Donna Wielbo, Ph.D. and Ian R. Tebbett, Ph.D.

The Use of Microcrystal Tests in Conjunction with Fourier Transform Infra Red Spectroscopy for the Rapid Identification of Street Drugs

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ABSTRACT: Microcrystal tests have long been used for the rapid identification of particulate matter, although this technique has now been largely abandoned in favor of sophisticated instrumentation. The sensitivity and specificity of a microcrystal test make it an attractive method for the initial screening of powders for drugs of abuse. By combining the techniques of crystal microscopy and micro-FTIR, both preliminary and confirmatory tests for the presence of drugs in solid dosage forms are able to be carried out. This technique has been used to unequivocally identify cocaine, heroin, morphine, codeine, and phencyclidine.

KEYWORDS: toxicology microcrystal tests, FTIR, drug abuse, drug identification

The ever increasing problem of drug abuse in the United States is well recognized and has been a major concern of recent government administrations. The analysis for drugs of abuse now accounts for a major proportion of the workload of major-city crime laboratories. Amongst the most commonly encountered drugs are cocaine, phencyclidine, heroin and amphetamines, including the amphetamine-derivative designer drugs. These compounds may be encountered in their pure form, or in street preparations, adulterated with diluents such as caffeine, strychnine, sugars, etc., which complicate the analysis.

Unfortunately, automated methods used in clinical drug identification and therapeutic drug monitoring are not directly applicable to the identification of illicit drugs commonly encountered in the crime laboratory because of the variation of adulterants and diluents present in the samples. Forensic-science examination of street drugs is therefore time consuming and often complicated, involving the use of several tests. These tests include an initial screening of the sample for the presence of controlled substances. This usually entails the use of non specific but highly sensitive color tests. Spot tests as such give an indication of a class of compounds but not specific compounds within that class. Further screening methods may involve the use of thin layer chromatography (TLC) or UV spectroscopy or both. If preliminary tests prove to be positive then the presence of the specific drugs must be confirmed, and generally, as a legal requirement, the structure of the illicit substance must be proven. This can only be achieved by mass spectroscopy

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[1]Graduate student and assistant professor, respectively, University of Illinois at Chicago, Dept. of Pharmacodynamics, Chicago, IL.

(MS) or by infra-red analyses (IR). Since street drugs are rarely encountered in their pure form, it is usually necessary to extract the drug of interest from any interfering compounds or diluents before GCMS or IR analysis. The extractions may be complicated, involving back extraction, filtration and evaporation stages, which increase the analysis time for a single sample. Using these methods, the time taken to examine a single drug sample can take from between 90 min to 4 h. In view of the drug caseload encountered by the crime laboratories and the subsequent backlog of cases, it is important that alternative, more efficient and less costly procedures for drug identification be investigated. Any new approach to drug analysis should be as reliable and accurate as the present methods so as not to compromise the testimony of the drug chemist. The ultimate aim is to improve the efficiency of the forensic-science laboratory in the "war against drugs."

Modern microcrystal tests are highly developed chemical precipitation tests [*1*,*2*]. The attractiveness of microcrystal tests lies in the fact that they are sensitive, and have some degree of selectivity and specificity. They also work rapidly and are simple to use. Many of the crystal tests were developed in the late 19th century for the identification of alkaloids. Over the years, these tests have been modified by various analysts and are now applicable to the majority of drugs. Despite the fact that they were developed over 100 years ago, microcrystal tests still have a role to play in modern drug analysis. In many crime laboratories crystal tests are used with color tests for screening samples. In some laboratories, it has been argued that microcrystal tests are so specific that no further confirmatory test is necessary. While this approach certainly reduces the turnover time of the analysis, the specificity of the technique is still in question. In recent years, microcrystal tests have received a certain amount of criticism and have largely been superseded by more sophisticated, although more time consuming, methods of analysis.

The principle disadvantages of microcrystal tests are that the presence of other compounds in the sample may interfere with the formation of the microcrystalline precipitate causing distortions of the expected crystal form, which may lead to difficulty in identification by anyone inexperienced in chemical microscopy. Considerable training and experience in chemical microscopy is required to be able to identify drugs based purely on the crystal form of the reaction products of particular microcrystal reagents [*3*]. In addition, a degree of subjectivity is inherently associated with the technique. Distortions of crystal form are also induced by changes in ambient temperature and humidity.

The past decade has witnessed a revolution in IR spectroscopy with the development of Fourier transform infra-red spectroscopy (FTIR). FTIR spectrometers have a number of advantages over the dispersive IR instruments that they have replaced. They can scan the sample up to 10 times per second, and all frequencies can be detected simultaneously. A better signal-to-noise ratio is obtained and the sensitivity is increased. The combination of this highly sensitive IR spectrometer with a microscope attachment now enables complete IR spectra to be obtained from microscopic particles [*4*]. We have combined the two techniques of microcrystal tests and FTIR spectroscopy in an attempt to reduce the number of tests and time taken to identify a drug in a street sample. The procedure described below allows both a preliminary identification and structural confirmation of a drug to be obtained in a matter of minutes.

Methods

The following microcrystal reagents, as described by Fulton [*1*], were selected for investigation:

Chloroauric acid—Gold chloride (1 g) in 20 mL of glacial acetic acid/concentrated sulfuric acid (1:1).

Chloroauric acid in HCl—Gold chloride (1 g) in 20 mL of concentrated hydrochloric acid/distilled water (1:3).

Chloroauric acid in H_3PO_4—Gold chloride (1 g) in 20 mL of concentrated orthophosphoric acid/distilled water (1:2).

Bromoauric acid—Gold chloride (1 g) and sodium bromide (0.764 g) in 5 mL of glacial acetic acid and 15 mL of concentrated sulfuric acid/distilled water (2:3).

