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**Author(s):** Monica Joshi

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A SYSTEMATIC EVALUATION OF THE ANALYSIS OF DRUG MICROCRYSTALS USING  
INFRARED MICROSCOPY

**Final Summary Overview**

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810 Seventh St., NW  
Washington, DC 20531

**Prepared by:**

Monica Joshi  
Associate Professor, Chemistry  
750 S. Church St., Schmucker Science South  
West Chester University of Pennsylvania  
[mjoshi@wcupa.edu](mailto:mjoshi@wcupa.edu)  
Phone: 610-436-2880

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## Project Purpose:

In recent years, the extraordinary surge of psychoactive substances has completely transformed the landscape of recreational drugs. Families of synthetic cathinones, synthetic cannabinoids and phenethylamines multiplied both in number and variety posing serious public health risks. These and other emerging drugs have also created significant analytical challenges to forensic laboratories. One of the major challenges is the ability to detect and discriminate structurally similar compounds in complex mixtures. As a consequence, a substantial amount of research in recent years has been directed towards developing new techniques and improving existing analytical methods for these novel psychoactive substances.

This project focused on two existing analytical techniques: microcrystalline tests and infrared microspectroscopy. The purpose of this project was to develop an infrared microspectroscopy method for drug microcrystals with a focus on emerging drugs. The combination of the two uncorrelated techniques is aimed at being a rapid, cost-effective and sensitive analytical scheme where each individual technique is strengthened by the other. Microcrystal tests have stood the test of time even with the spread of sophisticated instrumentation. In recent years, there has been renewed attention on these tests. They are the subject of a recent compendium published by the McCrone Research Institute<sup>1</sup>. The compendium verifies and validates the established tests for 19 illicit drugs and adulterants. The project discussed here, funded by NIJ Award: 2014-R2-CX-K008, is a systematic study of microcrystalline tests for emerging drugs and the infrared spectra of the resulting microcrystals.

## Project design

The project was divided into smaller specific objectives to achieve the overall purpose of the project in the proposed two-year period. These objectives were conducted in the phases listed below.

1. Perform a systematic study of microcrystalline tests for synthetic drugs
2. Apply infrared microspectroscopy to the microcrystals and obtain molecular spectra of the crystals
3. Develop a reference library of microcrystal photomicrographs and infrared spectra
4. Perform infrared microspectroscopy analysis of microcrystals of typical forensic drug samples

Analytes were chosen for this study based on trends observed over 2015-2016 in literature, DEA trend alerts and other online resources. Thirty substances were chosen from five classes of psychoactive substances: cathinones (ring, side chain and amino group modification), phenylethylamines (substituted hallucinogenic and psychedelic phenethylamines) aminoindanes, opioids and piperazines. The substances and their class are listed in Appendix A of this report. Each of these substances was subjected to a systematic study using the reagents described in the section below. The results of these tests guided the infrared spectroscopy method.

### Microcrystalline tests

Microcrystalline tests were conducted with reference standards purchased as 1 mg or 5 mg hydrochloride salts from Cayman Chemical (Ann Arbor, MI). The standards were either methanolic solutions or in powder form. The standards in powder form were dissolved in 1 mL

methanol. Tests were conducted by placing 1-5  $\mu\text{L}$  methanolic solutions on a clean glass slide to achieve 5-25  $\mu\text{g}$  of the test substance. The dried residue was dissolved in either 5  $\mu\text{L}$  of  $\text{H}_2\text{O}$ ,  $\text{HCl}$  or  $\text{CH}_3\text{COOH}$ . The acidic solvents were only used for the aqueous gold and platinum chloride reagents. To this drop 5  $\mu\text{L}$  of the reagent was added. The drop was then mixed with a glass capillary to initiate crystallization. The glass slide was set aside and observed periodically for crystal growth. A Leica DM 750P polarized light microscope or an Olympus BX43F polarized light microscope were used to observe the resulting microcrystals. Characteristic microcrystals were documented as photomicrographs in the Olympus cellSense Entry imaging software at 100x magnification.

