# Washington State Patrol

# Crime Laboratory Division

## Materials Analysis
## Seized Drug Training Manual

June 2023

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 seized drug 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 seized drug 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 EXPECTATIONS

Trainees who have prior related training and experience can progress through the training program at an accelerated pace or skip certain study segments. The required documentation of such related training and/or experience shall be left to the supervisor in coordination with the technical lead(s) or their designee.

The instructor shall be experienced in the area of Seized Drug Analysis. The instructor's casework and courtroom experiences, both prior and present, provide a unique aspect to the trainee's learning process that is impossible to duplicate in this training program. The instructor shall share such experiences with the trainee. The instructor shall also discuss with the trainee the training and reference materials (if any) available on the MA shared drive. Although the trainee's primary interaction shall be with the assigned instructor, this program promotes and encourages discussions with other experienced examiners. When possible, the trainee should also take outside courses related to Seized Drug Analysis.

The trainee shall maintain a notebook or multiple notebooks throughout the duration of this training program and shall record notes and observations for each study segment. The trainee notebook should be maintained in a neat and current fashion and should be present during conversations with the trainer. Upon completion of training, the trainee shall maintain the training notebook for the duration of their career. The form of the notebook(s) can be written, electronic, or a combination thereof.

The trainee is continuously evaluated throughout the training for comprehension and competency in theoretical knowledge, basic practical skills, and critical thinking skills. Training is progressive and continuously builds on and reinforces prior learning. Deficiencies on any of the training steps during the course of the training shall be rectified. It is important that these deficiencies be openly and promptly discussed among the trainee, trainer, technical lead, and/or supervisor, as appropriate. If necessary, training steps and testing can be repeated to satisfactorily complete this training program.

In order to successfully complete this training program the trainee shall, after completion of all topic areas, successfully complete a closed book written exam passed with 80%, a competency exam passed with a 100%, and an oral testimony exam with a pass/fail. The completion of these steps shall be documented. The competency exam shall take the form of a mock case with multiple items, which shall include a draft report. The oral testimony exam can either be a full

moot court or an oral examination of testimony type questions between the trainer and the trainee. Supervised casework may commence upon approval through the chain of command and will consist of a variety of cases. The number of supervised cases will be determined as appropriate for each trainee.

The technical review chapter may be covered before or after supervised casework.

Module 3 covers quantitative analysis of cannabis 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 seized drug analysts will complete this module.

The trainer is responsible for writing an interoffice communication (IOC) to the trainee's supervisor when the trainee has successfully completed the Seized Drug Training program. Training records, including training IOCs and authorizations, will be maintained in accordance with QOM requirements. Individual scientists are strongly encouraged to maintain copies of their own training records and their training notebook(s).

## 1.3 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 five parts:

- The *Objectives* summarize the purpose of each training segment.
- The *Topic Areas* designates topics to be included in the training segment.
- The *Readings* section lists required and/or suggested reference material that will be helpful 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 seized drugs:

- Seized drug analysis overview
- Chemistry principles
- Chemical screening
- Microcrystalline testing
- References & resources
- Measurement uncertainty

Module 2 covers qualitative examination of drugs of abuse:

- Amphetamine Type Stimulants (ATS)

- Cannabis – qualitative
- Cocaine
- GHB/GBL/1,4-Butanediol & analogs
- Hallucinogens
- Novel Psychoactive Substances (NPS)
- Opioids
- Pharmaceuticals & Legend Drugs
- Steroids and Other Prohibited Doping Compounds
- Seized Drug Case Approach
- Seized Drug Report Writing
- Technical Review

Module 3 covers quantitative analysis of cannabis:

- Cannabis – quantitative
- Pipettes

## 1.4 SAFETY

The analyst should have an understanding of the hazards associated with solvents, chemicals and compounds of interest in the analysis of any seized drugs. 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.

Opioids, especially fentanyl and analogs, are highly dangerous substances which can be lethal at small doses. Appropriate safety equipment including gloves, lab coats, safety goggles, and fume hoods should be employed when analyzing evidence or reference materials. It is recommended that another person be present in the relative vicinity when analyzing opioids should exposure occur and emergency measures are required. Narcan training must be completed before handling any opioids. Special care should be taken to ensure evidence samples are packaged appropriately to minimize potential exposure of anyone who handles the evidence. Affixing a brightly colored sticker indicating the item contains fentanyl is recommended prior to return of the evidence to the main evidence vault and/or return to the customer.

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 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.

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.

UV radiation can be harmful to the eyes and care should be exercised to avoid direct exposure to UV radiation.

All one time use glassware should be disposed of properly following use. Reusable glassware should be washed and stored appropriately.

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

# 2 SEIZED DRUG 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 seized drug analysis.
- To introduce general concepts related to seized drugs.

## 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 246-946 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. Seized drug evidence
   a. Solid dosage forms versus body fluids containing seized drugs
      i. Materials Analysis section analyzes for solid dosage forms
      ii. Toxicology analyzes body fluids for drugs of abuse 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 seized drugs
   f. Disposal of seized drugs
3. Seized drug analysis case approach
   a. Overview of case approach. Additional time will be spent on this topic in a later module.
   b. Qualitative versus quantitative examination
   c. Variable nature of materials received means no single approach or set of methods will adequately address all contingencies
   d. Conclusive identification of a seized drug 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 Materials Analysis Technical Procedures (MATP).
   e. 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.
   f. Sampling versus sample selection.
   g. Total number of items 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 READINGS

1. Required
    a. Revised Code of Washington (RCW) 69.41 & 69.50
    b. Washington Administrative Code (WAC) 246-945
    c. Title 21 United States Code
    d. WSP FLSB Forensic Services Guide
2. Suggested
    a. DEA.gov– Drug Information
    b. Saferstein, R. (2004). Forensic science handbook, Vol. II, 2nd Ed.: Prentice Hall.
    c. Smith, F. (2004). *Handbook of forensic drug analysis*. Elsevier.

## 2.4 STUDY QUESTIONS

1. What is a Drug – define and discuss?
2. What is meant by a Drug of Abuse?
3. What is a diluent?
4. What is an adulterant?
5. In what forms (presentations) could you see drugs of abuse?
6. What is an agonist?
7. What does IUPAC stand for?
8. Explain advantage and disadvantages of IUPAC names.
9. List the IUPAC name and any other general name used for the most common drugs of abuse
10. Draw the chemical structures of the following substances: Methamphetamine, Cocaine, Heroin.
11. List 'street names' of common drugs.
12. How and when are other naming systems used in classification of drugs (synonyms)?
13. What drug (active ingredient) is present in commonly abused pharmaceuticals?
14. Can drugs can be classified by pharmacological effects? If so, what are the basic pharmacological classifications of drugs? Group the common drugs of abuse by basic pharmacological classifications.
15. Describe the relationship between drug structure (i.e. heroin) and its pharmacological effect.
16. Describe how the form of a drug (i.e. salt form to base drug) can cause different pharmacological effects.
17. What form (i.e. salt form or base) of each common illicit drug is present and why?
18. Describe the common routes of administration and their effects for - Heroin, Methamphetamine, MDMA, Cocaine, ketamine, LSD, Psilocin or Psilocybin and testosterone.
19. What does the term anabolic mean?
20. What does the term androgenic mean?
21. Explain the difference between a natural, semi-synthetic and synthetic drug.
22. Classify the common drugs of abuse as natural, semi-synthetic or synthetic drugs.
23. Identify common sources of naturally occurring drugs.
24. What would be the source of synthetic drugs?
25. What functional groups classify a drug as an acid, base or neutral?
26. Identify 10 of the most common drugs as acid, base or neutral.
27. Why is it important to know why a drug is an acid, base, neutral?
28. What is the term “Alkaloid” used to describe?
29. Name two plant species that produce alkaloids of forensic interest.

30. Define (chemically) the terms isomer, derivative and analogue.
31. How does your drug legislation define isomer, derivative and analogue? What are the strengths and weaknesses of the legal definitions of these terms.
32. Why is heroin a schedule I controlled substance? Why is cocaine a schedule II controlled substance?
33. What criteria are used to determine the schedule of a drug?
34. Is there a difference between the controlled substance list at the state and federal levels?
35. List all of the controlled substances which are listed in more than one schedule.
36. Are there differences in charges/penalties for varying amounts of controlled substances in Washington? In the Federal system?

## 2.5 PRACTICAL EXERCISES

1. Observe your instructor or other qualified employee during the analysis of a seized drug case.
2. Review a minimum of 10 seized drug case files from several experienced forensic scientists and discuss them with your trainer. If possible review case files from a laboratory other than your own.

# 3 CHEMISTRY PRINCIPLES

## 3.1 OBJECTIVES

- To understand the significance of isomerism as related to seized drug 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 seized drugs.

## 3.2 TOPIC AREAS

1. ISOMERISM
    a. Review of isomers and stereoisomerism
        i. Isomers - compounds with the same molecular formula.
            1. Functional (e.g., CH3OCH3 versus CH3CH2OH).
            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 - $2^n$ 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. Diastereomers - 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 diastereomers 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).
                - 0.1N 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).
    - 0.1 N 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. 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 READINGS

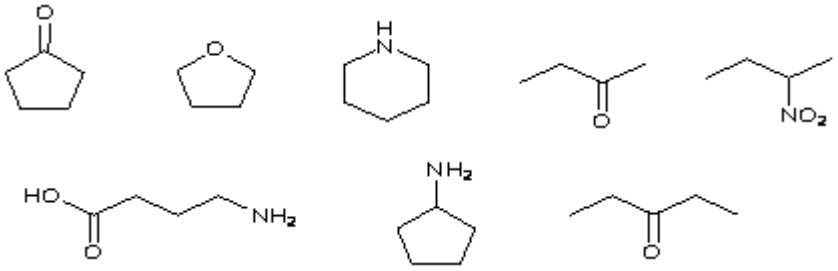
1. Required
a. Adair, A., Noggle, F. T., Odom, M. S., & Rhodes, M. A. (1983). The ANOR (alternate non-aqueous organic ratio) extraction procedure. *Micrograms*, *16*(1), 220-228.
b. Allen, A. C., Cooper, D. A., Kiser, W. O., & Cottrell, R. C. (1981). The cocaine diastereoisomers. *J Forensic Sci*, *26*, 12-26.
c. Analytical Extractions and Techniques Document \\FileShare\WSP\FLSB\CLD\Functional Areas\Materials Analysis\Training & Reference\Subdisciplines\Seized Drugs
d. Lin, D. L., Wang, S. M., Wu, C. H., Chen, B. G., & Liu, R. H. (2008). Chemical derivatization for the analysis of drugs by GC-MS-A conceptual review. *Journal of Food and Drug Analysis*, *16*(1), 1.
e. Moore, J. M. (1990). The Application of Chemical Derivatization in Forensic Drug Chemistry for Gas and High-Performance Liquid Chromatographic Methods of Analysis. *Forensic Science Review*, *2*(2), 79-124.
2. Suggested

a. Knapp, D. R. (1979). *Handbook of analytical derivatization reactions*. John Wiley & Sons.
b. Kranenburg, R. F., Ramaker, H. J., & van Asten, A. C. (2022). Portable near infrared spectroscopy for the isomeric differentiation of new psychoactive substances. *Forensic Science International*, *341*, 111467.
c. Kranenburg RF, Verduin J, Stuyver LI, de Ridder R, van Beek A, Colmsee E, van Asten AC, 2020. Benefits of derivatization in GC–MS-based identification of new psychoactive substances, Forensic Chemistry, Volume 20,
d. Moffat, A. C., Osselton, M. D., Widdop, B., & Watts, J. (2011). *Clarke's analysis of drugs and poisons*. London: Pharmaceutical press.
e. Schmid, M. G., & Hägele, J. S. (2020). Separation of enantiomers and positional isomers of novel psychoactive substances in solid samples by chromatographic and electrophoretic techniques–A selective review. *Journal of Chromatography A*, *1624*, 461256.
f. Thermo Scientific Pierce Reagents, Solvents and Accessories catalog available at www.thermo.com
g. Organic Chemistry, text of your choosing.

## 3.4 STUDY QUESTIONS

1. List any controlled substances (and their schedule) for which the isomer must be determined.
2. How are the terms homolog and analog related?
3. Define the following terms:
    a. Isomer
    b. Constitutional Isomer
    c. Stereoisomer
    d. Configurational Isomer
    e. Conformational Isomer
    f. Enantiomer
    g. Diastereomer
    h. Chirality, chiral center
    i. R (rectus), S (sinister)
    j. Optical activity, optical rotation
    k. Dextrorotatory, levorotatory
    l. Specific rotation [α]
    m. Optical Rotatory Dispersion (ORD)
4. Are enantiomers or diastereomers more difficult to conclusively identify? Why?
5. How can we distinguish d- and l- versus d,l- isomers using techniques available in the lab?
6. Why do different stereoisomers have different biological properties?
7. Draw the chemical structures for the following compounds and identify the chiral centers: Methamphetamine , Ephedrine, Methcathinone"
8. The specific rotation of a molecule [α] is dependent on what 4 factors?
9. How does the salt form of a drug affect its analysis?
10. How does pH affect the solubility of drugs?
11. How does the functional group affect the analysis procedure?
12. How could the form of the drug (i.e. salt or base) affect analysis?
13. What two characteristics make a molecule an excellent candidate for circular dichroism measurements?
14. There are 12 structural isomers with the formula $C_5H_{12}O$:
    a. Draw two with a straight carbon chain and an alcohol group.
    b. Draw two with a straight carbon chain and an ether group.
    c. Draw two with a branched carbon chain and either an ether or alcohol group.
    d. How do the isomers in a and b above differ?

15. From the structures below identify:
    a. The three pairs of structural isomers.
    b. The two structures that are not structural isomers.




16. 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

17. Why is derivatization useful for forensic chemical analysis?
18. 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
19. You are analyzing a methamphetamine sample by GC/MS and observe acetylated methamphetamine but you did not use a derivatizing agent as part of your sample preparation. How could this have happened?
20. Name an organic solvent generally suitable for the separation of the free base from a salt form for a controlled substance.
21. Describe the term “solubility”.
22. Define the following terms: very soluble, freely soluble, soluble, sparingly soluble, slightly soluble, very slightly soluble and practically insoluble.
23. Describe the term “dry extraction”.
24. Describe the term “liquid-liquid extraction”.
25. List some of the factors to consider when trying to separate a particular compound from a mixture.
26. What is a "basic" drug?
27. To what do the terms “cocaine base,” “crack,” "freebase" and “rock” refer to?

28. List three different general extraction schemes used to separate different classes of drugs.
29. List three different literature resources when trying to determine solubility of different chemical species.
30. What is pKa? How does it relate to pH?
31. What is the general scheme for basic extractions? List some reagents that can be used for such extractions.
32. What is the general scheme for acidic extractions? List some reagents that can be used for such extractions
33. What is an "amphoteric" extraction? Why is it more difficult to perform than an acidic or basic extraction?
34. Some drugs do not have an acid/base or salt form. Explain why these drugs can be extracted using a basic/acidic scheme.
35. 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)

## 3.5 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 anextraction scheme to isolate each component for analysis by FTIR.
3. Prepare a sample of methamphetamine in methanol.
    a. Analyze the sample by GC/MS. What issues exist with the chromatography?
    b. Add saturated sodium bicarbonate to the methanol preparation. Extract into pentane (or hexanes) and analyze by GC/MS. Did this improve the chromatography?
4. 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-bis(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?

