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1 Examination of Controlled Substances, Dangerous Drugs and Related Compounds

Scope

- To establish standards for reporting the results from the analysis of controlled substances and dangerous drugs, clandestine laboratory chemicals and other substances examined by chemists in the APD Forensic Chemistry section. This manual is not all-inclusive, and it is not possible to anticipate every situation that may arise or to prescribe a specific course of action for every case. Therefore, the analyst must exercise good judgment based on experience and common sense, especially when processing evidence. However, any portion of a procedure explicitly qualified by use of the word “shall,” may not be modified for use in casework without prior approval by the Technical Leader.

Safety

- Review the Forensic Science Division and Sectional manuals as needed.
- For hazardous materials or possible hazardous materials, use appropriate personal protective equipment.
- Material Safety Data Sheets are available if additional information is needed about a material.
- Use proper lifting techniques and caution when handling heavy items.
- Use caution and proper technique when using sharp instruments to cut into evidence packaging.

Procedure

- All observations and examinations shall be documented in the Laboratory Information Management System (LIMS).

Retrieval and Initial Examination of the Evidence

- Refer to the Forensic Science Division Standard Operating Procedures concerning retrieval, transfer, and handling of evidence.
- Separate (sub-divide) exhibits with multiple items if the contents appear to be different and they are to be analyzed.
- Care must be exercised to avoid cross-contamination if more than one item of evidence is open at the same time.
- A brief description of each item analyzed and its condition shall be documented in the case record.
- The use of abbreviations is acceptable as long as they have been defined.
- Logos and/or significant markings may be documented and compared with approved pharmaceutical reference literature (**Appendix B)**.
  - References used for comparison shall be documented in the case record.
  - Information found on untampered pharmaceutical packaging may be used as pharmaceutical identification.
  - References are recommended for tablets if no further analysis will be conducted.

- Bulk items that are to be altered such that it is no longer recognizable to the original description; photographs shall be taken of the original condition and attached to case record.
- If an item is used in its entirety during analysis it shall be documented in the case record.

Exam Counting Guidelines

- Items within a case may contain multiple samples. Number of samples shall be documented in the case record, and refers to the actual number of individual samples received (e.g., 10 tablets = 10; two baggies of marihuana = 2; 1 sheet of 25 squares of LSD = 25; baggie of 10 crack rocks = 1 item).
- If a sampling plan is used the number of individual samples that were analyzed shall be recorded in the case record as well as the report.
- Each spot test, instrumental examination; microscopic or visual examination shall be counted as one (1) examination each.
- Per item, preliminary pharmaceutical examinations will be counted as one (1) examination only, no matter how many dosages are received or how many pharmaceutical references are searched. For example, if 500 tablets are received in a particular item, only one (1) pharmaceutical examination can be counted and if several references are used, only one (1) pharmaceutical examination can be counted.

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Drug Item Reduction

- Drug Item Reduction allows for the analysis of key items within a case to maximize the resources of the laboratory. In every case, the most significant items in terms of quantity and penalty group are analyzed. This “rule of thumb” cannot address every drug case. Consideration must be given to the information provided in each request. This includes things such as the specific charges or types of offense, items unique to a single suspect, the statement of fact and examinations requested and the descriptions of evidence submitted as well as the chemist’s visual inspection of the items.
  - Syringes should only be analyzed if they are the only item in the case, or are tied to probable cause.
  - No further analysis is required for misdemeanor offense of intact, marked pharmaceutical preparations (e.g., tablets or untampered capsules) indicated as non-controlled or Penalty Group 3 or 4 preparations.
  - Plant material consistent with marihuana weighing less than 4oz.
  - Residues in drug paraphernalia, cigarettes or cigarette butts will not be analyzed when measurable quantities of the associated drugs are also included among the items submitted.
  - If items are not analyzed per this procedure, case record and report shall indicate this by a notation of “No Analysis”

Procedure for Weighing Sample

- Select the appropriate balance for the amount of sample to be weighed.

- If the estimated uncertainty is equal or larger than the weight, a more accurate balance shall be used.
- The balance used will be documented in the case record.
- A before analysis weight shall be documented:
  - Not including packaging (net).
    OR
  - Including packaging (gross).
    - If before analysis weight includes weight of packaging a tare weight shall be documented in order to calculate a net weight.
      - Document a tare weight when used
      - Tare weight shall be in same units as before analysis weight
      - If source of tare weight is not obvious, make notes describing how the tare was calculated.
      - Calculations involving weights will be done using the weights as they are recorded.
  - Conversion factors shall be 28.35g/oz or 454g/lb

- Documenting a net weight:
  - A net weight shall be documented in the case record for each item analyzed. Exceptions are listed in the Forensic Chemistry Section SOP's
  - In instances where statutory requirements or state sentencing guidelines designate weight thresholds, sufficient specimens will be weighed and analyzed to exceed the threshold.

- Documenting an after analysis weight
  - If after analysis weight is less than 0.01 grams, "Trace" may be documented.
  - If the entire sample is consumed in analysis, "0" should be documented.

Evidence Sampling Techniques (ISO 5.7.1)

- Sampling evidence is the most important initial step in forensic drug analysis. One must be sure that what is sampled is truly representative of the total population. The analyst must take into consideration the homogeneity (or lack thereof) among drug packaging (bags, packets, capsules, etc.) and its contents. Careful visual inspections and personal experience are essential in determining the proper sampling procedure.

- For cases in which sample selection (non-statistical sampling methods) is used, the report should state what was received, what was tested, and must be clear that the result/conclusion pertains to that which was tested (e.g., "100 baggies of white powder were received in item 1. Contents of six of the baggies were tested and found to contain cocaine.")
  - The gross weight of the entire item *may* be reported.
  - The weight of the samples actually tested **shall** be reported (e.g., "100 baggies of white powder with a total weight of 212.20 grams were received in item 1. **Contents of six of the baggies were tested and found to contain cocaine. The net weight of the contents of the six tested baggies was 13.60 grams**.")

- Within any sampling scheme, Statistical or Non-Statistical, if the first set of observations determines that more than one population is present, further samples from each population must be taken.

- **Single pharmaceutical tablets/capsules:**

  - Due to the unique physical identifiers present in pharmaceutical preparations, a consistent sample population can easily be determined. The thoroughness represented by the sampling scheme used for street drugs is not required for pharmaceutical preparations which are clearly visually consistent with each other.
  - If there is no reason to suspect tampering or counterfeiting, then one tablet or capsule may be analyzed to confirmation (report must state the number of items sampled for analysis and the weight of what was sampled).
  - If tampering is suspected, analyze dosage units utilizing the administrative, statistical, or non-statistical schemes.

- **For tablets or items that appear to not be homogeneous or that appear to be clandestinely manufactured:**

  - A statistical sampling plan may be used to select samples for confirmation.
  - Alternatively, a non-statistical method may also be used for exhibits containing clandestine tablets.
  - Information about the sampling plan shall be documented.
  - Where possible items should be grouped, subsampled, and analyzed by color, imprint, or other markings.

- **Non-statistical methods:**

  - For items where an inference to a population is ***not*** being made.

    - **All Items (Administrative)**
      - When all samples within an item are tested and individually confirmed no documentation of the sampling plan is necessary.

    - **Weight Thresholds**
      - A sufficient number of items may be selected to exceed the maximum applicable weight limit as delineated in the Texas Controlled Substances Act.
      - The selected evidence will be individually confirmed and separately identified.

- **Statistical sampling plan**

  - For items where an inference to a population ***is*** being made:

    - The statistical sampling plan is based on the hypergeometric distribution that will be used to determine the minimum number of items or samples to be tested and individually confirmed in order to prove that 90% of the total number of items in an evidence exhibit or population will be positive for the respective drug with a 95% confidence level.

- The hypergeometric model may be used for specimens with no significant markings or labels (e.g., the contents of plastic bags, bag corners, vials). The following table prescribes the minimum number of items randomly selected from a population to be tested.

	Required Number of Consecutive Postives
Number Samples	**90%**
5	**All**
6-7	**All**
8-10	**All**
11-13	**10**
14	**11**
15-16	**12**
17	**13**
18	**14**
19	**15**
20-26	**16**
27	**17**
28-29	**18**
30-31	**19**
32-37	**19**
38-39	**20**
40-48	**21**
49-58	**22**
59-69	**23**
70-88	**24**
89-109	**25**
110-159	**26**
160-279	**27**
280-939	**28**
940+	**29**

- Samples shall be marked such that data can be associated with each specific sample.
- If one or more negative samples(s) are encountered with the other samples containing a controlled substance, or if preliminary testing does not give consistent results, then testing the entire population or non-statistical sampling will be necessary.
- If all samples are not analyzed, the number of those that are fully analyzed will be recorded in the case record.
- For items consisting of samples that are obviously non-controlled such as gum, candy or vitamins, a single representative sample may be used.

- **Sampling for Quantitative Analysis**

  - Quantitative analyses require homogenized representative samples. Generally, a relatively large sample is homogenized with a mortar and pestle prior to taking two smaller samples required by the quantitative method to make the solutions. The remainder of the homogenized portion should be returned with the evidence in a suitably labeled plastic bag provided by the laboratory and clearly marked as a composite.
  - Large single sample items such as kilos of cocaine, either a core sample or combined samples taken from multiple locations will be used for the homogenizing process. This process shall be described in the case notes.
  - Items with multiple specimens may be analyzed qualitatively using either the statistical or non-statistical sampling plans. The multiple specimens will be individually quantitated and a value will be reported for each individual result.

- **Residue Samples**

  - Residues are samples which are either too small to be weighed accurately or that which remains after the bulk has been removed. Residues can be sampled by mechanical means (e.g., shaking or scooping) or chemical means (e.g., rinsing with solvent). Case record shall reflect the method by which the sample was removed.
  - When possible, a sample should be removed while leaving a portion of the residue intact.

Basic Analytical Scheme

- The basic analytical scheme for the analysis of suspected controlled substances, dangerous drugs and other related compounds consists of sample preparation and extraction or isolation procedures in various combinations with the following tests and instrumentation. The analyst must determine the appropriate sampling techniques, methods of recovery, extraction procedures and instrumental analysis to be used for identification of a compound on a case-by-case basis. When sample size allows, testing should be applied on separate samplings of the material. Minimum analysis requirements for confirmation of a controlled substance include:

  - One positive **confirmatory** test (either FTIR, GC/MS, or derivative procedure for GC-MS) and at least one *different,* **supplemental** positive test.

- **Supplemental** tests that may be used in combination with confirmatory tests include:

  - LC-MS
  - LC Retention Time
  - LC-Photodiode Array
  - GC-MS
  - GC retention time from a different instrument or column.
  - A derivative procedure on GC/MS.
  - FTIR (Confirmatory **or** reduced resolution)
  - UV/VIS
  - Pharmaceutical identification
  - Preliminary color test
  - TLC

- Alternatively, a combination of three of the following **supplemental** tests will also be acceptable:

  - LC-MS
  - LC Retention Time
  - LC-Photodiode Array or UV-Vis
  - GC retention time
  - Pharmaceutical identification
  - Modified Duquenois-Levine Test (Suspected hashish samples only)

- FTIR sample spectra of reduced resolving power, and spectra subtractions are not sufficient as the *confirmatory* test, and are only adequate as a *supplemental* test. Refer to FTIR section on interpretation of this manual.
- Microscopic identification is required, and at least one other positive test (Duquenois-Levine, GC-RT, GC/MS, or FTIR) is required to indicate the presence of THC and/or Cannabinoids in marihuana/plant samples, excluding seeds. The salt form or base form of the drug may be identified using FTIR or other scientifically accepted procedures.
- Each test shall be documented in the case record, including extractions and sample preparations used.

- **Negative or inconclusive results**

  - If a sample was subjected to the minimum amount of testing (listed below) and no controlled substance was identified, the sample will be reported as “No Controlled Substance Detected”.

  - The sample shall be subjected to a minimum of one confirmatory test (FTIR or GC-MS) and at least one ***different*** test, including:

    - LC-MS
    - LC-Photodiode Array
    - Splitless injection on a different GC-MS instrument or GC column.
    - FTIR
    - UV/VIS
    - Attempted derivatization of sample

- Analysts must use discretion and context clues when selecting appropriate analytical techniques. The techniques performed should take into account the nature of the sample, and its suitability for analysis with respect to instrumentation.

Quantitation

- Except for the below listed guidelines, drug items will not be routinely quantitated:
    - Samples from clandestine laboratories may be quantitated as needed for prosecution.
    - Samples to be released as training aids (dog dope) or department sanctioned narcotic reversal operations.
    - A special request from APD management for investigative purposes
    - Cocaine Hydrochloride and Heroin may be quantified for federally adopted cases upon request by the United States Attorneys' or Probation Office.

Concluding Examination and Return of the Evidence

- All original exhibits should be re-packaged per Forensic Science Division SOPs.
- Samples that are tested shall be clearly marked and repackaged separately from those not tested.

Plant Material

- Initially, all plant material shall be screened via stereomicroscopy for cystolithic hairs.
    - In the presence of cystolithic hairs or marihuana seeds, the analytical scheme for marihuana shall be completed.
    - In the absence of cystolithic hairs, a negative result from a Modified Duquenois-Levine Test or in conjunction with contextual clues from the evidence, samples shall be screened for controlled substances including, but not limited to Cathinones and Synthetic Cannabinoids.
    - Fungal material shall be screened as per the Mushroom or Peyote Procedures.

Marihuana, Hashish, and Tetrahydrocannabinols

Scope and Introduction

- To establish an analytical procedure for the examination of marihuana, hashish, and tetrahydrocannabinols.

Safety

- The plant material, and the dust and mold often present in botanical substances, may trigger allergic reactions. Susceptible personnel should take precautionary measures such as wearing masks, respirators, and gloves.

Equipment, Materials, and Reagents

- Microscope/Stereoscope
- Watch glass or microscope slide
- Hexane or other suitable solvent
- Duquenois Reagent
- Concentrated HCl
- BSTFA with 1% TMCCMS reagent

Macroscopic Identification

- Gross morphological characteristics that may be observed include the palmate arrangement of the leaflets, the pinnate leaf venation, the serrated edges of the leaflet, the buds (with or without seeds) and, if present, fluted stems and stalks. Mature stalks are considered those greater than one-quarter-inch in diameter. Stems, a support structure for another part of the plant such as a leaf or flower, are also fluted and may have hairs on the surface. Although not required for the identification of marihuana macroscopic identification can still prove useful.
- As a rule of thumb and common practice, stalks/stems less than one quarter of an inch in diameter are not considered mature and are considered to be usable marihuana that need not be culled from the sample.
- Due to the compressed or mutilated nature of many samples, many of these characteristics may not be discernible.
- Positive macroscopic examination results shall be recorded in the analytical notes as ‘consistent with marihuana”. A result is considered positive when sufficient characteristics are observed and are specified in the case notes. Negative observations may be recorded in a similar fashion.

Microscopic Identification

- Microscopic identification of the physical characteristics of marihuana constitutes a required examination for the confirmation of marihuana.
- View the sample at varying magnifications (approximately 10 – 40x) using a stereomicroscope.
- Leafy plant material should exhibit cystolithic hairs (”bear-claw” hairs) and glandular hairs on the upper side of the leaf, conical trichomes or filamentous hairs on the lower side of the leaf. Larger segments may also exhibit serrated edges with pinnate leaf venation.
- Seeds are coconut shaped, veined (with lacy markings) and have a ridge around the circumference.
- The observation of the presence of appropriate cystolithic hairs is sufficient for a positive test. The observation of additional characteristics is considered supportive.
- Positive microscopic examination results shall be recorded in the analytical notes as “consistent with marihuana” along with the characteristics observed.
- Negative observations will be recorded in a similar fashion.
- An inconclusive or negative determination may be made at the discretion of the analyst based on the overall appearance of the material.

Hashish, Oil Extracts, Ashes, Charred Material, or Residue

- Microscopically examine the substance for the presence of plant material.

- If the characteristics of marihuana **are not** found:

  - Suspend the sample in a suitable solvent.
  - Place the suspension a watch glass or microscope slide
  - Examine under appropriate magnification.
  - Observe the solubility of any resinous material and document any observations.
  - An inconclusive or negative determination may be made at the discretion of the analyst based on the overall appearance of the material.
  - Place suspected hashish sample into a GC vial (the same amount that would be normally used to for analysis by GC-MS.
  - Add enough BSTFA-TMCS reagent to dissolve the sample and allow to react for 15 minutes.
  - Fill the rest of the GC vial with chloroform after reacted.
  - Run sample on an AUTO method on the GC-MS.
  - At least one tetrahydrocannabinol, and at least two of the following: cannabinol, cannabidiol, or cannabichromene shall be identified if item is subject to federal reporting guidelines for "Hashish"

Modified Duquenois-Levine

- Extract sample into a suitable solvent (e.g., hexane, petroleum ether).
- Add approximately equal amounts of Duquenois reagent and concentrated HCl to extract.
- A positive reaction to the Duquenois portion is a purple color.
- Add sufficient $CHCl_3$ to form two discernible layers and mix.
- For a positive reaction to the Levine portion of the test, the bottom layer turns purple in the presence of cannabinoids.
- Positive results shall be indicated in the case record as "POS"
- Negative reactions shall be recorded in similar fashion.

Rapid Duquenois-Levine Procedure

- Place a small amount of plant material in a culture tube, add Duquenois reagent and concentrated HCl in approximately equal proportions. Observe a purple color. Add $CHCl_3$ and observe extraction of purple color into the $CHCl_3$ layer.
- Results must be recorded in the case record as "POS"
- Negative reactions may be recorded in similar fashion.

Gas Chromatography/Mass Spectrometry (GC/MS)

- GC/MS shall be performed if the Duquenois test is inconclusive/negative for a suspected marihuana sample.

Liquid Chromatography/Mass Spectrometry (LC/MS)

- Hashish, oil extracts, ashes, charred Material, or residue samples may be confirmed using a combination of LC-MS, LC-retention time, and a positive Modified Duquenois Levine Test.

Mushroom Procedure (Psilocybin/Psilocin)

Scope

- To establish a procedure for preparation and analysis of mushrooms.

Safety

- The plant material and the dust and mold often present in botanical materials may trigger allergic reactions, requiring susceptible personnel to take precautionary measures, such as wearing masks, respirators and gloves.

Equipment, Materials and Reagents

- Sonicator
- Vortex mixer
- Centrifuge
- Hot plate or other heating apparatus with magnetic stirring capability.
- Magnetic stir bar
- Mortar and pestle
- Reagents:
  - Fast Blue B Reagent
  - P-DMAB Reagent
  - Glacial Acetic Acid
  - NaOH
  - $CHCl_3$
  - Methanol
  - 0.2N H_2SO_4
  - Concentrated HCl

P-DMABA Test

- 1. Place a small piece of sample in test tube or spot plate and add reagent
- 2. A slow developing purple color is a positive test.

Weber Test

- Place sample in spot plate and add a few drops of reagent.
- A positive reaction for psilocin in the case notes if a red color forms after adding the Fast Blue B reagent, and if after adding HCl the color changes to blue.

Extraction Methods:

- Mushroom Extraction: Method 1
  - Grind 1-5 grams of dried mushrooms.

- Transfer powdered mushrooms to a beaker and add 30-50 ml of 10% acetic acid.
- Add magnetic stir bar, cover, and heat beaker on hot plate (medium heat) at a low boil with constant stirring.
- Filter or centrifuge mixture, discarding solids.
- Make aqueous solution basic with NaOH and extract to $CHCl_3$. Discard aqueous layer.
- Filter $CHCl_3$ and reduce volume with air.
- Use reduced $CHCl_3$ for UV and GC/MS

- Mushroom Extraction: Method 2

  - Grind 1-5 grams of dried mushrooms.
  - Add enough methanol to dampen mixture and grind again.
  - Squeeze mass with pestle and decant methanol through filter into centrifuge tube. Repeat process until 3-4 ml of methanol is collected.
  - Add ether to methanol at the ratio of 3 parts ether to 1 part methanol and shake. A white precipitate must form if psilocybin or psilocin is present.
  - Centrifuge and discard liquid.
  - Add 1-2 ml methanol to tube. Use spatula to dislodge precipitate and shake to re-dissolve in methanol.
  - Use Methanol extract for UV and GC/MS

- Mushroom Extraction: Method 3

  - Place a portion of the mushrooms in methanol.
  - Soak, vortex, or sonicate the extract.
  - Filter out the solids and concentrate the methanol extract.
  - Methanol extract may be used for UV, and TLC
  - GC/MS Clean up:
    - Option A
      - Add 3 ml to diethyl ether to methanol extract, agitate, yellow precipitate will form
      - Centrifuge the extract and discard the liquid
      - Wash the yellow precipitate with 2 ml diethyl ether
      - Centrifuge, discard liquid
      - Add 2-3 ml methanol and continue with desired tests
    - Option B
      - Evaporate Methanol extract to dryness
      - Add 1 ml H_2O, two drops of conc HCl and 2 ml $CHCl_3$
      - Vortex mixture for 2 minutes and centrifuge
      - Transfer aqueous layer to a clean test tube and add concentrated NH_4OH drop wise until the solution is basic check with pH paper, then add 0.5 ml $CHCl_3$ Vortex mixture and centrifuge.
      - Remove the $CHCl_3$ and dry by passing through anhydrous sodium sulfate.
      - Reduce volume of $CHCl_3$ with air and run on GC/MS

- Mushroom Extraction: Method 4

  - Place a portion of the mushrooms in 0.2 N H_2SO_4. Soak at least 30 minutes.
  - Filter out solids, add NaOH, and $CHCl_3$ extract.
  - $CHCl_3$ may be used for UV, TLC or GC/MS.

- Extraction of Mushroom in Chocolate Candy

  - Shave off approximately 1.5-2.5 grams of sample
  - Place in centrifuge tube and add 5-8 ml of 10% Acetic acid
  - Heat to low boil for 30 minutes, then cool to room temperature.
  - Decant into test tube and centrifuge, 4 to 5 layers will appear:
    - Chocolate precipitate
    - Acid layer (yellow to orange yellow)
    - Watery chocolate precipitate
    - Hard white wax
    - A small yellow oil layer.
  - Decant acid layer and add CHCl3, agitate, then centrifuge
  - Remove Acid layer and repeat one more times to remove remaining oils and chocolate.
  - Make aqueous layer basic with NaOH.
  - Extract with $CHCl_3$.
  - Evaporate $CHCl_3$ to small volume.
  - $CHCl_3$ ready for UV, TLC and GC/MS.

- Psilocybin

  - Dry solvent extraction, Method 2 or 3 above, must be used when it is desirable to identify psilocybin. Basic extractions will cause dephosphorylation to psilocin.

    - UV Analysis
      - Take methanol extract from Method 2 or 3, run UV.
      - Absorbances at 267 and 290 are characteristic of psilocybin in methanol.
    - TLC
      - Spot methanol from Method 2 extraction on TLC plates beside psilocybin and psilocin standards.
      - Develop plates in the following solvent systems:
      - N-Butanol:acetic acid:water (2:1:1)
      - N-Propanol:5% NH_4OH (2:1)
      - Location Reagent – p-DMAB spray
    - GC/MS
      - Inject 1 µL methanol from Method 2 extraction into GC/MS.
      - Only psilocin can be identified by GC/MS. Note retention time of eluting psilocin.
      - Perform library searches and comparisons to standard data. Print copy of MS data for case file

- Psilocin:

  - May be identified using Method 1 or 2 extractions; however Method 1 seems best in quantity of psilocin recovered.

    - UV Analysis

        - Take up residue from Method 1 extraction in methanol and run UV analysis. Absorbances at 268, 285, and 294 are characteristic of psilocin in methanol.
    - TLC
        - Use same solvent systems and method described for psilocybin TLC's.
    - GC/MS
        - Inject 1-3 µl of methanol, used to re-dissolve residue from Method 1 extract, into GC/MS.

- Derivatization Technique

  - Method 1
    - Soak mushrooms in methanol overnight.
    - Centrifuge, and collect the supernatant liquid.
    - Methanol extract may be analyzed by TLC
    - Place liquid extract in micro vial
    - Evaporate the liquid extract to dryness with a stream of dry air.
    - Add1-2 drops µl BSTFA to the dried residue.
    - Cap Vial and incubate for about 5-10 minutes at 90-100ºC
    - Hand inject for analyze by GC/MS.
    - After injection of the sample, Clean the syringe with ethyl acetate

  - Method 2
    - Soak mushrooms in methanol overnight.
    - Centrifuge, and collect the supernatant liquid.
    - Add 2-4 ml acetone to liquid and freeze overnight.
    - Use a stream of dry air to reduce the volume of the methanol –acetone to 0.5 -1 ml
    - Methanol-acetone extract may be analyzed by TLC
    - Place liquid extract in micro vial
    - Evaporate the liquid extract to dryness with a stream of dry air.
    - Add 100 µl BSTFA to the dried residue.
    - Cap Vial and incubate for about 30 minutes at 90-100 ºC
    - Hand inject for analyze by GC/MS.
    - After injection of the sample, clean the syringe with ethyl acetate
    -

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2 Clandestine Drug Laboratory Evidence Analysis

Scope

- To provide guidance on the sampling and analysis of items and samples related to suspected clandestine drug laboratories. This document provides general recommendations, and does not address scene attendance, processing, and safety in their entirety.

Introduction

- We consider an understanding of clandestine laboratory synthetic routes and the techniques used in the analysis of related samples to be fundamental to the interpretation and reporting of results. This understanding assures that results and conclusions from methods are reliable and analytical schemes are fit for purpose.

- The qualitative and quantitative analyses of clandestine laboratory evidence can require different approaches relative to routine seized drug analyses. Analysts shall understand the limitations of the procedures used in their qualitative and quantitative analyses.

Safety

- Only certified clandestine lab response personnel shall collect samples from clandestine laboratories.

- Many items seized at clandestine laboratories may be intrinsically dangerous. These may include items of unknown composition and chemicals that have not been fully characterized and whose specific hazards are not known. Therefore, caution must be exercised and routine safety protocols may not be sufficient.

Procedure

Sample selection for analysis

- The primary purpose of analysis is to prove or disprove allegations of clandestine drug syntheses.

- Ideally, the submitted items of evidence should collectively contain the necessary components to fully demonstrate either the intent to manufacture or the successful manufacture of a controlled substance. In addition to the controlled substance which is suspected to be the target product, precursors and essential chemicals should be identified when present.

- Not all items seized at a clandestine laboratory site may need to be analyzed. It is recommended that information be shared between the analyst and on-scene personnel to aid in sample selection.

- Items that are readily obtained from local retail stores and are sold from reputable manufacturers/distributors may not need to be collected or analyzed, particularly if in sealed and labeled containers. These include:
    - solvents (e.g. toluene, mineral spirits)
    - acids (e.g. hydrochloric acid, sulfuric acid)
    - bases (e.g. sodium hydroxide, ammonia water)

- Some of the following types of items may be collected and analyzed as they can assist in determining the chemical reaction(s) undertaken and the scope of the clandestine laboratory:
    - materials that appear to be waste
    - unlabeled materials that appear to be contaminated solvents, acids, or bases
    - samples from contaminated equipment
    - unlabeled single or multi-phasic liquids

Sampling procedure for analysis

- In the event that the entire item is not collected
    - The weight or volume should be measured or estimated and a representative sample may be collected. The representative sample should be sufficient for analysis, court presentation and defense analysis.
    - If the item is a multi-phasic liquid, the proportion of each layer should be estimated and a representative sample of each layer should be collected.

