CHROM. 5649

ROUTINE IDENTIFICATION OF DRUGS OF ABUSE IN HUMAN URINE

II. DEVELOPMENT AND APPLICATION OF THE XAD-2 RESIN COLUMN METHOD

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SUMMARY

The methods described in this report were developed and applied to the detection of drugs of abuse in human urine in a laboratory analyzing in excess of one thousand urines per day.

Routinely 2 ml of urine were analyzed fluorimetrically for morphine and quinine. The positive morphine and/or quinine samples were confirmed by subjecting 15-ml aliquots of urine to acid hydrolysis, extracting and applying the extract to thin-layer chromatograms followed by specific chromogenic spray reagents. 25 ml of urine were applied to polypropylene columns containing 1.1 g of Amberlite XAD-2 resin. The flow rates were controlled with the hydraulic flow control apparatus and the resin eluted with 15 ml chloroform–isopropanol (3:1). The organic solvent extracts were shaken with 1 ml of saturated sodium bicarbonate and the separated organic phases evaporated to dryness in a water bath under a stream of air. The residues were dissolved in methanol and applied equally to silica gel plates and sheets. The plates were developed in ethyl acetate–methanol–water–ammonia (85:10:3:1) and the sheets in chloroform–methanol–ammonia (90:10:1). The plates were sprayed with 0.3 % ninhydrin–acid, 5 % H_2SO_4, iodoplatinate and *p*-nitroaniline followed by NaOH. The Polygram sheets were sprayed with 0.4 % ninhydrin–acetone, $HgSO_4$, diphenylcarbazone, NaI and iodoplatinate. The opiates, opioids, barbiturates, amphetamines, phenothiazines, antihistamines and minor tranquilizers were detected by specific color reactions and R_F values. The plates and sheets were cross-compared for final interpretation of the results.

These methods were unusually simple, extremely rapid, inexpensive and very effective in detecting psychoactive drugs at concentrations ranging from 0.1 to 3 μg/ml of urine.

INTRODUCTION

The ever increasing usage of psychoactive drugs within our society and the subsequent effort to control abuse of these drugs has required the analytical laboratory to develop new, specific, sensitive, rapid and inexpensive techniques to meet the demand for screening an incredibly large number of urine samples on a daily basis.

The use of such analytical techniques as spectrophotometry, gas–liquid chromatography and fluorimetry for the detection of drugs in biological material[1–3] remains essential but the effectiveness and/or usefulness is somewhat dependent on the number of samples analyzed daily. In a laboratory required to analyze thousands of urines daily for psychoactive drugs and/or metabolites such methods unless automated are simply too time-consuming. The use of thin-layer chromatography (TLC)[4–6] for rapid reliable screening of a large number of samples containing several different chemical classes of drugs subject to abuse is essentially the present method of choice. The major objection concerning this technique is the time required (usually solvent–solvent extraction) to prepare the samples for the thin-layer chromatograms.

Recently, FUJIMOTO AND WANG[7] reported on the use of an Amberlite XAD-2 resin for the separation of narcotic analgesics from urine, which were subsequently detected by TLC. The resin, a styrene-divinylbenzene copolymer, has the capability of adsorbing many water-soluble organic compounds principally by Van der Waals forces. The feasibility of using the XAD-2 resin to adsorb narcotic analgesics and their surrogates, barbiturates, sympathomimetic amines, phenothiazines, antihistamines, tranquilizers, and other psychoactive compounds in the routine identification of drugs from urine was vigorously evaluated. This communication describes in detail the results of the methods developed and currently applied in our laboratory for the analysis of drugs of abuse in human urine.

MATERIALS AND METHODS

The urinalysis laboratory requires a 50-ml urine sample from which 2-ml aliquots are routinely analyzed by the ATS method[2] and 15-ml aliquots are subjected to acid hydrolysis and analyzed by TLC[4]. 25-ml aliquots of urine are analyzed by the recently developed XAD-2 resin column method.

Resin

The Amberlite XAD-2 resin (20–50 mesh) was obtained from Rohm and Haas Co., Philadelphia, Pa. The resin was washed with stirring four times with four bed volumes of acetone, three times with three bed volumes of methanol and three times with three bed volumes of distilled water. The XAD-2 resin remained in distilled water until transferred to the columns.

Columns

The polypropylene columns (135 × 10 mm) without flow regulator* (see Fig. 1) were obtained from Whale Scientific Co., Denver, Colo. The columns were plugged with fine glass wool and an aqueous slurry of the resin was poured into the column to provide a resin bed of 45 × 10 mm, thus filling up the lower thin portion of the column. A small glass wool plug was placed on top of the resin and the columns were placed in distilled water until transferred to the troughs.

Hydraulic flow control apparatus (HFCA)

A metal trough (83 × 5 × 5.5 in., Fig. 1) containing two draining taps at one end was used to control the rate of urine flow for a batch of columns (130–150). The trough supported a plexiglass rack with holes for the columns.

* The flow regulator that may be purchased with the columns contains an organic soluble component that interferes directly with the TLC detection of drugs.

