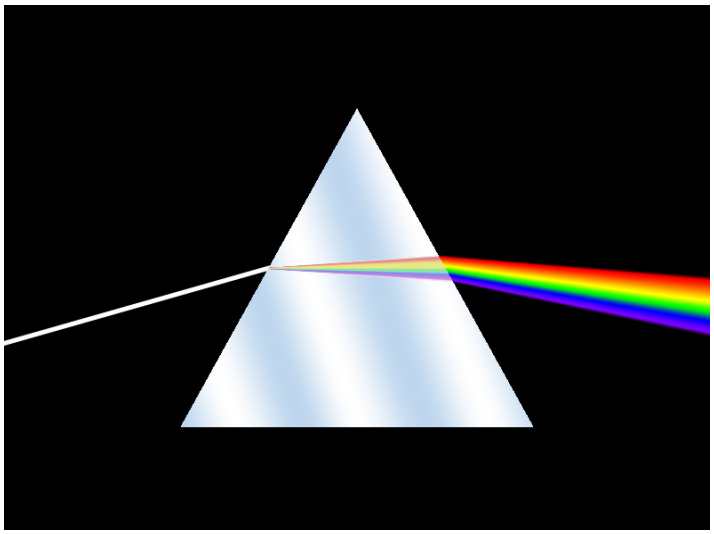
Washington State Patrol



Crime Laboratory Division

WASHINGTON STATE PATROL

Materials Analysis
Controlled Substances Training Manual

February 2016

Contents

1 INTRODUCTION

1.1 PURPOSE AND SCOPE

1. This manual contains an outline for training and/or assessing a forensic scientist in the area of controlled substance analysis. Each scientist will have a unique training program depending on the individual's strengths and weaknesses, previous background, the needs of the laboratory, and available personnel to provide the training. The sequence in which the various sections are presented should not necessarily be considered as a mandatory order of training.
2. This manual endeavors to promote and maintain consistency and quality among forensic scientists performing controlled substance analyses across the Crime Laboratory Division. Certain inherent aspects of chemical analysis prohibit the establishment of a rigid set of standard procedures to cover every case. Sufficient latitude should be given to allow for independent thought and individual freedom in selecting alternative courses of action. Upon completion of this training program, the trainee will be thoroughly familiar with the options available to perform an examination of most types of evidence that may be received.

1.2 ORGANIZATION OF THE TRAINING MANUAL

The training manual consists of several study segments, each covering different aspects of chemical analysis.

Each study segment is comprised of six parts:

- The *Objectives* summarize the purpose of each training segment.
- The *Topic Areas* designates topics to be included in the training segment.
- The *Safety* section indicates specific safety information relating to the training segment.
- The *Suggested Readings* section lists the reference material that should be read to successfully complete the study segments. The reading assignments are cumulative; comprehension of prior readings may be required to successfully complete study/discussion questions and exercises of subsequent study segments. It may not be necessary or practical to read every reference listed. The trainee will work with the trainer for specifics.
- The *Study Questions* have a number of purposes:
  - To assist reading comprehension by providing a focus on certain concepts prior to completing the Reading section;
  - To evaluate understanding of relevant concepts after completing the Readings; and
  - To promote active discussions between the trainer, trainee and trainee's co-workers using the questions as a starting point.
  - To document comprehension and/or application of Topic Areas.
  - Written answers to these questions will be maintained in the training notebook as documentation of training.
- The *Practical Exercises* are designed to provide the trainee first-hand experience with the main concepts of each study segment. Data or written explanation for each Practical Exercise must be maintained in the training notebooks.

Module 1 covers principles and techniques used in the analysis of controlled substances:

- Controlled substances analysis overview
- Chemistry principles
- Chemical screening
- Microcrystalline testing
- References & resources
- Measurement uncertainty

Module 2 covers qualitative examination of drugs of abuse:

- Cocaine
- Designer drugs
- GHB/GBL/1,4-Butanediol & analogs
- Hallucinogens
- Marijuana
- Opioids
- Pharmaceuticals & Legend drugs
- Phenethylamines
- Steroids

A written/oral examination will be given after Modules 1 and 2. Competency testing will follow Module 2. Supervised casework for Module 2 may begin following successful completion of all examinations and competency testing and upon approval from the Quality Manager. An IOC will be written by the trainer upon the successful completion of training recommending the trainee for supervised casework. Once the IOC has been approved through the chain of command, the trainee will be allowed to perform-supervised casework for Controlled Substances. Following successful supervised casework, an IOC will be routed through the chain of command to the Laboratory Manager recommending independent casework for Controlled Substances. A copy of the IOC will be provided to SAS.

Module 3 covers quantitative analysis of marijuana products. Module 3 is optional training that will be completed by analysts based on the needs of the Crime Laboratory Division. It is not expected that all controlled substance analysts will complete this module. A written/oral examination and competency testing will be given following the completion of this module. Upon the successful completion of the examination and competency testing an IOC will be written by the trainer recommending the trainee for supervised casework. Once the IOC has been approved through the chain of command, the trainee will be allowed to perform-supervised casework for marijuana quantitation. Following successful supervised casework, an IOC will be routed through the chain of command to the Laboratory Manager recommending independent casework for Controlled Substances. A copy of the IOC will be provided to SAS.

The instructor is responsible for ensuring that the trainee is prepared to testify as an expert witness. This can be done with mock trials; prearranged, as well as impromptu, question and answer sessions; and observation of courtroom testimony given by experienced forensic scientists. One or more mock trials will be scheduled during the training program.

2 CONTROLLED SUBSTANCE ANALYSIS OVERVIEW

2.1 OBJECTIVES

- To familiarize the trainee with drug scheduling and legal definitions of controlled substances.
- To familiarize the trainee with the types of materials accepted by the Division for controlled substance analysis.

2.2 TOPIC AREAS

1. Legal issues
    a. Controlled Substances Act
        i. Washington State Uniform Controlled Substances Act
            1. Chapter 69.50 Revised Code of Washington (RCW)
            2. Chapter 246-887 Washington Administrative Code (WAC)
        ii. Federal Controlled Substances Act
            1. Title 21 United States Code (21USC)
    b. Criteria for placement in drug schedules (refer also to the RCW)
        i. Schedule I
            1. has high potential for abuse;
            2. has no currently accepted medical use in treatment in the United States; and
            3. lacks accepted safety for use in treatment under medical supervision.
        ii. Schedule II
            1. the substance has high potential for abuse;
            2. the substance has currently accepted medical use in treatment in the United States, or currently accepted medical use with severe restrictions; and
            3. the abuse of the substance may lead to severe psychological or physical dependence.
        iii. Schedule III
            1. the substance has a potential for abuse less than the substances included in Schedules I and II;
            2. the substance has currently accepted medical use in treatment in the United States; and
            3. abuse of the substance may lead to moderate or low physical dependence or high psychological dependence.
        iv. Schedule IV
            1. the substance has a low potential for abuse relative to substances in Schedule III;
            2. the substance has currently accepted medical use in treatment in the United States; and
            3. abuse of the substance may lead to limited physical dependence or psychological dependence relative to the substances included in Schedule III.
        v. Schedule V
            1. the substance has low potential for abuse relative to the controlled substances included in Schedule IV;
            2. the substance has currently accepted medical use in treatment in the United States; and
            3. abuse of the substance may lead to limited physical dependence or psychological dependence relative to the substances included in Schedule IV.

vi. Schedule II to IV, in general, have accepted medical use and require a prescription (if available as a commercial pharmaceutical)

c. Legend drugs
   i. Legend drugs means any drugs which are required by state law or regulation of the state board of pharmacy to be dispensed on prescription only, or are restricted to use by practitioners only.
   ii. RCW 69.41

d. Paraphernalia
   i. Drug paraphernalia means all equipment, products, and materials of any kind which are used, intended for use, or designed for use in planting, propagating, cultivating, growing, harvesting, manufacturing, compounding, converting, producing, processing, preparing, testing, analyzing, packaging, repackaging, storing, containing, concealing, injecting, ingesting, inhaling, or otherwise introducing into the human body a controlled substance.
   ii. RCW 69.50.102

2. Controlled substance evidence
   a. Solid dosage forms versus body fluids containing controlled substances
      i. Materials Analysis section analyzes for solid dosage forms
      ii. Toxicology analyzes body fluids for controlled substances and metabolites
   b. Materials not accepted by the Materials Analysis section
      i. Hypodermic needles
      ii. Razor blades
      iii. Sharps
      iv. Used field test kits
   c. Packaging considerations
      i. Protecting fragile items such as paraphernalia
      ii. Liquids
      iii. Green or wet plant material
      iv. Residues
   d. Drug evidence recovered from body cavities
   e. Bulk controlled substances
   f. Disposal of controlled substances
3. Controlled substance case approach
   a. Qualitative versus quantitative examination
   b. Variable nature of materials received means no single approach or set of methods will adequately address all contingencies
   c. Conclusive identification of a controlled substance requires two uncorrelated analytical techniques.
      i. One of the techniques must provide molecular structural data (Category 1).
      ii. The second test does not need to provide molecular structural data but should be sufficiently specific for the analyte in question (Category 2).
      iii. See Controlled Substances Technical Procedures.
   d. Maintain at least half of the sample for future testing. Letter of consumption required if more than half the sample will be needed in testing.
   e. Sampling versus sample selection.
   f. Total number of exhibits analyzed per request is dependent on several factors:
      i. Possession versus delivery
      ii. Controlled buys
      iii. Multiple types of substances
      iv. Multiple suspects
      v. Specific agency request

2.3 SUGGESTED READING

1. Revised Code of Washington (RCW) 69.41 & 69.50
2. Washington Administrative Code (WAC) 246-887
3. Title 21 United States Code
4. WSP FLSB Forensic Services Guide

2.4 STUDY QUESTIONS

1. Discuss the considerations to be given regarding the number of exhibits to be analyzed. Include the importance of examining at least one exhibit per suspect.
2. Discuss the policies regarding the submission of large quantities of controlled substances.
3. Five exhibits were received in a single suspect case (a vial containing syringe rinse, three bindles of similar looking white powder and a balloon of brown sticky substance). What exhibits would you analyze? What if the first two exhibits you analyze are non-controlled?
4. You receive a phone call from an officer with a syringe. What do you tell him/her?
5. Why is heroin a schedule I controlled substance? Why is cocaine a schedule II controlled substance?
6. What criteria are used to determine the schedule of a drug?
7. Is there a difference between the controlled substance list at the state and federal levels?
8. List all of the controlled substances which are listed in more than one schedule.
9. Are there differences in charges/penalties for varying amounts of controlled substances in Washington? In the Federal system?
10. You have a residue case and will need to consume the entire sample for your analysis. What should you do? How would you phrase your final report if you conclusively identify methamphetamine in the residue?

2.5 PRACTICAL EXERCISES

1. Observe your instructor or other qualified employee during the analysis of a controlled substance.
2. Review a minimum of 10 controlled substance case folders from several experienced forensic scientists and discuss them with your trainer.

3 CHEMISTRY PRINCIPLES

3.1 OBJECTIVES

- To understand the significance of isomerism as related to controlled substance analysis.
- To evaluate techniques for the isolation and purification of organic compounds.
- To review use of derivatization to assist in the separation and identification of controlled substances.

3.2 TOPIC AREAS

1. ISOMERISM
    a. Review of isomers and stereoisomerism
        i. Isomers - compounds with the same molecular formula.
            1. Functional (e.g., CH_3OCH_3 versus CH_3CH_2OH).
            2. Positional (e.g., meta- versus ortho-dichlorobenzene)
            3. Geometric (e.g., cis- versus trans- 1,2-dichloroethylene)
        ii. Stereoisomers - isomers that differ only in the way the atoms are oriented in space (but are like one another with respect to which atoms are joined to which other atoms).
    b. Origins of stereoisomerism in organic compounds
        i. Asymmetric (chiral) carbons
        ii. Number of stereoisomers possible - 2n where n equals the number of asymmetric carbons (e.g., cocaine, which has four asymmetric carbons, has a maximum of 16 [24] possible stereoisomers; only eight are sterically possible, however).
    c. Types of stereoisomers
        i. Optical isomers (enantiomers) - non-superimposable mirror images of each other.
            1. d- and l- nomenclature
            2. + and – nomenclature
            3. R- and S- nomenclature
        ii. Diastereoisomers - stereoisomers that are not enantiomers, that is, not superimposable and not mirror images (e.g., cocaine, allococaine, pseudococaine, and pseudoallococaine).
    d. Optical isomers and their differentiation
        i. Differentiation
            1. Optically, by polarimetry
            2. Formation of diastereoisomers by chiral derivatization
            3. Chromatographically and electrophoretically by chiral media
        ii. Racemic mixtures (d,l compounds)
            1. Eutectic conglomerates (e.g., Pasteur's discovery of enantiomers from sodium ammonium tartrate).
            2. Racemates or mixed crystals (e.g., amphetamine with gold chloride)
                - d- and l- versus d,l
            3. Differentiation of amphetamine from dextroamphetamine.
            4. Clandestine production of d,l-methamphetamine from phenyl-2-propanone and methylamine.
            5. Clandestine production of d-methamphetamine from l-ephedrine or d-pseudoephedrine.
2. SEPARATIONS
    a. Physical separations (e.g., viewing the mixture with a stereomicroscope and physically removing particles from a mixture with forceps or other tool).

b. Chemical separations
    i. Solubility rules ("like dissolves like").
        1. Non-polar substances are soluble in non-polar solvents and generally insoluble in polar solvents such as water; analogous rules apply for polar substances such as salts of drugs (e.g., cocaine base is soluble in ether but not in water, and cocaine hydrochloride is soluble in water but not in ether).
        2. Intermediate type solvents (methanol, etc.) may often dissolve both the free compounds and their salts (e.g., cocaine base and cocaine hydrochloride are both soluble in methanol).
    ii. Acid - base separation
        1. pH
        2. Basis of acidity or basicity and correlation with structure:
            - Basic - amines (most drugs)
            - Neutral or weak base - imines (e.g., diazepam, methaqualone)
            - Neutral or very weak acids - amides and carbamates (e.g., methyprylone, diazepam, meprobamate, ethinamate)
            - Weak acids - phenolic drugs (morphine, hydromorphone, psilocyn)
            - Acidic - imides (glutethimide, barbiturates)
        3. pKa and its significance in extractions
    iii. Ion-pairing: salts of a few drugs (generally the hydrochloride salt of some large amines) may be soluble in solvents such as chloroform or methylene chloride due to the formation of ion pairs.
    iv. Filtering and centrifugation
    v. Distillation
    vi. Extraction/separation procedures:
    In order to purify a particular drug for identification, various extraction/separation methods are normally employed. These are based primarily on differences in solubilities of the various components in a given mixture and differences in solubility that can be created by changing the form of the drug and/or the combination of solvents used. In particular, the difference in solubility behavior between a drug in its "free" form and its salt (if applicable) is often used. Some examples of separations are given below to illustrate these principles. There are many variations that can be employed in these separations.
        1. Removal of excipients in some pharmaceutical products and diluted street drugs. Many filler/diluents are either insoluble in water (e.g., starch, talc) or most organic solvents (e.g., sugars) and may thus be removed by:
            - Centrifuging or decanting (using aqueous or organic solvents; alternatively, the powder can be placed on a filter and washed with solvent – this is often referred to as a “dry” extraction since water is not involved).
            - Aqueous - organic partitions.
        2. Separation of acidic and neutral compounds from basic drugs (e.g., this procedure is used in formulations with APC - aspirin, phenacetin, and caffeine).
            - 0.lN HCl (basic drug in form of water soluble HCl salt).
            - Centrifuge (to remove insoluble diluents).
            - $CHCl_3$ washes (to remove APC).
            - Basify (to form free bases).
            - $CHCl_3$ extract (to transfer free base to organic layer).

3. Separation of an acidic, basic, and neutral drug mixture (e.g., amphetamine, barbital, and caffeine).
    - 0.lN HCl/Et_2O Partition (amphetamine HCl in aqueous layer; barbital and caffeine in ether layer).
    - Remove ether layer, mix with 3N NaOH solution (caffeine in ether layer, sodium barbital in aqueous layer).
    - Remove aqueous layer and acidify with HCl, extract with ether (barbital in ether layer).
4. Separation of two basic drugs: Since most drugs are amines, it is not uncommon to have to separate two basic drugs from each other. A few possibilities include:
    - One of the drugs occurs as the free base and the other as an HCl salt. A dry extract (non-aqueous organic solvent) may be used to remove the free base.
    - Both drugs occur as either the free base or as HCl salts. Differences in solubilities may sometimes be used to remove one component (e.g., in many cases, procaine HCl can be removed from mixtures of this drug with cocaine HCl by washing with acetone).
    - One of the drugs (but not the other) forms a chloroform extractable ion pair (e.g., heroin HCl can be extracted into chloroform from a mixture of heroin and ephedrine in 3N HCl).

c. Chromatographic separations
    i. Gas chromatography
    ii. Thin Layer Chromatography
    iii. Other chromatographic techniques (e.g., SFC, HPLC, CE)

3. DERIVATIZATION
    a. Theory: Converting a compound to one having some adduct group, using a reagent which will react with the analyte at some active site.
    b. Why use derivatization?
        i. Separation of compounds in difficult mixtures;
        ii. To improve chromatography;
        iii. To increase sensitivity for specific detectors, such as UV or fluorescence;
        iv. Allow possible chiral separations;
        v. Increase volatility for GC.
    c. Desirable qualities of a derivatization reaction:
        i. Derivatization reagent should not react with solvent used;
        ii. Mild conditions;
        iii. Quick, easy, reproducible;
        iv. No side reactions;
        v. High percent yield;
        vi. Post-reaction: either no cleanup or very simple, such as allowing solvent to evaporate.
    d. Most common derivatization techniques
        i. Silylation
            1. Typically the replacement of active hydrogens from acids, alcohols, thiols, amines, amides, and enolizable ketones and aldehydes
            2. Examples of silylation reagents
                - Hexamethyldisilazane (HMDS)
                - N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)
                - N-O-bis(trimethylsilyl)acetamide (BSA)
                - N-O-bis(trimethylsilyl)trifluoroacetamide (BSTFA)

ii. Alkylation
    1. Typically represents the replacement of an active hydrogen by an aliphatic or aliphatic aromatic group
    2. Examples of alkylation reagents
        - Diazomethane
        - Borontrifluoride-Methanol (BF3-Methanol)
        - N,N-Dimethylformamide Dimethylacetal
        - Trimethylanilinium Hydroxide (TMPAH)
        - Pentafluorobenzyl Bromide (PFBBr)

iii. Acylation
    1. Typically used for the conversion of compounds containing active hydrogens into esters, thioesters, and amides.
    2. Examples of acylation reagents
        - Acetic Anhydride
        - Perfluoro Acid Anhydrides
            - Trifluoroacetic Acid Anhydride (TFAA)
            - Pentafluoropropionic Acid Anhydride (PFAA or PFPAA)
            - Heptafluorobutyric Acid Anhydride (HFBA or HFBAA)
        - Perfluoroacylimidazoles
            - Trifluoroacetylimidazole (TFAI)
            - Pentafluoropropionylimidazole (PFPI)
            - Heptafluorobutyrylimidazole (HFBI)
            - N-Methyl-bis-(trifluoroacetamide) (MBTFA)

e. Things to avoid:
    i. A+B→C and D+B→C; two different compounds (A or D) yielding the same product;
    ii. On-column derivatization (on-column derivatization would be, for example, adding reagent to the sample in solution in a GC vial, capping the vial, and using it as is for injection) in which the products of the reaction produce acidic chemicals which adversely affect the GC.
    iii. Reagents not fully reacting or remaining in the injection port or below, and reacting with subsequently injected analytes (e.g., see phenylboronic acid below).