3 Chemical Spot Tests

Scope

- To describe the chemical screening procedures, commonly referred to as chemical spot tests, for preliminary tests of controlled and non-controlled substances.

Safety

- Chemical spot tests may use a variety of corrosive, caustic, or other dangerous chemicals. Caution should always be practiced and appropriate personal protective equipment used. Refer to MSDS for additional safety information for specific chemicals.

Equipment, Materials and Reagents

- Spot plates, pipettes, or other appropriate containers/items
- Reagents appropriate to the specific chemical spot tests.

Standards, Controls and Calibration

- Quality check process
    - All stock and bench reagents are to be checked with the quality control substrate as listed for each reagent
    - Refer to respective sections in this manual for each reagent.
    - Stock reagents are to be quality checked at the time of creation then checked monthly.
    - Bench reagents will be quality checked monthly and upon refill from stock reagent.
    - All bench and stock reagents expire one year from the creation of the corresponding stock reagent.

Limitations

- All spot tests are presumptive in nature and serve only as a guide for an analyst's analytical scheme.
- Adulterants and complex mixtures may produce reactions that interfere with the interpretations.

Advantages

- Spot tests provide a quick and easy method for determining what a sample might contain.

- Spot tests can assist in the determination of appropriate analytical processing, collection of appropriate samples, and grouping samples for uniformity testing.

Marquis Test

Scope

- To establish test procedures for the presumptive detection of a range of compounds using the Marquis Test.

Reagents/Chemicals

- Conc. Sulfuric acid
- Formaldehyde Solution (approx. 37% Formaldehyde)

Marquis Reagent

- Add 1 mL formaldehyde solution to 9 mL conc sulfuric acid.
- Shelf life is 1 year from date of preparation.
- Quality-test reagent with amphetamine, methamphetamine, or an opiate upon preparation, and each calendar month thereafter.
- A negative control test shall also be performed using cocaine or other suitable substance.

Procedure

1. Combine a small amount of sample with a few drops of Marquis Reagent.
2. Record any resulting color reaction(s).

Interpretation

- Various colors representing the whole of the visible spectrum may be given by a large number of compounds. Additional results or interpretations may be found in Stevens (1986).
- A reaction which forms an orange color indicates the possible presence of amphetamine or methamphetamine.
- A reaction which forms a black color indicates the possible presence of Dextromethorphan, MDA or its analogues.
- A reaction which forms a dark purple color indicates the possible presence of heroin, opiates, methocarbamol, or guaifenesin.
- A reaction which forms a red color indicates the possible presence of salicylates.
- The color which appears must be documented on the examination worksheet.

Literature and Supporting Documentation

- H. M. Stevens, 1986. "Colour Tests" in *Clarke's Isolation and Identification of Drugs*,ed.
- A.C. Moffat (London: The Pharmaceutical Press) 128-147.
- Johns, S. H. et. al. "Spot Tests: A Color Chart Reference for Forensic Chemists," Journal of Forensic Sciences 24 (1979) 631-649.

Cobalt Thiocyanate (CoSCN) Test

Scope

- To establish test procedures for the presumptive detection of cocaine base and cocaine salts with the Cobalt Thiocyanate Test.

Reagents/Chemicals

- Cobalt thiocyanate
- Purified H_2O
- Concentrated HCl or other acid
- Chloroform ($CHCl_3$)

CoSCN Reagents:

- **2% Cobalt Thiocyanate Reagent**
  - Dissolve 2 g cobalt thiocyanate in 100 mL H_2O.

- Quality-test all reagents before use with cocaine standard.
- A negative control test shall also be performed using methamphetamine or other suitable substance.

Procedure

1. Combine a small amount of sample with the reagent. If a positive result is obtained, the analyst may stop and record any observations.
2. Add acid drop-wise to the sample until color disappears. The analyst may stop and record any observations.
3. Approximately five drops of $CHCl_3$ may be added to extract any soluble complexes. Record any observations.

Interpretation

- An appropriate notation (POS or +) in the case record that the addition of the reagent resulted in a blue color (+). This indicates a cocaine salt may be present.
- An appropriate notation in the case notes the addition of the reagent resulting in no color change (NEG or -) until a drop of acid has been added to the solution, which then resulted in a blue color (+). This indicates that cocaine base may be present.

Literature and Supporting Documentation

- L. J. Scott, “Specific Field Test for Cocaine,” Microgram 6 (1973): 179-181

Sodium Nitroprusside (SNP) Test

Scope

- To establish test procedures for the presumptive detection of secondary amines with the Sodium Nitroprusside Test.

Reagents/Chemicals

- Sodium Nitroprusside (Sodium Ferracyanide)
- Purified H_2O
- Acetaldehyde
- 1*M* Sodium Hydroxide (NaOH)
- 1*M* Sodium Carbonate (Na_2CO_3)

SNP Reagent

- Dissolve 0.09 g sodium nitroprusside in a mixture of 1 mL acetaldehyde and 9 mL H2O.
- Quality-test reagent with a methamphetamine standard.
- Negative controls may be performed similarly with any suitable compound that contains no secondary amine.

Procedure

1. Combine a small amount of sample with a few drops of SNP Reagent.
2. Add a few drops of 1*M* Na_2CO_3 (or NaOH) to the sample.
3. Record any observations.

Interpretation

- A positive indication (POS or +) in the case record means a reaction that forms a blue color (+) which indicates the possible presence of secondary amines, such as methamphetamine.

Literature and Supporting Documentation

- H. M. Stevens, “Colour Tests” in Clarke's Isolation and Identification of Drugs, ed. A. C. Moffat (London: The Pharmaceutical Press) 128-147.
- Johns, S. H. et. al. “Spot Tests: A Color Chart Reference for Forensic Chemists,” Journal of Forensic Sciences 24 (1979) 631-649.
- Basic Training Program for Forensic Drug Chemists, U. S. Dept. of Justice., 2nd Edition.

Sodium m-Periodate (SMP) Test

Scope

- To establish test procedures for the presumptive detection of a range of compounds using the Sodium m-Periodate spot test.

Reagents/Chemicals

- Sodium Metaperiodate
- 1 M NaOH
- 1 M Sodium Carbonate
- Quality-test with ephedrine or pseudoephedrine.

Procedure

1. Combine a small amount of sample and dry $NaIO_4$.
2. Add a few drops of 1 M Na_2CO_3 or NaOH.
3. Record any observations.

Interpretation

- A positive indication in the case record means the reaction resulted in a cherry odor (+),which is due to the formation of benzaldehyde and indicates the possible presence of ephedrine, pseudoephedrine, or phenylpropanolamine.
- A brown color indicates the possible presence of acetaminophen.

Literature and Supporting Documentation

- H. M. Stevens, “Colour Tests” in Clarke's Isolation and Identification of Drugs, ed. A. C. Moffat (London: The Pharmaceutical Press) 128-147.
- Johns, S. H. et. al. “Spot Tests: A Color Chart Reference for Forensic Chemists,” Journal of Forensic Sciences 24 (1979) 631-649.

Ferric Chloride Test

Scope

- To establish test procedures for the presumptive detection of a range of compounds using the Ferric Chloride Test.

Reagents/Chemicals

- Ferric Chloride, $FeCl_3 \cdot 6H_2O$
- Purified H_2O

5% Ferric Chloride Reagent:

- Dissolve 0.83 g $FeCl_3 \cdot 6H_2O$ in 10 mL H_2O.
- Quality-test reagent with GHB or aspirin.

Procedure

1. Combine a small amount of sample and a few drops of 5% Ferric Chloride reagent.
2. Record any observations.

Interpretation

- A reaction that forms an orange-brown color indicates the possible presence of GHB.
- A reaction that forms a dark purple color indicates the possible presence of salicylates.
- A reaction that forms a bluish-gray color indicates the possible presence of acetaminophen.
- The resulting color must be indicated in the case record.

Literature and Supporting Documentation

- H. M. Stevens, “Colour Tests” in Clarke's Isolation and Identification of Drugs, ed. A. C. Moffat (London: The Pharmaceutical Press) 128-147.
- Johns, S. H. et. al. “Spot Tests: A Color Chart Reference for Forensic Chemists,” Journal of Forensic Sciences 24 (1979) 631-649.

Froehde's Test

Scope

- Spot test procedure for the presumptive detection of narcotics

Reagents/Chemicals

- Add 0.5g ammonium molybdate per 100mL concentrated H_2SO_4.
- Quality-test reagent with a heroin, codeine or morphine standard.

Procedure

1. Combine a small amount of sample and a few drops of reagent in well
2. Record any observations.

Interpretation

- heroin - purple → green
- codeine - green → red/brown
- morphine - deep purple → slate
- aspirin - blue → purple
- phenoxymethylpenicillin - blue
- pentazocine – blue
- acetaminophen – pale blue

Literature and Supporting Documentation

- H. M. Stevens, 1986: "Colour Tests" in *Clarke's Isolation and Identification of Drugs*, ed. A.C. Moffat (London: The Pharmaceutical Press), 128-147.

Mecke's Test

Scope

- Spot test procedure for the presumptive detection of narcotics

Reagents/Chemicals

- Add 1g selenious acid per 100mL concentrated H_2SO_4.
- Quality-test reagent with a heroin, codeine or PCP standard.

Procedure

1. Combine a small amount of sample and a few drops of reagent in well
2. Record any observations.

Interpretation

- heroin – green/blue
- codeine – bright-green/blue green
- PCP – light yellow
- Quinine – light yellow

Literature and Supporting Documentation

- H. M. Stevens, 1986: "Colour Tests" in *Clarke's Isolation and Identification of Drugs*, ed. A.C. Moffat (London: The Pharmaceutical Press), 128-147.

Cobalt Nitrate Test

Scope

- To establish test procedures for the presumptive detection of gamma-hydroxybutyrate (GHB) and barbiturates using the Cobalt Nitrate Test.

Reagents/Chemicals

- Cobalt nitrate
- Isopropylamine
- 95% ethanol
- **1% Cobalt nitrate in ethanol reagent**: Add 1 g cobalt nitrate to 100 mL ethanol.
- **5% Isopropylamine in ethanol reagent**: Add 5 g isopropylamine to 100 mL ethanol.
- Quality-test reagent with a gamma-hydroxybutyrate or barbiturate standard.

Procedure

1. Combine a small amount of sample and a few drops of 1% cobalt nitrate in ethanol reagent.
2. Record any observations.
3. Add a few drops 5% isopropylamine to sample.
4. Record any observations.

Interpretation

- A purple color upon addition of 1% cobalt nitrate in ethanol indicates the possible presence of gamma-hydroxybutyrate (GHB+).
- A purple color which only forms after also adding 5% isopropylamine in ethanol indicates the possible presence of barbiturates (Barb+).

Literature and Supporting Documentation

- W. J. Stall, “The Cobalt Nitrate Color Test,” Microgram 13 (1980): 40-43.

Duquenois-Levine Test

Scope

- Spot test to determine the possible presence of components unique to marihuana, marihuana residue, or hashish.

Reagents/Chemicals

- Vanillin
- 95% Ethanol
- Acetaldehyde
- Conc. HCl
- Chloroform
- Hexane/Petroleum ether

Duquenois Reagent:

- Add 0.4 g vanillin and 5 drops acetaldehyde to 20 mL 95% ethanol.
- Quality-test the reagent with a known sample of marihuana or tetrahydrocannabinol.
- A negative control test shall also be performed using blank chloroform.

Procedure

1. Place a small amount of plant material in a testing container.
   Proceed directly to the next step or extract the plant material with hexane. If extracted, discard the plant material, and evaporate to dryness.
2. Add one volume of the Duquenois reagent and wait approximately one minute.
   (It is not necessary to wait as long with the extract.)
3. Add one volume of concentrated HCl.
4. Add one volume of $CHCl_3$.
5. Record any observations.

Interpretation

- A blue to violet color after the addition of HCl to the mixture of Duquenois reagent and plant material or extract is a positive reaction and indicates the possible presence of cannabinoids
- After adding $CHCl_3$ and mixing, a purple color in the organic (lower) layer is a positive reaction for the possible presence of cannabinoids
- A positive result indicates that the components (cannabinoids, including THC) unique to marihuana, marihuana residue, or hashish are present.
- A positive (+) indication in the case notes means the test resulted in a blue to violet color after the addition of the HCl and Duquenois reagents and that the $CHCl_3$ layer yielded a purple color.

Literature and Supporting Documentation

- C. G. Pitt, et. al. “The Specificity of the Duquenois Color Test for Marijuana and Hashish,” Journal of Forensic Science, 17 (1972): 693-700.

Liebermann Test

Scope

- To establish test procedures for the presumptive detection of a range of compounds using the Liebermann Test.

Reagents/Chemicals

- Sodium nitrite
- Sulfuric Acid

Liebermann Reagent

- Carefully add 5 g sodium nitrite to 50 mL sulfuric acid with cooling and swirling.
- Perform the addition in the hood, as toxic nitrogen oxides are produced.
- Quality-test the reagent with a known sample of codeine, methylphenidate, ephedrine, mescaline, or d-propoxyphene.

Procedure

- Combine a small amount of sample and a few drops of Liebermann Reagent.
- Heat to approximately 100°C for approximately one minute.
  (Note: This step is only necessary for certain compounds.)
- Record any observations.

Interpretation

- Various colors may be produced by a large number of different compounds. Additional results or interpretations may be found in Stevens (1986).

Literature and Supporting Documentation

- H. M. Stevens, 1986: “Colour Tests” in *Clarke's Isolation and Identification of Drugs*, ed. A.C. Moffat (London: The Pharmaceutical Press), 128-147.

p-DMABA Test

Scope

- To establish test procedures for the presumptive detection of procaine, benzocaine, LSD, and psilocybin/psilocin using the p-DMABA Test.

Reagents/Chemicals

- 95% Ethanol
- p-Dimethylaminobenzaldehyde
- Conc. HCl

p-DMABA reagent:

- Dissolve 0.1 g p-dimethylaminobenzaldehyde in 9.5 ml ethanol.
  Add 0.5 ml conc. HCl.
- Quality-test reagent with benzocaine, procaine, or LSD.

Procedure

1. Combine a small amount of sample and a few drops of p-DMABA reagent.
2. Record any observations

Interpretation

- A reaction which forms a bright yellow color indicates the possible presence of procaine or benzocaine.
- A reaction which forms a purple color indicates the possible presence of LSD, and psilocybin/psilocin.
- The resulting color must be indicated on the examination worksheet.

Literature and Supporting Documentation

- H. M. Stevens, “Colour Tests” in *Clarke's Isolation and Identification of Drugs*, ed. A. C. Moffat (London: The Pharmaceutical Press) 128-147.
- Johns, S. H. et. al. “Spot Tests: A Color Chart Reference for Forensic Chemists,” *Journal of Forensic Sciences,* 24 (1979): 631-649.
- *Basic Training Program for Forensic Drug Chemists*, U. S. Dept. of Justice., 2nd Edition.

Chlorophenol Red: Modified Schweppe's Test

Scope

- To establish test procedures for the presumptive detection of gamma-hydroxybutyrate(GHB) using the Modified Schweppe's Test.

Reagents/Chemicals

Chlorophenol Red solution

- 40 mg Chlorophenol Red in 100 mL of water, adjusting the pH to 7.0 with 0.1 *N* Sodium Hydroxide.

Modified Schweppe's Reagent - Solution A

- 2 g Dextrose in 20 mL water

Modified Schweppe's Reagent - Solution B

- 2.4 g Aniline Hydrochloride in 20 mL Methanol

Procedure for Modified Schweppe's Reagent

1. Mix Solution A and Solution B and dilute to 80 mL with Methanol.
2. Store the Modified Schweppe's Reagent in an amber bottle and refrigerate to retard decomposition.

Procedure for mixed reagent

1. Test the individual solutions against a blank tap-water sample.
2. Mix the Chlorophenol Red solution and the Modified Schweppe's Reagent using a 3:1 ratio (Chlorophenol Red solution:Modified Schweppe's Reagent).
3. The mixed reagent is stable for up to 3 weeks in an amber bottle on the bench.
4. Quality test reagent with GHB standard.
5. Test the mixed reagent against a blank tap-water sample. If the result of the test with the blank tap-water sample is a color change to brown, the solutions will need to be remade.

Procedure

1. Add approximately 0.5 mL of liquid sample or a small amount of powder sample to a test tube.
2. Check the pH. It is necessary for the pH to be carefully adjusted to 5-8.
3. Add 2 drops of the mixed reagent and swirl.
4. Record any observations.

Interpretation

A reaction that forms an orange or red color indicates the possible presence of GHB.

Literature and Supporting Documentation

- Smith, P. R., and J. S. Bozenko, Jr. 2002. *New presumptive tests for GHB.* Microgram, 35:10-15.

Sulfuric Acid Test

Scope

- To establish test procedures for the presumptive detection of a steroid.

Reagents/Chemicals

- Concentrated (96%) sulfuric acid (H_2SO_4)
- Quality-test reagent with a known sample of steroid.

Procedure

1. Combine a small amount of sample and a few drops of (H_2SO_4)
2. Record any observations
3. A UV light may be used to aid visualization of a color change.

Interpretation

- An orange or yellow color may indicate the possible presence of a steroid.
- The resulting color must be indicated in the case notes.

Literature and Supporting Documentation

- H. M. Stevens, “Colour Tests” in *Clarke's Isolation and Identification of Drugs*, ed. A. C. Moffat (London: The Pharmaceutical Press) 128-147.

Weber Test

Scope

- To establish test procedures for the presumptive detection of psilocin using the Weber Test.

Reagents/Chemicals

- Fast Blue B
- Conc. HCl
- Deionized H_2O

0.1% Fast Blue B:

- Dissolve 0.1 g Fast Blue B in 100 mL H_2O.
- Prepare this reagent fresh and quality-test with psilocin or a sample of mushroom shown to contain psilocin before use.

Procedure

- Combine a small amount of sample or methanol extract of the sample and a few drops of 0.1% Fast Blue B wait approximately one minute.
- Add one volume of conc. HCl.
- Record any observations.

Interpretations

- A positive reaction for psilocin is indicated (POS or +) in the case notes if a red color forms after adding the Fast Blue B reagent, and if after adding HCl the color changes to blue.

Literature and Supporting Documentation

- S. Garrett, Clemens, J. Gaskill, “The Weber test: a color test for the presence of psilocin in mushrooms,” *SWAFS Journal,* 15 (1993): 44-45.

Wintergreen Test

Scope

- To establish test procedures for the presumptive detection of cocaine using the Wintergreen Test.

Reagents/Chemicals

- Sodium Hydroxide
- Potassium Hydroxide
- Methanol

10% Sodium Hydroxide in methanol:
- Dissolve 1 g sodium hydroxide in 10 mL methanol.

10% Potassium Hydroxide in methanol:
- Dissolve 1 g potassium hydroxide in 10 mL methanol.
- Quality-test reagents with a cocaine standard.

Procedure

1. Combine a small amount of sample to a few drops of either 10% NaOH in methanol or 10% KOH in methanol reagent.
2. Record any observations.

Interpretations

- A positive indication (POS or +) on the worksheet means the reaction resulted in a wintergreen odor (+),which is due to production of methyl benzoate, and which indicates the possible presence of cocaine.

Literature and Supporting Documentation

- H. M. Stevens, “Colour Tests” in Clarke's Isolation and Identification of Drugs, ed. A. C. Moffat (London: The Pharmaceutical Press) 128-147.
- Johns, S. H. et. al. “Spot Tests: A Color Chart Reference for Forensic Chemists,” Journal of Forensic Sciences 24 (1979) 631-649.

4 Thin Layer Chromatography

Scope

- To describe the use of thin-layer chromatography as an analytical method.

Related Sections

- Marquis Reagent
- p-DMABA Reagent

Safety

- Use appropriate eye protection, gloves and lab coat to avoid any contact with the chemicals that are involved with this technique. This technique should be performed in a fume hood.
- Care should be used when spraying the TLC plates to avoid accidental ingestion of the reagent or exposure of the skin and eyes to the reagent. Refer to the appropriate MSDS for the safe handling of the solvents and reagents used in this technique.
- Developing solvents and indicator reagents should be discarded in an appropriate manner.

Equipment, Materials and Reagents

- Silica gel thin-layer chromatography plates
- Developing chamber
- Micropipettes (1-5 µL) or equivalent
- UV light box (long and short wave)

Reagents

- Approved TLC solvent systems

System ID	Solvent System	Typical Drugs Analyzed
1	50:25:15:10 (cyclohexane:toluene:acetone:diethylamine)	Marihuana
2	18:1 (chloroform, sat. with ammonia:methanol)	LSD
3	9:2 (chloroform:methanol)	LSD
4	9:1 (acetone:chloroform sat. with ammonia)	LSD
5	2:1:1 (n-butanol:acetic acid:water)	Psilocybin/Psilocin
6	**T-1** 1.5:100 (ammonium hydroxide:methanol)	General

7	**Davidow** 85:10:5 (ethyl acetate:methanol:ammonium hydroxide)	General
8	13:1.9: 0.1 (methyl ethyl ketone: dimethylformamide: NH4OH)	Mescaline

- **Approved indicating reagents:**

Indicating Reagents	Typical Drugs Analyzed
Fast Blue RR	marihuana and THC
p-DMABA	LSD, psilocybin mushrooms, and indoles
Ninhydrin	mescaline and amines
Acidified Iodoplatinate	mescaline, opiates, and tertiary amines
Marquis Test	general substances
Potassium permanganate	general substances

- **Preparation of select indicating reagents**
  - ***Fast Blue RR reagent***:
    - Purified H_2O, methanol, or ethanol
    - Fast Blue RR salt
    - Dissolve 0.25 g Fast Blue RR salt in 50 mL solvent. Developed spot for THC appears red.
  - ***Ninhydrin reagent:***
    - Ninhydrin
    - Acetone
    - Dissolve 0.5 g ninhydrin in 100 mL acetone. Developed spots appear red to purple.
  - ***Acidified Iodoplatinate reagent***:
    - 10% Platinic chloride solution
    - 4% Potassium iodide solution
    - Purified H_2O
    - Concentrated (37%) HCl
    - Mix 5 mL 10% platinic chloride solution with 125 mL 4% KI solution. Dilute to 250 mL with purified water. Add 12.5 mL conc. HCl. Developed spots appear purple or blue.
  - ***Potassium permanganate reagent (1%):***
    - 0.5 *N* sulfuric acid
    - Potassium permanganate ($KMnO_4$)
    - Add 1 g potassium permanganate to 100 mL 0.5 *N* sulfuric acid. Developed spots appear lighter than the background.

Standards, Controls and Calibration

- An appropriate known reference standard will be used to test the system and indicating reagents. A standard will be analyzed on all plates. If the expected result of the standard is not obtained, the issue should be resolved before the analysis is repeated.

Procedure

1. Extract the sample with an appropriate solvent.
2. Spot a suitable amount of extract from the sample and at least one standard on the TLC plate approximately 1.5 cm above the bottom of the plate.
3. Allow the sample to dry after application.
4. Place the plate vertically into a developing chamber with enough solvent mixture to cover 0.5 to 1.0 cm of the sample-end of the plate.
5. Allow the solvent front to rise near the top of the TLC plate.
6. Remove the plate from the solvent and allow it to air dry. Systems containing ammonia may be gently heated to remove the excess ammonia before spraying.
7. Apply an appropriate indicator spray and/or view under UV light to visualize the component(s) of interest.
8. Compare the migration of the sample spot to that of the standard.
9. Document the solvent or extraction procedure used to prepare the samples, the solvent system used to analyze the samples, and the results of analysis.

Interpretation

- A positive determination is made when the spot(s) of the unknown substance matches the color and migration of the standard.

Limitations

- TLC is not considered a confirmatory test and further analysis is necessary for the positive identification of a questioned substance.
- Various factors limit the determination of Rf values in TLC analysis, including the length of the plate, bleeding of the sample, temperature and developing time. However, the use of multiple systems and chemical locating reagents make it a more specific technique.

Advantages

- Relatively quick and easy technique.
- Can be used as a clean-up procedure for complex mixtures.
- Requires no expensive instrumentation.

Literature and Supporting Documentation

- J. M. Bobbitt, A. E. Schwarting, and R. J. Gritter. 1968. *Introduction to Chromatography*.
- John A. Miller and E. F. Neuzil. 1979. *Organic Chemistry, Concepts and Applications*. D. C. Heath & Company, Lexington, Mass, 555.
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- “Chromatographic Data, Thin Layer Chromatography Tables, Volume I, Sec. II.IV”, *CRC*

Handbook of Chromatography, Volume I, edited by Robert C. Weast, CRC Press, Division of the Chemical Rubber Company, 1972, 477-487.

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- E. Buel, C. N. Plum, and S. K. Frisbie. 1982. An Evaluation of a Partition Thin Layer Chromatography System for the Identification of Cannabinoids. *Microgram*, 15:145-157.
- R. B. Hughes and R. R. Kessler. 1979. Increased Safety and Specificity in the Thin-layer Chromatographic Identification of Marihuana, J. *Forensic Science*, 24:842-846.
- R. B. Hughes and V. J. Warner, Jr., 1978. A Study of False Positives in the Chemical Identification of Marihuana. J. Forensic Science, 23:304-310.

5 Instrumental Analysis

Ultraviolet/Visible Spectrophotometry (UV/VIS)

Scope

- A nondestructive technique for the preliminary identification of controlled substances, dangerous drugs and other substances.

Safety

- Use appropriate safety equipment when preparing reagents and pouring liquids. Refer to the MSDS for additional safety information for specific chemicals.

Equipment, Materials and Reagents

- Double-beam UV/visible spectrophotometer
- Quartz cuvettes, matched pair or equivalent
- An appropriate solution for the sample.
- Acidic solutions, such as 0.2 N H_2SO_4 or 0.1 N HCl
- Basic solutions, such as concentrated NaOH *or* 1.0 M Na_2CO_3
- Methanol or ethanol

Standards, Controls and Calibrations

- Refer to Chemistry Section SOP for information on quality control, acceptable standards, and reference materials.
- Appropriate blanks should be analyzed as deemed necessary by the chemist.

Procedure

- **Spectrophotometer Operating Conditions**
  - The wavelength range used for the UV/VIS analysis of most drug samples is 340 to 220 nm.
  - The range may be expanded to accommodate certain substances, such as LSD, alkyl nitrites, and GHB.

- **Sample Preparation**
  - Dissolve the sample in a solution appropriate for the substance.
  - Depending on the concentration of the sample, it may be necessary to dilute the solution.
  - Plant materials and mixtures may require extraction prior to analysis.

- **Sample Analysis**
  - Collect a spectrum of the sample in the appropriate solution.
  - A “pH shift” may be performed on samples in acidic solutions by adding concentrated sodium hydroxide until the solution is basic.
  - Each spectrum will be printed, labeled with laboratory case number, exhibit number, date, analyst’s initials, method of sample preparation (if not listed on the worksheet), instrument operating conditions.

