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Note

Determination of underivatized CNS-stimulants and methadone in urinary extracts by glass capillary gas chromatography

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Recently, reported methods for the determination of stimulants in biological fluids have employed conventional gas chromatography[1–8]. Quartz capillary columns have not yet been applied to the determination of underivatized stimulants or related drugs. Our method, previously used for the detection of stimulants in urine, involves extraction of alkalinized urines with large solvent volumes (diethyl ether), an additional clean-up extraction, back-extraction into 15% formic acid solution and chromatographic analysis of the formic acid extract on an alkalinized Carbowax column (5%KOH, 5% Carbowax 20M TPA on Chromosorb W HP). This technique is time consuming and laborious. Furthermore, the separation power of the column is not sufficient to resolve all the stimulants.

The frequency of use or abuse of amphetamine-related drugs was studied by Jain *et al.*[9]. Amphetamine, phentermine, ephedrine and methamphetamine were the most common stimulants detected in 10,000 screened samples. Aggarwal *et al.*[10] reported a very rapid procedure using a tiny volume of a solvent mixture for the extraction of basic drugs from alkalinized urines. We have now adapted their extraction technique, without any derivatization procedure, and combined it with chromatographic analysis on fused silica capillary columns.

EXPERIMENTAL

Apparatus

A Packard Model 427 gas chromatograph equipped with a flame ionization detector and a split injector was connected to a Sigma 10 Chromatography Data Station (Perkin-Elmer). A fused silica capillary column (25 m × 0.20–0.21 mm I.D., Hewlett-Packard No. 19091-60025) deactivated with Carbowax 20 M and with SP 2100 as the stationary phase was applied.

The glass capillary gas chromatographic (GCGC) conditions were as follows: injection port 230°C; detector 230°C; oven temperature isothermal at 110°C for 4 min, programmed from 110°C to 220°C at 16°/min and then isothermal at 220°C for 4 min. Nitrogen was employed both as carrier gas (flow-rate, 0.8 ml/min) and as the make-up gas (flow-rate, 10 ml/min). Splitting ratio = 1:10.

Reagents

The solid buffer was a mixture of $NaHCO_3$ and K_2CO_3 (3:2, w/w). The extraction solvent was chloroform–isopropanol (4:1, v/v). The internal standard solution

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was prepared by dissolving 10 mg of diphenylamine in 100 ml of ethanol. Drug standards in urine (1–20 μg/ml, as free base) were prepared by adding the drugs amphetamine, phentermine, chlorophentermine, ephedrine, phenmetrazine, diethylpropione, methylphenidate and methadone to a drug-free urine.

Procedure

Approximately 5 ml of urine were saturated with solid buffer (*ca.* 2 g). The mixture was shaken by vortexing and centrifuged for 5 min to remove any insoluble material. A 4-ml volume of the supernatant urine was transferred to a conically tipped culture tube with a PTFE-lined screw cap, followed by 100 μl of the internal standard solution. After mixing, 100 μl of the extraction solvent were added. The mixture was vortexed for 1 min, then centrifuged for 5 min. The supernatant urine was then aspirated and discarded. A 1-μl volume of the organic phase was injected on the gas chromatograph.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms obtained in this procedure. In the upper chromatogram the stimulants, methadone, nicotine and caffein (the last two compounds may be present in many urines) are resolved under the present chromatographic conditions. No interfering peaks were found in chromatograms from blank urines run through the procedure.

Calibration graphs of peak height (or peak area) ratios of the drug and internal standard *versus* the drug concentration were linear in the range 1–10 μg/ml for all the drugs. All the correlation coefficients were greater than 0.97. Table I gives precision data obtained by repeated analysis of a urine to which all the drugs had been added. Detection limits were as follows: stimulants, *ca.* 0.3 μg/ml (except for ephedrine, 1 μg/ml); methadone, *ca.* 0.6 μg/ml.

No interference from barbiturates was found. Other basic or neutral drugs or their metabolites may interfere; however, this problem is easily overcome by adapting the technique to mass spectrometry, which yields additional structural information. No metabolites were studied. Positive samples for each drug should be confirmed by mass spectrometry.

