CHROM. 5155

ROUTINE IDENTIFICATION OF DRUGS OF ABUSE IN HUMAN URINE

I. APPLICATION OF FLUOROMETRY, THIN-LAYER AND GAS-LIQUID CHROMATOGRAPHY

S. J. MULÉ
New York State Narcotic Addiction Control Commission, Testing and Research Laboratory, Brooklyn, N. Y. 11217 (U.S.A.)

(Received November 2nd, 1970)

SUMMARY

The methods described in this report were developed for the rapid analysis of over 500 urines per day for psychoactive drugs. These techniques involve extraction of the drugs from biological material, scanning the extract by automated spectrofluorometry, extensive use of thin-layer chromatography coupled with sequential chromogenic spraying and application of gas-liquid chromatography as an adjunct method for positive identification and confirmation.

The urinalysis laboratory requires a 50-60 ml sample from which a 2 ml aliquot is subjected to fluorometric analysis. The positive morphine and/or quinine samples were then acid hydrolyzed, extracted at pH 9 and the extracts applied to thin-layer plates and the presence of morphine and quinine confirmed by R_F values and reactions with specific chromogenic spray reagents. A 15 ml aliquot of the urine was extracted at pH <1 for barbiturates, diphenylhydantoin and glutethimide. The extract was applied to chromagram sheets developed and sprayed with reagents that provide reactions with these acidic drugs. A 25 ml aliquot of the urine was extracted at pH 10-11 for opiates, opioids, amphetamines, phenothiazines and tranquilizers. The organic extract was divided into A and B fractions, and these fractions developed on separate thin-layer silica gel plates. The A fraction was sprayed with chromogenic reagents primarily to detect amphetamine and analogues. The B fraction was sprayed with reagents to detect opiates, opioids, tranquilizers and phenothiazines. However, the reactions and R_F values on the A and B plates were usually cross compared for the various drugs of abuse.

The methods and techniques were relatively simple to perform and the psychoactive drugs could be detected in the range of 1 to 5 μg/ml of urine.

INTRODUCTION

The techniques[1] used for the determination and identification of psychoactive drugs are essentially those routinely used in analytical chemistry for the character-

ization of chemical structures. Unfortunately, many of the analytical techniques are only effective with pure material. This, of course, is seldom the situation with drugs and/or metabolites extracted from biological material. Therefore, although many methods[1-8] are available for the determination of drugs of abuse none completely fulfills the requirements of a rather large urine monitoring control program. In essence the only technique that can fully meet the needs of a control program monitoring urines for drugs of abuse is complete automation, consisting of continuous flow extraction, photometric detection and computerized data processing. Until technological development can achieve this goal the laboratory today must develop rapid methodology for the detection of drugs of abuse to meet the present requirements of the drug abuse problem.

This communication describes the methods utilized and developed in our laboratory to analyze 500 or more urines per day for psychoactive drugs. These techniques include: extraction from biological material; semiautomated spectrofluorometry; extensive use of thin-layer chromatography (TLC) with sequential chromogenic spraying for detection and use of gas-liquid chromatogrpahy (GLC) as an adjunct tool for positive identification and confirmation.

METHODS AND MATERIALS

The urinalysis laboratory requires a 50–60 ml urine sample from which the following aliquots and analyses are performed:

Spectrofluorometric analysis

2 ml of urine were placed in 15 ml glass stoppered centrifuge tubes and pH adjusted to 9–10 with 3.7 N NH_4OH. 4 ml of chloroform–isopropanol (3:1) was added to each tube and the samples shaken by hand for 30 sec–1 min. A two-thirds aliquot of the lower organic phase was removed for the morphine assay and the remaining one-third for the quinine assay. The automated turret spectrofluorometric (ATS) assay for morphine and quinine was then performed as described by MULÉ AND HUSHIN[9]. Those urine samples positive for morphine and/or quinine were subsequently acid hydrolyzed and analyzed by TLC as described below.

