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EXTRACTION TECHNIQUES FOR NARCOTICS, BARBITURATES, AND CENTRAL NERVOUS SYSTEM STIMULANTS IN A DRUG ABUSE URINE SCREENING PROGRAM

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SUMMARY

Several different extraction techniques, each of which is well suited to large scale screening of urines for drugs of abuse, are presented. Each technique has special advantages, depending on the needs of a clinical operation. In the paper we have compared three basic approaches.

(I) Extraction of drugs from urines by absorbing them on cation-exchange resin loaded paper and then eluting the drugs from the paper with two consecutive buffer-solvent systems. Sedative-hypontics are extracted at pH 1.0 using citrate buffer, and amphetamines and opiates at pH 10.1 using NH_4Cl–NH_4OH buffer.

(II) Direct extraction of drugs from an urine specimen first at pH 1 using HCl for sedative-hypnotics and then at pH 10.1 using NH_4Cl–NH_4OH for amphetamines and opiates. When the goal is limited to the detection of amphetamine and congeners only, the urine is extracted at pH 12 using NaOH.

(III) Acid hydrolysis of urine specimens followed by direct extraction of drugs at pH 10.1 using NH_4Cl–NH_4OH buffer. Our standard procedure involves the use of ion-exchange extraction technique (procedure I); procedure II is used for urine specimens of less than 40 ml volume. Procedure I seems best if (a) urine collection is to be paced at 2–3 day intervals; (b) volume of urine available is 40–50 ml or more; (c) pooling of ion papers of the same patient representing different urine specimens is desired to cut down the cost of analysis and work load without diluting the sensitivity of the test. This procedure has been compared with procedures II and III and some of the relative advantages and disadvantages of each procedure are presented.

INTRODUCTION

It is now generally accepted that programs for management of drug dependent individuals should include provisions for determining the extent of drug use by those in the program, even if such information is used only to assess the efficacy of the

management technique. The chemical analysis of urine is one approach to determine drug taking by individuals in such programs. At present it seems to be the predominant technique. Many methods for detecting commonly abused drugs in urine are available[1–17], however, these methods vary greatly with respect to their suitability for use in large scale urine monitoring programs. Among the criteria by which methods must be judged are: (1) Rapidity of analysis—the entire procedure from acquisition of urine specimen to reporting of results should not take more than 24 h; (2) sensitivity—the extraction procedure and spraying techniques must be able to detect the presence of ingested drug and/or its metabolites for at least 24–72 h following the use of drugs at commonly used doses; (3) convenience—laboratory personnel with minimal formal training should be capable of completing the entire analysis including the interpretation of results; (4) economy.

The direct extraction method proposed by MULÉ[9] involved differential pH extraction and required three separate extractions on three 15 ml aliquots of urine at pH's 2.2, 9.3 and 11.0 for barbiturates, opiates and amphetamines, respectively. MULÉ also reported[9] that the method of BECKETT AND ROWLAND[13] for the detection of amphetamine in his hands yielded poor results. HEATON AND BLUMBERG[10] described direct extraction of barbiturates from a 5 ml aliquot of urine at pH 6 with which they were able to detect secobarbital for 4–6 days after a single dose of three grains (about 195 mg). DAVIDOW *et al.*[8] described a single step direct extraction procedure for narcotics, amphetamines and barbiturates at pH 9.5 using a 10 ml aliquot of urine but the reported sensitivity for amphetamines and barbiturates was only 5 μg/ml of urine.

DOLE *et al.*[7] used cation-exchange resin loaded paper to absorb the drugs from urine and then eluted the drugs from the paper with three consecutive extractions at pH's 2.2, 9.3, and 11, respectively. This method when applied by HEATON AND BLUMBERG[10] yielded poor recoveries for barbiturates and amphetamines. Similarly MULÉ[9] using this procedure after modifications reported poor recoveries for barbiturates, methadone, and amphetamines.

The purpose of this report is to present our comparison of several extraction techniques applicable to large scale screening programs. These extraction techniques involve three basic approaches.

