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GAS CHROMATOGRAPHIC DETERMINATION OF COCAINE IN WHOLE BLOOD AND PLASMA USING A NITROGEN-SENSITIVE FLAME IONIZATION DETECTOR

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SUMMARY

A procedure is described for the determination of as little as 20 ng of cocaine from 1 ml of whole blood or plasma. Methods are also given for the storage of whole blood or plasma containing cocaine as well as for whole blood or plasma extracts. Blood levels in patients receiving intranasal cocaine for topical anesthesia while undergoing rhinoplasty are also presented.

INTRODUCTION

Cocaine, in addition to being a major drug of abuse, is also used as a local anesthetic by otolaryngologists and plastic surgeons[1,2]. Despite its wide use, little data is available on blood levels of cocaine in humans following its administration. This lack of data is due to the fact that until recently sensitive techniques were not available for the measurement of cocaine in blood or plasma. The colorimetric method of Woods *et al.*[3] was for many years the only procedure available for measuring cocaine in biological fluids and tissues. In 1975, Medzihradsky and Dahlstrom[4] published a gas–liquid chromatographic (GLC) method for the measurement of cocaine in plasma. Both these techniques however lack the sensitivity required to measure cocaine levels in blood or plasma following its topical application to mucous membranes. Because of this problem, reports on cocaine usage in man have been confined to measurements of cocaine and/or its major metabolite, benzoylecognine, in urine[5–7]. Jatlow and Bailey[8] published a GLC method for the measurement of cocaine in human plasma following topical application to the nasal mucosa. According to their procedure plasma samples had to be extracted immediately and the dried extracts were stable for 72 h only if stored in a desiccator at −15°. In this paper we describe a

specific GLC method for the determination of cocaine in whole blood or plasma as well as methods for storage of the biological fluid or its extract.

MATERIALS AND METHODS

Gas–liquid chromatography

A Hewlett-Packard Model 7620A gas chromatograph equipped with a nitrogen-sensitive flame ionization detector (NFID) and a Spectrum 10121 Filter and Amplifier (Spectrum Scientific, Valley Forge, U.S.A.) were used. The columns were coiled glass tubes, 3 ft. × 2 mm I.D. and packed with 100–120 mesh Chromosorb W AW DMCS (Supelcoport; Supelco, Bellefonte, Pa., U.S.A.) coated with 3% SP-2100-DB (Supelco, 01-1877). The column was pre-conditioned at 250° for 24 h with a helium flow-rate of about 20 ml/min. The operating conditions were: injection port, 260°; detector, 320°; air flow-rate, 185 ml/min; hydrogenflow-rate,25 ml/min; helium flow-rate, 45 ml/min; auxiliary gas (helium) flow-rate, 13 ml/min. The column temperature was programmed at 30°/min starting at 200° to a final level of 230°. The oven was held at this temperature for 3 min before cooling back to 200°.

Tuning of detector

To achieve optimum performance, the detector should be tuned daily. This was achieved by rotating the adjusting nut clockwise until the distance between the collector, which contains the rubidium bromide crystal, and the flame was greatest. The adjusting nut was then rotated counterclockwise until maximum pen deflection (MPD), corresponding to maximum background current, was observed. This was usually about 50–80% full scale deflection at range 10^2 and attenuation 64. When the MPD was less than 20% full-scale deflection the detector was removed and the crystal cleaned with a cotton swab moistened with distilled water.

Reagents

Benzene, distilled in glass (Burdick & Jackson, Muskegon, Mich., U.S.A.) and acetone, 99% molecular pure (Fisher Scientific, Fair Lawn, M.J., U.S.A.) were used without re-distillation. Isopropyl alcohol, AR grade, was re-distilled in glass and stored in a glass-stoppered amber reagent bottle. Carbonate buffer was prepared by the addition of 21 g Na_2CO_3 plus 0.42 g $NaHCO_3$ to 250 ml distilled water. The volume was brought up to 500 ml with distilled water and adjusted to pH 11–11.5 by the addition of 1.0 *N* NaOH. All other reagents were of analytical grade. Ethylmorphine·HCl (Merck, Rahway, N.J., U.S.A.) was utilized as the internal standard.

Standard solutions

Standard stock solutions of cocaine and ethylmorphine containing 1 mg/ml of the respective HCl salts were prepared in ethanol. These solutions were stored at 5° without notable decomposition. Working cocaine standards were prepared by various dilutions of the stock solution. Standard solutions in whole blood or plasma were prepared by adding 20 μl of the appropriate working cocaine standard to 1 ml of blood or plasma which were on ice.

