Technical Procedure for Extractions and Separations

1.0 Purpose - This procedure specifies the extraction and separation techniques used to analyze suspected controlled substances in solid dosage form.

2.0 Scope - This procedure applies to all separations and extractions performed in the Drug Chemistry Section at the Raleigh location of the State Crime Laboratory by Forensic Scientists who analyze solid dosage forms of suspected controlled substances.

3.0 Definitions - N/A

4.0 Equipment, Materials and Reagents

4.1 Equipment –** N/A

4.2 Materials

- Fume hood
- Heat source
- Eye protection
- Laboratory coat
- Gloves
- Beaker(s), sample vial(s), or other glass container(s)
- Centrifuge with centrifuge tubes or Vortex mixer
- Filter paper
- Funnel
- Glass stirring rod
- Graduated cylinder
- Mortar and pestle (optional)
- Pipettes with bulb
- Reagent or stock bottle(s)
- pH test paper
- Separatory funnel (optional)
- Syringe (disposable with needle)
- Spatula
- Test tube(s)
- Water (deionized)

4.3 Commercial Reagents

4.3.1 Suitable acid for example:

- Hydrochloric acid (concentrated or dilute)
- Acetic acid (glacial or dilute)

4.3.2 Suitable organic solvent(s) for example:

- Acetone
- Ethyl ether
- Chloroform
- Heptane

- Hexane
- Methanol
- Methylene chloride
- Isopropanol

4.3.3 Suitable base for example:

- Sodium hydroxide
- Sodium bicarbonate
- Ammonia/ammonium hydroxide

4.3.4 Drying agent (optional) for example:

- Sodium sulfate (anhydrous)
- Magnesium sulfate (anhydrous)

5.0 Procedure - Formula for Preparing Reagents - Reagents may be prepared in any amount provided that the component ratios are kept constant.

5.1 Reagents shall be labeled and stored according to the Drug Chemistry Section Technical Procedure for Receipt and Quality Assurance of Supplies, Equipment, Reference Collections, Standards and Reagents.

5.2 Solvents shall be labeled with name of solvent, date prepared and initials of preparer.

5.3 Ammoniated Solvents (Hexane or Chloroform)

5.3.1 Mix 10 milliliters ammonium hydroxide and 100 milliliters hexane (or chloroform) in a stock bottle and shake.

5.3.2 Allow layers to separate and draw off solvent for use.

5.4 0.6 N Hydrochloric Acid

5.4.1 Carefully add 5 milliliters of concentrated hydrochloric acid to 95 milliliters of water. (SAFETY NOTE: Always add the acid to the water.)

5.5 Ethyl Ether saturated with Hydrochloric Acid Reagent

5.5.1 Mix ethyl ether and concentrated hydrochloric acid in a test tube or other glass container in an approximate 1:5 (HCl:Ethyl ether) ratio.

5.5.2 Gently shake to mix the layers.

5.5.3 Allow layers to separate then remove ethyl ether for use.

5.5.4 Prepare fresh as needed.

5.6 Concentrated Sodium Hydroxide

5.6.1 Add desired amount of water to a beaker or other glass container.

5.6.2 Add sodium hydroxide pellets with stirring until solution is saturated (i.e., no more pellets will dissolve).

5.7 5 % Sodium Hydroxide

5.7.1 Dissolve 5 grams of sodium hydroxide pellets in 100 milliliters of water, with stirring.

5.8 20 % Sodium Hydroxide

5.8.1 Dissolve 20 grams of sodium hydroxide pellets in 100 milliliters of water, with stirring.

5.9 5 % Sulfuric Acid

5.9.1 Carefully add 5 milliliters of sulfuric acid to 95 milliliters of water, with stirring.

5.10 3:1 Chloroform:Isopropyl Alcohol Reagent

5.10.1 Mix 60 milliliters of chloroform and 20 milliliters isopropyl alcohol in a glass container. (Approximate 3:1 chloroform:isopropyl alcohol ratio.)

5.10.2 Gently shake to mix the layers before each use.

5.11 Potassium Permanganate

5.11.1 See Drug Chemistry Section Technical Procedure for Preliminary Color Tests for preparation instructions.

5.12 Expiration date

5.12.1 There is no expiration date for these solvents and reagents; however, quality control rechecks shall be performed on the reagents according to the Drug Chemistry Section Technical Procedure for Receipt and Quality Assurance of Supplies, Equipment, Reference Collection, Standards and Reagents to ensure reagent reliability.

