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First analytical chemistry study on drug abuse in the Buenos Aires (Argentina) University students

Patricia N. Quiroga [a], Rosa I. Panzuto [b], Gloria B. Alvarez [a], Daniel J.E. Mirson [a], Cecilia F. Ochoa [a], Estrella M. Assem [c], Clara M. López [a], Luis C. Schkolnik [b], Edda C. Villaamil [a], Otmaro E. Roses [a,*]

[a] *Cátedra de Toxicologia y Química Legal, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 7 piso, 1113 Buenos Aires, Argentina*

[b] *Dirección General de Salud y Asistencia Social, Universidad de Buenos Aires, Buenos Aires, Argentina*

[c] *Secretaría de Programación para la Prevención de la Drogadicción y la Lucha contra el Narcotráfico, Buenos Aires, Argentina*

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Abstract

One hundred samples were randomly selected from urine specimens collected from Buenos Aires University students, 50 males and 50 females, whose ages ranged from 19 to 47 years. Cocaine (COC), cannabinoids (CNNs), amphetamines (AMs), benzodiazepines (BZDs), barbiturates (BBTs), opiates (OPs) and salicylates (SAs) were searched for by ELISA, FPIA, normalized TLC, HPLC and GC/MS techniques. The presence of COC was detected in five samples, CNN in two and SA in twelve. No evidence of AMs, BZDs, BBTs or OPs was found.

Keywords: Drug analysis; Urine analysis; Student drug abuse

1. Introduction

In order to evaluate drug intake liable to generate addictive behavior in a given society, it is essential to work on the most accurate possible data. The mode of sample selection is therefore crucial. Specimens must be: qualitatively suitable, as homogeneously representative as possible, adequate in distribution and in frequency, quantitatively large enough; or at least the degree of reliability of the specimen should be known. The collected specimens must meet certain basic criteria related to two distinct aspects [1]: willingness and veracity.

In surveys carried out by analyzing biological samples, both the above criteria are likewise represented. The former, as regards sample collection, though compulsory, is performed for a different purpose and is entirely anonymous. This aspect plays a key role, guaranteed by the double-blind system and only the attendance at a given school, together with sex and age, was taken into account in order to avoid any other identification data.

Quantitative limitations on sample processing, related to the cost of reagents, apparatus and staff, make it difficult to study very large populations.

Among the potential consumers of abuse substances, university students are obvious candidates due to their age and the fact that they attend lecture halls and training areas, which readily allows them to elude parental control. For this reason, in this first survey on the intake of addictive drugs such a population has been chosen. To this end, advantage has been taken of urine samples provided by students at the University of Buenos Aires for routine clinical analysis.

For a first study and as an initial approximation, an estimated 50% incidence was adopted as the working hypothesis, as supported by Jenicek and Cleroux [2].

The student population of the University of Buenos Aires is roughly 170 000 strong and around 40 000 urine samples are collected annually.

Research on the presence of salicylates (SAs) was performed to find out whether cases of self-dosing or acetylsalicylic acid abuse were likely to have taken place, indicating behavior prone to the intake of pharmacologically active substances, inasmuch as it is the contention of one of the classical schools of medical toxicology in Argentina that there is not

* Corresponding author.

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only a correlation but also an established ratio between acetylsalicylic acid consumption and opioid consumption.

During the confirmation tasks on substances that had been found, GC/MS for cannabinols (CNNs) could not be performed due to the fact that material was no longer available. Inasmuch as two different methodologies had been resorted to, confirmation was deemed as positive, to statistical effect, when two results were found to be coincident.

As regards opiates, no hydrolysis of glucoronoids was performed since negative screening made hydrolysis unnecessary.

2. Materials and methods

2.1. Population studies

The urine donors were 100 university students belonging to 11 different schools, whose ages range from 19 to 47 years; 50 were male and 50 female.

2.2. Biological material

Samples were prepared by fractionation of the specimen provided by students for routine clinical analysis, according to the following criteria.

1. The sample was collected on Monday.
2. A form was completed with pertinent data (age, sex and school) taken from the medical order given to all subjects.
3. Random selection of 50 samples from 100 consecutive specimens for each gender, by means of a table of random numbers.
4. Absolute anonymity of the specimen donor.
5. Equal quantities were collected from either sex, taking into account the sex distribution for the days samples were collected.

20-ml aliquots of urine were sent to the laboratory and preserved at −20°C for analytical processing.

