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RESEARCH ARTICLES

TLC Techniques for Identification of Narcotics, Barbiturates, and CNS Stimulants in a Drug Abuse Urine Screening Program

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Abstract □ TLC identification techniques well suited to large-scale screening of urine samples for drugs of abuse are presented. Chromatographic developing solvent systems are described that can differentiate: (*a*) drugs of abuse and their adulterants from drugs used in treatment, and (*b*) methadone and/or cocaine from methapyrilene, diphenhydramine, pentazocine, cyclazocine, pipradrol, propoxyphene, thioridazine, promazine, and chlorpromazine. In addition, highly reliable spraying techniques for the identification of amphetamine, methamphetamine, and phenmetrazine are suggested. The combination of sprays described here reliably detects barbiturates at relatively low concentrations. A spraying technique to differentiate methadone from cocaine is also described.

Keyphrases □ Narcotics, barbiturates, and CNS stimulants—TLC identification, drug abuse urine screening program □ Barbiturates, narcotics, and CNS stimulants—TLC identification, drug abuse urine screening program □ CNS stimulants, narcotics, and barbiturates—TLC identification, drug abuse urine screening program □ Drug abuse urine screening program—TLC identification, narcotics, barbiturates, and CNS stimulants □ TLC—identification, narcotics, barbiturates, and CNS stimulants, drug abuse urine screening program

At present, TLC is the most suitable technique for large-scale screening of drugs of abuse in human urine. This technique meets all the criteria (*e.g.*, minimum instrumentation, low cost, minimum laboratory space, rapidity of analysis, excellent sharpness of separation, sensitivity to a wide variety of drugs of abuse, specificity, and ease of interpretation of results by laboratory personnel with minimal formal training) for its selection as a routine method for a large-scale urine screening program.

In addition, this technique permits the simultaneous identification of a wide range of substances in a single run. The monitoring of a urine specimen by TLC can alert the operator immediately of the number of drugs present in a specimen. Furthermore, the sensitivity of the technique can be easily adapted according to the purpose of screening, *e.g.*, screening of urines from patients in treatment for specific drugs of abuse or preemployment screening of urines. Large-scale drug abuse treatment programs require such a versatile and low cost screening procedure (currently, more than 1600 individuals are being treated in this institution and it is projected that 3000 will be treated within 18 months). Furthermore, an attempt is being made to rehabilitate a wide variety of drug users, opiate as well as nonopiate users. A significant percentage of patients are also taking prescribed tranquilizers or antibiotic drugs. It was, therefore, necessary to develop a mass screening technique capable of detecting a wide variety of substances and of differentiating illicit drugs and their adulterants from legitimate and prescribed drugs and their metabolites.

The only other technique that can permit simultaneous screening of a mixture of drugs is GLC but it has the inherent disadvantage of running a single specimen at a time; thus, it becomes time consuming and more expensive than TLC. A single specimen, using GLC, requires 20–30 min. for the complete screening of amphetamines and opiates, whereas 12–15 different urine specimens can be detected for a wide variety of drugs on a single thin-layer chromatoplate. GLC is used only for research and developmental work, and for validation of some results obtained by TLC.

Another potentially useful technique for mass screening of urines for morphine and conformationally related narcotic analgesics was recently reported (1). This technique is called the "free radical assay technique" (FRAT). When morphine, spin-labeled at the phenolic hydroxyl position, is added to a morphine–antibody preparation, it becomes bound to the antibody, immobilizing the spin-label and broadening the electron spin resonance (ESR) spectral signal. When a urine specimen containing morphine is mixed with the complex, some of the spin-labeled morphine is displaced and

Table I—R_f Values of Various Drugs[a]

Drug	$R_f \times 100$ Solvent A	Solvent B	Solvent C	Solvent E
Acetylmethadol	81,93[b]	95	98	96
Amphetamine	31	53	52	83
Chlordiazepoxide	55	64	72	74
Chlorpheniramine	44	58	70	91
Chlorpromazine	71	87	88	92
Cocaine	88	93	95	99
Codeine	24	34	42	76
Cyclazocine	60	70	72	97
Diazepam	89	92	92	93
Diphenhydramine (Benadryl)	71	83	86	91
Ephedrine	17	26	34	68
Hydromorphone	15	15	24	25
Imipramine	62	77	85	96
Iproniazid	25,50[b]	60,74[b]	62,89[b]	36,67[b]
Isoniazid	22	28	35	38
Lysergic acid diethylamide	55	64	66	—
Meperidine (Demerol)	62	66	72	95
Mescaline	15	15	22	—
Methadone	83	91	94	98
Methamphetamine	22	46	48	81
Methapyrilene (Histadyl)	70	78	86	97
Methaqualone	88	91	95	—
Methylphenidate (Ritalin)	80	80	85	96
Morphine	13	14	24	45
Naloxone	78	74	69	76
Pentazocine (Talwin)	90	85	90	98
Phenmetrazine (Preludin)	43	48	55	91
Pipradrol	91	96	98	99
Promazine	60	73	77	86
Propoxyphene (Darvon)	88	95	98	93
Quinine	34	38	50	71
Tetracycline	0	0	0	0
Thioridazine (Mellaril)	73	80	82	97
Trifluoperazine (Eskazine, Stelazine)	77	79	83	85

[a] Gelman precoated silica gel glass microfiber sheets, with a layer thickness of 250 μ, were used. Each developing solvent was allowed to travel a distance of 10 cm. [b] Showed two spots.

the ESR peaks are sharpened. This technique is reported to be many times more sensitive than TLC, to be sensitive to both free morphine and its glucuronide conjugate, and to require only about 30 sec. to complete a test. The cost for each narcotic test has not yet been fully worked out, but it is likely that it will eventually be less than $1.

The technique is specific and takes far less time than TLC, but the cost and time of analysis start rising if the aim is to screen drugs other than morphine and its conformationally related narcotic analgesics (*e.g.*, adulterants used to cut heroin, amphetamine and related stimulants, cocaine, sedative–hypnotics, and drugs used in treatment like methadone, cyclazocine, and naloxone). In addition, free radical–antibody preparations for detecting other than morphine-like drugs are not yet available. At present, we are able to decrease the workload and cost of analysis in this laboratory by adsorbing the drugs in a urine specimen on a piece of cation-exchange resin-loaded paper and then combining several ion papers of the same patient representing different urine specimens. Different specimens cannot be pooled using FRAT without the risk of diluting a positive specimen with several negative specimens. Thus, the cost of analysis for performing a number of urine tests on the same patient increases. Therefore, for the immediate future, TLC remains the technique best suited for use in large-scale screening programs.

There are several papers describing different extraction techniques, solvent systems, and spraying techniques for the identification of stimulants, narcotic analgesics, and sedative–hypnotics in urine using TLC (2–21). The solvent systems and spraying techniques developed by Davidow *et al.* (6, 7) and Dole *et al.* (8, 9) are the ones most commonly employed in monitoring treatment programs. While using these solvent systems in these laboratories, some difficulties were encountered in differentiating drugs of abuse and their adulterants from drugs used in treatment. In addition, in our hands at least, spraying techniques published to date for the detection of amphetamines (and congeners like phenmetrazine and methylphenidate) gave inconsistent results, even for standards carried through urines. Similarly, spraying techniques for the detection of sedative–hypnotics yielded unsatisfactory results due to poor visualization of spots. Some of these difficulties have been resolved in this laboratory, and the purpose of this article is to present some useful thin-layer developing solvent systems and reliable and sensitive spraying techniques for use in large-scale urine screening programs. Thin-layer developing solvent systems are described for routine use to differentiate: (*a*) drugs used in treatment (*e.g.*, methadone, acetylmethadol, cyclazocine, and drugs such as isoniazid for tuberculosis) from both the drugs of abuse and their adulterants (*e.g.*, antihistamines and quinine); (*b*) drugs such as cocaine, *d*-propoxyphene, pentazocine, and pipradrol from methadone; (*c*) methamphetamine, quinine, and phenmetrazine from each other; and (*d*) amphetamine from methamphetamine. Reproducible and reliable spraying techniques for the identification of amphetamine, methamphetamine, phenmetrazine, and barbiturates are proposed. In addition, a spraying technique to differentiate methadone from cocaine is described.