Bromoauric acid in H_3PO_4/H_2SO_4—Gold chloride (1 g) and sodium bromide (0.764 g) in 10 mL concentrated sulfuric acid/distilled water (2:3), and 20 mL of concentrated orthophosphoric acid.

Bromoauric acid in H_3PO_4/HOAc—Gold chloride (1 g) and sodium bromide (0.764 g) in 20 mL concentrated orthophosphoric acid/glacial acetic acid (1:5).

Platinic chloride—Platinic chloride (1 g) in 20 mL of distilled water.

Platinic iodide in H_3PO_4—Platinic chloride (0.5 g) in 10 mL distilled water, add sodium iodide (0.2 g), 2.5 mL distilled water and 9 mL concentrated orthophosphoric acid.

Platinic bromide solution—Platinic chloride (1 g) and sodium bromide (10 g) in 20 mL distilled water.

Platinic bromide in H_3PO_4—Platinic chloride (1 g) and sodium bromide (0.866 g) in 20 mL of concentrated orthophosphoric acid/distilled water (1:7).

Acidified mercuric iodide solution—Mercuric iodide (1 g) in 20 mL concentrated hydrochloric acid/distilled water (27:73).

5% Potassium iodide solution—Potassium iodide (5 g) in 100 mL distilled water.

2% Potassium permanganate solution—Potassium permanganate (2 g) in 100 mL distilled water.

2% Potassium permanganate solution with H_3PO_4—Potassium permanganate (2 g) in 100 mL distilled water, acidified with a few drops concentrated orthophosphoric acid.

Picric acid in acetic acid/magnesium acetate solution—Picric acid (0.03 g) in 1 mL glacial acetic acid and 5 ml of a 40% magnesium acetate solution.

Silver nitrate/Potassium iodide solution—Silver nitrate (1 g) and potassium iodide (15 g) in 20 mL of distilled water.

Sodium anthraquinone sulfonate solution—Sodium anthroquinone-sulfonate (1 g) in 20 mL distilled water.

Sodium anthraquinone sulfonate in HCl—Sodium anthroquinone-sulphonate (1 g) in 20 mL of concentrated hydrochloric acid/distilled water (1:3).

Naphthaquinone Sulphonate in HCl—1,2-Naphthoquinone-4-sulfonic acid sodium salt (1 g) in 20 mL of concentrated hydrochloric acid/distilled water (1:3).

The sodium salts of anthraquinone and naphthaquinone sulphonic acid were obtained from Eastman Kodak (Rochester, N.Y.). All other chemicals were obtained from Sigma (St. Louis, Mo.). Microcrystal reactions were initially carried out on glass microscope slides. Approximately 1 μL of reagent was added to a few micrograms of drug or drug mixture on the glass slide and reagent-drug complexes were seen to form within 1 to 2 min. Mixtures that were not soluble in a test reagent were first dissolved in approximately 1 μL of distilled water. Crystals were examined using a Nikon SKE Polarizing Light Microscope. Microcrystal reaction products were observed without a coverslip at 100

times magnification in plane polarized light, with crossed polars and with a first order red compensator.

After initial microscopical tests, the following reagents were chosen for further investigation with specific drugs. Cocaine:reagents 1, 2, 6, 8, and 10. Phencyclidine:reagents 1, 2, 4, 8, 13, 16, 17, and 18. Heroin, morphine, and codeine:reagents 4, 7, 8, 13, and 19. Amphetamine and methamphetamine:reagents 3, 6, 8, 11, 12, 20, 21, 22, and 24.

A photographic record of the resulting crystals was obtained using the Nikon microscope in conjunction with an Olympus PM6 camera and Kodak Ektachrome daylight film.

Micro-FTIR

A Spectra-Tech IR Plan, analytical grade microscope in conjunction with a Mattson, Galaxy 4020, bench FTIR Spectrometer, was used to obtain IR spectra of the crystal complexes. Samples for Micro-FTIR analysis were prepared on 13 mm by 1 mm, BaF_2 salt windows (Spectra-Tech Inc.). After crystallization of the reaction products, the sample was allowed to dry at room temperature. The apperture on the microscope stage was adjusted to exclude as much light as possible, and FTIR spectra were obtained in the transmission mode directly from the microscope stage. The sample was scanned 128 times at a resolution of 4.0 cm^{-1}. IR spectra were obtained over the range 900 cm^{-1} to 4000 cm^{-1}, with a mercury, cadmium tellurium (MCT) detector. All spectra were plotted using a Hewlett-Packard, HP Colorpro, Graphics Plotter.

For the optimum reagent, infrared spectra were obtained using drug standards in conjunction with their commonly encountered diluents, and on actual street drugs.

Results

The optimal reagent for each drug was established based on its selectivity and compatibility with the FTIR technique. Most of the reagents proved unsuitable for FTIR microscopy as they did not dry rapidly at room temperature or did not dry completely. Residual water in the samples tended to interfere with the FTIR spectrum. Other reagents crystallized, masking the drug crystals or resulted in the production of crystals that did not give good spectra. In the latter case, this was because either too much light was transmitted between the crystals, or the crystals were too small (<5 μm) to allow an infrared spectrum to be obtained. Based on these criteria, the following reagents, although sensitive, proved unsuitable for Micro-FTIR : Chloroauric acid (1), Bromoauric acid (4), Bromoauric acid in H_3PO_4/H_2SO_4 (5), Bromoauric acid in H_3PO_4/HOAc (6), Platinic Chloride solution (7), Platinic Bromide in H_3PO_4 (10), 5% potassium iodide solution (12), 2% Potassium Permanganate with H_3PO_4 (14) and Picric acid in acetic acid/magnesium acetate (15).

