History for the Cocaine SOP

Revision #	Issue or review date	History	Author or Reviewer
0	4/1/01	Original Issue	D.C. Sincerbeaux
1	8/27/02	Scope, add #	D.C. Sincerbeaux

Approval

Technical Leader _____ Date: 8-27-02
David Sincerbeaux

QA/QC Manager _____ Date: 8-27-02
Rick Groff

#4
Cocaine
Standard Operating Procedures

1.0.0 Background

Cocaine is one of many related alkaloids that can be extracted from the coca plant (Erythroxylon coca). Cocaine is a DEA controlled substance (CII) and can be identified using several different analytical techniques. General information about cocaine can be found in, "Erythroxylon Coca" a lecture by J.T. Maher 1976, DEA
"Cocaine" by C.Van Dyke & R.Byck, Scientific American Vol. 246 number 3, 1982.
"Topics in the Chemistry of Cocaine" by H.L. Schlesinger, Bulletin on Narcotics, Vol.XXXVII, No.1, 1985
"Drug Identification Bible", 4th Edition, 1999.

2.0.0 Scope

The following analytical procedures are used to confirm the presence of cocaine in samples.

3.0.0 Equipment and Reagents

The following pieces of equipment can be used in any combination to identify the analytes of interest.

3.1.0 A GC/MS and appropriate analytical software. Reference GC/MS SOP.
3.2.0 FTIR and appropriate analytical software. Reference FTIR SOP.
3.3.0 Polarizing microscope and reagents. Reference General Drug SOP.

4.0.0 Color Spot Tests

Cobalt thiocyanate is the most common spot test for cocaine. The base form of cocaine will not react with the cobalt thiocyanate. If the base form is suspected then a drop of HCl must be added to the sample. If cocaine is present then the turquoise precipitate will form.
Recipes for this reagent can be found in "Clarke's Isolation and Identification of Drugs" 2nd Edition, 1986.

5.0.0 GC/MS Sample Preparation and Analysis

5.1.0 Sample preparation.
- 5.1.1 Samples and standards are extracted directly using reagent grade solvent.
- 5.1.2 Samples and standards are dissolved in water, or weak acid, and then made basic with Na2CO3 or other strong base. Finally the solution is extracted using petroleum ether or hexane.

5.2.0 GC/MS analysis. The retention time of the sample should be within 0.04 minutes of a valid MS scan from the daily standard. **NOTE** The GC/MS is sensitive

to cocaine and care must be given to not overload the column and detector.

6.0.0 FTIR Sample Preparation Methods

6.1.0 Pick and Stick. Under a microscope cocaine appears as flat, mica like crystals. The cocaine can be separated from the cutting agent, added to KBr, and then formed into a pellet. This technique will often yield an IR pure spectra.

6.2.0 Cobalt Thiocyanate Derivative. To the sample add 2mls of the cobalt thiocyanate spot test reagent. Add 0.5 ml of concentrated HCl and mix well. Extract with chloroform and dry through Na2SO4 onto KBr. Let the chloroform evaporate and then make a pellet. Analyze and compare with a standard that was prepared the same way.
Reference: Naylor, Phillips, McCurdy, and Koers "A Simple Procedure for the Separation and Identification of Cocaine", Midwest Assoc. of Forensic Scientists Spring 1975 Meeting.

6.3.0 Direct. Grind some of the sample with KBr, and form a pellet.

6.4.0 Basic extraction and cleanup. Dissolve sample in water or weak acid. Make basic. Extract with appropriate non-polar solvent, and dry through NaSO4. Bubble HCl through extract and filter precipitate. Let dry and then mix with KBr, grind, and form a pellet.

6.5.0 Extract with chloroform, or methylene chloride, filter, and then recrystalize.

History for the Marijuana SOP

Revision #	Issue or review date	History	Author or Reviewer
0	8/17/01	Original Issue	Stuart Jacobson
1	8/27/02	Scope, add #	D.C. Sincerbeaux
2	11/05/04	Small changes in 6.2.3 and 3.4.0, dropped the use of benzene. 8.2.0 red from scarlet. 8.1.0 dropped need for unicellular hairs based on Note inserted into 5.1.2. 6.1.0, 4.1.2, and 4.2.1 appropriate solvent vs. pet ether.	D.C. Sincerbeaux

Approval

Technical Leader *David Sincerbeaux* Date: 11-10-04

QA/QC Manager *Rick Groff* Date: 11-5-2004

5
Marijuana
Standard Operating Procedures

1.0.0 Background

Marijuana (Cannabis Sativa) has been used for its sedative, euphoriant and hallucinogenic properties for over 3000 years. Written references to it date back to 2700 BC. It is primarily smoked but can be taken orally. The active compound, delta-9-tetrahydrocannabinol (THC) is most concentrated in the resin that is obtained from the flowers of the female plant. It is imperative that the analyst be familiar with the current Idaho code as it pertains to the legal definition of marijuana.

