Determinacion by direct injection into HPLC of cocaine, in urine samples, cocaine and crack unit samples.

Manoel Lima de Menezes*
Andréa Sanchez*
Priscila Raquel Martins*
Márcia Aparecida Zeferino Garcia**
Arnaldo Alves Cardoso
Pedro Lessi***
Oduvaldo Câmara Marques Pereira****

* UNESP, School of Sciences, Department of Chemistry. Av. Edmundo Coube S/N, Bauru, SP – 17033-60
** University of the Sacred Hearth-USC, Department of Chemistry. Rua Ir. Arminda 10-50, Bauru-SP 17044-160.
*** UNESP, Institute of Chemistry, Department of Analytical Chemistry. Rua Prof. Francisco Degni S/N, Araraquara-SP-14801-970.
**** UNESP, Institute of Biological Sciences. Department of Pharmacology, Rubião Jr.- Botucatu – SP. 18618-000

MENEZES, Manoel Lima de, et al. Determination by direct injection intro HPLC of cocaine, in urine samples, cocaine and crack samples. *Salusvita,* Bauru, v. 19, n. 2, p. 81-88, 2000.

ABSTRACT

A method has been developed for the extraction and determination of cocaine in urine samples. The method involved direct injection of urine on to an ISRP-C_8 (100mm x 4.6 mm DI) column and the use of 0.05mol L^1 sodium dibasic phosphate and acetonitrile (70:30 v/v) as mobile phase. The recovery of cocaine present in urine samples was higher than 98.8 ± 5.1%. with 2.7% relative standard deviation. The limit of detection was 0.10mg mL^{-1} and the range linearity 0.10 to 15.00mg mL^{-1} for cocaine. In the crack sample and in two cocaine samples concentrations of cocaine of 867, 894 and 65mg g^{-1} were found, respectively.

Key Word: cocaine, urine, HPLC.

INTRODUCTION

The determination and quantification of cocaine is important in forensic toxicology. It is an alkaloid of *Erythroxylum coca*, a native plant of Sri Lanka, Bolivia, Colombia and Peru. The content of cocaine in the leaves of coca varies from 0.5 to 2.0%. Indians of the Andes region use this plant with the purpose of gettting a sensation of well-being and to

diminish fatigue. The leaves are chewed in addition to some calcium oxide since the alkaline medium favors the liberation of the compound in its free form, becoming easier to be absorbed due to its greatest liposolubility (Larini, 1993).

Cocaine is readily biotransformed mainly by hydrolysis of esther and N-demethylation connections. This process leads to the formation of esther methylecgonine (EME), benzoilecgonine (BEC), ecgonine and norcocaine (Fig.1). Although cocaine is readily hydrolyzed to benzoylecgonine, several studies revealed that 0.5 mg L^{-1} of cocaine can be detected in urine, prior to hydrolysis, after 12 hours of inhalation (Larini, 1993).

