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Urinary Excretion of Cocaine, Ecgonine Methyl Ester and Benzoylecgonine Following Ingestion of Coca Leaves Decoction

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Introduction

Several vegetables from the gender *Erythroxylon*, *Erythroxylaceae* family, are known with the popular name of Coca. Evans (Evans, W.C.; 1989) mentions the existence of about 200 species, among which he considers *Erythroxylon coca* (Coca from Bolivia or huanuco) and *Erythroxylon truxillense* (Perú, Bolivia, Colombia e Indonesia) the most important.

The same Evans describes the Coca leaves and reports total alkaloids in quantities between 0.7 and 1.5 g % in them. The most important are Cocaine (**CO**), Cinnamoyl- cocaine and Truxilline.

Cocaine is well absorbed by several routes: nasal mucous, intravenous injection, lungs and gut. The intravenous and inhalatory routes are the best ones and **CO** is quickly hydrolyzed in the liver and in the plasma producing several metabolites: Benzoylecgonine (**BE**) and Ecgonine Methyl Ester (**EME**).

Coca leaves are employed mainly for extraction of **CO**, for chewing and as decoction with different medical purposes by people living in South American countries (Bolivia, Paraguay and north of Argentina). Chewing and decoction in that countries are very common not banned practices.

Many researchers have mainly reported Cocaine metabolites **BE** and **EME** in the urine of Coca leaf decoction tea consumers, whereas presence of cocaine is reported only occasionally, in very small amounts and in short periods of time (Riviere, L. and Saugy, M.; 1995) (Jackson, G.F.; Saadi, J.J. and Poklis A.;1991).

The scope of this study is to determine the amount and time of urinary excretion of **CO**, **BE** and **EME** from healthy volunteers after the ingestion of Coca leaf decoction.

Materials and methods

Decoction : Decoction was made using small water permeable paper bags containing 1 g of chopped Coca leaves in each one (Mate de Coca Windsor™) of Bolivian origin. The way to use it is one bag in approximately 150 ml. of tap water.

In our work, decoction was prepared with six bags in 950 ml of tap water in the condition prescribed by Codex Medicamentarius Argentino (Farmacopea Argentina VI edition). Each bag was removed and squeezed into the decoction. The volume was completed to 950 ml with tap water: 900 ml. were employed for the test with the voluntaries and 50 ml for quantitation of **CO**, **BE** and **EME** in coca decoction.

Subjects: six healthy volunteers (four male and two female) without a history of Coca or Cocaine use. Women were 25 and 29, and men 27, 27, 29 and 64 years old. Each of them drank 150 ml of the decoction.

Sample collection: a predose (time 0) urine sample was collected to ensure that no interfering substances were present.. Other urine specimens were collected to 2, 4, 6, 8, 12, 20, 30 and 48 hours after the ingestion of Coca leaf decoction. Urine pH was measured with Multistix SG™ strips and specific gravity with a refractometer Atago. Samples were stored at -18°C until analysis.

Drugs: Cocaine HCl Sigma # c5776; Ecgonine methylester monohydrate HCl Sigma #e5386; Benzoylecgonine Radian# e004; Cocaine- D3 HCl Radian # c014;Ecgonine methyl ester-D3 HCl Radian # e002; Benzoylecgonine-D3 HCl Radian # b008; Pentafluoropropionic anhydride (PFPA) Aldrich # 25238-7; Hexafluoroisopropyl alcohol (HFIP) Aldrich #10522-8.

Reactives: Solvents mentioned in text were of analytical grade; Solid Phase Extraction Columns: SPE Worldwide # CSDAU303; Stock solutions of **CO**, **BE**, and **EME** (each one 1 mg/ml in methanol); Internal Standard Solution (**IS**): **CO-D3**, **BE-D3** and **EME-D3** (each one 10 µg/ml in methanol).

Calibration solutions: each stock solution of **CO**, **BE** and **EME**, was diluted in urine (Cocaine and metabolites free) and mixed to obtain solutions containing 5000, 2000 and 800 ng/ml, and 5000, 2000, 800, 320, 128, and 51 ng/10 ml. Each one was spiked with 50 µl of **IS** and treated as described below for the urine samples.

