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IDENTIFICATION OF NARCOTICS, BARBITURATES, AMPHETAMINES, TRANQUILIZERS AND PSYCHOTOMIMETICS IN HUMAN URINE

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SUMMARY

This study was undertaken to evaluate the ion-exchange paper technique and rapid direct extraction methods for obtaining drugs of abuse from urine and the subsequent identification of these drugs or metabolites by thin-layer chromatography coupled with sequential chemical reagent spraying.

Recoveries from urine ± S.D. of 21.7 ± 5%, 2.4 ± 0.8% and 21 ± 1% were obtained for labeled morphine, pentobarbital and amphetamine, respectively, using the ion-exchange paper technique. With this method most narcotic analgesics could be detected at a level of 1 μg/ml urine (50 ml urine sample). The method in general was unacceptable for detecting either barbiturates, amphetamines or certain psychoactive drugs (psilocybin, glutethamide, chlorpromazine, marihuana). Percentage recoveries obtained with direct extraction from urine ± S.D. were 61 ± 4% for morphine-^{14}C, 86 ± 6% for pentobarbital-^{14}C, and 61 ± 21% for amphetamine-^{3}H. Utilizing direct extraction methods almost all the drugs of abuse could be detected at levels ranging from 1 to 2 μg/ml of urine (15 ml of urine sample).

The methods described in this report provided some specificity through differential pH extraction. The subsequent use of thin-layer chromatography and sequential spray reagents allowed identification of specific drugs or metabolites. All methods and techniques could be completed within 24 h. The direct extraction in all cases and the ion-exchange technique for narcotics were sufficiently sensitive to provide detection of drug usage 24 h after the last administration of drug. Both techniques were extremely simple to perform and did not require expensive equipment thus keeping the cost of analysis at a minimum. These techniques may readily be adapted to a urine monitoring program screening for drugs of abuse, provided the limitations as described are well understood.

INTRODUCTION

Although many methods are available for the detection of drugs of abuse[1–6] none meets all the requirements of a urine monitoring program. These requirements

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are: (1) *Specificity*. The drugs must be separated by groups (narcolic analgesics, barbiturates, psychotomimetics, "tranquilizers", amphetamines) and then individually identified. More than one analytical test is required to qualify for medicolegal validity. (2) *Rapidity of analysis*. The entire procedure from acquisition of the biological sample to recording of the results should not exceed 24 h. (3) *Sensitivity*. The methods must be able to detect the presence of the administered drug or metabolites in biological material for at least 24 to 48 h following drug usage. (4) *Simplicity*. The methods should not require skills or training beyond that normally achieved by a laboratory technician. (5) *Cost*. The cost must be kept to a minimum and therefore all procedures, equipment, and tests must be viewed economically. However, in the final analysis the cost should be evaluated in terms of the cost to society should the tests not be performed.

The method developed by DOLE *et al.*[7] using ion-exchange paper to absorb the drugs, followed by elution with a series of buffer-solvent systems seemed best to fulfill the requirements described above. This report describes the experience, modifications and data obtained identifying narcotic analgesics, barbiturates, amphetamines, "tranquilizers" and psychotomimetics in human urine using ion-exchange papers, resins, direct extraction, thin-layer chromatography and a series of spray reagents.

METHODS AND MATERIALS

Ion-exchange extraction of drugs

50 ml of undiluted urine (pH 5–6) were transferred to 4 oz. jars containing Reeve Angel ion-exchange paper SA-2 (5 cm × 5 cm). The samples were shaken for 30 min at 80 r.p.m. on an International Shaker machine. The urines were decanted and the ion-exchange papers washed twice with distilled water.

Barbiturates. To each jar containing the SA-2 paper 20 ml of citrate buffer, pH 2.2 and 10 ml of $CHCl_3$ were added. The samples were shaken for 10 min either intermittently by hand or on the International Shaker. The lower organic phases were separated from the aqueous phases by a separatory funnel. The papers were re-extracted with 10 ml of $CHCl_3$ by shaking the samples by hand for 1 min. The organic phases were combined and evaporated to dryness under N_2 or air on a water bath. The residues were dissolved in 25 to 50 μl of either methanol or $CHCl_3$.