5. Prepare samples of the mixtures describe in question seven of the study/discussion questions. Isolate each compound and confirm using an appropriate instrumental technique.
6. 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 seized drug color screening tests are listed in the MATP.
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 READINGS

1. Required
    a. Colour Tests for Precursor Chemicals of Amphetamine-Type Substances. United Nations Scientific and Technical Notes, SCITEC/20, 2005.
    b. Elkins, K. M., Weghorst, A. C., Quinn, A. A., & Acharya, S. (2017). Colour quantitation for chemical spot tests for a controlled substances presumptive test database. *Drug Testing and Analysis*, *9*(2), 306-310.
    c. Garrett AS, Siemens SR, Gaskill JH. (1992). The Weber Test: A Color Test for the Presence of Psilocin in Mushrooms. NWAFS Journal. 18(4).
    d. Johns, S. H., Wist, A. A., & Najam, A. R. (1979). Spot tests: A color chart reference for forensic chemists. *Journal of Forensic Science*, *24*(3), 631-649.
    e. Kovar KA, Laudszun M. (1989).Chemistry and Reaction Mechanisms of Rapid Tests for Drugs of Abuse and Precursors Chemicals. United Nations Scientific and Technical Notes, SCITEC/6.
    f. Nagy G, Szollosi I, Szendrei K. (2005). Colour Tests for Precursor Chemicals of Amphetamine-Type Substances. United Nations Scientific and Technial Notes, SCITEC/20.

g. Philp, M., & Fu, S. (2018). A review of chemical 'spot'tests: A presumptive illicit drug identification technique. *Drug testing and analysis*, *10*(1), 95-108.
h. (a) Ruybal R. 1972. Microgram. 5(3). (b) Ruybal C. Microgram. 6(2).
i. United Nations International Drug Control Programme. (1994). *Rapid Testing Methods of Drugs of Abuse: Manual for Use by National Law Enforcement and Narcotics Laboratory Personnel* (Vol. 13). United Nations Publications.

2. Suggested
   a. Butler WP.(1967). 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).
   b. Chen KK, Kao CH. 1926. Ephedrine and Pseudoephedrine, their isolation, constitution, isomerism, properties, derivatives, and synthesis. J. Am. Pharm. Assoc. 15(8):625-639.
   c. Clancy, L., Philp, M., Shimmon, R., & Fu, S. (2021). Development and validation of a color spot test method for the presumptive detection of 25-NBOMe compounds. *Drug Testing and Analysis*, *13*(5), 929-943.
   d. Feigl F. (1966). Spot Tests in Organic Analysis, 7th ed. Amsterdam: Elsevier.
   e. Kosecki PA, Brooke P, Canonico E, 2022. Fentanyl as a potential false positive with color tests commonly used for presumptive cocaine identification. J For Sci. 67: 2082-2088.
   f. Moffat AC. (1986). Clarke's Isolation and Identification of Drugs, 2nd Ed. London: Pharmaceutical Press.
   g. Moffat AC, Osselton MD, Widdop B. (2004). Clarke's Analysis of Drugs and Poisons, 3rd Ed. London:Pharmaceutical Press.
   h. Morris, J. A. (2007). Modified cobalt thiocyanate presumptive color test for ketamine hydrochloride. *Journal of forensic sciences*, *52*(1), 84-87.
   i. Philp, M., Shimmon, R., Tahtouh, M., & Fu, S. (2018). Color spot test as a presumptive tool for the rapid detection of synthetic cathinones. *JoVE (Journal of Visualized Experiments)*, (132), e57045.
   j. Siegel, J. A. (1988). Forensic identification of controlled substances. *Forensic science handbook*, *2*, 68-160.
   k. Toole, K. E., Fu, S., Shimmon, R. G., Kraymen, N., Taflaga, S., & Forensic, A. F. (2012). Color tests for the preliminary identification of methcathinone and analogues of methcathinone. *Microgram J*, *9*(1), 27-32.
   l. United Nations International Drug Control Programme. (1989). Recommended Methods for Testing Lysergide (LSD), Manual for use by National Narcotics Laboratories. United Nations Division of Narcotic Drugs, ST/NAR/17.


## 4.4 STUDY QUESTIONS

1. What is the practical value of color tests compared to other analytical techniques?
2. Name three different spot tests, and what drug(s) they would be used for.
3. List another color test that gives different results for different analytes.
4. In what ways are they inferior to instrumental techniques?
5. Define the following terms: presumptive, conclusive, chromophore, reagent, primary amine, secondary amine.
6. What two factors may inhibit the accurate description of spot/color test results?
7. What recommendations does Clarke's make regarding the ideal composition of a color test blank and how to make a final decision on the result of a test?
8. In regards to color tests, Suzanne Bell states, "…a small amount of the questioned powder is placed in a well of the plate and the reagent is added." Why would it be better to add the reagent first and then the powder to the reagent?
9. How are color test blanks documented/recorded?

10. Why should you use distilled water or deionized water when making aqueous reagents rather than tap water?
11. Name three situations where color tests would be commonly used.
12. What color is produced when the Marquis reagent reacts with methamphetamine or amphetamine?
13. Using Kovar and Laudzun's United Nations Technical Note SCITEC/6 February 1989 as reference, draw the general mechanism for the reaction of methamphetamine/amphetamine derivatives with the Marquis reagent.
14. At a clandestine laboratory site, a white powder is found on a piece of filter paper next to a bottle labeled "sodium cyanide." Assuming that the powder came from the bottle and that the bottle was correctly labeled, what reaction will occur if the powder is tested with the Marquis reagent? Explain.
15. The sodium nitroprusside-acetaldehyde/sodium carbonate test is also referred to as the _____ test.
16. A blue color with the sodium nitroprusside-acetaldehyde / sodium carbonate test indicates the presence of a _____ amine.
17. Using only the Marquis reagent and the sodium nitroprusside-acetaldehyde / sodium carbonate reagent, determine how you would distinguish between the following drugs: amphetamine, methamphetamine, MDMA, and MDA.
18. Opium derivatives such as codeine, morphine and heroin turn what characteristic color in the Marquis reagent?
19. Name three other terms for the para-Dimethylaminobenzaldehyde (p-DMAB) test.
20. Find which reagent you would use to screen for the following drugs: lysergic acid diethylamide (LSD), psilocybin, dimethyltryptamine? What structural unit do these three drugs have in common?
21. Look up the structure of the amino acid tryptophan in a reference book. Based on its structure, predict whether or not you would expect a positive purple reaction using the p-DMAB color test.
22. Chemically, what is the cause of the blue complex formed when cocaine hydrochloride is added to the $Co(SCN)_2$ reagent?
23. Give at least two examples of drugs that fluoresce under UV light.
24. Using Fiegl's Spot Tests in Organic Analysis and Spot Tests in Inorganic Analysis, find color tests for creatine and boric acid. Photocopy and save the relevant pages for future reference.
25. A series of color tests are negative. What additional tests should you perform, if any?
26. Why does crack react with cobalt plus acid but not cobalt alone?
27. What chemical moiety causes a purple color reaction with the Ehrlichs reagent.
28. How would you describe color tests to a jury?
29. Are color tests considered a category 1 or category 2 test? Why?
30. How long should a sample be allowed to react with the color test reagent before interpreting the results of the test?
31. Describe the difference between sensitivity and selectivity as it relates to color tests.
32. What quality control measures should be taken when performing a color test?
33. After issuing a report of findings indicating the submitted item was negative for seized drugs, the submitting officer calls and says he had a positive field test for heroin. How could this be?

## 4.5 PRACTICAL EXERCISES

1. Your trainer will select compounds from the following list for you to perform selected color tests listed in the MATP Appendix A – Common Sized Drug Color Screening Tests (or others as directed by your trainer) on the following substances, as available, and describe the results in a chart.

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

2. Obtain or prepare mixtures of several substances in the list in question 1. How does the combination of substances affect color tests?
3. Use available commercial field test (NIK, NARC, etc.) to test a variety of casework type samples and reference materials. What are some situations that could lead officers to report a false positive with field tests?

# 5 MICROCRYSTALLINE TESTING

## 5.1 OBJECTIVES

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

## 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 seized drugs.
    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 seized drugs.
    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 and it can be useful to separate potentially volatile components from non-volatile ones.
        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 to 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 Seized Drug Analysis 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. Use for the identification of methamphetamine
        ii. 1 gram of gold chloride is dissolved in a 20 milliliters of 1:2 phosphoric acid:water.
    c. Gold chloride in acetic acid
        i. Use for the identification of cocaine.
        ii. 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 on 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 CLD-Materials Analysis shared drive -Training/Reference Materials folder.

## 5.3 READINGS

1. Required
    a. ASTM E 1968. Current Edition. Standard Guide for Microcrystal Testing in the Forensic Analysis of Cocaine.
    b. ASTM E 1969. Current Edition. Standard Guide for Microcrystal Testing in the Forensic Analysis of Methamphetamine and Amphetamine.
    c. MATP Microchemical Testing chapter.
2. Suggested
    a. Allen, A. C., Cooper, D. A., Kiser, W. O., & Cottrell, R. C. (1981). The cocaine diastereoisomers. *J Forensic Sci*, *26*, 12-26.
    b. ASTM E 2125. Current Edition. Standard Guide for Microcrystal Testing in the Forensic Analysis of Phencyclidine and Its Analogues.
    c. Brinsko, K. M., Golemis, D., King, M. B., Laughlin, G. J., & Sparenga, S. B. (2015). A modern compendium of microcrystal tests for illicit drugs and diverted pharmaceuticals. *McCrone Research Institute: Chicago*.
    d. Clarke EGC. (1978). The Isolation and Identification of Drugs. p. 135-141.
    e. Davis, J. E. (1961). Barbiturate differentiation by chemical microscopy. *The Journal of Criminal Law, Criminology, and Police Science*, *52*(4), 459-468.
    f. Elie, M. P., & Elie, L. E. (2009). Microcrystalline tests in forensic drug analysis. *Encyclopaedia of Analytical Chemistry, John Wiley & Sons Ltd., Larkspur, USA*.
    g. Fulton, C. C. (1969). Modern Microcrystal Tests for Drugs.
    h. Nelson, H. C., Gardner, E. A., & Matteo, D. (2011). Microcrystal analysis of cocaine hydrochloride and added adulterants. *Journal of forensic sciences*, *56*(3), 736-740.
    i. Nichols, R. G. (1997). Drug proficiency test false positives: a lack of critical thought. *Science & justice: journal of the Forensic Science Society*, *37*(3), 191-196.

j. Saferstein R. (1982). Forensic Science Handbook, Vol. 1. Englewood Cliffs(NJ):Prentice Hall. p. 518-520.
k. Saferstein R. (1988). Forensic Science Handbook, Vol. 2. Englewood Cliffs(NJ):Prentice Hall. p. 69-160.Simpson BJ, Simpson TP, Liu RH.(1991). Microcrystaloscopic Differentiation of 3,4-Methylenedioxyamphetamine and Related Amphetamine Derivatives. *Journal of forensic sciences, 36*(3):908-914.
l. Wielbo D, Tebbet IR. (1992). The Use of Microcrystal Tests in Conjunction with FTIR for the Rapid Identification of Street Drugs. *Journal of forensic sciences 37*(4):1134-1148.

## 5.4 STUDY QUESTIONS

1. What are some of the advantages and disadvantages of microcrystalline tests?
2. What quality control measures are taken to ensure microcrystalline tests are working effectively?
3. How should results from the microcrystalline test be documented in case notes?
4. Can microcrystalline tests differentiate cocaine enantiomers and diasteriomers? Why would this be difficult to do?
5. Describe a procedure for obtaining a good microcrystal exam on a mixture of methamphetamine and Phenobarbital.
6. Why is the quarter wave retardation plate used in microcrystal exams (especially for cocaine)?
7. How would you describe the microcrystal examination in court?
8. Are crystals for a particulate substance unique? Explain.
9. How do crystal tests work? (i.e. basic theory)
10. What three metals are most commonly used in crystal reagents?
11. How does a volatility test work?
12. What shape crystal is obtained from cocaine HCl with the gold chloride reagent?
13. A suspected methamphetamine sample was submitted to the laboratory for analysis. How would you determine unequivocally the optical isomer using crystal tests? Explain your procedure carefully and logically.
14. Why do we use acids as solvents in crystal tests?

## 5.5 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.
- This training is a prerequisite for cannabis-quantitative analysis training.

## 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 READINGS

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? 2013. London: Laboratory News; Available on Materials Analysis Isilon folder>Training & Reference >Subdisciplines>Seized Drugs> Pipettes.
2. AccuTek Laboratories Guide to Pipetting. Available on Materials Analysis Isilon folder>Training & Reference >Subdisciplines>Seized Drugs> Pipettes.
3. ARTEL 10 tips to improve your pipetting technique. Available on Materials Analysis Isilon folder>Training & Reference >Subdisciplines>Seized Drugs> Pipettes.Calibry software users guide.
4. Gilson Microman® users guide. Available on Materials Analysis Isilon folder>Training & Reference >Subdisciplines>Seized Drugs> Pipettes.Millet, F., & Barthlen, T. (2007). Securing accuracy and precision when pipetting hot and cold liquids with Microman®.

## 6.4 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?

## 6.5 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 that are available regarding the analysis and identification of seized drugs.
- 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 that are commonly used in conjunction with seized drug 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. WA State 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 READINGS

1. Required
    a. Feeney, W., Moorthy, A. S., & Sisco, E. (2022). Spectral trends in GC-EI-MS data obtained from the SWGDRUG mass spectral library and literature: A resource for the identification of unknown compounds. *Forensic Chemistry*, *31*, 100459.
    b. Revised Code of Washington Chapter 69.50 Chapter 69.50 RCW: UNIFORM CONTROLLED SUBSTANCES ACT (wa.gov)
2. Suggested
    a. Cayman Chemical GC-MS Drug Identification Tool
    b. CFSRE NPS Discovery Monographs

c. Clandestine Laboratory Investigating Chemists Association Journal
d. Feigl F. (1966). Spot Tests in Organic Analysis, 7th ed. Amsterdam: Elsevier.
e. Instrument libraries
f. Marnell T. Drug Identification Bible, recent edition
g. Mills III T, Roberson J C. Instrumental Data for Drug Analysis, 3rd ed. Volumes 1- 6.
h. Moffat AC. Clarke's Isolation and Identification of Drugs. London: The Pharmaceutical press.
i. Schultes, R. E., & Hofmann, A. (1980). *The botany and chemistry of hallucinogens* (No. 1025). Charles C Thomas Pub Limited.
j. SWGDRUG Monographs
k. The Merck Index, Merck and Company, Inc., recent edition.
l. United States Pharmacopeia/National Formulary

## 7.4 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) include Kovats indices?
    e. 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. Excipient
    f. Immediate precursor
    g. Drug paraphernalia
3. Discuss what information is available in the CLIC Journal and "CLIC list".
    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?

## 7.5 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 Seroquel tablets controlled? What are they used to treat?
4. How are Dexedrine Spansule Capsules and tablets controlled? What are they used to treat?
5. What is the structure of Methenolone?
6. What are the legitimate uses for the following:
    a. Ketamine
    b. Xylazine
    c. Dimethylsulfone
    d. 1,4-Butanediol
7. What is the estimated minimum lethal dose of fentanyl?
8. What are the principal MS peaks and MW of Ethyl Caprate?

9. A compound has a molecular weight of 112 and the largest MS peak is 43. What could it be?
10. Locate and copy the IR spectrum of cocaine hydrochloride and cocaine base.
11. Locate and copy the MS spectrum of Oxycodone.
12. Define dioecious.
13. What Schedule of control does tilidine appear on?
14. What Schedule does barbital appear on?
15. 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 seized drugs.
- To be able to determine measurement uncertainty for balances used for weighing seized drugs.
- 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 items 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. (Remember, we consider each bag weighed to be two weighing events.)

         U = $\sqrt{N*(u_b)^2}$ = $\sqrt{10*0.0062^2}g$ = 0.020 g

## 8.3 READINGS

1. Required
   a. Bell, S. (1999). Measurement good practice guide no. 11. A beginner's guide to uncertainty of measurement. *Institute of Measurement & Control*.
   b. Birch, K. (2003). Measurement good practice guide No. 36: Estimating uncertainties in testing. *British Measurement and Testing Association, Crown*.

c. Miller, V. R. (2002). *Recommended guide for determining and reporting uncertainties for balances and scales*. US Department of Commerce, Technology Administration, National Institute of Standards and Technology.
d. Taylor, B. N., & Kuyatt, C. E. (1994). *Guidelines for evaluating and expressing the uncertainty of NIST measurement results* (Vol. 1297). Gaithersburg, MD: US Department of Commerce, Technology Administration, National Institute of Standards and Technology.

