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ACKNOWLEDGMENTS AND ADDRESSES

Received August 30, 1974, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication October 30, 1974.

Supported in part by a Merck Grant for Faculty Development (to H.-L. Fung) from the Merck Co. Foundation, and in part by General Research Support Grant 5-S01RR-05454-12 from the General Research Support Branch, Division of Research Facilities and Resources, National Institutes of Health.

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Systematic Identification of Drugs of Abuse I: Spot Tests

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Abstract □ More than 40 of the most commonly encountered street drugs were subjected to several spot tests. These tests were carried out in a special sequence leading to the construction of a flowsheet. Thus, with a limited number of simple tests, it is possible to identify tentatively or narrow down the drug. Since each drug investigated was subjected to all selected tests, whether such tests were developed for this type of compound or not, some unexpected and undocumented results were obtained.

Keyphrases □ Abuse drugs—systematic identification, spot tests, flow chart □ Drugs, abuse—systematic identification, spot tests, flow chart □ Spot tests—drugs of abuse, systematic identification

Spot tests still serve a valuable role in the identification of abused drugs today. Since many of the abused drugs are nonproprietary preparations and are prepared and marketed without any standards or quality control by any authority, they often contain substances other than what they are alleged to contain. Therefore, a fast simple battery of tests to provide preliminary information concerning the drug is needed.

In spite of the limitations of spot tests, such as the occurrence of false positives or false negatives, the lack of specificity, and the difficulty of interpreting some results, they still provide speedy answers to preliminary questions pertaining to these substances. The useful information provided by spot tests includes: *(a)* the definite absence of a compound or a group of compounds, and *(b)* the possible presence of a compound or compounds that belong to a certain group. The positive information provided by these tests will help in the selection of the specific confirmatory tests necessary.

The purpose of this work was to increase the usefulness of spot tests. More than 40 of the most common street drugs were subjected to nine different reagents. The results obtained provided a basis for the systematic classification of these drugs on an identification scheme. They also produced a number of unexpected and undocumented uses for conventional tests. Many false negatives and false positives were encountered and documented. These results increase the value of the tests once they are recognized and considered.

EXPERIMENTAL

Materials—The 43 drugs[1] investigated in this work are grouped in Table I according to their chemical nature and some of the results obtained in this work.

Preparation of Samples—The preparation of the standard references for the spot tests varied according to the test and the form of the drug provided. In general, the spot tests were performed directly on the powdered or liquid forms. For specific tests where no moisture is desired and where the drug has to be in a powdered form, solvents were removed from drugs available only in solution.

Generally, 1–2 mg was used for the tests; however, lysergide was detected in quantities as low as 5 μg. The tests were performed in 10-ml clear glass test tubes, and observation continued for approximately 30 min after the tests were completed.

Reagents and Procedures—The following reagents were prepared as indicated.

Mayer's Reagent (1)—This reagent consists of: mercuric chloride, 0.68 g; potassium iodide, 2.5 g; and distilled water to make 100 ml.

Dragendorff's Reagent (2)—Solution A consists of: bismuth subnitrate, 0.85 g; distilled water, 40 ml; and acetic acid, 10 ml. Solution B consists of: potassium iodide, 8.0 g; and distilled water, 20 ml. To prepare the concentrate, 5 volumes of A and 2 volumes of B are mixed. In this investigation, 20 ml of acetic acid was added to 10 ml of the concentrate, which was then diluted with 100 ml of distilled water (3).

Wagner's Reagent (4)—This reagent consists of: iodine, 1.27 g; potassium iodide, 2 g; and distilled water to make 100 ml. A small amount of the drug is dissolved in a few drops of 10% HCl. The reagent is then added dropwise to the acidic solution. A precipitate is

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

Table I—Investigated Drugs

Alkaloids
Atropine
Caffeine
Cocaine hydrochloride
Codeine phosphate
Diacetylmorphine hydrochloride (heroin)
Ephedrine sulfate
Lysergide (lysergic acid diethylamide tartrate)
Mescaline hydrochloride
Morphine sulfate
Nicotine salicylate
Opium
Papaverine
Physostigmine salicylate
Psilocin
Psilocybin
Quinine sulfate
Scopolamine hydrobromide
Strychnine
Yohimbine hydrochloride
Compounds that Give Positive Alkaloidal Reaction
Lidocaine hydrochloride
Meperidine[a]
Methadone hydrochloride
Methaqualone
Methylphenidate hydrochloride
Pentazocine hydrochloride[b]
Phencyclidine hydrochloride
Procaine hydrochloride
Propoxyphene napsylate[c]
Methapyrilene hydrochloride[d]
Barbiturates
Amobarbital
Phenobarbital sodium
Secobarbital
Amphetamines
Amphetamine sulfate
Methamphetamine hydrochloride
Miscellaneous
Aspirin
Benzocaine
Cannabidiol
Cannabinol
Diphenylhydantoin sodium[e]
Glutethimide[f]
Meprobamate
Δ^9-Tetrahydrocannabinol (II)
Thiopental

[a] Demerol. [b] Talwin. [c] Darvon. [d] Dormin. [e] Dilantin. [f] Doriden.

formed with most alkaloids and some other organic nitrogenous compounds. This procedure is followed with the above three alkaloidal reagents.