Thin-layer chromatographic analysis

Acid hydrolysis of urine. 15 ml of urine in a 40 ml glass stoppered centrifuge tube was autoclaved at 120° for ½ h in 2.3 N HCl (final normality) at 18–20 lb. pressure. The samples were rapidly cooled in dry ice and filtered. The filtrate was washed at the acidic pH with 15 ml of ethyl acetate by shaking for 5 min in an Eberbach shaker. The upper organic phase was aspirated off and the pH of the aqueous phase adjusted to about 9 with 9.5 N NaOH. 5 ml of 2.3 M K_2HPO_4, pH 9.3 (1 of NaCl) and 15 ml of chloroform–isopropanol (3:1) were added to each tube and the tubes shaken for 10 min in the Eberbach shaker. Following centrifugation at 2500 r.p.m. the upper aqueous phase was aspirated off. The organic phase was filtered and evaporated to dryness in a water bath at 85° under a stream of air. The residue was dissolved in 25–50 μl of methanol and applied to 0.25-mm silica gel TLC plate (E. Merck A.G., Darmstadt, G.F.R.). The plates were developed in ethyl acetate–methanol–ammonia (85:10:10) and oven dried at 100° for 15 min. Chromogenic sequential spraying

consisted of 0.5% H_2SO_4 (v/v), followed by viewing the plates under short and long wave UV light; iodoplatinate reagent, followed by mild heating and lastly ammoniacal silver nitrate followed by heating the plates for 10–15 min at 100°.

Urine extraction for barbiturates, diphenylhydantoin and glutethimide at pH <1. To 15 ml of urine in a 40 ml glass stoppered centrifuge tube was added 0.3 ml of 9 *N* H_2SO_4 and 15 ml chloroform. The samples were shaken for 10 min on the Eberbach shaker, centrifuged and the upper aqueous phase removed by aspiration. The organic phase was filtered and the filtrate evaporated to dryness on a water bath at 85° under a stream of air. The residue was taken up in 25–50 μl of methanol or chloroform and applied to Eastman Chromagram sheets (No. 6060 silica gel with fluorescent indicator). The Chromagram sheets were developed in ethyl acetate–methanol–ammonia (85:5:2.5). The sheets were air dried and subjected to the following chromogenic spray sequence: 10% NH_4OH (v/v) followed by visualization with short and long wave UV light; 0.1% $KMnO_4$; 1.0% silver acetate and finally with 0.1% diphenylcarbazone in chloroform.

Urine extraction at pH 10–11 for opiates, opioids, tranquilizers, phenothiazines, phenethylamines and related analogues. Transfer a 25 ml aliquot of urine to a 50 ml glass stoppered centrifuge tube, adjust pH to 10–11 with 6.2 *N* NaOH. Add 5 ml of potassium phosphate pH 10.3 and 12 ml of 25% ethanol in chloroform (v/v), shake for 10 min in the Eberbach shaker, aspirate most of the upper aqueous phase, add 12 ml of 25% ethanol in chloroform to each tube, shake by hand for 1 min and centrifuge if required. Remove the remaining aqueous phase by aspiration, add 100 μl of 6 *N* HCl in ethanol to the organic extract. Filter the organic phase and divide the filtrate into equal fractions (A and B). Evaporate the organic extracts to dryness in a water bath at 75° under a stream of air. Dissolve the residue in 25–50 μl of methanol and apply *fraction A* to 0.25 mm Silica Gel F_{254} thin-layer plates (E. MERCK A.G.). Develop the thin-layer plates in chloroform–methanol–ammonia (90:10:1). The residues labeled *fraction B* were applied to 0.25 mm Silica Gel F_{254} plates and developed in ethyl acetate–methanol–water–ammonia (85:10:3:1). The plates following development were dried in an oven for 15 min at 100° and *fraction A* plates treated as follows: viewed under short and long wave UV light; sprayed with 0.4% ninhydrin in acetone and irradiated for 5–10 min under long wave UV light; followed by 0.5% H_2SO_4 (v/v); 1.0% iodine in methanol; 0.5% H_2SO_4 (clears TLC plate); and iodoplatinate reagent. *Fraction B* plates were treated as follows: viewed under short and long wave UV light; sprayed with 5.0% H_2SO_4 (v/v) and the plates viewed under long wave UV; sprayed with iodoplatinate reagent and lastly ammoniacal silver nitrate followed by heat. In order to detect meprobamate the A and/or B plates were sprayed with furfural followed by conc. HCl and heat.

Gas–liquid chromatographic analysis

The GLC detection of drugs of abuse in the urinalysis laboratory was utilized only as an adjunct or confirmatory technique following routine TLC analysis of the urine extracts.

