene, thus unfairly labeling the person as a cocaine user. Factors such as the brand and batch of the candies, the number of candies consumed, and possibly the manner of consumption—whether sucking, chewing, or swallowing them—as well as the alcohol content of any accompanying beverage, may influence these results. Additionally, the sticky nature of the candies can lead to residues being retained between teeth, further extending the detection window, especially in oral fluid-Although in urine it is more difficult to make predictions because, as mentioned, there are multiple factors that can influence the concentration of analytes in a single urine sample.

In our increasingly connected world, we should consider the consumption of products from the Andean region (candies, cereal bars, liqueurs, pastries, cookies, among others) made with coca flour when addressing any user who might justifiably contest a positive drug test result.

Compliance with ethical standards

The use of biological samples has been necessary in the development of this research. This use is in agreement with the ethical standards of the current Helsinki Declaration and the recommendations of the Council of Europe for all human experimental investigations.

The authors state that they have obtained written informed consent from all individual participants involved in the study.

The owner (IP and / or institution) is the legal responsible of the samples, which neither will not be ceded to third parties nor used in other projects. All of the researchers participating in the study have enough experience and scientific capacity.

CRediT authorship contribution statement

N.C. Rubio: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft. **P. Herbello-Hermelo:** Formal analysis, Validation, Writing – review & editing. **I. Álvarez-Freire:** Formal analysis, Methodology. **P. Cabarcos-Fernández:** Investigation, Visualization. **M.J. Tabernero-Duque:** Methodology, Resources. **I. Sánchez-Sellero:** Formal analysis, Validation. **P. Bermejo-Barrera:** Project administration, Funding acquisition. **A.M. Bermejo-Barrera:** Conceptualization, Data curation, Supervision. **Antonio Moreda-Piñeiro:** Conceptualization, Supervision, Resources, Project administration, Funding acquisition, Writing – review & editing.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

