Journal of Chromatography, 152 (1978) 105–113

CHROM. 10,624

DETERMINATION OF COCAINE IN HUMAN URINE, PLASMA AND RED BLOOD CELLS BY GAS–LIQUID CHROMATOGRAPHY

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(Received September 12th, 1977)

SUMMARY

This paper describes a sensitive and reliable method for the determination of cocaine in human urine, plasma and red blood cells. Cocaine is extracted into cyclohexane from the biological materials at slightly alkaline pH, reduced with lithium aluminium hydride, acylated with pentafluoropropionic anhydride and detected by an electron capture detector. When compared with a gas chromatography–mass spectrometry method the results of cocaine determination correlated highly ($r = 0.986$). When cocaine was given intravenously to volunteer subjects only 0.2–1.4% of the administered dose was excreted as unmetabolized cocaine in the first 9 h after administration. Plasma and red blood cell levels of cocaine were also determined by this method after intravenous administration.

INTRODUCTION

Cocaine is a short-acting central nervous stimulant, the abuse of which has increased in recent years. This has prompted several investigators to develop new and more sensitive methods for the determination of cocaine and its metabolites in biological materials. Fish and Wilson[1] studied the urinary excretion of cocaine in humans using a gas chromatographic method for the determination of cocaine. Wallace *et al.*[2] have reported a method for the determination of cocaine and benzoylecgonine in urine after intranasal application of cocaine. Jatlow and Bailey[3] have described a gas chromatographic method for the determination of cocaine in human plasma using a nitrogen detection system. However, each method has been used for cocaine determination in either urine or plasma but not for both or for other biological materials.

We have described a gas chromatographic method for the determination of cocaine, benzoylecgonine and ecgonine using an electron capture detector[4]. The method was extended to determine the cocaine in urine, plasma and red blood cells (RBC) of the volunteer subjects who were given different doses of cocaine intra-

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venously. This paper reports the details for the determination of cocaine in human urine, plasma and RBC.

MATERIALS AND METHODS

All reagents used were of analytical grade. Nanograde cyclohexane and nanograde diethyl ether were obtained from Mallinkrodt (St. Louis, Mo., U.S.A.). Lithium aluminum hydride ($LiAlH_4$) and pentafluoropropionic anhydride (PFPA) were purchased from Chemical Procurement (College Point, N.Y., U.S.A.). ^{3}H-Cocaine (*levo*-(−)benzoyl-3,4-^{3}H; specific act. 35.2 Ci/mole) was obtained from New England Nuclear (Boston, Mass., U.S.A.).

Cocaine determination

Cocaine was determined by the method of Javaid *et al.*[4]. Briefly, 25–250 ng of cocaine standard in 0.5 ml of cyclohexane were reduced with 25 μl of $LiAlH_4$ suspension in ether (5 mg/ml). Eexcess $LiAlH_4$ was hydrolyzed by the addition of 25 μl of water and the reduced cocaine in the same tube was derivatized by the addition of 25 μl of PFPA. One ml of saturated sodium borate solution was added to this mixture. After shaking, the cyclohexane layer was removed and 1 μl injected into the Packard gas chromatograph equipped with 150 mC-^{3}H as the electron source. The chromatographic conditions were: column: 10 ft. coiled column packed with 5% OV-225 on Gas-Chrom Q (100–120 mesh) (Supelco, Bellefonte, Pa., U.S.A.); injection port temperature: 150°; column temperature: 110°; detector temperature: 150°.

Cocaine extraction

Cocaine·HCl, in concentrations ranging from 10 to 1000 ng/ml as free base, was added to normal human urine. One ml of saturated sodium borate solution was added to 5 ml of the urine sample. This resulted in a pH of 8.9 for the sample. The sample was extracted with 1 ml of cyclohexane by shaking for 5 min. After centrifugation the cyclohexane layer was separated and 0.5 ml was reduced and derivatized as described above.