Procedure I is the extraction of drugs from urines by absorbing them on a 6 × 6 cm piece of cation-exchange resin loaded paper and then eluting the drugs from the paper in two steps. This is a modification of the method developed by DOLE *et al.*[7]. The modification described here elutes sedative-hypnotics at pH 1, and opiates and amphetamines at pH 10.1 using NH_4Cl–NH_4OH buffer. This procedure is used routinely in our laboratories for all urine specimens that require simultaneous screening of opiates and amphetamines. Urine specimens submitted for screening of opiates only are also extracted by this procedure, but borate buffer, pH 9.3 (ref. 7) is used.

Procedure II is a direct extraction of drugs from a urine specimen, and like procedure I is a two-step procedure (*i.e.* sedative-hypnotics are extracted at pH 1, and amphetamines and opiates at pH 10.1). This procedure is a modification of single step direct extraction procedure developed by DAVIDOW *et al.*[8]. Urine specimens of less than 40 ml volume are monitored using procedure II.

Procedure III involves acid hydrolysis of urine specimens followed by a single step direct extraction of drugs at pH 10.1 using NH_4Cl–NH_4OH buffer; it is a modifi-

cation of the technique used by KOKOSKI *et al.*[11,12]. This procedure is not routinely used in our laboratories.

METHODS

Procedure I. Ion-exchange extraction of drugs followed by two-step elution

A 6 × 6 cm piece of Reeve Angel SA-2 cation-exchange resin loaded paper (marked with patients' I.D. number or name with lead pencil) is soaked in 40–50 ml of undiluted urine (pH 5–6) with intermittent shaking. After 30 min or more, ion paper is transferred into a plastic bag and sent to the laboratory for the desired analysis. To decrease the work load and cost of analysis, the routine procedure used in the laboratory, at present, is to pool several ion papers of the same patient representing different urine specimens. The single or pooled ion papers are transferred to 4 oz. wide mouth screw capped jars, rinsed twice with distilled water (rinsing is important to prevent emulsion formation in the extraction procedure) and extracted for different groups of drugs as follows:

(a) Sedative-hypnotics, benzodiazepines, and other drugs. To each jar containing ion-exchange paper, 15 ml each of sodium citrate buffer, pH 1.0* and chloroform are added (20 ml of each are used if the jar contains more than one ion paper). After shaking for 10 min on a reciprocating shaker, the lower organic phase is pipetted out into a plain 15 ml conical centrifuge tube. The aqueous phase is discarded and ion paper is saved for step (b). The solvent is evaporated in an oven (65° for the first 3 h, then 85° until dry) having horizontal air flow. The residue along the sides of the tube is washed with 0.5 to 1 ml of methanol and methanol evaporated to dryness as above. The residue thus obtained is redissolved in 25–30 μl of methanol and the entire extract is spotted on the chromatographic plate.

(b) Narcotic analgesics, amphetamine and congeners, and selected psychotropic drugs. Ion paper left after the extraction of sedative-hypnotics is then extracted at pH 10.1** by adding 15 ml each of chloroform–isopropanol (3:1) and NH_4Cl–NH_4OH buffer (20 ml of each are used if the jar contains more than one ion paper). After shaking for 10 min, the lower organic phase is transferred to a 15 ml plain conical centrifuge tube containing two drops (about 50 μl) of sulfuric acid in methanol (0.5% H_2SO_4 in methanol). The evaporation process is completed as described above (procedure Ia). Sulfuric acid is omitted if amphetamines are not to be detected.

Procedure II. Direct extraction of drugs from urine

As we shall point out below, ion-exchange extraction of drugs from urine requires at least 40 ml of urine if the likelihood of detection of positives is to remain comparable to direct extraction. Therefore, urine specimens of less than 40 ml volume are not analyzed by an ion-exchange extraction procedure, but instead are extracted directly for various groups of drugs as desired.

(a) Sedative-hypnotics, benzodiazepines, and other drugs. To a 15 ml aliquot of urine in a 50 ml screw capped round bottom centrifuge tube, 5 ml of 3.7% HCl

* Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) 296 g in water, followed by 256 ml concentrated HCl, diluted to 2,000 ml with water (pH 1.0 ± 0.1).