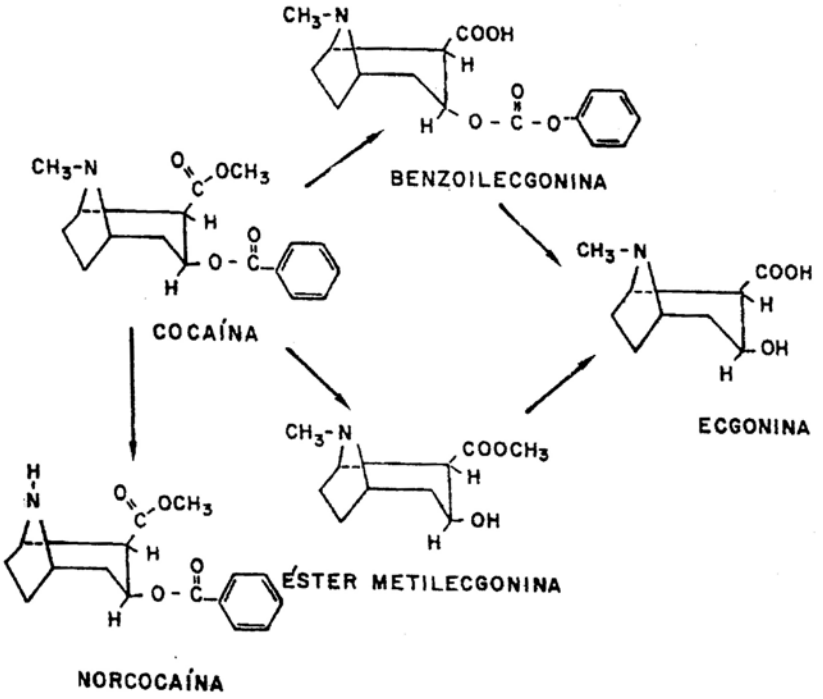

FIGURE 1 – Biotransformation of cocaine.

Urine and blood samples are the commonest physiological fluids used for the detection of cocaine. The preparation of the sample is an important pre-requisite to the determination of cocaine by HPLC (*High Performance Liquid Chromatography)* in biological fluid samples. The extraction is usually made by partition liquid-liquid, with variations of pH in the sample, or by solid phase extraction (SPE). By increasing the samples' pH (adding solutions of sodium carbonate or sodium hydroxide), cocaine can be easily extracted from the sample with the help of organic solvents such as hexane, diethyl ether or chloroform. The organic phase is usually evaporated; the remains are dissolved with the mobile phase and injected into the HPLC. High Performance Liquid Chromatography is the technique most used in the determination of cocaine in a variety of

samples (Nakahara, 1999., Höld et al., 1998, Moeller et al., 1998, Ma et al., 1997). Separation and determination of cocaine, benzoylecgonine and norcaine are usually made in chromatographic column C_{18} using mobile phases of methanol-acetonitrile-sodium acetate 0.026 mol L^{-1}, pH 2.2 with 1.29 x 10^{-4}mol L^{-1} tetrabutylamonium phosphate (12.5:10:77.5 v/v/v) (Ma et al., 1997) and acetonitrile phosphate buffer 0.02 mol L^{-1}, pH 6.0 (55:45 v/v) (Schwartz & David, 1985).

Tagliaro et al., 1998 used the technique of capillary electrophoresis in free solution in the determination of abuse and illicit drugs, but this technique also requires the preparation of samples before introduction in the equipment of zone capillary electrophoresis.

Several analytic methods using the technique of direct injection of the sample in columns ISRP (*internal surface reverse phase*) have been developed and evaluated for the same purpose, such as, extraction and separation of pesticides in row milk (Menezes et al., 1998) and the determination of caffeine in urine samples by direct injection in HPLC (Menezes et al., 1999).

The main purpose of this study was to develop a simple and rapid analytical method for the direct determination of cocaine in urine samples based in the analytic toxicology, particularly the forensic and clinical toxicology.

MATERIALS AND METHODS

Materials

Acetonitrile was obtained from Carlo Erba (Milan, Italy), dibasic sodium phosphate and chloridric acid (p.a) were purchased from Merck (E. Merck, Darmstadt, Germany). Water was processed in the Milli-Q purification system (Millipore, Bedford, MA, USA). The chair of the Police for Drug Investigation in Bauru donated the standards of cocaine, as well as the samples for cocaine and crack.

Enhancement of the urine samples containing cocaine and preparation of the standard curve for calibration.

Urine sample was diluted to 1:100 (v/v) with purified water and known quantities of cocaine were added in order to obtain concentrations of 3.00; 6.00 and 9.00mg mL^{-1}. Standard solutions containing 0.56; 1.12; 2.25; 4.50 and 9.00mg mL^{-1} of cocaine were prepared by dilution of a mother solution containing 500mg mL^{-1} of cocaine. This solution was previously prepared by dilution of 0.05g of the cocaine standard in 1.0 mL of a chloridric acid 1.00mol L^{-1} , completing the final volume of 10.0 mL with purified water.

Preparation of the samples of crack and cocaine.