Extraction conditions for plasma and RBS were established by adding cocaine·HCl (10–1000 ng/ml as free base) to plasma and RBC separated from outdated and freshly drawn blood samples. To 1 ml of plasma sample 0.5 ml of saturated sodium borate solution was added (except in experiments where the effect of pH on extraction of cocaine was studied). The plasma pH after the addition of borate solution was 9.2. Plasma was then extracted twice with 1 ml of cyclohexane each time, as above. The cyclohexane layers were combined and extracted with 2 ml of 0.1 *N* H_2SO_4. The cyclohexane layer was discarded and solid sodium carbonate (*ca.* 2 mg) was carefully added to the acid layer. After mixing, the pH of the solution was 9.8. This was then extracted twice, with 0.5 ml cyclohexane each time and the combined cyclohexane layers were dried under nitrogen. To this, 0.3–0.5 ml of cyclohexane were added and the samples were reduced and derivatized as above.

The extraction procedure from RBC was similar to that of plasma except the following two steps were added. One ml of water was added to 1 ml of RBC before the addition of borate solution. This reduced the interphase between RBC and cyclo-

hexane during extraction. Also, the acid layer was washed with 1 ml of cyclohexane once more after discarding the first cyclohexane layer.

Recoveries of cocaine were also calculated by adding ^{3}H-cocaine (0.2–1.0 μCi per 10-sample) to the samples. After extraction, the radioactivity in the aliquots of the organic phase was determined by a liquid scintillation counter (Nuclear-Chicago, Des Plaines, Ill., U.S.A.).

Cocaine administration and sample collection

Cocaine was administered intravenously[5] and blood samples were collected and transferred to heparanized tubes containing 2.5 mg/ml NaF. The samples were mixed and centrifuged to separate the plasma and RBC. Cocaine was extracted and determined as described before.

Three urine samples from subjects who received cocaine intravenously were collected over a period of 9 h as described elsewhere[6]. Sodium metabisulphite (0.5 mg/ml) was added to urine as a preservative and the samples stored at −16° until analysis.

RESULTS

Urine

Recoveries of cocaine from urine ranged between 95 and 102% by the extraction procedure described above. A typical chromatogram obtained from urinary cocaine determination is shown in Fig. 1C. Effect of temperature on the stability of cocaine in urine *in vitro* is shown in Table I. In 8 h 40% of cocaine was lost at 37°, whereas 80% was lost after 20 h.

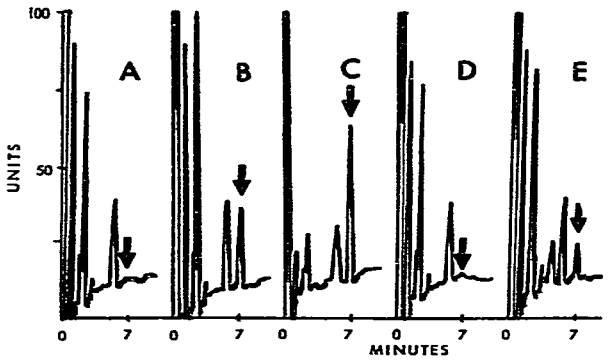

Fig. 1. A typical chromatogram of cocaine determination in human urine and plasma. Cocaine samples from urine (5 ml) and plasma (1 ml) were extracted and derivatized as described in Methods. One μl of sample was injected. Arrows indicate the retention time of cocaine. A, Reagent blank; B, cocaine standard; C, urine sample collected in first 3 hours after i.v. administration of 16 mg of cocaine. The sample was diluted 1:20 (v/v) before injection in the chromatograph; D, plasma blank; E, plasma sample, 5 min after administration of 16 mg of cocaine.

The results of urinary excretion of cocaine from three different subjects after intravenous (i.v.) administration are shown in Table II. The total urinary excretion over a 9 h period ranged from 0.2 to 1.4% of the administered cocaine. There was no correlation between the dose administered and urinary excretion of cocaine over the

TABLE I

STABILITY OF COCAINE IN URINE *IN VITRO*

Normal human urine was supplemented with cocaine. Aliquots of 5 ml in duplicate were analyzed for cocaine by the method described here.

