Journal of Pharmaceutical Sciences

MAY 1972
VOLUME 61 NUMBER 5

REVIEW ARTICLE

Drug Abuse Screening Programs: Detection Procedures, Development Costs, Street-Sample Analysis, and Field Tests

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Keyphrases ☐ Drug abuse screening programs—detection procedures, development costs, and field tests, review ☐ Narcotics—detection procedures in urine screening programs, review ☐ Barbiturates—detection procedures in urine screening programs, review ☐ Amphetamines—detection procedures in urine screening programs, review ☐ Chromatography—analysis, drug abuse screening programs, review ☐ TLC—analysis, drug abuse screening programs, review, analysis of street samples and field tests

This review is a modest effort toward providing a guide for new investigators in the field of drug abuse who are involved in setting up toxicology laboratory facilities in a large- or small-scale drug abuse urine screening program. Due to the increased usage of drugs, more and more outpatient and inpatient treatment programs for drug-dependent individuals are being established throughout the country. It is now generally recognized that these therapeutic programs should include provisions for determining illicit drug usage objectively, even if such information is used only to assess the efficacy of the management technique. Although saliva is also a vehicle for the excretion of certain drugs, *e.g.*, alcohol and morphine (1), the chemical analysis of urine is at present the predominant technique to determine drug taking by individuals in drug abuse screening programs. Although this review primarily concerns the detection of abuse drugs in human and/or animal urine, the techniques applicable to other biological materials also are included. In addition, development costs of a toxicology laboratory facility pertaining to drug abuse detection, the cost of analysis per urine test using different techniques, and analysis and field tests for some illicit drugs of abuse are discussed.

Methods currently available for the detection of drugs of abuse in urine can be broadly categorized as follows: spectrophotometry, radioisotope tracer techniques, GLC, radioimmunoassay and free radical assay techniques, TLC, simplified chemical screening and color reactions, and miscellaneous techniques and new instrumentation. Included among the miscellaneous techniques are: polarographic techniques, biological luminescence (bacterial luminescence), microdiffusion analysis, microcrystal tests, and optical crystallographic methods. These methods vary greatly with respect to their suitability for use in large-scale urine monitoring programs. Some of the criteria by which a method should be judged are: (*a*) rapidity of analysis, (*b*) sensitivity, (*c*) convenience—laboratory personnel with minimal formal training should be capable of completing the entire analysis including the interpretation of results, and (*d*) economy.

The requirements of a clinical program for drug detection can vary according to the clinical and administrative needs of the particular program and the aims of a management technique. For example, programs that use drug monitoring procedures as a deterrent to drug use could be far more concerned with accuracy and elimination of false positives than programs that use results primarily to gauge the clinical progress of voluntary patients or of different treatment approaches. Programs dealing primarily with narcotic users who do not commonly abuse other drugs are less concerned about

detection of barbiturates and amphetamines. However, programs where a large proportion of patients are taking prescribed tranquilizers or antibiotic drugs require sensitive, accurate, and low cost detection procedures. The detection systems used should be capable of differentiating prescribed medications and their metabolites from illicit substances and their metabolites and adulterants.

The number of urine specimens collected from each patient each week and the nature of the investigational drugs used to treat the patients can influence the decision of the investigator about the choice of the detection technique. In some situations, economy may have to be sacrificed for rapidity of analysis, *e.g.*, analysis of urine specimens dropped 2 hr. before embarkation by homebound soldiers in Vietnam. But in treatment programs where time is not a critical factor, where the results are not required instantaneously, and where the detection of a wide range of substances is desired, low cost, versatile detection techniques would be preferred. Even the techniques used for TLC can vary considerably in the extraction and detection procedures from program to program. This review focuses on the advantages and disadvantages of each detection and/or extraction procedure; it is hoped that this information will facilitate the decisions regarding the selection of these procedures for a particular clinical program.

DETECTION PROCEDURES

Spectrophotometry—Spectrophotometric techniques can be subdivided into the following categories: (*a*) UV and colorimetric spectrophotometry, (*b*) fluorometry; (*c*) atomic absorption spectrophotometry, (*d*) IR spectroscopy, and (*e*) mass spectrometry.

All of these techniques have widespread use in pharmaceutical research and analysis as well as in the broad area of organic and inorganic analysis. These techniques also provide valuable information on the structural identification and elucidation of unknown molecules. Of the various spectroscopic techniques mentioned, UV and fluorometric techniques are the ones that have been applied for the qualitative and quantitative detection of some abuse drugs in biological materials. Among the drugs of abuse, the biological disposition of morphine and its surrogates has been the main focus of study by the biological scientists since the early 1920's. The major problem encountered in the qualitative and quantitative determination of morphine in biological materials usually concerns the isolation and separation of morphine from body tissues and fluids. The term "free morphine" is used for the unchanged parent compound; the terms "bound," "combined," and "conjugated" are used to indicate the morphine before acid or enzymic hydrolysis. Most methods for the estimation of morphine follow a general pattern in that an effort is made to recover morphine selectively from biological contaminants by suitable extraction and/or absorption methods. The extraction procedures involve the partition of morphine between different solvent phases under a suitable pH.

UV and Colorimetric Spectrophotometry—Way *et al.* (2), while studying pharmacological effects of heroin and its rate of biotransformation, determined morphine from mouse tissue extracts colorimetrically by treating the aqueous buffer phase with the Folin–Ciocalteu reagent. Woods *et al.* (3) determined morphine in biological materials by esterification of morphine with *p*-nitrobenzoyl chloride, extracting the ester from a strongly alkaline aqueous solution by ethylene dichloride. The organic extract was then complexed with methyl orange according to the procedure of Brodie and Udenfriend (4) or with bromcresol purple as described by Woods *et al.* (5) for the determination of cocaine and mescaline in urine and other biological materials. This procedure was also applied by Woods *et al.* (3) for the determination of morphine in animal urines; the urines were carried through an acid–base extraction procedure and then the acid extract was carried through the esterification and extraction procedure with *p*-nitrobenzoyl chloride and the methyl orange complex formation.

Fujimoto *et al.* (6) described a photometric estimation of morphine from urine and other biological materials; the urine was carried through an acid–base extraction procedure and the morphine was finally extracted into a buffer solution (phosphate buffer pH 5.8). Siminoff and Saunders (7) applied the procedures of Woods *et al.* (3) and Fujimoto *et al.* (6), with a few modifications, to the determination of free and conjugated morphine in brain and other tissues of rabbits[1]. Szerb *et al.* (8), while determining morphine in blood and tissues, avoided the acid–base extraction procedures and instead used anhydrous benzene to precipitate the impurities. He then used ion-exchange resin (IRC-50 H) for the purification of morphine. The morphine, after eluting with 0.05 *N* HCl, was treated with the Folin–Ciocalteu reagent and the color developed was measured photometrically.

Mulé (9) described a UV spectrophotometric procedure for the determination of narcotic analgesics in human urine and other biological materials; the extracted drugs were scanned over the 360–225-nm. region. Harms (10) reported a UV spectrophotometric method for the determination of morphine and codeine in human urine. He utilized TLC for the separation of extracted drugs, followed by the elution of appropriate chromatographic spots. Oberst (11) proposed a colorimetric procedure for the determination of morphine in the urine of morphine addicts. He used permutit to purify morphine from a urinary extract. Preliminary extraction of morphine was carried out by the Pierce and Plant (12) procedure. Morphine concentration in the permutit was determined colorimetrically by the addition of sodium carbonate and the Folin–Denis (13) phenol reagent. Deckert (14) described a method in which morphine was extracted from urine by means of ethyl acetate and was determined nephelometrically as a morphine–molybdate complex. This method was further modified by Oberst (11)[2] to detect the presence of small amounts of morphine. An excellent review of biological disposition of morphine and the techniques used until 1960 was presented by Way and Adler (15).

[1] Readers interested in the applications of dye methods for the estimation of basic drugs in biological materials are advised to read the article by Axelrod (7A).

[2] References cited in this paper are quite useful for the detection of morphine in urine.

Stevenson (16) and Street and McMartin (17) described a UV spectrometric method for the qualitative estimation and identification of barbiturates in blood. Wallace (18) reported a spectrophotometric method for the quantitative determination of diphenylhydantoin in the presence of phenobarbital in whole blood or urine. The method involved hydrolysis of the hydantoin ring in strong alkali, followed by permanganate oxidation of the resulting amide to benzophenone and then steam distillation of benzophenone. Wallace (19) later modified this procedure by extracting the benzophenone into heptane and avoided the tedious step of steam distillation. In his subsequent report, Wallace (20) proposed a simultaneous spectrophotometric determination of diphenylhydantoin and phenobarbital in biological specimens including urine.

Axelrod (21), in his study on the biotransformation and physiological disposition of dextroamphetamine, estimated amphetamine in the urine and plasma of dogs spectrophotometrically by a modification of the methyl orange reaction of Brodie *et al.* (22). Wallace *et al.* (23) reported a UV spectrophotometric procedure for the determination of amphetamine and related drugs in human urine, oxalated blood, or serum. The method involved refluxing of the aqueous acid extract of amphetamines with anhydrous cerium sulfate. After cooling, the reaction product was extracted with *n*-hexane and the absorbance was measured at 287 nm. 2,5-Dimethyl-4-methylamphetamine (STP, DOM) has been found to give excellent yields of reaction product. Goldbaum and Domanski (23A) also described UV spectrophotometric methods for the estimation of amphetamine and congeners and other basic drugs in biological specimens.

The UV and colorimetric techniques reviewed here are primarily for the determination of drugs in biological materials and cannot be adapted to a large- or small-scale urine monitoring program for narcotic analgesics and other drugs of abuse because these techniques lack simplicity and rapidity.

Fluorometry—Increasing demands by biological scientists for the estimation of microgram and nanogram amounts of drugs in biological materials have led to the rapid development of fluorometric techniques. Among the drugs of abuse, only morphine, codeine, LSD, and quinine (quinine is widely used as a diluent in a heroin fix) have been tried fluorometrically. Fulton (24) described a spot test for morphine in which an intense fluorophor was produced by treating the drug with concentrated sulfuric acid followed by ammonia. In 1958, this observation was applied by Nadeau and Sobolewski (25) for the determination of morphine in raw opium. Balatre *et al.* (26) showed that a similar procedure can be used to produce fluorescent derivatives from codeine and ethylmorphine hydrochloride (codethyline). A spectrophotofluorometric method for the microdetection and estimation of morphine and codeine was also described by Brandt *et al.* (27). Kupferberg *et al.* (28) described a fluorometric method for the estimation of submicrogram quantities of morphine in biological materials excluding urine. The method involved acid–base extraction of morphine and then oxidation to pseudomorphine by the use of potassium ferricyanide in a weakly alkaline solution. Takemori (29) modified the procedure of Kupferberg *et al.* (28), claiming a 10-fold increase in sensitivity. Although the Nadeau–Sobolewski (25) method was cited by Udenfriend (30), the method lay dormant until Dal Cortivo and Matusiak (31) applied the procedure for morphine estimation in various biological materials including urine. The procedure was further modified by Dal Cortivo *et al.* (32) for morphine estimation in a urine screening program using an automated turret spectrofluorometer. Recently, Mulé and Hushin (33) applied this procedure, with certain modifications, for daily monitoring of urine specimens for morphine and quinine. Two milliliters of the sample (urine, plasma, or tissue homogenate) is adjusted to pH 9–10 with 3.7 *N* NH_4OH, and morphine and quinine are extracted with chloroform–isopropanol (3:1). A portion of the chloroform–isopropanol extract is evaporated to complete dryness. The residue, after treating with concentrated H_2SO_4 and NH_4OH, is autoclaved for 15 min. at 120° under 15–18 lb. of pressure. The fluorescence is ascertained in the automated turret spectrofluorometer[3]. The minimum emission wavelength is obtained by setting the monochromator drum dial at 410 nm. and scanning each sample through the 100–510-nm. range.

Quinine is determined by extracting another portion of the chloroform–isopropanol extract with 0.1 *N* H_2SO_4, measuring the fluorescence of the acid extract in the automated turret spectrofluorometer[4], and scanning as described for morphine.

Meperidine also was determined fluorometrically in urine specimens by Dal Cortivo *et al.* (34). They treated the extracted residue with formaldehyde and concentrated sulfuric acid solution and incubated the resulting solution at elevated temperatures. The emission was recorded over the 350–500-nm. range with the exciter monochromator set at 275 nm.

Methaqualone can be measured fluorometrically by reducing both free methaqualone and its hydrochloride with lithium borohydride to tetrahydroquinazolinone (35). This technique has been applied for measuring therapeutic plasma levels of this hypnotic drug.

Lysergic acid diethylamide (LSD) can also be estimated fluorometrically (36) in biological tissues. The homogenized tissue, serum, or urine (up to 5 ml.) is shaken with *n*-heptane (containing 2% isoamyl alcohol) and 1 *N* NaOH, and the mixture is saturated with sodium chloride. An aliquot of the heptane extract is shaken with 0.004 *N* HCl. After 10 min., the fluorescence of the acid phase is determined in a standard cell using a spectrofluorometer (Farrand or Bowman) (λ_{ex} 325, λ_{fl} 445 nm.). Metabolites of LSD do not interfere in the estimation. The method of Axelrod *et al.* (36) was slightly modified by Aghajanian and Bing (37) for the determination of LSD in human plasma. They recommended the use of an Extractomatic shaker (Virtis) for the extraction of LSD into the heptane layer because violent agitation caused elevation of control readings.

Recently, Bullock *et al.* (37A) developed a spectrofluorometric method for the detection and quantitation

[3] Utilizing the Corning No. 4-77 filter with the Farrand 400-nm. interference filter.
[4] Utilizing the Corning No. 7-60 filter.

of the constituents of marijuana in mammalian materials. The method is based on the fluorescence produced with certain Cannabis constituents by condensation with malic acid.

A fluorometric method of analysis for amphetamines based on the interaction between aliphatic and/or cyclic amines and 3-carboxy-7-hydroxycoumarin to yield highly fluorescent coumarin–amine salts was reported by Stewart and Lotti (37B).

