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FINAL SUMMARY OVERVIEW

DEVELOPMENT OF A MODERN COMPENDIUM OF MICROCRYSTAL TESTS FOR ILLICIT DRUGS AND DIVERTED PHARMACEUTICALS

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1.0 Purpose

The ideal microanalytical method for forensic drug analysis is an automated procedure operable by a technician covering all known drugs. This certainly applies to many analytical techniques today including, for example, the combination of gas chromatography and mass spectroscopy (GC-MS). Light microscopy and microcrystal tests have been in use for more than 100 years but are not often regarded as a modern or ideal method; however, they are useful when the automated equipment is not available or when one wishes to check for the presence of one or several specific drugs. And while laboratories may lack analytical capabilities, most laboratories still have microscopes and microscopists with proper training. If the analyst wishes to know whether a specific drug is present, the polarized light microscope (PLM) will answer this question very quickly, and compared to other methods, very inexpensively. It might be added that certain methods of analysis for drug identification, for example those specified by SWGDRUG, require the use of multiple uncorrelated techniques. This indicates that a good use of the light microscope would be to check and confirm the results obtained by other methods. Microcrystal tests brought up to date to include optical properties of resultant crystals and compiled in a modern compendium, would be an excellent confirmatory method to give that added degree of confidence in the procedures and in the courtroom.

2.0 Project Design and Methods

The purpose of this project was to compile a comprehensive compendium of microcrystal tests which have previously been developed for illicit drugs and diverted pharmaceuticals by determining, locating, and compiling analytical data and literature reference material from numerous sources (many of which are out-of-print or difficult to locate) spanning past decades. Table 1 lists the 19 drugs that were selected, based off of the NFLIS report as stated in the cooperative agreement, to be included in the project.

Table 1: List of Drugs Included in Project

List of Drugs	
Amphetamine	Methadone
Benzylpiperazine (BZP)	Methamphetamine
Clonazepam	Methylphenidate
Cocaine	MDMA
Codeine	Morphine
Diazepam	Oxycodone
Ephedrine	Phencyclidine (PCP)
Heroin	Pseudoephedrine
Hydrocodone	Psilocin
Hydromorphone	

The project was broken down into a literature review period and four distinct research phases as follows:

Literature Review

The literature review period allowed for the compilation of microcrystal tests which have previously been developed for illicit drugs and diverted pharmaceuticals by

determining, locating, and collecting analytical data and literature material from numerous sources spanning past decades.

Phase 1: Survey of Labs

In order to provide the most useful and relevant information to the forensic laboratories, an online survey was conducted (funded by McCrone Research Institute) to determine which microcrystal tests are currently in use by most forensic laboratories. Because the literature contains numerous microcrystal tests that could be used to identify a single compound, it was most efficient to compile commonly used protocols for the compendium. The results of the survey were reviewed and used to determine which tests would be vetted and appraised by McCrone Research Institute microscopists along with practicing forensic scientists in other collaborative laboratories.

Phase 2: Evaluation of Microcrystal Tests

This phase evaluated and documented the microcrystal tests chosen in Phase 1 for the following characteristics:

- The amount of time required for the formation of crystals
- The sensitivity of the test
- How well the test worked in the presence of common excipients, diluents, and adulterants
- How well the microcrystal test worked on drugs within modern delivery devices such as transdermal patches

Phase 3: Optical Properties of Microcrystals

This phase described, in detail, the microcrystals formed from selected reagents and included additional photomicrographs for illustration purposes. Optical properties of the microcrystals were also documented including: refractive indices, birefringence, extinction, color/pleochroism, and sign of elongation. Infrared microspectroscopy of the microcrystals was also performed to aid in identification. These spectra will be included in the Compendium.

Phase 4: Compilation of the Microcrystal Compendium

This phase involved editing and compiling all the photomicrographs, illustrations, data and results into the compendium for publication and dissemination to the forensic science community. “A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals will be accessible on McCrone Research Institute’s website at <http://www.mcri.org/v/777/Modern-Compendium-of-Microcrystal-Tests-for-Illicit-Drugs-and-Diverted-Pharmaceuticals> and the NCJRS website. Note: Due to the large file size of the compendium PDF, which exceeds the GMS’ file size limit of 10MB, the program manager of this reward requests that the Final Summary Overview be submitted as the in the GMS and the PDF not be attached or included with it, but mailed on a thumb drive to: Frances Scott, Office of Justice Programs, National Institute of Justice, 810-7th Street, NW, 7th Floor, Washington, DC 20531.

3.0 Data Analysis and Project Findings

Although the microcrystal tests analyzed in this project are all well known and documented, the optical properties of the resulting microcrystals have never been documented. While the main goal was to compile the microcrystal tests into one

convenient compendium, another objective was to use the polarized light microscope to acquire new data about these crystals after they were formed. This information will help in their identification, as well as increase the confidence of the analyst in declaring a positive versus a negative result. Below are details of the optical properties that were measured on the resulting microcrystals. (In some cases, optical properties such as refractive index were difficult to measure, and thus were not of practical use.) The full detail of this data is available in the online compendium.

Limit of Detection

This project sought to determine each microcrystal test's limit of detection (LOD), or the minimum amount of sample required to obtain a positive result. Many of the reference texts refer to the tests being very sensitive, and some mention a limit of detection using a single crystal the size of a period on a printed page. In order to be as practical and useful as possible for forensic scientists in the laboratory, an analogous unit of measurement was established for this project: throughout the Compendium, sample size is measured in units of PPP, or a "period on a printed page." This unit represents a quantified amount of sample filling the area of a period printed using 10 point font in the Times New Roman style. The estimated weight of a PPP was approximated at about 0.1 mg. All microcrystal tests within the Compendium have LODs from 1PPP up to 3PPP for the drugs analyzed. The LOD is determined using the pure substance and does not reflect the amounts needed when other adulterants, excipients, or drug forms (tablets, liquids, etc.) are used. Nevertheless, it functions as a means to compare relative sensitivities between microcrystal tests, and also gives the lab analyst an indication of required sample quantity.

