Reliability of Identification Techniques for Drugs of Abuse in a Urine Screening Program and Drug Excretion Data

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Abstract □ Results of a double-blind study regarding the validation of extraction and identification techniques for abuse drugs in 50 control urines to which were added known concentrations of drugs are presented. Data on the excretion of some commonly abused drugs in human urine are also reported.

Keyphrases □ Abuse drugs—reliability of urine screening program, excretion data, validation of extraction and identification techniques □ Screening program, urine—for abuse drugs, reliability and validity of extraction and identification techniques □ Excretion data—abuse drugs □ Drugs, abuse—urine screening program identification techniques, validity, excretion data

Recently, extraction and thin-layer identification techniques were reported for the detection of sedative-hypnotics, narcotics, and CNS stimulants and of some drugs used in the treatment of narcotic users (1-3). Operating costs of a toxicology laboratory facility in a Drug Abuse Urine Screening Program were also reported (4). This article discusses the results of a double-blind study performed to validate the efficacy of the extraction and identification techniques previously reported and also summarizes some data on the excretion of important drugs in human urine.

DOUBLE-BLIND STUDY

Control urines were collected from the staff personnel and pooled together. These staff members were not taking any drugs and were not involved in the analysis of coded samples. The person who chose the drug combinations and designed the study was not himself involved in the analysis or interpretation of results. The drugs chosen for this study were primarily those that are routinely tested in a drug abuse urine monitoring laboratory. Pentazocine and meperidine were the only drugs not routinely tested. There was no criterion followed in choosing various combinations, except that only one drug belonging to the sedative-hypnotic group was added to a urine specimen.

The combined urine was divided into fifty 120-ml. aliquots. Forty-two of these samples were individually spiked with a mixture of drugs having the concentrations shown in Table I. The remaining eight samples were chosen at random and left unspiked. The concentration of each drug added was at the lower limit of detectability for each procedure. (For lower limits of detectability for each procedure, see *References 1* and *2*.) After numbering all 50 bottles and compiling a list of drugs added to each bottle, the bottles and list were given to an impartial party. This party removed the labels and marked the bottles with her own numbers. From then on, this person was responsible for holding and breaking the codes and for the comparison of results. The samples, after coding, were sent back to the laboratory for complete analysis.

METHODS

The coded urine specimens were extracted for sedative-hypnotics, narcotic analgesics, and amphetamines and congeners both by direct extraction and ion-exchange extraction procedures (1-3). A 50-ml. aliquot of urine was used for the ion-exchange extraction procedure. The drugs were adsorbed on cation-exchange resin loaded paper, and the ion paper was first extracted at pH 1 with chloroform for sedative-hypnotics and then for opiates and amphetamines at pH 10.1 (using NH_4Cl-NH_4OH buffer) with chloroform-isopropanol (3:1). A 15-ml. aliquot of urine was used for the direct extraction procedure. Sedative-hypnotics were extracted at pH 1 with benzene-chloroform (8:2); opiates and amphetamines were extracted at pH 10.1 (using NH_4Cl-NH_4OH buffer) with chloroform-isopropanol (9:1).

Precoated silica gel glass microfiber sheets (Gelman) with a layer thickness of 250 μ were used for TLC. Opiates and amphetamines were chromatographed using ethyl acetate-cyclohexane-ammonium hydroxide-methanol-water (70:15:2:8:0.5) as a developing solvent, and sedative-hypnotics were chromatographed using ethyl acetate-cyclohexane-methanol-ammonium hydroxide (56:40:0.8:0.4) as a developing solvent (1, 3). Opiates and amphetamines were simultaneously detected by spraying the developed chromatogram in succession with ninhydrin, 0.5% H_2SO_4, iodoplatinate, and ammoniacal silver nitrate detection reagents; sedative hypnotics were detected by spraying the developed chromatogram sequentially with diphenylcarbazone, silver acetate, and mercuric sulfate reagents (1-3). Methadone, which was found to be completely extracted at pH 1 along with sedative-hypnotics, was detected by overspraying the plate with I_2-KI solution after the $HgSO_4$ spray.

