Journal of Chromatography, 81 (1973) 170–173
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CHROM. 6730

Note

Detection of biotransformed cocaine in urine from drug abusers

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(First received March 19th, 1973; revised manuscript received April 7th, 1973)

Cocaine (benzoyl methyl ecgonine) is commonly used by drug addicts but is infrequently detected in urine screening programs[1] because it is rapidly metabolized to benzoyl ecgonine and, to a lesser extent, ecgonine, which are present in the urine[2,3]. Both metabolites are highly water-soluble compounds and are not effectively extracted by toxicologic screening methods employing traditional solvents: chloroform, ether or isopropyl alcohol[4–9]. Further, the small percentage of cocaine that may be excreted is hydrolyzed to benzoyl ecgonine on standing in urine. This report describes solvent systems and techniques devised to extract, isolate, identify and confirm cocaine and its metabolites. Benzoyl ecgonine, ecgonine and cocaine are readily distinguished from other drugs and their metabolites that may also be present in the urine.

EXPERIMENTAL

Materials

EMIT enzyme immunoassay system and benzoyl ecgonine and ecgonine reagents were supplied by Dr. R. S. Schneider and Dr. R. Bastiani of the Syva Corporation, Palo Alto, Calif.

Thin-layer chromatographic plates. The plates were precoated with Silica Gel G, glass support, 20 × 20 cm, 0.25 mm layer thickness (from E. Merck, Darmstadt).

Developing solvents. The following solvents were used: (I) ethyl acetate–methanol (170:20) and 20 ml of 50% ammonia in a beaker; (II) chloroform–methanol (100:100) and 20 ml of 50% ammonia in a beaker.

Visualizing reagents. The following reagents were prepared:

(I) Modified Dragendorff's reagent[10]: Boil 2.6 g of bismuth subnitrate and 7 g of potassium iodide for a few minutes with 25 ml of glacial acetic acid. Allow to stand overnight. Filter off the precipitated potassium acetate and add 80 ml of ethyl acetate to 20 ml of the filtrate. The solution should be protected from light. Before using, dilute 10 ml with 25 ml of glacial acetic acid and 60 ml of ethyl acetate.

(II) Iodoplatinate solution: Dissolve 10 g of potassium iodide and 1 g of platinum chloride separately. Mix and dilute to 500 ml with water.

(III) Lead iodide reagent: Dissolve lead acetate ($Pb(OAc)_2 \cdot 3H_2O$) 0.24 g; potassium iodide 0.27 g; acetic acid 1 ml and magnesium acetate 40 g in 100 ml of

water. 5 ml of the above solution are added to potassium acetate slowly until PbI_2 dissolves.

Methods

Analytical procedure. The pH of 25 ml of urine is adjusted to 8.5 with 10 *N* NaOH and 2 ml of bicarbonate buffer (pH 8.5). The urine is then extracted twice by shaking vigorously with 75 ml of a solvent containing a mixture of chloroform–isopropanol–1,2-dichloroethane (8:1:3). The solvent layer is then allowed to separate for 5 min in a separatory funnel and the organic phase is filtered through Whatman No. 1 filter paper into a 150-ml beaker. The solvent layer is then completely evaporated and the residue containing cocaine, ecgonine and benzoyl ecgonine is saved for thin-layer chromatographic (TLC) analysis.

Thin-layer chromatography. The residue is dissolved in 0.2 ml of ethanol and applied with a 10-μl micropipette 3 cm from the lower edge of the TLC plate. One-dimensional chromatography is used but the plate is developed in two different solvent systems. Desaga rectangular tanks (external dimensions 8.5 × 4.5 × 8.5 in.) are saturated with the solvents for 30 min. Developing system I is used with the beaker containing 20 ml of 50% ammonia placed in the center of the tank[5]. The plate is developed to 15 cm for 45 min at room temperature. It is then air-dried for

TABLE I

THIN-LAYER CHROMATOGRAPHIC DATA OF BASIC DRUGS EXTRACTED FROM URINE

Developing systems: (I) Ethyl acetate–methanol (170:20) and 20 ml of 50% ammonium hydroxide solution in a beaker; (II) chloroform–methanol (100:100) and 20 ml of 50% ammonium hydroxide in a beaker.

Drugs	$R_F \times 10$	
	I	*II*
D-Amphetamine	3.1	Solvent front
D-Methamphetamine	2.3	Solvent front
Benzoyl ecgonine	origin	5.0
Caffeine	4.9	Solvent front
Cocaine	7.3	*
Codeine	1.8	Solvent front
Dihydrocodeine	0.9	Solvent front
Ecgonine	origin	2.0
Imipramine	4.9	Solvent front
Nicotine	5.1	Solvent front
Meperidine	3.5	Solvent front
Methadone	6.8	*
Morphine	0.8	Solvent front
Quinine	1.8	Solvent front
Procaine	5.2	Solvent front
Propoxyphene	7.4	*
Pentazocine	5.8	Solvent front
Thorazine	1.7	Solvent front
	4.0	Solvent front
	5.0	Solvent front

* Above solvent front.