Interpretation

- Evaluate the sample's UV spectrum by comparing it to reference literature, or to one or more UV spectra derived from known standards.
- Sample preparation or extraction methods should be documented in the case record or directly on the spectra.
- The interpretation of the spectra may be reflected directly on the spectrum and by Positive ("POS" or +), Negative ("NEG" or -) or "INC" (inconclusive) in the case record.
- If the sample spectrum is interpreted as a positive test, document the source of any reference spectrum employed.
- Use the approved list of reference abbreviations if applicable; if some other reference is used, provide a citation.

Limitations

- An ultraviolet spectrum is not specific, and a positive identification cannot be made exclusively on the basis of UV/VIS analysis.
- Not all substances absorb ultraviolet light; therefore the lack of absorbance or a flat-line spectrum is not necessarily an indication that a sample contains no controlled substances.
- The absorbance of a substance at any given wavelength may be modified by the presence of other compounds that also absorb at that wavelength. Additional sample preparation may be required to remove interfering compounds.

Advantages

- The test is quick and easy to perform.
- Usually very little sample preparation is required.
- UV analysis is a good screening tool and routine analysis may provide information regarding the general concentration of the sample (strong, average or weak) and the presence or absence of some dilutants and adulterants.
- This is usually a non-destructive technique and the sample can be recovered for other testing procedures, if necessary.
- May provide a quick and easy quantitation of some drugs/dilutants.

Literature and Supporting Documentation

- Douglas A. Skoog and Donald M. West, *Principles of Instrumental Analysis* (New York:Holt, Rinhart, and Winston, Inc., 1971).
- Terry Mills III and Conrad J. Roberson, *Instrumental Data for Drug Analysis*, (New York:Elsevier Science Publishing Co., Inc., 1987).
- F. Fell, *Clarke's Isolation and Identification of Drugs*, (London: The Pharmaceutical Society of Great Britain, 1986).
- Galen W. Ewing, *Instrumental Methods of Chemical Analysis*, Fifth Edition, ISBN 0-07-019857-8
- Stanley Manahan, *Quantitative Chemical Analysis*, ISBN 0-534-05538-9.
- Kenneth A. Rubinson, *Chemical Analysis*, ISBN 0-316-76087-0.

- Donald L. Pavia, Gary M. Lampman, George S. Kriz Jr., *Introduction to Spectroscopy*, ISBN 0-7216-7119-5.
- Robert M. Silverstein, G. Clayton Bassler, Terence C. Morill, *Spectrometric Identification of Organic Compounds*, 4th edition, ISBN 0-471-02990-4.
- S. Sternhell and J. R. Kalman, *Organic Structures from Spectra*, ISBN 0-471-90647-6.

Fourier Transform Infrared (FTIR) Spectrophotometry

Scope

- A non-destructive analytical technique used for the characterization and identification of suspected controlled substances, dangerous drugs and other substances. The infrared spectrum of the majority of controlled substances and other substances routinely identified is specific to a single compound and may be used for identification.

Safety

- Use appropriate safety equipment when preparing reagents. Refer to the MSDS for additional safety information for specific chemicals.

Equipment, Materials and Reagents

- Fourier transform infrared spectrophotometer
- Attenuated Total Reflectance (ATR) attachment
- Agate mortar and pestle
- Hydraulic press and KBr die, or hand press
- Potassium bromide (KBr), dry
- NaCl or KBr windows (*e.g.*, 2mm x 13 mm)
- Nujol
- Laboratory oven
- D.R.I.F.T.S attachment

Standards, Controls, and Calibration

- Refer to Forensic Chemistry Section SOP for information on quality control, acceptable standards, and reference materials.

Procedure

- **Sample Preparation**
  - Use appropriate extraction and clean-up procedures as necessary to isolate the sample. This may require the conversion of the sample to a suitable salt form prior to analysis.
  - Methods of introducing the sample into the instrument for analysis include the following:
    - No sample preparation is typically needed for the ATR attachment.
    - Liquid samples can be analyzed as a thin film between two NaCl or KBr (salt) cells.
    - Solid samples can be milled with dry KBr, KCl, NaCl or a similar matrix to
    - produce a fine powder. The powder is pressed into a thin pellet using a die and a hydraulic or hand press.
    - For cast film solid samples, dissolve a small amount in a suitable solvent

and place the solution on a single NaCl or KBr cell. Evaporate the solvent and scan the thin film remaining.

- For smeared solid samples, mix a small amount of the powdered substance with a drop of Nujol to form a mull and smear it on a NaCl or KBr cell.

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Sample Analysis

- Collect and print spectra with a resolution of at least 4 cm^{-1} from 4000 cm^{-1} to 400 cm^{-1} (or 600 cm^{-1} with NaCl) versus % transmittance (0-100). Spectral peaks should be of sufficient intensity to make an accurate comparison to known reference standards or published spectral data.

Interpretation

- Commercially available library searches can be used to provide useful information pertaining to the identity of a compound, but should not be used as a replacement for verifying positive identification, due to the abridged nature of the spectra found in search libraries.
- When using FTIR as the primary confirmation technique, the sample spectrum should compare favorably with a spectrum of a known standard in both its overall appearance and in the presence and location of the major peaks.
- Additional peaks that do not mask or interfere with important peaks or regions of interest are acceptable.
- Some sample spectra may compare favorably with a known standard, but may contain minor permutations. These spectra are considered to be of *reduced resolving power*, and should be prefixed with the word “Contains”, (e.g. “Contains Cocaine, “Contains Methamphetamine”, etc.) These spectra are not suitable as a confirmatory test, and the analytical scheme should be adjusted as outlined in the Basic Analytical Scheme of this manual.
- Sample preparation or extraction methods should be documented in the case record or directly on the spectra.
- Document the confirmation of the unknown spectra to a known reference and indicate the source of the reference in the case record (published or otherwise lab generated).

Limitations

- The sample must be relatively pure for positive identification.
- For an accurate comparison of an unknown spectrum to a standard spectrum, both samples (the sample and reference) must be in the same salt form. Some compounds may produce different crystal structures that can result in slightly different infrared spectra.
- Infrared cannot usually be used to distinguish optical isomers

Advantages

- Infrared is specific for the identification of controlled substances, dangerous drugs, and dilutants and can be used as a confirmatory test.
- Infrared is normally not a destructive test and the sample can be recovered for additional testing procedures, if necessary.
- An unknown infrared spectrum can be quickly compared to known compounds found in drug libraries stored in the computer and then confirmed using published data from a reliable source or in-house spectra produced from known standards.

Literature and Supporting Documentation

- Standard Practice for Describing and Measuring Performance of Fourier Transform Infrared (FT-IR) Spectrophotometers: Level Zero and Level One Tests, ASTM E 1421-91,1991.
- F. Fell, Clarke's Isolation and Identification of Drugs, (London: The Pharmaceutical Society of Great Britain, 1986).
- Forensic Science Handbook, Volume III, ed. by Richard Saferstein, (Englewood Cliffs, N. J.:Regents/Prentice Hall, 1993).
- Douglas A. Skoog, Principles of Instrumental Analysis, 3rd Edition, (New York: Saunders College Publishing, 1985) 148-149.
- John Coates, Interpretation of Infrared Spectra, A Practical Approach, Encyclopedia of Analytical Chemistry (Wiley and Sons, 2000), pp. 10815-10837.

Gas Chromatography/ Mass Spectrometry (GC/MS)

Scope

- An analytical technique for the characterization and identification of suspected controlled substances, dangerous drugs and other substances.

Safety

- Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the MSDS for additional safety information for specific chemicals.
- Properly secure high-pressure gas cylinders.
- Use caution around hot surfaces such as oven interiors and injection and detector ports.

Equipment, Materials and Reagents

- Gas chromatograph/mass spectrometer analytical instrument
- Helium or Hydrogen Gas
- Auto-sampler vials and caps (where applicable)
- Microliter syringe (where applicable)

Standards, Controls and Calibration

- Quality assurance of the mass spectrometer is accomplished by tuning the instrument to ensure that the mass-to-charge ratios (m/z) are assigned correctly and to provide leak detection.
- The instrument should be tuned according to the manufacturer's specifications.
- Refer to Chemistry Section SOP's for quality control schedule and tune schedule.
- Before every sample the analyst shall inject control blanks consisting of the same solvent used in sample preparation to verify sample integrity.
- Any contaminants or ghost peaks present in a control blank shall be inspected to verify that they will not interfere or invalidate a sample run. For example column/septa bleed, solvent impurities, and raised GC baselines are common and do not necessarily invalidate a sample blank.
- If narcotic peaks elute at any point during a control blank; the blank and subsequent injections will be considered invalid and not acceptable for analysis. The control blank and respective samples should be rerun to determine the source of, if any, contamination.

Procedure

- **GC/MS Operating Conditions**
  - Use appropriate temperature programs and adjust other critical parameters to ensure that the suspected substance(s) will elute during data collection. The program should allow a reasonable time for unknown or unexpected compounds to elute.
  - Print and retain the program parameters in the case record.

- **Sample Preparation and Analysis**

  - Extract samples into a suitable solvent before they are injected into the instrument. As a general rule, avoid highly polar solvents that can cause degradation of the GC column lining.
  - Analyze sample extracts and other controls, blanks, and/or reference material as appropriate.
  - Analysts shall evaluate the GC/MS total ion chromatogram (TIC) and spectra of reported substances and other compounds of interest. Analyst discretion will be used when selecting peaks for observation based on analytical scheme and circumstances of the case. Samples which contain multiple controlled substances require the identification of all controlled substances.
  - Document the following:
    - Complete gas chromatogram of the sample
    - Each sample mass spectrum that is used to confirm the identification of a reported substance.
    - Mass spectra of compounds of interest as determined by the analyst.
    - Peaks of interest not corresponding to any known reference may be documented as NCS either on the spectra or in the case record.

- **Retention Time Analysis**

  - Select the appropriate drug reference material for comparison with the unknown substance.
  - Analyze the prepared drug reference material, prepared unknown sample(s) and compare the retention time of the peaks.
  - APD Drug reference chromatograms must also contain a traceable lot number of the standard.

Interpretation

- The approved library references listed in the Chemistry Section SOPs will be used to indicate the source of the reference.
- Library searches can be used to provide useful information pertaining to the identity of a compound, but should not be used as a replacement for verifying positive identification, due to the abridged nature of the spectra found in search libraries.
- The difference between retention times of the known and unknown samples must be equal to or less than 1.0%. Calculated as follows:

$$\textit{Percent Different} = \frac{|\textit{retention}_{std} - \textit{retention}_{unk}|}{\textit{retention}_{std}} \times 100$$

Limitations

- When analysis by GC/MS is unable to provide positive identification in some instances, another technique (FTIR, derivatization, etc.) must be utilized to provide positive identification For example, certain stereo- and geometric isomers give identical or very similar results.

- Some compounds may not be suitable for GC/MS analysis due to variety of factors; for example, high injection port temperatures cause some compounds to break down before they are ionized, preventing their identification.
- It may be difficult to identify individual compounds in a homologous series.

Advantages

- Generally, mass spectra of controlled substances are specific to single compounds and may be used for identification.
- It may be possible to separate and identify complex mixtures that are difficult to separate through ordinary clean-up procedures.
- The technique is useful for analyzing small sample amounts that may be difficult to identify using other techniques.
- An autosampler, which increases the efficiency of analysis of numerous samples and functions unattended, may be attached to the GC/MS.

Literature and Supporting Documentation

- Douglas A. Skoog, *Principles of Instrumental Analysis*, 3rd Edition, (New York: Saunders College Publishing, 1985) 523-535, 554.
- F. W. McLafferty, *Interpretation of Mass Spectra*, 3rd Edition, (Mill Valley, California: University Science Books, 1980).
- J. Throck Watson, *Introduction to Mass Spectroscopy: Biomedical, Environmental, and Forensic Applications*, (New York: Raven Press Books, 1140 Avenue of the Americas,1976).

Liquid Chromatography/ Mass Spectrometry (LC-MS)

Scope

- An analytical technique for the characterization and identification of suspected controlled substances, dangerous drugs and other substances.

Safety

- Use appropriate safety equipment when preparing reagents and handling volatile chemicals. Refer to the MSDS for additional safety information for specific chemicals.
- Use caution around hot and cold surfaces such as automated liquid injection system and detector ports.

Equipment, Materials and Reagents

- Liquid chromatograph/mass spectrometer/ photodiode array analytical instrument
- Nitrogen gas generator or other source of N_2 Gas.
- LC-MS grade solvents
- Ultrapure or 18 Ω water.
- Auto-sampler vials and caps (where applicable)
- Microliter syringe (where applicable)

Standards, Controls and Quality Assurance

- Quality assurance of the mass spectrometer is accomplished by tuning the instrument to ensure that the mass-to-charge ratios (m/z) are assigned correctly.
  - The instrument should be tuned according to the manufacturer's specifications and may be tuned more frequently as deemed necessary by the analyst and/or the laboratory supervisor.
  - Maintain records of the tune in a file in the laboratory. If the tune is not successful, the instrument should be taken out of service until corrective action is taken.
- The analyst shall run a blank to verify that the column, solvent and laboratory glassware used are clean prior to the analysis of evidence samples.
  - A blank shall be run when trace samples are analyzed and as deemed necessary by the analyst. If contamination is indicated, the problem must be resolved before the analysis is repeated.
  - Maintain the resulting chromatograms in the case folder or in a retrievable form.

Procedure

- LC/MS Operating Conditions
  - Use appropriate solvent gradients, temperature programs and adjust other critical parameters to ensure that the suspected substance(s) will elute during data collection. The program should allow a reasonable time for unknown or unexpected compounds to elute.
  - Print and retain the program parameters in the case folder or in a retrievable form.

- Sample Preparation and Analysis
  - Extract samples into a suitable LC-MS grade solvent before they are injected into the instrument.
  - Print and retain the charts depicting the results of the LC/MS analysis in the case file. Include the following if collected:
    - The complete liquid chromatogram.
    - Chromatograms for all peaks corresponding to controlled substances and/or other substances reported.
    - Photodiode array absorbances for all peaks corresponding to controlled substances and/or other substances reported.
    - Mass spectra for all peaks corresponding to controlled substances and/or other substances reported
    - Mass spectra for any other peaks deemed relevant by the analyst
    - Laboratory case number, exhibit number, date, examiner's initials, and method of sample preparation (if not shown on the worksheet).

Interpretation

- To date no transferable LC/MS libraries exist. An in house library will be constructed from traceable, verified standards and stored for comparisons.
- Results from library searches need to be printed.

Limitations

- When analysis by LC/MS is unable to provide positive identification in some instances, another technique (FTIR, derivatization, etc.) must be utilized to provide positive identification For example, certain stereo- and geometric isomers give identical or very similar results.
- It may be difficult to identify individual compounds in a homologous series

Advantages

- It may be possible to separate and identify complex mixtures that are difficult to separate through ordinary clean-up procedures.
- The technique is useful for analyzing small sample amounts that may be difficult to identify using other techniques.
- LC-MS is capable of identifying thermally labile compounds and many others not readily analyzed by GC-MS.
- LC-MS runtimes are a fraction of their GC-MS counterparts.

Literature and Supporting Documentation

- Richard Saferstein, *Forensic Science Handbook Vol. 1*, 2nd Edition, (New Jersey: Prentice Hall, 2002) 41-68.

6 QUANTITATIVE ANALYSIS

Scope

- To outline acceptable methods for quantitation of narcotics.

Quantitation by Ultraviolet Spectrophotometry

Equipment, Materials and Reagents

- Double-beam UV/visible spectrophotometer
- Quartz cuvettes, matched pair or equivalent
- Class "A" Volumetric glassware
  - All glassware shall be inspected for suitability before use.

Standards, Controls, and Quality Assurance

- Performance verification run as delineated in Chemistry Section SOP.
- Absorptivities (E-Values) for compounds of interest are obtained from approved literature or determined in house using reference materials.
  - The following E-values are acceptable for UV drug quantitation:

Substance	E-Value	Wavelength	Source
Cocaine	430	233	Clarke
Heroin	46	279	Clarke
LSD	225	315	Clarke
Methamphetamine	12.1	257	Clarke

- Note that the E-values in Clarke's are at 1.0% and must be divided by 10 in order for the resultant calculation to yield a concentration value of mg/ml (0.1 %).
- These values may also be determined by laboratory reference material on the instrument prior to using this technique for quantitation. The results shall be documented in retrievable format and include the manufacturer and lot or batch number of the drug reference material.
- Reference Solvent Blank
- The concentration as the salt may be reported only if the analyst has identified the salt form by an accepted analytical procedure.
- See methods below for drug specific sample calculations.
- Calculations to determine the concentration should be included in the case record.
  - In order to ensure homogeneity two samples quantitated by UV shall have a concentration range ±3σ of the control chart standard deviation in order to be acceptable.

$$3\sigma \geq \left| \frac{\textit{Quantification 1} - \textit{Quantification 2}}{\textit{Avg. (Q1 \& Q2)}} \right| X100$$

- The control chart values for 3σ are located on the respective uncertainty budget for each procedure/drug of abuse.
- If the range between 2 quantifications exceeds this value the analyst shall resample and/or rehomogenize the item before additional quantifications are performed.

- Analysts may average the sample concentrations or report the lowest value.

Quantitation of Cocaine by Ultraviolet Spectrophotometry

Procedure

- Accurately weigh sample on analytical balance (4 decimal or higher) and bring to volume with 0.2N H_sSO_4 in a Class "A" volumetric flask.
- Pipette 1 ml of solution into an additional Class "A" volumetric flask; bring to volume with 0.2N H_2SO_4.
- Zero UV with 0.2N H_2SO_4 solvent blank and check absorbance at 233 nm. It should be zero.
- Scan sample on UV and print absorbance at 233 nm.
- Calculate percent as follows:

$$\frac{(\text{Abs. @ 233nm})}{430} \times \frac{1000}{100} \times 50 \text{ ml} \times 50 \text{ ml} = \text{mg Cocaine base}$$

$$\frac{\text{mg cocaine base}}{\text{mg sample}} \times 100 = \text{\% Cocaine as base}$$

- To determine % as HCl, divide by ratio of base/HCl MW
- MW Cocaine HCl = 339.8, MW Cocaine = 303.4, Ratio = 0.89

$$\frac{\text{\% base}}{0.89} = \text{\% Cocaine as HCl}$$

- Significant figures shall be carried through for all calculations. The final reported value shall be truncated (not rounded) to a whole percent.

Quantitation of Heroin by Ultraviolet Spectrophotometry

Procedure

- Grind sample in mortar and weigh out 20-30 mg of powder, assuming heroin is usual street purity (4-7%). For more potent heroin samples, use proportionally less sample.
- Place weighed sample in separatory funnel and add 10-15 ml of 10% HCl.
- Extract the HCl with three 10 ml portions of morphine solvent, combining the solvent extracts.
- Extract morphine solvent with two 5 ml portions of 0.2 N H_2SO_4.
- Zero UV instrument using 0.2 N H_2SO_4 as blank.
- Combine the H_2SO_4 extracts into a class "A" volumetric flask and run UV scan.

- Calculate the percent heroin using the following formula:

$$\frac{[(\text{Abs. @ 279nm}) - (\text{Abs@300nm})]}{46} \times \frac{1000}{100} \times (\text{Volume HCl extract}) = \text{mg Heroin}$$

$$\frac{\text{mg Heroin}}{\text{mg sample}} \times 100 = \%\ \text{Heroin as base}$$

- Significant figures shall be carried through for all calculations. The final reported value shall be truncated (not rounded) to a whole percent.

Quantitation of LSD by Ultraviolet Spectrophotometry

Procedure

- Soak one or more hits in a known volume of 0.2 N H_2SO_4 for several minutes.
- Remove carrier, squeezing solution out with applicator.
- Scan solution on UV spectrophotometer from 370-220 nm.
- Compare absorption spectra with standard spectra.
- By using "Peak Pick" (F4) function, the absorbance value for the wavelength will be displayed.
- Calculations:

Calculate quantity of LSD per square as follows:

$$\frac{[(\text{Abs.@315nm})-(\text{Abs.@370nm})]}{225} \times \frac{1000}{100} \times \text{Volume} = \text{mg LSD}$$

$$\text{mg LSD} \times \frac{1000\ \mu\text{g LSD}}{1\ \text{mg LSD}} = \frac{\mu\text{g LSD}}{\#\ \text{squares used}}$$

- Record LSD concentration per square on worksheet, not to be reported on lab report.
- If analysis is for federal prosecution calculate number of hits of liquid LSD
  - Note: Record number of drops used for analysis on worksheet

$$\frac{[(\text{Abs.@315nm}) - (\text{Abs.@370nm})]}{225} \times \frac{1000}{100} \times \text{Volume} = \text{mg LSD}$$

$$\frac{\text{mg LSD}}{\text{mg sample used}} \times \frac{1000\ \mu\text{g LSD}}{1\ \text{mg LSD}} \times \text{mg sample (net wt)} = \text{total } \mu\text{g LSD}$$

- Federal and conversion factor: 40 µg LSD / hit

Quantitation of Methamphetamine by Ultraviolet Spectrophotometry

Procedure

- Accurately weigh sample on analytical balance (4 decimal or higher) and bring to volume with 0.2N H_sSO_4 in a class "A" volumetric flask.
- Zero the UV with 0.2 N H_2SO_4 and check the absorbance at 257nm.
- Scan the sample, recording the absorbance at 257 nm.
- Calculate percent as follows:

$$\frac{\text{abs @ 257nm}}{12.1} \times \frac{1000}{100} \times 25 \text{ ml} = \text{mg Methamphetamine base}$$

$$\frac{\text{mg Methamphetamine}}{\text{mg Sample used}} \times 100 = \text{\% Methamphetamine as base}$$

- To determine % as HCl, divide by ratio of base/HCl molecular weights.

$$\frac{\text{\% Methamphetamine base}}{0.80} = \text{\% Methamphetamine HCl}$$

- Significant figures shall be carried through for all calculations. The final reported value shall be truncated (not rounded) to a whole percent.

Determination of Optical Isomer for "ICE" by Derivatization

Scope

- A substance containing *d*-methamphetamine HCl of at least 80% purity is defined as "ICE" by federal guidelines. Samples submitted for federal prosecution that meet these criteria shall be derivatized to determine the optical isomer(s) present.
- Individual samples (i.e. baggies, packets, etc.) shall each have qualitative analysis and isomer determination.

Procedure

- Dissolve approximately 2 mg of unknown sample in 1M Na_2CO_3 in test tube.
- Add approximately 1 ml of $CHCl_3$, mix well.
- Place the $CHCl_3$ in an auto sampler vial and add approximately 2 drops of (S)-(-)-N-TPC (S-(-)-N-(Trifluoroacetyl)-Prolyl Chloride).
- Repeat steps above for 3 known samples (*l*- methamphetamine, *d*-methamphetamine, and *d,l*-methamphetamine).
- Allow all 4 mixtures to react for at least 10 minutes.
- Inject each mixture into the GC/MS using the **"ISOMER"** program and record the retention times. (Note: Unknown mixture should be run first to determine if the

sample is racemic. l-methamphetamine elutes before d-methamphetamine by about 0.2 minutes.)

Interpretation

- Analysts shall examine all isomer chromatographs to confirm presence of *d*-methamphetamine.
- Presence of small amounts of *l*-methamphetamine *does not* preclude an analyst from reporting a sample as *d*-methamphetamine. However, the sample should contain predominantly *d*-methamphetamine. Otherwise, analysts are encouraged to report the sample as *d,l*-methamphetamine.

Quantitation by Gas Chromatography with Internal Standard

Quantitation of Cocaine by GC-FID

Equipment, Materials and Reagents

- GC with Flame Ionization detector
- Hydrogen carrier gas
- ACS grade chloroform or higher.
- Class "A" Volumetric glassware
  - All glassware shall be inspected for suitability before use.
- Analytical balance (Readability shall be 0.0001 or greater)
- 1mL Transfer pipette

Standards, Controls, and Quality Assurance

- N-tetracosane internal standard
- Guide 34 Cocaine HCl reference material (Calibrator)
- Traceable Cocaine HCl reference material (Quality Check Standard)
- Internal standard stock solution shall be labeled with a lot number which will correspond to the date the solution was prepared.
  - Solution shall be sealed and refrigerated when not in use.
  - Solution expires 3 months from date of preparation.

- Quality Check Standard (Cocaine/Tetracosane in CHCl3)

  - A new check standard shall be prepared each time a new internal standard solution is prepared.
    - 0 The reference material used to create the check standard must be a different lot, batch, or reference number than the material used as a calibrator.
  - Check standard lot number shall correspond with the internal standard stock solution.
  - The check standard concentration shall fall within limits of the standard curve.
  - Initially, any new check standard shall be run and analyzed along with the corresponding standard curve.
  - Determine the experimental concentration by performing a minimum of 5 injections on method "APDMID-FID".
  - The mean concentration of these 5 injections shall be calculated and documented on the GC-FID Quantitation Worksheet along with the original standard curve data.
  - All 5 injections must fall within ±10% (absolute) of the mean to be acceptable.

- Standard curves shall be considered valid for use with casework until:

  - Certain changes in the condition of the GC-FID hardware (including any major repairs to the FID, or replacement of key components.)
  - Internal standard solution is depleted.
  - Check standard falls outside of ±10% (absolute) tolerance of the mean.

- A period of one month has passed since the curve has been run.

- While is use for casework, approved standard curve excel workbooks will be saved electronically.
- Once a curve is no longer suitable for case work the respective excel workbook will be moved to the appropriate electronic folder and archived.

- Case Samples

  - The laboratory has determined that the purity difference between these two results must be equal to or less than the control limits (± 3 standard deviations) from the control chart standard deviation. This process is used to establish the homogeneity of the sample.
  - Analysts may average the sample concentrations or report the lowest value.

Procedure

- Generation of Standard Curve

1. Internal Standard Solution: make an internal standard solution of approximately 0.1 mg/mL concentration of N-tetracosane in chloroform. This solution will be the solution used to make at least five standard cocaine solutions and to make the unknown analyte solutions.

2. Make a series of standards using cocaine HCl in the internal standard solution ranging in cocaine concentration from 0.20 mg/mL to 2.00 mg/mL (as cocaine HCl). Each standard should be prepared by diluting a known mass to 10mL. Also, a minimum of three data points (injections) should be collected for each standard and unknown analyte.

3. Inject each of the standards into the GC-FID using the method APDMID-FID. The method uses the back injection port, column, and detector.

4. After the standards have run, launch the Data Analysis portion of ChemStation. Open the chromatogram for a standard. Select Chromatogram and then click Select Integrator. On that window, select RTE Integrator. Then, Select Integrate from the Chromatogram menu. Then, after integrating, select Generate Percent Report from the Chromatogram menu. A window will pop open on the bottom running a report. Right click that window and select print.

   This will print out a copy of the integrated areas of each peak. Look for the peak area column on the report. Divide the cocaine number by the N-tetracosane number. This number will be the Area Ratio. (Cocaine peak will be at approximately 4.78 minutes and N-tetracosane at 5.87 minutes.)