** Saturated solution of ammonium chloride (2,500 ml) adjusted to pH 10.1 ± 0.1 with concentrated ammonium hydroxide (about 2,400 ml).

(producing pH 1) and 15 ml of benzene–chloroform (8:2) are added. The tube is *shaken gently* by hand in a vertical direction for 1 min, and centrifuged for 5–7 min if needed to break emulsion. If emulsion still persists after centrifuging, it is cleared by adding 1–2 ml of absolute ethanol. The upper organic phase is then pipetted out into a 15 ml plain, conical centrifuge tube and evaporation process is completed as described above (procedure Ia). The residual aqueous acidic phase is saved if amphetamines and opiates are to be tested.

(b) Single stage extraction of narcotic analgesics, amphetamine and congeners, and selected psychotropic drugs. To the residual aqueous phase remaining after extraction of sedative-hypnotics (procedure IIa) or to a 15 ml aliquot of urine (if barbiturates analysis is not desired) in a 50 ml screw capped round bottom centrifuge tube are added 10 ml of NH_4Cl–NH_4OH buffer (pH 10.1) and 15 ml of chloroform–isopropanol (9:1). The tube is *shaken gently* for 1 min, centrifuged (if necessary), and the lower organic phase is pipetted out into a 15 ml plain conical centrifuge tube containing two drops (50 μl) of sulfuric acid in methanol (0.5% H_2SO_4 in methanol). The evaporation process is completed as described above (procedure Ia). Sulfuric acid is omitted if amphetamines are not to be tested.

(c) To extract amphetamine and congeners, and certain narcotic analgesics other than morphine. This method has been designed primarily for monitoring urines for amphetamine, methamphetamine, and phenmetrazine when it is not necessary to screen simultaneously for morphine. The method appears to be specific, sensitive, and reproducible. To a 15 ml aliquot of urine in a 50 ml screw capped round bottom centrifuge tube, 10 ml of 0.75% sodium hydroxide and 15 ml of benzene–chloroform (8:2) are added. The process is completed as above (procedure IIb) except that it is the upper organic phase which is pipetted out into a 15 ml plain conical centrifuge tube containing two drops (50 μl) of H_2SO_4 in methanol.

Procedure III. Acid hydrolysis of urine specimens

Since as much as 83% of the total morphine[18] and 88% of the total codeine[19] may be excreted as their glucuronides, acid hydrolysis of urine specimens collected infrequently becomes of great value. In such cases urine specimen is first hydrolyzed and then drugs are extracted directly at a pH 10.1 as described below.

(a) Sedative-hypnotics, benzodiazepines, and other drugs. This group of drugs is extracted without subjecting the urine to acid hydrolysis as described under procedure IIa using a 15 ml aliquot of urine.

(b) Hydrolysis of glucuronic acid conjugate of morphine and codeine. To a 10 ml aliquot of urine in a 50 ml screw capped round bottom centrifuge tube is added 1 ml of conc. HCl (more than 1 ml of conc. HCl is added for specimens showing effervescence). The tube (uncapped) is placed in a boiling water bath*. After 1 h, the tube is cooled, 15 ml each of NH_4Cl–NH_4OH buffer (pH 10.1) and chloroform–isopropanol (9:1) are added and contents shaken gently for 1 min. After centrifuging (if necessary), the lower organic phase is pipetted out into a 15 ml plain conical centrifuge tube containing two drops (50 μl) of sulfuric acid in methanol (0.5% H_2SO_4 in methanol). The evaporation process is completed as described above (procedure Ia). Sulfuric acid is omitted if amphetamines are not to be detected.

TABLE I

SENSITIVITY OF EXTRACTION TECHNIQUES

Gelman precoated silica gel glass microfiber sheets with a layer thickness of 250 μ have been used for TLC because of the ease with which they can be handled. The specific color reactions presented here may not be obtainable on glass plates coated with silica gel.
+ (positive), — (negative), ± (trace) and o (test not performed).