1.0 mL of a solution of chloridric acid 1.00mol.L^{-1} and 5.0 ml of pure water was added to an amount of 0.005g of the sample of crack and or cocaine. The mixture was homogenized and filtered in Whatman qualitative filter paper and transferred to a 10.0 mL capacity volumetric balloon, the volume being completed with pure water. These solutions were diluted to 1:200 (v/v) prior to the injection in the liquid chromatographic system.

Instrumentation

The chromatographic experiments were conducted under isochratic conditions in a high efficiency liquid chromatographic system (Varian model 2510) equipped with reciprocating pump, a detector for UV with variable wavelength (Varian 2550), with wavelength adjusted to 235 nm and an SP 4400 Chromajet integrator (Variant Associates, Inc; Sunnyvale, CA,USA). The samples and standard solutions were injected in a column ISRP-C_8 with a manual injection valve (Rheodyne 7125, Cotati, CA, USA), using a loop of 10mL.

Chromatographic conditions

The chromatographic column ISRP-C_8 (100 mm x 4.6 mm DI) was prepared according to Menezes & Felix, 1998.

The determination of cocaine was conducted under room temperature with a flow of mobile phase adjusted to1.0mL min^{-1}. The mobile phase used was a mixture of the dibasic sodium phosphate solution 0.05mol L^{-1} pH 8.0 and acetonitrile (70:30 v/v).

Evaluation of the direct injection of the enhanced urine sample with cocaine.

The experiments were conducted by triple injection of each concentration of the urine sample previously enhanced containing 3.00, 6.00 and 9.00 mg mL^{-1} of cocaine.

Results were evaluated according to the percentage of retention and the relative odds ratio (relative odds ratio, ROD = odds/mean of results x 100)*.

Determination of the concentration of cocaine in the cocaine and crack samples.

The real samples of cocaine and crack were injected three times and the results evaluated according to the relative odds ratio.

MENEZES, Manoel Lima de, et al. Determination by direct injection intro HPLC of cocaine, in urine samples, cocaine and crack samples. *Salusvita*, Bauru, v. 19, n. 2, p. 81-88, 2000.

*Relative odds ratio is also known as variance coefficient (SKOOG et al., 1998).

RESULTS AND DISCUSSION

This method of direct injection allows the determination of the cocaine concentration in urine samples without previous treatment of the sample. The urine sample was diluted to 1:100 (v/v) to assure that metabolites and small concentration of proteins could be readily eluted from the chromatographic column allowing a retention period of 2.50±0.02 minutes.