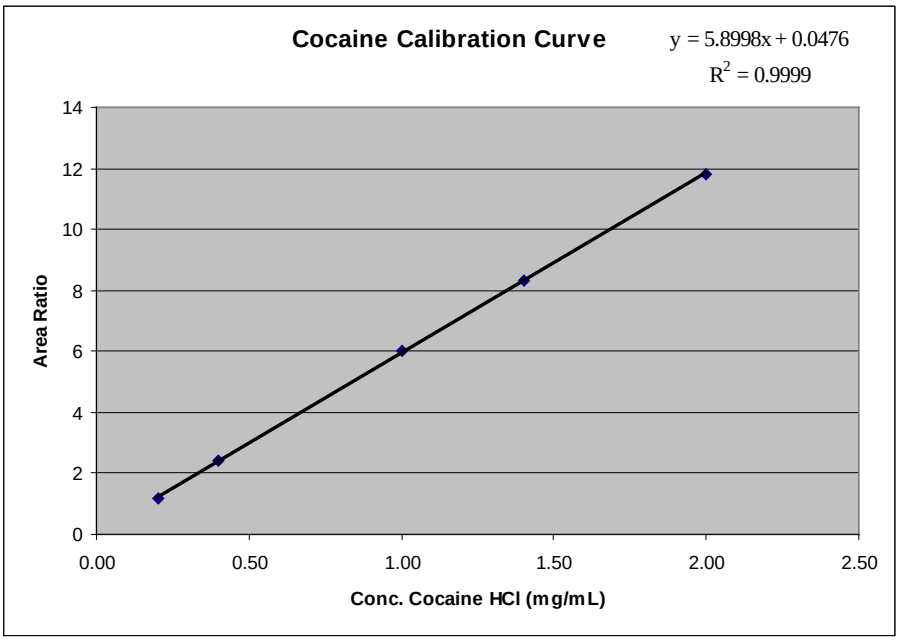
   Generate the standard curve in Excel by plotting the concentration of the standards on the x-axis and the corresponding area ratio on the y-axis. A linear trendline should be calculated. The R^2 correlation number should be a minimum of .9800 for the standard curve.

   Experimental and theoretical values for standard concentrations shall fall within 10% (absolute) of each other in order to be acceptable. If a standard does not meet criteria a

new standard dilution may be prepared and injected. It is not necessary to immediately prepare an entirely new series of dilutions. However, if more than one standard falls out of tolerance the analyst shall prepare a new curve.

To plot and get the equation of the trend line, click on the chart. Go up to the main menu, select Chart and then Add Trendline. Select Linear and go to the options tab. Select the 'Display Equation on Chart' option and the 'Display R-squared on chart' option.

Conc Cocaine HCl	Cocaine Area	Tetracosane Area	Area Ratio	Average Ratio	σ	2 σ	3 σ
0.20	152325	129620	1.1751659	1.1869433	0.0066503	0.01330058	0.01995087
0.20	152398	128035	1.1902839				
0.20	152575	128086	1.1911919				
0.20	152666	128359	1.1893673				
0.20	151622	127552	1.1887074				
0.40	308831	128333	2.4064816	2.4045057	0.0028286	0.00565725	0.00848588
0.40	307636	127757	2.4079776				
0.40	307538	128013	2.4023966				
0.40	308682	128555	2.4011668				
0.40	304260	126498	2.4052554				
1.00	765855	127622	6.0009638	6.0054653	0.0097916	0.0195832	0.02937481
1.00	764303	127556	5.9919016				
1.00	765502	127219	6.0171987				
1.00	769062	127923	6.0119134				
1.00	765682	127500	6.005349				
1.40	1112295	133290	8.3449246	8.3403222	0.0098866	0.01977323	0.02965985
1.40	1097639	131437	8.3510655				
1.40	1101925	132371	8.3245197				
1.40	1099959	131857	8.34206				
1.40	1099661	131869	8.339041				
2.00	1557622	132177	11.784365	11.799683	0.0241255	0.04825107	0.07237661
2.00	1521175	128915	11.799829				
2.00	1519049	128344	11.835762				
2.00	1517932	128941	11.772299				
2.00	1508532	127775	11.806159				



5. The corresponding standard curve shall be linear with a correlation of .98 or greater. Save the equation and continue on to the unknown samples.

- Quantifying the unknowns using Standard Curve

1. Place a homogenized,known mass of the unknown sample into a volumetric flask and dilute to mark using the same internal standard solution you prepared in Part I, solution #1. Use approximately 1 mg of the sample per 1 mL of internal standard solution.

2. Inject that solution into the GC-FID and use the method APDMID-FID.

3. Launch the Data Analysis portion of ChemStation. Open the chromatogram for a standard. Select Chromatogram and then Percent Report from the menu. A window will pop open on the bottom running a report. Right click that window and select print.

   Divide the cocaine peak's corr. area by the N-tetracosane peak's corr. area to calculate the area ratio.

4. Using the Standard Curve from Part I, solve for the concentration of cocaine (x-value). The y-value is the area ratio. This will be the concentration of cocaine in the unknown solution in mg/mL.

   To calculate the percent cocaine base (for reporting for federal cases):

$$\frac{\text{mg/mL Cocaine} \quad x \quad \text{mL of Internal Standard Solution}}{\text{mg unknown sample used}} \times 100 = \%\text{Cocaine HCl}$$

% Cocaine HCl x 0.89 = % Cocaine base

5. Save the Excel workbook to e-mail to the technical reviewer after case is completed.

Reference:

Pinero and Casale, Microgram Journal, Vol. 4, January-December 2006.
http://www.justice.gov/dea/programs/forensicsci/microgram/journal_v4_num14/pg7.html

Quantifying Heroin by GC-FID with Internal Standard

Equipment, Materials and Reagents

- GC with Flame Ionization detector
- Hydrogen carrier gas
- ACS grade chloroform or higher.
- Class "A" Volumetric glassware
  - All glassware shall be inspected for suitability before use.
- Analytical balance (Readability shall be 0.0001 or greater)
- 1mL Transfer pipette

Standards, Controls, and Quality Assurance

- N-Octacosane internal standard
- Guide 34 Heroin reference material (Calibrator)
- Traceable Heroin reference material (Quality Check Standard)
- Internal standard stock solution shall be labeled with a lot number which will correspond to the date the solution was prepared.
  - Solution shall be sealed and refrigerated when not in use.
  - Solution expires 3 months from date of preparation.

- Quality Check Standard (Heroin/Octacosane in $CHCl_3$)
  - A new check standard shall be prepared each time a new internal standard solution is prepared.
    - The reference material used to create the check standard must be a different lot, batch, or reference number than the material used as a calibrator.
  - Check standard lot number shall correspond with the internal standard stock solution.
  - The check standard concentration shall fall within limits of the standard curve.
  - Initially, any new check standard shall be run and analyzed along with the corresponding standard curve.
  - Determine the experimental concentration by performing a minimum of 5 injections on method "APDHIGH-FID".
  - The mean concentration of these 5 injections shall be calculated and documented on the GC-FID Quantitation Worksheet along with the original standard curve data.
  - All 5 injections must fall within ±10% (absolute) of the mean to be acceptable.
  - To ensure suitability of an existing standard curve the check standard shall be injected a minimum of 5 times with each subsequent batch of samples. The concentration will be documented and calculated on the Quantitation Worksheet, and shall fall within 10% (absolute value) of the initial experimentally determined mean.

- Standard curves shall be considered valid for use with casework until:

  - Certain changes in the condition of the GC-FID hardware (including any major repairs to the FID, or replacement of key components.)

- Internal standard solution is depleted.
- Check standard falls outside of 10% (absolute) tolerance.
- A period of one month has passed since the curve has been run.

- While is use for casework, approved standard curve excel workbooks will be saved electronically.
- Once a curve is no longer suitable for case work the respective excel workbook will be moved to the appropriate electronic folder and archived.

- Case Samples

  - The laboratory has determined that the purity difference between these two results must be equal to or less than the control limits (± 3 standard deviations) from the control chart. This process is used to establish the homogeneity of the sample.
  - Analysts may average the sample concentrations or report the lowest value.

- Generation of Standard Curve

1 Internal Standard Solution: Make an internal standard solution of approximately 0.2 mg/mL concentration of n-octacosane in chloroform. This solution will be the solution used to make at least five standard heroin solutions and to make the unknown analyte solutions.

2 Make a series of standards using heroin base in the internal standard solution from solution #1 ranging in heroin concentration from 0.2 mg/mL to 1.00 mg/mL. (You can go higher, you just need to show that the method is linear to that amount). Each standard should be prepared by diluting a known mass to 10mL. Also, a minimum of three data points (injections) should be collected for each standard and unknown analyte.

<u>Example:</u>
A series of standards- 0.20 mg/mL, 0.50 mg/mL, 0.70 mg/mL, 0.90mg/mL 1.00 mg/mL.

3 Inject each of the standards into GC-FID using the method APDHIGH-FID. The method uses the back injection port, column, and detector.

4. This will print out a copy of the integrated areas of each peak. Look for the peak area column on the report. Divide the heroin number by the octacosane number. This number will be the Area Ratio. (Heroin peak will be at approximately 6.39 minutes and Octacose at 7.18 minutes.)

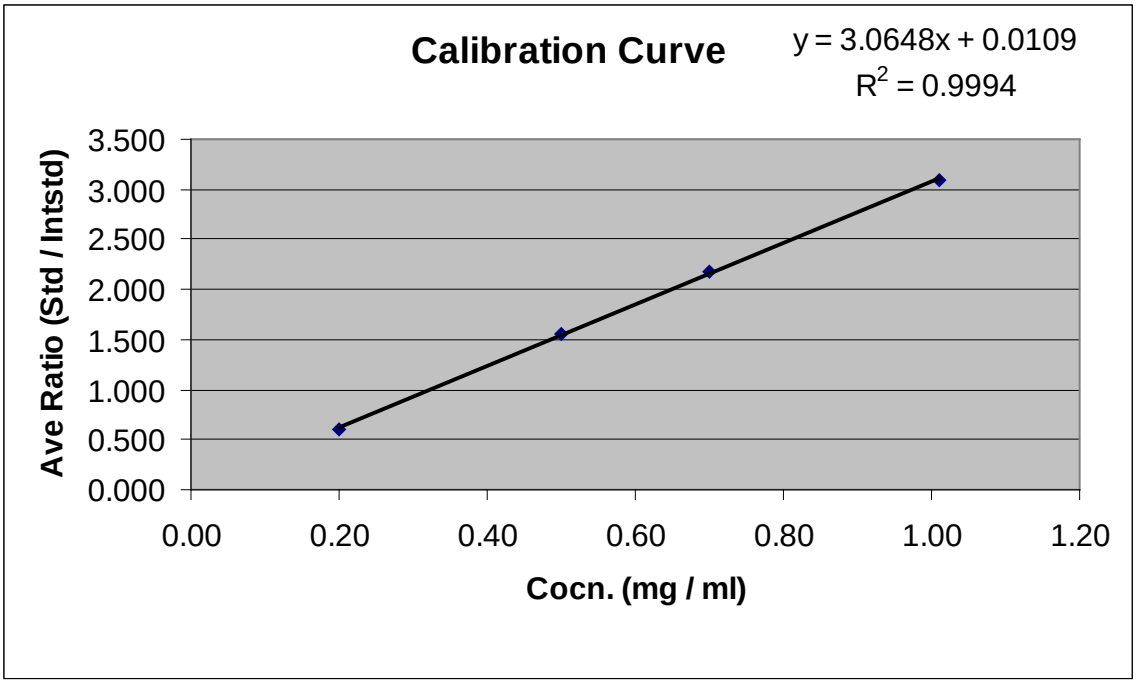
Generate the standard curve in Excel by plotting the concentration of the standards on the x-axis and the corresponding area ratio on the y-axis. A linear trendline should be calculated. The R^2 correlation number should be a minimum of .98 for the standard curve.

Experimental and theoretical values for calibration standard concentrations shall fall within 10% of each other in order to be acceptable. If a standard does not fall in this range a new standard dilution may be prepared and injected. It is not necessary to immediately prepare an entirely new series of dilutions. However, if more than one standard falls out of tolerance the analyst shall prepare a new curve.

To plot and get the equation of the trend line, click on the chart. Go up to the main menu, select Chart and then Add Trendline. Select Linear and go to the options tab. Select the 'Display Equation on Chart' option and the 'Display R-squared on chart' option.

All Calibration Data Collected

Run	Heroin Area	C28 Area	Ratio	Average Ratio	Retention Time (min)	
0.20 mg / ml					Heroin	C-28
1	173138	287288	0.60266352928	0.60394452749	6.384	7.177
2	174587	288415	0.60533259366		6.381	7.173
3	174324	288365	0.60452551454		6.381	7.172
4	172985	286752	0.60325647249		6.381	7.172
Average RT					**6.38**	**7.17**
0.50 mg / ml						
1	432484	275054	1.57236033652	1.55871482431	6.388	7.175
2	435620	282230	1.54349289587		6.390	7.175
3	437479	281010	1.55680936621		6.389	7.176
4	436195	279219	1.56219669865		6.389	7.177
Average RT					**6.39**	**7.18**
0.70 mg / ml						
1	617729	281718	2.19272108988	2.18319840576	6.392	7.174
2	617495	282747	2.18391353401		6.392	7.175
3	622840	285970	2.17799069833		6.393	7.175
4	625803	287307	2.17816830081		6.393	7.176
Average RT					**6.39**	**7.18**
1.01 mg / ml						
1	870844	287562	3.02836953422	3.08411297507	6.396	7.176
2	876475	283513	3.09148081393		6.397	7.176
3	869764	279995	3.10635547063		6.396	7.176
4	871718	280273	3.11024608150		6.394	7.174
Average RT					**6.40**	**7.18**



All QC Data Collected

Sample	Heroin Area	C28 Area	Ratio	Average Ratio	Retention Time (min)	
0.40 mg / ml					Heroin	C-28
1	353076	286328	1.23311726412	1.23734348840	6.387	7.175
2	357122	287936	1.24028256279		6.386	7.173
3	356483	288273	1.23661598554		6.386	7.175
4	360999	291279	1.23935814116		6.386	7.173
Average RT					**6.39**	**7.17**

Results of Calculations

Expected	x value Heroin (mg / ml)	Calculated Average (mg / ml)	Percent Difference of Expected & Calculated	Standard Deviation (1 σ) (mg / ml)	3 Standard Deviations (3 σ) (mg / ml)	3 Standard Deviations (3 σ) (%)
0.40	0.40	0.40	0.0%	0.001	0.003	0.8%
	0.40					
	0.40					
	0.40					

Maximum	**0.0%**	**0.001**	**0.003**	**0.8%**

5. The corresponding standard curve shall be linear with a correlation of at least .98. Save the equation and continue on to the unknown samples.

- Quantifying the unknowns using the Standard Curve

1. Place a known mass of the unknown sample into a volumetric flask and dilute to mark using the same internal standard solution you prepared in Part I, solution #1. Use approximately 0.5 mg of the sample per 1 mL of internal standard solution.

2. Inject that solution into the GC-FID, and use the method APDHIGH-FID.

3. From the printout, divide the heroin peak's corr. area by the octacosane peak's corr. area to calculate the area ratio.

4. Using the Standard Curve from Part I, solve for the concentration of heroin (x-value). The y-value is the area ratio. This will be the concentration of heroin in the unknown solution in mg/mL.

   To calculate the percent heroin base (for reporting for federal cases):

   $$\frac{\text{mg/mL Heroin} \times \text{mL of Internal Standard Solution}}{\text{mg unknown sample used}} \times 100 = \%\text{Heroin}$$

5. Save the Excel workbook to e-mail to the technical reviewer after case is completed.

Reference:

Pinero and Casale, Microgram Journal, Vol. 4, January-December 2006.
http://www.justice.gov/dea/programs/forensicsci/microgram/journal_v4_num14/pg7.html

7 ESTIMATION OF UNCERTAINTY (ISO 5.4.6)

Scope

- An uncertainty of measurement takes into consideration all the potential variables that contribute to the measured result. Sources contributing to the uncertainty may include, but are not limited to, the reference standards or materials used, the procedure or equipment used, the environmental conditions, the properties or condition of the item being tested and the analyst performing the test. All components that may contribute to the measured uncertainty will be taken into consideration when estimating the uncertainty of measurement. The Forensic Chemistry Section will have procedures for estimating the uncertainty of measurement where required. This section will attempt to identify all the components of uncertainty and make a reasonable estimation and will ensure that the form of reporting of the result takes into consideration any applicable measurement uncertainty.

Definitions

- Uncertainty of Measurement: A parameter associated with a measured result that characterizes the possible range of values that could, under a specified level of confidence, be attributed to the result or method.
- Measurand: Quantity intended to be measured.
- Bias: Also known as systematic error, this type of variation is non-random and is caused by recurring influence of one or more factors. For example, if the scale was not level, this could cause a systematic over-weighing or under-weighing of the sample.
- Type A Evaluation: These uncertainties are evaluated by statistical analysis of a series of observations
- Type B Evaluation : method of evaluation of uncertainty by means other than the statistical analysis of a series of observations
- Readability: the smallest increment which the balance displays (ranges 0.01g to 0.00001g in our laboratory)
- Repeatability/Reproducibility : closeness of the agreement between the results of successive measurements of the same measurand carried out under the same conditions (example: a balances ability to consistently deliver the same weight for a given mass)
- Linearity : the quality of delivering a significantly identical sensitivity throughout the weighing capacity of a balance
- Standard Uncertainty (u): a component of uncertainty, represented by an estimated standard deviation equal to the positive square root of the estimated variance.
- Distribution:
  - Normal : A pattern of frequency of values arrayed around a central mean value, such that the pattern is consistent with a Gaussian distribution

- Rectangular **:** A distribution of values that that there is equal probability that a value lies anywhere within the interval.

- Combined Standard Uncertainty **:** (u_c**)** square root of the sum of the squares of the uncertainty factors, used to express the uncertainty of many measurement results.
- Coverage factor **(k):** when applied to the combined uncertainty allows for the definition of the confidence interval; (k = 2 allows for approximately a 95.4% confidence interval, k = 3 allows for approximately a 99.7% confidence interval)
- Expanded Uncertainty **($U_{k=n}$):** quantity defining an interval within which the result of a measurement may be expected to encompass.
- Relative Contribution: demonstrates the individual factor's contribution to the event uncertainty.
- Standard Deviation: A value associated with a normal, or Gaussian distribution describing an average departure from the mean value.

Procedures (ISO 17025:2005, 5.4.6.2)

- Qualitative procedures such as identifying the presence or absence of a controlled substance do not require a measurement of uncertainty.

- The following test procedures have been identified as requiring an uncertainty of measurement estimation:

  - Controlled substance drug weights (Laboratory Balances)
  - Cocaine quantifications (UV and GC-FID)
  - Heroin quantifications (UV and GC-FID)
  - Methamphetamine quantification (UV)

- The Forensic Chemistry section Technical Leader in controlled substances is responsible for assessing and determining the uncertainty of measurement for the test procedures listed above.

  - Balance uncertainties shall be revisited annually after calibration by an approved vendor as needed. This may be documented in an Excel spreadsheet.
  - The Excel spreadsheet for each balance will be archived electronically.
  - Quantification uncertainties shall be revisited as needed.

Balance Uncertainty Determination

- Procedure for developing an uncertainty budget associated with weighing a controlled substance (An Excel spreadsheet may be used to compile data and perform all the needed calculations).
- Multiple analysts should participate in the test procedure. Ideally, all analysts proficient in the controlled substances discipline should participate.
- Uncertainties are grouped by model, and precision of balance.

- **Measurement Traceability** is established through the annual calibration of the balances, and the mass references standards used to confirm the continued calibration.
- **Measurement Assurance** is determined through the intermediate checks of the balances and mass reference standards.

- Identification and evaluation of components of uncertainty in the weighing procedure **(ISO 17025:2005, 5.4.6.2)**:

  - **Weighing Events**

    - A weighing event is defined as:

      - The taring of the balance followed by addition of the measurand (Single event). *Note: This method shall be used for collection of any data involving preparation of the uncertainty budget and monthly quality checks.*

      - Removal of the tared weighing vessel, filling with material, and then returning to the balance would constitute 2 weighing events.

    - Analysts have discretion with the weighing process in casework, but shall record the number of weighing events in the case record.

  - **Measuring Equipment (Balance)**

    - **Multiple equipment of the same model** –Type A Evaluation of process repeatability data.
    - **Readability** – Type B evaluation. Obtained from manufacturer's specifications. The purpose of this component is to account for the rounding that is automatically performed by the balance.
    - **Linearity** – Type B evaluation. Obtained from manufacturer's specifications.
    - **Balance Bias** – Mass reference standards are used to confirm the continued calibration status of the balances. This provides the laboratory with an ongoing evaluation of bias.
    - **Repeatability** – Type A evaluation. Determined in the laboratory by one of the following methods:

      - Use the historical data obtained from the monthly quality assurance checks to determine the standard deviation. The process for monthly balance checks is outlined in the Forensic Chemistry section SOP's. The monthly checks should attempt to cover the working range of the balance, or normal use of the instrument.

      - For new balances with no historical data, weigh several mass reference materials multiple times (minimum of 20 per mass) then calculate the standard deviation for the readings. Again, it is preferred to use masses throughout the range of the instrument or normal use of the instrument.

        - It is also acceptable to use a combination of historical data and data obtained specifically for the purpose of determining the uncertainty budget to calculate the standard deviations.
        - The standard deviations are calculated in grams.
    - **Balance calibration uncertainty –** Type B evaluation. This is obtained from the report of the annual calibration of balances by an approved vendor. A coverage factor of k=2, or 3 is acceptable.
    - **Buoyancy -** Samples with a density less than that of steel (8000 kg/m^3) have a "negative" effect on the difference between the "true" mass and the measured mass on the balance. So the balance actually shows a mass that is less than the "true" mass. As a result, any uncertainty due to buoyancy is neglected.
- **Staff**
    - **Multiple Analysts** –Type A evaluation of process repeatability data.
    - **Training** –Type A evaluation of process repeatability data.
    - **Experience**–Type A evaluation of process repeatability data.
- **Test Method**
    - **Eccentricity** - Type A evaluation of process repeatability data.
    - **Buoyancy**- Type B Evaluation. Buoyancy is difficult to account for in seized drug cases because the density of the material being weighed must be known. However, for material that has a lower density than the steel calibration weights (8.0 g/cm^3) the bias imparted is always negative and the weight displayed by the balance will be less than the true weight of the material. As a result any uncertainty due to buoyancy is negligible and may be ignored.
- **Facility**
    - **Location –** The location of the equipment has not been altered since calibration, and is covered by the calibration uncertainty. This becomes invalid if the balance is moved due to variations in gravitational forces.
    - **Temperature Variation -** Type A evaluation of process repeatability data.
    - **Air Flow -** Type A evaluation of process repeatability data.
    - **Vibration -** Type A evaluation of process repeatability data.
    - **Humidity -** Type A evaluation of process repeatability data.
    - **Static Electricity -** Type A evaluation of process repeatability data.

- Quantify standard uncertainty components:
  - Repeatability data is evaluated as a normal distribution and expressed as a standard deviation in grams:
    - $u_{(repeat)} = a / 1$
  - Readability data is evaluated as a rectangular distribution and is calculated by:
    - $U_{(read)} = a / \sqrt{3}$
    - Where $a = {}^{1}/_{2}$ (Readability of the balance in grams)
  - Balance Calibration Uncertainty
    - The uncertainty will be divided by the coverage factor, 2, to arrive at a standard uncertainty in grams.
    - $u_{(balcal)} = a / 2$
  - Balance Linearity is evaluated as a rectangular distribution:
    - Standard uncertainty = $a / \sqrt{3}$
    - $u_{(linear)} = {}^{1}/_{2}$ (linearity of the balance in grams)

- Calculate the relative contribution: Determine to what extent the factor affects the overall uncertainty budget. An item that contributes less than 1/3 of greatest relative contributor may be considered "Negligible".
  - $U^2 / (\sum(u_n)^2) *100$ This value is used to determine which factors are significant.
- Calculate the Combined Standard Uncertainty: $\mathbf{U_c} = (\sum(u_n)^2)$
  - U_c (single weighing event) = $\sqrt{u(read)^2 + u(repeat)^2 + u(linear)^2 + u(balcal)^2}$
- Calculate the Expanded Combined Uncertainty using the desired coverage factor.
  - The Forensic Chemistry section will typically use a 99.7% confidence interval (k=3).
  - $U_{ck=3} = 3*u_c$

- It is acceptable to determine the uncertainty associated with each balance individually, or to pool data based on make, model, and precision of balance.

Example Uncertainty Budget Sheet				
Factor	Value (x), g	Standard Uncertainty (u), g	Distribution	Relative contribution to u in %
Readability (Type B)	From manufacturer	(X/2)/√3	**Rectangular**	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties **$(u_n)^2/(\sum(u_n)^2)$**
Repeatability (Type A)	Determined in house. This is the SD determined as listed above (k=3)	x/1	**Normal**	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties **$(u_n)^2/(\sum(u_n)^2)$**
Linearity (Type B)	From manufacturer	(x/2)/√3	**Rectangular**	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties **$(u_n)^2/(\sum(u_n)^2)$**
Uncertainty from balance calibration report (U, coverage factor k=2 or3) (Type A)	Determined annually by approved vendor.	x/1	**Normal**	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties **$(u_n)^2/(\sum(u_n)^2)$**
Subtotal of the uncertainty ($\sum(u_n)^2$)		Sum of the square of each of the uncertainty factors		
Uc = square root of ($\sum(u_n)^2$)	Square root of the sum of the squared uncertainty components	**grams**		
Expanded Uncertainty (U); where (k) = 3	$U_{k=3}$ =(u_cx3)	**gram/weighing event**		

Uncertainty of Measurement Calculation for Drug Weights

- An uncertainty of measurement shall be determined for sample weights that are reported.
- The case record will indicate the uncertainty value (or values) for the balance(s) used. The current uncertainty values for each balance shall be made available to each analyst.
- The number of weighing events shall be recorded in the case record.

- Significant Figures

  - The number of significant figures in the reported value must be less than or equal to the number of significant figures in the precision of the balance.

  - Significant figures will be conserved during calculations and reporting. In other words, when combining measurements with different precision, the number of significant figures of the final reported result can be no greater than the number of significant digits to the right of the decimal of the least precise balance. See Table 1.

Table 1	
Balance	**Weight**
Readability 1 = 0.01	10.04
Readability 2 = 0.001	10.036
Total Weight	10.076
Reported Weight	10.07

Single Weighing Event

- The calculated expanded uncertainty of the scale used is the uncertainty for the measurement.

- Examples:
  - The reading from the scale is 1.23 grams and the uncertainty value is 0.04g. The reported value would be 1.23 grams ±0.04g.
  - The reading from the scale is 1.0 pounds and the uncertainty value is 0.2lbs. The reported value would be 1.0 pounds ±0.2lb

Multiple Measurements with the Same Balance

- To determine the uncertainty value for multiple measurements, one cannot simply add all of the uncertainties together and report the combined uncertainties as the total uncertainty. This is because the error is random; sometimes the measured weight will be too high. Other times it will be lower than the true value.
- The Root Sum Squares method must be used for this calculation.

Equation 1

$$U = \sqrt{N * (u_b)^2}$$

U = total Uncertainty for the sum value
N = number of measurements
u_b = Uncertainty of the balance

Equation 1 can be simplified to Equation 2.