5.13 Application of Procedures on Evidence

5.13.1 Extraction of Organic Acids and Bases

5.13.1.1 This procedure shall be used to isolate and purify acidic and basic compounds for further analysis. The chemical properties of the organic acid or base being extracted, and the properties of other substances mixed with the sample will determine which acids, bases, and solvents shall be used**.**

5.13.1.2 Dissolve the sample in 0.6 N hydrochloric acid or other suitable acid.

5.13.1.1.1 Check the pH of the solution with test paper to ensure the solution is acidic**.**

5.13.1.2 Extract the acid solution with a suitable organic solvent.

5.13.1.3 If an organic base is the compound of interest, discard the solvent washings.

5.13.1.4 If acidic drugs such as barbiturates or common diluents are compounds of interest, retain the solvent washings and evaporate for further analysis.

5.13.1.5 Make the acidic solution basic by adding a suitable base.

5.13.1.5.1 Check the pH of the solution with test paper to ensure the solution is basic.

5.13.1.6 Extract the basic solution with a suitable organic solvent.

5.13.1.7 If the organic base being extracted is not volatile, evaporate the solvent under the hood, leaving the extracted organic base.

5.13.1.7.1 The solvent may also be used for Gas Chromatograph-Mass Spectrometry (GC-MS) analysis if approved for use in the GC-MS instrument.

5.13.1.8 If the organic base is volatile (e.g., phenethylamines), or if the salt form of the organic base is desired, add the ethyl ether saturated with hydrochloric acid reagent drop wise until precipitation is complete.

5.13.1.8.1 Check with pH test paper to avoid excess hydrochloric acid being added.

5.13.1.9 Evaporate the solvent under the hood and/or filter to isolate the organic salt.

5.13.1.9.1 Organic solvent extracts may be dried using magnesium sulfate, sodium sulfate, or other drying agent.

5.13.1.9.2 Re-crystallization with an organic solvent such as ethyl ether or methanol may be performed.

5.13.1.10 Note: The extractions may be performed using separatory funnels, test tubes, glass sample vials, beakers, or reaction vessels collected from crime scenes.

5.13.1.11 Suggested uses:

5.13.1.11.1 Cold concentrated sodium hydroxide made to a paste and ethyl ether may be used to extract propoxyphene from pharmaceutical preparations of acetaminophen.

5.13.1.11.2 Sodium bicarbonate and chloroform may be used to extract LSD.

5.13.1.11.3 Cocaine HCl and nicotinamide may be separated by adding sodium hydroxide to an aqueous solution, decant aqueous. Wash solid material with water, extract with ethyl ether and evaporate the ether.

5.13.2 Dry Solvent Extractions of Drugs Using Ammoniated Solvents

5.13.2.1 This procedure is used to remove a variety of drugs from pharmaceutical preparations and clandestine mixtures.

5.13.2.2 Place a small amount of sample in filter paper over a small beaker and wash with ethyl ether.

5.13.2.3 Discard washings.

5.13.2.4 Allow sample to air dry briefly, and wash with several small portions of ammoniated hexane.

5.13.2.5 Evaporate solvent over moderate heat in a fume hood.

5.13.2.6 Note: The ammoniated solvent shall NOT be used directly for GC-MS analysis.

5.13.2.7 Suggested uses for ammoniated hexane:

5.13.2.7.1 Common diluents (e.g., caffeine, diltiazem, levamisole) may be removed from cocaine base.

5.13.2.7.2 Organic bases may be removed from acetaminophen and nicotinamide.

5.13.2.7.3 Basic drugs (e.g., opiates, diethylpropion, and diazepam) may be removed from pharmaceutical and clandestine preparations.

5.13.2.7.4 Phenethylamines (e.g., methamphetamine, etc.) may be removed from commercial and clandestine preparations, but volatile extracts shall be converted back to acidic pH with acidic ether before being evaporated.

5.13.2.8 Suggested uses for ammoniated chloroform:

5.13.2.8.1 Hydromorphone, morphine, diazepam, lorazepam, flurazepam, phentermine, chlordiazepoxide, cocaine, pentazocine, methaqualone, benzodiazepines from pharmaceutical and clandestine preparations.

