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SIMULTANEOUS DETECTION OF A WIDE VARIETY OF COMMONLY ABUSED DRUGS IN A URINE SCREENING PROGRAM USING THIN-LAYER IDENTIFICATION TECHNIQUES

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SUMMARY

A single-step extraction method and thin-layer identification techniques capable of testing a wide variety of drugs of abuse are presented. These techniques are well suited for large and/or small drug programs involved in urine testing because they provide substantial economic benefits and improve clinical functioning. The drugs are absorbed on a 6 × 6 cm piece of paper loaded with cation-exchange resin and then eluted from the paper at pH 10.1 using ammonium chloride–ammonia buffer. The simultaneous thin-layer detection of sedatives, hypnotics, narcotic analgesics, central nervous system stimulants and miscellaneous drugs is accomplished by spotting the solution of extracted residue on a 20 × 20 cm Gelman pre-coated silica gel glass microfiber sheet (ITLC Type SA). A two-stage solvent system is used in order to obtain a chromatogram with optimum separation of a wide range of drugs. This system can separate methadone and/or cocaine from propoxyphene, methaqualone, methylphenidate, pentazocine, pipradrol, Doxepin, chlorpromazine, phenazocine, naloxone, naltrexone, imipramine and trimeprazine; amphetamine from phenylpropanolamine and dimethyltryptamine; codeine from dextromethorphan; methamphetamine from dimethyltryptamine, etc. Different detection reagents are then applied in succession to different marked areas of the developed chromatogram. This elegant method of extraction and spraying has enabled us to detect morphine base at a sensitivity level of 0.15 μg/ml, amphetamine sulfate at 1.0 μg/ml, methamphetamine hydrochloride at 0.5 μg/ml, phenmetrazine hydrochloride at 0.5 μg/ml, codeine phosphate at 0.5 μg/ml, methadone hydrochloride at 1.0 μg/ml, secobarbital at 0.36 μg/ml and phenobarbital at 0.5 μg/ml in urine. The minimum volume of urine needed to achieve these sensitivities is 20 ml. The cost of analysis per urine specimen using these techniques for concomitant screening of these drugs is less than US$ 1.

INTRODUCTION

Owing to the increasing usage of drugs, urine screening for drugs of abuse has

become a necessary adjunct in drug abuse prevention and treatment programs. Urine analysis provides a clinician with an objective measure of drug abuse among his clients. In addition, frequent collection of urines for a drug-dependent individual has a strong deterrent effect on drug use. We feel that psychologically it helps to stop the craving for the occasional or covert drug use. Recent shift in emphasis from heroin abuse to poly-drug use has caused much concern among the drug abuse prevention programs and law enforcement agencies. Although many methods for detecting commonly abused drugs are available[1-60], they vary greatly in their suitability for use in large-scale urine monitoring programs. Some of the present methods, such as immunoassay techniques[52-60], are very sensitive but prohibitive in cost, and usually selective in the drugs that they are able to test. Although radioimmunoassay[56-58], free radical assay technique[52,54,55] and hemagglutination inhibition test[59-60] have a sensitivity at nanogram levels for the detection of morphine and structurally related narcotics, the chances of cross-reactivity with other drugs are enhanced at this sensitivity level. Enzyme multiplied immunoassay technique[53], the sensitivity of which is the same as that of thin-layer chromatography (TLC) requires individual testing of each drug in a urine specimen, and a urine specimen to be tested for morphine, methadone, amphetamine, barbiturates and cocaine metabolites will cost US$ 2.50–3.85 depending upon the volume of reagents purchased. Furthermore, antibodies have not yet been developed to test for other drugs of abuse such as methylphenidate (Ritalin), phenmetrazine (Preludin) and phencyclidine (PCP) or for drugs used in the treatment of heroin addicts such as acetylmethadol and naltrexone. At present, the only suitable technique that has the versatility for testing an entire array of drugs of abuse in one run is TLC. Even this technique varies considerably in the extraction and detection procedures from laboratory to laboratory. The extraction of drugs from a urine specimen is a necessary prerequisite to TLC. The superiority of a reported TLC technique as applied to the detection of drugs in a biological fluid can be attributed to the efficiency of the pre-chromatographic extraction step and the specificity and the sensitivity of the detection techniques used. The results of surveys by the Center for Disease Control* definitely demonstrated that the use of TLC as a general approach to the identification of drugs of abuse has the highest proficiency. The only other technique that can permit simultaneous screening of a mixture of drugs is gas–liquid chromatography (GLC), but it has inherent disadvantage of running a single specimen at a time; thus, it becomes time consuming and more expensive than TLC. GLC is useful when the analysis of an unconscious patient's physiological fluids for a particular drug is required. We use GLC only for research and development work, and for the validation of some results obtained by TLC. Different extraction techniques currently used prior to TLC or GLC involve three basic approaches: direct extraction of drugs from a urine specimen at various pHs; acidic or enzymatic hydrolysis of urine specimens followed by direct extraction of drugs; and extraction of drugs from urine by absorbing them on a resin column (ionic or non-ionic) and then eluting with organic solvents or absorbing the drugs and/or their metabolites on a paper loaded with cation-exchange resin and then eluting with different buffer–

* The Center for Disease Control, Department of Health, Education and Welfare, Atlanta, Ga., conducts a Proficiency Testing Program of Clinical Laboratories in Drug Abuse Detection for Special Action Office for Drug Abuse Prevention.

solvent systems. These extraction techniques with relative advantages and disadvantages for each procedure as regards to their suitability in a large-scale urine screening program were discussed in depth by Kaistha[50]. A comparison of data obtained by using several extraction techniques was also reported by Kaistha and Jaffe[45].

The purpose of this paper is to present a single-step extraction method and thin-layer identification techniques for concomitant screening of a wide variety of drugs of abuse. The technique is simple, cheap, reliable, sensitive and easily adaptable to small- or large-scale urine screening programs. This is a modification of a two-step extraction technique reported by Kaistha and Jaffe[45,46] for the extraction of sedatives and hypnotics at a pH of 1 and amphetamines and narcotic analgesics at a pH 10.1. The drugs are first absorbed on a 6 × 6 cm piece of paper loaded with cation-exchange resin and then eluted from the paper at pH 10.1 using ammonium chloride–ammonia buffer. Drugs of abuse such as sedatives, hypnotics, benzodiazepines, narcotic analgesics, central nervous system stimulants, phenothiazines and drugs used in the treatment of heroin users were found to be extractable at this pH, equalling the highest sensitivity previously reported[45]. A two-stage thin-layer development solvent has been used to obtain a chromatogram with optimum separation of a wide range of drugs. The detection techniques previously reported[46] have been elegantly and ingeniously incorporated in order to detect an entire array of drugs of abuse at maximum sensitivity. Further, the cost of the analysis of a single urine specimen for complete screening of these drugs is less than $ 1.