Storage of samples

Samples of whole blood collected in heparinized glass syringes were immediately placed on ice. Aliquots (1 or 2 ml) of whole blood or plasma were transferred to 50-ml round-bottom glass centrifuge tubes and immediately quick-frozen by immersion into liquid nitrogen while shaking the tubes. The frozen samples were stored at −15°. Samples frozen and stored in this manner are stable for at least 6 weeks.

Extraction

The frozen samples were thawed by immersion into a 60° water bath for 10 sec and immediately iced. Twenty microliters of ethylmorphine standard (75 ng/μl in ethanol), 2 ml of carbonate buffer and 10 ml benzene–isopropanol (9:1) were added and the samples extracted by shaking for 10 min at 2,500 oscillations per min. The phases were separated by centrifugation for 5 min at 1300 *g*. The clear upper solvent phase was transferred to clean 50-ml round-bottom glass centrifuge tubes, 8 ml 0.01 *N* HCl added and the samples shaken for 3 min at 2500 oscillations per min followed by centrifugation for 10 min at 1300 *g*. The clear upper layer was aspirated and discarded. Care was taken to remove as little of any emulsion phase which formed in some samples. The aqueous phase was adjusted to pH 7–7.3 by the addition of 200 μl 0.5 *N* NaOH and 1.0 ml 0.01 *M* HEPES buffer (pH 7.4). At this point the extraction procedure may cease and the samples stored at room temperature for at least 5 days.

Extraction was completed by addition to the aqueous phase of 100 μl 10 *N* NaOH and 8 ml benzene–isopropanol (9:1). The samples were shaken for 5 min at 2500 oscillations per min and centrifuged for 5 min at 1300 *g*. The clear upper solvent phase was transferred to concentratubes (Laboratory Research Co., Los Angeles, Calif., U.S.A.) and evaporated to dryness under nitrogen in a 60° water bath. The residue was dissolved in 20 μl acetone (99% pure) and 2–3 μl injected into the chromatograph.

RESULTS AND DISCUSSION

Typical chromatographs of whole blood extracts from a patient before and 90 min after topical application of cocaine are shown in Fig. 1. The peak eluting between cocaine and ethylmorphine is apparently due to a contaminant in the solvent. Under our laboratory conditions the retention times for cocaine and ethylmorphine were 100–120 sec and 170–200 sec, respectively.

A series of standard solutions of cocaine containing the internal standard were prepared in ethanol and in whole blood. Standard curves were prepared by injecting 2 μl of ethanol standard solutions (or extract) into the gas chromatograph and plotting the ratio of the peak height of cocaine to that of ethylmorphine against the concentration of cocaine. The curves were linear over the range of 20–640 mg cocaine per ml whole blood (Fig. 2). Assuming that the extraction of ethylmorphine from blood is similar to that of cocaine, one may estimate the percent recovery of cocaine by comparing the slopes of the two curves. Analysis of the data in Fig. 2 indicates that the mean analytical recovery was 65%.

Studies by Van Dyke *et al.*[9] suggest that cocaine is hydrolyzed, at least in part, by plasma cholinesterases. The rate of enzymatic hydrolysis of cocaine appears to be

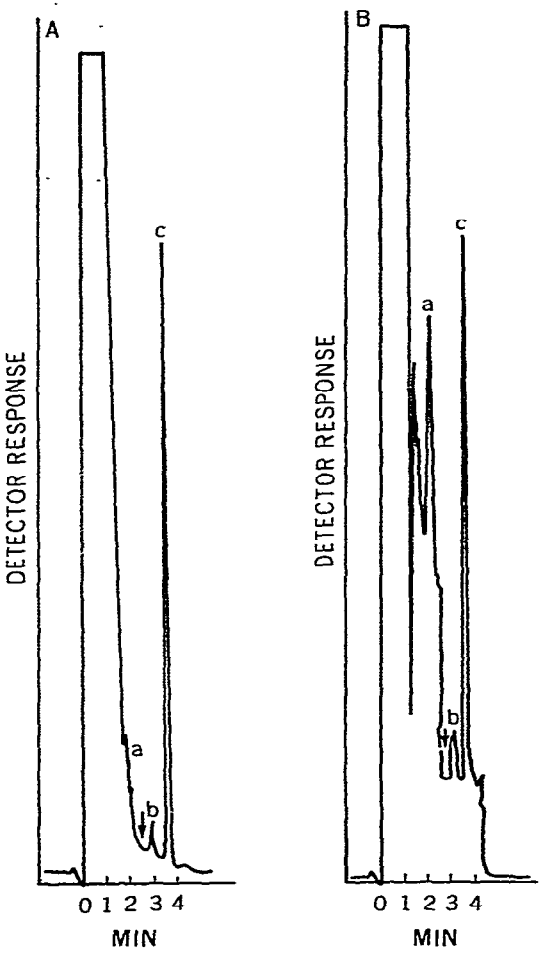

Fig. 1. Chromatograms of an extract of whole blood before (A) and 90 min after intranasal administration of cocaine (B). Attenuation, 8; range, 10^2. Arrow signifies attenuation changed to 32. a = Cocaine; b = contaminant; c = ethylmorphine.