3.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

3.4 SUGGESTED READING

1. Adair, A.R., et al., 1983. The ANOR (Alternate Non-Aqueous Organic Ratio) Extraction Procedure. Microgram.16(1):220-224.
2. Allen, A.C., et al. 0981. The Cocaine Diastereomers. J. For Sci. 26(1):12-26.
3. Knapp, DR. 1979. Handbook of Analytical Derivatization Reactions. New York:John Wiley & Sons.
4. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
5. Thermo Scientific Pierce Reagents, Solvents and Accessories catalog available at www.thermo.com

6. Organic Chemistry, text of your choosing.

3.5 STUDY QUESTIONS

1. Define the following and give an example:
    a. Functional Isomer
    b. Positional Isomer
    c. Geometric Isomer
    d. Enantiomer
    e. Diastereomers
2. Are enantiomers or diastereomers more difficult to conclusively identify? Why?
3. How can we distinguish d- and l- versus d,l- isomers using techniques available in the lab?
4. Give two examples of molecules in which one isomer is controlled and the other is not. Be sure to cite the applicable schedules.
5. Who do different stereoisomers have different biological properties?
6. For each of the pairs below draw the structures, describe the structural/functional relationships, and determine the control status:
    a. Methamphetamine, phentermine
    b. Cocaine, allo-cocaine
    c. Cocaine, Pseudococaine
    d. Δ9-tetrahydrocannabinol, Δ8-tetrahydrocannabinol
    e. Codeine, 6-acetylcodeine
    f. Methamphetamine, 1-phenyl-1-(N-methylamino)propane
    g. Dextropropoxyphene, levopropoxyphene
    h. Psilocyn, Bufotenine
    i. Cathine, Phenylpropanolamine
    j. Prilosec, Nexium
7. How could the compounds in the following mixtures be isolated:
    a. Cocaine HCl & Nicotinamide
    b. Methamphetamine & Dimethyl Sulfone
    c. MDMA & Caffeine
    d. Diazepam & Lactose
    e. Cocaine & Procaine
    f. Hydrocodone & Acetaminophen
    g. Aspirin, Caffeine, Butalbital, Codeine (aka Fiorinal with Codeine)
8. Draw the structure of the following compounds. Indicate which functional group (if any) can be derivatized and suggest the most effective derivatizing agent.
    a. Amphetamine
    b. Methamphetamine
    c. Dimethylamphetamine
    d. 1-Benzylpiperazine
    e. Secobarbital
    f. Cocaine
    g. Δ9-tetrahydrocannabinol
    h. Morphine
    i. Diacetylmorphine
    j. Oxycodone
    k. LSD
    l. PCP
9. You dissolve a white powder in an alcohol and analyze using GC/MS. You detect acetylated methamphetamine. How could this have happened?
10. Why is it best to derivatize barbiturates for analysis by GC/MS? Which derivatizing agent works best for barbiturates?

3.6 PRACTICAL EXERCISES

1. Measure out two 10 mg portions of methamphetamine hydrochloride. Dissolve one of these in 10 mL of water and the other in 10 mL of aqueous base (using whichever is customary in your laboratory).
    a. Remove 1 mL of each of these and extract with 10 mL of methylene chloride. Obtain GC/MS data of the organic phase.
    b. Remove 1 mL of each of these and extract with 10 mL of pentane (or hexanes). Obtain GC/MS data of the organic phase.
    c. Explain the results of the GC/MS data. How important is the pH of the aqueous solution for the extraction of methamphetamine into the organic phase?
    d. Repeat the exercise, substituting cocaine for methamphetamine.
2. Prepare a mixture of methamphetamine, pseudoephedrine (or ephedrine), and dimethyl sulfone.
    a. Base extract the sample into methylene chloride and analyze by GC/MS.
    b. Add one to two drops of acetic anhydride to the previously analyzed sample and re-analyze by GC/MS.
    c. Base extract the sample into pentane (or hexanes) and analyze by GC/MS.
    d. Develop and extraction scheme to isolate each component for analysis by FTIR.
3. Prepare 1 mg/mL solutions of heroin, morphine, codeine, and acetylmorphine.
    a. Analyze each solution by GC/MS.
    b. Derivatize each solution using the following and analyze by GC/MS:
        i. Acetic Anhydride
        ii. Trifluoroacetic anhydride (TFAA)
        iii. N,O-bix(trimethylsilyl)trifluoroacetamide (BSTFA)
    c. Any other derivatizing agent(s) your lab may have.
    d. Which derivatizing agent was most useful? Which was the least useful?
4. Prepare samples of the mixtures describe in question seven of the study/discussion questions. Isolate each compound and confirm using an appropriate instrumental technique.
5. Any other exercises deemed appropriate by the trainer.

4 CHEMICAL SCREENING

4.1 OBJECTIVES

- To familiarize the trainee the theory and application of chemical screening tests in drug analysis.
- To familiarize the trainee with safety and quality assurance issues related to the use of chemical screening tests.

4.2 TOPIC AREAS

1. Screening tests
    a. Tests designed to eliminate some drugs from consideration or narrow down the possibility of an unknown sample's contents.
    b. Color tests are often subjectively interpreted.
2. Color tests
    a. History
        i. According to Feigl, chemical spot tests were first reported in 1859,
        ii. The use of chemical spot tests was more fully realized in the 1920's.
    b. Theory
        i. Color tests generally target functional groups or a molecular moiety.
        ii. Color tests are largely empirical.
    c. Reagents and Procedure
        i. Formulations and testing procedures for common controlled substance color screening tests are listed in the Materials Analysis Technical
3. UV/Fluorescence Testing
    a. Exposure of ergot alkaloids to long-wave UV (360 nm) results in blue fluorescence.
    b. Effect screening technique for LSD on many substrates or methanol extracts.
4. TLC
    a. Can be used for screening.
    b. Refer to the TLC training module for more information on this technique.
5. Instrumental techniques
    a. Can be used for screening
    b. Refer to the technique specific module for more information.

4.3 SAFETY

1. It is important to note that some of the ingredients in color test reagents, TLC developing baths, and TLC visualizing reagents pose significant health hazards and before making or using any of these reagents, the appropriate Material Safety Data Sheet should be consulted. Good chemical safety practices should be employed when working with reagents. TLC should be performed in a functional fume hood. When the TLC plate has been reviewed and observations recorded, the plate should be disposed of properly and should not be kept as part of the case record.
2. Care should be taken to use a minimal amount of unknown material when performing color tests. Suspected controlled substances could actually contain oxidizers or other reactive substances.
3. UV radiation can be harmful to the eyes and care should be exercised to avoid direct exposure to UV radiation.

4.4 SUGGESTED READING

1. Butler WP. Methods of Analysis for Alkaloids, Opiates, Marijuana, Barbiturates, and Miscellaneous Drugs. Internal Revenue Service (Reprinted by the Bureau of Narcotics and Dangerous Drugs, U.S. Department of Justice), rev. 6-67.
2. Moffat AC. 1986. Clarke's Isolation and Identification of Drugs, 2nd Ed. London: Pharmaceutical Press.
3. (a) Ruybal R. 1972. Microgram. 5(3). (b) Ruybal C. Microgram. 6(2).
4. Moffat AC, Osselton MD, Widdop B. eds.2004. Clarke's Analysis of Drugs and Poisons, 3rd Ed. London:Pharmaceutical Press.
5. Garrett AS, Siemens SR, Gaskill JH. 1992. The Weber Test: A Color Test for the Presence of Psilocin in Mushrooms. NWAFS Journal. 18(4).
6. Chen KK, Kao CH. 1926. Ephedrine and Pseudoephedrine, their isolation, constitution, isomerism, properties, derivatives, and synthesis. J. Am. Pharm. Assoc. 15(8):625-639.
7. Feigl F. 1966. Spot Tests in Organic Analysis, 7th ed. Amsterdam: Elsevier.
8. Rapid Testing Methods of Drugs of Abuse, Manual for use by National Law Enforcement and Narcotics Laboratory Personnel. United Nations International Drug Control Programme, ST/NAR/13/REV.1,1994.
9. Chemistry and Reaction Mechanisms of Rapid Tests for Drugs of Abuse and Precursors Chemicals. United Nations Scientific and Technical Notes, SCITEC/6, 1989.
10. Colour Tests for Precursor Chemicals of Amphetamine-Type Substances. United Nations Scientific and Technical Notes, SCITEC/20, 2005.
11. Recommended Methods for Testing Lysergide (LSD), Manual for use by National Narcotics Laboratories. United Nations Division of Narcotic Drugs, ST/NAR/17, 1989.
12. Siegel JA. 1988. Forensic Identification of Controlled Substances. In: Saferstein R. ed. Forensic Science Handbook, Vol. II, Englewood Cliffs(NJ):Prentice Hall.
13. Johns SH, Wist AA, Najam AR. 1979. Spot Tests: A color Chart Reference for Forensic Chemists. J For Sci. 24(3):631-649.

4.5 STUDY QUESTIONS

1. How would you describe color tests to a jury?
2. Describe the difference between sensitivity and selectivity as it relates to color tests.
3. What quality control measures should be taken when performing a color test?
4. Are color tests considered a category 1 or category 2 test? Why?
5. How long should a sample be allowed to react with the color test reagent before interpreting the results of the test?
6. After issuing a report of findings indicating the submitted item was negative for controlled substances, the submitting officer calls and says he had a positive field test for heroin. How could this be?
7. How could you distinguish procaine HCl and cocaine HCl using a color test? How could you distinguish cocaine HCl and cocaine base using a color test?

4.6 PRACTICAL EXERCISES

1. Your trainer will select compounds from the following list for you to perform selected color tests. Perform the above listed color tests on the following substances, as available, and describe the results in a chart.

- Acetaminophen
- Alprazolam
- Amphetamine
- Aspirin
- Barbital
- Benzoylecgonine
- Caffeine
- Cathine
- Cathinone
- Clonazepam
- Cocaine Base
- Cocaine HCl
- Codeine
- Dextromethorphan
- Diazepam
- Diethyltryptamine
- Dimethylamphetamine
- Dimethyl sulfone
- Ephedrine
- Ethylbenzylamine
- Guaifenesin
- Heroin
- Hydrocodone
- Hydromorphone
- Inositol
- Lidocaine
- Lorazepam
- Methamphetamine
- Methylbenzylamine
- MDA
- MDMA
- Methylphenidate
- Morphine
- Noscapine
- Opium powder
- Oxycodone
- Papaverine
- Phencylidine
- Phenobarbital
- Procaine HCl
- Pseudoephedrine
- Secobarbital
- Sodium bicarbonate
- Tetramisole
- Thebaine

2. Perform the Marquis, Simon's, Van Urk's and Weber tests on LSD, LAMPA, psilocin, psilocybin, and bufotenine.
3. Select a color test (it doesn't have to be on the list above) to demonstrate how a color test can distinguish cocaine HCl from procaine HCl and cocaine base.
4. Obtain or prepare mixtures of several substances in the list in question 1. How does the combination of substances affect color tests?

5 MICROCRYSTALLINE TESTING

5.1 OBJECTIVES

- To familiarize the trainee with microcrystal tests used for the identification of controlled substances.
- To have the trainee demonstrate the ability to perform microcrystalline tests on controlled substances.

5.2 TOPIC AREAS

1. Basic concepts
    a. Microcrystal tests are simple, highly sensitive, and rapid.
    b. They are used as a category 2 test only and are not used by themselves for identification of controlled substances.
    c. Most commonly used for basic nitrogenous drugs but also has been used for some neutral and acidic compounds.
    d. This technique can be used to differentiate the enantiomers of various controlled substances.
    e. Microcrystal tests are primarily empirical.
        i. Most are probably due to the formation of a metal complex.
        ii. Most mechanisms are unknown.
2. Techniques
    a. Direct crystal tests
        i. The sample is dissolved in an aqueous solution, generally a dilute acid.
        ii. The precipitating reagent is added.
        iii. Crystal formation is observed.
    b. Hanging microdrop
        i. This method is based on the volatility of the sample and is useful as it tends to purify the sample during the process.
        ii. First method
            1. The sample is placed on a slide with a well.
            2. A drop of base is applied to the sample.
            3. A cover slip with a drop of acid on it is placed over the well and allowed to sit for 10-30 minutes.
            4. The cover slip is removed and placed onto a slide with the precipitating reagent.
            5. Crystal formation is observed.
        iii. Second method
            1. The sample is placed on a slide with a well.
            2. A drop of base is applied to the sample.
            3. A drop of the precipitating reagent is applied a cover slip and placed over the sample in the well.
            4. Crystals are observed in the hanging drop.
3. Reagents
    a. While dozens of crystal tests reagents have been documented and used in forensic testing, this training manual will focus on the two microcrystal tests commonly employed by the WSP CLD Materials Analysis Controlled Substances sub-discipline. Other tests are available (such as those using platinic chloride) and can be used with consideration for selectivity, specificity, and safety.
    b. Gold chloride in phosphoric acid
        i. Used for the methamphetamine identification.
        ii. 1 gram of gold chloride is dissolved in a 20 milliliters of 1:2 phosphoric acid:water.
    c. Gold chloride in acetic acid

   d. Use for the identification of cocaine.
   e. 1 gram of gold chloride dissolved in 20 milliliters of 20% acetic acid.
4. Procedure
   a. Gold chloride in phosphoric acid
      i. A drop of the gold chloride in phosphoric acid reagent is placed on a microscope slide. If any precipitation forms, this would indicate contamination of either the slide or reagent. If no precipitation forms, this serves as an acceptable blank for the test.
      ii. A small amount of sample (less than 1 milligram) is transferred to the drop of reagent on the slide.
      iii. Crystal formation is observed using a polarized light microscope.
      iv. A description or sketch of the observed crystals is documented along with the results of the blank.
   b. Gold chloride in acetic acid
      i. Two drops of dilute acid (either 10% acetic or 10% hydrochloric acid) are placed a slide.
      ii. A small amount of sample (less than 1 milligram) is transferred to one drop of the acid on the slide.
      iii. A drop of the gold chloride in acetic acid reagent is added to each drop of dilute acid on the slide.
      iv. If precipitation forms in the drop without sample, this would indicate contamination of either the slide or reagents. If no precipitation forms, this serves as an acceptable blank for the test.
      v. Crystal formation of the sample is observed using a polarized light microscope.
      vi. A description or sketch of the observed crystals is documented along with the results of the blank.
5. Expected results
   a. Refer to the Microcrystal Test Training - Expected Results PowerPoint presentation located on The Portal under CLD-Materials Analysis-Training/Reference Materials.

5.3 SAFETY

It is important to note that some of the ingredients in microcrystalline reagents pose significant health hazards and before making or using any of these reagents, the appropriate Material Safety Data Sheet should be consulted. All glassware should be disposed of appropriately following analysis.

5.4 SUGGESTED READING

1. Allen AC. et al. 1981. The Cocaine Diastereoisomers. J For Sci. 26(1):12-26.
2. ASTM E 1968. Current Edition. Standard Guide for Microcrystal Testing in the Forensic Analysis of Cocaine.
3. ASTM E 1969. Current Edition. Standard Guide for Microcrystal Testing in the Forensic Analysis of Methamphetamine and Amphetamine.
4. ASTM E 2125. Current Edition. Standard Guide for Microcrystal Testing in the Forensic Analysis of Phencyclidine and Its Analogues.
5. Clarke EGC. 1978. The Isolation and Identification of Drugs. p. 135-141.
6. Davis JE. 1961. Barbiturate Differentiation by Chemical Microscopy. J Criminal Law, Criminology, & Police Science. 52(4):459-468.
7. Fulton CC. 1969. Modern Microcrystal Tests for Drugs. John Wiley & Sons. p. 1-49.
8. Microgram, various articles
9. Nichols RG. 1997. Drug Proficiency Test False Positives: A Lack of Critical Thought. Science and Justice. 37:191-196.
10. Saferstein R. 1982. Forensic Science Handbook, Vol. 1. Englewood Cliffs(NJ):Prentice Hall. p. 518-520.
11. Saferstein R. 1988. Forensic Science Handbook, Vol. 2. Englewood Cliffs(NJ):Prentice Hall. p. 69-160.

12. Simpson BJ, et al 1991. Microcrystaloscopic Differentiation of 3,4-Methylenedioxyamphetamine and Related Amphetamine Derivatives. J For Sci. 36(3):908-914.
13. Wielbo D, Tebbet IR. 1992. The Use of Microcrystal Tests in Conjunction with FTIR for the Rapid Identification of Street Drugs. J For Sci. 37(4):1134-1148.

5.5 STUDY QUESTIONS

1. What are some of the advantages and disadvantages of microcrystalline tests?
2. Since microcrystalline tests are primarily empirical, why is this not a problem for use in forensic case samples?
3. What quality control measures are taken to ensure microcrystalline tests are working effectively?
4. How should results from the microcrystalline test be documented in case notes?
5. How can microcrystal tests be utilized to differentiate stereoisomers?
6. Can microcrystalline tests differentiate cocaine enantiomers and diastereoisomers? Why is would this be difficult to do?
7. Describe a procedure for obtaining a good microcrystal exam on a mixture of methamphetamine and Phenobarbital.
8. Why is the quarter wave retardation plate used in microcrystal exams (especially for cocaine)?
9. How would you describe the microcrystal examination in court?

5.6 PRACTICAL EXERCISES

1. Observe your trainer perform microcrystalline tests on several compounds. It is helpful to use a microscope with a training head or a video camera/monitor when observing your trainer demonstrate these tests. If this is not available, be prepared to change spots at the microscope quickly!
2. Perform the gold chloride in phosphoric acid microcrystal test using the direct crystal test technique on the following:
    a. All available standards of amphetamine stereoisomers
    b. All available standards of methamphetamine stereoisomers
    c. All available standards of ephedrine stereoisomers
    d. All available standards of pseudoephedrine stereoisomers
    e. All available standards of hallucinogenic phenethylamines such as MDA, MDMA, PMA, etc.
    f. Cathine
    g. Cathinone
    h. Methcathinone
    i. Phenylpropanolamine
    j. Phenylephrine
    k. Methylphenidate
    l. Dimethyl sulfone
    m. Caffeine
    n. Cocaine HCl
    o. Cocaine Base
    p. Heroin
    q. Mixtures of a number of the compounds listed above
    r. Any other compounds suggested by your trainer
3. Perform the gold chloride in phosphoric acid microcrystal test using the hanging drop technique on the following:
    a. Methamphetamine HCl (d or l)
    b. Methamphetamine HCl (d or l) mixed with dimethyl sulfone
    c. Methamphetamine HCl (d or l) mixed with cocaine HCl
    d. Methamphetamine HCl (d or l) mixed with a barbiturate
4. Perform the gold chloride in acetic acid microcrystal test using the direct crystal test technique on the following:
    a. Cocaine hydrochloride

b. Cocaine base
c. Procaine hydrochloride
d. Procaine base
e. Lidocaine (salt and/or base)
f. Any other “caine” standards available
g. Methamphetamine HCl
h. Amphetamine HCl
i. Caffeine
j. Inositol
k. Dimethyl sulfone
l. Nicotinamide
m. Diltiazem
n. Mixtures of a number of the compounds listed above
o. Any other compounds suggested by your trainer

5. Obtain a sample of an unknown methamphetamine isomer from your trainer.
   a. Test the sample using the gold chloride in phosphoric acid reagent. Note any crystals observed.
   b. Mix a small amount of the unknown sample with a d-methamphetamine standard on a microscope slide. Perform the microcrystalline test and note any crystals observed.
   c. Mix a small amount of the unknown sample with an l-methamphetamine standard on a microscope slide. Perform the microcrystalline test and note any crystals observed.
   d. Which stereoisomer of methamphetamine is present in the unknown sample?

6 PIPETTES

6.1 OBJECTIVES

- To familiarize the trainee with the operation of variable volume positive displacement pipettes.
- To improve the trainee's skill and expertise in pipetting to increase accuracy and precision.