EXTRACTION PROCEDURE

A 6 × 6 cm piece of H. Reeve Angel (Clifton, N.J., U.S.A.) SA-2 cation-exchange resin-loaded paper (marked with a patient's identity number or name with a lead pencil) is soaked in 20–50 ml of fresh undiluted urine (pH 5–6) with intermittent shaking*. After 60 min, the paper is transferred into a plastic bag and sent to the laboratory for the desired analysis. In order to decrease the work load and cost of analysis, the routine procedure used in the laboratory at present is to pool several ion-exchange papers of the same patient representing different urine specimens taken during 1 week. The single or pooled ion-exchange papers are transferred to a 4-oz. wide-mouthed screw-capped jar and rinsed with 10–20 ml of water (deionized or distilled; ordinary cold tap water does not interfere in the subsequent extraction and identification of drugs; however, rinsing is important in order to prevent emulsion formation in the extraction procedure). To each jar containing single or many ion-exchange papers, 3 ml of ammonium chloride–ammonia buffer [saturated solution of ammonium chloride (2500 ml) adjusted to a pH 10.1 ± 0.1 with concentrated ammonia solution (about 2400 ml)] are added, the contents swirled and 5 ml of water

* Our investigation revealed that a minimum volume of 20 ml of urine is needed for a sensitivity of 0.15 μg of morphine base (0.20 μg of morphine · HCl · H_2O), 1.0 μg of amphetamine sulfate, 0.5 μg of methamphetamine · HCl, 0.5 μg of codeine phosphate, 0.5 μg of phenobarbital and 0.36 μg of seconal per millilitre of urine. The paper needs to be soaked in urine for 1 h with intermittent shaking; however, to accomplish better location of morphine, the paper may be soaked for 1 h with constant shaking, or kept overnight or for 24 h with no shaking, or shaken for 10 min and then kept overnight.

(deionized or distilled) and 15 ml of chloroform–isopropanol (3:1) are added. When the purpose is to test for opiates only, extraction is accomplished by using 15 ml each of borate buffer, pH 9.3 [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 are required)] and chloroform–isopropanol (3:1). After shaking for 10 min on a reciprocating shaker (Eberbach table model shaker), the lower organic phase* is pipetted out into a 15-ml conical centrifuge tube containing two drops (about 50 μl) of 0.5% sulfuric acid in methanol. The solvent is evaporated in an oven maintained at 85–90° with a horizontal air flow. The residue along the sides of the tube is washed with 0.5–1 ml of methanol, vortexed and methanol is evaporated to dryness as above. The residue thus obtained is re-dissolved in about 30–50 μl of methanol and the contents are vortexed again (the volume of methanol added varies according to the amount of residue in a test-tube; one should be able to spot about two 5-μl capillaries of the extract).

THIN-LAYER CHROMATOGRAPHY (TLC)

Gelman pre-coated silica gel glass microfiber sheets (ITLC type SA) with a layer thickness of 250 μm were used throughout. We prefer these sheets because of the convenience with which they can be handled. These plates can be subjected to varying heat treatments in order to detect selectively certain drugs such as methamphetamine, phenmetrazine, methylphenidate, morphine and codeine; furthermore, they can be cut to any desired size. For urine specimens to be tested for opiates only, a 20 × 20 cm sheet is cut into two 10 × 20 em pieces; three standards (one at each edge and one in the center) and 10–12 samples are spotted. Urine specimens to be monitored for simultaneous detection of sedatives, hypnotics, amphetamine and congeners, opiates and other drugs of abuse are spotted on a 20 × 20 cm sheet, and a two-stage solvent system is used to achieve the optimum separation of all drugs.

Solvent systems

The solvent systems C, D, E and F below were the same as the corresponding solvents used by Kaistha and Jaffe[46].

C: ethyl acetate–cyclohexane–ammonia–methanol–water (70:15:2:8:0.5).

D: ethyl acetate–cyclohexane–methanol–ammonia (56:40:0.8:0.4).

E: ethyl acetate–cyclohexane–methanol–ammonia (70:15:10:5).

F: ethyl acetate–cyclohexane–ammonia (50:40:0.1).

It is recommended that solvents D and F be used fresh or within 24 h. Solvents C and E should preferably be used after storage overnight; they both keep well for 3–4 weeks.

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* After pipetting out the lower organic layer (chloroform–isopropanol) the ion-exchange paper and aqueous phase are discarded. This single extraction is capable of extracting more than 80% of absorbed drugs. For mass screening purposes, the ion-exchange papers are extracted only once; however, when quantitative recovery of the absorbed drug is desired, the ion-exchange paper is extracted with three 10-ml volumes of chloroform–isopropanol. Each extract is transferred to the same 50-ml conical centrifuge tube containing four drops of 0.5% sulfuric acid in methanol.

Detection reagents

The following detection reagents were used, each as described by Kaistha and Jaffe[46]:

(a) ninhydrin, 0.5% (w/v) solution in *n*-butanol; this solution can be used for 24–48 h if stored in a refrigerator.
(b) diphenylcarbazone (DPC), 0.01% in equal parts of acetone and water.
(c) silver acetate, 1% (w/v) solution in water.
(d) mercury(II) sulfate solution.
(e) sulfuric acid, 0.5% (v/v) solution in water.
(f) iodoplatinate.
(g) iodine–potassium iodide.
(h) ammoniacal silver nitrate.
(i) potassium permanganate.

Procedure

The sample solution is prepared by vortexing the residue obtained under Extraction Procedure in about 30–50 μl* of methanol. Marks are drawn on a 20 × 20 cm thin-layer plate at 1.8, 9.0 and 14.5 cm from the bottom. Using a 5-μl capillary tube**, three standards***, one at each edge (1 cm away from the edge) and one in the center of plate and 10–12 samples each 1.2 cm apart are spotted on the line drawn 1.8 cm away from the bottom of the plate. 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 the point of application is kept as small as possible in order to achieve the maximum resolution and all spots of samples and standards are kept virtually of 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 to the thin-layer plate, the spots are air dried and the plate is dried for 3–5 min at 85–90° before it is placed in the standard rectangular tank. Drying of the plate has been found to be important especially when there is high humidity in the atmosphere; it is a necessary prerequisite in order to achieve the optimum separation of all drugs. A minimum of 5 min heating

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* Residues to be tested for opiates alone using sodium borate buffer require about 30 μl of methanol; residues to be tested for an entire array of drugs using ammonium chloride–ammonia buffer require 40–50 μl of methanol. After vortexing the residue, about 2–2½ 5-μl capillaries are available for spotting and are spotted entirely. The residue remaining in the test-tube is used for re-checks of the results when re-check requests are made by clinics.

