

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  $1.5 \times 10^{-1}$  for the 100- $\mu$ m diffusion layer case and  $1.15 \times 10^{-1}$  for the 500- $\mu$ m 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  $\mu$ m. 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<sup>3</sup> of the diffusion layer is equal to that of the membrane when the diffusion layer thickness ( $h_{aq}$ ) is 100  $\mu$ m and is 10-fold greater when  $h_{aq}$  is 1000  $\mu$ m. 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  $\mu$ m and the resistances are about equal when  $h_{aq}$  is 1000  $\mu$ m. 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 hy-

drocortisone, 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  $\mu$ m.

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.

## REFERENCES

- (1) T. Yotsuyanagi, A. Molokhia, S. Hwang, N. F. H. Ho, G. L. Flynn, and W. I. Higuchi, *J. Pharm. Sci.*, **64**, 71(1975).
- (2) S. Hwang, E. Owada, T. Yotsuyanagi, L. Suhardja, N. F. H. Ho, G. L. Flynn, and W. I. Higuchi, *ibid.*, **65**, 1574(1976).
- (3) T. J. Roseman and W. I. Higuchi, *ibid.*, **59**, 353(1970).
- (4) T. J. Roseman, *ibid.*, **61**, 46(1972).
- (5) T. J. Roseman and S. H. Yalkowsky, *ibid.*, **63**, 1639(1974).
- (6) G. L. Flynn, N. F. H. Ho, W. I. Higuchi, and J. Kent, *ibid.*, **65**, 157(1976).
- (7) K. Desai, Ph.D thesis, University of Michigan, Ann Arbor, Mich., 1976.
- (8) K. T. Kirton, T. J. Roseman, and A. D. Forbes, *Contraception*, **8**, 561(1973).

## ACKNOWLEDGMENTS AND ADDRESSES

Received October 6, 1975, from the College of Pharmacy, University of Michigan, Ann Arbor, MI 48104

Accepted for publication December 22, 1975.

Supported by Contract NO1-HD-3-2740, National Institute of Child Health and Human Development, Bethesda, Md.

The authors thank Dr. Theodore Roseman, The Upjohn Co., Kalamazoo, Mich., for the generous supply of Silastic 382 membranes.

\* To whom inquiries should be directed.

<sup>3</sup> 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.

# 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 <sup>a</sup> |             |              |    |    | Spray Reagents <sup>b</sup> |   |   |                              |               |                                            |           |                         |                                     |                      |
|------------------------------------------------------|-----------------------------|-------------|--------------|----|----|-----------------------------|---|---|------------------------------|---------------|--------------------------------------------|-----------|-------------------------|-------------------------------------|----------------------|
|                                                      |                             |             |              |    |    | Visibility <sup>b</sup>     |   |   | Ninhydrin-Phenylacetaldehyde | Iodoplatinate | Ninhydrin-Phenylacetaldehyde Iodoplatinate | Ehrlich's | Ehrlich's-Iodoplatinate | Mercuric Chloride-Diphenylcarbazone | Diazotized Benzidine |
|                                                      | Visible                     | Longwave UV | Shortwave UV |    |    |                             |   |   |                              |               |                                            |           |                         |                                     |                      |
| 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 <sup>c</sup>                              | 65                          | 65          | 53           | 60 | 0  |                             |   | + |                              | +             | +                                          |           |                         |                                     |                      |
| Methadone hydrochloride                              | 64                          | 77          | 38           | 72 | 0  |                             |   | + |                              | +             | +                                          |           |                         |                                     |                      |
| Methapyrilene hydrochloride <sup>d</sup>             | 28                          | 46          | 62           | 48 | 0  |                             |   | + |                              | +             | +                                          | +         |                         |                                     |                      |
| Methaqualone                                         | 85                          | 80          | 81           | 91 | 0  |                             |   | + |                              |               |                                            |           | +                       |                                     |                      |
| Methylphenidate hydrochloride                        | 68                          | 73          | 65           | 74 | 0  |                             |   | + |                              | +             | +                                          |           |                         |                                     |                      |
| Pentazocine hydrochloride <sup>f</sup>               | 50                          | 83          | 60           | 82 | 0  |                             |   | + |                              | +             | +                                          |           |                         |                                     |                      |
| Phencyclidine hydrochloride                          | 83                          | 82          | 48           | 70 | 0  |                             |   | + |                              | +             | +                                          |           |                         |                                     |                      |
| Procaine hydrochloride                               | 31                          | 60          | 71           | 54 | 0  |                             |   | + |                              |               |                                            | +         | +                       |                                     |                      |
| Propoxyphene napsylate <sup>g</sup>                  | 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 <sup>h</sup>                            | 77                          | 86          | 85           | 95 | 0  |                             |   | ± |                              |               |                                            |           |                         |                                     | +                    |
| Meprobamate                                          | 30                          | 76          | 72           | 86 | 0  |                             |   |   |                              |               |                                            |           | +                       |                                     |                      |
| Phenytoin sodium <sup>i</sup>                        | 48                          | 83          | 86           | 95 | 0  |                             |   | ± |                              |               |                                            |           |                         |                                     | +                    |
| Δ <sup>9</sup> -Tetrahydrocannabinol                 | 83                          | 85          | 83           | 98 | 42 |                             |   |   |                              |               |                                            |           |                         |                                     | +                    |
| Thiopental                                           | 82                          | 82          | 90           | 94 | 10 |                             |   | + | +                            | +             |                                            | +         | +                       | +                                   |                      |