Table I—Double-Blind Study: Validation of Extraction and Identification Techniques

Mixture of Drugs and Their Concentration (mcg./ml.) in Urine	Number of Specimens Spiked[a]
Morphine (1 mcg.), codeine (1 mcg.), methapyrilene (1 mcg.), methadone (1 mcg.), and quinine (0.5 mcg.)	3
Morphine (1 mcg.) and methadone (1 mcg.)	3
Morphine (0.5 mcg.), phenmetrazine (0.5 mcg.), and methamphetamine (1 mcg.)	3
Morphine (1 mcg.), codeine (1 mcg.), methapyrilene (1 mcg.), methadone (1 mcg.), quinine (0.5 mcg.), and amobarbital (0.5 or 1 mcg.)	3
Morphine (1 mcg.), methapyrilene (1 mcg.), and quinine (0.5 mcg.)	2
Morphine (0.5 mcg.), amobarbital (0.5 mcg.), or phenobarbital (1 mcg.)	6
Morphine (1 mcg.), meperidine (1 mcg.), and secobarbital (1 mcg.)	1
Amphetamine (1.5 mcg.), pentobarbital (1 mcg.), or phenobarbital (1 mcg.)	4
Methamphetamine (0.5 mcg.) and glutethimide (1 mcg.)	3
Amphetamine (2 mcg.) and phenmetrazine (1 mcg.)	3
Codeine (1 mcg.) and secobarbital (1 or 0.5 mcg.)	3
Methadone (1 mcg.), methapyrilene (1 mcg.), and diphenylhydantoin (1 mcg.)	3
Methadone (1 mcg.) and phenobarbital (0.5 mcg.)	2
Methadone (1 mcg.), pentazocine (1 mcg.), and secobarbital (0.5 mcg.)	1
Methamphetamine (1 mcg.) and pentobarbital (0.5 mcg.)	2
Blank urines	8

[a] All urine specimens were extracted both by ion-exchange and direct extraction procedures and results were 100% in agreement with the list of drugs added to each specimen. Methadone was found to be completely extracted at pH 1 along with sedative-hypnotics and was identified by overspraying the plate with I_2-KI detection reagent after applying diphenylcarbazone, silver acetate, and mercuric sulfate sprays (1-3).

Table II—Excretion of Drugs in Human Urine

Drug	Dose	Initial Detection of Drug[a], hr.	Last Detection of Drug (Ion-Exchange Extraction Procedure)[a], hr.
Morphine sulfate	15 mg. intramuscularly	6	72–84[b]
Codeine sulfate	30 mg. orally	4	34[c]
Pentazocine hydrochloride	50 mg. orally	24[d]	60–70[d]
Methadone hydrochloride	38 mg. orally	7.5	56[e]
α-Acetylmethadol hydrochloride[f]	60 mg. orally	6	71–76
Sodium phenobarbital	30 mg. orally	6	24
Sodium secobarbital	60 mg. orally	12[g]	36–40
Amphetamine sulfate	5 mg. orally	3.5	29
Methamphetamine hydrochloride	5–6 mg. orally	3.5	23[h]
Phenmetrazine hydrochloride[i]	8 mg. orally	5	22

[a] Although drugs listed in this column were extracted by both ion-exchange extraction and direct extraction procedures, the number of hours the drugs could be detected was found to be the same for both procedures; any differences have been recorded as footnotes. A 50-ml. aliquot of urine was used for the ion-exchange extraction procedure, and a 15-ml. aliquot was used for the direct extraction procedure. [b] Direct extraction procedure could detect morphine from 24 to 30 hr. [c] Direct extraction procedure could detect codeine until 24 hr. Since codeine is partially metabolized to morphine (10), the presence of morphine could be detected from 15 to 36 hr. [d] The first urine specimen could not be collected earlier than 24 hr. after ingestion of the drug. The direct extraction procedure did not detect pentazocine after 24 hr. [e] The urine was not collected after 56 hr. The person who ingested this drug was not exposed to methadone previously. The metabolite of methadone (11) could be detected if the chromatogram was developed in a solvent consisting of ethyl acetate-cyclohexane-*p*-dioxane-methanol-distilled water-concentrated ammonium hydroxide (50:50:10:10:1.5:0.5). The developed chromatogram was sprayed sequentially with sulfuric acid (0.5% v/v in water) and iodoplatinate detection reagents. [f] α-Acetylmethadol was detected as its metabolites by developing the chromatoplate in ethyl acetate–cyclohexane-*p*-dioxane–methanol–water–ammonium hydroxide (50:50:10:10:1.5:0.5) and spraying the developed chromatogram in succession with 0.5% H_2SO_4, I_2–KI, and iodoplatinate detection reagents (3). [g] Urine specimen could not be collected earlier than 12 hr. [h] Direct extraction procedure could detect this drug up to 17 hr. [i] Preludin.

EXCRETION OF DRUGS IN HUMAN URINE

Several single-blind studies were conducted on staff personnel by administering orally therapeutic doses of amphetamine, methamphetamine, and phenmetrazine. Urine specimens were collected at various time intervals (Table II) and were extracted by both direct extraction and ion-exchange extraction procedures. Codeine, methadone, α-acetylmethadol, morphine sulfate, pentazocine, phenobarbital, or secobarbital was administered to seven human volunteers. Each volunteer received only one drug. Urine was collected at various time intervals and extracted (Table II).