Narcotic analgesics and psychoactive drugs. To each jar containing the papers 20 ml of borate buffer pH 9.3 and 20 ml of $CHCl_3$–isopropanol (3:1, v/v) were added. The samples were shaken for 10 min intermittently by hand or on the International Shaker. The phases were separated by a separatory funnel and the aqueous phases discarded. The organic phases were evaporated to dryness and the residues dissolved in 50 μl of methanol.

d-Amphetamine and analogues. To each jar containing the papers 20 ml of carbonate buffer, pH 11.0 and 20 ml of $CHCl_3$ were added. The samples were shaken intermittently by hand for 10 min or on the International Shaker, phases were separated and 50 to 100 μl of glacial acetic acid were added to each organic phase. All organic phases were evaporated to dryness and the residues dissolved in 25 to 50 μl of either methanol or $CHCl_3$.

Direct extraction of drugs

Barbiturates. To 15 ml of urine in 40 ml glass stoppered centrifuge tubes 10 ml of citrate buffer, pH 2.2 and 10 ml of $CHCl_3$ were added. The samples were shaken at 300 oscillations/min for 15 min, centrifuged and $CHCl_3$ phases transferred to conical flasks. The samples were re-extracted with 10 ml $CHCl_3$ as described above and the organic phase added to the original $CHCl_3$ extract. The organic extracts were evaporated to dryness in a water bath under a stream of N_2 or air. The residues were dissolved in 25 μl of methanol.

Narcotic analgesics and psychoactive drugs. To 15 ml of urine in 40 ml glass stoppered centrifuge tubes 10 ml borate buffer, pH 9.3 and 10 ml of chloroform–isopropanol (3:1) were added. The samples were extracted, organic solvents evaporated and the residues dissolved in 25 μl methanol.

d-Amphetamine and analogues. To 15 ml of urine in 40 ml glass stoppered centrifuge tubes 10 ml of carbonate buffer, pH 11.0 and 10 ml of $CHCl_3$ were added. The samples were extracted, organic solvents evaporated and the residues dissolved in methanol. However, prior to evaporation of $CHCl_3$, 50 μl of glacial acetic acid was added to the organic extracts.

Recovery experiments

In order to ascertain the effectiveness of these methods from 0.1 to 2 μg of either morphine-^{14}C (0.21 mC/mmole) or pentobarbital-^{14}C (0.30 mC/mmole) and 0.25 to 10 μg of *d*-amphetamine-^{3}H (0.37 mC/mmole) were added to 10 ml of BRAY's phosphor solution[8]. The radioactivity was determined in a Packard TriCarb liquid scintillation spectrometer. Counts/min were plotted against concentration following correction of the data for quenching[9]. From this relationship an absolute ng/c.p.m. factor was derived. All calculations of the percentage of each drug recovered from urine were made using the factor following correction of the data for quenching and aliquots used in the extraction procedure.

Utilizing the *ion-exchange paper method*, the following recoveries were obtained after adding 1 to 20 μg of the drug to undiluted urine: morphine-^{14}C, 21.7 $\pm$ 5% (S.D.); pentobarbital-^{14}C, 2.4 $\pm$ 0.8% (S.D.); *d*-amphetamine-^{3}H, 2.1 $\pm$ 1% (S.D.). Following *direct extraction* of the same quantity of these drugs from undiluted urine, the following recoveries were obtained: morphine-^{14}C, 61 $\pm$ 4% (S.D.); pentobarbital-^{14}C, 86 $\pm$ 6% (S.D.); *d*-amphetamine-^{3}H, 61 $\pm$ 21% (S.D.).

Thin-layer chromatography

All chromatography was performed on 6 $\times$ 6 cm or 10 $\times$ 20 cm glass chromatoplates which contained an adsorbent layer of 250 μ of Silica Gel G. The chromatoplates were made as described by STAHL *et al.*[10] as follows: 34 g of Silica Gel G were dissolved in 80 ml of doubly distilled water. The gel was spread on the plate to provide a thickness of 250 μ. The plates were left at room temperature for 10 to 15 min, then activated at 100° for 1 h. After cooling, the plates were placed in a desiccator until used.