EXPERIMENTAL

TLC—Gelman precoated silica gel glass microfiber sheets[1], with a layer thickness of 250 μ, were used throughout.

A 20 × 20-cm. sheet is cut into either four pieces of 10 × 10 cm. or two pieces of 10 × 20 cm. Ordinary rectangular battery jars are used to develop 10 × 10-cm. sheets; about 30–40 ml. of developing solvent is used and allowed to travel a distance of about 6–8 cm. If a standard size rectangular developing tank with double groove is used, about 120–150 ml. of developing solvent is added and two sheets of 10 × 20 or 20 × 20 cm. or four sheets of 10 × 10 cm. are allowed to develop in each tank.

Preparation of Sample—The extraction techniques used are essentially the same as were reported recently (19, 20). A 6 × 6-cm. piece of cation-exchange resin-loaded paper[2] is soaked in 40–50 ml. of undiluted urine (pH 5–6) with intermittent shaking. After 30 min. or more, the ion paper is transferred to a 120-ml. (4-oz.) wide-mouth screw-capped jar, rinsed twice with distilled water (washing prevents emulsion formation during extraction), and extracted for barbiturates, opiates, and amphetamines. Sedative–hypnotics, some benzodiazepines, and other drugs are extracted first with 15 ml. each of

[1] I.T.L.C. Type SA.
[2] Reeve Angel SA-2.

Table II—R_f Values and Color Reactions of Sedative–Hypnotics and Benzodiazepine Compounds[a]

Drug	$R_f \times 100$, Solvent D	Color[b] Diphenylcarbazone	Silver Acetate	Mercuric Sulfate	Iodine–Potassium Iodide
Amobarbital[c]	87	OrPk[d]	BlPu	PuF	Nc
Sodium barbital[e]	73	Nc[f]	BlPu	Pu	Nc
Diphenylhydantoin[c] (Dilantin)	70	OrPk[d]	BlPu	Pu	Nc
Glutethimide[c] (Doriden)	89	OrPk[f]	BlPu	PuF	Nc
Pentobarbital[c]	87	OrPk[d]	BlPu	Pu	Nc
Phenobarbital[c]	66	OrPk[d]	BlPu	Pu	Nc
Secobarbital[c]	88	OrPk[d]	BlPu	Pu	Nc
Chlordiazepoxide[g] (Librium)	27	Nc	Nc	Nc[h]	DBr
Chlordiazepoxide metabolite (Ro5-2092)	27	Nc	BlPu (2.5 mcg. or above)	Nc[h]	DBr
Diazepam (Valium)	86	OrPk (2.0 mcg. or above)	BlPu (1.5 mcg. or above)	Nc[h]	DBr
Diazepam metabolite (Ro5-5345)[i]	65	Nc	BlPu (0.5 mcg. or above)	Nc[h]	LBrF
Diazepam metabolite (Ro5-6789)[j]	31	Pu	BlPu (0.5 mcg. or above)	Lv or Bl (0.5 mcg.)	DBrF
Diazepam metabolite (Ro5-2180)	71	Nc	BlPu (0.5 mcg. or above)	BlPu (0.5 mcg.)	DBr
Oxazepam	31	LPu (2 mcg. or above)	BlPu (0.5 mcg. or above)	Lv or Bl (0.5 mcg.)	DBrF

[a] Color reactions reported were obtained on Gelman precoated silica gel glass microfiber sheets (I.T.L.C. Type SA), with a layer thickness of 250 μ, following consecutive spraying. Solvent D was used as a developing solvent and was allowed to travel a distance of 10 cm. Barbiturates can be differentiated from benzodiazepine compounds and their metabolites by overspraying the plate with iodine–potassium iodide solution. Spots due to barbiturates do not form any color with iodine–potassium iodide spray. Methadone and its metabolite are also extractable at pH 1 along with sedative–hypnotics and can be identified by overspraying the plate with iodine–potassium iodide. [b] Bl, blue; Br, brown; Ch, charcoal; D, dark; Dis, disappears; Dz, decolorized zone forms within a few seconds; F, fades away; Gn, green; Go, gold; Gy, gray; L, light; Lv, lavender; NBl, navy blue; Nc, no color; Or, orange; Pk, pink; Pu, purple; Re, reddish; Tn, tan; Tq, turquoise; W, weak; Wr, weak reaction; Yl, yellow. [c] The standards of these drugs were detected with the proposed combination of sprays at the level of 0.1 mcg. [d] Sometimes orange-pink spots for barbiturates may not appear after the diphenylcarbazone spray, but this spray must be applied to make the spots visible with subsequent sprays. [e] The standard of sodium barbital was detected with the proposed combination of sprays at the level of 0.5–1 mcg. [f] Barbital and glutethimide in urine can be seen with subsequent sprays only if the diphenylcarbazone spray was applied initially. To achieve the best results for these two drugs, the spray should be stored in a refrigerator and used within 24 hr. This spray, if stored in a refrigerator, can be used for a week for sedative–hypnotics other than barbital and glutethimide. [g] Patients on this drug showed in their urine a metabolite which behaved like barbiturates; but on spraying the chromatoplate with iodine–potassium iodide, these specimens showed a dark-brown spot at an R_f value of about 0.18–0.27, indicating the presence of unchanged drug and/or its metabolite (Ro5-2092) (lactam of chlordiazepoxide). [h] The standards of these drugs did not give any color with mercuric sulfate up to the level of 4.5–5 mcg. Diazepam gave a light-purple color with mercuric sulfate at the 5.0-mcg. level. [i] This metabolite of diazepam is the same as oxazepam. [j] This metabolite showed another spot at an R_f value of about 0.39, which gave a bluish-purple spot with mercuric sulfate.

sodium citrate[3] buffer (pH 1) and chloroform. After shaking for 10 min., the lower organic phase is pipeted out into a plain 15-ml. conical centrifuge tube[4] and solvent is evaporated to dryness in an oven[4] (65° for the first 3 hr. and then 85–90° until dry) with horizontal flow of air. The residue along the sides of the tube is washed with 0.5–1 ml. of methanol, and the methanol is evaporated to dryness.

The aqueous buffer (pH 1) is discarded and the ion paper is then extracted at pH 10.1 for narcotic analgesics, amphetamine and congeners, and selected psychotropic drugs. Extraction is accomplished by using 15 ml. each of ammonium chloride–ammonium hydroxide buffer[5] (pH 10.1) and chloroform–isopropanol (3:1). Ammonia fumes can be a problem[6]. Therefore, when there is no interest in amphetamines, a borate buffer (pH 9.3)[7] (8, 9) is used. The process is completed as already described, except that the lower organic phase is pipeted out into a plain 15-ml. conical centrifuge tube[4] containing two drops (about 50 μl.) of sulfuric acid (0.5%) in methanol. Sulfuric acid is omitted if amphetamines are not to be detected.