Atomic Absorption Spectrophotometry, IR Spectroscopy, and Mass Spectrometry—These methods have not been used for detection of abuse drugs in urine, although they can be used for identification purposes once the drug has been purified after its extraction. Recently, Law *et al.* (37C) reported the identification of abuse drugs in biological material such as gastric lavage, serum, or urine using a mass spectrometric technique alone or in combination with GC. Bellman (38) reported a general method for the mass spectral identification of some hallucinogenic drugs such as LSD, 2,5-dimethyl-4-methylamphetamine (STP, DOM), *N,N*-dimethyltryptamine (DMT), psilocin, and psilocybin in powders, solutions, and tablets. The drugs are converted into their free base form before scanning by carrying them through the acid–base extraction procedure. The organic solvent is removed at room temperature in a current of nitrogen. The residue, before being transferred to the probe, is dried in a vacuum desiccator to remove any condensed moisture. Psilocybin and psilocin cannot be differentiated using this technique without recourse to TLC. Mass spectrometry and other physical and chemical techniques were used by Phillips and Mesley (39) for the identification of 2,5-dimethyl-4-methylamphetamine.

Radioisotope Tracer Techniques—Radioactive methods for the detection of drugs of abuse in human urine are impractical in terms of cost and time for routine analysis. Yeh and Woods (40) determined codeine, morphine, dihydromorphine, and their metabolites in biological materials including urine. Using tracer methodology, Mulé and Woods (41) described a procedure for the estimation of *N*-methyl-^{14}C-labeled morphine in biological materials sensitive to a level of 4–5 ng. Adler *et al.* (42) used the tracer technique to find the biological disposition of small doses of morphine in rats. Using tracer methodology together with photometric procedures and countercurrent techniques, Adler *et al.* (43) provided quantitative data for the excretion of codeine metabolites following administration of codeine to normal human adults. The metabolic fate of cyclazocine in dogs was also studied by Mulé *et al.* (44) using tracer techniques.

Dring *et al.* (45), using ^{14}C-labeled amphetamine and its isomers, studied the metabolic fate of amphetamine in the urine of man and other mammals. In their subsequent detailed publication (46), they determined amphetamine and its metabolites by the isotope-dilution procedure using ^{14}C-labeled amphetamine. Beckett *et al.* (47), using ^{14}C-labeled amphetamine, reported a relation between blood levels and urinary excretion of amphetamine under controlled acidic pH values and under fluctuating urinary pH values. They found that the apparent rate of urinary excretion of amphetamine was proportional to its plasma concentration only under the controlled acidic urinary conditions.

Quinn *et al.* (48) reported a method for the estimation of phenmetrazine in plasma based on the introduction of a radioactive label into the molecule. The method involved isolation of the drug from the plasma by extraction into an organic solvent, followed by a reaction of the compound with tritiated acetic anhydride. The excess acetic anhydride was hydrolyzed and extracted into a basic solution, and the amount of radioactive compound in the organic solvent was assayed in a liquid scintillation spectrometer (Packard Tri-carb).

Kuntzman *et al.* (49), using labeled pentobarbital-^{14}C, reported a sensitive method for the determination and isolation of pentobarbital-^{14}C metabolites and its application to *in vitro* studies of drug metabolism. Strolin-Benedetti *et al.* (50), using liquid scintillation counting, reported quantitative evaluation of the urinary metabolites in a study of labeled barbiturates and hydantoins.

Radioactive tracer techniques were recently used to study the disposition and metabolism of Δ^1-tetrahydrocannabinol (also called Δ^9-tetrahydrocannabinol) and $\Delta^{1,6}$-tetrahydrocannabinol (also called Δ^8-tetrahydrocannabinol) in animals and man (51–55).

GLC—GC is essentially a technique of separating a mixture into its components. This technique has proved to be a valuable tool for the separation of drugs and/or their metabolites in biological material. Scientists and medical technologists working in the field of toxicology, forensic chemistry, and drug abuse detection are advised to refer to the chapter on GC by Leach (56) regarding the choice of stationary phases, support materials, and other pertinent information. Readers interested in practical details about GC are also advised to refer to the "Handbook of Analytical Toxicology" (57) and the references cited therein. The preparation, scope, and limitations of columns also were discussed by McMartin and Street (58).

Parker *et al.* (59) reported a rapid GC method for the screening of toxicological extracts of various drug groups. They used 60–80-mesh Chromosorb W, acid washed, coated with either SE-30 (5%) or Carbowax 20M (1%) as column packings to resolve the mixture of cocaine, morphine, codeine, *etc.* Kazyak and Knoblock (60) presented data obtained from a 1% SE-30 (on Chromosorb W) column at different temperatures and inlet pressures. The column performance with 5% SE-30 and 3% QF-1 (FS-1265) was compared to demonstrate the effect of polarity and a substrate concentration on retention time and operating parameters. The biological specimens were extracted first at pH 4–7.5, then at pH <3.0, and then at pH 9.0 for various groups of drugs.

Jain and Kirk (61–64) reported a rapid, simple, and systematic procedure for extracting and isolating most of the drugs from the blood. In their systematic applications of GLC in toxicology, they used 1% Hi-Eff-8B (cyclohexane–dimethanol succinate) as the column substrate. Blood (500 μl.) without any pH adjustment was extracted with 3 × 1-ml. portions of acetone and ethyl ether (equal volumes of acetone and ether mixed together). The combined extracts were evaporated to dryness, and the residue was dissolved in 95% ethanol for

injection into the gas chromatograph. They were able to detect alkaloids (*e.g.*, morphine, heroin, codeine, cocaine, and quinine), antihistamines, barbiturates (short, ultrashort, and long acting), and tranquilizers in the blood after administering these drugs subcutaneously or orally to female rats.

Street (65, 66) reported a method for the preparation of column packing (Chromosorb G coated with SE-52 or SE-30) whereby the adsorption of polar compounds (alkaloids) was reduced by diatomaceous earth (Chromosorb G). He suggested (65) the use of acetic anhydride for primary and secondary amines, the use of acetone for amphetamine, and the use of benzaldehyde (formation of Schiff's base) for high molecular weight alkaloids by formation of on-column derivatives of the drugs by drawing the solution of these reagents in the syringe followed by the solution of the compound. This is a simple and cheap procedure for the identification of peaks. Anders and Mannering (67) and Mulé (9) proposed a similar peak-shift technique by preparing derivatives on the column by following the injection of the parent compound with an injection of acetic or propionic anhydride.

Street (66) described a procedure for the isolation of basic and amphoteric alkaloids such as morphine from a 5-ml. aliquot of urine. The urine was carried through the acid–base extraction procedure. The basic alkaloids were extracted with ether at pH 10 using NaOH; then the amphoteric alkaloids were extracted from the aqueous acidic phase by rendering it alkaline with a saturated solution of $NaHCO_3$ and then extracting with ether. A procedure for isolation of alkaloids from blood and liver samples was also described. Wilkinson and Way (68) described a specific GLC method for the determination of nanogram amounts of morphine in plasma and cerebrospinal fluid. The procedure required the conversion of morphine, after extraction and concentration, to its trimethylsilyl ether derivative. Bis(trimethylsilyl)trifluoroacetamide plus 1% trimethylchlorosilane was used as the solvent and silylating agent in the derivatization. The column packing used was 3% OV-1 on 100–120-mesh Gas Chrom Q.

Fish and Wilson (69) proposed a GC procedure for routine monitoring of morphine and cocaine in urine using bis(trimethylsilyl)acetamide for derivatization. The column packing used was Chromosorb W-AW-DMCS coated with OV-17. A 5-ml. aliquot of urine was carried through the acid–base extraction. Morphine was extracted with ether at pH 9 using NH_4OH, and cocaine was extracted with ether at pH 8 using $NaHCO_3$. Ikekawa *et al.* (70) determined morphine and codeine in urine by GC after trimethylsilylation with bis(trimethylsilyl)acetamide. They recommended the use of two or three kinds of column packings for precise identification of morphine; the column packings used were 1.5% OV-1, 1.5% SE-30, 1.5% OV-17, 1.5% QF-1, and 1.5% XE-60[5]. Martin and Swinehart (71) used hexamethyldisilazane, trimethylchlorosilane, and bis(trimethylsilyl)acetamide as silylation reagents. Bis(trimethylsilyl)acetamide proved to be an excellent reagent for silylation of morphine alkaloids. Hexamethyldisilazane was used by Brochmann-Hanssen and Svendsen (72, 73) as a silylation reagent for phenolic alkaloids and for quantitative determination of morphine in opium by GLC. Gas Chrom P, after treatment with concentrated hydrochloric acid, methanolic potassium hydroxide, and hexamethyldisilazane, was coated with 0.1% polyethylene glycol and 1% SE-30 and used as column packing.

Fenimore and Davis (74) suggested the use of GLC for confirmation and identification of results obtained by ion-exchange or TLC procedures. Drugs were extracted from the urine by means of cation-exchange paper, as described by Dole *et al.* (75), and conjugates were hydrolyzed directly on the paper using β-glucuronidase. The free alkaloids were eluted from the paper at pH 9.3 into chloroform–isopropanol (3:1); after evaporation of the solvent, the residue was silanized using *N,O*-bis(trimethylsilyl)trifluoroacetamide. A portion of the silanizing mixture was injected into the GC column, and the instrument was programmed from 180 to 230° at a 3° rise/min.

Numerous methods for the detection and identification of barbiturates in biological fluids by GLC have appeared since 1960. Janak (76) identified barbiturates by GC separation of their pyrolytic products by heating the sample to 800°; a similar procedure was described later by Nelson and Kirk (77). Separation of barbiturates by GC by preparing dimethyl derivatives of barbiturates after reaction with diazomethane was described by Cook *et al.* (78) and Cook (79). Parker and Kirk (80) and Parker *et al.* (59, 81) attempted to use GC for the separation of barbiturates. They tried 5% SE-30, 1% Carbowax 20M, or a mixed liquid phase of 1.5% SE-30 and 2% Carbowax 20M as stationary phases in an attempt to achieve better resolution of barbiturates without tailing. Cieplinski (82) attempted to prevent tailing of barbiturates by the incorporation of high molecular weight organic acids into the liquid phase [*e.g.*, dimer acid (0.75%) with SE-30 (1.5%)]. Martin and Driscoll (83) minimized tailing by reducing the polarity of the barbiturate nucleus by treatment with dimethyl sulfate. VandenHeuvel *et al.* (84) tried QF-1 for the detection of barbiturates but recommended the use of more than one column for satisfactory resolution of mixtures. Brochmann-Hanssen and Svendsen (85) used four different stationary phases to separate barbituric acid derivatives. They reported that all compounds could be distinguished by using two columns, one with a nonpolar liquid phase and another with a moderately polar polyester phase.

McMartin and Street (86) used columns packed with Chromosorb W which had been treated and coated with a mixture of SE-30 (2%) and tristearin (0.1%) or QF-1 tristearin. While applying their technique for the identification of barbiturates in blood in poisoning cases, they cautioned about the interfering compounds like bemegride, glutethimide, aspirin, salicylic acid, and caffeine which could be present in the extract. On the SE-30–tristearin column, caffeine showed the same retention time as secobarbital, but resolution of the two

[5] Using this technique, Ikekawa *et al.* (70) were able to detect morphine for up to 72 hr. in 100 ml. of urine following subcutaneous injection of 10 mg. of morphine hydrochloride in human volunteers. Morphine could be detected in more than 500 ml. of urine even 5 days after injection.

drugs was achieved by using QF-1–tristearin. Street (87) also described a direct derivative formation technique within the gas chromatograph for the identification of barbiturates, hydantoins, amides, *etc.* Urine, blood, and liver samples were extracted as described earlier by Street (65, 66) and McMartin and Street (88). One to five microliters of reagent *N,O*-bis(trimethylsilyl)acetamide was drawn into a 10-μl. syringe, followed by 1 or 2 μl. of the pure solution of the residue of the biological extract, and injected directly into the gas chromatograph. This treatment increased the sensitivity of the method and also resulted in the formation of symmetrical peaks. Recently, Street (88A) recommended the use of unmodified barbiturates for the quantitative GLC recovery while still retaining "on-column" formation of methyl and trimethylsilyl derivatives for qualitative identification.

Kazyak and Knoblock (60) extracted barbiturates from a urine specimen with chloroform at pH 4.0–7.5. The residue obtained after the evaporation of the solvent was dissolved in chloroform or an ethanol–chloroform mixture and injected into the chromatographic column containing 1 or 5% SE-30. Jain *et al.* (89) and Jain and Kirk (63) reported a GLC procedure for the rapid detection of barbiturates in blood samples in barbiturate poisoning and also in blood samples from patients who were given therapeutic levels of the commonly used barbiturates. Blood (400–500 μl.) was extracted, without any pH adjustment, with 3 × 1-ml. portions of acetone–ether (equal volumes). The residue, after evaporation of solvent, was dissolved in 95% ethanol and injected into the chromatographic column containing either acid-washed firebrick coated first with 1.5% SE-30 and then with 2% Carbowax 20M or 1% Hi-Eff-8B on Gas Chrom P.

Machata and Battista (90) described the GC of barbiturates using Apiezon M and Carbowax 20M as the stationary phases. Parker *et al.* (91) reported determination of barbiturates in blood and urine using GLC with a peak-shift technique for qualitative confirmation. They extracted blood or urine specimens in chloroform or ethylene dichloride at pH 5.5. Tetramethylammonium hydroxide in methanol was used for derivatization; QF-1 and SE-30 mixed liquid phase columns were used for the separation of derivatives. Leach and Toseland (92) described the GLC of barbiturates in blood using a 10% Apiezon L column. They were able to differentiate cyclobarbital (cyclobarbitone) from phenobarbital (phenobarbitone) using Apiezon columns or 3% XE-60 columns. Reith *et al.* (93) also used Apiezon columns but could not achieve the separation of cyclobarbitone from phenobarbitone; therefore, they used a permanganate oxidation method to differentiate these two compounds. Anders (94) also described a rapid micromethod for the GC determination of blood barbiturates by injecting chloroform extracts of small quantities of blood. Toxicological specimens were also extracted with chloroform after rendering the pH to 5.7 with sodium dihydrogen phosphate. Silicone fluid (3.5%) DC-200 was used as the stationary phase on Gas Chrom Q.