slowed if the freshly collected samples are immediately iced. Studies in our laboratory indicate that freshly collected blood or plasma samples may be stored on ice for at least 1 h without any detectable loss of cocaine.

In many cases it is usually desirable to collect and store the blood samples for analysis at a later time. Simple freezing of whole blood or plasma at −15° does not appear to inhibit totally the enzymatic hydrolysis of cocaine[7]. One solution is to add a plasma cholinesterase inhibitor to the samples[8]. We have found that if one quick-freezes the sample in liquid nitrogen, while shaking the tubes, storage at −15° is possible for at least 6 weeks without any apparent loss of cocaine (Table I).

Another problem with respect to cocaine is the storage of whole blood or plasma extracts. Jatlow and Bailey[8] reported that plasma extracts were stable for at least 72 h if stored in a desiccator at −15°. Fig. 3 depects our results following ex-

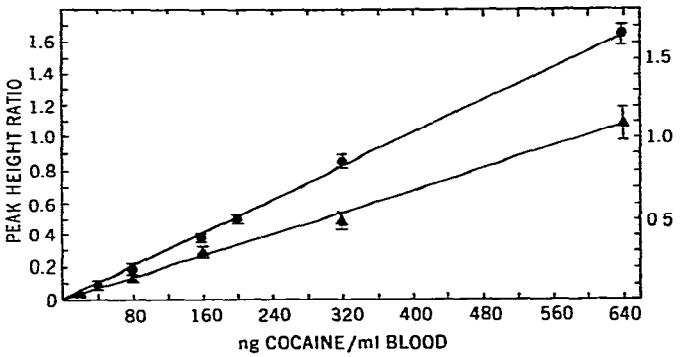

Fig. 2. Calibration curve for cocaine. Each point is the mean ± S.D. of four determinations done on four different days. Ethanol standards (●) were prepared assuming 100% recovery of cocaine and ethylmorphine from whole blood. ▲ = Blood extracts.

traction and storage of plasma extracts of cocaine at −15°. One should note that these samples were not stored in a desiccator. Since all these samples were spiked and extracted at the same time the decrease in the concentration of cocaine, as judged by the decrease in the slope of the calibration curves, cannot be due to enzymatic hydrolysis but may be due to an non-enzymatic acid or base catalyzed reaction.

The data in Table I presents changes which occurred when whole blood spiked with cocaine was taken through the extraction procedure up to and including the acid wash followed by adjustment to pH 7.2. Analysis of the data indicates that under these conditions the aqueous extracts could be stored for at least 5 days at room temperature without any loss of cocaine.

TABLE I

SLOPE OF THE CALIBRATION CURVE RELATING PEAK HEIGHT RATIO TO CONCENTRATION OF COCAINE IN WHOLE BLOOD AFTER STORAGE OF SAMPLE UNDER DIFFERENT CONDITIONS

— = Not determined.

Length of storage (days)	*Slope ($\cdot 10^3$)*	
	*Frozen**	*Aqueous wash****
0	1.71 ± 0.08***	1.71 ± 0.08***
1	1.62	1.64
2	1.80	1.58
3	—	1.59
5	1.59	1.86
10	1.80	—
17	1.65	—
44	1.68	—

* Samples containing various concentrations of cocaine were quick-frozen in liquid nitrogen and stored at −15° until analysis. Each value is the mean slope from two calibration curves.

** Samples containing various concentrations of cocaine were extracted through the acid wash. The aqueous acid wash was adjusted to pH 7.2 and stored at room temperature until analysis. Each value is the mean slope from two calibration curves.