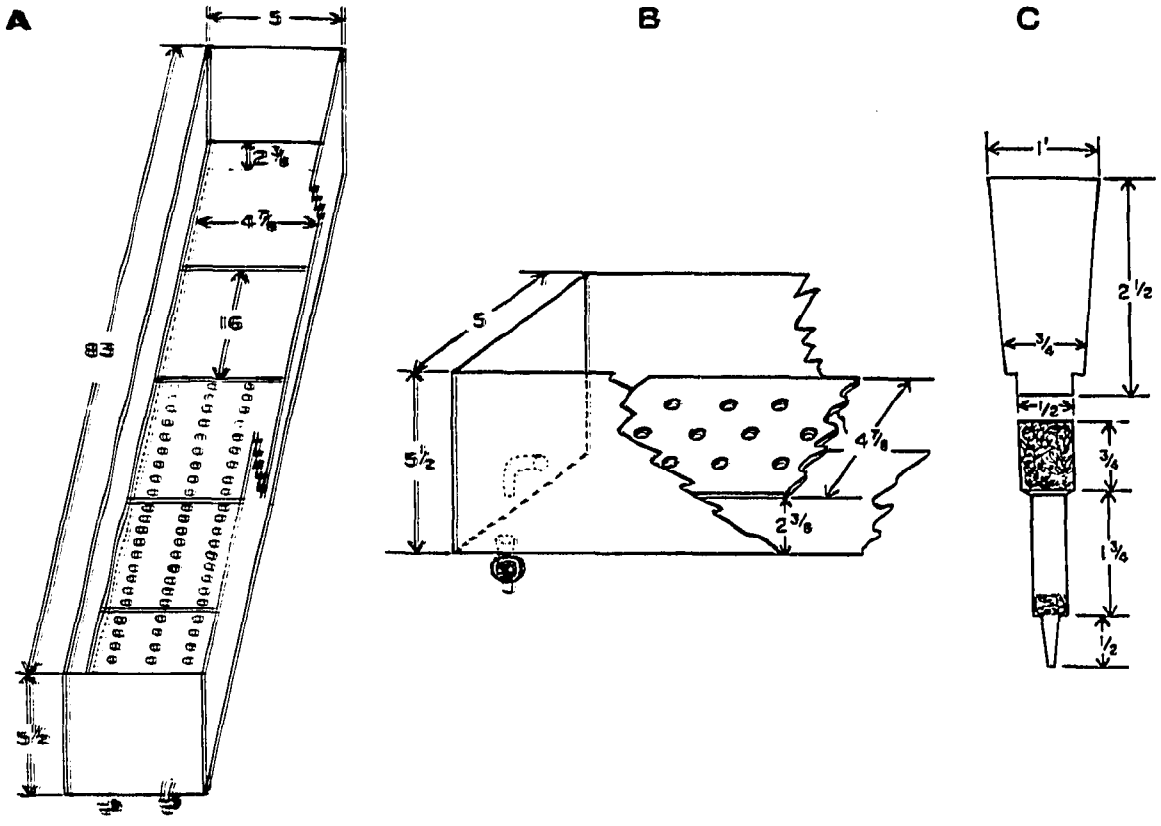

Fig. 1. Schematic diagrams of the hydraulic flow control apparatus (HFCA) (A, B) and the polypropylene column (C) used in this study.

Procedure

The columns containing the XAD-2 resin were transferred to the HFCA and partially submerged in water while 25 ml of urine were poured into the columns. At this point the water level in the HFCA was close to the upper ridge of the columns and the small tap on the HFCA was opened. About 15 min were required for the urine to pass through the wider portion of the columns. At this time the large tap on the HFCA was opened and the water in the trough was allowed to drain rapidly (about 5 to 8 min). To slow draining columns air pressure was applied intermittently to induce a normal flow rate. All the columns were then transferred to 50-ml centrifuge tubes which contained 1 ml of a freshly prepared saturated aqueous solution of sodium bicarbonate. The resins were eluted with 15 ml of chloroform–isopropanol (3:1) in two divided portions of 5 ml followed by 10 ml. Following elution, the columns were removed and the organic–aqueous phases in the centrifuge tubes shaken in a Genie Vortex mixer. The two phases separated on standing and the upper aqueous phases were removed by aspiration. About 100 μl of 6 N HCl in ethanol were added to each tube and the organic solvent evaporated in a water bath at 80° under a stream of air.

Thin-layer chromatography

The residues in the tubes were dissolved in about 50 μl of methanol and applied with disposable capillary tubes in equal parts to two different chromatographic plates, viz. (A) 0.25-mm Silica Gel F_{254} thin-layer plates made by E. Merck A.G.

Darmstadt, G.F.R. and (B) 0.25-mm Polygram silica gel sheets made by Macherey, Nagel and Co., Düren, G.F.R. These plates were obtained from Brinkmann Instruments, Inc., Westbury, N.Y., U.S.A.

The Silica Gel F_{254} plates were developed in ethyl acetate–methanol–water–ammonia (85:10:3:1) (S_1). The Polygram silica gel sheets were developed in chloroform–methanol–ammonia (90:10:1) (S_2). All the chromatograms following development were dried in an oven for 15 min at 100°.

Reagents

All chemicals were of reagent grade and were obtained through J. T. Baker or Fisher Scientific Co. The specially prepared spray reagents were:

(1) *0.3 % Ninhydrin–acid* — 0.3 g of ninhydrin was dissolved in 99 ml of isopropanol and 1 ml of glacial acetic acid was added. The reagent may be stored in the refrigerator.

(2) *0.4 % Ninhydrin–acetone* — 0.4 g of ninhydrin was dissolved in 100 ml of acetone. This reagent must be freshly prepared prior to use.