RESULTS AND DISCUSSION

The results of the double-blind study obtained by both procedures were submitted back to the impartial party for breaking the codes. Each drug was reported by its chemical name except the drugs belonging to the sedative-hypnotic group which were reported as barbiturates. On comparison, the results obtained by both procedures were found 100% in agreement with the list of drugs added to each specimen. The technicians analyzing these coded samples were told that all the urines had been "spiked" with routinely tested drugs but that they should record the presence of any additional drug noticed.

Although phenobarbital and diphenylhydantoin could be differentiated from amobarbital, pentobarbital, secobarbital, and glutethimide, no instructions were given to do so. The last four drugs could not be differentiated from each other, except glutethimide whose purple color after $HgSO_4$ fades away rapidly. The purple coloration in the case of the phenobarbital spot after $HgSO_4$ spray does not appear immediately in all cases. Sometimes the appearance of a purple-colored spot takes 30–60 sec., depending upon the concentration of drug present.

An interesting finding was that methadone was almost completely extracted at pH 1 along with barbiturates. However, when two or more ion papers of the same patient are pooled together, methadone is not completely extracted with barbiturates and is detected again along with opiates. Simultaneous detection of amphetamines and congeners and of opiates was achieved using a ninhydrin spraying technique. By using ninhydrin spray, sometimes two or three purplish spots are seen after heating the plate in the oven for 4 min. at 90° (step ii of ninhydrin spraying technique, *References 1–3*), but these spots are well below the level of amphetamine and methamphetamine. These spots, believed due to biogenic amine metabolites, virtually disappear after respraying with ninhydrin and heating on a hot plate maintained at a low temperature for 10–30 sec. (amphetamine and methamphetamine spots undergo different color changes in the first few seconds, and phenmetrazine appears as a bright-pink spot within 10–30 sec.). None of the additional spots seen during step ii of the ninhydrin procedure is stained with iodoplatinate. Only methadone, methapyrilene[1], morphine, codeine, quinine, and psychotropic drugs form characteristic colored spots. The presence or absence of morphine and codeine is based primarily on a positive reaction to ammoniacal silver nitrate spray. After spraying *heavily*, the chromatogram is heated for 30–60 sec. on a hot plate maintained at a medium temperature. Morphine and codeine, which become bleached during the application of the spray, reappear as *distinct* dark-brown or black spots after heat treatment. Spots of questionable existence and spots that behave like morphine and codeine after ammoniacal silver nitrate and heat treatment, but having slight variations in color and R_f values as compared to the known standards, are verified by spraying with potassium permanganate (0.02 *M* in water). The chromatogram is then heated on the hot plate as already described for a few seconds. The spots other than morphine and codeine disappear, thus reducing further the possibility of false positives for morphine. If desired, the plate may be oversprayed with ammoniacal silver nitrate and heated.

The identification of methadone along with barbiturates is very specific because the drugs like cocaine, pipradrol, and pentazocine[2] which can give a false test for methadone when sprayed along with opiates are not extracted at pH 1.

The value of the results of the double-blind study lies in validating the efficacy and reliability of the extraction and identification techniques previously reported by the authors.

The data on the excretion of some drugs in human urine as given in Table II have been included as a guideline for clinicians evaluating the urine reports of drug-dependent individuals. Although adequate information is available in the literature and in some reference books (5, 6) on the metabolism of the drugs listed in Table II, very little is known about the number of hours or days that these drugs can be detected in human urine by routine methods of detection after a minimum single therapeutic dose. This information is particularly of great value to clinicians working for a methadone maintenance treatment program for pacing the collection of urine from the drug-dependent individuals attending the outpatient clinics.

Mulé (7), using the direct extraction procedure and collecting urines at time intervals of 6 through 24 hr., was able to detect the presence of amphetamine or methamphetamine from patients who had received 15 mg. of either of these drugs. Heaton and Blumberg (8) reported that by using the direct extraction procedure, they could detect secobarbital in human urine for 4–6 days after a single 3-gr. (195 mg. approximately) dose. Ikekawa *et al.* (9), using GC analysis, were able to detect morphine up to 72 hr. (if 100 ml. of urine was used for analysis) in five of seven cases after a 10-mg. s.c. injection.

[1] Histadyl.
[2] Talwin.

In the single-blind study reported here, a minimum single therapeutic dose of each drug, without disclosure of its name, was given to each subject. Urines were collected from each individual before the ingestion of the drug to serve as control specimens. Several urine specimens were collected from each individual at regular time intervals of 6–10 hr. between the initial and last detection of the drug. The data on the first and last detection of each drug in human urine in Table II can vary from one investigator to another, depending upon the sensitivity of the extraction and identification procedures used. These data are based on the detection of the unchanged drug, except α-acetylmethadol which was detected as its metabolite. Spraying techniques used to identify each drug were reliable and specific.

