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The occurrence of cocaine and metabolites in hair of drug abusers. Possible determination of cut-off values

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Introduction

The abuse of cannabis and cocaine is extending among adolescents and young adults across Chile and generating a big concern in our community.

Almost 300 thousand Chileans age 12 to 64 have tried cannabis and almost 100 thousand cocaine during last year (1). Regarding to cocaine this drug is specially used in the cheap semi-refined form "free base"[1] known to be highly addictive.

This paper deals with our efforts to learn how the "state of the art" in hair analysis was and with the development of a reliable hair analysis including methods for washing, extracting, detecting and quantitating cocaine and its application in forensic science, criminal justice and high security employee screening. Kintz's recommendations on preparation of samples, decontamination procedure, application of two different analytical methods, determination of cut-off values were fully adopted (2).

Seventy men black hair specimens were collected and analysed, age ranged from 24 to 48, all of them proceeding from known dealers and users under arrest. Samples were first screened by enzyme linked immunosorbent assay (Elisa) (3), technique adapted for the detection of cocaine in hair and then cocaine and metabolites identified and quantitated by GC/MS.

At the present hair analysis is a useful analytical tool for forensic science, criminal justice and follow up of addicts producing historic quantitative results, from weeks to months and informing about severity and pattern of repetitive use of drugs (4).

In this study we've adapted the classic extraction system after a mild acid hydrolysis for the detection of cocaine in hair (2) with some modifications to allow a better sensitivity.

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[1] free base(pasta base) is consumed straight, in mixture with marijuana(marciano) or with tobacco(tabacazo).

Generally drug control system consists in an immunological test for the screening of drugs and the presumptive positives are all confirmed by a second independent test. In our case initial screening test was by an Elisa kit for cocaine, choice based upon low cost and high sensitivity. The confirmation was carried out by GC/MS, with lower limits of quantification of 0.025 ng/mg and 0.020 ng/mg of hair with a signal to noise ≥ 10 for cocaine and benzoylecgonine respectively.

The present study characterises the profile of cocaine and its metabolites in the hair matrix and after arrangement of the results concludes in the establishment of cut-off values and benzoylecgonine/cocaine (bze/coc) ratios as a first approach to define active use of cocaine.

Experimental

Equipment

GC/MSD parameters

Analysis were carried out on a combined GC/ MSD system Hewlett Packard 5890/5972

Carrier gas	Helium flow rate 0.9 mL/min
Split	Splitless mode
Column	HP- Ultra 1 fused silica capillary column, 25m, 0.20mm i.d., 0.11μm film thickness
Temperature Transfer Line	300°C
Temperature MS Source	230°C
Temperature MS Quad	150°C
Temperature program	150°C, 20°/min till 310° held 10 min

Mode SIM:

	Ions	dwell time
Group 1	82,96,271	20
Group 2	82,182,303	20
Group 3	82,196,317	20
Group 4	82,240,361	20

Hair samples

Hair samples were collected from the posterior vertex of donors, as close to the scalp as possible. Seventy hair specimens, all of them from known dealers and abusers under arrest.

The samples nearly 200 mg were collected and placed in aluminium foil carriers, identifying clearly the root, then in collection envelopes sealed with a plastic numbered code and transported to the laboratory.

Preparation of samples

Samples were removed from the envelopes one at a time, code number registered and integrity verified. It was used a 4 cm segment corresponding to an approximate growth period of 3 months. Approximate 100 mg were subjected to the following washing step before the analysis: a) 3 times 4 mL of warm bidistilled water; b) 3 times 4 mL of acetone; c) 3 times 4 mL of ethanol and d) 3 times 4 mL of dichloromethane and cut into segments between 1 to 3 mm.

Ethanolic and dichloromethane washings were evaporated just to dryness under a stream of N_2 in a heating block at 37°C. Dry residues were reconstituted with 100 µL of pH 6 phosphate buffer and submitted to Elisa immunological testing of high sensitivity (0.4 ng/mL). If the tests were positive, washing has to be analysed with the same procedure used for the sample.

The washed hair sample is dried, pulverised, weighing exactly 50 mg, placed in a labelled tube of 16×100 mm, then addition of 1 mL of 0.1N HCl and incubation overnight at 37°C. Take a 50 µL aliquot evaporate just to dryness under N_2 stream in a heating block at 37°C, reconstitute the residue with 100 µL of pH 6 phosphate buffer and submitted for Elisa testing according to manufacturer instructions using negatives and positives controls (4).