Drug	*Drug added per ml of urine (µg)*							
Sedative-hypnotics	*Ion-exchange extraction of drugs (procedure Ia using 50 ml of urine)*[a]				*Direct extraction of drugs (procedure IIa using 15 ml of urine)*[b]			
	0.5	*1*	*1.5*	*2*	*0.5*	*1*	*1.5*	*2*
Amobarbital	+	o	o	o	+	o	o	o
Barbital sodium[a]	—	—	—	—	—	—	—	±
Diphenylhydantoin (Dilantin)	±	+	o	o	±	+	o	o
Glutethimide (Doriden)	—	+	o	o	—	+	+	o
Pentobarbital	+	o	o	o	+	o	o	o
Phenobarbital	±	+	o	o	±	+	o	o
Secobarbital	+	o	o	o	+	o	o	o

Amphetamine and congeners	*Ion-exchange extraction of drugs (procedure Ib using 50 ml of urine)*[c]				*Direct extraction of drugs (procedure IIb using 15 ml of urine)*[d]				*Direct extraction of drugs (procedure IIc using 15 ml of urine)*[e]				
	0.5	*1*	*1.5*	*2*	*0.5*	*1*	*1.5*	*2*	*0.5*	*1*	*1.5*	*2*	*2.5*
Amphetamine[f]	—	+	o	o	—	—	+	+	—	+	o	o	o
Methamphetamine[f]	±	+	o	o	±	+	o	o	±	+	o	o	o
Phenmetrazine[f]	+	+	o	o	+	+	o	o	+	+	o	o	o
Ephedrine[f]	—	—	—	+	—	—	—	+	—	—	—	±	+
Pipradrol[f]	—	+	o	o	—	+	o	o	—	+	o	o	o
Methylphenidate[g] (Ritalin)	+	o	o	o	—	+	o	o	—	+	o	o	o
Narcotic analgesics their adulterants, and miscellaneous drugs													
Morphine[f]	+	o	o	o	+	o	o	o	o	o	o	o	o
Codeine[f]	+	o	o	o	±	+	o	o	o	o	o	o	o
Methadone[f]	±	+	o	o	±	+	o	o	—	+	o	o	o
Methapyrilene[f] (used as an adulterant with heroin)	±	+	o	o	±	+	o	o	—	+	o	o	o
Quinine[f] (used as an adulterant with heroin)	+[h]	o	o	o	+[h]	o	o	o	+	o	o	o	o
Hydromorphone[f]	—	+	o	o	—	+	o	o	o	o	o	o	o
Meperidine[f] (Demerol)	+	o	o	o	—	+	o	o	o	o	o	o	o
Pentazocine[f] (Talwin)	+	o	o	o	—	+	o	o	o	o	o	o	o
Cyclazocine[f]	+	o	o	o	—	+	o	o	o	o	o	o	o
Propoxyphene[f] (Darvon)	—	+	o	o	—	+	o	o	o	o	o	o	o
Cocaine[f]	±	+	o	o	—	+	o	o	o	o	o	o	o

(continued on p. 88)

TABLE I *(continued)*

Drug	*Drug added per ml of urine (μg)*												
Narcotic analgesics their adulterants, and miscellaneous drugs	*Ion-exchange extraction of drugs (procedure Ib using 50 ml of urine)*[c]				*Direct extraction of drugs (procedure IIb using 15 ml of urine)*[d]				*Direct extraction of drugs (procedure IIc using 15 ml of urine)*[e]				
	0.5	*1*	*1.5*	*2*	*0.5*	*1*	*1.5*	*2*	*0.5*	*1*	*1.5*	*2*	*2.5*
Lysergic acid-diethylamide[f] (LSD)	±	+[i]	o	o	±	+[i]	o	o	o	o	o	o	o
Mescaline[f]	±	+[i]	o	o	—	+[i]	o	o	o	o	o	o	o
2,5-Dimethoxy-4-methylamphetamine[f] ("STP"; DOM)	±	+[i]	o	o	—	+[i]	o	o	o	o	o	o	o
Chlordiazepoxide[g] (Librium)	—	—	±	+[i]	—	—	—	+[i]	o	o	o	o	o
Chlorpromazine[f] (Largactil, Thorazine)	+	o	o	o	—	+	o	o	o	o	o	o	o
Trifluoperazine[f] (Stelazine, Eskazine)	+	o	o	o	—	+	o	o	o	o	o	o	o