Chromatographic solvents. The composition of the various solvents (v/v) was:

CS_1 Ethanol–dioxane–benzene–NH_4OH (5:40:50:5)
CS_2 Ethyl acetate–methanol–NH_4OH (85:10:5)
CS_3 Methanol–*n*-butanol–benzene–water (60:15:10:15)

CS_4 Ethanol–pyridine–dioxane–water (50:20:25:5)
CS_5 *tert.*-Amyl alcohol–*n*-butyl ether–water (80:7:13)
CS_6 Chloroform–acetone (90:10)
CS_7 Benzene–glacial acetic acid (90:10)
CS_8 Dioxane–benzene–NH_4OH (20:75:5)
CS_9 Isobutanol–glacial acetic acid–cyclohexane (80:7:10)
CS_{10} Ethanol–NH_4OH (80:20)
CS_{11} Phenol–water (80:20)
CS_{12} *n*-Amyl alcohol–glacial acetic acid–water (40:10:50)
CS_{13} Isopropanol–NH_4OH–chloroform (45:10:45)
CS_{14} Cyclohexane–ethanol (85:15)

Spray reagents. The spray reagents and their composition were: (a) Iodoplatinate, prepared by adding 10 ml of a 10% solution of platinum chloride to 250 ml of 4% potassium iodide and diluted to 500 ml with distilled water. (b) Ammonical silver nitrate, prepared by mixing just prior to use 30 ml of 5 *N* NH_4OH and 30 ml of 50% $AgNO_3$. If cloudy add drop by drop 5 *N* NH_4OH until solution clears. (c) 0.5% H_2SO_4. (d) 0.02 *M* $KMnO_4$. (e) 0.4% ninhydrin, in acetone prepared just prior to use. (f) 1% silver acetate. (g) 2% mercurous nitrate. (h) Dragendorff's reagent, solutions were prepared as follows: (1) 1.7 g of bismuth nitrate in 100 ml 20% acetic acid; (2) 40 g of potassium iodide in 100 ml water. The reagent is made by mixing 20 ml of (1) with 5 ml of (2) and adding 70 ml of water.

Buffers. (a) Citrate, pH 2.2, 980 ml of 0.1 *M* citric acid were mixed with 20 ml of 0.2 *M* Na_2HPO_4. (b) Borate, pH 9.3, 950 ml of a saturated borax solution were mixed with 50 ml of 0.3 *N* NaOH. (c) Carbonate, pH 11.0, 12.5 ml of 0.2 *M* $NaHCO_3$ were mixed with 500 ml of Na_2CO_3.

Standard solutions of authentic drugs. In general the standard stock solutions were made from the commercially available salts of the drugs to provide a final concentration of 1 μg/ml in methanol, calculated as the free base or acid of the drug.

Procedure. (a) Residues of urines extracted at pH 2.2 dissolved in the organic solvent were applied to the chromatoplate with a 10 μl Hamilton syringe. Authentic standards of the barbiturates in concentrations of 5 to 10 μg were applied to the same plate. Fifty to seventy-five milliliters of solvent was transferred to the developing tank and allowed to rise 6 cm from the origin. The chromatoplates were usually air dried 10 to 30 min. The following sprays were applied in succession: (1) 1% silver acetate; (2) 0.02 *M* potassium permanganate. In some instances 2% $HgNO_3$ was used in place of 1% silver acetate. After spraying with silver acetate or mercurous nitrate the plates were allowed to dry in the hood for 10 to 15 min then placed in the oven for a few minutes. Color changes were observed and the plates subjected to U.V. light, then sprayed with $KMnO_4$, color changes noted and the R_F values of reactive compounds recorded. (b) Residues of urines extracted at pH 9.3 dissolved in the organic solvent were applied to the chromatoplates with a 10 μl Hamilton syringe. Authentic standards of the narcotic analgesics, or psychoactive drugs ranging in concentration from 5 to 20 μg were applied to the same plate. About 50 to 75 ml of the solvent was transferred to the developing tank and the solvent allowed to rise 6 cm from the origin. After development the chromatoplates were air dried for 10 to 30 min or placed in an oven for 10 min. The following sprays were applied in succession to the chromatoplate: (1) iodoplatinate; (2) ammoniacal silver nitrate; (3) po-

tassium permanganate. On occasion 0.5% sulfuric acid was initially sprayed on the plates and the Dragendorff reagent was applied after the iodoplatinate reagent. Following the iodoplatinate spray, color changes were observed and the chromatoplate allowed to dry for 10 min, sprayed with ammoniacal silver nitrate and placed in the oven at 100° for 5 min and color changes noted. Plates were then sprayed with potassium permanganate, color changes observed and R_F values determined. (c) Residues of urines extracted at pH 11.0 dissolved in the organic solvent were applied to plates developed and dried as described above. The plates were sprayed in succession with 0.4% ninhydrin, subjected to short-wave U.V. light, for 10 to 15 min and color changes noted. The plates were then sprayed with the iodoplatinate reagent, color changes observed, and the R_F values of the reactive compounds determined. In some cases the plates were sprayed with only the iodoplatinate reagent.