REFERENCES

(1) K. K. Kaistha and J. H. Jaffe, paper presented at the 33rd Meeting of the Committee on the Problems of Drug Dependence, National Academy of Sciences, National Research Council, Toronto, Ontario, Canada, Feb. 1971; vol. 1, pp. 576–600.

(2) K. K. Kaistha and J. H. Jaffe, *J. Chromatogr.*, **60**, 83(1971).

(3) K. K. Kaistha and J. H. Jaffe, to be published.

(4) K. K. Kaistha and J. H. Jaffe, to be published.

(5) E. G. C. Clarke, "Isolation and Identification of Drugs," Pharmaceutical Press, London, England, 1969.

(6) "The Pharmacological Basis of Therapeutics," 4th ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N. Y., 1970.

(7) S. J. Mulé, *J. Chromatogr.*, **39**, 302(1969).

(8) A. M. Heaton and A. G. Blumberg, *ibid.*, **41**, 367(1969).

(9) N. Ikekawa, K. Takayama, E. Hosoya, and T. Oka, *Anal. Biochem.*, **28**, 156(1969).

(10) G. J. Mannering, A. C. Dixon, E. M. Baker, and T. Asami, *J. Pharmacol. Exp. Ther.*, **111**, 142(1954).

(11) A. H. Beckett, J. F. Taylor, A. F. Casy, and M. M. A. Hassan, *J. Pharm. Pharmacol.*, **20**, 754(1968).

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Preparation of Pure *meso*-Tetraphenylporphine and Two Derivatives

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Abstract ☐ Optimal conditions for the preparation and purification of *meso*-tetraphenylporphine, methyl-*meso*-tetraphenylporphine, and methoxy-*meso*-tetraphenylporphine were studied. The reaction of equimolar concentrations of pyrrole and the respective aldehyde in propionic acid gave the best yield of porphine compounds. These compounds were further purified by column chromatography and, according to elemental analyses, found to be of high purity.

Keyphrases ☐ *meso*-Tetraphenylporphine, derivatives—preparation, purification ☐ Methyl-*meso*-tetraphenylporphine—preparation, purification ☐ Methoxy-*meso*-tetraphenylporphine—preparation, purification ☐ Column chromatography—purification of *meso*-tetraphenylporphine and derivatives

Porphyrin compounds elicit a wide range of pharmacologic activities. The addition of various groups to the porphine ring may alter both the biologic activity and distribution. To study the pharmacology of these compounds accurately, it is necessary to obtain products of high purity. *meso*-Tetraphenylporphine derivatives have been prepared by the condensation of pyrrole and an aldehyde under pressure (1, 2) or by refluxing in acetic acid (3). The isolation of a pure product from these reaction mixtures was found to be difficult and of a low yield. Adler *et al.* (4, 5) prepared *meso*-tetraphenylporphine by refluxing pyrrole and benzaldehyde in propionic acid. On cooling, *meso*-tetraphenylporphine crystallized from the reaction mixture and was easily isolated. The optimum conditions for the preparation in propionic acid of *meso*-tetraphenylporphine, *p*-methoxy-*meso*-tetraphenylporphine, and *p*-methyl-*meso*-tetraphenylporphine are reported here.

MATERIALS AND METHODS

Determination of Optimum Reaction Times—*meso*-Tetraphenylporphine, *p*-methoxy-*meso*-tetraphenylporphine, and *p*-methyl-*meso*-tetraphenylporphine were prepared by reaction of equimolar concentrations (0.1 mole) of freshly distilled pyrrole[1] and the respective aldehyde (benzaldehyde[2], anisaldehyde[3], or *p*-tolualdehyde[4]) in 500 ml. of propionic acid[2]. The reactions were refluxed for 12 hr.

One-milliliter aliquots of the hot refluxing reactions were obtained during the 12-hr. period. Each aliquot was added to 19 ml. of benzene, and the visible spectra were determined on a spectrophotometer[5]. The most prominent spectral peak of each of the three compounds occurred at 515 nm., which is in agreement with the spectra reported by Badger *et al.* (6). The absorbance of each aliquot of the reaction mixture was determined at 515 nm. The results are presented in Fig. 1.

Molar Ratio Studies—Four different molar concentrations of pyrrole and aldehyde were used for the preparation of *meso*-tetraphenylporphine, *p*-methoxy-*meso*-tetraphenylporphine, and *p*-methyl-*meso*-tetraphenylporphine. The molar ratios of pyrrole to aldehyde were 1:1, 1:2, 1:4, and 2:1. Each reaction was carried out in 500 ml. of propionic acid. The appropriate quantities of

[1] Mallinckrodt.
[2] Matheson, Coleman and Bell.
[3] K & K Laboratories.
[4] Eastman Kodak.
[5] Perkin-Elmer 123.