2. Suggested
   a. A Statistics textbook of your choosing.

## 8.4 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?

## 8.5 PRACTICAL EXERCISES

1. A surrogate weight 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, 40.00g. The uncertainty of measurement for the balance used for the measurements in question 10 is 0.02 g @k=2.
Using a blank copy of the measurement uncertainty spreadsheet, calculate the measurement uncertainty for this balance.

# 9 AMPHETAMINE TYPE STIMULANTS (ATS)

## 9.1 OBJECTIVES

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

## 9.2 TOPIC AREAS

1. ATS are a large class of compounds that include the following drug categories:
    a. Neurotransmitters
    b. Hormones
    c. Stimulants
    d. Entactogens
    e. Anorectics
    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. 3.4-Methylenedioxyamphetamine (MDA)
    r. 3.4-Methylenedioxymethamphetamine (MDMA)
    s. Phenethylamine
    t. Phenylephrine
    u. Selected Synthesis Precursors
        i. Phenylpropanolamine (PPA)
        ii. Phenyl-2-propanone (P2P)
    v. Diluents and Excipients
        i. Dimethyl Sulfone
        ii. Caffeine
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
        1. Amphetamine crystal forms
        2. MDMA HCl hydration forms
    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 H2SO4 or 0.1NHCl 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

## 9.3 READINGS

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

1. Required
    a. ASTM E 1969. Current edition. Standard Guide for Microcrystal Testing in the Forensic Analysis of Methamphetamine and Amphetamine.
    b. Drug Identification Bible, Amera-Chem, Inc. (ATS related content)
    c. History Channel. 2017. History of Meth. https://www.history.com/topics/crime/history-of-meth
    d. Irsfeld, M., Spadafore, M., & Prüß, B. M. (2013). β-phenylethylamine, a small molecule with a large impact. *Webmedcentral*, *4*(9).
    e. Rasmussen, N. (2015). Amphetamine-type stimulants: the early history of their medical and non-medical uses. *International review of neurobiology*, *120*, 9-25.
    f. Segawa, H., Kumisaka, K., Sugita, R., Iwata, Y. T., Yamamuro, T., Kuwayama, K., ... & Inoue, H. (2018). Comparison and evaluation of the quick purification methods of methamphetamine hydrochloride from dimethyl sulfone for spectroscopic identification. *Forensic science international*, *282*, 86-91.
    g. United Nations Office on Drugs and Crime (UNODC). (2006). Recommended Methods for the Identification and Analysis of Amphetamine, Methamphetamine and their Ring-Substituted Analogues in Seized Materials.
    h. Lee MM. 1995. The Identification of Cathinone in Khat (Catha edulis): A Time Study. J For Sci. 40(1):116-121.
    i. United Nations Office on Drugs and Crime (UNODC). (2015). Recommended methods for the identification and analysis of synthetic cathinones in seized materials.

j. U.S. Department of Justice Drug Enforcement Administration Diversion Control Program, Drugs and Chemicals of Concern Khat, Quat, Tschat, Miraa (Cathinone, Cathine), August 2001.

2. Suggested
   a. Analytical Profiles of Amphetamines and Related Phenethylamines, CND Analytical, Inc.
   b. Feigl F. 1966. Spot Tests in Organic Analysis, 7th ed. Amsterdam: Elsevier.
   c. Fulton CC. 1969. Modern Microcrystal Tests for Drugs. John Wiley & Sons. p. 1-49.
   d. Gough TA. 1991. The Analysis of Drugs of Abuse: Separation Science Series. Chichester, (England):John Wiley and Sons. p. 3 - 22.
   e. Johns SH, et al. 1979. Spot Tests, A Color Chart Reference for Forensic Scientists. J For Sci. 24(3):631-649.
   f. Mills III T, Roberson J C. Instrumental Data for Drug Analysis, 3rd ed. Volumes 1- 6.
   g. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
   h. Sanderson RM. 2008. Identification of N-Methylbenzylamine Hydrochloride, N-Ethylbenzylamine Hydrochloride, and N-Isopropylamine Hydrochloride. Microgram Journal. 6(1-2).
   i. Shulgin A, Shulgin A. 1991. PIHKAL: A Chemical Love Story. Transform Press.
   j. Smith, F. (2004). *Handbook of forensic drug analysis*. Elsevier.
   k. 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.
   l. Kalix P. 1992. Cathinone, a natural amphetamine. Pharmacol. Toxicol. 70:77-86.
   m. LeBelle MJ, et al. 1993. Gas chromatographic-mass spectrometric identification of chiral derivatives of the alkaloids of KHAT. For Sci Intern. 61:53-64.
   n. Szendrei, K. (1980). The chemistry of khat. *Bull Narc*, *32*(3), 5-35.


## 9.4 STUDY QUESTIONS

1. What common drugs of abuse could be included as ATS?
2. What is a phenethylamine?
3. Draw the structures for the following ATS:
   a. Amphetamine, methamphetamine, cathine, cathinone, methcathinone, fenetylline are non ring-substituted amphetamines.
   b. MDA, MDMA, MDEA, FLEA, MDBD are methylenedioxy-substituted amphetamines.
   c. Other ring-substituted amphetamines include 2,4,5-ring-substituted amphetamines (e.g. TMA-2, STP/DOM, DOB, DOC, DOI, DOET) and 2,4,5-ring-substituted phenethylamines (e.g. 2C-B, 2C-T, 2C-T-2, 2C-T-7, 2C-C, 2C-I).
   d. Other ring substitution patterns include Mescaline, PMA, PMMA, DMA, TMA, 4-MTA.
4. Identify the differences and similarities between the substances in Questions 3.
5. Are the substances in Questions 3 commonly seen in your jurisdiction?
6. In what physical form (e.g. powder, liquid) are ATS compounds frequently seen your laboratory?
7. What are the general effects of ATS? Are there differences between the classes of compounds?
8. Describe the activity and effects of the substances above.
9. In what general classification do each of the ATS fall? (e.g., Stimulant, Depressant)?
10. Describe the structural relationship between an amphetamine and a phenethylamine.
11. What configuration is the chiral center of d-methamphetamine, R or S?
12. Is the levo-methamphetamine in Vicks Inhalers controlled? Explain.
13. What is an entactogen?
14. What are the street names used to describe MDMA?
15. Name four drugs besides MDMA which are used at "rave" clubs.
16. When and why was MDMA first introduced?
17. What compounds are typically found in ecstasy tablets?
18. What drug is commonly referred to as 'speed'?
19. What is 'ice'?
20. Describe the differences between Methamphetamine HCl ( MA.HCl ) and MA Base.

21. What is the most commonly seen ATS in the laboratory?
22. What are commonly used street terms to describe ATS products?
23. Describe the common forms of ATS.
24. Determine the proper protocol for the analysis of each of commonly seen ATS compounds.
25. Are these compounds considered acidic, basic or neutral compounds and why?
26. Can these compounds exist as a salt form and how does this affect its analysis?
27. Do these compounds exist as isomers and how does it affect analysis? (interpretation)
28. How does volatility of the salt or base form of the ATS compound affect the analysis?
29. Identify what form of the drug (salt or base) is most suited to particular analytical techniques.
30. How can you differentiate the salt and base forms of the common ATS drugs?
31. What methods of analysis provide the best level of discrimination for ATS compounds?
32. Describe an extraction process for ATS commonly seen mixtures.
33. What molecular structure gives rise to the m/z 58 ion in the mass spectrum of methamphetamine?
34. What gives rise to the m/z 44 ion in amphetamine?
35. What are the key m/z ions seen in the mass spectrum of commonly seen ATS compounds?
36. Which ring-substituted beta-phenethylamine may be indicated by a rapidly developing very bright green color when treated with Marquis reagent?
37. Will EI mass spectrometry alone effectively distinguish 2,3-methylendioxyamphetamine from 3,4-methylenedioxyamphetamine?
38. What is the effect on the apparent mass spectral base ion, when 2-CB is scanned from 30 – 400 amu as compared to 40 – 400 amu?
39. Why do you often see a small amount of amphetamine and dimethylamphetamine when you analyze residue in a methamphetamine smoking device?
40. Which analytical techniques do not distinguish phentermine and methamphetamine? Which techniques will distinguish these compounds?
41. What is the structural relationship of ephedrine and pseudoephedrine? How can they be distinguished from one another?
42. Why is dimethyl sulfone a common diluent/cutting agent of methamphetamine? Name at least two strategies sufficient for forensic identification for chemically separating a mixture of methamphetamine and dimethyl sulfone.
43. Discuss the physical appearance of methamphetamine manufactured by different routes. How is “ice” created?
44. What is CMP/150 compound? What are the challenges of separating this compound from methamphetamine on the GC?
45. What schedule are methamphetamine and amphetamine? What are the legitimate uses of each compound?
46. 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?
47. The pKa of methamphetamine is 10. When performing an acid-base extraction of methamphetamine using water and hexane, what pH should you adjust the aqueous layer to so that all of the methamphetamine extracts into hexane?
48. What is the main psychoactive ingredient of Khat?
49. What are the three main alkaloids in Khat?
50. What Drug Class is Khat?
51. Cathinone converts to what substance? How can the cathinone be preserved?
52. What is ibogaine?
53. Where can DMT be found?
54. Describe an extraction procedure for the analysis of Khat.
55. Describe the physical characteristics of Khat.

## 9.5 PRACTICAL EXERCISES

1. Separate a mixture of pseudoephedrine and methamphetamine and confirm instrumentally, preferably by FTIR.
2. Separate a mixture of methamphetamine and dimethyl sulfone and confirm instrumentally, preferably by FTIR.
3. Analyze compounds listed in the Topic Areas by FTIR and GC/MSD. Discuss the differences in data obtained compare to the structures of the molecule, especially concerning the limitations of the analytical data to provide structural information on particular compounds.
4. Derivatize amphetamine, methamphetamine, and a variety of other phenethylamines. Discuss the applicability of this technique in an analytical scheme.
5. Practice base extracting a salt form of an ATS. Salt out the analyte and analyze by FTIR.
6. Conduct microcrystalline tests on substances listed in the Topic Areas which have not been previously analyzed in the Microcrystalline Testing chapter of this manual.
7. Analyze unknown samples provided by your trainer.

# 10 CANNABIS (MARIJUANA) – QUALITATIVE ANALYSIS

## 10.1 OBJECTIVES

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

## 10.2 TOPIC AREAS

1. Definitions
    a. Definitions associated with cannabis are frequently updated. Refer to RCW 69.50.101 for the most current definitions.

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. Cannabis 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. Cannabis 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 $CO_2$
        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 cannabis, 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
    h. Conversions of cannabinoids
        i. CBD → THC - in plants and synthetically
            1. THC Isomers
        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 cannabis 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 affect 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 cannabis)
    ii. Negative control (blank or known non-cannabis)
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. Likely an electrophilic substitution of the phenolic ring by protonated aldehyde groups of vanillin and acetaldehyde
        2. Empirical formula of colored product for the Duquenois reaction of THC is: THC + VANILLIN + 2ACETALDEHYDE − 3H2O

8. Training Topics
    a. Cannabis 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)
iii. Synthetic conversion of CBD to THC
1. Expected tetrahydrocannabinols
2. Analytical limitations associated with CBD conversion

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 cannabis 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 246-945-052 Schedule II, 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 cannabis
i. Marrubium vulgare (white horehound)
1. Marketed as a tobacco alternative.
2. Looks similar macroscopically to cannabis.
3. Does not have cystolithic hairs

4. Extracts analyzed by GC/MS indicate the presence of marrubiin

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 cannabis

## 10.3 READINGS

1. Required

a. Bailey MA. 1979. The Value of the Duquenois Test for Cannabis – A Survey. J For Sci. 24(4):817-840.
b. Desiderio, D. M. (Ed.). (1993). *Mass spectrometry: Clinical and biomedical applications*. Springer Science & Business Media. Chapter 6 – Cannabinoids.
c. Drug Identification Bible. Current edition. Amera-Chem, Inc. (cannabis related info)
d. Duggan, P. J. (2021). The chemistry of cannabis and cannabinoids. *Australian Journal of Chemistry*, *74*(6), 369-387.
e. Feliciani, G. (2022). Cannabis Drug Development and the Controlled Substances Act. *Duke J. Const. L. & Pub. Pol'y Sidebar*, *18*, 153.
f. Hazekamp, A., Peltenburg, A., Verpoorte, R., & Giroud, C. (2005). Chromatographic and spectroscopic data of cannabinoids from Cannabis sativa L. *Journal of liquid chromatography & related technologies*, *28*(15), 2361-2382.
g. RCW 69.50
h. Tsujikawa, K., Okada, Y., Segawa, H., Yamamuro, T., Kuwayama, K., Kanamori, T., & Iwata, Y. T. (2022). Thermal decomposition of CBD to Δ9-THC during GC-MS analysis: A potential cause of Δ9-THC misidentification. *Forensic science international*, *337*, 111366.
i. Williams, JB, et al. (2023). GC Separation for Identification of *iso*-THC Contaminants and Accurate Quantification of $\Delta^8$-THC and $\Delta^9$-THC in *Cannabis* Samples. Cayman Chemical Application Note.


2. Suggested

a. Acosta, A., & Almirall, J. (2021). Differentiation between hemp-type and marijuana-type cannabis using the Fast Blue BB colorimetric test. *Forensic Chemistry*, *26*, 100376.
b. Bernard G. 1984. Identification of Cannabis by Thin Layer Chromatography. Microgram. 17(5).
c. Bouquet RJ. 1950. Cannabis. Bulletin on Narcotics. 2:14-30.
d. Brenneisen R, et al. 1988. Chromatographic and Spectroscopic Profiles of cannabis of Different Origins, Part I. J For Sci. 33(6):1385-1404.
e. Buel E, et al. 1982. An Evaluation of a Partition TLC System for the Identification of Cannabinoids. Microgram. 15(9).
f. Butler WP. 167. Methods of Analysis, Internal Revenue Service, publication No. 341. p. 105.
g. Churchill KT. 1983. Synthetic Tetrahydrocannabinol. J For Sci. 24(3):762-772.
h. Coutts RT, Jones GR. 1979. A Comparative Analysis of Cannabis Material. J For Sci. 24(2):291-302.
i. De Pasquale A, et al. 1974. Micromorphology of epidermic surfaces of female plants of Cannabis sativa L. Bulletin on Narcotics.4:27-40.
j. Fairbairn JW. 1972. The Trichomes and Glands of Cannabis sativa L. Bulletin on Narcotics.24(4):29-33.
k. Forester DE. 1997. The Duquenois Color Test for Marijuana: Spectroscopic and Chemical Studies. Ph.D. Thesis from Georgetown University, Vol. 1 and 2.
l. Giroud C, et al. 2000. Salvia divinorum: An hallucinogenic mint which might become a new recreational drug in Switzerland. For Sci Intern.112(2-3):143-150.

m. Gülck, T., & Møller, B. L. (2020). Phytocannabinoids: origins and biosynthesis. *Trends in plant science*, *25*(10), 985-1004.
n. Hughes RB, Kessler RR. 1979. Increased Safety and Specificity in the Thin-Layer Chromatographic Identification of Marijuana. J For Sci. 24(4):842-846.
o. Hughes RB, Warner, Jr. VJ. 1978. A Study of False Positives in the Chemical Identification of Marijuana. J For Sci. 23(2):304-309.
p. Kovar KA, et al. 1989. Concerning the Duquenois-Levine Test for the Identification of Hashish and Marijuana. Microgram. 22(7).
q. Kruger, J. S., & Kruger, D. J. (2022). Delta-8-THC: Delta-9-THC's nicer younger sibling?. *Journal of cannabis research*, *4*(1), 1-8.
r. Langenheim JH. 1982. Botany: Plant Biology and Its Relation to Human Affairs. New York:John Wiley & Sons, Inc. p 49-79.
s. Lewis, K., Wagner, R., Rodriguez-Cruz, S. E., Weaver, M. J., & Dumke, J. C. (2021). Validation of the 4-aminophenol color test for the differentiation of marijuana-type and hemp-type cannabis. *Journal of Forensic Sciences*, *66*(1), 285-294.
t. Mahmoud AE, Holley JH, Lewis G, Russell MH, Turner CE. 1984. Constituents of Cannabis Sativa L. XXIV: The Potency of Confiscated Marijuana, Hashish and Hash Oil Over a Ten Year Period. J For Sci. 29(2):500-513.
u. Marijuana Its Identification. U. S. Treasury Department. 1948. pp. 1-37.
v. Mason AP, McBay AJ. 1985. Cannabis: Pharmacology and Interpretation of Effects. J For Sci. 30(3):615-631.
w. Nakamura GR. 1969. Forensic Aspects of Cystolith Hairs of Cannabis and Other Plants. Journal of AOAC. 52(1):5-16.
x. Nakamura GR, Thornton JI. 1977. The Forensic Identification of Marijuana: Some Questions and Answers. Journal of Police Science and Administration.1(1):102-112.
y. Nigam M C, et al. 1965. The Essential Oil of Marijuana: Composition of Genuine Indian Cannabis Sativa L. Canadian Journal of Chemistry. 43:3372-3376.
z. Pitt CG, Hendron RW, Hsia RS. 1972. The Specificity of the Duquenois Color Test for Marijuana and Hashish. J For Sci. 17(4):693-699.
aa. Reisfield AS. 1993. The botany of Salvia divinorum (Labiatae). SIDA Contributions to Botany 15(3):349-366.
bb. Smith RN. 1974. A Brief Note on the Response of Some Essential Oils and Extracts of Vegetable Origin to the Duquenois-Levine Test for Cannabis. J For Sci Society.14(3):191-194.
cc. Thornton JI, Nakamura GR. 1972. The Identification of Marijuana. J For Sci Society. 12(3):461-519.