(3) *Iodoplatinate* — 1 g of platinic chloride was dissolved in 10 ml of water and was mixed with 10 g of potassium iodide dissolved in 200 ml of water. The mixture was diluted to 500 ml with water and stored in a refrigerator.

(4) *10 % Sodium iodide* — 10 g of sodium iodide was dissolved in 100 ml of water and stored in a refrigerator.

(5) *p-Nitroaniline (PNA)* — 2.5 g of PNA was dissolved in 250 ml of 1 *N* HCl and diluted to 500 ml with ethanol. Prior to use 20 ml of 5 % sodium nitrite solution (5 g/100 ml in water) was added to 100 ml of the PNA. The mixture was cooled to 5–10° (refrigerate) for 10 min and used only when cold.

(6) *25 % Sodium hydroxide* — 50 g of sodium hydroxide was dissolved in 100 ml of water. 50 ml of this solution was diluted to 100 ml with ethanol and stored in a refrigerator until it turned pale yellow.

(7) *5 % Sulfuric acid* — 5 ml of concentrated sulfuric acid was diluted to 100 ml with water.

(8) *0.1 % Diphenylcarbazone* — 0.1 g of diphenylcarbazone was dissolved in 100 ml of chloroform. The reagent was stored in a refrigerator.

(9) *Mercuric sulfate* — 0.5 g of mercuric oxide was dissolved in 20 ml of concentrated sulfuric acid. The acid solution was diluted slowly with 200 ml of water.

Spray reagent sequence

The TLC plates following development and drying were viewed under short- and long-wave UV light. The chromatograms developed in the solvent system S_1 were treated and sprayed as follows: (1) sprayed with 0.3 % ninhydrin–acid solution and heated at 100° in an oven for 5 min; (2) while hot sprayed with 5 % H_2SO_4, returned to the oven for 5 min and viewed under short- and long-wave UV light; (3) sprayed with the iodoplatinate reagent and air-dried; (4) sprayed lightly with the PNA reagent, after which a black or grey background should appear promptly; (5) then sprayed with 25 % alcoholic NaOH solution until the *d*-amphetamine reference standard appears reddish orange.

The Polygram sheets developed with the S_2 solvent system were sprayed and treated as follows: (1) sprayed with 0.4 % ninhydrin–acetone and irradiated under

long-wave UV light for 5 to 10 min, then transferred to an oven at 100° for 5 min; (2) after cooling, sprayed with the mercuric sulfate solution; (3) following drying, viewed under short- and long-wave UV light and then sprayed with 0.1 % diphenylcarbazone and heated for a few minutes at 100°; (4) sprayed with 10 % sodium iodide and air-dried; (5) finally sprayed with the iodoplatinate reagent.

Reference standards

Three primary groups were prepared as follows: (A) a solution with a final concentration of 2 mg/ml in methanol of *d*-methorphan, codeine, morphine, quinine, meperidine, chlorpromazine, *dl*-methadone, and *d*-propoxyphene; (B) a solution containing 2 mg/ml in methanol of *d*-amphetamine, methamphetamine, phenylpropanolamine, methylphenidate and phenmetrazine; (C) a solution containing 2 to 5 mg/ml in methanol of phenobarbital, pentobarbital, amobarbital, secobarbital, glutethimide and diphenylhydantoin. All standards were prepared from the commercially available drugs. Approximately 10 to 20 μg of each drug were applied to the respective TLC plates.

Urine internal standards

Four control urines containing known drugs of abuse in concentrations of 1 to 2 μg/ml were added to every 100 unknown urines analyzed each day.

Recovery experiments

The following drugs were evaluated: *d*[^{3}H]amphetamine (3.23 mCi/mmole); l-[^{3}H]methadone (92.3 mCi/mmole); [^{14}C]pentobarbital (3.23 mCi/mmole); [^{14}C]-caffeine (4.4 mCi/mmole); [^{14}C]morphine (3.69 mCi/mmole); [^{14}C]mescaline (16.4 mCi/mmole); [^{14}C]meperidine (5.0 mCi/mmole); [^{14}C]cocaine (3.13 mCi/mmole); [^{14}C]meprobamate (8.19 mCi/mmole); [^{14}C]phenobarbital (4.69 mCi/mmole); [^{14}C]-nicotine (20 mCi/mmole); and [^{14}C]morphine glucuronide (0.008 mCi/mmole). The drugs were obtained from either the New England Nuclear Corp., Boston, Mass., the Mallinckrodt Chemical Works, St. Louis, Mo., or the Amersham/Searle Co., Arlington Heights, Ill. Morphine glucuronide was a gift from Dr. A. L. MISRA. All labeled drugs were diluted with the appropriate non-labeled drugs to provide final specific activities ranging from 0.008 to 1.27 mCi/mmole. A standard stock solution of 10 μg/ml was made of each drug from which aliquots were taken and added to 10 ml of BRAY's phosphor solution[8]. The radioactivity was determined in a Nuclear Chicago Unilux III scintillation spectrometer. An absolute μg/c.p.m. factor was obtained from a plot of c.p.m. *vs.* concentration. All calculations of the percentage of each drug recovered were made using the absolute factor and correcting the data for aliquots and quenching using the external standardization channels ratio method and the internal standardization technique.

