CHROM. 5782

Mini thin-layer chromatography in the detection of narcotics in urine from subjects on a methadone maintenance program

In recent years great interest has developed in the detection of narcotics in urine. Chromatographic methods have been widely used[1–11]. Despite the numerous procedures available, a rapid and more sensitive method is still desirable for routine urine analysis of narcotics. By using the mini thin-layer plate (3 × 3 cm), we have been able to detect the presence of narcotics, *viz.* morphine, codeine, methadone, meperidine, in concentrations as low as 100 ng/ml in an unhydrolyzed urine sample. The entire procedure for 16 samples can be performed by one person in 90 min.

Materials

Extraction solvent. Chloroform–ethyl acetate–methanol (3:1:1).

Thin-layer plates. Chromatogram sheets (6061, silica gel, 20 × 20 cm, Eastman) were cut into 3 × 3 cm plates and heated at 120° for 10 min before use.

Chromatographic solvent. Ethyl acetate–methanol–ammonia (85:10:5).

Color reagent. Iodoplatinate reagent was prepared by adding 0.5 ml of 10% platinum chloride and 0.5 g of potassium iodide in 25 ml of water and diluted with 3 volumes of 2 *N* HCl before use.

Procedure

The urine was extracted by a modification of the method of DAVIDOW[3]. A 5-ml aliquot was made ammoniacal with 2–3 drops of concentrated ammonium hydroxide and extracted for 15 min with 5 ml of the extraction solvent. The organic phase was separated by centrifugation and then evaporated to dryness under a stream of air. The residue was dissolved in 20 μl of methanol and a 1–2 μl aliquot was removed by means of a self-drawing capillary pipette for application on a mini thin-layer plate.

Five samples were spotted on a single plate; these usually included four different extracts of urine plus a marker which consisted of a mixture containing approximately 0.5 μg each of morphine, methadone, meperidine, codeine and amphetamine.

The plate was developed in a petri dish (diameter 5.5 cm, height 3.5 cm) containing 1.5–2 ml of chromatographic solvent. Four plates may be developed at the same time in one container. When the solvent reached 2 mm from the top (developing time usually 1.5 min), the plate was removed, dried with an electric hair dryer and sprayed with the iodoplatinate reagent.

Results

The R_F values and iodoplatinate color reaction of five drugs developed according to the above procedure are shown in Table I. At a drug concentration of 0.1 μg/ml they were easily detected in 5-ml standard samples.

The usefulness and applicability of this procedure was established by tests on the urine samples taken from subjects on a methadone maintenance program. The specimens were kindly provided by Hine Laboratories who also confirmed the presence of methadone and morphine in the samples. Fig. 1 shows the chromatographic pattern of the plates each spotted with extracts of four urine samples and the standard. All

TABLE I

R_F VALUES AND COLOR REACTIONS OF NARCOTICS DEVELOPED ON A MINI THIN-LAYER PLATE

Developing solvent: ethyl acetate–methanol–ammonia (85:10:5); color reagent: iodoplatinate solution.

Narcotics	*R_F values*	*Colors*
Methadone	0.91	brown
Amphetamine	0.66	blue turning to white
Codeine	0.58	purple
Morphine	0.40	dark blue
Meperidine	0	purple

were positive for methadone. Both C and D were positive for morphine and quinine. Sample A was positive for morphine only and sample B for quinine but not for morphine.

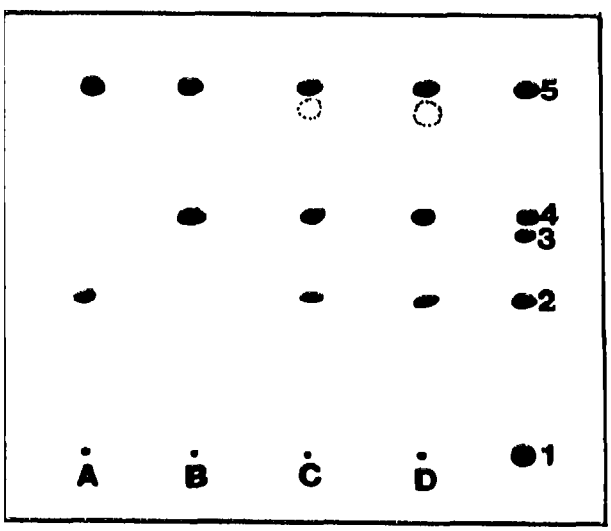

Fig. 1. Thin-layer chromatogram of urine obtained from four subjects (A–D) on a methadone maintenance program. The reference marker is in the extreme right on each plate and consists of a mixture of approximately 0.5 μg each of meperidine, morphine, codeine, quinine and methadone (1–5, respectively).