Apparatus. A Perkin-Elmer Model 900 gas chromatograph equipped with dual flame ionization detectors including dual channel wide dynamic range amplifier. The chromatograph was connected to a Leeds and Northrup model W/L dual channel potentiometer recorder with 1 mV range.

Columns. (1) A 6-ft. borosilicate glass coiled column of 1/4 in. O.D. was packed with Gas-Chrom Q (100–200 mesh) and coated with 3% SE-30; (2) A second glass column (6 ft. × 1/4 in. O.D.) was packed with Chromosorb G A/W DMCS (80–100 mesh) and coated with 10% Apiezon-L and 10% KOH. Both columns were conditioned at 200° for a period of 12 h.

For the *barbiturate analysis* the operating conditions with the 3% SE-30 column were: column 180°; injector 250°; manifold 225°; hydrogen at 20 lb. $in.^{-2}$; air at 30 lb. $in.^{-2}$; nitrogen carrier flow rate at 40 ml/min.

For the *opiate analysis* the operating conditions with the 3% SE-30 column were the same as for the barbiturate analysis except a column temperature of 205° was maintained.

For the *phenethylamine and related analogues analysis* the operating conditions with the 10% Apiezon-L, 10% KOH column were: column 160°; injector 200°; manifold 200°; hydrogen at 20 lb. $in.^{-2}$; air at 30 lb. $in.^{-2}$; nitrogen carrier flow rate at 40 ml/min.

Procedure. A 0.1% solution of the commercially available drug was prepared in either methanol, ethyl acetate or acetone. 2–25 µg of the drug was directly injected into the gas chromatograph with a Hamilton microliter syringe. GC analysis of the drugs extracted from urine was accomplished as described for the pure drug by injecting a suitable aliquot (1–5 µl) of the extract dissolved in 50 µl of methanol. In some cases cochromatography was performed by adding the suspected drug to the unknown extract and gas chromatographing the mixture.

Controls. Composite drug standards ranging in concentrations from 10–20 µg of each drug was directly applied to the TLC plates. A urine extractable drug standard consisting of 2–5 µg/ml was utilized with every 50 unknown urines analyzed.

Sensitivity. The limiting sensitivity of most drugs detected was in the range of 1–5 µg/ml of urine.

Reagents. All chemicals were of reagent grade and obtained through J. T. Baker Chemical Company or Fisher Scientific Company. Specially prepared spray reagents were: (1) iodoplatinate. 1 g of platinic chloride in 10 ml of water was mixed with 10 g of potassium iodide in 200 ml of water. The mixture was diluted to 500 ml with water and stored in a refrigerator; (2) ammoniacal silver nitrate, prepared by mixing just prior to use 30 ml of 5 *N* NH_4OH and 30 ml of 50% $AgNO_3$. If cloudy add drop by drop 5 *N* NH_4OH until solution clears. All other spray reagents described were simple percentages either (v/v) or (w/v) of the commercial reagent.

Potassium phosphate buffer, pH 10.3. Prepared by mixing 350 g of anhydrous K_2HPO_4 with 50 g of $K_3PO_4 \cdot H_2O$ and dissolving in 1 l of distilled water.

Thin-layer chromatographic plates. The 0.25 mm silica gel plates with or without fluorescent indicator (F_{254}) were made by E. Merck A.G. Darmstadt, G.F.R. and distributed by EM Reagents, Division of Brinkman Instruments, Inc., Westbury, N.Y. The Eastman Chromagram sheets (silica gel) were made by Eastman Kodak Company, Rochester, N.Y., U.S.A.

RESULTS AND DISCUSSION

Acid hydrolysis of urine and extraction at pH 9.0

Table I summarizes the data obtained following acid hydrolysis of the urine

TABLE I

ACID HYDROLYSIS OF URINE AND EXTRACTION OF DRUGS AT pH 9

Drug	*R_F × 100*	*Spray reagent color reactions*		
	Ethyl acetate–methanol–ammonia (85:10:10)	*0.5% H_2SO_4 followed by long wave UV visualization*	*Iodoplatinate*	*Ammoniacal silver nitrate*[d]
Morphine	40	green[c]	blue-purple	black
Codeine	75	—	blue	—
Quinine[a]	88	bright blue fl.	blue	—
Nicotine[b]	34	—	blue-black	—
d-Propoxyphene (Darvon)	95	—	red-blue	—
Meperidine (Demerol)	95	—	red-blue	—
Methadone (Dolophine)	96	—	red-blue	—

[a] Quinine provides several products in this fraction with R_F × 100 values of 92, 88, 85, 77, 73, 69, 62, 54, 42, 27, 23 and 15. The colors observed under UV following 0.5% H_2SO_4 range from bright blue through orange, yellow and green. Some of these products were metabolites and some degradation products due to acid hydrolysis.