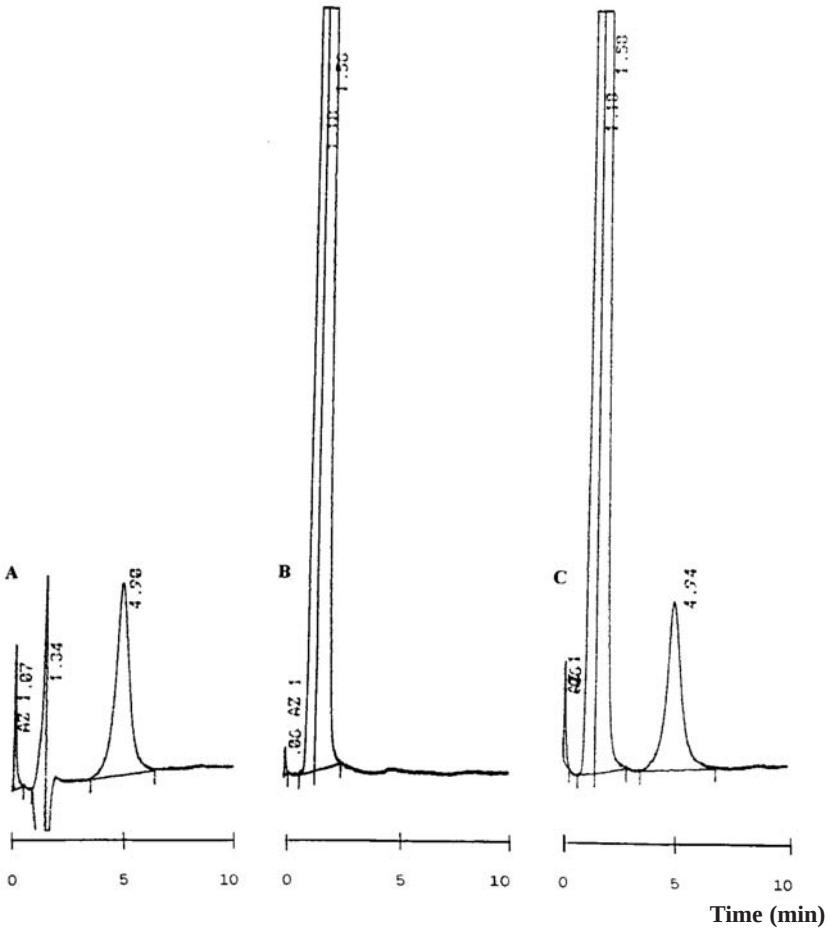

FIGURA 2 - Chromatograms obtained with (A) standard solution contaning 9,00mg mL^{-1} of cocaine with a retantion period of 4.90 ± 0.04 minutes; (B) non-enhanced urine sample; (C)chromatogram obtained with an enhanced sample of urine with 8,00mg mL^{-1} of cocaine.

FIGURE 2 shows the chromatograms of the standard solution of cocaine: (A) 9.00 mg mL^{-1}, (B) urine sample and (C) enhanced sample of urine with 8.00mg $mL^{-1}$of cocaine. In the chromatogram C it was observed that the cocaine is extracted from the matrix without interference peaks. This is due to the use of a mobile phase constituted of an aqueous solution of dibasic sodium phosphate 0.05mol L^{-1} pH 8.0 and acetonitrile (70:30 v/v). It is important to stress that in basic solution, other

products commonly found in urine, with acid or slightly acid properties such as benzoic acid, hypuric acid, methilhypuric acid, acetic acid, citric acid, TLA acid and aminoacids. are partially shown in ionized form and partially in the molecular form; phenols, hydroquinone and catechol are shown in the ionic form. On the other hand, cocaine does not show an ionic form in a range of pH, having a structure slightly non-polar. Thus, in pH 8.4, due to their ionic forms, the compounds mentioned as "commonly found in urine" do not undergo retention in a stationary non-polar phase (ISRP-C_8) whereas the cocaine is retained.

The chromatographic experiments to evaluate the efficiency of the retention were made by enhancement of the urine sample containing cocaine with concentrations of 3.00, 6.00 and 9.00 mg mL^{-1} were injected three times. The retrievals obtained were high showing values higher than 98.8±5.1%. The repeatability of results was verified by averages and calculations of relative odds ratio obtaining very significant values as can be seen in TABLE 1.

TABLE 1 – Evaluation of the cocaine recuperation in the enhanced samples of urine and respectives relative odds ratio.

level of enhancement (mg mL^{-1})	Recuperation (%)	ROD (%)
3.00	98.8 ± 5.1	2.7
6.00	99.3 ± 2.9	1.6
9.00	99.7 ± 2.3	1.2

The limit of detection was determined by measuring the minimal concentration of detected cocaine, taking into consideration the height of the peak which corresponded twice the noise. Thus, the detection limit was 0.10mg mL^{-1} of cocaine which was detected with an UV-visible detector adjusted to a wavelength of 235 nm and 0.04 units of absorbancy and the integrator adjusted with one unit of attenuation. The linearity of the detector was established determining the concentration of cocaine in an aqueous solution of cocaine, varying from 0.10 to 15.000 mg mL^{-1} of cocaine.

The use of the chromatographic column ESRP-C_8 was also effective to the determination of concentrations of cocaine in real samples such as: cocaine unit samples and cracks samples. The samples were previously prepared, according to item 2.3, and diluted to 1:200(v/v) and afterwards injected in the liquid chromatographic system. Concentrations of 894 and 65mg g^{-1} of cocaine were detected in the samples. In the crack samples the concentration was 867 mg g^{-1} of cocaine. The significant difference between the two samples (cocaine and crack – TABLE 2) may be due to the fact that cocaine is rarely found in pure form – it is usually mixed to sugar, starch or wheat flour. By filtration, wheat and starch are removed during the pre-treatment process and the dissolved sugar will not be detected during the monitoring of chromatograms with the detector in the region of the visible UV.