Apparatus: Visiprep™ Supelco. Thermovap™ Pierce Evaporator. Refractometer ATAGO. The GCMSD System was Hewlett Packard 5890/5972 equipped with capillary column HP1 ultra (length: 25 m; ID: 0.25 mm; film thickness: 0.25µm) and Autosampler HP

Temperature: Injector: 250 °C. Transfer line: 280 °C Oven temperature program: T1: 92 °C during 2 min; Temp. Rate 7 °C/min up to 270 °C; T2: 270 °C during 5 min.
Mode: SIM. Selected ions: **CO** (182), **BE** (318), **EME** (182), **CO-D3** (185), **BE-D3** (321) and **EME-D3** (185).

Methodology: 50 µl **IS** were added to 10 ml of each one of 54 urine samples collected and to 3 aliquots of 10 ml of Coca decoction. All samples (urine and decoction) were prepared by solid phase extraction according to Worldwide Technical Manual (Worldwide; 1990), as is described below.

Samples were prepared adding 0.5 ml H_2SO_4 0.2 N to 10 ml urine (pH should be 3.5), and the mixture poured into the SPE column (conditioned as prescribed by manufacturer) and pulled through at a flow of 1-2 ml/minute. Columns were washed passing successively 2 x 3 ml distilled water, 2 x 3 ml HCl 0.1 N, air during 2 minutes (vacuum 15-20 inches of Hg), 2 x 3 ml methanol and finally air 2 minutes as before described.

Cocaine and metabolites were eluted with 2 x 3 ml of "basic elution solvent": methylene chloride/isopropyl alcohol/ammonium hydroxide (80:20:2)

Eluates were evaporated under nitrogen stream at 40°C and derivatized with PFPA and HFIP as follows: to each dry extract were added 70 µl PFPA and 30 µl HFIP; vortexed during 15 seconds, heated for 10 minutes at 70 °C and evaporated to dryness under nitrogen stream at 50 °C. Dry residue was dissolved in 50 µl of ethyl acetate and injected in GCMS.

Relationship between non deuterated and correspondent deuterated compounds areas in samples were obtained, and data interpolated in the respective calibration curve. Calibration curves were made processing calibration solutions as the urine samples and plotting values of **CO**, **BE** and **EME** (in ng/ml) in "X" axis, and area relationships **CO/CO-D3**, **BE/BE-D3** and **EME/EME-D3** in "Y"axis. Samples containing **EME** and **BE** concentration exceeding the upper limit of respective calibration curve (14 samples) were appropriately diluted with distilled water to fit in them, and processed as before described for the original urine samples.

Limits of Detection and Limits of Quantitation were 15.9 and 18.5 for **EME**; 4.1 and 4.6 for **CO**, and 54.3 and 62.5 for **BE** (in all cases ng/ml) respectively. Linearity for all the three substances was between 4 and 10000 ng/ml sample using the technique described.

Results

Media and SD of **CO**, **BE** and **EME** urine concentrations in the samples collected at same

time are specified in table 1. **CO**, **BE** and **EME** levels in biological samples of the six subjects are shown in table 2 and there respective graphics in figures 1 (1a to 1f) . Maximum urinary concentrations (between brackets number of subjects) of **EME** were found at 2 (2), 4(3) and 6(1) hours, **BE** at 2(1), 4(3), 6(1) and 8(1) hours, and **CO** at 2(3), 4(2), and 20(1) hours after ingestion. Presence of **CO**, **EME** and **BE** was observed in all samples.
CO, **BE** and **EME** concentrations in decoction were 29.3; 19.3 and 8.7 μg/ml respectively.

Discussion and Conclusions

In this study we found **CO**, **BE** and **EME** in all urine samples collected up to 48 hours. Maximum media urinary concentrations of **EME**, **CO** and **BE** were found at 2 hours after Coca tea ingestion. Highest urinary concentrations of **CO** and metabolites (in ng/ml) were found in women: **CO**, 133; **EME**, 13887 and **BE**, 10710 at 2 hours after ingestion, last two in the same sample (see tables 1 and 2). Up to the moment we do not find explanation for the unexpected high **CO** excretion from subject IV at 20 hours.
Riviere, L. and Saugy, M.(1995) report maximum levels of **EME**, **CO** and **BE** at 4 (with practically a "plateau" up to 13 hs), 2 and 13 hours after the Coca decoction intake, respectively. Jenkins et al (1996) found, in an experience in two subjects who drank Coca tea of Bolivian and Perú origin, maximum urinary excretion (after began experience) of **EME** and **BE** in one of them at 10 hs (2250 and 3368 ng/ml respectively) and in the other one at 3.5 hs (2314 and 4155 ng/ml in the same order). For highest **CO** concentration was found at 5 hs (196 ng/ml) and 3,5 hours (585 ng/ml) for Bolivian and Peruvian Coca drinker respectively. **EME** and **BE**, but no **CO**, were found up to 48 hours.
In both papers (Jenkins and Riviere-Saugy) are not included tables and as a consequence is not possible a deep study of data. In general data from that authors are compatible with that from this paper. Some differences with the above mentioned (Jenkins; Rivier Sagy), may be because of we used Coca tea in the proportion is normally prepared by people (1 g/150 ml), while in Riviere and Sagy study, subjects drank a more concentrated decoction: 10 g per liter. Jenkins used Coca from different origin, so comparison is not easy.
In this study all the six subjects drank the same decoction, prepared in the proportion that generally people use do and with a more numerous casuistic than reported in all previous papers we found.