In order to ascertain the effectiveness of the XAD-2 resin column technique the radioactively labeled drugs in concentrations of 1 to 30 μg/ml of fresh urine were resin extracted in duplicate as described previously. Following elution, the organic phases were separated from the aqueous sodium bicarbonate and each phase transferred to scintillation counting vials and evaporated on a Fisher slide warmer at 65°. 10 ml of BRAY's phosphor solution were added to each vial and the radioactivity as well as quenching determined as described. The labeled drugs adsorbed on the XAD-2

resin were determined by thoroughly mixing the resin after drying in an oven and transferring 1/10 volume to scintillation counting vials.

Comments on the procedure

It is not necessary depending on the requirements of the program to analyze the urines by the spectrofluorimetric or acid hydrolysis method. It is certainly feasible to analyze the urines for drugs of abuse using only the XAD-2 resin method. However, where legal evidence is required, it is suggested that additional analytical techniques be employed.

RESULTS

Characteristics of the XAD-2 resin column method

Table I presents the data obtained using the XAD-2 resin column method following the analysis of all fractions for radioactive drugs. The percentage recovery of the drugs of abuse in chloroform–isopropanol (3:1) ranged between 49.3 % and 91.6 %. Nicotine was poorly recovered in this fraction (29 %). Morphine glucuronide, the major conjugated metabolite of morphine, was not recovered in the organic solvent. The urine effluent contained a large percentage of the drugs (range 1.8 to 79.1 %) and was the major source of loss with the method; thus a percentage of the drugs were simply not adsorbed to the XAD-2 resin. Losses in the $NaHCO_3$ solution were generally 3 % or less except for the water-soluble morphine glucuronide. The

TABLE I

FUNCTIONAL CHARACTERISTICS OF THE XAD-2 RESIN COLUMN METHOD

Drug[a]	*Concentration (μg/ml urine)*	% *Loss*[b]			% *Recovery*[c]
		Urine	*$NaHCO_3$*	*Resin*	
d-[^{3}H]Amphetamine	2.00	45.0	2.1	3.5	49.3
[^{14}C]Caffeine	11.00	6.1	0.9	1.7	91.3
[^{14}C]Cocaine	1.00	4.4	0.1	3.9	91.6
[^{14}C]Meperidine	1.00	1.8	0.3	9.1	88.9
[^{14}C]Mescaline	1.00	19.1	2.9	5.1	72.9
1-[^{3}H]Methadone	10.40	26.9	0.1	17.5	55.5
[^{14}C]Morphine	1.25	32.2	1.3	2.4	64.0
[^{14}C]Morphine glucuronide	30.00	79.1	9.1	11.7	0.0
[^{14}C]Meprobamate	1.00	15.9	0.9	8.8	74.4
[^{14}C]Nicotine	11.00	68.1	0.6	2.0	29.0
Quinine[d]	10.00	—	—	—	78.4
[^{14}C]Pentobarbital	2.00	6.7	0.6	4.3	88.4
[^{14}C]Phenobarbital	1.00	7.9	1.6	7.4	83.1

[a] Duplicate 25-ml urines containing the labeled drugs were applied to the columns and resin extracted as described under MATERIALS AND METHODS.

[b] The saturated $NaHCO_3$ solution as well as the resin itself were analyzed (see MATERIALS AND METHODS) to determine the percentage of the drugs not recovered in the eluting solvent. The percentage in urine was calculated as the difference.

[c] Percentage recovery of the labeled drugs in the chloroform–isopropanol (3:1) eluting solvent.

[d] Non-labeled quinine was assayed spectrofluorimetrically[2].

TABLE II

EFFECT OF VARIOUS SOLVENT SYSTEMS ON THE RECOVERY OF [^{14}C]MORPHINE FROM THE XAD-2 RESIN

Eluting solvent	*% Recovery from resin*[a]	*% Loss through partitioning in $NaHCO_3$ phase*[b]	*% Loss in urine effluent water wash or resin*[c]
Ethylene dichloride	0.32	0.18	99.5
Ethylene dichloride–isopropanol (9:1)	21.5	4.9	73.6
Ethylene dichloride–isopropanol (3:1)	31.6	3.8	64.6
Ethylene dichloride–ethyl acetate (2:3)	4.3	3.0	92.7
Ethyl acetate	15.3	5.2	79.5
Chloroform–methanol (9:1)	16.4	5.6	78.0
Chloroform–isopropanol (3:1)[d]	40.1	0.9	59.0

[a] The XAD-2 resin was washed with 25 ml of distilled water and eluted with 10 ml of the solvent by the technique described under MATERIALS AND METHODS. The [^{14}C]morphine concentration in urine was 1.25 μg/ml and a total of 25 ml of urine was applied to the column (31.2 μg).

[b] Percentage of [^{14}C]morphine remaining in the aqueous $NaHCO_3$ solution.

[c] Percentage of [^{14}C]morphine remaining in the urine effluent (not adsorbed on resin), in the water wash, or not eluted from the column with the appropriate solvent.

[d] Following adjustment of the urine to pH 9, a 52.7% recovery was obtained. Using fresh urine (pH about 6.5), eluting the resin with 15 ml of solvent and eliminating the 25-ml water wash provided a 64% recovery.

XAD-2 resin was a major source of loss for certain drugs, *i.e.* methadone, meperidine, meprobamate, and morphine glucuronide. The resin trapped 7 % or less of the other drugs tested.