2.3. Apparatus

For high-pressure liquid chromatography (HPLC), a Jasco PU 980 model with a Rheodyne 7125 injector having a 20 μl loop, a Jasco UV 975 detector, wavelength 236 nm, and a Lichrospher column 100 RP-18 (5 μm) in Lichrocart 135-A was used. The mobile phase was 75:25 deionized water/acetonitrile carried to pH 3.0 with orthophosphoric acid at a flow rate of 1.2 ml/min in the isocratic mode [3].

For GC/MS, a Hewlett Packard system, consisting of a GC HP 5890 and an MSD 5972 selective mass detector, a fused silica HP-5 (methylphenyl silicone) column, 30 m in length and 0.25 mm in inner diameter was used. The carrier gas was helium; the column flow was 0.7 ml/min in the split mode at a 1:10 ratio. The temperature program has been described elsewhere (Roses et al. [4]).

Spectrophotometry was performed with a Merck Vitalab ECLAIR model spectrophotometer for polarized immunofluorescence.

2.4. Chemicals

The chemicals used were: solid phase extraction (SPE) columns with hydrophobic cation exchange bonded silica copolymer (World Wide brand Catalog No. WSDAU 020) to screen abuse drugs; SPE JT Baker C18 columns (Catalog No. 7020-01). The chemicals mentioned in the text were of analytical quality.

2.5. Kits

The reagent kit for enzymoimmunoanalysis (ELISA) was obtained from ELISA Technologies: cocaine/benzoylecgonine (Catalog No. 101310); generic benzodiazepine (Catalog No. 100210); generic barbiturate (Catalog No. 100110); amphetamine (Catalog No. 105210); tetrahydrocannabinol (Catalog No. 105010); and generic opiate (Catalog No. 100610).

The reagent kit for fluorescence polarization immunoassay (FPIA) was obtained from Merck Diagnostics (DAU TRAK): cocaine (Catalog No. 6131); benzodiazepines (Catalog No. 6116), opiates (Catalog No. 6125), barbiturates (Catalog No. 6123); amphetamines (Catalog No. 6114); and cannabinols (Catalog No. 6112).

TOXILAB® THC II Plus was obtained from Toxilab.

2.6. Screening

Screening was performed on unprocessed samples by ELISA and later using FPIA and TOXILAB®, according to techniques described in each kit, for the following substances: opiates, amphetamines, barbiturates, benzodiazepines and cannabinols, as well as cocaine and its metabolites. Salicylate assay was carried out by the classic Trinder reaction [5].

2.7. Sample processing for cocaine and its metabolites

CSDAU 202 copolymer columns were employed. The sample was prepared by adding 0.5 ml of 0.2 N sulfuric acid to 5 ml of urine. Sample pH ranged from 2.0 to 5.0. The column was prepared with 3 ml methanol and 3 ml deionized H_2O in sequential fashion. The sample was then applied to the column at a 1 ml/min flow rate. The column was washed with 3 ml deionized water and 3 ml 0.2 N hydrochloric acid, then dried for 2 min under maximum vacuum (15–20 inches of Hg). 3 ml of methanol were then added and the column dried again for 2 min under maximum vacuum. Cocaine and benzoylecgonine were eluted with 3 ml of the mixture methylene chloride/isopropanol/ammonium hydroxide, ratio 80:20:2. The eluate was dried under nitrogen and minimal heat (<40°C). The sample was derivatized with *N,N*-dimethyl-formamide diethyl acetal (Merck Catalog No.

803066) as described by Thenot et al. [6] and 1 μl was injected into the GC/MS.

2.8. Sample processing for salicylates

The sample was prepared by adding 1 ml of 8% (vol./vol.) orthophosphoric acid solution in water, pH 3.0 or lower, to 1 ml of urine. The C18 SPE column was activated by passing through 3×1 ml of methanol, followed by 3×1 ml of deionized water. The sample was passed through the column at a flow rate of 1 ml/min and washed with two 0.5-ml aliquots of 8% (vol./vol.) orthophosphoric acid solution in water. Elution was performed with three 100-μl aliquots of methanol and taken to a 2-ml volume with the mobile phase (deionized water/ acetonitrile 75:25, pH 3.0), then 20 μl were injected into the HPLC.

2.9. Sample processing for cannabinoids

Sample processing was performed according to the manufacturer's instructions (TOXILAB®), covering steps of extraction with special solvent, concentration and TLC resolution.