6.2 TOPIC AREAS

1. Pipette Terms
   a) Air displacement pipettes
   b) Aspirate
   c) Blow-out
   d) Dispense
   e) Positive displacement pipette
2. Accuracy and precision in pipetting
3. Forward and reverse pipetting
4. Pipetting ergonomics
5. Quality Control
   a) Calibry software
   b) Evaporation trap

6.3 SAFETY

Maintaining good posture and taking frequent breaks will reduce the likelihood of repetitive strain injuries which are frequently attributed to repetitive pipetting.

6.4 SUGGESTED READING

The following are a list of possible sources on pipetting techniques, ergonomics and improved efficiency. There are many worthwhile websites and free online guides to pipetting which will provide valuable information.

1. Are you applying good pipetting practice? [Internet]. 2013. London: Laboratory News; [cited 2013 July 8]. Available from http://www.labnews.co.uk/features/are-you-applying-good-pipetting-practice/
2. AccuTek Laboratories Guide to Pipetting. Available on the Portal under Materials Analysis Training & Reference Material – Pipettes.
3. ARTEL 10 tips to improve your pipetting technique. Available on the Portal under Materials Analysis Training & Reference Material – Pipettes.
4. Gilson Microman® users guide. Available on the Portal under Materials Analysis Training & Reference Material – Pipettes.
5. Calibry software users guide.

6.5 STUDY QUESTIONS

1. What is the difference between air displacement and positive displacement pipettes?
2. Describe forward and reverse pipetting? Which technique will be used for quantitative analysis?
3. How will the position and depth of the pipette tip in the solvent affect accuracy?
4. Why should the pipette tip be pre-rinsed before aspiration of the sample aliquot?
5. Describe how you will set the volume on the pipette to maximize the accuracy.
6. What quality assurance measures are required for pipettes?
7. How can you be certain the correct volume is being pipetted?
8. What systematic and random error parameters are used for monitoring the pipettes in the Calibry software?
9. Why is an evaporation trap necessary when monitoring the pipettes using the Calibry software?

10. What environmental conditions must be included when monitoring the pipettes using the Calibry software?

6.6 PRACTICAL EXERCISES

1. Practice aspirating and dispensing samples using three different solvents.
2. Aspirate and dispense ten aliquots of water and weigh each aliquot on a balance. Calculate the average weight for these ten samplings. Repeat this exercise without pre-rinsing the tip. Repeat this exercise holding the tip deep into the water or at an angle when aspirating the sample. Record and evaluate results. Was there a difference in accuracy when the tip was not pre-rinsed or positioned improperly?
3. Check pipettes using the Calibry software.

7 REFERENCES & RESOURCES

7.1 OBJECTIVES

- To develop an understanding of the many references and resources available regarding the analysis and identification of controlled and non-controlled substances.
- To be able to visually screen tablets and compare to the various tablet and capsule databases that are available in the laboratory.
- To review the definitions of words commonly used in conjunction with controlled substance analysis.
- To understand what training and resources may be available to the scientist.

7.2 TOPIC AREAS

1. References and resources available
    a. in-house hard copy references
    b. other FLSB laboratory resources
    c. FLSB Librarian
    d. other scientists and other laboratories
    e. internet
    f. instrument libraries
    g. journals and professional publications
    h. professional associations and societies
        i. AAFS (American Academy of Forensic Sciences)
        ii. NWAFS (Northwest Association of Forensic Scientists)
        iii. CLIC (Clandestine Laboratory Investigating Chemists Association)
        iv. ACS (American Chemical Society)
        v. SWGDRUG (Scientific Working Group for the Analysis of Seized Drugs)
        vi. ASTM (American Society for Testing and Materials)
    i. Poison Control
2. Visual identification of pharmaceutical products
    a. proper documentation of appearance and markings
    b. references for comparison
3. Verification of drug reference materials
    a. Analytical requirements
    b. Appropriate verification data
        i. Structural elucidation
        ii. Peer reviewed publications
        iii. Exceptions
4. Training opportunities
    a. workshops and papers at professional meetings
    b. instrument manufacturer workshops

7.3 SAFETY

There are no specific safety considerations related to this training module.

7.4 SUGGESTED READING

1. The Merck Index, Merck and Company, Inc., recent edition.
2. Moffat AC. Clarke's Isolation and Identification of Drugs. London:The Pharmaceutical press.
3. United States Pharmacopeia/National Formulary
4. Marnell T. Drug Identification Bible, recent edition.
5. Microgram, recent edition.
6. Clandestine Laboratory Investigating Chemists Association Journal, recent edition.

7. Revised Code of Washington Chapter 69.50 http://search.leg.wa.gov/pub/textsearch/default.asp
8. Franzosa ES, Harper C W. The Logo Index for Tablets and Capsules. U.S. Department of Justice, recent edition.
9. Johnson J, Chapman K. The Med-Scan Manual, Med-Scan International, Inc. recent edition.
10. Physician's Desk Reference, Medical Economics Company, Inc., recent edition.
11. Saferstein R. 1990. Criminalistics: An Introduction to Forensic Science, 4th ed. Simon and Schuster Company. p. 233-244.
12. Mills III T, Roberson J C. Instrumental Data for Drug Analysis, 3rd ed. Volumes 1- 6.
13. Schultes RE, Hofmann A. 1980. The Botany and Chemistry of Hallucinogens. Springfield(IL):Charles C Thomas.
14. Feigl F. 1966. Spot Tests in Organic Analysis, 7th ed. Amsterdam: Elsevier.
15. Internet access
16. Instrument libraries

7.5 STUDY QUESTIONS

1. Review the analytical references listed above.
    a. What reference(s) would you use to develop an extraction scheme?
    b. What reference(s) would you use to find the expected value of an UV maximum in a basic solution?
    c. What reference(s) include solubility information on various drugs and their salts?
    d. What reference(s) contain analytical data on IR and MS?
2. Define the following terms; give an example of each.
    a. Depressant
    b. Hallucinogen
    c. Stimulant
    d. Narcotic
    e. Opiate
    f. Salt
    g. Diluent
    h. Excipient
    i. Immediate precursor
    j. Drug paraphernalia
3. Discuss what information is available in Microgram and the CLIC Journal.
    a. How can we use this information?
4. What limitations do reference materials and libraries have?
5. What physical characteristics are available for comparison when identifying pharmaceutical products?
6. What are the criteria for a journal or resource to be used for the verification of a drug reference material?
7. You receive a new synthetic cannabinoids drug reference from Cayman Chemical. What must be done to verify the reference material is acceptable for use? Name acceptable sources of verification data.

7.6 PRACTICAL EXERCISES

This exercise is designed to familiarize the trainee with the technical literature used in the field of forensic science. The trainee is required to answer the questions and properly cite any references used. (Note: there may be more than one possible answer).

1. How many crystalline modifications are there to the molecule oligomycin a?
2. You have an unknown white tablet marked "3367" on one side and "WPI" on the other. Who makes the tablet, and what is the principal active ingredient?
3. How are Mevacor tablets controlled?
4. SmithKline Beecham products manufacture Dexedrine Spansule Capsules and tablets. How are they controlled? What are they used to treat?

5. What is the address to contact Nutramax Laboratories, Inc.?
6. What three indexes are found in the Physicians Desk Reference?
7. What is the structure of Methenolone?
8. What color tests are there for Codeine N-oxide?
9. How is Ketamine extracted from a product?
10. What is the estimated minimum lethal dose of talbutal?
11. Give a brief overview of how cocaine is extracted from coca leaves.
12. What are the principal MS peaks and MW of Ethyl Caprate?
13. A compound has a molecular weight of 112 and the largest MS peak is 43. What could it be?
14. Locate and copy the IR spectrum of Prazepam.
15. Locate and copy the MS spectrum of Oxycodone.
16. Define dioecious.
17. What Schedule of control does tilidine appear on?
18. What Schedule does barbital appear on?
19. I have a solution of 200 milligrams of codeine per 100 milliliters of solution. Is it controlled? If so, how?

8 MEASUREMENT UNCERTAINTY

8.1 OBJECTIVES

- To develop an understanding of the concept of measurement uncertainty as it relates to weights of controlled substances.
- To be able to determine measurement uncertainty for balances used for weighing controlled substances.
- To understand the definitions and statistics associated with measurement uncertainty.

8.2 TOPIC AREAS

1. Definitions
    a. Significant figures
    b. Precision
    c. Accuracy
    d. Measurement uncertainty
    e. Standard uncertainty
    f. Expanded uncertainty
    g. Confidence level
    h. True value
    i. Measurement error
    j. Standard deviation
    k. Arithmetic mean
    l. Variance
    m. Normal probability distribution
    n. Rectangular (uniform) probability distribution
2. Error versus Uncertainty
    a. Error implies doubt in results
    b. Uncertainty implies confidence in results
3. Estimation of uncertainty
    a. “Type A” method
    b. “Type B” method
4. Standard Deviation
    a. Establishing from pool of data
    b. Establishing from a calibration certificate
    c. Establishing from manufacturer’s specifications
5. Eight Step Process for Calculating Uncertainty
    a. Specify the process and equation.
    b. Identify & characterize the uncertainty sources
    c. Quantify uncertainty estimates
    d. Convert factors to standard uncertainties
    e. Calculate combined standard uncertainty
    f. Expand the uncertainty by k
    g. Evaluate the expanded uncertainty
    h. Report the uncertainty
6. An Organized Approach for Determining Measurement Uncertainty
    In order to establish measurement uncertainty, an analyst must first identify the influences that have the potential to impact the measurement result. Each identified element that could impact measurement must be evaluated. Arbitrarily deciding to include or not include these elements in the establishment of uncertainty is unacceptable. The Guide to the Expression of Uncertainty of Measurement and NISTIR 6919 outline the following organized procedure for determining uncertainty of measurement as it relates to balances and weighing.

It may be appropriate to determine and high and low range uncertainty for the balance. The split between these ranges may be dictated by the balance lower and upper range readability or may be arbitrary for balances with only one readability. For balances that do not change readability the lowest 10% of the balance's range is a good starting point for the low range estimation.

a. Specify the process and equation.
The relationship between what is being measured and the parameters that affect that measurement need to be defined. The following will be used for balances:
y=(mx+b)+U
where:
y is the balance indication;
m is the sensitivity of the weighing device;
x is the applied load;
b is the zero offset, and
U is the assigned measurement uncertainty (2* uc)
(uc is the combined standard uncertainty)

b. Identify and characterize the uncertainty sources.
   i. A list of contributors of uncertainty must be established. This list is known as the "budget" and is easily documented using a list or a "Cause and Effect" diagram. Each contributor must be evaluated and documentation must be maintained to show how the contributor will be accounted for in the uncertainty calculations. Type A evaluation (of uncertainty) is a method of evaluation of uncertainty by the statistical analysis of a series of observations (GUM 2.3.2). These elements will be covered in the process standard deviation. Type B evaluation (of uncertainty) is a method of evaluation of uncertainty by means other than the statistical analysis of a series of observations. These elements are covered by certificates of calibration or manufacturers specifications. The list of elements contributing to uncertainty and type of evaluation for each element will be documented with the Measurement Uncertainty data.
   ii. Ultimately, the elements of uncertainty that we are going evaluate are the balance calibration, the balance readability at zero and load, the balance linearity and the "process".
      1. The measurement process reproducibility will be established using a surrogate samples representing approximately 5% of the balance capacity and approximate balance capacity. Refer to the MATP for more information about the use of surrogates and quantifying the measurement process reproducibility.

c. Quantify the resulting uncertainty components.
   i. A value must be assigned to each element of uncertainty.
   ii. A graphical means of comparing the individual components of uncertainty is the Pareto chart.

d. Convert the influences of the uncertainty components on the measurement to standard deviation equivalents.
   i. All values determined in step three must be in the same units. We are fortunate that all of our measurements are already in grams and no further conversions must be made.
   ii. The uncertainty component associated with the process is already represented by a standard deviation as calculated in step three. The uncertainty components associated with the balance calibration, readability and are not represented by a standard deviation and must therefore be converted to such terms. It is most conservative to assume the distribution for the balance linearity and readability is rectangular (uniform). The Certificate of Calibration for the balance will indicate the expanded uncertainty which should be k=2; therefore, this component has normal distribution and the divisor is 2.00. The divisor used to convert

components with rectangular (uniform) distribution is $\sqrt{3}$. Now that we know what type of distribution and divisor to use, convert both the individual uncertainties to standard deviation equivalents.

iii. Example:
From the certificate of calibration for the balance the uncertainty is reported to be ±0.02 gram assuming a normal distribution, k=2. To convert this to a usable term for our future calculations the following equation is used:
0.02 g/2.00 = 0.01g

e. Calculate the combined standard uncertainty (uc).
   i. In step four we established standard deviation equivalents for each of the elements of uncertainty. Now we used the root sum squared method to combine these elements. We will use the following formula:

$$uc = \sqrt{s_p^2 + r_z^2 + r_l^2 + u_b^2 + u_l^2}$$

Where: sp = measurement process reproducibility from one surrogate in the appropriate range for the uncertainty estimation
rz = balance readability at zero
rl = balance readability at load
ub = uncertainty of the balance
ul = balance linearity

   ii. Example:

$$uc = \sqrt{(0.005)^2 + (0.005)^2 + (0.005)^2 + (0.01)^2 + (0.02)^2}$$

$= 0.02$ g

f. Calculate the expanded uncertainty (U).
   i. In step five we determined the combined standard uncertainty which represents one standard deviation. We now must expand the combined standard uncertainty by the appropriate coverage factor. Normally, k = 2 is used for expanded uncertainty which represents approximately a 95% confidence interval. Throughout the world k = 2 is the standard value used for expanding uncertainty and NIST has adopted this value as well.
   ii. k = 2 actually is a 95.45% confidence interval.
   iii. A 95% confidence interval would be k = 1.960.
   iv. The following formula is used to calculate the expanded uncertainty:
   (U) = 2 uc
   v. Example:
   (U) = 2 *0.02 g
   = 0.04 g

g. Evaluate U for appropriateness.
   i. This is the step in the process in which we stand back and look at what we've done so far. This is our error checking step and we must address the following three questions before we report uncertainty:
      1. Does the expanded uncertainty make sense?
      2. Is the expanded uncertainty at least two (the k factor that we used) times the largest standard uncertainty component?
      3. Is the expanded uncertainty large enough to encompass the normal indication errors that experience tells you are possible?
   ii. If "yes" is the answer to all three questions, we have successfully determined a number that represents an estimation of uncertainty for our balance. If "no" is the answer to one or more questions, re-check your math for each calculation.

h. Report the uncertainty.
   i. When we report uncertainty we need to provide our customers the basic information about uncertainty. This includes stating the uncertainty, the k value

used for expanding the uncertainty and indicating the approximate confidence interval.

ii. Example:
The measured result is 40.5000 grams ± 0.0062 gram. The reported uncertainty is expanded using a coverage factor k=2 for a level of confidence of approximately 95%, assuming a normal distribution.

7. Maintaining Measurement Uncertainty Values
    a. Once measurement uncertainty has been established, it is not necessary to weigh the surrogate samples monthly.
    b. If a new analyst joins the group, they must complete this training module and then may commence the weighing of the surrogate sample. After six weighing events the measurement uncertainty will be recalculated. It is not necessary to start from scratch with uncertainty calculations when an analyst joins or leaves the group.
8. Significant Figures and Rounding
    a. The number of significant figures must, at a minimum, correspond to the uncertainty in the measurement and must not be more than the precision of the measuring device. Excel rounding rules will be followed. Rounding up will only apply to uncertainty calculations and not to reporting of case samples. If truncation is required for reporting purposes, truncation will occur after calculation to the appropriate significant figures and calculation of the measurement uncertainty.
9. Uncertainty Calculations
    - Multiple Weighing events
        - When combining weights to report a total net weight of multiple packages, one cannot simply add together the individual uncertainties and report the combined uncertainty. All calculations that are necessary to calculate combined uncertainty and the total weight of exhibits must be documented in the case notes. If weights are to be combined and one uncertainty reported, the same balance will be used for weighing each package.

            U = $\sqrt{N*(u_b)^2}$ or $\sqrt{N}$ * ub
            U = total Uncertainty
            N = number of measurements
            ub = Uncertainty of the balance
        - Dynamic and static weighing are both considered two weighing events and the reported uncertainty must account for the two weighing events. This calculation may be done manually with the above listed formula or the MU spreadsheet can be updated to automatically perform this calculation.
        - Examples:
            - A bag of leaf material weighed 39.8 grams.

                U = $\sqrt{N*(u_b)^2}$ = $\sqrt{2*0.0062g^2}$ = 0.0088 g
            - Five plastic bags of marijuana were tested and the total net weight was determined to be 42.5000 grams. The same balance was used for each of the five bags of marijuana.

                U = $\sqrt{N*(u_b)^2}$ = $\sqrt{5*0.0062g^2}$ = 0.014 g

8.3 SAFETY

Calculating measurement uncertainty for weighing is primarily an administrative task and involves minimal laboratory activity. Refer to the Balances sections of the training manual and technical procedures for additional safety information.

8.4 SUGGESTED READING

1. Bell S.1999. Measurement Good Practice Guide No. 11, A Beginner's Guide to Uncertainty of Measurement National Physical Laboratory.
2. Birch K. 2003. Measurement Good Practice Guide No. 36, Estimating Uncertainties in Testing, British Measurement and Testing Association.
3. Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results. NIST Technical Note 1297. 1994.
4. Recommended Guide for Determining and Reporting Uncertainties for Balances and Scales. NIST IR6919, NIST. 2002.
5. A Statistics textbook of your choosing.

8.5 STUDY QUESTIONS

1. Define the following terms.
    a. Significant figures
    b. Precision
    c. Accuracy
    d. Measurement uncertainty
    e. Standard uncertainty
    f. Expanded uncertainty
    g. Confidence level
    h. True value
    i. Measurement error
    j. Standard deviation
    k. Arithmetic mean
    l. Variance
    m. Normal probability distribution
    n. Rectangular (uniform) probability distribution
    o. Cause and effect diagram
    p. Pareto chart
2. How are error and uncertainty different when it comes to scientific measurements?
3. Are uncertainties estimated or unequivocally calculated? Why?
4. When establishing MU, data can be gathered in several ways. What type of data is gathered using the "Type A" evaluation? What type of data is gathered using the "Type B" evaluation?
5. What is the purpose of a cause and effect diagram when establishing MU?
6. The greatest contributions to uncertainty are the balance and the human use of the balance. How are these elements represented in uncertainty calculations?
7. Why do we say that k = 2 is a level of confidence of approximately 95%?
8. Air buoyancy is an element that could impact uncertainty. Did we consider air buoyancy when determining uncertainty for our balances?
9. You are establishing measurement uncertainty for a new balance. The balance has a range of 0.001 gram to 310 grams. What weight of surrogates should be used to collect data for determining the measurement process reproducibility? How many weighing events should be undertaken with the surrogate before the measurement process reproducibility is determined? What information might be considered when deciding if a low range and high range uncertainty estimation are appropriate for this balance?
10. You are in the process of weighing the forty gram mass standard and your phone rings. In your haste to answer the phone you did not return the mass standard to its storage container and hold the mass standard in your gloved hand while you have a twenty minute phone conversation. At the end of your conversation you return to the balance to continue weighing your mass standard. What problem has arisen in this situation? What is the correct way to deal with this problem?
11. A check weight with an uncertainty of measurement of +/- 0.0249 mg @k=2 is used to gather the following data:40.00g, 40.00g, 39.99g, 39.99g, 40.00g, 40.00g, 40.00g, 40.00g, 40.01g, 40.00g, 39.99g, 40.00g, 40.00g, 40.00g, 39.99g, 40.00g, 39.99g, 40.01g, 40.00g, 40.00gThe uncertainty of measurement for the balance used for the measurements in question 10 is 0.02 g @k=2.

12. Calculate the process standard deviation, the standard uncertainty and the expanded uncertainty using k=2.

8.6 PRACTICAL EXERCISES

This training module has no practical exercises.