Alternatively, drugs can be directly extracted from the urine specimen by adding 5 ml. of 3.7% hydrochloric acid (producing a pH of 1) and 15 ml. of benzene–chloroform (8:2) to 15 ml. of urine in a 50-ml. screw-capped round centrifuge tube. The tube is shaken gently by hand in a vertical direction for 1 min. and centrifuged if necessary, and the upper organic layer is pipeted out into a plain 15-ml. conical centrifuge tube[4]. The process is completed as already described for sedative–hypnotics. The residue obtained is tested for sedative–hypnotics, benzodiazepines, and other drugs.

To the residual aqueous acidic phase remaining after extraction of sedative–hypnotics (or to a 15-ml. aliquot of urine if barbiturate detection is not desired) are added 10 ml. of ammonium chloride–ammonium hydroxide buffer and 15 ml. of chloroform–isopropanol (9:1). The process is completed as already described, except that the lower organic phase is pipeted out into a plain 15-ml. conical centrifuge tube[4] containing 2 drops (50 μl.) of sulfuric acid (0.5%) in methanol. Sulfuric acid is omitted if amphetamines are not to be detected. The residue obtained is tested for opiates, amphetamines, and psychotropic drugs.

Sensitivities of ion-exchange and direct-extraction techniques were described in detail elsewhere (19, 20).

Solvent Systems—The following solvent systems proved useful:

A. Ethyl acetate–cyclohexane–*p*-dioxane–methanol–water–ammonium hydroxide (50:50:10:10:1.5:0.5)
B. Same as A but ratio of water to ammonium hydroxide is reversed, *i.e.*, 0.5:1.5
C. Ethyl acetate–cyclohexane–ammonium hydroxide–methanol–water (70:15:2:8:0.5)
D. Ethyl acetate–cyclohexane–methanol–ammonium hydroxide (56:40:0.8:0.4)
E. Ethyl acetate–cyclohexane–methanol–ammonium hydroxide (70:15:10:5)
F. Ethyl acetate–cyclohexane–ammonium hydroxide (50:40:0.1)

[3] Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), 296 g. in water, followed by 256 ml. concentrated hydrochloric acid, diluted to 2000 ml. with water (pH 1.0 ± 0.1).

[4] When there are not many samples to be tested or when results are required faster, the lower organic phase is pipeted out into a plain 40- or 50-ml. conical centrifuge tube and the solvent is evaporated to dryness on a boiling water bath. By using this modification, complete analysis of 12–13 samples can be performed within 30–40 min. after the receipt of the ion-exchange paper or urine. This modification is used for all samples that require results to be reported within 30–60 min. after complete screening.

[5] Saturated solution of ammonium chloride (2500 ml.) adjusted to pH 10.1 ± 0.1 with concentrated ammonium hydroxide (about 2400 ml.).

[6] Due to ammonia fumes, certain steps like preparation of buffer and discarding of buffer and ion papers after extraction are carried out in a fume hood. Ion papers should be first dumped into a beaker containing a large volume of water before being discarded.

[7] Saturated solution of sodium borate, $Na_2B_4O_7 \cdot 10H_2O$ (2850 ml.), adjusted to pH 9.3 with sodium hydroxide solution (150 ml. of 1.2% solution of sodium hydroxide is required).

Table III—R_f Values of Various Combinations of Drugs

Drugs	$R_f \times 100$ Solvent A[a]	Solvent B[a]	Solvent C[a]	Solvent F[b]
Amphetamine–methamphetamine	30, 23	51, 45	57, 48	—
Amphetamine–phenmetrazine–quinine	30, 38, 31[c]	48, 52, 48[c]	57, 58, 48[c]	—
Amphetamine–quinine	31, 31[c]	45, 40	55, 46	—
Methamphetamine–phenmetrazine–quinine	28, 45, 34	37, 49, 40	44, 56, 44	—
Methamphetamine–quinine	28, 34	37, 40	43, 45	—
Methadone–acetylmethadol	80, 82[d], 93	92, 96	97[e]	28, 47[d], 37
Methadone–chlorpromazine (Largactil, Thorazine)	80, 70	92, 84	94, 86	28, 15
Methadone–cocaine	80, 89	92[e]	94[e]	28, 46
Methadone–cyclazocine	80, 57	91, 70	93, 73	—
Methadone–diphenhydramine (Benadryl)	80, 67	90, 84	92, 84	28, 14
Methadone–imipramine	79, 64	90, 79	92, 76	—
Methadone–meperidine (Demerol)	80, 57	90, 68	92, 71	—
Methadone–methapyrilene (Histadyl)	82, 68	90, 77	92, 78	—
Methadone–pentazocine (Talwin)	82, 75	90, 82	95, 91	27, 23
Methadone–pipradrol	82, 94	91, 94	97[e]	27, 59
Methadone–promazine	82, 52	91, 70	95, 78	—
Methadone–propoxyphene (Darvon)	83, 95	91, 96	95[e]	27, 55
Methadone–methylphenidate (Ritalin)	83, 74	91, 77	94, 85	—
Methadone–thioridazine (Mellaril)	83, 72	91, 82	94, 84	27, 8
Methadone–trifluoperazine (Eskazine, Stelazine)	83, 57	93, 70	94, 76	—
Morphine–hydromorphone	12[e]	14[e]	15[e]	—
Morphine–isoniazid (INH)	13[e]	15, 23	16, 24	—
Phenmetrazine–quinine	32, 27	47, 35	51, 38	—
Cocaine–chlorpromazine (Largactil, Thorazine)	89, 70	95, 85	97, 86	46, 20
Cocaine–diphenhydramine (Benadryl)	87, 69	96, 85	97, 84	46, 15
Cocaine–pentazocine (Talwin)	88, 76	96, 88	96, 90	46, 27
Cocaine–pipradrol	88, 93	98[e]	99[e]	46, 58
Cocaine–promazine	88, 62	96, 71	96, 77	46, 7
Cocaine–propoxyphene (Darvon)	88, 87	96[e]	97[e]	46, 60
Cocaine–thioridazine (Mellaril)	88, 74	95[e]	96, 88	46, 7

[a] Gelman precoated silica gel glass microfiber sheets, with a layer thickness of 250 μ, were used. Each developing solvent was allowed to travel a distance of 10 cm. [b] This is a special solvent designed to resolve mixtures of drugs that could give false positives for methadone and/or cocaine. [c] Mixtures of these drugs could be resolved by allowing the developing solvent to travel a distance of about 13 cm. [d] Acetylmethadol seen as two spots in Solvents A and F. [e] The mixture of these compounds showed only one spot.

All solvents except C should be used within 24 hr. of their preparation to achieve good results.

Solvent C should preferably be used after storing overnight; it keeps well for 3–4 weeks.

Detection Reagents—The following were used:

(*a*) Ninhydrin (0.5% w/v) in *n*-butanol; solution can be used for 24–48 hr. if stored in a refrigerator.

(*b*) Sulfuric acid (0.5% v/v) in water.

(*c*) Iodoplatinate: (i) Stock solution 5 ml. of 5% platinum trichloride solution mixed with 45 ml. of 10% potassium iodide and 50 ml. of water. (ii) Spray solution, equal volumes of stock solution and 2 *N* hydrochloric acid, mixed before use (2 *N* hydrochloric acid, approximately 17 ml. of concentrated hydrochloric acid diluted to 100 ml. with water) (9).

(*d*) Ammoniacal silver nitrate. Mix 15 ml. each of 5 *N* ammonium hydroxide and 50% solution of silver nitrate, and add more 5*N* ammonium hydroxide until the solution becomes clear (about 20 ml. of 5 *N* ammonium hydroxide required); 5 *N* ammonium hydroxide approximately, prepared by mixing 19.4 ml. concentrated ammonium hydroxide with 80.6 ml. water (9).