[b] A minor metabolite of nicotine may be observed at R_F (× 100) 68 following the iodoplatinate spray. The R_F × 100 of the nicotine standard (unhydrolyzed, unextracted) was 91.

[c] A green fluorescence was observed with visualization under long wave UV light when morphine was present in high concentrations.

[d] Heat at 100° for several minutes was required after spraying.

and extraction at pH 9 as described under methods. The urines in this fraction were analyzed provided a positive result with the fluorometric assay for morphine or quinine was obtained[9]. The principle emphasis in this fraction was thus placed upon confirmation of the routine fluorometric screen for morphine and/or quinine. It is quite obvious that a clear separation of the narcotic analgesics, meperidine, methadone and *d*-propoxyphene was not achieved with this solvent system. This, of course, is not important in this system since an effort to identify these drugs is made in the basic pH 10–11 extraction. Codeine, however, may be readily identified following the iodoplatinate reaction. Morphine, the most important drug in this fraction, was easily identified especially after treating the plate with ammoniacal silver nitrate. Quinine is also confirmed in this system, however, hydrolysis does degrade this compound. Nicotine and its metabolites are important in this system since a vast majority of the urines contain this compound and its metabolites which react with the iodoplatinate spray and thus might cause some confusion with the positive identification of a narcotic analgesic.

Urines extracted at pH <1

The data obtained on R_F values and color reactions following sequential chromogenic spraying for barbiturates, diphenylhydantoin and glutethimide extracted from urine appears in Table II. It is quite obvious that difficulty was encountered in separating amobarbital, pentobarbital and secobarbital by TLC. Secobarbital, however, is readily identified by the bright yellow reaction observed following the 0.1% $KMnO_4$ spray. Amobarbital and pentobarbital metabolites may be

TABLE II

EXTRACTION OF DRUGS FROM URINE AT pH < 1

Drug	$R_F \times 100$[a]	*Spray reagent color reactions*			
	Ethyl acetate–methanol–ammonia (85:5:2.5)	*10% NH_4OH, UV visualization*[b]	*0.1% $KMnO_4$*[c]	*1.0% Silver acetate*[d]	*0.1% Diphenyl-carbazone*[e]
Phenobarbital (Luminal)	40	blue	—	white	blue
Amobarbital (Amytal)	77	blue	—	white	blue
Pentobarbital (Nembutal)	75	blue	—	white	blue
Secobarbital (Seconal)	72	blue	yellow	white	blue
Diphenylhydantoin (Dilantin)	61	—	pale spot	white	—
Glutethimide (Doriden)	89	—	pale spot	white	—

[a] Metabolites of pentobarbital and amobarbital occur at R_F (× 100) 39, 28 (observed as white spots after the silver acetate spray). Metabolites of secobarbital occur at 42 and 32 (observed after $KMnO_4$ spray). A metabolite of glutethimide occurs at R_F 81 (observed after silver acetate spray).

[b] No reaction observed with the nonfluorescent indicator TLC plates. However, with the chromagram fluorescent indicator plates the barbiturates appear blue on an orange background under short wave UV light.

[c] The permanganate spray also provided light yellow spots with the metabolites of secobarbital. Glutethimide and diphenylhydantoin appeared as pale spots on a pink background.

[d] The silver acetate reacting compounds quench the fluorescent indicator TLC plates when visualized under short wave UV light.

[e] The barbiturates and metabolites appear blue on a yellow background after diphenylcarbazone spray (DPC). The metabolite of glutethimide R_F 81 turns blue following the DPC spray. Glutethimide itself, however, does not.