9 COCAINE

9.1 OBJECTIVES

- To become familiar the methods and procedures used to identify cocaine and their benefits and limitations.
- To demonstrate analytical protocols on “casework” type samples.

9.2 TOPIC AREAS

1. Legal definition and scheduling
    a. Schedule II
        i. RCW 69.50.206 (b) (4) Coca leaves and any salt, compound, derivative, or preparation of coca leaves including cocaine and ecgonine, and their salts, isomers, derivatives, and salts of isomers and derivatives, and any salt, compound, derivative, or preparation thereof which is chemically equivalent or identical with any of these substances, but not including decocainized coca leaves or extractions of coca leaves which do not contain cocaine or ecgonine.
            1. The latter section was added in the 1980s to circumvent the issue of whether “cocaine” only referred to the l optical isomer which is what is found in the coca plant.
    b. Base vs. salt in federal scheduling/sentencing (historically vs. currently)
2. Structure and isomers
    a. Coca plant (Erythroxylum coca.)
    b. Enantiomers
    c. Diastereomers
    d. Salt vs. base forms
3. Commonly encountered forms of cocaine
    a. White or beige compressed powder (hydrochloride salt).
    b. White, off-white or tan chunks (base form)
    c. Residues
        i. Smoking devices (pipes) – usually glass tubes containing metal turnings; base form
        ii. Containers, scales, razor blades, mirrors, spoons, currency, etc.
        iii. Residue remaining from conversion to base form
    d. Solutions
        i. Syringe contents possibly mixed with heroin or methamphetamine
        ii. Solutions from conversion to base form
4. Related Alkaloids
    a. Ecgonine
    b. Methylecgonine (ecgonine methyl ester)
    c. Benzoylecgonine
    d. trans-cinnamoylcocaine
    e. cis-cinnamoylcocaine
    f. Methylecgonidine
    g. Norcocaine
    h. Tropacocaine
    i. Ethylcocaine (Cocaethylene)
    j. Tropane
    k. Atropine
    l. Scopolamine
5. Common diluents and/or substitutes
    a. Powders
        i. Sugars (sucrose, mannitol, lactose, inositol, etc.)
        ii. Sodium bicarbonate (baking soda)

iii. Starch, flour, cake mix, etc.
iv. Other “caines” (procaine, benzocaine, lidocaine, tetracaine)
v. Nicotinamide
vi. Diltiazem and hydroxyzine
vii. Levamisole

b. Chunks
i. Nicotinamide
ii. Wax or wax mixed with baking soda, flour, etc.
iii. Acetaminophen, aspirin, ibuprofen, etc. (crumbled tablets)
iv. Soap

6. Analysis
a. Screening tests
i. Color tests
1. Blue ppt forms with CoSCN; with the addition of $SnCl_2$ the color remains for cocaine, ppt dissolves for many other “caines” such as lidocaine and procaine
2. Marquis to screen cocaine from methamphetamine
b. Common separation / isolation techniques
i. Aqueous base, extract to organic solvent
ii. Water (for salt forms) or dilute aqueous acid, basify, extract to organic solvent
iii. Dilute aqueous acid, organic wash, basify, extract to organic solvent
iv. Organic solvent (base form), wash with water
v. Dry organic extraction (e.g., wash hydrochloride salt with acetone, CH_2Cl_2 to separate from sugars)
vi. Particle picking
vii. Ion pairing (i.e., concentrated aqueous acid, extract to organic solvent)
viii. CoSCN extract: add dilute acid and CoSCN, extract to organic solvent, basify, dry organic. (useful for separation from other caines)
c. Identification methods, including differentiating capability of each, especially with respect to diastereomers and other similar molecules (ethyl cocaine, tropacocaine, atropine, etc.).
i. IR
1. Cocaine contains two esters, the carbonyl of one of which is conjugated with a phenyl ring. The latter will thus experience a weakening of the carbonyl bond and this will be reflected in its infrared absorption. Two strong carbonyl absorptions are thus seen in the infrared spectrum of cocaine (as the free base or any of its salts), with the lower frequency absorption arising from the benzoyl conjugated carbonyl group.
ii. GC/MS
1. The two polar ester groups are both attached to chiral carbons. The cocaine diastereomers, which result from shifting these esters groups from equatorial to axial (or vice versa), thus will have quite different polarities. This will have a pronounced affect on their volatilities, which explains why the four cocaine diastereomers have very different GC retention times.
iii. Microcrystal tests
1. Gold chloride in acetic acid
2. Refer to the microcrystal test training chapter
iv. TLC
1. 4:1 Chloroform:methanol works well for cocaine
2. Develop with acidified iodoplatinate or other reagent as appropriate
v. Raman

9.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment

including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

9.4 SUGGESTED READING

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8. Feigl F. 1966. Spot Tests in Organic Analysis, 7th ed. Amsterdam: Elsevier.
9. Fulton CC. 1969. Modern Microcrystal Tests for Drugs. John Wiley & Sons. p. 1-49.
10. Elsherbini SH. 1998. Cocaine Base Identification and Quanitification. Forensic Sci. Rev.10(1):1-12.
11. Microgram, a monthly DEA publication (many articles, see annual index).
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18. Washington State Board of Pharmacy, Pharmacy Lawbook, current year.

9.5 STUDY QUESTIONS

1. Using published information and/or laboratory databases compare data from analysis of "caine" compounds, ecgonines, cocaine metabolites and isomers of cocaine using:
    a. IR (base and salt forms)
    b. GC/MS
    c. Microcrystal tests
    d. TLC (using different solvent systems)
2. Which compounds have infrared spectra most similar to that of cocaine? What does this imply about the quality of infrared spectra that must be obtained in order to be able to distinguish it from these compounds?
3. What is the main basis for differentiating between the cocaine diastereomers using GC/MS?
4. How could you demonstrate which isomer of cocaine is present in the sample? Why isn't this necessary in our casework?
5. What is a diluent or cutting agent? Why are they routinely seen in controlled substance cases?
6. Briefly describe how cocaine is produced starting from the coca plant.
7. Explain the difference between cocaine hydrochloride and cocaine base as you would to a jury. Describe how each is primarily introduced into the body and why.
8. Why is cocaine base called crack?
9. Cocaine is often referred to as a narcotic. Should it be?

9.6 PRACTICAL EXERCISES

1. Prepare the following samples for analysis, with goal of isolating cocaine
    a. Base mixed with lidocaine
    b. Base mixed with procaine
    c. Hydrochloride mixed with nicotinamide
    d. Hydrochloride mixed with diltiazem
    e. Marijuana pipe with cocaine
2. Practice converting cocaine base to the salt form and/or salt to the base. Analyze using FTIR and/or Raman.
3. Prepare mixtures of cocaine and inositol in concentrations varying from 10% to 90%. Analyze on FTIR.
4. Prepare a mixture of cocaine and heroin. Isolate each compound and identify using available analytical techniques.

10 DESIGNER DRUGS

10.1 OBJECTIVES

- To become familiar the methods and procedures used to identify designer drugs and their benefits and limitations.
- To become familiar with the legal status of designer drugs.
- To demonstrate analytical protocols on “casework” type samples.

10.2 TOPIC AREAS

1. Legal definitions and scheduling
    a. Unique to each category of compounds
    b. Updated frequently
    c. Varies between State and Federal
    d. RCW vs. WAC
    e. Analog laws
2. Emerging compounds
    a. Popularity changes frequently
    b. Influx of compounds from foreign countries
    c. Lack of appropriate reference materials
    d. Important to stay current with trends and sub-culture websites
3. Substituted phenethylamines
    a. Dimethoxyamphetamines
    b. Dimethoxyphenethyamines
    c. Trimethoxyamphetamines
    d. 2C compounds
    e. Cathinones
    f. NBOMes
    g. Mescaline
    h. Others
4. Piperazines
    a. 1-benzylpiperazine (BZP)
    b. 1-(3-Chlorophenyl)piperazine (mCPP)
    c. 1-(4-Methoxyphenyl)piperazine (MeOPP)
    d. 1-(3,4-Methylenedioxybenzyl)piperazine (MDBP)
    e. 1-(3-Trifluoromethylphenyl)piperazine (TFMPP)
5. Synthetic Cannabinoid Agonist
    a. Generally referred to as “synthetic cannabinoids”.
    b. These compounds are CB1 and CB2 receptor agonists which mimic the psychoactive effects of THC.
    c. Product names include Spice, Spice Gold, Spice Silver, Spice Diamond, Spice Arctic, PEP Spice, K2, Yucatan Fire, Spike99, Spicey XXX, Genie, Pulse, Buzz, Fire ‘N Ice, Skunk, Ex-Ses, Tribal Warrior, Mojo, Spirit, Cosmic Haze, Hawaiian Hayze, Serenity Now, and many many more…
    d. Preparations are usually a plant based mix sprayed with the synthetic cannabinoids. May be marketed as an incense product not intended for human consumption.
    e. Large amounts of synthetic tocopherol (vitamin E) are reported to be present which may be intended to mask the analytical detection of the active ingredients.
    f. Important acronyms
        i. HU – Hebrew University.
        ii. JWH – John W. Huffman, a professor and researcher at Clemson University that synthesized and researched many CB1 and CB2 receptor compounds.
        iii. CP – Cyclohexylphenol structures that also bind to CB1 and CB2 receptors. Originally created by Pfizer.

g. More extensive training material including current legal updates is available on The Portal under Chemistry: Training/Reference Material:The Spice Rack

10.3 SUGGESTED READING

1. See "References for Verification of Designer Drugs" located on the Portal under Chemistry: Training/Reference Material: Names and Verification References of Designer Drugs
2. Allen A, Cooper D. 1979. Structural Elucidation of Low Molecular Weight Primary and Secondary Amines (via Phenylisothiocyanate Derivatives). Microgram. 12(2):24-52.
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5. BZP and TFMPP: Chemicals Used to Mimic MDMA's Effects. Microgram Bulletin. 200. 35(5):123.
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14. Shulgin A, Shulgin A. 1991. PIHKAL: A Chemical Love Story. Transform Press.Soine WH, et al. 1984. Differentiation of Side Chain Positional Isomers of Amphetamine. J For Sci. 29(1):177-184.
15. Soine WH, et al. 1983. Differentiation of 2,3-Methlyenedioxyamphetamine from 3,4-Methylenedioxyamphetamine. J For Sci. 28(2):386-390.
16. Structure-Activity relationships, Synthesis, Precursor Preparation and Analysis of Methylenedioxyamphetamine and its Analogs and Homologs: A four volume collection presented at the 4th Annual Technical Training Seminar. Clandestine Laboratory Investigating Chemists (CLIC) Meeting, September 7-10, 1994.
17. Zhingel KY, Dovensky W, Crossman A, Allen A. 1991. Ephedrone: 2-Methylamino-1-Phenylpropan-1-One (Jeff). J For Sci. 36(3):915-920.

10.4 STUDY QUESTIONS

1. How are synthetic cannabinoid agonists scheduled in the state of Washington? In Federal law?
2. Which synthetic cannabinoid agonists are scheduled federally but are not included in Washington law?
3. List the six classes of synthetic cannabinoid agonists listed in the WAC. Draw their general chemical structure and give at least one example of a compound for each class.
4. Are there other recognized classes of synthetic cannabinoids agonists other than those listed in the WAC? If so, list them, draw their general chemical structure and give at least one example of a compound for each class.

5. Give examples of designer drugs which are known to have positional isomers. Does the isomer impact the legal status? How can we correctly identify which positional isomer is present in a case sample?
6. What is the legal definition of an analog? What is our policy for reporting analogs?
7. What are the 2C compounds? What is their control status?
8. What are NBOMes? What is their control status?
9. What is the control status of piperazines?
10. Is BZP a hallucinogen? Is TFMPP a hallucinogen? Why are they often seen in combination in tablets?
11. How would you report the following substances?
    a. XLR11
    b. JWH-018
    c. HU-210
    d. PB-22
    e. 2C-H
    f. Methylone
12. You have identified UR-144 in a case sample. The prosecuting attorney calls to ask whether this substance is controlled or not. How do you respond?

10.5 PRACTICAL EXERICES

1. Analyze a mixture of BZP, MDMA, and TFMPP by GC/MS or GC/FID on different functionality columns. Derivatize the sample using acetic anhydride and trifluoroacetic anhydride. Which functionality column is most effective at separating the BZP, MDMA, TFMPP and associated derivatives?
2. Analyze any Ecstasy tablets available. What compounds were present in these tablets?
3. If available, analyze a synthetic cannabinoid sample.

11 GHB/GBL/1,4-BUTANEDIOL

11.1 OBJECTIVES

- To become familiar the methods and procedures used to identify GHB, GBL, and 1,4-Butanediol and their benefits and limitations.
- To understand the effects metabolism has on the conversion of GHB, GBL, and 1,4-Butanediol.
- To demonstrate analytical protocols on “casework” type samples.

11.2 TOPIC AREAS

1. Legal definitions and scheduling
2. Structures
3. Synthesis
    a. Base-catalyzed hydrolysis of GBL
4. Metabolism
    a. In aqueous solutions, GHB is found in equilibrium with gamma-butyrolactone (GBL).
    b. 1,4-butanediol is another closely related compound that along with GBL is metabolized to GHB in the body.
5. Commonly encountered forms
    a. Solid salt
    b. Liquid – often in relatively high concentrations
        i. cleaning solutions
        ii. dietary supplements
    c. Clandestine labs
    d. Commercial solvents
    e. Beverages
        i. alcohol
        ii. sports drink (e.g. GatoradeTM)
        iii. tea
        iv. other
6. Analysis
    a. Form
        i. solid – direct IR
        ii. liquid – direct IR or dry to solid and direct IR
    b. Screening tests
        i. pH – if aqueous and acidic, expect to find both GHB and GBL
        ii. color tests
            1. chlorophenol red with modified Schweppes
            2. bromocresol purple and bromothymol blue with modified Schweppes
            3. bromocresol green with modified Schweppes
    c. Crystal test
        i. copper nitrate / silver nitrate
    d. IR
        i. direct on liquids or solids
    e. GC/MS
        i. direct for GBL and 1,4-butanediol
        ii. GHB is converted to GBL
        iii. GHB does not chromatograph well; can be derivatized (TMS derivatives are water-sensitive)
    f. Capillary Electrophoresis (CE)
        i. micellar method – GHB and GBL easily separated; 1,4-butanediol has poor detection limits
        ii. indirect UV detection method
    g. Raman

i. direct on liquids or solids

h. Other

11.3 SUGGESTED READING

All reading should use the most current edition available, unless otherwise noted by the instructor as an historical reference.

1. Andrea KM, et al. 2000. Microchemical Identification of GHB. J For Sci. 45(3):665-668.
2. Baldacci, A, et al. 2003. Determination of γ –Hydroxybutyric Acid in Human Urine by Capillary Electrophoresis with Indirect UV Detection and Confirmation with Electrospray Ionization Ion-Trap Mass Spectrometry. J. of Chromatography A. 990:99-110.
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18. Vose, J, Tighe T, Schwartz M, Buel E. 2001. Detection of Gamma-Butyrolactone (GBL) as a Natural Component in Wine. J For Sci. 46(5):1164-1167.
19. Washington State Board of Pharmacy, Pharmacy Lawbook, current year.
20. Witkowski MR, et al. 2006. GHB Free Acid: II. Isolation and Spectroscopic Characterization for Forensic Analysis. J For Sci. 51(2):330-339.

11.4 STUDY QUESTIONS

1. Describe the equilibrium formed GHB and GBL in aqueous solutions of various pH values. How does this affect analysis?
2. What is Xyrem? What is it prescribed for?
3. List the physical properties of GHB, GBL, and 1,4-butanediol.

11.5 PRACTICAL EXERCISES

1. Perform color tests and microcrystal tests on standards of GHB, GBL, and 1,4-Butanediol..

2. Analyze samples of GHB, GBL, and 1,4-Butanediol on FTIR and GC/MS.
3. Prepare a derivatized sample of GHB for GC/MS analysis.
4. Carry out a series of evaluations on compounds supplied by the instructor using the CE system (if available).
5. Carry out Raman analyses (if available) of standards.
6. Analyze samples of GHB mixed in a variety of matrices.

12 HALLUCINOGENS

12.1 OBJECTIVES

- To become acquainted with suspected hallucinogenic controlled substances, analogs and non-controlled hallucinogens that may be submitted to the laboratory.
- To demonstrate an understanding of the methods and procedures used to identify hallucinogens.
- To demonstrate analytical protocols on “casework” type samples.

12.2 TOPIC AREAS

6. Hallucinogens and the law
    a. Scheduled compounds
    b. Interpretation of the statutes
7. Indoles
    a. LSD and ergot alkaloids
        i. isomers and derivatives of lysergic acid
        ii. syntheses of LSD and clan labs
            1. Hawaiian woodrose
            2. Morning glory seeds
        iii. analysis of LSD
        iv. forms
            1. blotter paper (and DEA data base of logos)
            2. window panes
            3. sugar cubes and candy
            4. liquid in dropper bottles
        v. non-controlled compounds
            1. Lysergic acid methylpropylamide
            2. Lysergic acid methylisopropylamide
            3. Iso-LSD
            4. Other closely related isomers
    b. Synthetic Tryptamines
        i. Dimethyltryptamine (DMT)
        ii. Diethyltryptamine (DET)
        iii. Alpha-ethyltryptamine
        iv. Other tryptamines
        v. Chemical structures, synthesis, isomers and analysis
    c. Psilocybin/Psilocyn
        i. Chemical structures and isomers
        ii. Decayed material: proper storage requirements
        iii. Analysis of mushrooms
        iv. Psilocybin degradation to psilocin
            1. natural existence in the plant material
            2. documentation of which compound is initially present in the mushroom versus which compound is identified
            3. reporting considerations (conversion of psilocybin to psilocyn during the analysis)
    d. Related compounds
        i. baeocystin
        ii. norbaeocystin
        iii. bufotenine
8. Phencyclidine, analogs and derivatives
    a. PCP (phencyclidine, 1-(1-phenylcyclohexyl) piperidine)
        i. in solution
        ii. on vegetable material

iii. crystal form
b. TCP (1-(1-thiophenecyclohexyl)piperidine)
c. PCE (1-(1-phenylcyclohexyl)ethylamine)
d. PHP (1-(1-phenylcyclohexyl)pyrrolidine)
e. Ketamine
f. Other scheduled and non-scheduled compounds
g. Precursor compounds and reagents
    i. 1-phenylcyclohexylamine
    ii. 1-piperidinocyclohexanecarbonitrile (PCC)
h. Syntheses and clan labs

9. Other Controlled Hallucinogens
    a. Benzilates (JB compounds)
    b. Ibogaine
    c. THC and isomers, cannabinoids
    d. Other compounds as listed in the RCW and/or WAC
    e. β-Carbolines
        i. harmaline
        ii. harmine
10. Non-controlled Hallucinogens
    a. Amanita Muscaria
        i. Mushroom contains:
            1. muscimol
            2. ibotenic acid
            3. muscarine
        ii. Amanita mushrooms are chewed, ground and mixed with food or drink, or brewed into a tea.
        iii. Mushrooms grow wild in Europe, Asia, and North American, often under birches, firs, and larches.
    b. Ayahuasca
        i. A combination of extracts of Banisteriopsis caapi and Psychotria viridis
        ii. Both types of vines grow in the Amazonian forests of South America.
        iii. Also known as Yagé
        iv. Active ingredients are harmaline and DMT
    c. Calamus
        i. Acorus calamus is a wild plant grown in North America, Europe, and Asia.
        ii. An iris-like plant that grows 5-6 feet tall and is often found among cattails near streams and ponds.
        iii. The active ingredient is asarone and the effects are said to be similar to LSD.
    d. Catnip
        i. Nepeta cataria is best known for its stimulating effect on cats.
        ii. When smoked or made into a tea is supposed to have an effect similar to marijuana and in large doses like LSD.
    e. Damiana
        i. Turnera diffusa a shrub available in gardening stores.
        ii. The berries are sold in some health food stores.
        iii. The dried leaves or berries are smoked which is reported to produce marijuana-like effects.
    f. Datura
        i. Datura stramonium, commonly known as Jimson weed, grows wild throughout Asia, Europe and North America.
        ii. It can be used in a tea or smoked.
        iii. The effects last from 36 hours to several days. Excessive use can cause amnesia or permanent brain damage.
        iv. The seeds are toxic and as little as half a teaspoon can cause death.
    g. Dextromethorphan
        i. Common ingredient in OTC cold medicine.

ii. When taken in doses of 300 mg or larger it may produce auditory and visual disturbances similar to LSD or psilocybin mushrooms.

h. Doña Ana
   i. A cactus, Coryphantha macromeris, which grows wild in southern Texas and northern Mexico.
   ii. Contains macromerine which is similar to mescaline but a fifth as potent.

i. Henbane
   i. Hyoscyamous niger is similar to Datura.
   ii. A hairy, sticky plant which grows along roadsides.
   iii. Principle ingredients are hyoscyamine, atropine, and scopolamine.
   iv. Can be smoked or eaten.

j. Mandrake
   i. Mandragora officinarum grows wild in fields in southern Europe.
   ii. Contains mandragorine, atropine, scopolamine and hyoscamine.

k. Nutmeg
   i. Twenty grams of ground nutmeg contains:
      1. 210 mg of myristicin (similar to MMDA)
      2. 70 mg of elemicin (similar to TMA)
      3. 39 mg of safrole (similar to MDA)

l. Salvia divinorum
   i. A wild member of the mint family.
   ii. Crushed leaves may be chewed or mixed with water to drink. The leaves can also be smoked.
   iii. Contains salvinorin A which produces effects similar to psilocybin mushrooms, PCP or ketamine.

m. Sceletium tortuosum
   i. A plant common in South Africa.
   ii. Also known as Kanna, Channa, and Kougoed.
   iii. Contains mesembrine, mesembrenone, and mesembrenol which are all mildly psychoactive compounds.