In addition the concentration of hydrochloric acid described in Fulton's reagents as 1 part concentrated acid to 3 parts water, was found to etch the barium fluoride disc. To avoid this problem, we substituted 1M HCl in the microcrystal reagents, with no loss in sensitivity or selectivity.

Cocaine

Although all gold chloride reagents produced characteristic crystals with the cocaine HCL, the optimum reagent for Micro-FTIR was found to be chloroauric acid in HCl (2). This reagent proved to be sensitive, selective, dried down rapidly at room temperature, and showed little or no interference in the FTIR Spectra. Figure 1 shows a photomicrograph of cocaine crystals formed on reaction with gold chloride reagent. The infrared

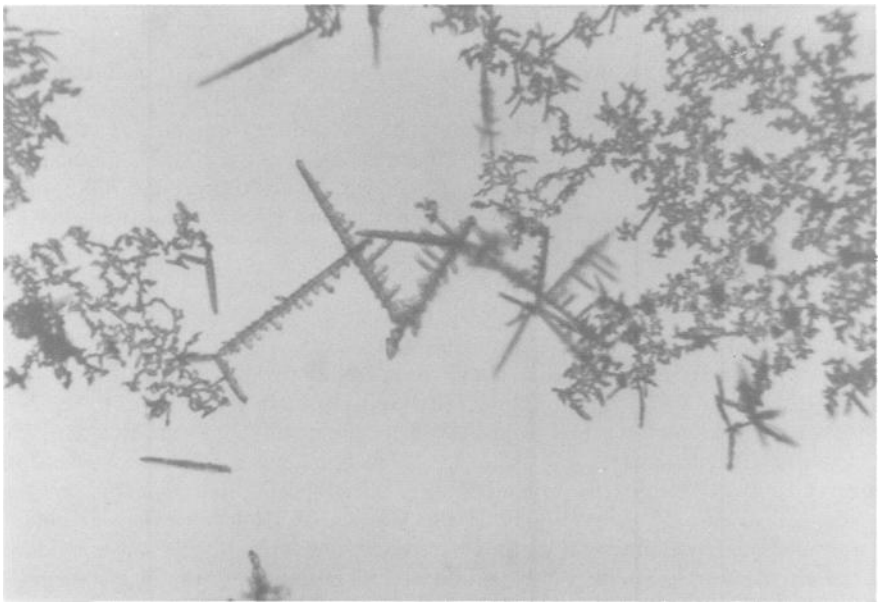

FIG. 1—*Cocaine HCl with chloroauric acid in 1M HCl.*

spectrum of an area of these crystals is shown in Fig. 2. Although some peak shifts are apparent in the fingerprint region, the spectrum is similar to that of cocaine.

Heroin, Morphine, Codeine

Although several reagents produced characteristic crystals with the opiates, most were unsuitable for infrared analysis, either because they did not dry down or because the resulting crystals were too small (for example, platinic chloride) to allow a good IR spectrum to be obtained. The optimum reagent was seen to be acidified mercuric iodide solution (13), which was selective, sensitive and dried rapidly at room temperature. The crystal complexes formed with the opiates were all quite similar in form when viewed under plane polarized light (Figs. 3, 4, and 5). However the lack of interference by mercuric iodide in the IR spectrum allowed morphine, codeine and heroin to be easily distinguished spectroscopically. Figure 6 shows the FTIR spectrum of the heroin, mercuric iodide complex.

Phencyclidine

The following reagents were shown to produce characteristic crystals, or precipitates, with phencyclidine, allowing spectra to be obtained: chloroauric acid in HCl (2); platinic chloride solution (8); acidified mercuric iodide solution (13) (Fig. 7) and 2% potassium permanganate solution (17). All other reagents tested with this drug were unsuitable for FTIR microscopy. Figure 8 shows the FTIR spectrum obtained from the PCP, mercuric iodide complex.

Amphetamine, Methamphetamine

Platinic bromide (11) was a suitable reagent for the identification of dl-Methamphetamine, but produced no reaction with l- and d-amphetamine. Acidified sodium anthra-