Balasubramanian *et al.* (95) reported a method for the estimation of amobarbital (amylobarbitone) in serum and hydroxyamylobarbitone in serum and urine by GLC using NPGA (3%) with trimer acid (0.75%) as the stationary phase on Chromosorb W. The samples were acidified, saturated with ammonium sulfate, and extracted with ether. The concentrated ether extracts were chromatographed on TLC plates, the barbiturate spots were extracted into acetone, and the acetone extract was concentrated and evaporated on stainless steel gauzes for GC.

Diphenylhydantoin can be determined in blood specimens by the procedures described by Sandberg *et al.* (96) and Sabih and Sabih (97). The procedure described by Sandberg *et al.* involved the conversion of diphenylhydantoin to its methyl ether by treatment with freshly prepared diazomethane, while the procedure described by Sabih and Sabih required simple extraction with chloroform after acidification with 0.5 *N* HCl. Gas Chrom Q coated with 2% XE-60, 3% SE-30, or 5% DC-200 was used as the packing material. The determination of glutethimide[6] in biological fluids by GLC also was reported (98, 99). Recently, Baselt and Casarett (100) reported GLC methods for the routine identification of barbiturates and amphetamines; these methods are adaptations of techniques reported by Lebish *et al.* (101) and Stewart *et al.* (102). Barbiturates are extracted with chloroform at pH 5, chloroform is evaporated to dryness, and the residue is treated with dimethyl sulfate for derivatization. Dimethyl derivatives of barbiturates are extracted with heptane, and the heptane extract is concentrated and injected into a chromatographic column packed with Gas Chrom Q coated with 1% SE-30.

GLC methods for the identification and quantitative determination of amphetamine and its congeners and some of the related CNS stimulants in biological fluids have been reported in the literature. Kazyak and Knoblock (60) suggested GLC of compounds of general toxicological interest using 1% SE-30 at different temperatures. Parker *et al.* (59) proposed a rapid GC method for the screening of toxicological extracts, including sympathomimetic amines, using SE-30 (5%) or Carbowax 20M as the stationary phases. Anders and Mannering (67, 103) proposed GLC for some of the sympathomimetic amines, alcohols, and phenols by forming their acetates and propionates directly on the column following the injection of the parent compound with an injection of either acetic or propionic anhydride. Brochmann-Hanssen and Svendsen (104) found that a number of amines reacted with ketone solvents such as acetone to produce derivatives which gave sharp, symmetrical peaks on the chromatograms.

The reaction between ketones and primary amines was studied by Bergel and Lewis (105) and Bergel *et al.* (106). According to these authors, the reaction involves an addition followed by loss of water to form an azomethine. With secondary amines, no such loss of water can occur; tertiary amines like benzheptamine gave no reaction with ketone. Beckett and Rowland (107, 108) identified amphetamine by the alteration of its retention time by conversion to its acetone derivative; this technique was a modification of the procedure originally

[6] Doriden (Ciba).

described by Brochmann-Hanssen and Svendsen (104). The determination of amphetamine in urine was based on a modification of the method of Cartoni and deStefano (109). The column used was 100–120-mesh acid-washed Celite 545, onto which was coated 5% KOH and 10% Carbowax 6000. Beckett (110) stressed the importance of this technique in the identification of aralkyl amines in man. By this technique it was possible to differentiate methylephedrine from ephedrine, because the former did not form any acetone derivative; methylamphetamine could be separated from amphetamine and from β-phenethylamine (an amine often present in nonfresh biological fluids). Beckett *et al.* (111) described a general procedure for the analysis of basic drugs (and their metabolites) in human urine. Some of the basic drugs studied could be misused as stimulants in sports to increase performance. They recommended that international control of drug taking in sports be based primarily upon urine analysis by GLC systems. Different column packings were suggested for CNS stimulants having small molecular weights (small molecules based on the amphetamine structure) and for compounds having high molecular weights like codeine, methadone, and cocaine. The use of derivative formation with acetone, carbon disulfide, and benzyl methyl ketone for primary amines, with oxazolidines and carbinolamines for secondary amines, and with acetyl, propionyl, and heptafluorobutyryl derivatives for primary and secondary amines was recommended for conclusive identification of "positives."

Steele *et al.* (111A) recently described an improved modification of the method of Beckett *et al.* (111) for the rapid screening of common stimulants, antihistamines, and local anesthetics in urine samples from athletes. Campbell (112) reported an improved procedure for the measurement of therapeutic levels of amphetamine in human plasma based on the GC technique of Cartoni and deStefano (113) and of Beckett and Rowland (108). Formation of derivatives of amines and alkaloids within the gas chromatograph was also reported by Street (65, 87) using acid anhydrides, ketones, and aldehydes. Gordis (114) reported GC resolution of optical isomers of amphetamine using trifluoroacetyl-l-prolyl chloride for derivative formation. The column packing used was 3% SE-30 siloxane polymer on Gas Chrom P. Gunne (115), using the method of Gordis (114), measured the urinary output of two optical isomers of amphetamine after administration of racemic amphetamine or methylamphetamine to human subjects. Using the same technique, Gunne and Gallard (116) were able to report the stereoselective metabolism of amphetamine in the urines of albino rats after intraperitoneal administration of *d,l*-amphetamine and *d*- or *l*-amphetamine. Noonan *et al.* (117) reported a method for detecting amphetamine in biological fluids of horses using GC of a halogenated derivative. Amphetamines were extracted from alkaline biological fluid into a mixture of hexane and isooctane. The trichloroacetamide of amphetamine was made with trichloroacetyl chloride in the organic phase. The derivative was chromatographed on methylsilicone (JXR) and detected by electron capture. This technique utilized the extreme sensitivity of the electron-capture detector to a halogenated derivative.

Cartoni and Cavalli (118) reported an analytical procedure for the detection of amphetamine and related CNS stimulants in urine. The method consisted of a preliminary screening by TLC, followed by scraping of the suspicious spots from the plates for confirmation by GLC on different columns (Carbowax 20M and Apiezon L were used for column packings). GC of some hallucinogenic drugs sold for illicit use was reported by Genest and Hughes (119).

GLC has not been applied to the detection of LSD and/or its metabolites in biological fluids, but Radecka and Nigam (120) described a procedure to identify hydrogenated LSD by GC. Katz *et al.* (121) reported a simple GC procedure for the identification of LSD from a sugar cube, filter paper, or bicarbonate capsule. They obtained a symmetrical LSD peak using a 0.3% SE-30 column, with helium as the carrier gas.

Although several GLC procedures have been reported in the literature for the analysis of Cannabis constituents, no GC method has been reported for the detection of natural cannabinoids and/or their metabolites in biological fluids. GLC, using a 3% SE-30 column, was first applied by the Canadian Food and Drug Directorate in the early 1960's (122, 123). The columns in use today are SE-30 (124–126), XE-60 (127), Carbowax 20M (128), and OV-17 (129, 130). By using these columns, good separation has been achieved between cannabinol, Δ^1-tetrahydrocannabinol, cannabidiol, cannabichromene, and cannabigerol. However, Δ^1-tetrahydrocannabinol and $\Delta^{1,6}$-tetrahydrocannabinol, having close retention times, appear as a doublet; hence, quantitative differentiation between these two is frequently difficult. Claussen *et al.* (124) recommended the use of trimethylsilyl ether derivatives for better separation of Δ^1-tetrahydrocannabinol from $\Delta^{1,6}$-tetrahydrocannabinol.

Radioimmunoassay and Free Radical Assay Techniques—A radioimmunoassay procedure for detecting extremely low concentrations of morphine (0.5 ng.) was recently reported by Spector and Parker (131). Morphine, after conversion to a 3-*O*-carboxymethyl derivative, was coupled to bovine serum albumin and the resulting complex was utilized for immunization of rabbits. This radioimmunoassay technique has not yet been applied for detecting opiates in urine.

Recently, Adler and Liu (132) confirmed the findings of Spector and Parker (131) and reported a hemagglutination-inhibition test for the detection of morphine. They claimed the sensitivity of this test to be equal to or greater than radioimmunoassay. According to their report, this technique appears to be adaptable to the screening of a large number of urine specimens with a minimum of effort and equipment. Another potentially useful immunoassay technique (133) involves the use of spin labeling or tagging of morphine with extra electrons rather than the use of radioactive morphine. Electron spin resonance (ESR) spectrometers rather than liquid scintillation spectrometers are used for the analysis of morphine or other spin-labeled compounds. This technique is called the "free radical assay technique," FRAT, and has been applied for mass screening

of urines for opiates including heroin and its natural and synthetic equivalents conformationally related to morphine. 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 ESR spectral signal. When a urine specimen containing morphine is mixed with the complex, some of the spin-labeled morphine is displaced and the ESR peaks are sharpened. This technique is reported to be many times more sensitive than TLC, sensitive to both free morphine and its glucuronide conjugate and requiring only about 30 sec. to complete a test. Free radical antibody preparations for detecting cocaine, methadone, amphetamines, barbiturates, meperidine, and glutethimide are under development and will be offered as part of the FRAT system as soon as they become commercially available. The FRAT system will also prove a valuable tool for hospital emergency rooms where instant test results are desirable. This technique is currently being used to detect heroin users among servicemen homebound from Vietnam.

Chromatographic Techniques—Chromatography is a suitable technique for analyzing very small amounts of drugs and/or their metabolites in biological materials. It can also be scaled up for isolation purposes. By using chromatographic techniques, closely related substances can be easily separated without resorting to traditional methods of separation, which are often laborious. The literature is replete on applications of these techniques to toxicological problems[7]. Among the techniques commonly employed are paper chromatography, column chromatography, and TLC.

Paper Chromatography—Although the development of TLC (in 1960 and later years) has provided the advantages of speed and sensitivity, paper chromatography has the merits of simplicity and its capacity to handle greater quantities of materials. Asatoor *et al.* (134) utilized paper chromatography for the identification of amphetamine and *p*-hydroxyamphetamine in rat and human urine. These were examined by two-dimensional chromatography on Whatman No. 1 paper, using mixtures of isopropyl alcohol–aqueous ammonia–water (8:1:1) and butanol–acetic acid–water (12:3:5) as the solvents. Diazotized *p*-nitroaniline was used as the spraying reagent.

Clarke (135) reported the combined use of paper chromatography and TLC as adjuncts to each other for the identification of some proscribed psychedelic drugs. He found that tryptamine derivatives and dimethyltryptamines are better differentiated using paper chromatography, while lysergide (LSD), methylsergide, and methylergonovine (methylergometrine) are well separated by TLC. Similarly, trimethoxyamphetamine is well separated from methoxamine using TLC. The paper chromatographic technique used was a modification of the method reported by Curry and Powell (136).

Mannering *et al.* (137) used paper chromatography for the identification of morphine in human urine. Urines were acidified and autoclaved for 30 min. at 15 lb. pressure. The hydrolyzed urines were extracted at pH 11 and 8–9. Morphine was extracted at pH 8–9 with equal volumes of ethanol (10%) and chloroform. The solvent was removed and the solution of the residue in ethanol was chromatographed using Whatman No. 1 filter paper. Isoamyl alcohol–acetic acid (10:1), butanol–acetic acid (10:1), and isoamyl alcohol–ammonium hydroxide (10:1) were used as solvents. After the development of the chromatogram, the paper was sprayed lightly with platinate reagent. Colored areas were cut from the paper and extracted with chloroform after wetting with ammonium hydroxide. Chloroform was removed, and the solution of the residue in ethanol was treated with Frohde's, Marquis', and Mecke's reagents. The colored reactions were compared with standard morphine. Out of 1103 urines, 412 gave positive morphine tests.

Using paper chromatography, Waddell (138) reported a rapid method for the identification of barbiturates in biological fluids. Barbiturates were extracted into ethyl ether from 2 ml. of serum, oxalated plasma, urine, or gastric contents at pH 6. A measured volume of ether extract was impinged to dryness, and the solution of the residue in methanol was applied to Whatman No. 2 paper which had been previously dipped in alkali and dried. After development of the chromatogram, the paper was removed and dried in air and the barbiturates were located on the paper by viewing in a dark room with a mercury vapor lamp. Quantitation, if desired, was done by extracting the spot with carbonate buffer of pH 11 and then measuring UV absorbance at several pH's.

Nicholson (139) used paper chromatography to study the urinary excretion rates of phenobarbital (phenobarbitone), pentobarbital (pentobarbitone), and their metabolites in horse urine. Ion-exchange paper chromatography and ionophoresis as applied to toxicological analysis were dealt with in detail by Street (140). Paper chromatography was used for the identification of powdered LSD samples in a collaborative study by Look (141). Agurell *et al.* (51) recently reported the use of paper chromatography for the presence of metabolites of Δ^1-tetrahydrocannabinol (a major psychotomimetically active constituent of Cannabis) in the urine of rabbits.

Column Chromatography—In column chromatography, the fixed phase is packed in a glass column having a sintered disk. In the absence of a sintered disk, the base of the column is packed with a mass of glass wool. The fixed phase may be an adsorbent such as alumina, Celite, silica gel, charcoal, Florisil, starch, talc, cellulose, sucrose, Sephadex, and Amberlite XAD-2 (a nonionic polymeric adsorbent) or an ion-exchange resin. In drug abuse detection, column chromatography has been used as a cleanup procedure to purity the unknown mixture prior to its examination by spectrophotometric or other analytical techniques. With urine and other biological fluids, it is used as a prechromatography extraction procedure for the isolation of drugs and their metabolites. Nakamura and Meuron (142) used a Celite column as a cleanup procedure preceding quantitative UV determination of heroin in illicit preparations. Tompsett (143) reported the use of a cation-exchange resin to separate alkaloids and related substances from urine prior to their identification and determination by paper chro-

[7] Readers interested in updated literature are advised to consult *Reference 57*, p. 984.

matography. Dowex 50X12 (200–400 mesh) was used as the cation-exchange resin. A system of gradient elution using different concentrations of hydrochloric acid was used for the separation of various alkaloids.

