day, the fluxes decrease very slowly and, perhaps from a practical viewpoint, may be considered to be fairly constant between the 10th and 80th day.

For a given concentration of 50 or 100 mg/ml, the initial flux per unit area of the cylinder is 1.5×10^{-1} for the 100-μm diffusion layer case and 1.15×10^{-1} for the 500-μm layer case; however, the fluxes converge in 6 hr. The corresponding time changes in the total amount of drug released from the matrix and the receding boundary zone thickness are found in Figs. 9 and 1, respectively.

Physical Significance between Progesterone and Hydrocortisone Simulation Studies—A comparison between the progesterone and hydrocortisone simulation studies gives an interesting mechanistic insight into the transport processes involved. It is believed that these model simulations, which encompass the interactions among the drug delivery device, the vaginal membrane, and the aqueous diffusion layer between the device and membrane, are the first of their kind.

The flux per unit area of the cylinder *versus* time profiles in Fig. 11 typify the differences in the transport mechanisms of progesterone and hydrocortisone. The concentration is fixed at 50 mg/ml, but the diffusion layer thickness is varied from 100 to 1000 μm. The pertinent physically meaningful parameters of these steroids (C_s, K_s, P_m, and P_{aq}) are given in Table I.

Considering only the aqueous diffusion layer and the vaginal membrane, one finds that for progesterone the resistance[3] of the diffusion layer is equal to that of the membrane when the diffusion layer thickness (h_{aq}) is 100 μm and is 10-fold greater when h_{aq} is 100 μm. Correspondingly, for the more polar hydrocortisone, the resistance of the diffusion layer is 12-fold less than that of the membrane when h_{aq} is 100 μm and the resistances are about equal when h_{aq} is 1000 μm. Thus, in general, the transport of progesterone across the aqueous and membrane layers tends to be more on the aqueous diffusion-controlled side and the transport of hydrocortisone is more membrane controlled.

When one now brings in the steroid–silicone device, the additional resistance in the matrix, which increases with the recession of the boundary with time and is in series with the aqueous layer and membrane resistances, must be considered. With the large matrix–aqueous partition coefficient, K_s, for progesterone, the change in the net flux with time is largely influenced by the aqueous diffusion layer in the first 20 days. In comparison with the small K_s of 0.05 for hydrocortisone, the net flux changes quite rapidly with time from membrane control to matrix control.

In conclusion, the *in vivo* studies of progesterone-containing silicone vaginal devices in Rhesus monkeys (8) support the physical model simulations in this present paper. In particular, it was noted (8) that the amount of steroid released was independent of progesterone concentration in the silicone device at the high 10 and 30% dose levels (in other words, pseudo-zero-order release rates); these observations were compatible with the rate-controlling process being the diffusion of the steroid across the aqueous boundary layer and the vaginal membrane until the depleted layer in the matrix is large. In another *in vivo* situation involving the matrix release of medroxyprogesterone in the human female, Roseman and Higuchi (3) estimated the aqueous layer between the device and the vaginal membrane to be about 500 μm.

Studies are continuing in which the steroid–silicone matrix is interfaced *in situ* in the rabbit vagina to demonstrate the concept of the systems model approach to drug delivery in the vagina.

[3] The resistance is defined as the reciprocal of the permeability coefficient; hence, the resistances of the diffusion layer and membrane are $1/P_{aq}$ and $1/P_m$, respectively.

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Systematic Identification of Drugs of Abuse II: TLC

ASAAD N. MASOUD

Abstract □ A limited number of spray reagents and solvent systems were selected or developed to separate and identify over 40 of the most commonly encountered drugs of abuse. A new reagent is reported, and new uses were developed for well-known reagents. A flowsheet for the systematic utilization of the spray reagents is given, and use of this sequence made it possible to identify systematically an unknown drug using only two to four TLC plates, providing that the drug was one of the compounds investigated. This TLC system also can be used to complement and confirm results obtained from spot tests.

Keyphrases □ Drugs of abuse—TLC identification, spray reagents and solvent systems selected and developed □ Abuse drugs—TLC identification, spray reagents and solvent systems selected and developed □ TLC—identification, drugs of abuse, spray reagents and solvent systems selected and developed

TLC is presently considered one of the most suited techniques for drug analysis. It is fast, requires minimal equipment, can be carried out using a minimal amount of sample, and provides highly reliable results (1).