[a] Barbiturates are extracted at pH 1.0 using citrate buffer and chloroform. Sensitivities presented were obtained by spraying the developed chromatogram in succession with detection reagents diphenylcarbazone[8], silver acetate[7] and mercuric sulfate[8]. Sodium barbital was detected as a bluish purple spot after silver acetate. However, on application of mercuric sulfate spray, the spot faded but reappeared within a minute as a faint purple spot (detection level 3–4 μg/ml of urine after silver acetate). Methadone, if present in a urine specimen, would be extracted along with the barbiturates and can be detected by overspraying the plate with the detection reagent I_2–KI[20] after $HgSO_4$ spray.

[b] This procedure involves direct extraction of urine at pH 1.0 using 3.7% HCl and benzene–chloroform (8:2). Using detection technique as described above, sodium barbital could be detected at a level of 2–3 μg/ml of urine.

[c] This procedure is capable of extracting amphetamines and opiates simultaneously at pH 10.1 using NH_4Cl–NH_4OH buffer and chloroform–isopropanol (3:1).

[d] This procedure involves direct extraction of urine at pH 10.1 using NH_4Cl–NH_4OH buffer and chloroform–isopropanol (9:1). Like ion-exchange extraction procedure, it is capable of extracting amphetamines and opiates simultaneously.

[e] This procedure involves direct extraction of urine at pH 11–12 and has been designed primarily for monitoring urines for amphetamine, methamphetamine, and phenmetrazine when it is not neccesary to screen simultaneously for morphine.

[f] Sensitivities presented for these drugs were obtained by spraying the developed chromatogram in succession with detection reagents ninhydrin (0.5% w/v) in *n*-butanol, sulfuric acid (0.5%) in H_2O, iodoplatinate[7] and ammoniacal silver nitrate[7]. After ninhydrin spray, the following steps have been found necessary to detect amphetamine and congeners: (i) The plate is irradiated under short wave length for 5 min. Amphetamine may appear as a light greyish spot; (ii) the plate is then heated at 90° for 4 min; methamphetamine, pipradrol and methylphenidate can be seen at this stage; (iii) re-irradiating under short-wave length for 5 min increases the intensity of amphetamine spot if it had appeared earlier or causes amphetamine to appear as a light grey or greyish blue spot. This step is omitted if amphetamine becomes visible after steps (i) and/or (ii); (iv) respraying with ninhydrin solution and heating for a second on a hot plate maintained at a low temperature causes amphetamine and methamphetamine to undergo different color changes. Sometimes methamphetamine appears only at this stage. Heating is continued for 10–30 sec until phenmetrazine appears as a bright pink spot, sometimes it is necessary to respray and reheat for a few seconds to see phenmetrazine[21]. Detection of morphine and codeine is based primarily on a positive reaction to the ammoniacal silver nitrate spray. After applying this spray, 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 spray reappear as distinct dark brown or brown or black spots after heat treatment[21].

[g] Sensitivities presented for these drugs were obtained by spraying the developed chromatogram in succession with detections reagents I_2–KI[20] and iodoplatinate[7].

[h] Quinine can be detected under short UV light at a level as low as 0.05 μg/ml of urine if the chromatogram is first sprayed with 0.5% H_2SO_4.

[i] These drugs were extracted as described under procedures Ib and IIb but no sulfuric acid was added to the chloroform–isopropanol extract.