Despite concentration **BE** in decoction was higher than **EME**, in urine 2 hours samples **EME** levels were higher than **BE** in 4 subjects (2 males and 2 females) were found. **CO** was identified in all samples. This topic must be take in account in next studies.

We think that urinary excretion of **EME**, could be used in some cases to determine the time of assumption of cocaine tea and as a clue of its absorption through the gastrointestinal tract.

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TABLE 1 – Media and Standard Deviation of EME, CO and BE excreted in different hours (in ng/ml)

Hour	EME	CO	BE
2	6007.8 ± 4228.1	77.2 ± 39.6	4956 ±3165.8
4	4718.7 ± 1715.9	51.2 ± 23.2	4653.2 ± 1600.1
6	3516.3 ± 1658.5	31.3 ± 10.9	4043.5 ± 1581.1
8	3014.0 ± 2205.0	31.7 ± 21.2	3182.0 ± 1469.0
12	1651.0 ± 668.8	21.3 ± 8.0	2704.5 ± 713.1
20	579.5 ± 289.9	26.2 ± 33,3	1477.2 ± 435.7
36	135.2 ± 85.9	7.6 ± 2.2	378.8 ± 315.5
48	59.0 ± 29.2	7.3 ± 2.0	127.2 ± 93.2

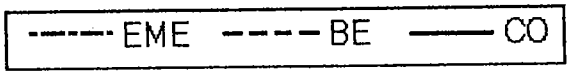
TABLE 2 - Urinary excretion (ng/ml) of EME, CO and BE by GCMS in 6 subjects.

Subject I				Subject II			
Hour	EME	CO	BE	Hour	EME	CO	BE
2	1716	50	2063	2	6743	58	4205
4	2973	61	4001	4	2877	57	2869
6	3717	24	6532	6	5387	49	4428
8	430	8	1082	8	5259	21	4857
12	2799	36	3992	12	1430	15	2733
20	266	8	1311	20	704	12	1974
36	49	7	385	36	100	7	315
48	22	6	93	48	41	8	84

Subject III				Subject IV			
Hour	EME	CO	BE	Hour	EME	CO	BE
2	5499	133	6059	2	3464	49	2462
4	6641	72	7610	4	5735	6	4555
6	3063	36	4014	6	2761	30	3401
8	3144	51	3717	8	1182	17	1692
12	1069	21	2343	12	1305	17	1937
20	474	15	1329	20	700	94	1463
36	102	7	168	36	98	7	250
48	94	7	103	48	89	6	104

Subject V				Subject VI			
Hour	EME	CO	BE	Hour	EME	CO	BE
2	13887	123	10719	2	4738	50	4228
4	3750	60	4023	4	6336	51	4861
6	956	17	1654	6	5214	32	4232
8	2147	30	3629	8	5923	63	4116
12	1198	15	2340	12	2105	24	2882
20	302	13	828	20	1031	15	1958
36	170	12	157	36	292	6	998
48	40	6	64	48	68	11	315

Figures 1 – Excretion of EME and BE (left scale) and CO (right scale)