2.0.0 Scope

The following analytical procedures are used to confirm the presence of marijuana in plant material and residue samples. The procedure is composed of a series of tests, none of which by themselves are specific for marijuana or THC, but taken in combination are considered specific for the presence of marijuana or its resins. GC/MS is not routinely applied to marijuana analysis but may be used and is considered specific for THC.

3.0.0 Equipment and Reagents

3.1.0 Stereomicroscope.
3.2.0 Thin layer chromatography tank and plates.
3.3.0 Aqueous Fast Blue BB solution. (Fast Blue B salt may be used as a substitute)
3.4.0 Petroleum ether, hexane, diethyl ether, methanol, toluene, and chloroform.
3.5.0 GC/MS and analytical software.

4.0.0 Solvent Extraction

4.1.0 Plant material
- 4.1.1 Place approx. 0.1g of plant material in test tube.
- 4.1.2 Cover with appropriate solvent.
- 4.1.3 Use extract for thin layer and/or modified Duquenois-Levine.
- 4.1.4 Retain small amount of unused solvent as blank.

4.2.0 Residues
- 4.2.1. Flush pipe or item(s) containing suspected residue with appropriate solvent and collect solvent in test tube (item(s) may also be swabbed).
- 4.2.2. Use extract for thin layer and/or modified Duquenois-Levine.
- 4.2.3. Retain small amount of unused solvent as blank.

5.0.0 Microscopic Examination

5.1.0 Plant material is examined using a stereo microscope for the following characteristics:
- 5.1.1 Cystoliths and/or Cystolithic hairs – Small "bear claw" shaped hairs with bases of calcium carbonate. The cystoliths and hairs are located on the

topside of the leaf or leaf- fragment.

5.1.2 Unicellular hairs – Fine hairs located on the underside of the leaf or leaf-fragment. **Note** Unicellular hairs are not always observed on the leaves from the budding parts of the marijuana plant.

5.2.0 Seeds are examined using a stereomicroscope for the following characteristics:

5.2.1 Veined shell.

5.2.2 Ridged edges.

5.2.3 Point on one end and dint on the end of plant attachment.

6.0.0 Thin Layer Chromatography

6.1.0 Spot a small amount of solvent extract onto a thin layer plate along side of a marijuana standard and a solvent blank.

6.2.0 Develop the plate using one or more of the following mobile phases:

6.2.1 Hexane/diethyl ether 4:1 (petroleum ether may be substituted for hexane).

6.2.2 Chloroform or Toluene.

6.2.3 Petroleum ether/methanol 95:5 (if PCP is suspected)

6.3.0 Visualize by spraying the plate with Fast Blue BB salt solution.

6.3.0 Compare results of unknown to those of standard. Photocopy the plate for the case file.

7.0.0 Modified Duquenois-Levine

7.1.0 In a test tube containing a portion of the evaporated solvent extract, mix 2-10 drops of Duquenois reagent and an equal amount of concentrated HCl.

7.2.0 Let stand ½ to 3 minutes and observe color change.

7.3.0 Add chloroform.

7.4.0 Observe if the purple color transfers into chloroform layer. * Note* Transferring the solution from step 7.2.0 into a clean test tube before the addition of chloroform will decrease the color interference from chlorophyll.

8.0.0 Results and Reporting

A positive test shall be defined as the following:

8.1.0 Microscopic

8.1.1 Observation of cystolithic hairs on the leaf and/or the presence of characteristic seeds.

8.2.0 Thin Layer

8.2.1 Presence of a red spot with migration distance consistent with the red THC spot of the standard.

8.2.2 Negative blank.

8.3.0 Modified Duquenois-Levene

8.2.1. A purple* color developing after the addition of the HCl (*color may very from blue to reddish purple depending on the sample).

8.2.2. Transfer of the color into the organic layer after the addition of chloroform.

A positive result shall be defined as the following:

8.4.0 Positive microscopic, single TLC system, and modified Duquenois-Levine.

8.4.1 Report as "contains marijuana. Schedule I, non-narcotic".

8.5.0 Negative microscopic. Positive modified Duquenois-Levene and two positive TLC systems.

8.5.1 The conclusion should contain the words "contains, marijuana, and resins."

9.0.0 Germination

Marijuana seeds without THC are only controlled if they are fertile. The germination test should only be performed if it has been determined that the seeds do not contain THC. *Note* In determining the presence of THC, soaking the seeds for up to thirty minutes in petroleum ether /hexane, does not effect germination rates.

