A Modern Compendium of Microcrystal Tests

for Illicit Drugs and Diverted Pharmaceuticals

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Microcrystal tests

- Specific reagent is added to a suspected drug, and results in formation of a recognizable crystalline precipitate
- Inexpensive, fast, reliable, confirmatory
- Most microcrystal tests have been in use for decades

The Problem

- Most microcrystal references are old, out of print, or not easily accessible
  - Few photomicrographs
  - Reagent recipes and procedures may be difficult to understand
  - Lack information
    - Potential interferences
    - Optical properties of the microcrystals
  - New, alternative delivery devices (*e.g.* gels, dermal patches) may present challenges

Solution: Compendium

- Compilation of Current Microcrystal Tests
- Procedures vetted by McRI and practicing forensic scientists in collaborative labs
- Produce Compendium:
  - Recommended protocols
  - Morphologies of crystals (photomicrographs)
  - Infrared spectra
  - Optical and crystallographic properties
    - Missing in many references

Compilation of Current Tests

- National Forensic Laboratory Information System (NFLIS) Report
  - National and regional estimates for top 25 drugs
- Literature Review
- Survey of Labs (funded by McRI)

Drug Enforcement Administration

Office of Diversion Control

YEAR 2009 ANNUAL REPORT

NFLIS

NATIONAL FORENSIC LABORATORY INFORMATION SYSTEM

Table 1.1 NATIONAL AND REGIONAL ESTIMATES FOR THE 25 MOST FR

Estimated number and percentage of total analyzed drug items, 2009.

Drug	National		West		Midwest	
	Number	Percent	Number	Percent	Number	Percent
Cannabis/THC	590,791	33.96%	82,606	29.03%	198,918	49.95%
Cocaine	449,523	25.84%	44,704	15.71%	74,480	18.70%
Methamphetamine	134,891	7.75%	72,603	25.51%	19,241	4.83%
Heroin	118,136	6.79%	15,164	5.33%	29,784	7.48%
Oxycodone	47,098	2.71%	5,853	2.06%	7,819	1.96%
Hydrocodone	46,153	2.65%	5,669	1.99%	8,732	2.19%
Alprazolam	37,380	2.15%	2,154	0.76%	6,281	1.58%
MDMA	23,358	1.34%	6,061	2.13%	4,629	1.16%
1-Benzylpiperazine (BZP)	13,397	0.77%	1,069	0.38%	3,960	0.99%
Methadone	10,774	0.62%	1,915	0.67%	1,837	0.46%
Clonazepam	10,494	0.60%	1,130	0.40%	2,210	0.56%
Noncontrolled, non-narcotic drug[2]	8,745	0.50%	*	*	4	0.00%
Buprenorphine	8,172	0.47%	606	0.21%	881	0.22%
Diazepam	7,711	0.44%	1,340	0.47%	1,726	0.43%
Morphine	7,362	0.42%	1,585	0.56%	1,686	0.42%
Amphetamine	6,498	0.37%	772	0.27%	1,688	0.42%
Phencyclidine (PCP)	5,700	0.33%	813	0.29%	334	0.08%
Pseudoephedrine[3]	5,678	0.33%	245	0.09%	2,475	0.62%
Carisoprodol	4,863	0.28%	*	*	424	0.11%
Codeine	4,160	0.24%	686	0.24%	599	0.15%
Psilocin/psilocibin	4,063	0.23%	1,287	0.45%	1,073	0.27%
Methylphenidate	2,401	0.14%	323	0.11%	673	0.17%
Lorazepam	2,369	0.14%	436	0.15%	632	0.16%
Hydromorphone	2,232	0.13%	323	0.11%	413	0.10%
TFMPP	1,707	0.10%	*	*	355	0.09%
Top 25 Total	1,553,657	89.30%	250,120	87.89%	370,857	93.13%
All Other Analyzed Items	186,249	10.70%	34,455	12.11%	27,349	6.87%

The Drugs evaluated

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

Literature Review

- 41 references
- Listed all known microcrystal tests
  - No spot tests
- Spreadsheet broken down by:
  - Drug
  - Reagent/recipe
  - Comments
  - Results
  - Reference