RESULTS AND DISCUSSION

Sensitivities found and presented in Table I are based on tests in which pure drugs were added to control urine (obtained from laboratory personnel). Several single blind studies were conducted on staff personnel by administering orally therapeutic doses of amphetamine (5 mg), methamphetamine (5–6.5 mg), phenmetrazine (8 mg), phenobarbital (29 mg), and secobarbital (60 mg). Urine specimens were collected at various time intervals beginning from 3.50 h through 24 h. Phenobarbital and secobarbital could be detected by procedure Ia and IIa from 6 h until 24 h; amphetamine and methamphetamine by procedure Ib and IIb from 3.50 h until 17–24 h, and phenmetrazine by procedure Ib and IIb from 6 h until 14–22 h. Double blind studies on amphetamine (6.5 and 7.5 mg), methamphetamine (4 and 5 mg), phenmetrazine (5 and 8 mg), and pentobarbital (28 mg) were also performed by administering orally single dosage of these drugs to the clinical staff personnel. Urines were collected within 12 h of drugs usage and analyzed by proposed extraction techniques. All drugs could be detected satisfactorily.

All of the extraction and thin-layer identification techniques described in this report can be satisfactorily applied in a large scale clinical program involving urine monitoring of drug addicts and abusers. Yet, which technique is optimally useful depends on the needs of a clinical operation. Both procedures (procedure I, using cation-exchange resin loaded paper and procedure II, using direct extraction of drugs) are capable of extracting different groups of drugs in two steps. Sedative-hypnotics, benzodiazepine compounds, some methadone* and its metabolite are extracted at pH 1 (procedures Ia and IIa); amphetamines and opiates are extracted simultaneously at pH 10.1 (procedures Ib and IIb).

MULÉ[9] eluted different groups of drugs from the ion paper with three consecutive extractions and reported that barbiturates could be detected at levels of 1–5 μg/ml of urine (phenobarbital and sodium barbital could not be detected at levels below 5 μg/ml of urine) at a pH of 2.2. He was able to detect morphine and other narcotic analgesics at a level of 0.5–1 μg/ml of urine at a pH of 9.3 (methadone at a level of 5 μg/ml) and amphetamines at a level of 10 μg/ml of urine at a pH of 11.0. However, using direct extraction of drugs from urine specimen at the same three pH's he was able to increase the sensitivity for barbiturates (except sodium barbital), methadone and amphetamine to yield positive results at a concentration of 1 μg/ml of urine. We found that extracting barbiturates at pH 1 and using the spraying technique described here (Table I, footnotes), we were able to detect barbiturates at a concentration of 0.5–1 μg/ml of urine (sodium barbital at a level of 2–4 μg/ml of urine) both by using ion-exchange paper technique and direct extraction of drugs. However, chlordiazepoxide has been found to give a metabolite that behaved like barbiturates and interfered with the detection of phenobarbital and/or sodium barbital and still remains a problem to be solved.

* Extraction of control urines having concentration of methadone at a level of 1 μg/ml of urine showed that methadone is completely extracted at pH 1 along with barbiturates by both procedures. Hence urine specimens required to be monitored for barbiturates and opiates should be checked for the presence of methadone along with barbiturates by the spraying technique proposed in this report (see footnotes, Table I). The extraction of methadone at pH 1 appears to be a specific procedure for the identification of this drug. Drugs like propoxyphene (Darvon), pipradrol, diphenhydramine (Benadryl), pentazocine (Talwin), and cocaine, which can give a false test for methadone using thin-layer techniques, are not extracted at this pH.

TABLE II

COMPARISON OF SENSITIVITY OF TWO ALTERNATE EXTRACTION PROCEDURES IN DETECTING BARBITURATES AND RELATED COMPOUNDS IN URINE SAMPLES OBTAINED FROM A NARCOTICS TREATMENT PROGRAM

Number of specimens compared	*Ion-exchange extraction of drugs (procedure Ia)*[a]			*Direct extraction of drugs (procedure IIa)*[b]
	Barbiturate positive specimens using 25 ml of urine	*Barbiturate positive specimens using 40–50 ml of urine*	*Total barbiturates positive specimens using ion-exchange extraction technique*	*Barbiturate positive specimens using 15 ml of urine*
122	8	4	8 + 4 = 12[c]	12[c]

[a] Procedure same as (a), Table I.
[b] Procedure same as (b), Table I.
[c] Later investigations revealed that one of the metabolite of chlordiazepoxide (other than lactam and open lactam) behaved like barbiturates if the developed chromatogram is sprayed in succession with diphenylcarbazone, silver acetate and mercuric sulfate solutions. We have not excluded the possibility of the presence of chlordiazepoxide in these samples.