RESULTS AND DISCUSSION

The primary purpose of the study was to obtain methods which would allow rapid and accurate identification of drugs of abuse from human urine. The chemical principle of differential extraction, based on the ionization of acidic and basic drugs at various pH's, was utilized.

Urines extracted at pH 2.2

Table I summarizes the data obtained with barbiturate drugs extracted from urine by the ion-exchange paper technique[7] or direct solvent–solvent extraction. The silver acetate reagent reacted with the barbiturates and/or metabolites to provide bright white spots. The plates were then usually placed under U.V. light. If the plates were sprayed with 2% mercurous nitrate instead of silver acetate, the compounds appeared white on a grayish background. It was also possible to initially spray the

TABLE I

EXTRACTION OF BARBITURATES AT pH 2.2[a]

Drug	$R_F \times 100$									*Color*[c]		
	CS_1[b]	CS_2	CS_3	CS_4	CS_5	CS_6	CS_7	CS_8	CS_{13}	*Silver acetate*	*U.V. irradiation*	*Potassium permanganate*
Phenobarbital	52	41	97	92	99	32	25	14	40	Chalky white	White	Light pink
Pentobarbital	86	81	95	93	90	48	—	41	80	Chalky white	White	Light pink
Sodium barbital	70	46	95	93	100	90	—	19	54	Chalky white	White	White
Amobarbital	77	66	99	92	97	49	—	39	74	Chalky white	White	Light yellow
Secobarbital	84	72	97	95	97	51	36	44	78	Chalky white	White	Bright yellow

[a] The standard barbiturates were detected with the silver acetate reagent at a level of 0.5 μg. Using the ion-exchange paper technique phenobarbital and sodium barbital could not be detected below levels of 5 μg/ml of urine, whereas the other barbiturates were observed at levels between 1 and 5 μg/ml of urine. All the barbiturates except sodium barbital were detected at concentrations of 1 μg/ml of urine using the direct extraction method.

[b] For solvents, see text.

[c] Solvent systems primarily used were CS_1 and CS_2 and the color reactions reported were obtained with these solvent systems after development, following consecutive spraying.

plates with potassium permanganate, followed by mercurous nitrate which caused the compounds to turn black. U.V. irradiation did not provide a color change with either the free barbiturates or their metabolites. The plates were then sprayed with $KMnO_4$ which caused the barbiturate spots to turn light pink, remain the same or in the case of secobarbital turn bright yellow.

Urines obtained from patients admitted to the Clinical Research Center in Lexington, Ky. and shown to be positive for barbiturates by the method of COCHIN AND DALY[4] as performed in the chemistry laboratory of the Addiction Research Center (ARC), were subjected to the ion-exchange paper technique described under METHODS AND MATERIALS. Only 47% of these urines were found to be positive by ion-exchange extraction. The quantity of barbiturates ingested by the individuals was unknown, but it was quite obvious that the ion-exchange paper technique was not sensitive enough to detect all the barbiturates or their metabolites in each case.

Urines extracted at pH 9.3

Tables II and III show the data obtained on R_F values and the color reactions observed with narcotic analgesics and psychoactive drugs following consecutive spraying with various reagents. The narcotic analgesics could effectively be separated from one another upon choosing the proper solvent system. The consecutive spraying technique then allowed for further identification of a specific narcotic drug. Morphine quite characteristically appeared purple after spraying with the iodoplatinate reagent. Ammoniacal silver nitrate reacted with morphine to turn the spot black (precipitation

TABLE II

EXTRACTION OF NARCOTIC ANALGESICS AT pH 9.3[a]