5.13.3 Separation of Organic Acids and Bases by Solvent Wash

5.13.3.1 This procedure uses the solubility differences between organic acids, bases, and diluent materials to separate the desired components for further analysis.

5.13.3.2 Place a small amount of sample in filter paper over a small beaker or other glass container.

5.13.3.3 Wash sample with several small portions of suitable solvent.

5.13.3.4 Evaporate solvent over heat source in a fume hood to yield compounds.

5.13.3.5 Note: This procedure may be carried out in glass beakers or vials when a “backwash” is needed to help purify an extracted material.

5.13.3.6 Suggested uses:

5.13.3.6.1 Sample preparation for FT-IR analysis:

- Diazepam may be removed from commercial preparations with acetone or ethyl ether washes.
- Methylphenidate may be removed from commercial preparations with chloroform washes.

5.13.3.6.2 Common diluents (mannitol, inositol) may be removed from cocaine hydrochloride with chloroform washes. The cocaine remains in the chloroform wash.

5.13.3.6.3 Lidocaine HCl may be removed from cocaine HCl with acetone washes. The cocaine HCl remains in the filter paper and the lidocaine remains in the acetone wash.

5.13.3.6.4 Methamphetamine/dimethylsulfone mixtures may be separated with successive and multiple washes of ethyl ether, acetone, and chloroform. The methamphetamine remains in the chloroform wash and the dimethylsulfone remains in the acetone wash.

5.13.3.6.5 Clorazepate may be removed from commercial preparations using chloroform:methanol (3:1) followed by subsequent chloroform washes.

5.13.3.6.6 Sample preparation for GC-MS analysis:

5.13.3.6.6.1 Alprazolam, lorazepam, diazepam, etc.: Add several drops of solvent to an intact (uncrushed) tablet. Allow the tablet to soak for a short time. Transfer the solvent through a filter to a sample vial or insert and add more solvent for analysis.

5.13.3.6.6.2 Coated tablets: Remove coating before adding several drops of solvent to the remaining intact tablet, prepare as described above. Pharmaceutical tablets shall be extracted to remove large amounts of acetaminophen or aspirin prior to running on the GC-MS.

5.13.3.6.6.3 Sulfates need to be extracted/converted before they are analyzed via GC-MS.

5.13.3.6.6.4 Syringes: Wash with methanol and extract if necessary. (If excessive quantities of blood or other liquids are present in a syringe, an extraction is required).

5.13.3.6.6.5 Sugar cubes or blotter with LSD may be washed with methanol to obtain samples for color tests.

Sugar cubes or blotter may then be soaked in methanol overnight in the refrigerator and then analyzed via GC-MS.

5.13.4 Extraction of Psilocybe Mushrooms - *Using Acetic Acid:*

5.13.4.1 Break up approximately 1-2 grams of psilocybe mushrooms and place in a small beaker.

5.13.4.2 Add enough deionized water to moisten the sample.

5.13.4.3 Add 1-2 milliliters of glacial acetic acid.

5.13.4.4 Check with pH test paper to ensure solution is acidic.

5.13.4.5 Stir 1-2 minutes. (DO NOT leave in acidic solution for extended period of time.)

5.13.4.6 Decant liquid to large test tube.

5.13.4.7 Carefully add concentrated ammonium hydroxide drop-wise until a pH of 8 is obtained.

5.13.4.8 Gently extract with ethyl ether or 3:1 chloroform/isopropyl alcohol reagent.

5.13.4.9 Solvent may be dried using magnesium sulfate or sodium sulfate.

5.13.4.10 Evaporate under nitrogen or air flow with NO HEAT.

5.13.4.11 Reconstitute precipitate in chloroform or methanol to inject on GC-MS.

5.13.5 Extraction of Psilocybe Mushrooms - *Using Sodium Bicarbonate:*

5.13.5.1 Break up approximately 1-2 grams of psilocybe mushrooms and place in a small beaker.

5.13.5.2 Add water and sodium bicarbonate (approximately 10 grams) until an off-white paste forms.

5.13.5.3 Check with pH test paper to ensure solution is weakly basic or a pH of 8 is obtained.

5.13.5.4 Add ethyl ether and stir.

5.13.5.5 Decant off ethyl ether.

5.13.5.6 Solvent may be dried using magnesium sulfate or sodium sulfate.

5.13.5.7 Evaporate solvent under nitrogen or air flow with NO HEAT.

5.13.5.8 Reconstitute precipitate in chloroform or methanol to inject on GC-MS.

5.13.6 Extraction of Anabolic Steroids from Vegetable Oils

5.13.6.1 This procedure is used to isolate anabolic steroids from various vegetable oil preparations.

5.13.6.2 Withdraw 1 milliliter of oil from multi-injection sample vial using a disposable syringe, and transfer the oil to a centrifuge tube or test tube.

