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#### ABSTRACT

The objectives of present work are twofold. First, we want to verify that hygrine and cuscohygrine are good markers to distinguish between chewing coca leaves and cocaine abuse. Secondly, we try to develop a quick and easy qualitative method to determine the two mentioned markers.

We analyzed two kinds of urine samples: the first group consisted of twenty-four (24) subjects: urine samples were obtained from various types of workers (e.g. doctors, chemists, nurses, technicians, painters, contractors, employees and some retired persons) who admitted chewing coca leaves. Frequency of the habit of chewing coca leaves was variable. They practiced "coqueo" between two (2) and forty-four (44) years. Sixteen (16) of them used alkaline substances to enhance the extraction of cocaine from the leaves The second group of urine samples consisted on thirty-eight (38) cocaine abusers, from forensic cases from Spain and Argentina. A GC/MS qualitative method, performed after liquid–liquid extraction, was developed and validated (the parameters studied were selectivity/ specificity, LOD and stability), and then applied to the urine samples.

Hygrine and cuscohygrine are good markers to distinguish between chewing coca leaves and cocaine abuse, and the qualitative method presented can be used successfully in workplace drug testing and forensic cases.

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#### 1. Introduction

Coca leaves have traditionally been used for chewing and brewing tea for centuries among the indigenous population of the Andean region. This practice causes no harm and is known for the health benefits such as overcoming altitude sickness, suppressing pain and fatigue, and aiding digestion.

The traditional method of chewing coca leaf, called "acullico" or "coqueo", consists of keeping a coca leaves ball soaked with saliva

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http://dx.doi.org/10.1016/j.forsciint.2014.02.024 0379-0738/© 2014 Elsevier Ireland Ltd. All rights reserved. in the mouth together with an alkaline substance that assists in extracting cocaine from the leaves.

Coca leaf was included in the UN Single Convention of Narcotics Drugs in 1961 and therefore subject to international control, however the practice of the so-called "coqueo" (coca leaf chewing) is legal in Argentina [1] and recently also in Bolivia. Today coca leaf chewing is widespread in all sectors of society not only among some of the indigenous Latin American populations or in the Andean countries (which due to their high geographical locations above sea level). The use is widely regarded to be beneficial to health and thus does not represent a legal issue.

The practice of the "coqueo" is not only circumscribed to the northern of the Argentina but is widespread in all the countries. New social groups such as urban middle classes in Latin America, at the moment regularly chew coca leaves. This fact makes it more difficult to distinguish between the "coqueo" and the cocaine abusers. The first is legal in Argentina but the second one is illegal and both are positive for cocaine and their metabolites in urine.







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In a previous paper [2] two derivatives of pyrrolidine: hygrine and cuscohygrine were proposed as possible markers for distinguish between "coqueros" and cocaine abusers. During coca chewing a person can in fact absorb a large number of substances present in the leaves. These alkaloids are presumably lost during the process of preparation of cocaine.

It is very important to be able to distinguish these different practices from a forensic perspective. The practice of "coqueo" is not considered an addiction in many of the regions where it is customarily used whereas abuse of the hydrochloride form is an illegal addiction.

The objectives of this paper are twofold. Firstly, we wanted to verify that hygrine and cuscohygrine are good markers to distinguish between chewing coca leaves and cocaine abuse. Secondly, we tried to develop a quick and easy qualitative method to determine the two mentioned markers.

#### 2. Materials and methods

### 2.1. Biological samples

Twenty-four (24) urine samples were obtained from people who chewed coca leaves. All of them signed an informed consent form before the study.

Volunteers were four (4) women and twenty (20) men aged between 18 and 73 years, and with very different types of work, such as doctors, chemists, nurses, technicians, athletes, painters, contractors, employees and some retired persons.