Presented in Table II are the data on the effectiveness of various solvent systems on eluting [^{14}C]morphine from the XAD-2 resin. Ethylene dichloride was the least effective solvent for recovering [^{14}C]morphine from fresh urine using the XAD-2 resin column method. Most of the drugs were not adsorbed to the resin (remained in urine effluent) or were lost in the $NaHCO_3$ solution, water-wash or remained adsorbed to the resin. Ethylene dichloride–isopropanol or ethyl acetate mixtures were more effective than ethylene dichloride alone, but significant quantities of the drug were not recovered. The most effective eluting solvent mixture analyzed was chloroform–isopropanol (3:1). This mixture provided a 54.1 % recovery of [^{14}C]morphine from a fresh urine and an unwashed resin (Table III) eluted with 10 ml of solvent. A recovery of 52.7 % was obtained by adjusting the urine pH to 9, washing the resin with water and eluting with 10 ml of solvent. A recovery of 64 % was obtained from fresh urine (pH about 6.5), eliminating the water-wash and eluting with 15 ml of the solvent (Tables I and III). The lowest recovery (40.1 %) for [^{14}C]morphine (Table II) was obtained from fresh urine, washing the resin with 25 ml of water and eluting with 10 ml of solvent.

Table III summarizes the results obtained concerning aqueous elution of the resin and organic solvent volumes on the recovery of a representative group of drugs subject to abuse. It is quite clear that washing the column with 25 ml of distilled water after application of the urines containing the drugs to the columns, elutes a percentage of the drugs' (4.3 to 14.0 %). Increasing the volume of chloroform–isopropanol (3:1) from 10 ml to 15 ml also significantly increased the percentage recovery of the drugs in this solvent.

TABLE III

PERCENT RECOVERY OF DRUGS ADSORBED ON THE XAD-2 RESIN WASHED OR UNWASHED FOLLOWED BY ELUTION WITH CHLOROFORM–ISOPROPANOL (3:1)[a]

Drug	*Concentration (μg/ml urine)*	*% Recovery (elution volume 10 ml)*			*% Recovery (elution volume 15 ml) Resin unwashed*
		Resin unwashed	*Resin washed*	*% Loss in wash*	
d-[^{3}H]Amphetamine	1.7	35.4	23.2	12.2	43.7
l-[^{3}H]Methadone	0.04	53.6	48.8	4.3	57.6
[^{14}C]Morphine	1.25	54.1	40.1	14.0	64.0
[^{14}C]Morphine glucuronide[b]	30.0	7.0	2.6	4.4	—
[^{14}C]Pentobarbital	2.0	79.1	66.0	13.1	89.4

[a] The urines (25 ml) in duplicate containing the labeled drugs were applied to the columns and eluted with 10 or 15 ml of solvent as described under MATERIALS AND METHODS. Prior to elution with solvent the resin was washed with 25 ml of distilled water or not washed. The concentration of the radioactive drugs in the solvent or water wash was determined by liquid scintillation spectroscopy as described under MATERIALS AND METHODS.

[b] This compound was eluted from the resin with 5 ml of methanol following elution with chloroform–isopropanol (3:1). Methanol elution of this compound was not used in the experiments with 15 ml of the organic solvent.

TABLE IV

EFFECT OF CONTROLLED AND UNCONTROLLED FLOW RATES ON PERCENTAGE RECOVERY OF DRUGS FROM XAD-2 RESIN[a]

Drug	*With controlled flow rate (HFCA)*	*Without controlled flow rate*	*% Increase with controlled flow rate (HFCA)*
[^{14}C]Morphine	62.4	39.8	22.6
Quinine[b]	62.5	52.8	9.7
[^{14}C]Phenobarbital	81.9	51.1	30.8
[^{14}C]Meprobamate	74.4	61.9	12.5

[a] 25 ml of urine in duplicate containing the labeled drugs (1 to 1.25 μg/ml), were applied to the columns and eluted with 15 ml of chloroform–isopropanol (3:1) under controlled (HFCA) or uncontrolled flow rates. The recovery of labeled drugs was determined by liquid scintillation spectroscopy as described under MATERIALS AND METHODS.

[b] Quinine was assayed spectrofluorometrically[2]. This drug was eluted from the resin with chloroform–methanol (9:1).

The effect of controlling the flow rates of the urine with HFCA as compared with not controlling the flow rates appears in Table IV. It is quite obvious that controlling flow allows for an increased recovery of these drugs which ranged from 9.7 to 30.8 % over uncontrolled conditions.

Comparison of the XAD-2 resin column method with other methods

The data concerning the comparison of the XAD-2 resin column technique with currently available methods for extracting drugs of abuse appear in Table V. The resin method for recovering drugs from urine compares favorably with the usual solvent extraction techniques. For the narcotic analgesics recoveries of 55.5 to 89.0 % were achieved. The recovery of *d*-[^{3}H]amphetamine was 49.3 % as compared

TABLE V

COMPARISON OF THE XAD-2 RESIN COLUMN TECHNIQUE WITH OTHER ANALYTICAL METHODS OF EXTRACTING DRUGS[a]

