CHROM. 3892

Gas chromatographic determination of morphine and cocaine in urine

Recent British legislation[1] has necessitated the establishment of treatment centres for persons dependent on diacetylmorphine and cocaine. In the U.S.A., drug monitoring systems based on thin-layer chromatographic analyses of urine have superceded the nalorphine test[2,3] but methods reported[3–5] give qualitative or semi-quantitative excretion data only. Recent gas chromatographic methods have been described for the identification and, in some cases, the determination of morphine[6–8] and cocaine[8,9] in biological fluids but our method is more sensitive, accurate and rapid and, therefore, preferable in routine measurements of the urinary output of these compounds. Data obtained from such assays may be useful in the objective assessment of dose requirements in drug-dependent persons.

Experimental

Apparatus

A Perkin Elmer Model F11 (Mark 1) dual glass column gas chromatograph with flame ionisation detector and a Leeds and Northrup 'Speedomax W' 2.5 mV recorder was used. Support coating was effected by a filtration technique[10] and the packing dried in a Hi-Eff* fluidiser[11]. Columns (1 m × 3 mm internal diameter) were packed with Chromosorb W-AW-DMCS, 100/120 mesh, coated with OV-17** (2.9%). Glass injection liners were used with "hot zone" injection.

Reagents

Diethyl ether (Analar) washed successively with sodium hydroxide solution (5% aqueous) and water, then dried over calcium chloride, and re-distilled freshly.

Chloroform (purified by passing through silica gel and alumina) to which ethanol (3%) was added.

Strong solution of ammonia, concentrated hydrochloric acid, ammonium sulphate and sodium bicarbonate, all Analar grade.

Solutions of benzhexol hydrochloride and nalorphine hydrobromide in distilled water (≡ 1 mg base/100 ml).

Bis(trimethylsilyl) acetamide (BSA) (Applied Science Laboratories Inc.) diluted, as required, 1 in 4 with dried, re-destilled carbon tetrachloride. Care must be taken to avoid contact with rubber from which BSA removes certain components, one of which has a retention time, on OV-17 columns, identical with that of morphine trimethylsilyl ether.

Procedure

Morphine. To each sample of urine (5 ml) was added concentrated hydrochloric acid (0.1 ml), nalorphine hydrobromide solution (1 ml) and ammonium sulphate (4 g): the mixture was shaken for 5–10 min. Ether was added portionwise (3 × 5 ml) and extraction achieved by shaking each time for 5 min, centrifuging and pipetting

* Applied Science Laboratories Inc., per Kodak Ltd., Liverpool.

** OV-17 is a phenyl substituted dimethylpolysiloxane polymer manufactured by Supelco Inc., Bellafonte, Pa. (U.S.A.).

off the ether layers which were rejected. Strong solution of ammonia (0.3 ml) was added to adjust to pH 9 (approx.) and further ether extraction (3 × 5 ml) was carried out. The bulked ether solution was evaporated to dryness, in a stream of nitrogen, at 45° and the residue dissolved in the diluted BSA reagent (100 μl). The solution (2 μl) was injected on to the column.

Cocaine. To each sample of urine (5 ml) was added concentrated hydrochloric acid (0.1 ml) and benzhexol hydrochloride solution (1 ml). Preliminary ether extraction was carried out (3 × 5 ml) rapidly and the ether rejected. Saturated solution of sodium bicarbonate (2 ml) was added to the aqueous layer to give pH 8 (approx.) and further ether extraction, followed by evaporation, was performed as above. The residue was dissolved in chloroform–ethanol (100 μl) and the solution (2 μl) chromatographed.

Results

Typical chromatograms, obtained under the conditions noted, are illustrated in Fig. 1. Values for the ratios of peak heights morphine TMSi ether: nalorphine TMSi ether and cocaine: benzhexol were plotted against concentrations for samples of urine to which morphine or cocaine, respectively, had been added. Linear regressions were obtained for duplicate determinations at several points over the ranges

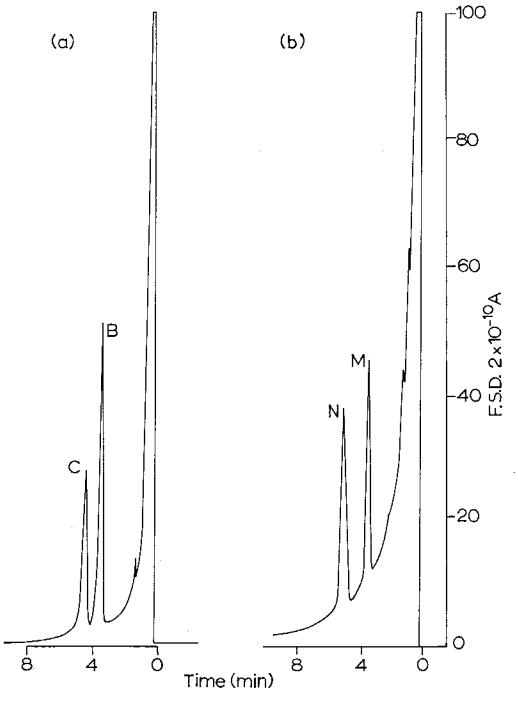

Fig. 1. Typical gas chromatograms of: (a) Cocaine, C, and benzhexol (internal standard), B. Conditions: oven, 185°; injection block, 250°; nitrogen (oxygen free), 53 ml/min; air, 40 p.s.i.; hydrogen, 23 p.s.i.; chart speed, 15 in./h. (b) Morphine trimethylsilyl ether, M, and nalorphine trimethylsilyl ether (internal standard), N. Conditions: as above, except oven 205°.