## 10.4 STUDY QUESTIONS

1. Define the terms cannabis and marijuana.
2. Are cannabis and marihuana the same thing?
3. What is hemp, and what is the primary difference between it and marijuana?
4. How many species of cannabis are known?
5. What is the primary psychoactive chemical in cannabis?
6. What are the primary chemical constituents found in cannabis?
7. Are these compounds considered alkaloids?
8. Name the principal cannabinoids.
9. Draw the structures of delta-9-tetrahydrocannabinol, cannabinol, and cannabidiol.
10. How is cannabinol formed?
11. What are different nomenclature systems (i.e. delta-9, delta-1) used to describe THC?
12. For a positive conclusion, what morphological features are essential to be observed in the macro/ microscopic examination suspected cannabis?
13. What are examples of unicellular and multi-cellular hairs on Cannabis?
14. What hair combinations are selective/unique to cannabis? When was this research completed?
15. Are there any other plants that have cystolithic hairs?

16. Discuss the taxonomy of cannabis.
17. Name the gross physical and microscopic characteristics of the *Cannabis sativa L.* plant.
18. Are cystolithic hairs glandular or non-glandular?
19. Does the male plant produce flowers?
20. Does the male plant produce THC?
21. What is sensimilla?
22. What are the typical characteristics of cannabis seeds?
23. List some features of cultivated cannabis that non-cultivated cannabis lacks.
24. What are other cannabis products?
25. What are the most common cannabis products submitted to your lab?
26. How are these cannabis products used?
27. What is the name for the extracted resin of cannabis?
28. Describe the range of physical forms in which cannabis plant material presented in casework submissions.
29. What is the difference between cannabis resin, hashish and hash oil?
30. What is the goal of cannabis breeding/cultivation?
31. How is this resin produced?
32. Ditchweed marijuana is a term that describes uncultivated marijuana of very low potency. Can this be cultivated into a plant producing very potent buds?
33. What are Thai sticks?
34. What is the difference between Thai sticks and Thai pills?
35. Can cannabis be baked into brownies without losing most of its potency?
36. Describe the preparation of “butane honey oil”.
37. What is cannabis butter?
38. What was the original purpose for the development of 'spice'-type compounds?
39. What has been found in herbal products marketed as “Spice,” “Spice Gold,” “Spice Diamond,” etc.?
40. In your jurisdiction, are chemical compounds found in spice products controlled as specific chemical compounds or as categories of chemical functional groups?
41. What color tests are used in the analysis of cannabis?
42. What instrumental techniques are considered most suitable for the analysis of cannabis?
43. Is a conclusive identification of cannabis accomplished by obtaining positive results with both a microscopic examination and with an appropriate color test?
44. What has been reported to yield positive color changes with the Duquenois-Levine Test?
45. How can TLC be used as part of an analytical protocol for the identification of cannabis?
46. What are the challenges in finding cannabinoids in young cannabis plants vs. mature plants?
47. What is the role of the hydrochloric acid in the Duquenois test?
48. What is the difference between the Duquenois test, the Duquenois-Levine test, the Rapid Duquenois Levine test, and the Modified Duquenois test?
49. Are color tests suitable for the determination of 'spice'-type compounds?
50. Should you run a blank with TLC and Duquenois?
51. List 3 analytical tests used to identify cannabis and the expected results.
52. Are there any substances that will give a positive Duquenois-Levine test (characteristic violet chloroform layer) other than THC and other cannabinoids?
53. Describe the analytical criteria necessary to identify THC in each of the following: Cannabis, Cannabis resin (Hashish), Hash Oil Food Products.
54. Which parts of the cannabis plant are controlled?
55. Is hemp legal in your jurisdiction?
56. How many species of Cannabis are controlled?
57. Must cannabis seeds be viable to be controlled?
58. Is cannabis controlled in your jurisdiction? Under what legislative Schedule is cannabis and why?
59. Does cannabis have approved medical uses?
60. Are human deaths commonly attributed directly to the toxic effect of smoking cannabis?

61. Twelve 5 mm long, burnt, hand-rolled cigarette remnants containing suspected cannabis are submitted as an exhibit for seized drug analysis. Cannabis is identified. Is this sufficient to constitute a usable amount? Explain this according to your jurisdictional requirements.
62. Does EI-GCMS alone easily distinguish delta-9-THC from delta-8-THC without derivatization?
63. THC is suspected to be contained in an exhibit consisting of a dozen Gummy Bears. The Duquenois-Levine Test corroborates this. Suggest an effective procedure to prepare an extract for injection into a GCMS.
64. Does EI-GCMS easily distinguish between all of the synthetic cannabinoids?
65. How much weight loss in a cannabis seizure can be attributed to natural causes? Are cystolithic hairs unique to cannabis? If not, name some other common plants that have them.
66. Is the Duquenois-Levine test specific for cannabis? Is the Duquenois-Levine test specific for THC?
67. What is the base of a cannabis cystolithic hair made of?
68. Describe the morphology of a cannabis leaf? Plant? Seed?
69. List some of the materials that give false positive results for the Duquenois-Levine test.
70. What is hashish? What is kif?
71. Define the following
    a. “cannabis”
    b. “cannabis-infused products”
    c. “usable cannabis”
    d. “THC concentration”
72. Describe the possession limits of cannabis and products.
73. Discuss the history of cannabis use and criminalization in the United States. Include the recent legal change from the term marijuana to cannabis.

## 10.5 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 cannabis.
    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 cannabis 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 cannabis plants, if available.
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 cannabis.
5. Discuss with your trainer what tests and results need to be present to identify a sample as cannabis.
6. Examine a sample of mechanically prepared cannabis concentrate 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 a cannabis concentrate. Discuss how the sample would be reported. Repeat with a cannabis concentrate oil, wax or other similar form.

7. Add a small amount of dilute acid to a cannabis sample and observe the result under a stereomicroscope. Explain what happened.
8. Use QuEChERs or other appropriate extraction technique to prepare an infused product for analysis by GC/MS. Analyze and discuss the effectiveness of the extraction technique.

# 11 CANNABIS – QUANTITATIVE ANALYSIS

## 11.1 OBJECTIVES

- To understand the quantitation of THC as it relates to identifying cannabis .
- 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 cannabis as defined by the Revised Code of Washington.

## 11.2 TOPIC AREAS

1. Legal scheduling of cannabis
    a. I-502
    b. HB2056
    c. HB2136
    d. SB5052
    e. SB5367
    f. 0.3% THC necessitates quantitative analysis
    g. 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

## 11.3 READINGS


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.
3. ElSohly MA, et al. 1984. Constituents of Cannabis sativa L. XXIV: The Potency of Confiscated Marijuana, Hashish and Hash Oil over a Ten-Year Period. J For Sci. 29(2):500-514.
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
9. Lercker G, Bocci F, Frega N. 1992. Cannabinoid acids analysis. Il Farmaco 47(3):367-378.
10. Mehmedic Z, et al. 2010. Potency Trends of Delta-9-THC and Other Cannabinoids in Confiscated Cannabis Preparations from 1993 to 2008. J For Sci. 55(5):1209-1217.
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.
13. Poortman-van der Meer AJ, Huizer H. 1999. A contribution to the improvement of accuracy in the quantitation of THC. For Sci Int 101:1-8.

14. Quantitative Chromatographic Analysis by Raymond P.W.Scott, part of the Chrom-Ed Series. The Internal Standard Method.
15. Quantitative Chromatographic Analysis by Raymond P.W.Scott, part of the Chrom-Ed Series. The External Standard Method.
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.
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.

## 11.4 STUDY QUESTIONS

1. Describe the possession limits of cannabis and products.
2. Why is 0.3% THC a critical value for cannabis?
3. Compare and contrast methods of GC quantitative analysis.
4. What are the advantages of the internal standard method for quantitative analysis?
5. Why is tribenzylamine used as the internal standard for quantitation of THC?
6. How are THCA and THC related? How does this relationship impact the quantitative analysis of leaf cannabis ?
7. What is the purpose of the internal standard solution (ISS)? Calibration verification solution (CVS)? Resolution verification solution (RVS)?
8. 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?
9. How are the blanks evaluated during quantitative analysis?
10. How long can calibrators, ISS, CVS, and RVS be stored and used?
11. Why is the THC value determined by quantitative analysis not reported?
12. Why is moisture content of the leaf material not evaluated?
13. What is a "sample set" when setting up a sequence table?
14. How frequently must you run a Continuing Calibration Verification solution (CCV)?
15. Why do we perform replicate injections of the CVS and samples?
16. You are evaluating your sequence and notice that the final CVS (CCV2) has a CV of 5.67. What should you do?
17. You are working ten, one-item cannabis cases. List what your sequence will look like.
18. One of the case samples you analyze is greater than the upper limit of quantitation. What should you do?
19. Describe the appropriate means of reading a meniscus for volumetric glassware.
20. 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?
21. For each step in the quantitation process, identify elements which could contribute to measurement uncertainty.

## 11.5 PRACTICAL EXERCISES

1. Extract a sample of leaf cannabis 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 ~~RVS~~ TCS and CVS in triplicate
   f. Evaluate the curve, RVS and the triplicate injections of the ~~RVS~~ TCS and CVS.

   ***Be sure to use chemistry form 5032 – THC Quant Stock Solution Prep and form 5034 – Calibration Curve Worksheet.
5. Analyze cannabis 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.

# 12 COCAINE

## 12.1 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.

## 12.2 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 SnCl2 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, CH2Cl2 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 effect 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

## 12.3 READINGS

1. Required
    a. Allen, A. C., Cooper, D. A., Kiser, W. O., & Cottrell, R. C. (1981). The cocaine diastereoisomers. *J Forensic Sci*, *26*, 12-26.
    b. Broséus, J., Gentile, N., Pont, F. B., Gongora, J. M. G., Gasté, L., & Esseiva, P. (2015). Qualitative, quantitative and temporal study of cutting agents for cocaine and heroin over 9 years. *Forensic science international*, *257*, 307-313.
    c. Broséus, J., Gentile, N., & Esseiva, P. (2016). The cutting of cocaine and heroin: a critical review. *Forensic science international*, *262*, 73-83.
    d. Casale JF, Klein RFX. (1993). Illicit Production of Cocaine. *Forensic Science Review,* 5(2): 96-104.
    e. Drug Identification Bible, Amera-Chem. Inc., most current edition. (cocaine related info)
    f. Poupko, J. M., Hearn, W. L., & Rossano, F. (2018). Drug contamination of US paper currency and forensic relevance of canine alert to paper currency: a critical review of the scientific literature. *Journal of forensic sciences*, *63*(5), 1340-1345.
    g. Schlesinger, H. L. (1985). Topics in the chemistry of cocaine. *Bulletin on Narcotics*, *37*(1), 63-78.
    h. Smith, R. M. (1997). The mass spectrum of cocaine. *Journal of forensic sciences*, *42*, 475-480.
2. Suggested
    a. Aaron, R., & Lewis, P. (1987). Cocaine residues on money. *Crime Laboratory Digest*, *14*(1), 18-18.
    b. Analytical Profiles of Cocaine, CND Analytical, Inc.
    c. Casale, J. F., Boudreau, D. K., & Jones, L. M. (2008). Tropane ethyl esters in illicit cocaine: isolation, detection, and determination of new manufacturing by-products from the clandestine purification of crude cocaine base with ethanol. *Journal of forensic sciences*, *53*(3), 661-667.
    d. Casale, J. F., Hays, P. A., Toske, S. G., & Berrier, A. L. (2007). Four new illicit cocaine impurities from the oxidation of crude cocaine base: formation and characterization of the diastereomeric 2, 3-dihydroxy-3-phenylpropionylecgonine methyl esters from cis-and trans-Cinnamoylcocaine. *Journal of forensic sciences*, *52*(4), 860-866.
    e. Feigl F. 1966. Spot Tests in Organic Analysis, 7th ed. Amsterdam: Elsevier.
    f. Fulton CC. 1969. Modern Microcrystal Tests for Drugs. John Wiley & Sons. p. 1-49.
    g. Jenkins, A. J. (2001). Drug contamination of US paper currency. *Forensic Science International*, *121*(3), 189-193.
    h. Mills III T, Roberson J C. Instrumental Data for Drug Analysis, 3rd ed. Volumes 1- 6.
    i. Moffat AC. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
    j. Moore, J. M., & Casale, J. F. (1994). In-depth chromatographic analyses of illicit cocaine and its precursor, coca leaves. *Journal of Chromatography A*, *674*(1-2), 165-205.
    k. Moore, J. M., Meyers, R. P., & Jimenez, M. D. (1993). The anatomy of a cocaine comparison case: a prosecutorial and chemistry perspective. *Journal of Forensic Sciences*, *38*(6), 1305-1325.
    l. Morelato, M., Franscella, D., Esseiva, P., & Broséus, J. (2019). When does the cutting of cocaine and heroin occur? The first large-scale study based on the chemical analysis of cocaine and heroin seizures in Switzerland. *International Journal of Drug Policy*, *73*, 7-15.

m. Negrusz, A., Perry, J. L., & Moore, C. M. (1998). Detection of cocaine on various denominations of United States currency. *Journal of Forensic Science*, *43*(3), 626-629.
n. Oyler, J., Darwin, W. D., & Cone, E. J. (1996). Cocaine contamination of United States paper currency. *Journal of analytical toxicology*, *20*(4), 213-216.
o. Sleeman, R., Burton, F., Carter, J., Roberts, D., & Hulmston, P. (2000). Drugs on Money.
p. Yuksel, B., & Sen, N. İ. L. G. Ü. N. (2018). Development and validation of a GC-FID method for determination of cocaine in illicit drug samples. *Journal of research in pharmacy*, *22*(4).

