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Detection of drugs in urine for methadone treatment programs*

Since the publication by DOLE *et al.*[1] of a method for detection of selected "drugs of abuse" in urine by thin-layer chromatography (TLC), several authors[2-4] have raised doubts as to the sensitivity and reliability of the method in practice. This report describes a procedure which is being used successfully in the Honolulu Methadone Treatment Program for screening of urine samples. The method is derived from that of DOLE and others. TLC is used for the qualitative identification of the opiates; gas–liquid chromatographic (GLC) techniques[5,6] are adapted to provide the necessary sensitivity for detection of amphetamine and barbiturate usage by patients.

Methods and materials

Narcotic analgesics and related bases. Urine samples are extracted onto SA-2 (Reeve-Angel) ion-exchange paper according to the DOLE procedure[1]. The paper is then back-extracted by placing it into 20 ml of borate buffer (pH 9.3) and 25 ml of chloroform–isopropanol (3:1) for 30 min with occasional shaking. The organic layer is filtered through Whatman No. 1 paper into a 50 ml beaker and evaporated to dryness. Excessive heat must be avoided during evaporation. The residue is dissolved in 100 μl of methanol and 10–20 μl are spotted on a 20 × 20 cm Silica Gel G thin-layer plate of 250 μ thickness. Standards of nicotine, morphine, cocaine, quinine, and methadone are placed on the same plate. The plate is placed into butanol–acetic acid–water (4:1:5) (top layer) and the solvent allowed to rise 10 cm. After evaporation of the butanol, the plate is viewed under UV light for drugs which fluoresce, then sprayed with iodoplatinate reagent for visualization of the drugs. R_F values and colors are shown in Table I. If morphine is detected, further confirmation may be obtained by spraying with ammoniacal silver nitrate, which turns the morphine spot black with the application of heat.

TABLE I

IDENTIFICATION OF NARCOTIC ANALGESICS AND RELATED BASES

Drug	R_F	*Color with*	
		UV	*Iodoplatinate*
Nicotine	0.08	Dark blue	Black
Morphine	0.20	—	Blue-green
Codeine	0.20	—	Blue
Cocaine	0.22	Dark blue	Violet
Trifluoperazine	0.32	Yellow	Dark Blue
Methadone metabolite	0.36	—	Violet
Quinine	0.45	Blue-white	Blue-black
Methadone	0.50	—	Violet
Chlorpromazine	0.50	Blue-yellow	Dark Blue
Propoxyphene	0.50	—	Violet
Pentazocine	0.63	—	Violet

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Barbiturates. A 5 ml aliquot of a fresh urine sample is adjusted to pH 5 with 1 ml of saturated potassium dihydrogen phosphate solution and extracted with 25 ml $CHCl_3$. The $CHCl_3$ layer is filtered through Whatman No. 1 paper into a 50 ml beaker and taken to dryness on a steam bath. 2 ml of 50 % saturated potassium carbonate solution and 0.2 ml dimethyl sulfate are added to the beaker and heated on a steam bath for 5 min. After cooling, the solution is poured into a 15 ml centrifuge tube. The beaker is rinsed with 1 ml of heptane which is added to the tube. After mixing the contents of the centrifuge tube on a Vortex mixer, the heptane layer is transferred to a clean tube and evaporated under a stream of nitrogen to approximately 100 μl. 1–5 μl is injected into the gas–liquid chromatograph. Currently in use is a Wilkens Model 400 D gas chromatograph equipped with a 3 mm × 2 m glass column containing 1 % SE-30 on Gas-Chrom Q (Applied Science Labs., State College, Pa.) and a hydrogen flame detector. The conditions are as follows. Temperatures: inlet, 200°; column, 150°; detector, 180°. Nitrogen flow rate is 30 ml/min. The following are the retention times for the methylated derivatives of the common barbiturates: amobarbital, 1.4 min; pentobarbital, 1.6 min; secobarbital, 1.9 min; phenobarbital, 4.2 min. A control sample which contains 20 μg/ml of each of the above drugs is analyzed with each group of unknown samples.