11. Analytical Considerations
   a. Extraction/purification difficulties
      i. isolation from plant material
      ii. low concentration/matrix interference
      iii. break down of fragile compounds
         1. LSD/Psilocybin
            a. thermal
            b. ultraviolet
   b. Chromatographic separation
      i. temperature programs
      ii. inlet systems
      iii. use of absolute retention times or use of internal reference standard
      iv. use of standards

12.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

12.4 SUGGESTED READING

This is not meant to be a comprehensive list but a starting point of useful references for the background and analysis of hallucinogens.

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    a. Blackledge RD, Phelan CP. 2006. Identification of Bufotenine in Yopo Seeds via GC/IRD. Microgram Journal. 4(1-4):3-11.
    b. Phelan CP. 1999. Identification of psilocin and bufotenine via GC/IRD. Microgram. 32(2):83-89
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12.5 STUDY QUESTIONS

13. What is the difference between psilocin and psilocybin? How would you distinguish then analytically? How do we report these substances?
14. Discuss the differences in the morphology of the different types of mushrooms that contain psilocybin.
15. What is synesthesia?
16. What is the medicinal use of hallucinogens?
17. What is ecstasy? Is this a legal, scientific, or street term?
18. List the structures of the indoles and phencyclidine, analogs, and derivatives listed in the outline.
19. How are dopamine and serotonin related to hallucinogens?
20. How can LSD and LAMPA be distinguished using GC/MS?
21. The base peak in the MS of psilocin is m/z 58. Methamphetamine's MS also has a base peak of 58. Why do psilocin, a tryptamine, and methamphetamine, a phenethylamine, have the same base peak. Illustrate with structures.

12.6 PRACTICAL EXERCISES

1. Obtain a sample of peyote. Extract and confirm the mescaline.
2. Obtain samples of psilocybin mushrooms and chocolate covered 'shrooms. Test both using the Weber color test. Discuss extraction techniques with your trainer and perform the most appropriate extraction to isolate and confirm psilocin. Use TLC or CE, GC/MS and FTIR to analyze the samples.
3. Analyze a variety of samples (e.g. sugar cubes, candies, blotter paper) impregnated with LSD. Use UV as a screening technique.
4. Analyze a mixture of PCP and marijuana.

13 MARIJUANA – QUALITATIVE ANALYSIS

13.1 OBJECTIVES

- To develop an understanding of the various forms of marijuana.
- To demonstrate an understanding of the methods and procedures used to identify the various forms of marijuana.
- To observe and demonstrate the potential false positives.
- To become familiar with other botanical specimens which may be submitted as marijuana.

13.2 TOPIC AREAS

1. Definitions
    a. "Marijuana" or "marihuana" means all parts of the plant Cannabis, whether growing or not, with a THC concentration greater than 0.3 percent on a dry weight basis; the seeds thereof; the resin extracted from any part of the plant; and every compound, manufacture, salt, derivative, mixture, or preparation of the plant, its seeds or resin. The term does not include the mature stalks of the plant, fiber produced from the stalks, oil or cake made from the seeds of the plant, any other compound, manufacture, salt, derivative, mixture, or preparation of the mature stalks (except the resin extracted therefrom), fiber, oil, or cake, or the sterilized seed of the plant which is incapable of germination.

    b. "Marijuana-infused products" means products that contain marijuana or marijuana extracts and are intended for human use. The term "marijuana-infused products" does not include useable marijuana.

    c. "THC concentration" means percent of delta-9-tetrahydrocannabinol content per dry weight of any part of the plant *Cannabis*, or per volume or weight of marijuana product, or the combined percent of delta-9 tetrahydrocannabinol and tetrahydrocannabinolic acid in any part of the plant *Cannabis* regardless of moisture content.

    d. "Useable marijuana" means dried marijuana flowers. The term "useable marijuana" does not include marijuana-infused products.

2. Botanical Characteristics
    a. Entire plant
        i. Green in color and normally grows to a height of 4 to 6 feet when mature. Some plants have been known to grow as tall as 15 feet.
        ii. Generally conical shaped – size and number of branches and leaves are influenced by the proximity of the plants during growth.
    b. Stalks and stems
        i. Fluted lengthwise
        ii. Covered with hair
        iii. Opposite branching
    c. Leaves
        i. Shape – compound palmate
            1. Compound – usually 5 to 9 leaflets per leaf; almost always an odd number
            2. Palmate (hand like) – leaflets originate from a common point (apex)
        ii. Vein structure – best seen on underside of leaf, alternate venation ending in a sharp point at each serration.
    d. Leaflets
        i. Lanceolate (shaped like a lance)
        ii. Serrated edges (saw tooth margins)
    e. Upper surface of leaves

i. Dark green
ii. Cystolithic hairs
    1. Crystal of calcium carbonate at the base
    2. Shaped like a bear claw (curved, tapered)
    3. Marijuana is not the only plant with cystolithic hairs or hairs that look similar to cystolithic hairs.

f. Lower surface of leaves
    i. Lighter green than upper surface
    ii. Simple hairs
        1. More numerous than cystolithic hairs
        2. Usually longer than cystolithic hairs
        3. Not tapered – approximately constant in cross section
        4. Marijuana is not the only plant with simple hairs.
    iii. Guard hairs – found along the veins, may look somewhat like cystolithic hairs
g. Glandular hairs
    i. multi-cellular
    ii. bulbous tip and covered with sticky residues
    iii. found on upper and lower surfaces of leaves
h. Flowers
    i. Sex determined after flowers appear.
    ii. Normally dioecious (separate sexes) – staminate (male) and pistillate (female) function in separate plants.
        1. Male flower (staminate inflorescence)
            a. Sprays about six inches in length at the top of stalks and branches.
            b. Five sepals (outermost row of petals) make up the calyx (outermost series of flower parts).
            c. Each flower has five stamens opposite each sepal
                i. Stamen – the pollen producing structure of a flowering plant.
            d. Pollen is shed from two pore-like openings in the tips of the anthers (pollen sacs).
                i. The pollen is light yellow, sheds profusely and is quite buoyant.
        2. Female flower (pistillate inflorescence)
            a. Appear in dense clusters near the top (apex of the plant) and usually occur in pairs.
            b. The pistil is the plant organ that is fertilized and develops a seed.
            c. Bracts are hairy and rich in resin secreting glands.
                i. Bracts cover the pistil and make it difficult to observe.
            d. The stigma, a long, brown fuzzy-looking structure, protrudes from the bract, usually in pairs.
                i. Stigmas trap the pollen from the male plant as part of the fertilization process. In some samples of marijuana, the plant material may look brown because of the numerous stigmas.
    iii. “cola” (entire top of female flowering plant) vs. “bud”
i. Seeds
    i. Covered by bract in plant
    ii. Naked seeds are:
    iii. Ovoid in shape
    iv. Flattened on one end
    v. Distinct ridge around greatest circumference (raphe)
    vi. Mottled with tortoise shell-like appearance (reticulated)
    vii. Light gray to gray-brown in color when mature

3. Taxonomy

a. Kingdom – Plantae
b. Division – Spermatophyta (seed plants)
c. Sub-Division (class) – Angiospermae (flowering plants)
d. Subclass – Dicotyledoneae (2 seed leaves)
e. Order – Urticales
    i. This describes the type of flower. Marijuana flowers, which lack petals, have a perianth (ovary and sepal) that is surrounded by a sheath called a “bract”. This order also includes elms, mulberries, and nettles.
f. Family – Cannabinacea (Hemp family)
    i. Only two plants fall into this category, hops and marijuana.
g. Genus – Cannabis
h. Species - sativa
i. Closest botanical relative is genus Humulus (hops)
j. Question of number of species of Cannabis
    i. Most experts agree there is only one, Cannabis sativa L., within which there exists a number of variations.
    ii. Some reportedly feel there may be more than one species.
    iii. This question should not be a problem due to the wording of our statute.

4. Chemical Constituents
    a. Effervescence test for the cystolithic nature of hairs (Greek: cysto, bladder, pouch; lithic, stone).
        i. Addition of HCl to cystolithic hairs
        ii. Evolution of bubbles of CO2
        iii. $CaCO_3 + 2HCl \rightarrow CaCl_2 + H_2O + CO_2\uparrow$
        iv. An indication of cystolithic hairs – not specific for marijuana
5. Cannabinoids
    a. Definition: Compounds of synthetic or natural origin from Cannabis whose structure comprises a 5-alkylresorcinol moiety covalently bonded to a mono-terpenoid or analogous moiety.
    b. Found only in marijuana, as far as anyone knows.
    c. More than 60 known, though most of these are minor components (Carlton Turner, 1979).
    d. Some of the major cannabinoids include tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), cannabigerol and cannabichromene.
    e. Monoterpenoid and dibenzopyran nomenclature
    f. Active ingredients
        i. (-) Δ9 - trans tetrahydrocannabinol (Δ9 THC)
        ii. (-) Δ8 - trans tetrahydrocannabinol (Δ8 THC) Typically, there is 10 to 20 times as much Δ9 THC as Δ8 THC in plant material.
    g. RCW Schedule 1 cannabinoids
        i. Tetrahydrocannabinols, synthetic equivalents of the substances contained in the plant, or in the resinous extractives of Cannabis, species, and/or synthetic substances, derivatives, and their isomers with similar chemical structure and pharmacological activity such as the following:
        ii. Delta 1 - cis - or trans tetrahydrocannabinol, and their optical isomers, excluding tetrahydrocannabinol in sesame oil and encapsulated in a soft gelatin capsule in a drug product approved by the United States Food and Drug Administration;
        iii. Delta 6 - cis - or trans tetrahydrocannabinol, and their optical isomers;
        iv. Delta 3,4 - cis - or trans tetrahydrocannabinol, and its optical isomers;
    h. Conversions of cannabinoids
        i. CBD → Δ 9 THC (in plants)
        ii. Δ9 THC → CBN (old marijuana samples, therefore, often contain predominantly CBN)
    i. Concentration of resin/distribution of THC in plants:
        i. Concentration of cannabinoids

ii. (Highest to Lowest): Bracts (seed covers) → Flowers → Leaves → Stems → Roots and Seeds

6. Microscopic Botanical Observations
    a. The defining microscopic characteristic of marijuana is the presence of "bearclaw" shaped cystolithic hairs (on the top leaf surface) and simple hairs on opposite sides of the same leaf fragment.
    b. No other plants are known to have the identical microscopic morphology of marijuana. This microscopic observation, combined with a positive Duquenois-Levine test (see below), serves to identify marijuana to the exclusion of all other known plants.
    c. Stereoscopic examination, approximately 8X to 40X magnification.
7. Duquenois-Levine (chemical color test for cannabinoids)
    a. Reagents for Duquenois-Levine test
        i. Duquenois Reagent
            1. 20 mL of ethanol
            2. 0.4 gram vanillin
            3. 5 drops acetaldehyde
        ii. Concentrated hydrochloric acid (HCl)
        iii. Chloroform ($CHCl_3$)
    b. Lifetimes of reagents
        i. HCl will slowly lose its strength (due to evaporation of HCl gas) and is the most likely reagent to go bad. The potency of the HCl will effect the development of color in the chemical test.
        ii. Duquenois reagent may also lose its potency. It has a long shelf life if it is refrigerated. If the solution doesn't yield positive results with known marijuana, the reagent should be discarded. A distinct yellowing of the reagent is a good indication that it may be losing its effectiveness.
        iii. When these reagents lose their strength, little or no color change will result.
    c. Periodic check of reagents
        i. Positive control (known marijuana)
        ii. Negative control (blank or known non-marijuana)
    d. Duquenois-Levine Procedure
        i. Several methods are published for performing the Duquenois-Levine test.
        ii. One method commonly used among WSP Chemists is described:
            1. Add Duquenois reagent to the sample and mix well. Typically, it is not necessary to let the mixture soak for any longer than about two minutes. This solution may or may not be decanted prior to the next step. If the mixture includes leaf material, allowing the mixture to stand too long may extract the green pigments from the plant. This may mask the color development when the acid is added.
            2. Add an approximately equal volume of concentrated HCl to the Duquenois solution. Within two minutes, a blue to blue/purple color should form, but may be weak or faint with weak samples. If the sample is weak or the color change is difficult to interpret, the Duquenois and/or Duquenois/HCl solution should be decanted from the plant material. For very weak samples, it is advantageous to not mix the HCl with the Duquenois solution, but rather let it sit undisturbed. If a color band forms, the colored layer can be isolated with a pipette and transferred to another test tube prior to adding the chloroform (see next step).
            3. Once a color has formed, but after no more than 3 minutes, add an approximately equal volume of chloroform (or a quantity sufficient to distinctly observe the separate organic and aqueous layers) to the solution and mix thoroughly. As the layers separate, the blue to purple color should transfer to the lower chloroform layer.
    e. Chemistry and specificity of the Duquenois-Levine Test
        i. Tests for cannabinoids as a group, not a test for just THC.

ii. Various individual cannabinoids give different colors, in various shades of purple, violet or blue, e.g. THC purple, CBN blue, etc. Old marijuana samples often give the blue of CBN.
iii. Chloroform step (Levine Modification) increases the specificity of the test.
iv. A test for certain types of 2,5-dialkylresorcinols
v. Length of alkyl chains determine solubility in chloroform
    1. Resorcinol → pink; clear chloroform layer
    2. 5-Methylresorcinol → red; slight pink in chloroform layer
vi. Mechanism
    1. For a proposed detailed mechanism, see reading reference #26.
    2. Likely an electrophilic substitution of the phenolic ring by protonated aldehyde groups of vanillin and acetaldehyde
    3. Empirical formula of colored product for the Duquenois reaction of THC is: THC + VANILLIN + 2ACETALDEHYDE $-3H_2O$

8. Training Topics
    a. Marijuana derivatives, cannabinoids and structural relationships
    b. Legal scheduling
        i. State possession limits
        ii. Federal law
    c. Extraction from sample matrix
    d. Analysis
        i. Qualitative Analysis
            1. Microscopic characterization
            2. Duquenois-Levine
            3. Thin layer chromatography
                a. Mobile phases: hexane:diethyl ether (4:1); toluene
                b. Visualization: Fast Blue B (carcinogenic), or Fast Blue BB
                c. Differentiating THC from THCA
            4. Gas chromatography/mass spectrometry
                a. Derivatization using MSTFA
        ii. Quantitative analysis (covered in the next chapter)
    e. Forms encountered in casework
        i. Leaf material
        ii. Non-leaf material
            1. Pipe residues
                a. Potential for multiple drug residues
            2. Vegetable material with no microscopic characteristics available:
                a. Residues
                b. Stalks
                c. Burned/partially charred material
                d. Decayed plant material
                e. Solvent extracted marijuana leaves
            3. Extracts
                a. Compressed material (hashish)
                b. Oil extracts (hash oil)
                c. Kif, Kief, Kef, or Keef
                    i. Refers to the loose, dried resin glands (or trichomes) of Cannabis which accumulate on containers or have been removed with a kiefing screen or sieve.
                    ii. Kief can be smoked in a number of ways, including using smoking pipes, bongs, and vaporizers.
            4. Seeds
                a. Since sterilized seeds which are incapable of germination are not controlled, in cases where only seeds are present, it may be necessary to attempt to germinate the seeds and examine the product plant.

iii. Pharmaceutical preparations
1. Dronabinol (synthetic) in sesame oil and encapsulated in a soft gelatin capsule is a United States Food and Drug Administration (FDA) approved drug product. (Dronabinol is the synthetic form of (-)-delta-9-(trans)-tetrahydrocannabinol.)
a. WAC 246-887-160 Schedule III, DEA Schedule III
b. Marinol, is the commercial name for a product containing dronabinol, Δ9- THC.
c. The FDA approved Marinol to treat nausea and vomiting associated with cancer chemotherapy in patients who have failed to respond adequately to conventional treatments. The FDA also approved Marinol to treat appetite loss associated with weight loss in people with acquired immunodeficiency syndrome (AIDS).

f. Other botanical samples submitted as marijuana
i. Marrubium vulgare (white horehound)
1. Marketed as a tobacco alternative.
2. Looks similar macroscopically to marijuana.
3. Does not have cystolithic hairs
4. Extracts analyzed by GC/MS indicate the presence of marrubiine
ii. Salvia divinorum
1. A wild member of the mint family.
2. Crushed leaves may be chewed or mixed with water to drink. The leaves can also be smoked.
3. Contains salvinorin A which produces effects similar to psilocybin mushrooms, PCP or ketamine.
4. Leaves do not have structural similarities of marijuana

13.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

13.4 SUGGESTED READING

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13.5 STUDY QUESTIONS

1. What is the active ingredient in marijuana? List some of the other cannabinoids that are present in marijuana.
2. Are cystolithic hairs unique to marijuana? If not, name some other common plants that have them.
3. Is the Duquenois-Levine test specific for marijuana? Is the Duquenois-Levine test specific for THC?
4. What is the base of a marijuana cystolithic hair made of?
5. Describe the morphology of a marijuana leaf? Plant? Seed?
6. List some of the materials that give false positive results for the Duquenois-Levine test.
7. What is hashish? What is kif?
8. How would you report the following if you received it in the lab?
    a. A pressed ball of a brown, resinous substance?
    b. A smoking device with charred residue but no visible plant material?
9. Define the following
    a. “marijuana” or “marijuana”
    b. “marijuana-infused products”
    c. “usable marijuana”
    d. “THC concentration”
10. Describe the possession limits of marijuana and products.
11. What are synthetic cannabinoids?
12. Which synthetic cannabinoids are controlled substances in Washington? In Federal law?

