



Designation: E1968 – 19

Standard Practice for Microcrystal Testing in Forensic Analysis for Cocaine¹

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INTRODUCTION

Microcrystal tests are primarily chemical-precipitation tests in which a light microscope is used to observe and distinguish the different types of crystals formed. These tests require skill and expertise on the part of the analyst that can be gained adequately only through appropriate training and experience in their use. These tests should not be attempted by those who are unfamiliar with them for use in the analysis of cocaine.

1. Scope

1.1 This practice describes procedures applicable to the analysis of cocaine using multiple microcrystal tests **(1-6)**.²

1.2 These procedures are applicable to cocaine, which is present in solid form or an injectable liquid form. They are not typically applicable to the analysis of cocaine in biological samples.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *These procedures could generate observations indicating a positive test for cocaine or its enantiomers which could be incorporated into the analytical scheme as defined by the laboratory.*

1.5 *This standard cannot replace knowledge, skills, or abilities acquired through appropriate education, training, and experience (see Practice E2326) and is to be used in conjunction with professional judgment by individuals with such discipline-specific knowledge, skills, and abilities.*

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the*

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² The boldface numbers in parentheses refer to a list of references at the end of this standard.

Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 ASTM Standards:³

E1459 Guide for Physical Evidence Labeling and Related Documentation

E1492 Practice for Receiving, Documenting, Storing, and Retrieving Evidence in a Forensic Science Laboratory

E1732 Terminology Relating to Forensic Science

E2326 Practice for Education and Training of Seized-Drug Analysts

E2329 Practice for Identification of Seized Drugs

E2548 Guide for Sampling Seized Drugs for Qualitative and Quantitative Analysis

E2764 Practice for Uncertainty Assessment in the Context of Seized-Drug Analysis

3. Terminology

3.1 Definitions:

3.1.1 For definitions of terms used in this standard, refer to Terminology E1732.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *aggregation, n*—the collecting of units or parts into a mass or whole.

3.2.2 *birefringence, n*—property of some crystals, those having more than one refractive index; this property will result in interference colors, which are viewed through a polarized light microscope.

³ For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3.2.2.1 *birefringent, adj*—material exhibiting birefringence.

3.2.3 *cocaine, n*—either *d*- or *l*-cocaine; it should be noted that *l*-cocaine is the naturally occurring isomer found in the coca plant.

3.2.4 *habit, n*—the external morphology of the crystal.

3.2.5 *microdrop, n*—a small drop of liquid that would fit on the end of a standard size, flattened toothpick; the approximate volume of this drop would be 10 to 25 μL .

3.2.6 *needles (acicular), n*—long, thin crystals with pointed ends.

4. Summary of the Technique

4.1 A small amount of test material containing the suspected cocaine is dissolved in a dilute acid and the appropriate precipitating reagent is added. The crystals that are formed are observed and distinguished utilizing a light microscope.

5. Significance and Use

5.1 This technique involves a chemical-precipitation reaction between cocaine and the precipitating reagent. The habit and the aggregation of the crystals formed could be used to distinguish cocaine from other drugs (6).

5.2 This technique can be utilized on cocaine present in either the salt or free base form.

5.3 This technique does not distinguish between the salt and free base forms.

6. Interferences

6.1 *Diluents/Adulterants*—Diluents/adulterants, such as lidocaine or benzocaine, present in combination with cocaine in the sample to be tested could inhibit crystal formation or could generate crystals that are distorted or otherwise rendered unidentifiable (7). Diluting the sample could reduce the interference. The higher the concentration of the adulterant, the more difficult it will be to observe characteristic crystals. There could be cases where diluting the sample would not work. In these instances, it will be necessary to separate the cocaine from the diluents/adulterants or to use other testing methods to analyze for cocaine.

7. Apparatus

7.1 *Standard Light Microscope*, capable of varying magnifications including 100 \times is needed for viewing the crystals. This is the minimum equipment required. A polarized light attachment is not essential, but is desirable, because the heavy metal crystals of cocaine are birefringent.

7.1.1 *Polarized Light Microscope (PLM)*, capable of varying magnifications from 40 \times to 400 \times . The following are typical accessories on a PLM and could be useful, but are not required, to conduct microcrystalline testing: specialized rotating stage (360 $^\circ$) and compensator (retardation plate). Cross-polarizers are verified by observing a black background when the polarizer and analyzer are in the optical path at 90 degrees to one another (for example, polarizer is in the east-west direction and the analyzer is in the north-south direction).

7.1.2 The best practice for documenting the crystal formation results is to take a digital photograph. It is advised that the minimum equipment required also has the capability of digital photography.

8. Reagents and Materials

8.1 10 %–20 % *Solution of Acetic Acid* (hereafter, dilute acetic acid).

8.2 *Cocaine Standard*.

8.2.1 *l-Cocaine Standard*.

8.3 5 % *Gold Chloride (HAuCl₄)*, in reagent grade water.

8.4 10 % or 0.5 N *Solution of Hydrochloric Acid* (hereafter, dilute HCl).

8.5 *Platinum Chloride (H₂PtCl₆)*, in reagent grade water.