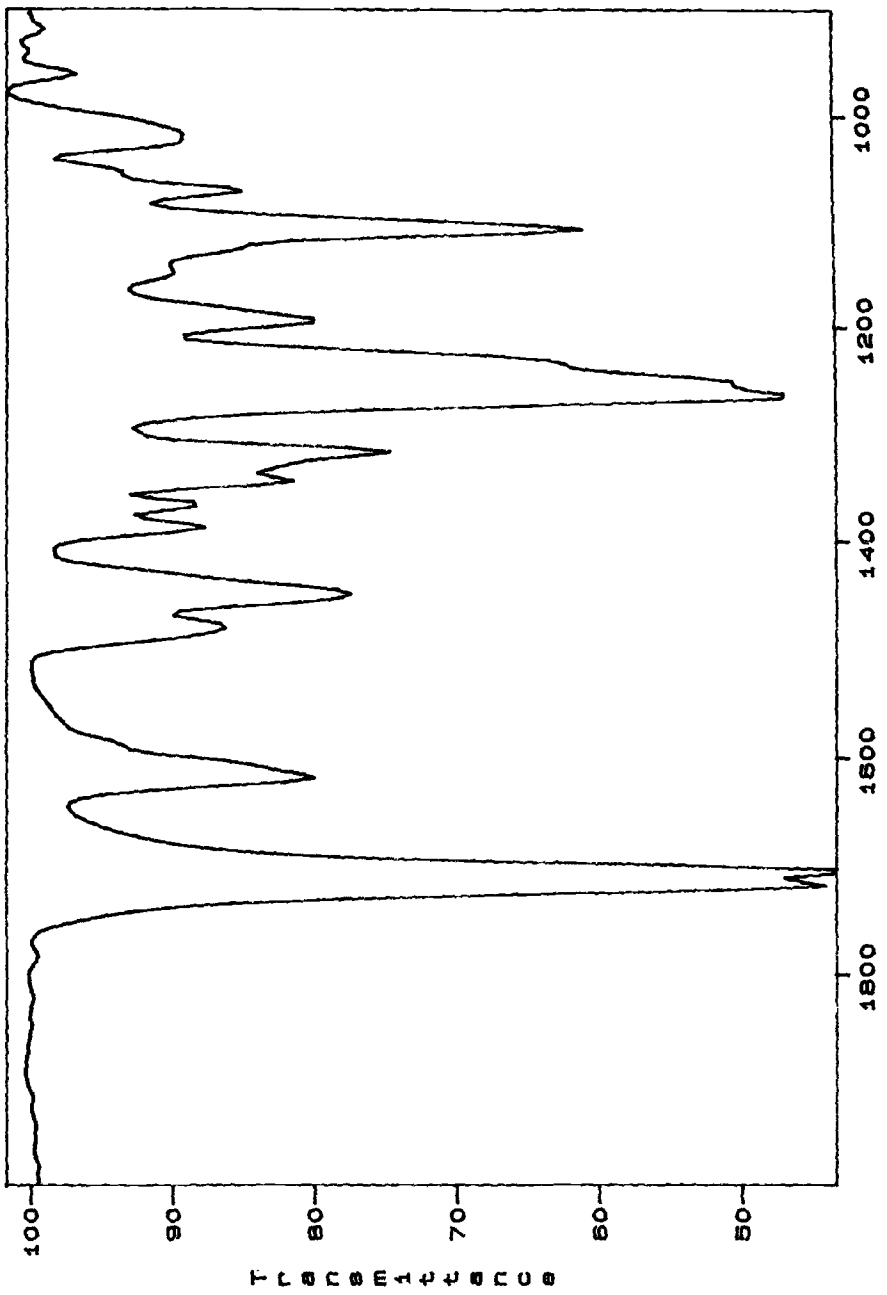

FIG. 2—*Cocaine HCl with chloroauric acid in 1M HCl.*

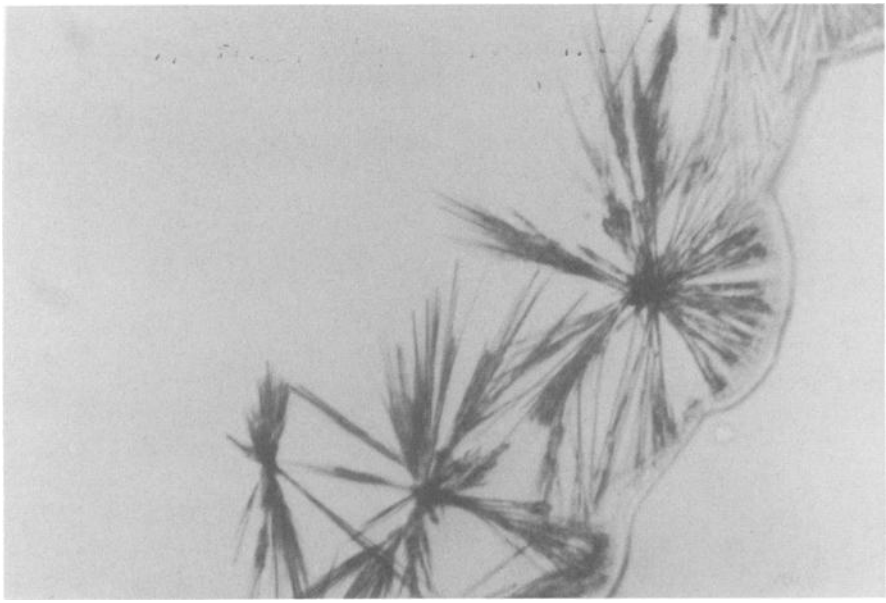

FIG. 3—*Morphine sulfate with acidified mercuric iodide.*

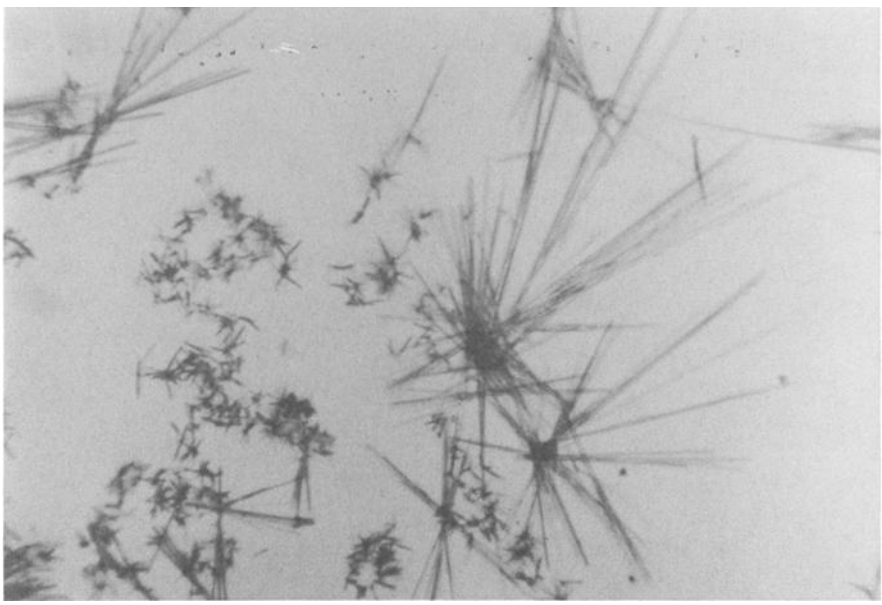

FIG. 4—*Codeine sulfate with acidified mercuric iodide.*

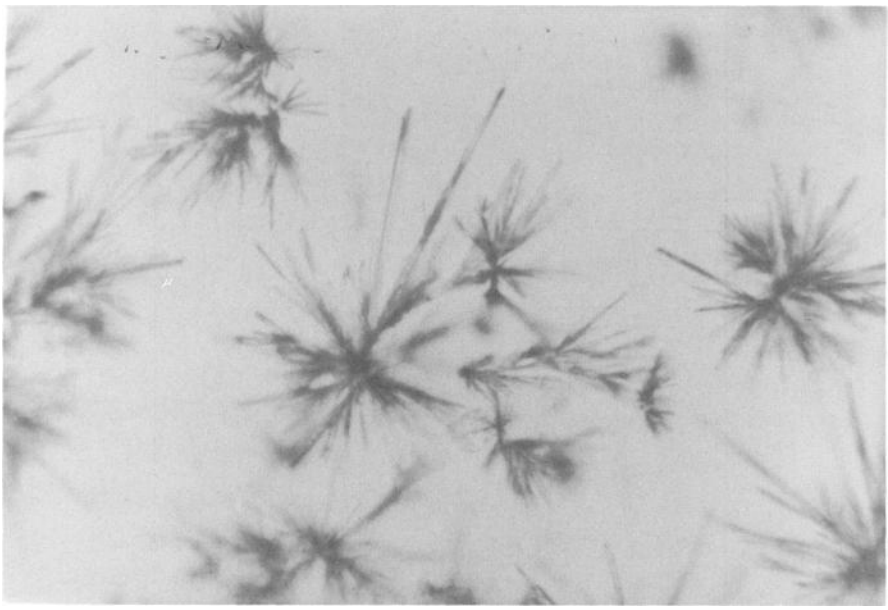