Drug	*Concentration (μg/ml urine)*	*% Recovery*					
		XAD-resin method	*Selective pH solvent extraction at*[b]			*Ethanol–K_2CO_3 extraction*[5]	*Single extraction at pH 9.5 (ref. 6)*
			pH 1	*pH 9*	*pH 10–11*		
d-[^{3}H]Amphetamine	2.0	49.3			63.5	58.9	64.5
[^{14}C]Caffeine	11.0	91.3			89.2	32.8	91.9
[^{14}C]Cocaine	1.0	91.6			86.0	73.8	93.1
[^{14}C]Meperidine	1.0	89.0			93.5	90.7	98.7
[^{14}C]Mescaline	1.0	73.0			73.0	32.3	93.0
l-[^{3}H]Methadone	10.4	55.5			64.7	85.7	71.0
[^{14}C]Morphine	1.25	64.0		70.7	38.0	82.0	75.3
[^{14}C]Meprobamate	1.0	74.4	77.2		54.6	89.3	84.5
[^{14}C]Nicotine	11.0	29.2			87.0	82.7	91.5
Quinine[c]	10.0	78.4			90.4	92.8	98.0
[^{14}C]Pentobarbital	2.0	88.4	81.6			92.0	63.2
[^{14}C]Phenobarbital	1.0	83.1	89.5			72.2	39.3

[a] The labeled drugs were extracted by the XAD-2 resin technique described under MATERIALS AND METHODS and by solvent extraction methods reported by MULÉ[4], BASTOS *et al.*[5] and DAVIDOW *et al.*[6].

[b] The data reported concern primarily the narcotic analgesics which in general were more effectively extracted at pH 9. The pH 10–11 extraction was developed to obtain a large number of basic organic drugs including amphetamines, phenothiazines and antihistamines. See MULÉ[4] for details.

[c] Quinine was determined spectrofluorimetrically[2].

to a range of 58.9 to 64.5 % for the other methods. [^{14}C]Pentobarbital and [^{14}C]-phenobarbital were effectively recovered at 88.4 % and 83.1 %, respectively, with the resin technique. It must be noted that nicotine was poorly recovered from the resin (29.2 %), a desirable feature of the resin since nicotine interferes with the TLC detection of drugs in urine.

In Table VI appear the TLC results obtained with various psychoactive drugs. Both solvent systems were used to obtain maximal information for positive identification of these drugs or their metabolites. Typically the residue obtained following evaporation of the organic extract was taken up in about 50 μl of methanol and applied to both the silica gel plate and the sheet simultaneously in approximately equally divided volumes. Following development in their respective solvent systems, the plates and sheets were dried and viewed under long- and short-wave UV light prior to spraying with the specific sequence of chromogenic reagents described. This procedure (UV visualization) allows for preliminary detection of those drugs that fluoresce under long-wave UV light (*i.e.* quinine, chlorpheniramine, chlorpromazine, etc.). Quenching of the background fluorescence occurred with almost all the drugs under short-wave UV light when the silica gel plates (F_{254}) were viewed.

The following general comments were applicable to the data obtained with the silica gel plates (system S_1): (1) ninhydrin–acid followed by heating was effective in detecting methamphetamine (secondary amine) as well as some primary amines, *i.e.* mescaline, phenylpropanolamine; (2) 5 % H_2SO_4 allowed for the detection of pheno-

TABLE VI

TLC hR_F VALUES AND COLOR REACTIONS OF DRUGS

Solvent systems: S_1 = ethyl acetate–methanol–water–ammonia (85 : 10 : 3 : 1); S_2 = chloroform–methano ammonia (90 : 10 : 1).

Drug	*Silica gel plate/S_1*					
	hR_F	RR_F[a]	*Color reactions*			
			Ninhydrin–acid + heating	*5% H_2SO_4 + heating*	*Iodo-platinate*	*PNA + NaOH*
Cocaine[c]	90	5.00	—	—	red-blue	—
Codeine	30	1.67	—	—	blue	—
Meperidine (Demerol)	62	3.44	—	—	blue	—
Methadone[c] (Dolophine)	80	4.44	—	—	red-blue	orange
l-Methorphan	10	0.55	—	—	blue	—
Morphine	18	1.00	—	—	blue	—
Pentazocine (Talwin)	77	4.28	—	—	red-blue	—
d-Propoxyphene[c] (Darvon)	94	5.22	—	—	red-blue	—
d-Amphetamine (Dexedrine)	42	2.33	—	—	[e]	orange-re
Ephedrine	19	1.05	purple	—	[e]	—
Mescaline	22	1.22	light purple	—	purple	orange-re
Methamphetamine (Methedrine)	28	1.55	purple	—	[e]	—
Methylphenidate (Ritalin)	76	4.22	—	—	purple	—
Phenylpropanolamine	31	1.72	purple	—	[e]	orange-re
Phenmetrazine (Preludin)	46	2.55	purple	—	purple	—
Cyclohexylamine	20	1.11	light purple	—	purple	orange-re
Phenylethylamine	35	1.94	—	—	[e]	orange-re
Nicotine[c]	56	3.11	—	—	blue	—
Quinine[f]	42	2.33	—	—	blue	—
Amitriptyline (Elevil)	79	4.36	—	—	purple	—
Chlordiazepoxide (Librium)	68	3.78	—	—	red-blue	—
Chlorpheniramine (Chlortrimeton)	33	1.83	—	—	blue	—
Chlorpromazine (Thorazine)	72	4.00	—	reddish pink	purple-red	—
Diazepam (Valium)	92	5.11	—	—	light red	—
Prochlorperazine (Compazine)	52	2.89	—	blue	purple	—
Promethazine (Phenergan)	70	3.89	—	pink	purple	—
Thioridazine (Mellaril)	73	4.05	—	blue	purple	—
Tripelenamine (Pyribenzamine)	60	3.33	—	blue	blue	—
Perphenazine (Trilafon)	52	2.88	—	light purple	purple	—
Allylisobutylbarbital	36	2.00	—	—	—	—
Amobarbital (Amytal)	86	4.78	—	—	—	—
Aprobarbital (Alurate)	84	4.67	—	—	—	—
Barbital (Veronal)	78	4.33	—	—	—	—
Diphenylhydantoin[c] (Dilantin)	82	4.55	—	—	—	—
Glutethimide[c] (Doriden)	92	5.11	—	—	—	—
Hexobarbital (Ortal)	84	4.66	—	—	—	—
Meprobamate[h] (Equanil-Miltown)	81	4.50	—	—	—	—
Phenobarbital (Luminal)	73	4.05	—	—	—	—
Pentobarbital (Nembutal)	87	4.83	—	—	—	—
Secobarbital (Seconal)	87	4.83	—	—	—	—