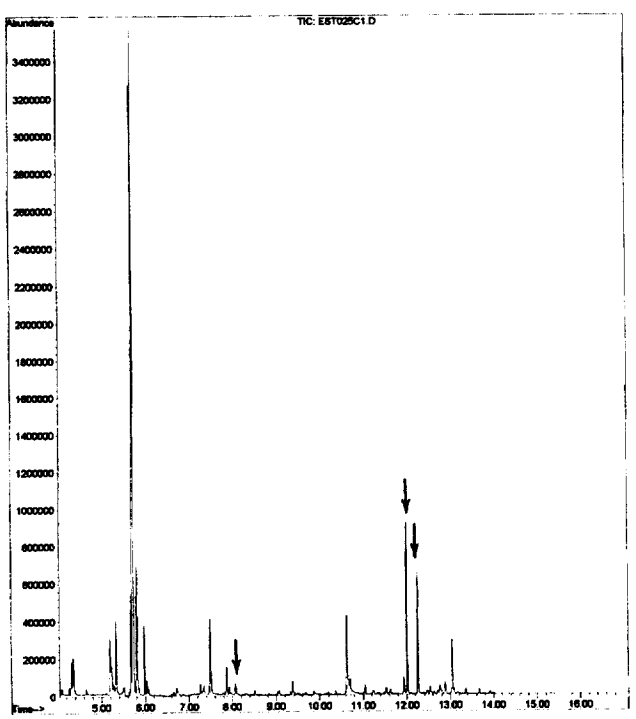

Fig. 1. Total ion chromatogram of an illustrative sample. Peaks relevant to methylecgonine (8.071 min), cocaine (12.010 min) and benzoylecgonine (12253) can be seen.

3. Results

Fig. 1 shows the results of gas chromatography analysis (total ion chromatogram) in an illustrative sample and in Figs. 2–4 the mass spectra of methylecgonine, cocaine and benzoylecgonine detected in the same sample are compared with the library spectra.

Fig. 5 shows an HPLC exhibiting a salicylate anion peak.

The presence of cocaine was confirmed in seven samples, that of cannabinols in two and that of salicylates in twelve.

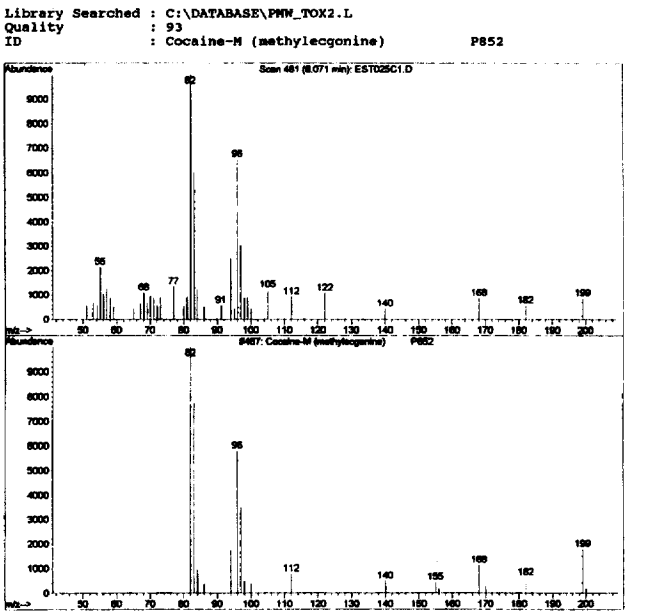

Fig. 2. Methylecgonine mass spectrum (93% quality match) corresponding to the peak at 8.071 min.

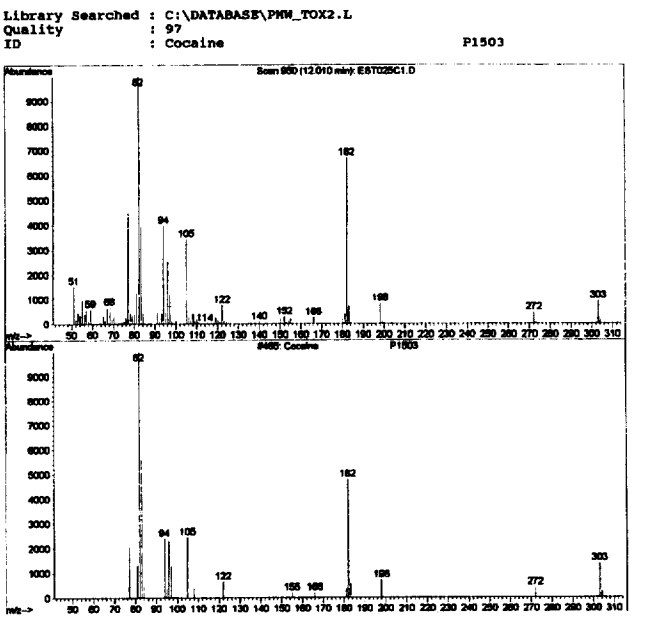

Fig. 3. Cocaine mass spectrum (97% quality match) corresponding to the peak at 12.010 min.