Hours	*μg Cocaine/l urine at*		*Percent loss at*
	4°	*37°*	*37°*
0	100	100	—
1	—	80	20
4	93	82	18
8	94	60	40
20	86	20	80

9 h period. Fish and Wilson[1] have reported that the excretion of cocaine increased with lower urinary pH, whereas at higher urinary pH the excretion decreased. In the present studies there was no attempt made to control the diet of these subjects and the differences may be due to the differences in urinary pH. Furthermore, since the samples were stored for a long period of time before analysis, there may have been non-specific hydrolysis of cocaine even when frozen. Recently Jatlow and Bailey[3] have reported up to a 20% loss of cocaine in plasma even when stored frozen and we observed a 14% loss of cocaine in urine in 20 h at 4°.

TABLE II

URINARY EXCRETION OF COCAINE AFTER INTRAVENOUS INJECTION*

Averages of three subjects.

Dose (mg)	*μg cocaine** excreted in*			*Total 9-hour excretion (μg)*	*Percent of the dose*
	First three hours	*Second three hours*	*Third three hours*		
4	36.9	13.8	5.6	56.3	1.4
8	30.4	7.3	5.4	43.1	0.5
12	30.4	11.4	3.2	45.0	0.4
16	33.0	88.7	19.6	141.3	0.9
24	11	13.2	17.1	41.3	0.2

* Subjects voided before the cocaine administration. After intravenous injection of cocaine each three-hour urine was collected separately and volume recorded.

** Cocaine in 5-ml aliquots, in duplicate, was determined by the method described here.

Plasma

Fig. 1E shows a typical chromatogram obtained from cocaine determination in plasma. Recoveries of cocaine from plasma by the above extraction procedure ranged from 65 to 80%. The range of cocaine recoveries was greater (65–85%) when plasma from different individuals was used, whereas when the same plasma was used to determine the recoveries, day to day variation was small. Hence, when the samples from different individuals were analyzed, the blood samples withdrawn before the administration of cocaine were used for blanks and recoveries.

When different solvent systems were used to determine the recoveries by

counting radioactivity the recoveries with cyclohexane, benzene and heptane–isoamyl alcohol (98:2, v/v) were 65, 72 and 78%, respectively. Changing the pH between 7.4 to 9.5 did not affect the recoveries. Although extraction with heptane–isoamyl alcohol was slightly better than cyclohexane, in the present studies the latter solvent was used for extraction, because heptane–isoamyl interfered with the $LiAlH_4$ reduction step.

The reliability of the method over a wide range of concentrations was checked by determining cocaine in human plasma supplemented with cocaine. Each sample was analysed in quadruple. These results are given in Table III. The method shows reliability and good reproducibility over a wide range of concentrations. In another set of experiments, the plasma samples supplemented with cocaine were analyzed by the method described above and compared with a flame ionization detector–gas chromatographic method and a gas chromatography–mass spectrometry method[7]. These results are shown in Table IV. The correlation coefficient between the three methods was 0.986.

TABLE III

DETERMINATION OF COCAINE IN PLASMA SAMPLES SUPPLEMENTED WITH COCAINE

Normal human plasma was supplemented with different amounts of cocaine. One-ml aliquots of plasma were used to determine the cocaine by the method described in this paper.

Sample	*No. of detns.*	*ng/ml*	$\bar{x}$	*S.E.M.*
F	1	9,953	9,971	±255
	2	10,368		
	3	9,257		
	4	10,305		
W	1	2,173	2,141	±35
	2	2,173		
	3	2,037		
	4	2,183		
P	1	235	223	±6
	2	235		
	3	207		
	4	227		
	5	210		
M	1	194	199	±8
	2	207		
	3	221		
	4	198		
	5	175		
H	1	95	103	±13
	2	82		
	3	95		
	4	140		

Stability of cocaine in plasma

As mentioned earlier, cocaine is hydrolysed to benzoylecgonine and finally to ecgonine. Pseudocholine esterase catalyses this reaction[3]. To establish the stability of cocaine in plasma *in vitro* cocaine was added to plasma and the sample kept frozen

TABLE IV

DETERMINATION OF COCAINE IN PLASMA BY THREE DIFFERENT METHODS

EC–GC is the method for the determination of cocaine described in this report. FID–GC and GC–MS method for the determination of cocaine were that of Lin *et al.*[7].