[a] RR_F = R_F value relative to morphine.

[b] The chromatograms were viewed under UV light prior to spraying with the sequence chromogenic reagents and after spraying with 5% H_2SO_4 or H_2SO_4. All the drugs except mepr bamate quench under the short-wave UV light.

[c] The metabolites of cocaine (ecgonine and benzoylecgonine) if extracted would provi R_F = 0 in both systems. The metabolites of the following drugs were useful in the identificati of the parent compound: methadone gave hR_F values of 58 and 55 in solvent system S_1 and S nicotine gave hR_F values of 36 and 22 in S_1 and 37 and 27 in S_2; glutethimide gave hR_F values of in S_2; diphenylydantoin gave hR_F values of 10 in S_2; *d*-propoxyphene provided a streak fro 56 down (up to 1 in. long) in S_1.

ng-wave / light[b]	Polygram silica gel sheets/S_2						
	hR_F	RR_F	Color reactions				
			Ninhydrin–acetone		$HgSO_4$	Diphenyl-carbazone	NaI + iodo-platinate
			UV +	heating			
·	94	4.95	—	[d]	—	—	purple
·	55	2.89	—	—	—	—	purple-brown
·	85	4.47	—	[d]	—	—	purple
·	84	4.42	—	[d]	—	—	brown
·	69	3.63	—	green-blue	—	—	purple
·	19	1.00	—	yellow	—	—	blue
·	56	2.95	—	blue	—	—	purple
·	93	4.89	—	—	—	—	purple
·	50	2.63	purple	—	red	—	[e]
·	14	0.74	—	blue	red-purple	—	[e]
·	37	1.95	purple	—	red-brown	—	purple
·	47	2.47	—	purple	red	—	[e]
reen	85	4.77	—	[d]	—	—	purple
·	16	0.84	light purple	—	red	—	[e]
·	69	3.63	—	[d]	—	—	purple
·	23	1.21	purple	—	red	—	green
·	41	2.16	purple	—	red	—	[e]
·	82	4.31	—	—	—	—	blue-grey
right blue	43	2.26	—	—	[f]	—	purple
·	89	4.68	—	light blue	—	—	purple
reen-yellow	77	4.05	—	yellow	—	—	purple
reen	67	3.53	—	blue	—	—	purple
lue	90	4.74	—	—	red-purple[g]	—	purple
reen-yellow	93	4.89	—	—	—	—	purple[i]
lue	90	4.74	—	—	red-purple[g]	—	blue
·	89	4.68	—	blue	brown[g]	—	purple
lue	88	4.63	—	green	yellow-green[g]	—	green-purple
lue	80	4.21	—	blue	—	—	purple
lue	72	3.79	—	—	—	—	purple
—	62	3.26	—	—	white[j]	white	[i]
—	63	3.31	—	—	white[j]	purple	—
—	58	3.05	—	—	white[j]	white	[i]
—	50	2.63	—	—	white[j]	purple	—
—	54	2.84	—	[d]	white[j]	light purple	[i]
—	91	4.79	—	—	white[j]	purple	—
—	86	4.53	—	—	white[j]	purple	—
—	34	1.79	—	—	white	—	—
—	46	2.42	—	—	white[j]	purple	—
—	59	3.10	—	—	white[j]	purple	—
—	70	3.68	—	—	white[j]	white	[i]

[d] Large concentrations of this drug may be partially decomposed and react.

[e] May react when present in high concentration (50 μg or more) and provide a blue color.

[f] The fluorescence of the spot intensifies after spraying with 5% H_2SO_4 or $HgSO_4$ solution. The metabolic products of quinine with hR_F values were: 30, 23, 7 in S_1 and 33 in S_2.

[g] Color observed after heating at 100° for a few minutes.

[h] Using a 25-ml sample of urine a grey spot was observed between 5 μg/ml and 25 μg/ml and a white spot above 25 μg/ml. This drug may be confirmed by finally spraying the sheet (S_2) with 10% furfural–alcoholic solution followed by con. HCl. A dark black spot appears after subjecting the sheet to heat (100°) for a few minutes.

[i] After sodium iodide spray a yellow color reaction may be observed.

[j] At low concentrations the white spot may fade into the grey background.

thiazine and some antihistamine compounds and intensified the fluorescence observed with quinine and/or metabolites when viewed under long-wave UV light; (3) iodoplatinate reagent provided reactions with opiates, opioids, amines, tranquilizers and antihistamine compounds; (4) PNA plus NaOH were sprayed on the plates to identify and confirm the presence of primary amines (*e.g. d*-amphetamine).