** These capillaries are used in these laboratories for routine spotting of more than 4000 samples and are preferred to the Hamilton microsyringe, as we are interested primarily in the progress of a treatment program, and therefore mainly in qualitative information.

*** Morphine·HCl, codeine phosphate, quinine sulfate, methadone·HCl and methapyrilene·HCl, dissolved together in methanol (each 1 μg/ml); amphetamine sulfate, methamphetamine·HCl, phenmetrazine·HCl (Preludin), methylphenidate·HCl (each 1 μg/ml); and barbiturate mixture (seconal, amobarbital, pentobarbital, phenobarbital, glutethimide and diphenylhydantoin (each 1 μg/ml); one capillary of opiate mixture is spotted first, followed by 1.5 capillaries of each amphetamine and its congeners, one capillary of cocaine·HCl (1 μg/ml in methanol) and finally one capillary of barbiturate mixture is over-spotted. 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 as used for the extraction of urine samples. Although we do not carry the standards through the extraction procedure for routine use, the operator may do so if desired.

is necessary to resolve cocaine from methadone if the air has high humidity; 3 min heating is sufficient if the air is dry and less humid. Pre-equilibration of the tank with solvent vapor has not been found necessary for routine work as the identification of drugs is based primarily on a comparison with known standards spotted beside the unknown, rather than on absolute R_F values. The plate is first developed in 100 ml of solvent E (150 ml of solvent E are used if two plates are placed in the tank) until the solvent reaches a level of about 9.0 cm (development time about 20 min). The operator may use solvent C in place of solvent E. The plate is air dried for about 10 min and then dried in an oven at 85–90° for 3–5 min. Solvent E (or C) is discarded and the plate is then developed in 100 ml of solvent F (180 ml of solvent F are used if two plates are placed in the tank) until the solvent reaches a level of 14.5 cm (development time 45–50 min).

Each technician should be given about 3% coded positive and blank internal controls (positive controls can be prepared by adding minimum known concentrations of opiates, amphetamines and barbiturates to control urines) of the total number of urine samples analyzed by each technician per week.

Detection techniques

Detection reagents (a)–(h) are applied in succession to the specified areas of the same plate. The plate is air dried for 10 min or until the smell of ammonia disappears.

(a) Ninhydrin spray. The lower 4.5 cm and upper 5.0 cm area of the plate are covered with glass plates and the uncovered middle portion is sprayed with ninhydrin. After ninhydrin spray, the following steps are necessary in order to detect amphetamine and congeners.

(i) The plate is irradiated with shortwave light for 7 min; amphetamine appears as a purple or light graypurple spot. This step is necessary although sometimes amphetamine may not be visible at this stage. Methamphetamine may appear at this stage as a grayish purple spot when the amount spotted is approximately 7 μg or more.

(ii) The plate is then heated in the oven at 85–90° for 5 min; methamphetamine appears as a purple spot; methylphenidate and other drugs can be seen at this stage (see Table I).

(iii) Re-irradiation with shortwave light for 7 min increases the intensity of the amphetamine spot if it appeared earlier or causes amphetamine to appear as a light gray or grayish purple spot if it did not appear earlier under step (i).

(iv) Re-spraying the same middle portion with ninhydrin and heating on a hot plate at about 246–249° for a few seconds causes methamphetamine and amphetamine to undergo different color changes. The methamphetamine spot changes to dark purple in the initial stages of this heat treatment; in some instances, methamphetamine appears at this stage if it did not appear earlier under step (ii). Heating is continued for 10–30 sec until the phenmetrazine (Preludin) standard appears as a bright pink spot and the methylphenidate (Ritalin) spot changes from purple to light yellow (methapyrilene does not form a light yellow spot). This step is designed primarily to detect methamphetamine, phenmetrazine and methylphenidate and to differentiate methylphenidate from methapyrilene.

(b) Diphenylcarbazone (DPC), (c) silver acetate and (d) mercury(II) sulfate. The lower 9.5 cm portion of the plate is covered and the upper 5.0 cm uncovered

portion is sprayed with DPC and silver acetate in rapid succession, resulting in the formation of bluish purple spots. Then, covering the lower 4.5 cm portion, the uncovered 10.0 cm portion is immediately sprayed lightly with mercury(II) sulfate; purple spots due to barbiturates, glutethimide (Doriden) and diphenylhydantoin (Dilatin) are circled. Any additional colored spots due to other drugs and changes in color for amphetamine, methamphetamine and phenmetrazine are noted.

The plate is then heated in the oven at 85–90° for 5 min; changes in color for amphetamine and methamphetamine spots, additional colored spots due to phenothiazines and/or their metabolites and other drugs are noted. This heat treatment is also necessary in order for methadone, cocaine and propoxyphene spots to appear distinctly after the iodoplatinate spray. Codeine appears as a light orange spot, which proved to be more specific than the conventional brown color with iodoplatinate. After the heat treatment, the plate is also examined under shortwave and longwave UV light for characteristic fluorescence given by various drugs (see Table I).

(e) Sulfuric acid spray. Covering the upper 5.5 cm portion of the plate, sulfuric acid is applied lightly to the lower uncovered portion and the plate is examined under shortwave UV light for quinine and one of its major metabolites for their characteristic brilliant blue fluorescence. The codeine spot becomes more obvious while applying the acid spray, but it later becomes lighter in color and starts to disappear.

(f) Iodoplatinate spray. This spray is applied over all of the plate and the color changes for various drugs are noted; cocaine gives a characteristic grayish brown spot, methaqualone (not passed through the body) brown (Table I), and morphine blue or navy blue*. Amounts of morphine below 1 μg give a blue spot on keeping the plate at room temperature for 15–30 min; however, this spot can be made to appear earlier by holding the chromatogram very near to a hot plate at 300–370° for 30–60 sec. It is recommended that the chromatogram should be kept at room temperature for a few minutes for the morphine spot to become more distinct and to reappear if it faded away during the heat treatment. If the background of the chromatogram turns grayish blue due to the heat treatment, the chromatogram can be sprayed with 0.5% sulfuric acid in order to clear the background.