Equation 2

$$U = \sqrt{N} * u_b$$

Example 1:

A balance used to obtain the weight for five bindles has an uncertainty of 0.01 gram. Table 3 shows the weights of the 5 bindles using this balance.

Table 3	
Bindle #	**Weight (g)**
1	0.52
2	0.67
3	0.78
4	1.02
5	0.21
Total	**3.20 g**

The uncertainty from each weighing must be considered. To determine the combined uncertainty from all five weighings use Equation 2.

Using Equation 2, the combined uncertainty would be calculated as follows:

$$U = \sqrt{N} * u_b = \sqrt{5} * 0.01 = 0.02 \ g$$

Since uncertainty values will have the same precision as the balance being used and will be rounded up; the calculated total uncertainty value of 0.022 gram would be rounded up to 0.03 gram. Reported weight would be 3.20grams ±0.03g

Multiple Measurements: Two or More Balances or Multiple Uncertainty Values for a Single Balance

- At times, individual items within an exhibit will necessitate using two different balances, or, different weights may fall within different calculated uncertainty values for a particular balance. The example below would apply to either of these situations. These calculations also apply to subtraction calculations; although the difference is found through subtraction, the combined uncertainty is the sum of the separate uncertainties.

Example:

A submission came into the laboratory that consisted of four plastic bags and three paper bags all containing material that was visually consistent. Due to the different sizes of the containers, the analyst decided to weigh the contents of the plastic bags on a 400-gram capacity balance with an uncertainty value of 0.01 gram and weigh the contents of the paper bags on a larger capacity balance with an uncertainty value of 0.4 gram.

As an aside, if the analyst made all the measurements on a single 600-gram capacity scale that had separate uncertainty values for different ranges of weights, the “d” value would be the same for both weight levels.

Table 4. Balance B1	
Plastic bags	Weight (g)
1	10.56
2	14.66
3	28.50
4	31.27
Total	84.99 g

Uncertainty = 0.01g, readability = 0.01

Table 5. Balance B2	
Paper bags	**Weight (g)**
1	450.6
2	422.8

3	575.2
Total	1448.6 g

Uncertainty = 0.4 g, readability =0.2

For this calculation Equation 2 will be used twice – once for B1 and once for B2. The combined equation (Equation 3) will be as follows:

Equation 3

$$U = \left(\sqrt{N_{B1}} * u_{B1}\right) + \left(\sqrt{N_{B2}} * u_{B2}\right)$$

U: total uncertainty
N_{B1}: number of measurements on Balance 1
u_{B1}: uncertainty of Balance 1
N_{B2}: number of measurements on Balance 2
u_{B2}: uncertainty of Balance 1

Using the above numbers for the weights obtained in Tables 3 and 4 the following measurement of uncertainty is obtained.

$$U = \left(\sqrt{4} * 0.01\right) + \left(\sqrt{3} * 0.4\right) = 0.71g$$

The combined net weight (total of balance B1 plus total of balance B2) is 1533.59 grams. When combining measurements with different degrees of precision, *the precision of the final answer can be no greater than the least precise measurement.* With this rule in mind, the total weight would be 1533.5 grams (one decimal place). The weight itself is never rounded up, but rather is rounded down. The calculated uncertainty (U) equaled 0.71 gram, which would be rounded up to 0.8 gram (uncertainty sum rounded up to the nearest increment of the larger readability value). The reported net weight would be 1533.5 ± 0.8 grams

Uncertainty Determination for Quantification by Ultraviolet Spectrophotometry

Scope

- To outline procedures for developing an Uncertainty Budget Associated with Ultraviolet Spectrophotometry quantifications (purity determinations) of Cocaine, Methamphetamine, and Heroin respectively. **(ASCLD/LAB Policy on Measurement Uncertainty, 4.1)**
- An Excel spreadsheet may be used to perform all the needed calculations

Test Method Information

- Specific methods are delineated in the Quantitative Analysis section of this manual.

- Multiple analysts perform these methods.

  - One balance is used for these methods.
  - Analysts use the same class "A" glassware for these methods.
  - Analysts use the same dilute acid solution for preparing their measurands.
  - Analysts use the same reference materials (measurands) of known purity for the test method.
    - The measurands are traceable reference materials accompanied by a Certificate of Analysis that the laboratory will retain.
    - The measurands are considered to be homogeneous.

- A method uncertainty is established by analyzing the measurands multiple times.

  - The measurands are analyzed over several days to establish figures of merit such as repeatability.

- The laboratory shall retain all existing data from the measurement process.
- Uncertainties and control charts are grouped by drug of abuse.
- Method Uncertainty will be reevaluated as deemed necessary by the technical leader.

- **Measurement Traceability** is established through the equipment and materials used in the test method. The following that have a significant effect on the accuracy of the test method **(ISO 17025:2005, 5.4.6.2)**:

  - **Analytical balance (weighing of sample) -**Type A evaluation of method process reproducibility

    - External calibration performed annually by a vendor that meets Section 3.2.3 of the ASCLD/LAB Policy on Measurement Traceability

  - **Reference material (Measurand)** –Type B Evaluation

    - Material may be acquired from an accredited Reference Material Producer (RMP) that is accredited to ISO Guide 34:2009.

- Material may be acquired from an RMP that is not to ISO Guide 34:2009 accredited but the laboratory will evaluate the competence and traceability of the RMP and maintain objective evidence
- Purity of Reference Material – Type "B" Evaluation

- **Staff**
  - **Multiple Analysts** -Type A evaluation of method process reproducibility data.
  - **Training** - Type A evaluation of method process reproducibility data.
  - **Experience** – Type A evaluation of method process reproducibility data.
- **Preparation of Samples**
  - **Balance (Weighing of samples)** - Type A evaluation of method process reproducibility data.
  - **Volumetric Flasks -** Type A evaluation of method process reproducibility data. Includes:
    - Uncertainty of bringing flask to volume (reading flask meniscus)
    - Uncertainty of volume of flasks
    - Class "A" flasks were included in the uncertainty of measurement evaluation However, there is no significant impact on the accuracy of the method and is documented on each respective uncertainty budget.
    - The lab has determined that calibration of flasks is not necessary,
- **Homogeneity** – Type B evaluation controlled through the requirement for the agreement of replicates.
- **Analysis -** Type A evaluation of method process reproducibility data. Includes:
  - Instrument parameters (wavelength ranges, choppers, filters, mirrors, etc.)
  - Interference from solvent/matrix (Noise)

- **<u>Measurement Assurance</u>**
  - The lab has determined that the monthly checks of the calibration status of the analytical balance are required. The lab uses calibrated mass reference standards with established measurement traceability to perform the monthly checks of the calibration status of the balance.
  - The spectrophotometers are quality checked monthly.

- **<u>Evaluation of uncertainty components:</u>**

  - Method Process Reproducibility

    - A control chart is derived from the analysis of the measurand.
    - Long term variability associated with method performance is captured by the control chart.
    - Ideally, a minimum of 5 points from every analyst will be collected annually, or as deemed necessary by the Technical Leader.
    - The standard deviation obtained from the control chart is calculated as a relative percentage from a minimum of 25 points.

$$\text{Relative Standard Deviation} = \frac{|\text{Standard Deviation } \%Purity_{lab}|}{\text{Mean } \%Purity_{lab}} \times 100$$

      - Expressed as a relative percent.

  - This value represents the Method Process Reproducibility

  - Homogeneity

    - Controlled through the procedure administrative requirement for agreement of replicates. The difference between 2 samples may not be greater than the method variability.
    - This value is equal to the relative standard deviation calculated from the control chart.

  - Purity of reference material

    - The purity specification listed on the Certificate of Analysis will determine the purity range, and shall be listed on each respective uncertainty budget.

  - Uncertainty associated with volumetric flasks (Class A)

    - This value is the tolerance in mL as listed by the vendor.
    - To be conservative the value used will be taken from the largest volumetric flask used (currently 25mL).

- **Conversion of quantities to standard uncertainties**

  - The measurement unit will be a relative standard uncertainty expressed as a percentage.

  - Method process reproducibility

    - This value is in the correct unit but will be divided by the square root of the number of data points (N) from the control chart. This yields the relative standard deviation of the mean.

      - $RSD_{mean} = \frac{RSD}{\sqrt{N}}$

  - Method Process Reproducibility is evaluated as a normal distribution since all data points are within 3 standard deviations of the mean

    - $U_{reproducibility} = RSD_{mean} / 1$

  - Homogeneity

    - Lab procedure requires that two replicate samples both be within a 99% confidence interval (k=3, or 3σ).
    - The value (3σ) is also represented by the method process reproducibility (3 x RSD).
    - Since the value is a coverage factor of 3 it will divided by three to achieve a standard uncertainty (3/RSD).
    - This component is evaluated as a rectangular distribution.

    - For a rectangular distribution, the standard uncertainty is calculated by:

    - $U_{homogeneity} = a / \sqrt{3}$

      - Where a = RSD

      - Std. Uncertainty = $\frac{A}{\sqrt{3}}$

  - Purity of reference material
    - This component is evaluated as a rectangular distribution.

    - For a rectangular distribution, the standard uncertainty is calculated by:
      $U_{RMpurity} = a / \sqrt{3}$

      - Where a = the outside limit of the purity

      - Std. Uncertainty = $\frac{A}{\sqrt{3}}$

- Uncertainty associated with volumetric flasks (Class A)
  - Review of specifications from the vendor shows that the tolerance of 25mL volumetric flask is ±0.03mL.
  - The relative percentage is given by:
    - (0.03 mL/ 25mL) * 100 = 0.14
  - This component is evaluated as a rectangular distribution.
  - For a rectangular distribution, the standard uncertainty is calculated by:
    $U_{vol} = a / \sqrt{3}$
    - Where a = the outside limit of the tolerance (relative)
    - Std. Uncertainty = $\frac{A}{\sqrt{3}}$
    - $= \frac{0.14}{\sqrt{3}}$
    - = 0.08%

- Calculation of combined standard uncertainty (relative percentages)
  - Combined standard uncertainty (U_{rel}) is the positive square root of the variance of all components combined.
  - $U_{rel} = \sqrt{u(_{reproducibility})^2 + u(_{homogeneity})^2 + u(_{RMpurity})^2 + u(_{vol})^2}$

- Calculation of expanded uncertainty
  - In this case the u_{rel} is calculated as a relative percentage and must be converted to an absolute percent of the experimentally determined mean value:
    $(U_{rel} /100)$ * Experimentally determined purity = U_{abs}
  - The expanded uncertainty is expressed mathematically as:

$$U = k * U_{abs}$$

  - Using a coverage factor k = 3 (confidence level of approximately 99% assuming the %purity follows a normal distribution):

$$U_{k=3} = 3 * U_{abs}$$

- Calculate the relative contributions/Evaluation of Uncertainty
  - Determine to what extent each factor affects the overall uncertainty budget.
  - This calculation is used to determine which factors are significant:
    - $\frac{U^2}{(\sum(u_n)^2)} * 100$
    - Where U is an individual standard uncertainty component.
    - U_n is the subtotal of squared U values.
  - An item that contributes less than 1/3 of greatest relative contributor (%) may be considered "Negligible".

Example Quantification Uncertainty Budget Sheet				
Factor	Value (x),	Distribution	Standard Uncertainty (u), relative %	Relative contribution to u in %
Method Process Reproducibility	Determined in house.	Normal	x/1	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_n)^2/(\sum(u_n)^2$
Homogeneity	Determined in house.	Rectangular	x/1	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_n)^2/(\sum(u_n)^2$
Purity of reference material	From manufacturer	Rectangular	x/√3	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_n)^2/(\sum(u_n)^2$
Uncertainty of volumetric flask volume	From manufacturer	Rectangular	x/√3	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties $(u_n)^2/(\sum(u_n)^2$
Subtotal of individual u values		$\sum(u_n)$:		
Subtotal of squared u values		$(\sum(u_n)^2)$		
Combined Standard Uncertainty		U_{rel}		

Uncertainty Determination for Quantification by Gas Chromatography

Scope

- To outline procedures for developing an Uncertainty Budget Associated with Gas Chromatography Flame Ionization Detector quantifications (purity determinations) of Cocaine and Heroin respectively. **(ASCLD/LAB Policy on Measurement Uncertainty, 4.1)**
- An Excel spreadsheet may be used to perform all the needed calculations

Test Method Information

- Specific methods are delineated in the Quantitative Analysis section of this manual.

- Multiple analysts perform these methods.

    - One balance is used for these methods.
    - Analysts use the same class "A" glassware for these methods.
    - Analysts use the same internal standard solution for preparing their measurands.
    - Analysts use the same calibrators and quality control measurands for the test method.
    - A method uncertainty is established by analyzing the respective quality control measurands multiple times.
    - The measurands are analyzed over several days to establish figures of merit such as repeatability.

- The laboratory shall retain all existing data from the measurement process.
- Uncertainties and control charts are grouped by drug of abuse.
- Method Uncertainty will be reevaluated as deemed necessary by the technical leader.

- **Measurement Traceability** is established through the equipment and materials used in the test method. The following that have a significant effect on the accuracy of the test method **(ISO 17025:2005, 5.4.6.2)**:

    - **Analytical balance (weighing of sample) -**Type A evaluation of method process reproducibility

        - External calibration performed annually by a vendor that meets Section 3.2.3 of the ASCLD/LAB Policy on Measurement Traceability

    - **Calibrators** –Type B Evaluation

        - Material may be acquired from an accredited Reference Material Producer (RMP) that is accredited to ISO Guide 34:2009.

- Material may be acquired from an RMP that is not to ISO Guide 34:2009 accredited but the laboratory will evaluate the competence and traceability of the RMP and maintain objective evidence.

- Purity of Calibrator – Type "B" Evaluation

- Administrative Requirement for agreement – Type B Evaluation of 10% requirement for agreement between theoretical and experimental values.

➢ **Quality Control Measurands --**Type B Evaluation

- The quality control measurands are composed of drugs of abuse that the A.P.D has on hand.
- The quality control measurands have a purity established by third party analysis as well as historical data.
- The quality control measurands are considered to be homogeneous.
- Purity of quality control measurand – Type "B" Evaluation

➢ **Staff**

- **Multiple Analysts** -Type A evaluation of method process reproducibility data.
- **Training** - Type A evaluation of method process reproducibility data.
- **Experience** – Type A evaluation of method process reproducibility data.

➢ **Preparation of Samples**

- **Balance (Weighing of samples)** - Type A evaluation of method process reproducibility data.
- **Volumetric Flasks -** Type A evaluation of method process reproducibility data. Includes:
  - Uncertainty of bringing flask to volume (reading flask meniscus)
  - Uncertainty of volume of flasks
  - Class "A" flasks were included in the uncertainty of measurement evaluation However, there is no significant impact on the accuracy of the method and is documented on each respective uncertainty budget.
  - The lab has determined that calibration of flasks is not necessary,

➢ **Homogeneity** – Type B evaluation controlled through the requirement for the agreement of replicates.

- **Analysis -** Type A evaluation of process repeatability data. Includes:
  - Instrument parameters (oven temperatures, split ratios, gas flow, column condition, autosampler, FID, etc.)
  - Interference from matrix (Noise)
  - Data processing through integration parameters.

- **Measurement Assurance**

  - The lab has determined that the monthly checks of the calibration status of the analytical balance are required. The lab uses calibrated mass reference standards with established measurement traceability to perform the monthly checks of the calibration status of the balance.

- **Evaluation of uncertainty components:**

  - Method Process Reproducibility

    - A control chart is derived from the analysis of the measurand.
    - Long term variability associated with method performance is captured by the control chart.
    - Ideally, a minimum of 5 points from every analyst will be collected annually, or as deemed necessary by the Technical Leader.
    - The standard deviation obtained from the control chart is calculated as a relative percentage from a minimum of 25 points.

$$\text{Relative Standard Deviation} = \frac{\text{Standard Deviation } \%Purity_{lab}}{\text{Mean } \%Purity_{lab}} \times 100$$

      - Expressed as a relative percent.

    - This value represents the Method Process Reproducibility

  - Homogeneity

    - Controlled through the procedure administrative requirement for agreement of replicates. The difference between 2 samples may not be greater than the method variability.
    - This value is equal to 3 times the relative standard deviation calculated from the control chart.

  - Purity of calibrators

    - The purity specification listed on the Certificate of Analysis will determine the purity range, and shall be listed on each respective uncertainty budget.

- Quality Control Measurands
  - The purity specification listed on the Certificate of Analysis or corresponding lab report will determine the purity, and shall be listed on each respective uncertainty budget.
  - This value is the difference between the purity experimentally determined by the lab, and the purity determined by a third party (relative percent).

$$\textit{Q.C. Measurand Uncertainty} = \frac{|\%Purity_{std} - \%Purity_{lab}|}{\%Purity_{std}} \times 100$$

    - Expressed as a relative percent.

- Uncertainty associated with volumetric flasks (Class A)
  - This value is the tolerance in mL as listed by the vendor.
  - To be conservative the value used will be taken from the largest volumetric flask used (currently 25mL).

- **<u>Conversion of quantities to standard uncertainties</u>**
  - The measurement unit will be a relative standard uncertainty expressed as a percentage.
  - Method process reproducibility
    - This value is in the correct unit but will be divided by the square root of the number of data points (N) from the control chart. This yields the relative standard deviation of the mean.
      - $RSD_{mean} = \frac{RSD}{\sqrt{N}}$
    - Method Process Reproducibility is evaluated as a normal distribution since all data points are within 3 standard deviations of the mean
    - $U_{reproducibility} = RSD_{mean} / 1$
  - Homogeneity
    - Lab procedure requires that two replicate samples both be within a 99% confidence interval (k=3, or 3σ).
    - The value (3σ) is also represented by the method process reproducibility (3 x RSD).
    - Since the value is a coverage factor of 3 it will divided by three to achieve a standard uncertainty (3/RSD).
    - This component is evaluated as a rectangular distribution.

- For a rectangular distribution, the standard uncertainty is calculated by:
- $U_{homogeneity} = a / \sqrt{3}$
  - Where a = RSD
  - Std. Uncertainty = $\frac{A}{\sqrt{3}}$

- Quality Control Measurands
  - This component is evaluated as a rectangular distribution.
  - $U_{measurand} = a / \sqrt{3}$
    - Where a = the outside limit of the uncertainty of the purity.
    - Std. Uncertainty = $\frac{A}{\sqrt{3}}$

- Uncertainty associated with volumetric flasks (Class A)
  - Review of specifications from the vendor shows that the tolerance of 25mL volumetric flask is ±0.03mL.
  - The relative percentage is given by:
    - (0.03 mL/ 25mL) * 100 = 0.14
  - This component is evaluated as a rectangular distribution.
  - For a rectangular distribution, the standard uncertainty is calculated by
  - $U_{vol} = a / \sqrt{3}$
    - Where a = the outside limit of the tolerance.
    - Std. Uncertainty = $\frac{A}{\sqrt{3}}$
    - $= \frac{0.14}{\sqrt{3}}$
    - = 0.08%

- Calibrators
  - Purity of reference material is evaluated as a rectangular distribution.
  - $U_{calib} = a / \sqrt{3}$
    - Where a = the outside limit of the purity.
  - Quantification method also requires that theoretical concentrations of calibration samples be within 10% agreement of experimental concentrations
  - This administrative requirement is evaluated as a rectangular distribution.
  - $U_{admin} = a / \sqrt{3}$
    - a = 10.0%
    - $10.0 / \sqrt{3}$
    - = 5.77%

- Calculation of combined standard uncertainty (relative percentages)
  - $U_{rel} = \sqrt{u_{(reproducibility)}^2 + u_{(homogeneity)}^2 + u_{(measurand)}^2 + u_{(vol)}^2 + u_{(calib)}^2 + u_{(admin)}^2}$

- Calculation of expanded uncertainty
  - In this case the u_{rel} is calculated as a relative percentage and must be converted to an absolute percent of the experimentally determined mean value:

    $(U_{rel} /100)$ * Experimentally determined purity = U_{abs}
  - The expanded uncertainty is expressed mathematically as:

$$U = k * U_{abs}$$

Using a coverage factor $k = 3$ (confidence level of approximately 99% assuming the %purity follows a normal distribution):

$$U_{k=3} = 3 * U_{abs}$$

- Calculate the relative contributions/Evaluation of Uncertainty
  - Determine to what extent each factor affects the overall uncertainty budget.
  - This calculation is used to determine which factors are significant:
    - $\frac{U^2}{(\sum(u_n)^2)} * 100$

- Where U is an individual standard uncertainty component.
- U_n is the subtotal of squared U values.

- An item that contributes less than 1/3 of greatest relative contributor (%) may be considered "Negligible".

Example Quantification Uncertainty Budget Sheet				
Factor	Value (x),	Distribution	Standard Uncertainty (u), relative %	Relative contribution to u in %
Calibrator Uncertainty	Purity provided from manufacturer	Rectangular	x/√3	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties **$(u_n)^2/(\sum(u_n)^2)$**
Administrative Calibrator Uncertainty	10.0%	Rectangular	x/√3	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties **$(u_n)^2/(\sum(u_n)^2)$**
Repeatability (Method Uncertainty)	Determined in house.	Normal	x/1	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties **$(u_n)^2/(\sum(u_n)^2$**
Control Chart	Determined in house.	Normal	x/1	The standard uncertainty for the factor divided by the subtotal of the standard uncertainties **$(u_n)^2/(\sum(u_n)^2$**
Subtotal of individual u values		$\sum(u_n)$:		

Subtotal of squared u values	$(\sum(u_n)^2)$	

References

SWGDRUG: Supplemental Document SD-3, **For Part IVC - Quality Assurance/Uncertainty, Measurement Assurance for Weight Determinations in Seized Drug Analysis**. Available at www.swgdrug.org.

SWGDRUG: Supplemental Document SD-4, **Measurement Uncertainty for Purity Determinations in Seized Drug Analysis For Part IVC – Quality Assurance/Uncertainty**. Available at www.swgdrug.org.

ASCLD/LAB International: **Estimating Uncertainty of Measurement Policy** (AL-PD-3060) (Garner, North Carolina:ASCLD/LAB, 2013). Available at www.ascld-lab.org

ASCLD/LAB International:**Guidance on the Estimation of Measurement Uncertainty – Annex B Drug Chemistry Discipline Three Examples – Weight, Volume and Purity Determination** (AL-PD-3063) (Garner, North Carolina:ASCLD/LAB, 2013). Available at www.ascld-lab.org

National Institute of Standards and Technology, SOP29 – **Standard Operating Procedure for the Assignment of Uncertainty**, (Gathersburg, Maryland, February 2012)

http://www.nist.gov/pml/wmd/labmetrology/upload/SOP_29_20120229.pdf

Joint Committee for Guides in Metrology (JCGM), **Evaluations of Measurement Data – Guide to the Expression of Uncertainty in Measurement (GUM)** Available at www.bipm.org

8 DRUG ANALYSIS NOTES

Scope

- The following is a collection of notes on the analysis of selected drugs. Included in this section are general physical and chemical properties and extraction techniques used by the Austin Police Department Chemistry Section. This section is not exhaustive as it is not possible to anticipate every situation that may arise or to prescribe a specific course of action for every case; therefore, the examiner must exercise good judgment based on experience and common sense.

Extraction Techniques

Dry Extraction

- Dry extraction is the use of organic solvents to isolate or remove substances based on their solubility. This technique can be very useful and must not be overlooked. Dry extraction can be versatile in that a sample may be changed in form to make it more suitable for using this technique. For example, a drug may be converted to its base form, to a salt form, or to a different salt form in order to obtain the desired solubility or insolubility of a substance for dry extraction. Dry extraction is most useful when:
  - A single drug is present and the drug is soluble in an organic solvent while the excipients are not.
  - The drug of interest is soluble in an organic solvent while other drugs and excipients are not.
  - The contaminant(s) is soluble in an organic solvent while the drug of interest is not.

Immiscible Solvent Extraction

- This is the most common extraction method used by drug analysts. Mixtures of substances are isolated or separated according to their acidic or basic nature using the proper pH and an organic solvent such as chloroform, hexane, or ether.

Extraction of Acidic Drugs

- Acidic drugs will extract from aqueous acidic solutions into organic solvents and from organic solvents into aqueous alkaline (basic) solutions. e.g.:

  1. Add 0.2 N H_2SO4 to separatory funnel or test tube.
  2. Add sample and shake to dissolve.
  3. Add an immiscible organic solvent and shake. Drain or transfer solvent layer into another separatory funnel, test tube, or evaporation dish.

4. The solvent can now be evaporated for IR or GC/MS analysis or can be reconstituted into an acidic solution such as 0.2 N H_2SO4 for UV analysis.

Extraction of Basic Drugs

- Basic drugs will extract from alkaline (basic) aqueous solutions into an immiscible organic solvent and from immiscible organic solvent into acidic solutions.

  1. Add 0.2 N H_2SO4 to separatory funnel or test tube.
  2. Add sample and shake to dissolve.
  3. Make basic with aqueous base, such as concentrated NaOH.
  4. Add an immiscible organic solvent and shake. Drain or transfer solvent layer into another separatory funnel, test tube, or evaporation dish.
  5. The solvent can now be evaporated for IR analysis or can be extracted to and acid solution such as 0.2 N H_2SO4 for UV analysis.

Extraction of Neutral Drugs

- Neutral drugs are usually more soluble in organic solvents than aqueous solutions.
- Neutral drugs will usually extract from acidic, alkaline, or neutral solutions into chloroform or ether.

Common Contaminants

- Most drug samples require purification prior to instrumental analysis. Even when chromatographic techniques are used, it is sometimes desirable to isolate or concentrate the compound of interest. The removal of contaminants will greatly facilitate the identification of the controlled substance.

  - **Nicotinamide**

    - Nicotinamide is not very soluble in hexane and can be removed from basic drugs by one or more extractions as follows:

      - Base → Hexane → H2SO4

  - **Acetaminophen**

    - Acetaminophen is not very soluble in dilute H2SO4 and can be removed from basic drugs as follows:

      - Sample + Base → $CHCl_3$ (water wash if necessary)→ 0.2 N H_2SO_4

  - **Aspirin**
    - Aspirin extracts well from acidic solutions and very slightly from basic solutions.
    - Separation of a basic drug from aspirin:

- Sample + 1 M Na_2CO_3 → $CHCl_3$ → 3 $NaHCO_3$ washes
- This will move the aspirin to the NaHCO3, leaving basic drugs in the CHCl3.

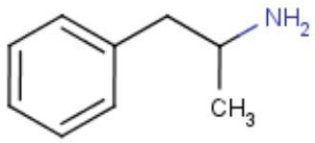
- Alternate method:
  - Sample + 0.2N H_2SO_4 → 3-4 $CHCl_3$ washes
  - Basic drugs will remain in the acidic solution while the aspirin is removed by the CHCl3 washes.
- Separation of an acid drug from aspirin:
  - Sample + 0.2 N H_2SO_4 → $CHCl_3$ → $NaHCO_3$ washes
  - Strongly acidic drugs, such as aspirin, can be washed from $CHCl_3$ with 2-3 bicarbonate washes. Weak acidic drugs will remain in $CHCl_3$ and may be extracted with 0.1 N NaOH if desired.

➢ **Caffeine**

- Caffeine is a neutral drug that will extract from acidic, basic or neutral aqueous solutions with chloroform.
- Caffeine is only slightly soluble in Hexane.
- Caffeine can be removed with water from hexane or chloroform.

