**1.0 Purpose** - This procedure specifies the required elements for the preparation and use of microcrystalline reagents, and viewing suspected marijuana/hemp/hashish samples with the polarized microscope.

**2.0 Scope** - This procedure applies to all polarized light microscopy techniques used in the Drug Chemistry section of the Pitt County Sheriff's Office Forensic Services Unit.

**3.0 Definitions**

- **Hashish** - Common name for the extracted resin of marijuana.
- **Performance verification –** The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Quality control (QC) check -** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **Reference Material** – Material sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties.

**4.0 Equipment, Materials and Reagents**

**4.1 Equipment**

- *Leica* DM750P Polarized Light Microscope

**4.2 Materials**

- Beakers or other glass vessels
- Gloves
- Graduated cylinder
- Glass stirring rod
- Microscope slides
- Objective centering screws
- Reagent bottles and/or stock bottles
- Reference materials
- Spatula
- Suspected controlled substance exhibit
- Weigh vessel

**4.3 Reagents**

- Gold Chloride (ACS grade or higher)
- Mercuric Chloride (ACS grade or higher)

**5.0 Procedure**

**5.1 Sampling -** Plant material shall be sampled according to the Technical Procedure for Sampling.

### 5.2 New Microscopes

**5.2.1** New polarized microscopes shall be installed by an approved vendor according to the manufacturer's instructions, and documented in the Document Management (DM).

### 5.3 Maintenance

**5.3.1** Polarized microscopes shall be serviced yearly by an approved vendor, and service visits documented in DM.

**5.3.2** When a microscope has been placed out of service (e.g., maintenance, malfunction, leaving the direct control of the Laboratory), correct operation shall be verified by a successful cocaine crystal QC check.

**5.3.3** Laboratory personnel shall examine the effect(s) if any, of a malfunction on analysis and implement the Laboratory Procedure for Corrective Action as required.

**5.3.4** If the amount of light passing through the optics decreases significantly so that a sample cannot be seen, steps shall be taken to correct this. This may include, but is not limited to, checking the Kohler illumination parameters.

### 5.4 Operation of the Polarized Light Microscope

**5.4.1** Switch on the light source. (Refer to the operator manual for location/description of specific parts mentioned below.)

**5.4.2** Place the specimen slide on the stage.

**5.4.3** Adjust the desired light intensity with the control lever.

**5.4.4** Make sure the field diaphragm is open to the edge of the field view.

**5.4.5** Focus with the coarse and fine adjustments for the desired objective.

**5.4.6** Move the microscope slide around to view the entire specimen, adjusting the focus accordingly.

**5.4.7** Push the filter in to view the specimen with polars crossed, or pull it out to view with uncrossed polars.

**5.4.8** If the objective is changed:

**5.4.8.1** Adjust the fine focus adjustment.

**5.4.8.2** Set the field diaphragm to just inside the field of view.

**5.4.8.3** Adjust the aperture diaphragm for optimum contrast and resolution.

**5.5 Standards and Controls -** Quality control checks of all reagents shall consist of a negative check and a positive check. Both checks shall be acceptable according to the procedure listed for each reagent, and shall be recorded together as a quality control check in the Reagent Log.

**5.5.1** Negative quality control checks shall be performed according to the procedure listed with no sample present.

**5.5.1.1** Acceptable results are: no crystal formation.

**5.5.1.2** If crystals do form, steps will be taken until no crystals are formed. This includes retesting with a new microscope slide, re-cleaning any utensils used, or making a new reagent.

**5.5.2** Positive and negative quality control checks shall be performed at six month intervals according to the procedure listed for each reagent using the reference material listed. See each procedure for acceptable results.

**5.5.2.1** The result of the quality control check shall be recorded in the Reagent Log according to the Drug Chemistry Technical Procedure for Quality Assurance.

**5.5.3** Microcrystalline reagents may be prepared in any amount provided that the component ratios are kept constant.

**5.5.4** Microcrystalline reagents shall expire three years from date of preparation.

**5.5.5** Reagent bottles shall be labeled and checked as described in the Drug Chemistry Technical Procedure for Quality Assurance.

**5.6 Application of Procedures on Evidence**

**5.6.1 Dry Sample Method**

**5.6.1.1** A small portion of sample shall be placed on a microscope slide and a drop of the reagent or solvent shall be mixed with the sample.