Refractive Index

The refractive indices of the resulting microcrystals was sometimes a difficult optical property to determine because the reagent needed to be washed off the crystals and dried before the Cargille certified refractive index liquid could be added for measurement. Crystals in ordinary aqueous reagents were easily dried at room temperature, while those in acidic reagents needed to be washed with a solvent, such as ethanol or chloroform, and dried before their refractive indices could be measured.

In many instances, crystals have refractive index values greater than 1.700. This is a very high refractive index, and above the limit of what most crime laboratories are capable of measuring with their available liquids. Therefore, when samples exhibited a refractive index greater than 1.700, we simply recorded that observation and did not pursue it any further.

Birefringence

Birefringence (B) was determined by estimating the thickness of the crystal using a calibrated ocular scale and applying that measurement to the retardation colors observed between crossed polars. The birefringence value B was then calculated using a Michel-Levy interference color chart and/or the classic birefringence equation, $B=R/(1000 \times T)$ where R equals retardation and T equals thickness. These B values were described as low (less than 0.010) or high (greater than 0.050) for the microcrystals that were precipitated by the reactions.

Sign of Elongation

Due to the elongated nature of many of the resulting microcrystals from these tests for drugs, a shape dependent optical property known as sign of elongation was recorded for identification purposes. If the refractive index parallel to a crystal's long axis, or length, is greater than the refractive index perpendicular to the long axis, or width, the crystal has a positive sign of elongation. If the opposite is true, it has a negative sign of elongation.

Some crystals have a radial arrangement of a needle-like habit. In those cases, the sign of elongation test can also be performed, but it is known instead as the sign of sphericity.

Interference Figures

Interference figures are one of the more difficult optical properties to determine, because the sample has to be of appropriate size and aligned in the proper orientation. Many of the microcrystals throughout the study were much too small for such conoscopic observations. Others were simply not sitting in the correct orientation to obtain an interference figure. Subsequent attempts to orient the crystals into the proper position were futile as the crystal shape caused the sample to have a preferential sitting position.

There were however, a few samples that did show good interference figures. For example, d- and dl-amphetamine with gold chloride reagent gives microcrystals that display well-oriented and easily identifiable interference figures: d-amphetamine crystal precipitates are biaxial (+) while dl-amphetamine is biaxial (-).

Fourier Transform Infrared Microspectroscopy

Spectra of the resulting crystals obtained by Fourier-transform infrared microspectroscopy (IMS) were not originally available in historical data. By collecting and including the infrared spectra from all of the resultant microcrystals in the new compendium, microcrystal tests will carry additional weight and build confidence in positively identifying drugs by these alternate techniques.

In reviewing the spectral data, small differences are seen between different microchemical tests for the same drug. These variances may simply be small peak shifts in wavenumbers, but nonetheless constitute distinct differences. The spectra collected from the microcrystals are also different from the spectrum of the original pure drug substance. Thus the chemical reaction that occurs when the microcrystals precipitate out of solution results in an observable change in the resulting spectra.

Adulterants, Excipients, and Modern Delivery Devices

Most of the drugs submitted to crime laboratories as evidence today are not pure substances and may be diluted or “cut” with adulterants and/or excipients. In addition, pharmaceutical formulations may contain mixtures and multiple drugs. When appropriate, the most commonly encountered adulterants and excipients were tested in various ratios alongside the drugs to determine the impact on the final microcrystalline form. Usually, the drug of interest was easily identified, even when present in minute quantities compared to a large amount of excipient. However, in rare cases, the drug of interest was completely undetectable in the presence of the excipient.

Several types of pharmaceuticals employing modern delivery devices were also tested to determine whether or not the crystal tests would provide results including gels, liquids, extended-release formulations, and transdermal patches. In some cases the

microcrystal test produced no positive results and micro-scale extractions were required to better facilitate a reaction. After some manipulations, many microcrystal tests were successful on modern delivery devices.

4.0 Implications for Criminal Justice Policy and Practice in the United States

Microcrystal tests for the analysis of controlled substances provide a fast, reliable, and inexpensive method of identifying substances. It is often one of the primary tests used by labs when GC-MS is not available. However, in instances where an established microcrystal test is not known by the lab or analyst, the ramifications can be far reaching.

Microcrystal tests have a number of positive attributes to recommend their use in any laboratory. Highlighting these uses should encourage crime laboratories to employ microchemical tests whenever possible. As shown throughout the compendium, most of these tests require only about 0.1mg of test substance, which is about the diameter of a period on a printed page, and most tests generate crystals almost instantly. Combining the sensitivity and speed of microcrystal tests with the new optical properties determined for each microcrystalline precipitate, together with the infrared spectra collected for each tests' precipitate equips analysts with a very powerful set of data for drug identification.

Having all of this data available in one place in the compendium is convenient and will save forensic scientists a lot of time. Not only are all of the tests simply described together with many photomicrographs, the reagents and their recipes are

easy to understand and prepare. This is a significant improvement because many of these recipes were convoluted and cryptic when described in the original texts.

Combining this information together with numerous photomicrographs and the resulting IMS spectra should make microcrystal tests much more straightforward to perform.

The significance and magnitude of giving analysts this modern and validated method for identification cannot be overstated, and it is hoped that the impact of the compendium on the criminal justice field will be profound.