(g) Iodine–potassium iodide spray. Covering the lower 4.5 cm portion of the chromatogram, this spray is applied to the upper uncovered area of the plate; spots due to methadone, cocaine, propoxyphene (Darvon) and methaqualone become more distinct; any additional spots for drugs such as ethchlorvynol, hydrochlorothiazide, hydroflumethiazide, phencyclidine and phendimetrazine that may appear are also noted.

(h) Ammoniacal silver nitrate spray. This spray is applied only to the lower 4.5 cm portion of the chromatogram; the blue color of morphine and the brown color

* Navy blue or varying hues of blue or bluish brown spots that appear immediately at the morphine level should be circled. The chromatogram is then kept for 15 min in order to (i) allow the low concentration of morphine to appear, (ii) permit the spots that are not due to morphine to change to brown or to other colors or even to disappear, and (iii) enhance the intensity of morphine spots. The chromatogram should be sprayed with ammoniacal silver nitrate in order to confirm the doubtful spots of varying hues of blue or bluish brown, which will disappear upon immediate heating —see *(h) Ammoniacal silver nitrate spray.* GLC may be performed on the remaining residue or on another aliquot of the same urine in order to validate the result of doubtful cases if legal evidence is needed.

of codeine* are bleached immediately on spraying, but reappear as dark brown or black spots after the chromatogram is heated for 30–60 sec on a hot plate maintained at 300–370 °.

(i) Potassium permanganate spray. This spray can be used as an adjunct to the ammoniacal silver nitrate spray if needed[46].

Performance evaluation of detection techniques

The Center for Disease Control has been conducting a Proficiency Testing Program in Toxicology Drug Abuse since 1972 and this laboratory has been one of the participants for the last 2 years and has been consistently earning a cumulative average of 100% since 1973. In this survey, ten urine specimens are shipped every 3 months to each participant for the detection of morphine, methadone, barbiturates, amphetamine and/or methamphetamine and cocaine and/or its metabolites. The matrix of these samples are human urines, some are addict's urines and some urines are spiked with the above drugs and also could contain drugs and/or metabolites other than the above drugs. These laboratories have been using the ion-exchange paper technique to absorb the drugs from these urines. Two alternative methods are used to extract the drugs from the ion-exchange paper. The drugs are either extracted by using a single-step extraction technique, as proposed in this paper, or the barbiturates are extracted first at pH 1.0 using sodium citrate–hydrochloric acid buffer as previously reported by Kaistha and Jaffe[45], and opiates, amphetamines and other drugs at pH 10.1 as proposed in this paper. When a two-stage extraction process is employed, barbiturates are developed by using solvent C or D (methadone, cocaine and propoxyphene are also detectable together with barbiturates as they are partially extracted by chloroform at this pH, which makes their detection very specific when solvent D is used); opiates, amphetamines and other drugs are separated using a two-stage thin-layer development system as proposed in this paper (solvents E and F).

RESULTS AND DISCUSSION

Tables I–III summarize the results for the listed drugs. Using single-step extraction and thin-layer identification techniques, we have been able to achieve the same sensitivities that we reported previously[45,46], *i.e.*, extracting barbiturates at pH 1.0 and opiates and amphetamines at pH 10.1. The sensitivities achieved using 20 ml of urine are: morphine $HCl \cdot H_2O$, 0.20 μg/ml (morphine base 0.15 μg/ml**); amphetamine sulfate, 1.0 μg/ml; methamphetamine·HCl, 0.5 μg/ml; phenmetrazine, 0.5 μg/ml; methylphenidate, 1.0 μg/ml; codeine phosphate, 0.5 μg/ml; phenobarbital, 0.5 μg/ml; seconal, 0.36 μg/ml; and methadone, 1.0 μg/ml. Laboratories other than the highly proficient can also achieve the above sensitivities if the techniques proposed in this paper are followed.

Solvents C and D when used alone are excellent for the separation of barbi-

* Codeine gives a blue-brown spot depending upon the age of the iodoplatinate spray solution and its exposure to light.

** Sensitivities of 0.1 μg/ml and 0.07 μg/ml if volumes of urine used are 30–35 ml and 43–50 ml, respectively.

TABLE I

COLOR REACTIONS USING THE PROPOSED SPRAYING TECHNIQUES

Gelman pre-coated silica gel glass microfiber sheets with a layer thickness of 250 μm were used. The specific color reactions presented here may not be obtainable on glass plates coated with silica gel. The amount of each drug used for these color reactions was about 5 μl (1 μg/μl) unless otherwise specified. Using larger amounts than 5 μg, drugs may show up at earlier stages of the reported spraying sequence with the detection reagents, and may give varying hues of the colors recorded in the table. Color codes: Bl, blue; Br, brown; Ch, charcoal; D, dark; Dis, disappears; Dz, decolorized zone forms within a few seconds; F, fades away; Ft, faint; Gn, green; Go, gold; Gy, gray; L, light; NBl, navy blue; Or, orange; Pk, pink; Pu, purple; Re, reddish; Tn, tan; Tq, turquoise; Vt, violet; Yl, yellow. N.S., not sprayed.

The color reactions reported were obtained using the described two-stage development system by developing the plate first in solvent E up to 9.0 cm and then in solvent F up to 14.5 cm following consecutive spraying with detection reagents as listed in table.

Drug	*Ninhydrin procedure*				*DPC*	*Silver acetate*	*Mercury(II) sulfate*	*Mercury(II) sulfate and heat*	*Fluorescence, longwave UV*	*0.5% H_2SO_4 and shortwave UV*	*Iodoplatinate*	*Iodine–potassium iodide*
	UV, 7 min	*Heat at 90° for 5 min*	*UV, 7 min*	*Respray and heat at 246° on a hot plate*								
α-Acetylmethadol	–	–	–	–	–	–	–	–	–	–	Br	Br
Amitriptyline	–	LGyBl	Gy	Gy, distinct at back	N.S.	N.S.	–	Dis	–	–	Br	Br or DBr
Amobarbital	N.S.	N.S.	N.S.	N.S.	–	BlPu	Pu	Dis	Tn	Tn, shortwave UV	–	–
Amphetamine sulfate	LPu Pu (7 μg)	FtPu Pu (7 μg)	LPu Pu (7 μg)	Dis at front Gy (7 μg)	N.S.	N.S.	Pk or ReOr	LOr or Gy	LOr or ReOr	Dis	–	–
Barbital	N.S.	N.S.	N.S.	N.S.	–	BlPu	LPu	Dis	–	–	–	–
Benzheptamine	N.S.	N.S.	N.S.	N.S.	–	–	–	–	–	–	Br or GyBr	Br
Butabarbital	N.S.	N.S.	N.S.	N.S.	–	BlPu	Pu	Dis	Tn	Tn, shortwave UV	–	–