Discussion

The utilization of mini thin-layer plates speeds up the steps in the chromatographic procedures considerably. Not only is this method more simple, rapid and sensitive than conventional ones, but these advantages are attainable without sacrificing accuracy. The saving in time and material is considerable. Only 1.5 min are required for the development of four plates with 1.5–2 ml of solvent, and the spotting time for 1–2 μl of extract is less than 1 min. At drug concentrations of 0.1 μg/ml of urine, 16 determinations can be completed within 90 min on extracts from 5-ml samples of urine. At concentrations lower than 50 ng/ml, sensitivity can be maintained using larger volumes of urine and spotting the entire solvent extract. However, for routine analysis, 5 ml of urine should be adequate.

The enhanced sensitivity of the procedure allows for estimation of the drugs

without need to subject the urine to acid hydrolysis. Urinary salts and pigments did not appear to influence the R_F values of the narcotics and all spots were nicely separated. Under the conditions described, no color spots were detected with the iodoplatinate reagent in the urine taken from normal subjects or persons known to be on amphetamine or barbiturates maintenance programs.

While the R_F values for drugs were found to vary somewhat from plate to plate, the relative position of drugs on the plate is consistent. Therefore, standards are recommended for each separation.

Although the purpose of this communication is to report a rapid and sensitive procedure for routine screening of narcotics in urine, the procedure could be modified for detection of other drugs of abuse such as amphetamines and barbiturates using different extraction conditions, developing solvent systems and visualizing reagents. Hence, the present study indicates that mini thin-layer chromatography (TLC) has wide applicability. Any drug which can be detected by conventional TLC can also be estimated on mini plates and this appears to have many advantages for qualitative purposes in routine clinical tests.

Mini TLC is adaptable also for quantitative analysis. We are currently studying the applicability of the procedure for the estimation of morphine in plasma and cerebral spinal fluid using a densitometer to scan the spots. It is to be emphasized, however, that the present procedure is designed primarily for routine surveillance of narcotic addicts such as those on a methadone maintenance program. The test alone cannot be considered conclusive for medical–legal purposes. In such instances other confirmatory tests need to be applied.

This study was supported by a research grant from the National Institutes of Health, GM-01839. The urine specimens were kindly provided by Mr. COLIN KEHE of Hine Laboratories.

Department of Pharmacology,
University of California,
School of Medicine,
San Francisco, Calif. 94122 (U.S.A.)

I. K. HO
H. H. LOH

Drug Dependence Research Center,
Mendocino State Hospital,
Talmage, Calif. 95481 (U.S.A.)

E. LEONG WAY

1 J. COCHIN AND J. W. DALY, *Experientia*, 18 (1962) 294.
2 J. COCHIN AND J. W. DALY, *J. Pharmacol. Exp. Ther.*, 139 (1963) 160.
3 B. DAVIDOW, *Psychopharmacol. Bull.*, 3 (1966) 30.
4 V. P. DOLE, W. K. KIM AND I. EGLITIS, *Psychopharmacol. Bull.*, 3 (1966) 45.
5 R. J. KOKOSKI, *Psychopharmacol. Bull.*, 3 (1966) 34.
6 H. J. KUPFERBERG, A. BURKHALTER AND E. L. WAY, *J. Chromatogr.*, 16 (1964) 558.
7 S.-C. LIN AND E. L. WAY, *J. Forensic Sci.*, 8 (1963) 209.
8 G. MANNERING, A. C. DIXON, N. CARROL AND O. COLE, *J. Lab. Clin. Med.*, 44 (1954) 292.
9 S. J. MULE, *Psychopharmacol. Bull.*, 3 (1966) 37.
10 W. M. MCISAAC, *Psychopharmacol. Bull.*, 3 (1966) 40.
11 K. D. PARKER, C. H. HINE, N. NOMOF AND H. W. ELLIOTT, *J. Forensic Sci.*, 11 (1966) 152.

First received September 6th, 1971; revised manuscript received October 20th, 1971