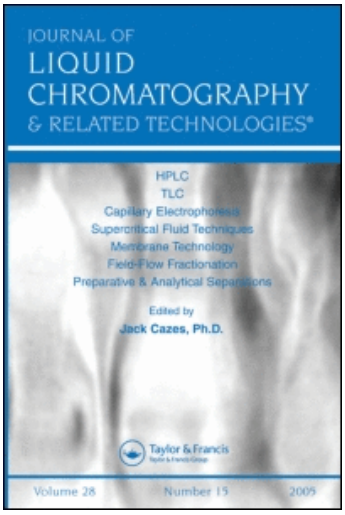

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Determination of Cocaine in Samples of Hair Using the Chromatographic Column, ISRP-C_{18}

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ABSTRACT

A method has been developed for the determination of the cocaine levels in samples of hair, using a chromatographic column internal surface reverse phase (ISRP)-C_{18}, for direct injection of the extracts of hair, without purification or derivation of the sample. The method allows monitoring an individual stopped for using or making cocaine. This method allowed the determination of levels of cocaine concentration in 75% of the analyzed samples of chemical dependents' hair, with cocaine detected at levels of 0.37–16.85 $\mu g\, g^{-1}$. In the other analyzed samples (25%), the drug was not detected, because the corresponding individuals told us that they consumed cocaine infrequently and in small amounts. The detection

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limit determined was 0.003 $\mu g\,L^{-1}$. The method is adequate for the determination of cocaine concentrations present in samples of hair.

Key Words: Cocaine; ISRP-Column; Hair; 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 getting a sensation of well being and to diminish fatigue. The leaves are chewed after addition of 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.

Cocaine is readily biotransformed, mainly by hydrolysis of ester and *N*-dimethylation connections. This process leads to the formation of esther methylecgonine (EME), benzoilecgonine (BEC), ecgonine, and norcocaine. Although, cocaine is readily hydrolyzed to benzoylecgonine, several studies revealed that 0.5 $\mu g\,L^{-1}$ of cocaine can be detected in urine, prior to hydrolysis, after 12 hr of inhalation.[1]

Urine and blood samples are the most common physiological fluids used for the detection of cocaine. Today, hair is recognized as a third fundamental biological specimen for drug testing besides urine and blood. The fields in which hair analysis has, so far, been applied are mainly forensic toxicology and drug abuse studies, followed by clinical toxicology and clinical chemistry. The most important consideration in hair sampling is collecting hair from a preferable anatomical location (posterior vertex) where hairs are relatively uniform.[2]

The preparation of the sample is an important prerequisite to the determination of cocaine by HPLC in biological fluid and hair samples. The extraction is usually made by liquid–liquid partition, with variations of pH in the sample, or by solid phase extraction (SPE). By increasing the sample's 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.[1–5] Separation and determination of cocaine, benzoylecgonine, and norcaine are usually made in C_{18} chromatographic columns, using mobile phases of methanol–acetonitrile–sodium acetate 0.026 $mol\,L^{-1}$, pH 2.2 with 1.29×10^{-4} $mol\,L^{-1}$, tetrabutylammonium

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phosphate (12.5 : 10 : 77.5 (v/v/v),[5] and acetonitrile phosphate buffer 0.02 mol L^{-1}, pH 6.0 (55 : 45 v/v).[6]

Several analytic methods using the technique of direct injection of the sample in internal surface reverse phase (ISRP) columns have been developed and evaluated for the same purpose, such as, extraction and separation of pesticides in raw milk[7] and the determination of caffeine in urine samples by direct injection in HPLC.[8]

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

EXPERIMENTAL

Chemicals and Solvents

Acetonitrile was obtained from Carlo Erba (Milan, Italy), diacid sodium phosphate and perchloric acid (p.a) were purchased from Merck (E. Merck, Darmstadt, Germany). Water was processed in the Milli-Q purification system (Millipore, Beldford, MA). The chair of the Police for Drug Investigation in Bauru donated the cocaine standards. The hair samples were collected from individuals in chemical dependent treatment, Squadron of the Life Clinic from Bauru, SP, Brazil.

Standard solutions containing 0.003 at 0.070 μg L^{-1} of cocaine were prepared by dilution of a mother solution containing 500 mg L^{-1} of cocaine. This solution was previously prepared by dilution of 0.05 g of the cocaine standard in 10 mL of a perchloric acid 0.4 mol L^{-1}.

Samples

A group of 16 chemical dependents, previously in treatment, was selected from the Center of Recuperation Squadron of the Life, in Bauru, SP, Brazil, from which it was possible to collect 16 patient samples during the month of June 2002. The 16 collected samples were stored tightly in plastic packings, shut, and properly labeled with the patient's initials, age, and the month when the cocaine was used.