Amphetamine

- **Drug Name** - Amphetamine --- Common Names - Speed, Crank, Go-Fast
- **Chemical Formulas**
  - ➢ Empirical - C_9H_{13}
- **Molecular Weight** - 135



Amphetamine
CAS 300-62-9

- **Properties**
  - ➢ Color - White to brown.

- Form - Powder, liquid, sticky paste, etc.
- Solubility
  - Free Base - Slightly soluble in water. Soluble in alcohol, ether, chloroform, and acids.
  - Hydrochloride - Soluble in water, alcohol, and chloroform.
  - Sulfate - Soluble in water. Slightly soluble in alcohol.

- **Analysis Procedure**

- Color Tests
  - Marquis - Orange to orange-brown color; immediate reaction.
  - Sodium Nitroprusside Test – Negative

- UV Analysis
  - Direct in 0.2 N H_2SO_4.
  - Extracted: Sample + Base → $CHCl_3$ → 0.2 N H_2SO_4
  - TLC (when necessary)
    Same as methamphetamine.

- GC/MS Analysis
  - Direct in CHCl3
  - Extracted: Sample + Base → $CHCl_3$

- IR Analysis
  - Sample can be run direct using:
    - ATR
    - Mixed with KBr in drift cell
  - Isolate the amphetamine base or amphetamine salt for IR analysis by extracting the sample as follows:
    - Extracted: Sample + Base → $CHCl_3$ → evaporate under low heat for amphetamine base oil.
  - Place oil on KBr Salt cell or direct on ATR
  - The $CHCl_3$ from the above extraction is filtered into an evaporation dish and HCl bubbled in.
  - Amphetamine HCl is hygroscopic, therefore it is usually best to bubble HCl gas through $CHCl_3$ to convert to HCl salt rather than adding HCl as liquid.
  - Place salt on KBr Salt cell or direct on ATR.

BZP Derivatization with TMS

Purpose

- To establish a method for derivatization and subsequent characterization of N-Benzylpiperazine via GC-MS.
- TMS derivatization is known to react with amines and hydroxyls to produce different ion fragmentation pattern for identification.

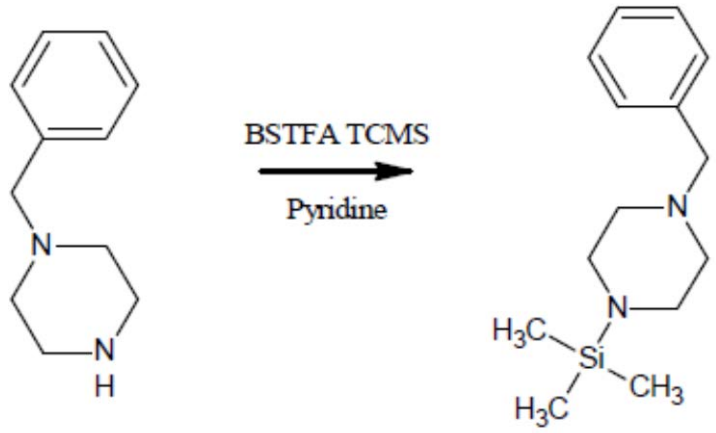
- Bistrifluoroacetamine with 1% Trimethylchlorosilane is a derivatizing agent capable of forming stable trimethylsilyl adducts to hydroxyl groups and primary and secondary amines. By using it as a form of sample preparation for analysis of solid state drugs by GC-MS, it is possible to create two different uniquely identifiable mass spectra for the same compound.

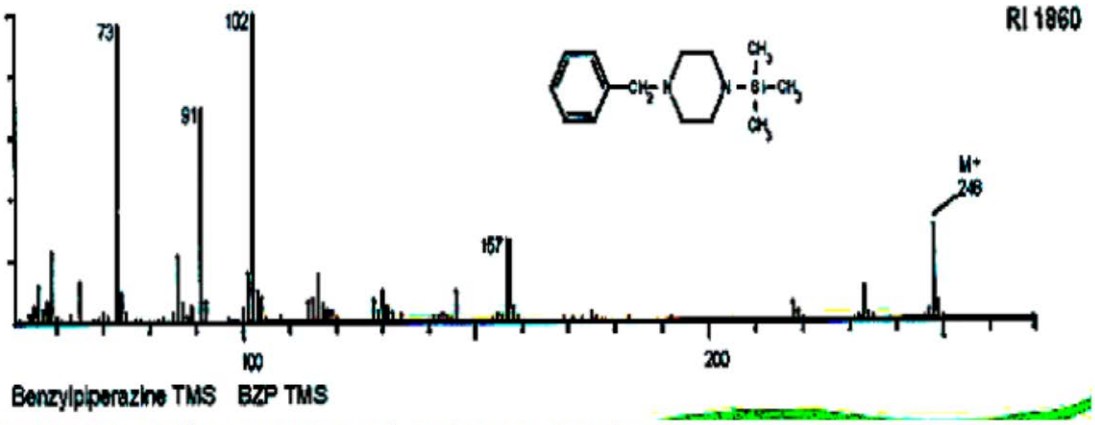
Equipment, Materials and Reagents

- Pyridine ACS Reagent >99% SIGMA [CAS 110-86-1]
- N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA TMCS) Fluka [CAS 25561-30-2]
- GC vials
- Chloroform ACS grade [CAS67-66-3]

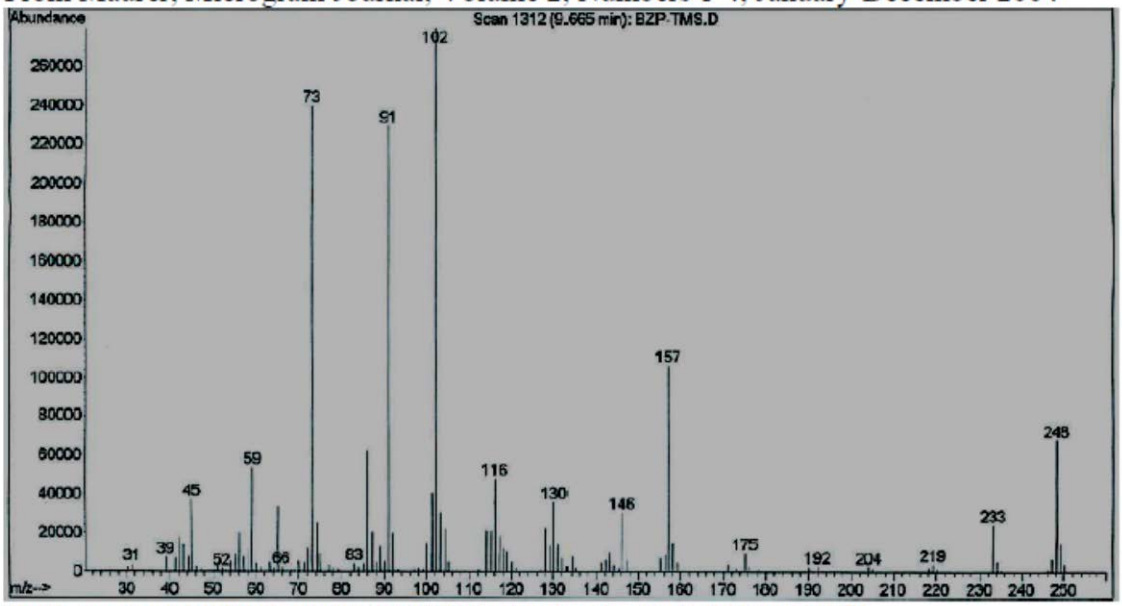
Procedure

1. Extract BZP tablet base to chloroform.
2. Place the chloroform layer from step 1 into a GC-MS vial.
3. Add to vial approximately 0.25 to 0.5 mL of BSTFA-TMCS reagent and 200 µL of pyridine. Cap tightly, mix, and allow to react for 15 minutes.
4. Inject into GC-MS using methods APDLOW or APDSLOW.



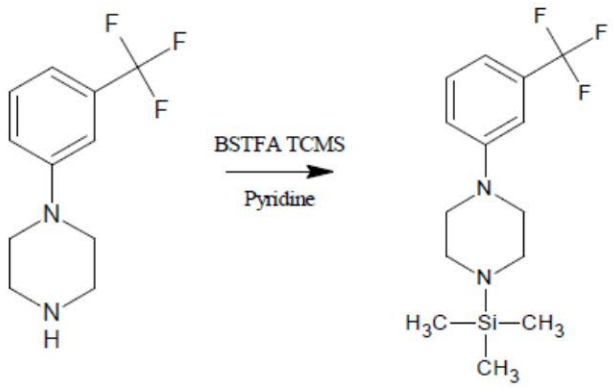


From Maurer, Microgram Journal, Volume 2, Numbers 1-4, January-December 2004

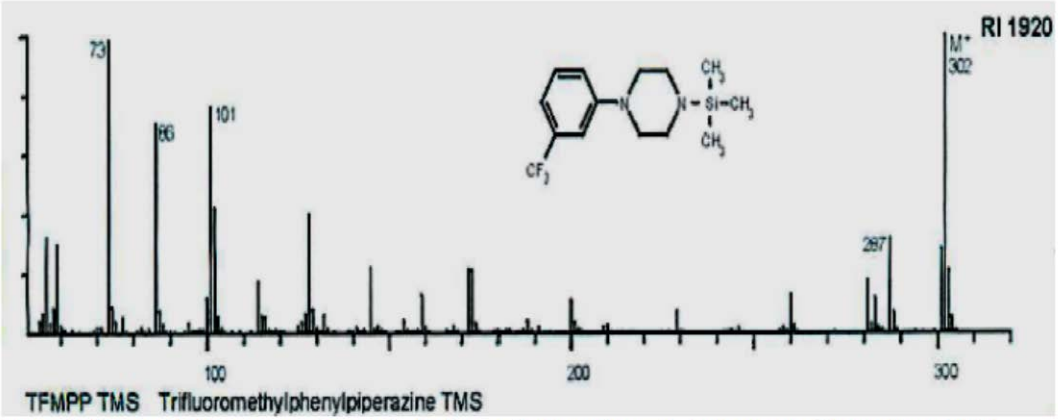


BZP standard (APD Lot# 06252010) derivatized with BSTFA-TMCS

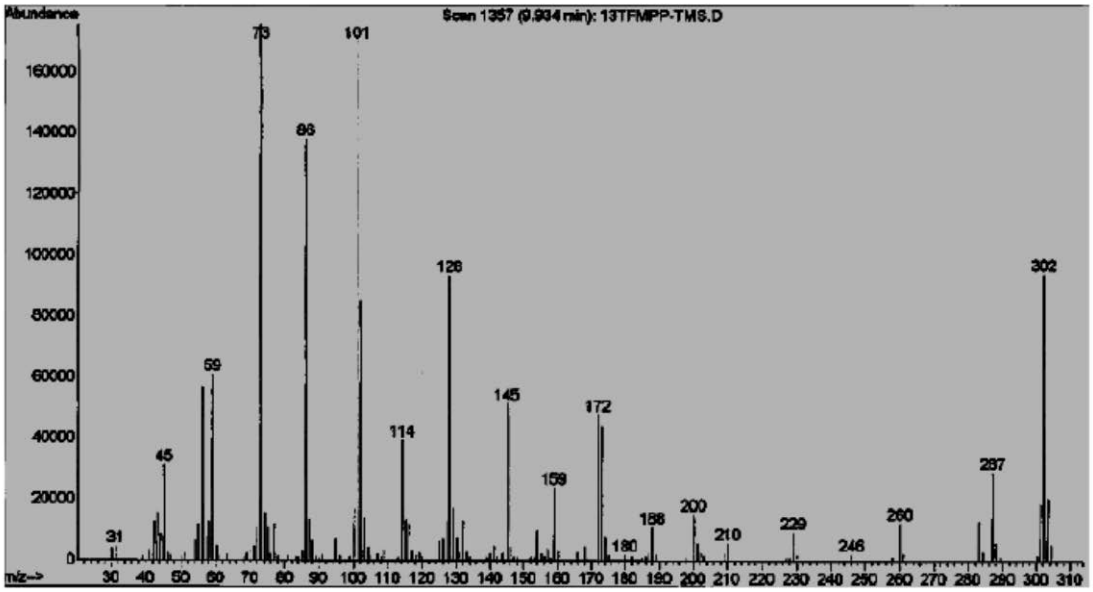
Derivatization reaction of TFMPP with BSTFA-TMCS



Since most BZP tablets contain TFMPP, the BZP-TCMS derivatizing reagent also reacts with the active hydrogen on TFMPP as well.



From Maurer, Microgram Journal, Volume 2, Numbers 1-4, January-December 2004



TFMPP standard (Sigma 102K3678) derivatized with BSTFA-TMCS

The TFMPP derivatives do not give unique, identifiable mass spectra, so retention time will be necessary to determine which TFMPP has been identified (1,2; 1,3; 1,4).

References

- Maurer; Microgram Journal, Volume 2; Numbers 1-4, January-December 2004 http://www.justice.gov/dea/programs/forensicsci/microgram/journal2004/page22.html
- Pierce Handbook for Derivatization for GC

BZP Derivatization with Trifluoroacetyl

Purpose

- To establish a method for derivatization and subsequent characterization of N-Benzylpiperazine via GC-MS.

- Trifluoracetic anhydride is a derivatizing agent capable of forming stable trifluoroacetyl adducts to hydroxyl groups and primary and secondary amines. By using it as a form of sample preparation for analysis of solid state drugs by GC-MS, it is possible to create different mass spectra for compounds that would normally give similar mass spectra (ex: methamphetamine and phentermine).

Equipment, Materials, and Reagents

- Pyridine ACS Reagent >99% SIGMA [CAS 110-86-1]
- Trifluoroacetic anhydride (TFAA) Fluka [CAS 407-25-0]
- GC vials
- Chloroform ACS grade [CAS67-66-3]

Procedure

1. Place 1-2 mg of the sample to be analyzed into a GC-MS vial and cap it tight.
2. Using a syringe, add approximately 200 µL of TFAA and 100 µL of pyridine to the vial.
3. Mix slightly and allow solution to react for 15 minutes.
4. Add chloroform to the vial.
5. Take the derivatized substance in chloroform and wash with equal volume of dilute base (pH 10 or greater) and place chloroform in a fresh GC vial to be injected. [this step is used to neutralize any leftover TFAA- to avoid column bleed]
6. Inject into GC-MS using APDAUTO on the 15m columns or APDLOW on the 20m column.

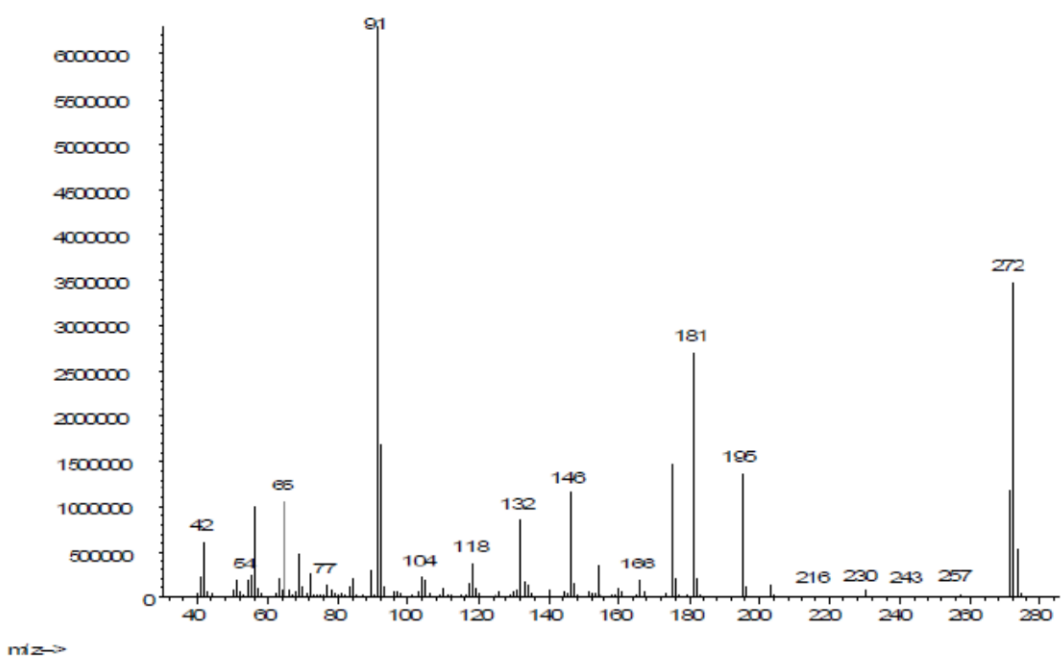
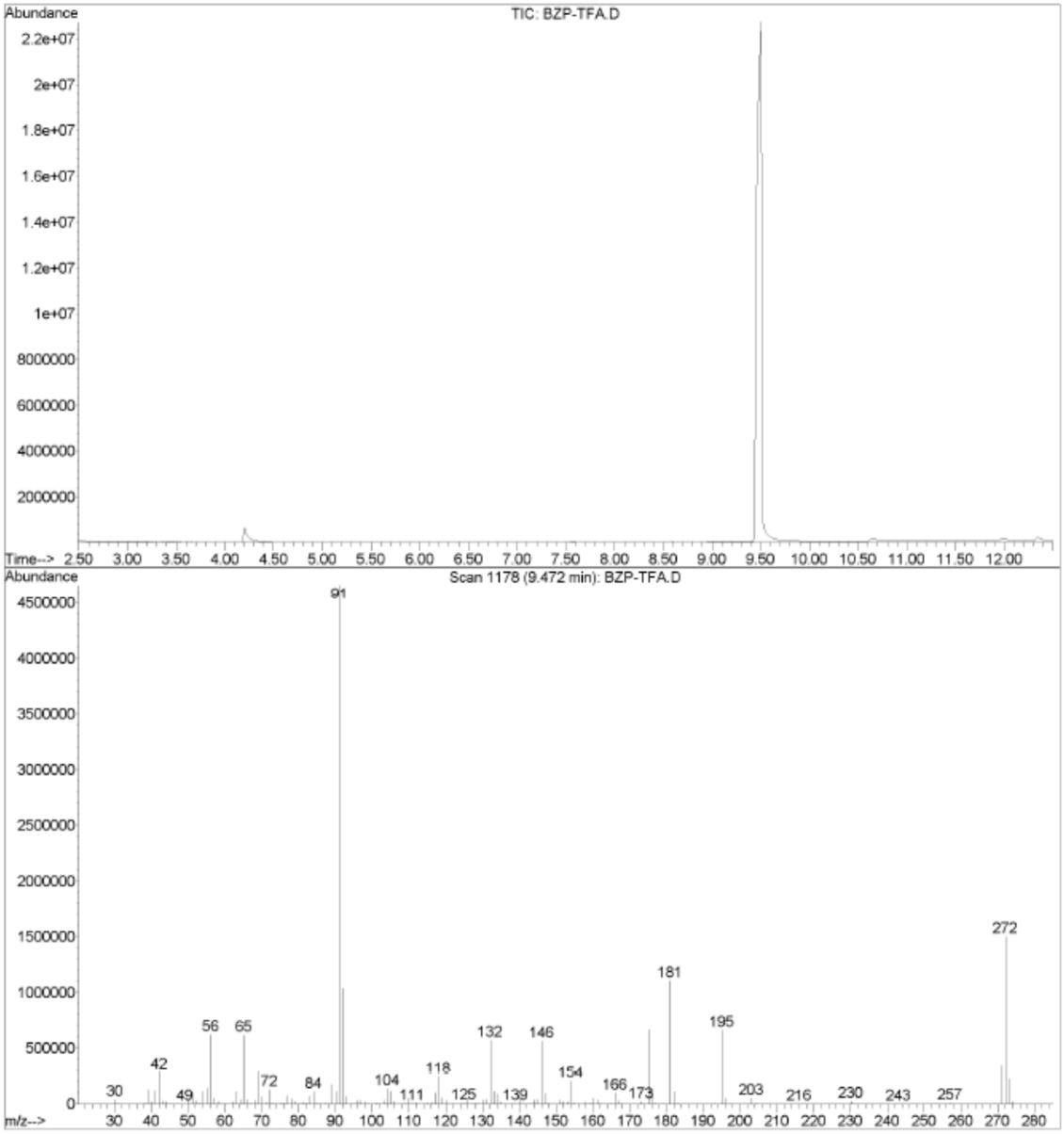


Figure 9. Mass spectrum of BZP, TFA derivative

From reference #1

File :D:\Chris\2010\BZP-TFA.D
Operator :
Acquired : 24 Feb 2010 10:51 using AcqMethod APDLOW.M
Instrument : US 70820483
Sample Name: BZP-TFA derivative
Misc Info : CKiyak 6524
Vial Number: 88



References

- Heegel and Trigg; JCLIC vol. 18(3); p. 9-16
- Pierce Handbook Guide to Derivatization Reagents for GC
- Gan et. al.; J. Forensic Sci.; vol 36(5); p. 1331-1341

Cannabinoids-Synthetic

- Contained in herbal incense products or a powder.

- **Drug Names** --- Common Names – JWH, CP, and WIN series. UR-144, XLR.
- **Chemical Formulas**

  - They mimic the actions or have similar structure to THC, naphthoylindoles, benzoylindoles, phenylacetylindoles

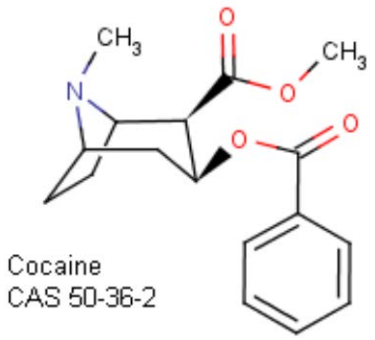
- **Analysis Procedure**

  - Use a non-polar solvent to extract plant materials for instrumental analysis.
  - Mixtures of synthetic cannabinoids are extremely difficult to separate for UV or IR analysis due to similar chemical properties.

Cocaine

- **Drug Name** - Cocaine--- Common Names - Coke, Snow, Crack, Rock, Free Base
- **Chemical Formulas:**

  - Empirical - $C_{17}H_{21}NO_4$



  - Molecular Weight – 303

- **Properties**
  - Color – White, off-white, or cream colored.
  - Form – Powder, compressed powder bricks, waxy chunks (rocks)
  - Solubility
    - Free base: Soluble in alcohol, acetone, chloroform, ether, hexane, and acids; Insoluble in water.
    - Hydrochloride: Soluble in water, alcohol, chloroform; slightly soluble in acetone; practically insoluble in ether and hexane.

- **Analysis Procedure**
  - Color Tests
    - Marquis: No color change.
    - Cobalt Thiocyanate
      - Immediate blue – Cocaine HCl
      - Blue after adding conc. H_2SO_4 – Cocaine base
  - UV Analysis
    - Run sample direct in 0.2N H_2SO_4 solution.
    - Dilute sample approximately 10:1 with additional H_2SO_4 and run UV again to obtain absorbance at 233.
    - Overlay scans and compare results with standard spectra.
    - If contaminants are present, perform basic extraction.
    - Run UV on H_2SO_4 from extraction direct and dilute.
  - GC/MS Analysis
    - Place small amount of sample in vial.
    - Add approximately 1 ml of $CHCl_3$ or Hexane, mix well to dissolve.
    - Inject 1 µl of solvent into GC/MS.
    - Compare data to standard spectra.
  - IR Analysis
    - Place sample on IR.
    - Compare spectra to standard spectra.
    - If contaminants are present, perform extraction.
    - Place crystallized sample from extraction on IR.

- **Cocaine Cleanup**
  - Dry Extraction Cocaine HCl
    - Acetone wash removes Benzocaine and Lidocaine from sample.
    - Hexane wash removes impurities from sample.
    - Chloroform extract leaves behind impurities like sugars in filter.
  - Dry Extraction Cocaine base
    - Hexane extraction leaves behind Procaine.
    - $CHCl_3$ extract removes excess water from sample.
  - Cobalt Thiocyanate Extraction
    - Place sample in a test tube and add 10 to 15 drops of CoSCN reagent.

- Add 3-5 drops of conc. HCl. Blue precipitant should form.
- Extract solution twice with small volume of $CHCl_3$.
- Wash $CHCl_3$ with base
- Split $CHCl_3$ into two portions,
- To one portion extract with acid for UV
- Second portion place in GC/MS vial for analysis or take to dryness for FTIR

➢ $KMnO_4$ Extraction
- Dissolve sample in 0.2N H_2SO_4.
- Add dropwise 1% $KMNO_4$ to acid until solution remains pink.
- Make solution basic (NaOH) and extract with Hexane or $CHCl_3$.
- Wash solvent 3 times with water.

- **Conversion of Cocaine HCl to Cocaine Base**

➢ Add equal parts of cocaine HCl and sodium bicarbonate in a beaker.
➢ Add enough water to dissolve all powder.
➢ Heat to near boiling.
➢ Cocaine base will be an oil on top.
➢ Remove from heat and allow cooling.
➢ Liquefied cocaine base will sink to the bottom.
➢ Decant the water mixture while still warm. If left to cool, you will not be able to get the “cookie” out.
➢ Transfer cocaine “cookie” onto filter paper.
➢ Cut into rock size pieces while damp.
➢ Place Cocaine rocks onto filter paper to finish drying.

Codeine Cough Syrup

- **Analysis Procedure**

➢ **METHOD 1:** (G. Harbison June 2004)

- Place 2 drops of syrup in GC-MS vial and add $CHCl_3$, seal vial and shake
- Analyze on GC-MS
- If extraction is necessary:
  1. Place approx. 1 ml of syrup in a clean test tube (use more if dilute).
  2. Make the solution acidic with 0.2 N H_2SO_4
  3. Wash with $CHCl_3$
     - Keep the $CHCl_3$ layer for extraction later
  4. Make the aqueous layer basic with Conc. NaOH and extract with $CHCl_3$ The organic layer is suitable for GC-MS and contains Codeine.
  5. Extract the remaining $CHCl_3$ with 0.2 N H_2SO_4
     - Analyze acid on UV.
     - If the UV or the GC-MS shows the presence of Caffeine, wash with $CHCl_3$ and re-run the UV.
  6. Analyze a portion of the $CHCl_3$ from Step 3 on GC/MS
     - Should contain methylparaben and Promethazine
  7. Evaporate the $CHCl_3$ from Step 3

        - The CHCl3 contains the Promethazine
    8. Reconstitute the residue with 0.2 N H_2SO_4
    9. Wash the acid with Diethyl Ether (removes methyl and propylparaben)
        - Analyze the acid on UV
        - If the UV or the GC-MS shows the presence of Caffeine, wash with $CHCl_3$ and re-run the UV.
    10. Compare the retention times from the GC, the spectra from the UV and from the MS to known standards.