A	B	C	D	E	F	G
Controlled substance	Drug class	Reagent	Reagent recipe	Comments	Result	Reference
Cocaine	Stimulant	1% Potassium permagnate	1 g KMnO4 in 5 drops H3PO4 adding water to make 100 mL	treat acid solution of the sample with a drop of 1% KMnO4 (until solution remains pink) then extract into a small amount of chloroform. Chloroform is then evaporated on to 2 slides to run the microcrystal tests	used in presence of procaine or benzocaine to purify sample	1
Cocaine	Stimulant	Di-p-toluoyl-d-tartatic acid in dil. Alcohol with glycerin (TDTA)	10 mg of TDTA taken up in 10 mL volumetric flask dissolved in 1 mL ethanol then made to volume by adding 8 mL of water and 1 mL of glycerin	drop of reagent placed on slide, small quantity of sample added to reagent and stirred	symmetrical rosettes with l-cocaine; needles to tufts, to fan shaped, to sheaves with d-cocaine	18
Cocaine	Stimulant	Di-p-toluoyl-l-tartatic acid in dil. Alcohol with glycerin (TLTA)	10 mg of TLTA taken up in 10 mL volumetric flask dissolved in 1 mL ethanol then made to volume by adding 8 mL of water and 1 mL of glycerin	drop of reagent placed on slide, small quantity of sample added to reagent and stirred	grayish white crystals from needles to tufts to fan shaped, to sheaves with l-cocaine; symmetrical rosettes with d-cocaine	18
Cocaine	Stimulant	Gold bromide	1 g gold chloride +1.5 mL HBr (40%)+28.5 mL of (2+3)H2SO4 is added to 30 mL of glacial acetic acid	Reagent added directly to test substance then coverslipped	Plates and variously skeletonized crystals, crosses or X's with ragged blade-arms, dichroic, salmnoy or orange to colorless (pg 300)	12
Cocaine	Stimulant	Gold chloride	1g gold chloride in 20 mL water	Test substance placed on slide. 1 drop of 1% HCl added (1% HOAc can be substitute). Small drop of reagent placed on coverslip. Edge of coverslip used to mix reagent and test substance. Coverslip dropped directly over sample.	X's with a bar through them	1
Cocaine	Stimulant	Gold chloride	dissolve 5g gold chloride in sufficient water to produce 100 mL	microdrop of test substance added to coverslip then a microdrop of reagent is added, the drop is stirred and inverted over a well slide	serrated needles	4
Cocaine	Stimulant	Gold chloride	5% solution gold chloride in reagent grade water	Dissolve test substance in small drop of 10% hydrochloric acid or 10 % acetic acid. Add small drop of reagent to edge of acid drop.	NA	10
Cocaine	Stimulant	Gold chloride	5% solution gold chloride	Dissolve test substance in small drop of reagent then add drop of 20% acetic acid.	White and forms 2 long rods that cross at 90 degree angle. Each axis has shorter branches perpendicular to the main axis. Variations based on adulterants present	11
Cocaine	Stimulant	Gold chloride	1g gold chloride in 20mL or 60 mL of 1:1 sulfuric acid or 1:3 HCl	Dissolve test substance in small drop of 1:3 HCl. Add reagent (Method 2?).	Highly skeletonized crystals, feathered X's, combs or ladders with branches/needles	12
Cocaine	Stimulant	Gold chloride	5% solution gold chloride in reagent grade water	Small drop of 5% acetic acid used to dissolve test substance. Add small drop of reagent as added to the drop.	2 long rods each containing smaller branches running perpendicular to eahc other like 4 fern leaves	16
Cocaine	Stimulant	Gold chloride	5% solution gold chloride in reagent grade water	2 drops of test substance (2-3 mg of drug/5 drops of 10% HCl) placed on slide. 2 drops of reagent placed nearby. Procede as method 2	Radiating clusters of fine needles with perpendicular branches	17
Cocaine	Stimulant	Gold chloride	5% solution gold chloride in reagent grade water	From LAPD - they use 20% acetic acid to dissolve the sample	Radiating clusters of fine needles with perpendicular branches	LAPD
Cocaine	Stimulant	Gold chloride (acid)	3g of auric chloride per 100 mL of water acidified with a few drops of phosphoric acid	I assume this works the same way as other gold chloride tests but couldn't find a direct mention of a procedure or results	I assume this works the same way as other gold chloride tests but couldn't find a direct mention of a procedure or results	19
Cocaine	Stimulant	Lead iodide	adjust a 30% w/v solution of potassium acetate in water to pH 6 with 2N acetic acid and saturate with lead iodide	microdrop of test substance added to coverslip then a microdrop of reagent is added, the drop is stirred and inverted over a well slide	feathery rosettes	4
Cocaine	Stimulant	Lead iodide	adjust a 30% w/v solution of potassium acetate in water to pH 6 with 2N acetic acid and saturate with lead iodide	From LAPD - they use 20% acetic acid to dissolve the sample	feathery rosettes	LAPD
Cocaine	Stimulant	Platinum bromide	1g H2PtCl6.6H20, 1.7ml of 40% HBr diluted to 40 mL with (1+7) H3PO4	Test substance dissolved in water, reagent added (Method 2?)	Skeletonized and feathery crystals	12
Cocaine	Stimulant	Platinum chloride	10% platinum chloride solution in water (w/v)	Test substance placed on slide. 1 drop of 1% acetic acid is added. Small drop of reagent placed on coverslip. Edge of coverslip used to mix reagent and test substance. Coverslip dropped directly over sample.	Asymmetrical feathered fronds	1
Cocaine	Stimulant	Platinum chloride	5% solution platinum chloride in reagent grade water	Dissolve test substance in small drop of 10% hydrochloric acid or 10 % acetic acid. Add small drop of reagent to edge of acid drop.	NA	10
Cocaine	Stimulant	Platinum chloride	1g platinum chloride in 10 mL or 20 mL of G-W(1+9)	Test substance dissolved in water, reagent added (Method 2?)	Skeletonized and feathery crystals	12
Cocaine	Stimulant	Platinum chloride	5% solution platinum chloride in reagent grade water	Small drop of 5% acetic acid used to dissolve test substance. Add small drop of reagent as added to the drop.	Feathery K shaped crystals	16
Cocaine	Stimulant	Platinum chloride	5% solution platinum chloride in reagent grade water	2 drops of test substance (2-3 mg of drug/5 drops of 10% HCl) placed on slide. 2 drops of reagent placed nearby. Procede as method 2	V-shaped long, thin needles with branching	17
Cocaine	Stimulant	Platinum chloride	5% solution platinum chloride in reagent grade water	From LAPD - they use 20% acetic acid to dissolve the sample	V-shaped long, thin needles with branching	LAPD
Cocaine	Stimulant	Potassium ferrocyanide		test substance added to drop of water which is lightly acidified then procede as method 2?	large rosettes	31