Fenimore and Davis (74) used a simple resin column for extraction of drugs in human urine. This was accomplished by using a disposable Pasteur pipet containing an approximately 1-cm. column of Dowex 50W-X2 cation-exchange resin supported by a small plug of glass wool. After converting the resin to the sodium form and then washing with distilled water, 20 ml. of urine was forced slowly through the tube with a hypodermic syringe. The resin, after washing with distilled water and acetone, was dried and tested immediately for the presence of narcotic alkaloids. A few drops of Marquis' reagent, prepared by adding 0.5 ml. of 40% formaldehyde solution to 10 ml. of concentrated sulfuric acid, were added to the resin. A dark coloration, ranging from reddish brown to deep violet, indicated the presence of narcotic alkaloids. The primary objective of this test was to eliminate negative samples from further confirmatory testing. Fenimore and Davis (74) reported that in a preliminary study of 108 urine samples, positive tests were obtained on 20 samples by means of the Dole *et al.* (75) TLC procedure and on 37 samples by use of the resin test. This apparent increase in sensitivity was attributed to the extraction of both free and conjugated alkaloids by the ion-exchange resin. Since a test of this type does not identify a specific alkaloid, GC was recommended for confirmation and identification of alkaloids.

TLC—At present, TLC is the most suitable technique for the rapid detection of drugs of abuse in human urine. This technique meets all the criteria (*e.g.*, minimum instrumentation, low cost, simplicity, minimum laboratory space, rapidity of analysis, excellent resolution of components, high sensitivity to a wide variety of abuse drugs, specificity, and ease of interpretation of results by laboratory personnel with minimal formal training) for selection as a routine method for a large- or small-scale urine screening program. In addition, this technique permits the simultaneous detection of a wide range of substances in a single run. The monitoring of a urine specimen by TLC alerts the operator immediately of the number of drugs present in a specimen. Furthermore, the sensitivity of the technique can be easily adapted to the purpose of screening, *e.g.*, screening of urines from patients in treatment for specific drugs of abuse, preemployment screening of urines, or identification of illicit preparations seized by narcotic agencies. Large-scale drug abuse treatment programs require such a versatile and low cost screening procedure. This technique is also capable of differentiating illicit drugs and their adulterants from legitimate and prescribed drugs and their metabolites.

Many TLC methods for detecting commonly abused drugs in urine are available (144–175); however, these methods vary greatly with respect to their suitability for use in large-scale urine monitoring programs. The extraction of drugs from a urine specimen or biological material is a necessary prerequisite to TLC. It is due to this prechromatography extraction step as well as to the variety of detection procedures used that TLC techniques vary considerably from program to program. In fact, 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 prechromatographic extraction step and the specificity and sensitivity of the visualization techniques used. Several different extraction techniques involving three basic approaches have been reported in the literature: (1) direct extraction of drugs from a urine specimen at various pH's, (2) acid or enzymatic hydrolysis of urine specimens followed by direct extraction of drugs, and (3) extraction of drugs from urine by absorbing them on a resin column (ionic or nonionic) and then eluting with organic solvents or absorbing the drugs and/or their metabolites on a cation-exchange resin-loaded paper and then eluting with different buffer–solvent systems. These three basic approaches are discussed here.

1. Direct Extraction of Drugs from Urine and Other Biological Fluids: A comprehensive extraction procedure for commonly abused drugs involving acid–base extraction and fractionation of various groups of drugs in different organic solvents was reported by Sobolewski and Nadeau (147). Sunshine (148, 149) suggested a multiple-extraction procedure for the extraction of acidic, neutral, and basic drugs from biological fluids at two different pH's, using chloroform as a solvent. Beckett and Rowland (107) and Beckett *et al.* (111) described procedures for the direct extraction of urine (1–5 ml.) for amphetamine and other stimulants. Marks and Fry (150) proposed a similar method for the extraction of amphetamines but used 20 ml. of urine. Cartoni and Cavalli (118) extracted amphetamine and related drugs from 5–10 ml. of urine after making it alkaline with NaOH. Cochin and Daly (151, 152) reported methods for the direct extraction of analgesic drugs, phenothiazines, and antihistamines using a larger volume of urine. A sample of urine containing at least 100 mcg. of drug was adjusted to pH 9 with sodium hydroxide and extracted with ethylene chloride containing 10% isoamyl alcohol (volume of extracting solvent used was four times greater than the volume of urine). Cochin (153) also suggested the direct spotting of urines on a TLC plate and thus bypassed the extraction procedure. McIsaac (154) used a 25-ml. aliquot of urine for the extraction of barbiturates and a 50-ml. aliquot for amphetamines. Barbiturates were extracted at pH 5 and amphetamines at pH 9. Mulé (155) reported the direct extraction of narcotic analgesics in human biological materials using 6-ml. samples. The specimen was adjusted to pH 10.0 with sodium hydroxide, buffered with pH 10.4 potassium phosphate buffer, and extracted with 15 ml. of ethylene dichloride. Later, Mulé (156) proposed differential pH extraction of barbiturates, opiates, and amphetamines at pH 2.2, 9.3, and 11.0 using three separate 15-ml. aliquots of urine. Recently, he (157) proposed extraction of barbiturates at pH 1.0 (using a 15-ml. aliquot of urine and extracting with 15 ml. of chloroform) and extraction of opiates, phenothiazines, phenethylamines, and related analogs at pH 10–11. A 25-ml. aliquot of urine was adjusted to pH 10–11 with sodium hydroxide; potassium phosphate buffer, pH 10.3, was added and extracted with 2 × 12-ml. portions of 25% ethanol in chloroform. To the combined organic ex-

tract, 6 *N* HCl in ethanol (100 μl.) was added and filtered. The filtrate was divided into two equal fractions and each fraction was evaporated to dryness in a water bath at 75°. One fraction was tested for phenethylamines and related analogs; the second was tested for opiates, phenothiazines, *etc.*

Heaton and Blumberg (158) proposed direct extraction of barbiturates from a 5-ml. urine specimen. The urine specimen was buffered to pH 6 with a potassium phosphate buffer and extracted with diethyl ether. Using this technique, they detected secobarbital for 4–6 days after a single dose of 3 grains (about 195 mg.). Street and Perry (159) extracted stimulants and other alkaloids with anhydrous peroxide-free ethyl ether at pH 9 using 10 ml. of gastric contents or a urine specimen. Barbiturates were extracted from 3 ml. of plasma or gastric contents by deproteinizing with sodium tungstate and acidifying with sulfuric acid. After centrifugation, the protein-free supernate was extracted with chloroform. Itiaba *et al.* (160) described a method for the direct extraction of barbiturates from a 2-ml. aliquot of serum. The sample was brought to pH 4.0 and extracted with 3 × 20-ml. portions of chloroform. The pooled extract was decolorized with activated charcoal, filtered, and evaporated to dryness on a steam bath. Bastos *et al.* (161) proposed that the basic and neutral organic drugs could be extracted into ethanol from urine saturated with potassium carbonate. Drugs in the ethanol concentrate were purified by extraction into ether at pH 8.5. The ether extract was evaporated and applied to several thin-layer chromatograms. Davidow (162) and Davidow *et al.* (163) extracted morphine and other basic drugs with 50 ml. of extracting solvent (chloroform–ethyl acetate–methyl or ethyl alcohol in the ratio of 3:1:1) after adding 1 ml. of concentrated ammonium hydroxide to 10 ml. of a urine specimen. Davidow *et al.* (164) later described a single-step direct extraction procedure for narcotics, amphetamines, and barbiturates at pH 9.5 using a NH_4Cl–NH_4OH buffer. A 10-ml. aliquot of urine was extracted with 50 ml. of chloroform–isopropanol (96:4) at pH 9.5; the reported sensitivity for amphetamines and barbiturates was only 5 mcg./ml. of urine. Kaistha and Jaffe (165–167) proposed a two-step extraction procedure using a 15-ml. aliquot of urine. Barbiturates were extracted at pH 1 and amphetamines and opiates at pH 10.1 using a NH_4Cl–NH_4OH buffer. The reported sensitivity for barbiturates was 0.5–1 mcg./ml. of urine (except sodium barbital); for amphetamines it was 1–2 mcg./ml. of urine.

2. Acid or Enzymatic Hydrolysis of Urine: Since as much as 83% of the total morphine (168) and 88% of the total codeine (43) may be excreted as their glucuronides, acid hydrolysis of urine specimens collected infrequently is of great value. Beckett *et al.* (111) suggested acid hydrolysis of urine specimens for glucuronides of ring-hydroxy amphetamines after the preliminary extraction of free drug. Urine (10 ml.) was rendered alkaline (pH 9–10) by the addition of solid sodium carbonate and then extracted with freshly distilled diethyl ether (Analar). The extracted urine was then neutralized and heated at 80–100° with 2 ml. of 6 *N* HCl for 1 hr. to hydrolyze the conjugated drug. After cooling, the pH was adjusted to 9–10 by the addition of 2 ml. of 6 *N* NaOH and solid Na_2CO_3. The urine was then extracted as before. Cochin and Daly (151) suggested acid hydrolysis of urine specimens to split the free base from the glucuronide, the form in which the majority of the morphine derivatives and synthetic analgesics are excreted in man. A portion of the urine sample was heated for 1 hr. at 100° with one-tenth volume of concentrated hydrochloric acid before being made alkaline and extracted. They found that most of the alkaloids, including morphine, levorphanol, codeine, and propoxyphene, were stable under their conditions of acid hydrolysis. Heroin was, however, converted to morphine, and phenazocine yielded a small amount of a breakdown product. Cochin (153) also suggested enzymatic hydrolysis of urine specimens using β-glucuronidase. Interesting findings were the appearance of the morphine in increasing amounts with increasing time of hydrolysis of the codeine urine and the disappearance of the codeine spot. According to Cochin, β-glucuronidase is capable of rupturing the *O*-methyl bond of codeine. Also of interest was the disappearance of several of the quinine spots with enzymatic hydrolysis.

Dole *et al.* (75, 170) suggested that the drugs be first adsorbed on Reeve Angel SA-2 cation-exchange resin-loaded paper and that this paper be covered with 10 ml. of glucuronide reagent (β-glucuronidase, 4000 fishman units in 10 ml. of 0.2 *M* sodium acetate, pH 5.2) and incubated at 37° overnight. After enzymatic hydrolysis, the paper is extracted by a standard procedure. McIsaac (154) suggested acid hydrolysis of the urine specimen for the identification of alkaloids. A 25–50-ml. aliquot of urine, after acidification with 10% concentrated HCl, was autoclaved at 15 lb. pressure for 15 min. and cooled, and the pH was brought to 8.5 with solid sodium carbonate. The urine was then extracted with four volumes of 1,2-dichloroethane and isoamyl alcohol (9:1). Mulé (155) suggested autoclaving of an aliquot of urine with one-tenth of its volume of concentrated hydrochloric acid for 25 min. at 15 lb. pressure for determination of total morphine (free plus conjugated). In his later publication (156), he recommended that either acid or enzymatic hydrolysis should precede extraction of drugs at pH 9.3. Recently, Mulé (157) reported that a 15-ml. aliquot of urine may be autoclaved at 120° for 30 min. in 2.3 *N* HCl at 18–20 lb. pressure. After acid hydrolysis, the sample was 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. on an Eberbach shaker. The upper organic phase was aspirated off, and the pH of the aqueous phase was adjusted to about 9 with 9.5 *N* NaOH. The aqueous phase was then buffered to pH 9.3 with potassium phosphate buffer and extracted with 15 ml. of chloroform–isopropanol (3:1).

Ikekawa *et al.* (70) determined total morphine in urine by passing 50 ml. of a specimen through a column packed with charcoal. Morphine and its glucuronide were eluted with 20 ml. of glacial acetic acid. The acetic acid was evaporated in a vacuum; the residue was dissolved in a mixture of 4 ml. water and 4 ml. concentrated hydrochloric acid, and the solution was heated at 100° for 15 min. The hydrolysate was adjusted to pH

2.5 with 5 *N* NaOH and extracted with four volumes of chloroform–isopropanol (9:1). The aqueous phase was adjusted to pH 9.0 and extracted twice with four volumes of chloroform–isopropanol (9:1). The combined organic extract was dried over anhydrous sodium sulfate and evaporated to dryness. Kokoski *et al.* (173) and Kokoski (174) reported that only hydrolyzed urines are routinely screened for narcotics and amphetamine detection in their laboratory. A 10-ml. aliquot of urine, after addition of 1 ml. of 12 *N* hydrochloric acid, is autoclaved at 15–18 lb. pressure for 15 min. or placed in a water bath at 80–100° for 1 hr. After cooling in ice water, 1.5 ml. of 15 *N* ammonium hydroxide is added to the acid-hydrolyzed urine or the pH may be adjusted to 9, using 10 *N* NaOH and 6 *N* H_2SO_4 as required. Forty milliliters of extraction solvent (chloroform–isopropanol, 9:1) is added and the capped tube is shaken by hand for a few seconds. After the separation of two phases, 20 ml. of the organic phase is poured into a 50-ml. beaker, 6–10 drops of hydrochloric acid in methanol are added to form the hydrochlorides, and the mixture is stirred. The extract is evaporated to dryness in a vacuum oven at not over 100°. Barbiturates and acidic compounds are extracted without acidic hydrolysis.

Kaistha and Jaffe (165, 166) used a 10-ml. aliquot of urine for acid hydrolysis. The urine, in a 50-ml. round-bottom centrifuge tube, after addition of 1 ml. of concentrated hydrochloric acid, was hydrolyzed in a boiling water bath. After 1 hr., the tube was cooled and the contents were extracted with 15 ml. each of NH_4Cl–NH_4OH buffer, pH 10.1, and chloroform–isopropanol (9:1). The lower organic phase was pipeted out into a 15-ml. plain conical centrifuge tube containing 2 drops (50 μl.) of sulfuric acid in methanol (0.5% H_2SO_4 in methanol). Sulfuric acid was omitted if amphetamines were not to be detected. The data suggested (165, 166) that it would be advisable to extract amphetamines before acid hydrolysis. Acid hydrolysis is not used routinely in our laboratories.

Burnett *et al.* (171) used acid as well as enzymatic hydrolysis of urine specimens for the detection of methaqualone and its metabolites. Urine, 10 ml., after addition of 1.5 ml. of concentrated hydrochloric acid, was heated in a boiling water bath for 2 hr. The mixture was then adjusted to pH 10–11 with ammonium hydroxide and extracted with ether. For enzymatic hydrolysis, the urine specimen (10 ml.), after addition of 1 ml. acetate buffer (pH 5.0) and 1 ml. β-glucuronidase[8], was incubated overnight at 37°. The mixture was adjusted to pH 10–11 with ammonium hydroxide and extracted with 25 ml. of ether. The ether phase was separated and evaporated to dryness in a vacuum.