9.1.0 Wrap a minimum of 10, to a maximum of 100 seeds, in a moist paper towel and place in a covered container. The container is then placed in a safe place for 14 days.

9.2.0 Check seeds daily making sure they do not dry out. Also watch out for mould.

9.3.0 Report how many seeds sprouted as a percentage of the original total.

10.0.0 GC/MS Confirmation

10.1.0 Extract sample as in section 4.0.0

10.2.0 Run extract according to GC/MS SOP along with a known standard containing THC.

10.3.0 Compare retention time and ion chromatograph of sample with THC standard.

10.4.0 Report positive results using the words "Contains marijuana. Schedule I, non-narcotic" if cystolithic hairs or characteristic seeds were also found, otherwise use 8.5.1.

11.0 References

Identification of Marijuana, by J.I. Thornton and G.R. Nakamura
Journal Forensic Science (1972), 12, 461

History for the Psilocyn/Psilocybin Mushrooms SOP

Revision #	Issue or review date	History	Author or Reviewer
0	7/22/02	Original Issue	D.C. Sincerbeaux
1	8/27/02	Scope & #	D.C. Sincerbeaux

Approval

Technical Leader _____ Date: 8-27-02
David Sincerbeaux

QA/QC Manager _____ Date: 8-27-02
Rick Groff

#8
Psilocyn/Psilocybin Mushrooms
Standard Operating Procedures

1.0.0 Background

Psilocyn and psilocybin are related tryptamines that are found in many species of mushrooms. The mushrooms have been used in religious ceremonies for at least 3000 years by the native peoples of Mexico and Central America. Psilocyn and psilocybin are schedule one hallucinogens. More information is available through the "*Drug Identification Bible 2002".*

2.0.0 Scope

The following procedures are used to identify psilocyn and or psilocybin from mushrooms.

3.0.0 Equipment and Reagents

The following equipment can be used to identify the analytes of interest.

3.1.0 A GC/MS and appropriate analytical software. Reference GC/MS SOP.

3.2.0 Reagent grade solvents, methanol and acetone.

4.0.0 Color Spot Test

4.1.0 "Weber test"

4.1.1 Grind mushroom sample with mortar and pestle.

4.1.2 Add powdered sample to well of spot plate. Add 1% Fast blue BB solution. Should turn orange-red within a couple of minutes.

4.1.3 Remove some of the liquid to another well and then add a drop of concentrated HCl. A positive test is one that turns a blue-green color.

5.0.0 GC/MS Sample Preparation and Analysis

3.1.0 Extraction.

3.1.1 Grind up sample with mortar and pestle.

3.1.2 Place approximately 0.25 grams of sample into a test tube, add 2-3 mls of methanol, cap, shake, and let stand for at least 30 minutes. **NOTE** At this stage the methanolic extract may be injected into the GC/MS.

3.1.3 Centrifuge and decant solution into clean test tube. Cap and place into freezer for at least one hour.

3.1.4 Remove from freezer and immediately add equal volume of acetone and mix.

3.1.5 Centrifuge, decant, and concentrate (1ml) supernatant.

3.2.0 Analysis.

3.2.1 Run samples on GC/MS using a split or splitless data acquisition method

depending on the sensitivity of the instrument.

3.2.2 Compare with standard of either psilocyn or psilocybin. NOTE psilocybin breaks down into psilocyn in the hot injection port of a GC.

3.3.0 Conclusions and Reporting.

3.3.1 Confirmation. The retention time must be within 0.04 min of a valid scan of the standard and the MS spectra must match. If both conditions are satisfied then confirmation can be reported as "Contains psilocyn and/or psilocybin".

4.0.0 Thin Layer Chromatography

If differentiation of psilocyn and psilocybin is required then a T1 system (10mls methanol + 7 drops of NH4OH), developed with PDMAB, works well.

History for the Lysergic Acid Diethylamide SOP

Revision #	Issue or review date	History	Author or Reviewer
0	11/02/01	Original Issue	D.C. Sincerbeaux
1	8/27/02	Scope & add #	D.C. Sincerbeaux

Approval

Technical Leader _____ Date: 8-27-02
David Sincerbeaux

QA/QC Manager _____ Date: 8-27-02
Rick Groff