TLC analysis with different solvent systems and different modes of detection presents a reinforced selectivity. The corroborative findings show that this technique, when properly performed, achieves effectual specificity. Thus, the multiple TLC identity test is considered equivalent in its relevancy, analytical power,

Table I—Summary of R_f Values and Detection Modes

Compound	Solvent System[a] 1	2	3	4	5	Visibility[b] Visible	Longwave UV	Shortwave UV	Spray Reagents[b] Ninhydrin–Phenylacetaldehyde	Iodoplatinate	Ninhydrin–Phenylacetaldehyde Iodoplatinate	Ehrlich's	Ehrlich's–Iodoplatinate	Mercuric Chloride–Diphenylcarbazone	Diazotized Benzidine
Alkaloids															
Atropine	0	11	18	47	0					+	+				
Caffeine	45	46	72	68	0			+					+		
Cocaine hydrochloride	81	81	65	47	0			+		+	+				
Codeine phosphate	27	25	38	47	0			+		+	+				
Ephedrine sulfate	2	14	32	63	0			+					+		
Heroin	48	40	46	52	0			+		+	+				
Lysergide	42	56	73	69	0		+	+				+			
Mescaline hydrochloride	22	17	26	69	0			+	+	+	+		+		
Morphine sulfate	5	15	36	53	0			+		+	+				
Nicotine salicylate	68	47	64	34	0			+		+	+				
Opium	6	16	36	53	0			+		+	+				
Papaverine	86	72	72	65	0		+	+		+	+				
Physostigmine salicylate	64	52	62	55	0			+		+	+				
Psilocin	22	33	36	60	0	+		+	+	+	+	+	+		
Psilocybin	0	41	5	31	0			+					+		
Quinine sulfate	19	52	53	63	0		+	+		+	+				
Scopolamine hydrobromide	21	27	67	52	0					+	+				
Strychnine	28	77	16	51	0			+		+	+				
Yohimbine hydrochloride	43	79	83	71	0		+	+		+	+				
Nonalkaloids that give positive alkaloidal reactions															
Lidocaine hydrochloride	80	82	84	60	0			+		+	+				
Meperidine[c]	65	65	53	60	0			+		+	+				
Methadone hydrochloride	64	77	38	72	0			+		+	+				
Methapyrilene hydrochloride[d]	28	46	62	48	0			+		+	+[e]	+			
Methaqualone	85	80	81	91	0			+					+		
Methylphenidate hydrochloride	68	73	65	74	0			+		+	+				
Pentazocine hydrochloride[f]	50	83	60	82	0			+		+	+				
Phencyclidine hydrochloride	83	82	48	70	0			+		+	+				
Procaine hydrochloride	31	60	71	54	0			+				+	+		
Propoxyphene napsylate[g]	77	81	80	73	0			±		+	+				
Barbiturates															
Amobarbital	65	82	86	95	0			+						+	
Phenobarbital sodium	49	77	85	95	0			+						+	
Secobarbital	70	84	84	94	0			+					+	+	
Amphetamines															
Dextroamphetamine sulfate	15	30	41	69	0			+	+						
Methamphetamine hydrochloride	26	34	26	70	0			+		+	+			+	
Miscellaneous															
Aspirin	0	25	71	92	10		±	+						+	
Benzocaine	79	78	84	92	14			+				+	+		
Cannabidiol	84	86	82	98	50			+							+
Cannabinol	82	86	81	98	45			+							+
Glutethimide[h]	77	86	85	95	0			±						+	
Meprobamate	30	76	72	86	0							+			
Phenytoin sodium[i]	48	83	86	95	0			±						+	
Δ^9-Tetrahydrocannabinol	83	85	83	98	42										+
Thiopental	82	82	90	94	10			+	+[j]	+		+	+	+	

[a] The composition of the solvent systems is given under *Experimental*. The R_f values are averages of three determinations. [b] + = easily detected spot, and ± = very faint spot. The empty spaces indicate negative reactions with the exception of the last three columns. These reagents were not attempted on the alkaloids, the compounds that give positive alkaloidal reactions, and the cannabinoids. [c] Demerol. [d] Histadyl, Dormin. [e] The intensity of the color produced by methapyrilene hydrochloride was less than that produced with iodoplatinate spray on a fresh plate. [f] Talwin. [g] Darvon-N. [h] Doriden. [i] Dilantin. [j] Thiopental produced a faint white spot 15-20 min after the application of iodoplatinate.

and utility to the IR identity test for regulatory drug analysis (2).