Methods

Preparation of Standard Solutions of Cocaine and Spiked Hair

Stock standard solutions were prepared by dissolving known amounts of cocaine in 0.4 mol L^{-1} to give solutions containing between 0.003 and

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0.30 μg L^{-1}. To the test tube, was added 0.1 g of the hair sample, spiking cocaine by addition of 5.0, 10.0, and 20.0 μL of the most concentrated solution containing 250 μg mL^{-1} in 2.0 mL acetonitrile. The test tube was maintained at room temperature for 24 hr, to obtain 1.25, 0.5, and 5.0 μg of cocaine in the hair. The hair in the tube was washed with 2.0 mL of acetonitrile, evaporated, and the residue diluted with 1.0 mL perchloric acid 0.4 mol L^{-1}. An aliquot of the 500 μL was injected into an HPLC for determination of cocaine adsorbed in the hair. The evaluation of cocaine extraction was accomplished by transferring 5.0 mL of perchloric acid 0.4 mol L^{-1} and 0.1 g of the hair sample to a test tube, maintaining it closed and under temperature at 60°C, in a water bath for 2 hr. After, 500 μL of the extract were injected into HPLC. The same procedure was made with the control sample.

Construction of the Calibration Curve

The determination of the cocaine masses present in the samples was made using the method of external pattern. The calibration curve was obtained by injected solutions pattern containing 0.0075, 0.015, and 0.03 cocaine μg mL^{-1}, prepared by dilution with acid perclóric. A solution of 0.4 mol L^{-1} of cocaine contains 25.0 mg mL^{-1}. The extracts of the spiked and real samples of hair were injected in the liquid chromatography system.

Instrumentation

The chromatography assay was performed with a Varian model 2510 liquid chromatograph isocratic pump, equipped with a Varian model 2550 variable-wavelength UV-Visible detector set at 235 nm, and a SP 4400 Chromajet integrator system (Varian Associates, Sunnyvale, CA). Samples and standard solutions were injected onto a 250 mm × 2.0 mm ID ISRP-C_{18} column (prepared according to Menezes and Felix)[7] by means of a manual injector (Rheodyne 7125, Cotati, CA) fitted with a 500-μL loop.

Chromatographic Conditions

The mobile phase was 88 : 12 (v/v) 0.025 mol L^{-1} phosphate buffer pH 8.0–acetonitrile, and the flow-rate was 0.80 mL min^{-1}. Separations were conducted at room temperature. The system was equilibrated for 15 min at the initial mobile phase composition before sample injection; the injection volume was 500 μL. Detection was performed at 235 nm.

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Evaluation of Hydrochloric Acid 0.03 mol L^{-1}, Perchloric Acid 0.04 mol L^{-1}, and of Perchloric Acid 0.4 mol L^{-1} in the Recovery of the Cocaine Added to Samples of Non Users' Hair

In the first test, 5.0 mL of hydrochloric acid 0.03 mol L^{-1} was added to the test tubes containing samples of cocaine. These test tubes, tightly shut, were maintained in a water bath at 60°C for 2 hr. The same procedure was made in the following tests, using perchloric acid 0.04 mol L^{-1} and perchloric acid 0.4 mol L^{-1}. The results are in Tables 1–3, respectively.

Preparation of the Real Samples of Hair for Cocaine Determination

The samples of the chemical dependents' hair were pricked off to obtain 0.1 g and those samples were transferred to test tubes, to which 5 mL of perchloric acid, 0.4 mol L^{-1}, was added. The results in the previous tests were described above. These tubes were tightly shut and maintained in a water bath at 60°C, for 120 min. The extracts (500 μL) were injected, without previous treatment, in the liquid chromatography system, equipped with a ISRP-C_{18} column.

Table 1. Recoveries (%) of cocaine from spiked hair samples, employing 0.03 mol L^{-1} chloridric acid.

Amount added (μg)	Determined cocaine (μg)	Recovery (μg)	Recovery (%)	Mean recovery (%) ± (s)	RSD (%)
1.25 a	1.03	0.86	83.5	83.4 ± 2.6	2.2
1.25 b	1.01	0.82	81.2		
1.25 c	1.04	0.89	85.6		
2.50 a	2.37	0.58	24.5	25.3 ± 80.6	15.0
2.50 b	2.37	0.71	29.9		
2.50 c	1.99	0.43	21.6		
5.00 a	4.00	1.88	47.0	43.2 ± 26.5	11.4
5.00 b	4.44	2.32	52.3		
5.00 c	4.02	1.22	30.3		

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Table 2. Recoveries (%) of cocaine from hair samples spiked, employing 0.04 mol L^{-1} perchloric acid.