The frequency of the habit of chewing coca leaves was variable, ranging from once a week (about 10 g of coca leaves) to several times a day (with amounts ranging from 10 to 60 g per day and exceptionally up to 300 g per day). The period of the practice of coca leave chewing ranged from two (2) to forty-four (44) years. Sixteen (16) of them used alkaline substances to enhance the extraction of cocaine from the leaves. Regarding their medical history: three had hypertension, two both diabetes and hypertension, one was diagnosed with diabetes and the other with depression. None showed signs of cocaine abuse (Table 1).

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**Fig. 1.** Coca leaves: large leaf (bottom) and small leaf (top)

People who chew coca leaves usually consume two different types of coca leaves: both contain hygrine and cuscohygrine. The most frequently consumed coca leaf in Argentina is the large-leafed variety (Fig. 1). A botanical analysis of the leaves to determine to which variety or species of Erythroxylon the consumed leaves belonged to was not undertaken. [3]

Twenty-nine (29) cocaine abuser urine samples (two (2) women and twenty-seven (27) men, aged 23–56 years) were submitted from forensic cases of Spain. Most of them had been arrested for drug trafficking and/or robbery, and many had a history of polydrug abuse. Also nine (9) cocaine abuser urine samples were submitted from forensic cases of Argentina (seven men and two women; Table 2).

Table 1	
Coca leaves	consumption.

NRO	Age/gender	Years of consumption	Frequency of use	Amount (g)	Leaf	Alkaline substance	Cocaine	EME	Ну	СН	C-coc	Other
1	43/m	23	Several time a day	10-20	Large	Yes	+	+	+	+	+	Nicotine-cotinine
2	73/f	44	Once a week	5	Large	No	+	+	+	+	+	Nicotine-cotinine
3	45/m	20	Several time a day	10-20	Large	No	+	+	+	+	+	-
4	18/m	2	Once a week	10	Large	No	_	+	+	+	_	Nicotine-cotinine
5	70/f	10	Several time a day	20-25	Large	No	+	+	+	+	+	Nicotine-cotinine
6	43/f	3	Once a week	5	Large	No	-	+	+	+	_	
7	29/m	?	Once a week	5	Large	No	_		+	+	_	Nicotine-cotinine
8	42/m	26	Several time a day	10-20	Small-large	Yes	+	+	+	+	+	Nicotine-cotinine
9	59/m	30	Once a day	5	Large	Yes	_	+	+	+	_	-
10	60/m	20	Several time a day	50	Large	Yes	+	+	+	+	+	Nicotine-cotinine
11	25/m	6	Several time a day	60	Large	Yes	+	+	+	+	+	Nicotine-cotinine
12	20/m	2	Several time a day	50	-	Yes	+	+	+	+	+	
13	58/m	24	Several time a day	50	Large	Yes	+	+	+	+	+	-
14	27/m	10	Once a day	5	Large	Yes	_	+	+	+	_	-
15	29/m	10	Once a day	4	Large	Yes	+	+	+	+	+	-
16	48/m	36	Once a day	4	Large	No	+	+	+	+	+	Nicotine-cotinine
17	58/m	30	Several time a day	10-20	Large	Yes	+	+	+	+	+	Nicotine-cotinine
18	64/m	5	Several time a day	10-20	Large	Yes	+	+	+	+	+	-
19	29/m	10	Several time a day	10-20	Large	Yes	+	+	+	+	+	-
20	32/m	16	Several time a day	10-20	Small	Yes	+	+	+	+	+	Metamizol
21	60/m	10	Once a day	5	Large	Yes	+	+	+	+	+	-
22	37/m	10	Several time a day	30	Large	No	+	+	+	+	+	Nicotine-cotinine
23	66/f	12	Several time a day	300	Large	Yes	+	+	+	+	+	Fluoxetine
24	?/m	?	Several time a day	20	Small-large	Yes	+	+	+	+	+	-

EME, methylecgonine; Hy, hygrine; CH, cuscohygrine; C-coc, cinnamoylcocaine.

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