TABLE III

THIN-LAYER CHROMATOGRAPHIC DATA ON VARIOUS BARBITURATES

All chromatography was performed on Eastman Chromagram Sheets with fluorescent indicator. Detection of drugs was as described under METHODS AND MATERIALS. The concentration of each drug was usually 10 µg. Solvent systems utilized (v/v) were: S_1, chloroform–acetone (90:10); S_2, ethyl acetate–methanol–ammonia (85:10:5); S_3, ethanol–dioxane–benzene–ammonia (5:40:50:5); S_4, hexane–ethanol (90:10); S_5, ethyl ether–chloroform (90:10); S_6, chloroform–isopropanol–ammonia (45:45:10); S_7, ethyl acetate–methanol–ammonia (85:10:25).

Barbiturate	$R_F \times 100$						
	S_1	S_2	S_3	S_4	S_5	S_6	S_7
Hexobarbital (Ortal)	85	89	87	62	90	86	89
Phenobarbital (Luminal)	67	57	45	18	96	54	66
Amobarbital (Amytal)	81	83	81	29	96	84	83
Pentobarbital (Nembutal)	80	81	82	31	98	80	84
Secobarbital (Seconal)	82	83	85	31	97	82	82
Aprobarbital (Alurate)	82	76	76	27	91	84	87
Allylisobutylbarbituric acid	83	75	76	32	91	84	87
Barbital (Veronal)	75	66	66	20	91	67	82

observed as white spots following the silver acetate spray ($R_F \times 100$ of 39 and 28). Diphenylhydantoin (Dilantin) as well as glutethimide (Doriden) provide discernible reactions with the silver acetate spray (white) and may be differentiated from barbiturates on the basis of R_F values. Furthermore, a metabolite of glutethimide ($R_F \times 100$ of 81) appears *blue* after the diphenylcarbazone spray reaction.

A small study on the detection and metabolism of barbiturates was initiated with two human volunteers following an ingestion of 100 mg of pentobarbital at 11 p.m. in the evening and urines obtained at about 7 a.m. the following morning. The parent compound (pentobarbital) was easily detected along with metabolites at $R_F \times 100$ of 38 and 27. A similar experiment was conducted whereby 100 mg of amobarbital was ingested and the parent drug as well as a metabolite at $R_F \times 100$ of 39 was easily detected following extraction and analysis for barbiturates.

In Table III the data appears on several barbiturates with various chromatographic solvent systems. It is quite obvious that no single solvent system was available in separating amobarbital, secobarbital and pentobarbital. Phenobarbital, however, is quite easily separated from the other barbiturates and usually exhibits a lower R_F value.

Urines extracted at pH 10–11

In Table IV appear the results obtained with amphetamine and related drugs (A fraction) as well as the data on opiates, tranquilizers and phenothiazines (B fraction). The S_3 solvent system was primarily used with the A fraction extracts and the S_1 solvent system with the B fraction extracted compounds. The S_2 solvent system was used occasionally for amphetamine and related analogues and proved to be effective in separating ecgonine from cocaine and benzoylecgonine.

Normally the TLC plates following development were viewed under short wave and long wave UV light. This provided for an initial evaluation of those drugs that fluoresce under long wave UV light and those that quench under short wave UV light with the fluorescent indicator plates (F_{254} nm). This procedure was followed whether the plates were sprayed with the A or B series of chromogenic reagents.

The drugs primarily extracted from urine in the A fraction and detected with the A series of spray reagents allows for the following comments: (1) Ninhydrin followed by UV irradiation for 10 min was only effective in detecting the primary amines, amphetamine and phenylpropanolamine; (2) Spraying with 0.5% H_2SO_4 usually intensified the ninhydrin reaction; (3) Iodine was in general a universal reagent so that almost all the compounds present on the plate provided a yellow-brown spot with this reagent; (4) Careful use of known standard reference drugs was required to identify the iodine reacting compounds; (5) The second application of 0.5% H_2SO_4 was used to clear the plate following the iodine spray; (6) The thin-layer plates were then sprayed with the iodoplatinate reagent which provided relatively good reaction with the opiates, opioids, phenothiazines and tranquilizers, but in general no reaction with amphetamine and related analogues. A reaction with iodoplatinate for the amphetamine-like compounds appears to depend upon levels of the drug present.