Amount added (μg)	Determined cocaine (μg)	Recovery (μg)	Recovery (%)	Mean recovery (%) ± (s)	RSD (%)
1.25 a	1.04	0.47	45.2	41.5 ± 10.8	4.5
1.25 b	1.15	0.42	36.5		
1.25 c	0.96	0.41	42.7		
2.50 a	2.10	1.13	53.8	48.7 ± 9.9	4.8
2.50 b	2.37	1.14	48.1		
2.50 c	2.08	0.92	44.2		
5.00 a	4.69	3.59	76.5	76.2 ± 5.9	4.6
5.00 b	4.24	3.03	71.5		
5.00 c	4.74	3.82	80.6		

RESULTS AND DISCUSSION

The method developed allowed pre-concentration, separation, and determination of the cocaine, after injection of the obtained extracts of the samples of chemical dependents' hair, in an ISRP-C_{18} (250 mm × 2.0 mm ID) chromatographic column.

Table 3. Recoveries (%) of cocaine from hair samples spiked, employing 0.4 mol L^{-1} perchloric acid.

Amount added (μg)	Determined cocaine (μg)	Recovery (μg)	Recovery (%)	Mean recovery (%) ± (s)	RSD (%)
1.25 a	0.59	0.62	105.1	101.2 ± 7.36	7.5
1.25 b	1.08	1.00	92.6		
1.25 c	0.84	0.89	105.9		
2.50 a	1.76	2.05	116.5	109.6 ± 5.48	6.0
2.50 b	1.61	1.72	106.8		
2.50 c	1.82	1.92	105.5		
5.00 a	3.63	3.80	104.6	97.2 ± 7.17	6.9
5.00 b	3.94	3.80	96.4		
5.00 c	3.24	2.94	90.7		

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The on-line pre-concentration was accomplished by injecting 500 μL of the sample extract. Considering the fact that the chromatographic column has a small diameter, the aqueous phase of the sample conditions the head of the column, thus moving the organic phase and a constituent, acetronitrile, of the mobile phase, leading to pre-concentration of cocaine at the head of the column.

After the elution of 500 μL of the sample, the mobile phase, comprising an acetronitrile : aqueous solution of sodium phosphate, pH 8 (88 : 12 v/v), completely eluted the cocaine, which was adsorbed by the stationary phase in the ISRP-C_{18} column within 9.07 ± 0.01 min; this can be observed in Fig. 1.

Figure 1 presents the chromatogram obtained after the injection of 500 μL of a standard solution containing $0.015\ \text{mg L}^{-1}$ of cocaine (chromatogram A), and a chromatogram obtained after the injection of 500 μL of the extract of a chemical dependent's hair whose sample is number 2 (chromatogram B). Finally, $5.64\ \mu\text{g g}^{-1}$ of cocaine was found. In chromatogram B, a change is observed in the base line; this is caused by metabolites in the hair extract, but this leads to no interference in the integration of the cocaine peak.

Limit of Detection, Limit of Quantification, and Linearity of the Detector

The detection limit was determined by measuring the minimum amount injected that produced a peak two times the peak to peak noise. The value obtained was $0.003\ \text{g L}^{-1}$ and limit of quantification obtained was $0.006\ \mu\text{g L}^{-1}$ for cocaine, respectively. The response was found to be linear between 0.003 and $0.304\ \mu\text{g L}^{-1}$.

The accuracy indicates the agreement of the experimental values with the true value, and linearity is one of the parameters to be considered.

Parameters of the Analytic Curve

The parameter of the analytical curve regarding the chromatography method, which was developed for the cocaine determination in extracts of hair is expressed in the ($Y = -1943.78 + 392575.29X$) equation curve. The coefficient of correlation (r^2) is higher than 0.99, indicating that linearity between the concentrations (x) and the areas (y), in the intervals used, is satisfactory.

The relationship of x and y in the curve are expressed with r^2, where the expected ideal values are 1 and -1, that is to say, the closer of the unit, the larger the probability of a very defined linear relationship existing.

The linearity of the detector for the cocaine determination was adapted for concentrations in the interval of 0.006 and $0.152\ \mu\text{g L}^{-1}$.