13.6 PRACTICAL EXERCISES

1. Take the herbal samples provided by your trainer and conduct visual and microscopic examinations of each sample. Also, take these herbal samples and conduct the Duquenois-Levine color tests directly on the material and then on a dried petroleum ether extract on each sample. Record the results of each test. Which method has fewer false positives?
    a. For plants that contain cystolithic hairs, note the microscopic similarities and differences in comparison to marijuana.
    b. Note carefully the colors (or lack of colors) produced in both steps of the Duquenois-Levine test for those materials sometimes reported as “false positives”: eucalyptus, rosemary, oregano, marjoram, mace, Western red cedar, coffee.
    c. Run some old marijuana samples and note the colors they give for the Duquenois-Levine (which cannabinoid is expected to be predominant in these?)
    d. Run some freshly harvested marijuana plants.
2. Duquenois-Levine for some phenolic compounds
    a. Oils: Eugenol and Patchouli oil
    b. Solids: Resorcinol, Orcinol (5-methylresorcinol), Olivetol (5- pentylresorcinol), Catechin, Napthoresorcinol
3. Marinol (Δ9 THC/Dronabinol capsules) (If available)
    a. Compare the colors obtained and the structures of the former compounds with those of the cannabinoids.
4. Observe your trainer or other experienced forensic chemist as they conduct an analysis of suspected marijuana.
5. Discuss with your trainer what tests and results need to be present to identify a sample as marijuana.
6. Examine a sample of hash and attempt to find the cystolithic hairs in the mass of material. Discuss with your trainer the criteria that must be met to identify the material as hash. Discuss how the sample would be reported. Repeat with hash oil, if available.
7. Test marijuana seeds for viability.
8. Add a small amount of dilute acid to a marijuana sample and observe the result under a stereomicroscope. Explain what happened.

14 MARIJUANA – QUANTITATIVE ANALYSIS

14.1 OBJECTIVES

- To understand the quantitation of THC as it relates to identifying marijuana.
- To become familiar with the accreditation requirements related to traceability and measurement uncertainty related to quantitative analysis.
- To demonstrate an understanding of the methods and procedures used to identify marijuana as defined by the Revised Code of Washington.

14.2 TOPIC AREAS

1. Legal scheduling of marijuana
    a. I-502
    b. HB2056
    c. HB2136
    d. SB5052
    e. 0.3% THC necessitates quantitative analysis
    f. Other THC percentages requiring quantitative testing (concentrates)
2. Types of quantitation methods
    a. Internal standard method
    b. External standard method
    c. Area percent method
    d. Others
3. Limits of detection
4. Quality control and quality assurance
5. Measurement uncertainty

14.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

14.4 SUGGESTED READING

1. De Backer B, et al. 2012. Evolution of the Content of THC and Other Major Cannabinoids in Drug-Type Cannabis Cuttings and Seedlings During Growth of Plants. J For Sci 57(4):918-922.
2. Dussy, FE et al. 2005. Isolation of Delta9-THCA-A from hemp and analytical aspects concerning the determination of Delta9-THC in cannabis products. For Sci Int 149:3-10.
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4. European Food Safety Authority. 2011. Scientific Opinion on the safety of hemp (Cannabis genus) for use as animal feed. EFSA Journal 2011 9(3); Pages 1- 8 and 19-22. http://www.efsa.europa.eu/en/efsajournal/doc/2011.pdf
5. GC Solutions #19: Calibration Curves – Part 1. www.sepscience.com/techniques/gc/articles
6. GC Solutions #20: Calibration Curves – Part 2, Internal Standard Approach. www.sepscience.com/techniques/gc/articles
7. GC Solutions #21: Calibration Curves – Part 2, Standard Addition. www.sepscience.com/techniques/gc/articles
8. Health Canada - Industrial Hemp - Technical and Policy Documents. http://www.hc-sc.gc.ca/hc-ps/substancontrol/hemp-chanvre/docs/index-eng.php
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11. NIST GMP 3. 2013. Good Measurement Practice for Method of Reading a Meniscus Using Water or Other Wetting Liquid.
12. NIST GMP 7. 2006. Good Measurement Practice for Cleaning Precision Glassware.
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14. Quantitative Chromatographic Analysis by Raymond P.W.Scott, part of the Chrom-Ed Series. The Internal Standard Method. www.chromatography-online.org/quant
15. Quantitative Chromatographic Analysis by Raymond P.W.Scott, part of the Chrom-Ed Series. The External Standard Method. www.chromatography-online.org/quant
16. Suurkuusk G. 2010. Validation of the Gas Chromatographic Method for THC, CBD, and CBN Determination. University of Tartu Master's Thesis.
17. United Nations Office on Drugs and Crime. 2009. Recommended Methods for the Identification and Analysis of Cannabis and Cannabis Products. http://www.unodc.org/documents/scientific/ST-NAR-40-Ebook.pdf
18. Zoller O, et al. 2000. High-performance liquid chromatographic determination of delta-9-tetrahydrocannabinol and the corresponding acid in hemp containing foods with special regard to the fluorescence properties of delta-9-tetrahydrocannabinol. J Chrom A 872:101-110.
19. Any Analytical Chemistry textbook which includes quantitative analysis.

14.5 STUDY QUESTIONS

1. How would you report the following:
    a. Leaf material with an offense date after May 1, 2013, with a THC concentration greater than 0.3%? less than 0.3%
    b. Less than 0.1 gram of leaf material.
2. Define the following
    a. "marijuana" or "marijuana"
    b. "marijuana-infused products"
    c. "usable marijuana"
    d. "THC concentration"
3. Describe the possession limits of marijuana and products.
4. Why is 0.3% THC a critical value for marijuana?
5. Compare and contrast methods of GC quantitative analysis.
6. Why are the advantages of the internal standard method for quantitative analysis?
7. Why is tribenzylamine used as the internal standard for quantitation of THC?
8. How are THCA and THC related? How does this relationship impact the quantitative analysis of leaf marijuana?
9. What is the purpose of the internal standard solution (ISS)? Calibration verification solution (CVS)? Resolution verification solution (RVS)?
10. What are the lower and upper limits of quantitation for THC in our validated method? What will you do if a case sample is outside these ranges?
11. How are the blanks evaluated during quantitative analysis?
12. How long can calibrators, ISS, CVS, and RVS be stored and used?
13. Why is the THC value determined by quantitative analysis not reported?
14. Why is moisture content of the leaf material not evaluated?
15. What is a "sample set" when setting up a sequence table?
16. How frequently must you run a Continuing Calibration Verification solution (CCV)?
17. Why do we perform replicate injections of the CVS and samples?
18. You are evaluating your sequence and notice that the final CVS (CCV2) has a CV of 5.67. What should you do?
19. You are working ten, one-item marijuana cases. List what your sequence will look like.
20. One of the case samples you analyze is greater than the upper limit of quantitation. What should you do?
21. Describe the appropriate means of reading a meniscus for volumetric glassware.

22. How should glassware be evaluated prior to preparing solutions? What conditions would result in a flask not being used for the preparation of a solution?
23. For each step in the quantitation process, identify elements which could contribute to measurement uncertainty.

14.6 PRACTICAL EXERCISES

1. Extract a sample of leaf marijuana with methanol, chloroform and petroleum ether. Run each extract on the MS. Identify as many components as possible. Which solvent works best? Repeat this exercise derivatizing with MSTFA or BSTFA.
2. Calibration curve preparation and familiarization with quantitation in ChemStation
    a. Prepare 50 ml of Internal Standard Solution as described in the Technical procedures. Be sure to verify this solution on GC/MS.
    b. Prepare 20 ml of a 1 mg/ml caffeine solution in methanol. Be sure to verify this solution on GC/MS.
    c. Prepare calibration level standard solutions as described in the Technical Procedures using the caffeine solution instead of THC.
    d. Run a sequence and inject each calibration level in triplicate.
    e. Populate a calibration curve in ChemStation.
    f. Evaluate the triplicate injections – calculate the average for each level, standard deviation and coefficient of variance.
    g. Repeat steps “c” through “f”.
    h. Review the results with your trainer before attempting the next practical exercise.
3. Extract a sample of tea leaves or coffee grounds using methanol. Analyze the sample to determine the caffeine content.
4. THC calibration curves
    a. Prepare a set of THC calibrators.
    b. Prepare RVS.
    c. Prepare CVS.
    d. Prepare TCS.
    e. Run a sequence consisting of the THC calibrators, RVS, TCS and CVS. Run the TCS and CVS in triplicate
    f. Evaluate the curve, RVS and the triplicate injections of the TCS and CVS.

    ***Be sure to use chemistry form 5032 – THC Quant Stock Solution Prep and form 5034 – Calibration Curve Worksheet.
5. Analyze marijuana reference materials available in your lab as if they were an actual case sample. Use chemistry form 5031 – FID Quant Worksheet.
6. Your trainer will provide you with a sample which will be greater than the upper limit of quantitation. Analyze this sample. Prepare a dilution and reanalyze.
7. Obtain the unknown sample from your trainer and analyze the sample as if it were an actual case sample. Compare your quant results with other trainees and/or trained analysts.

15 OPIOIDS

15.1 OBJECTIVES

- To become familiar with the methods and procedures used to identify opioids and their benefits and limitations
- To demonstrate analytical protocols on “casework” type samples.

15.2 TOPIC AREAS

1. Opiate versus opioid
    a. Opiate describes alkaloids directly derived from opium. These are naturally occurring compounds.
    b. Opioid describes alkaloids that are opiates, semi-synthetic opiates or fully synthetic. This is a more generic term which may refer to naturally occurring compounds (morphine), semi-synthetic compounds (heroin), or fully synthetic compounds (tramadol).
2. Opium and its alkaloids (Opiates)
    a. Derived from Papaver somniferum L.
    b. A naturally occurring product
    c. Schedule II
    d. Most of the world production is grown in the following areas of the world:
        i. Golden Triangle
            1. Burma
            2. Laos
            3. Thailand
        ii. Golden Crescent
            1. Afghanistan
                a. Largest producer of opium
            2. Pakistan
            3. Iran
        iii. Mexico
        iv. Columbia
    e. Production methods
        i. Labor intensive slicing of the pod and scraping of the opium gum or latex
        ii. Pod may be sliced on multiple occasions
    f. Major opium alkaloids
        i. Morphine
            1. Most prevalent opium alkaloid constituting 10-16% of the total mass.
            2. Generally encountered in tablets or other pharmaceutical preparations though may be seen as an impurity in some heroin samples.
            3. Schedule II
        ii. Codeine
            1. Can range from 1-3% of the total mass.
            2. Generally encountered in tablets and is often in combination with acetaminophen.
            3. Schedule II, III or V depending on preparation
        iii. Thebaine
            1. The most poisonous opium alkaloid
            2. Schedule II
        iv. Papaverine
            1. Not controlled
        v. Noscapine
            1. Has no analgesic properties

2. Not controlled

g. Tincture of opium
h. A contemporary form of laudanum
i. Deordorized or “denarcotized” prepared by removing the noscapine
j. Used in the treatment of severe diarrhea that does not respond to other forms of therapy

3. Semi-synthetic opioids
   a. Heroin
      i. Derived from morphine
      ii. Schedule I
      iii. Synthesis
         1. Morphine extracted from opium
         2. Morphine is reacted with acetic anhydride, sodium chloride and hydrochloric acid.
         3. Generally a 1:1 conversion of morphine to heroin
      iv. Appearance
         1. Dependent on the manufacturing process and cutting agents
      v. Most commonly encountered forms
         1. Bulk:
            a. China White – seen often in the Eastern and Central United States
            b. Black tar – seen most commonly in the Western United States
         2. Residues:
            a. Spoons
            b. Syringes
            c. Foil
            d. Filters such as cotton balls.
      vi. Considered a “prodrug” and is not active in the body. It deacetylates to monoacetylmorphine and then to morphine in vivo. Heroin is delivered to the brain quicker than an equivalent dose of morphine, hence the reason for abuse.
   b. Monoacetylmorphine (MAM)
      i. Exists as two isomers. 6-MAM tends to exist in higher concentration than 3-MAM.
      ii. May be present in black tar heroin samples due to incomplete acetylation of morphine or due to a degradation of heroin by chemical and/or enzymatic hydrolysis.
      iii. Not specifically listed in the RCW but can be considered a Schedule II substance.
   c. Acetylcodeine
      i. Formed from codeine during the acetylation of opium.
      ii. Not specifically listed in the RCW but can be considered a Schedule II substance.
   d. Buprenorphine
      i. Used as an analgesic and in anti-addiction therapy for other opioids
      ii. Generally encountered in tablets in combination with naloxone but may be seen in transdermal patches and sublingual films
      iii. Naloxone is an opioid antagonist which deters the IV use of buprenorphine
      iv. Derived from thebaine
      v. Schedule III
   e. Dihydrocodeine
      i. Used as an analgesic or cough suppressant
      ii. Generally encountered in tablets or other pharmaceutical preparations
      iii. Schedule II, III or V depending on preparation
   f. Hydrocodone
      i. Also known as dihydrocodeinone
      ii. Used as an analgesic or cough suppressant

iii. Generally encountered in tablets or other pharmaceutical preparations and is commonly seen in combination with acetaminophen or NSAID
iv. Schedule II or III depending on preparation in Washington. As of October 2014 all hydrocodone preparations are Federal schedule II.
v. Review the reporting requirements for hydrocodone/dihydrocodeinone preparations in the Materials Analysis Technical Procedures

g. Hydromorphone
i. Also known as dihydromorphinone
ii. Used as an analgesic or cough suppressant
iii. Generally encountered in tablets or other pharmaceutical preparations
iv. Schedule II

h. Oxycodone
i. Used as an analgesic
ii. Generally seen in tablets and may be seen in combination with acetaminophen or ibuprofen.
iii. OxyContin tablets sold in the US are available in 10 mg (round white), 15 mg (round grey), 20 mg (round pink), 30 mg (round brown), 40 mg (round yellow), 60 mg (round red), and 80 mg (round green). A 160 mg (oblong blue) tablet is available in Canada. Traditional "OC" tablets produced by Purdue Pharmaceuticals were replaced in 2010 by "OP" tablets which are intended to impede misuse of the extended release tablets.
iv. Schedule II

i. Oxymorphone
i. Used as an analgesic
ii. Can be produced from thebaine or morphine
iii. Generally encountered in tablets or other pharmaceutical preparations
iv. Schedule II

4. Commonly abuse synthetic opioids
a. Diphenoxylate
i. Used for the treatment of diarrhea
ii. Usually seen in tablet form in combination with atropine.
iii. Schedule V when combined with atropine

b. Dextropropoxyphene
i. Optical isomer (levopropoxyphene) is not scheduled
ii. Used as an analgesic and cough suppressant
iii. Schedule II or IV depending on preparation
iv. Generally encountered in tablets or other pharmaceutical preparations and is commonly seen in combination with acetaminophen
v. May be seen as the hydrochloride or napsylate salt

c. Fentanyl
i. Highly potent analgesic in which dosages are often measured in micrograms.
ii. Approximately 80 times more potent than morphine or 40 times more potent than oxycodone
iii. May be encountered as injectible liquids, time release patches, oral lozenges (lollipops), oral sprays or inhalers.
iv. Schedule II
v. Analogs of fentanyl include:
1. Alfentanil
2. Sufentanil
3. Remifentanil
4. Carfentanil
a. 10,000 times more potent than morphine
b. Used in veterinary medicine to immobilize large animals such as elephants
5. Numerous clandestinely produced fentanyl analogs are Schedule I substances:

a. Acetyl-alpha-methylfentanyl (N-[1-(1-methyl-2-phenethyl)-4-piperidinyl]-N-phenylacetamide)
b. Alpha-methylfentanyl (N-[1-(alpha-methyl-beta-phenyl) ethyl-4-piperidyl] propionanilide)
c. Alpha-methylthiofentanyl (N-[1-methyl-2-(2-thienyl)ethyl-4-piperidinyl]-N-phenylpropanamide)
d. Beta-hydroxyfentanyl (N-[1-(2-hydroxy-2-phenethyl)-4-piperidinyl]-N-phenylpropanamide)
e. Beta-hydroxy-3-methylfentanyl (N-[1-(2-hydrox-2-phenethyl)-3-methyl-4-piperidinyl]-N-phenylpropanamide)
f. 3-Methylfentanyl (N-[3-methyl-1-(2-phenylethyl)-4-piperidyl]-N-phenylprop anamide)
g. 3-Methylthiofentanyl (N-[(3-methyl-1-(2-thienyl)ethyl-4-piperidinyl]-N-phenylpropanamide)
h. Para-fluorofentanyl (N-(4-fluorophenyl)-N-[1-(2-phenethyl)-4-piperidinyl] propanamide)
i. Thiofentanyl (N-phenyl-N-[1-(2-thienyl)ethyl-4-piperidinyl]-propanaminde)

d. Levorphanol
    i. Used as an analgesic
    ii. Generally seen as tablets but may also be seen as an injectable
    iii. Schedule II
e. Methadone
    i. Used as an analgesic, cough suppressant, and in anti-addiction therapy for other opioids.
    ii. Exists as optical isomers but only the racemic form is available in the US.
    iii. Generally encountered in tablets.
    iv. Schedule II
f. Pentazocine
    i. Opioid agonist-antagonist due to the two enantiomers
    ii. Used as an analgesic
    iii. Most commonly seen as an injectable but may also be seen in tablets
    iv. Schedule IV
g. Pethidine (Meperidine)
    i. Used as an analgesic
    ii. Generally seen as tablets but may also be seen as a syrup or injectable
    iii. Schedule II
    iv. Derivatives
        1. Hydroxypethidine – Schedule I
        2. Pethidine—Intermediate-A, 4-cyano-1-methyl-4-phenylpiperidine – Schedule II
        3. Pethidine—Intermediate-B, ethyl-4-phenylpiperidine-4-carboxylate – Schedule II
        4. Pethidine—Intermediate-C, 1-methyl-4-phenylpiperidine-4-carboxylic acid – Schedule II
h. Tramadol
    i. Used as an analgesic
    ii. Chemically unrelated to opioids but appears to have action on opioid receptors
    iii. Generally seen as the hydrochloride salt form in tablets or capsules but may be seen as an injectable preparation or suppositories
    iv. Federally scheduled as of 08/18/14. In Washington tramadol is not a controlled substance but is a legend drug.