Survey of Labs (McRI Funded)

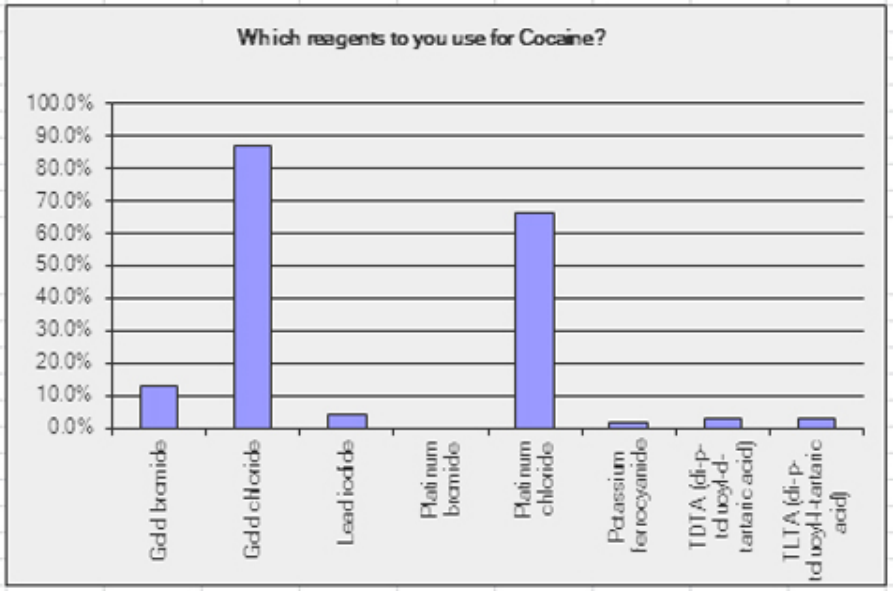
- 161 individuals involved
- 90 different labs
- What test used
- What reagent used
- Any other reagents
- SOPs

Microcrystal Tests for Drugs

Which reagents to you use for Cocaine?

Answer Options	Response Percent	Response Count
Gold bromide	13.0%	10
Gold chloride	87.0%	67
Lead iodide	3.9%	3
Platinum bromide	0.0%	0
Platinum chloride	66.2%	51
Potassium ferrocyanide	1.3%	1
TDTA (di-p-toluoyl-d-tartaric acid)	2.6%	2
TLTA (di-p-toluoyl-l-tartaric acid)	2.6%	2
Other (please specify)		3
answered question		77
skipped question		83

Number	Response Date	Other (please specify)	Categories
1	May 4, 2012 5:11 PM	TDTA and TLTA previously used but not currently.	
2	Apr 24, 2012 5:16 PM	sample with dil.acetid acid solution	
3	Apr 24, 2012 5:01 PM	Hydrochloric acid	



Evaluation of Tests

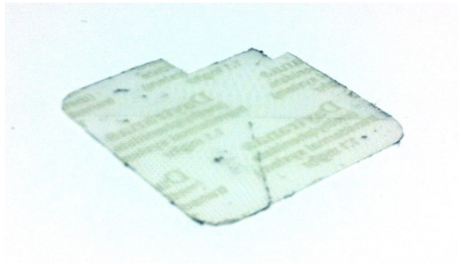
- Time required for formation of crystals
- Sensitivity of test
- How test works in presence of excipients, diluents, adulterants
  - Sugars, starch, caffeine, etc.
- How test works on modern delivery devices
  - Transdermal patches
  - Gels

Micro Extractions

- May be necessary to isolate drug of interest due to:
  - Interferences from other drugs
  - Matrix inhibitions (gels, patches)
  - Low concentration
- Solubility
- Acid/Base extraction