10 min and run in the developing system II with the beaker containing 20 ml of 50% ammonia placed in the center of the tank; it is developed to 8 cm for 20 min. The plate is thoroughly dried in an oven at 50° for 30 min, cooled to room temperature, then sprayed with the modified Dragendorff's reagent.

Cocaine, benzoyl ecgonine and ecgonine give distinct red spots. The plate is then lightly resprayed with iodoplatinate reagent and the same compounds now appear as violet-red spots. The R_F value of ecgonine, benzoyl ecgonine, cocaine and other drugs in solvent I and the combined R_F values when developed in both solvent systems are given in Table I. Benzoyl ecgonine and ecgonine standards are prepared simply by adding cocaine to a urine specimen at pH 8.5 and leaving it at room temperature for 24 to 48 h.

Microcrystal tests

The suspected benzoyl ecgonine spot is scraped from the TLC plate and eluted with absolute methanol, filtered into a 5-ml beaker and evaporated to dryness on a steam-bath.

The unknown residue is taken up in one drop of 5% acetic acid solution and applied to a micro slide with a micro spatula and allowed to dry on a steam-bath or low temperature hot plate. The procedure is repeated until the 5% acetic acid solution containing the unknown residue is exhausted.

The crystallizing reagent lead iodide–potassium acetate in acetic acid–magnesium acetate (see ref. 11) solution is applied to a 12-mm cover glass and dropped over the residue on the slide. If benzoyl ecgonine is present, rosettes of needles with blue and gray birefrigence and positive elongation appear within 15 min. Ecgonine produces straight and individual needles with positive elongation; cocaine produces needles that show dendritic branching and positive elongation.

Benzoyl ecgonine can alternatively be confirmed from the TLC plates by enzyme immunoassay[12] by dissolving the eluted residue obtained from the TLC plate in 0.5 ml of bicarbonate buffer, pH 8.5.

RESULTS AND DISCUSSION

Using the above described techniques, benzoyl ecgonine was identified in 15% of 1000 randomly selected urines from drug abuse treatment programs in New York City; in less than 1% of these cases there was sufficient cocaine present in the urine to permit its identification. Wherever ecgonine was present, benzoyl ecgonine was also identified; however, benzoyl ecgonine was often present in the absence of ecgonine. In 80% of the benzoyl ecgonine positive cases other drugs of abuse were also identified.

The extraction, purification and confirmation of cocaine, benzoyl ecgonine and ecgonine from urine of addicts is described in these studies. Quinine, methadone, phenothiazines, meperidine, propoxyphene, codeine, morphine, amphetamines, barbiturates and their metabolites and many other drugs of abuse do not interfere with this identification. Cocaine and its metabolites can be easily distinguished by microcrystal tests that are described. The sensitivity for benzoyl ecgonine extracted by this procedure is 3–5 μg/ml on TLC plates and 1 μg/ml by EMIT.

ACKNOWLEDGEMENTS

We wish to thank Dr. R. S. Schneider and Dr. R. Bastiani of the Syva Corporation for their cooperation in supplying EMIT equipment and reagents; and Miss Frances Plotnik and Miss Marilyn Rosenfeld for secretarial assistance.

REFERENCES

1 C. D. Chambers, W. J. Rusell Taylor and A. D. Moffett, *Int. J. Addict.*, 7 (1972) 427.
2 F. Fish and W. D. C. Wilson, *J. Pharm. Pharmacol.*, 21 (1969) 135.
3 L. S. Woods, F. G. McMahon and M. H. Seevers, *J. Pharmacol. Exp. Ther.*, 101 (1951) 200.
4 N. N. Valanju, M. M. Baden, S. N. Valanju and S. K. Verma, *Int. Microfilm J. Leg. Med.*, 5 (1970) 4.
5 M. M. Baden, N. N. Valanju, S. K. Verma and S. N. Valanju, *Amer. J. Clin. Pathol.*, 57 (1972) 43.
6 B. Davidow, N. Li Petri and B. Quame, *Amer. J. Clin. Pathol.*, 50 (1968) 714.
7 S. J. Mulé, *J. Chromatogr.*, 55 (1971) 255.
8 V. P. Dole, A. Growther, J. Johnson, M. Monsalvatge, B. Biller and S. S. Nelson, *N.Y. State J. Med.*, 72 (1972) 471.
9 K. K. Kaistha, J. H. Jaffe, *J. Chromatogr.*, 60 (1971) 83.
10 P. Majlát and I. Bayer, *J. Chromatogr.*, 20 (1965) 187.
11 C. C. Fulton, *Modern Microcrystal Tests for Drugs*, Wiley-Interscience, New York, 1969, pp. 29, 392, 393.
12 K. E. Rubenstein, R. S. Schneider and E. F. Ullman, *Biochem. Biophys. Res. Commun.*, 47 (1972) 846.