(*e*) Potassium permanganate, 0.02 *M* in water.

(*f*) Bromcresol green sodium salt, 0.2% w/v in 50% ethanol (use within 24 hr.).

(*g*) Sodium bicarbonate, 1% w/v in water.

(*h*) Diphenylcarbazone (7), 0.01% in equal parts of acetone and water (see *Footnote f*, Table II).

(*i*) Silver acetate, 1% w/v in water.

(*j*) Mercuric sulfate solution. Mercuric oxide (HgO), 0.50 g., is dissolved in 20 ml. of concentrated sulfuric acid. The acid solution is added slowly to water and then made to 200 ml. with water (7).

(*k*) Iodine–potassium iodide (21). Iodine (2 g.) is added to 50 ml. of 95% ethanol and shaken; potassium iodide (2 g.) is dissolved in 16.2 ml. of water, and both solutions are mixed together and shaken until a clear solution is formed; then 33.8 ml. of concentrated hydrochloric acid is added and mixed to form the final solution, which is stored at room temperature.

Procedure—Sample solution is prepared by dissolving the residues obtained under *Preparation of Sample* in 30–50 μl. of methanol[8]. Using a plain 5-μl. disposable capillary tube[9], one capillary (5 μl.) of standard solution of opiates[10] at each edge (1 cm. away from edge and 1.5 cm. up from the bottom of plate) and five samples each 1.2 cm. apart are spotted on a 10 × 10-cm. chromatoplate[11]. Depending on the needs of the program, either the entire extract of sample solution is transferred in 5-μl. aliquots to the thin-layer plate or only one or two capillaries are transferred and the remaining solution of residue is saved for the identification of drugs like cocaine or for validation of results by GLC technique. If cocaine is to be detected, then the remaining solution (if no solution is left over after spotting for opiates and amphetamines, another 30 μl. of methanol is added to the residue) is spotted on a separate thin-layer plate adjacent to methadone and cocaine standards (5 μl. of methadone standard solution is first applied and then 5 μl. of cocaine standard solution is overspotted on the methadone standard). The use of an air blower to evaporate the sample solution on the thin-layer plate is avoided when amphetamines are to be detected. The diameter of the spot at

[8] Residues to be tested for opiates alone using sodium borate buffer and residues to be tested for barbiturates using sodium citrate buffer require about 30 μl. (3 drops) of methanol; residues to be tested for opiates and amphetamines using ammonium chloride–ammonium hydroxide buffer require 40–50 μl. of methanol due to extraction of some ammonium salt with chloroform–isopropanol.

[9] These capillaries are used in these laboratories for routine spotting of about 3000 samples and are preferred over the Hamilton microliter syringe since we are interested primarily in the progress of a treatment modality and, therefore, mainly in qualitative information. When these findings are required for legal toxicological work, the Hamilton microliter syringe should be used for spotting.

[10] For the best comparison of R_f values of unknowns with the standards, these standards may be added to control urine and carried through the same extraction procedure used for the extraction of urine samples. Although we have found that the carrying of standards through the extraction procedure is not necessary for routine use, the operator may do so if desired.

[11] Three standards (one at each edge and one in the center) and 11–12 samples are spotted if a 10 × 20-cm. chromatoplate is used.

Table IV—Color Reactions[a] Using Spraying Techniques (*a*) and (*b*)[b]

Drug	Spraying Technique (*a*) — Ninhydrin Procedure — UV 5 min. and Heat at 90° for 4 min.	Spraying Technique (*a*) — Ninhydrin Procedure — Respray and Heat at Low Temperature on a Hot Plate	Spraying Technique (*a*) — Sulfuric Acid, 0.5%	Spraying Technique (*a*) — Iodoplatinate	Spraying Technique (*a*) — Ammoniacal Silver Nitrate and Heat	Spraying Technique (*a*) — Sensitivity	Spraying Technique (*b*) — Bromcresol Green (BCG)	Spraying Technique (*b*) — Sodium Bicarbonate	Spraying Technique (*b*) — 0.5% Sulfuric Acid	Spraying Technique (*b*) — Sensitivity
Amphetamine	Gy after UV and LGy after heat[c]	GyF	BlGy	Dis	—	1 mcg.	Pu after 4 min.	Bl	—	1 mcg. (BCG), 0.5 mcg. ($NaHCO_3$)
α-Acetylmethadol	—	—	Gy	PuBr	—	1 mcg.	Pu after 2 min.	DPu	Dz but Pu at back	1 mcg. (BCG), 0.5 mcg. ($NaHCO_3$)
Chlordiazepoxide	—	—	—	—	—	—	—	—	—	—
Chlordiazepoxide metabolite (Ro5-2092)	—	—	—	—	—	—	—	Dz	Dz after 2 min.	1 mcg. ($NaHCO_3$)
Chlorpheniramine	—	LGy	LBr or LGy	Ch	Ch while spraying, Br or Tn after heat	0.5 mcg. (iodoplatinate)	Pu after 3 min.	DPu	Dz after 2 min.	0.5 mcg. (BCG)
Chlorpromazine (Largactil, Thorazine)	—	—	Tq or LBl	GyBr or Gy	Gy or Br after heat	0.5 mcg.	LPu at back after 6 min.	LPu	Dz after 5 min.	1 mcg. (BCG)
Cocaine	—	—	—	Br or Tn	LBr after heat	1 mcg.	—	—	—	—
Codeine	—	—	—	NBl	DBr after heat	2 mcg. (iodoplatinate), 0.5 mcg. (ammoniacal silver nitrate and heat)	—	—	—	—
Cyclazocine	—	—	LGy	Br	DBr after heat	2.5 mcg.	Pu after 1 min.	Pu	Faint	1 mcg. (BCG), 0.5 mcg. ($NaHCO_3$)
Diazepam	—	—	—	Br or Tn	—	1 mcg.	Dz at back after 5 min.	Dz	Dz	1 mcg.
Diazepam metabolite (Ro5-6789)[d]	—	Tn or Yl	Tn or Or	LTn or Or	Gy while spraying, Dis after heat but Tn at back	1.5 mcg.	LPu at back after 5 min.	LPu	Dz	1 mcg.
Diazepam metabolite (Ro5-2180)	—	—	—	Tn	Dis but Tn at back after heat	2 mcg.	—	Dz	Bl at back	1 mcg. ($NaHCO_3$)
Diazepam metabolite (Ro5-5345)	—	Pu	Pu	Br	Dis but Tn at back after heat	1 mcg.	—	Dz	Bl at back	1 mcg. ($NaHCO_3$)
Diphenhydramine (Benadryl)	—	—	Gy	Tn (1 mcg.), GyBr (2 mcg.)	—	1 mcg.	Pu after 3 min.	LPu (0.5 mcg.), DPu (1 mcg.)	Pu at back	1 mcg. (BCG), 0.5 mcg. ($NaHCO_3$)
Ephedrine	LPu (streak) after heat at 90°	DPu	RePu	Dis	—	1 mcg.	—	—	—	—

(*Continued*)

Table IV—(*Continued*)