The drugs extracted from urine in the B fraction and detected with the B series of chromogenic reagents allows for the following comments: (1) With 5% H_2SO_4 the phenothiazine compounds appeared quite readily as redish-pink to blue spots; (2) Quinine and metabolites after this reagent when viewed under long wave UV light

TABLE IV

EXTRACTION OF DRUGS FROM URINE AT pH 10–11

Solvent systems used (v/v) were: S_1, ethyl acetate–methanol–water–ammonia (85:10:3:1); S_2, methanol–ammonia (99:1); S_3, chloroform–methanol–ammonia (90:10:1).

Drug	$R_F \times 100$			UV light		Spray reagents color reactions[a]						
						A fraction					B fraction	
	S_1	S_2	S_3	Long wave	Short wave	Ninhydrin + UV irradiation	0.5% H_2SO_4	1% Iodine	0.5% H_2SO_4	Iodoplatinate	5% H_2SO_4	Iodoplatinate
Morphine	18	44	23	green fl.	Q[e]	—	—	yellow-brown	—	purple	—	blue
Codeine	30	46	70	—	Q	—	—	yellow-brown	—	purple	—	blue
Methadone (dolophine)	80	83	80	—	Q	—	—	yellow-brown	—	purple	—	red-blue
Meperidine (Demerol)	62	63	87	—	Q	—	—	yellow-brown	—	purple	—	blue
d-Propoxyphene (Darvon)	94[d]	82	97	—	Q	—	—	yellow-brown	—	purple	—	red-blue
Pentazocine (Talwin)	77	59	60	—	—	—	—	yellow-brown	—	red-blue	—	red-blue
Cyclazocine	57	49	52	green fl.	—	—	—	yellow-brown	—	red-blue	—	red-blue
l-Methorphan	10	13	61	—	Q	—	—	yellow-brown	—	blue	—	blue-black
Hydroxyzine (Vistaril)	96	—	90	blue fl.	Q	—	—	yellow-brown	—	blue	—	blue
Quinine	42[d]	63	49	blue fl.	Q	—	—	yellow-brown	—	purple	bright blue[f]	blue
Cocaine	90	77	92	—	Q	—	—	yellow-brown	—	red-blue	—	red-blue
Ecgonine[b]	—	32	—	—	—	—	—	—	—	blue		blue
Benzoylecgonine[b]	—	78	—	—	Q	—	—	—	—	red-blue	—	red-blue
d-Amphetamine (Dexedrine)	42	52	73	—	Q	purple	increased intensity	yellow-brown	—	—	—	—[g]
Methamphetamine (Methedrine)	28	40	66	—	Q	—	—	yellow-brown	—	—	—	—[g]
Ephedrine	19	38	25	—	Q	—	—	yellow-brown	—	—	—	—
Phenmetrazine (Preludin)	46	67	83	—	Q	—	—	yellow-brown	—	—	—	—
Methylphenidate (Ritalin)	76	78	96	green fl.	Q	—	—	yellow-brown	—	—	—	—

Phenylpropanolamine	31	54	27	—	Q	light purple	increased intensity	yellow-brown	—	—	—	—
Chlorpromazine (Thorazine)	72	60	90	blue fl.	Q	—	—	yellow-brown	—	blue-brown	redish-pink	purple-red
Promethazine (Phenergan)	70	69	91	—	Q	—	—	yellow-brown	light yellow	blue-brown	pink	purple
Prochlorperazine (Compazine)	52	68	87	blue fl.	Q	—	—	yellow-brown	light yellow	blue-brown	blue	purple
Thioridazine (Mellaril)	73	—	88	blue fl.	Q	—	—	dark-brown	dark yellow	blue-yellow	blue	purple
Nicotine	56[d]	66	81	—	Q	—	—	yellow-brown	yellow brown	black-brown	—	blue
Diazepam (Valium)	92	84	92	green fl.	Q	—	—	yellow-brown	—	light red-blue	—	red-blue
Chlordiazepoxide (Librium)	68	82	75	green fl.	Q	—	—	yellow-brown	—	brown-blue	—	red-blue
Meprobamate[c] (Equanil)	81	85	41	—	—	—	—	—	—	—	—	—
Amitriptyline (Elevil)	79	77	95	—	Q	—	—	yellow-brown	—	purple	—	purple
Tripelenamine (Pyribenzamine)	60	51	80	blue fl.	Q	—	—	yellow-brown	—	dark-blue	blue	blue
Chlorpeniramine (Chlortrimeton)	33	34	57	green fl.	Q	—	—	yellow-brown	—	blue	—	blue

[a] Chromogenic sprays utilized under A were primarily for the detection of amphetamines and analogues and used only with the A extract. Those utilized under B were primarily for the detection of opiates and used only with the B extract.

