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Note

Gas chromatographic–mass spectrometric determination of morphine, codeine and 6-monoacetylmorphine in blood extracted by solid phase

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After intake, heroin is metabolized almost instantaneously to 6-monoacetylmorphine (MAM), which is then converted into morphine. The parent substance cannot, therefore, be spotted in the blood, and to evaluate the cause of death in drug addicts, the toxicologist is usually limited to testing the specimen for morphine. Since morphine, however, represents a second generation of heroin metabolites, no simple relation exists between its concentration in blood and the toxic effects of heroin. However, blood concentrations of the initial metabolite, MAM, might provide acceptable data for toxicological interpretations.

Gas chromatography (GC) or gas chromatography–mass spectrometry (GC–MS) has been used for testing for MAM in the urine [1–3]. Only one report [4] of its measurement in blood, based on a high-performance liquid chromatographic (HPLC) method, has appeared.

This paper describes a simple, comprehensive method that can be used to determine MAM along with morphine and codeine in the blood. It embraces purification and enrichment of the opiates by solid-phase extraction of the body fluid, followed by capillary GC–MS analysis of the extracts with deuterium-labelled analogues of the opiates as internal standards. The method has been tried on the blood of addicts who had died from overdoses of heroin.

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EXPERIMENTAL

Instrumentation and software

The following Hewlett-Packard devices, specified with their model numbers, were used: 7673A automatic sampler, 5890A gas chromatograph, 5970A mass-selective detector, 16 computer, 9133 Winchester disc drive and 59974 MS-MSD operating software. The chromatographic separation was done in a DB-5 capillary (15 m × 0.25 mm I.D.) with 0.25-μm stationary phase (cross-linked 95% dimethyl–5% diphenylpolysiloxane). For automatic regulation of the apparatus and processing of the analytical data to yield a final printout of the results, we devised a macro program.

Chemicals[a]

We purchased morphine·HCl·$3H_2O$ and codeine·H_2PO_4·$\frac{1}{2}H_2O$ from the Swedish National Drug Company, MAM·HCl, [$^2H_3$]MAM·HCl, [$^2H_3$]morphine·H_2O, [$^2H_3$]codeine and pentafluoropropionic anhydride (PFPA) from Reagenta (Uppsala, Sweden) and Bond Elut C_{18} columns (LR07204) from Sorbent (V. Frölunda, Sweden).

Biological materials

Everyday biological materials, sent to our laboratory for toxicological investigation, formed the pool of blood specimens from which we selected test materials. These were from heroin addicts who had been found dead with a needle in their arms or under circumstances otherwise indicating that they had died from injecting an overdose. The hospital blood bank provided the fresh blood.

Extraction of blood

A 0.5-ml volume of ethanol containing deuterium-labelled opiates as internal standards (50 ng of [$^2H_3$]morphine, 50 ng of [$^2H_3$]codeine and 10 ng of [$^2H_3$]MAM) was added to 1.0 g of blood. The mixture was vigorously shaken and left on an ice-bath for 10 min before 6.5 ml of ice-chilled 0.1 *M* sodium carbonate buffer (pH 9) were added. After centrifugation of the suspension at 5900 *g* for 10 min at 6 °C, the clear supernatant was decanted and rapidly sucked through a methanol-activated C_{18} column. To remove interfering substances in the biological matrix, the column was washed with 3 ml of distilled water. By sucking air for at least 10 min through the column with a vacuum of ca. 7 p.s.i. at the outlet, complete removal of water was assured. The target substances were finally eluted into silanized vials with 1.3 ml of dichloromethane–acetone (1:1).

[a]The deuterium-labelled opiates carried three deuterium atoms in the N-methyl group.

Derivatization of opiates

The dichloromethane–acetone extract was evaporated to dryness at 65°C under a stream of nitrogen, and 0.05 ml of PFPA was added to the residue. The derivatization reaction was carried out at 65°C for 30 min. The mixture was again taken to dryness under a stream of nitrogen at 65°C, and the residue was finally reconstituted in 0.04 ml of ethyl acetate.

GC–MS determination

A 3-μl volume of the derivatized extract was injected splitless (1 min) at an injector temperature of 210°C. The oven temperature, initially set at 150°C, was maintained for 1 min after the sample injection, and was then raised at 50°C/min. When 240°C had been reached, the rate was adjusted to 5°C/min until the final temperature was 256°C. The whole chromatographic run-time was 6 min.

The temperature of the interface connecting the gas chromatograph to the mass spectrometer was 250°C. The spectrometer was operated in the multiple-ion detection (MID) mode and focused the following mass fragments: *m/z* 361 and 577 (morphine–PFPA); *m/z* 414.2 (morphine–PFPA and MAM–PFPA); *m/z* 417.2 ([$^2H_3$]morphine–PFPA and [$^2H_3$]MAM–PFPA); *m/z* 282 and 445 (codeine–PFPA); *m/z* 285 and 448 ([$^2H_3$]codeine–PFPA); *m/z* 473.2 (MAM–PFPA); and *m/z* 476.2 ([$^2H_3$]MAM–PFPA). The scan rate was 3.7 cycles per second.