Drug	Spraying Technique (*a*) —Ninhydrin Procedure— UV 5 min. and Heat at 90° for 4 min.	Respray and Heat at Low Temperature on a Hot Plate	Sulfuric Acid, 0.5%	Iodoplatinate	Ammoniacal Silver Nitrate and Heat	Sensitivity	Spraying Technique (*b*) Bromcresol Green (BCG)	Sodium Bicarbonate	0.5% Sulfuric Acid	Sensitivity
Hydromorphone	—	Tn (1 mcg.)	GyPu	GyPu	Bl while spraying and DBr after heat	1 mcg.	Pu at back after 6 min.	Bl	Dis but Pu at back	0.5 mcg. (BCG)
Imipramine	Yl after after heat at 90°	—	Tq	Br	LGy or Tn after heat	1 mcg.	LPu after 3 min.	LPu	Pu at back	0.5 mcg. (BCG)
Iproniazid	—	Pu	LGy	—	—	1 mcg.	—	—	—	—
Isoniazid (INH)	Yl after UV (5 mcg.) and Pu after heat at 90° (1 mcg.)	DPu	LPu or DPu	—	—	1 mcg.	—	—	—	—
Lysergic acid diethylamide (LSD)	Pu fluorescence under UV	GyPu or DPu	DGyPu	DBr	LBr after heat	1 mcg.	LPu at back (5 mcg.)	DBl after 1 min. (2 mcg.)	Dis but Dz at back	2 mcg. ($NaHCO_3$)
Meperidine (Demerol)	—	—	—	GyPu	—	1.5 mcg.	Pu at back after 3 min.	Pu	Dis but Dz at back	1 mcg. (BCG), 0.5 mcg. ($NaHCO_3$)
Methamphetamine	Pu after heat at 90°	GyPu or PuF	Gy or LPu	Dis	—	1 mcg.	Pu after 6 min.	Bl or Pu	Dis	0.5 mcg.
Methadone	—	—	LBl	LTn, Br, or PuBr	Tn or LBr at back (1.5 mcg.)	0.5 mcg. and 1 mcg.	LPu or Pu after 3 min.	Pu	Dz changes to LPu after 3 min.	0.5–1 mcg.
Mescaline	Pu after UV and heat at 90°	LPu or DPu	RePu or ReOr	Pu (Dis) (1 mcg.), Pu or ReOr (3 mcg. or more)	Tn after heat	0.5 mcg. and 1 mcg.	Pu after 4 min.	Bl	Dis	1 mcg. (BCG), 0.5 mcg. ($NaHCO_3$)
Methapyrilene (Histadyl)	—	—	—	Ch	Dis after heat	0.5 mcg.	LPu at back after 6 min.	Bl or LPu	Dis	1 mcg. ($NaHCO_3$)
Methylphenidate (Ritalin)	Pu after heat at 90°	Gy (weak)	LGy	LTn	Dis after heat	2 mcg.	—	Pu or LPu	Pu at back	1.5 mcg. ($NaHCO_3$)
Morphine	—	—	—	NBl (2 mcg.)	Bleaches while spraying and Br or DBr after heat (0.5 mcg.)	2 mcg. (iodoplatinate) 0.5 mcg. (ammoniacal silver nitrate and heat)	—	—	—	—
Naloxone	—	—	—	—	Br or DBr after heat	1.5 mcg.	—	—	—	—
Pentazocine (Talwin)	—	Pu or LPu	Pu or LPu or BlPu	BrPu, Gy, or LBr	Br or LBr after heat	1.5–2 mcg.	Pu while spraying	Pu	Dis	0.5 mcg.
Phenmetrazine	—	Pk	DPk	LPk	—	0.5 mcg.	Pu after 6 min.	DPu or Bl	Pu at back	0.5 mcg. ($NaHCO_3$)

Table IV—(*Continued*)

Drug	Spraying Technique (*a*) —Ninhydrin Procedure— UV 5 min. and Heat at 90° for 4 min.	Respray and Heat at Low Temperature on a Hot Plate	Sulfuric Acid, 0.5%	Iodoplatinate	Ammoniacal Silver Nitrate and Heat	Sensitivity	Spraying Technique (*b*) Bromcresol Green (BCG)	Sodium Bicarbonate	0.5% Sulfuric Acid	Sensitivity
Phenylpropanolamine	Pu after UV and DPu after heat	DPu or Pu	RePu	Dis	—	0.5 mcg.	—	LPu	Dis	2 mcg.
Pipradrol	—	Pu or very LPu	LPu	Dis or Tn	Dis, Br at back after heat	0.5–1 mcg.	LPu after 3–4 min.	LPu	LPu at back	1 mcg.
Promazine	—	—	LBl or Bl	Ch or Gy	Gy or D while spraying and Br or D after heat	0.5–1 mcg.	Pu after 5 min.	Pu	Dis but Pu at back	1 mcg. (BCG), 0.5 mcg. ($NaHCO_3$)
Propoxyphene (Darvon)	—	—	LBl	LBr or Bl	Br while spraying, Dis after heat	1 mcg.	LPu after 3 min.	LPu	Dz	0.5 mcg.
Quinine	—	—	LGy or BlGy; also examined under UV for fluorescence	Tn (0.5 mcg.), LBr (1 mcg.)	—	Less than 0.5 mcg. under UV, 0.5 mcg. (iodoplatinate)	—	—	Nc but examine under UV for fluorescence	0.5 mcg. or less under UV after H_2SO_4
Tetracycline	—	—	—	—	—	—	—	—	—	—
Thioridazine (Mellaril)	—	—	LBl or Tq	Ch or GyBr	Gy while spraying and DBr or LCh after heat	0.5–1 mcg.	NBl after 1 min.	Pu	Pu at back	0.5 mcg.
Trifluoperazine (Eskazine, Stelazine)	—	—	Bl	Ch or GyPu	Gy while spraying and DBr after heat	0.5–1 mcg.	LPu after 6 min.	LPu	Dz	1 mcg. (BCG), 0.5 mcg. ($NaHCO_3$)

[a] Bl, blue; Br, brown; Ch, charcoal; D, dark; Dis, disappears; Dz, decolorized zone forms within a few seconds; F, fades away; Gn, green; Go, gold; Gy, gray; L, light; Lv, lavender; NBl, navy blue; Nc, no color; Or, orange; Pk, pink; Pu, purple; Re, reddish; Tn, tan; Tq, turquoise; W, weak; Wr, weak reaction; Yl, yellow. [b] Color reactions reported were obtained with Solvent C after development, following consecutive spraying with detection reagents described under spraying techniques (*a*) and (*b*). Color reactions and sensitivities using iodoplatinate and ammoniacal silver nitrate detection reagents were not included under spraying technique (*b*) as these are virtually identical for both spraying techniques. Gelman precoated silica gel glass microfiber sheets (I.T.L.C. Type SA) were used due to the ease with which these can be handled and subjected to varying heat treatments. Certain specific color reactions reported using these chromatoplates may not be obtainable on glass plates. [c] Reirradiating for 5 min. increases the intensity of spot. [d] This metabolite showed two spots; the upper spot seems to be minor in nature and did not form any colored complex with iodoplatinate.

the point of application is kept below 1 cm., and all spots of samples and standards are kept virtually of the uniform size so that none of the spot touches the solvent when the plate is placed in the developing solvent.

After the standards and samples have been applied over the thin-layer plate, the spots are air dried before the plate is placed in an appropriate solvent in a covered rectangular battery jar or tank[12]. The solvent is allowed to travel a distance of 6–8 cm.[13] (time required is 8–10 min.); the plate is then removed, allowed to dry at room temperature, and sprayed as described under *Detection Techniques*.

Each technician should be given coded positive and negative internal controls (positive controls may be prepared by adding known concentrations of opiates, amphetamines, and/or barbiturates to control urines) along with urine samples to be tested. These internal controls should form at least 1% of the total number of samples analyzed by each technician per week.