Drug	$R_F \times 100$					*Color*[b]			
	CS_1	CS_2	CS_3	CS_4	CS_5	*Iodoplatinate*	*Ammoniacal silver nitrate*	*Potassium permanganate*	*Dragendorff's reagent*
Morphine	12	33	20	29	10	Dark purple	Black	Black-yellow	Rust-orange
Codeine	44	52	23	29	10	Purple	Purple	Purple-yellow	Rust-orange
Dihydrohydroxycodeinone	82	82	26	55	10	Purple	Red-brown	Yellow	Brown-orange
Dihydrocodeinone	36	47	17	12	7	Dark purple	Light black	Yellow	Rust-orange
Oxymorphone	57	59	27	53	10	Purple	Black	Black-yellow	Brown-orange
Methadone	94	96	13	37	20	Red-brown	Red-brown	Yellow	Rust-orange
Meperidine	79	92	44	49	20	Purple	Yellow	Yellow	Brown-orange
Propoxyphene	97	97	49	91	36	Red-purple	Red-purple	Light yellow	Brown-orange
Nalorphine	35	49	71	85	44	Dark blue	Black	Black-yellow	Brown-orange
Naloxone	60	65	—	—	—	Light blue	Blue-black	Yellow	Rust-orange
Quinine	40	67	31	45	32	Purple	Yellow	Yellow	Rust-orange

[a] With the iodoplatinate reagent most narcotic analgesic standards were detected at a minimum level of 0.1 to 1.0 μg. The narcotic analgesics were detected at a concentration of 0.5 to 1 μg/ml of urine with the ion-exchange paper technique, except for methadone where a minimum of 5 μg/ml of urine was required.

[b] The color reactions recorded were those obtained primarily with solvent systems CS_1 and CS_2, following consecutive spraying.

TABLE III

EXTRACTION OF PSYCHOACTIVE DRUGS AT pH 9.3[a]

Drug	$R_F \times 100$						*Color*[b]			
	CS_1	CS_2	CS_3	CS_4	CS_5	CS_{14}	*Iodoplatinate*	*Ammoniacal silver nitrate*	*Potassium permanganate*	*Dragendorff reagent*
Marihuana	96	98	97	97	96	14	Tan	Tan	Brown	—
Mescaline	12	40	10	5	7	—	Dark purple	Purple	Dark purple	—
LSD-25	59	76	82	87	40	—	Blue-purple	Blue	Rust-brown	—
Glutethamide	90	95	—	—	89	16	Brown	Dark brown	Dark brown	—
Psilocybin	—	57	5	2	2	2	Blue-purple	Purple	Purple	—
Diazepam	90	98	—	—	85	19	Reddish-brown	Brown	Dark brown	—
Chlordiazepoxide	40	53	94	92	77	15	Light purple	Purple	Purple	Orange
Chlorpromazine	90	94	40	39	13	14	Dark purple	Purple	Yellow	Orange
Cocaine	78	92	34	71	12	9	Purple	Purple	Purple	—

[a] The sensitivity of detection achieved with the authentic standards using the iodoplatinate reagent was 0.5 to 1 μg, except for glutethamide and psilocybin, which required about 20 μg, and marihuana, which required 100 μg. These drugs were detected, at a level of 1 μg/ml of urine, except for psilocybin, glutethamide, chlorpromazine and marihuana, which required 5 to 20 μg/ml of urine.

[b] The color reactions observed were primarily those obtained with solvent systems CS_1 and CS_2, following consecutive spraying.

of the silver salt of morphine). Spraying with potassium permanganate provided a yellow fringe around the black morphine spot. Codeine, methadone and meperidene could readily be differentiated from morphine by R_F values as well as the absence of converting the purple color reaction obtained with the iodoplatinate reagent to black following the ammoniacal silver nitrate spraying.

Methadone was difficult to extract using the ion-exchange technique and could not be detected below 5 to 10 μg/ml of urine. However, using the direct extraction method described by MULÉ[1] or the direct extraction described in this report, methadone could be extracted and detected at levels of 1 μg/ml of urine.

Urines obtained from patients admitted to the Clinical Research Center in Lexington, Ky., were analyzed for narcotic analgesics using the ion-exchange technique. In all cases the urines found positive by the chemistry laboratory of the ARC using a modification of the method described by MANNERING *et al.*[2], were also found to be positive using the ion-exchange extraction technique.

All the urines were analyzed without prior hydrolysis[1,11] (either acid or enzymatic). However, in some cases aliquots of the same urine were directly extracted, ion-exchange extracted or subjected to glucuronidase hydrolysis and then ion-exchange extracted. The methods were obviously more sensitive following hydrolysis due to liberation of the free drug from the glucuronide conjugate. It is recommended that either acid or enzymatic hydrolysis precede extraction of the drugs at pH 9.3.