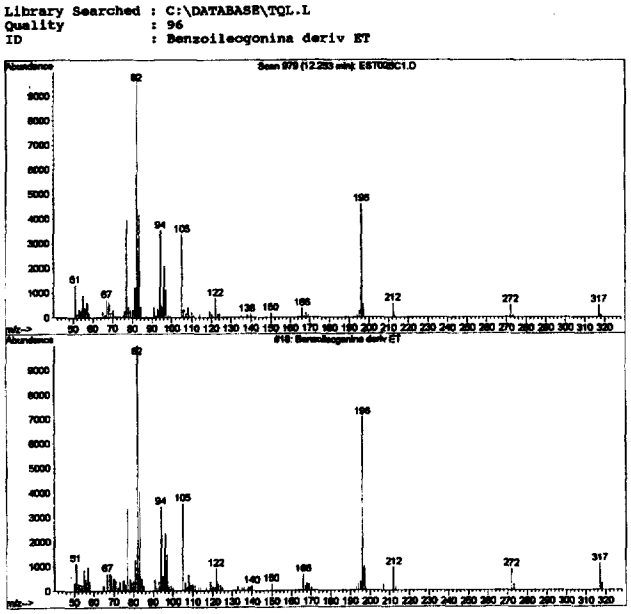

Fig. 4. Benzoylecognine mass spectrum (96% quality match) corresponding to the peak at 12.253 min.

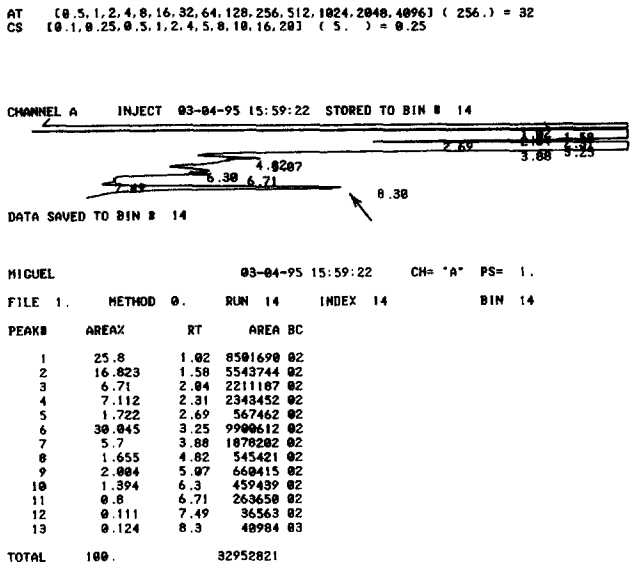

Fig. 5. Chromatogram from HPLC. The arrow indicates the salicylate peak.

The correlations between substances found and gender and age, and gender and school are shown in Tables 1 and 2, while Table 3 lists analytical findings for detected substances.

4. Discussion and conclusions

No benzodiazepines, barbiturates, amphetamines or opiates were found in the analyzed samples.

Cocaine was detected in five samples and cannabinoids in two, with a single specimen presenting both substances.

In samples where screening indicated cocaine and/or its metabolites, GC/MS confirmed their presence. However, the same technique was not practicable for cannabinols as there was not enough sample left for processing. In spite of this limitation, the fact that findings disclosed by the two immunological reactions agreed with normalized TLC (TOXILAB®) partly confirmed the presence of cannabinols.

It should be pointed out that, in our experience, there was a remarkable agreement between positive data from immunoanalysis and those from mass spectrometry, TOXILAB® and the Trinder reaction with HPLC.

Table 3 highlights the good agreement between the content of cocaine, opiates, benzodiazepines, barbiturates and cannabinoids by means of the diverse procedures. Such was not the case with amphetamines, which proved positive by ELISA screening but not by FPIA, TOXILAB® or GC/MS. This suggests that their lack of detection is attributable to an immunological non-specific reaction or they were at the threshold level.

In positive cases, statistical analysis by the χ^2 test indicated non-significant differences for gender, age and school ($p > 0.4$, $p > 0.5$, and $p > 0.5$, respectively) [7].