Detection Techniques—(a) *For Narcotic Analgesics, Amphetamine and Congeners, and Selected Psychotropic Drugs*—Detection reagents (*a*)-(*d*) are applied in succession to the same plate. After ninhydrin spray, the following steps are necessary to detect amphetamine and congeners:

1. The plate is irradiated under short wavelength for 5 min.; amphetamine may appear as a light-grayish spot. This step is necessary even though sometimes amphetamine may not be visible at this stage.
2. The plate is then heated at 90° for 4 min.; amphetamine may appear now if it has not appeared earlier. Methamphetamine, pipradrol, and methylphenidate can be seen at this stage (see Table IV for sensitivities).
3. Reirradiating under short wavelength for 5 min. increases the intensity of the amphetamine spot if it appeared earlier or causes amphetamine to appear as light-gray or grayish-blue spot if it did not appear earlier. Color changes for methamphetamine and other drugs are noted (Table IV). This step is omitted if the amphetamine spot becomes visible after Steps 1 and/or 2.
4. Respraying with ninhydrin solution and heating in the oven maintained at 170–180° for 3–5 sec. or on a hot plate maintained at 250–260° for 1 sec. cause methamphetamine and amphetamine to undergo different color changes. Sometimes methamphetamine appears at this stage if it did not appear earlier under Step 2. 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. Although this step is designed primarily to detect phenmetrazine, color changes for drugs listed in Table IV are noted. Spots[14] believed due to biogenic amine metabolites and sometimes seen after Step 2 virtually disappear after this brief heating treatment.

Spraying the plate with sulfuric acid (0.5%) causes the formation of spots of varying shades for methadone, pentazocine, acetylmethadol, and phenothiazines and their metabolites. Spots due to amphetamine, quinine, methamphetamine, pipradrol, methylphenidate, and phenmetrazine undergo different color changes (Table IV). Amphetamine, if not seen earlier after the ninhydrin spray, can be seen now as a faint grayish or bluish-gray spot. Quinine, which also forms a grayish or bluish-gray spot, can be confirmed readily under short UV light by its brilliant blue fluorescence, and its metabolites are seen as a series of blue, yellow, and red bands.

Iodoplatinate spray is then applied and color changes are noted. The sensitivity of this spray to morphine and codeine is 2 mcg. if the plate was previously sprayed with ninhydrin. When iodoplatinate spray is used first or after sulfuric acid only, it detects less than 1 mcg. of morphine (Table V). In interpreting the plates for the presence of morphine, it is not essential that one see colored spots after the iodoplatinate spray. In these laboratories the presence or absence of morphine and codeine is based primarily on a positive reaction to ammoniacal silver nitrate spray. After applying this spray *heavily*, the chromatogram is heated for 30–60 sec. on a hot plate maintained at a medium temperature (300–370°) or for 5–10 min. in an oven maintained at 170–180°. Morphine and codeine, which become bleached during the application of spray, reappear as *distinct* dark-brown or black spots after heat treatment (sensitivity 0.5 mcg.). A dark-brown or brown or black streak without a distinct spot is not indicative of positive morphine. Drugs such as phenothiazines and their metabolites, iproniazid, isoniazid[15], oxytetracycline, penicillin, and hydromorphone (hydromorphone and morphine have the same R_f values) do not become bleached during the application of this spray but instead form yellow, brown, or dark-brown colored spots. After application of heat, color changes for various drugs are noted (Table IV).

Potassium permanganate spray (*e*) proved to be a very useful adjunct to the confirmatory spray. Spots of questionable existence and spots that behave like morphine and codeine after ammoniacal silver nitrate and heat treatment, but having slight variations in color and R_f values as compared to the known standards, are verified by spraying with potassium permanganate. The chromatogram is then heated on the hot plate as already described for a few seconds. The spots other than morphine and codeine disappear, thus reducing further the possibility of false positives for morphine. If desired, the plate may be oversprayed with ammoniacal silver nitrate and heated.

(b) *For Narcotic Analgesics, Amphetamine and Congeners, and Selected Psychotropic Drugs Using Bromcresol Green Spraying Technique*—Alternatively, the plate is sprayed with detection reagent (*f*) followed by (*g*) and then with (*b*)-(*d*). After spraying with bromcresol green, the chromatogram is kept at room temperature for 2–8 min. to allow the formation of colored spots for amphetamine, methamphetamine, phenmetrazine, and other drugs. Overspraying with sodium bicarbonate causes these drugs to appear as more distinct spots of blue to purple shades. Sometimes spots due to amphetamines appear only after the sodium bicarbonate spray. Morphine, codeine, and quinine are relatively insensitive to bromcresol green in concentrations less than 5 mcg. Spraying with sulfuric acid results in the formation of colored complexes for phenothiazines and their metabolites against a pale-yellow background. The iodoplatinate spray gives colored complexes for alkaloidal drugs, opiates, antihistamines, phenothiazines, and their metabolites but not for amphetamine and congeners. Thus, phenothiazine metabolites and other drugs, if any, having the same R_f values as amphetamine and congeners can be differentiated by formation of colored complexes with iodoplatinate (see Table IV for color reactions and sensitivities).

(c) *For Narcotic Analgesics and Psychotropic Drugs in the Absence of Amphetamine and Congeners*—When the goal is only to detect narcotic analgesics, the plate is sprayed in succession with detection reagents (*b*)-(*d*); (*e*) is used if needed for verification of doubtful cases. The chromatoplate is subjected to heat treatment after the application of ammoniacal silver nitrate and potassium permanganate sprays as described under spraying technique (*a*).

(d) *For Amphetamine and Congeners*—The plate is sprayed with detection reagents (*a*) and (*b*), and the various steps described under spraying technique (*a*) after ninhydrin spray are strictly followed. Ephedrine also can be reliably detected if color changes under Steps 2 and 4 followed by sulfuric acid spray are carefully noted (Table IV).

Alternatively, the chromatogram may be sprayed with detection reagent (*f*) followed by (*g*). If this technique is used, the plate is also sprayed with detection reagents (*b*) and (*c*) to differentiate these drugs from phenothiazines and their metabolites and other alkaloids. In these laboratories, the use of ninhydrin spraying technique (*a*) is preferred.

(e) *For Differentiating Cocaine from Methadone*—The chromatogram is sprayed in succession with detection reagents (*c*) and (*d*). Application of the ammoniacal silver nitrate spray (sprayed heavily)

[12] Preequilibration of jar or tank with solvent vapors has not been found necessary for routine work, since the identification of drugs is based primarily on a comparison with known standards spotted beside the unknowns rather that on absolute R_f values.

[13] A 20 × 20-cm. plate is used only when a mixture of drugs cannot be resolved into separate drug entities. Then solvent is allowed to travel a distance of 10–15 cm. as desired (time taken is 20–30 min. for 10 cm. and 50–60 min. for 15 cm.).

[14] Two or three purple spots seen after Step 2 of the ninhydrin spraying technique are well below the level of amphetamine and can be easily distinguished from amphetamines by their different color reactions. None of these spots gives positive reaction when oversprayed with iodoplatinate and ammoniacal silver nitrate reagents.

[15] With the ninhydrin spraying technique, isoniazid does not form any tan or brown spot on heating after the application of ammoniacal silver nitrate spray. The iodoplatinate spray, when used first or after sulfuric acid only, does not form any colored complex with isoniazid up to 12 mcg., but a tan or brown spot appears on heating after ammoniacal silver nitrate at a concentration of about 5 mcg.