0.5–10 μg/ml (morphine) and 0.5–15 μg/ml (cocaine). Ten determinations were made at points from the upper and lower parts of each range and standard deviations for these are given in Table I.

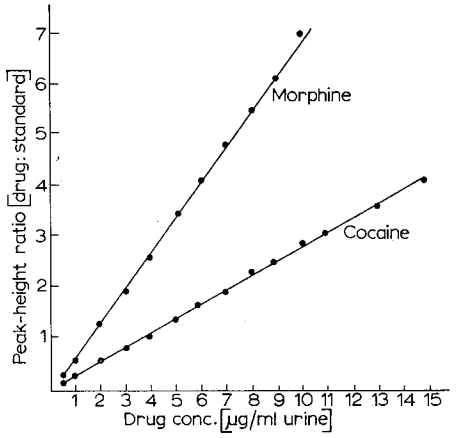

Fig. 2. Standard curves for peak height ratios of morphine trimethylsilyl ether:nalorphine trimethylsilyl ether (internal standard) and cocaine:benzhexol (internal standard) *vs.* concentrations of morphine and cocaine, respectively, added to urine.

TABLE I

REPRODUCIBILITY OF THE GAS–LIQUID CHROMATOGRAPHIC DETERMINATION OF MORPHINE AND COCAINE EXTRACTED FROM URINE

Compound	*Concentration (μg/ml)*		*Number of determinations*
	Actual	*Found (± S.D.)*	
Morphine	1.0	0.92 ± 0.02	10
Morphine	9.0	9.01 ± 0.15	10
Cocaine	3.25	3.01 ± 0.09	10
Cocaine	13.0	12.91 ± 0.39	10

Discussion

In any routine monitoring of drug excretion the analytical method should be as rapid and simple as possible consistent with accuracy and specificity. Gas chromatography offers a more accurate means of quantifying results than thin-layer chromatography and has advantages of speed, specificity and sensitivity as compared with spectrophotometric determinations of morphine[12–15] and cocaine[16–18] extracted from biological media.

Blood concentrations are more meaningful than excretion data in terms of drug effects and blood is easier to extract than urine, giving relatively clean solutions which resolve well on GLC analysis[8]. However, where several successive samples are necessarily required from drug-dependent persons in order to establish their individual excretion profiles over a period of time, willing co-operation of the donors is essential and urine is easier to obtain than blood!

Numerous combinations of solvents have been tried for the extraction of morphine from urine but the use of ether alone has obvious practical advantages[7]. It is an excellent solvent for many basic drugs although difficulties have been encountered with the amphoteric compounds morphine and nalorphine[19,20], recoveries of morphine from aqueous solution (pH 8–8.5) being only about 50%[7]. However, CURRY[21] and KRAMARENKO[22] recommended prior saturation of urine with ammonium sulphate and, using this technique, we have achieved upwards of 80% extraction. Conflicting reports have appeared on the efficiency of ether as a solvent for cocaine[23,24] but our experience confirms that good recoveries are obtained without prior saturation[22]. However, rapid extraction and minimal contact with acidic solutions is essential to prevent loss of cocaine, by hydrolysis, in aqueous media.

The gas chromatographic behaviour of morphine has been reported by numerous authors[7,8] and good peak shape for small amounts of free base have been obtained. Particularly with small amounts of base quantitation is facilitated by the use of trimethylsilyl ethers which give improved peak shape and eliminate sorption–desorption equilibria[25]. Using BSA as the silylating reagent gives the advantages of immediate reaction and the absence of ammonium chloride as a reaction product. Acetylation to diamorphine prior to chromatography is also advantageous[6] but this process is relatively time consuming.

Morphine, both free and conjugated, is excreted in the urine of persons taking either morphine or heroin, the amounts of free base varying in different individuals from 1–14% of the dose ingested[12]. Our lower limit of detection (0.5 μg/ml) is well below the amounts excreted even by patients receiving therapeutic doses of morphine[14] and the method has been applied successfully in analysing the urine of persons dependent on heroin taken alone or in combination with other drugs including cocaine, pethidine, methadone, methaqualone and diphenylhydramine.

Previously reported gas chromatographic methods for the detection of cocaine in urine have not been quantitative and, although there is a report on excretion of ecgonine by coca chewers[26], there are no data on the disposition of cocaine or its metabolites in humans taking the drug parenterally. Animal studies have shown marked species variation in metabolism of cocaine but it is thought that the 1–12% excretion of unchanged drug, over 24 h, in dog may be paralleled in man[27]. On this assumption our procedure is adequate to detect and estimate the drug in urine of cocaine-dependent individuals.

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