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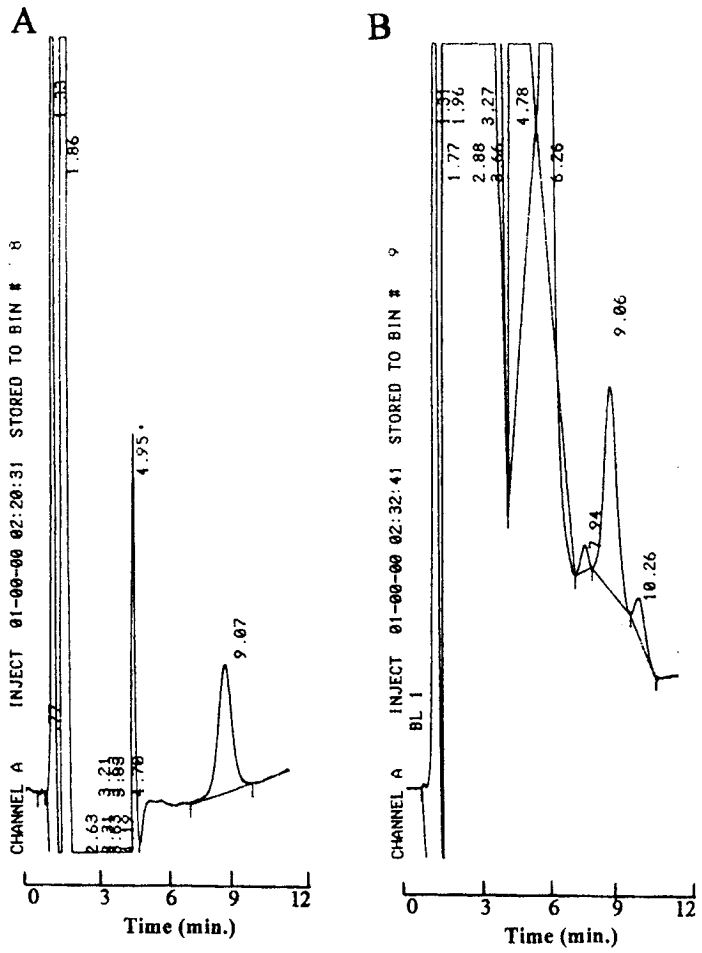

Figure 1. Chromatograms obtained with: (A) the injection of 500 μL of a standard solution containing 0.015 μg L^{-1} cocaine, with retention time of 9.07 ± 0.01 min; (B) the injection of 500 μL by a extract of hair sample of a cocaine dependent, with retention time of 9.06 ± 0.01 min.

Recovery of Added Cocaine in Samples of Non Users' Hair, with Hydrochloric Acid and Two Levels of Perchloric Acid

The main objective, this work was to improve the methods already described in the literature for the extraction of cocaine from hair. It used hydrochloric acid 0.03 mol L^{-1} and perchloric acid 0.04 mol L^{-1} for extraction.

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Tables 1 and 2 show that the use of these acids was not efficient, showing variances of 25.3–83.4% recovery, with RSD varying from 2.2% to 15.0%.

Perchloric acid 0.4 mol L^{-1} was used in the pre-concentration technique. In agreement with the results presented in Table 3, it was observed that the employment of the acid perchloric 0.4 mol L^{-1} in the recovery of the cocaine was satisfactory, obtained recovery percentages varying from 90% to 116% and relative standard deviation from 6.0% to 7.4%.

Application of the Analytical Method

The proposed analytical method was employed for the determination of cocaine in samples of chemical dependents' hair during treatment.

In agreement with the results obtained in Table 4, it is observed that in 75% of the analyzed samples of chemical dependents' hair, cocaine was detected from 0.37 to 16.85 μg g^{-1}. In the other analyzed samples (25%) the drug was not detected, because the corresponding users said that they consumed cocaine infrequently and in small amounts.

Table 4. Determination of cocaine from real chemical dependents hair samples.

Sample number	Chemical dependent age	(2002) Month used	Cocaine determined (μg)
1	22	April	nd
2	20	April	5.64
3	20	April	nd
4	17	April	2.30
5	22	January	nd
6	16	June	1.05
7	17	June	nd
8	16	April	3.84
9	18	April	0.37
10	20	April	0.65
11	25	April	16.85
12	20	February	0.60
13	18	April	0.54
14	16	March	2.13
15	22	June	13.65
16	17	June	2.54

Note: nd, Not determined.

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CONCLUSION

Evaluation of the ISRP column, for the separation and determination of cocaine in hair samples, has shown that the column is suitable for efficient quantification and precise analysis. The 0.4 mol L^{-1} perchloric acid for extraction of cocaine from hair samples was very efficient. The certain cocaine levels in the analyzed samples, indicates that the individuals are indeed users of drugs.

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