## 12.4 STUDY QUESTIONS

1. Cocaine is often referred to as a narcotic. Should it be?
2. If research shows cocaine can be detected on currency, should we test currency submitted for testing of drug residue?
3. What is the name of the plant (genus, species) in which cocaine is a naturally occurring alkaloid?
4. To what do the terms, “cocaine base,” “crack,” "freebase" and “rock” refer?
5. Describe the chemical process to produce crack or freebase out of cocaine powder.
6. What are common adulterants and diluents found in illicit cocaine samples?
7. What are some degradation products that can result from cocaine?
8. Cocaine has four enantiomeric/diastereomeric forms. Name them. Can they be distinguished with FTIR and GC/MS? Does your jurisdiction control the base form of cocaine, differently than it controls cocaine salt?
9. Does your jurisdiction impose enhancements to penalties for quantities of cocaine?
10. What countries are typical sources for the coca plant?
11. In what form is cocaine typically transported?
12. Describe the process of isolating cocaine from the coca plant.
13. In general terms, how is cocaine hydrochloride converted into cocaine base?
14. How does the salt form of cocaine affect color tests, solubilities and extraction procedures?
15. Does EI-GCMS differentiate among the various diastereomers of cocaine? If so, which ion fragments or relative ion fragment ratios are particularly discriminating?
16. Under what circumstances would GCMS be a preferred analytical technique for the identification of cocaine?
17. What are some other alkaloids from the cocaine plant, besides cocaine, that commonly appear in GCMS analyses?
18. Does conversion of the salt form of cocaine into the base form of cocaine constitute manufacturing?
19. Contrast the application of $Co(SCN)_2$ to cocaine hydrocholoride and to cocaine base?
20. Will $Co(SCN)_2$ produce similar results with benzocaine, lidocaine, and procaine, as it does with cocaine?
21. Will infrared spectrometry distinguish cocaine hydrochloride from cocaine base? From pseudococaine hydrochloride?
22. Explain why the presence of cis- and trans-cinnamoylcocaine may be problematic when quantitation of cocaine is performed by UV spectrophotometry.
23. What color tests are typically used for the presumptive identification of cocaine?
24. Suggest an extraction scheme to isolate suspected cocaine from shampoo, in preparation for subsequent GCMS injection
25. How is cocaine hydrochloride converted into “crack”?
26. What common feature does “caine” refer to in the terms cocaine, benzocaine, lidocaine and procaine?
27. True or false? Placing cocaine hydrochloride in a basic aqueous solution, will cause it to be mostly uncharged and more soluble in nonpolar (e.g. organic) solvents.
28. True or false? Placing cocaine hydrochloride in an acidic aqueous solution, will cause it to be mostly charged and more soluble in polar (e.g. aqueous) solvents.

29. Why would FTIR be considered a preferred analytical technique for the identification of cocaine?
30. Since the cocaine molecule contains two carbonyl groups, would you expect the IR of the carbonyl region to have one or two absorption peaks? Why?
31. What are other materials that can contain cocaine?
32. What street drug term refers to a mixture of cocaine and heroin?
33. Can cocaine hydrochloride be effectively smoked in a pipe? Explain.
34. Is cocaine easily detectable in residue left in a pipe after smoking?
35. What is the melting point of cocaine base?
36. What is the melting point of cocaine hydrochloride?
37. How many asymmetrical centers are present in the cocaine molecule? How many stereoisomers does this number of asymmetric centers predict? How many diastereomeric pairs of cocaine are observed?
38. Does your analytical protocol require that “cocaine base” be distinguished from salt forms of cocaine?
39. What are the physical differences between cocaine HCl and cocaine base?
40. Describe the differences in use between cocaine HCl and cocaine base. Why would one be used over the other? What form would typically have more 'cuts'? Why?
41. Does cocaine have any legitimate medicinal use?
42. Draw the structures of the four cocaine isomers and show the equatorial/axial relationship of the carbomethoxy- group and the benzoyloxy- group for each isomer.
43. What are four properties (chemical & physiological) of cocaine base (aka smokable cocaine) that have contributed to its popularity?
44. A sample submitted to you for analysis (labeled as suspected cocaine, and described as "rock-like substance") is a thick brown liquid. Provide an explanation for what happened.
45. How does the salt form of cocaine affect the administration of cocaine in the body?
46. If procaine hydrochloride is present with cocaine hydrochloride when it is converted into cocaine base, is the procaine removed from the product or does it form the free base form of procaine?
47. Discuss the “cocaine isomer defense.”
48. How is the Scott Test more selective than using aqueous $Co(SCN)_2$ alone?
49. Describe an analytical protocol sufficient for conclusive qualitative identification of cocaine.
50. Is this protocol also adequate for reporting a conclusive qualitative identification of “cocaine base”?
51. When and how was the “isomer problem” for cocaine prosecutions resolved?

## 12.5 PRACTICAL EXERCISES

1. Devise a dry extraction scheme to separate a mixture of cocaine base, cocaine hydrochloride and inositol. Illustrate the separation by a diagram.
2. Prepare the following samples for analysis. Analyze by FTIR & GCMS as mixtures. Attempt to isolate the components of the mixture and analyze by FTIR & GC/MS.
    a. Base mixed with lidocaine
    b. Base mixed with procaine
    c. Hydrochloride mixed with nicotinamide
    d. Hydrochloride mixed with diltiazem
3. Practice converting cocaine base to the salt form and/or salt to the base. Analyze using FTIR and/or Raman.
4. Prepare mixtures of cocaine and inositol in concentrations varying from 10% to 90%. Analyze on FTIR.
5. Prepare a mixture of cocaine and heroin. Isolate each compound and identify using available analytical techniques.

# 13 GHB/GBL/1,4-BUTANEDIOL

## 13.1 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.

## 13.2 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

## 13.3 READINGS

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

1. Required
    a. Andera, K. M., Evans, H. K., & Wojcik, C. M. (2000). Microchemical identification of gamma-hydroxybutyrate (GHB). *Journal of Forensic Science*, *45*(3), 665-668.
    b. Chappell JS. 2002. The Non-Equilibrium Aqueous Solution Chemistry of GHB. CLIC.12(4):20-27.
    c. Ciolino, L. A., Mesmer, M. Z., Satzger, R. D., Machal, A. C., McCauley, H. A., & Mohrhaus, A. S. (2001). The chemical interconversion of GHB and GBL: forensic issues and implications. *Journal of Forensic Science*, *46*(6), 1315-1323.
    d. Mercer, J. W., Oldfield, L. S., Hoffman, K. N., Shakleya, D. M., & Bell, S. C. (2007). Comparative analysis of gamma-hydroxybutyrate and gamma-hydroxyvalerate using GC/MS and HPLC. *Journal of forensic sciences*, *52*(2), 383-388.
    e. Smith PR, Bozenko JS. 2002. New Presumptive Tests for GHB. Microgram. 35(1):10-15.
    f. Witkowski, M. R., Ciolino, L. A., & DeFrancesco, J. V. (2006). GHB free acid: II. Isolation and spectroscopic characterization for forensic analysis. *Journal of forensic sciences*, *51*(2), 330-339.
2. Suggested
    a. Bishop, S. C. (2004). *Advanced Capillary Electophoretic Techniques for the Detection of Date-Rape and Club Drugs for a Forensic Setting* (Doctoral dissertation, Ohio University).
    b. Dahlén, J., & Vriesman, T. (2002). Simultaneous analysis of γ-hydroxybutyric acid, γ-butyrolactone, and 1, 4-butanediol by micellar electrokinetic chromatography. *Forensic science international*, *125*(2-3), 113-119.
    c. DeFrancesco, J. V., Witkowski, M. R., & Ciolino, L. A. (2006). GHB free acid: I. Solution formation studies and spectroscopic characterization by 1HNMR and FT-IR. *Journal of forensic sciences*, *51*(2), 321-329.
    d. Meyers, J. E., & Almirall, J. R. (2004). A study of the effectiveness of commercially available drink test coasters for the detection of “date rape” drugs in beverages. *Journal of analytical toxicology*, *28*(8), 685-688.
    e. Vose, J., Tighe, T., Schwartz, M., & Buel, E. (2001). Detection of gamma-butyrolactone (GBL) as a natural component in wine. *Journal of Forensic Sciences*, *46*(5), 1164-1167.
    f. Philp, M., & Fu, S. (2018). A review of chemical ‘spot’tests: A presumptive illicit drug identification technique. *Drug testing and analysis*, *10*(1), 95-108.

## 13.4 STUDY QUESTIONS

1. Describe the equilibrium formed GHB and GBL in aqueous solutions of various pH values. How does this affect analysis?
2. List the physical properties of GHB, GBL, and 1,4-butanediol.
3. What was the original medical use of GHB?
4. GBL and butanediol are known as prodrugs. What does this mean?
5. Can IR be used to distinguish GHB from GBL?
6. How can be GHB and GBL be separated chemically for analysis?
7. Is NaGHB hygroscopic?
8. Can GHB (free acid) and GBL be differentiated by NMR analysis?
9. Name two analogs of GHB.
10. What special challenges arise in the identification of GBL?

## 13.5 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 trainer 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.

# 14 HALLUCINOGENS

## 14.1 OBJECTIVES

- To become acquainted with suspected hallucinogenic substances 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.

## 14.2 TOPIC AREAS

1. Hallucinogens and the law
    a. Scheduled compounds
    b. Interpretation of the statutes
2. 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
3. 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

4. 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
5. 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.

6. 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
v.

## 14.3 READINGS

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

1. Required
a. Casale JF. 1985. An Aqueous-Organic Extraction Method for the Isolation and Identification of Psilocin from Hallucinogenic Mushrooms. J For Sci. 30(1):247-250.

b. Drug Identification Bible, Amera-Chem. Inc., most current edition. (hallucinogen related info)
c. Garrett AS, Siemens SR, Gaskill JH.1992. The Weber Test A Color Test for the Presence of Psilocin in Mushrooms. NWAFS Journal. 18(4).
d. Siefert JH, Collins, DL. 1984. Distinguishing Between LSD and LAMPA by Capillary GC/MS. Microgram. 17(7):100-104.
e. United Nations Office on Drugs and Crime (UNODC). (1989). Recommended Methods for Testing Peyote Cactus (Mescal Buttons)/Mescaline and Psilocybe Mushrooms/Psilocybin.
f.

2. Suggested
   a. Hallucinogens, General
      i. Gahlinger PM. 2004. Illegal Drugs: A Complete Guide to Their History, Chemistry, Use and Abuse. New York:Penguin(Plume).
      ii. Inaba DS, Cohen WE 2000. Uppers, Downers, All Arounders: Physical and Mental Effects of Psychoactive Drugs, 4th ed. CNS Publications.
      iii. Laing R. ed. 2003. Hallucinogens A Forensic Drug Handbook. Academic Press.
      iv. Schultes RE. 1976. A Golden Guide: Hallucinogenic Plants. Golden Press.
      v. Shulgin A, Shulgin A. 1991. PIHKAL: A Chemical Love Story. Transform Press.
      vi. Shulgin A, Shulgin A. TIHKAL: The Continuation. Transform Press.
      vii. Tripsitter.com/psychedelics
      viii. Tsujikawa K, et al. 2006. Analysis of hallucinogenic constituents in *Amanita* mushrooms circulated in Japan. For Sci Int. 164: 172-178.
      ix. Więckiewicz, G., Stokłosa, I., Piegza, M., Gorczyca, P., & Pudlo, R. (2021). Lysergic acid diethylamide, psilocybin and dimethyltryptamine in depression treatment: a systematic review. *Pharmaceuticals*, *14*(8), 793.
   b. Bufotenine
      i. Barry, T. L., Petzinger, G., & Zito, S. W. (1996). GC/MS comparison of the West Indian aphrodisiac" Love Stone" to the Chinese medication" chan su": bufotenine and related bufadienolides. *Journal of forensic sciences*, *41*(6), 1068-1073.
      ii. Blackledge RD, Phelan CP. 2006. Identification of Bufotenine in Yopo Seeds via GC/IRD. Microgram Journal. 4(1-4):3-11.
      iii. Phelan CP. 1999. Identification of psilocin and bufotenine via GC/IRD. Microgram. 32(2):83-89
   c. Dimethyltryptamine (DMT)
      i. Dunlap, L. E., & Olson, D. E. (2018). Reaction of N, N-dimethyltryptamine with dichloromethane under common experimental conditions. *ACS omega*, *3*(5), 4968-4973.
      ii. Fasanello, J. A., & Placke, A. D. (2007). The isolation, identification, and quantitation of dimethyltryptamine (DMT) in Mimosa hostilis. *Microgram*, *5*, 41-52.
      iii. Pires, A. P. S., De Oliveira, C. D. R., Moura, S., Dörr, F. A., Silva, W. A. E., & Yonamine, M. (2009). Gas chromatographic analysis of dimethyltryptamine and β-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. *Phytochemical Analysis*, *20*(2), 149-153.
      iv. Rossi, G. N., Crevelin, E. J., de Oliveira Silveira, G., Eugênia Costa Queiroz, M., Yonamine, M., Cecilio Hallak, J. E., & Guimarães dos Santos, R. (2019). Internet method for the extraction of N, N-dimethyltryptamine from Mimosa hostilis roots: Does it really extract dimethyltryptamine?. *Journal of Psychedelic Studies*, *3*(1), 1-6.
   d. LSD
      i. Clark CC. 1989. The Differentiation of Lysergic Acid Diethylamide (LSD) from N-Methyl-N-Propyl and N-Butyl Amides of Lysergic Acid. J For Sci. 34(3):532-546.
      ii. Hida, M., & Mitsui, T. (1999). Rapid identification of lysergic acid diethylamide in blotter paper by microscope FT-IR. *Analytical sciences*, *15*(3), 289-291.

iii. Jacobs JL. 1984. A Simplified Method for the Clean-up and Identification of LSD. Microgram. 17(6):89.
iv. Japp M, et al. 1987. The Separation of Lysergide (LSD) from Related Ergot Alkaloids and Its Identification in Forensic Science Casework Samples. J For Sci. 32(4):933-940.
v. Morgan PL. 1982. Prep-TLC of LSD, DEA Laboratory Notes. Microgram. 15(1):6-10.
vi. Nichols HS, et al. 1983. Capillary GC Separation of LSD and LAMPA. Journal of High Resolution Chromatography and Chromatography Communications. 6:101-103.

e. Peyote
i. Bauer, B. E. The Compounds in Psychedelic Cacti. Accessed 030323
ii. Gambelunghe, C., Marsili, R., Aroni, K., Bacci, M., & Rossi, R. (2013). GC-MS and GC-MS/MS in PCI mode determination of mescaline in peyote tea and in biological matrices. *Journal of forensic sciences*, *58*(1), 270-278.
iii. Lum PW, Lebish P. 1974. Identification of Peyote via Major Non-Phenolic Peyote Alkaloids. Journal of the Forensic Science Society. 14(1):63-69.

f. Phencyclidine (PCP)
i. A Review of the Syntheses and Analyses of Phencyclidine and Its Analogs. Clandestine Laboratory Investigating Chemists 5th Annual Training Seminar. September 6-9, 1995.
ii. Alvarez JJ. 1977. Thiophene Analog of Phencyclidine. Microgram. 10(9):120-133.
iii. Bailey K, et al. 1976. Identification of Some Analogs of the Hallucinogen Phencyclidine. Journal of the Association of Official Analytical Chemists. 59(1):81-89.
iv. Lodge BA, et al. 1992. New Street Analogs of Phencyclidine. For Sci Intern. 55(1):13-26.
v. Shulgin A T, MacLean D E. 1976. Illicit Synthesis of Phencyclidine (PCP) and Several of Its Analogs. Clinical Toxicology. 9(4): 553-560.

g. Psilocyn-Psilocybin
i. Beug MW, Bigwood J. 1982. Quantitative Analysis of Psylocybin and Psilocin Levels in Twenty Species from Seven Genera of Wild Mushrooms in the Pacific Northwest. Journal of Ethnopharmacology. 5:271-285.
ii. Beug MW, Bigwood J. 1982. Variation of Psilocybin and Psilocin Levels with Repeated Flushes (Harvests) of Mature Sporocaps of Psilocybe Cubensis (Earle)Singer. Journal of Ethnopharmacology. 5:287-291.
iii. Gross, S. T. (2000). Detecting psychoactive drugs in the developmental stages of mushrooms. *J Forensic Sci*, *45*(3), 527.
iv. Koçak, A., De Cotiis, L. M., & Hoffman, D. B. (2010). Comparative study of ATR and transflection IR spectroscopic techniques for the analysis of hallucinogenic mushrooms. *Forensic science international*, *195*(1-3), 36-41.
v. Lee RE. 1985. A Technique for the Rapid Isolation and Identification of Psilocin from Psilocin/Psilocybin-Containing Mushrooms. J For Sci. 30(3):931-941.
vi. Leung AY, Paul AG. 1968. Baeocystin and Norbaeocystin: New Analogs of Psilocybin and Psilocin in Psilocybe baeocystis. Journal of Pharmaceutical Sciences. 57(10):1667-1701.
vii. Sarwar M, McDonald JL. 2003. A Rapid Extraction and GC/MS Methodology for the Identification of Psilocyn in Mushroom/Chocolate Concoctions. Microgram Journal. 1(3-4):177-183.
viii. Stamets PE, et al. 1980. A New Species and a New Variety of Psilocybe from North America. Mycotaxon. 11:476-484.
ix. Watling R. 1983. Hallucinogenic Mushrooms. Journal of the Forensic Science Society, 23:53-66.

