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TECHNICAL NOTE

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Further Studies on Spot Tests and Microcrystal Tests for Identification of Cocaine

ABSTRACT: The presence of cocaine in illicit drug samples is still being determined in some laboratories using spot tests and microcrystal tests. Seventeen chemical species were tested using three different spot tests (Wagner, Marquis, and cobalt thiocyanate followed by stannous chloride reactions) and two microcrystal tests (gold chloride and platinic chloride) to determine whether the results could be differentiated from the results of these tests on cocaine. The data obtained indicated that nine of the 17 compounds gave results similar to those from cocaine using the three spot tests, but that the results from microcrystal testing allowed for differentiation of all nine compounds from cocaine.

KEYWORDS: forensic science, criminalistics, spot tests, microcrystal tests, cocaine

The proper techniques to be used for positive identification of illicit drugs continue to be a topic of active discussion. The use of microcrystal tests for identification of drugs has been criticized (1), and SWGDRUG[2] is recommending that when identification of chemical species is performed using microcrystal tests and spot tests that these tests should be supplemented with an uncorrelated test such as gas chromatography or thin layer chromatography (2). Others, however, argue that microcrystal testing is perfectly reliable (1,3–5).

It is generally recognized that spot (color) tests, though quickly performed and useful for narrowing the number of possible drug classes to which the unknown sample belongs (6), lack specificity and can result in false positive or false negative conclusions (7–9). These tests are also subject to interference by adulterants and diluents commonly found in illicit drug samples (9,10). The elimination of several drug classes using spot tests, however, then allows the analyst to choose reagents for follow-up microcrystal testing. Recently, the ASTM Forensic Sciences subcommittee on criminalistics (E 30.01) revised the standards for microcrystal testing of cocaine and of amphetamine-drugs, and also issued standards for microcrystal testing of phencyclidine. In these standards and elsewhere (6,11–13), it is stated that the presence of adulterants and/or diluents may inhibit crystal formation or distort or otherwise render the crystals unidentifiable. Also, identification of closely related compounds with subtle differences requires the skill of highly trained and experienced analysts (6,14).

Crime laboratories that analyze large numbers of seized drug samples, such as those in the City of New York Police Department (NYCPD) and in the Drug Enforcement Administration (DEA), have traditionally used a selected sequence of a limited number of spot tests as a practical means for rapidly screening samples for the presence of cocaine or opioids. A sequence of spot tests used by NYCPD for at least the past 10 years and still in use today consists of Wagner, Marquis and cobalt thiocyanate reagents (Personal Communication, 2002). Individual scientists at the DEA laboratory in New York have used for at least the past 10 years and still use today the sequence of Marquis and cobalt thiocyanate spot tests (Personal Communication, 2002). In each laboratory, if the Marquis test yields a positive result, then spot tests other than cobalt thiocyanate are employed. We recently searched the literature to determine whether others have reported on any compound that could provide false positive results concerning the identification of cocaine using the sequence of Wagner, Marquis and cobalt thiocyanate reagents, and we found five compounds that reportedly give the same color reactions as cocaine (7,8,15). The five compounds are atropine, methadone, nicotine, phencyclidine and scopolamine. Though one report indicated no reaction of either methadone or atropine with the Marquis reagent (7), another described a red color with methadone and an orange-brown color with atropine (8). In addition, we identified from the literature 12 other compounds that give results similar to those from cocaine using the Marquis and cobalt thiocyanate reagents (8). It remained to be determined whether these 12 chemical species would also give Wagner spot test results similar to cocaine. Also, since we are not aware of any studies comparing the results of cocaine to those from the 17 compounds using the cobalt thiocyanate reaction followed by the addition of stannous chloride (16), we included this additional test in our studies. Those chemical species that gave results similar to cocaine on all four tests would then be compared to cocaine using the gold chloride and platinic chloride microcrystal tests, two reagents that are routinely used to identify cocaine in forensic science laboratories and for which test procedures have been standardized (13).

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[2] SWGDRUG: Scientific Working Group on Drugs.