TABLE 2 – Concentration of cocaine in a real sample of crack and in two real samples of cocaine.

samples	Cocaine (mg g^{-1})	DPR (%)
Cocaine	1 894 ± 0,7	0.03
Cocaine units	65 ± 1,5	0.76
"*Crack*"	867 ± 6,0	0.23

CONCLUSION

This method is simple, ready and the ISRP-C_8 column may be used to the direct determination of the concentrations of cocaine in urine samples or in cocaine and crack samples.

It was observed that the concentration of cocaine found in crack is superior to those of cocaine detected in samples of cocaine units. Taking into consideration that the concentration of cocaine in crack was near or superior to that found in the non diluted samples of cocaine, it is easy to understand why crack users become readily addicts and, thus, are more liable to show acute intoxication and exposure to drug overdose.

ACKNOWLEDGMENT

The authors wish to thank the FAPESP, the Universidade Estadual Paulista-UNESP by financial support and also the Labors Health Institute in Bauru for the laboratory infrastructure. The authors also whishes to thanks to Mr.Edson Cardia, chief of police officer for the donation of cocaine and crack samples.

BIBLIOGRAPHIC REFERENCES

HÖLD, K. D., HUBBARD, L. D., DIANA, G. W., ROLLINS, D. R. Quantification of cocaine in human hair: the effect of centrifugation of hair digests., *J.Anal. Toxicol.* v. 22, p.414, 1998.

LARINI,L. Toxicologia. 2. Ed. São Paulo, Manole, 1993.

MA, F., ZHANG, J. LAU, C. E. Determination of cocaine and its metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats., *J. Chromatogr.B. Biomed Sci Appl.*, v.693, n.2, p.307-312 1997.

MENEZES, M.L. DEMARCI, A. A. C., FÉLIX,G., On-line extraction and determination of carbofuran in raw milk by direct HPLC injection on an ISRP column., *Chromatographia*. v.47, n.1/2, p.81, 1998.

MENEZES, M.L. FÉLIX ,G. On-line extraction and separation of bendiocarb, methomyl, methylparathion and pentachlorophenol pesticides from raw milk, *J.Liq.Chromatogr.Rel. Technol*. v.21, n.18, p.2863, 1998.

MENEZES, M. L. SANCHEZ, A. MARTINS, P. R., GARCIA, M. Z., PEREIRA, O. C. M., CARDOSO, A. A. Determinação de cafeína em amostras de urina por injeção direta no HPLC., *Salusvita*, v.18, n.2, p.35, 1999.

MOELLER, M.R. STEINMEYER, S., KRAEMER., T. Determination of drugs of abuse in blood-Review., *J.Chromatogr.B.* v.713**,** p.91, 1998.

NAKAHARA, Y. Hair analysis for abused and therapeutic drugs., *J. Chromatogr.B.* v.733, p.161, 1999.

SCHWARTZ, R., DAVID, O. K. Liquid chromatography of opium alkaloids, heroin, cocaine and related compounds using electrochemical detection. *Anal. Chem*., v.57, p.1362, 1985.

SKOOG, D. A., HOLLER, F. J. NIEMAN, T. A. Principles of instrumental analysis 5.ed. San Francisco, Harcourt Brace College Publishing, appendix (A-7), 1998.

TAGLIARO, F., TURRINHA, S., PISI, S., SMITH, P., MARIAGO, M. Determination of illicit and/or abused drugs and compounds of forensic interest in biosamples by capillary electrophoretic/electrokinetic methods-Review. *J.Chromatogr.B* v.713, p.27,1998.

MENEZES, Manoel Lima de, et al. Determination by direct injection intro HPLC of cocaine, in urine samples, cocaine and crack samples. *Salusvita*, Bauru, v. 19, n. 2, p. 81-88, 2000.