Christiansen and Rafaelsen (172) used β-glucuronidase–aryl sulfatase for the enzymatic hydrolysis of the urine of human volunteers for the detection of Cannabis metabolites after oral administration of Cannabis. Agurell *et al.* (51) used enzymatic hydrolysis for the detection of the metabolites of Δ^1-tetrahydrocannabinol in rabbit urine.

3. Extraction of Drugs from Urines Using Non-ionic Polymeric Resin Columns and/or Ion-Exchange Resins or Resin-Loaded Papers: Marks and Fry (150) used Zeocarb (SRC 13) sodium form, ion-exchange resin for the extraction of morphine from urine specimens. After extraction of amphetamines, a 20-ml. aliquot of urine was diluted with 20-ml. of water and adjusted to pH 5–6 with 6 *N* HCl. To the diluted urine were added 2–3 g. of Zeocarb 225 (SRC 13); then the tube was stoppered and mixed[9] for 15 min. The urine was decanted and discarded. The resin, after washing with distilled water, was extracted with 20 ml. each of borate buffer, pH 9.3, and chloroform–isopropanol (3:1). After shaking for 10 min., the upper aqueous layer was aspirated off and discarded and the lower organic phase was filtered through Whatman No. 1 phase-separating paper into a boiling tube and evaporated down in a boiling water bath. Fujimoto and Wang (175) used an Amberlite XAD-2 resin column for the separation of narcotic analgesics in human urine. The Eastman Kodak Co., using a modification of the method of Fujimoto and Wang, has put on the market a "Kodak Chromat/O/Screen Extraction Kit." This kit consists of a disposable plastic column, prepacked with Amberlite XAD-2 polymeric adsorbent. A 20-ml. portion of urine is mixed with 1–2 ml. of pH 9.5 buffer and passed through the column. Alkaloids, amphetamines, and barbiturates are eluted from the column using 15 ml. of a mixture of dichloroethylene–ethyl acetate (4:6). The eluant is collected in a 25-ml. beaker or centrifuge tube, 1 drop of 0.1 *N* HCl is added, and the mixture evaporated to dryness in a water bath. The Eastman Kodak Co. also suggested an alternate extraction procedure using Amberlite XAD-2 resin. A 10-ml. portion of urine, after adjusting to pH 5–6, is treated with 2 g. of resin in a 50-ml. beaker and shaken for 5 min. Urine is decanted, resin is washed twice with distilled water and once with 5% solution of methanol in water, and washings are discarded. The drugs (all three groups) are extracted with 10 ml. of methanol by shaking for 5 min. The methanol is decanted to a 25-ml. beaker and is evaporated to dryness in a water bath maintained at 60°.

Brinkmann Instruments Inc. (176) has also marketed a disposable plastic column utilizing Amberlite XAD-2 nonionic adsorbent resin. The company modified the disposable plastic column suggested by Quame (177) for simultaneous extraction of alkaloids, barbiturates, amphetamines, glutethimide, phenothiazines, and methadone using Amberlite resin. The procedure requires that only 20 ml. of urine be passed through the plastic column. The drugs are eluted with 15 ml. of dichloroethylene–ethyl acetate (4:6). One drop of 0.1 *N* HCl is added to the eluant before evaporation to dryness in a water bath (at 85°).

Dole *et al.* (75, 170) suggested the use of Reeve Angel SA-2 cation-exchange resin-loaded paper to absorb the drugs from urine and then eluted the drugs from the paper with three consecutive extractions at pH 2.2, 9.3, and 11, respectively. The use of Amberlite IR 120 (sodium form) cation-exchange resin-loaded paper was a major breakthrough in testing drugs of abuse in meth-

[8] Ketodase (Warner-Chilcott).

[9] Rolamix.

adone maintenance programs. The adsorption of drugs onto the paper can be done in a clinic or field station, and the paper (with the patient's name, date, and relevant clinical data typed on it or written in with a lead pencil) rather than a urine specimen is sent to the laboratory; this procedure also provides a useful preliminary fractionation of the urinary constituents. Pigments and various other interfering substances present in urine are not adsorbed by the ion paper. Furthermore, with a few modifications the method could be applied to a wide range of diagnostic problems and epidemiological studies. A sample of urine, usually 50–100 ml., is diluted with an equal volume of water. A 6 × 6-cm. piece of cation-exchange paper is marked with the patient's name or identification number and soaked in the diluted urine with intermittent shaking. If the urine is unusually alkaline, it is acidified to a pH between 5 and 6 before ion paper is added to the urine. After 30 min. or more, it is removed, rinsed in water, folded into plastic film, and sent to the laboratory for analysis. The ion paper is transferred to a 120-ml. (4-oz.) screw-capped bottle, washed with water, and extracted with three consecutive buffer–solvent systems. Barbiturates and acidic drugs are extracted first at pH 2.2 using 20 ml. each of citrate buffer and chloroform; opiates are extracted at pH 9.3 using 20 ml. each of borate buffer and chloroform–isopropanol (3:1); amphetamines are extracted at pH 11 using 20 ml. each of carbonate buffer and chloroform.

Jaffe and Kirkpatrick (178), using the same SA-2 Amberlite IR-120 cation-exchange paper, extracted opiates, amphetamines, and phenothiazines simultaneously with 20 ml. each of borate buffer, pH 9.5, and chloroform–isopropanol (9:1). They eliminated the use of carbonate buffer, pH 11.0, for the extraction of amphetamines. Barbiturates were adsorbed on Reeve Angel SB-2 Amberlite IRA-400 anion-exchange resin-loaded paper and were extracted at pH 2.2 with 20 ml. each of tartaric acid solution (2%) and chloroform–isopropanol (9:1). Heaton and Blumberg (158) modified the procedure reported by Dole *et al.* (75) and extracted narcotics, amphetamines, and psychotropic drug metabolites from cation-exchange paper at pH 9.3–9.4 using 20 ml. each of borate buffer and chloroform–isopropanol (3:1). They reported that the procedure of Dole *et al.* (75) yielded poor recoveries for barbiturates and amphetamines. Mulé (156) modified the procedure of Dole *et al.* (75); he recommended the use of 50 ml. of *undiluted urine*. Using this modified technique, he still reported poor recoveries for barbiturates, methadone, and amphetamine.

Kaistha and Jaffe (165–167) recently reported a modification of the method developed by Dole *et al.* (75). The modification elutes sedative–hypnotics at pH 1 and opiates and amphetamines at pH 10.1 using NH_4Cl–NH_4OH buffer. By using this modified technique, barbiturates (except sodium barbital), amphetamine, methamphetamine, phenmetrazine, and opiates including methadone could be detected at a level of 0.5–1 mcg./ml. of urine. A 6 × 6-cm. piece of Reeve Angel SA-2 cation-exchange resin-loaded paper is soaked in 40–50 ml. of undiluted urine with intermittent shaking. After 30 min. or more, the ion paper is transferred into a plastic bag and sent to the central laboratory for the desired analysis. To decrease the workload and cost of analysis, ion papers of the same patient for 1 week are pooled together. The single or pooled ion papers are transferred to 120-ml. (4-oz.) wide-mouth screw-capped jars, rinsed twice with distilled water, and extracted for different groups of drugs. Sedative–hypnotics, benzodiazepines, and other acidic drugs are extracted at pH 1 using 15 ml. each of sodium citrate buffer and chloroform (20 ml. of each is used if the jar contains more than one ion paper). After shaking for 10 min. on a reciprocating shaker, the lower organic phase is pipeted out into a plain 15-ml. conical centrifuge tube. The aqueous phase is discarded, and ion paper is then extracted at pH 10.1 by adding 15 ml. each of chloroform–isopropanol (3:1) and NH_4Cl–NH_4OH buffer (20 ml. of each is used if the jar contains more than one ion paper). After shaking for 10 min., the lower organic phase is transferred to a 15-ml. plain conical centrifuge tube containing 2 drops (about 50 μl.) of sulfuric acid in methanol (0.5% H_2SO_4 v/v in methanol). Sulfuric acid is omitted if amphetamines are not to be detected. The use of the NH_4Cl–NH_4OH buffer, pH 10.1, permits the simultaneous extraction of opiates and amphetamines without losing the sensitivity of either group of drugs. In our laboratories, this buffer system is routinely used for those urine specimens that require either simultaneous screening of opiates and amphetamines or of amphetamines only. Urine specimens submitted for screening of opiates are extracted with borate buffer, pH 9.3.

TLC Identification Techniques—The papers already cited (144–178) described many useful developing solvent systems and spraying techniques for the detection and identification of stimulants, narcotic analgesics, and sedative–hypnotics in urine using TLC. In addition, TLC data for various individual drugs and/or groups of drugs appeared in many publications (9, 10, 118, 179–192). Sunshine *et al.* (193) reported chromatographic data for 138 commonly used therapeutic agents in seven solvent systems. Emmerson and Anderson (194) described a method for the TLC of analgesic drugs and related compounds in nonaqueous solvent systems. Although varying solvent systems and spraying techniques are used by different workers, the solvent systems and spraying techniques developed by Davidow *et al.* (163, 164) and Dole *et al.* (75, 170) are the ones most commonly employed in monitoring treatment programs. The single-step solvent system developed by Davidow *et al.* (163, 164) for the simultaneous separation of barbiturates, glutethimide, amphetamines, phenothiazines, and opiates consisted of ethyl acetate–methyl alcohol–concentrated ammonium hydroxide (85:10:5). The same solvent system was used by Dole *et al.* (75, 170) for the individual separation of barbiturates, amphetamines, and opiates; in addition, they proposed another developing system for barbiturates consisting of chloroform–acetone (90:10). Recently, Brinkmann Instruments came out with their drug screening system (176) and recommended the solvent system and spraying techniques originally suggested by Davidow *et al.* (164).

In recent reports (165–167), Kaistha and Jaffe pointed out that some difficulties were encountered

using the above solvent system in differentiating drugs of abuse and their adulterants from drugs used in treatment. Two solvent systems, consisting of ethyl acetate–cyclohexane–*p*-dioxane–methanol–water–NH_4OH (50:50:10:10:1.5:0.5) and ethyl acetate–cyclohexane–NH_4OH–methanol–water (70:15:2:8:0.5), were proposed for the simultaneous separation of opiates, amphetamines, and phenothiazines. The former solvent system was recommended for routine use of TLC separation of opiates if a procedure similar to the one of Dole *et al.* (75, 170) was used for extraction, where no sulfuric or hydrochloric acid was added to the chloroform–isopropanol extracts. This system is also capable of separating a mixture of amphetamine and methamphetamine; methamphetamine, phenmetrazine, and quinine; methamphetamine and quinine; and methadone and methapyrilene[10]. The latter solvent system was recommended for routine TLC separation of opiates, amphetamine and congeners, and psychotropic drugs and should preferably be used after storing overnight. Another useful solvent system, consisting of ethyl acetate–cyclohexane–NH_4OH (50:40:0.1), was developed to differentiate methadone and/or cocaine from acetylmethadol, *d*-propoxyphene[11], pipradrol, diphenhydramine[12], chlorpromazine[13], promazine, thioridazine[14], and pentazocine[15]. These drugs can give a false test for methadone and/or cocaine if the proper solvent is not used to separate them. A solvent system specially formulated for the TLC separation of sedative–hypnotics consisted of ethyl acetate–cyclohexane–methanol–NH_4OH (56:40:0.8:0.4). By using this solvent, acidic bodily metabolites and drugs like phenylbutazone, tolbutamide, and chlorpropamide, which are also extractable along with barbiturates at acidic pH, stay at the origin; thus, their possible interference with the detection of barbiturates (due to identical tinctorial properties) is eliminated.

The Eastman Kodak Co. has also come out with a drug screening procedure and is selling separate kits for the identification of opiates, barbiturates, and amphetamines (195). The solvent systems used are different for each group of drugs, and the premixed solutions are sold in tubes in the form of a gel. The gel for amphetamines contains butanol–acetic acid–water and takes about 45–60 min. to travel to the solvent front.

The shortcomings of currently used detection techniques for the identification of barbiturates, amphetamine, methamphetamine, and phenmetrazine were also pointed out recently (165–167). Dole *et al.* (75) recommended the following procedure for the detection of barbiturates, amphetamine, and opiates. Barbiturates are detected by spraying the developed chromatogram with sulfuric acid (0.5% in water). The components can be seen as white spots against a grayish-white background. The confirmation is obtained by drying the plate with warm air and then spraying with silver acetate (1% in water). After irradiation for a few minutes with UV light, the barbiturates appear as brown spots against a gray background. If that is followed by permanganate (0.02 *M* potassium permanganate in water), the barbiturates change to yellow spots against a reddish background. Amphetamine is detected by spraying the developed chromatogram with ninhydrin (0.4% ninhydrin in acetone prepared within 30 min. before use) and then irradiating the plate under UV light for 15 min. Opiates are detected by spraying the developed chromatogram in succession with the following reagents: (*a*) sulfuric acid, (*b*) iodoplatinate, (*c*) ammoniacal silver nitrate or Marquis' reagent, and (*d*) permanganate. Quinine and its metabolites yield a brilliant set of bands under UV light after the sulfuric acid spray. An iodoplatinate spray reveals a variety of narcotic drugs and tranquilizers. After the ammoniacal silver nitrate spray, the chromatoplate is heated on a hot plate for 1–2 min. Morphine and hydromorphone appear as black spots. Alternatively, morphine can be confirmed by spraying with Marquis' reagent (a solution of 0.5 ml. of formaldehyde and 10 ml. of concentrated sulfuric acid), but it is less sensitive and is incompatible with any further testing on the same plate. If needed, potassium permanganate provides another confirmation.