Table V—Color Reactions[a] Using Iodine–Potassium Iodide and Iodoplatinate Sprays Either Alone or in Combination[b]

Drug	Iodine–Potassium Iodide Spray	Iodoplatinate Spray	Sensitivity	Iodoplatinate Spray Alone	Sensitivity
Amphetamine	NC	NC	—	NC	—
α-Acetylmethadol	LBr	DBr or Br	0.5 mcg.	Tn	1 mcg.
Chlordiazepoxide	LBr	DBr or Br	0.5 mcg.	LPu	12 mcg.
Chlordiazepoxide metabolite (Ro5-2092)	LBr	DBr or Br	0.5 mcg.	LPu	12 mcg.
Chlordiazepoxide metabolite (Ro5-4383)	LBr	DBr or Br	0.5 mcg.	LPu	12 mcg.
Chlorpheniramine	LBr	Ch	0.5 mcg.	Ch	0.5 mcg.
Chlorpromazine	LBr	Br	0.5 mcg.	Ch	0.5 mcg.
Cocaine	Tn	Br	0.5 mcg.	Br	1 mcg.
Codeine	Tn	DBr	0.5 mcg.	Ch	1 mcg.
Cyclazocine	Br	DBr	0.5 mcg.	GyBr	1 mcg.
Diazepam	DBr	DBr	0.5 mcg.	Br	0.5 mcg.
Diazepam metabolite (Ro5-6789)	DBr[c]	DBr	0.5 mcg.	DBr	1 mcg.
Diazepam metabolite (Ro5-2180)	DBr	DBr	0.5 mcg.	DBr	1 mcg.
Diazepam metabolite (Ro5-5345)	LBr	Tn	0.5 mcg.	Go	2 mcg.
Diphenhydramine (Benadryl)	LBr	Tn	0.5 mcg.	Ch	0.5 mcg.
Ephedrine	NC	Br (disappears immediately)	6 mcg.	NC	—
Hydromorphone	DBr	Ch	0.5 mcg.	Ch	1 mcg.
Imipramine	DBr	Ch	0.5 mcg.	Ch	0.5 mcg.
Iproniazid	NC	Gy	0.5 mcg.	Gy	10 mcg.
Isoniazid (INH)	Dz (Yl at back)	Dis (1 mcg.), LGy (1.5–2 mcg.)	1.5 mcg.	LPu	12 mcg.
Lysergic acid diethylamide (LSD)	DBr	DBr	0.5 mcg.	ReBr	1 mcg.
Meperidine (Demerol)	LBr	DBr	1 mcg. (I_2–KI), 0.5 mcg (iodoplatinate)	Ch	1 mcg.
Methamphetamine	NC	Br	0.5 mcg.	Gy	7 mcg.
Mescaline	Tn	Ch	0.5 mcg. (iodoplatinate), 1 mcg. (I_2–KI)	LGy	0.5 mcg.
Methadone	Br	DBr	0.5 mcg.	Br	1 mcg.
Methapyrilene (Histadyl)	Br	DBr	0.5 mcg.	Ch	0.5 mcg.
Morphine	Tn	Ch or Gy	1.5 mcg. (I_2–KI), 0.5 mcg. (iodoplatinate)	Ch or Gy (turns NBl on keeping)	0.5 mcg.
Naloxone	Br	Dis (0.5 mcg.), Tn (1–2 mcg.)	0.5 mcg. (I_2–KI)	NC	—
Pentazocine	Go	Br	0.5 mcg.	Br	0.5 mcg.
Phenmetrazine	Br	Br	1.0 mcg. (I_2–KI), 2.0 mcg. (iodoplatinate)	Pu	13 mcg.
Pipradrol	Br	DBr	0.5 mcg.	Br	3.5 mcg.
Promazine	Br	Ch	0.5 mcg.	Ch	0.5 mcg.
Propoxyphene (Darvon)	Tn	DBr	0.5 mcg.	Br	0.5 mcg.
Quinine	Br	Ch	0.5 mcg.	Ch	0.5 mcg.
Methylphenidate (Ritalin)	LBr	Ch (disappears after 1 min.)	0.5 mcg. (I_2–KI), 1.0 mcg. (iodoplatinate)	Br	5 mcg.
Tetracycline	NC	NC	—	NC	—
Thioridazine (Mellaril)	Br	Ch	0.5 mcg.	Ch	1 mcg.
Trifluoperazine (Eskazine, Stelazine)	Br	Ch	0.5 mcg.	Ch	0.5 mcg.

[a] Bl, blue; Br, brown; Ch, charcoal; D, dark; Dis, disappears; Dz, decolorized zone forms within a few seconds; F, fades away; Gn, green; Go, gold; Gy, gray; L, light; Lv, lavender; NBl, navy blue; Nc, no color; Or, orange; Pk, pink; Pu, purple; Re, reddish; Tn, tan; Tq, turquoise; W, weak; Wr, weak reaction; Yl, yellow. [b] Color reactions reported were obtained after developing the chromatoplate with Solvent C. The plate was then either sprayed in succession with detection reagents I_2–KI and iodoplatinate or with iodoplatinate alone. Drugs like chlordiazepoxide, methamphetamine, methylphenidate, naloxone, and phenmetrazine are relatively insensitive to iodoplatinate spray, but these can be satisfactorily detected if the plate is sprayed first with I_2–KI and then followed by iodoplatinate. [c] This metabolite showed two spots; the upper spot was brownish in color and appears to be minor in nature. The upper spot did not form any colored complex when the plate was sprayed with iodoplatinate alone.

over the iodoplatinate spray bleaches the spot for methadone (sometimes the color reappears), while the spot for cocaine immediately changes to a yellowish color (color appears while spraying). Procaine hydrochloride[16], if used as an adulterant, does not interfere with the identification of cocaine as the proposed developing solvent, F, can resolve the mixture of methadone, cocaine, and procaine. Cocaine travels higher than methadone while procaine, if present, stays below methadone. Procaine can also be differentiated by the formation of a charcoallike color after the iodoplatinate spray and a light yellow color after the ammoniacal silver nitrate spray.

(f) *For Drugs like Chlordiazepoxide, Naloxone, and Methylphenidate*—These drugs are relatively insensitive to the iodoplatinate spray (Table V). However, they can be satisfactorily detected if the plate

[16] Novocaine.

is sprayed first with the iodine–potassium iodide solution (21) and then the iodoplatinate spray (Table V). The application of this combination of sprays does not interfere with the subsequent use of the ammoniacal silver nitrate spray for the identification of morphine and codeine. Thus, there appears to be a useful application of this spraying technique in abstinence programs using naloxone as an antagonist. Chlordiazepoxide[17], diazepam[18], and oxazepam can also be detected, along with barbiturates using spraying technique (*g*).

(g) *For Sedative-Hypnotics, Benzodiazepine Compounds, and Other Drugs*—The developed chromatogram is sprayed in succession with detection reagents (*h*)–(*j*). The silver acetate spray is used between diphenylcarbazone and mercuric sulfate sprays. The diphenylcarbazone and mercuric sulfate spraying technique as used currently (7) produces a spot of weak intensity and transient nature. However, these shortcomings are overcome by the use of the proposed combination of sprays. Barbiturates like barbital, phenobarbital, pentobarbital, amobarbital, and secobarbital and drugs like glutethimide[19] and diphenylhydantoin[20] form characteristic colored complexes with silver acetate and/or mercuric sulfate (Table II). The purple coloration in the case of the phenobarbital spot after the mercuric sulfate spray does not appear immediately in all cases. Sometimes the appearance of a purple-colored spot takes 30–60 sec., depending upon the concentration of phenobarbital present.