The purpose of this study was to develop systems, reagents, and specific techniques suited for the identification of the most commonly encountered street drugs. Forty-three drugs were considered along with these goals: (*a*) to limit the number of spray reagents needed, (*b*) to reduce the number of TLC plates run per sample, and (*c*) to limit the number of solvent systems to a minimum.

EXPERIMENTAL

Materials—The 43 drugs investigated in this work had been subjected to spot tests previously (3). Based on the results obtained from the alkaloidal precipitating agents (3), drugs that give a positive test to Mayer's, Dragendorff's, or Wagner's reagents are grouped to-

Table II—Results Produced by Ninhydrin–Phenylacetaldehyde Spray

Compound	Color	Remarks
Mescaline hydrochloride	Fluorescent yellow	Visible with other reagents
Psilocin	White spot with dark center	Visible upon standing and with other reagents
Dextroamphetamine sulfate	Fluorescent yellow	Becomes pink with Ehrlich's reagent; not visible with any other reagent investigated

gether and referred to as nonalkaloids that give a positive alkaloidal reaction. Otherwise, the drugs[1] listed in Table I are grouped according to their chemical nature.

Preparation of Reference Standards—Examination of the solubility of the compounds investigated showed that most were soluble in alcohol USP except for the following (4). Lysergide was obtained as an aqueous solution and was diluted using distilled water. Yohimbine hydrochloride and dextroamphetamine sulfate were dissolved in 50% ethanol. A solution of 2 mg/ml was prepared in the appropriate solvent, with the exception of lysergide where a concentration of 0.1 mg/ml was used.

These solutions were kept in tightly closed vials in the refrigerator. They were stable for several months under these conditions, with the exception of the alkaloid psilocin. Psilocin, once in solution, had to be used within a few days. It decomposed completely, even in the refrigerator, to a dark-brown solution which did not contain any of the original reference alkaloid. However, it is recommended that fresh reference standards be prepared every 3–4 months.

Preparation of Unknown Samples—If a sample is suspected to contain a certain compound or compounds, it is dissolved in the same solvent as the standards. But if it is completely unknown, the sample is divided into three parts and three solutions are prepared using alcohol USP, 50% ethanol, and water as solvents.

To assure dissolution of the drug, the test tube is shaken thoroughly using a vortex apparatus and is also heated for a few minutes over a steam bath. Then the sample is either filtered or allowed to sit so that the suspended material can settle prior to spotting.

Chromatography Equipment—Silica gel GF-254 TLC plates[2], 0.25 mm, were activated by warming at 110° for 1 hr.

Standard size development tanks were lined[3] and allowed to equilibrate. The paper lining should be wetted with solvent before the chromatography is started (5). Solvent systems were changed frequently for reproducible results.

Application of Drugs—Approximately 5 μl of the standard solution was applied on the TLC plates. When the concentration of the sample is unknown, it is advisable to apply larger quantities to assure detection of compounds present in low concentrations.

Solvent Systems—Five solvent systems were satisfactory for the screening and identification of all drugs studied. These solvents were: 1, chloroform–ether–methanol–concentrated ammonium hydroxide (75:25:5:1) (6); 2, ethyl acetate–1-propanol–concentrated ammonium hydroxide (40:30:3) (7); 3, methanol–concentrated ammonium hydroxide (100:1.5) (8); 4, alcohol USP–acetic acid–water (60:30:10) (8); and 5, benzene (9).

Systematic Utilization of Solvent Systems—If the substance is completely unknown, two plates are prepared and developed in Solvent Systems 1 and 2. After detection and tentative identification, one or two more plates may be prepared and developed in one or more of the other solvent systems.

If the substance is suspected to be a specific compound, it is sufficient to select two appropriate systems based on the information in Table I.

Solvent System 5 was used previously for the identification of marijuana constituents (9) and was incorporated in this study specifically to separate cannabinoids.

[1] These drugs were obtained from the United States Pharmacopeial Convention, Inc., the National Institute of Mental Health, and miscellaneous pharmaceutical and chemical companies.

[2] Prepared in-house using Desage equipment.

[3] Using Whatman No. 1 chromatography paper.