(Continued on p. 368)

TABLE I *(continued)*

Drug	*Ninhydrin procedure*				*DPC*	*Silver acetate*	*Mercury(II) sulfate*	*Mercury(II) sulfate and heat*	*Fluorescence, longwave UV*	*0.5% H_2SO_4 and shortwave UV*	*Iodoplatinate*	*Iodine–potassium iodide*
	UV, 7 min	*Heat at 90° for 5 min*	*UV, 7 min*	*Respray and heat at 246° on a hot plate*								
Caffeine	–	–	–	–	N.S.	N.S.	–	–	–	–	–	Br or GyBr
Chlorpromazine (Largactil, Thorazine)	Dz	FtYl	FtYl	LPu	N.S.	N.S.	Pk	Pk*, RePk (7 μg)	Br	Br, shortwave UV	GyBr or Ch, LBr (7 μg)	Ch, Br at back
Chlorpheniramine	–	LYL	FtYl	LPu	N.S.	N.S.	Pk	Dis	–	Bl, shortwave UV	Ch	Ch
Chlordiazepoxide	–	FtPu (7 μg)	FtPu (7 μg)	Dis	N.S.	Bl, 2 spots (7 μg)	Dis	Dis	2-4 spots of Bl and GnYl or Yl	shortwave UV same as long-wave UV	Dis or Tn at front or Gy at back	Br to Tn at back for GnYl spots seen under UV
Chlordiazepoxide metabolite (RO5-2092)	–	–	–	–	N.S.	N.S.	–	–	2 spots, Gn and GnYl	Shortwave UV same as longwave UV	–	Br to Tn
Cocaine·HCl	–	–	–	–	N.S.	N.S.	–	–	–	–	Br to GyBr	GyBr
Codeine phosphate	–	Gy (7 μg)	Gy (7 μg)	Gy (7 μg)	N.S.	N.S.	–	Or	Or	Or, Dis after 30 sec	LBr at front and Bl at back, and Bl Br (7 μg)	LBr, Br (7 μg)
Cyclazocine	–	–	FtLPu	LPu or Pu	N.S.	N.S.	Dis	Dis	–	–	BrGy or Br	Br
Desimpramine	–	Pu or	DPu	DPu	N.S.	N.S.	DPk	LGy at	LBl or	shortwave	Br or	Br

		DPu						front and GyGn at back	LGn	UV same as longwave UV	LBr	
Dextromethorphan	–	LPu (7 μg)	LPu (7 μg)	Gy or LPu	N.S.	N.S.	Dis	–	–	–	GyBr, LBr (7 μg)	Br, DBr (7 μg)
Diazepam	N.S.	N.S.	N.S.	N.S.	–	–	–	–	YlGn	Shortwave UV same as longwave UV	Tn	Br
Diazepam metabolite (RO5-5345)	N.S.	N.S.	N.S.	N.S.	–	–	–	–	YlGn or Bl	Shortwave UV same as longwave UV	Tn	Br
Diazepam metabolite (RO5-6789)	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	–	–	2 spots, Bl and Br	shortwave UV same as longwave UV	–	–
Diethylpropion (Tenuate)	N.S.	N.S.	N.S.	N.S.	–	–	–	–	–	–	Tn (7 μg)	Br
2,5-Dimethoxy-4-methylamphetamine (STP, DOM)	–	LPu GyPu (7 μg)	Pu	Pu or LPu	N.S.	N.S.	–	–	Br	–	Tn at front, Pk at back	Tn
N,N-Dimethyltryptamine (DMT)	FtPu, Gy (7 μg)	Pu, GyPu (7 μg)	DPu, GyPu (7 μg)	DPu, PuYl (7 μg)	N.S.	N.S.	Pk	GyPu at front, GyGn at back and RePu (7 μg)	Gn or OrGn Br (7 μg)	–	Tn	Br, DBr (7 μg)
Diphenhydramine	LTq	GyTq	GyPu	GyPu	N.S.	N.S.	Re or Pk	Tn	Or	–	–	Tn
Diphenylhydantoin	N.S.	N.S.	N.S.	N.S.	–	BlPu	Pu	Dis	–	–	–	–
Diphenylpyraline	–	FtPu	LPu	LPu or DPu	N.S.	N.S.	Re or Pk	Dis	LOr	–	–	Tn
Doxepin	Dz	Gy	Gy or LGy	Pu or PuGy	N.S.	N.S.	Re or PuRe	Pk	Or	–	BrPu	Tn

(Continued on p. 370)

TABLE I *(continued)*

Drug	*Ninhydrin procedure*				*DPC*	*Silver acetate*	*Mercury(II) sulfate*	*Mercury(II) sulfate and heat*	*Fluorescence, longwave UV*	*0.5% H_2SO_4 and shortwave UV*	*Iodoplatinate*	*Iodine–potassium iodide*
	UV, 7 min	*Heat at 90° for 5 min*	*UV, 7 min*	*Respray and heat at 246° on a hot plate*								
Ephedrine	FtPu (7 μg)	Pu, PkPu (7 μg)	Pu	Dis or LPu, Pu (7 μg)	N.S.	N.S.	Pk	Dis	LOr (7 μg)	–	Tn at back (7 μg)	Tn at back (7 μg)
Ethchlorvynol (Placidyl)	N.S.	N.S.	N.S.	N.S.	–	BlPu	GnPu	LPu or Tn	Re Or (7 μg)	–	–	–
Fluphenazine	–	–	FtPu	LPu or Pu at back	N.S.	N.S.	Pk	Pk, LtOr	Br	–	2 spots, BrPu and LGy	Gy or GyBr
Glutethimide	N.S.	N.S.	N.S.	N.S.	Pk (7 μg)	BlPu	Pu	Dis	Tn	Tn, shortwave UV	–	Tn (7 μg)
Heroin	–	–	–	–	N.S.	N.S.	–	–	–	–	2 spots, BrBl	Tn, changes to Bl on keeping
Hydrochlorothiazide	–	–	–	–	N.S.	N.S.	–	–	LGn or Gn	Tn, shortwave UV	–	–
Hydrocodone	–	–	–	–	–	–	–	–	–	–	BlBr	DBr
Hydroflumethiazide	LPu	Dis or LPu	Dis	Dis	N.S.	N.S.	N.S.	–	PuVt at back	–	–	–
Hydromorphone	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	–	lGn	Gn, short-wave UV	BrB lor BrNBl	LBr or DBr
Imipramine (Tofranil)	–	GyPu, Gy (7 μg)	GyPu, LGy (7 μg)	GyPu, Gy (7 μg)	–	–	Pk or Re	Dis**, Tq (7 μg)	YlGn, Br (7 μg)	–	LBr	Br, DBr (7 μg)
Lysergic acid diethylamide (LSD)	LPu	Pu	Pu	DPu	N.S.	N.S.	Pk	GyPu	Or	–	TnOr at front, Gn at back	Tn