TABLE III

COMPARISON OF SENSITIVITY OF PROPOSED EXTRACTION PROCEDURES IN DETECTING AMPHETAMINES IN URINE SAMPLES OBTAINED FROM A NARCOTICS TREATMENT PROGRAM

Number specimens compared	*Ion-exchange extraction of drugs (procedure Ib)*[a]			*Direct extraction of drugs*		*Direct extraction of drugs after acid hydrolysis*
	Amphetamines positive using 25 ml of urine	*Amphetamines positive using 40–50 ml of urine*	*Total amphetamines positive using ion-exchange extraction technique*	*Amphetamines positive using 15 ml of urine (procedure IIb)*[b]	*Amphetamines positive using 15 ml of urine (procedure IIc)*[c]	*Amphetamines positive using 10 ml of urine (procedure IIIb)*[d]
126	4	3	4 + 3 = 7	7	7	3

[a] Procedure same as (c), Table I.
[b] Procedure same as (d), Table I.
[c] Procedure same as (e), Table I.
[d] Although this procedure involving acid hydrolysis is intended for the detection of total morphine, its feasibility to detect amphetamines was assessed. Data suggest that it will be advisable to detect amphetamines before acid hydrolysis.

In our effort to extract amphetamines and opiates in a single step by using NH_4Cl–NH_4OH buffer (pH 10.1), we were able to detect morphine at a level of 0.5 μg/ml of urine, codeine 0.5–1 μg/ml of urine, methadone 1 μg/ml of urine and amphetamines 0.5–2 μg/ml of urine. We were able to obtain these sensitivities (determined by working with control urines to which these drugs had been added) both by ion-exchange extraction and direct extraction procedures (procedures I and II). In fact, concentration of morphine as low as 10 μg in 50 ml of urine could be detected by proposed ion-exchange extraction procedure and spraying techniques (same sensitivity for morphine was obtained at pH 9.3).

Data presented in Tables II, III and IV show that ion-exchange extraction technique is capable of yielding satisfactory results if an adequate volume of urine (40–50 ml) is available. Direct extraction of drugs from a urine specimen (procedures IIa, IIb and IIc) is certainly superior if the volume of urine available is less than 40 ml. When the goal is the detection of morphine only and the volume of urine available is less than 40 ml, acid hydrolysis on a 10 ml aliquot of urine seems to be a better choice (procedure IIIb). A study of morphine excretion in human urine after 15 mg dosage (administered I.M. to a single volunteer) showed that procedure Ib (ion-exchange extraction procedure) is capable of detecting morphine until 72–84 h if the

TABLE IV

COMPARISON OF SENSITIVITY OF THREE ALTERNATE EXTRACTION PROCEDURES IN DETECTING MORPHINE IN URINE SAMPLES OBTAINED FROM A NARCOTICS TREATMENT PROGRAM

Number of specimens compared	*Ion-exchange extraction of drugs (procedure Ib)*[a]			*Direct extraction of drugs (procedure IIb)*[b]	*Direct extraction of drugs after acid hydrolysis (procedure IIIb)*[c]
	Morphine positive specimens using 25 ml of urine	*Morphine positive using 40–50 ml of urine*	*Total morphine positive using ion-exchange extraction technique*	*Morphine positive using 15 ml of urine*	*Morphine positive using 10 ml of urine*
118	31	33	31 + 33 = 64	55	64

[a] Procedure same as (c), Table I.
[b] Procedure same as (d), Table I.
[c] This procedure involves acid hydrolysis of glucuronic acid conjugate of morphine on a 10 ml aliquot of urine followed by direct extraction of total morphine.

volume of urine used is 50 ml. Procedure IIb (direct extraction procedure) could detect morphine only up to 30 h due to limitation of the volume of urine used.