5.13.6.3 Add 2 milliliters of heptane or hexane and mix well.

5.13.6.4 Add 1 milliliter of methanol and mix well.

5.13.6.5 Centrifuge or vortex for 2-3 minutes to separate the layers.

5.13.6.6 Transfer the methanol layer to a small beaker or vial and evaporate methanol with a heat source.

5.13.7 Separation of Cocaine Base and Diluents Utilizing Hexane/Water

5.13.7.1 This procedure is used to separate procaine base, benzocaine base, levamisole, and other hexane insoluble compounds.

5.13.7.2 Crush a portion of the sample and place in a test tube.

5.13.7.2.1 The size of the portion used will be dictated by the ratio of the diluents(s) to cocaine base present in the sample. Suggested 20-30 milligrams if available.

5.13.7.3 Add approximately 2 milliliters of hexane to the test tube.

5.13.7.4 Vortex or agitate test tube.

5.13.7.5 Allow layers to separate and any insoluble material to settle to the bottom.

5.13.7.6 Remove hexane to a second test tube.

5.13.7.7 Add approximately 10 milliliters water to hexane. (Fill rest of test tube.)

5.13.7.8 Vortex or agitate test tube.

5.13.7.9 Remove hexane layer and evaporate hexane over moderate heat to obtain sample.

5.13.7.9.1 A drying agent such as sodium sulfate may be used prior to evaporating hexane.

5.13.7.9.2 If a large amount of diluent is present in the sample, additional washings of hexane may be performed prior to evaporation. Remove the hexane layer to a new test tube and add additional aliquots of water. Repeat until diluent is removed.

5.13.7.10 Note: Keep top of test tubes pointed away from face or covered while vortexing to avoid splashing in eyes or face.

5.13.7.11 If an infrared of the diluents is desired, extract the water solution with approximately 25 milliliters of methylene chloride. Sodium sulfate or other drying agent may be used if needed.

5.13.8 Separation of Cocaine Base Utilizing Potassium Permanganate

5.13.8.1 This procedure is used to remove cinnamoyl cocaines, nicotinamide, procaine, caffeine, stearic acid, sodium bicarbonate, sodium borate, and "field test blue."

5.13.8.2 Note: This procedure is less efficient at removing anhydroecgonine methyl ester and will not remove methylbenzoate which appear similar to cinnamoyl cocaines by infrared analysis.

5.13.8.3 Crush a small portion of the sample (approximately 30 mg) and place in a test tube.

5.13.8.4 Add 3 milliliters of hexane and vortex approximately 30 seconds.

5.13.8.5 Add 1 milliliter of potassium permanganate reagent and vortex 1 minute.

5.13.8.6 Squirt approximately 10 milliliters of deionized water through the mixture.

5.13.8.7 Allow layers to separate.

5.13.8.8 Remove hexane layer and evaporate over moderate heat.

5.13.8.9 Note: Keep top of test tubes pointed away from face or covered while vortexing to avoid splashing in eyes or face.

5.14 Sampling - See Drug Chemistry Section Technical Procedure for Sampling.

5.15 Calculations – N/A

5.16 Uncertainty of Measurement – N/A

6.0 Limitations – See individual procedures listed above.

7.0 Safety – See State Crime Laboratory Safety Manual.

8.0 References

Adair, A., et al. "The ANOR (Alternate Non-aqueous Organic Ratio) Extraction Procedure." *Microgram,* Volume XVI, Issue 1 (Jan. 1983): 220-224.

Adams, R. and J. R. Johnson. *Laboratory Experiments in Organic Chemistry*. 4th Edition New York: MacMillan, 1949: 103-115.

Analytical Profiles of Cocaine, Local Anesthetics and Common Diluents Found With Cocaine. Auburn, Alabama: CND Analytical, Inc., 1990.

Berg, E. W. *Physical and Chemical Methods of Separation*. New York: McGraw-Hill, 1963: 6-7.