In Table III the data appear obtained with the various psychoactive drugs extracted at pH 9.3. The ion-exchange extraction method was not very sensitive for several of these drugs (glutethamide, psilocybin, chlorpromazine, marihuana). However, even in the case of LSD where the drug could be detected in urine at a level of

TABLE IV

EXTRACTION OF AMPHETAMINE AND ANALOGUES AT pH 11.0[a]

Drug	$R_F \times 100$									*Color*[b]			
	CS_1	CS_2	CS_3	CS_4	CS_5	CS_8	CS_{10}	CS_{11}	CS_{12}	*Ninhydrin*	*U.V. irradiation*	*Iodoplatinate*	*Iodoplatinate*[c]
d-Amphetamine	67	74	13	11	14	13	90	12	20	Light pink	Red-purple	Purple	Purple-yellow
Methamphetamine	68	75	12	12	13	8	93	—	13	Light blue	Blue	Blue	Purple-grey
Ephedrine	35	—	—	—	17	—	89	43	13	—	—	Light purple	Light purple
Phenmetrazine	68	91	47	53	27	15	96	—	12	—	—	Blue	Purple

[a] The minimal sensitivity of detection achieved with ninhydrin or the iodoplatinate reagent for authentic standards of these drugs was 5 μg. Using ion-exchange paper a minimum of 10 μg/ml of urine was required for detection of these drugs except for ephedrine, which could not be detected. By directly extracting the urines as described under METHODS AND MATERIALS, *d*-amphetamine could be detected at a level of 1 μg/ml of urine.

[b] Solvent systems primarily used were CS_1 and CS_2 and the color reactions reported were obtained with the solvents after development, following consecutive spraying.

[c] Color reactions were those obtained with the iodoplatinate reagent alone without prior spraying.

1 to 5 μg/ml the method was not sufficiently sensitive to detect LSD in human urine following ingestion of the drug. This was primarily due to the relatively small quantity of LSD ingested (100 to 150 μg) and the subsequent extensive metabolism[12,13] of the drug.

Urines extracted at pH 11.0

Table IV summarizes the data obtained with *d*-amphetamine and analogues extracted at pH 11.0. It was quite difficult to separate *d*-amphetamine from methamphetamine (methadrine) with the chromatographic solvents used in this study. Methamphetamine did not react readily to the ninhydrin spray reagent, however, the color reaction was somewhat different from that observed for amphetamine. The ion-exchange extraction was totally inadequate for ephedrine or phenmetrazine and the ninhydrin spray reagent was ineffective for the detection of these drugs. The iodoplatinate reagent, however, was most effective in detecting *d*-amphetamine and its analogues.

Urines obtained from patients admitted to the Clinical Research Center, Lexington, Ky., who had received 15 to 30 mg of *d*-amphetamine and/or 15 to 30 mg of methamphetamine, were extracted using the ion-exchange technique and the extracts were subjected to thin-layer chromatography. In comparison to standards it was quite doubtful that the drugs were detected at all. Urines were also obtained from patients at time intervals of 6 h through 24 h following the ingestion of 7.5 mg each of *d*-amphetamine and methamphetamine. The urines were ion-exchange extracted and chromatographed. Neither *d*-amphetamine nor methamphetamine could be detected in the urine of these patients at each time interval tested.

Five milliliters of urine obtained at each time interval following the mixture of *d*-amphetamine and methamphetamine were extracted using the method described by BECKETT AND ROWLAND[6] and subjected to thin-layer chromatography. The drugs were not detected using the ninhydrin reagent but very faint blue color reactions were noted at certain time intervals following the iodoplatinate spray reagent. In another experiment urine samples were obtained from patients which had received either 15 mg of *d*-amphetamine or 15 mg of methamphetamine and were directly extracted as follows: In 40 ml glass stoppered centrifuge tubes 15 ml of urine, 10 ml of carbonate buffer, pH 11.0 and 10 ml of $CHCl_3$ were placed. The samples were shaken for 15 min (300 oscillations/min), centrifuged and the $CHCl_3$ layers removed. The aqueous phases were re-extracted with 10 ml of $CHCl_3$ as described and the $CHCl_3$ extracts combined. About 50 μl of glacial acetic acid was added to the $CHCl_3$ and the organic solvent evaporated on a water bath under N_2. The residues were dissolved in about 25 μl of methanol and chromatographed. *d*-Amphetamine and methamphetamine were detected at each time interval using ninhydrin, U.V. and the iodoplatinate spray reagent. It would appear that the direct extraction method as described provided the best results and in essence was the only sensitive technique for detecting *d*-amphetamine and its analogues in urine.

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