Obviously the sensitivity of direct extraction procedure can be increased and would be greater than that of the ion-exchange technique if a comparable large volume of urine is used. However, the formation of emulsion necessitating routine centrifugation, the use of large size centrifuge tubes necessitating repeated washings

are some of the problems that have to be worked out. In the procedures reported here we are extracting barbiturates, opiates and amphetamines using the same 15 ml aliquot of urine to keep the use of glassware like 50 ml and 10 ml centrifuge tubes and pipettes etc. at a minimum. We feel that clinical operations pacing the collection of urines at 48–72 h intervals can satisfactorily employ ion-exchange extraction procedure for all urines having volumes larger than 40 ml. The relative advantages and disadvantages of this procedure as compared to the direct extraction procedure are presented in Table V.

It is suggested that each batch of cation-exchange resin loaded SA-2 paper

TABLE V

COMPARISON OF ION-EXCHANGE EXTRACTION AND DIRECT EXTRACTION PROCEDURES

Factor	*Direct extraction procedure*	*Ion-exchange extraction procedure*
Shipping of samples	Liquid urine shipped, preservative needed if more than 12 h are required to reach destination.	No liquid urine to be shipped, ion-exchange paper after treatment with undiluted urine at collection station is shipped in an envelope.
Storage of urines	Urines require cold storage until analysis. Sometimes space problems do arise on a large scale screening program.	No cold storage and space problems. Thousands of ion papers can be stored (in a refrigerator to prevent fungus growth) using minimum space until ready to be processed.
Pooling of different urine specimens of the same patient	Not possible without diluting the sensitivity of the test.	Several ion papers of the same patient representing different urine specimens can be pooled without any loss of sensitivity.
Cost of analysis	As the pooling of different urine specimens of the same patient is not possible, the cost of analysis incurred per urine test per patient will be more, especially for clinical programs where patients are required to leave 2–3 urines per week in the early phase of treatment and where a patient population receiving treatment is more than 1500.	Cost of analysis can be cut down substantially by pooling several ion papers representing different urine specimens of the same patient.[22]
Minimum volume of urine required for analysis	10–15 ml is adequate for testing all three groups of drugs as the same aliquot of urine is used for the complete analysis. This procedure is excellent for situations where urine can not be collected in volumes more than 10–15 ml.	40–50 ml of undiluted urine is recommended to achieve the best results.
Maximum volume of urine that can be used	Maximum of 15 ml of urine using 50 ml centrifuge tube can be tested.	No limit, up to 100 ml can be used.
Preliminary treatment of urine	None, operator can proceed directly with the desired procedure.	Although pH 5–6 of urine is recommended before adding ion paper to urine, the pH 7–7.2 has not shown any adverse effects for the detection of opiates. Morphine was absorbed by the ion paper even when ion paper was added to a control urine at pH 7.2.

TABLE V *(continued)*

Factor	*Direct extraction procedure*	*Ion-exchange extraction procedure*
Sensitivity	Direct extraction procedures for barbiturates and amphetamines are sensitive if the volume of urine provided by the patient is less than 40 ml. For morphine detection, direct extraction of urine on a 10 ml aliquot after acid hydrolysis is the method of choice for urine specimens of less than 40 ml volume.	This investigation showed that ion-exchange extraction technique is the method of choice for the detection of barbiturates, amphetamines, and opiates for urine specimens of more than 40 ml volume. Acid hydrolysis of urines followed by direct extraction of morphine need not be adopted as a routine monitoring procedure. Using ion-exchange extraction technique, urine collection can be paced at 48–72 h intervals.
Simplicity and rapidity of analysis	Simple and rapid, no special supplies and equipment needed except a centrifuge machine. All chemicals and solvents easily available.	Simple and rapid but dependent upon the supply of SA-2 cation-exchange resin loaded paper. No special equipment needed except a shaker. All chemicals and solvents easily available.
Reproducibility and reliability.	Reproducible and reliable.	Reproducible and reliable.

should be checked for sensitivity for amphetamines, barbiturates and opiates by adding these drugs to control urines and then carrying through assay procedures.

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