FIG. 5—*Heroin hydrochloride with acidified mercuric iodide.*

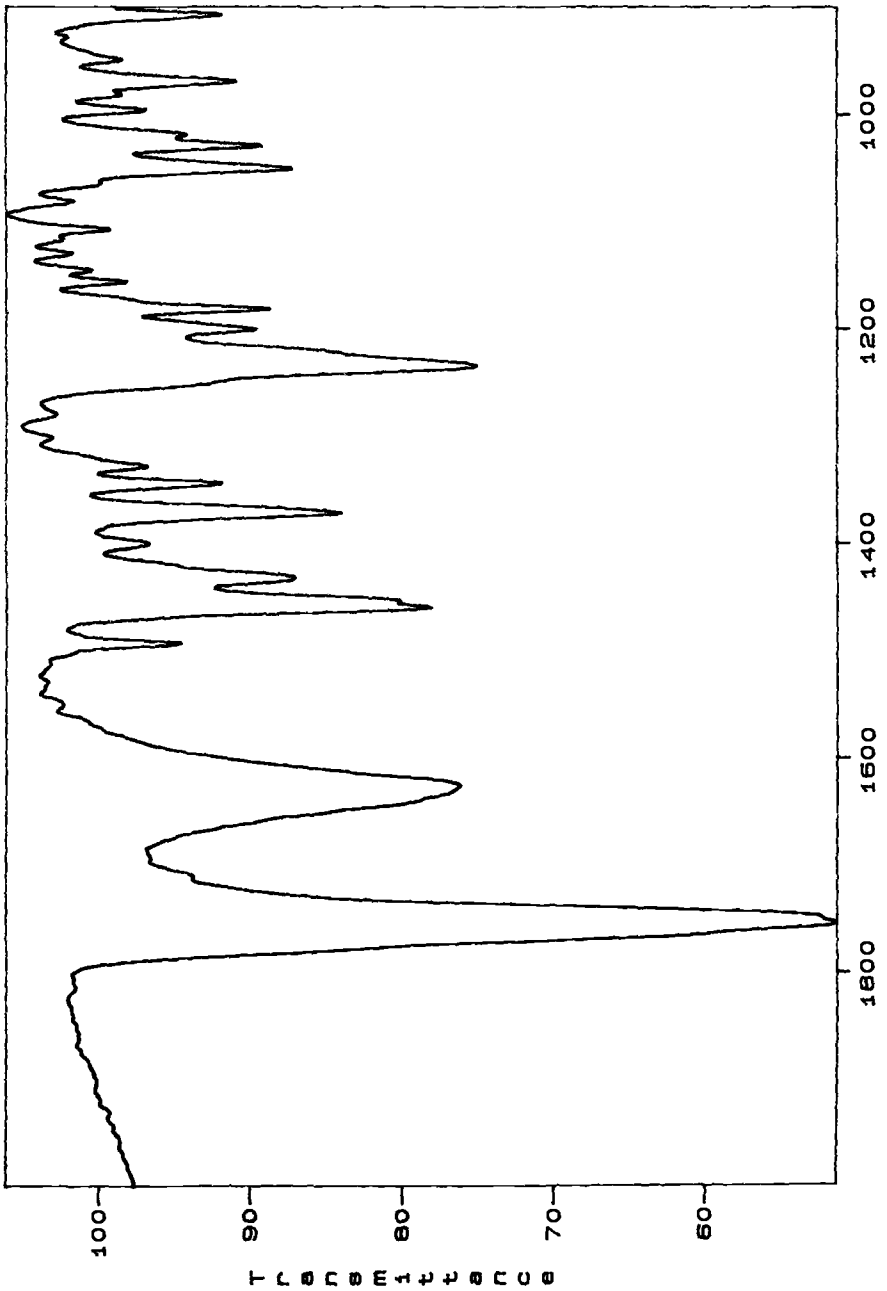

FIG. 6—*Heroin hydrochloride with acidified mercuric iodide.*

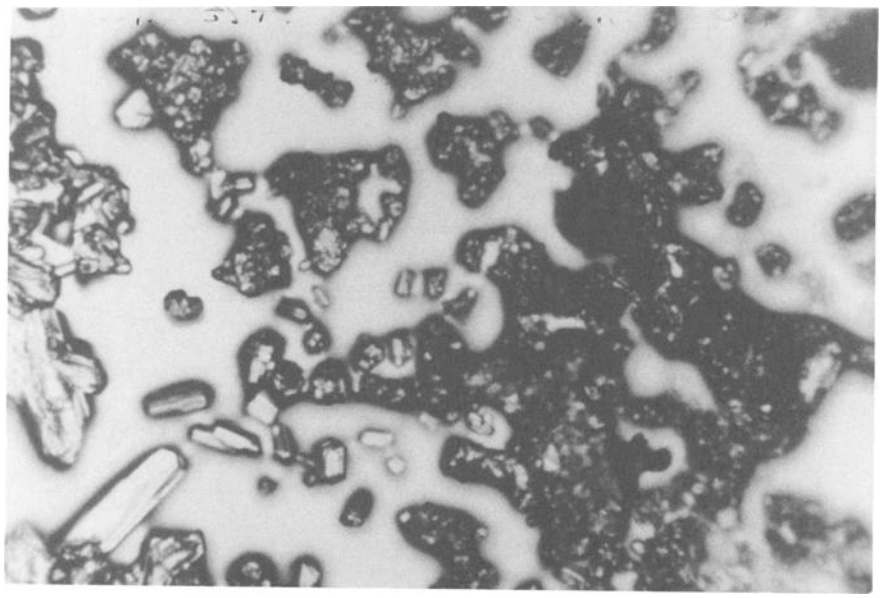

FIG. 7—*PCP hydrochloride with acidified mercuric iodide.*

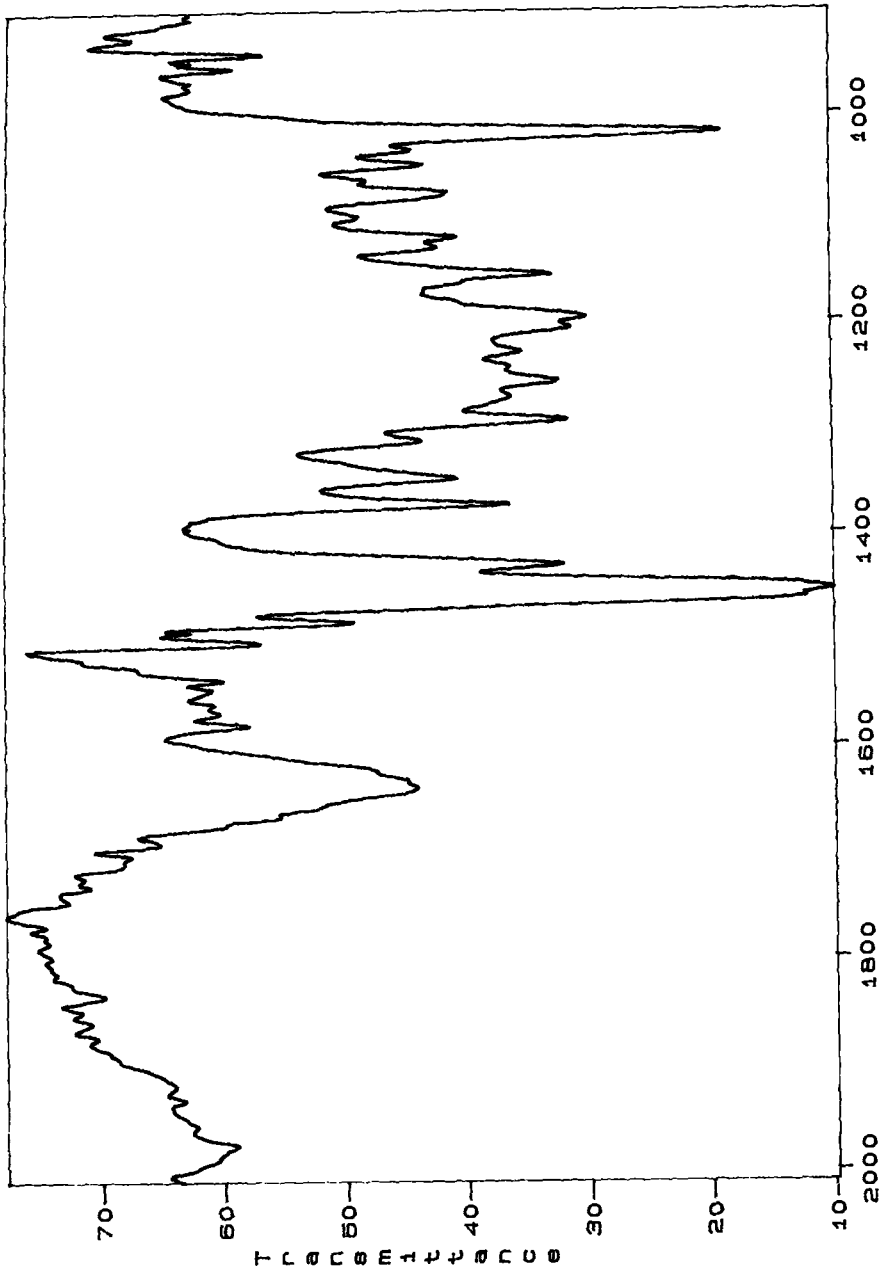

FIG. 8—*PCP hydrochloride with acidified mercuric iodide.*

quinone sulfonate (22) and naphthaquinone sulfonate (24) (Figs. 9, 10, and 11) reagents produced reaction products with all three substances which were compatible with FTIR. The sodium anthraquinone sulfonate did however cause some etching of the barium fluoride disc.