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Materials and Methods

Chemicals—Atropine sulfate, procaine hydrochloride, and sodium pentobarbital were obtained from Amend Drug and Chemical Company, Inc., Irvington, NJ, and New York, NY; cocaine hydrochloride was obtained from Merck Chemical Division, Rahway, NJ; acetylsalicylic acid, benoxinate hydrochloride, (+) brompheniramine maleate, carbinoxamine maleate, chlorpheniramine maleate, dibucaine hydrochloride, diethylpropion hydrochloride, (+/−)-methadone hydrochloride, nicotine hydrogen tartrate, phencyclidine hydrochloride, phendimetrazine bitartrate, pheniramine maleate, (−) scopolamine hydrochloride, (−) scopolamine methylbromide, (−) scopolamine methylnitrate and (−) sparteine sulfate were obtained from Sigma Chemical Co., St. Louis, MO.

Reagents—The following reagents were prepared as indicated.

Spot test reagents:

Cobalt Thiocyanate: Cobalt chloride, 6.8 g, and ammonium thiocyanate, 4.3 g, dissolved in sufficient distilled water to produce 100 mL of solution (11).

Stannous Chloride: stannous chloride, 5.0 g, and concentrated hydrochloric acid, 10 mL, plus sufficient distilled water to produce 100 mL (16, modified).

Wagner's Reagent: Iodine, 1.27 g, and potassium iodide, 2.0 g, dissolved in sufficient distilled water to produce 100 mL of solution (7).

Marquis Reagent: 8–10 drops of 40% formaldehyde solution were added to 10 mL concentrated sulfuric acid (7).

Microcrystal test reagents:

Gold Chloride ($HAuCl_4 \cdot 3H_2O$): Gold chloride, 5.0 g, dissolved in sufficient distilled water to produce 100 mL (11).

Platinic Chloride ($H_2PtCl_6 \cdot 5H_2O$): Platinic chloride, 5.0 g, dissolved in sufficient distilled water to produce 100 mL (11).

Test Procedures—Two of the authors were present for each test to review and corroborate the results.

Spot tests—Three drops of reagent were first added to a well of a porcelain spot test plate. A few crystals of drug were then added to the well and the color was recorded. To the wells containing blue product after addition of the chemical to cobalt thiocyanate reagent, three drops of the stannous chloride reagent were added and the persistence or disappearance of the blue color was recorded. Wells containing reagent only served as controls.

Microcrystal tests—Two drops of drug solution (approximately 2–3 mg of drug/5 drops of 10% HCl) were put on a clean glass slide. Two drops of reagent were placed near the drops of drug and a glass rod was used to create a tiny channel connecting the solutions (17). The reaction was observed without a cover slip, at 100 or 200 × magnification, using an Olympus BH-2 polarized light microscope, with an Olympus C-35AD-4 camera attached, to detect and document crystal formation. Kodak Plus-X 125 Pro DX film was used.

Results

Spot Tests

Wagner's Reagent: Upon the addition of cocaine, the reagent quickly turned brown and then a black film formed. Benoxinate, chlorpheniramine, dibucaine, diethylpropion, and pentobarbital did not react as cocaine. The other drugs, similar to cocaine, turned brown and then formed a black film within 1 min. Thus, the Wagner reagent could not discriminate cocaine from 12 other compounds.

Marquis Reagent: Cocaine did not produce a colored product when reacting with this reagent. This result is in agreement with other reports (7,8). After 15 mins, atropine produced a faint pink tint and methadone produced a yellowish-brown color. Using this spot test for deciding rapidly whether the drug is present or not, this reagent cannot be used to differentiate any of the 17 drugs used in this study from cocaine. Acetylsalicylic acid, which develops a pink-red color within 5 min, was used as a positive control.

Cobalt Thiocyanate and Stannous Chloride: Cocaine, when reacted with cobalt thiocyanate, turned blue immediately. Phencyclidine and pentobarbital did not turn blue. All of the other compounds turned blue quickly with the exception of benoxinate, methadone, nicotine and scopolamine methylbromide, which turned blue after several minutes. After stannous chloride was added to the wells, the blue color remained in all of the wells except atropine and nicotine. Procaine hydrochloride was used as a positive control, since the blue color product that formed upon addition of cobalt thiocyanate dissolved on addition of stannous chloride.