Micro Extractions (Pharms)

- Tablet
  - Ephedrine
  - Hydromorphone
  - Codeine
- Oral suspension (syrup)
  - Codeine
  - Methylphenidate
  - Methadone
- Rectal gel
  - Diazepam
- Gel
  - Oxycodone
  - Diazepam
- Transdermal patch
  - Methylphenidate



Final Compendium

- 1 drug per page
  - Reagent/recipe
  - Photomicrograph of microcrystals
  - FTIR of microcrystals
  - Optical/crystallographic properties
  - Additional reagents
- Available on www.mcri.org as PDF
  - ASTEE
  - MAFS

d-Amphetamine: Gold Chloride

2 reliable reagents

REAGENT 1: Gold Chloride ($HAuCl_4$)

1 g $HAuCl_4$, make up to 20 mL using (1+2) H_3PO_4, which is a dilute phosphoric acid made by combining one part (e.g. 6.67 mL) of concentrated H_3PO_4 with two parts (e.g. 13.33 mL) of water.

Test Method

There are two test methods, volatility and direct; both give the same crystals.
Volatility: Glass ring on slide, place sample at center and add a drop of 10 N (4 g/10 mL, 40%) NaOH. Invert 5 µL hanging drop of reagent on coverslip over glass ring for 5 minutes (longer if oily drops do not form at edge of hanging drop). Remove coverslip and place faceup to expose hanging drop to air.
Direct: Add 10 µL of reagent directly to sample (gentle stirring is optional), or dissolve sample in 2 µL of concentrated H_3PO_4 then add 2–10 µL of reagent.

References

1. ASTM E1969-11. Standard Guide for Microcrystal Testing in Forensic Analysis of Methamphetamine and Amphetamine, ASTM International: West Conshohocken, PA, 2011.
2. AOAC Official Methods, 13th Edition, William Horwitz, Ed., Association of Analytical Chemists: Washington, D.C. 1980.

Limit of Detection (LOD)

2 PPP for both test methods

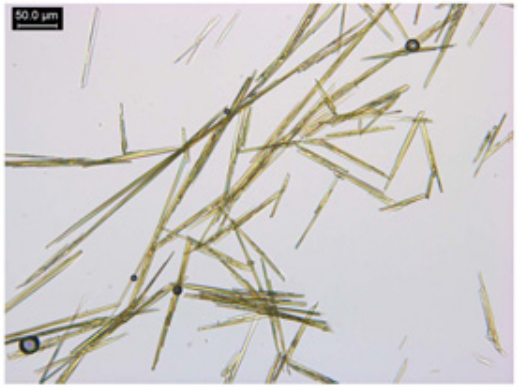
Time Lapsed for Crystal Formation

Volatility: 5 minutes
Direct: Instant

Crystal Morphology and Test Notes

Long yellow rods, sometimes segmented. Ends are blunt or slightly angled, though some taper.

Photomicrograph of Typical Crystals

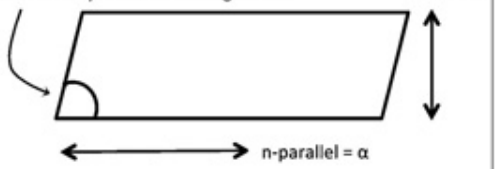


Crystals in a hanging drop. 2 PPP of d-amphetamine + one drop of 40% NaOH under a 5 µL hanging drop of reagent. See more images in Appendix.

Pharmaceuticals, Adulterants or Other Drug Interactions

Microcrystal test was successful on a single prill from a 10 mg capsule and on a single prill from a 10 mg "XR" extended release formulation.

PLM Optical Properties

Size	10 µm – 1+ mm
Color/Pleochroism	Yellow, not pleochroic
Refractive Indices	n-parallel = 1.658 (25° C) n-perpendicular > 1.70
Morphology Illustration Interfacial angles vary (may be 90° or less), and are likely due to uneven growth.  	
How Were Crystals Dried for RI?	Excess reagent was wicked with a lab tissue, then washed with chloroform via a tungsten needle. Residual reagent remained on some crystals but others were relatively dry.
Estimated Birefringence	Moderate
Extinction	Parallel only
Sign of Elongation	Negative (–)
Interference Figure	Biaxial (+), 2V ≈ 64°