The method is adequate for the screening of samples from suspected stimulant abusers. As the technique is very simple the saving of labour and time is considerable compared to the methods previously used.

TABLE I

PRECISION DATA

In each case $n = 6$.

Compound	*Mean (μg/ml)*	*CV (%)*
Amphetamine	8.9	8.4
Phentermine	2.8	10.0
Chlorophentermine	5.2	3.1
Ephedrine	4.8	10.8
Phenmetrazine	4.9	5.5
Diethylpropione	5.8	4.1
Methylphenidate	3.1	1.9
Methadone	2.6	11.5

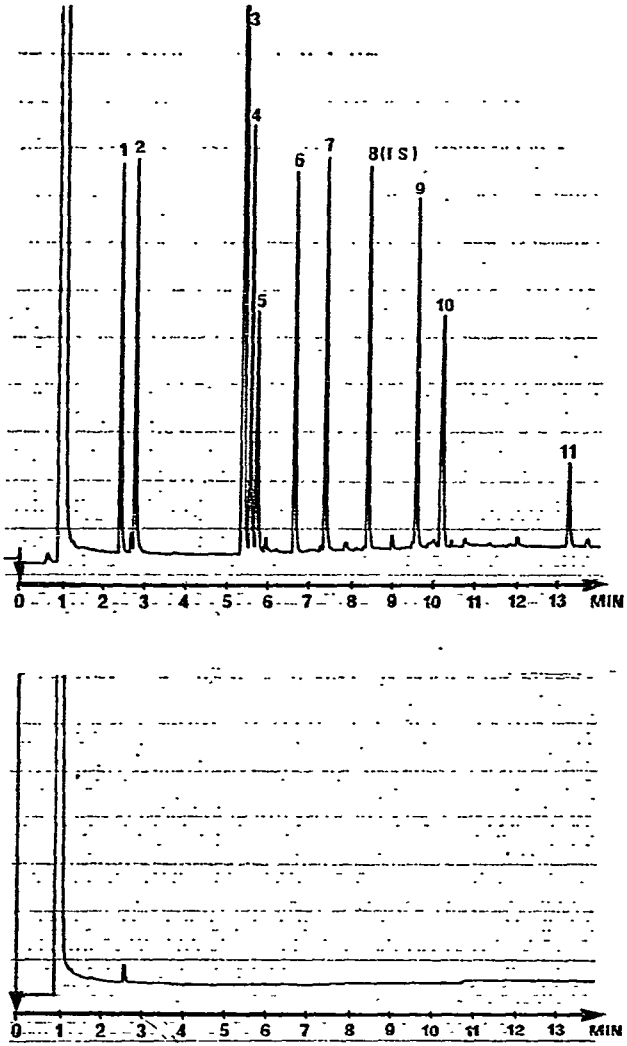

Fig. 1. Upper chromatogram: an analysis of a urine drug standard (5 μg/ml of each drug, except ephedrine 20 μg/ml, as free base). Peaks: 1 = amphetamine; 2 = phentermine; 3 = nicotine; 4 = chlorophentermine; 5 = ephedrine; 6 = phenmetrazine; 7 = diethylpropione; 8 = diphenylamine (internal standard); 9 = methylphenidate; 10 = caffein; 11 = methadone. Retention times are given in Table II. Lower chromatogram: a urine blank without addition of the internal standard.

TABLE II

RETENTION TIMES OF THE STIMULANTS

Peak no.	*Compound*	*Retention time (min)*	*Relative retention*
1	Amphetamine	2.59	0.30
2	Phentermine	2.90	0.34
3	Nicotine	5.57	0.65
4	Chlorophentermine	5.78	0.68
5	Ephedrine	5.93	0.69
6	Phenmetrazine	6.83	0.80
7	Diethylpropione	7.54	0.88
8	Diphenylamine (I.S.)	8.55	1.00
9	Methylphenidate	9.70	1.13
10	Caffein	10.36	1.21
11	Methadone	13.47	1.58

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