The following comments were applicable to the data obtained with the Polygram silica gel sheets (system S_2): (1) ninhydrin–acetone with UV irradiation allowed for the detection of primary amines, *i.e. d*-amphetamine and phenylpropanolamine; heating the sheets provided reactions with secondary amines; (2) $HgSO_4$ was used primarily for the detection of barbiturates and other acidic drugs, *i.e.* glutethimide and diphenylhydantoin; (3) heating and viewing the plates under UV light provided evidence for the presence of phenothiazines and antihistamines; (4) 0.1 % diphenylcarbazone reacted with barbiturates and acidic drugs to provide additional evidence for detection; (5) NaI and iodoplatinate reacted with most of the basic drugs present. Following the specific sequence of chromogenic reagents sprayed on both plates, the color reactions and R_F values were cross-compared for the final identification of the drugs.

The limits of detection for the various drugs from urine following XAD-2 resin extraction and chromatography were: 0.1 μg/ml for quinine, 0.5 to 1 μg/ml for morphine, *dl*-amphetamine, methamphetamine, methadone, and pentobarbital, 2 μg/ml for secobarbital, glutethimide, and diphenylhydantoin, and 3 μg/ml for meprobamate and methylphenidate.

DISCUSSION

The Amberlite XAD-2 resin is a styrene-divinylbenzene copolymer that adsorbs a wide variety of organic compounds by Van der Waals forces[9]. The ionic species of the organic molecule did not appear to be important with this method. However, the volume of the eluting solvent, the flow rate of urine and eluting solvent, aqueous washing of the resin as well as the organic solvent mixture were all very significant in obtaining good recoveries of the psychoactive drugs.

The chloroform–isopropanol (3:1) solvent was selected as the most efficient solvent for eluting the drugs from the resin. 10 ml of the solution are not as efficient in recovering [^{14}C]morphine from the resin as were 15 ml of the solvent. It is also suggested that the resin be eluted in a two-step process: first with 5 ml of solvent followed by 10 ml of solvent for best recovery results. In order to improve recovery, the resin was not washed (see Table III). Washing the column with water removed residual urine as well as water-soluble drugs. Aqueous sodium bicarbonate was shaken with the eluting solvent to remove some acidic drugs such as acetylsalicylic acid and metabolites as well as residual urine which interfered with the subsequent chromatographic detection of drugs of abuse.

The recoveries obtained with the drugs utilizing the XAD-2 resin were quite satisfactory in comparison to other analytical methods (Table V). The low recovery of nicotine was advantageous since tobacco alkaloids and their metabolites interfere with subsequent chromatography. The chromatograms were quite clear with this technique and allowed for effective interpretation of the color reactions. Typically the two plates were cross-compared both for R_F values and for color reactions. The silica gel plates with the S_1 system and sequence of chromogenic sprays was used primarily to detect

secondary and *primary amines*, phenothiazine derivatives, quinine and metabolites as well as *opiates*, *opioids*, and antihistamine compounds. The Polygram silica gel sheet with S_2 system and sequence of chromogenic reagents was used to detect primarily *barbiturates*, glutethimide, primary amines, opiates, opioids, and phenothiazine derivatives.

The ninhydrin–acid reagent used with the silica gel plates and the S_1 system was effective in detecting about 5 μg of the secondary amines. A few primary amines such as mescaline, methoxamine and phenylpropanolamine may also react with this reagent. The R_F values of methamphetamine and phenylpropanolamine were quite similar so that final interpretation as to the presence of a primary amine should be made following the PNA plus NaOH spray. The sulfuric acid spray followed by heating provided suitable conditions for the oxidation of phenothiazine compounds and intensified the fluorescence of quinine and metabolites. The iodoplatinate reagent was an excellent general reagent for organic bases. The use of PNA followed by the sodium hydroxide reagent increased the sensitivity for detecting primary amines, *i.e.* 1 to 5 μg of amphetamine, methoxamine, metaraminol[5]. These reagents also provide a strong reaction with an apparently N-demethylated metabolite of methadone.

Ninhydrin–acetone followed by UV irradiation for 10 min as used with the Polygram sheets and the S_2 system was effective in detecting about 3 to 5 μg of the primary amines. Heating the plate for 5 min increased the sensitivity and allowed the detection of methamphetamine as well as certain other secondary amines such as desipramine and mephentermine.

The mercuric sulfate diphenylcarbazone (DPC) spray sequence for the barbiturates, glutethimide, diphenylhydantoin, and meprobamate was chosen over the previous silver acetate DPC system[4] because of the incompatibility with ninhydrin and the iodide reagents in the present spray sequence. The spraying of the mercuric sulfate reagent provided sufficient sulfuric acid to enhance the fluorescence of quinine and oxidize the phenothiazine derivatives during the heating of the chromatogram. The spraying of diphenylcarbazone provided additional evidence for the presence of barbiturate drugs. Sodium iodide was sprayed on the plates prior to the iodoplatinate reagent primarily to complex with mercury ions. Characteristic reactions with organic bases were then achieved with the iodoplatinate reagent.

The incorporation of the XAD-2 resin method as well as the TLC detection technique described has allowed the urinalysis laboratory to significantly increase the daily analysis for drugs of abuse with a decrease in cost per sample as well as a decrease in the time required to report final results. The resin method, therefore, quite simply provides for totally effective surveillance of drug usage within a treatment, aftercare or chemotherapeutic maintenance program.

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