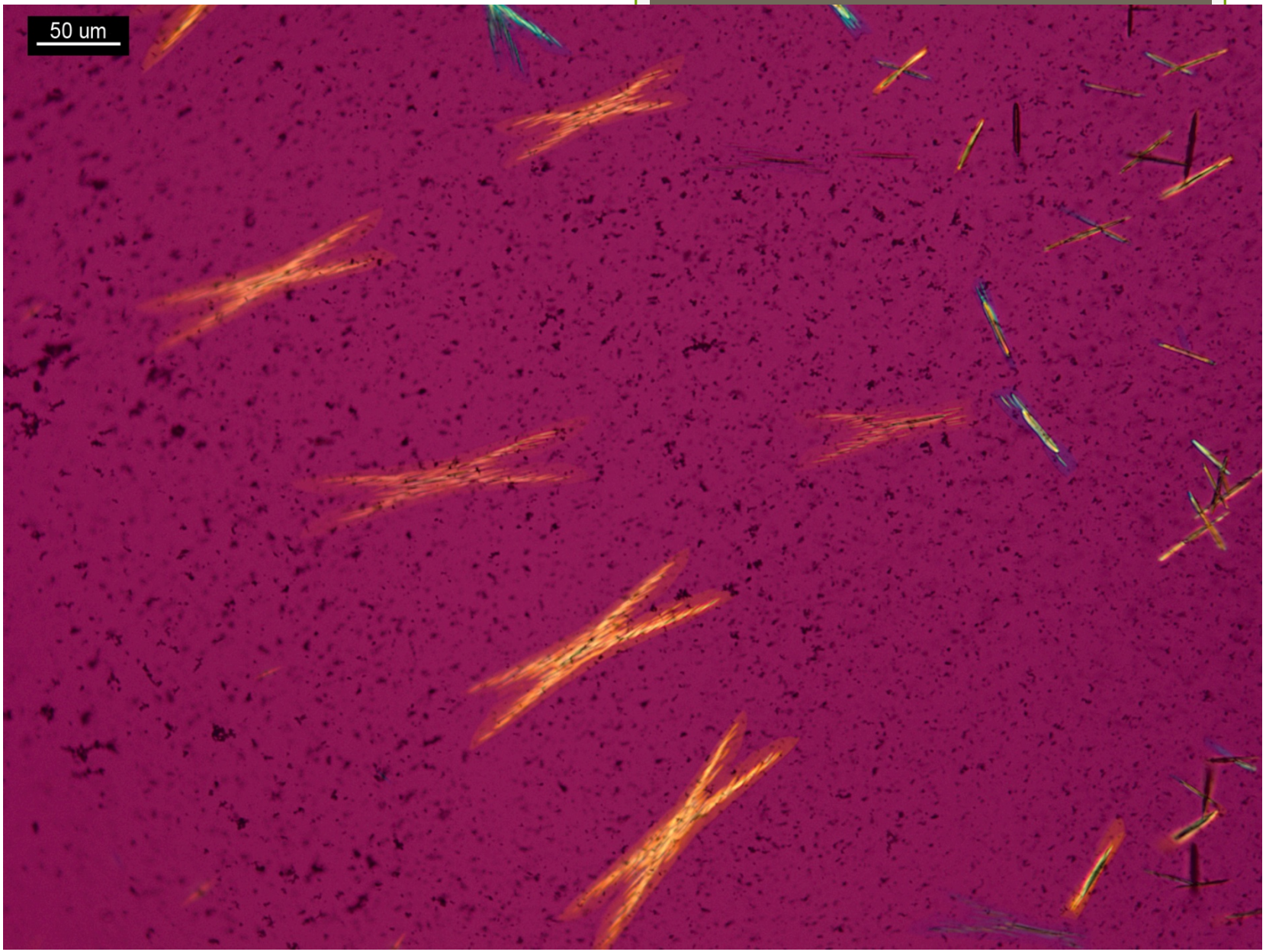
IR Spectrum

See spectrum image in Appendix

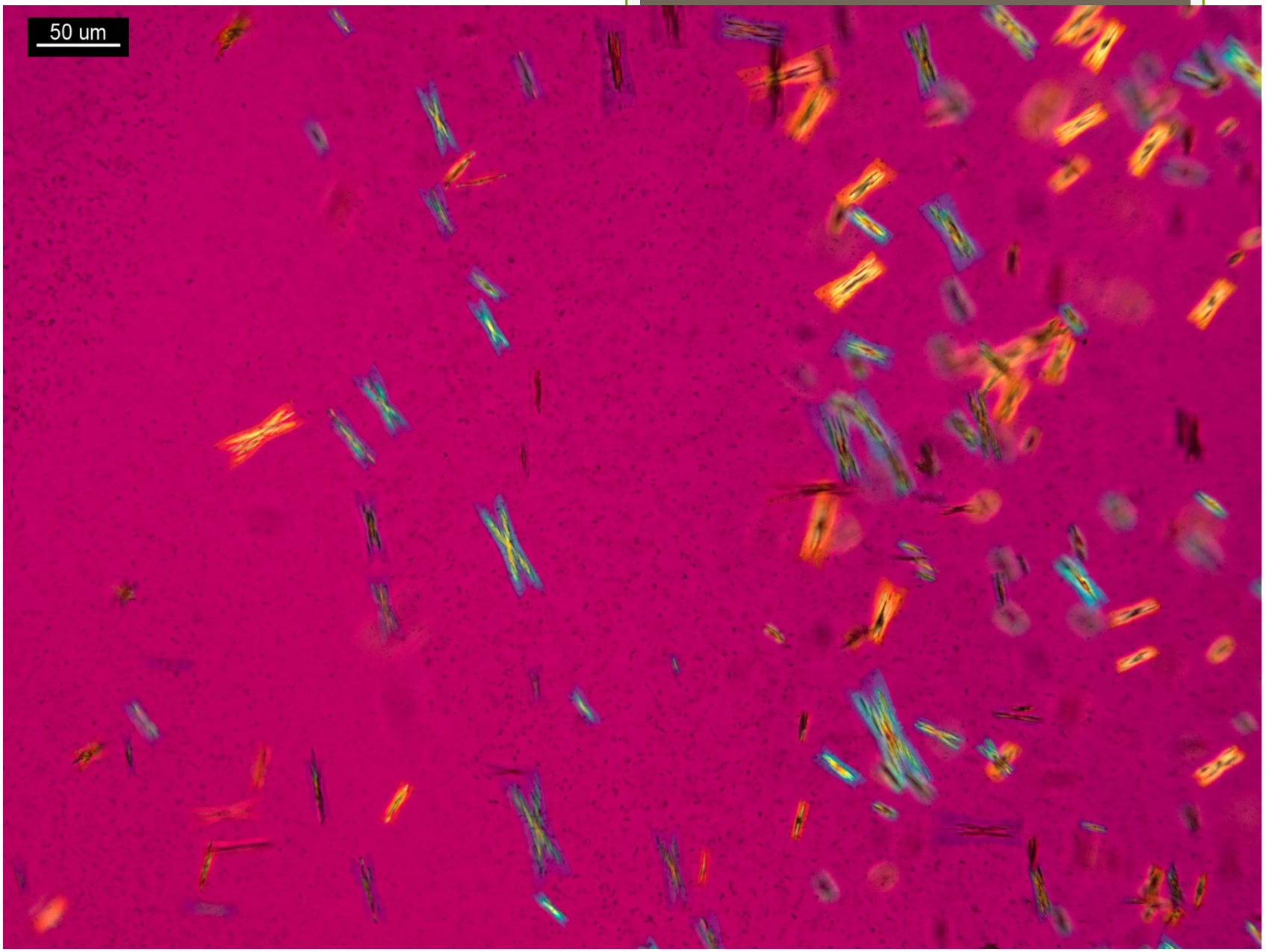
Codeine with I-KI