- **METHOD 2:** (D.L. Stephens June 2004)

  - GC/MS Analysis:
    - Undiluted Samples: Place 2 drops of sample in a sample vial, add 2 drops of base (NaOH or Na_2CO_3), and then add $CHCl_3$. Mix contents prior to analysis on GC/MS.
    - Diluted Samples: Fill Autosampler vial approximately half full with sample. Add 2 drops of base (NaOH or Na_2CO_3), and then add enough $CHCl_3$ to fill the bottom quarter volume of the vial with solvent.
  - UV Analysis:
    - Extract two portions of Sample to obtain spectra for both Codeine and Promethazine.
    - Codeine Extraction: Add a portion of sample to a test tube and make basic with Na_2CO_3. Extract into CHCl3. Discard the upper aqueous layer. Add 0.2N H_2SO_4 and extract sample into upper acid layer for UV analysis.
    - Promethazine Extraction: Add a portion of sample to a test tube and make acidic with 0.2N H_2SO_4. Extract into $CHCl_3$. Discard the upper aqueous layer. Add 0.2N H_2SO_4 and extract sample into upper acid layer for UV analysis.

- **METHOD 3:** (G Rodriguez June 2004)

  - If the sample appears to be cough syrup:
    - GC/MS - Analyze the sample direct in $CHCl_3$
    - UV - Analyze the sample direct in acid and pH shift
  - UV Extraction for Codeine:
    - Dilute the sample in acid.
    - Make the sample basic and extract with Hexane.
    - Wash the Hexane with water.
    - Extract the Hexane with acid to recover the Codeine.
    - Extract basic solution with $CHCl_3$
    - Extract $CHCl_3$ with acid to recover Promethazine
  - If the sample appears to be in carbonated drink or neutral solution:
  - Sample extraction for GC/MS:
    - If sample is in an acidic solution, wash with $CHCl_3$, then make acidic solution basic and extract with $CHCl_3$.
    - Run both $CHCl_3$ wash and $CHCl_3$ extract samples
    - (acid→ $CHCl_3$ wash will contain methylparaben, propylparaben and promethazine)
  - UV: direct in acid and extracted - see above

Dihydrocodeinone (Hydrocodone)

- **Analysis Procedure**

  - If there are pharmaceutical markings on the tablet:
    - Reference the source for pharmaceutical identification.
    - Analyze on UV to identify peak for Acetaminophen.
    - Analyze sample plus Na_2CO_3 in $CHCl_3$ on GC/MS to show Acetaminophen and Dihydrocodeinone. (Strong base will remove acetaminophen)
    - Extract tablet to obtain UV of Dihydrocodeinone or
    - Obtain GC retention of Dihydrocodeinone
    - Use the appropriate footnote in report.
  - If the sample is a powder, or the pharmaceutical markings are not identified:
    - Analyze on UV to identify peak for Acetaminophen.
    - Analyze sample plus Na_2CO_3 in $CHCl_3$ on GC/MS to show Acetaminophen and Dihydrocodeinone. (Strong base will remove acetaminophen)
    - Obtain GC retention time for Dihydrocodeinone
    - Dissolve the sample in 0.2 N H_2SO_4.
    - Make basic with NaOH extract to Hexane to acid for UV of Dihydrocodeinone.

Gamma-Hydroxybutyrate (GHB)

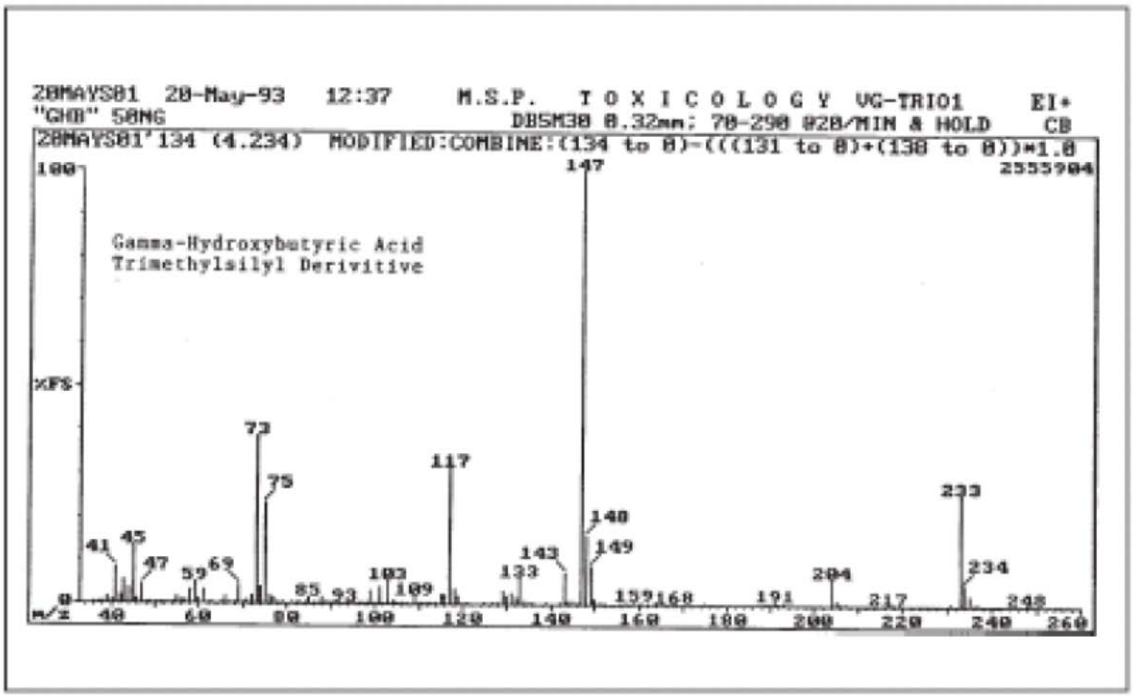
- **Analysis Procedure**

  - Color Tests
    - Chlorophenol Red and Modified Schweppe's reagent procedure
      1. Mix both Chlorophenol Red and Modified Schweppe's reagent together (3:1).
      2. Add 0.5 ml liquid or a small amount of powder to test tube.
      3. Check pH of liquid and adjust to 5-8 if necessary.
      4. Add 2 drops of the mixed reagent and gently swirl.
      5. An immediate red color indicates the presence of GHB.
      6. Weaker solutions will be from orange to red.
      7. No color change is negative

  - Analysis of GHB by TMCS Derivatization
    - Materials:
      - Generic oil for oil bath
      - Chloroform (ACS Grade)
      - N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane [CAS25561-30-2]
    - Procedure:
      1. Place 1 mg of dried GHB salt (any salt form) in autosampler vial and add 0.05-0.1 cc (use syringe) of the BSTFA with 1% TCMS derivatizing agent.
      2. Place in an oil bath (50 -70 □C) for 30 minutes.

3. Add solvent to vial and inject into GC/MS using GHB method. (NOTE: Chloroform preferred, do not use any solvent with hydroxyl or amine groups)

- Reference Spectra:



From JCLIC- Volume 3 Number 3- Bommarito (1993)

From JCLIC- Volume 3 Number 3- Bommarito (1993)

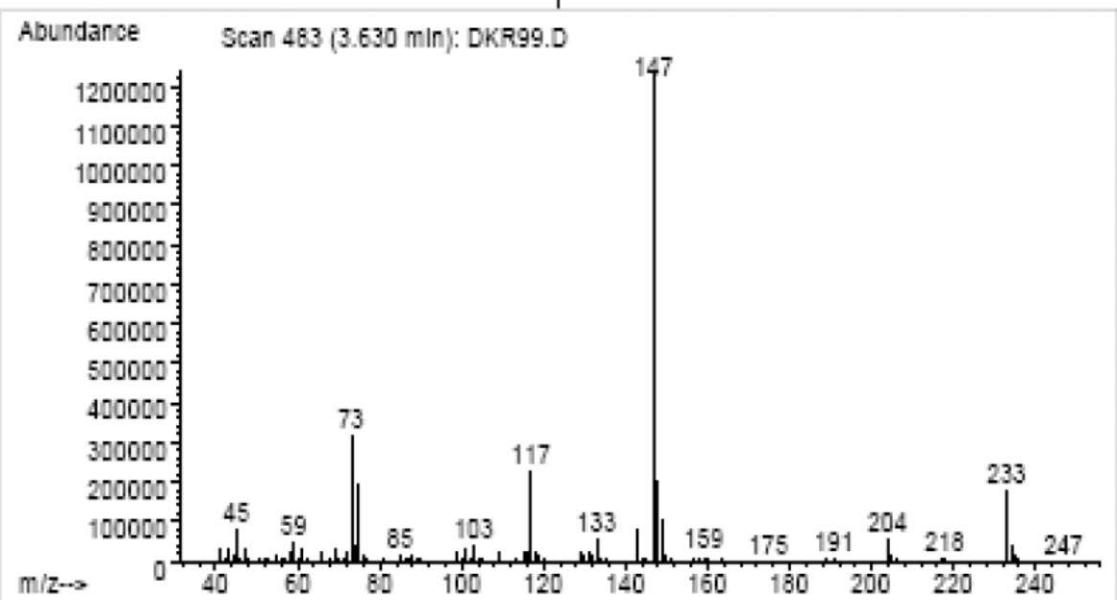


Figure 8: Mass spectrum of di(trimethylsilyl) derivative of GHB

From JCLIC- Volume 12, Number 1- Rees, et.al. (2002)

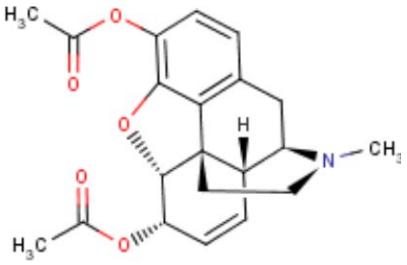
- Analysis of GHB in water:
  - Wash the water with CHCl3. GBL will extract into CHCl3 and may be analyzed by GC/MS or evaporated for FTIR.
  - Dry the aqueous layer by adding Ethanol and place on heating block until dissolved.
  - Filter and cool, then refrigerate. Crystals will form.
  - Filter crystals and wash with hexane and dry. Crystalize again if necessary.
  - Analyze direct on FTIR and GC/MS. Note: GHB converts to GBL in the injection port and will only give the mass spectrum of GBL.
- Synthesis of GHB from Gamma-Butyrolactone (GBL):
  - As a clear liquid, GBL is odorless and appears to have the approximate density of water.
  - Dissolve 60 grams of NaOH in 875 ml of 95% Ethanol
  - Add 120 ml of GBL
  - Allow to stand. Crystals will form. No heat is needed.
  - Filter the GHB crystals, refrigerate the Ethanol and filter again.
- Analysis of GBL or 1,4-Butanediol:
  - Analyze direct in CHCl3 using a low temperature method on the GC/MS. (40º starting temperature, 35º/minute ramp to 290º).
  - Analyze direct for FTIR. If spectrum is weak, heat to remove some of the water and retest.

Literature References

- Meyers, Almirall. *Journal of Forensic Science* 2005; 50(1): 31-36
- Rees, et al. JCLIC 2002; 12(1): 17-25
- Bommarito, JCLIC 1993; 3(3): 10-13

Heroin

- **Drug Name**-Heroin, Diacetylmorphine--- Common Name -H, Smack, Junk
- **Chemical Formulas:**
  - Empirical - $C_{21}H_{23}NO_5$



Heroin (diacetylmorphine)
CAS 561-27-3

  - Molecular Weight - 369

- **Properties**
  - Color - From light brown to dark brown or black.
  - Form - Powder; hard, rock-like; gummy or pliable mass.
  - Odor - Usually has acetic acid odor.
  - Solubility:
    - Free Base: Soluble in chloroform, ether, and alcohol.
    - Hydrochloride: Soluble in water, alcohol, and chloroform. Insoluble in ether.

- **Procedure**
  - Color Tests
    - Marquis: Purple; slow reaction <30 seconds for color to develop.
  - UV Analysis
    - Qualitative
      - Run sample direct in 0.2 N H_2SO_4 to obtain 278 absorbance.
      - Make solution basic and run UV again to obtain pH shift, if desired.
  - TLC
    - TLC System - T1
    - Location Reagent - acidified iodoplatinate

- GC/MS Analysis:
  - Direct Method
    - Place sample in vial and add morphine solvent to dissolve.
    - Inject into GC/MS
    - Perform library searches and compare to standard MS data.
    - Print copy of spectra data for case file.

  - Extracted Method:
    - Take a sample and place in vial, add 2 drops of weak base and add chloroform.
    - Cap vial and shake, Run on GC-MS

  - Alternate method:
    - If heroin quantitation is performed, the extracted sample for UV analysis may be utilized for GC/MS.
    - Take the H2SO4 solution containing sample and make basic.
    - Extract with 0.5 to 1 ml of morphine solvent and inject solvent into GC/MS.

- IR Analysis
  - Heroin extraction for IR analysis is the same as described under UV quantitation and GC/MS (Extracted Method). The basic steps are:
    - Sample + 10% HCl → Mor. Sol. → 0.2 N H_2SO_4
      Then: H_2SO_4 + $NaHCO_3$ → Morphine Solvent
    - Evaporate the solvent to dryness on hotplate (low heat) with warm air from heat gun to speed up the process.
    - Allow dish to cool. Residue should begin to crystallize on cooling.
    - Scrape residue from dish and make KBr pellet. Run IR and compare results to standard spectra.

Iodine Identification

Scope

- To establish analytical procedures for the identification of Iodine.

Safety

- Material Safety Data sheets are available in the laboratory if additional information is needed about any of the chemicals used in the analytical procedure.

Procedure

- Color Test using Starch

  - Equipment, Materials and Reagents:
    - Soluble starch solution. Canned laundry spray starch will work.
    - Cotton swabs and/or filter paper.
    - Other equipment and supplies as needed.

- Procedure:
  - If the sample is solid crystals, place some on the filter paper or swab the container with a clean cotton swab and spray with the starch.
  - If the sample is a stain cut a small piece and place on the filter paper or swab the area with a clean cotton swab and spray with the spray starch.
- Interpretation
  - Observance of a blue color is indicative of the presence of Iodine.

- This method was tested against the following chemicals for reactivity:

  - Nicotinamide (Aldrich lot 03913PS) **No Reaction**
  - Sodium Fluoride (JT Baker lot 247090) **No Reaction**
  - Potassium Permanganate (JT Baker lot V14627) **Purple**
  - Sodium Chloride (General Lab Supply lot 326809) **No Reaction**
  - Inositol (Aldrich lot 08519BF) **No Reaction**
  - Sodium (meta) Periodate (Fisher lot 865876) **No Reaction**
  - Ephedrine HCl (Aldrich lot 04128BR) **No Reaction**
  - Pseudoephedrine HCl (Aldrich lot 04003MQ) **No Reaction**
  - Potassium Iodide (JT Baker lot 41398) **No Reaction**
  - Potassium Bromide (EMD Chemicals lot 44240506) **No Reaction**
  - Methamphetamine HCl (Sigma 78F0325) **No Reaction**
  - Methamphetamine base (from the HCl above) **No Reaction**
  - Sodium Hydroxide (Sigma 119H0169) **No Reaction**
  - Red Phosphorus (Aldrich 02930PV) **No Reaction**
  - Iodine (JT Baker lot 401874) **Blue**

- UV Analysis

  - Procedure:
    - A sample is placed in a volumetric flask. Chloroform is added to make a dilution to about 0.2 mg/ml.
    - Repeat previous step using methanol.
    - Place a sample of each in a clean quartz UV cell.
    - Scan from 400–700 nm (chloroform is 511 nm and methanol is 443 nm).
  - Interpretation:
    - Compare to a traceable commercial iodine standard.

- GC/MS ANALYSIS
  - Equipment, Materials and Reagents:
    - Disposable MS vials and caps
    - Disposable test tubes with caps
    - 3N NaOH
    - Acetone
    - Chloroform
    - Ethanol (absolute)
    - Red Phosphorus
  - Direct Method: (Note: this method is very destructive to the injection syringe.)
    - Place a spatula tip of sample in a MS vial.
    - Fill with $CHCl_3$ and cap.

        - Analyze by GC/MS using a low temperature (35 – 300 degree) program.
    - Iodoform Reaction Method:
        - Place one spatula tip of sample in a test tube.
        - Add a few drops of Acetone.
        - Add 3N NaOH drop wise until clear yellow.
        - Extract with chloroform.
        - Analyze by GC/MS using a normal temperature (70 – 300 degree) program.

    - Iodoethane (ethyl iodide) Reaction Method: (Note: The sample in step 1 can be replaced with a sample of a reaction mixture and omit adding the red phosphorus (step 3).
        1. Place about 75 mg of sample in a clean MS vial.
        2. Add just enough absolute ethanol to cover the sample (about 3 drops).
        3. Add about 5 mg of Red Phosphorus then cap the vial.
        4. Allow over night reaction at room temperature.
        5. Analyze a sample of the headspace by GC/MS using a 40 degree isothermal program with no solvent delay.

    - Interpretation:
        - Compare to a traceable commercial or in-house standards

Literature and Supporting Documentation

- Skoog, Douglas A. and West, Donald M. *Analytical Chemistry*. 3rd Edition New York: Holt, Rinehart and Winston, 1979. Page 333 – 334.
- Jim Schieferecke B.S. *GC/MS Identification of Iodine – Part Two*.
- *Microgram*, Volume XXXIV, No. 5, May 2001. Pages 112 – 117.

Lisdexamphetamine Dimesylate

- **Drug name:** Lisdexamphetamine dimesylate is the active ingredient in an ADHD drug which was approved by FDA in February of 2007. Trade name is “Vyvvanse” and manufactured by New River Pharmaceutical, Inc and distributed by Shire Pharmaecutical. It is a Federal Schedule II drug. Tablets contain 30mg, 50mg or 70mg

- **Chemical Formula**

    - Empirical: $C_{12}H_{25}N_3O\ [SCH_4O_3]_2$
    - M.W. = 455.60

- **Analysis Procedure**

    - Color Test:
        - Marquis: burnt orange
        - Liebermann’s: orange
        - Sodium Nitroprusside: negative
        - Acidified Cobalt thiocyanate: negative

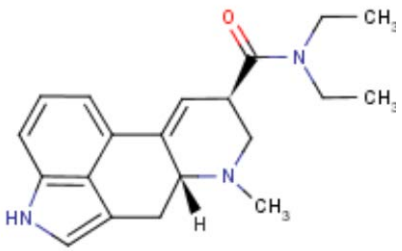
    - GC/MS:

- o extract with methanol
- o See Designer Drug Binder: Analytical profile of Lisdexamphetamine dimesylate for reference spectra

- FTIR:
  - o Direct in ATR
  - o See Designer Drug Binder: Analytical profile of Lisdexamphetamine dimesylate for reference spectra.

Lysergic Acid Diethylamide (LSD)

- **Drug Name** - Lysergic Acid Diethylamide --- Common Names - LSD, Acid
- **Chemical Formulas**
  - Empirical - $C_{20}H_{25}N_3O$



LSD
CAS 50-37-3

  - Molecular Weight – 323
- **Properties**
  - Color - N/A
  - Form - usually soaked onto perforated paper with printed designs, drawings, etc. Also found on candy that is tightly sealed with foil
  - Solubility:
    - o Free Base: Soluble in alcohol, $CHCl_3$, and ether.
    - o Tartrate: Soluble in dilute acids.
- **Analysis Procedure**
  - Presumptive tests:
    - o p-DMABA Test: Purple color, slow reaction < 30 seconds.
    - o LSD fluoroesces under UV light

- UV Analysis
  - Procedure:
    - Soak one or more hits in a known volume of 0.2 N H_2SO_4 for several minutes.
    - Remove carrier, squeezing solution out with applicator.
    - Scan solution on UV spectrophotometer from 370-220 nm.
    - Compare absorption spectra with standard spectra.
    - By using "Peak Pick" (F4) function, the absorbance value for the wavelength will be displayed.

- TLC (when necessary)
  - The following TLC systems are recommended for LSD:
    18:1, 9:2, or 2:1 solvent system as found in Clark
  - Location Technique
    - UV light
    - p-DMABA Spray - This visualization spray is used after UV light exam.

- GC/MS Analysis
  - Elute LSD from media by soaking 5-10 min. in 0.2 N H_2SO_4. Usually 50-100 µgm of LSD is required for GC/MS. Remove paper, squeezing solution from paper with applicator.
  - Blank acid solution is to processed in same manner as sample
  - Place solution in centrifuge tube and make basic with 1M Na2CO3.
  - Add 3-4 drops of $CHCl_3$ and shake.
  - Centrifuge to force $CHCl_3$ to bottom.
  - Using GC/MS method for LSD, inject several µl of $CHCl_3$ (>5µl).

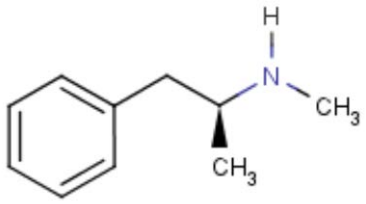
- IR Analysis (Not usually performed)
  - Elute LSD from media by soaking 5-10 min. in 0.2 N H_2SO_4. Usually 500-1000 µgm of LSD is required for IR analysis. Remove paper, squeezing solution from paper with applicator.
  - Wash H_2SO_4 solution once with $CHCl_3$, discarding $CHCl_3$ in waste solvent container.
  - Make solution basic with Na_2CO_3 and extract with $CHCl_3$.
  - Filter $CHCl_3$ into an evaporating dish and evaporate in fume hood using warm air from heat gun to speed up process.
  - Add KBr to dish and "scratch" with spatula to mix LSD residue with KBr.
  - Run KBr as background, then run sample mixed in KBr

Methamphetamine

- **Drug Name** – Methamphetamine – Common Names – Speed, Crank, Go-fast

- **Chemical Formulas**

  - Empirical – $C_{10}H_{15}N$ (CAS: 537-46-2)



- Molecular Weight – 149

- **Properties**
  - Color – White, yellowish, brown, etc.
  - Form – Powder, liquid, sticky paste, etc.
  - Solubility
    - Free Base: Soluble in alcohol, ether, chloroform, hexane, and acids
    - Hydrochloride: Soluble in water, alcohol, chloroform, and acids. Slightly soluble in acetone. Insoluble in ether and hexane.

- **Analysis Procedure**
  - Color Tests
    - Marquis: Orange to orange-brown color; immediate reaction.
    - Sodium Nitroprusside: Deep blue color; immediate reaction.
  - UV Analysis
    - Run sample direct in 0.2N H_2SO_4 solution
    - If sample appears clean, add a few drops of conc. NaOH for pH shift
    - Compare results to standard spectra
    - If contaminants are present, perform extraction.
  - GC/MS Analysis
    - Place small amount of sample to vial.
    - Add 2 drops of 1M Na_2CO_3.
    - 1 to 2 ml of $CHCl_3$ or Hexane, cap and mix well.
    - Inject solvent layer into GC/MS.
    - Compare data to standard spectra.
  - IR Analysis
    - Place sample on IR.
    - Compare spectra to standard spectra.
    - If contaminants are present, perform extraction.
    - Run IR on crystallized sample from extraction.

- Methamphetamine Cleanup
  - Dry Extraction:
    - Acetone wash will remove impurities from Methamphetamine HCl for IR analysis, even though Methamphetamine is slightly soluble in acetone.
  - Conway Diffusion:
    - Diffusion cleans up methamphetamine for UV analysis.

Methamphetamine and MDMA Separation Techniques for UV Analysis

Scope

- A procedure for preparation and analysis of samples with both methamphetamine and MDMA. MDMA absorbs UV radiation about 15 times stronger than methamphetamine. Because of this, obtaining a UV of methamphetamine in a mixture with MDMA is difficult at best. Potassium permanganate is used to remove the MDMA so that the UV spectrum of methamphetamine can be obtained.

Equipment, Materials and Reagents

- Disposable test tubes
- Disposable pipettes
- GC-MS vials with crimp caps
- Quartz cuvette for UV analysis
- Reagents:
  - 0.2 N H_2SO_4
  - Conc. NaOH [40% aqueous]
  - $CHCl_3$
  - 1 M Na_2CO_3
  - 10% $KMnO_4$ [10% aqueous solution]
  - Hexane

Procedure

- Place approximately 10 – 15 mg of the sample in about 2 ml acid. Scan with UV (dilute if necessary). MDMA UV is obtained. (If the sample contains Caffeine, wash the acid with Chloroform and repeat)
- To the acid mixture, add 1 drops of 10% $KMnO_4$ at a time until pink or purple color persists. Make this solution basic with conc. NaOH and extract with hexane.
- Extract the hexane portion with 0.2 N H_2SO_4. Scan the acid portion with UV and a UV spectrum of methamphetamine is obtained. (If the methamphetamine is very weak, increase sample size)

Methcathinone and analogues

- **Properties:**
  - Color: White to cream powder
  - Solubility: Non-polar solvent

- **Analysis:**
  - Mixtures of methcathinones are difficult to separate for UV and IR analysis due to similar chemical properties.
  - Color Tests
    - Marquis: Yellow color; immediate reaction for 3,4-Methylenedioxymethcathinone(MDMC), 3,4-Methylenedioxypyrovalerone(MDPV), and Butylone.
  - UV Analysis for MDPV
    - Run sample direct in methanol (abs at 233, 282, and 315)
    - If contaminants are present, perform extraction.
  - GC/MS Analysis
    - Place small amount of sample to vial.
    - Add 2 drops of 1M Na_2CO_3.
    - 1 to 2 ml of $CHCl_3$ or Hexane, cap and mix well.
    - Inject solvent layer into GC/MS.
  - IR Analysis
    - Place sample on IR.
    - Compare spectra to standard spectra.
    - If contaminants are present, perform extraction.
    - Run IR on crystallized sample from extraction.

Literature Reference

- *The Characterization of 3,4-Methylenedioxypyrovalerone (MDPV)*, Microgram Journal, Volume 7, Number 1 (March 2010)
- *Color Tests for the Preliminary Identification of Methcathinone and Analogues of Methcathinone*, Microgram Journal, Volume 9, Number 1

Morphine Tablets

- **Extraction Method:**
  - Grind the tablets with Na_2CO_3
  - Dry in the oven
  - Add a small amount of morphine solvent ($CHCl_3$:Isobutanol, 80:20).
  - Perform GC/MS analysis
  - Evaporate solvent and reconstitute in 0.2N H_2SO_4 for UV analysis.

- **Direct Method:**

- Grind the tablet and dry extract the powder with $CHCl_3$.
- Filter and perform GC/MS analysis.
- Evaporate solvent and reconstitute in 0.2N H_2SO_4 for UV analysis

N-Hydroxy-3, 4-Methylenedioxyamphetamine

- N-hydroxy MDA tends to undergo pyrolytic disproportionation in the heated injection port of the GC/MS resulting in oxidation of the N-hydroxy MDA molecule to 3,4-Methylenedioxyphenyl-2-propanone-2-oxime (MDP-2-P oxime) coupled with the reduction of N-hydroxy MDA to 3,4-methylenedioxyamphetamine (MDA).
- This disproportionation phenomenon could result in the misidentification of N-hydroxy MDA as MDA, since this controlled substance alone is detected in routine GC/MS analysis.
- Sample Preparation:
  - Small amount of sample is added to the sample vial
  - Add appropriate organic solvent (Chloroform)
  - Add one or two drops of base (Na_2CO_3)

Opium

Scope

- To establish a procedure for preparation and analysis of Morphine, Codeine and other constituents found in opium and opium poppies.