A novel technique to differentiate barbiturates from drugs like chlordiazepoxide and metabolites and/or artifacts of phenylbutazone, methadone, diazepam, and oxazepam is to overspray the plate with the iodine–potassium iodide spray (*k*) after the mercuric sulfate spray. All these drugs except barbiturates, glutethimide, and diphenylhydantoin appear as varying shades of brown (Table II). Only one metabolite of chlordiazepoxide (other than lactam and open lactam) did not form any colored complex with the iodine–potassium iodide spray. This metabolite of chlordiazepoxide behaved like barbiturates with silver acetate and mercuric sulfate spray and interfered with the detection of phenobarbital and/or sodium barbital and still remains a problem to be solved. However, chlordiazepoxide can be differentiated from barbiturates if, on spraying with the iodine–potassium iodide spray, a brown spot appears at an R_f of about 0.18–0.27. The appearance of this lower spot is indicative of the presence of chlordiazepoxide in a urine specimen, because this is due to either unchanged parent drug or its lactam (Ro5-2092).

RESULTS AND DISCUSSION

Tables I–V summarize the data accumulated for the listed drugs. Many useful TLC developing solvent systems applicable to a large-scale rapid screening of urines have been formulated. Table I gives the R_f values for drugs of abuse and their adulterants, drugs used in the treatment of addicts and miscellaneous drugs that could interfere with the test. The R_f values were tabulated to give an idea to the operator about the solvent to be chosen, depending on the needs of a clinical operation. Although these values may vary from day to day and even from plate to plate because of factors like humidity, adsorbent activity of layers, uniformity of layer thickness, and changes in temperature, the resolving pattern remains the same. Solvent A is recommended for routine use of thin-layer separation of opiates if a procedure similar to the one described by Dole *et al.* (8, 9) is used for the extraction where no sulfuric acid or hydrochloric acid is added to the chloroform–isopropanol extracts. Solvent B is suggested where sulfuric acid or hydrochloric acid is added to the chloroform–isopropanol extracts. Solvent A is capable of separating mixtures of amphetamine and methamphetamine; methamphetamine, phenmetrazine, and quinine; methamphetamine and quinine; and methadone and methapyrilene (Table III). Both solvent systems can separate amphetamine from quinine and phenmetrazine if the solvent is allowed to travel a distance of about 13 cm. Solvent C is recommended for routine thin-layer separation of opiates, amphetamine and congeners, and psychotropic drugs. It can differentiate methadone from methapyrilene, cyclazocine, pentazocine, meperidine, methylphenidate, imipramine, promazine, and trifluoperazine; and it can differentiate isoniazid from morphine (Table III).

Sedative–hypnotics, benzodiazepine compounds, and methadone and its metabolite are best detected using Solvent D. This solvent has been specially formulated only for this group of drugs because acidic bodily urine metabolites and drugs like phenylbutazone, tolbutamide, and chlorpropamide, which are also extractable at pH 1 and could possibly interfere because of identical tinctorial properties, stay at the origin (see Table II for R_f values and color reactions).

Solvent E is recommended only for those urine specimens that have been subjected to acid hydrolysis; this would help in the interpretation of results by elimination of some background interference. Solvent F is the solvent that most efficiently differentiates methadone and/or cocaine from acetylmethadol, *d*-propoxyphene[21], pipradrol, diphenhydramine[22], chlorpromazine[23], promazine, thioridazine[24], and pentazocine[25]. These drugs can give a false test for methadone and/or cocaine if the proper solvent is not used to separate them (see Table III for mixture of these drugs).

Spraying techniques developed in these laboratories for the detection of amphetamine and congeners and sedative–hypnotics and for differentiating drugs such as cocaine from methadone have proved very useful for detecting these drugs in urines of drug users attending the methadone outpatient treatment program. Currently, spraying technique (*a*) is used for the simultaneous detection of amphetamines and opiates, and spraying technique (*c*) is used for the detection of opiates when simultaneous screening of amphetamines is not required. By following the steps outlined under the ninhydrin spraying technique, amphetamine, methamphetamine, and phenmetrazine (phenmetrazine forms a characteristic pink color after heat treatment) can be differentiated from each other.

The cost of analysis per urine specimen using ion-exchange extraction techniques and the data on the reliability of thin-layer identification techniques were reported elsewhere (22, 23).

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Versatile System for Partition Chromatography of Corticosteroids and Prediction of Their Elution Curves

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Abstract □ The versatile solvent system of *n*-hexane–chloroform–dioxane–water in the ratio 90:10:40:5 was used for the partition chromatographic isolation of corticosteroids. The relative amounts of the components may be adjusted if necessary to give a convenient partition coefficient on a diatomaceous earth column. Data are presented for 17 steroids, showing the close agreement between partition coefficients predicted from chromatographic elution curve data and those calculated from solvent extraction data. A procedure for the experimental determination of column parameters is given, and the necessary equations are outlined. The prediction of peak elution volumes and solute bandwidths as a function of partition coefficients from the derived equations is demonstrated, and the quantitative effect of varying the stationary phase volume and column length is calculated. The effect of steroid side-chain structure and fluoride substitution on the partition coefficient was studied. The effect of the substituents can be estimated from their expected polarity.

Keyphrases □ Corticosteroids—separation by partition chromatography, versatile solvent system, prediction of elution curves □ Partition chromatography, 17 corticosteroids—versatile solvent system, prediction of elution curves from partition coefficients, substituent effect □ Chromatography, partition—versatile solvent system for corticosteroids, prediction of elution curves

A common problem in the partition chromatographic separation of steroids is the choice of an efficient procedure from among the variety of solvent systems and column designs quoted in the literature (1, 2). It would be convenient if a versatile solvent system, coupled with a column of predictable characteristics, was available. Other chromatographic systems for the analysis of corticosteroids are available such as TLC (3, 4), GLC (5, 6), and paper chromatography (7). The problems of high temperature operation, long analysis times, and uncertain recoveries of these methods make the column partition chromatographic method a useful alternative. An efficient three-component partition system was described for a selection of four corticosteroids (8). Attempts to evaluate the usefulness of the system for a wider variety of steroids resulted in inconveniently large elution volumes for many steroids. Much more convenient elution times and a more versatile system were obtained after addition of chloroform to produce a four-component system.

The mathematical basis of partition chromatography was first demonstrated by Martin and Synge (9). Glueckauf (10) later extended the theory to nonequilibrium conditions such as exist in columns at normal flow rates. A truly versatile partition system must have properties that can be predicted by theory if its full usefulness is to be realized. Therefore, an outline of the pertinent equations and the demonstration of their applicability to the present system is presented.

EQUATIONS

Column Parameters—A dry-packed column was found to be more convenient and rapid to prepare in this laboratory than a slurry-packed column. This presents a problem in the determination of the effective volumes of stationary and mobile phases on the column, since not all of the open space between particles is taken up by the mobile phase on a dry-packed column. If a column of standardized inside diameter, weight of diatomaceous earth, and length is prepared using a slurry- and a dry-packing technique, then the total volumes occupied by the two columns are given by:

$$V_{ST} = V_{SS} + V_{SM} + V_{Si} \qquad \text{(Eq. 1)}$$

and:

$$V_{DT} = V_{DS} + V_{DM} + V_{Di} + V_{DO} \qquad \text{(Eq. 2)}$$

for the slurry- and dry-packed columns, respectively, where V_{DT}, V_{DS}, V_{DM}, V_{Di}, and V_{DO} are the total, stationary, mobile, inert, and void volumes for the dry-packed column and V_{ST}, V_{SS}, V_{SM}, and V_{Si} are the total, stationary, mobile, and inert volumes for the slurry-packed column. The sum of V_{SS} and V_{SM} is determined by measuring the volume needed to slurry pack a column with mobile phase only. The total volume of the slurry-packed column, V_{ST}, is equal to V_{DT} and is calculated from the length and cross-sectional area of the packed portion of the column. The inert volume of the