5. Opioid antagonists
    a. Most bind to opioid receptors more readily than opioids but do not activate the receptor. This blocks the receptors and prevents the body from responding to opioids. They are routinely used in the treatment of opioid addiction or overdose.

i. Nalbuphine
ii. Nalorphine
iii. Naloxone
iv. Naltrexone

6. Analysis
    a. General considerations
        i. Illicit preparations are rarely pure substances.
        ii. Many pharmaceutical preparations are mixed with other active ingredients such as acetaminophen, NSAIDs, expectorants, or decongestants. Generally the opioid is the active ingredient in the lowest concentration.
        iii. Time release, continuous release, extended release, or sustained release preparations are capsules or tablets formulated to dissolved slowly and release the active ingredient over time. The active ingredients are layered, compartmentalized, or imbedded in a matrix of insoluble material.
        iv. Polymorphism
            1. Some materials can exist in more than one crystal structure.
            2. These substances will often give different IR spectra
            3. Heroin and Oxycodone are known to be polymorphic
        v. Heroin may contain an excess of 6-MAM, 3-MAM, or acetylcodeine which can cause difficulties in confirmation with IR. This is particularly common with heroin in solution such as exhibits recovered from syringes or syringe rinses. Other naturally occurring opiates such as morphine and noscapine may interfere with the identification of heroin when utilizing IR. Using the appropriate extraction method can improve the likelihood of isolating and identifying heroin with IR.
        vi. The solubility of the compound of interest should be considered in the selection of solvent for GC. The presence of two free hydroxyl groups decreases morphine's solubility in non-polar solvents such as pentane. Morphine and most of its salts are soluble in alcohol, while morphine base is soluble in methylene chloride.
        vii. Codeine and morphine do not recrystallize well which can make isolation for IR challenging.
        viii. Tablet and capsule binders and excipients can interfere with analysis especially when an alcohol extraction is used for GC. Base extraction into methylene chloride will remove some of these materials. Some of the colored dyes used in tablets and capsules can be difficult to remove through the extraction process. Filtering the sample through a plugged pipette layered with activated charcoal may remove dyes.
        ix. A mixture of methylene chloride and ethanol is an effective solvent for extracting opium to identifying the naturally occurring opiates by GC/MS.
    b. Color tests
        i. The sulfuric acid series is particularly helpful in screening many opioids
    c. Microcrystal tests – generally not specific and are not routinely used.
    d. TLC
        i. Systems TA, TB, or TC are useful for most opioids
        ii. Develop with acidified iodoplatinate or other reagent as appropriate
    e. CE
        i. Compared to other constituents of black tar, heroin often will give a weak peak.
        ii. The technique of choice for determining which optical isomer is present in a sample.
    f. GC
        i. 3-MAM and acetylcodeine co-elute on HP-5 equivalent GC columns
        ii. Buprenorphine and noscapine are late eluters and the appropriate method should be selected to ensure detection of these compounds.
    g. MS
        i. The technique of choice for the confirmation of many opioids.
    h. IR

i. Polymorphism of heroin and oxycodone should be considered when utilizing this technique. Appropriate standards are necessary for identification of heroin and oxycodone when polymorphism is an issue.
ii. Methadone base is an oil at room temperature. For IR sampling, the HCl salt can be formed to give a white solid or the oil can be deposited directly on the accessory.
iii. Isolated fentanyl will be an oil and can be deposited directly on the IR accessory for analysis.

i. Extractions
    i. Ion-Pairing
        1. Works well for many opioids.
        2. Acetylcodeine, noscapine and thebaine will ion-pair and extract with heroin.
        3. For heroin dissolve in ~3N HCl or in water and add concentrated HCl after dissolution to make ~3N HCl. The acidic solution is extracted with CH_2Cl_2 and the organic layer is back extracted with water. The aqueous layer is basified with $NaHCO_3$ and extracted with pentane. Do not use a strong base such as NaOH or NH_4OH as this will result in the hydrolysis of heroin to morphine. Allow the pentane to slowly evaporate without disturbance and without blowing the solvent off. Alternatively, a small amount of CH_2Cl_2 can be added to the pentane during evaporation.
        4. Noscapine tends to extract with heroin using the ion-pairing technique. Washing the aqueous layer briefly with pentane may aid in the reduction of noscapine. When evaporating the pentane, do not blow down the sample with air. As the pentane level drops, noscapine will reveal itself in a bloom of cobwebby white material. Gather this cobwebby material on the tip of a probe and remove it from the pentane as it continues to evaporate. The heroin that results is usually form II (rosettes) when it is pure. If the rosettes are not formed or if they are buried in cobwebby crystals, a series of quick pentane rinses, slow evaporations, and gathering/removal of noscapine cobwebs can remove the noscapine from the heroin. This may take several rounds of this process if enough material is available.
    ii. ANOR (Alternate Non-aqueous Organic Ratio) extraction procedure
        1. A dry extraction technique which utilizes solvents such as chloroform, petroleum ether or hexane that have been made acidic or basic to obtain the free acid, ion-pair or free base form a drug.
        2. The standard base into CH_2Cl_2 extraction works well for many opioids in tablet form and may also be effective for some liquid preparations.
        3. Pharmaceutical preparations containing an opiate with excess acetaminophen may require the first step of extracting with 0.1N HCl into CH_2Cl_2 to remove the acetaminophen. The resulting aqueous solution can be made basic with the addition of NH_4OH and then extracted with pentane. Acetaminophen is less soluble in pentane than in CH_2Cl_2.
        4. The amine and phenol functional groups in morphine make it amphoteric. Aqueous solutions of morphine require a narrow pH range of approximately 9.5 for extraction. Saturated borate solution or concentrated sodium $NaHCO_3$ work well.
        5. Morphine or hydromorphone can be isolated from tablets using a 3% trimethyamine solution in methanol. The tablet should be dissolved and filtered for GC analysis.

j. Derivatization
    i. A trimethylsilyl (TMS) group derivatization agents such as BSA and BSTFA are effect for many opioids.

k. Reporting

i. Opium should not be reported as opium. Examples of phrases that could be used for suspected opium include:
    1. a mixture of compounds that occur in opium
    2. morphine and other compounds found in opium.
    3. a mixture of compounds consistent with opium.
ii. Compounds in which the optical isomer determines whether the substance is controlled or not should be carefully reported to indicate what was identified and if the optical isomer was specifically identified.
iii. Bulk hydrocodone is a schedule II substance. Hydrocodone is generally mixed with a non-narcotic in tablets and can be reported as hydrocodone or dihydrocodeinone. Refer to the Pharmaceutical Identification section of the technical procedures for specific reporting criteria.

15.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

15.4 SUGGESTED READING

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12. Saloom JM. 1983. Use of the ANOR Method for Separation of Propoxyphene from Propoyxphene & APC. Microgram. 16(1).
13. The Merck Index. Rahway(NJ):Merck and Co., Inc.
14. Microgram, Vol.18, No. 8, August 1985 (describes 9:1 diethylether: diethylamine TLC system for separating heroin and acetylcodeine).
15. DEA website (www.dea.gov).

15.5 STUDY QUESTIONS

1. What is a narcotic? Is there a difference between the legal and scientific definition of a narcotic?
2. Which compounds ion pair?
3. Which opioids will derivatize with a TMS derivatizing compound?
4. What is an opiate? What is an opioid?
5. Define synthetic and semi-synthetic opiates. Give examples of each.
6. What are the polymorphs of heroin base? How can these affect analysis and data interpretation?
7. Discuss reporting hydrocodone versus dihydrocodeinone (see RCW: Scheduled Drugs)
8. How can hydration of codeine or oxycodone affect analysis and data interpretation?
9. Discuss the manufacture of heroin.

10. Why are black tar heroin and China White heroin different?
11. Review opiates in the UCS (RCW 69.50). Why are some opiates listed in more than one schedule?

15.6 PRACTICAL EXERCISES

1. Hydrolyze heroin to mono-acetylmorphine (which isomer?)
2. Acetylate codeine, purify, and obtain an infrared spectrum. Analyze by GC/MS.
3. Acetylate morphine and analyze by GC/MS.
4. Use a TMS derivatizing compound to derivatize several opioids for analysis by GC/MS.
5. Obtain hydrocodone/acetaminophen tablets. Isolate each of the active ingredients and analyze with sulfuric acid color tests and on IR and GC/MS.
6. Analyze a sample of violin rosin.
7. Extract black tar heroin and obtain an IR of heroin.
8. Analyze heroin, morphine, codeine, and thebaine with TLC. Compare the Rfs and the color of the spots.
9. Analyze a morphine sulfate tablet. Which extraction technique is most effective? Is one functionality column better than the other for this compound?
10. Analyze a hydrocodone or codeine syrup, if available.

16 PHARMACEUTICALS & LEGEND DRUGS

16.1 OBJECTIVES

- To become familiar with various types of pharmaceutical preparations that may be submitted to the laboratory.
- To understand how the various preparations are controlled by the Uniform Controlled Substances Act or by prescription for legend drugs
- To become familiar with and understand the limitations of the various reference sources that can be used to assist in the identification of pharmaceuticals.
- To become familiar with some common trade names of pharmaceuticals and their active ingredients, e.g. Valium - Diazepam.

16.2 TOPIC AREAS

1. Legal Control
    a. Controlled by Uniform Controlled Substance Act
        i. Many substances fall into more than one schedule depending on dosage form or concentration.
    b. Controlled by Prescription (Legend Drug)
2. General Drug Categories
   The classes of compounds specifically listed below are those most commonly abused and therefore seen in casework. The Other category lists other classes of drugs that may be seen in casework but are not as commonly abused.
    a. Analgesics
        i. Narcotic
        ii. Non-Narcotic
    b. Antipsychotics
    c. Sedatives
        i. Barbiturates
        ii. Benzodiazepines
        iii. Nonbenzodiazepine sedatives
        iv. Uncategorized sedatives
    d. Stimulants
    e. Others
        i. Antacids
        ii. Antiarrhythmics
        iii. Antibacterials
        iv. Antibiotics
        v. Anticoagulants and thrombolytics
        vi. Anticonvulsants
        vii. Antidepressants
        viii. Antidiarrheals
        ix. Antiemetics
        x. Antifungals
        xi. Antihistamines
        xii. Antihypertensives
        xiii. Anti-inflammatories
        xiv. Antineoplastics
        xv. Antipyretics
        xvi. Antivirals
        xvii. Beta-Blockers
        xviii. Bronchodilators
        xix. Corticosteroids
        xx. Cytotoxics

xxi. Decongestants
xxii. Diuretics
xxiii. Expectorants
xxiv. Hormones
xxv. Hypoglycemics
xxvi. Immunosuppressives
xxvii. Laxatives
xxviii. Vitamins

3. Analysis
    a. Initial examination
        i. Physical condition
        ii. Appearance
        iii. Weight/volume/count
        iv. Logo or factory labeling
    b. Imprint Identification
        i. Use of reference materials
        ii. Documentation requirements
        iii. Tablets/capsules from other countries
        iv. Counterfeit tablets and possible adulteration of capsule contents
    c. Sampling
        i. Homogeneity
        ii. Note: Some extended release preparations may be layered or compartmentalized in the tablet. This needs to be considered when preparing the sample for analysis.
        iii. Representative sampling
        iv. Amount available and amount to be used for analysis
    d. Isolation from pharmaceutical preparation
        i. Extraction (Many pharmaceuticals are acidic or neutral.)
        ii. Liquid/solid
            1. alternate non-aqueous organic ratio procedure (ANOR)
        iii. Liquid/liquid
        iv. Other
            1. Note: Many barbiturates and some benzodiazepines are polymorphic, exhibiting two or more crystal forms. An extract often results in a glass rather than a crystalline residue.
    e. Particle picking
            1. May be useful for some capsule powders
    f. Tablet binder/filtration
    g. Screening tests
        i. Tablet, capsule markings or factory labeling
        ii. Color tests
            1. Barbiturates:
                a. Dille-Koppanyi
                b. Zwikkers
                c. formation of a colored barbiturate – $CuSO_4$-pyridine complex
            2. As a whole, the benzodiazepines do not give definitive results with the commonly employed color tests that we use in our laboratory system. Most will give a positive Mayers, and a few that contain a third nitrogen (as an amine, such as chlordiazepoxide and flurazepam) may give a positive cobalt thiocyanate and/or Ruybals; some will produce a positive test with dinitrobenzene (the Zimmerman test).
    h. Identification methods
        i. TLC
            1. Consider acid/base property of the compound of interest when selecting a development system.

2. Acidified iodoplatinate is an effective developer for many neutral or basic nitrogenous drugs. Acidified potassium permanganate works well for many acidic drugs.

ii. CE
1. Particularly useful for isomer separation

iii. IR
1. Binders/fillers/excipients
2. Low concentration of compound of interest especially in some benzodiazepines
3. Polymorphism

iv. GC/MS
1. Isomers (3-methylfentanyl vs. alpha-methylfentanyl)
2. High molecular weights
   a. Long retention times
   b. MS Scan range
3. Compound of interest may break down in the injection port
   a. Carisoprodol →Meprobamate
   b. Methocarbamol→guaifenesin
   c. Clorazepate→N-desmethyldiazepam

i. Reporting
i. Qualified report when using only pharmaceutical identification
ii. Report only on what was analyzed
iii. The report should be clear if the isomer was determined or not.

16.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

16.4 SUGGESTED READING

1. Analytical Profiles of Barbiturates and Other Depressants. CND Analytical, Inc. 1991.
2. Analytical Profiles of the Benzodiazepines. CND Analytical, Inc. 1989.
3. Analytical Profiles of the Narcotic Analgesics. CND Analytical, Inc. 1991Cole M. 2003.The Analysis of Controlled Substances: A Systematic Approach. Wiley.
4. Drug Identification Bible. Amera-Chem, Inc.
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14. Physician's Desk Reference. Medical Economics Company, Inc.
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16.5 STUDY QUESTIONS

1. What is the difference between an anxiolytic, sedative, and tranquilizer?
2. Give three examples of narcotic analgesics and three examples of non-narcotic analgesics. Indicate which are controlled, which are legend drugs, and which are available over-the-counter.
3. Discuss the pharmaceutical forms of fentanyl. What are fentanyl analogs? What are the safety concerns regarding fentanyl and analogs?
4. Which of the stereoisomers of propoxyphene is controlled? How would you analyze propoxyphene? How would you report propoxyphene?
5. What is the barbital structure? How are barbiturates derived from this basic structure?
6. Discuss analytical challenges in the analysis of barbiturates.
7. Discuss analytical challenges in the analysis of antibiotics.
8. Why is a logo identification more definitive for tablets than for capsules?
9. The term “pharmaceuticals” is rather broad. Discuss the forms of “pharmaceuticals” that could be seen in the lab and give an example of each type.
10. For each of the following determine:
    a. Trade and/or generic name
    b. Principle usage
    c. Drug class
    d. Control status
        i. Oxycodone
        ii. Hydrocodone
        iii. Codeine
        iv. Diazepam
        v. Alprazolam
        vi. Flunitrazepam
        vii. Zolpidem
        viii. Tramadol
        ix. Carisoprodol
        x. Pethidine/Meperidine
        xi. Sildenafil Citrate
        xii. Tadalafil
        xiii. Naproxen sodium
        xiv. Methylphenidate
        xv. Quetiapine Fumarate
        xvi. Eszopiclone (Zopiclone)
        xvii. Sertraline hydrochloride
        xviii. Cyclobenzaprine Hydrochloride
        xix. Gabapentin
        xx. Hydroxyzine
        xxi. Atorvastatin
        xxii. Adderall
        xxiii. Dilaudid
        xxiv. Cipro
        xxv. Modafinil
        xxvi. Bupropion
        xxvii. Haldol
        xxviii. Bactrim
        xxix. Tridil
        xxx. Aspirin

16.6 PRACTICAL EXERCISES

1. Obtain fifteen to twenty pharmaceutical samples from the trainer. Record packaging information, inventory and describe imprint of each sample. Obtain a logo identification from two sources for each sample. Using the PDR, describe if the substance is controlled and the pharmaceutical indication(s). Determine how each substance would be isolated and instrumentally analyzed.
2. Extract lorazepam tablets with chloroform or methylene chloride. Dry down and obtain an infrared spectrum. Repeat, this time with trituration using a little pentane. This may prompt crystallization. Compare the infrared spectra of the glass and the crystalline forms.
3. Crush a tablet and try to obtain an IR of the compound of interest. Use an appropriate extraction technique to isolate the compound of interest and obtain an IR.
4. Analyze a methocarbamol tablet on IR and GC/MS. What analytical challenges are encountered?
5. Obtain an extended release tablet and isolate the active ingredient, if available.

17 PHENETHYLAMINES

17.1 OBJECTIVES

- To become familiar with the chemical structures of commonly encountered phenethylamine class drugs, their general properties and chemical characteristics.
- To become familiar with the methods and procedures used to identify phenethylamines and their strengths and limitations
- To demonstrate analytical protocols on “casework” type samples.

17.2 TOPIC AREAS

1. Substituted phenethylamines are a large class of compounds that include the following drug categories:
    a. Neurotransmitters
    b. Hormones
    c. Stimulants
    d. Entactogens
    e. Anoretics
    f. Bronchodialators
    g. Antidepressants
2. Compounds commonly and historically seen in casework:
    a. Amphetamine
    b. Methamphetamine
    c. N,N-dimethylamphetamine
    d. N-ethylamphetamine
    e. Ephedrine/Pseudoephedrine
    f. Phenylpropanolamine (norephedrine)
    g. Cathine (norpseudoephedrine)
    h. Cathinone
    i. Methcathinone
    j. Phentermine
    k. Chlortermine/chlorphentermine
    l. Phenmetrazine
    m. Phendimetrazine
    n. Benzphetamine
    o. Fenfluramine
    p. Methylphenidate
    q. Diethylpropion
    r. Phenethylamine
    s. Phenylephrine
    t. Propylhexadrine
    u. Fenproporex
    v. Fenethylline
    w. Encamfamin
    x. Selected Synthesis Precursors
        i. Phenylpropanolamine (PPA)
        ii. Phenyl-2-propanone (P2P)
    y. Diluents and Excipients
        i. Dimethyl Sulfone
        ii. Caffeine
        iii. Benzylamines
        iv. N-isopropylbenzylamine
        v. N-Methylbenzylamine
        vi. N-Ethylbenzylamine

3. History of Amphetamine/Methamphetamine Use/Abuse
    a. Ephedra bush steeped as a tea and used as a stimulant in America in mid-19th century.
    b. Ephedrine isolated from Ephedra in 1892.
    c. Eli Lilly Company developed production of ephedrine in the 1920s.
    d. Amphetamine synthesized in Germany in 1887 but received little attention until 1927.
    e. Pure d-amphetamine was synthesized in the 1930s for medical use. Shortly after methamphetamine was produced.
    f. Amphetamine marketed as an OTC inhaler, Benzedrine, in 1932.
    g. Amphetamine prescriptions available for narcolepsy in 1937.
    h. Amphetamine pills were handed out along with food and cigarette rations to Allied, German and Japanese forces during World War II.
    i. Leaders including Churchill and Hitler used stimulants.
    j. FDA banned OTC sales of amphetamine in 1959.
    k. Federal food and drug laws were changed in 1965 to remove many amphetamine products from the market which resulted in clandestine manufacturing, especially of methamphetamine on the West Coast.
4. Methods of Abuse
    a. Smoking
    b. Injection
    c. Inhalation
    d. Ingestion
5. Legal Definitions and scheduling
    a. Many stimulants listed as schedules I-V controlled substances
        i. Amphetamine and methamphetamine along with the salts, isomers, and salts of the isomers are equally controlled.
    b. Phenethylamines may be legend drugs
    c. Numerous OTC phenethylamines
6. Commonly Encountered Forms/Visual Identification
    a. Licit preparations are primarily tablets but historically were available in injectable forms.
    b. Illicit preparations
        i. Manufacturing route can affect appearance
        ii. Powders
        iii. Chunky material
        iv. “Ice”
        v. Tablets/Capsules
        vi. Residues in snorting or smoking devices
        vii. Plant material for khat
7. Analysis
    a. General Considerations
        i. Illicit preparations are often not pure.
        ii. Time release, continuous release, extended release, or sustained release preparations are capsules or tablets formulated to dissolved slowly and release the active ingredient over time. The active ingredients are layered, compartmentalized, or imbedded in a matrix of insoluble material.
        iii. Hydrochloride salts often make analysis by GC challenging. Converting the salt to the base form will generally improve chromatography and eliminate the “HCl saddle”.
    b. Color tests
        i. Marquis reagent is a useful preliminary screening test for many phenethylamines.
        ii. The sodium nitroprusside test (sometimes referred to as the Simon-Awe test) is useful to distinguish between amphetamine-based homologs.
        iii. The Chen-Kao test is specifically for ephedrine/pseudoephedrine.
    c. Microscopic and Microcrystalline Methods
        i. Microcrystalline Techniques – refer to the Microcrystalline Testing Training Section
        ii. Stereoisomer determinations

d. Thin Layer Chromatography
    i. 4:1 Chloroform:methanol works well for most phenethylamines
    ii. Develop with acidified iodoplatinate or other reagent as appropriate
e. Extraction Methods
    i. Particle picking
    ii. Aqueous base, extract to organic solvent.
    iii. Water or dilute aqueous acid, basify, extract to organic solvent.
    iv. Dilute aqueous acid, organic wash, basify, extract to organic solvent.
    v. Dry organic extraction (e.g., acetone will effectively separate methamphetamine from MSM).
    vi. Ion pairing (e.g., concentrated acid, extract to organic solvent).
    vii. ANOR (Alternate Non-Aqueous Organic Ratio) extractions.
f. Spectroscopic Identification
    i. FTIR/Raman
    ii. GC/MSD
        1. Spectra Considerations
            a. Distinguishing ephedrine from pseudoephedrine
            b. Methamphetamine Combined with Reduced Methamphetamine from synthesis
        2. Derivatives
g. Enantiomeric Determinations
    i. Chiral CE and LC
    ii. Polarimetry
    iii. Chiral derivatizations for GC/MS