Davidow *et al.* (164) suggested a detection procedure for the simultaneous identification of amphetamine, barbiturates, glutethimide, phenothiazine drugs, and opiates. The developed chromatoplate is air dried and then heated in an oven at 75° for 10 min. The plate, while still hot, is sprayed with the ninhydrin solution and placed under UV light for 2 min.; the pink spot due to amphetamine is marked. The plate at room temperature is first sprayed with diphenylcarbazone solution and then with mercuric sulfate reagent. They recommended that the mercuric sulfate spray should be applied rather heavily until the barbiturates and glutethimide in the positive control specimen appear as blue to pink spots. The plate is reheated in an oven at 75° for 2 min. Phenothiazine drugs appear as violet to orange-red spots after this brief heating treatment. The plate is then examined under UV light for fluorescence due to quinine. The cooled plate is then sprayed with the iodoplatinate, air dried, and sprayed with Dragendorff's solution. The authors (164) pointed out specifically that the deviations from the suggested procedure with respect to oven temperature, time that the chromatoplates are left in the oven, or length of exposure to UV light would adversely affect the sensitivity of morphine to the iodoplatinate–Dragendorff reagent. Brinkmann Instruments Inc., in its drug screening system (176), recommended the above spraying procedure with a few modifications for the simultaneous identification of amphetamine, barbiturates, and opiates.

Eastman Kodak Co., in its drug screening procedure (195), recommended the following visualization technique for the identification of different groups of drugs. Alkaloids are detected by spraying the developed chromatogram consecutively with a series of reagents. After *thoroughly* air drying the developed chromatogram for 10–20 min. (Eastman Kodak uses TLC plates made of acetate, precoated with silica gel, and develops the plate in a solvent made in the form of gel):

[10] Histadyl (Lilly).
[11] Darvon (Lilly).
[12] Benadryl (Parke-Davis).
[13] Thorazine (Smith Kline & French).
[14] Mellaril (Sandoz).
[15] Talwin (Winthrop).

1. Spray with sulfuric acid (0.5% in water) and examine under UV light for quinine.
2. Spray with iodoplatinate solution furnished with the kit. Do not overwet the chromatogram. Dry the sheet for 10 min. before proceeding with additional sprays.
3. Spray with freshly prepared ammoniacal silver nitrate. The morphine spot disappears when sprayed with this reagent but reappears when the chromatogram is heated for several minutes at 100°.
4. Spray with potassium permanganate solution.
5. Spray with Dragendorff's reagent.

Cocaine and methadone cannot be resolved with the Kodak Chromat/O/screen analysis kit for alkaloids. They recommended the use of Eastman Chromagram sheet 6060 and a solvent mixture of ethyl acetate–methanol–water (7:2:1). Operators using this kit or TLC sheets manufactured by Eastman Kodak Co. should keep in mind that since these sheets are made of acetate precoated with silica gel, they cannot be subjected to heat treatment higher than 100°. It is imperative that after the application of ammoniacal silver nitrate, these plates are not heated on the hot plate or at a temperature higher than 100°—a treatment suggested by Dole *et al.* (75) and Kaistha and Jaffe (165–167) for the immediate reappearance of a black spot due to morphine. Amphetamines are detected with ninhydrin. The developing solvent supplied in the form of a gel contains ninhydrin which stains amphetamines. After air-drying the developed chromatogram for 10–20 min., it is placed under UV light for 5 min. and the spots for amphetamines are marked. Then carbonate (supplied with the kit) is sprayed; spots due to amino acids disappear or turn pink. After spraying, the plate is again examined under UV light; dextroamphetamine and methamphetamine appear as fluorescent spots. Additional confirmation of the presence of amphetamines is possible using iodoplatinate reagent. Barbiturates are detected by spraying the air-dried developed chromatogram in succession with silver acetate, diphenylcarbazone, and mercuric sulfate solutions. A potassium permanganate solution may be used after these sprays.

Recently, Rosenthal *et al.* (195A) reported a TLC procedure for the simultaneous detection and identification of a number of barbiturates (*e.g.*, secobarbital, pentobarbital, and amobarbital) and glutethimide in chloroform extracts of serum.

Kaistha and Jaffe (165–167) recently reported highly sensitive and reliable spraying techniques for the identification of barbiturates, amphetamines, and opiates. They used Gelman glass microfiber sheets precoated with silica gel (ITLC type SA) for the development of TLC techniques. TLC plates made of glass microfiber were preferred because of the ease with which they can be handled; they can be cut into any desired size and can be subjected to varying heat treatments. Barbiturates and drugs like glutethimide[6] and diphenylhydantoin[16] are best detected by spraying the developed chromatogram in succession with diphenylcarbazone, silver acetate, and mercuric sulfate reagents, because these form characteristic colored complexes with silver acetate and/or mercuric sulfate. The other combination of sprays reported in the literature either produce colored spots of weak intensity and transient nature (164, 176) or result in the formation of white spots (195) or brown spots after irradiation with UV light (75). A novel technique to differentiate barbiturates from drugs like chlordiazepoxide[17], metabolites and/or artifacts of phenylbutazone, methadone, diazepam[18], and oxazepam is to overspray the plate with an iodine–potassium iodide spray after a mercuric sulfate spray. All these drugs except barbiturates, glutethimide, and diphenylhydantoin appear as varying shades of brown. Opiates, amphetamine, methamphetamine, and phenmetrazine are simultaneously detected by spraying the developed chromatogram in succession with ninhydrin, sulfuric acid, iodoplatinate, and ammoniacal silver nitrate. It is imperative that the steps outlined by the authors after the ninhydrin spray are followed for the satisfactory identification of amphetamine, methamphetamine, and phenmetrazine. After the application of the iodoplatinate spray, it has been found essential that the chromatogram should be sprayed heavily with ammoniacal silver nitrate and then subjected to heat treatment (heated for 30–60 sec. on a hot plate maintained at a medium temperature) for the presence or absence of morphine. If necessary, the plate may be resprayed and reheated. This spray reduces the possibility of false positives for morphine and has proved to be a reliable confirmatory test as compared to other combinations of sprays reported in the literature, such as the use of Dragendoff's reagent after the iodoplatinate spray. The potassium permanganate spray has proved to be a very useful adjunct to the ammoniacal silver nitrate spray. The spots which 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 for a few seconds on the hot plate maintained at a medium temperature. The spots other than morphine and codeine disappear, thus reducing further the possibility of false positives for morphine. If desired, the plate is oversprayed with ammoniacal silver nitrate and heated, and morphine and codeine reappear as characteristic brown or dark-brown spots. When the goal is limited to the detection of only narcotic analgesics, the ninhydrin spray is omitted and the developed chromatogram is sprayed in succession with sulfuric acid, iodoplatinate, and ammoniacal silver nitrate as described above.

Potassium permanganate spray is used if needed for verification of doubtful spots. If the interest is only to detect amphetamine and congeners, the developed chromatogram is sprayed with ninhydrin followed by sulfuric acid. Spraying with sulfuric acid is suggested because spots due to amphetamine and congeners undergo different color changes and sometimes amphetamine, if not seen earlier, appears at this stage as a faint grayish or bluish-gray spot. An alternate spraying technique was also described (165, 167) for narcotic

[16] Dilantin (Parke-Davis).

[17] Librium (Roche).
[18] Valium (Roche).

analgesics and amphetamines using bromcresol green followed by sodium bicarbonate, sulfuric acid, iodoplatinate, and ammoniacal silver nitrate. A spraying technique to differentiate cocaine from methadone was also proposed. The reliability of these detection and extraction procedures was reported recently (196). TLC techniques have also been used for the separation of metabolites of Cannabis (172, 197) and Δ^1-tetrahydrocannabinol, the major psychotomimetically active constituent of Cannabis (51).

Recently, a mini TLC procedure for the detection of narcotics in the urine, using dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride), was proposed by Ho *et al.* (197A). By using this procedure, the dried organic solvent extract of urine is treated with sodium bicarbonate and dansyl chloride reagent and the mixture is heated at 37° in the dark for 2 hr. The fluorescent reaction mixture is used for direct spotting on a mini thin-layer plate. The authors claim they can detect less than 1 ng. of morphine, amphetamine, and methamphetamine. Although the procedure appears tedious, it does have potential for the detection of many drugs in biological fluids.

Simplified Chemical Screening and Color Reactions—Many drugs give distinct colors when brought into contact with various chemical reagents. These color reactions or spot tests are not confined to a single drug but are produced by most of the drugs in a given class or group of drugs or even by drugs not in that group. Spot tests can be performed directly on blood, urine, homogenized tissue samples or tissue distillates, and other fluids. Sunshine (198) has described in detail the spot tests for the detection of various drugs and/or groups of drugs in biological materials. Colors formed with various hallucinogens using *p*-dimethylaminobenzaldehyde reagent have been listed. Stevens (199) has classified the color reactions according to the main component of the reagent, describing the different colors formed with various drugs or groups of drugs. Sobolewski and Nadeau (147) reported a scheme for rapid identification of commonly used sedatives, hypnotics, and tranquilizers in urine using different color reagents. The drugs were extracted in four extraction steps with various organic solvents; each organic extract was concentrated or evaporated to dryness and then treated with suitable reagents. It is recommended that the analyst should run both a "positive" control (normal urine to which has been added traces of suspected drug) and a reagent control every time a test is performed.

Miscellaneous Techniques—*Polarographic Techniques*—The theory and applications of polarographic analysis of biological materials were discussed by Feldstein (200). As applied to the detection of ethanol in biological materials, the technique involves polarographic identification of acetaldehyde; hence, it appears to be more specific than the conventional dichromate oxidation procedures. The polarographic techniques apparently have not been applied to the detection of abuse drugs in biological materials.

Biological Luminescence (Bacterial Luminescence)—McElroy and Seliger (201) reported that some fungi and bacteria can emit light. The light emitted by luminous bacteria is usually a broad band in the blue or blue-green region of the spectrum (between 480 and 500 nm). The requirements for luminescence are a reduced form of riboflavin, an aldehyde, oxygen, and an enzyme. Luminous bacteria have been favored for studying the action of drugs because the effects can be observed externally by means of a photoelectric cell. It is also possible to obtain mutant strains of luminescent bacteria that are nonluminous, and then one can examine the ability of various drugs to restore luminescence. The applications of this technique for the detection of abuse drugs in biological materials could not be traced.

Microdiffusion Analysis—This technique permits the detection of volatile substances in biological materials. The interested readers are advised to refer to the articles by Feldstein (202) and Sunshine (203) for the detection of volatile alcohols and other volatile poisons in biological materials.

Microcrystal Tests—The crystal or microcrystal test is a most effective aid to identification following TLC, UV, and color tests. The test consists of mixing a drop of a solution of the test material with a drop of the reagent on a microscope slide and observing the crystals formed under the microscope. The unknown and known must be compared using the same reagents and the same conditions. Identification is made by comparing the microscopic appearance of the crystals formed. Crystal tests are performed on reasonably purified substances. The tests can be performed on the eluates of paper and TLC bands. The applications of these tests for the identification of drugs of abuse and other drugs were described in detail by Fulton (204), Clarke (205), and Eisenberg (206). Clarke (205) gave specific examples such as the differentiation of phenmetrazine from phendimetrazine, of levorphanol and racemorphan from dextrorphan, of racemethorphan from dextromethorphan and levomethorphan, of racemic propoxyphene from dextropropoxyphene and levopropoxyphene, and of the (+), (−), and racemic forms of amphetamines. Clarke (135) also reported that crystal tests provided excellent means of differentiating trimethoxyamphetamine from *p*-methoxyphenethylamine and methoxamine. The former gave bunches of serrated needles with gold bromide solution, while the latter compounds gave no crystals at all.

Optical Crystallographic Methods—Many drugs can be readily and specifically identified from their optical crystallographic properties, which can be determined within certain limits by means of a polarizing microscope. The applications of optical crystallographic techniques for the identification of some abuse drugs were cited by Eisenberg (206) and Sunshine (207).

New Instrumentation—Syva Corp.'s[19] FRAT ESR instrument used with the FRAT free radical assay technique was discussed in this article (133). Micro-Now Instrument Co.[20] also has available an ESR machine which can be used for the same purpose. Quantum Assay Corp.[21] markets a semiautomatic extraction apparatus (Auto-Assay model 1000) for mass screening for drug presence in urine. It can make 240 extractions/-

[19] Palo Alto, CA 94304
[20] Chicago, IL 60646
[21] Fairfield, NJ 07006

day. The model consists of a base mounting a rack of ten 100-ml. capacity (combined fluid volume) hydrodynamically designed extraction tubes, the motor-drive assembly, timing circuits, an integral temperature-controlled water bath, twin blowdown assemblies for solvent concentration, air control valving, and three snap-in racks, each capable of holding ten 15-ml. glass conical centrifuge tubes. Ten discrete samples may be prepared simultaneously during all steps of the wet chemistry process. This model is specially designed for laboratories engaged in mass drug screening where drugs are first adsorbed on cation-exchange paper and then the cation-exchange paper is extracted with different solvent systems (75, 165–167). The Auto-Assay model is suitable for the simultaneous extraction of amphetamines and opiates or amphetamines alone from cation-exchange paper, using pH 10.1 NH_4Cl–NH_4OH buffer (165–167).

Scientific Products[22] introduced the S/P Toxichron Drug Analyzer System. It is claimed that this system offers the clinical laboratory an effective means of dealing with the urgency of drug overdose by providing very rapid qualitative and quantitative analyses of serum or urine for the most common abuse drugs. Barbiturates, alcohols, narcotic and nonnarcotic analgesics, stimulants, tranquilizers, and many other compounds can be analyzed in minutes. Technicon Instruments Corp.[23] developed an automated screening system for the detection of morphine in urine specimens based on the work of Kupferberg *et al.* (28), Takemori (29), and Blackmore *et al.* (207A). The system accepts fresh, untreated urine samples and completes the assay in less than 6 min. It can screen 40 samples/hr. The test appears to be specific since it is based on the conversion of morphine, which is weakly fluorescent, to pseudomorphine, which is highly fluorescent.