PCP with Potassium Permagnate

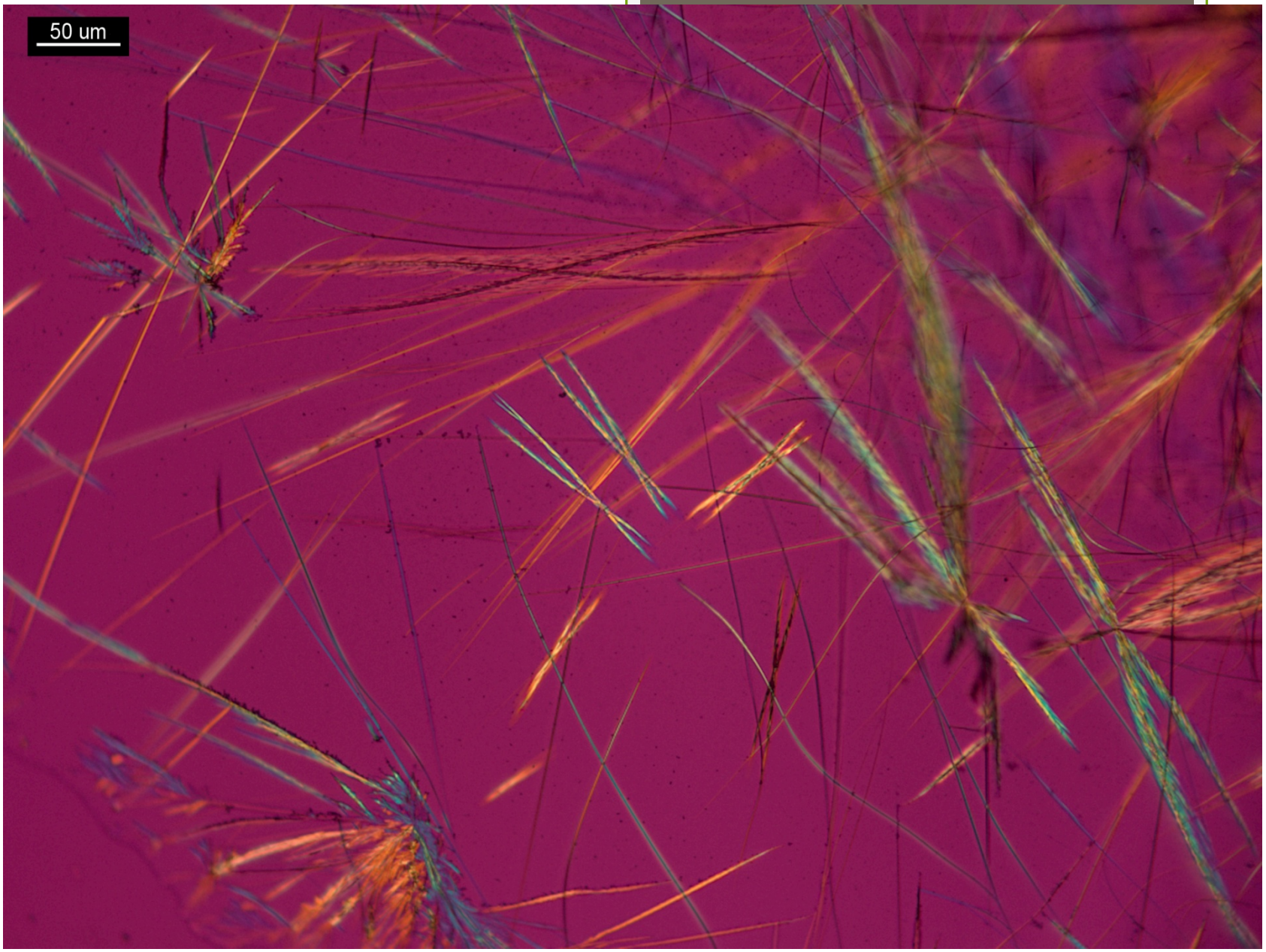
MDMA with Gold Chloride



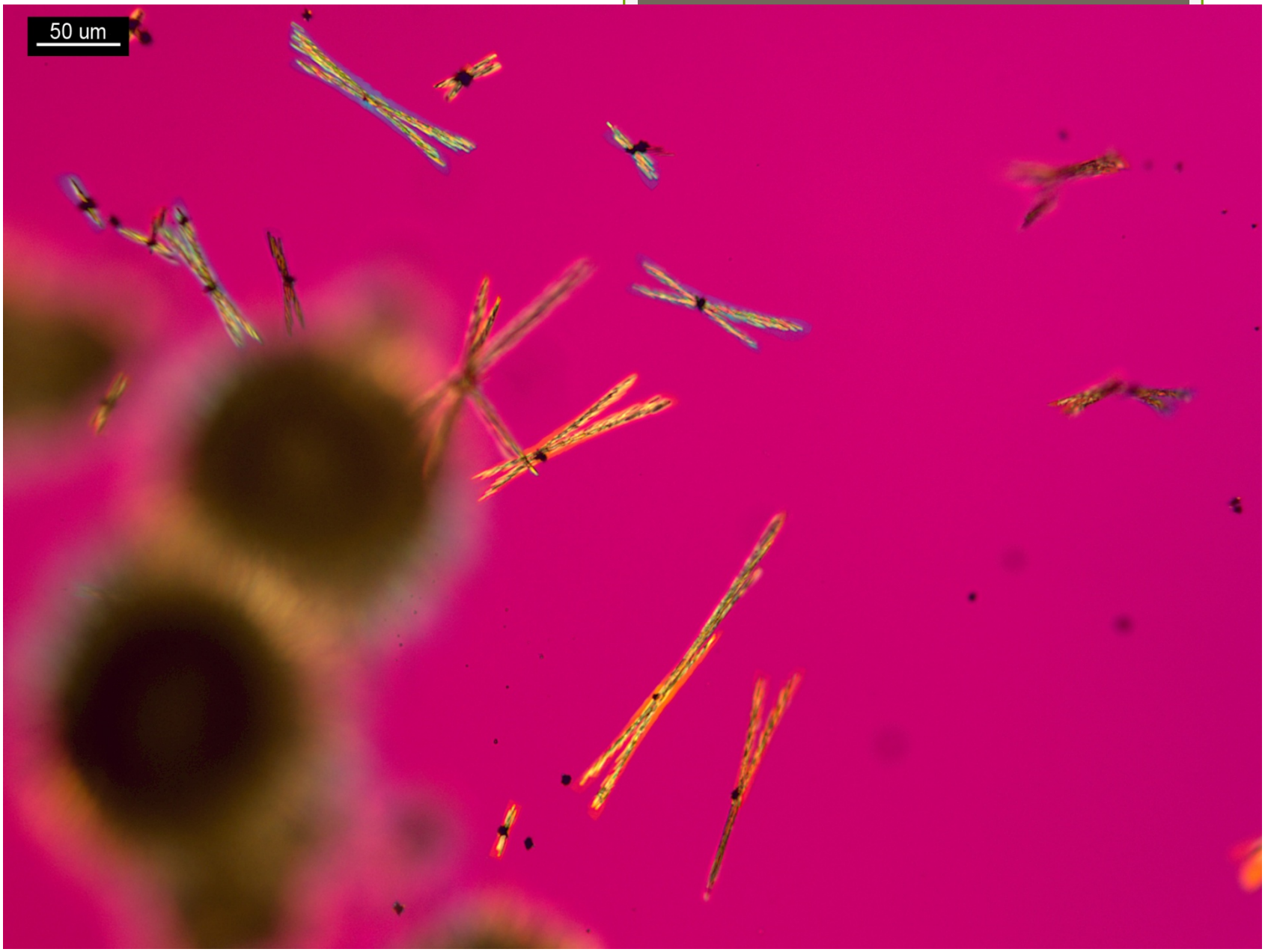
MDMA and dl-Amphetamine