## 14.4 STUDY QUESTIONS

LSD

1. What does LSD stand for?
2. Name two ergot alkaloids.
3. What is the common name and the taxonomical name (genus & species) for the mold found on wheat/rye and used to make LSD?
4. In what form is LSD usually sold/distributed?
5. How many micrograms are considered a full psychedelic dose of LSD?
6. What are starting materials for the synthesis of LSD?
7. What does prolonged exposure to UV light do to LSD?
8. Why are illicit LSD manufacturing laboratories easy to conceal?
9. Sunlight degrades LSD. (True or False)
10. Describe a rapid method for the detection of LSD on your hands.
11. What color tests can be used for the presumptive identification of LSD?
12. Why are LSD and chemically related compounds considered especially hazardous?
13. Why is manufacture of LSD so limited?
14. What are the primary pharmacological effects of LSD and mescaline?
15. List some medicinal uses (if any) of LSD.
16. Why are penalties associated with LSD sometimes not in terms of weight?
17. Why is better to analyze LSD sooner rather than later?
18. How might the presence of LSD on blotter paper be recognized?
19. Describe several matrices (forms or types of illicit products) in which LSD in found?
20. Has the cultivation of ergot fungi proven to be a reliable means of synthesizing lysergic acid?
21. Has ergocristine been observed to be used effectively as a precursor in illicit manufacture of a common hallucinogen? Explain.
22. Name two screening tests for LSD. Describe how they are used.
23. Will LSD be mostly charged or uncharged (neutral) at acidic pH?
24. At acidic pH, will LSD be more soluble in nonpolar (organic) solvents or polar (aqueous) solvents?
25. Why can a dirty injection port liner be particularly problematic for GCMS analyses of LSD?
26. Will infrared spectrometry distinguish d-lysergic acid diethylamide (LSD) from l-LSD?
27. Will infrared spectrometry distinguish LSD from iso-LSD?
28. Will several TLC systems and visualization techniques effectively distinguish LSD from its isomers? Explain.
29. What distinguishes the structure of LAMPA from LSD? How is this difference distinguished analytically?
30. Will the infrared spectrum of an optically pure isomer of LSD be the same as that of a racemic mixture?
31. If an infrared spectrum of a sample does not match a reference spectrum of LSD, to the degree required to establish identity, does that rule out that the analyte is, in fact LSD? Explain why this is or is not true.
32. If only a single piece of LSD blotter paper is received, is it necessary or appropriate to extract the entire exhibit in an attempt to recover sufficient analyte for instrumental analysis by GCMS? (Assume a 1 ul injection volume and a GC detection limit of 0.1 ug for LSD.) Describe an appropriate sample preparation procedure. |
33. You open a case that contains 150 different items, all suspected LSD, but the submission form warns that it is likely that not all items contain LSD. How would you decide which items to analyze?
34. What techniques may be used to distinguish LSD from iso-LSD? Is GC-MS an appropriate technique?
35. How does your laboratory's protocol direct you to report the quantity of material received, in a case where an exhibit consists of a single sheet of perforated LSD blotter paper? The blotter paper is ten square centimeters in size. Perforations divide the sheet into ten rows and ten columns, or 100 one square centimeter pieces. The paper weighs 2.20 grams.

MUSHROOMS - PSILOCYBIN / PSILOCIN and CACTUS - MESCALINE

1. What are the primary pharmacologically active constituents of *Psilocybe* mushroom and peyote cactus?
2. Is it possible to synthesize psilocybin? Why is this not typically done?
3. Describe the physical appearance of peyote cactus, and the associated 'buttons'.
4. What are some typical macroscopic characteristics of *Psilocybe* mushrooms?
5. How are *Psilocybe* mushrooms used?
6. How are peyote buttons used?
7. What is the major psychoactive component of the cactus Peyote?
8. What are the major psychoactive components found in *Psilocybe* Mushrooms?
9. What does a characteristic blue coloration on the stems or caps of *Psilocybe* mushrooms suggest?
10. How many species of mushrooms contain psilocybin and psilocyn? Does this impact their control?
11. Which color tests are useful for indicating the presence of psilocybin/psilocin in mushrooms?
12. Describe an effective procedure to extract psilocybin/psilocin from mushrooms for identification by GCMS.
13. Is the elevated temperature of the GC injection port sufficiently hot to cause degradation of psilocybin?
14. If so, what degradation results? If so, how should this be reflected in an analytical result based on GCMS data?"
15. Explain why an extraction of the mycelium of *Psilocybe* mushrooms for the purpose of identifying psilocybin or psilocin by GCMS may not be successful.
16. Why does the analysis of *Psilocybe* mushroom frequently involve derivatization?
17. Describe some extraction techniques which may be used for the analysis of psilocin and psilocybin. Include any precautions that must be taken.
18. What are the active ingredients of the Amanita Mushrooms?
19. Under what schedules are Peyote, Psilocybin and Psilocin controlled?
20. What is the control status of psilocybin mushroom spores?
21. How are mushrooms preserved after harvesting?
22. Where can psilocybin mushrooms be found?
23. What happens to psilocybin when analyzed by GC techniques?
24. What color tests can be used for the presumptive identification of psylocin/psylocybin?
25. Organize the following terms in order of the life cycle of a mushroom:
    a. spores germinate to become mycelium
    b. mycelium branch to form a compact hardened fungal mass (sclerotia)
    c. formation of fruiting bodies
26. What equipment might be related to psilocybin mushroom growing operations?
27. 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.Are all parts of the plant classified botanically as *Lophophora williamsii Lemaire*, whether growing or not, controlled as peyote? |
28. Describe an effective sample preparation procedure GCMS identification of the principal alkaloid found in peyote.
29. Describe an effective extraction procedure to recover the principal psychoactive component in peyote.
30. Describe an extraction technique which may be used to isolate the mescaline in peyote for analysis.
31. What is the principle active ingredient of peyote?
32. What color tests can be used for the presumptive identification of mescaline?
33. What is peyote? Describe its physical appearance.
34. Are peyote cacti available legally?

SALVIA

1. What is sage/diviner's sage?
2. What is the current local legal status of *Salvia divinorum*?
3. Describe the extraction process employed by Giroud for the analysis of fresh *Salvia divinorum* leaves.

PCP

1. PCP and its analogs contain how many rings in their structure?
2. Describe three effects of PCP.
3. What does PCP stand for?
4. In what sort of form(s) would you typically find PCP?
5. What makes PCP especially dangerous, compared with other drugs of abuse?
6. PCP was originally used as a large animal sedative. Why was it discontinued for use in this fashion?
7. Name a starting material needed for the synthesis of PCP.
8. What are the safety hazards that a chemist faces when synthesizing PCP? At what point in the synthesis does this hazard occur?

GENERAL

1. Define the term indole. Name some common indole hallucinogens.
2. Define the term catechol. Name some common catechol hallucinogens.
3. What is synesthesia?
4. What is the medicinal use of hallucinogens?
5. Hallucinogens may present in many physical forms. List examples of physical forms which could contain hallucinogens and the type of hallucinogen they might contain.
6. What botanical samples contain naturally occurring DMT?
7. How much ketamine needs to be taken to produce the full psychedelic experience?
8. Does ketamine have any legitimate uses? If so, what are they?
9. What is Yohimbe?

## 14.5 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 other hallucinogenic substances as available.

# 15 NOVEL PSYCHOACTIVE SUBSTANCES (NPS)

## 15.1 OBJECTIVES

- To become familiar the methods and procedures used to identify novel psychoactive substances (NPS) and their benefits and limitations.
- To become familiar with the legal status of NPS.
- To demonstrate analytical protocols on “casework” type samples.

## 15.2 TOPIC AREAS

7. 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
8. 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
9. Substituted phenethylamines
    a. Dimethoxyamphetamines
    b. Dimethoxyphenethyamines
    c. Trimethoxyamphetamines
    d. 2C compounds
    e. Cathinones
    f. NBOMes
    g. NBOHs
    h. Mescaline
    i. Others
10. 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)
11. 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.

12. Opioids
    a. Nitazenes
    b. Fentanyl analogs
13. Benzodiazepines (BZDs)

## 15.3 READINGS

1. Required
    a. Bruni, A. T., Rodrigues, C. H. P., dos Santos, C., de Castro, J. S., Mariotto, L. S., & Sinhorini, L. F. C. (2022). Analytical Challenges for Identification of New Psychoactive Substances. *Brazilian Journal of Analytical Chemistry*, *9*(34), 52-78.
    b. Marin, M., Maximo, J., van Wijk, P., & DABCC, X. (2019). The evolution of designer benzodiazepines: challenges for detection and monitoring. *Clinical Laboratory News: Washington, DC*.
    c. Vandeputte, M. M., Van Uytfanghe, K., Layle, N. K., St. Germaine, D. M., Iula, D. M., & Stove, C. P. (2021). Synthesis, chemical characterization, and μ-opioid receptor activity assessment of the emerging group of “nitazene” 2-benzylbenzimidazole synthetic opioids. *ACS Chemical Neuroscience*, *12*(7), 1241-1251.
    d. Zawilska, J. B., & Wojcieszak, J. (2019). An expanding world of new psychoactive substances—designer benzodiazepines. *Neurotoxicology*, *73*, 8-16.
2. Suggested
    a. Abiedalla, Y., Almalki, A. J., DeRuiter, J., & Clark, C. R. (2021). GC–MS and GC–IR analysis of methylenedioxyphenylalkylamine analogues of the psychoactive 25X-NBOMe drugs. *Forensic Chemistry*, *23*, 100314.
    b. Almalki, A. J., Clark, C. R., & DeRuiter, J. (2019). GC–MS analysis of regioisomeric substituted N-benzyl-4-bromo-2, 5-dimethoxyphenethylamines. *Forensic Chemistry*, *14*, 100164.
    c. Bishop SC, McCord BR, Gratz SR, Loeliger JR, Witkowski MR. 2005. Simultaneous Separation of Different Types of Amphetamine and Piperazine Designer Drugs by Capillary Electrophoresis with a Chiral Selector. J For Sci. 50(2):1-10.
    d. Clark, C. R., Auburn University, & United States of America. (2019). Forensic Chemistry of Substituted N-Benzylmethoxy (N-BOMe) Phenethylamines: Isomeric Designer Drugs Related to 25I-N-BOMe. *Annotation*.
    e. Dal Cason TA. 1989. The Characterization of Some 3,4-Methylenedioxyphenylisopropylamine (MDA) Analogs. J For Sci. 34(4):928-961.
    f. Davidson, J. T., & Jackson, G. P. (2019). The differentiation of 2, 5-dimethoxy-N-(N-methoxybenzyl) phenethylamine (NBOMe) isomers using GC retention indices and multivariate analysis of ion abundances in electron ionization mass spectra. *Forensic Chemistry*, *14*, 100160.
    g. de Boer D, Bosman IJ, Hidvegi E, Manzoni C, Benko AA, dos Reys LJAL, Maes RAA. 2001. Piperazine-like compounds: A new group of designer drugs-of-abuse on the European market. For Sci Intern. 121(1-2):47.
    h. Heegel RA, Trigg JJ 2008. Different Approaches to the Separation of 1-Benzylpiperazine and 1-(3-Trifluoromethylphenyl)piperazine Found in Illicit Ecstasy Tablets. J. CLIC.19(3): 9-16.
    i. Kranenburg, R. F., García-Cicourel, A. R., Kukurin, C., Janssen, H. G., Schoenmakers, P. J., & van Asten, A. C. (2019). Distinguishing drug isomers in the forensic laboratory: GC–VUV in addition to GC–MS for orthogonal selectivity and the use of library match scores as a new source of information. *Forensic Science International*, *302*, 109900.
    j. Machado, Y., Coelho Neto, J., Lordeiro, R. A., Alves, R. B., & Piccin, E. (2020). Identification of new NBOH drugs in seized blotter papers: 25B-NBOH, 25C-NBOH, and 25E-NBOH. *Forensic Toxicology*, *38*, 203-215.

k. Manchester, K. R., Lomas, E. C., Waters, L., Dempsey, F. C., & Maskell, P. D. (2018). The emergence of new psychoactive substance (NPS) benzodiazepines: A review. *Drug testing and analysis*, *10*(1), 37-53.
l. Mesley RJ, Evans WH. 1970. Infrared Identification of Some Hallucinogenic Derivatives of Tryptamine and Amphetamine. Journal of Pharm. Pharmac. 22:321-332.
m. Neto, J. C. (2015). Rapid detection of NBOME's and other NPS on blotter papers by direct ATR-FTIR spectrometry. *Forensic science international*, *252*, 87-92.
n. Noggle FT, et al. 1991. Methods for the Analysis of 1-(3,4-Methylenedioxyphenyl)-2-Butanamine and N-Methyl-1-(3,4-Methylenedioxyphenyl)-2-Propanamine (MDMA). Journal of Chromatographic Science. 29:103-106.
o. NPS Discovery Monographs.
p. Ntoupa, P. S. A., Papoutsis, I. I., Dona, A. A., Spiliopoulou, C. A., & Athanaselis, S. A. (2021). A fluorine turns a medicinal benzodiazepine into NPS: the case of flualprazolam. *Forensic Toxicology*, *39*, 368-376.
q. 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.
r. Soine WH, et al. 1983. Differentiation of 2,3-Methlyenedioxyamphetamine from 3,4-Methylenedioxyamphetamine. J For Sci. 28(2):386-390.
s. Strano Rossi, S., Odoardi, S., Gregori, A., Peluso, G., Ripani, L., Ortar, G., ... & Romolo, F. S. (2014). An analytical approach to the forensic identification of different classes of new psychoactive substances (NPSs) in seized materials. *Rapid Communications in Mass Spectrometry*, *28*(17), 1904-1916.
t. 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.
u. Vandeputte, M. M., Krotulski, A. J., Walther, D., Glatfelter, G. C., Papsun, D., Walton, S. E., & Stove, C. P. (2022). Pharmacological evaluation and forensic case series of N-pyrrolidino etonitazene (etonitazepyne), a newly emerging 2-benzylbenzimidazole 'nitazene'synthetic opioid. *Archives of Toxicology*, *96*(6), 1845-1863.
v. Vandeputte, M. M., Krotulski, A. J., Papsun, D. M., Logan, B. K., & Stove, C. P. (2022). The rise and fall of isotonitazene and brorphine: two recent stars in the synthetic opioid firmament. *Journal of Analytical Toxicology*, *46*(2), 115-121.
w. Wallach, J., & Brandt, S. D. (2018). Phencyclidine-based new psychoactive substances. *New Psychoactive Substances: Pharmacology, Clinical, Forensic and Analytical Toxicology*, 261-303.