- **Properties:**

  - Botanical Information**:** Opium Poppy
  - Family: Papveraceae; Genus: Papaver; Species: Somniferum
    - Papaver somniferum is an annual that with brilliant white or red flowers growing on a central bulbous pod.
    - When scratched, the pod produces a milky latex called opium.
  - Chemical Properties
    - This latex contains a variety of opiates including codeine, morphine, noscapine, thebaine and papaverine.
    - Morphine is converted to Heroin

- **Equipment, Materials and Reagents**

  - Disposable test tubes
  - 0.2 N H_2SO_4
  - 1 M Sodium Carbonate
  - Conc. NaOH [40% aqueous solution]
  - Conc. sulfuric acid
  - $CHCl_3$
  - Morphine Solvent (isobutyl alcohol:chloroform, 20:80)

- **Analysis:**

  - Grind 3-4 grams of the dried bulbs (top) from the plant.

- Cover with 0.2 N H_2SO_4 and heat for about an hour then cool to room temperature.
- Strain the mass through gauze and squeeze out excess.
- Filter this liquid if necessary to remove any plant material.
- Wash with $CHCl_3$ to remove acidic and neutral compounds.
- Discard the $CHCl_3$ layer.
- Make basic with NaOH and extract with $CHCl_3$
- Save $CHCl_3$ layer, it contains Codeine, Thebaine, Papaverine and other opiates.
- Take aqueous layer, make acidic with Conc. Sulfuric acid, and then carefully back titrate to a pH of 10 with 1 M sodium carbonate.
- Extract with Morphine Solvent
- Save solvent layer, it contains morphine.
- Combine $CHCl_3$ and solvent layers; then evaporate.
- Reconstitute with about 0.5-1 ml of $CHCl_3$.
- Analyze on GCMS.

- **Interpretation**

  - Report as opium if morphine, codeine and at least one of the following alkaloids is detected: papaverin, noscapin or narceine.
  - Can be reported as Codeine, Morphine and name of one of the other alkaloids detected with footnote "These are commonly detected constituents of opium"

Literature and Supporting Documentation

- "Basic Training Program for Forensic Drug Chemists". US Dept of Justice, Drug Enforcement Administration, pp. 6-95 through 6-96.

Pemoline

- Cylert tablets from Abbott Labs contain Pemoline.

- **Properties:**

  - Classification: Schedule 4, penalty group 3 controlled substance in Texas.
  - Solubility: Pemoline is practically insoluble in most solvents. However, soluble UV.

- **Procedure:**

  - One tablet is crushed and allowed to soak in 2-3 ml of ethanol overnight.
  - Filter the ethanol.
  - Take a sample for GC/MS via pulsed injection
    - Compare data and retention time to known standard.
  - Evaporate the remainder of $CHCl_3$ extract
  - Run sample on FTIR
    - Use DRIFTS or ATR attachment

  - If the GC/MS is unsuccessful, add a sample of the residue from the FTIR analysis in ethanol and reanalyze using GCMS pulsed injection mode.

Peyote (Mescaline)

- **Properties**
  - Scientific Name -- *Lophophora williamsii cactus*
  - Active Ingredient - Mescaline
  - Common Names - Peyote, Cactus, Buttons
  - Chemical Formulas
    - Empirical - $C_{11}H_{17}NO_3$
    - Molecular Weight - 211
  - Color - Green when fresh; brown when dried
  - Form - Cactus plant
  - Solubility
    - Free Base: Soluble in water, alcohol, $CHCl_3$. Almost insoluble in ether.
    - Sulfate: Soluble in hot water, methanol; sparingly soluble in cold water, alcohol
    - Hydrochloride: Soluble in water, alcohol.

- **Procedure:**
  - Morphological Examination - Comparison of physical characteristics of plant material to literature descriptions, photographs, etc.

  - Color Tests
    - Marquis - Orange; test peyote by cutting a thin slice of plant material and placing in spot plate well containing reagent.
    - Nitric Acid - Red; use same technique as described under Marquis test above.

  - Mescaline Extraction
    - Cut peyote buttons into small pieces or slices. Use several grams of peyote if available.
    - Place cut peyote into a small beaker and soak in strong NaOH solution for 30 minutes. Use just enough solution to cover peyote. Very low heat may increase the quantity of mescaline recovered.
    - Filter NaOH solution through glass wool into a separatory funnel. Wash aqueous solution once with ether, discarding the ether.
    - Extract with $CHCl_3$, being careful to avoid an emulsion by shaking gently.
    - Discard the aqueous layer and extract $CHCl_3$ layer with 0.2 N H_2SO_4.

  - UV Analysis
    - Run UV scan on 0.2 N H_2SO_4 solution from above extraction.
    - A characteristic absorbance at 268 should be seen.

  - TLC
    - If TLC is desired, take the 0.2 N H_2SO_4 from UV analysis, make basic, and extract to $CHCl_3$. Place $CHCl_3$ in evaporation dish and reduce volume on hot plate. Spot $CHCl_3$ on TLC plate beside mescaline standard.
      - Solvent Systems - T1, 18:1 listed in Clark.
      - Location Reagent - Acidified iodoplatinate

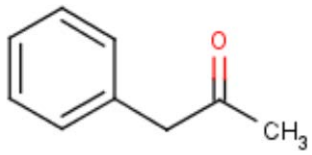
- GC/MS Analysis
  - Take H_2SO_4 solution (after UV analysis) and make basic. Extract to $CHCl_3$ and reduce volume.

- IR Analysis
  - Using the previously described extraction method for peyote, mescaline can be isolated for IR analysis.
  - Take 0.2 N H_2SO_4 solution of UV analysis and make basic with conc. NaOH. Extract solution with $CHCl_3$.
  - Place $CHCl_3$ into an evaporation dish. Add 1-2 drops of conc. HCl or bubble HCl gas through solvent to form mescaline HCl.
  - Evaporate to dryness on hot plate.

Phenylacetone (P2P)

- **Properties**

  - Chemical Name – Phenylacetone, Phenyl-2-Propanone
  - Common Names - P-2-P
  - Chemical Formulas
    Empirical - $C_9H_{10}O$



Phenyl-2-propanone (P2P)
CAS 103-79-7

  - Molecular Weight – 134

  - Color - Pale yellow to brown
  - Form - Liquid
  - Solubility - Soluble in most organic solvents. Insoluble in water.

- **Procedure**

  - Color Tests - None

  - UV Analysis
    - UV analysis of clandestine manufactured phenylacetone can be difficult due to contaminants. The information to be gained may not be worth the extractions required.

- First attempt attaining a reasonably clean UV spectra by adding a small amount of sample to 0.2 N H_2SO_4 or alcohol and making a scan.
- If UV spectra is not acceptable, extraction techniques must be explored.

- TLC - None

- GC/MS Analysis
  - Sample can usually be placed directly in a suitable solvent for injection. If P-2-P is concentrated, it should be diluted approximately one drop per 5-10 ml before injection.
  - Phenylacetone, being neutral, will extract from aqueous solutions, regardless of pH, to $CHCl_3$. When P-2-P is highly contaminated, it may be helpful to purify prior to injection. This can be accomplished by placing sample in $CHCl_3$ or hexane and washing organic layer with acidic, alkaline, and neutral solutions.
  - Inject solvent containing sample into GC/MS.

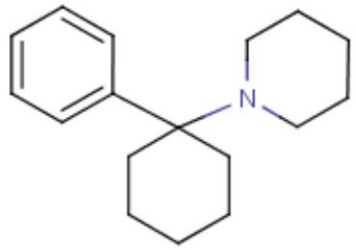
.

- IR Analysis
  - Clandestine manufactured phenylacetone will rarely yield acceptable IR spectra when run direct. The organic phase/aqueous phase washes as described in GC/MS analysis will sometimes yield acceptable IR spectra.
  - After washes, the organic layer is reduced to a low volume and the oily residue is run on IR.

Phencyclidine (PCP)

- **Properties**

  - Chemical Name – Phencylidine, 1(1-Phenylcyclohexyl)piperidine
  - Chemical Formulas
    - Empirical – $C_{17}H_{25}N$, $C_{17}H_{25}N{\cdot}HCl$



Phencyclidine (PCP)
CAS 77-10-1

    - Molecular Weight – 243.4, 279.9
    - CAS # 77-10-1, 956-90-1

  - Color - Pale yellow to white
  - Form – Liquid, Powder
  - Solubility
    - Base form: Soluble in most organic solvents, very soluble in ether

- HCl salt: Soluble in ethanol, chloroform and in water, very slightly soluble in ether

- **Procedure**

  - Color Tests – HCl salt form – p-Dimethylaminobenzaldehyde - red

  - UV Analysis
    - Ultraviolet Spectrum: Aqueous acid – 252nm, 258 nm, 263nm
    - Absorptivities for UV Quantitation 12.9 @ 262
    - UV analysis of clandestinely manufactured PCP is relatively easy
      1. Place a drop in 0.2 N H2SO4.
      2. Run scan from 340 to 210 nm
      3. Compare results to standard spectra.
      4. If results are acceptable, print spectra for case file.
      5. If UV spectra is not acceptable,
      6. Make basic and extract with chlorofom
      7. Extract with 0.2 N H_2SO_4, and rerun sample

  - TLC
    - System: TA as listed in Clark
    - Developer: Acidified Iodoplatinate Solution

  - GC/MS Analysis
    - Liquid Sample can usually be placed directly in a suitable solvent for injection.
    - Solid sample should be placed in base, extract with Chloroform
      1. Inject solvent containing sample into GC/MS.
      2. Compare data and retention time to known standard.

  - IR Analysis
    - Solid sample use Drift attachment or ATR
      1. Run directly on IR
      2. If spectra is not acceptable, perform appropriate extraction technique and re-run.
    - Liquid Sample use Salt Cell for FTIR
      1. Run directly on IR
      2. If spectra is not acceptable, perform appropriate extraction technique and re-run

Red Phosphorus

- **Scope**
  To establish a procedure for the identification of Red phosphorus samples.

- **Safety**

  - Always use Nitric acid in the fume hood.

- **Equipment, Materials and Reagents**

  - Materials:

- Disposable test tubes
- Disposable glass pipettes
- Glass wool
- GCMS vials and crimp caps
- Filter paper and funnels

- Acids:
  - Ammonium Molybdate
  - Conc. Nitric Acid
  - 0.2 N Sulfuric acid

- Solvents:
  - Chloroform
  - Acetone
  - Carbon Disulfide
  - Deionized H_2O

- Phosphorus Detection Kit, which contains Acid Molybdate Solution, Fiske & Subbarow Reducer Solution and Phosphorus Standard Solution
  - Quality test reagent with a known sample of phosphorus

- Bromine

- **Analysis**

  - Presumptive:

    1. Combine approximately 0.20 g sample, 1 ml 0.2 N Sulfuric acid, and 1 ml deionized H_2O.
    2. Mix and allow 2-3 minutes for reaction.
    3. Centrifuge the solution until a relatively clear liquid is present.
    4. In a separate test tube, combine 1 ml of the supernatant, 1.5 ml deionized H_2O, and 0.5 ml Acid Molybdate Solution.
    5. Mix the contents of the test tube.
    6. Add 4 drops Fiske & Subbarow Reducer Solution.
    7. Mix the contents by inversion and let stand.
    8. Record any observations.

    - Interpretation
      - A positive result for the presence of phosphorus is the formation of a pale blue color followed by a more intense royal blue color (+). (NOTE: Reaction may occur over a period of about ten minutes.)

  - FTIR Analysis

    1. If the Red phosphorus is clean and unused, skip to step 3.
    2. If the Red phosphorus is obviously used or from a reaction mixture or from matchbook scrapings, wash with Chloroform, then acetone, then water, then acetone a second time and allow to dry.
    3. Place a few milligrams of the sample in a test tube.
    4. In the fume hood carefully dilute the conc. nitric acid 1:1 with deionized water then add approximately 1 ml of the dilute Nitric acid drop wise to

the test tube. (NOTE: Some impurities from matchbooks and reactions can cause the nitric acid to react violently and may even ignite a small flame in the test tube. If this happens, let the reaction subside and add one more drop of Nitric acid. Continue until the whole aliquot of acid has been added.)

5. Place the test tube in the test tube heater block and allow at least 30 minutes for reaction or until the solution turns a clear pale yellow.
6. As soon as the test tubes from step e have been placed in the heater block, place approximately 1 gram of ammonium molybdate in a clean test tube and fill to about ¾ full with water. Place this test tube in the heater block also. Agitate frequently.
7. After about 30 minutes, the Nitric acid solutions should be a clear yellow, filter through a glass pipette with a glass wool plug into a clean test tube.
8. Add approximately 1 ml of the ammonium molybdate solution to each of the Nitric acid test tubes and place back in the heater block for about 30 minutes to an hour. A yellow precipitate will form in the presence of red phosphorus (ammonium phosphomolybdate). Allow the test tubes to cool to room temperature.
9. Filter and rinse the yellow precipitate and allow to air dry. (NOTE: Sometimes, especially if oven dried, the yellow precipitate will turn a greenish color. The precipitate can still be analyzed.)
10. Analyze the ammonium phosphomolybdate with FTIR.
11. Compare data to known standard spectra.

- GC/MS Analysis
  1. If the Red Phosphorus is clean and unused, skip to step 3.
  2. If the Red Phosphorus is obviously used or from a reaction mixture or from matchbook scrapings, wash with Chloroform, then acetone, then water, then acetone a second time and allow to dry.
  3. Place a few milligrams of the sample in a test tube.
  4. In the fume hood, carefully add approximately 1 ml of Carbon Disulfide to the test tube which makes a suspension
  5. Add 1 - 2 drop of Bromine and cap the test tube. Agitate for several seconds. If Carbon Disulfide turns clear, then Phosphorus tribromide is formed.
  6. Analyze with GCMS on a low temperature method (50º isothermal for 6 minutes).
  7. Compare the retention time from the GC and the spectra from the MS to the standard phosphorus tribromide.

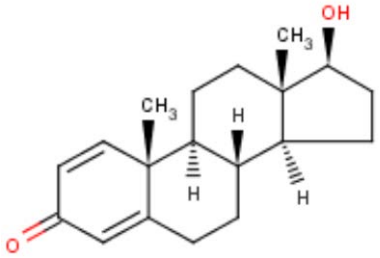
- **Literature and Supporting Documentation**
  Sigma Diagnostics, Phosphorus, Inorganic, Procedure No. 670

Steroids

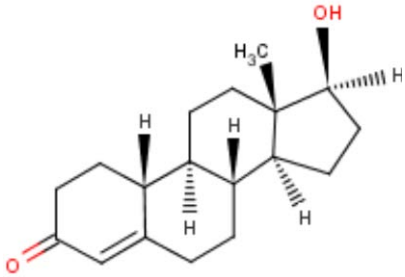
- **Properties**

  - Common Name – Anabolic Steriods, Boldenone (CAS 846-48-0), Nandrolone (CAS 434-22-0), Testosterone (CAS 58-22-0) – Testosterone esters, Trenbolone (CAS 10161-33-8), Stanolonone (CAS 521-18-6)

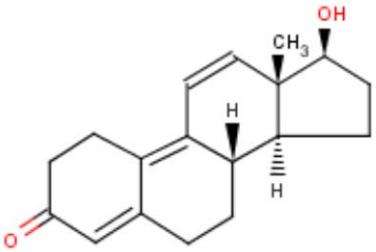
- Chemical Formulas



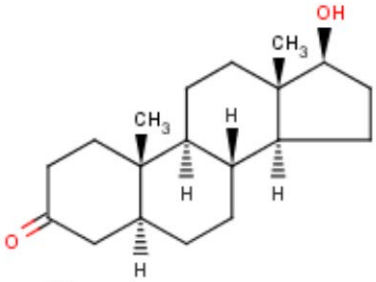
Boldenone
C9H26O2 MW=286.4



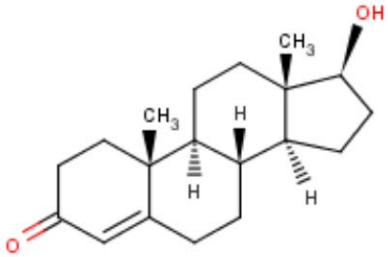
Nandrolone
C18H26O2
MW=274.4



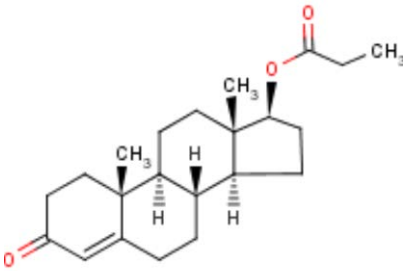
Trenbolone
C18H22O2
MW=270.4



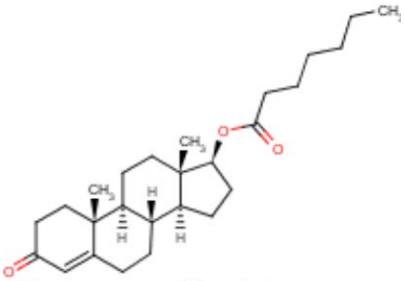
Stanolone
C19H30O2
MW=290



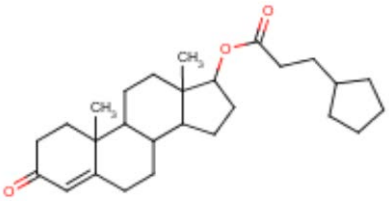
Testosterone
C19H28O2
MW=288.4



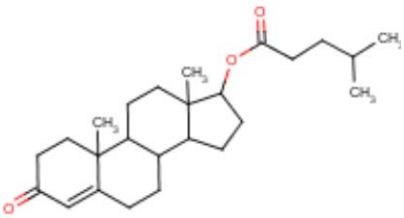
Testosterone Propionate
C22H32O3 MW=344.5
CAS 57-85-2



Testosterone Enantate
CAS 315-37-7 MW=400.6
C26H40O3



Testosterone Cypionate
C27H40O3
MW=412.6
CAS 58-20-8



Testosterone isocaproate
C25H38O3
MW=386.6
CAS 15262-86-9

- Color - Various
- Form – Liquid, Powder, Tablets
- Solubility:
  - Base form: Soluble in most organic solvents, very soluble in ether
  - HCl salt: Soluble in ethanol, chloroform and in water, very slight soluble in ether

- **Properties of steroid esters**

  - An ester is a chain composed primarily of carbon and hydrogen atoms. This chain is typically attached to the parent steroid hormone at the 17th carbon position.
  - Esterification of an injectable anabolic/androgenic steroid basically accomplishes one thing; it slows the release of the parent steroid from the site of injection. This happens because the ester will notably lower the water solubility of the steroid and increase its lipid (fat) solubility by causing the drug to form a deposit in the muscle tissue and slowly enter into circulation as it is picked up in small quantities by the blood. Generally, the longer the ester chain, the lower the water solubility of the compound, and the longer it will take to for the full dosage to reach general circulation.
  - Esterification temporarily deactivates the steroid molecule. With a chain blocking the 17th beta position, binding to the androgen receptor is not possible (it can exert no activity in the body). In order for the compound to become active the ester must therefore first be removed. This automatically occurs once the compound has filtered into blood circulation, where esterase enzymes quickly cleave off (hydrolyze) the ester chain. This will restore the necessary hydroxyl

(OH) group at the 17th beta position, enabling the drug to attach to the appropriate receptor. Now and only now will the steroid be able to have an effect on skeletal muscle tissue.

- **Ester Profiles**

  - Acetate: Chemical Structure $C_2H_4O_2$.
    - Also referred to as Acetic Acid; Ethylic acid; Vinegar acid; vinegar; Methanecarboxylic acid.
    - Acetate esters delay the release of a steroid for only a couple of days. This ester is used on oral primobolan tablets (metenolone acetate), Finaplix (trenbolone acetate) implant pellets, and occasionally testosterone.

  - Propionate: Chemical Structure $C_3H_6O_2$.
    - Also referred to as Carboxyethane; hydroacrylic acid; Methylacetic acid; Ethylformic acid; Ethanecarboxylic acid; metacetonic acid; pseudoacetic acid; Propionic Acid.
    - Propionate esters will slow the release of a steroid for several days.

  - Phenylpropionate: Chemical Structure $C_9H_{10}O_2$.
    - Also referred to as Propionic Acid Phenyl Ester.
    - Phenylpropionate will extend the release of active steroid a few days longer than propionate. Durabolin is the drug most commonly seen with a phenylpropionate ester (nandrolone phenylpropionate), although it is also used with testosterone in Sustanon and Omnadren.

  - Isocarpoate: Chemical Structure $C_6H_{12}O_2$.
    - Also referred to as Isocaproic Acid; isohexanoate; 4-methylvaleric acid. Isocaproate begins to near enanthate in terms of release.
    - The duration is still shorter, with a notable hormone level being sustained for approximately one week. This ester is used with testosterone in the blended products Sustanon and Omnadren.

  - Caproate: Chemical Structure $C_6H_{12}O_2$.
    - Also referred to as Hexanoic acid; hexanoate; n-Caproic Acid; n-Hexoic acid; butylacetic acid; pentiformic acid; pentylformic acid; n-hexylic acid; 1-pentanecarboxylic acid; hexoic acid; 1-hexanoic acid; Hexylic acid; Caproic acid.
    - This ester is identical to isocarpoate in terms of atom count and weight, but is laid out slightly different (Isocaproate has a split configuration, difficult to explain here but easy to see on paper).. Caproate is the slowest releasing ester used in Omnadren.

  - Enanthate: Chemical Structure $C_7H_{14}O_2$.
    - Also referred to as heptanoic acid; enanthic acid; enanthylic acid; heptylic acid; heptoic acid; Oenanthylic acid; Oenanthic acid.
    - Enanthate is one of the most prominent esters used in steroid manufacture. Enanthate will release a steady level of hormone for approximately 10-14 days.

- Cypionate: Chemical Structure $C_8H_{14}O_2$.
  - Also referred to as Cyclopentylpropionic acid, cyclopentylpropionate.
  - Cypionate's release duration is almost identical to enanthate (10-14 days), and the two are likewise thought to be interchangeable in U.S. medicine. The enanthate ester is slightly smaller than cypionate, and it therefore releases a small (a few milligrams) amount of steroid more in comparison.

- Decanoate: Chemical Structure $C_{10}H_20O_2$.
  - Also referred to as decanoic acid; capric acid; caprinic acid; decylic acid, Nonanecarboxylic acid.
  - The Decanoate ester is most commonly used with the hormone nandrolone (as in Deca-Durabolin). Testosterone decanoate is also the longest acting constituent in Sustanon, greatly extending its release duration. The release time with Decanoate compounds is listed to be as long as one month, although most recently we are finding that levels seem to drop significantly after two weeks.

- Undecylenate: Chemical Structure $C_{11}H_20O_2$.
  - Also referred to as Undecylenic acid; Hendecenoic acid; Undecenoic acid.
  - This ester is very similar to decanoate, containing only one carbon atom more. Its release duration is likewise very similar (approximately 2-3 weeks). Undecylenate seems to be exclusive to the veterinary preparation Equipoise (boldenone undecylenate).

- Undecanoate: Chemical Structure $C_{11}H_{22}O_2$.
  - Also referred to as Undecanoic Acid; 1-Decanecarboxylic acid; Hendecanoic acid; Undecylic acid.
  - Undecanoate is not a commonly found ester, and only appears to be used in the nandrolone preparation Dynabolan, and oral testosterone undecanoate (Andriol). Since this ester is chemically very similar to undecylenate, it has a similar release duration (approximately 2-3 weeks). Andriol in fact works very poorly at delivering testosterone, bolstering the idea that oral administration is not the idea use of esterified androgens.

- Laurate: Chemical structure $C_{12}H_{24}O_2$.
  - Also referred to as Dodecanoic acid, laurostearic acid, duodecyclic acid, 1-undecanecarboxylic acid, and dodecoic acid.
  - Laurate is the longest releasing ester used in commercial steroid production, although longer acting esters do exist. Its release duration would be closer to one month than the other esters listed above, although realistically we are probably to expect a notable drop in hormone level after the third week. Laurate is exclusively found in the veterinary nandrolone preparation Laurabolin.

- **Procedure**

  - Color Tests – HCl salt form – p-Dimethylaminobenzaldehyde - red
  - TLC
    - Mobile System: TP, and TQ
    - Plates: Silica gel G, 250 um thick

- TP: Methylene chloride:ether:methanol:water (77:15:8:1.2)
- TQ: Dichloroethane:methanol:water (95:5:0.2)

- Developer
  - DPST solution: sulphuric acid-ethanol reagent
  - Spray the plate and heat at 105 ºC for 10 minutes.

- UV Analysis

  - Steroids are generally in water, methanol or oil

  - Sample in Methanol
    1. Run UV scan from 340 to 210 nm
    2. Compare spectra to standard spectra.

  - Aqueous Samples
    1. Evaporate sample and take up residue in Methanol
    2. Run UV scan from 340 to 210 nm
    3. Compare results to standard spectra.

  - Sample in Oil
    1. Place sample in test tube
    2. Gently add Methanol so that it lays on top
    3. Place sample in freezer for 20-30 minutes
    4. Slowly pipette methanol to new test tube
    5. Run UV scan from 340 to 210 nm
    6. Compare results to standard spectra.

  - Tablets
    1. Take a small portion of tablet and place in methanol
    2. Filter methanol if needed
    3. Run UV scan with same parameters as above samples.

- GC/MS Analysis
  - Liquid samples that are in an alcohol need only be diluted for GC-MS analysis.
  - Oil samples needed to be extracted to Methanol or Chloroform and placed in freezer to solidify oil
  - Aqueous samples can be air dried and taken up in methanol or basified and extracted to chloroform
  - Solid samples should be placed in base to Chloroform for injection
  - Compare data and retention time to known standard.

- IR Analysis
  - Solid sample use Drift attachment or ATR
    1. Run directly on IR
    2. Compare spectra to standard spectra.
    3. If spectra is not acceptable, perform appropriate extraction technique and re-run

  - Liquid Sample use Salt Cell for FTIR
    1. Run directly on IR
    2. Compare spectra to standard spectra.

3.If spectra is not acceptable, perform appropriate extraction technique and re-run.

APPENDIX A

All software libraries and library entries shall be reviewed by the technical leader prior to installation in an instrument.

Accepted instrumental data libraries for drug identification include:

- APD Drug Standards (GCMS)
- APD ATR Standards (FTIR)
- APD Standards (FTIR)
- Aldrich Condensed Phase sample Library (FTIR)
- Aldrich Vapor Phase Sample Library (FTIR)
- American Academy of Forensic Sciences (AAFS) (GCMS)
- Georgia State
    - Georgia State Forensic Drugs (GCMS)
    - George State Sample Library (FTIR)
    - HR Georgia State Forensic Drugs (FTIR)
- HR Nicolet Sample Library (FTIR)
- Hummel Polymer Sample Library (FTIR)
- National Institute of Standards and Technology (NIST) (GCMS)
- Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Monographs (All instrumentation)
- Sigma Biological Sample Library
- Wiley07 (GCMS)

NOTE: Data from any library not listed above may be included in the case record for informational purposes only. This data shall not be used in drug identification/confirmation, and shall be clearly annotated as such.

APPENDIX B

Accepted pharmaceutical reference sources for drug identification include:

- Amera-Chem (Rx-ID)
- Micromedix
- Pharmer.org
- Drugs.com
- Ident-a-drug
- Physician's Desk Reference (Any volume)
- Pharmaceutical packaging that is intact (i.e. unopened bottles or blister packs)

- Information obtained directly from manufacturer or manufacturer's website.