- Optional step indicated in some procedures uses an acid solution (B) which may be used to dissolve the sample before addition of the reagent solution (A).

**5.6.1.2** Any crystals formed or hairs present shall be observed under non-filtered and/or filtered light.

**5.6.2 Criteria for Establishing Matches**

**5.6.2.1** Test samples that have evaporated to dryness may not be used for evaluation of crystals.

**5.6.2.2** Crystals are identified by morphology (i.e., shape).

**5.6.2.3** Crystals or cystolithic hairs shall be compared to those of standards tested in the same manner unless frequently observed.

**5.6.2.4** Final comparison shall be with actual crystals given by the standards listed.

## 5.7 Formulas for Preparing Reagents

### 5.7.1 5 % Mercuric Chloride

**5.7.1.1** This reagent is used for heroin and caffeine.

**5.7.1.2 5 % Mercuric Chloride (A)**

**5.7.1.2.1** Add 1.5 grams of mercuric chloride to 30 milliliters of distilled water (5% weight volume solution).

**5.7.1.2.2** Suggested Lot number format:
year/month/day/MerCur5/Initials of preparer.

**5.7.1.3 0.05 N Hydrochloric Acid (B)**

**5.7.1.3.1** Add 1 milliliter of concentrated hydrochloric acid to 250 milliliters of distilled water.

**5.7.1.4 Application of Procedure on Evidence**

**5.7.1.4.1** Mix directly with a drop of 5% mercuric chloride (A).

**5.7.1.4.2** Optional step: Dilute a small portion of the sample on a microscope slide in a drop of 0.05N hydrochloric acid (B) before mixing with a drop of the 5% mercuric chloride (A).

**5.7.1.5** QC check: Heroin forms fans/dendrites

**5.7.1.6** Results: Heroin forms fans/dendrites
Caffeine – dendrites, but longer and less dense than heroin dendrites.

### 5.7.2 Gold Chloride in 20 % Acetic Acid With Optional 0.05 N Hydrochloric Acid

**5.7.2.1** This reagent is used for cocaine and phencyclidine.

**5.7.2.2 Gold Chloride/20 % Acetic Acid (A)**

**5.7.2.2.1** Add 10 milliliters glacial acetic acid to 40 milliliters of water.

**5.7.2.2.2** Dissolve 1.0 gram of gold chloride in the 50 milliliters of 20 % acetic acid, with stirring.

**5.7.2.2.3** Suggested Lot number format:
year/month/day/GdCl20/Initials of preparer.

**5.7.2.3 0.05 N Hydrochloric Acid (B)**

**5.7.2.3.1** Add 1 milliliter of concentrated hydrochloric acid to 250 milliliters of distilled water.

**5.7.2.4 Application of Procedure on Evidence**

**5.7.2.4.1** Mix directly with a drop of gold chloride/20% acetic acid (A).

**5.7.2.4.2** Optional step: Dilute a small portion of the sample on a microscope slide in a drop of 0.05N hydrochloric acid (B) before mixing with a drop of gold chloride/20% acetic acid (A).

**5.7.2.5** QC Check: Cocaine forms cross shaped crystals.

**5.7.2.6** Results: Cocaine forms cross-shaped crystals.
Phencyclidine forms squares with diagonal markings, often elongated along one axis.

**5.7.2.7** Limitations: This technique does not distinguish between the salt and free base forms or the d-/l- forms of cocaine. It can however distinguish cocaine from its diastereoisomers.

**5.7.3 50 % Acetic Acid and Gold Chloride in 50 % Acetic Acid**

**5.7.3.1** This reagent is used for enantiomer determination for propoxyphene.

**5.7.3.2 Gold Chloride in 50 % Acetic Acid (A)**

**5.7.3.2.1** Carefully add 20 milliliters glacial acetic acid to 20 milliliters of distilled water.

**5.7.3.2.2** Mix the contents of a one gram ampoule of gold chloride into the 50 % acetic acid solution.

**5.7.3.2.3** Suggested Lot number format: year/month/day/GdCl50/Initials of preparer.

**5.7.3.3** **50 % Acetic Acid (B) –** Carefully add 20 milliliters glacial acetic acid to 20 milliliters of distilled water.