Table III—Compounds Reacting with Ehrlich's Reagent and Ehrlich's–Iodoplatinate Combination Reagent

Compound	Color Produced with Ehrlich's Reagent	Color Produced with Combination Reagent
Lysergide	Purple	Color disappears
Psilocin	Blue	Violet purple
Procaine hydrochloride	Yellow	Dark brown
Thiopental	Chalky white	Orange
Benzocaine	Yellow	Dark brown
Meprobamate	White; becomes yellow with heating	Color disappears

Detection—Developed plates were placed in a fume hood and allowed to dry for 10 min or until the odor of ammonium hydroxide disappeared. The plates were examined for visible spots and then viewed under both longwave and shortwave UV light and marked.

If the compound is completely unknown, the systematic utilization scheme of the spray reagents (Scheme I) should be followed. If the substance is suspected to be a certain compound, the appropriate spray reagent or combination of reagents can be selected from Table I. The composition and application of the spray reagents used are discussed here.

Ninhydrin–Phenylacetaldehyde Spray—Solution A is 0.4% ninhydrin in pH 9 phosphate buffer. Solution B is 0.5% phenylacetaldehyde in alcohol USP.

Spray lightly with Solution A and then apply a heavier spray with Solution B. Let the plate sit for about 10 min. Heating the plate in an oven at 100° for a few minutes speeds the reaction.

This reagent has not been reported in the literature. However, the use of the condensation products of ninhydrin with aldehydes for the detection of amines was reported (10). Fluorescamine, which forms the same fluorophores with amines, was reported (11) for the assay of minute quantities of amines and later was used for the detection of amphetamines in urine (11, 12).

Iodoplatinate Spray (13)—Solution A is 10% hexachloroplatinic(IV) acid solution. Solution B is 6% aqueous potassium iodide solution.

Mix 3 ml of Solution A with 100 ml of Solution B and add 97 ml of distilled water.

Ehrlich's Spray (14)—Prepare 0.2% solution of *p*-dimethylaminobenzaldehyde in 10% hydrochloric acid. To each 100 ml, add 0.2 ml of 5% ferric chloride solution. The reagent decomposes with time and is light sensitive. Therefore, it should be prepared weekly and protected from light.

Mercuric Chloride–Diphenylcarbazone Spray (15)—Solution A is 0.2% (w/v) diphenylcarbazone in 95% ethanol. Solution B is 2.0% (w/v) mercuric chloride in 95% ethanol.

Mix equal volumes of the two solutions prior to use.

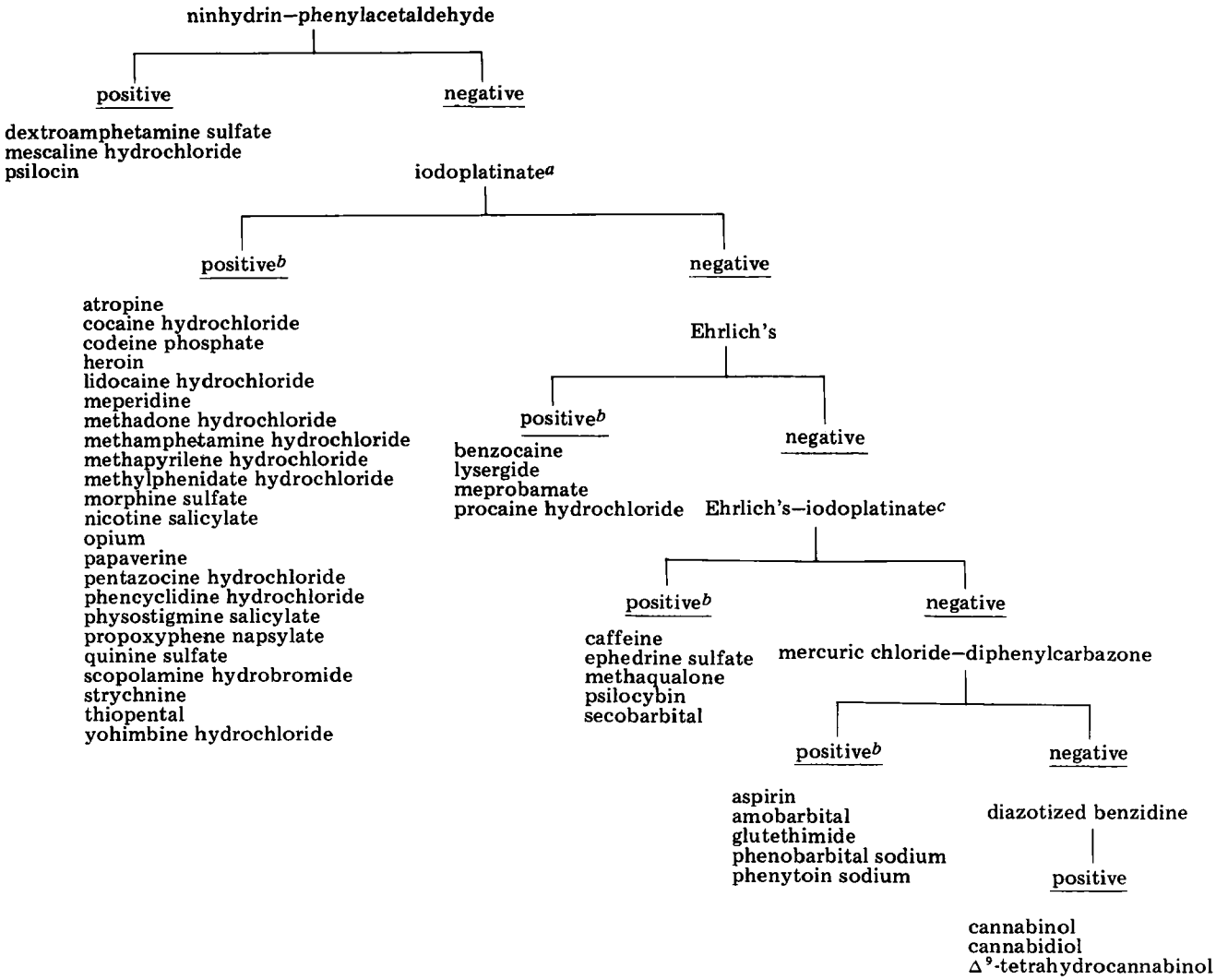
Diazotized Benzidine Spray (9, 16)—Solution A is 5 mg of benzidine dihydrochloride in 14 ml of concentrated hydrochloric acid diluted to 1 liter with distilled water. Solution B is 10% sodium nitrite solution.