[b] These metabolites of cocaine may be detected by following the iodoplatinate reagent with 5% H_2SO_4 and mild heating. In the ethyl acetate–methanol–NH_3 (85:10:10) solvent system, R_F (× 100) values of 95, 3 and 12 were obtained for cocaine, ecgonine and benzoylecgonine, respectively.

[c] Meprobamate is detected by finally spraying the plates with furfural followed by conc. HCl. A dark black spot appears after subjecting the plates to heat (100°) for a few minutes.

[d] In the S_1 solvent system metabolites of quinine were observed at R_F (× 100) 30, 23, 07; for nicotine at 36, 22; for methadone at 58, and for *d*-propoxyphene a streak was observed from the origin that provided an R_F of 38. Methadone standard generally provided an R_F of 95 in the S_1 or S_3 solvent system, however, in the composite standard the R_F values were identical to the urine extracted compound. Furthermore in the S_3 system a metabolite of methadone was observed at R_F 40. Combining the data from S_1 and S_3 provided excellent confirmatory data for methadone.

[e] The fluorescent indicator TLC plates fluoresce at 254 nm under short wave UV light. Q denotes the drugs which quench the fluorescence. The plates were viewed under UV light prior to spraying with either the A or B sequence of chromogenic reagents.

[f] Must be viewed under long wave UV light.

[g] *d*-amphetamine and methamphetamine may react with iodoplatinate when present in high concentrations (50 μg or more) and provide a blue color reaction.

vividly fluoresced as bright blue, green or orange spots. The presence of quinine was quite characteristic following the sulfuric acid spray; (3) The primary detecting reagent, however, with this group of drugs was iodoplatinate and it was quite effective in detecting the opiates, phenothiazines, tranquilizers, cocaine and its metabolites.

It is important to note that in some instances a different R_F value was obtained with a drug extracted from urine as compared to the nonextracted reference standard drug. An example of this was methadone which could in effect not be separated from *d*-propoxyphene when applied separately on a TLC plate. However, methadone and metabolite extracted from urine provided R_F values different from the standard reference methadone as well as *d*-propoxyphene (see Table IV). The urinary extracted methadone did agree with methadone in the composite standard which contains several narcotic drugs.

Meprobamate was easily detected by spraying the A fraction with furfural and conc. HCl after the iodoplatinate reagent. The presence of this drug was confirmed with the B fraction TLC plate that was sprayed with ammoniacal silver nitrate (detection of morphine) and then followed by furfural and conc. HCl. A characteristic brown-black reaction (spot) for meprobamate was obtained with furfural reagent.

Cocaine presented a rather difficult problem. In order to effectively detect the usage of this drug it was decided to attempt to identify the primary metabolites, ecgonine and benzoylecgonine[10]. If the urine was acid hydrolyzed and then extracted, neither cocaine nor any degradation product was detected indicating destruction under the conditions of acid hydrolysis. However, cocaine and metabolites may be extracted through the procedure used for the extraction of urine at pH 10–11. Neither ecgonine nor benzoylecgonine moves from the origin in solvent systems S_1 and S_3. In solvent system S_2 ecgonine may be separated from benzoylecgonine and cocaine. The metabolite, benzoylecgonine, may be separated from cocaine and ecgonine by using the solvent system ethyl acetate–methanol–ammonia (85:10:10) (see Table IV).

Methamphetamine and codeine also present a rather difficult problem with the solvent systems used in Table IV. A good iodoplatinate reaction with the A sequence of reagents would indicate codeine, however, high levels of methamphetamine may also provide a reaction with iodoplatinate. It is necessary to observe the metabolite of methamphetamine (amphetamine), or the metabolite of codeine (morphine) or resort to either GLC and/or other solvent systems for a definitive identification of these two drugs.