Meperidine (Demerol)	–	Gy (7 μg)	LPu	Gy (7 μg)	N.S.	N.S.	–	–	–	–	GyBr, LBr (7 μg)	Br, DBr (7 μg)
Mephentermine	GyPu (7 μg)	FtPu shadow, Pu (7 μg)	Dis	Pu, GyPu (7 μg)	N.S.	N.S.	Pk	Dis	LOr (7 μg)	–	BrGy, LBr (7 μg)	Tn
Mescaline	N.S.	N.S.	N.S.	N.S.***	N.S.	N.S.	PkOr (7 μg)	Tn (7 μg)	Yl, LOr (7 μg)	–	Br	Br, DBr (7 μg)
α-Methadol	–	–	–	–	N.S.	N.S.	–	–	–	–	Br	Br
Methadone	–	GyPu	GyPu	GyPu	N.S.	N.S.	–	–	–§	–	Br	Br
Methamphetamine	LPu (7 μg)	Pu, DPu (7 μg)	Pu	DPu	N.S.	N.S.	PkRe	Dis, PuOr (7 μg)	LOr	–	Tn (7 μg)	GyBr, DBr (7 μg)
Methapyrilene (Histadyl)	–	–	–	–	–	–	–	PuGy	PuGy	–	Ch	Ch
Methaqualone	–	–	–	–	Pu	Vt	Dis	–	–	–	Br	Br
Methylphenidate	–	GyPu (7 μg)	FtPu	LPu, changes to Yl	N.S.	N.S.	–	LPu	Yl, LGn (7 μg)	–	Tn after 30 sec, LBr (7 μg)	LBr, DBr§§ (7 μg)
Morphine	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	–	GnYl	–	NBl	Br, changes to Bl on keeping
Naloxone	–	LPu at back, Gy (7 μg)	–	–	N.S.	N.S.	–	Tn (7 μg)	LTn	–	LTn after 30 sec, LBr (7 μg)	Tn
Naltrexone	Gy (7 μg)	LPu at back, Pu (7 μg)	–	FtPu at back, Pu (7 μg)	N.S.	N.S.	–	Tn (7 μg)	LTn	–	LTn after 30sec, LBr (7 μg)	Tn
Nortriptyline	Gu (7 μg)	DPu or Pu	DPu	DPu, GyPu (7 μg)	N.S.	N.S.	PkRe	Tn at front, Or at back, TnGy (7 μg)	Or	–	LBr	Br, DBr (7 μg)

(Continued on p. 372)

TABLE I *(continued)*

Drug	*Ninhydrin procedure*				*DPC*	*Silver acetate*	*Mercury(II) sulfate*	*Mercury(II) sulfate and heat*	*Fluorescence, longwave UV*	*0.5% H_2SO_4 and shortwave UV*	*Iodoplatinate*	*Iodine–potassium iodide*
	UV, 7 min	*Heat at 90° for 5 min*	*UV, 7 min*	*Respray and heat at 246° on a hot plate*								
Oxazepam	LPu, streak	Dis	–	–	N.S.	N.S.	–	LBl (7 μg)	–	–	–	–
Papaverine	Dz	Dis	–	Gy	N.S.	N.S.	Yl	Yl	Or	–	Br	LBr
Pentazocine	–	Gy (7 μg)	–	LPu	N.S.	N.S.	–	–	–	–	LBr	LBr
Pentobarbital	N.S.	N.S.	N.S.	N.S.	–	BlPu	Pu	Dis	Br	–	–	Br (7 μg)
Phenazocine	–	–	–	–	–	–	–	–	–	–	Br	Br
Phencylidine	N.S.	N.S.	N.S.	N.S.	–	–	–	–	–	–	Br	Br
Phendimetrazine	–	–	–	–	–	–	–	–	–	–	LBr (7 μg)	Tn, DBr (7 μg)
Phenethylamine	DPu	DPu	DPu	DPu	N.S.	N.S.	Pk	Tn	Or	–	Br	Br
Phenmetrazine	–	LPu or FtPu	FtPu	Pk	N.S.	N.S.	Pk	Pk	–	–	–	Tn
Phenobarbital	N.S.	N.S.	N.S.	N.S.	PkPu	BlPu	Pu	Dis	–	–	–	–
Phentermine	FtPu (7 μg)	LPu, GyPu (7 μg)	LPu	LPu, LGy (7 μg)	N.S.	N.S.	–	–	LYl (7 μg)	–	–	Tn
Phenylpropanolamine	DPu	PkPu	DPu	Mixture of 2 spots; upper Dis, lower Pk	N.S.	N.S.	Upper Dis, lower Pk	Dis	–	–	–	–
Pipradrol	N.S.	N.S.	N.S.	N.S.	–	–	–	–	–	–	Br	1 addional spot, upper Tn, lower LBr
Prochlorperazine	Dz	Dis	–	LPu	N.S.	N.S.	–	Pk	Br	–	ChBr	ChBr

Promazine	Dz	GyPu	GyPu at back	GyPu	N.S.	N.S.	Pk at back	PkOr	Br	Pk, Br, shortwave UV	Ch	ChBr
Propoxyphene	N.S.	N.S.	N.S.	N.S.	–	–	–	–	–	Tn	Br	Br
Pseudoephedrine	–	LPu, streak	LPu	Dis	N.S.	N.S.	–	–	–	–	–	–
Pyribenzamine	–	Gy	LPu, streak	DPu	N.S.	N.S.	–	Pu	Pu	–	GyBr	Br
Pyrilamine	–	GyPu	GyPu	DPu	N.S.	N.S.	–	Pu	Pu	–	NBl	Br
Quinine	–	–	–	–	–	–	–	–	BlPu	Shortwave UV, bright Bl	Br	DBr
Secobarbital	N.S.	N.S.	N.S.	N.S.	Pk	BlPu	Pu	Dis	Br	–	–	–
Thioridazine (Mellaril)	Dz	YlGn	GnPu	GyPu	–	–	Pk	Tq§§§	Br	Tq, Br, shortwave UV	DBr	ChBr at front and Or at back, plus additional lower spot, LBr
Trifluoperazine (Stelazine, Eskazine)	Dz	Dis	LGyPu	Dis at front, GyPu at back	N.S.	N.S.	–	LPu, GyOr	Br, BrPu	–	GyPu or BrPu	DBr
Trimeprazine	—	–	–	–	–	–	Pk	LOr, PkRe	Br	PkRe, Br, shortwave UV	Br	DBr

* Chlorpromazine (Thorazine) passed through the body showed 4–5 pink spots after spraying with mercury(II) sulfate and then heating.