Microcrystal Tests

Gold Chloride: The description of the crystal shape is based on terms by Clarke (11). Cocaine formed radiating clusters of fine needles with perpendicular branches (Fig. 1). These crystal shapes are similar to those reported by Wielbo and Tebbett (6), and could be distinquished from the crystals produced by brompheniramine (rosettes, Fig. 2), phendimetrazine (fans, Fig. 3), pheniramine (bundles of blades, Fig. 4), scopolamine hydrochloride (long thin spear-shaped crystals, Fig. 5), scopolamine methylbromide (clusters of rods and blades, Fig. 6) and sparteine (clusters, Fig. 7). Carbinoxamine, methadone and scopolamine methylnitrate did not form distinct crystals.

Platinic Chloride: Cocaine formed V-shaped long, thin needles with branching (Fig. 8). None of the other compounds formed crystals using this reagent.

Discussion

The data indicated that, using just the three specified spot tests, including the use of stannous chloride reagent, cocaine could not be differentiated from nine other compounds, which may lead to false positive conclusions. These other chemical species are brompheniramine, carbinoxamine, methadone, phendimetrazine, pheni-

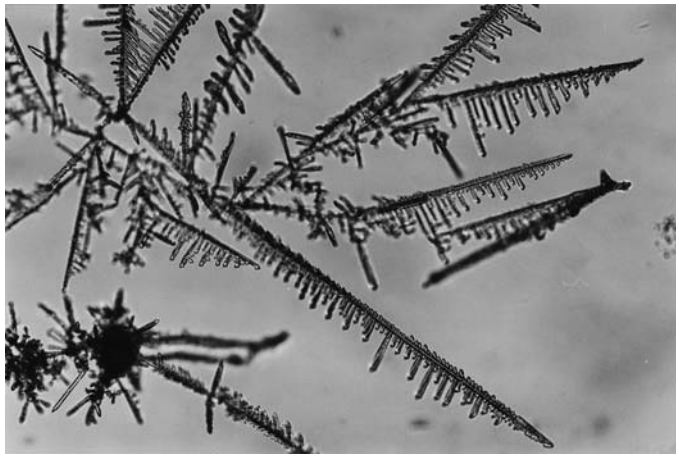

FIG. 1—*Cocaine and Gold Chloride, 200×.*

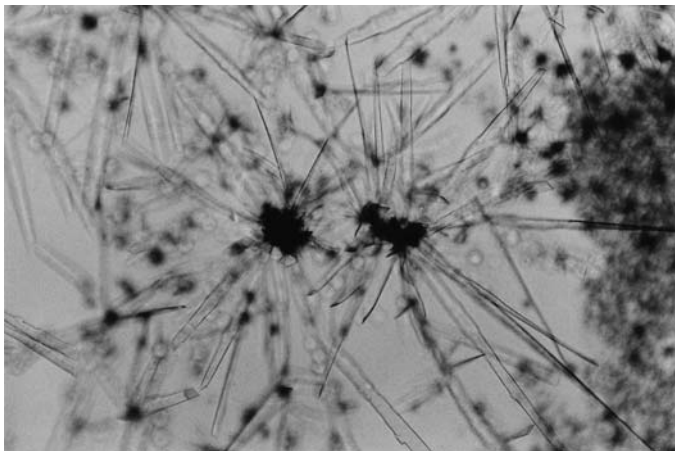

FIG. 2—*Brompheniramine and Gold Chloride, 100×.*

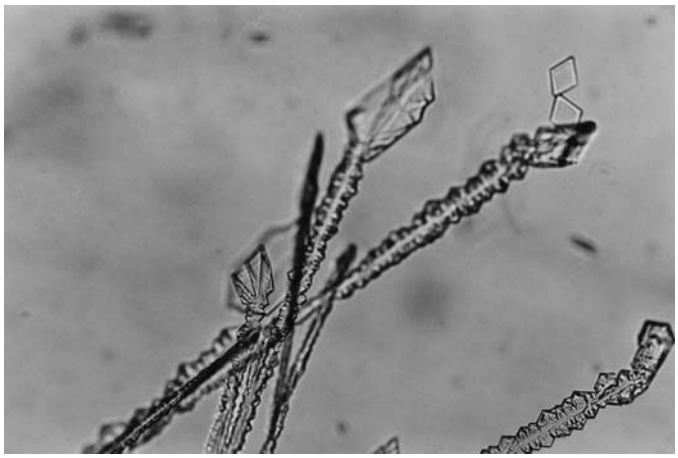

FIG. 5—*Scopolamine Hydrochloride and Gold Chloride, 200×.*

FIG. 3—*Phendimetrazine and Gold Chloride, 200×.*

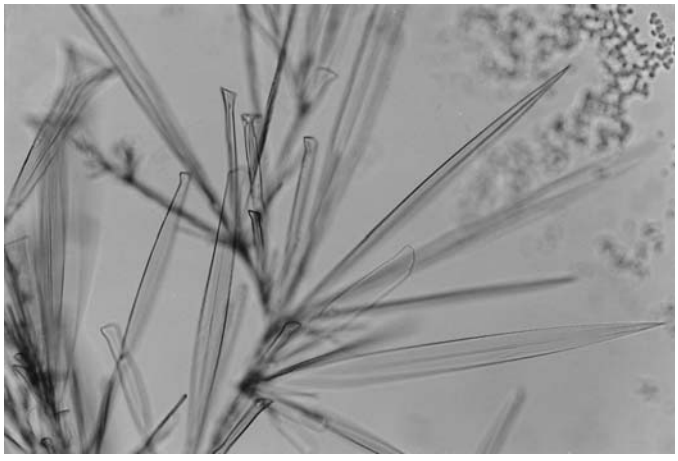

FIG. 6—*Scopolamine Methylbromide and Gold Chloride, 200×.*

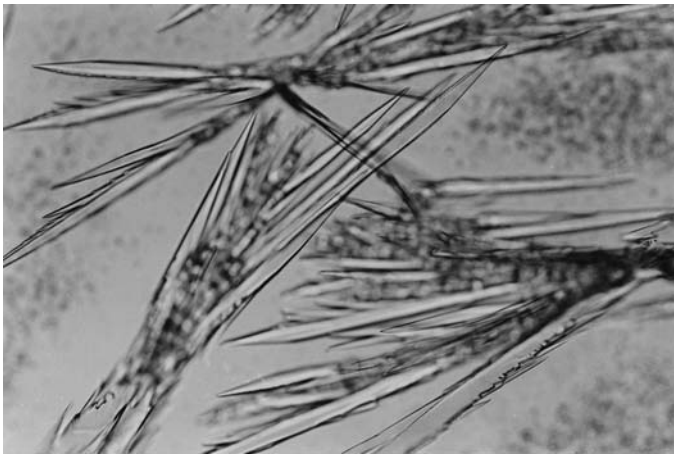

FIG. 4—*Pheniramine and Gold Chloride, 200×.*

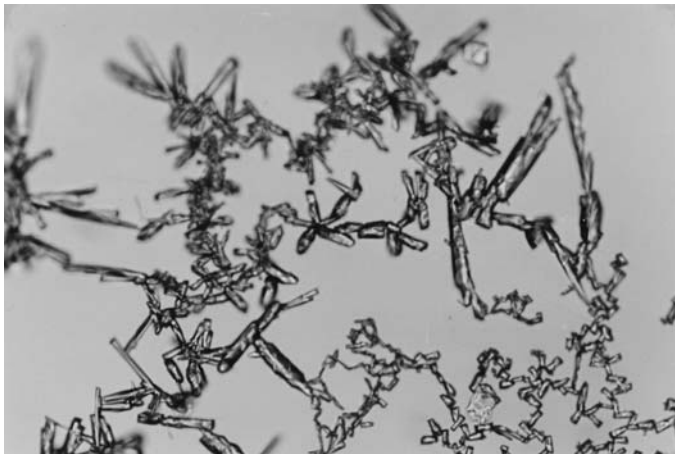

FIG. 7—*Sparteine and Gold Chloride, 200×.*

FIG. 8—*Cocaine and Platinic Chloride, 100×.*

ramine, scopolamine hydrochloride, scopolamine methylbromide, scopolamine methylnitrate and sparteine. Our spot test results using methadone were different from those reported by others (7,8). The differences may be due to variations in drug concentration and in time allotted for the reaction to take place. Also, our results using phencyclidine were different from those reported by others (7,8). These few examples point out the difficulty in reaching conclusions using spot tests alone.