Gas–liquid chromatography of drugs of abuse

The retention time data obtained with various drugs of abuse appears in Table V. The barbiturates may be effectively separated by GLC whereas complete separation by TLC (*i.e.* amobarbital and pentobarbital) was not achieved. In all cases the retention time for the barbiturates was quite short ranging from 1.10 for barbital to 4.31 for hexobarbital. The change in retention time for the barbiturates did not appear to be directly correlated with the size or chain length substitution on barbituric acid. Glutethimide, a sedative hypnotic drug, may also be detected by GLC (retention time of 3.75).

Amphetamine and related analogues were chromatographed (Table V) on an Apiezon column. A fairly good separation was achieved with this column for these

TABLE V

GAS–LIQUID CHROMATOGRAPHIC RETENTION TIME DATA ON VARIOUS DRUGS OF ABUSE[a]

Drug	*RT*	*RRT*[b]
Barbiturates		
Pentobarbital (Nembutal)	2.62	1.00
Barbital (Veronal)	1.19	0.45
Secobarbital (Seconal)	3.43	1.31
Amobarbital (Amytal)	2.25	0.86
Phenobarbital (Luminal)	5.75	2.19
Aprobarbital (Alurate)	1.75	0.67
Hexobarbital (Ortal)	4.31	1.64
Allylbarbituric acid (Sandoptal)	2.25	0.86
Glutethimide (Doriden)[c]	3.75	1.43
Amphetamine and related analogues		
d-Amphetamine (Dexedrine)	4.12	1.00
Methamphetamine (Methedrine)	5.62	1.36
Methylphenidate (Ritalin)	—	—
Phenylpropanolamine	6.37	1.55
Phenmetrazine (Preludin)	3.87	0.94
Ephedrine	5.15	1.25
Narcotic analgesics		
Codeine	8.87	1.00
Morphine	10.50	1.18
d-Propoxyphene (Darvon)	5.19	0.58
Methadone (Dolophine)	5.25	0.59
Meperidine (Demerol)	1.31	0.15
Pentazocine (Talwin)	6.75	0.76
Cyclazocine	5.75	0.65
Cocaine[d]	7.50	0.84
Benzoylecgonine[d]	15.12	1.70
Ecgonine[d]	3.37	0.38

[a] The conditions whereby the drugs were analyzed appear under METHODS AND MATERIALS.
[b] The RRT (relative retention time) refers to pentobarbital for the barbiturates, *d*-amphetamine for the amphetamines and codeine for the narcotic analgesics.
[c] Not a barbiturate.
[d] Not a narcotic analgesic.

drugs. The retention time of each drug except for phenmetrazine was greater than *d*-amphetamine.

Data on the retention time of narcotic analgesics also appears in Table V. The data obtained was quite similar to that reported previously[1] where a much larger number of narcotic drugs were separated by gas chromatography. In essence all the drugs had a shorter retention time than codeine except for morphine and benzoylecgonine. It is of interest to mention that cocaine was easily separated from its metabolites (ecgonine and benzoylecgonine). However, relatively large concentrations of ecgonine and benzoylecgonine (10–20 μg) were required for sufficient detection with the SE-30 column.

ACKNOWLEDGEMENTS

The author expresses his appreciation for the technical assistance provided by P. L. HUSHIN, H. SHAH, E. SAFFER, R. MAST and R. GEISER.

REFERENCES

1 S. J. Mulé, *Anal. Chem.*, 36 (1964) 1907.
2 S. J. Mulé, *J. Chromatog.*, 39 (1969) 302.
3 J. Cochin and J. W. Daly, *Experientia*, 18 (1962) 294.
4 B. Davidow, N. LiPetri and B. Quame, *Amer. J. Clin. Pathol.*, 50 (1968) 714.
5 J. M. Fujimoto and R. I. H. Wang, *Toxicol. Appl. Pharmacol.*, 16 (1970) 186.
6 G. F. Phillips and J. Gardner, *J. Pharm. Pharmacol.*, 21 (1969) 793.
7 A. H. Beckett, G. T. Tucker and A. C. Moffat, *J. Pharm. Pharmacol.*, 19 (1967) 273.
8 I. Sunshine, *Amer. J. Clin. Pathol.*, 40 (1963) 576.
9 S. J. Mulé and P. L. Hushin, *Anal. Chem.*, in press.
10 F. Fish and W. D. C. Wilson, *J. Pharm. Pharmacol.*, 21 (1969) 135S.