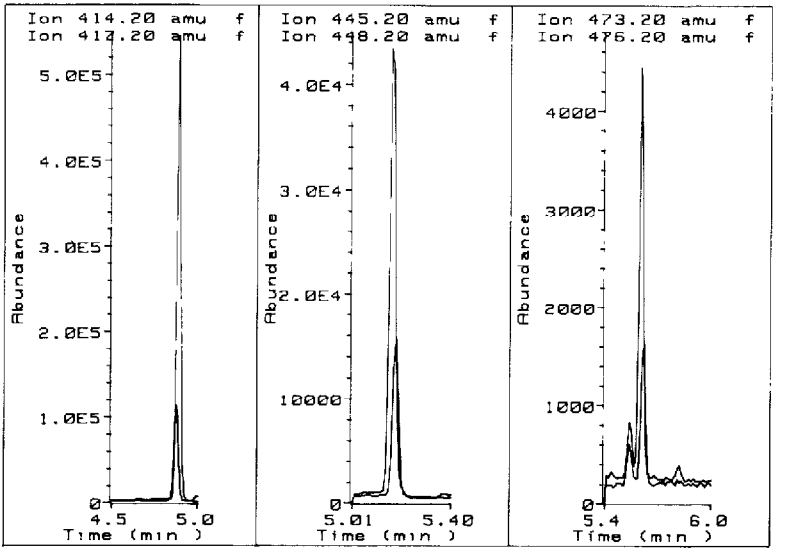

Fig. 1. Chromatogram of blood from a deceased person, showing 243 ng/g morphine (left), 10 ng/g codeine (middle) and 4.6 ng/g MAM (right). The heading peak on each segment of the chromatogram is the deuterated analogue used as an internal standard (50 ng/g [$^2H_3$]morphine, 50 ng/g [$^2H_3$]codeine, 10 ng/g [$^2H_3$]MAM).

Quantification and recovery studies

To generate standard curves, different concentrations of 1–2000 ng of morphine and codeine and 0.5–50 ng of MAM, added to 1 g of fresh blood, were measured. The ratio of the peak areas of the substance and the internal standard, determined from the opiate mass fragments listed on the chromatograms in Fig. 1, was calculated as a function of the concentration of the substance. This equation was then stored in a data file and subsequently used for the automated quantification.

To determine the recoveries of morphine, codeine and MAM, 0.5 ml of the internal standard solution was added in four replicate experiments to fresh blood, which was then processed as described above. The peak area for each substance was measured and calculated as a percentage of the corresponding value obtained from the internal standards in ethanol taken to dryness by evaporation before derivatization and GC–MS determination.

RESULTS

Fig. 1 shows typical chromatograms of the main fragments of morphine, codeine and MAM, together with their deuterated analogues, in post-mortem blood. These substances were well separated from one another and from interfering components in the biological materials. The deuterated variants of morphine, codeine and MAM appeared 11–17 ms ahead of their unlabelled counterparts.

Data in Table I summarize the standard curve constants for morphine, codeine and MAM, the coefficients of variation (C.V.) for the peak-area ratios and the recoveries.

Table II shows the results from applications of the method to a determination of the concentrations of morphine, codeine and MAM in the blood of

TABLE I

STANDARD CURVES, PRECISION AND RECOVERY FOR DETERMINING MORPHINE, CODEINE AND MAM IN BLOOD

r expresses the correlation coefficients and $S(yx)$ the standard deviations as a percentage of the mean value of the peak-area ratio of substance to internal standard, with number of experiments in parentheses.

Substance	Range (ng/g)	Slope	Intercept	r	$S(yx)$ (%)	Recovery (mean ± S.D.) (%)
Morphine	1–2000	0.017	0.067	0.9985	10.6 (18)	68 ± 0.1 (4)
Codeine	1–2000	0.018	0.162	0.9976	13.5 (18)	93 ± 0.1 (4)
MAM	0.5–50	0.080	−0.012	0.9993	6.1 (11)	96 ± 0.1 (4)

TABLE II

CONCENTRATIONS OF MORPHINE, CODEINE AND MAM IN THE BLOOD OF SIX DECEASED HEROIN ADDICTS

Case No.	Concentration (ng/g of blood)		
	Morphine	Codeine	MAM
1	250	151	3.5
2	1592	221	2.0
3	1452	142	3.0
4	243	10	4.6
5	463	48	1.6
6	835	50	6.1
C.V. (%)	74	77	49

deceased drug addicts. The concentration of MAM showed a smaller variation between individuals than did those of morphine and codeine.

DISCUSSION

This paper presents a method for measuring simultaneously morphine, codeine and MAM in blood. Blood specimens taken from suspected heroin addicts possess an increased risk for transmitting the acquired immunodeficiency syndrome (AIDS). In the first step of the work-up, we therefore added the internal standards dissolved in a sufficiently large volume of ethanol to reach 20% in the blood; 10 min exposure to this ethanol concentration has been shown to deactivate completely the AIDS-generating virus [5]. By using solid-phase extraction, deuterium-labelled variants of the opiates as internal standards and GC–MS optimally programmed for the opiate analysis, concentrations as low as 0.5 ng of MAM per gram of blood could be determined. This sensitivity limit allowed measurement of MAM in unhydrolysed blood. More importantly, the final extract could, as a result, be taken up in a volume sufficiently large to permit the use of an autosampler in the fully automated GC–MS determination. The precision and recovery of the method seem to make it suitable for use even in toxicological investigations of forensic cases.

The presence of morphine, codeine and MAM was confirmed by the appearance of all the appropriate m/z numbers in the spectra. Another indicator was the difference in retention times between the deuterated and unlabelled opiates. For positive testing, we required the target substance to appear 10–17 ms after its ^{2}H-labelled counterpart.

Blood samples from addicts who had died from an overdose of heroin tested positive for MAM and did so in a smaller range of concentration than mor-

phine or codeine. A likely explanation for this is that the concentration of MAM in blood may have some bearing on the cause of death. Before this possibility is more closely investigated, the stability of MAM in blood should be studied.

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