8. Khat (Catha edulis)
    a. A shrub from North Africa
    b. Leaves and tender stems from the tops of the plant are harvested and often wrapped in banana leaves and sprinkled with water to preserve potency
    c. Fresh leaves and tender stems are chewed or steeped as a tea which releases cathinone. As the leaves mature or dry, cathinone is converted to cathine.
    d. GRABA is dried preparation of khat with a similar appearance to marijuana. This dried form of khat is said to preserve the cathinone content.
    e. Legal
        i. Cathinone is schedule I
        ii. Cathine is schedule IV
    f. Analysis
        i. Extractions
            1. Acid/base extraction of plant material:
                a. Harvest leaf material and cut or otherwise divide into small pieces.
                b. Cover plant cuttings with 0.1N HCl and soak or (preferably) sonicate for about 15-30 minutes.
                c. Decant or filter to remove extracted plant material from the acidic fraction.
                d. Wash the acid extract (red in color) with chloroform or dichloromethane until washes are clear and emulsions are reduced (~4 washes).
                e. Basify the aqueous layer with sodium bicarbonate or NaOH – the solution will often turn greenish-brown when basified.
                f. Extract the aqueous layer immediately with dichloromethane.
                g. The dichloromethane fraction should be suitable for GC/MS analysis.
            2. MeOH extraction followed by acid/base extraction:
                a. Harvest leaf material and cut or otherwise divide into small pieces.

b. Cover plant cuttings with MeOH and soak or (preferably) sonicate for about 15-30 minutes.
c. Decant or filter to remove extracted plant material from the extract (the extract will be green).
d. Evaporate as much MeOH as is practical under a stream of gas without using heat (heat may degrade compounds of interest)
e. Add 0.2N H_2SO_4 or 0.1N HCl to the extract (the solution will be red or red-brown)
f. Wash the acid solution with chloroform or dichloromethane until washes are clear and emulsions are reduced (~4 washes).
g. Basify the aqueous layer with sodium bicarbonate or NaOH – the solution will often turn greenish-brown when basified.
h. Extract the aqueous layer immediately with dichloromethane.
i. The dichloromethane fraction should be suitable for GC/MS analysis.

ii. GC/MS
1. direct
2. achiral derivative (e.g. acetic anhydride or TMS) for cathinone
3. chiral deriviative (to differentiate (+)-norpseudoephedrine (cathine) from (-)-norephedrine)

iii. Capillary Electrophoresis (CE)
1. Chiral analysis method to differentiate(+)-norpseudoephedrine (cathine) and (-)-norephedrine

iv. Infrared spectroscopy
1. May provide limited information

v. TLC
1. Solvent - ethyl acetate : methanol : ammonia (85:10:5).
2. Visualization – 0.5% ninhydrin, followed by heat
a. Cathine Rf ~0.25 – purple spot
b. Cathinone Rf ~0.46 – orange spot

17.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

17.4 SUGGESTED READING

All reading should use the most current edition available, unless otherwise noted by the trainer as an historical reference.

1. Adair, A.R., et al., 1983. The ANOR (Alternate Non-Aqueous Organic Ratio) Extraction Procedure. Microgram.16(1):220-224.
2. Analytical Profiles of Amphetamines and Related Phenethylamines, CND Analytical, Inc.
3. ASTM E 1969. Current edition. Standard Guide for Microcrystal Testing in the Forensic Analysis of Methamphetamine and Amphetamine.
4. Designer Drug Reference Binder (located in each lab)
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19. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
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23. Shulgin A, Shulgin A. 1991. PIHKAL: A Chemical Love Story. Transform Press.
24. U.S. Department of Justice Drug Enforcement Administration Diversion Control Program, Drugs and Chemicals of Concern Khat, Quat, Tschat, Miraa (Cathinone, Cathine), August 2001.
25. Velapoldi RA, Wicks MS. 1974. The use of chemical spot test kits for the presumptive identification of narcotics and drugs of abuse. J For Sci.19(3):636-656.
26. Wielbo D, Tebbet IR. 1992. The Use of Microcrystal Tests in Conjunction with FTIR for the Rapid Identification of Street Drugs. J For Sci. 37(4):1134-1148.

17.5 STUDY QUESTIONS

1. List the structure (including isomer and optical activity considerations), properties, and current legal control of the above listed compounds.
2. Define the drug category terms listed above.
3. Which analytical techniques do not distinguish phentermine and methamphetamine? Which techniques will distinguish these compounds?
4. Which isomer of methamphetamine is used as a stimulant? Which is used as a vasoconstrictor?
5. What is the structural relationship of ephedrine and pseudoephedrine? How can they be distinguished from one another?
6. Why is dimethyl sulfone a common diluent/cutting agent of methamphetamine?
7. Discuss the physical appearance of methamphetamine manufactured by different routes of analysis. How is "ice" created?
8. What is CMP/150 compound? What are the challenges of separating this compound from methamphetamine on the GC?
9. What schedule are methamphetamine and amphetamine? What are the legitimate uses of each compound?
10. Pseudoephedrine in no longer readily available over the counter. Research the history behind this legislation. Which substance has replaced pseudoephedrine in many over the counter cold and allergy preparations?
11. Discuss the enzymatic conversion of cathinone to cathine and its impact on evidence handling procedures for khat submissions.

17.6 PRACTICAL EXERCISES

1. Separate a mixture of pseudoephedrine and methamphetamine and confirm instrumentally, preferably by FTIR.
2. The trainee will carry out FTIR and GC/MSD determinations of available standards listed in the outline above and complete a report concerning the differences in data obtained as compared to the structure of the molecules, especially concerning any limitations of the analytical data to provide structural information on particular compounds.
3. The trainee will create structural derivatives using available compounds and reagents for GC/MSD determination.
4. The trainee will receive or prepare a series of unknowns containing listed compounds in combination with common diluents and excipients, and will carry out separation techniques as needed to obtain FTIR spectra on the isolated phenethylamines suitable for case identification. Extraction/preparation of the mixtures will then be completed and GC/MSD analysis carried out.
5. The trainee will carry out applicable microcrystalline tests for the above substances.

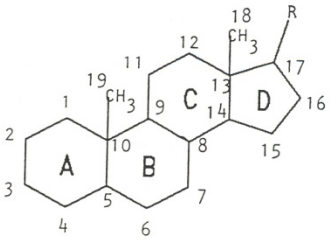
18 STEROIDS

18.1 OBJECTIVES

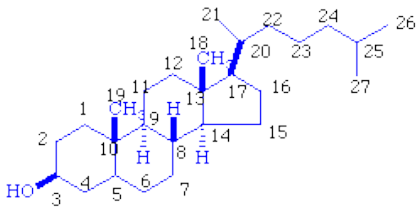
- To become familiar with the chemical structures of anabolic steroids, their general properties and chemical characteristics.
- To become familiar with the methods and procedures used to identify anabolic steroids and their benefits and limitations.
- To demonstrate analytical protocols on “casework” type samples.

18.2 TOPIC AREAS

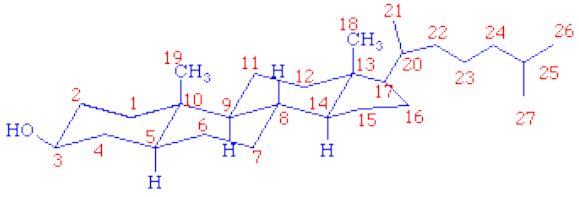
1. Legal Considerations
    a. RCW 69.50.208
    b. WAC 246-887-160
    c. According to the DEA there are over 100 different types of anabolic steroids that have been developed, and each requires a prescription to be used legally in the United States.
    d. Exceptions: “…does not include an anabolic steroid which is expressly intended for administration through implants to cattle or other nonhuman species.” Nine such commercial products are cited (F-TO, Finaplix-H, Finaplix-S, Heifer-oid [three types], Implus, Revalor-s and Synovex H).
    e. Several products containing both an anabolic steroid and a female hormone are also controlled and are specifically listed, although these probably are not likely to be abused since they are not intended to promote muscle
2. Structures and Ring Numbering System
    a. Steroids are based on a tetracyclic ring system consisting of a reduced phenanthrene ring fused with a cyclopentane ring at the end. The four rings are designed A-D as indicated below:



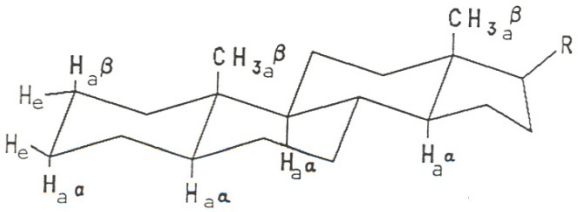
    b. The numbering system for substituents attached to the rings is:



5α-cholestan-3β-ol



c. The four rings are normally all trans-fused giving a planar configuration (see above structure). Substituents can therefore be designed as either axial or equatorial (pointing perpendicular to the plane of the rings or projecting out from the rings in the same plane, respectively). Substituents that point up from the plane (in the orientation of the molecular normally drawn) are designated as beta, while those that point down are called alpha (whether they are axial or equatorial).



3. Encountered Forms and Use
    a. Forms include tablets, injectable liquids, gels/creams and less often patches. Clandestinely-produced tablets may also be encountered, as can powders.
    b. According to the DEA website, these drugs are often used in patterns called cycling, which involves taking multiple doses of steroids over a specific period of time, stopping for a period, and starting again. Users also frequently combine several different types of steroids in a process known as stacking. By doing this, users believe that the different steroids will interact to produce an effect on muscle size that is greater than the effects of using each drug individually. Another mode of steroid use is called "pyramiding." With this method users slowly escalate steroid use (increasing the number of drugs used at one time and/or the dose and frequency of one or more steroids), reach a peak amount at mid-cycle and gradually taper the dose toward the end of the cycle.
4. Analysis
    a. Pharmaceutical Identification
        i. Pharmaceutical products with unambiguous identification markings or unopened ampoules (Analysis: One Category 1 test plus pharmaceutical identification)
            1. Tablets and capsules
            2. Injection ampoules with factory seals intact
            3. Time release patches in sealed packages
        ii. Powders, liquids in an unmarked containers, or injection ampoules that have been opened and do not have factory seals intact (Analysis: Two tests required).
        iii. One problem associated with illegal steroids is the language barrier. A number of these items are smuggled into the United States from foreign countries. The label on the bottle may be in Spanish or Portuguese, and may or may not accurately reflect the contents. Quite often, the oil submitted contains nothing at all, or a complex mixture of steroids. In such a case the pharmaceutical identification is invalid and another analytical method must be used to satisfy the two test requirement.
    b. Color tests
        i. Sulfuric Acid: Apply sulfuric acid directly to the sample on a white non-reactive tile or test tube. Observe the color produced. Many of the colors, usually yellow or orange, fluoresce under 350 nm UV light, either immediately or after dilution.
        ii. Naphthol-Sulfuric Acid: Mix 1 gram of naphthol with 40 ml of sulfuric acid, until dissolved (this may require heating and stirring in a water bath). To use, mix sample with 1 ml of reagent, and heat in a water bath at 100°C for 2 minutes, and note any color produced.
    c. TLC

i. Solvent Systems
    1. Methylene chloride: ether: methanol: water (77:15:8:1.2)
    2. Dichloroethane: methanol: water (95:5:0.2)
    3. Chloroform: acetone (8:2)
    4. Chloroform: methanol (4:1)

ii. Visualization
    1. Sulfuric acid/ethanol reagent, prepared by gradually adding 10 ml of concentrated sulfuric acid to 90 ml of ethanol, using silica gel plates. After spraying the plate, heat the plate to 105°C for 10 minutes to complete the visualization.

d. Extraction methods
    i. Recommended for oils
        1. Two reagents are prepared before the extraction procedure. Reagent A: place 500 ml of acetonitrile in a bottle. Add approx. 50 ml of hexane and shake. Reagent B: place 500 ml of hexane in a bottle. Add approx. 50 ml of acetonitrile and shake. These reagents are then used for the actual steroid extraction procedure. They are pre-saturated as a small amount of hexane will be miscible with acetonitrile. Fresh reagents that are not pre-saturated will not separate into layers when used for the steroid extraction.
        2. In a clean test tube, mix approx. 2 ml of Reagent A with 2 ml of Reagent B and mix with a vortex mixer. When the layers separate, a portion of the lower acetonitrile layer is removed for the GC/MS blank. Then about 8-10 drops of the oil from the exhibit is added to the mixture and vortex mixed. The oil will collect in the hexane layer and the steroid in the lower acetonitrile layer. The acetonitrile layer can be analyzed by GC/MS or evaporated down to be analyzed by IR.
    ii. Other extraction options
        1. The powder sample or oil is dissolved in petroleum ether and forced through an Alltech Sep-Pak (silica 900 mgs) disposable filter. Oils will wash out with the liquid phase, the steroid(s) will remain on the solid phase. Wash 3 times with petroleum ether. Sample should be oil free. Elute Sep-Pak with acetone to remove the steroid(s) from the solid phase. Discard the first few drops of acetone, and collect the rest. The acetone may be dried down to obtain IR or used directly for GC/MS.
        2. Liquid phase extraction for oils -- may be extracted directly with acetonitrile. The mixture of the oil and acetonitrile is placed in a freezer for approximately 30 minutes. The two phases will separate. The acetonitrile may be directly used for GC/MS or dried down for IR analysis.
        3. Liquid phase extraction for tablets -- tablets may be extracted directly; however, other methanol or chloroform soluble compounds may also be extracted. The methanol or chloroform extract may be directly examined using GC/MS or it may be dried down for IR analysis assuming that only one component is present.

e. GC/MS
    i. Most steroids are large molecules which will require a run long enough to ensure elution of the compound of interest. The MS parameters also need to scan to a high enough amu to accommodate large steroid esters which may have molecular weights above 450 amu.

f. IR
    i. Isolation of pure compound from solutions in oil is necessary for analysis by IR.
    ii. Products containing mixtures of steroids (usually, these are mixtures of different esters of the same steroid) can be challenging, if not impossible, to isolate for analysis by IR.

iii. Infrared analysis is usually not a problem for tablets containing a single steroid, for which a water/methylene chloride partition may serve to isolate the steroid.
iv. At least two of the listed controlled steroids, Stanozolol and Oxymetholone, can exhibit in either of two tautomeric forms and this appears to affect the infrared spectra of these steroids. For these, infrared spectra of tablet extracts may match spectra of residues of standards dissolved in methylene chloride (or whatever solvent is used to extract the tablets).

g. Derivatization of steroids
   i. Alcohols can be esterified or silated. All above listed steroids except for testolactone contain at least one free alcohol group. Five of the listed are diols: Fluoxymesterone, Formebulone, Methandriol, Oxymesterone, and Oxymetholone. Oxymetholone contains an enol hydroxyl group, so some of the keto tautomer may also be present. In theory, the diols may form three different products when esterified or silated, although more likely, only one or two may result. Results with some of the steroids indicate that they do not usually readily acetylate using acetic anhydride added to the GC vial. The best results are usually obtained using various silating agents. More than likely, you will have to prepare a silated steroid standard before you do your case since we do not have a lot of reference data on hand.
   ii. Although none of the steroids has an amine group, Stanozolol has an aromatic N-H group that might silate.
   iii. Esters can be hydrolyzed. Saponification reactions have been used to transform the ester into the parent compound to obtain a second “leg” for identification:
      1. Add 5 pellets of KOH to 2 ml of a methanol extract of a sample
      2. Allow 15 minutes at room temperature for hydrolysis
      3. Dry down the hydrolyzed methanol extract
      4. Dissolve the residue in diethyl ether
      5. Wash the ether solution with water, and dry with anhydrous sodium sulfate
      6. Evaporate to dryness
      7. Dissolve residue in methanol and evaluate by GC/MSD
   iv. Hydrolysis Procedure for Trenbolone Acetate in an Oil:
      1. Extract steroid from oil using “Extraction Methods—Recommended for Oils” listed above
      2. Evaporate acetonitrile in spotwell (white ceramic helps see the yellowish oil)
      3. Dissolve one pellet of KOH in 2-3 mL methanol in a test tube
      4. Heat KOH/MeOH until near boiling in water bath
      5. Add KOH/MeOH to spotwell
      6. Transfer to test tube and allow to cool
      7. Add DI water until volume doubled*
      8. Extract with methylene chloride for GC/MS
      9. Adding water addresses the concern that the methylene chloride would be miscible with the methanol which has been saturated with potassium hydroxide. An aqueous phase protects the MeCl from the KOH.

18.3 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any controlled substance. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence. Refer to the technical procedures manual for specific safety information relating to each analytical technique.

18.4 SUGGESTED READING

1. Analytical Profiles of Anabolic Steroids, Vol. 1-2, CND Analytical, Auburn, Alabama.
2. Chiong D M, Consuegra-Rodriguez E, Almirall JR. 1992. The Analysis and Identification of Steroids. J For Sci. 37(2):488-502.
3. Keller RJ, ed. 1986. Sigma Library of FTIR Spectra, Vol. 1-2. St. Louis(MO):Sigma Chemical Company.
4. Mills III T, Roberson J C. Instrumental Data for Drug Analysis, 3rd ed. Volumes 1- 6.
5. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
6. Pharmacy Law book, published by the Washington State Department of Health Board of Pharmacy (Most recent version).
7. Physicians Desk Reference, Medical Economics Co., Montvale, New Jersey.
8. Walters MJ, Ayers RJ, Brown DJ. 1990. Analysis of Illegally Distributed Anabolic Steroid Products by Liquid Chromatography with Identity Confirmation by Mass Spectrometry or Infrared Spectrophotometry. J AOAC. 73(6):904-926.

18.5 STUDY QUESTIONS

1. Several of the steroids specifically mentioned in the RCW are synonyms. Which steroids are listed "twice"?
2. List the structure of the above mentioned steroids and any associated esters.
3. What is the difference between an anabolic steroid and a corticosteroid?
4. What functional groups are common to most anabolic steroids?
5. You receive a vial of yellow liquid that is not factory sealed and is labeled as containing a mixture of testosterone esters. How will you proceed with your analysis?

18.6 PRACTICAL EXERCISES

1. Obtain a tablet containing a known anabolic steroid. Extract and confirm the steroid.
2. Perform the acetonitrile/hexane extraction and the acetonitrile/freezer extraction on several samples of steroids in oil and identify the contents using GC/MS and FTIR. Be sure to compare the label information to the actual contents.
3. Hydrolyze the esters of a steroid in oil and analyze by GC/MS and FTIR.

CONTROLLED SUBSTANCES TRAINING CHECKLIST – MODULE 1

Trainee:		Trainer:		
		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Chemistry Principles				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Chemical Screening				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Microcrystalline Testing				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Pipettes				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
References & Resources				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Measurement Uncertainty				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Written/Oral Exam				

CONTROLLED SUBSTANCES TRAINING CHECKLIST – MODULE 2 – PAGE 1

Trainee:		Trainer:		
		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Cocaine				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Designer Drugs				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
GHB/GBL/1,4-Butanediol & Analogs				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Hallucinogens				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Marijuana- Qualitative Analysis				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Opioids				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Pharmaceuticals & Legend Drugs				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			

CONTROLLED SUBSTANCES TRAINING CHECKLIST – MODULE 2 – PAGE 2

Trainee:		Trainer:		
		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Phenethylamines				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Steroids				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Written/Oral Exam				
Competency Samples				

CONTROLLED SUBSTANCES TRAINING CHECKLIST – MODULE 3				
Trainee:		Trainer:		
		Trainee Initials/Date	Trainer Initials/Date	Time for Completion
Marijuana – Quantitative Analysis				
	Reading			
	Demonstration/observation			
	Study questions			
	Practical exercises			
Written/Oral Exam				
Competency Samples				