**5.7.3.4** **Application of Procedure on Evidence - Mixed Crystal Testing (for enantiomer determination)**

**5.7.3.4.1** Mix a portion of the unknown sample directly with reagent (A), or dilute the sample on a microscope slide in a drop of 50 % acetic acid (B) before mixing with a drop of the Gold Chloride in 50 % acetic acid (A). Crystals formed are compared with crystals formed from the mixture(s) in the next step**.**

**5.7.3.4.2** Mix a second portion of the unknown sample with an equal portion of the d- or l- isomer of propoxyphene. Mix directly with reagent (A), or dilute the sample on a microscope slide in a drop of 50 % acetic acid (B) before mixing with a drop of the Gold Chloride in 50 % acetic acid (A). Crystals formed are compared with crystals formed from the sample in the previous step.

**5.7.3.4.3** Single isomer and mixed isomer crystals have different appearances. Compare the crystals formed from the straight unknown to the d- and l-mixed samples. Isomer determination may be made when racemic crystals are formed by one of the known isomer additions.

**5.7.3.4.4** QC Check: d,l-Propoxyphene forms small, curved, irregular needles almost at once.

**5.7.3.4.5** Results: d,l-Propoxyphene forms small, curved, irregular needles almost at once.
Single isomer propoxyphene (d- and l-) give large, straight needles which are very slow to form.

## 5.8 Microscopic Examination of Marijuana/Hemp, or Hashish Using Chloroform

**5.8.1** Chloroform can be used to aid in the visualization of plant particles of marijuana/hemp or hashish.

**5.8.1.1** Marijuana/hemp or hashish cystolithic hairs look like bear claws.

**5.8.1.2** For frequently seen cystolithic hairs, the marijuana/hemp or hashish standard is not required to be run with each test sample.

**5.8.2 Application of Procedure on Evidence**

**5.8.2.1** Add a drop of chloroform to the microscope slide. View to ensure no hairs are present.

**5.8.2.1.1** If hairs are present, steps will be taken until no hairs are present. This includes, but is not limited to, obtaining a new microscope slide or new solvent.

**5.8.2.2** Place a small sample of suspected marijuana/hemp or hashish onto the microscope slide and mix the chloroform with the material.

**5.8.2.3** Observe under a relatively low magnification (approximately 10x). Record results of the microscopic examination in the case notes.

**5.8.3** Microscopic characteristics present in the exhibit shall be documented in the case notes by checking the box beside the characteristic(s).

**5.8.3.1** An indication of hairs implies the observation of both cystolithic and glandular hairs for that exhibit, unless otherwise noted. Be sure to indicate which type of hairs is/are observed with the polarized microscope.

## 6.0 Limitations

**6.1** Diluents may interfere with crystal formation in some cases. Extraction or solvent washes may be needed to remove unwanted components before microcrystalline reagents are used.

**6.2** Concentration of samples may need to be increased or decreased to aid in crystal formation.

## 7.0 References

*Leica* DM750 User Manual (Downloaded .pdf from internet after purchase.)

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## 8.0 Records

- Reagent Log(s)
- Microscope maintenance records in DM.

| **REVISION HISTORY** | | |
|---|---|---|
| **CURRENT VERSION** | **EFFECTIVE DATE** | **SUMMARY OF CHANGES** |
| 1 | 2017/11/14 | Original Document. |
| 2 | 2018/04/01 | **Header** – Added "Drug Chemistry"<br>**Scope** – Updated "Illicit Drugs discipline" to "Drug Chemistry section" |
| 3 | 2020/01/15 | **Header – Updated "Instruments" to "Drug Chemistry"**<br>**1.0** – Added "hemp" and removed "THC"<br>**3.0** – Added definitions for hashish and performance verification<br>**4.0** – Rearranged and added reagents list, and added ACS grade or higher requirement<br>**5.1 Sampling** – added section<br>**5.2 New Microscopes** – moved **original Section 5.5**<br>**5.3 Maintenance** –moved **original Section 5.6**<br>**5.5 Standards and Controls** – moved **original Section 5.1**<br>**5.8 Microscopic Examination of Marijuana/Hemp, or Hashish Using Chloroform** – Updated wording to include hemp.<br>**References** – Updated ASTM E1968 to more current version.<br>**Note:** Changes in this update were made to more closely match the corresponding instructions in the new Drug Chemistry Technical Procedure for the Identification of Plant Material, which utilizes the stereo microscope. |