Butler, William P. *Methods of Analysis for Alkaloids, Opiates, Marihuana, Barbiturates, and Miscellaneous Drug, Publication #341*. Washington, D.C.: U.S. Treasury Department, Internal Revenue Service, December, 1966: 64.

Canaff, R. F. *A Basic Training Course for Forensic Drug Chemists*. U. S. Government Printing Office: BNDD publication, 1972.

Casale, J. "An Aqueous-Organic Extraction Method for the Isolation and Identification of Psilocin from Hallucinogenic Mushrooms." *Journal of Forensic Science* (January, 1985).

Casale, J.F., and R.F.X. Klein. "Illicit Production of Cocaine." *Forensic Science Review,* Volume 5, Issue 2 (1993): 95-107.

Chiong, D.M., E. Consuegra-Rodrigues and J.R. Almirall. "The Analysis and Identification of Steroids."*Journal of Forensic Science,* Volume 37. Issue 37, (March, 1992): 488-502.

Clark, C.C. "The GLC Quantitation of Some Anabolic Steroids in Vegetable Oil Preparations." *Microgram,* Volume XXV, Issue 10 (October, 1992): 255-268.

Forensic Chemist Seminar. Drug Enforcement Administration Special Testing and Research Laboratory. May 9, 2007.

Kerr, K. "A Simple Procedure for Separating Cocaine Base from Procaine Base." *Microgram,* Volume XXIII, Issue 5 (May 1990): 93-96.

Leffler, J. E. *A Short Course in Modern Organic Chemistry*. New York: MacMillan, 1973: 17-27.

Moffat, A.C., et al., eds. *Clarke's Isolation and Identification of Drug*. 2nd Edition. London: Pharmaceutical Press, 1986.

Moriwaki, W. and M. Lee. ADimethyl Sulfone in Methamphetamine Exhibits.@ *Microgram,* Volume XXIX. Issue 3 (March 1996): 58-60. **NOTE:** This article states that the methamphetamine is present in the acetone layer. Actually it is present in the chloroform layer.

Technial Procedure for Extraction of Psilocybe Mushrooms, modified by Chemist T.H. McSwain with the North Carolina State Bureau of Investigation Drug Chemistry Laboratory, in use in the laboratory since January, 1985.

Shriner, R.L., R.C. Fuson and D.Y. Curtin. *The Systematic Identification of Organic Compound.* 5th Edition. New York: Wiley and Sons, 1964: 67-106.

Stall, W. ASeparation of Darvon and APC.@ *Microgram, Volume* XI, Issue 11 (Nov. 1978): 204-206.

Zacharia, D. "Infrared Identification of LSD in Capsules, Liquid, Sugar Cubes and Tablet." *Microgram,* Volume I, Issue 4 (Jan. 1968): 23-24.

Clarke, E.G.C. and R.G. Todd, eds. *Isolation and Identification of Drugs*. 1st Edition. London: Pharmaceutical Press, 1969: 526-527.

Allcox, I.L. "A Separation of Psilocybe Mushroom Fragments from Chocolate Candy." Internal Communication, North Carolina State Bureau of Investigation Crime Laboratory, 2010.

Allcox, I.L. "Extraction of Chocolate Mushrooms." Internal Communication, North Carolina State Bureau of Investigation Crime Laboratory, 2007.

Sarwar, Mohammad and John McDonald. "A Rapid Extraction and GC-MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions." *Microgram Journal*, Issue 3-4 (July-December 2003).

Suzuki, E.M. and W.R. Gresham. "Identification of Some Interferences in the Analysis of Clorazepate." *Journal of Forensic Sciences*, Volume 28, Issue 3 (July 1983): 655-682.

9.0 Records – FA case file notes

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document for Conversion to ISO Standards. Technical Procedures D-1 through D-4, D-10, D-12 through D-15, D-17, D-19 were combined into **6.1 Extraction of Organic Acids and Bases**. D-5 and D-6 were combined to **6.2 Dry Solvent Extractions of Drugs Utilizing Ammoniated Solvents**. D-7, D-16 and D-18 were combined for **6.3 Separation of Organic Acids and Bases by Solvent Wash**. D-8 and D-23 were combined to **6.4 Extraction of Psilocybe Mushrooms**. D-20 and D-22 were rescinded. They may be reinstated by the Forensic Scientist Manager of Drug Chemistry.