## 15.4 STUDY QUESTIONS

1. How are synthetic cannabinoid agonists scheduled in the state of Washington? In Federal law?
2. Which cannabimimetic agents and synthetic cannabinoids are Schedule 1 substances in the WAC 246-945-051? Are the same substances included in RCW 69.50.204? Are they listed in Title 21 Code of Federal Regulations (CFR) Part 1300 to end (21CFR§1308)?
3. 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?
4. What is the legal definition of an analog? What is our policy for reporting analogs?
5. What are the 2C compounds? Are they controlled substances?
6. What are NBOMes? Are they controlled substances?
7. What are NBOHs? Are they controlled substances?
8. What analytical challenges exist for the identification of NBOH compounds?
9. What is the current legal status of nitazene compounds?

## 15.5 PRACTICAL EXERICES

1. Analyze any Ecstasy/Molly tablets or capsules available. What compounds were present in these tablets?
2. If available, analyze a synthetic cannabinoid sample.
3. Analyze available substituted phenethylamine training samples and/or reference materials. Can available equipment distinguish between the compounds? Does derivatization assist with distinguishing these compounds?
4. Analyze available nitazene compounds.

# 16 OPIOIDS

## 16.1 OBJECTIVES

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

## 16.2 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. Colombia
    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 Chemical 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 abused 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. Legitimate pharmaceutical preparations may be encountered as injectable liquids, time release patches, oral lozenges (lollipops), oral sprays or inhalers.
iv. Illicit preparations can include powders and pressed tablets
v. Schedule II
vi. 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. Fentanyl analogs
a. Fentanyl core with substitutions

b. Isomers
c. Control

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. Schedule IV

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 a large proportion 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 items 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.

x. Isomers generally need to be distinguished by retention time comparison to in-house reference materials. Elution order or separation of isomers is dependent on GC column functionality. Consider using a combination of different functionality columns to strengthen the retention time comparison.

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. Strength of this technique may be limited for isomers and needs to be coupled with retention time data.
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 CH2Cl2 and the organic layer is back extracted with water. The aqueous layer is basified with NaHCO3 and extracted with pentane or hexane. Do not use a strong base such as NaOH or NH4OH 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 CH2Cl2 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 CH2Cl2 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 CH2Cl2 to remove the acetaminophen. The resulting aqueous solution can be made basic with the addition of NH4OH and then extracted with pentane. Acetaminophen is less soluble in pentane than in CH2Cl2.
    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 NaHCO3 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 effective 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.

## 16.3 READINGS

1. Required
    a. Adair AR, et al. 1983. The ANOR (Alternate Non-aqueous Organic Ratio) Extraction Procedure. Microgram. 16(1).
    b. Borka L. 1988. The Polymorphism of Heroin and its Forensic Aspects. Acta Pharm. Suec. 14:210-212.
    c. Drug Identification Bible. Amera-Chem, Inc (opioid related info)
    d. Zerell, U., Ahrens, B., & Gerz, P. (2005). Documentation of a heroin manufacturing process in Afghanistan. *Bulletin on Narcotics*, *57*(1-2), 11-31.
2. Suggested
    a. Analytical Profiles of the Narcotic Analgesics, CND Analytical, Inc., 1991.
    b. Chambers, S. A., DeSousa, J. M., Huseman, E. D., & Townsend, S. D. (2018). The DARK side of total synthesis: strategies and tactics in psychoactive drug production. *ACS chemical neuroscience*, *9*(10), 2307-2330.
    c. Clark CC. 1977. A study of Procedures for the Identification of Heroin. J For Sci. 22(2):418-428.
    d. DEA website (www.dea.gov).
    e. Fulton CC. 1969. Modern Microcrystal Tests for Drugs. New York: Wiley-Interscience.
    f. Kanamori, T., Okada, Y., Segawa, H., Yamamuro, T., Kuwayama, K., Tsujikawa, K., & Iwata, Y. T. (2023). Analysis of highly potent synthetic opioid nitazene analogs and their positional isomers. *Drug testing and analysis*, *15*(4), 449-457.
    g. Manura JJ. 1978. The Forensic Identification of Heroin. J For Sci. 23(1):44-56.
    h. Microgram, Vol.18, No. 8, August 1985 (describes 9:1 diethylether: diethylamine TLC system for separating heroin and acetylcodeine).
    i. Mills T. Roberson JC. 2006. Instrumental Data for Drug Analysis, 3rd Edition. Boca Raton(FL):CRC Press Taylor & Francis Group, Volumes 1-6.
    j. Moffat AC, Osselton MD, Widdop B. eds. 2004. Clarke's Analysis of Drugs and Poisons, 3rd ed. Vol. 1 & 2, London:Pharmaceutical Press.
    k. Morelato, M., Franscella, D., Esseiva, P., & Broséus, J. (2019). When does the cutting of cocaine and heroin occur? The first large-scale study based on the chemical analysis of cocaine and heroin seizures in Switzerland. *International Journal of Drug Policy*, *73*, 7-15.
    l. O'Connor ME. 1985. Separation of Heroin from Acetylcodeine. Microgram. 18(8).
    m. Raffa, R. B. (Ed.). (2014). *Kratom and other mitragynines: the chemistry and pharmacology of opioids from a non-opium source*. CRC Press.
    n. Saad, H. E. A., El-Neketi, M., & Badria, F. A. (2018). Forensic analysis of a confiscated illicit heroin sample. *Curr Res Bioorg Org Chem: CRBOC-105. DOI*, *10*.
    o. Saloom JM. 1983. Use of the ANOR Method for Separation of Propoxyphene from Propoyxphene & APC. Microgram. 16(1).
    p. The Merck Index. Rahway(NJ):Merck and Co., Inc.

## 16.4 STUDY QUESTIONS

1. How are fentayl-related substances scheduled/listed in Title 21 Code of Federal Regulations (CFR) Part 1300 to end (21CFR§1308)? Give an example of a fentanyl-related substance, including structure, of each category.
2. What are the challenges associated with the identification of fentanyl isomers?
3. Discuss safety concerns associated with fentanyl and related substance. Include PPE, evidence packaging, and general safety considerations.

4. List a natural, semi-synthetic, and synthetic opiate compound.
5. What is the scientific name (i.e., genus/species) of the plant that produces opium?
6. What are the major chemical constituents of opium?
7. What are the major alkaloids found in the opium poppy
8. How does an opium poppy differ from an ornamental poppy?
9. Is heroin a naturally occurring alkaloid in *Papaver somniferum*?
10. What are the five natural opiates from *Papaver somniferum*?
11. Briefly describe the typical diluents/adulterants found in heroin in your jurisdiction.
12. Why is heroin also known as 'diacetylmorphine'?
13. What does the presence of papaverine and noscapine in a heroin sample signify?
14. What makes heroin more pharmacologically potent than morphine?
15. What compounds must be identified to report that a sample contains opium?
16. What are some common excipients and diluents found in heroin mixtures?
17. Distinguish the terms “narcotic” and “drug.”
18. What is the difference between opiates and opioids?
19. Name three opiates and three opioids.
20. How is opium used?
21. How is heroin used?
22. What are the most important alkaloids found in opium?
23. To what does the street drug term “speed ball” refer?
24. What areas of the world are typically associated with the cultivation of opium and the synthesis of heroin?
25. What does the color of opium signify?
26. Briefly describe the extraction and purification process of the alkaloids from opium.
27. Describe how heroin is synthesized.
28. How is opium obtained from the poppy plant?
29. Briefly describe the acetylation process of morphine to heroin
30. Briefly describe the possible products in heroin if the acetylation is incomplete or if acetylation is conducted on opium directly without isolation of the alkaloids
31. What chemical markers can be used to link two samples as to originating from the same source?
32. Under local legislation what chemicals are controlled that could be used in the manufacture or production of the opioids.
33. Under International Treaties what chemicals are controlled that could be used in the manufacture or production of the opioids.
34. Where is the greatest amount of opium produced?
35. Where is the greatest amount of heroin produced?
36. Give a practical example where a counterfeit opioid may appear in a seemingly legitimate pharmaceutical product.
37. Can opium alkaloids be found in juvenile opium plants or in old dried husks?
38. Draw the chemical structures of morphine, codeine, diacetylmorphine, acetylcodeine, thebaine, oxycodone, hydromorphone, pethidine, fentanyl, methadone.
39. What are the typical doses and duration of action of the above and when are they prescribed?
40. What chemical derivative(s) of morphine result(s) when it is reacted with acetic anhydride? With acyl chloride?
41. Is Oxycontin (oxycodone) derived from opium or morphine?
42. Is methadone derived from opium or morphine?
43. Is codeine derived from opium or morphine?
44. Is fentanyl derived from opium?
45. Is meperidine derived from opium?
46. Does Oxycontin (oxycodone) have similar physiological effects as opium narcotics?
47. Does methadone have similar physiological effects as opium narcotics?
48. What consequence may result if a strong base is used to extract a sample suspected to contain morphine? Heroin?
49. What process gives rise to O-6-Monoacetylmorphine?

50. Is morphine an amphoteric drug? If so, how does that impact it's preparation for analysis by GC-MS?
51. At acidic pH, basic drugs are mostly charged and more soluble in polar solvents (e.g. water). True or false?
52. At basic pH, are basic drugs mostly uncharged and more soluble in nonpolar solvents (e.g. organic) True or false?
53. What should be used to extract an amphoteric drug having a pKa of 8.02 and 9.85 from an aqueous layer into an organic layer?
54. What considerations are incorporated in GC the temperature program used when analyzing heroin? (HINT: consider diluents, adulterants and retention indices )
55. Discuss extraction schemes for heroin such as methanol, chloroform and ionic binding that are useful for extracting trace amounts of heroin from different matrices.
56. How can hydration of codeine or oxycodone affect analysis and data interpretation?
57. What are the polymorphs of heroin base? How can these affect analysis and data interpretation?
58. What common compounds are present with heroin in a syringe? Discuss breakdown products of heroin.
59. In your jurisdiction, what are some common non-controlled opiates?
60. Do laws in your jurisdiction define threshold quantities specific for heroin? What is the lowest threshold quantity defined for heroin in your jurisdiction?
61. In your jurisdiction, what distinguishes legal control of morphine vs. heroin?
62. Review opiates in the UCS (RCW 69.50). Why are some opiates listed in more than one schedule?
63. What processes, discoveries, or inventions have increased the addiction liability of opiates and opioids?
64. What is the main medical use of opioids?
65. Which class of opium alkaloids is most addictive?
66. What is the source of the vinegar-like odor that frequently emanates from black tar heroin?
67. Why do Columbian poppy farmers collect the opium as liquid latex rather than the gum?
68. A weak spectrum of heroin on a GC MS is observed. What further tests could be performed, including extractions?

## 16.5 PRACTICAL EXERCISES

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

# 17 PHARMACEUTICALS & LEGEND DRUGS

## 17.1 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, eg Valium - Diazepam.

## 17.2 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 (para-, meta-, and ortho-fluorofentanyl)
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.
iv. Reporting when pharmaceutical identification is inconsistent with other analytical results.

## 17.3 READINGS

1. Required
   a. Drugs.com Pill Identifier
   b. Jufer-Phipps, R. A., & Levine, B. S. (2020). Benzodiazepines. *Principles of forensic toxicology*, 317-331.
   c. Nguyen, L., Evans, A., Frank, G., Levitas, M., Mennella, A., & Short, L. C. (2022). Genuine and counterfeit prescription pill surveillance in Washington, DC. *Forensic science international*, *339*, 111414.
   d. United Nations Office on Drugs and Crime (UNODC). (2012). Recommended Methods for the Identification and Analysis of Barbiturates and Benzodiazepines under International Control
2. Suggested
   a. Analytical Profiles of Barbiturates and Other Depressants. CND Analytical, Inc. 1991.
   b. Analytical Profiles of the Benzodiazepines. CND Analytical, Inc. 1989.
   c. Analytical Profiles of the Narcotic Analgesics. CND Analytical, Inc. 1991Cole M. 2003.The Analysis of Controlled Substances: A Systematic Approach. Wiley.
   d. Drug Identification Bible. Amera-Chem, Inc.
   e. Fleischer D. 1976. Screening Tests for Diazepam. Microgram. 8(8):114 – 115
   f. López-Muñoz, F., Ucha-Udabe, R., & Alamo, C. (2005). The history of barbiturates a century after their clinical introduction. *Neuropsychiatric disease and treatment*, *1*(4), 329-343.
   g. Mills III T, Roberson J C. Instrumental Data for Drug Analysis, 3rd ed. Volumes 1- 6.
   h. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
   i. Miller PM. 1980. Extraction and Identification of 1,4 Benzodiazepines. Microgram. 13(6):104-106.

j. Noggle F T, Clark CR. 1979. Identification of Some Benzodiazepines of Forensic Interest. J AOAC. 62(4):799 - 807.
k. Popescu, A. M., Radu, G. L., Onisei, T., Raducanu, A. E., & Niculae, C. G. (2014). Detection by gas chromatography-mass spectrometry of adulterated food supplements. *Rom Biotechnolog Lett*, *19*(4), 9485-92.