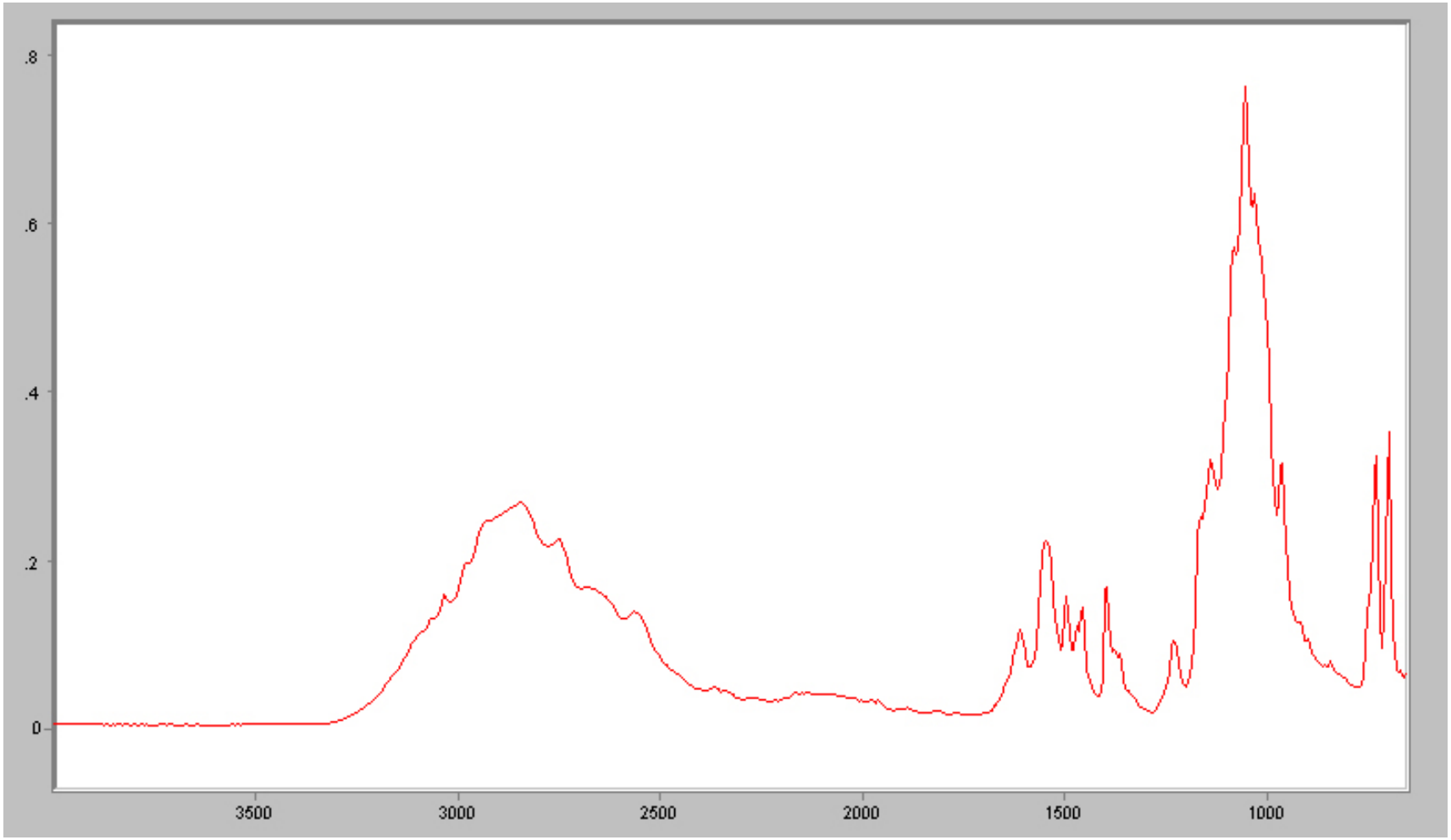
MDMA and Caffeine



MDMA Tablet



D-Amphetamine



Future Research

- New grant focused on pharmaceuticals and bath salts
- Develop tests for these substances
- Perform same analyses
- Add to existing compendium

Acknowledgements

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