Marquis Reagent (5)—This reagent consists of: concentrated sulfuric acid, 10 ml; and formaldehyde solution, 40%, 8–10 drops. It is freshly mixed prior to use. An intense purple color is considered a positive; this test is for opiates.

Cobalt Thiocyanate (5)—This reagent is an aqueous solution of 2% cobalt thiocyanate. A flaky blue precipitate is considered a positive; this test is for cocaine and related compounds.

Table II—Alkaloids that Give Negative Tests with One or More Reagents

Compound	Reagent		
	Mayer's	Dragendorff's	Wagner's
Caffeine	−	+	+
Heroin	+	+	−
Ephedrine	−	−	−
Lysergide	−	−	−
Mescaline	−	−	−
Morphine	+	+	−
Psilocin	−	−	+
Psilocybin	−	−	−

Table III—Nonalkaloids that Give Positive Alkaloidal Tests with One or More Reagents

Compound	Mayer's	Dragendorff's	Wagner's
Lidocaine hydrochloride	+	+	+
Meperidine	+	+	+
Methadone hydrochloride	+	+	+
Methaqualone	+	+	+
Methylphenidate hydrochloride	−	+	−
Pentazocine hydrochloride	+	+	+
Phencyclidine hydrochloride	+	+	+
Procaine hydrochloride	−	+	−
Propoxyphene napsylate	+	+	+
Methapyrilene hydrochloride	+	+	+

Zwikker's Test (6)—Solution A consists of 1% cobalt acetate in methanol, and Solution B consists of 5% isopropylamine in methanol. In the absence of water, the test is performed by adding to a small amount of the drug a few drops of A followed by a few drops of B. A blue-violet color is the positive test; this test is for barbiturates.

Mandelin's Test (7)—Ammonium vanadate, 1%, must be added gradually to concentrated sulfuric acid with continuous stirring. The reagent should acquire an orange color. In this investigation, only the formation of an instant conspicuous color is considered positive.

Ehrlich's Reagent (8)—A 0.2% solution of *p*-dimethylaminobenzaldehyde in 65% sulfuric acid is prepared, and 0.2 ml of 5% ferric chloride solution is added to each 100 ml. This reagent decomposes with light and should be prepared weekly. A purple to deep-blue color that develops slowly and becomes more intense with time is a positive; this test is for lysergide.

Duquenois Reagent (5)—Five drops of acetaldehyde and 0.4 g of vanillin are dissolved in 20 ml of alcohol USP. This reagent should be kept in a glass-stoppered bottle and protected from light; it should be discarded upon assuming a deep-yellow color.

To the drug or extract, a few drops of the reagent are added followed by a few drops of concentrated hydrochloric acid, and the mixture is shaken. Then a few drops of chloroform are added and the mixture is shaken again. The test is considered positive if a blue-violet color is developed upon the addition of the hydrochloric acid and the color migrates to the chloroform layer; this test is for marijuana and cannabinoids.