<sup>a</sup>The composition of the solvent systems is given under *Experimental*. The  $R_f$  values are averages of three determinations. <sup>b</sup>+ = 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. <sup>c</sup>Demerol. <sup>d</sup>Histadyl, Dormin. <sup>e</sup>The intensity of the color produced by methapyrilene hydrochloride was less than that produced with iodoplatinate spray on a fresh plate. <sup>f</sup>Talwin. <sup>g</sup>Darvon-N. <sup>h</sup>Doriden. <sup>i</sup>Dilantin. <sup>j</sup>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<sup>1</sup> 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<sup>2</sup>, 0.25 mm, were activated by warming at 110° for 1 hr.

Standard size development tanks were lined<sup>3</sup> 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.

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

<sup>2</sup> Prepared in-house using Desage equipment.

<sup>3</sup> 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*-dimethylnobenzaldehyde 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.

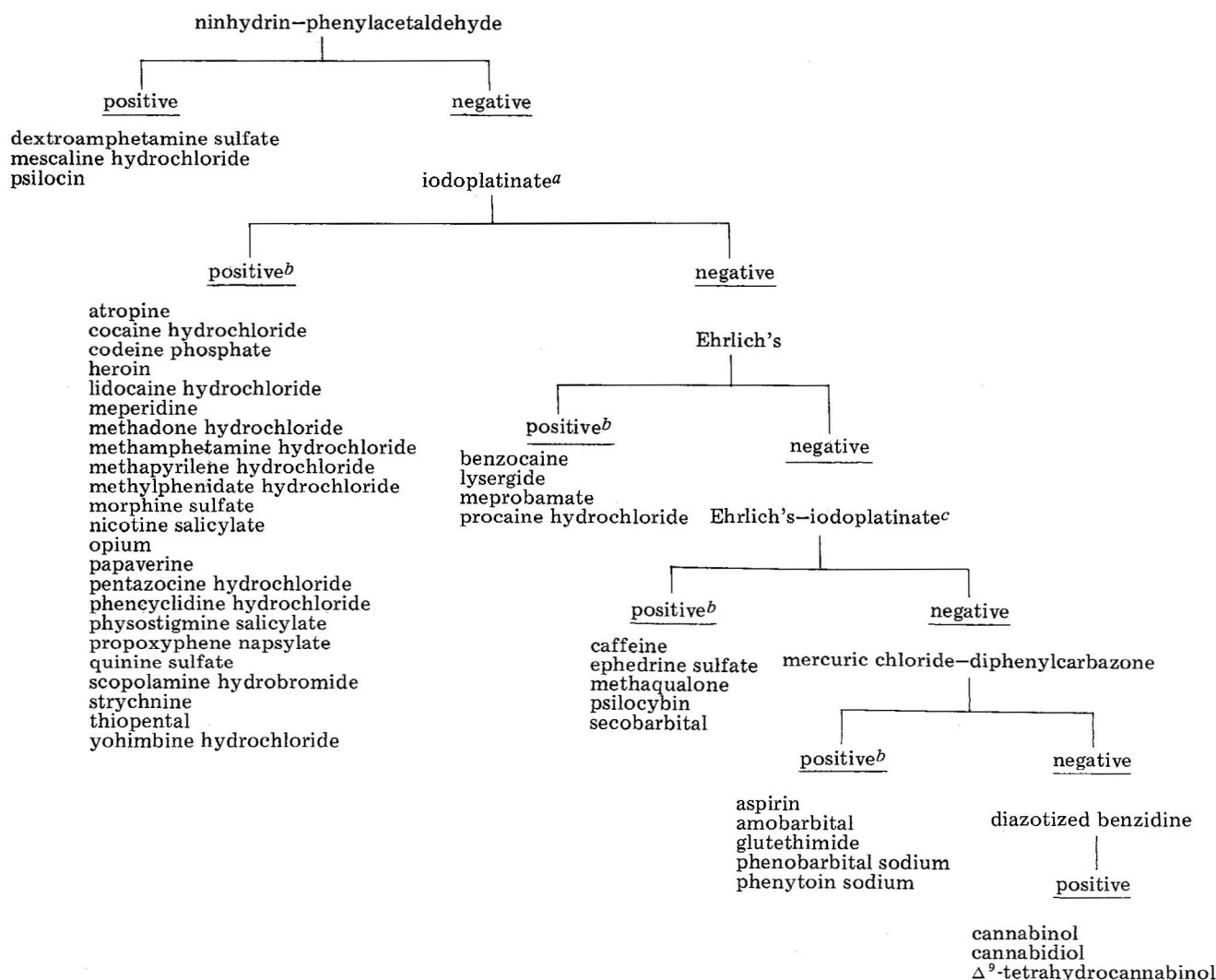
**Diazotized Benzidine Spray (9, 16)**—Solution A is 5 mg of benzidine dihydrochloride in 14 ml of concentrated hydrochloric acid diluted to 1 liter with distilled water. Solution B is 10% sodium nitrite solution.