## 17.4 STUDY QUESTIONS

1. Why are tablets sometimes treated differently than other drugs of abuse in legislation?
2. Why is the analysis of pharmaceutical tablets typically treated differently than analysis of other drugs of abuse (e.g. cocaine or methamphetamine)?
3. Name a presumptive test for pharmaceutical preparations that cannot be used for any other type of drug of abuse.
4. What are the trade names of the following substances: Diazepam, Flurazepam, Lorazepam, Alprazolam?
5. What are the two main classes of sedative-hypnotics?
6. What are the two types of depressants (legal use of the term depressant) and what are their effects?
7. Name several reliable reference sources useful for providing descriptions of physical appearance, morphological characteristics, and markings found on (a) pharmaceutical preparations and (b) illicit materials.
8. What is the forensic significance of the principal constituent found in Vicks brand inhalers?
9. Barbiturates are derived from what compound?
10. How are barbiturates categorized? What are the categories?
11. Name three barbiturates.
12. In the US, barbiturate drugs typically have been replaced by other pharmaceutical preparations. Why?
13. What two barbiturates are found in Tuinal?
14. Methadone is typically used in the treatment of heroin addicts. Why?
15. The chemical structure of Fentanyl does not resemble common constituents of opium. Why is it considered an opioid?
16. There are at least four different legal forms of medical fentanyl marketed in the US. Describe the different forms. Provide names of products for each form.
17. Assuming that Fentanyl is on the order of 100 times more potent than morphine; and 3-Methylfentanyl is estimated to be about 1000 times more potent than morphine; and Carfentanyl is about 10,000 times more potent than morphine.
    How likely is it that these substances will be detected and identified by GCMS, using your laboratory's routine sample preparation and analysis procedures for an unknown powder suspected to contain heroin?
18. What steps can be taken to ensure that each of these analytes will be detected and identified using GCMS? Assume powders are homogeneous mixtures for the purpose of this question. (Is this a realistic assumption for street samples?)
19. List three medical uses of benzodiazepines.
20. Does your laboratory's case acceptance policy distinguish between substances legislatively controlled for possession as compared to those controlled only for sales or distribution?
21. Since hydromorphone is amphoteric, will it be water soluble in strong acid or strong base?
22. How can time-release formulations be best prepared for injection into a GCMS?
23. Give several examples of routinely encountered drugs or pharmaceuticals that will not be identified conclusively by GCMS analysis alone. What additional analytical techniques will facilitate identification of the controlled substances present in them?
24. Describe how to prepare a sample for analysis by GCMS, where the exhibit consists of one tablet containing 250 mg of acetaminophen and 5 mg of hydrocodone.
25. A clear viscous liquid is submitted for analysis. What extraction scheme and tests would you conduct? If using a GC/MS what parameters are considered? (hint: MeOH, add one drop of hexane and the MeOH is insoluble in aqueous)

26. A reddish orange liquid is submitted. What could it be? What extraction scheme and tests would you perform?
27. A clear liquid is submitted. Weight is crucial. How would you weigh the item and present a scheme of analysis.
28. A coated tablet is submitted, the officer suspects MDMA. What is your initial suspicion?
29. A non-coated tablet is submitted having a design on one side. Color tests are negative. What additional tests are performed?
30. What appears to be a crushed tablet is submitted for testing. What extraction, tests and other considerations are deliberated?
31. Under what trade names was methaqualone sold?
32. What class of sedative-hypnotics is the most widely used in the United States?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.
33. Discuss the pharmaceutical forms of fentanyl. What are fentanyl analogs? What are the safety concerns regarding fentanyl and analogs?
34. Which of the stereoisomers of propoxyphene is controlled? How would you analyze propoxyphene? How would you report propoxyphene?
35. Discuss analytical challenges in the analysis of barbiturates.
36. Discuss analytical challenges in the analysis of antibiotics.
37. 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.
38. What are current common counterfeit pharmaceuticals containing fentanyl? What are they meant to mimic? What are legitimate pharmaceutical preparations containing fentanyl?
39. How would you report a pharmaceutical identifier that is inconsistent with other analytical results?
40. Give examples of five FDA approved benzodiazepines and five non-approved benzodiazepines.
41. How would you document pharmaceutical identifiers for the following:
    a. Tablets or capsules
    b. Sealed blister packs
    c. A sealed foil package containing a Suboxone film or a fentanyl transdermal patch
    d. An injectable vial labeled to contain “Morphine Sulfate Injectable”

## 17.5 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. Analyze a Suboxone film.

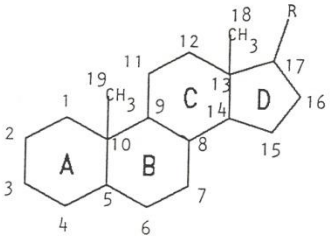
# 18 STEROIDS AND OTHER PROHIBITED DOPING COMPOUNDS

## 18.1 OBJECTIVES

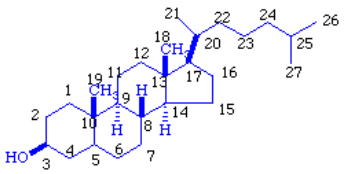
- To become familiar with the chemical structures of anabolic steroids, their general properties and chemical characteristics.
- To become familiar with other prohibited doping compounds which may be encountered in casework.
- To become familiar with the methods and procedures used to identify anabolic steroids and their benefits and limitations.
- To demonstrate analytical protocols on “casework” type samples.

## 18.2 TOPIC AREAS

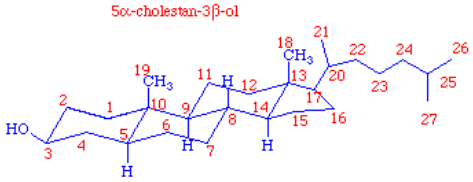
1. Legal Considerations
    a. RCW 69.50.208
    b. WAC 246-945-054
    c. According to the DEA there are over 100 different types of anabolic steroids that have been developed, and each requires a prescription to be used legally in the United States.
    d. Exceptions: “…does not include an anabolic 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:



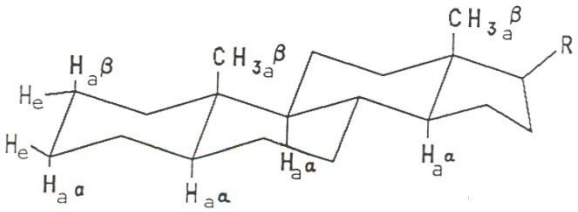

    b. The numbering system for substituents attached to the rings is:




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 degrees 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 degrees 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 item 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 READINGS

1. Required
   a. Berneira, L. M., Poletti, T., de Freitas, S. C., da Silva, C. C., Ortiz, R. S., & de Pereira, C. M. P. (2022). Extraction and analytical approaches in the forensic evaluation of anabolic androgenic steroid formulations. *Wiley Interdisciplinary Reviews: Forensic Science*, *4*(3), e1437.
   b. Chiong D M, Consuegra-Rodriguez E, Almirall JR. 1992. The Analysis and Identification of Steroids. J For Sci. 37(2):488-502.

c. da Justa Neves, D. B., & Caldas, E. D. (2017). GC–MS quantitative analysis of black market pharmaceutical products containing anabolic androgenic steroids seized by the Brazilian Federal Police. *Forensic science international*, *275*, 272-281.
d. Lurie, I. S., Sperling, A. R., & Meyers, R. P. (1994). The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC. *Journal of forensic sciences*, *39*(1), 74-85.
e. RCW 69.50


2. Suggested


a. Analytical Profiles of Anabolic Steroids, Vol. 1-2, CND Analytical, Auburn, Alabama.
b. Keller RJ, ed. 1986. Sigma Library of FTIR Spectra, Vol. 1-2. St. Louis(MO):Sigma Chemical Company.
c. Mills III T, Roberson J C. Instrumental Data for Drug Analysis, 3rd ed. Volumes 1- 6.
d. Moffat, A.C. Clarke's Isolation and Identification of Drugs. London: Pharmaceutical Press.
e. 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.


## 18.4 STUDY QUESTIONS

1. What is the difference between an anabolic steroid and a corticosteroid?
2. What functional groups are common to most anabolic steroids?
3. All natural steroids are chemically derived from what chemical?
4. Describe the differences between the steroid classes.
5. Steroids are typically found in what form?
6. What are the most commonly abused steroids?
7. What is (usually) the desired effect when using steroids?
8. Steroids are only illegal in the EU under certain circumstances. What are those circumstances?
9. In the United States, how are steroids scheduled?
10. What is HGH?
11. What are the special difficulties encountered when analyzing HGH or similar substances?
12. What are the three most common dosage formulations of steroids?
13. What determines the lipid solubility of a steroid?
14. What factors complicate the analysis of steroids by GC?
15. In the IR analysis of steroids what can be problematic?
16. 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?
17. What compounds other than anabolic steroids would be considered prohibited doping compounds?

## 18.5 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.

# 19 SEIZED DRUG CASE APPROACH

## 19.1 OBJECTIVES

- To become familiar with seized drug analysis case approach.
- To become familiar with some specific case approach requirements.
- To become familiar with note taking and documentation for seized drug anaylsis.
- To become familiar with interpretation of data for seized drug anaylsis.

## 19.2 TOPIC AREAS

1. Seized drug analysis case approach
    a. Secure evidence while it's in your possession.
    b. Review the RFLE to verify what testing was asked to be performed and that the evidence received matches that listed.
    c. Consider the appropriate PPE for handling the evidence.
    d. Contamination prevention measures
        i. Only handle and open one piece of evidence at a time.
        ii. Cleaning work surface
        iii. Disposable bench cover
        iv. Change gloves as needed
        v. Use disposable utensils and glassware when possible. Clean reusable glassware and utensils thoroughly.
    e. Evaluate the evidence packaging. Are the seals intact and appropriate? Document as you open the evidence and evaluate every layer of packaging?
    f. Assess the evidence. Is it a solid, liquid, gas? Does it look like a drug or something else? Is the evidence something we do not accept for analysis – syringes or sharps? Are there safety hazards such as sharp edges or broken glass?
    g. Qualitative versus quantitative examination
    h. Order of analysis – destructive vs. non-destructive testing.
    i. Variable nature of materials received means no single approach or set of methods will adequately address all contingencies
    j. Approach needs to preclude false positives and false negatives.
    k. Limitations to the methods must be considered and reported as appropriate.
        i. Thermolability
        ii. Isomers
        iii. Resolution
    l. Conclusive identification of a seized drug 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 Materials Analysis Technical Procedures (MATP).
    m. Sufficient material for testing
        i. Limited amounts of material, especially residue
            1. Foil with burn marks
            2. Pharmaceuticals with low concentration of analyte
            3. Other types of residue
        ii. 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. This permission MUST be obtained before any testing commences.
        iii. Requirements for consuming a sample.
    n. Sampling versus sample selection.
    o. Total number of items 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

p. Weighing and count estimation for tablets
i. Gross weight versus net weight
ii. Average tablet weight

q. Evidence repackaging considerations
i. Preserving evidence
ii. Safety considerations
1. Fentanyl
2. Sharps/broken glass
iii. Separating packaging from drug material for testing by other disciplines

r. Specific considerations for Seized Drugs
i. Analysis of Psilocybin-Containing Items
Psilocybin converts to psilocyn by thermal dephosphorylation when analyzed by gas chromatography. The analytical scheme should account for this limitation by the use of derivatization to protect the phosphate group for GC/MS or by GC/MS data without derivatization coupled with HPLC, TLC, or selective microchemical tests which do not result in the dephosphorylation of psilocybin. If an analytical scheme is selected that does not account for the dephosphorylation of psilocybin, the conclusions and report must reflect the item contains psilocybin and/or psilocyn.
ii. Samples where only one isomer is controlled
There are multiple examples of drugs in which one isomer is controlled and the other is not. Confirmation of the isomeric form may require consideration to analytical approach or may not be possible. Reporting should address times when it is not possible to confirm the isomer.

2. Data collection
a. Types of data included in case file versus case record
b. Documentation requirements for data

3. Data interpretation
a. GC/MS
i. Appropriate blanks
ii. Interfering compounds
iii. Sufficient resolution
iv. Sufficient ions present
v. Appropriate retention time

b. FTIR
i. Sufficient resolution
ii. Mixtures

c. GC/FID
i. Appropriate blanks
ii. Interfering compounds
iii. Sufficient resolution
iv. Appropriate retention time

d. HPLC
i. Appropriate blanks
ii. Interfering compounds
iii. Sufficient resolution
iv. Appropriate retention time
v. UV Spectrum

e. Microcrystalline Tests
i. Appropriate blanks
ii. Mixtures

f. Other analytical techniques
4. Notetaking
    a. Description of evidence
        i. Thorough description of what is received
        ii. Indicate what will be analyzed and what is not analyzed
        iii. Pharmaceutical descriptors
    b. Sample selection decisions
    c. Testing conducted
        i. Weight
            1. Balance used
            2. Gross weight vs. net weight
            3. Units of measurement
        ii. Pharmaceutical reference searching
        iii. Microcrystalline testing
            1. Microscope used
            2. Reagents
            3. Blanks
            4. Results of sample testing
            5. Interpretation of results/pictures/sketches/description of crystals
        iv. Stereomicroscopic examination
        v. Chromatographic testing
            1. Sample preparation
            2. Equipment used
            3. Vial positions
            4. Blanks
            5. Results
        vi. FTIR
            1. Extraction/sample preparation
            2. Equipment used
            3. Results
        vii. Other analytical techniques
        viii. Additional information in notes
            1. Safety information
            2. Repackaging information
    d. Conclusion(s)

## 19.3 READINGS

1. Required
    a. ASTM 2548 (current edition). Standard Practice for Sampling Seized Drugs for Qualitative and Quantitative Analysis.
    b. ASTM E3239 (current edition). Standard Practice for Identification of Seized Drugs.
    c. Brettell, T. A., & Lum, B. J. (2018). Analysis of drugs of abuse by gas chromatography–mass spectrometry (GC-MS). *Analysis of Drugs of Abuse*, 29-42.
    d. Materials Analysis Technical Procedures, Seized Drug chapter.
    e. SWGDRUG (2019). Construction of an Analytical Scheme Supplemental Document SD-7
2. Suggested
    a. Saferstein, R. (2004). Forensic science handbook, Vol. II, 2nd Ed.: Prentice Hall. Forensic Identification of Controlled Substances chapter.

## 19.4 STUDY QUESTIONS

1. Discuss the considerations to be given regarding the number of items to be analyzed.

2. Five items were received in a single suspect, possession case (a vial containing syringe rinse, three bindles of similar looking white powder, and a balloon of brown sticky substance). What items would you analyze? What if the first two items you analyze are non-controlled?
3. Under what circumstances can the packaging material be used as one of the required tests for seized drugs?
4. You receive a phone call from an officer with a syringe. What do you tell him/her?
5. What documentation is required for Pharmaceutical Identification?
6. 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?
7. Discuss which cannabis cases must be analyzed by quantitation and which can be analyzed qualitatively.
8. When is a Pharmaceutical Identification insufficient to be used as one of the required tests for seized drugs?

## 19.5 PRACTICAL EXERCISES

1. Your trainer will provide you with mock case data. Interpret the data and discuss the limitations with the data and conclusions that can be reached based on these limitations.
2. You will be provided with a series of mock cases. Take notes and analyze as you would a real case. Reports for these cases will be covered in the Report Writing chapter which may be worked simultaneously with this chapter.

# 20 SEIZED DRUG REPORT WRITING

## 20.1 OBJECTIVES

- To become familiar with specific reporting requirements for seized drug casework.
- To become familiar with writing reports in the seized drug service in LIMS-plus.

## 20.2 TOPIC AREAS

1. QOM Requirements for reports
2. MATP requirements for reports
    a. Conclusive results
        i. Positive identification of seized drugs
        ii. Negative identification of seized drugs
    b. Inconclusive results
        i. Must state why the result is inconclusive
    c. Evidence received versus evidence tested
    d. Amount/quantity of material
    e. Tests conducted
    f. Other information
3. LIMS workflow
    a. Itemization
    b. Data entry in LIMS Seized Drug Module
    c. Remarks
4. Analyst review of the report

## 20.3 READINGS

1. Required
    a. CLD LIMS Manual
    b. CLD Quality and Operations Manual
    c. LIMS Seized Drug Module report writing guide
    d. Materials Analysis Technical Procedures

## 20.4 STUDY QUESTIONS

1. Describe a situation in which a qualified conclusion would be reported for seized drugs? What must be said in the report?
2. What information must be included in the report for the description of an intact tablet with legible markings?
3. How would you convey your results if you did not identify a specific stereoisomer?
4. Should you report the schedule or status of a controlled substance?
5. What circumstances require the reporting of Measurement Uncertainty?
6. What are the reporting requirements for analytical results that are inconsistent with a Pharmaceutical Identifiers?
7. How would you report a tablet count determined by calculation including weight of the whole and weight of a subset?

## 20.5 PRACTICAL EXERCISES

1. Prepare reports in the LIMSCLD Test Environment for the mock cases worked in the Seized Drug Case Approach chapter.

# 21 TECHNICAL REVIEW

## 21.1 OBJECTIVES

- To become familiar with the requirements of technical review of seized drug casework.

## 21.2 TOPIC AREAS

1. QOM requirements
2. Technical requirements
    a. Necessary documentation present
    b. Analytical methods are appropriate
    c. Sample selection decisions appropriate and documented
    d. Data supports the conclusion
    e. Quality control appropriate and documented
3. Documenting technical review
4. Administrative review may be handled by the scientist conducting technical review.
5. Technical review dispute resolution procedure

## 21.3 READINGS

1. Required
    a. Materials Analysis Technical Procedures
    b. CLD LIMS Manual
    c. CLD Quality and Operations Manual

## 21.4 PRACTICAL EXERCISES

1. Your trainer will provide you with several mock cases to technically review. Discuss your findings and the process with your trainer.
2. You will be assigned a number of actual seized drug cases for review. Conduct your review and take notes of any observations, questions or concerns about the case. The case will be officially reviewed by another qualified scientist. Once their review is complete, discuss your observations, questions, or concerns about the case with the official reviewer. You and the official reviewer will discuss any concerns with the case with the case analyst.