The following series of reagents were used for the project:

1. Gold chloride: Two formulations were used
  - a. 5% Aqueous  $\text{HAuCl}_4$
  - b. 5%  $\text{HAuCl}_4$  in 1:2 concentrated  $\text{H}_2\text{SO}_4$ :  $\text{H}_2\text{O}$
2. Gold bromide: 1 g  $\text{HAuCl}_4$  + 0.76g  $\text{NaBr}$  in 5 mL glacial  $\text{CH}_3\text{COOH}$  + 15 mL 2:3 concentrated  $\text{H}_2\text{SO}_4$ :  $\text{H}_2\text{O}$
3. Platinum chloride: 5% Aqueous  $\text{H}_2\text{PtCl}_6$
4. Platinum bromide: 1 g  $\text{H}_2\text{PtCl}_6$  in 1.7 mL 40%  $\text{HBr}$  + 20 mL 2:3 concentrated  $\text{H}_2\text{SO}_4$ :  $\text{H}_2\text{O}$
5. Mercuric chloride: Two formulations were used depending on the length of time for crystal growth. The reagent crystallizes very quickly in the aqueous formulation and prevents growth of drug-reagent crystals.
  - a. 1 g  $\text{HgCl}_2$  in 100 mL water
  - b. 1 g  $\text{HgCl}_2$  2.5 mL 1:1 Glycerol: Water 14.2 mL water, 500  $\mu\text{L}$  3M  $\text{HCl}$

6. Mercuric iodide: 1 g HgI<sub>2</sub> in 20ml 27:73 HCl: H<sub>2</sub>O

Cocaine and heroin were used as reference compounds to check reagent validity on a monthly basis. Each of the 30 substances included in this project were studied with the reagents listed above. The resultant crystal growth was observed in brightfield and under crossed polars. The shape, habit, size, length of time for crystal growth, dimorphism, birefringence and other specific crystal characteristics were noted. Terms used to describe the crystals were consistent with the terminology described in literature<sup>2,3</sup>.

The performance characteristics of repeatability, reproducibility and selectivity were studied. High and low concentrations, varying room temperatures, varying humidity conditions and different analysts were used to determine the repeatability and reproducibility of the tests. The selectivity of the tests was studied with two-component within-class mixtures and between-class mixtures. For a mixture of two drugs A and B, 5 tests were set up: A, B, A=B, A>B and B>A. The influence of one drug over the crystal formation of the other and any deformations to the single drug reaction were documented.

### Infrared microspectroscopy

Vibrational microspectroscopy of drug microcrystals has been studied before<sup>1,4,5</sup>. Wielbo and Tebbett describe many challenges in collecting infrared spectra from drug microcrystals<sup>4</sup>. Laughlin et al., have not yet described the infrared spectra collection methods<sup>1</sup>. Elie et al., describe Raman microspectroscopy as an alternative technique to circumvent the reagent interferences in collecting spectra of drug microcrystals. Raman microspectroscopy instrumentation is expensive and is not readily available in all forensic laboratories. In this

project, we studied several infrared spectroscopy methods and recommend some practices for collection of spectra of drug microcrystals.

1. Instrumentation: All spectra were collected by a Thermo Scientific™ Nicolet™ iN™10 Infrared Microscope with a liquid N<sub>2</sub> Mercury, Cadmium, Tellurium (MCT) detector. All data was processed and analyzed in the Thermo Scientific™ OMNIC™ Picta™ software.
2. Method parameters: Wavenumber range: 3500-700 cm<sup>-1</sup>, No. of scans: 128, Resolution: 4 cm<sup>-1</sup>, average aperture size 50 x 50 μm
3. Sampling techniques: This study compares spectra from reflection and transmission modes. In each mode, a traditional and a novel substrate were studied for feasibility. The sampling that consistently worked across various reagents and crystal shapes was chosen for the final document. In reflection mode, the commonly used gold slides were compared to the novel MirrIR low-e microscope slides (Kevley Technologies, Chesterland, OH). For transmission, BaF<sub>2</sub> salt windows were compared to the novel Amorphous Material Transmitting IR Radiation (AMTIR) windows (Pike Technologies, Madison, WI).
4. Crystal preparation: The microcrystalline tests were carried out on glass microscope slides. When characteristic crystals were observed under a polarized light microscope, single crystal manipulation techniques were used to separate the crystal from the matrix. This technique was suitable for crystals that were sturdy and retained their shape. Microcrystalline tests for a substance that yielded microcrystals with these characteristics were chosen for the infrared microspectroscopy studies. MicroTools and liquid wicks (MiTeGen LLC, Ithaca, NY) were used to separate and clean the crystals.