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<sub>f</sub>* 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<sub>f</sub>* 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. <sup>a</sup> 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. <sup>b</sup> Compounds producing color reactions with earlier reagents not included. See Table I for more complete information. <sup>c</sup> 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,  $\Delta^9$ -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<sub>f</sub> Values**—The data pertaining to the R<sub>f</sub> values of all compounds

**Table V—Results of Mercuric Chloride-Diphenylcarbazone Spray**

| Compound                          | Color Produced                 |
|-----------------------------------|--------------------------------|
| Aspirin <sup>a</sup>              | White                          |
| Amobarbital <sup>a</sup>          | Purple                         |
| Phenobarbital sodium <sup>a</sup> | Purple                         |
| Phenytoin sodium <sup>a</sup>     | Purple                         |
| Glutethimide <sup>a</sup>         | Purple                         |
| Methamphetamine hydrochloride     | Blue                           |
| Secobarbital                      | White center and purple margin |
| Thiopental                        | Purple                         |

<sup>a</sup> These compounds did not produce colors with any other reagent.

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.

## REFERENCES

- (1) E. Stahl, "Drug Analysis by Chromatography and Microscopy," 1st ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1973, p. 3.
- (2) D. Banes, "A Chemists Guide to Regulatory Drug Analysis," The Association of Official Analytical Chemists, Benjamin Franklin Station, Washington, D.C., 1974, p. 63.
- (3) A. N. Masoud, *J. Pharm. Sci.*, **64**, 841(1975).
- (4) "Handbook of Analytical Toxicology," I. Sunshine, Ed., The Chemical Rubber Co., Cleveland, Ohio, 1969, pp. 2-123.
- (5) J. Bobbitt, A. Schwarting, and R. Gritter, "Introduction to Chromatography," Reinhold, New York, N.Y., 1968, pp. 54, 58.
- (6) R. A. Van Welsum, *J. Chromatogr.*, **78**, 237(1973).
- (7) J. K. Brown, L. Shapazian, and G. D. Griffin, *ibid.*, **64**, 129(1972).
- (8) E. G. C. Clarke, "Isolation and Identification of Drugs," Pharmaceutical Press, London, England, 1969, pp. 46, 51, 192.
- (9) A. N. Masoud and N. J. Doorenbos, *J. Pharm. Sci.*, **62**, 313(1973).
- (10) K. Samejima, W. Dairman, J. Stone, and S. Udenfriend, *Anal. Biochem.*, **42**, 237(1971).
- (11) S. Udenfriend, S. Stein, P. Bohlen, and W. Dairman, *Science*, **178**, 871(1972).
- (12) B. Klein, J. Sheehan, and E. Grunberg, *Clin. Chem.*, **20**, 272(1974).
- (13) E. Stahl, "Thin-Layer Chromatography, A Laboratory Handbook," 2nd ed., Springer-Verlag, New York, N.Y., 1969, pp. 883, 893, 520, 869.
- (14) S. K. Sim, "Medicinal Plant Alkaloids," 2nd ed., University of Toronto Press, Toronto, Canada, 1965, p. 97.
- (15) D. J. Berry and J. Grove, *J. Chromatogr.*, **80**, 205(1973).
- (16) H. Aramaki, N. Tomiyasu, H. Yoshimura, and H. Tsukamoto, *Chem. Pharm. Bull.*, **16**, 822(1968).
- (17) K. K. Kaistha and J. H. Jaffe, *J. Pharm. Sci.*, **61**, 679(1972).

## ACKNOWLEDGMENTS AND ADDRESSES

Received August 23, 1974, from the *School of Pharmacy, Creighton University, Omaha, NE 68178*

Accepted for publication January 12, 1976.

Presented at the joint meeting of the American Society of Pharmacognosy and the Pharmacognosy and Natural Products Section, APHA Academy of Pharmaceutical Sciences, Chicago meeting, August 1974. The presentation of this paper at the meeting was made possible by the award granted to the author by Lederle Co.

The author expresses his appreciation to Dr. Irving Sunshine, Chief Toxicologist, Cuyahoga County Coroner's Office, Cleveland, Ohio, for suggesting the use of the reagent ninhydrin-phenylacetaldehyde and for his encouragement to publish the method described in this paper.

Present address: Department of Anesthesiology, University of Nebraska Medical Center, Omaha, NE 68105