Mix equal volumes of the two solutions immediately prior to use. The mixed spray deteriorates in a few hours.

RESULTS AND DISCUSSION

The two main parameters involved in the identification of unknown substances by TLC are related to detection and R_f values. Detection involves the visibility of the substance or substances with visible light, under long- or shortwave UV light, or by the chromogenic reaction produced by a certain spray reagent or a combination of spray reagents. The R_f values are only approximate values and are affected by many variables. Therefore, it is absolutely necessary to use different solvent systems and different modes of detection to identify unknown substances.

Detection—The visibility characteristics of substances and the color reactions that they produce with certain spray reagents are an important part of the identification process by TLC. The results pertaining to each detection mode are discussed in the sequence of actual utilization during identification procedures. Utilization of these detection modes in this particular sequence is particularly useful if



Scheme I—Systematic utilization of spray reagents. [a] Iodoplatinate may be sprayed on the same plate on which ninhydrin–phenylacetaldehyde has been sprayed. However, the color reactions produced by thiopental and methapyrilene hydrochloride may become difficult to detect. [b] Compounds producing color reactions with earlier reagents not included. See Table I for more complete information. [c] Iodoplatinate should be sprayed on the same plate previously sprayed with Ehrlich's reagent.

the substance is of a completely unknown nature. Furthermore, this sequence provides maximum information from each TLC plate, thus reducing the number of plates necessary for the identification of the unknown.

Scheme I provides the sequence in which the spray reagents are utilized, and Table I includes data pertaining to color reactions and R_f values.

Visible Compounds—Psilocin was the only compound visible under visible light. The alkaloid developed a gray color which darkened with time. The color developed in a few minutes in a 100° oven or in 15–20 min at room temperature. This color was produced by the decomposition product(s) of the compound.

Visibility under UV Light—All compounds investigated were visible under shortwave UV light except atropine, scopolamine hydrobromide, Δ^9-tetrahydrocannabinol, and meprobamate. However, only lysergide, papaverine, quinine, yohimbine hydrochloride, and aspirin were visible under longwave UV light. The visibility of these compounds was due to their fluorescent nature or to their quenching of the fluorescent background of the adsorbent material used in this work.

Ninhydrin–Phenylacetaldehyde Spray—Dextroamphetamine sulfate has been a very difficult compound to detect by TLC. Several techniques including potassium permanganate spray (8), fast blue B followed by sodium hydroxide and again by fast blue B (13), acid–base extraction of the dextroamphetamine salt to produce the base prior to the application of the iodoplatinate (7), and iodine solution in methanol (6) were attempted but did not produce satisfactory results.