At present, naphthaquinone sulfonate seems to be the best reagent we have found for the identification of amphetamines by micro-FTIR. However, because of the instability of this solution, further work is continuing to improve on the analytical technique for these particular compounds. Figure 12 shows the FTIR spectrum obtained from the d-amphetamine, acidified naphthaquinone sulfonate complex.

All other reagents were unsuitable for infrared analysis. The micro FTIR technique was shown to be reproducible by repeated analysis of each drug 10 times. The applicability of the technique to actual case samples was verified by the examination of street drugs submitted to the Illinois State Police laboratory, Maywood, Illinois.

Conclusion

Microcrystal reagents in conjunction with Fourier Transform Infra Red Spectroscopy, offer a rapid method for the examination of street drugs. A preliminary test and structural confirmation can be achieved in a few minutes, without the need for extraction of the drug from any excipients. Microcrystal tests are sensitive, allowing the detection of microgram quantities of drug. Common diluents such as sugars, starch, and other drugs did not interfere with the analysis even though in some instances the drug of interest was only present as 1 or 2% by weight of the sample. Microcrystal tests have been used for over a century and have received criticism in recent years as an out dated technique having no place in the modern crime laboratory. Conversely, some drug chemists argue that microcrystal tests are so specific that there is no need to perform a confirmatory test. Indeed, some crime laboratories will testify purely on the basis of a crystal test. This work suggests that microcrystal tests do still have a very valuable role to play in