The addition of microcrystal tests, however, allowed the differentiation of cocaine from the nine other compounds. Using gold chloride, three of the compounds did not produce distinct crystals and the remaining six chemicals produced distinctly different crystalline structures. None of the compounds except cocaine produced distinct crystals with platinic chloride.

Reaching an accurate conclusion using microcrystal tests will depend on the level of experience of the analyst, the proper use of standards and controls, the presence of adulterant and/or diluent in the seized samples, the reaction pH, the temperature and humidity, and the concentration of the reagent and of the chemical (6,11). The description of the crystals formed after reacting cocaine in hydrochloric acid with gold chloride has variously been described as combs and rosettes of needles (18) and serrated needles (11). The use of photomicrographs or drawings of the crystals for comparing the unknown sample to standards would be valuable and would address one of the criticisms raised against microcrystal tests regarding limits of documentation as compared to that of modern instrumentation.

Our work demonstrates the value of the combined use of spot and microcrystal tests to differentiate cocaine from other chemical species. Clearly, the basis of the microcrystal test is empirical, the result of years of accumulated experience by many scientists. To date, we are not aware of any chemical that produces a false positive relative to cocaine provided that the correct set of tests is performed properly. Yet, the current list of chemicals is extensive, and not all of the chemicals have been compared to cocaine; also, new chemicals are continuously being synthesized. Further, because a number of variables discussed above could prevent an analyst from reaching an accurate conclusion, the use of a more analytical procedure should be considered. In this regard, Wielbo and Tebbett (6) have proposed the combined use of microcrystal testing with Fourier transform IR spectrophotometry being applied to the product of the microcrystal test.

Gas chromatography/mass spectrometry (GC/MS) testing identifies compounds based on specific chemical structures, rather than on empirical and not clearly understood chemical reactions. GC/MS analysis can separate and identify each component in mixtures of either drugs or drugs mixed with adulterants and diluents. For example, a substance may contain two drugs that, in combination, give positive results with the Wagner, Marquis and cobalt thiocyanate plus stannous chloride tests, such as a "speedball" containing both cocaine and heroin. Alternatively, a mixture consisting of non-controlled substances may give spot test results indicative of cocaine, as our findings indicate. Microcrystal testing of such drug mixtures may not allow for clear identification of the drugs, while GC/MS analysis would likely separate and accurately identify each component. In some situations, the identification of enantiomers, however, may be more easily accomplished using microcrystal tests (13).

When mixtures of drugs are present, GC/MS testing would be advantageous over IR spectroscopy, another analytical technique based on chemical structure, because GC/MS does not require fairly pure substances. GC/MS analysis can provide quantitative data. This information may be vital in those cases where the defendant sold non-controlled cocaine look-alike substances in previously used cocaine-containing containers. If the small amount of residue in the container is sufficient to produce positive spot test and microcrystal test results, analysis by GC/MS would confirm only minute amounts of cocaine and suggest that the sale was not for the alleged purpose of drug trafficking (albeit, sale of imitation controlled substances is unlawful and, in certain jurisdictions, detecting any quantity of cocaine in a sample may mean that the entire sample may be considered to be cocaine). However, an initial microscopic examination of the sample as part of an integrated microchemical approach should help the analyst in detecting such mixtures. Finally, since the results obtained by GC/MS analysis are validated with standards, and there is a "paper trail" of measurable results, it is more reliable for the analyst to present and defend the results in court.

In criminal matters, the defendant's guilt must be established "beyond a reasonable doubt." We believe it behooves the criminal justice system to use the best technology available to reach that level of proof. To that end, this paper presents new experimental findings and identifies several issues for consideration in determining the appropriate methods for identification of drugs.

Acknowledgments

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