Upon transfer to the substrate of choice, the crystals were further washed with chloroform to remove any residue of the reagent.

## Project findings

The project resulted in the development of previously unreported microcrystalline tests for several novel psychoactive substances. The project describes the development of an infrared microspectroscopy method for the chemical identification of the drug microcrystal in addition to the optical description of the crystals. The combination method was applied against six street samples and was successful in identification of the primary psychoactive substance in them.

### Microcrystalline tests

Thirty substances were studied with each of the reagents listed. Many of the substances gave more than one reaction resulting in drug-reagent crystals. Reactions that occurred reproducibly and within a practical time frame were chosen as the best tests. The cathinone parent structure substances gave the most number and varied reactions. The crystals were reproducible, sturdy and easy to grow. The substances classified as opioids were the most difficult to grow crystals for. They required larger amounts of substances and took over 3 hours to grow any crystals.

In mixture studies, the results were dependent on the concentrations of the individual components. Distortions ranged from size of the crystals, composites crystals of both individual components or the formation of an entirely new habit of crystal. All these findings will be discussed in one of the planned publications listed below.

The microcrystalline tests are summarized below:

<b>Class of psychoactive substances</b>	<b>Best microcrystalline test reagent</b>
Cathinones	HAuBr <sub>4</sub> HAuCl <sub>4</sub>
Phenylethylamines	H <sub>2</sub> PtBr <sub>6</sub> H <sub>2</sub> PtCl <sub>6</sub>
Aminoindanes	H <sub>2</sub> PtBr <sub>6</sub>
Opioids	HgCl <sub>2</sub>
Piperazines	H <sub>2</sub> PtCl <sub>6</sub> H <sub>2</sub> PtBr <sub>6</sub> HAuCl <sub>4</sub>

### Infrared microspectroscopy

The purpose of the infrared method development was to improve the quality of spectra obtained from drug microcrystals. Microcrystals resulting from drug reagent reactions are temporary addition compounds where the drug is incorporated intact into the crystal habit specific to its structure. Therefore, the IR spectrum of a drug microcrystal should closely resemble the drug only spectrum. In this project we compared microcrystal spectra for the same drug with different reagents and for drugs that were structurally similar. While the spectra did show some shifts from the drug only spectra, our efforts were focused on reducing interferences from the reagent that can cause difficulty in spectral interpretation.

Sampling mode: Overall, the transmission mode spectra collected on AMTIR windows gave the cleanest and most informative spectra. The AMTIR surface is more robust, reusable, is resilient to the high acidic conditions of reagents and does not interfere with the microcrystal spectrum.

The drug reagent reaction can be carried out directly on the surface and the reagent wicked

away after the crystal growth has occurred. Though the BaF2 and MirrIR windows allow for transmitted light visualization of crystal growth, they are both severely affected by the reagent.

Overall, the infrared microspectroscopy method allows for visualization of the crystal before collection of the spectra and is non-destructive to the crystal. The spectra have some shifts when compared to the drug only spectra but given a microcrystal from an unknown substance, the method can be used to add structural information to the crystal which strengthens the microcrystalline tests. This method was also successful in mixtures where more than one type of crystal was formed, thus strengthening the discriminatory power of the infrared spectroscopy when analyzing mixtures.