DEVELOPMENT COSTS OF A TOXICOLOGY LABORATORY AND COST PER URINE TEST

Recently, the initial development costs of setting up a toxicology laboratory facility in a drug abuse urine screening program using TLC techniques were reported (208). The breakdown cost per single urine test was also cited. This cost was calculated using ion-exchange paper techniques (75, 165–167, 170). When using ion-exchange paper techniques, drugs are first adsorbed on Reeve Angel SA-2 cation-exchange resin-loaded paper and then this paper is extracted with a series of buffer–solvent systems. Conventional procedures (75, 156) extract barbiturates at pH 2.2, opiates at pH 9.3, and amphetamines at pH 11. The modified procedure (165–167) extracts barbiturates at pH 1 and then extracts amphetamines and opiates simultaneously at pH 10.1, using a NH_4Cl–NH_4OH buffer. Furthermore, the use of cation-exchange resin-loaded paper to absorb the drugs from a urine specimen has cut considerably the cost of monitoring the progress of each patient by permitting the combination of several ion papers of the same patient representing different urine specimens. By such batching, gains in speed, convenience, and economy have been achieved without sacrificing any sensitivity of the extraction and identification techniques or affecting the efficacy of the treatment program, even though there is a delay in getting results back to the clinics. A technician with minimal formal training can screen 300 urine specimens (pooled or unpooled) in a 37.5-hr. work week, provided the analysis of this batch is started at the same time. This number can be increased to 350–400 specimens/week if some ancillary help is provided to wash the bottles and test tubes. The cost for complete screening of urine for opiates using borate buffer, pH 9.3 (75), or opiates[24] plus amphetamine and congeners[25] using NH_4Cl–NH_4OH buffer, pH 10.1 (165–167) was reported to be approximately \$0.75 per test based on 300 specimens per technician per week. This amount was inclusive of chemicals, breakage, chromatographic plates, disposable capillaries, and labor costs. An additional cost of \$0.54 per specimen was calculated for supervisory salary, laboratory rental cost, equipment depreciation, and overhead charges. When a barbiturate[26] analysis is desired along with opiates and amphetamines, it costs an additional \$0.48 per specimen because the extra labor spent will not be as much compared to the cost of a specimen monitored only for barbiturates.

Recently, Brinkmann Instruments Inc. (176) introduced a disposable plastic column prepacked with Amberlite XAD-2 polymeric adsorbent for the simultaneous extraction of opiates, amphetamines, and barbiturates from a urine specimen. The complete extraction kit consists of an adsorbent cartridge containing Amberlite XAD-2 resin, a sample reservoir, a filter cartridge, and a phase-separating filter. This complete extraction kit costs about \$0.89; the adsorbent cartridge containing Amberlite XAD-2 and the phase-separating filter cost about \$0.49 and have to be replaced for every urine specimen. However, the sample reservoir costs about \$0.11 and may be washed and reused approximately 50 times or until the snap joint wears out. The filter cartridge costs about \$0.28 and can be reused after washing and replacing the phase-separating filter with a new one (a special set of tools is available to replace the phase-separating filter). Although opiates, amphetamines, and barbiturates can be extracted simultaneously, the cost of analysis per urine test for screening these groups of drugs using this kit is still higher because from \$0.35[27] to \$0.49 must be spent per specimen just for replacing the adsorbent cartridge and the phase-separating filter. The sample reservoir and filter cartridge cost an additional \$0.39 whenever replaced. Furthermore, it appears that different extractions kits have to be used for different urine specimens of the same patient. Analogously, when using cation-exchange resin-loaded paper, the cost of ion-exchange paper is about \$0.05 and the screw-capped 120-ml. (4-oz.) glass bottle used for extraction (costing \$0.10) is reused until it breaks.

[22] McGaw Park, IL 60085
[23] Tarrytown, NY 10591

[24] The opiate test includes morphine, codeine, and structurally related narcotic analgesics and their adulterants like quinine and antihistamines as well as methadone, acetylmethadol, or cyclazocine (drugs used in treatment).
[25] This test includes amphetamine, methamphetamine, and phenmetrazine as well as methylphenidate and pipradrol if desired.
[26] This test includes all derivatives of barbituric acid as well as glutethimide and diphenylhydantoin.
[27] The author was told that the company could sell the adsorbent cartridge at a special rate of about \$0.35 if the total annual purchase of these extraction kits is approximately \$5000.

A similar extraction kit, introduced by Eastman Kodak Co., is still more expensive; the adsorbent cartridge alone costs $0.82. Eastman Kodak Co. has also put on the market separate analysis kits for TLC identification of alkaloids, amphetamines, and barbiturates (195). Each kit consists of a developing solvent in the form of a gel, chromatographic supplies, visualization spray (most of the sprays should be freshly prepared), forceps, *etc.* Each analysis kit is capable of carrying out 150 tests and costs about $73.00 (approximately $0.49 per test for each group of drugs). This cost of $0.49 per test does not include the price of the extraction kit. It appears that the use of these kits for routine TLC identification is expensive, but these kits will be valuable for field tests of these groups of drugs.

GLC is another technique that can be used for mass screening of urines in treatment programs. Like TLC, it can permit simultaneous screening of a mixture of drugs, but it has the inherent disadvantage that only a single specimen can be chromatographed 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 TLC chromatoplate. In the author's laboratories, GLC is used for research and developmental work and also for the validation of some results obtained by TLC (out of 3000 weekly specimens, about 1% are validated using a combination of both TLC and GLC techniques).

Another potentially useful technique, recently introduced by Syva Corp., for mass screening of urines for opiates (morphine and its natural and synthetic equivalents) is the FRAT system (133). This technique is reported to be many more times sensitive than TLC, sensitive to both free morphine and its glucuronide conjugate, and to require only about 30 sec. to complete a test. The author has been told that the price for the opiate reagent varies from $0.50 to $1.50 per test, depending on the yearly volume of tests performed. This 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 natural or synthetic equivalents (*e.g.*, adulterants used to cut heroin, amphetamine, and related stimulants; cocaine; sedative–hypnotics; and drugs used in treatment like methadone, acetylmethadol, cyclazocine, and naloxone). In addition, free radical antibody preparations for detecting other than morphinelike drugs are not yet commercially available. Because of the high speed with which analysis can be performed, this technique is currently used to detect heroin use among our homebound soldiers from Vietnam. But in treatment programs where time is not a critical factor and where detection of a wide range of substances is desired, this appears to be an expensive technique as compared to TLC.

TLC is a versatile technique capable of detecting a wide range of substances in a single run. Furthermore, clinical programs using cation-exchange resin-loaded paper to absorb the drugs from a urine specimen (75, 165–167, 170) can substantially decrease the workload and cost of analysis by combining several ion papers of the same patient representing different urine specimens. Different urine specimens cannot be pooled using the free radical assay technique (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- or small-scale screening programs.

ANALYSIS OF ILLICIT STREET-SAMPLE DRUGS OF ABUSE

Law enforcement agencies such as the Bureau of Narcotics and Dangerous Drugs, U. S. Department of Justice, and the Food and Drug Administration (FDA) have their own laboratories to test the samples seized by police and federal and state narcotic agents; they have their own manual for methods of analysis (209). These laboratories are continually devising new techniques for the identification of opiates, amphetamines, barbiturates, marijuana, and various hallucinogenic drugs; hence, the methods available for the identification of abuse drugs for the use of law enforcement agencies will not be reviewed here. However, experience has shown that the chemists in charge of drug abuse monitoring laboratories for treatment programs do receive illicit drug samples from drug addicts and other sources. The guidelines given below are designed for the chemist called upon to analyze the various drugs in a street sample. The samples received are in the form of tablets, capsules, sugar cubes, filter papers, aqueous solutions, and powders. If the sample is in powder form, it is carefully mixed to ensure a uniform specimen. In the case of tablets, capsules, or cubes, each piece should be individually tested. Liquids should be shaken well and filtered if necessary. The isolation methods for drug identification from tablets, capsules, and unknown powders as outlined by Sunshine (210) may be consulted for additional information.

General Examination—A small portion is dissolved in diluted hydrochloric acid and divided into two parts. One portion is tested with Mayer's reagent and the second with Wagner's reagent (211). Alkaloids and synthetic opiates give precipitates with these reagents. Caffeine is not precipitated with Mayer's reagent.

Microchemical and Spot Tests—These techniques were reviewed under *Detection Procedures* (198, 199, 204–206). If the quantity of "unknown" available is very small, spot testing using the iodoplatinate reagent (198) would be desirable to test the presence of alkaloids. Treating the unknown sample with a few drops of Marquis' reagent would give a purple-violet color if narcotic alkaloid is present (see under *Field Tests*).

TLC—TLC can be performed directly on the methanolic solution (approximately 0.1% solution in methanol or ethanol containing 1 drop of diluted hydrochloric acid, and filtering if necessary) of tablets, contents of capsules, powders, and cubes without resorting to extraction procedures (powders of vegetable origin cannot be directly spotted). Filter papers may be washed with methanol; an aqueous solution may be

treated just like a urine specimen. Acidic, neutral, and basic drugs may be separated by the direct extraction procedure from an aqueous solution preceding TLC, as proposed by Kaistha and Jaffe (165–167). Alternatively, the scheme outlined by Sunshine (210) for the isolation of drugs from fluids may be followed. TLC developing solvent systems and identification techniques reviewed under *TLC Identification Techniques*, particularly the ones proposed by Dole *et al.* (75, 170), Cochin (153), Cochin and Daly (151, 152), Davidow *et al.* (163, 164), Sunshine and coworkers (149, 161, 193), and Kaistha and Jaffe (165–167), are the best suited for drug identification in an unknown sample. In addition, references cited by Sunshine (212) may be consulted for the selection of appropriate solvent systems for a suspected drug. It is imperative that the known standards of the opiates, amphetamines, hallucinogenic compounds, and sedative–hypnotics are spotted beside the unknown sample.

UV Absorption Spectrophotometry—It is suggested that a UV absorption spectrum of an alcoholic or water extract be run simultaneously with TLC because it will prove to be a useful adjunct to the data generated by color and microcrystal tests and TLC. For example, morphine exhibits a bathochromic shift, showing an absorbance maximum at 285 nm. in neutral or acidic solution and at 296–297 nm. in alkaline solution; heroin shows a maximum at 280 nm. in an acid solution. Additional information on the UV spectrophotometric absorption data for various drugs of interest may be obtained from the tables compiled by Sunshine (213) and Daglish (214).

GLC—GLC may be used for the final identification of the compound after obtaining some preliminary data from the tests already discussed.

Free Radical Assay Technique—Although the FRAT system (133) is capable of detecting the presence of morphine and its natural and synthetic equivalents (heroin, codeine, ethylmorphine, *etc.*) in an unknown sample instantaneously, it is not able to differentiate morphine and its natural and synthetic equivalents from one another. Free radical antibody preparations for other drugs of abuse are not yet commercially available.

IR Spectrophotometry and NMR Spectroscopy—IR absorption spectrophotometry is an extremely useful technique for the identification of drugs since an IR spectrum is equivalent to a fingerprint of the drug being examined. To obtain useful information from an IR spectrum, it is imperative that the sample should not contain more than one drug and it must be as pure as possible. The purification is achieved by paper chromatography or TLC as desired; then the spot is eluted with a suitable organic solvent and the extract is evaporated to dryness. This technique may only be used if the methods previously discussed fail to identify the drug.

NMR can also prove to be a very valuable tool for the determination and verification of the molecular structure of a suspected drug. Readers interested in this technique are advised to consult "Interpretation of NMR Spectra" (215) and the references therein for various textbooks.

Derivative Formation—In some cases, preparation of a suitable derivative and its melting point can be useful in differentiating structurally similar compounds such as phenmetrazine from phendimetrazine (215A). When dealing with a mixture of drugs, a simple acid–base extraction procedure or a cleanup procedure using a Celite column followed by preparative TLC is desirable before derivatization.

Screening Procedures—The following screening procedures are used for some abuse drugs.

Identification of Heroin in Illicit Preparations—The adulterants commonly used in illicit heroin preparations are quinine, antihistamines such as methapyrilene, procaine, and carbohydrates such as starch, lactose, and mannitol. In addition, the presence of acetylcodeine (142, 216, 217), O^6-monoacetylmorphine (142, 218, 219), and caffeine has been reported in illicit preparations. TLC procedures capable of separating morphine from heroin (151, 209, 217, 222), heroin and/or morphine from monoacetylmorphine (9, 217, 220), and heroin from acetylcodeine (217, 221) were reported. Davis *et al.* (219) used paper chromatography for the separation of monoacetylmorphine from heroin. Steel (222) proposed several solvent systems for the identification of opiates by TLC in narcotic seizures. Levine (223) showed that many alkaloids, such as heroin, can be extracted as ion pairs by chloroform and other chlorinated hydrocarbons. Using this principle, Nakamura and Meuron (142) described a cleanup procedure for the removal of commonly used adulterants from the white and brown heroin specimens preceding UV determination. A solution of heroin in hydrochloric acid is poured over a Celite 545 column, and heroin is then eluted with chloroform. The eluate is collected in a volumetric flask containing a few drops of concentrated hydrochloric acid and a few milliliters of methanol. After a definite volume is achieved with chloroform, the extract is scanned for UV absorbance from 340 to 250 nm. The heroin concentration is calculated from the absorption maximum at 280 nm. The procedure is applicable for the determination of heroin in the presence of acetylcodeine. Heroin is very stable in acids and in boiling water, but it is readily hydrolyzed by alkali to its end-product, morphine. Hence, a UV absorption maximum at 297 nm. of a heroin specimen in an alkaline medium would be that of morphine and not heroin. Acetylcodeine shows no UV absorbance at 297 nm. in alkaline medium.

Grooms (218) suggested GLC of heroin in illicit samples by silylation with *N,O*-bis(trimethylsilyl)-acetamide. By using this technique, it was possible to separate heroin from lactose and procaine, the common diluents of heroin. But it appears that under the conditions described, heroin could not be determined in the presence of quinine, since both have the same retention time. Grooms (218) suggested that the presence of quinine may be checked under UV light after acidification with concentrated nitric acid.