Sample	*μg/ml plasma*		
	*EC–GC**	*FID–GC**	*GC–MS**
A	8.4	8.1	7.9
B	5.4	4.2	4.2
C	2.7	2.2	2.1
D	2.1	2.2	2.0
E	0.57	0.39	0.4

* Correlation coefficient = 0.986.

for a period of time. Just before the determination, the sample was thawed and an aliquot taken for cocaine analysis. The rest of the plasma was frozen again until the next determination. When the cocaine level was determined after the first 5 days of freezing, there was a 40% loss and over a period of 1 month, more than 60% of the cocaine was lost. Even when the cocaine plasma was kept in the cold room at 4°, more than 20% of the cocaine was lost in 1 h (Table IV). At room temperature the loss in 1 h was close to 45%. When NaF (2.5 mg/ml plasma) was added to the plasma containing cocaine, there was no appreciable loss in cocaine over a 2-h period (Table V).

TABLE V

STABILITY OF COCAINE IN PLASMA *IN VITRO*

Normal human plasma was supplemented with cocaine to give a final concentration of 450 ng/ml. To two flasks NaF (2.5 mg per millilitre plasma) was also added. One-ml plasma aliquots, in duplicate, from each flask were taken at different times for the determination of cocaine by the method described in this report.

Time (min)	*ng cocaine/ml plasma*			
	4°		*25°*	
	−NaF	*+NaF*	*−NaF*	*+NaF*
0	442	447	444	443
15	442	456	449	445
30	389	455	410	433
60	357	437	276	429
120	357	457	196	400
240	361	456	217	369

Red blood cells

The recoveries of cocaine ranged from 60 to 70% by the extraction procedure described above. The addition of equal amounts of water improved the extraction efficiency by breaking the cells and reducing the interphase when the samples were extracted with cyclohexane. Extra washing of the acid layer with cyclohexane improved the chromatogram by reducing the number of extraneous peaks. A typical chromatogram of cocaine determination in RBC is shown in Fig. 2C.

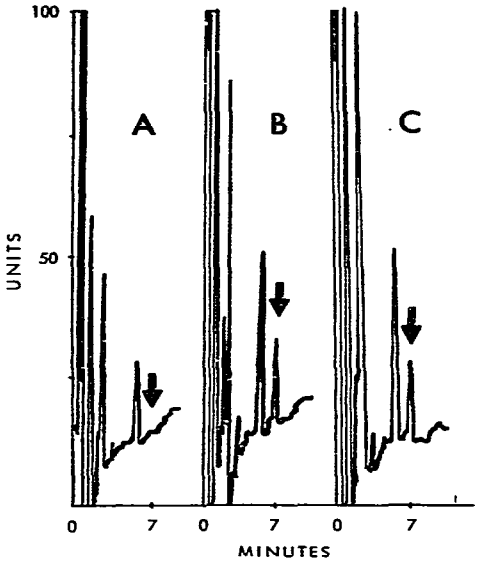

Fig. 2. A typical chromatogram of cocaine determination in human red blood cells. A, RBC blank; B, RBC supplemented with cocaine standard; C, RBC, 5 min after i.v. administration of 16 mg of cocaine.

Determination of cocaine in human blood

Cocaine was determined in the plasma and RBC by the method described above, after different doses of i.v. and intranasal administration of cocaine. Table VI shows the results of 16 mg i.v. administration of cocaine in one of the subjects (J.D.). Plasma cocaine levels of 184 ng/ml were found 5 min after i.v. injection. Two hours after cocaine administration, the plasma levels were 16 ng/ml. The half-life $T_{\frac{1}{2}}$ of cocaine disappearance in this subject was calculated to be 47 min. There were interindividual variations in the plasma levels and $T_{\frac{1}{2}}$ of cocaine disappearance. These results will be discussed separately.

RBC cocaine levels of 333 ng/ml were found 5 min after i.v. injection. The

TABLE VI

COCAINE IN PLASMA AND RBC AFTER 16-mg INTRAVENOUS ADMINISTRATION

Time (min) after i.v. injection	*ng cocaine*/ml*	
	Plasma	*RBC*
5	184	333
10	102	185
30	98	167
60	33	146
90	28	107
120	16	26

* Cocaine was determined by the method described in this report. These values are the averages of two determinations.

amount of cocaine per ml of RBC in the subject reported here was higher at each time point than that of plasma. Although Fish and Wilson[1] reported a RBC to plasma cocaine ratio of 1.2 after intramuscular (i.m.) administration, there were no cocaine levels given in that study. We found that in some individuals the RBC to plasma cocaine ratio was less than one.