### Implications for criminal justice policy and practice in the United States

Microcrystalline tests are a SWGDRUG Category B technique that are currently used sparingly because of the availability of chromatographic and other structural identification techniques. Their greatest strength lies in their ability to differentiate optical isomers based on crystal format. But their place in an analytical scheme is important because they are easy to do, require microgram quantities of samples, are cost effective, non-destructive and can very easily serve as a screening technique for unknowns. The findings of this project demonstrate that microcrystal tests can be easily applied to growing classes of novel psychoactive substances. For forensic laboratories using the microcrystalline tests for routine identification of traditional drugs such as cocaine and heroin, having a reference library of photomicrographs of other drugs can be a very useful tool when faced with mixtures. The project findings add another application for infrared microscopes in forensic labs. The methods developed show that there is

greater value in combination of the two techniques of microcrystalline tests and infrared microspectroscopy than use of either of them alone. The reference library of photomicrographs and infrared spectra is an extensive starting point for forensic laboratories to validate and apply.

## Scholarly Products

### Presentations

1. Sean Brady and Monica Joshi (February 2016) A Study of Microcrystal Tests for Emerging Psychoactive Substances, 68<sup>th</sup> American Academy of Forensic Sciences, Las Vegas, NV
2. Matthew Quinn and Monica Joshi (February 2017) Microcrystalline Tests for Emerging Drugs of Abuse, 69<sup>th</sup> American Academy of Forensic Sciences, New Orleans, LA
3. Michael Cain Jr. and Monica Joshi (February 2017) The Development of an Infrared Microspectroscopy Method for Microcrystals, 69<sup>th</sup> American Academy of Forensic Sciences, New Orleans, LA

### Planned Publications

1. Quinn, M. Joshi, M. Microcrystalline tests for emerging psychoactive substances, *Manuscript in preparation*. The manuscript is being prepared for publication in the journal Forensic Chemistry. The manuscript will be submitted for review in February 2017. The manuscript describes the characteristics of the crystals observed for each class of psychoactive substances and mixture studies.
2. Cain Jr., M. Joshi, M. Infrared microspectroscopy of drug microcrystals, *Manuscript in preparation*. The manuscript is being prepared for publication for review in the journal Forensic Chemistry or Drug Testing and Analysis. The manuscript is scheduled for

submission at the end of grant period in March 2017. The manuscript will describe the development of the infrared spectroscopy method for drug microcrystals and identification of unknown substances with a combination of microcrystalline tests and infrared microspectroscopy.

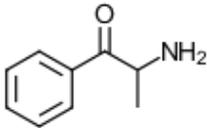
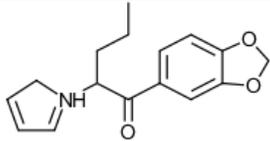
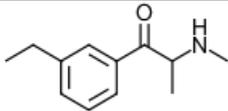
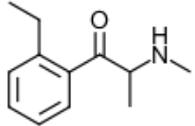
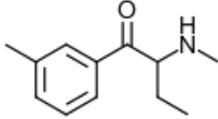
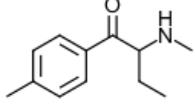
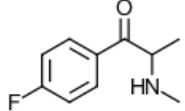
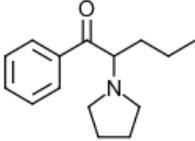
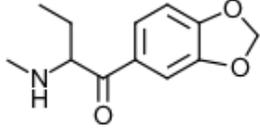
3. Reference database of microcrystalline tests and infrared spectra for novel psychoactive substances, *Database in preparation*. A simplified reference database of photomicrographs and infrared spectra is being prepared for availability through the National Institute of Justice website. The online access is expected in early 2017.