Identification of Lysergic Acid Diethylamide (LSD)—Look (141), while reporting the results of a collaborative study on the identification and determination of lysergic acid diethylamide, also reviewed the various qualitative and quantitative methods available for its analysis. Paper chromatography (135, 141, 224, 225), TLC (139,

165, 167, 220, 224, 226–229), and IR spectrophotometry (226, 227) are the techniques generally used for the identification of LSD samples. Genest (230) proposed a direct densitometric method on TLC plates for the determination of LSD and iso-LSD in morning glory seeds. Dal Cortivo *et al.* (228) used a filter fluorometer and chromatogram scanning apparatus for the direct fluorometric measurement of spots on a flexible silica gel coated chromatogram. Martin and Alexander (231) reported that a modification of the method of Dal Cortivo *et al.* is used in FDA laboratories (231A). The sample extract is spotted on TLC plates, and the developed spot is scraped off and eluted with hydrochloric acid–methanol. The resulting solution is activated at 325 nm. and read at 430 nm. Genest and Farmilo (232) suggested a 2-hr. acid hydrolysis followed by prolonged UV irradiation. This time-consuming technique was modified by Andersen (233) to a simple controlled UV degradation of alkaloids in a chloroform solution followed by TLC. The developing solvent used was chloroform–acetone (1:4), and spots were detected under short UV light. This system separated LSD from iso-LSD. He applied this procedure to other indole alkaloids also. Quantitative procedures are usually based upon UV spectrophotometry (144, 226, 227) or employ the common colorimetric method for ergot alkaloids based on reaction with *p*-dimethylaminobenzaldehyde (234). Spectrophotofluorometric methods for determining LSD were described by Axelrod *et al.* (36), Aghajanian and Bing (37), Dal Cortivo *et al.* (228), Genest and Farmilo (232), and Martin and Alexander (231).

The methods described by Martin and Alexander (226) have been found satisfactory for the identification of LSD in sugar cubes, aqueous liquid, gelatin capsules, powder, and volatile, nonaqueous liquid. The cleanup procedure using Celite and a 2% citric acid column can separate LSD from iso-LSD. LSD in illicit samples also has been detected by TLC techniques suggested by Kaistha and Jaffe (165–167). The presumptive test for LSD suggested by Look (141, 235), combined with GLC (121) and TLC techniques (165, 167, 226), would be the positive means of LSD identification. Lysergamide, lysergic acid, methysergide, ergonovine, and other ergot alkaloids can be identified by TLC techniques (135, 220, 229) or UV degradation followed by TLC (233).

Identification of Hallucinogenic Drugs Other than LSD—Mescaline (3,4,5-trimethoxyphenethylamine), *N*,*N*-dimethyltryptamine (DMT), and 5-hydroxy-*N*-dimethyltryptamine (bufotenine) have been identified by GLC methods (119). Mescaline and other alkaloids from tops or buttons of a peyote cactus can be extracted in a soxhlet apparatus, as described by Martin and Alexander (231), or extracted with chloroform and fractionated into phenolic and nonphenolic alkaloids by Amberlite IRA 400 (OH^-) ion-exchange resin (236). These alkaloids can then be identified by GC procedures as reported by Lundstrom and Agurell (236A) and Kapadia and Rao (236B). The mescaline itself can be identified by a microchemical test (236C) or by IR absorption. Clarke (135) suggested that the best means of differentiating mescaline and trimethoxyamphetamine from other methoxy derivatives of phenethylamine is their reaction with concentrated nitric acid, with which these two compounds give a dull-purple color. The test may be carried out directly on the paper chromatogram by holding it over nitrous fumes generated from copper and nitric acid. Confirmation may be made by means of crystal tests. Mescaline gives needles with styphnic acid; trimethoxyamphetamine gives serrated needles with gold bromide solution (*p*-methoxyphenethylamine and methoxamine give no crystals at all). Mescaline can also be identified by TLC techniques (119, 165, 167, 232, 237, 238).

N,*N*-Dimethyltryptamine (DMT) in an unknown sample can be identified by TLC techniques (135, 231) and UV absorption spectra (231). This compound exhibits maxima at about 290, 282, and 276 nm. and minima at about 287 and 278 nm. Diethyltryptamine (DET) can also be identified by TLC (231). Psilocybin can be identified by a UV absorption spectrum after purification on a Celite column (231). Paper chromatographic and TLC techniques were used by Clarke (135) for the identification of psilocybin, psilocin, and bufotenine. Interested readers are advised to consult the monographs by Clarke (239) on dimethyltryptamine, psilocybin, psilocin, bufotenine, phencyclidine (PCP), and 2,5-dimethyl-4-methylamphetamine for various identification tests. 2,5-Dimethyl-4-methylamphetamine (STP, DOM) can be identified by its UV spectrum in aqueous solution, followed by an IR identification of the extracted base (39, 231); it can be extracted with chloroform from an alkaline solution. Wallace *et al.* (23) reported that 2,5-dimethyl-4-methylamphetamine forms a reaction product with cerium sulfate, which can be extracted with hexane, and the UV absorbance can be measured at 287 nm. TLC can also be used for its identification (39).

Identification of Marijuana—The various *Cannabis sativa* preparations are the most widely used illicit drugs in the different parts of the world. Marijuana, as sold in the illicit drug traffic, consists of dried matured leaves and flowering tops of both male and female plants. It is also known as bhang. The pure resin which is scraped from the leaves and flowering tops of the female plant is called charas. Hashish (ganja) is a medium-range preparation consisting of the female flowering tops and stems with resin attached to their surfaces. The following procedures are suggested for the identification of Cannabis preparations.

1. Macroscopic: The color, odor, and form of Cannabis are very distinct from materials used as diluents.

2. Microscopic: The Cannabis plant has characteristic cystolith hairs on the stems and outside of leaves. Cannabis resin (charas and hashish) usually contains considerable amounts of plant material (leaves) having cystolith hairs. These hairs can be detected by mixing a small quantity of resin with *n*-hexane, transferring a drop of the suspension to a microscope slide, and examining under a microscope at 100× when solvent evaporates. Marijuana containing dried matured leaves does not need any mixing with hexane. This technique was recently used as a nonchemical field test for marijuana (240). The cystolith hair of Cannabis contains a calcium carbonate deposit which liberates carbon dioxide on treatment with diluted hydrochloric acid,

giving rise to effervescence. This may be used as an additional test to detect the presence of carbonate deposits on the suspected plant material. The reader is advised to study the forensic aspects of cystolith hairs of Cannabis and other plants by Nakamura (240A).

3. TLC: Preparation of sample—Green plant material containing leaves, stem, and seeds may be reduced to a coarse powder and extracted with petroleum ether or *n*-hexane (95% ethanol can also be used). The material (50–100 mg.) is transferred to a test tube and covered with petroleum ether (1–2 ml.) for 10–15 min. with intermittent shaking, filtered, and evaporated to dryness if desired. Larger volumes of extraction solvent may be used to ensure maximum extraction of the active constituents. Alternately, the sample may be prepared as described by Butler (240B) and Turk *et al.* (240C), and the residue obtained after evaporation of solvent may be used for color tests (240B) and TLC (240C). Cannabis resin (charas, hashish, *etc.*), about 2–10 mg., is shaken with 95% ethanol or *n*-hexane (0.5 ml.) and processed as above. A cigarette may be opened and the material suspected as marijuana may be physically separated and treated as for green plant material.

TLC identification—Many TLC developing solvent systems using kieselguhr G or silica gel have been reported (126–128, 240C, 240D, 241–246) for the qualitative analysis of Cannabis. Silica gel G impregnated with dimethylformamide and using cyclohexane as an eluent is a frequently used TLC system. In a simpler system, chromatoplates of silica gel and elution with a mixture of petroleum ether–ether (4:1) are used (247). The residue obtained under preparation of sample is dissolved in chloroform or 95% ethanol (alternatively, the sample extract solution can also be used for spotting) and is spotted beside the reference solution of standard Cannabis resin[28] in 95% ethanol [or ethanolic extract prepared from standard marijuana[28] or the reference solution of a mixture of Δ^1-tetrahydrocannabinol[28] (Δ^9-tetrahydrocannabinol), $\Delta^{1,6}$-tetrahydrocannabinol[28] (Δ^8-tetrahydrocannabinol), cannabinol[28], cannabidiol[28], cannibicyclol[28], and cannabichromene[28] in 95% ethanol]. The developed chromatogram is sprayed with a freshly prepared solution of Fast Blue RR (0.2% w/v in 50% ethanol). Tetrahydrocannabinols and the other compounds mentioned give varying shades of orange, reddish brown, and light pink. This spraying technique is currently used in these laboratories for the detection of unknown marijuana street samples (247A). The sensitivity of this spraying technique has been increased by slightly heating the chromatogram (Gelman glass microfiber sheet precoated with silica gel) on a hot plate at a low temperature. This brief heating treatment increases the intensities of colors formed for various compounds. Spraying techniques using *O*-dianisidinetetrazolium chloride (128), Fast Blue salt (246), Beam's reagent, Duquenois' reagent, diazotized benzidine (126), Duquenois–Negm's reagent (247), and various other reagents (241) have been used for the identification of Cannabis. The specificity of various color tests was investigated by the United Nations laboratory in Switzerland (248). Although Beam's test was found to be relatively specific, the test is negative for Δ^1-tetrahydrocannabinol (Δ^9-tetrahydrocannabinol). The Duquenois–Negm reaction, although less specific than the Beam test, is more sensitive. The specificity of this test has been enhanced by the modification suggested by Levine, as reported by Butler (240B). The chemical basis of the Beam test is oxidation of cannabidiol, cannabigerol, and their acids to hydroxyquinones, the anions of which are violet (249). The use of both the Beam and Duquenois–Levine tests on the specimen offers a high probability for the positive identification of the drug. For additional information pertaining to the qualitative and quantitative analysis of Cannabis constituents, readers should consult the recent review article on the chemistry and pharmacology of marijuana (247B).

4. GLC: GLC offers a positive means of identification of Cannabis preparations. All preparations (except pure compounds) should be subjected to a cleanup procedure prior to GLC. About 1 g. of powdered sample may be extracted with 95% ethanol, as described for green plant material, and passed through a Florisil column (126) using benzene as the effluent solvent (about 25 ml. is sufficient). The eluate is evaporated to dryness, and the residue is dissolved in a minimum volume of methanol and injected. Alternatively, 1 g. of ground sample may be placed on a silica gel column and extracted with about 25 ml. of benzene (128). The solvent is removed on a water bath, and the residue is dissolved in a minimum volume of methanol and injected. GLC procedures using various columns were already discussed (122–130). Satisfactory resolution of various cannabinols has been obtained using a 3% SE-30 column (247A).

FIELD TESTS FOR SOME ILLICIT DRUGS OF ABUSE

Microtest for Hallucinogens—Treating a small quantity of sample in one of the depressions in a spot plate or in a micro test tube with *p*-dimethylaminobenzaldehyde test solution gives color reaction for various hallucinogens, *e.g.*, LSD, lysergic acid, *N,N*-dimethyltryptamine, diethyltryptamine, bufotenine, ibogaine, psilocin, psilocybin, ergonovine, tryptamine, and 2,5-dimethyl-4-methylamphetamine (198). Similarly, treating another portion with ethanolic *p*-dimethylaminobenzaldehyde reagent gives colors different than those obtained with the *p*-dimethylaminobenzaldehyde reagent.

Field Test for Hallucinogens Using Filter Paper—Alliston *et al.* (250) suggested that a small amount of material may be placed on a filter paper and a drop of *p*-dimethylaminobenzadehyde reagent [5% solution of *p*-dimethylaminobenzaldehyde in hydrochloric acid–methanol (1:1)] be added. By chromatographic action the material responding to the reagent is carried away from the bulk of the sample and is concentrated into striations. They claimed that is was possible to obtain a response with weak samples of lysergide which had failed to produce a fluorescence with a UV lamp. Drugs responding to this test were listed in the article (250). This procedure is claimed to be an improved modification of a field test described by Dechert (251).

Presumptive Test for LSD—Look (141, 235) described

[28] Marijuana or Cannabis resin or reference compounds can be obtained from the National Institute of Mental Health.

a presumptive test for LSD utilizing an indicator paper. Whatman No. 1 filter paper is saturated with a 2% solution of *p*-dimethylaminobenzaldehyde in alcohol, air dried, and cut into strips 3.81–5.08 cm. (1.5–2 in.) wide. The strips are stored in a tightly capped amber glass bottle. LSD (about 40 mcg.) is extracted from ground powder with enough methanol to provide about 5 drops of liquid extract. One to two drops of liquid extract are transferred to indicator paper, and methanol is allowed to evaporate; 1 drop of hydrochloric acid is added. A violet-red or violet-blue spot develops if LSD is present. The test is sensitive to 1 mcg. of LSD.

Nonchemical Field Test for Marijuana—The sample is examined under a microscope for the presence of cystolith hairs (240, 240A). This test was already discussed under *Identification of Marijuana*.

Microtest for Marijuana—A drop of sample extract solution (see under *Preparation of Sample for TLC*) is placed on a white porcelain plate or a micro test tube and the solvent is allowed to evaporate. One drop of Duquenois' reagent (126, 240B) is added, followed by 1 drop of concentrated hydrochloric acid. The color is noted and a few drops of chloroform are added; if a violet-to-purple color is transferred to the chloroform layer, the test is positive. This test also can be performed on the suspected material directly, as recently reported by Fochtman and Winek (252). The Beam test also may be performed simultaneously.

Microtest for Narcotic Alkaloids—A small quantity of the suspected material is placed on a white porcelain spot plate, and a few drops of Marquis' reagent (2 drops of formaldehyde solution mixed with 1 ml. of concentrated sulfuric acid) are added. An intense purple color indicates the presence of an opium alkaloid such as diacetylmorphine (heroin), morphine, normorphine, desomorphine, hydromorphinol (oxymorphone), and codeine; other narcotic alkaloids give varying shades of yellow to purple. Clarke (253) tabulated the data available on colors formed by treatment of various drugs with Marquis' reagent. He suggested that this test should only be performed if the suspected material gives a positive test for the presence of alkaloid with Mayer's and Wagner's reagents. Fenimore and Davis (74) suggested the application of this test for the presence of narcotic drugs in a urine specimen. The test is based on the extraction of narcotic drugs and their metabolites from urine by means of ion-exchange resin, with subsequent development of a characteristic color directly on the resin.

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ACKNOWLEDGMENTS AND ADDRESSES

Received from the *Department of Psychiatry, University of Chicago, Chicago, IL 60637,* and the *Department of Mental Health, State of Illinois.*

The author expresses his gratitude to Dr. Jerome H. Jaffe,

Director of the Special Action Office for Drug Abuse Prevention, and Associate Professor, Department of Psychiatry, University of Chicago, and to Dr. Edward C. Senay, Director, Illinois Drug Abuse Program, and Associate Professor, Department of Psychiatry, University of Chicago, for their valuable suggestions in the preparation of this article.

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