** Impramine passed through the body showed three Tq spots, one of which was also seen with the standard solution at the solvent front and was of strong intensity.

*** When the Mescaline spot travels 4.5 cm or higher, it gives a purple color after first exposure to UV light and after heating the plate at 90° for 5 min, and light purple on re-spraying with ninhydrin and heating at 246°.

§ Major metabolite of methadone (2-ethyl-1,5-dimethyl-3,3-diphenyl-1-pyrroline) emits characteristic LGn fluorescence which may be used to differentiate it from methadone.

§§ Methylphenidate in amounts greater than 5 μg gave an additional LBr spot of higher R_F value.

§§§ Thioridazine passed through the body showed 3 or 4 Tq spots.

TABLE II

R_F VALUES OF DRUGS ALONE AND IN VARIOUS COMBINATIONS

Gelman pre-coated silica gel glass microfiber sheets with a layer thickness of 250 μm were used. A two-stage developing solvent system was used; the plate was developed in solvent E or C up to 9.0 cm and then in solvent F up to 14.5 cm. The chromatograms were heated for 3–5 min at 85–90° before being placed in the solvents. The results in this table were obtained by placing two thin-layer chromatograms in the tank using 150 ml of solvent C or E and 180 ml of solvent F.

Drug	$R_F \times 100$	
	Solvents E and F	*Solvents C and F*
α-Acetylmethadol	67	—
Amitriptyline	64	—
Amitriptyline + methadone	68 (one spot)	68, 71
Amobarbital	91	—
Amphetamine sulfate	47	—
Amphetamine + methamphetamine + phenmetrazine	58[a], 56[a], 61	40, 33, 44
Amphetamine + phenethylamine + methamphetamine	58[a], 49, 57[a]	40, 29, 33
Amphetamine + β-phenylethylamine	57, 48	36, 29[b]
Amphetamine + phenylpropanolamine + methamphetamine	57[a], 45, 55[a]	40[c], 33
Amphetamine + phenylpropanolamine	57, 44	35 (one spot)
Amphetamine + mephentermine	57 (one spot)	37, 30
Amphetamine + naloxone	45, 54	36, 58[d]
Amphetamine + naltrexone	45, 49	36, 50[e]
Amphetamine + nortriptyline	51, 55	36, 41
Amphetamine + N,N-dimethyltryptamine	48, 52	42, 43[f]
Barbital	78	—
Benzheptamine	88	—
Butabarbital	87	—
Caffeine	51	—
Chlorpheniramine	51	—
Chlorpromazine	63	—
Chlorpromazine + methadone	64[a], 67	65[a], 70
Chlordiazepoxide	45, 40[h]	—
Chlordiazepoxide metabolite (RO5-2092)	39, 27[i]	—
Cocaine	66	72
Cocaine + methadone + propoxyphene + methaqualone	70, 68, 78, 86	72, 69, 76, 85
Cocaine + methadone + methylphenidate	71, 68, 64	71, 69, 63
Codeine phosphate	37	—
Codeine + dextromethorphan	46, 64	26, 41
Cyclazocine	60	—
Desimpramine	41	—
Dextromethorphan	51	—
Diazepam	84	—
Diazepam metabolite (RO5-5354)	73	—
Diazepam metabolite (RO5-6789)	85	—
Diethylpropion	88	—
Diethylpropion + benzheptamine	87, 91	81, 91
2,5-Dimethoxy-4-methylamphetamine	48	—
N,N-Dimethyltryptamine	48	—
N,N-Dimethyltryptamine + phenmetrazine	50 (one spot)	43, 39
Diphenhydramine	62	—
Diphenylhydantoin	81	83
Diphenylhydantoin + phenobarbital	83, 89	83 (one spot)
Diphenylpyraline	56	—

TABLE II *(continued)*

Drug	$R_F \times 100$	
	Solvents E and F	*Solvents C and F*
Doxepin	63	—
Doxepin + methadone	63, 66	60, 68
Ephedrine	36	—
Ethchlorvynol	95	—
Fluphenazine	52	—
Glutethimide	92	—
Glutethimide + seconal	89, 93	90, 95
Heroin	53, 46[j]	—
Hydrochlorothiazide	38	—
Hydrocodone	31	—
Hydroflumethiazide	52	—
Hydromorphone	18	—
Imipramine	59	—
Lysergic acid diethylamide	50	—
Meperidine	57	—
Mephentermine	43	—
Mephentermine + amphetamine	57 (one spot)	30, 37
Mescaline	29	—
α-Methadol	68	—
Methadone	67	—
Methadone + propoxyphene + methylphenidate	63, 69, 60	64, 71, 56
Methadone + α-acetylmethadol + methadol	66, 71, 74	65, 70 (one spot for acetylmethadol and methadol)
Methadone + major metabolite of methadone (2-ethyl-1,5-dimethyl-3,3-diphenyl-1-pyrroline)	67, 64	66, 64
Methadone + pentazoine	67, 65	64, 60
Methadone + phenazocine	67, 73	67, 72
Methadone + pipradrol	65, 70[k]	65, 70
Methadone + trimeprazine	66, 70[l]	66, 70[l]
Methadone + phendimetrazine	66, 60	68, 59
Methamphetamine	44	33
Methamphetamine + nortriptyline	50, 56	33, 41
Methamphetamine + N,N-dimethyltryptamine	51, 55	33, 39
Methapyrilene	62	—
Methapyrilene + methylphenidate	62 (one spot)	58 (one spot)
Methaqualone (Qualude)	81	—
Methylphenidate	61	—
Methylphenidate + thioridazine	61, 63[m]	56, 58[m]
Methylphenidate + imipramine	63 (one spot)	59 (one spot)
Methylphenidate + chlorpromazine	61, 66	59, 63
Morphine	20	—
Morphine + hydromorphone	28 (one spot)	15 (one spot)
Morphine + hydrocodone	26, 42	14, 21
Naloxone	54	57
Naloxone + dextromethorphan	55, 51	57, 43
Naltrexone	49	52
Nortriptyline	48	—
Oxazepam	55	—
Papaverine	56	—

(Continued on p. 376)