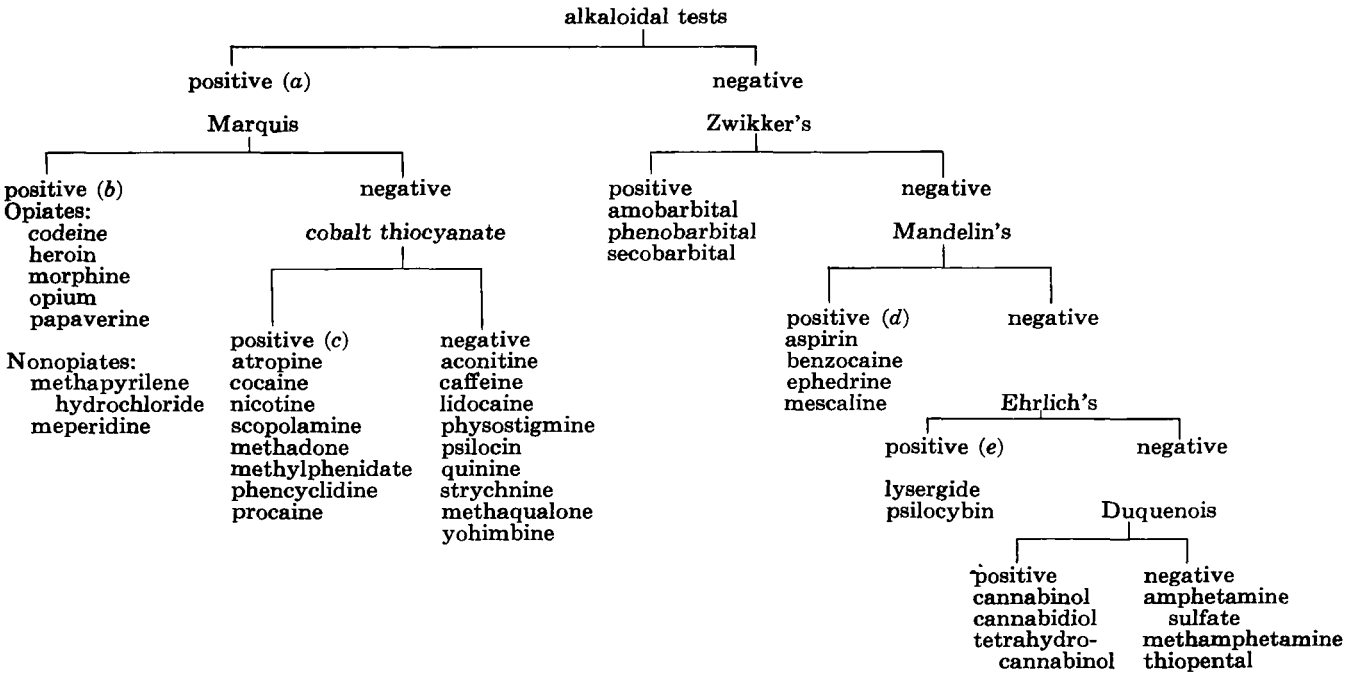
RESULTS AND DISCUSSION

Alkaloidal Reagents—All investigated drugs (Table I) were subjected to Mayer's, Wagner's, and Dragendorff's reagents, and a number of alkaloids gave negative reactions to one or more of them. These alkaloids are listed in Table II. All other alkaloids, namely atropine, cocaine, codeine, nicotine, opium powder, papaverine, physostigmine, quinine, scopolamine, strychnine, and yohimbine, gave a positive test to all three reagents.

A number of nonalkaloids gave positive reactions to one or more of the reagents used (Table III). These compounds have been designated as compounds that give a positive alkaloidal reaction. All

Table IV—Compounds that Give Positive Reaction with Cobalt Thiocyanate Reagent

Atropine	Meperidine
Cocaine hydrochloride	Methadone hydrochloride
Codeine phosphate	Methylphenidate hydrochloride
Heroin	Phencyclidine hydrochloride
Nicotine salicylate	Procaine hydrochloride
Opium	Methapyrilene hydrochloride
Scopolamine hydrobromide	Phenobarbital sodium



Scheme I—Systematic utilization of spot tests. Key: a, *compounds that gave a positive test with any of the three reagents used;* b, *other compounds given in text do not give positive alkaloidal tests;* c, *other compounds listed in Table IV do not give positive alkaloidal tests;* d, *see criteria for a positive Mandelin's reagent in the text; and* e, *psilocin also gives a positive for Ehrlich's reagent but it does give positive alkaloidal tests.*

other nonalkaloids tested gave negative results to these reagents.

Marquis Reagent—This reagent produces a characteristic intense purple color that becomes dark purple and then dark blue-violet with opiates (5–7). Such color was observed as expected with the five opiates tested, *i.e.*, codeine phosphate, heroin, morphine sulfate, papaverine, and opium powder. However, some nonopiates produced colors that are very similar to those produced by opiates. These false positives produced by ephedrine sulfate, methapyrilene hydrochloride, amphetamine sulfate, meperidine, and methamphetamine hydrochloride are not documented in other references known to the author and should be considered when interpreting the results of this test.

Cobalt Thiocyanate Test—This test is described (5) as an identification test for cocaine and procaine hydrochloride (5), but the characteristic blue flaky precipitate was also produced in this investigation with other drugs. Table IV lists all of the drugs that produced a positive test with this reagent. The lack of specificity of this test should be considered. However, these drugs are either alkaloids or compounds that give positive alkaloidal reaction with the exception of phenobarbital sodium.

Zwikker's Test—This test, also referred to as the alkaline cobalt test, was discussed in detail by Clarke (6). According to Clarke, a blue-violet color is produced by barbiturates as well as glutethimide. However, in this laboratory, utilizing the reagents described earlier, only the three barbiturates used (amobarbital, phenobarbital sodium, and secobarbital) produced such color. It was not possible to produce a positive test with glutethimide. The color produced by glutethimide reference standard was gray and was not considered a positive reaction. None of the other drugs investigated produced positive reactions with this test.