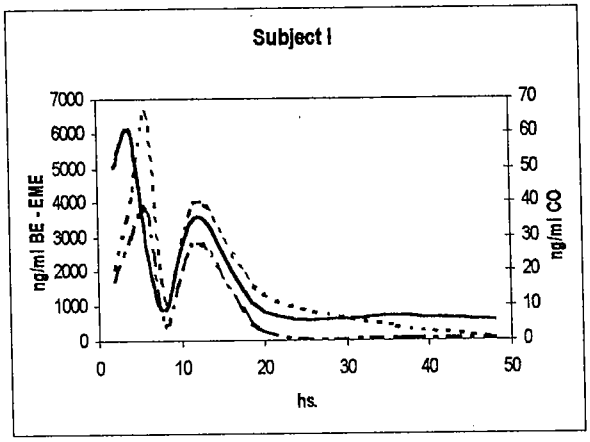


Fig. 1a

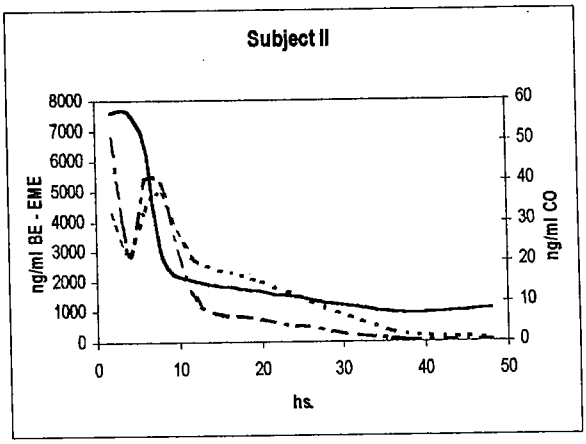


Fig. 1b

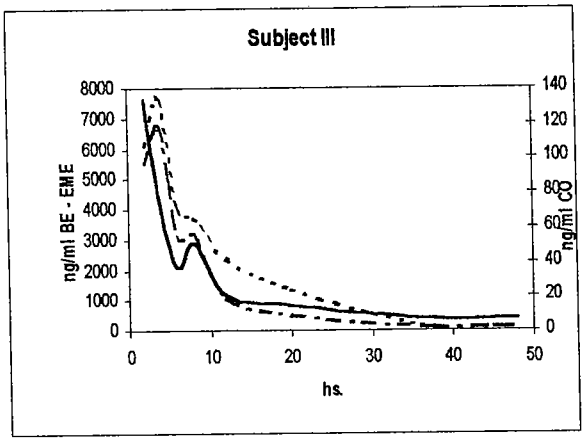


Fig.1c

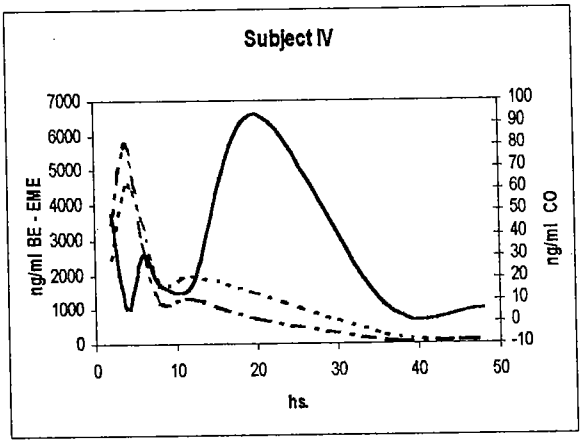


Fig. 1d

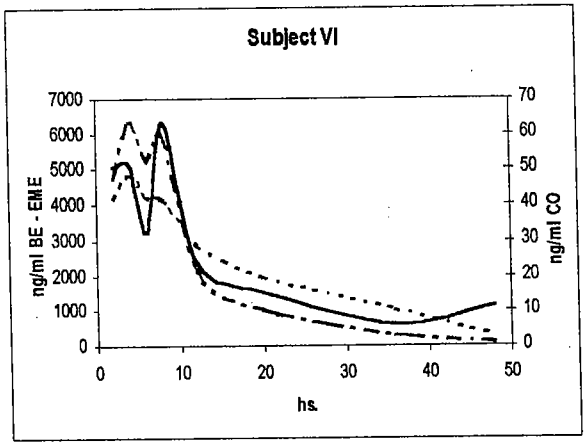


Fig. 1e

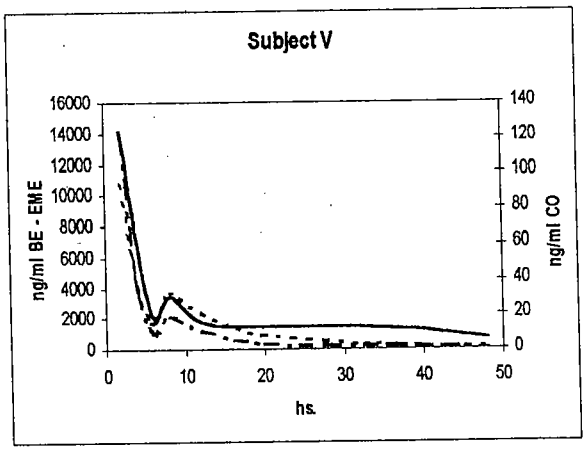


Fig. 1f