References

[1] Argentine Ley 23737/1989, art. 15. Honorable Congreso de la Nación de Argentina, October 10, 1989. ⟨https://www.argentina.gob.ar/normativa/⟩. Accessed January 2023.
[2] N.C. Rubio, D. Thurmann, F. Krumbiegel, F. Pragst, Behaviour of hygrine and cuscohygrine in illicit cocaine production establishes their use as markers for chewing coca leaves in contrast with cocaine abuse, Drug Test. Anal. 9 (2) (2017) 323–326, https://doi.org/10.1002/dta.1972.
[3] N.C. Rubio, S. Strano-Rossi, M.J. Tabernero, L. Anzillotti, M. Chiarotti, A. M. Bermejo, Hygrine and cuscohygrine as possible markers to distinguish coca chewing from cocaine abuse in workplace drug testing, Forensic Sci. Int. 227 (2013) 60–63, https://doi.org/10.1016/j.forsciint.2012.09.005.
[4] N.C. Rubio, S. Strano-Rossi, M.J. Tabernero, J.L. Gonzalez, L. Anzillotti, M. Chiarotti, A.M. Bermejo, Application of hygrine and cuscohygrine as possible markers to distinguish coca chewing from cocaine abuse on WDT and forensic cases, Forensic Sci. Int. 243 (2014) 30–34, https://doi.org/10.1016/j.forsciint.2014.02.024.
[5] S. Laposchan, R.F. Kranenburg, A.C. van Asten, Impurities, adulterants and cutting agents in cocaine as potential candidates for retrospective mining of GC-MS data, Sci. Justice (2022) 60–75, https://doi.org/10.1016/j.scijus.2021.11.004.
[6] I. Bauer, Travel medicine, coca and cocaine: demystifying and rehabilitating Erythroxylum– a comprehensive review, Trop. Dis., Travel Med. Vaccin. 5 (1) (2019) 20, https://doi.org/10.1186/s40794-019-0095-7.
[7] M.A. Martínez González, Criterios cuantitativos en toxicología forense, Rev. Española De. Med. Leg. 40 (1) (2014) 30–38, https://doi.org/10.1016/j.reml.2013.03.002.
[8] N.T. Lappas, C.M. Lappas Forensic Toxicology Principles and Concepts. Elsevier (2016) ISBN: 9780128004647 https://doi.org/10.1016/C2018-0-04868-9.
[9] Mandatory Guidelines for Federal Workplace Drug Testing Programs–Oral Fluid70814Federal Register / Vol. 88, No. 196 / Thursday, October 12, 2023 / Rules and Regulations, Department of Health and Human Services, SAMHSA. ⟨https://www.federalregister.gov/documents⟩. Accessed sept. 2024.
[10] Mandatory Guidelines for Federal Workplace Drug Testing Programs–Urine70768Federal Register / Vol. 88, No. 196 / Thursday, October 12, 2023 / Rules and Regulations, Department of Health and Human Services, SAMHSA. ⟨https://www.federalregister.gov/documents⟩. Accessed sept. 2024.
[11] M. Brcak, O. Beck, T. Bosch, D. Carmichael, N. Fucci, C. George, M. Piper, A. Salomone, W. Schielen, S. Steinmeyer, S. Taskinen, W. Weinmann, European guidelines for workplace drug testing in oral fluid, Drug Test. Anal. 10 (3) (2018) 402–415, https://doi.org/10.1002/dta.2229.
[12] M. Brcak, O. Beck, T. Bosch, D. Carmichael, N. Fucci, C. George, M. Piper, A. Salomone, W. Schielen, S. Steinmeyer, S. Taskinen, W. Weinmann, European guidelines for workplace drug testing in urine, Drug Test. Anal. 9 (6) (2017) 853–865, https://doi.org/10.1002/dta.2178.
[13] B.K. Logan, A.L. D'Orazio, A.L. Mohr, J.F. Limoges, A.K. Miles, C.E. Scarneo, S. Kerrigan, L.J. Liddicoat, K. Scott, M.A. Huestis, Recommendations for toxicological investigation of drug-impaired driving and motor vehicle fatalities-2017 update, J. Anal. Toxicol. 42 (2) (2018) 63–68, https://doi.org/10.1093/jat/bkx082.
[14] N.C. Rubio, M. Hastedt, J. González, F. Pragst, Possibilities for discrimination between chewing coca leaves and cocaine abuse by hair analysis including hygrine, cuscohygrine, cinnamoylcocaine and cocaine metabolite/cocaine ratios, Int. J. Leg. Med. 129 (1) (2015) 69–84, https://doi.org/10.1007/s00414-014-1061-6.
[15] I. Álvarez-Freire, P. Cabarcos-Fernández, N.C. Rubio, A. Moreda-Piñeiro, M. J. Tabernero-Duque, I. Sánchéz-Sellero, P. Bermejo-Barrera, A.M. Bermejo-Barrera, Detection of coca alkaloids in oral fluid from coca leaf (tea) consumers: using solid phase extraction to improve validation parameters and widen the detection window, Anal. Methods 15 (45) (2023) 6177–6183, https://doi.org/10.1039/D3AY01298K.
[16] N.C. Rubio, A. Moreda-Piñeiro, I. Álvarez-Freire, P. Bermejo-Barrera, M. J. Tabernero-Duque, A.M. Bermejo, The probability to detect cocaine, methylecgonine, cinnamoylcocaine, hygrine and cuscohygrine in urine samples of coca leaves chewers after six years, Microchem. J. 151 (2019) 104215, https://doi.org/10.1016/j.microc.2019.104215.
[17] N.C. Rubio, P. Bermejo-Barrera, A. Bermejo, A. Moreda-Piñeiro, Development of reliable method for assessing coca alkaloids in oral fluid by HPLC-MS/MS, J. Anal. Toxicol. 43 (3) (2019) 196–202, https://doi.org/10.1093/jat/bky076.
[18] N.C. Rubio, A. Moreda-Piñeiro, P. Bermejo-Barrera, A.M. Bermejo, Perfil de alcaloides de la hoja de coca en el fluido oral de un mascador de hoja de coca y un bebedor de té de coca. Estudio preliminar. Coca alkaloids profile in oral fluid from people chewing coca leaves and drinking coca tea. Preliminary study, Acta Toxicol. ógica Argent. 27 (2) (2019) 72–80. ⟨https://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S1851-37432019000200004&lng=es&nrm=iso⟩. Accessed sept. 2024.