It was possible to detect amphetamines using 0.5% ninhydrin in butanol as described by Kaistha and Jaffe (17). However, many of the drugs used in this work produced similar chromogenic reactions to those of amphetamines, thus rendering the reagent less specific than ninhydrin–phenylacetaldehyde.

The ninhydrin–phenylacetaldehyde described in this work is highly sensitive, easy to use, and can be stored for months without deterioration. The yellow fluorescent color produced by the application of this reagent to dextroamphetamine sulfate was converted to a very characteristic pink color upon the application of Ehrlich's reagent. This new color adds to the value of the reagent in the identification process.

Mescaline hydrochloride produced a similar chromogenic reaction with the reagent, but the color was not converted to pink upon the application of Ehrlich's reagent. Psilocin produced a white spot with a dark center with this reagent. The results pertaining to the application of this reagent are summarized in Table II.

In conclusion, ninhydrin–phenylacetaldehyde is used only if dextroamphetamine or any of its salts are suspected. However, methamphetamine hydrochloride did not produce any color reaction with this reagent. After obtaining the information sought from the plates sprayed with this reagent, the plates can be sprayed with iodoplatinate spray without interference with the results, with the exception of methapyrilene hydrochloride and thiopental which may

Table IV—Compounds Reacting Only upon the Use of the Combination of Ehrlich's Reagent followed by Iodoplatinate Spray

Compound	Color Produced
Caffeine	Purple
Ephedrine sulfate	Brown
Mescaline hydrochloride	Orange
Psilocybin	Brown
Methaqualone	Brown
Methapyrilene hydrochloride	Deep blue
Secobarbital	Pink

become difficult to detect (Table I).

Iodoplatinate Spray—This reagent, as expected, reacted with all alkaloids investigated (Table I) except caffeine, ephedrine sulfate, and lysergide. It also reacted with all nonalkaloids that give a positive alkaloidal reaction except methaqualone and procaine hydrochloride (3). Of the drugs listed in other groups, thiopental and methamphetamine hydrochloride produced a color reaction.

Ehrlich's Spray—This reagent is known to produce blue or purple colors with ergot alkaloids and other indole compounds (8, 14). In this study, a number of other compounds produced various chromogenic reactions with this reagent. Those colors underwent certain changes upon the application of iodoplatinate spray following Ehrlich's spray. The color change is considered a valuable additional characteristic for the identification of these compounds (Table III). The compounds listed in Table III, except psilocin and thiopental, were not detected by ninhydrin–phenylacetaldehyde or iodoplatinate sprays.

Furthermore, a number of compounds that did not produce a color reaction with Ehrlich's reagent produced characteristic colors upon the application of iodoplatinate spray on a plate previously sprayed with Ehrlich's reagent (Table IV). Thus, Ehrlich's–iodoplatinate combination reagent made it possible to detect and identify some new compounds not visible by either spray separately. All of the compounds listed in Table IV, except mescaline hydrochloride, methapyrilene hydrochloride, and secobarbital, were not detected by any other reagent.

Mercuric Chloride–Diphenylcarbazone Spray—This reagent is used for the detection of barbiturates (15). It produced a characteristic purple color with these compounds, with the exception of secobarbital which developed a white spot with a purple margin. Other compounds that reacted with this reagent are listed in Table V.

Diazotized Benzidine Spray—This reagent is used to detect cannabinoids and marijuana (9, 16). It produced a characteristic orange color with the three cannabinoids studied.

R_f Values—The data pertaining to the R_f values of all compounds investigated in Solvent Systems 1–5 are summarized in Table I. These solvent systems were satisfactory in most cases.

If the unknown is suspected to be a certain chemical, two appropriate solvent systems may be chosen. However, if the sample is completely unknown, Solvent Systems 1 and 2 are recommended first; then one or more of the other solvents may be used if confirmatory tests are necessary.

Solvent System 5 is suggested only when cannabinoids or marijuana extracts are suspected.

Table V—Results of Mercuric Chloride–Diphenylcarbazone Spray

Compound	Color Produced
Aspirin[a]	White
Amobarbital[a]	Purple
Phenobarbital sodium[a]	Purple
Phenytoin sodium[a]	Purple
Glutethimide[a]	Purple
Methamphetamine hydrochloride	Blue
Secobarbital	White center and purple margin
Thiopental	Purple

[a] These compounds did not produce colors with any other reagent.

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