Hair samples tested negatives by Elisa immunoassay were pooled and the washing step repeated and submitted to GC/MS analysis. If the results were negative, the negative hair matrix was certified for use.

GC/MS

Positives samples by Elisa were extracted on solid phase extraction column Baker Bond SPE™ Narc-2 using an extraction procedure previously described for urine samples (5). After elution and evaporation to dryness the residue is derivatized with 100 µL of MSTFA / TMSCl 1% by heating 30 min to 75°C. The derivatized extracts were cooled to room temperature and 2 µL were injected into the GC/MS instrument using the parameters already indicated.

Selective ion monitoring (SIM) mode was used. Compounds are cited in order of elution. Retention times and specific ions were included in a modified Macro Deuser (ion for quantitation showed in brackets and two qualifier ions).

1. Ecgonine methyl ester: (96), 82, 271
2. Cocaine: (182), 82, 303
3. Cocaethylene: (196), 82, 317
4. Benzoylecgonine: (240), 82, 361

Results and Discussion

In table 1 appear cocaine concentrations published by different authors, including the results obtained in this study.

Table N° 1: Ranges of cocaine concentration (ng/mg) in hair

Reference	range	mean
Kintz **94**	0.4 - 78.4 (14)	8.30
Cone et al. **91**	6.4 - 19.2 (10)	10.05
Moeller et al. **93**	0.3 -127.0 (34)	20.06
Báez et al **98**	0.5 -137.0 (66)	16.30

The distribution of our results in positive hairs suggests a cut-off of 1 ng/mg of cocaine. Using these limits 11 out of the 66 positive samples will be negatives (Table 2). Reviewing of all data supporting drug intake like physician information and the detection of drug in blood or urine allowed us to reduce the above level to 0.5 ng/mg of cocaine in which case only three samples remained as negatives.

Table N° 2: Cocaine concentrations (*ng/mg*), ranges and number of samples, from November 97 until August 98.

Cocaine concentration (ranges)	Number of samples
0.00 - 0.50	3
0.50 - 1.00	8
1.00 - 5.00	19
5.00 - 20.00	26
>20.00	10
Totals	66

Parent drug was detected in the absence of any of its metabolites in 24 % of the positive samples with low concentrations ranging from 0.3 to 5.4 ng/mg of cocaine. These results are in agreement with those of Kintz (6) but not with the values of Moeller research (7) who detected cocaine and benzoylecgonine in all the positives.

In our study cocaine concentrations were higher than benzoylecgonine by a factor of 6 to 12. We never detected ecgonine methylester.

At the same time as cocaine concentration increases, it correlates with the possibility of metabolites detection specially cocaethylene. This compound was never detected when cocaine was less than 3 ng/mg.(Table 3)

Table 3: Cocaethylene concentrations (*ng/mg*), ranges and number of samples.

Cocaethylene concentration (ranges)	Number of samples
0.00 - 0.50	1
0.50 - 1.00	5
1.00 - 5.00	13
5.00 - 10.00	5
>10.00	2
Totals	26

Findings of cocaine and benzoylecgonine in hair are not as conclusive of cocaine consumption as it is cocaethylene presence in the matrix.

With our results ratios benzoylecgonine/cocaine (bze/coc) greater than 0.03 would indicate active use (Table 4). Cone *et al* (8) distinguish external contamination from active use with a ratio over 0.05 .

Table N°4: Ratios Benzoylecgonine/Cocaine

Number of samples	Ratio bze / coc
2	0.00 - 0.03
8	0.03 - 0.06
18	0.06 - 0.09
11	0.09 - 0.12
9	> 0.12

Conclusions

We adopted a simple and reliable hair analysis for the automated detection and quantitation of cocaine and its metabolites. The incorporation of enzyme linked immunosorbent assay (*Elisa*) proved to be successful as initial test for the presence of cocaine in hair.

In summary one could use the above criteria to define active use of cocaine but controversies on how drugs enter hair, time of appearance, factors influencing binding, risk of false positives and false negatives, potential for sex and ethnic bias should be solved before accepting the complete validity of hair analysis.

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