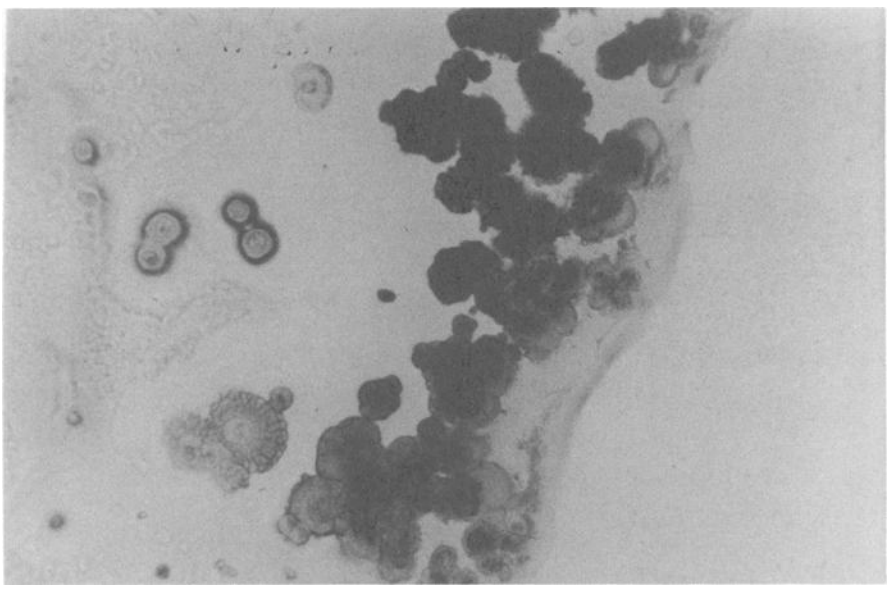

FIG. 9—*d-amphetamine sulfate with acidified NQS reagent.*

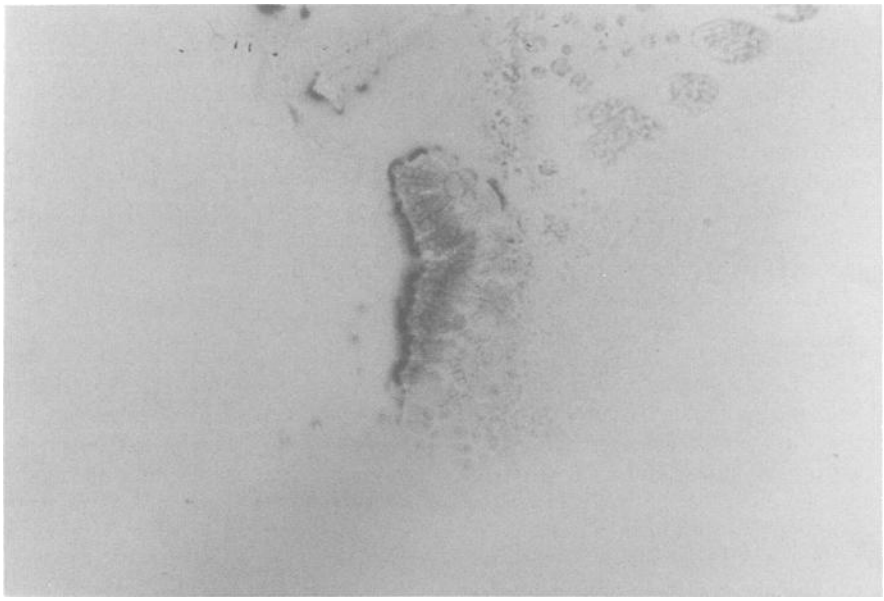

FIG. 10—*l-amphetamine sulfate with acidified NQS reagent.*

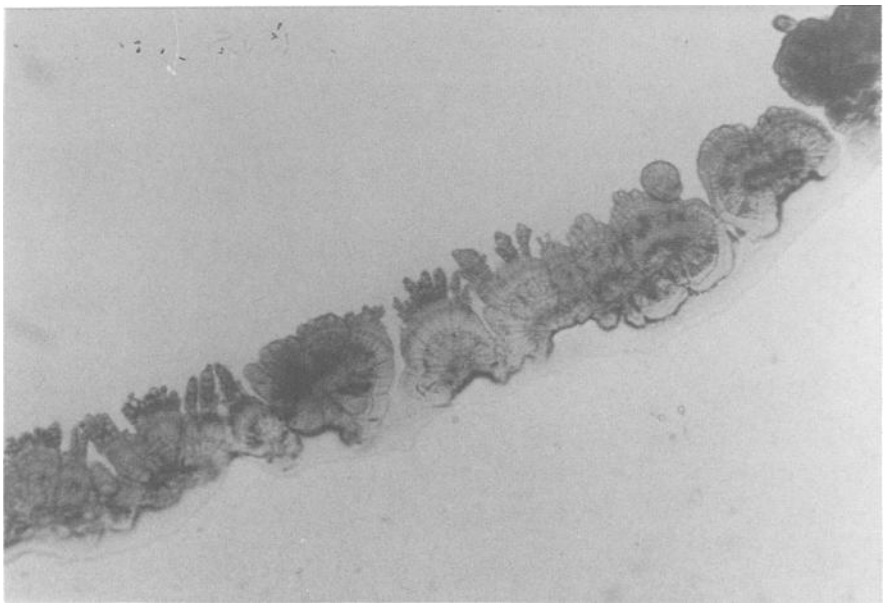

FIG. 11—*dl-methamphetamine with acidified NQS reagent.*

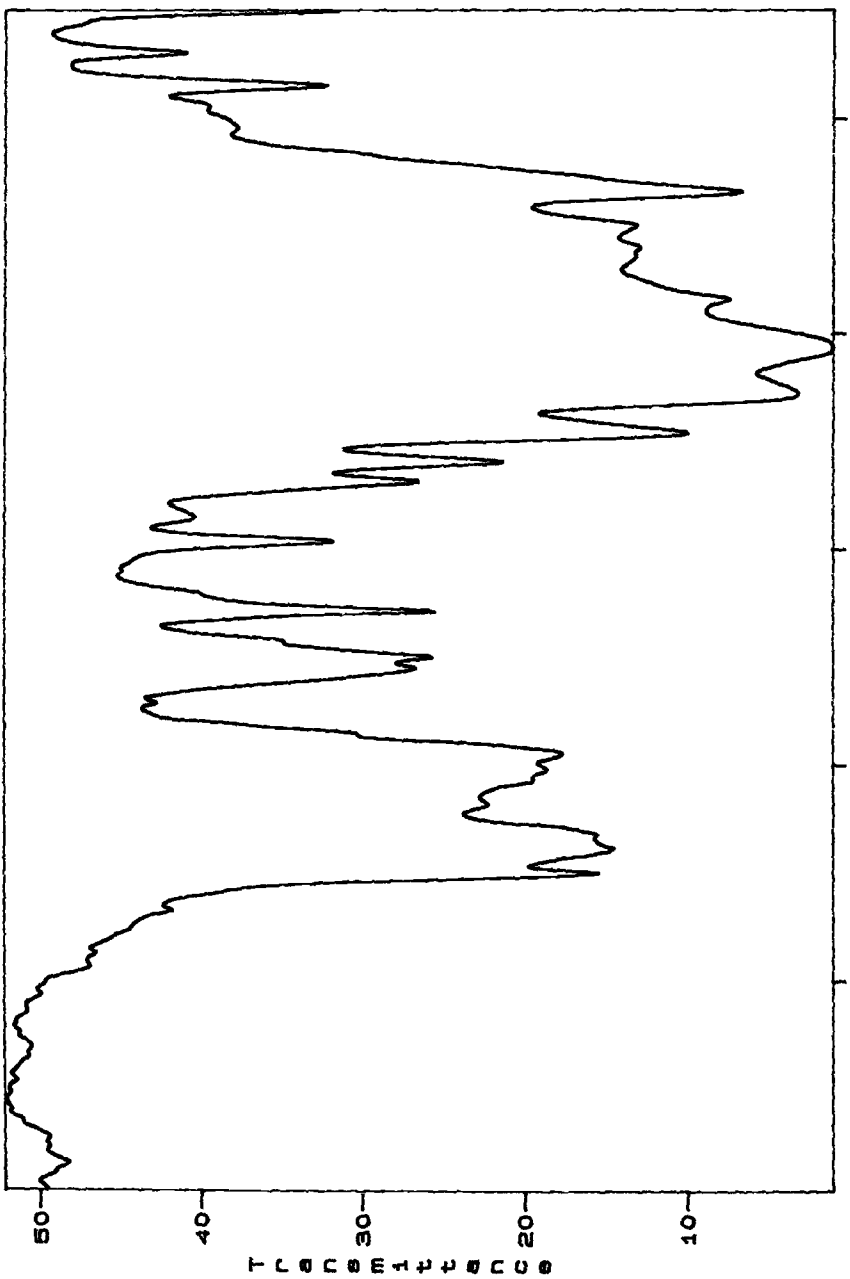

FIG. 12—*d-amphetamine sulfate with acidified NQS reagent.*

drug identification. While they do have a fairly high degree of specificity, the similarities in crystal forms produced by a reagent with closely related compounds, for example the opiates, would indicate that, unless the analyst is highly trained in chemical microscopy, a confirmatory test is necessary. The use of FTIR allows this confirmation to be achieved within a few minutes and after minimal training.

Acknowledgments

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References

[1] Fulton, C. C., Modern Microcrystal Tests for Drugs, Wiley-Interscience, New York, 1969.
[2] Clarke, E. G. C., Isolation and Identification of Drugs, 1st edition, The Pharmaceutical press, London, 1969, pp. 135–141.
[3] Palenik, S., Particle Atlas of Illicit Drugs Walter McCrone Associates, Chicago, 1974.
[4] Griffiths, P. R. and De Haseth, J. A., Fourier Transform Infrared Spectroscopy, Wiley-Interscience, New York, 1986.

Address requests for reprints or additional information to
Donna Wielbo, Ph.D.
Department of Pharmacodynamics
University of Illinois at Chicago
Box 6998
Chicago, IL 60680