8.6 10 mg *TDTA (+)-O,O'-Di-p-toluoyl-D-tartaric Acid Monohydrate [CAS 32634-68-7]* in 1 mL ethanol, 1 mL glycerin, and 8 mL distilled water (8).⁴

8.7 10 mg *TLTA (-)-O,O'-Di-p-toluoyl-L-tartaric Acid Monohydrate [CAS 32634-66-5]* in 1 mL ethanol, 1 mL glycerin, and 8 mL distilled water (8).⁴

8.8 *Methanol or Diethyl Ether*.

9. Sampling, Test Specimens, and Test Units

9.1 The general handling and tracking of samples should meet or exceed the requirements of Practice E1492 and Guides E1459 and E2548.

10. Performance Verification

10.1 Prior to casework, the reagents used for these microcrystal tests shall be tested for reliability using a cocaine standard and negative controls following the prescribed procedure. Only when it is determined that the reagents are producing the expected response could the reagents be used in the testing procedure.

10.2 The microscope should be inspected, adjusted, and aligned to ensure it is in proper working order. This can be confirmed during the testing of the cocaine standard. Perform the analysis of unknown samples and standards under the same microscope operating procedures (for example, use of cross polarizers).

11. Procedure

11.1 *Gold Chloride or Platinum Chloride Tests*:

11.1.1 Place a small amount (approximately 1 mg) of test material on a microscope slide.

11.1.1.1 While the test material can be placed directly onto the slide, it could also be introduced onto the slide from a dilute solution of methanol or diethyl ether and allowing the solvent to dry before continuing with the analysis.

11.1.2 Dissolve the sample in a few microdrops of dilute HCl or dilute acetic acid.

⁴ Crystals could form in the reagent after about three months. Prior to use, verify the reagents using the cocaine standard.


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11.1.3 *Optional*—Add a drop of dilute acetic acid to the dilute HCl on the slide.

11.1.4 Add a few microdrops of gold chloride reagent or platinum chloride reagent to the edge of the test solution on the microscope slide.

11.1.5 Observe the formation of the crystals using a properly aligned and adjusted standard light microscope or PLM. This observation can be done between crossed polarizers if desired. If crossed polarizers are to be used, verify cross-polarizers by observing a black background when the polarizer and analyzer are in the optical path at 90 degrees to one another (for example, polarizer is in the east-west direction and the analyzer in the north-south direction).

11.1.6 Formation of crystals corresponding to those obtained with standards is indicative of the presence of cocaine. The shape of these crystals could vary slightly depending on the concentration of the cocaine in the acid solution, or the acid/acid mixture used or diluents present.

11.1.7 If a dense cloud of precipitate is formed upon the addition of the precipitating reagent, it is possible that the crystals could not be readily visible. Allowing extra time for crystal formation is sometimes necessary. It could be necessary to repeat the test reducing the concentration of suspected cocaine in the acid solution by either decreasing the amount of test material or increasing the volume of solvent.

11.2 *TDTA and TLTA Optional Tests (Differentiation Between *l*- and *d*- Cocaine When Isomer Determination is Required)*:

11.2.1 Place a small sample of the test material on a microscope slide.

11.2.2 Add 1–2 drops of the TDTA or TLTA reagent to the test material.

11.2.2.1 If both tests are to be run on the same slide, use care to keep the reagent and test material areas separate.

11.2.3 Observe the crystals with a light microscope or PLM.

11.2.4 Crystal formation between *l*-cocaine and TDTA reagent is immediate. Crystal formation between *l*-cocaine and TLTA reagent is slower than for TDTA and crystals could form around the edge of the liquid.

11.2.5 Formation of crystals in a habit corresponding to those obtained with *l*-cocaine standard (*d*-cocaine is not readily commercially available) is indicative of the presence of *l*-cocaine.

11.2.5.1 If the test material contains *d*-cocaine, the TDTA and TLTA observations will be reversed (that is, the speed of reaction and crystal habits formed will be the same as observed with *l*-cocaine, but with the opposite reagents).

11.2.6 This procedure is optimized for the cocaine hydrochloride salt and does not work with cocaine base (“crack”) (7). Thus, the test material requires the addition of dilute HCl before conducting the test in order for crystals to form correctly.

12. Interpretation of Observations (9)

12.1 If crystals morphologically similar to those formed by a cocaine standard are formed by either precipitating reagents, the sample is considered positive by this technique for the presence of cocaine.

12.2 All observed crystalline precipitates shall be documented and included in the analyst’s notes for each item analyzed.

12.3 The forensic identification of cocaine requires the use of multiple techniques, see Practice E2329.

13. Precision and Bias

13.1 When used as described above, gold chloride is capable of distinguishing cocaine from its diastereoisomers and from other relevant analytes (9, 10). Limitations associated with the analysis shall be documented, as outlined in Section 5.2 of Practice E2764.

13.2 When used as described above, a combination of TDTA and TLTA tests are capable of distinguishing between enantiomers of cocaine (9). Limitations associated with the analysis shall be documented, as outlined in Section 5.2 of Practice E2764.

14. Keywords

14.1 analysis; cocaine; microcrystalline testing

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**E1968 – 19****RELATED MATERIAL**

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