Adopting a possible 50% incidence as advised by the authors of Ref. [2] in initial studies such as ours, the statistical range corresponding to recorded values may be calculated by the formula [2,8]

$$c = (\text{S.D.} \times 0.5)/n^{-2}$$

where c is the absolute precision level.

Table 1
Distribution of the studied population and of the positive samples according to sex and age

Population			Positive samples					
Age (years)	Sex		Cocaine		Cannabinoids		Salicylates	
	F	M	F	M	F	M	F	M
19–23	30	36	2	1	–	–	4	4
24–28	15	10	1	1 [a]	–	1 [a]	1	–
29–33	2	1	–	–	–	–	1	–
34–38	3	1	–	–	1	–	1	1
39–43	–	1	–	–	–	–	–	–
44–48	–	1	–	–	–	–	–	–
Total	50	50	3	2	1	1	7	5

F: females, M: males.
[a] Same person.

Table 2
Distribution of the studied population and the positive samples according to sex and school

School	Population		Positive samples					
	F	M	Salicylates		Cocaine		Cannabinoids	
			F	M	F	M	F	M
Medicine	23	12	3	1	2	1	1	–
Architecture	4	2	1	–	1	–	–	–
Psychology	11	6	2	1	–	1 [a]	–	1 [a]
Engineering	5	23	–	2	–	–	–	–
Veterinary	2	–	1	–	–	–	–	–
Exact Science	1	–	–	–	–	–	–	–
Social Science	1	1	–	–	–	–	–	–
Law	2	1	–	1	–	–	–	–
Agronomy	1	–	–	–	–	–	–	–
Pharmacy	–	4	–	–	–	–	–	–
Biochemistry								
Economy	–	1	–	–	–	–	–	–
Total	50	50	7	5	3	2	1	1

F: females, M: males.
[a] Same person.

Table 3
Screening methods for drug use among university students

Determination	ELISA		FPIA		TOXILAB®		Trinder	
	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.
Cocaine	95	5	95	5	95	5		
Opiates	100	–	100	–	100	–		
Benzodiazepines	100	–	100	–	100	–		
Amphetamines	97	3	100	–	100	–		
Barbiturates	100	–	100	–	100	–		
Cannabinoids	98	2	98	2	98	2		
Salicylates							88	12

Cocaine positives were confirmed by GC/MS.
Cannabinoid positives were confirmed by TOXILAB®.
Salicylate positives were confirmed by HPLC.

Table 4
Some worldwide statistics for drug use/abuse

Country	Year	Cocaine	Cannabis	Observations	Ref.
USA	1969	No data	22%	barbiturates 10%	[9]
USA	1972	8%	30%	analgesics 5%	[10]
Mexico	1976	7.03%	76.84%	barbiturates 25–97%	[11]
USA	1984	12.2	29.4	data in million of users	[12]
Peru	1986	0.2%	0.5%		[11]
USA	1987/88	20.0%	62.4%	percentage of total of positive samples	[13]
Ecuador	1988	0.3%	1.2%		[11]
Bolivia	1990	1.3%	1.8%	only college students	[14]
USA	1990	3%	10%		[14]
Panama	1991	1.9%	1.9%		[14]
Colombia	1992	0.3%	0.6%		[14]

Thus, at 95 and 90% confidence levels, the relative precision levels were 0.100 and 0.085, respectively.

The following values are for 90% confidence levels and are accompanied by 95% confidence levels in parentheses.

Subjects consuming either cocaine or cannabis, 0–14.5% (0–16%); cocaine alone, 0–13.5% (0–15%); cannabis alone, 0–10.5% (0–12%); both cocaine and cannabis, 0–9.5% (0–11%); and salicylates, 3.5–20.5% (2–22%).

Statistics from other countries indicate a predominance of cannabis addiction over cocaine, as shown in Table 4.

Interestingly, consumption patterns disclosed by this study feature relatively low figures as compared with those of other

countries, or with results obtained from written surveys in Argentina for the university population. However, it is certainly alarming that there should be a greater probable number of cocaine addicts than cannabis addicts, since the former is regarded as a hard drug. In contrast, general statistics worldwide disclose a greater percentage of cannabis than cocaine users.

The incidence of salicylates is low and most likely corresponds to therapeutic use through self-medication. Remarkably enough, none of the samples presenting salicylates disclosed evidence of any of the other abuse drugs investigated, contrary to what the Argentinean medical toxicology schools have maintained for many years.

It may be concluded that the data achieved by our study provide useful basal parameters for further evaluations, by applying the figures obtained to the prospective study of new populations.

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