DISCUSSION

Although there are several reports in the literature on the study of cocaine, general pharmacodynamics in humans have not been studied. One of the reasons for this has been the lack of specific and sensitive methods for the determination of cocaine and its metabolites in biological materials. The present report describes a reliable and sensitive method for the determination of cocaine in human urine, plasma and RBC. Cocaine after extraction at slightly alkaline pH is reduced to 2-hydroxymethyltropine and acylated by PFPA and detected by gas chromatography using an electron capture detector with greatly increased sensitivity.

The recoveries of cocaine from urine, plasma and RBC were the same at a pH range of 6.5 to 9.5. Above pH 10 the recoveries were greatly reduced due to the chemical hydrolysis of cocaine to benzoylecgonine. Wallace *et al.*[2] found that extraction of cocaine from urine between pH 5.5 and 9 ranged from 94 to 100% recovery. At pH 5 and 11.5 the recovery of cocaine from urine was 50 and 63%, respectively. Similarly, Woods *et al.*[8] were able to extract cocaine completely above pH 6.

A range of doses of i.v. cocaine was administered to volunteer subjects, and its excretion in urine was studied by the method described above. Free cocaine in the urine ranged from 0.2 to 1.4% of the dose administered. There was no relation between the dose of cocaine administered and the amount excreted as free cocaine in the urine. This is not very surprising because Fish and Wilson[1] have shown that amounts of cocaine excreted depended upon the urinary pH. We also found that when cocaine was incubated *in vitro* in normal urine at 37°, 20% was lost in the first hour from non-specific hydrolysis. This loss was much higher when the urine pH was higher than 9.5. This would suggest that varying amounts of non-specific hydrolysis in urine in the bladder prior to voiding could contribute to different amounts of cocaine excreted. In the present studies there was no attempt made to control the food intake of the volunteer subjects. Fish and Wilson[1] found that the amount of cocaine excreted in the urine varied between 1 and 9% after 120 mg of i.m. injection. Wallace *et al.*[2] found free cocaine in the range of 0–3 μg/ml urine over the first 8 h after the topical application to nasal mucosa of 250 mg of cocaine.

The recoveries of plasma ranged from 65 to 85%, although when plasma from the same subjects was used for the determination of recoveries, the variations were small. The interindividual variation in recoveries probably was due to the differences in the amounts of lipids because some of the plasma samples resulted in a large interphase between organic and aqueous phase.

One of the problems with cocaine determination in biological materials is that it is hydrolyzed even when stored at −16°. We found that when plasma samples containing cocaine were stored over a period of 1 month there was 60% loss. Price[9] reported that after 2 months of storage at 4° more than 90% of the cocaine was lost

in spleen and liver of a man who died of cocaine poisoning. Jatlow and Bailey[3] suggested that the loss in plasma cocaine was due to the hydrolysis by pseudocholine esterase since the loss could be prevented by NaF or physostigmine. We have similarly observed that in the presence of NaF (2.5 mg per millilitre plasma), the loss in plasma cocaine at 4° was negligible over a period of 2 h.

The recovery of cocaine from RBC varied between 60 and 70%. Recoveries were always better when the cells were first lysed with water before extraction. The method described here has been used to study the pharmacokinetics of cocaine in humans after i.v. and intranasal application in 12 volunteer subjects. The plasma levels varied from 86 to 309 ng/ml of plasma 5 min after 16 mg i.v. administration of cocaine. These results will be presented in a separate communication.

ACKNOWLEDGEMENTS

The authors thank Judith Balcer for her excellent technical assistance. Appreciation is also expressed to Drs. Lin and Narasimhachari for analysing the cocaine samples by mass spectrometry. Help of Barbara Duslak for tracing the chromatograms is also appreciated. This research was supported, in part, by National Institute on Drug Abuse contract No. ADM-45-74-165.

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