Amphetamines. After extraction of the barbiturates, the same 5 ml of urine is made pH 11 with concentrated sodium hydroxide and extracted with 25 ml $CHCl_3$. The $CHCl_3$ layer is filtered through Whatman No. 1 paper into a 50 ml beaker, 100 μl of acetic anhydride is added, and the extract is evaporated to 1–2 ml with gentle heating. The sample is then transferred to a 15 ml graduated centrifuge tube, and evaporated further to 100 μl under a stream of nitrogen. 1 μl of the concentrate is injected into the gas–liquid chromatograph, using the conditions as given above. Under these conditions, the retention times for the N-acetyl derivatives of amphetamine and methamphetamine are 0.8 and 1.2 min, respectively. Control samples, prepared by adding authentic amphetamine and methamphetamine to "clean" urine at concentrations of 5 μg/ml, are extracted and analyzed with each day's batch of samples.

Results and discussion

Although the use of ion-exchange paper results in a two-thirds reduction in morphine recovery compared to a direct extraction[2], it is a convenient means of concentrating the drug found in a large volume of urine (50–100 ml) and provides a cleaner final extract than a direct extraction of only 5 ml of urine. The chromatography solvent used has been found to yield more reproducible R_F values and more clearly defined drug spots than others suggested; its slower movement is less likely to cause busy technicians to overdevelop a chromatogram. The presence of acetic acid in the solvent makes initial spraying with 0.5 % sulfuric acid unnecessary. Detection of methadone using the ion-exchange paper technique is not always reliable; in a sampling of 793 urines from 25 patients on a daily maintenance dose of 80–180 mg of methadone hydrochloride, only 705 (89 %) of the urines were positive for methadone (minimum of 30 ml urine per ion-exchange paper). We have found that the methadone metabolite, whose R_F value is midway between morphine and methadone and less subject to interference by other drugs, is a more reliable indicator of methadone usage[7]. Preliminary results from this laboratory show that the metabolite, a cyclic

N-demethylated derivative of methadone[8], is excreted in the urine at concentrations 3–6 times that of the parent drug[7].

The color of morphine after spraying with iodoplatinate is quite distinctive in this system, and is not readily confused with codeine or cocaine, both with similar chromatographic mobilities. These drugs are further differentiated by the use of the silver reduction technique.

It has been realized[2,3] that the recovery of amphetamines and barbiturates from ion-exchange paper is unacceptable for the purposes of a urine monitoring program of this type. In addition, thin-layer procedures for amphetamines and barbiturates are plagued by poor limits of detectability, difficulty in separation of the various drugs within the two classes, and frequent interference by normal urine constituents and other drugs. In order to avoid these problems, we have turned to GLC methods which are fast, adaptable to batch analysis, and which will detect amphetamine or barbiturate usage for one or two days after the ingestion of therapeutic dosages, using a 5 ml sample of urine.

Derivative formation before chromatography serves two purposes: it reduces loss of the amphetamines by volatilization and, by decreasing the polarity of the barbiturates, allows the same GC conditions to be used in analysis of both classes of drugs.

After a dose of 10 mg of amphetamine or methamphetamine, urine concentrations of 1–4 μg/ml are commonly observed for a period of 30 h. It has been our experience with this method that methamphetamine is always excreted together with a lesser amount of its metabolite, amphetamine. Other basic drugs, such as methadone and morphine, extracted along with the amphetamines, are eluted from the gas chromatograph much later under the conditions stated and do not produce a noticeable rise in the base line at the concentrations normally encountered. Thus sequential injections may be made every 2–3 min.

GC methods exist for the analysis of narcotics and related bases[9,10]; however, for large-scale urine screening purposes the superiority of these methods over TLC has not been proven. It is our finding that in the analysis of the amphetamines and barbiturates, although GC requires a greater initial financial outlay and more sophistication on the part of the analyst, it has definite advantages in screening programs where these factors are not an obstacle.

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