Journal of Chromatography, 248 (1982) 318–320
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 15,150

Note

Study of the stability of cocaine and benzoylecgonine, its major metabolite, in blood samples

YAO LIU*, ROBERT D. BUDD* and ERNEST C. GRIESEMER
Los Angeles County Medical Examiner-Coroner, 1104 Mission Road, Los Angeles, CA 90033 (U.S.A.)
(Received June 30th, 1982)

Cocaine is a popular drug of abuse and its frequency of use in the Los Angeles area is growing each year (Table I)[1,2]. Thus, it is often necessary for toxicology laboratories to analyze for its presence in biological fluids, particularly blood.

Cocaine is extensively metabolized to benzoylecgonine in the body and this metabolite presents many analysis difficulties since it extracts poorly and cannot be analyzed by gas chromatography without derivatization.

It is also known that under basic conditions and heat cocaine breaks down into benzoylecgonine outside the body[3,4].

Since there may be a time delay before an obtained blood sample is analyzed for cocaine and since reanalysis may be requested at a much later date, the question of the stability of cocaine in refrigerated blood samples (pH $\approx$ 7.4) has arisen. This study has been done to determine the stability of cocaine and benzoylecgonine in stored, refrigerated blood samples.

TABLE I

LOS ANGELES COUNTY COCAINE ABUSE TRENDS

	1974	*1975*	*1976*	*1977*	*1978*	*1979*	*1980*
Findings in 10,000 probation samples	—	72	23	54	115	158	163
Findings in coroner's drug overdose death cases	1	7	11	10	11	16	23
Findings in coroner's drug related death cases	0	0	0	3	6	5	9

EXPERIMENTAL

Sample preparation and storage

A blood standard containing 0.100 mg% cocaine was prepared from blood

* Permanent address: Forensic Sciences Institute, Ministry of Public Security, Beijing, China.

0021-9673/82/0000–0000/$02.75 © 1982 Elsevier Scientific Publishing Company

TABLE II

COCAINE DEGRADATION

S.D. = Standard deviation; C.V. = coefficient of variation; $n = 7$.

Day analyzed	*Cocaine concentration in blood (mg%)*	*S.D.*	*C.V. (%)*	*Benzoylecgonine concentration in blood (mg%)*	*S.D.*	*C.V. (%)*	*Total cocaine and benzoylecgonine concentration (mg%)*
0	0.100	–	–	0	–	–	0.100
1	0.093	0.0018	1.9	0.009	0.0013	14.2	0.102
7	0.084	0.0023	2.7	0.013	0.0005	4.1	0.097
14	0.083	0.0028	3.4	0.016	0.0007	4.5	0.099
36	0.070	0.0030	5.2	0.027	0.0017	6.2	0.097

previously tested cocaine and benzoylecgonine negative. This blood standard was stored in a regrigerator kept at 16°C throughout the testing period.

Method of analysis

Cocaine was analyzed by a modified method based on that of Wallace *et al.*[5]. The cocaine and benzoylecgonine (cocaine metabolite) were extracted from blood samples with chloroform–ethanol (4:1) which was then separated and evaporated to dryness. The benzoylecgonine was then ethylated to cocaethylene by reacting the residue with ethanol–sulfuric acid (2:1) at 85°C. The solution was then washed with diethyl ether, made basic with sodium carbonate solution, and extracted with chloroform. An aliquot of the final extract was injected into a gas chromatograph–mass spectrometer equipped with a 3% OV-1 column and run at 230°C. Calculations were based on peak areas, an SKF-525 (*p*-diethylaminoethyldiphenylpropylacetate) internal standard, and a cocaine–benzoylecgonine quality control worked up simultaneously with the blood sample being tested.

RESULTS AND DISCUSSION

The stability of cocaine in a stored, refrigerated blood sample has been studied. The results (illustrated in Table II) show several interesting features: (1) Cocaine degradation occurred in the stored, refrigerated blood sample —a 30% loss occurred after only 36 days, and substantial losses of 7% occurred after only 1 day; (2) Once degradation to benzoylecgonine had occurred, further degradation did not occur. This is demonstrated by the fact that the total concentration of cocaine and benzoylecgonine remained virtually constant over the time period of the study.

These results have importance for toxicology laboratories —blood cocaine concentrations will decrease with passing time. Thus, if sufficient time passes, a blood specimen originally positive for cocaine may yield a negative result at a later time. Best procedures are those that detect both cocaine and benzoylecgonine since the total concentration remains constant.

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