TABLE II *(continued)*

Drug	$R_F \times 100$	
	Solvents E and F	*Solvents C and F*
Pentazocine	60	—
Pentobarbital	90	—
Phenazocine	69	—
Phencylidine	82	—
Phendimetrazine	61	—
Phenethylamine	39	—
Phenmetrazine	46	—
Phenmetrazine + lysergic acid diethylamide	50, 55	43, 51
Phenobarbital	80	—
Phenobarbital + diphenylhydantoin	89, 83	83 (one spot)
Phentermine	49	—
Pipradrol	74[k]	—
Pipradrol + methadone	70[k], 65	70, 65
Prochlorperazine	54	—
Promazine	54	—
Propoxyphene	73	—
Pseudoephedrine	34	—
Pyribenzamine	60	—
Pyrilamine	59	—
Quinine	43	—
Secobarbital	93	94
Thioridazine	60	—
Trifluoperazine	56	—
Trimeprazine	66	—

[a] Spots overlapped.
[b] This system showed two spots for phenethylamine.
[c] This system gave one spot for amphetamine and phenylpropanolamine.
[d] Naloxone showed two spots.
[e] Naltrexone showed two spots and the second spot had the same R_F value as naloxone.
[f] Spots overlapped.
[g] Chlorpromazine artifact seen as additional spot at lower level.
[h] Chlordiazepoxide showed 2 to 4 artifacts under long wave UV light during the spraying procedure (see Table I).
[i] This chlordiazepoxide metabolite showed two spots under longwave UV light during the spraying procedure (see Table I).
[j] Heroin gave one minor spot for monoacetylmorphine.
[k] One artifact of pipradrol seen at a higher R_F value.
[l] One artifact of trimeprazine seen at a lower R_F value. Trimeprazine can be differentiated from pipradrol by the pink color after spraying with mercury(II) sulfate and the light orange color after spraying with mercury(II) sulfate and heating.
[m] One artifact of thioridazine seen at a lower R_F value.

turates. Solvent C can separate barbiturate mixtures such as phenobarbital, diphenylhydantoin and seconal from glutethimide. It is also very useful for the separation of opiates as reported previously[46]. Solvent D can separate phenobarbital from diphenylhydantoin and seconal but can not resolve seconal from glutethimide (Table III). However, the advantage of using solvent D is its capacity to separate methadone from cocaine and propoxyphene. Solvent F, as reported previously[46], can also separate methadone from cocaine, and propoxyphene in addition to

TABLE III

R_f VALUES OF MIXTURES OF SEDATIVES AND HYPNOTICS

Gelman pre-coated silica gel glass microfiber sheets with a layer thickness of 250 μm were used. Each chromatogram was dried for 5 min before being placed in the solvent. Each developing solvent was allowed to travel a distance of 14.5 cm; 120 ml of developing solvent were used and two chromatograms were placed in each tank.

	$R_f \times 100$	
	Solvent C	*Solvent D*
Amobarbital + phenobarbital	84, 61	81, 61
Butabarbital + phenobarbital	84, 61	80, 61
Diphenylhydantoin + phenobarbital	76, 62	69, 61
Diphenylhydantoin + glutethimide + phenobarbital + seconal	77, 95, 63, 87	67, 88*, 61, 88*
Diphenylhydantoin + barbital	77*	63*
Glutethimide** + seconal	95, 86	86*
Phenobarbital + seconal	59, 87	63, 87

* Glutethimide and seconal, diphenylhydantoin and barbital gave one spot.

** Glutethimide can be differentiated from barbiturates by applying I–KI spray after mercury(II) sulfate, giving a brown color.

α-acetylmethadol and its metabolites. Solvent C when used alone can resolve a mixture of 2,5-dimethoxy-4-methylamphetamine ("STP", DOM) and dimethyltryptamine (DMT), which could not be separated using a twostage development system (solvents E and F or solvents C and F).

The two-stage development system proposed in order to achieve the optimum separation of a wide variety of drugs on the same plate has proved to be very useful for detecting amphetamines, opiates, barbiturates and other drugs in one step (Table II). Drugs that have the same R_f values and drugs that could possibly interfere in the individual detection of drugs of abuse were mixed and the mixtures were chromatographed using a two-stage development system with solvents E and F and solvents C and F (Table II). Using this two-stage development system, we were able to separate methadone from cocaine, propoxyphene (Darvon), methaqualone, methylphenidate, pentazocine (Talwin), pipradrol, Doxepin, chlorpromazine (Largactil, Thorazine), phenazocine, naloxone, naltrexone, imipramine and trimeprazine; amphetamine from phenylpropanolamine, β-phenethylamine, phendimetrazine, nortryptyline, naloxone, phenmetrazine and DMT; codeine from dextromethorphan; naloxone from dextromethorphan; methamphetamine from DMT; glutethimide from seconal; diethylpropion (Tenuate) from benzheptamine; and methylphenidate from chlorpromazine. However, using the two-stage system of solvents C and F, we were able to resolve mixtures of amphetamine from methamphetamine (Desoxyn) and phenmetrazine; amphetamine from naltrexone; methamphetamine from DMT; and phenmetrazine from DMT.

When using a two-stage development system, it is imperative that the plate be first dried in the oven for 3–5 minutes at 85–90° (3 min in dry weather and 5 min when there is high humidity), then developed up to 9.0 cm in solvent E or C, air dried for about 10 min after taking the plate out of the tank, and again dried for 3–5 min in the oven at 85–90° before developing in solvent F. It is recommended that a total distance of 14.5 cm should be traveled by solvent F in order to separate drugs listed in Table II. The instructions and sequence for spraying the detection reagents pro-

posed in this paper must be followed in order to achieve the maximum sensitivity for each class of drugs, *i.e.*, ninhydrin only on the middle portion, diphenylcarbazone and silver acetate only on the upper 5.0 cm portion and mercury(II) sulfate should cover the area of 10 cm leaving only the lower 4.5 cm. Spraying with mercury (II) sulfate over the upper uncovered area of 10 cm and then heating the chromatogram in an oven at 85–90° for 5 min permits the detection of codeine as a specific light orange-colored spot; phenothiazines are also detected as bright pink spots, and many drugs are able to emit specific fluorescence under longwave UV light. When the lower 4.5 cm is not sprayed with ninhydrin, the sensitivity of morphine detection after the iodoplatinate spray increases. This sensitivity can be enhanced further by holding the chromatogram near the hot-plate (300–370°), thus permitting the detection of morphine in urine specimens at the 0.07–0.15 μg level (morphine base).

The cost of analysis per urine specimen using the above techniques is $ 0.58 for detecting opiates and performing at least 4 or 5 tests and $0.82 for testing the entire array of drugs of abuse[61].

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