*** Slope ± standard deviation were obtained from 4 determinations done on 4 different days.

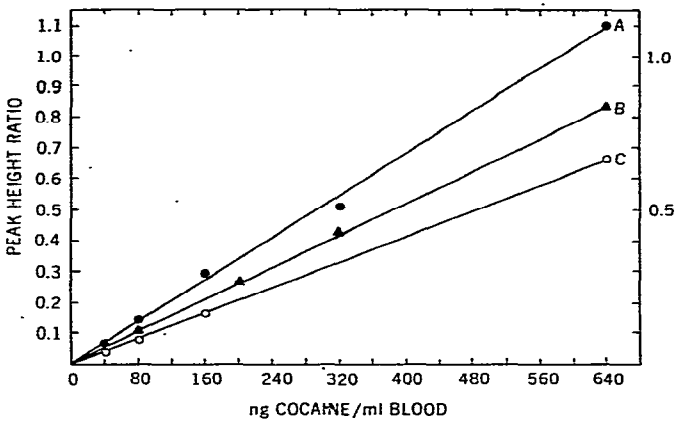

Fig. 3. Effect of storage of final extract residues at −15° on cocaine concentration. A = Extracted and analyzed on same day; B = storage for 2 days; C = storage for 4 days.

As a result of these studies we recommend the following procedures for the analysis of cocaine in whole blood or plasma. The sample should be placed on ice as soon as it is obtained. Within 1 h a 1- or 2-ml aliquot is transferred to a 50-ml glass-stoppered centrifuge tube and quick-frozen in liquid nitrogen with shaking. These samples are then stored at −15° until the day of analysis. On the day of analysis the samples are thawed, extracted through the acid wash, and the aqueous acid wash adjusted to pH 7.2. Batches of 5 tubes are then carried through the final extraction and evaporation procedure and analyzed. Aliquots of the patient's zero-time blood sample are removed and spiked with different amounts of cocaine. These spiked samples are frozen and extracted along with the rest of the blood samples and are utilized to prepare a standard curve on the day of analysis.

In the course of these studies we encountered some difficulties with the column packing and the silanized glass wool. The column packing, as purchased from Supelco, is deactivated for basic drugs and the agent(s) utilized for this purpose are subject to oxidation, especially if no carrier gas is passing through the heated column. Thus, when not in use, the oven temperature should be reduced to at least 50° and a flow of carrier gas maintained through the column.

On several occasions we noticed extreme tailing of the ethylmorphine peak. This appeared to be due to an interaction with the silanized glass wool and usually arose after 1 or 2 days of constant use. The problem was easily solved by leaving that portion of the column which is in the injector port devoid of packing material and glass wool.

Three patients undergoing rhinoplasty who received cocaine intranasally consented to have blood samples withdrawn during the procedure and postoperatively. Cotton strips (5 × 1/2 in.) were soaked in 10 ml of a 10% cocaine solution, rung almost dry, and one strip placed in each nostril for 10 min. Approximately 5 ml of the 10% cocaine solution was in both strips before insertion into the nostril and 2–3 ml were recovered upon squeezing the strips after removal from the nostrils. The average log (cocaine concentration)–time curve is shown in Fig. 4. The half-life obtained from

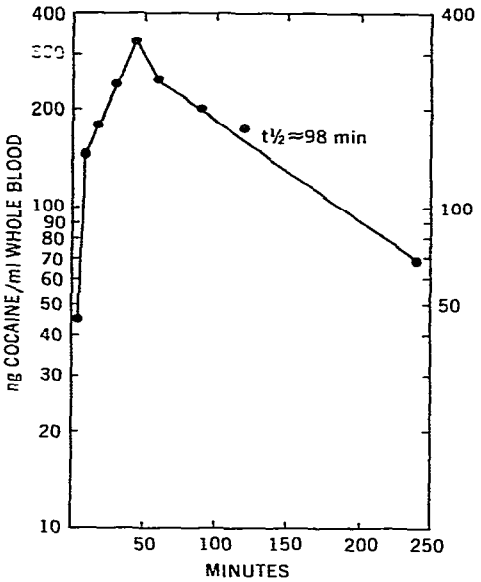

Fig. 4. The logarithm of the concentration of cocaine in whole blood *versus* time following intranasa exposure to cocaine. Cocaine was applied on cotton strips and left in both nostrils for 10 min. Each point is the mean from three different patients.

this data (98 min) may be an overestimate of the true half-life since cocaine has been shown to be present on the nasal mucosa up to 3 h after intranasal application[7].

The increased sensitivity and selectivity of the nitrogen detector as compared to the conventional flame ionization detector has allowed us to measure the concentration of cocaine in human blood or plasma following its topical application to the nasal mucosa. Under the conditions described in this paper we were able to measure as little as 20 ng cocaine from 1 ml of whole blood. It is reasonable to assume that concentrations less than 20 ng/ml can be measured with this method by (a) increasing the sample size, (b) decreasing the volume into which the blood extract is taken up, or (c) a combination of a and b.

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