Table V—Easily Characterized Colors Produced by Mandelin's Reagent

Compound	Color Produced Instantly	Comments
Ephedrine sulfate	Brick red	Stable
Mescaline hydrochloride	Orange color	Changes to yellow, then green
Aspirin	Green with blue tint	Changes to red-violet
Benzocaine	Red-violet	Stable

Mandelin's Test—This test, also referred to as the ammonium vanadate test, has been described for the analysis of more than 150 different drugs (6, 7, 9–11). Most of these drugs show slowly developing colors that are yellow, orange, green, gray, or shades of each or that change from one to another. The interpretation of such results for the purpose of identification is very difficult and should not be relied upon. Of the drugs that gave negative alkaloidal tests in this investigation, only four compounds gave sharp conspicuous colors instantly (Table V).

Ehrlich's Reagent—This reagent is also referred to as Van Urk's reagent or simply given the name of its main ingredient, *p*-dimethylaminobenzaldehyde (6, 8). It produces a characteristic purple to deep-blue color with lysergide. However, in this study, two other indole alkaloids (psilocin and psilocybin) gave similar colors to the colors produced by lysergide. Moreover, other ergot alkaloids produced similar positive reactions.

Duquenois Test—Only the cannabinoids gave a positive test.

Systematic Utilization of These Tests—By using the results obtained from the individual tests performed, it was possible to construct a flowsheet (Scheme I) giving the specific order for performing these tests. By following this flowsheet, it is possible, in a short time and with a few simple tests, to identify tentatively or to narrow down the possibilities of the drug being investigated. Such results can be extremely informative in eliminating the possibility of a suspected drug or determining the confirmatory tests necessary for absolute identification.

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ACKNOWLEDGMENTS AND ADDRESSES

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

Accepted for publication October 30, 1974.

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 the Lederle Co.

PHARMACEUTICAL TECHNOLOGY

Automated System for Analytical Microbiology V: Calibration Lines for Antibiotics

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Abstract □ The accuracy of an automated system for the microbiological assay of antibiotics was increased by improvement attendant to connection to an on-line computer. The system was used to investigate the suitability of four forms of interpolation formulas by assaying for chlortetracycline and erythromycin. The calibration lines were prepared as point-to-point straight-line approximations and as cubic equations. Cubic equations through four calibration points were preferred. Since the automated system was a four-channel instrument, a separate response line was prepared for each channel. Combining the four response lines into one could substantially degrade the accuracy and precision of assays. A new general equation relating the response of the test organism to concentrations of active materials was used to account for factors in addition to the antibiotic upon the dose–response line. Some of these factors were: diluents, growth substances, relative proportions of mixed antibiotics, pH and buffer capacities of the sample solution and assay broth, salts, and organic compounds in samples and not in standard solutions. The equation was used to show under what conditions the dose–response lines of mixtures and single-component antibiotics could be the same. It could also account for the nonspecific nature of turbidimetric assays. The equation showed assay biases to be caused not by differences in composition of antibiotics in standards and samples but by differences in other substances affecting growth of the test organism. A new dose–response line applicable to assays using *Klebsiella pneumoniae* was described.

Keyphrases □ Microbiology, analytical—automated system, calibration lines for antibiotics □ Automated analysis—system for analytical microbiology, calibration lines for antibiotics □ Antibiotics—automated system for analytical microbiology, calibration lines □ Chlortetracycline—automated analysis, evaluation of interpolation formulas, calibration lines □ Erythromycin—automated analysis, evaluation of interpolation formulas, calibration lines

Automation of critical steps in the turbidimetric microbiological assay (1, 2) resulted in a significant increase in the accuracy and precision of assays. A further increase came when interpolation of sample potency was performed by a computer (3). A limitation of the latter was caused by the three-digit resolution of the digital voltmeter used to measure output of the spectrophotometer. Connection of the spectrophotometer to the computer dedicated to analytical services effected a further increase in accuracy and precision (4). The on-line computer had the further advantage of providing a typed report of assay results within 5 min after the last assay tube had been measured. Computerization of reading and recording of turbidity and calculations of potencies completed automation of the operational parts of the assay system.

Operational aspects of automated assays are now of such precision that the form of the calibration line used for interpolating potencies of samples can significantly affect accuracy and precision. Attention will be directed to consequences of using several forms of the lines. One with a theoretical basis and four empirical ones will be considered.

The philosophy guiding the design of the automated system (1, 2) was to minimize variances caused by operation of the electrical and mechanical parts. The same philosophy is applied in this report to treatment of the data to extract potencies with a minimum of computational errors.

EXPERIMENTAL

Preparation of Tests—The five dose–response lines were ap-