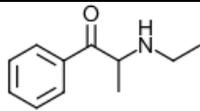
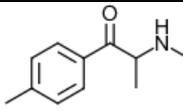
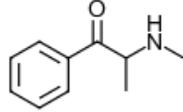
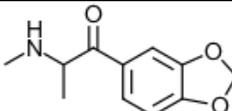
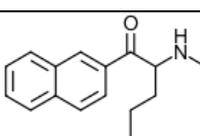
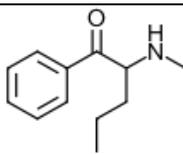
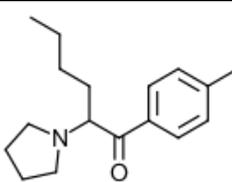
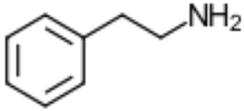
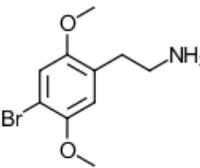
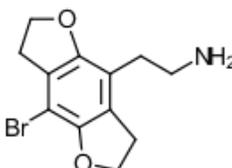
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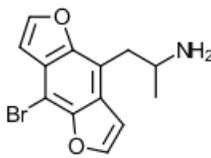
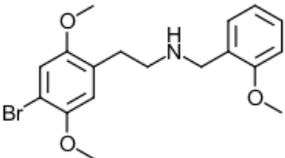
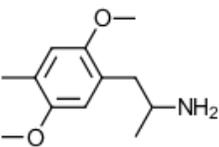
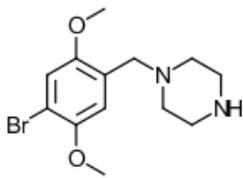
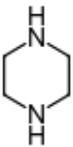
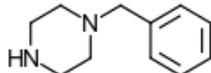
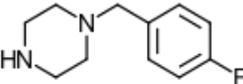
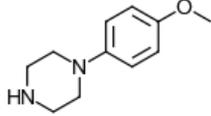
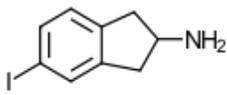
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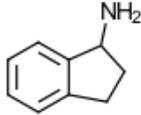
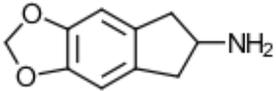
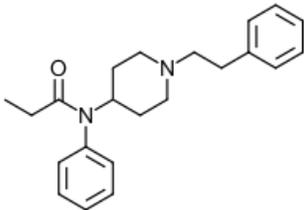
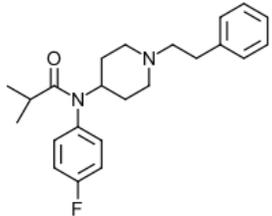
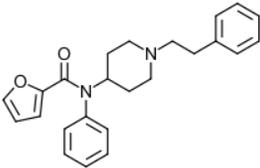
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## APPENDIX A: PSYCHOACTIVE SUBSTANCES STUDIED IN THIS PROJECT

CLASS	Name of substance	Molecular structure
<p style="text-align: center;">Cathinone</p> 	<p>3,4-Methylenedioxypropylammonium (3,4-MDPV)</p>	
	<p>3-Ethylmethcathinone</p>	
	<p>2-Ethylmethcathinone</p>	
	<p>3-Methylbuphedrone</p>	
	<p>4-Methylbuphedrone</p>	
	<p>4-Fluoromethcathinone</p>	
	<p><math>\alpha</math>-Pyrrolidinopentiophenone</p>	
	<p>Butylone</p>	

	Ethcathinone	
	Mephedrone	
	Methcathinone	
	Methylone	
	NRG-3	
	Pentadrone	
	4'-methyl- $\alpha$ - Pyrrolidinohexanophenone (MPHP)	
Phenethylamines 	2C-B	
	2C-B-fly	

	Bromo-DragonFLY	
	25B-NBOMe	
	DOM	
	2C-B-BZP	
Piperazines 	BZP	
	4-Fluoro BZP	
	1-(4-Methoxyphenyl) piperazine	
Aminoindanes	5-IAI	

	MDAI	
Opioids (various anallogs)	Fentanyl	
	FIBF	
	Furanyl Fentanyl	
	W-18	